Seroprevalences of Antibodies to *Toxoplasma gondii* in Stray Dogs in Taipei

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(Received: 8 August 2003, accepted: 6 January 2004)

**ABSTRACT**

*Toxoplasma gondii* infection is widespread and is of economic as well as public health importance. To provide the basic data necessary for studying the epidemiology of toxoplasmosis in stray dogs in Taipei, the seroprevalences of IgG and IgM antibodies to *T. gondii* were measured using a kinetics-based enzyme-linked immunosorbent assay in conjunction with immunoblotting. Immunoblotting is able to detect the presence of antibody to *T. gondii*-specific 30 kD-surface antigen. The results showed that total seropositivity was 8% (n=100) with a 95% confidence interval of (0.0535, 0.152). The prevalences of IgG and IgM antibody to *T. gondii* were 6% and 3% respectively. Nevertheless, this difference was not statistically significant (McNemar's test, P=0.0898). Only a small number of stray dogs had active *T. gondii* infection (IgM positive). The prevalences of *T. gondii* antibody were 11.4% (n=44) for males and 5.4% (n=56) for females. Antibody prevalence was not sex-dependent (Fisher's exact test, P=0.2951).

**Key words:** dog, *Toxoplasma gondii*, antibody

**Introduction**

*Toxoplasma gondii* is an obligate intracellular protozoa. *T. gondii* infection is widespread and is of economic as well as public health importance. *T. gondii* can cause death in infected neonatal and perinatal animals. In humans, congenital toxoplasmosis and fatal disseminated toxoplasmosis in acquired immunodeficiency syndrome patients are significantly important. Humans and animals become infected either by eating food contaminated with sporulated oocysts, or by consuming raw or undercooked meat containing cysts (Dubey, 1994; Davidson, 2000). IgG antibody is detectable at about 2 weeks after the infection (Lin and Bowman, 1991; Lin et al., 1992), and remains measurable 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 appears at about 1 week after the infection) permits the detection of recent infection (Lin and Bowman, 1991; Lin et al., 1992). The enzyme-linked immunosorbent assay (ELISA) for antibody detection has the advantages of screening a large number of samples, with good sensitivity and specificity (Lin, 1998). Moreover, the specificity of antibody detection can be dramatically improved if ELISA is used in conjunction with immunoblotting (Gross et al., 1992). Immunoblotting is able to detect the presence of antibody to *T. gondii*-specific 30 kD-surface antigen (Kasper and Khan, 1993).

It has been shown that dogs are involved in the mechanical transmission of *T. gondii* to humans after ingesting cat feces or by rolling in cat feces containing oocysts (Lindsay et al., 1997). Also, the prevalence of toxoplasmosis in stray dogs is a good indication of the extent of the infection in

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the environment (Ali et al., 2003). Therefore, the present study was performed using both kinetics-based ELISA and immunoblotting to survey the prevalence of antibodies to T. gondii, in order to provide the basic data necessary for studying the epidemiology of toxoplasmosis in stray dogs in Taipei.

Materials and Methods

Blood sample collection

Venous blood samples were obtained from stray dogs in Taipei animal shelters in 2001 whenever they were going to be euthanized. A total of 100 samples (44 males and 56 females) were collected and allowed to clot at 4°C overnight. Sera were then collected and stored at -30°C until use.

Toxoplasma gondii antigen preparations

T. gondii RH tachyzoites obtained from the peritoneal cavity of mice were washed twice with phosphate buffered-saline (pH 7.4) and purified by 3 µm 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 phosphate buffered-saline, and subjected to three cycles of freeze-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°C, and the supernatants were collected as soluble antigens for kinetics-based ELISA.

