

Sporulated *Toxoplasma gondii* oocysts from feces of domesticated and stray cats in Manila, Philippines

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ABSTRACT

Toxoplasma gondii has been an emerging public health concern due to its ability to infect a wide range of mammalian and non-mammalian host. This study aimed to gather evidence on the presence of *T. gondii* oocyst from feces of stray and domesticated cats in Manila, Philippines to provide a baseline data on the potentially infective oocysts that may be transmitted to the general public living in close proximity and in direct contact with the definitive host. A total of 70 cat feces (domesticated = 35; stray cats = 35) were investigated for the presence of *T. gondii* oocyst using Sheather's sucrose floatation technique and light microscopy examination. Two sample z-tests of proportion using STATA software was used to determine if there was a significant difference between the proportion of stray and domesticated cat feces positive for *T. gondii* oocysts with level of significance set at 0.05 ($\alpha=0.05$). A total of 6 stray cat feces were positive for *T. gondii* oocyst. The overall prevalence was 9% (6/70) while domesticated and stray cat prevalence were 0% (0/35) and 17.14% (6/35), respectively. The present study provides the baseline data of the prevalence of *T. gondii* infected cats in Manila and to the best of our knowledge, the first report of the potential number of cats shedding oocyst in a metropolitan environment in the Philippines.

Key words: Cat, Oocyst, Philippines, Sporulated, *Toxoplasma*.

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INTRODUCTION

Considered as one of the neglected infections of poverty in the United States,^[1] *Toxoplasma gondii* infection has become a global concern following its ability to infect different age groups of the population.^[2] *T. gondii* has also been a major concern for people who are immune compromised such as those living with HIV with reported fatal outcomes of infection resulting to cerebral toxoplasmosis.^[3] In the environment, *T. gondii* includes several mammalian and avian intermediate hosts that are closely living within human settlements^[4] which brings the population closer in the circle of

infection.^[5] The only known definitive hosts of *Toxoplasma gondii* are members of the cat family (Felidae).^[5] Cats are known to shed unsporulated oocyst which takes 48 to 72 hrs before becoming sporulated in the environment and become infective. Sporulated oocysts in the environment are then transferred to potential hosts through ingestion (CDC DPDx). Infection can also occur upon the ingestion of meat infected with trophozoites (CDC DPDx). Shedding of oocysts, however, may appear to be variable as some studies suggest.^[6] Because of the spaces shared by domesticated or stray cats and humans, knowledge on the shedding of oocysts by the former is of great significance. In the Philippines, studies concerning the prevalence of toxoplasmosis are directed towards seroprevalence in cats,^[7] in rats,^[8] pigs and humans.^[9]

The aim of the present study is to gather evidence on the presence of *Toxoplasma gondii* oocysts from feces of stray and domesticated cats in Manila, Philippines and provide a baseline data on the potentially infective

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oocysts that may be transmitted to the general public living in close proximity and in direct contact with the definitive host.

MATERIALS AND METHODS

Study population

Domesticated ($n=35$) and stray cats ($n=35$) coming from the Manila City Pound, Philippine Society for Prevention of Cruelty to Animals and four different barangays in Metro Manila. Cats were selected based on random sampling with inclusion criteria set based on age, neutered and spayed status. Cats whose ages fall between 3 to 48 weeks old, non-neutered and non-spayed female cats were included in this study. Neutered male and spayed female cats are excluded from the study. The sample size was computed through G*Power version 3.1, using an alpha level of 0.05, a power of 85% and setting an allocation ratio of 1. The minimum sample size computed was 36. The researchers increased the total sample size to 70 for rigor of results.

Sample collection, processing and microscopy

Fresh stool samples were collected and placed in wide-mouth, sterile-screw-capped containers and were processed and analysed at the Medical Technology Department, Institute of Arts and Sciences, Far Eastern University-Manila and processed within 48 hrs. Five (5) grams of cat feces were processed using Sheather's floatation method for the detection of *Toxoplasma gondii* oocysts. Briefly, 335-mL of sterile-distilled water and 454-g of granulated sugar was mixed over an open flame with constant stirring until the sugar was completely dissolved. Upon cooling to room temperature, 6-mL of formaldehyde (37%) was added. The solution's specific gravity was adjusted to 1.27. Cat feces were dissolved in 10-mL distilled water and filtered through 1.2 μm pore size glass-microfiber filter with the sediments eluted using 2-mL of sterile distilled water to make a suspension. In a 10-mL test tube, 2-mL of Sheather's sucrose solution was added to 1-mL of sediment suspension and centrifuged at 1000 g for 10 minutes. Each 10-mL test tube was then filled to the brim with Sheather's sucrose solution and a coverslip was placed on top in contact with the solution and left to stand for 15 min. Each coverslip from the Sheather's sucrose floatation set-up was carefully recovered and placed onto a clean glass slide (wet-side down) and was examined under 400X magnification using a Nikon Eclipse E100 light

microscope. 200 microscopic fields was scanned in a zigzag direction for a standardized microscopy reading.

