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Serological survey of *Toxoplasma gondii* infection among Urban (Manila) and Suburban (Dasmariñas, Cavite) Residents, Philippines

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ABSTRACT

Serological survey of *Toxoplasma gondii* infection was carried out in 68 urban (Manila) and 72 suburban (Dasmariñas, Cavite) residents, comprising 69 females and 71 males, age 16-56 years old. Using a survey questionnaire, the name and address, gender, age, weight, height, civil status, cat exposure, and additional information like history of pregnancy and current reproductive status of females were obtained. The presence of anti-*T.gondii* antibodies was confirmed using the Toxocell latex agglutination test. The pooled population revealed 38 (27.1%) seropositive (sero$^+$) cases, with higher infection rate among the suburban (30.6%) and female (32.0%) respondents. Differences in percent infection between the study groups, sexes and respondents with normal, overweight and underweight body mass index were insignificant. Of the 22 sero$^+$ females, 21 were in their reproductive age, including five cases in pregnant (23.81%) volunteers. Infection was appreciable among those who had apparent association with cats (32.3%), reinforcing the feline's primary role in the transmission of *T. gondii* to humans. Considering the dearth of baseline data on toxoplasmosis in the country, similar studies in other communities are highly recommended.

Key words: serological assay；*Toxoplasma gondii*; urban and suburban dwellers, Philippines

INTRODUCTION

Toxoplasma gondii is a coccidian apicomplexan parasite with a cosmopolitan distribution affecting a wide range of vertebrates, including humans (Dubey *et al*., 1998). The oocysts shed with the cat feces can contaminate soil, water, fresh fruits and vegetables, thru which the oocysts may be transmitted to animals and humans (Jones *et al*., 2005; Dubey and Beattie, 1988). Humans may also acquire the infection through undercooked infected meat or congenitally to the fetus (Song *et al*., 2005).

Documentation of human cases of *T. gondii* infection is worldwide. It is prevalent in Bangladesh (Ashrafunnessa *et al*., 1998), Indonesia (Konishi *et al*., 2000), Laos (Catar *et al*., 1992), Malaysia (Nissapatorn *et al*., 2004), Singapore (Lim *et al*., 1982), Thailand (Chintana, 1991), Vietnam (Sery *et al*., 1988), Japan (Konishi and Takahashi, 1987; Takahashi *et al*., 1985), and Korea (Lee *et al*., 2000). In the Philippines, the few earlier documentations relate to hogs (Manuel, 1982; Arambulo *et al*., 1974; Marbella, 1980) and cats (Dans, 2002). Recently, we reported high prevalence of Types I, II and III strains of *T. gondii* infecting *Rattus norvegicus* and *Rattus mindanensis* inhabiting agricultural, commercial and residential sites in Dasmariñas, Cavite, Philippines (Salibay and Claveria, 2006; 2005a; 2005b). To our knowledge, there exists only one published record of human toxoplasmosis in the country (Kawashima *et al*., 2000). In view of our recent findings of the endemicity of toxoplasmosis in *Rattus* spp. in Dasmariñas Cavite, and the paucity of documented data on *T. gondii* infection among the Filipinos, we attempted a serological investigation that targeted suburban and urban respondents residing in Dasmariñas, Cavite and Manila.

MATERIALS AND METHODS

Study group

Respondents comprised 68 and 72 persons from Manila (= urban) and Dasmariñas, Cavite (= suburban), respectively. They were amply briefed of the purpose of the study. The respondents were willing volunteers and as part of an earlier agreement, we are restricted in providing information other than those necessary in the study.

Prior to blood extraction intended primarily for anti-*T. gondii* antibodies (Abs) detection, each respondent was provided a questionnaire asking for the following personal data and other information: name and address, gender, age, weight, height, civil status and ownership and/or association with cat(s) within the vicinity of their residence and surroundings. To assess potential transmission of *T. gondii* infection to females, their reproductive status based on age and current/past pregnancies and their association with cats such as direct (i.e. via feeding and grooming of cats as pets) or indirect (via presence of cats in their surroundings) were also considered. The respondents were classified into four groups: ≤20, 21-30, 31-40 and ≥41 years old.

