Prevalences of Antibodies to *Toxoplasma gondii* in Cats and Humans in Taipei, Taiwan

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ABSTRACT

The prevalences of IgG and IgM antibodies to *Toxoplasma gondii* in cats and humans in Taipei, Taiwan were measured by using both kinetics-based enzyme-linked immunosorbent assay and immunoblotting. Stray cats had significantly higher seroprevalence (37.0%, n=100) than pet cats (14.0%, n=57). Also, stray cats had higher IgG (1:393 vs. 1:22), but similar IgM (1:23 vs. 1:20) geometric mean titers (GMT) compared to the pet cats. Stray female cats had a significantly higher seroprevalence (50.0%) than males (24.0%). For the comparisons of the pet animals, a logistic regression model with five factors (sex, age, weight, breed, domain) was analyzed. In pet cats, as the animals were growing older the odds ratio for them to be seropositive was getting higher. However, seroprevalence was not sex-, weight-, breed-, or indoor/outdoor activity-dependent. In humans, the prevalence of IgG antibody (which was not sex-dependent) was 7.0% (n=100) and the GMT was 1:177. Veterinarians did not have higher seroprevalence than that of nurses.

Key words: Cat, *Toxoplasma gondii*, Man-animal relationships

Introduction

Toxoplasma gondii infection is widespread and is of economic as well as public health importance. Humans and animals become infected either by eating food contaminated with sporulated oocysts, or by consuming raw or undercooked meat containing cysts (reviewed in Frenkel, 1988; Derouin, 1992; Dubey, 1994). Understanding the prevalence of toxoplasmosis in cats and humans is important, because 1) cat is a definitive host that sheds oocysts; and 2) there is an increase in the number of cases of acquired immune deficiency syndrome patients showing severe disseminated toxoplasmosis (Luft and Remington, 1992; Richards *et al.*, 1995).

Antibody detection has been used as an indicator of *T. gondii* infection. IgG antibody is detectable at about 2 weeks after the infection (Lin and Bowman, 1991; Lin *et al.*, 1992), and remains throughout the life of the host (Remington and Desmonts, 1983). Thus, detection of IgG antibody in a single sample cannot demonstrate the presence of active infection. Testing for IgM antibody, which

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appears at about 1 week after the infection, permits the detection of recent infection (Lin and Bowman, 1991; Lin *et al.*, 1992). Enzyme-linked immunosorbent assay (ELISA) for antibody detection has the advantages of screening a large number of samples, with good sensitivity and specificity. Moreover, the specificity of antibody detection can be dramatically improved if ELISA is used in conjuction with immunoblotting (Gross *et al.*, 1992) which is able to test the presence of antibodies to *T. gondii*-specific 30 kD-surface antigen (Kasper and Khan, 1993).

A survey using kinetics-based ELISA (KELA) of IgG antibodies to *T. gondii* in cats in Taiwan has been published (Lin *et al.*, 1990). However, that survey was performed mainly on animals kept at cat farms (70 out of 117) and only 16 stray cats were examined. In addition, observed indications of a relationship between animal contact and human toxoplasmosis in turn pose a further question regarding the risk of infection for veterinarians, who--by virtue of their occupation--are exposed to infected animals. Currently, no such data are available in Taiwan and the survey conducted on humans by using an IgG-immunofluorescence antibody assay only tested pregnant women and neonates (Yu, 1985).

According to the previous report (Lin and Su, 1997), "relative" true positive is defined as being positive in both KELA and immunoblotting. Thus, KELA or immunoblotting has 100% relative sensitivity. The relative specificities of KELA and immunoblotting for the detection of IgG antibody in cats are 95.8% and 93.7%, and in humans 98.9% and 100%. The relative specificities of KELA and immunoblotting for the measurement of IgM antibody in cats are 99.5% and 99.0% (Lin and Su, 1997). The present study was performed, using both KELA and immunoblotting to survey the prevalence of antibodies to *T. gondii* in humans and also in both stray and pet cats, in order to provide the basic data necessary for studying the epidemiology of *T. gondii* in Taipei, Taiwan. Similar study on the seroprevalences to *T. gondii* in privately-owned dogs in Taiwan has been published (Lin, 1998).

Materials and Methods

Sampling

Venous blood samples were randomly collected from 157 cats (100 stray and 57 pet) and 100 humans in Taipei, Taiwan in the period of 1995-1996. The pet animals used were all the cases of veterinarian W.-L. Su at the National Taiwan University Animal Hospital during 1995-1996. Blood samples of stray animals were collected randomly from cats captured on the streets or those kept in animal shelters. All human blood samples were obtained from the volunteers including 31 veterinarians, 58 nurses, and 11 individuals in other occupations. Veterinarians were those at the National Taiwan University Animal Hospital and other private animal clinics. Nurses were those working at the National Taiwan University Hospital. People in other occupations were the owners of pet animals. Blood samples were allowed to clot at

4℃ overnight, sera were collected and stored at -30℃ until later use.

