SEROPREVALENCE OF *Toxoplasma gondii* ANTIBODIES IN *Felis catus* FROM A TERTIARY PUBLIC HOSPITAL

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ABSTRACT

The study was conducted to determine the seroprevalence of antibodies against *Toxoplasma gondii*, a zoonotic parasite, in cats found within the vicinity of a tertiary pubic hospital. A total of 42 stray domestic short-haired cats of both sexes, six to 48 months of age were used in the study. The animals had no record of deworming and vaccination and no owners claimed the cats used in the study. Blood sera were tested for *Toxoplasma gondii* antibodies using commercially available ELISA kit. It was observed that 42.86% (18/42) cats had serologic evidence of *T. gondii* infection. Of these, 33.33% (6/18) were males and 66.67% (12/18) were females. With regards to age, 22.22% (4/18) were juveniles while 77.78% (14/18) were mature. Neutrophilia, eosinopenia and lymphocytosis were observed in all seropositive animals.

Keywords: cat, domestic, hospitals, toxoplasmosis

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INTRODUCTION

Toxoplasma gondii is an intracellular, protozoan parasite that affects almost all nucleated cells and tissues of the body (Quinn and McGraw, 1972; Dubey 1986). It can infect a wide variety of birds and mammals including humans (Lucas *et al.,* 1999; Dubey, 2005; Roberts and Schmidt, 2005; Little, 2008; Molina and Ridley-Dash, 2008; Thompson *et al.*, 2009; Weiss and Dubey, 2009) by ingestion of oocyst-contaminated soil and water, tissue cysts in undercooked meat, transplacental and lactogenic. No population is exempt from the possibility of contracting *T. gondii* considering that this parasite could be harboured by a large variety of reservoir hosts which serve as potential medium of transmission among populations (Zeibig, 1997).

Among the species infected, members of the family Felidae serve as the definitive hosts. Felids shed the parasite as sporulated oocysts through defecation (Breecher, 2006). These sporulated oocysts remain infective in the environment (Maharana *et al.,* 2010). The cat has gained popularity as a household pet (Earle *et al.,* 1990; Sevilla, 2007). Local statistics from 2000 to 2005 showed a visible increase in the number of these animals (Sevilla, 2007). With the rapid increase in number, the loss of confinement controls, straying and hunting predisposes these animals to contract and shed *T. gondii*. Toxoplasmosis may be asymptomatic in apparently healthy domestic cats. In most adults, it does not cause serious clinical illness, but it is considered a devastating disease in immunocompromised individuals (Ichhpujani and Mittal, 2011).

A tertiary public hospital, according to the Department of Health, is an institution that provides clinical care and management, as well as specialized and sub-specialized forms of treatments, surgical procedure and intensive care. These are major hospitals that usually have a full complement of services including paediatrics, obstetrics, general medicine, gynecology, surgery and psychiatry. They cater to patients of various and wide array of health needs including severely immunocompromised patients.

Clearly, *Toxoplasma gondii* is cosmopolitan in the human population, thus posing a risk for possible human infection (Acha and Szyfres, 1987) and it is considered one of the most common zoonoses occurring worldwide (Blood and Henderson, 1975; Belfort *et al.,* 2011). Several published and unpublished studies reporting the local seroprevalence of *T. gondii* antibodies have already been

conducted. These include Molina and Ridley-Dash (2008), Advincula *et al.* (2010) and Reyes *et al.* (2013). It is the interest of this study to determine the seroprevalence of antibodies against *Toxoplasma gondii* in domestic short-haired cats within the vicinity of a tertiary public hospital. The results of this study will be the first coming from a human tertiary public hospital and can be used to better assess the local epidemiology of *Toxoplasma gondii* in the country.

MATERIALS AND METHODS

An ocular inspection of the tertiary public hospital in Metro Manila was conducted to assess the presence of stray domestic short haired cats (Figure 1 and 2). The cats were captured using a drop trap (Reyes *et al.,* 2013) on specific pre-selected dates within an operating time-frame which was from 6 pm to 12 am to reduce the bias in the selection of the sample animals. The cats were placed in individual cages until owners claimed the captured cats. The owners were given 6 h to claim pets that have been caught during the operating frame. Unclaimed animals were transported back to the UP Veterinary Teaching Hospital-Maahas Station, Los Baños, Laguna.

Figure 1. Domestic short-haired cat (encircled) waiting to be fed just outside the maternity ward of a tertiary public hospital.