Determination of body mass index (BMI)

The respondent's BMI was determined using the formula: BMI = weight (kg)/height (meter^2) or BMI = kg/m^2 (Collin Bell *et al*., 2002). The BMI values were interpreted as follows: 18.5 or less (underweight); 18.5-24.0 (normal); 25.0-29.9 (overweight); 30.0-39.9 (obese); and 40.0 or greater (extremely obese).

Serum collection and serological assay and ABO blood type determination

Five ml blood was extracted from the arm vein, allowed to clot at room temperature for 30 min and centrifuged at 1,500 rpm for 5 min. Using pipettes, sera were individually transferred into properly-labeled tubes, stored in a 4-8°C refrigerator and were serologically processed within 24 hrs post-extraction. The respondent's ABO blood type was determined using a typing kit (Dunganon Company, United Kingdom). Blood extraction and tests were done at the Biology Laboratory, DLSU-Dasmariñas, Cavite, and Science Laboratory, Centro Escolar University, Manila, Philippines.

Sera were assayed for anti-*T. gondii* Abs using the Toxocell latex agglutination test (LAT) (BIOKIT S.A. Manufacturing Company, Barcelona Spain). The test kit contains a suspension of polystyrene latex particles of uniform size coated with soluble *T. gondii* antigen. On a disposable slide containing 50 µl of the serum, a drop of LAT suspension was added. Of the three slides, the last two served as positive control (= diluted human serum containing rabbit IgG anti-*Toxoplasma*) and negative control (= non-reactive diluted human serum). The serum was mixed with the reagent and the preparation gently rotated for 5 min on a shaker at 60-80 rpm. In a reactive serum the latex suspension showed clear agglutination (titre ≥15 IU/ml), while a non-reactive serum resulted to a suspension with a homogenous appearance.

Statistical method

The results according to age groups, gender and other parameters, including the respondent's association with cats were compared between urban and suburban study populations, and analyzed using the chi-square, student *t*-test and analysis of variance (ANOVA) at ≤ p=0.05.

RESULTS

The 140 respondents assayed for anti-*T. gondii* Abs comprised 69 females and 71 males, ranging from 16 to 56 years old**.** The urban group consisted of 68 respondents; 56 (82.3%) were 30 years old and younger (Table 1). In the suburban group, of the 72 volunteers, 44 (61.1%) were ≥31 years old. The ABO blood type profile of the pooled study groups revealed the preponderance of Type O (n=89), followed by

Type B (n=25), Type A (n=14) and Type AB (n=12) (Table 2). Based on the respondents' basal mass weight (BMI), 65 (46.4%) had normal weight (NW), 31 (22.1%) were overweight (OW), 29 (20.7%) underweight (UW), and 15 (10.7%) obese (OB).

Thirty-eight (27.1%) of the 140 respondents tested seropositive (sero$^+$) (Table 1). More sero$^+$ cases were detected among the suburban relative to the urban volunteers; the difference however, is insignificant. Infection was higher in females (32.0%), particularly among the ≥31 yrs age group compared to males (22.5%) (Fig.1). Of the 22 sero$^+$ females, 21 were in their reproductive age (≤ 20 to 41 years), including five cases (23.0%) among pregnant volunteers.

Table1. Comparison of results of serological assay for anti-*T. gondii* Abs between urban and suburban population across four age groups.

