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Seroprevalence and Risk Factors Associated with Seropositivity to *Toxoplasma gondii* among Stray and Domestic Cats (*Felis silvestris catus*) in Metro Manila

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ABSTRACT

Toxoplasma gondii is a protozoan parasite that causes toxoplasmosis. It is widespread in the environment and infects a variety of warm-blooded animals, causing miscarriages and birth problems. Previous studies in the Philippines have determined the seropositivity of *T. gondii* in humans. However, the seroprevalence of the parasite among household pets, particularly its feline definitive host, remains insufficient. This study aimed to: (1) determine the seroprevalence of *T. gondii* antibodies among domestic and stray cats in the Philippines; and, (2) to analyze the risk factors associated with seropositivity. Blood samples from 59 domestic and stray cats were collected and tested for *T. gondii* seropositivity using a commercially available indirect ELISA kit, while pet owners and handlers were given questionnaires about their cats. Thirteen or 22.03% of the cats were seropositive to *T. gondii*, and risk factor analysis revealed a significant difference between domestic and stray cats with regard to diet ($p = 0.026$, OR = 8.333, $\varphi c = 0.299$) and domestication ($p = 0.039$, OR = 5.000, $\varphi c = 0.276$). Cats fed with table food tested 31.43% seropositive compared to the 4.35% of those fed with cat food, whereas 33.33% of the stray cats were seropositive compared to 7.69% for domestic cats. Odds ratio test showed that the risk factors studied were associated with higher likelihood of *T. gondii* seropositivity. These results implicate diet and environment in the transmission dynamics of *T. gondii* among cats.

Keywords: *Toxoplasma gondii,* seroprevalence, risk factor analysis, indirect ELISA

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INTRODUCTION

Toxoplasma gondii is an intracellular parasite that infects a wide range of warm-blooded animals, including cats, dogs, and humans. It is present worldwide and is of medical and veterinary importance due to its ability to cause miscarriages and birth defects in intermediate hosts (Garcia and others 2012). Infection by this zoonotic parasite causes toxoplasmosis, occurring through the ingestion of cysts in undercooked and raw meat or the accidental ingestion of oocysts from the environment (Duan and others 2012).

T. gondii only undergoes sexual reproduction and gametogenesis within its definitive host, the members of Family Felidae; this serves as the only method for the production of oocysts (Webster 2007). Oocysts are environmentally stable and are shed in the cat's feces. These remain infectious for approximately two years, causing widespread contamination and providing a source of infection to humans and other intermediate hosts (Yan and others 2012).

Previous studies on *T. gondii* in the Philippines have covered toxoplasmosis in rats, cats, pigs, and humans (Advincula and others 2010). An earlier study on the seroprevalence of *T. gondii* among Filipinos in the Philippines revealed an overall seropositivity of approximately 11.1% in the Metro Manila area, which is a relatively low seropositivity compared to rural areas like Leyte (30.1%) and Mindoro (61.2%) (Kawashima 2000).

Domestic cats (*Felis silvestris catus*) are household pets that are exposed to similar environments as humans but are less cautious of the cleanliness of their immediate environment. This increases their exposure to *T. gondii* and should provide a more accurate estimate of the prevalence of the parasite in the environment. As the parasite's definitive host, cats are crucial for the parasite to reach maturity and complete its life cycle. Consequently, *T. gondii* is able to manipulate the behavior of its intermediate host to enhance transmission to the definitive host (Webster 2007). Domestic cats, therefore, represent a major source of contamination and infection for humans and other potential hosts.

T. gondii infection can be detected through several diagnostic methods. One of these is the indirect enzyme-linked immunosorbent assay (ELISA), a serological test that is one of the most widely used methods for the diagnosis of toxoplasmosis. Another technique is the polymerase chain reaction (PCR), a highly specific and

sensitive molecular test that enables detection of the parasite DNA (Castillo-Morales and others 2012). This method, however, requires the use of costly reagents.

This study aimed to (1) determine the seroprevalence of *T. gondii* antibodies among domestic and stray cats in the Philippines using indirect ELISA and (2) to analyze the risk factors associated with seropositivity. Findings from this study will be significant in monitoring and controlling the possible infection of intermediate hosts, particularly humans, because of their interaction with cats.

