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Original Article

Toxoplasma gondii Infection: Relationship between Seroprevalence and Risk Factors among Inhabitants in Two Offshore Islands from Taiwan

Chia-Kwung Fan[a]*, Chien-Wei Liao[b], Ting-Chang Kao[c], Jin-Lian Lu[a], and Kua-Eyre Su[d]

[a]Department of Parasitology, College of Medicine, Taipei Medical University, Taipei, Taiwan, Republic of China,
[b]Department of Parasitology, National Yaming University, Taipei, Taiwan, Republic of China,
[c]Department of Parasitology, National Defense Center College, Taipei, Taiwan, Republic of China, and
[d]Department of Parasitology, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China

A seroepidemiological study of toxoplasmosis among inhabitants of Penghu Island and Kinmen Island offshore of Taiwan was performed using the latex agglutination test from July 1999 to June 2000. In order to determine risk factors for *Toxoplasma gondii (T. gondii)* infection, the effects of a history of eating raw/undercooked meats and raising pets were focused on using a self-administrated questionnaire. The seroprevalence (28.2%; 190/673) in Kinmen Island was significantly higher than that (2.7%; 8/293) in Penghu Island ($P < 0.001$). A significant difference in seroprevalence between both sexes was found in Kinmen Island ($P < 0.05$), but not in Penghu Island. The results of multiple logistic regression analysis showed that the older the age, the higher the OR in both Islands, yet a significant difference in seroprevalence between children and adults or the elderly was observed in Kinmen Island ($P < 0.001$). Moreover, those who had histories of raising cats or eating raw/undercooked meats seemed to have greater opportunities to become infected with *T. gondii* (OR=2.9, 95% CI=1.9-4.5, $P < 0.001$; OR=1.5, 95% CI=1.1-2.1, $P < 0.05$). In Penghu Island, a significant association between seroprevalence and a history of raising cats was also observed (OR=4.6, 95% CI=1.1-20.1, $P < 0.05$). Furthermore, workers, farmers, and fishermen seemed to be more susceptible to *T. gondii* infection than students in Kinmen Island.

Key words: *Toxoplasma gondii*, seroprevalence, risk factors, Penghu and Kinmen offshore Islands, Taiwan

Although toxoplasmosis is a cosmopolitan infection, the disease appears to be overshadowed in the tropics by other endemic diseases such as malaria and enteric fever. Toxoplasmosis has not generated a great deal of attention in Taiwan, while in other parts of the world it is considered an important and, at times, devastating parasite [1]. Humans acquire the infection from ingestion of infected meat, especially pork and goat meat, containing encysted forms of the parasite, or food contaminated with oocysts [2].

Analysis of the world literature on seroprevalence revealed that over 50% of the US population is seropositive [1], in parts of Europe levels reach nearly 90% [3], and in some Asian countries from 17% to 48% have serologic evidence of previous exposure to the parasite [4, 5]. Most infections are asymptomatic, but in some persons it eventually becomes symptomatic. Toxoplasmic encephalitis is a major disease in AIDS

Received March 22, 2001; accepted May 28, 2001.
*Corresponding author. Phone: +886-2-22434856; Fax: +886-2-22434856
E-mail: tedfan@tmu.edu.tw (C-K, Fan)

patients. Prenatal infection may also occur, resulting in newborns with congenital toxoplasmosis [6]. In the US, the *Toxoplasma*-associated annual economic public health burden reportedly exceeds US $400 million [7].

In regard to Taiwan's mainland, reports on the seroprevalence of *Toxoplasma gondii (T. gondii)* infection in Taiwanese and aborigines are available [8, 9, 10], but the seroprevalence of *T. gondii* infection in inhabitants of certain offshore islands from Taiwan remains unknown to date.

Therefore, in the present project, we conducted a seroepidemiological study of toxoplasmosis screened for sera anti-*Toxoplasma* antibodies in inhabitants of Penghu Island and Kinmen Island offshore from Taiwan by means of latex agglutination test from July 1999 to June 2000. Additionally, a self-administrated questionnaire that requested various personal details was included, and in order to determine risk factors for *T. gondii* infection, investigation of individual's history of eating raw or undercooked pork, goat, beef, or chicken meat and raising cat or dog pet were especially addressed in this study.