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

Antibody detection by kinetics-based enzyme-linked immunosorbent assay

IgG and IgM, specific for T. gondii antigens, were detected by kinetics-based ELISA (Lin et al., 1990). Fifty µl of soluble whole tachyzoite antigens, prepared as 20 µg/ml in 0.1 M bicarbonate buffer (pH 9.6), were placed in each well of the MaxiSorp™ microtiter plates (Nunc, Roskilde, Denmark). The plate was incubated overnight at 4°C and washed with phosphate buffered-saline containing 0.05% Tween 20, by an ELISA washer (Dynatech Lab., Chantilly, VA, USA). One hundred µl of phosphate buffered-saline containing Tween 20 and 3% skim milk were then added as a blocking solution. After 40 min of incubation at 37°C, the plate was washed again. Fifty µl of a 1:20 sample diluted in blocking solution were then added to the wells, followed by incubation at 37°C for 40 min. Positive and negative sera were also run with each test. After washing, 100 µl of 1:8,000 (for IgG) or 1:5,000 (for IgM) peroxidase-labeled conjugates were added. Conjugates used were rabbit anti-dog IgG Fc or sheep anti-dog IgM (Biogenesis Ltd., England, UK). The plate was then incubated at 37°C 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 450 nm using a microplate reader (Dynatech Lab.). 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) was directly proportional to the quantity of antibody present in the sample. Sera were screened in triplicate and the mean kinetics-based ELISA value of ≥ 0.02 was considered positive. Samples, which were positive in both immunoblotting and kinetics-based ELISA, were run again with a serial dilution in kinetics-based ELISA. The last dilution giving a value ≥ 0.02 was the titer of the sample (Lin et al., 1990).

Immunoblotting assay

IgG or IgM kinetics-based ELISA positive samples were further tested by immunoblotting. A minigel apparatus (Biometra Inc., Tampa, FL, USA) was utilized for sodium dodecyl sulphate-polyacrylamide gel electrophoresis. All the reagents used were purchased from Bio-Rad
Antibodies to *T. gondii* in Stray Dogs

<table>
<thead>
<tr>
<th>Table 1. Prevalences of IgG and IgM antibodies to <em>T. gondii</em> in stray dogs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Positive in IgG Positive in IgM Positive in both Positive in either IgG or IgM</strong> No. % No. % IgG &amp; IgM No. % 95% confidence interval</td>
</tr>
<tr>
<td>100 12 6 6 3 2 1 16 8 (0.0352, 0.152)</td>
</tr>
</tbody>
</table>

| Table 2. IgG and IgM antibody titers to *T. gondii* in stray dogs. |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Antibody | No. Positive | Reciprocal of antibody titers | | | | | | | | | | |
|          | 20 | 40 | 80 | 160 | 320 | 640 | 1280 | 2560 | 5120 | geometric mean titer |
| IgG      | 12 | 0  | 4  | 2   | 0   | 0   | 2    | 0    | 4    | 0     | 285.1 |
| IgM      | 6  | 4  | 2  | 0   | 0   | 0   | 0    | 0    | 0    | 0     | 25.2  |

Lab. Nonidet-P40 extracted *T. gondii* membrane antigens were mixed with reducing sample buffer (5% of 2-mercaptoethanol in sample buffer) and heated for 4 min at 90°C. Five μl of the above buffer, containing 12.5 μg of protein or pre-stained low molecular weight standards (Bio-Rad Lab.), were then applied to 12% sodium dodecyl sulphate-polyacrylamide gel and electrophoresed at 120 volts for 1 hr. The gel was then transblotted onto a nitrocellulose paper (BA83, Schleicher & Schuell, Germany) by using a blotting apparatus (Biometra Inc.). After incubation with blocking solution overnight at 4°C, the nitrocellulose paper was incubated with a 1:100 sample diluted in blocking solution with 1% Triton X-100 (Sigma Chemical Co.) at room temperature for 1 hr. Positive and negative sera were also run with each test. The nitrocellulose paper was then washed and incubated with peroxidase-labeled conjugates (see antibody detection by kinetics-based ELISA) at room temperature for 1 hr. Once again, the nitrocellulose 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.

Only the sera which tested positive in both kinetics-based ELISA and immunoblotting were considered positive. In stray dogs, total seropositivity was 8% (n=100) with a 95% confidence interval of (0.0352, 0.152) (Table 1). The prevalences of IgG and IgM antibody to *T. gondii* were 6% and 3% respectively. Nevertheless, this difference was not statistically significant (McNemar’s test, P=0.0898). The geometric mean titers for IgM and IgG were 1:25.2 and 1:285.1 (Table 2). The prevalences of *T. gondii* antibody were 11.4% (n=44) for males and 5.4% (n=56) for females. There was no association of *T. gondii* antibody prevalence with sex (Fisher’s exact test, P=0.2951, Table 3).