Figure 2. Another stray domestic short-haired cat (encircled) resting in one of the parking areas of a tertiary public hospital.

A total of 42 stray domestic short-haired cats of both sexes (14 males and 28 females), with age ranging approximately from four to 48 months found within the vicinity of a tertiary public hospital were used in the study. The age of the animals were estimated through dental arcade examination and then grouped as either adults (28/42) or juveniles (14/42). The diet of these animals included, but not limited to table scraps from the hospital and other buildings around the area and smaller animals such as rodents and birds.

Chemical restraint was done using tiletamine hypochloride-zolazepam hypochloride (Zoletil® 50mg/ml Virbac Laboratories, Carros, France) given intramuscularly at a dose of 5 mg/kg body weight using a 23-guage hypodermic needle attached to a 3.0 ml sterile disposable syringe. Approximately 5.0 ml blood was collected from each animal via the jugular vein and the samples were placed in two different vacuum tubes. The first tube was a 3.0 ml lavender capped tube that contains the anti-coagulant, ethylenediamine tetraacetic acid (EDTA) and the other one, a 3.0 ml red capped tube with no anticoagulant. The former contained blood sample used for hematologic examination, while the latter for serum collection.

Routine physical examination was done. Direct fecal smear and hematologic examinations were performed similar to the

methods described by Coles (1986). Blood processing and serum collection techniques were similar to the methods described by Reyes *et al.,* (2013). The sera were tested using the commercially available *Toxoplasma* test kit (ImmunoComb® Feline *Toxoplasma gondii* and *Chlamydophila felis* IgG Antibody Test Kit, Biogal Laboratories, Galed, Israel). Interpretation of the colour reactions was made using digital scanning software (Biogal CombScan® 2000, Biogal Laboratories, Galed, Israel).

The procedures in the study have been approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños. The cats were humanely destroyed after blood collection.

The comparative serologic data between sex and age groups were analysed using Fisher's exact test of independence (α=0.05), a test used when there are two nominal variables of small sample size. Data analysis of the proportion of seropositive and seronegative was done using Approximate Z-test (α=0.05) since the samples were obtained only from one location.

RESULTS AND DISCUSSION

The study considered the stray domestic short-haired cats found within the vicinity of a tertiary public hospital. None of the animals caught in the given time frame was claimed by anyone, hence were transported to the UP VTH-Maahas Station. These hospitals, according to the Department of Health, are those that render a vast array of services from clinical treatment and management to intensive care, as well as specialized and sub-specialized forms of treatment. The number of patients presented in such facilities and the health status of these patients were important considerations in the study of zoonotic diseases, one of which is toxoplasmosis. These facilities are known to serve immunocompromised patients. Furthermore, geriatric and young patients, which are highly susceptible to infection are also presented in these hospitals.

A total of 42 stray domestic short-haired cats found within the vicinity of a tertiary public hospital were considered in the study. These animals were tested for *Toxoplasma gondii* antibodies. Direct fecal examination showed that none of these animals had

parasitic ova observed. Routine blood test (PCV, tWBC and absolute dWBC) results show neutrophilia, eosinopenia and lymphocytosis as the common observation for all test samples and are summarized in Table 1.

Table 1. Hematologic values of *Toxoplasma gondii* seropositive (n=18) and seronegative (n=24) stray domestic cats (*Felis catus*) within the vicinity of a tertiary public hospital.

Parameter	Seropositive (n=18)	Seronegative (n=24)	Reference values*
Packed Cell Volume (%)	33.0 ± 6.8	34.0 ± 6.1	38.0 ± 2.5
Total WBC (x 10^3 cells/µL)	14.8 ± 6.0	17.9 ± 7.2	11.28 ± 2.0
Absolute Differential WBC (x 10^3 cells/µL):			
Neutrophils	10.6 ± 4.4	12.42 ± 6.3	7.78 ± 0.37
Eosinophils	1.4 ± 1.5	1.2 ± 0.9	8.02 ± 0.30
Lymphocytes	2.6 ± 1.2	3.8 ± 2.2	2.45 ± 0.27
Monocytes	0.1 ± 0.2	0.4 ± 0.56	0.25 ± 0.12
Basophils	None observed	None observed	None observed

*Carlos *et al.,* 1970. Hematologic studies on normal dogs and cats. *Philippine Journal of Veterinary Medicine* 9: 35-50.