***Toxoplasma gondii* antigen preparations**

T. gondii RH tachyzoites obtained from the peritoneal cavity of mice were washed twice with phosphate buffered-saline (PBS) and purified by 3μm Nuclepore polycarbonate membranes (Costar Corporation, Cambridge, MA, USA). Soluble whole tachyzoite antigens were prepared as described (Lin and Bowman, 1991; Lin *et al.*, 1992). Briefly, tachyzoites were suspended in PBS of pH7.4, and subjected to three cycles of freezing-thawing and were ultra-sonicated 10 times, at 35 Watts/30 sec each (Heat Systems Inc., Farmingdale, NY, USA). The disrupted organisms were centrifuged at 10,000 xg for 40 min at 4℃, and the supernatants were collected as soluble antigens.

In case of soluble membrane antigens, tachyzoites were suspended in 1% Nonidet-P40 (NP40, Sigma Chemical Co., St. Louis, MO, USA)-50 mM Tris (pH 8.0). Following incubation at 4℃ for 12 h, the suspensions were centrifuged and the resultant supernatant was collected (Gross *et al.*, 1992). After determination of protein concentrations (DC protein assay, Bio-Rad Lab., Richmond, CA, USA), both the antigens were stored at -70℃.

Antibody detection by kinetics-based enzyme-linked immunosorbent assay

Fifty μl of soluble whole tachyzoite antigens, prepared as 20μg/ml in 0.1M bicarbonate buffer of pH9.6, were placed in each well of the MaxiSorp™ microtiter plates (Nunc, Roskilde, Denmark). The plate was incubated overnight at 4℃ and washed with PBS, containing 0.05% Tween 20 (PBST), by an ELISA washer (Dynatech Lab., Chantilly, VA, USA). One hundred μl of PBST containing 3% skim milk (Difco Lab., Detroit, MI, USA) were then added as a blocking solution. After 40 min of incubation at 37℃, the plate was washed again. Fifty μl of 1:20 diluted sample in PBST containing 3% skim milk were then added to the wells, followed by incubation at 37℃ for 40 min. Positive and negative sera were also run with each test. Samples obtained from naturally infected subjects were used as positive controls. After washing, 100μl of 1:8,000 (for IgG) or 1:5,000 (for IgM) peroxidase-labeled conjugates were added. Conjugates used here were either goat anti-human IgG Fc (Organon Teknika Co., Durham, NC, USA), goat anti-cat IgG Fc, or sheep anti-cat IgM (μ chain) (Biogenesis Ltd., England, UK). The plate was then incubated at 37℃ for 40 min, washed, and finally rinsed with distilled water. One hundred μl of the substrate containing *O*-phenylenediamine (Sigma Chemical Co.) were added. The plate was read at 450nm using a microplate reader (Dynatech Lab.). IgG and IgM, specific for *T. gondii* antigens, were detected by KELA (Lin *et al.*, 1990). Accordingly, the rate of reaction between the bound peroxidase conjugate and substrate was determined by recording three absorbance readings at 2-min intervals each. These intervals provide a linear relationship between absorbance values and times so that the

resulting sample regression coefficient (slope representing the rate of substrate conversion by enzyme) is directly proportional to the quantity of analyte present in the sample. Sera were screened in triplicate and the mean KELA value of ≥0.02 was considered positive. "Relative" true positive samples, which were positive in both immunoblotting and KELA, were run again at serial dilution in KELA. The last dilution giving value ≥0.02 was the titer of the sample.

Immunoblotting assay

IgG or IgM KELA positive sera were further confirmed by immunoblotting assay. A minigel apparatus (Biometra Inc., Tampa, FL, USA) was utilized for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and all the reagents used were purchased from Bio-Rad Lab. NP-40 extracted *T. gondii* membrane antigens were mixed with reducing sample buffer and heated for 5 min at 90℃. Five μl of the above buffer, containing 12.5μg of protein or pre-stained low molecular weight standards (Bio-Rad Lab.), was then applied to 12% SDS-polyacrylamide gel and electrophoresed at 120 volts for 1 hr. The gel was then transblotted onto a nitrocellulose (NC) paper (BA83, Schleicher & Schuell, Germany) by using a blotting apparatus (Biometra Inc.). After blocking with 5% skim milk-PBS overnight at 4℃, NC paper was incubated with the sample diluted to 1:100 in 5% skim milk-PBSTT (PBST with 1% Triton X-100) at room temperature for 1 hr. Positive and negative sera were also run with each test. The NC paper was then washed and incubated with various peroxidase-labeled conjugates (see antibody detection by KELA) at room temperature for 1 hr. Once again, the NC paper was washed and substrate containing 4-chloro-1-naphthol (Sigma Chemical Co.) was added. The positive result was visualized by the appearance of 30-kD band.