**Discussion**

The present report showed the prevalences of IgG and IgM antibodies to *T. gondii* in stray dogs in Taipei measured by a combination of both kinetics-based ELISA and immunoblotting. Although such serial testing may decrease sensitivity, it enhances specificity (Lin, 1998). This study revealed that only 3% of stray dogs had active *T. gondii* infection (i.e. IgM positive). Previous report has also shown a low prevalence of IgM antibody in 658 privately-owned dogs from the National Taiwan University Animal Hospital (2%, Lin, 1998). Interestingly, either stray (8%, Table 1) or pet dogs (7.9%; Lin, 1998) had similar seroprevalence as humans (7%, n=100; Lin et al., 1998). This similarity further supports the hypothesis that the prevalence of toxoplasmosis in stray dogs is a good indication.
Table 3. Association of *T. gondii* antibody prevalence with sex in stray dogs.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total No.</th>
<th>No. positive</th>
<th>% positive</th>
<th>Fisher's exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>44</td>
<td>5</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>56</td>
<td>3</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8</td>
<td>8.0</td>
<td><em>P</em> = 0.2951</td>
</tr>
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of the extent of the infection in the environment (Ali *et al.*, 2003). In contrast, Fan *et al.* (1998) indicated that stray dogs in Taipei had as high as 39.1% (n=110) of seroprevalence to *T. gondii*. The use of different assays and the improvement of the environmental hygiene from 1998 to 2001 may account for this difference. Similarly to privately-owned dogs (Lin, 1998), antibody prevalence was not sex-dependent in stray dogs (Table 3). Other published reports also have failed to detect any sexual variation in *T. gondii* infection in dogs (Childs and Seegar, 1986; Smielewska-Los *et al.*, 2002; Ali *et al.*, 2003).

In Taiwan, pig is a major food-animal harboring *T. gondii* organisms (Chang *et al.*, 1990). Nevertheless, pork is frozen before being sold in supermarkets and cooked well before being served. Both freezing and heating render *T. gondii* nonviable (Dubey, 1974; Dubey *et al.*, 1990). Recent investigations have also shown that pork, beef, mutton, and chicken flesh sold in the supermarkets in Taipei were not contaminated with live *T. gondii* organisms (DS Lin, unpublished data). These may account for the low prevalence of toxoplasmosis in both dogs and humans in Taipei reported by this and other papers (Lin, 1998; Lin *et al.*, 1998).

References


Childs JE and Seegar WS. 1986. Epidemiologic observations on infection with *Toxoplasma gondii* in three species of urban mammals from Baltimore, Maryland, USA. Int. J. Zoon. 13:249-261.


台北市流浪犬弓蟲血清抗體盛行率

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(投稿日期：2003.8.8，接受日期：2004.1.6)

摘 要

弓蟲感染非常普遍，在經濟及公共衛生上很重要。為了提供基本的數據做為台北市流浪犬
弓蟲症之流行病學研究，本調查以 kinetics-based enzyme-linked immunosorbent assay 合併
immunoblotting 併測弓蟲 IgG 及 IgM 血清抗體盛行率。Immunoblotting 可以檢測出弓蟲特異性
30 kD 表面抗原。結果顯示整體弓蟲抗體的血清陽性率為 8% (n=100)，而 95% confidence interval
為 (0.0352, 0.152)。IgG 及 IgM 抗體盛行率分別為 6% 及 3%，然而此差異統計上並未顯著
(McNemar's test, P=0.0898)。只有少數的流浪犬為活動性弓蟲感染 (即 IgM 陽性)。弓蟲抗體盛行
率在雌犬為 11.4% (n=44)，而在雄犬為 5.4% (n=56)。抗體盛行率和性別無關 (Fisher's exact test,
P=0.2951)。

關鍵詞：狗，弓蟲，抗體