MATERIALS AND METHODS

Consent Forms

An introductory letter indicating the purpose of the study and the assistance needed had been sent to pet owners, shelter administrators, and veterinarians from different sampling sites in Metro Manila for approval. A consent form explaining the purpose of the study and rights regarding participation had also been provided.

Questionnaire

Prior to blood extraction, information regarding the age, sex, breed, diet, location, presence of other cats, contact with the outdoor environment (for domestic cats), health condition, medical history, litter information, and domestication was obtained through questionnaires filled out by the owners, shelter administrators, and veterinarians.

Sampling Sites and Blood Collection

Blood samples from domestic cats were collected from the Makati Dog and Cat Hospital, Riverside Village, and Companion Animal Veterinary Clinic, whereas samples from stray cats were collected from the Philippine Society for the Prevention of Cruelty to Animals and the Marikina City Vet Office. One milliliter of whole blood sample was collected by licensed veterinarians via venipuncture of the cephalic veins (McCurnin and Bassert 2002). Proper animal restraint was accomplished before venipuncture. The elbow of the cats was kept extended and pressure was applied such that the vein remains filled with blood. After the venipuncture, firm pressure was applied to the puncture site for 60 s to prevent hematoma formation (McCurnin and Bassert 2002). Fifty-nine cat blood samples were successfully obtained. The blood was stored in red-topped vacutainer tubes

(contains no anticoagulant), labeled, and left in a slanting position for 10 min at room temperature (RT) to facilitate clotting. The tubes were then kept inside a Styrofoam box with ice (Stevens and others 2007) to keep the temperature low. The blood samples were then transported to the Medical Microbiology Laboratory in UP Diliman, where they were either stored in the refrigerator or immediately processed.

Serum Preparation

The vacutainer tubes were taken out of storage and left undisturbed at RT for 10 min, to aid in clotting (www.invitrogen.com). Three hundred microliters of serum was then taken from the tubes, and transferred into sterile 1.5 mL microcentrifuge tubes. The serum was centrifuged at 1000 x *g* for 10 min, and immediately transferred to a sterile 1.5 mL microcentrifuge tube in aliquots of 250 µL. The tubes were stored in the freezer (www.invitrogen.com) until a sufficient number of samples were obtained.

Serological Assay

IgG antibodies against *T. gondii* in the serum samples were detected through indirect ELISA using a commercially available kit (ID Screen® Toxoplasmosis Indirect Multi-species from ID.vet Innovative Diagnostics, Montpellier, France) following the manufacturer's instructions. Samples were prepared in a 96-well microplate coated with P30 antigen of *T. gondii*. To each well, 90 µL Dilution Buffer 2 was added, followed by 10 µL of the Negative Control in wells A1 and B1, and 10 µL of the Positive Control in wells C1 and D1. Serum samples were thawed and 10 µL were dispensed into the remaining wells. Microplates were then incubated for 45 min at RT. The wells were washed thrice with 300 µL Wash Solution, then 100 µL of Conjugate was added, followed by incubation for 30 min at RT. The wells were washed thrice with 300 µL Wash Solution, then 100 µL Substrate Solution was added, followed by incubation in the dark for 15 min at RT. The reaction was stopped by adding 100 µL Stop Solution. The optical density (OD) of the samples and controls were measured at 450 nm and recorded using a microplate reader. This assay was performed twice.

Interpretation and Validation of Results

The OD values were tested for validity using the readings of the positive and negative controls. The test was considered valid if the mean OD values of the

positive control was greater than 0.350 (OD_{PC}>0.350), and if the ratio of the OD values of the positive and negative controls was greater than 3.5 (OD_{PC}/OD_{NC}>3.5).

The Sample/Positive (S/P) percentage was computed for each sample using Equation 1. The results for each sample were labeled as either negative (S/P ≤ 40%), doubtful (40% < S/P < 50%), or positive (S/P ≥ 50%).

$$S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100 \qquad (1)$$

Analysis of Data

Statistical analysis of the prevalence of *T. gondii* across potential risk factors for infection was performed using Chi-square test in SPSS software (Release 20.0 standard version, SPSS Inc., Armonk, New York). Risk factors that were analyzed included sex, diet, domestication (domestic versus stray), and location (from veterinary clinics versus from animal shelters). P values less than 0.05 were considered to be statistically significant (Duan and others 2012). Otherwise, the null hypothesis was accepted, and the risk factor in question was concluded to play no role in *T. gondii* infection.