Materials and Methods

Study population and geographical aspects of islands. Penghu Island and Kinmen Island were chosen as sites for the present study due to their limited industrial development and lower standard of living relative to that of the Taiwan mainland. Penghu Island is located in the Taiwan Straits, which drains approximately 126.86 km^2, and is an offshore island near Taiwan's mainland with a total of approximately 89 thousand Taiwanese inhabitants. The Penghu archipelago consists of many islands and is located between latitudes 23°9′ to 23°47′N and longitudes 119°18′ to 119°42′E, with a mean elevation of 50–80 m, mean daily temperatures of 15–30 °C, and relative humidity varying between 15% and 40%. Average rainfall per year is about 1,000 mm. Kinmen Island is near *Fukin* Province of Mainland China, which drains approximately 150.456 km^2, is also an offshore island but far from Taiwan's mainland with a total of approximately 47 thousand Taiwanese inhabitants. Its location is between latitudes 24°9′ to 24°34′N and longitudes 118°8′ to 118°31′E, with a mean elevation of 50–150 m, a mean daily temperature of 10–25 °C, and relative humidity varying between 30% and 50%. Average rainfall per year is about 1,049 mm. Moreover, most inhabitants of both islands are workers, farmers or fishermen (Ministry of the Interior, ROC, 2000) (Fig. 1).

Serum Samples and Questionnaire. Inhabitants residing in 2 different geographical areas, including 5 villages in Penghu Island and 4 villages on Kinmen Island, were included in the present study. A total of 966 serum samples were obtained by venipuncture, of which 293 serum samples from inhabitants in Penghu Island and 673 serum samples from inhabitants of Kinmen Island were randomly collected from apparently healthy individuals who went to basic medical units for routine health examinations. The serum samples were collected from 128 males and 165 females from Penghu Island, and from 361 males and 312 females from Kinmen Island. The mean ages of inhabitants of Penghu Island were similar in both sexes and ranged between 7 and 78 yr in males and 6 and 80 yr in females. Mean ages were also similar in both sexes and ranged between 5 and 76 yr in males and 6 and 79 yr in females among inhabitants of Kinmen Island. This study was conducted from July 1999 to June 2000. Each subject completed a self-administrated questionnaire, the results of which were reviewed by trained public health nurses. Sociodemographic information was obtained directly from each individual through a self-administrated questionnaire that requested various personal details, including age, sex, weight, height, occupation, and residential district.

In addition, items regarding whether the subjects had histories of eating raw or undercooked meat (including pork, beef, goat and chicken) and raising pets (cats and dogs) were also included on the questionnaire. Informed consent was obtained from each subject before that person participated in the study.

Latex agglutination test (LA). In this study, all sera were screened using the *Toxoplasma* latex agglutination test employing commercial reagents (TOXO Test-MT, Eiken Co. Ltd, Tokyo, Japan). Compared to the Sabin-Feldman test, the sensitivity and specificity of this test were 96.3% and 97.1%, respectively [11]. The test was performed according to the manufacturer's instructions, with the help of a 96-well U-form microtiter plate (Nerbe, Germany), buffer solution, and latex solution. Sera found positive at titers $\geq 1/32$ (*i. e.*, 1:32 to 1:1024) were regarded as positive.