Age Groups	Urban		Suburban		Sub-Total	
	N	sero+ (%)	n	sero+ (%)	n	sero+ (%)
≤ 20	28	5 (17.8)	8	3 (37.5)	36	8 (22.2)
21-30	28	8 (28.6)	20	3 (15.0)	48	11 (22.9)
31-40	7	0 (0.0)	27	11 (40.7)	34	11 (32.4)
≥ 41	5	3 (60.0)	17	5 (29.4)	22	8 (36.4)
Total (%)	68 (48.6)	16 (23.5)	72 (51.4)	22 (30.6)	140	38 (27.1)

Among the 24 blood type B respondents, 12 (48.0%) were sero$^+$ compared to only 22 (24.7%) sero$^+$ cases among 89 type O volunteers (Table 2). Sero$^+$ cases in types O and B were appreciably higher compared to types A and AB respondents. Moreover, infection was detected in 13 (34.2%), 15 (39.5%), 9 (23.7%) and 1 (2.6%) normal weight, underweight, overweight and obese, volunteers, respectively (Table 3), revealing insignificant difference between the sero$^+$ NW, UW and OV volunteers.

Of the 65 respondents with either direct or indirect exposure/association with cats, 21 (32.3%) were sero$^+$, and among the 75 volunteers without close association, only 17 (22.7%) were infected (Table 4). Overall infection (55.3%) was significantly higher among respondents with close association with cats.

Table 2. Seroprevalence: ABO blood types of respondents.

Age Group	Blood Type			
	A	B	AB	O
≤20	0/2	4/8	0/3	4/23
21-30	2/8	2/3	0/3	7/34
31-40	0/2	4/9	1/4	6/19
≥41	0/2	2/5	1/2	5/13
Total (%)*	2/14 (14.3)	12/25 (48.0)	2/12 (16.7)	22/89 (24.7)
(% sero$^+$)**	5.3	31.6	5.3	57.9

* sero$^+$ cases/number respondent per blood type across four age groups

**sero$^+$ cases/ 38 total sero$^+$ cases

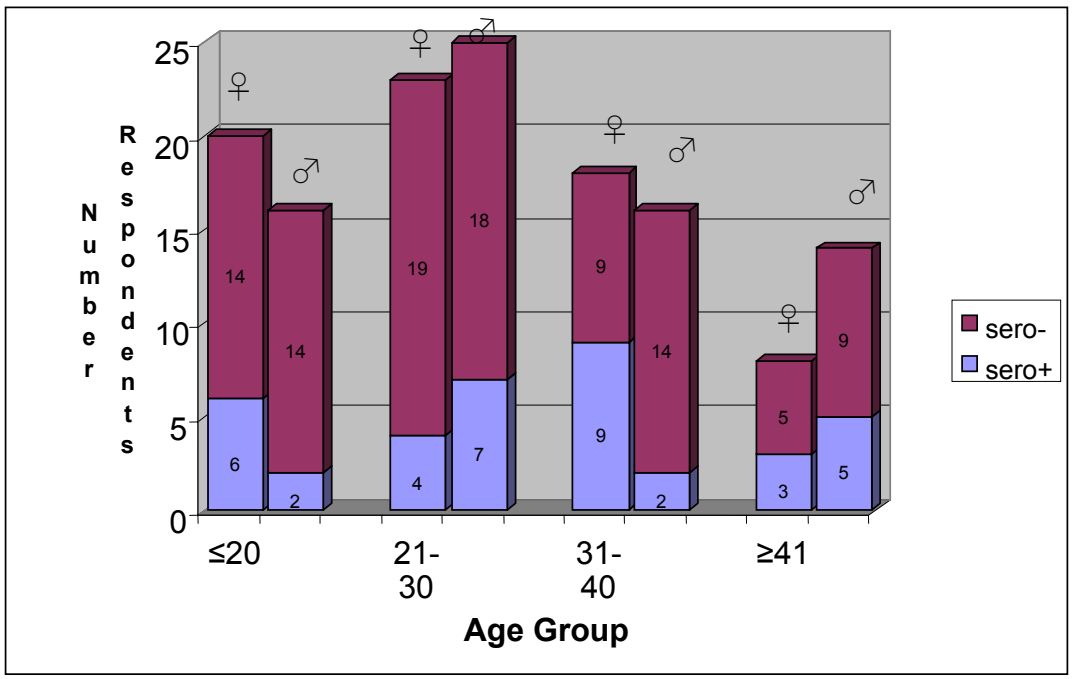

Figure1. Sex-associated distribution of *T. gondii* infection in pooled urban and suburban respondents according to age groups. N=140