Phi and Cramer's V values were also reported using Cramer's Phi test to determine the strength of association between *T. gondii* infection and the risk factors. Phi values from 0–0.30 indicated absence of relationship to weak relationship, 0.31–0.70 implied a moderate relationship, and 0.71–1.0 suggested a strong relationship (Release 20.0 standard version, SPSS Inc., Armonk, New York).

Odds ratios (ORs) were also noted to compare the relative odds of the occurrence of *T. gondii* infection across the risk factors involved. Odds ratio equal to 1 indicated that the risk factor does not affect the likelihood of infection, ORs greater than 1 indicated that the risk factor is associated with higher likelihood of infection, and ORs less than 1 indicated that the risk factor is associated with lower likelihood of infection (Szumilas 2010).

Waste Disposal

Potentially infectious materials were decontaminated using an autoclave prior to disposal (CDC 2009).

RESULTS

Validity of the Assay

Two separate assays were performed and both were deemed valid as each set met the conditions for validity. The ratios of the OD values of the positive and negative controls for the first and second batches were 3.51 and 3.53, respectively.

Seroprevalence

Out of the 59 blood samples collected, 22.03% were seropositive to *T. gondii* and 72.88% were negative for infection. Three samples exhibited a doubtful result. The seroprevalence of *T. gondii* in cats from Marikina, Manila, and Makati were 27.78%, 40%, and 14.29%, respectively (Table 1).

Table 1. Seroprevalence of *T. gondii* in cats (*Felis catus*) based on geographic location in the Philippines

	Positive	Negative	Doubtful	Total	Prevalence of *T. gondii*(%)
Makati City	2	10	2	14	14.29
Manila City	6	9	0	15	40
Marikina City	5	12	1	18	27.78
Pasig City	0	2	0	2	-
Antipolo City	0	10	0	10	-
Total	13	43	3	59	

Table 2. Seroprevalence of *T. gondii* infection in cats (*Felis catus*) across each risk factor

	Positive	Negative	Doubtful	Total	Sum	Details
Total	13	43	3	59		
Stray	11	21	1	33	59	Data
Domestic	2	22	2	26		completed
In Clinics	2	20	2	24	57	2 from
In Shelters	11	21	1	33		private homes
Male	6	15	2	23	56	3 without
Female	7	25	1	33		information
Table food	11	23	1	35	58	1 without
Cat food	1	20	2	23		information

The seroprevalence across risk factors are summarized in Table 2. Each risk factor was observed to indicate at least one cat to be positive. Those shown to be seropositive to *T. gondii* were 33.33% (11/33) in stray cats, 7.69% (2/26) in domestic cats, 8.33% (2/24) in cats kept in clinics, 33.33% (11/33) in cats from shelters, 26.09% (6/23) in male cats, 21.21% (7/33) in female cats, 31.43% (11/35) in cats fed with table food, and 4.35% (1/23) in cats fed with cat food.

Risk Factor Analysis

A statistically significant association with seropositivity was found with domestication and diet ($p<0.05$). The proportion of seropositive cats was found to be higher in stray cats and in cats fed with table food. Cramer's Phi, however, indicated that the correlation is not strong, but the OR test suggested that all risk factors are associated with higher odds of *T. gondii* infection (OR>1, Table 3).

Table 3. Summary of statistical analysis for potential risk factors associated with *T. gondii* infection

Risk Factor	Odds ratio(OR) *	*p*-value**	Phi value(φc)***
Sex	1.733	0.403	0.115
Diet	8.333	0.026	0.299
Domestication	5.000	0.039	0.276
Location	5.000	0.054	0.262

* OR=1 means that the risk factor does not affect the odds of infection, OR<1 means that the risk factor is associated with lower odds of infection, and OR>1 means that the risk factor is associated with higher odds of infection.

** P-values<0.05 are considered statistically significant.

*** Phi values from 0–0.30 indicate absence of relationship to weak relationship, 0.31–0.70 with a moderate relationship, and 0.71–1.0 with a strong relationship.

DISCUSSION

Cats are considered reservoirs of zoonotic diseases such as toxoplasmosis. These animals that live in close contact with humans increase the risk of transfer of potential infections, especially to immuno-compromised individuals (Grøndalen and others 2004). Whether domestic or stray, cats are exposed to the same environment as humans and may be used as sentinels that reflect the spread of *T. gondii* in the environment. A high seropositivity in cats would indicate a high risk of infection in the community. The seropositivity obtained in this study was 22.03% (13/59). This rate of *T. gondii* infection is rather low relative to those reported in other countries, which can be as high as 80% of tested cats (Alvarado-Esquivel and others 2007).