Statistical analysis. In the present study, the subjects were categorized into 3 age groups ($\leq$ 14-yr-old, children group; 15–59-yr-old, adult group; and $\geq$ 60-yr-

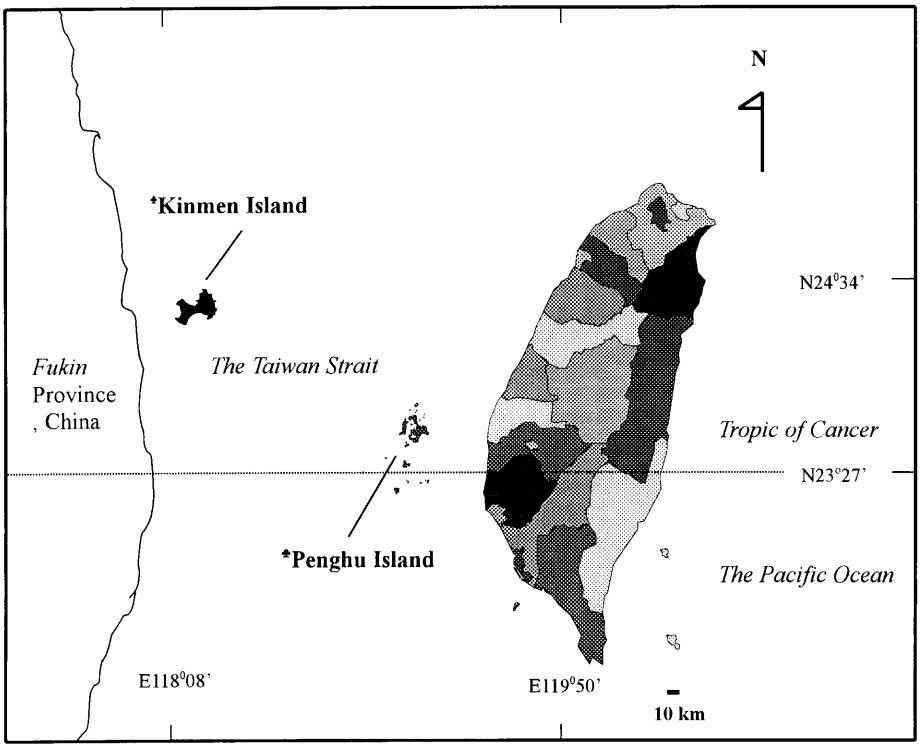

Fig. 1 Map of Taiwan showing study areas, (♣) indicates Penghu Island; (♠) indicates Kinmen Island. The large shaded area indicates the Taiwan mainland.

old, elderly group). In Taiwan, individuals are generally regarded as children until the age of 14, adults up to the age of 60, and elderly beyond the age of 60 (Ministry of the Interior of ROC, 1985). Serum samples that showed LA positivity were considered seropositive. Statistical analysis was performed using the SAS software system (SAS Institute, Inc., Cary, NC, USA). The increasing trend of age-specific seropositive rates was tested for statistical significance using the Chi-square test for trends. Multivariate-adjusted odds ratios (ORs) with their 95% confidence intervals were estimated by means of multiple logistic regression analysis. The statistical significance of differences in age-, sex-, risk factor- and occupation-adjusted seropositive rates among comparison groups was examined by testing the statistical significance of regression coefficients.

Results

Of the total 966 serum samples studied, 198 (20.5%; 198/966) were positive for *Toxoplasma* antibody as determined by LA test. Table 1 showed that the overall seropositive rates for toxoplasmosis were 2.7% (8/293) and 28.2% (190/673) for inhabitants in Penghu Island and Kinmen Island, respectively. According to sex groups, the prevalence was 3.6% (6/165) in females and the prevalence was 1.6% (2/128) in males for the inhabitants of Penghu Island. For inhabitants of Kinmen Island, the prevalence was 34.9% (109/312) in females and 22.4% (81/361) in males. According to age groups, the highest seropositive rate of 4.7% (2/43) was observed in the adult group, followed by 2.4% (4/167, 2/83) observed both in the child and elderly groups among inhabitants of Penghu Island. Regarding the inhabitants of Kinmen Island, the highest seropositive rate of 66.0% (33/50) was observed in the adult group, followed by 55.6% (55/99) in the elderly group, with the lowest of 19.5% (102/524) observed in the child group. With respect to occupation, seroprevalences were 3.8% (1/26), 3.4% (1/29), 3.2% (1/31), 2.9% (5/173), and 0.0% (0/34) for workers, farmers, unemployed, students, and fishermen, respectively, on Penghu Island. On Kinmen