Data analysis

Only the sera which tested positive in both KELA and immunoblotting assays were considered positive. Geometric mean titers (GMT) were compared by t-test. In case of humans, comparisons were made on the basis of sex and occupations. In stray cats, the association of seroprevalence with sex was analyzed. Fisher's exact test was used for the above comparisons. In pet cats, a logistic regression model with 5 factors [sex, age, weight, breed, domain (either kept indoors or outdoors)] was analyzed. The computation of logistic regression was done by using SAS GENMOD procedure (SAS Institute Inc., 1993). Significance in the model was tested using likelihood-ratio chi-square. The difference was considered to be significant for $P<0.05$, or marginally significant for $0.1>P>0.05$.

Results

The prevalences and titers of serum IgG and IgM antibodies to *T. gondii* in cats and humans are listed in Tables 1-3. Only small numbers of cats (1.8 - 5.0 %) were recently

Table 1. Prevalences of IgG and IgM antibodies to *T. gondii* in cats and humans in Taipei, 1995-1996.

Category	No.	Positive in IgG		Positive in IgM		Positive in both IgG & IgM		Positive in either IgG or IgM		
		No.	%	No.	%	No.	%	No.	%	95%C.I.
Cat										
Stray	100	37	37.0[a]	5	5.0	5	5.0	37	37.0[a]	(0.28, 0.47)
Pet	57	8	14.0[b]	1	1.8	1	1.8	8	14.0[b]	(0.05, 0.23)
Human	100	7	7.0	NA		NA		7	7.0	(0.02, 0.12)

C.I.: confidence interval.
a,b: Different superscript letters indicate P=0.001 in Fisher's exact test.
NA: not available.

Table 2. IgG antibody titers to *T. gondii* in cats and humans in Taipei, 1995-1996.

Category	No. positive	Reciprocal of IgG antibody titers									
		20	40	80	160	320	640	1280	2560	5120	GMT
Cat											
Stray	37	3	6	2	3	1	5	9	7	1	393
Pet	8	7	1	0	0	0	0	0	0	0	22
Human	7	1	0	1	2	1	2	0	0	0	177

GMT: Geometric mean titer.
Comparison by t-test (stray vs. pet cats; P<0.001, df=44).

infected (IgM positive), making it difficult to carry out detailed IgM analyses. Therefore, comparisons were made on the basis of those which tested positive in either IgG or IgM.

Stray cats had higher seroprevalence and higher IgG GMT than the pet cats (Tables 1, 2). Stray female cats (25/50=50.0%) had higher (Fisher's exact test, P=0.004) antibody prevalence than males (12/50=24.0%). For the pet animals, we compared five factors (sex, age, weight, breed, domain) which might affect the seroprevalence. A logistic regression model including all five factors was designed to fit to the cat data. The analysis showed that none of the five factors have significant effects on the positive response (data not shown). However, 1) the age factor with the least P value (P=0.26) might have some effect on the positive response; and 2) the previous study on the seroprevalences to *T. gondii* in pet dogs showed that the logistic regression model had significant effects for age, weight, and breed (Lin, 1998). Therefore, we constructed a three factor (age, weight, breed)-contingency table (Table 4) and fitted this table with a simple

Table 3. IgM antibody titers to *T. gondii* in cats in Taipei, 1995-1996.

Category	No. Positive	Reciprocal of IgM antibody titers			
		20	40	80	GMT
Stray	5	4	1	0	23
Pet	1	1	0	0	20

GMT: Geometric mean titer.
Comparison by t-test (P=0.70, df=5).

logistic regression model: $\log[\pi\ (1-\pi)] = \mu + A_i$. This model fitted the data of pet cats in Table 4 well (with $G^2=5.11$ and df=6) and we found that the age factor had marginally significant effect (P=0.093) on the positive antibody response. The maximum likelihood estimate of age effect was 1.26 with standard error of 0.68. This indicated that the odds for older pet cats having positive response was 3.53 times (3.53=exp1.26) higher than that of younger pet ones, though it was marginal significance ($0.1>P>0.05$). This result roughly showed that as the cats were growing older the odds for them to be infected was getting higher.

The human IgG antibody prevalence was not sex- or occupation-dependent (Table 5). Veterinarians did not have higher seroprevalence than nurses whose job is not dealing with cats. Because there were only 11 people in other occupations, this group was not used for analysis.

Table 4. The contingency table of antibody response to *T. gondii* in pet cats in Taipei, 1995-1996.