DISCUSSION

Currently obtained serological data on higher sero$^+$ cases among suburban residents reinforce those of Kawashima *et al*. (2000), who reported higher prevalence among Filipinos residing in rural areas compared to cities. Similar records of higher prevalence among the rural folks have been directly correlated with people's lifestyle and occupation, favoring higher chances of oocysts ingestion through contaminated soil and water, and intake of contaminated agricultural crops and animal produce and unpasteurized milk of infected farm animals (Ertug *et al*., 2005; Jones *et al*; 2005). The Similar risk of exposure to *T. gondii* faced

Table 3. Distribution of sero+ cases as to BMI of respondents.

Age Group	Body Mass Index			
	NW	UW	OW	OB
≤ 20	2/22	3/9	2/3	1/2
21-30	4/29	6/9	1/6	0/4
31-40	4/7	4/6	3/15	0/6
≥ 40	3/7	2/5	3/7	0/3
Total (%)*	13/65 (20.0)	15/29 (51.7)	9/31 (29.0)	1/15 (6.7)
(% sero$^+$)**	34.2	39.5	23.7	2.6

* sero$^+$ cases/# respondents per BMI across four age groups

**sero$^+$ cases/ 38 total sero$^+$ cases NW- normal weight UW- underweight OW-overweight OB-obese

by urban and suburban dwellers does not seem to corroborate earlier inference that the presumably high level of hygiene in urban relative to rural/suburban settings lowers the risk of human infection (Excler *et al*., 1988). The higher number of sero$^+$ cases among the underweight respondents is in agreement with slow or stunted growth in infected animals and humans (Roberts and McLeod, 1999; El Mossalamy *et al*., 1997). The susceptibility of both sexes to *T. gondii* is consistent with earlier studies (Konishi *et al*., 2000; Al-qurashi, 2004). Considering the association of increased incidence of certain diseases with particular blood types (Markell *et al*., 1999), the significant number of type B volunteers who tested sero$^+$ cases is worth noting.

The 95.4% infection among females in their reproductive age corroborates those of Al-qurashi (2004), Konishi *et al*. (2000) and Bobic *et al*. (1998). The higher rate of infection among those with direct or indirect association with cats further underpins the crucial role of cats in the transmission of infection to humans (Terazawa *et al*., 2003; DeFeo *et al*., 2002; Ashrafunnessa *et al*., 1998). Considering possible congenital transmission, females in their reproductive ages should be wary of possible infection.

Differences in socio-cultural practices, rat species population and distribution, topography, environmental factors, transmission route(s) and host age and sexes influence the prevalence and incidence of *T. gondii* infection (Yamaoka and Konishi, 1993; Bobic *et al*., 1998; Dubey and Beattie, 1988).

Table 4. Summary of serological assay among respondents with and without association/exposure to cats.

Age group (n)	With cat exposure•			Without cat exposure		
	sero$^+$	sero$^-$	n	sero$^+$	sero$^-$	n
≤20 (36)	6	15	21	2	13	15
21-30 (48)	7	16	23	4	21	25
31-40 (34)	5	7	12	6	16	22
≥41 (22)	3	6	9	5	8	13
Total	21	44	65	17	58	75
(%)	(32.3)	(67.7)	(46.4)	(22.7)	(77.3)	(53.6)

- direct exposure (feeding and grooming)
- indirect exposure (cats in the vicinity of households)

Considering the dearth of baseline data on human toxoplasmosis in the country, epidemiological studies are highly recommended targeting populations and communities in urban, suburban and rural settings where rats and cats are widespread.

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