Table 1 Demographic characteristics of the seroprevalence of the toxoplasma antibody among inhabitants of Penghu Island and Kinmen Island offshore of Taiwan (1999-2000)

Variable	Group	No. tested	No. positive	Positive rate (%)
Penghu Island				
Sex	Male	128	2	1.6
	Female	165	6	3.6
Age (Years)	≤14 (Children)	167	4	2.4
	15-59 (Adult)	43	2	4.7
	≥60 (Elderly)	83	2	2.4
Occupation	Worker	26	1	3.8
	Farmer	29	1	3.4
	Fisherman	34	0	0
	Student	173	5	2.9
	[a]Others	31	1	3.2
Mean		293	8	2.7
Kinmen Island				
Sex	Male	361	81	22.4
	Female	312	109	34.9
Age (Years)	≤14 (Children)	524	102	19.5
	15-59 (Adult)	50	33	66
	≥60 (Elderly)	99	55	55.6
Occupation	Worker	32	19	59.4
	Farmer	36	21	58.3
	Fisherman	53	28	52.8
	Student	529	113	21.4
	[a]Others	23	9	39.1
Mean		673	190	28.2

[a]unemployed individuals.

Island, the seroprevalences were 59.4% (19/32), 58.3% (21/36), 52.8% (28/53), 39.1% (9/23), and 21.4% (113/529) for workers, farmers, fishermen, unemployed, and students, respectively. Table 2 shows that the seropositivity for antibody against *T. gondii* in those who had histories of raising cats and dogs were 8.3% (3/36) and 4.4% (5/113), respectively, for inhabitants of Penghu Island. On Penghu Island, none of those participating in the study indicated on the questionnaire that they ate raw/undercooked meats, thus no seropositive subjects were available. On the other hand, of the inhabitants of Kinmen Island, the seropositive rates were 49.0% (47/96), 33.5% (66/197), and 28.6% (58/203) in those subject who raised cats, ate raw/undercooked meat (including pork, beef, goat, and chicken) and raised dogs, respectively. In the multiple logistic regression analysis, age, sex, occupation, and risk factor were included in the regression model. As shown in Table 3, sex, age, risk factor, and occupation remained significantly associated with seropositivity of *T. gondii* antibody for the inhabitants of Kinmen Island but not for those of Penghu Island. The multivariate-adjusted OR was 1.6 for females compared with males (OR = 1.6, 95% CI = 1.2-2.2, $P < 0.05$). The older the subject, the greater the multivariate-adjusted OR, which was as high as 5.0 for those older than 60 years (elderly group) as compared with those younger than 14 years (children group) on Kinmen Island (OR = 5.0, 95% CI = 3.2-7.8, $P < 0.001$). Moreover, the multivariate-adjusted ORs were 2.9, 1.5, and 1.0 for those who had histories of raising cats, eating raw/undercooked meats (including pork, goat, beef, and chicken) and raising dogs as compared with those without histories of raising cats, eating raw/undercooked meats, or raising dogs of the inhabitants of Kinmen Island, respectively (OR = 2.9, 95% CI = 1.9-4.5, $P < 0.001$; OR = 1.5, 95% CI = 1.1-2.1, $P < 0.05$; OR = 1.0, 95% CI = 0.7-1.4, $P = 0.898$). For the inhabitants of Penghu Island, the multivariate-adjusted OR was 4.6 for

Table 2 Seropositivity of antibody against *T. gondii* in individuals with histories of eating raw/undercooked meat or raising dogs or cats among the inhabitants of Penghu Island and Kinmen Island offshore of Taiwan