Group	Explanatory factors			No.	Total
	Age	Weight	Breed	positive	No.
1	1	1	1	1	6
2	1	1	2	0	16
3	1	2	1	0	3
4	1	2	2	1	6
5	2	1	1	2	6
6	2	1	2	1	8
7	2	2	1	1	5
8	2	2	2	1	3
				7	53

Age (years): 1) ≤1, 2) >1.
Weight (kg): 1) ≤3 2) >3
Breed: 1) mixed, 2) pure
Four of the 57 cats and one of the 8 seropositive cats are not listed, because their ages cannot be clearly determined.

Table 5. Prevalence of human serum IgG antibody to *T. gondii* with sex and occupations in Taipei, 1995-1996.

Category	No. positive/ No. tested	Percent positive	P value
Sex			
Female	4/74	5.4	
Male	3/26	11.5	P=0.128
Occupation			
Veterinarian	1/31	3.2	
Nurse	3/58	5.2	P=0.336

Fisher's exact test.

Discussion

The present paper is the first report concerning the prevalences of IgG and IgM antibodies to *T. gondii*, measured by combination of both KELA and immunoblotting assay, in cats and humans in Taipei, Taiwan. Although such serial testing may decrease sensitivity, it enhances specificity. The various comparisons presented here were not based only on IgM positive samples, because only a small number of cats had active infection. It is not understood at present why the infected stray animals had higher IgG antibody GMT than the infected pet animals.

The seroprevalence in cats was higher than that of previous report (7.7%, n=117) (Lin *et al.*, 1990). It is possible that in the previous report, only IgG antibody was measured and the survey was performed mainly on animals in the farms (70 out of 117). Indeed, we found antibody prevalence in stray cats was 37.0%. The stray cats are more reliant on hunting for survival and thereby are more implicated in the

transmission cycle of the parasites. The seroprevalence [7.0%, 95% confidence interval = (0.02, 0.12)] in humans observed in this study was close to that observed from the survey on pregnant women (9.2%, n=1,796) and neonates (10.3%, n=1,121) (Yu, 1985).

The present study showed female stray cats had higher seroprevalence than males. However, the other reports have failed to detect any sexual variation in antibody prevalence (Childs and Seegar, 1986). Nevertheless, female mice are known to be more susceptible than males to *T. gondii* infection (Kittas *et al.*, 1984). Female sex hormones (Kittas and Henry, 1979; 1980) and the failure of female animals to respond quickly in terms of T-cell proliferation and interferon-γ production (Roberts *et al.*, 1995) compared to their male counterparts, may account for their higher susceptibility. Concurrent with the previous report (Childs and Seegar, 1986), as the pet animals were getting older the odds for them to be infected was getting higher. This is because once animals are infected, IgG antibodies remain in the body throughout the life of the host (Remington and Desmonts, 1983). Unlike the findings in pet dogs (Lin, 1998), we failed to detect variation in seroprevalence with respect to body weight or breed in pet cats. This might be due to the small sample size examined.

In humans, there was no significant difference in IgG antibody prevalence between veterinarians and nurses. This result is similar to those noted in other reports that veterinarians--though frequently come in contact with animals--are not the major at risk group (Behymer *et al.*, 1973; Gupta *et al.*, 1985). In Taiwan, most pigs have been found seropositive in *T. gondii* (Chang *et al.*, 1990) and pork is a major meat source. However, meats are often frozen before sold and Taiwanese tend to cook meat well before eating. It is known that both freezing and heating render *T. gondii* nonviable (Dubey, 1974; Dubey *et al.*, 1990). Therefore, more investi-gations are required to understand the major source of *T. gondii* infection in Taiwan.

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臺北市貓及人之弓蟲抗體盛行率

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摘要

以 kinetics-based enzyme-linked immunosorbent assay 及 immunoblotting 偵測臺北市貓及人之弓蟲 IgG 與 IgM 抗體盛行率。街貓 (37.0%, n=100) 比家貓 (14.0%, n=57) 有顯著高的抗體盛行率。同時，街貓有比家貓高的 IgG geometric mean titer (GMT) (1:393 vs 1:22)，但兩者有相似的 IgM GMT (1:23 vs 1:20)。街貓之中，雌性 (50.0%) 比雄性 (24.0%) 有顯著高的抗體盛行率。家貓之比較，乃利用一 logistic regression model 分析五種因子 (性別，年齡，重量，品種，活動領域)。結果發現，當家貓逐漸年老，其抗體陽性之 odds ratio 也跟著升高。然而，抗體盛行率和性別，重量，品種，及室內外活動領域無關。在人類，IgG 抗體盛行率為 7.0% (n=100)，其 GMT 為 1:177，和性別無關。獸醫師之弓蟲抗體盛行率並沒比護士高。

關鍵詞：貓、弓蟲、人與動物之關係