To minimize the subjectivity in the interpretation of the test results, digital scanning software that interprets the colour reaction was employed. Among the 42 cats tested, 42.86% (18/42) cats tested positive for *T. gondii* antibodies (Table 2). The results of this study was found to be closely similar to previous studies conducted by Reyes *et al.,* 2013 which reported 46.67% (14/30) of the cats found within the vicinity of a wildlife facility exposed to *T. gondii*. Other studies were Molina and Ridley-Dash (2008) with a result of 33.3% and Advincula *et al.* (2010) with 46.67%. Detection of antibody titers of at greater than or equal to 1:32 signifies a previous history of infection or exposure to *T. gondii*. Ingestion of oocysts contaminated food or water, raw or undercooked meat is the main modes of transmission.

Table 2. Frequency distribution of stray domestic shorthaired cats (n=42) with serologic evidence of exposure to *Toxoplasma gondii* by sex.

Interpretation	Males (%)	Females (%)	Total (%)
Seronegative (≤1:16)	8/14 (57.14)	16/28	24 (57.14)
Seropositive (≥1:32)	6/14 (42.86)	12/28	18 (42.86)
Total	14	28	42

Compared to other countries, the seroprevalence in this study is quite high. Other studies reported a seroprevalence of 26.3% in Brazil (Silva *et al.,* 2002); 22.6% in Argentina (Lopez *et al.,* 2011) 30.2% in Sri Lanka (Kulasena *et al.,* 2011); and 33% and 25.5% in the upper and lower regions of South Korea (Lee *et al.,* 2011).

A prevalence of 42.86% for both male and female cats was observed in this study. Previous studies reported by Lucas *et al.* (1999), Pena *et al.* (2006), Advincula *et al.* (2010) and Reyes *et al.* (2013) showed that male cats were more likely to be exposed to the parasite than females. Male cats exhibit certain traits not usually exhibited by female cats. These include the natural roaming and territorial behaviour (Aiello, 1998; Lee *et al.,* 2002), aggression (Bendinelli *et al.,* 1995), biting and fighting (Mosallanejad *et al.,* 2010; Medeiros *et al.,* 2012). These behaviour increases the possible contact of these animals to oocysts contaminated soil and water. In this study, the prevalence for both male and female animals was similar and could be attributed to the higher number of female cats caught during the study. The higher number of females in turn, may be attributed to the female animal's behavior of being non-territorial and may co-exist with other females in a clowder.

With regards to age, 50% (14/28) were adults and 28.57% (4/14) were juveniles where there the seropositivity increases with age (Table 3). This is similar to what De Craeye *et al.* (2008) and Reyes *et al.* (2013) reported. Post natal infection through ingestion of infected cysts had been reported. A higher frequency in adult animals could be due to the increased tendency to roam, forage and hunt for food that includes smaller prey. This could be the same reason why a greater number of adult cats were captured in the study. Unlike the previous study by Reyes *et al.* (2013) where none of the juvenile animals were found to be seropositive, the

results of this study suggest that juvenile animals these were exposed to the infection at an early age. The presence of animals in a large clowder increases the possibility of exposure. However, the study is unable to determine the exact period of exposure or whether the antibodies were acquired transplacentally. It also suggests a high possibility of an active source of infection in the area.

Table 3. Frequency distribution of stray domestic shorthaired cats (n=42) with serologic evidence of exposure to *Toxoplasma gondii* by age.

Interpretation	Mature (%)	Juvenile (%)	Total (%)
Seronegative (≤1:16)	14/28 (50.00)	10/14 (71.43)	24 (57.14)
Seropositive (≥1:32)	14/28 (50.00)	4/14 (28.57)	18 (42.86)
Total	28	14	42

Statistical analysis using Approximate Z-test (α=0.05) showed no significant proportion of seropositive and seronegative animals. Furthermore, Fisher's Exact test (α=0.05) shows no significant correlation between the occurrence of *Toxoplasma gondii* antibodies with regards to sex and age.

Although there is no sufficient evidence that the amount of seropositive cats is statistically significant, the results of this study show that there is a large proportion of the population that is at risk. Therefore, the employment of an effective and long term control and prevention program for *T. gondii* is necessary. The study was not able to confirm clinical infection, but the detection of antibodies against *T. gondii* indicates its presence.

Therefore, the results of this study suggest the employment of an effective and long term control and preventive measures as the Philippine populations have already been exposed to the parasite (Kawashima *et al.,* 2000). Control protocols and biosecurity measures must be employed to prevent nosocomial infections and the spread of infection in immunologically challenged populations such as those in tertiary public hospitals. Personal hygiene and sanitation are the best ways to prevent infection.

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