Variable	Group	No. tested	No. positive	Positive rate (%)
Penghu Island				
Eating raw/under-	No	293	8	2.7
cooked meats	Yes	0	0	-
Raising dogs	No	180	3	1.7
	Yes	113	5	4.4
Raising cats	No	257	5	1.9
	Yes	36	3	8.3
Kinmen Island				
Eating raw/under-	No	496	124	25
cooked meats	Yes	197	66	33.5
Raising dogs	No	470	132	28.1
	Yes	203	58	28.6
Raising cats	No	577	143	24.8
	Yes	96	47	49

those who had histories of raising cats (OR = 4.6, 95% CI = 1.1-20.1, $P < 0.05$). However, no significant association between seropositivity and history of eating raw/undercooked meats and raising dogs were found among the inhabitants on Penghu Island in this study ($P > 0.05$). Additionally, the multivariate-adjusted ORs were 2.4, 4.1, 5.2, and 5.4 for unemployed, fishermen, farmers, and workers compared with students in Kinmen Island, respectively (OR = 2.4, 95% CI = 1.0-5.7, $P <$

Table 3 Multivariate-adjusted odds ratios for various risk factors associated with seropositivity of *T. gondii* antibody among the inhabitants of Penghu and Kinmen Islands offshore of Taiwan

Variable	Group	Multivariate-adiusted odds ratios (95% CI)	*P* value
Penghu Island			
Sex	Male	1.0[a] (referent)	
	Female	2.4 (0.5-12.0)	0.280
Age (yr)			
Children	≤14	1.0[b] (referent)	
Adults	15-59	2.0 (0.4-11.3)	0.428
Elderly	≥60	1.8 (0.2-16.8)	0.994
Risk Factors			
Raising dogs	No	1.0[c] (referent)	
	Yes	2.7 (0.6-11.5)	0.159
Raising cats	No	1.0[d] (referent)	
	Yes	4.6 (1.1-20.1)	<0.05
Occupation	Student	1.0[f] (referent)	
	Others	1.1 (0.1-9.8)	0.919
	Farmer	1.2 (0.1-10.9)	0.870
	Worker	1.3 (0.1-11.6)	0.790
Kinmen Island			
Sex	Male	1.0[a] (referent)	
	Female	1.6 (1.2-2.2)	<0.05
Age (yr)			
Children	≤14	1.0[b] (referent)	
Adults	15-59	6.0 (4.0-9.0)	<0.001
Elderly	≥60	5.0 (3.2-7.8)	<0.001
Risk Factors			
Raising dogs	No	1.0[c] (referent)	
	Yes	1.0 (0.7-1.4)	0.898
Raising cats	No	1.0[d] (referent)	
	Yes	2.9 (1.9-4.5)	<0.001
Eating raw/under-cooked meats	No	1.0[e] (referent)	
	Yes	1.5 (1.1-2.1)	<0.05
Occupation	Student	1.0[f] (referent)	
	Others	2.4 (1.0-5.7)	<0.05
	Fisherman	4.1 (2.3-7.3)	<0.001
	Farmer	5.2 (2.6-10.4)	<0.001
	Worker	5.4 (2.6-11.2)	<0.001
Island	Penghu	1.0[g] (referent)	
	Kinmen	14.0 (6.8-28.7)	<0.001

[a]Adjusted variables included, age, risk factor, occupation.
[b]Adjusted variables included, sex, risk factor, occupation.
[c,d,e] Adjusted variables included, age, sex, occupation.
[f] Adjusted variables included, age, sex, risk factor.
[g] Adjusted variables included, age, sex, risk factor, occupation.

0.05; OR = 4.1, 95% CI = 2.3-7.3, $P < 0.001$; OR = 5.2, 95% CI = 2.6-10.4, $P < 0.001$; OR = 5.4, 95% CI = 2.6-11.2, $P < 0.001$). Furthermore, the inhabitants of Kinmen Island had a 14.0-fold seropositivity rate as compared with the inhabitants of Penghu Island (OR = 14.0, 95% CI = 6.8-28.7, $P < 0.001$).

Discussion

The latex agglutination test is widely used as an effective screening test for toxoplasmosis [12]. We used the LA test because of its simplicity and qualitative agreement with the dye test (DT) [13], and close correlation with ELISA [14] and direct agglutination (DA) test [15]. Compared to the Sabin-Feldman test, the sensitivity and specificity of this test were 96.3% and 97.1%, respectively [11].