A third of the stray cats (33.33%) were seropositive compared to 7.69% in domestic cats. This difference in seropositivity was based on statistical tests ($p<0.05$). Studies conducted in other countries similarly reveal that stray cats generally have higher seropositivity rates and are more prone to infection (Meireles and others 2004, Miró and others 2004, Raeghi and others 2011). The differences in the lifestyles of stray cats and domestic cats affect their daily encounters with the potential parasite, which subsequently affect their health. Domestic cats are kept indoors, and are often personally carried or kept in cages when brought outside the house. They are generally exposed to clean, controlled environments free of *T. gondii* contamination. They may, however, get infected by the ingestion of oocysts that have been left by other cats, or the ingestion of cysts in raw or uncooked contaminated meat.

Stray cats, on the other hand, are exposed to environments that are not maintained to the same degree of sanitation compared to the inside of a house. The outside environment contains numerous possible sources of contamination, such as raw or uncooked food scraps from domestic garbage, and droppings from intermediate hosts like mice, birds, and reptiles, which may contain cysts. Interaction with other free-roaming stray cats also hastens the spread of infections. This is supported by the ORs for domestication, which suggested the risk factor is likely to increase the odds of seropostivity to *T. gondii* in cats (OR=5.000, Table 3).

A difference in seropositivity values was also seen in cats according to their diets. A higher percentage of positive result was seen in cats fed with table food or leftovers (31.43%), relative to cats fed with cat food (4.35%). The odds ratio test also indicated that diet has the highest OR value (8.333), corresponding to the increased chances of infection in cats fed with table food (Table 3). These results are similar to those obtained in studies conducted in Brazil and Mexico by Lucas and others (1999), and Alvarado-Esquivel and others (2007), respectively. Table food, when fed to cats, is often mixed with uncooked or undercooked meat and by-products that are easily contaminated. Cat food, on the other hand, is factory-processed and is less exposed to the environment prior to feeding, as it is kept in containers or packages. Companies that manufacture animal food undergo regular monitoring procedures to ensure the safety of their products. Protocols in these companies involve the application high temperatures (>100 °C) in the production of commercial cat food. This ensures the elimination of viable tissue cysts, as *T. gondii* becomes nonviable at temperatures above 66 °C (Meireles and others 2004). In addition, cats fed with cat food are most likely domestic, which as previously mentioned, are less prone to infection.

A larger percentage of males (26.09%) were seropositive to *T. gondii* compared to females (21.21%). The difference, however, was not statistically significant ($p>0.05$). This was also seen in studies conducted by Alvarado-Esquivel and others (2007), Hooshyar and others (2007), and Wu and others (2011) in Mexico, Iran, and China, respectively. This may be attributed to the territoriality and roaming behaviors associated with male cats. These allow them to cover a wider range of areas, which increases their chances of coming into contact with oocysts in contaminated meat and environments, and with other cats, particularly females (Reyes and others 2013). A study by Maruyama and others (1998) suggested that male cats may have increased chances of infection through aggressive encounters during estrus period. The OR test, however, confirmed that sex is a risk factor involved with higher likelihood of *T. gondii* infection (OR=1.733, Table 3).

Seropositivity was also higher in cats being cared for in animal shelters (33.33%) compared to those kept in veterinary clinics (8.33%). OR test confirmed that location as a risk factor can possibly increase the likelihood of *T. gondii* infection (OR=5.000). Generally, the cats from animal shelters were usually stray cats, whereas cats from veterinary clinics were usually domestic cats. In this study, the same holds true. The main difference was the sample size, with the risk factor for location having fewer samples due to incomplete data supplied by the participants. As it is, the higher seropositivity in cats from animal shelters may be explained by the same reasons justifying the higher seropositivity among stray cats.

In conclusion, the seroprevalence of *T. gondii* among cats in Metro Manila is relatively low compared to other countries, which may indicate that the parasite is not as widespread in the areas covered by the study. Results from the risk factor analysis also emphasize the role of diet and environment in the transmission dynamics of *T. gondii* among cats.

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