The major inhabitants in Taiwan's main and offshore islands, including Penghu Island and Kinmen Island, are Taiwanese whose ancestors migrated from the *Fukin* Province of mainland China 400 yr ago, and both inhabitants sharing the same traditional culture and food habits [16]. *Toxoplasma* infections reportedly vary with ethnicity and are attributed to traditional culture and food habits [17]. However, the present study indicated that the seroprevalence of toxoplasmosis among inhabitants of Kinmen Island was higher than that in Taiwanese [8, 9], yet lower than that in aboriginal people [10] of Taiwan's mainland. A logistic regression analysis revealed that ingestion of various raw/undercooked meats and raising cats seemed to be the main risk factors in acquisition of *T. gondii* infection for inhabitants of Kinmen Island in the present study. However, among the seropositive subjects with histories of eating raw/undercooked meats, the meat they indicated that they ate most frequently was pork (33.8%, 68/201), followed by goat (28.8%, 49/170), beef (28.0%, 58/207), and chicken (21.2%, 7/33). Nevertheless, no significant difference in seroprevalence between people that did and did not eat raw/undercooked pork, goat, beef or chicken was observed (data not shown). To our knowledge, in Kinmen Island most meat was imported from Taiwan's mainland and some meat was transported from China, especially the *Fukin* Province, and whether these meats from China contained the parasite was unclear, whereas, a previous report indicated the seroprevalence of *T. gondii* infection in swine was 27.7% (1,073/3,880) in Taiwan [18]. Reportedly, the seroprevalence of *T. gondii* infection in pigs, goats, cattle, or chickens ranged from 1.8% to 67.4% in the world [19]. However, no reports on seroprevalence in swine, goats, cattle, or chickens are available regarding Kinmen Island to date.

Furthermore, many reports have indicated the associ-

ation of cat ownership with an increased risk of *T. gondii* infection [20]. To our knowledge, the foods to most of cats were similar to what their owners, and only fewer cases were fed with canned food. In addition, cats were not always kept indoors, often the case the cats wandered outdoors, thus cats might have a greater opportunity to become infected with *T. gondii* when they were wandering around the environment, and oocysts shed from cat feces could contaminate the cat's environment in addition to that of their masters. However, contamination of drinking water with *T. gondii* oocysts in Canada has been reported, confirming that domestic cats or cougar feces contaminated a surface water reservoir with *T. gondii* oocyst [21].

Moreover, raising dogs seemed not to be a risk factor for acquisition of *T. gondii* infection among inhabitants in Kinmen Island, since no significant difference in seroprevalence was observed between the groups with and without experience of raising dogs. However, *T. gondii* does not replicate in the gut of dogs and no oocysts are shed, thus dogs are considered to be uninvolved in the transmission of *T. gondii* [19]. However, Frenkel *et al.* (1996) indicated that dogs might play a more important role in the transmission of *T. gondii* than do cats due to the dogs' xenosmophilia [22]. However, since no reports on the prevalence of dogs infected with *T. gondii* are available regarding Kinmen Island, whether dogs share a similar *T. gondii* transmission route to their masters as do cats remains unclear.

On the other hand, the seroprevalence of toxoplasmosis among the inhabitants of Penghu Island was much lower than that reported in Taiwanese [8, 9] or aboriginal people [10] in Taiwan's mainland, although the lower seroprevalence among inhabitants of Penghu Island might be explained due to people with fewer opportunities for exposure to various risk factors of *T. gondii*. Yet, people with histories of raising cats had higher seroprevalence than that did those without histories of raising cats, which is a result similar to that found in Kinmen Island. Moreover, no significant association between seropositivity and history of eating raw/undercooked meats or raising dogs was found among inhabitants on Penghu Island. Thus, it seemed that the 2 factors were not the main risk factors for people becoming infected with *T. gondii* on this island.

The primary reason that most of the inhabitants of both islands who participated in our study were children and elderly people was that most of the young adults in these areas migrate to the Taiwan mainland to seek employment. It is noteworthy that children and elderly people have weaker immunity to various pathogens, thus these 2 populations might be more susceptible to *T. gondii* infection than are young adults [23]. Therefore, in order to protect them from *T. gondii* infection, enhanced hygienic knowledge and education appeared very urgent for these populations in Penghu Island.

Moreover, females with higher seropositive rates than males were found in both Islands, yet only a significant difference in seroprevalence between both sexes was found on Kinmen Island in the present study. However, several studies have reported sex differences in the prevalence of toxoplasmosis. In some instances, the prevalence was found to be higher in females [24], while in others it was higher in males [25] though Griffin and Williams (1983) found no sex difference [26]. Sex differences in most studies have been attributed to greater exposure to infected meat by either females or males, depending on food habits and culture [19]. In Taiwan, including Penghu Island and Kinmen Island, most females often take charge of food preparation and cooking, thus it could alternatively be explained that the higher seroprevalence of females was due to their higher opportunity in exposure to *T. gondii* infection.

Furthermore, the difference of the seroprevalence of toxoplasmosis between children, adults and the elderly of both islands investigated in the present study was significantly ambiguous. Nevertheless, the mean seroprevalence (15.3%, 106/691) among children in both Islands was higher than that reported in South Yorkshire (6.0%, 15/249), Ireland (12.8%, 163/1276), or Korea (7.7%, 42/542) [27, 28, 29]. Moreover, the seroprevalence of toxoplasmosis among inhabitants either of Penghu Island or Kinmen Island by age revealed that positivity was seen to increase with age, which was similar to results reported in Nepal, Bolivia, France, and Japan [15, 30-32]. However, it is acknowledged that seroprevalence increases with age, as seen in studies conducted in various countries [19].

However, many reports have indicated that swine, goat, cattle, and chicken raiser and slaughtermen were high-risk populations for the acquisition of *T. gondii* infection because they had higher opportunities for exposure to risk factor-pork, goat, beef, and chicken meat [33]. However, due to no swine, goat, cattle, and chicken raiser or slaughtermen having been included in the present study, the immune status of *T. gondii* infection

for these populations on both islands remained unclear. Nevertheless, in the present study, workers and farmers seemed to have higher seroprevalences of *T. gondii* infection than other populations either on Kinmen Island or Penghu Island. Interestingly, most seropositive workers indicated that they had the habit of consuming various raw/undercook meats, especially the pork, goat and beef, since they believed that such foods would provide more strength for their work. Thus, the high levels of seroprevalence in some workers might be ascribed to their eating habits. Moreover, the reason for the high seroprevalence in farmers was unclear. However, ingestion of oocysts in contaminated soil seems to be the main mode of transmission of toxoplasmosis for farmers in Japan [34].

Additionally, a positive correlation has been demonstrated between the duration of latent toxoplasmosis and the intensity of superego strength decrease, and a decrease in the strength of an individual's superego strength (the willingness to accept group moral standards), suggesting that this alteration in behavior was induced by *T. gondii* infection [35]. It may be relevant to examine whether the instability of the *T. gondii*-infected inhabitants of Kinmen Island leads to an increase in social problems in the Kinmen Island is concerned. Therefore, not only an investigation of the prevalence of *T. gondii* infection in domestic animals and isolation of the parasite from the various meats is necessary, but also an investigation of contamination with oocysts in soils, foods, and drinking water should be considered in regard to Kinmen Island.

Taken together, the prevalence of antibodies to *Toxoplasma* in our study indicates a high level of transmission among the inhabitants of Kinmen Island, yet there are no records of cases of congenital or postnatal toxoplasmosis on the island. This may be explained by the lack of serologic tests for toxoplasmosis in the past and/or by a low degree of suspicion of this disease among clinicians. To our knowledge, it is the first seroepidemiological report on the status of *T. gondii* infection among the inhabitants of Penghu and Kinmen offshore islands from Taiwan to date.

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