Statistical analysis

Two sample z-tests of proportion using STATA software was used to determine if there was a significant difference between the proportion of stray and domesticated cat feces positive for *Toxoplasma gondii* oocysts with level of significance set at 0.05 ($\alpha=0.05$).

RESULTS

Microscopy returned with 9% (6/70) positivity for *Toxoplasma gondii* oocysts (Table 1) which were subspherical, measuring 10 to 12- μm and were all sporulated with each oocysts containing two visible sporocysts (Figure 1).^[10,11] There were no domesticated cat feces positive for *Toxoplasma gondii* oocysts while stray cat feces returned with 6 positive results. The overall prevalence was 9% (6/70) while domesticated and stray cat prevalence were 0% (0/35) and 17.14% (6/35), respectively. The computed p-value between the prevalence of *Toxoplasma gondii* in domesticated and stray cats was 0.010 which is less than $\alpha=0.05$ which points to no significant difference between the proportions of domesticated and stray cats positive for *Toxoplasma gondii* (Table 1). Other parasitic forms noted in the microscopic examination were *Toxocara* spp. ova and Hookworm ova and no co-infection with the noted parasites along with *Toxoplasma gondii* oocysts were noted (Results not shown).

DISCUSSION

To date, there is only one species in the genus *Toxoplasma* despite attempts to make this genus classification polyspecific.^[12] Although molecular and serological tests are invaluable in the detection of *T.gondii* from both environmental and clinical samples, the microscopic screening of the samples with the use of Sheather's sucrose floatation and light microscopy appears to be highly reliable and a readily available method for the detection of *Toxoplasma gondii* oocysts even in resource limited settings^[13], as well as providing visual evidence of the presence of intact sporulated oocysts as for the case of the present study. In this study, microscopic evidences and statistical analysis provided baseline data on the number of cats that are sources of potentially infectious *Toxoplasma gondii* oocysts in various areas in Manila. Oocysts that are shed from infected cats have been an important factor in the disease transmission of *T.gondii*.^[14] It is important to note that the environment

Table 1: *Toxoplasma gondii* oocyst detection in domesticated and stray cats.

Study population	<i>Toxoplasma</i> spp. oocysts in cat feces	Prevalence	Two sample z-tests of proportion: $\alpha=0.05$
Domesticated cats	0/35	0%	Computed p-value = 0.010
Stray cats	6/35 (M=2; F=4)	17%	Interpretation:
	Total positivity 6/70	Overall prevalence 9%	No significant difference between proportion of domesticated and stray cat feces positive for <i>Toxoplasma gondii</i> oocysts.

M = male; F = female

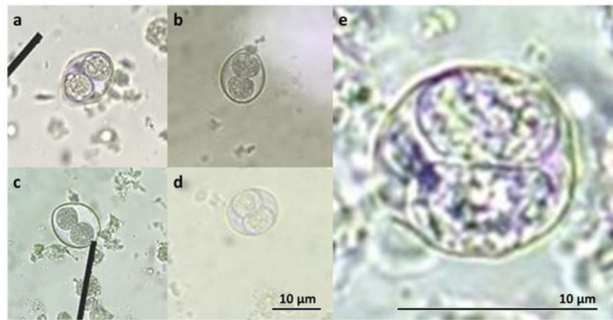


Figure 1: Sporulated *Toxoplasma* spp. oocysts. a to e, all *Toxoplasma gondii* oocysts observed were subspherical and sporulated, containing two sporocysts per oocyst with oocyst measurement of 10-12 µm; 400X magnification.

where the cats in the present study were collected were described as unpaved communities with large patches of exposed soil. Cats preferably defecate in soil to be able bury their feces which will potentially facilitate oocyst sporulation in the environment (CDC DPDx). During the temperate and dry days, the soil tends to become dust-like and by the dusk, children in the community would go out and get involved in various types of recreational activities which involves soil contact where *Toxoplasma gondii* oocysts may be present and be accidentally ingested mainly through hand to mouth transmission. Similarly, the general population is at risk by getting involved with the children's recreational activities or work which involves soil contact especially in depressed areas. Although the behaviour of residents on washing their hands or not after soil contact was no longer monitored as a limitation of the study, this needs to be placed under perspective as a potentially significant source of infection and may have implications which extends to other members of the household, in particular, pregnant women and immunocompromised individuals. In addition, the locations where cats were sampled are depressed areas of the capital with poor sanitation conditions which relates to studies in recent years suggesting that the majority of toxoplasmosis cases in humans mainly comes from environmental exposure rather than accidental ingestion

of infected meat.^[15] Further, the *Toxoplasma gondii* oocysts in the present study were observed to be sporulated which is evidence of its viability to contribute to the infections process. Lastly, results of the present study showed that there is a higher positivity rate between female stray cats ($n=4$) compared to male stray ($n=2$) cats. This means that infection from mother to offspring through placental transfer is likely to occur which may lead to an increasing population of definitive hosts for *Toxoplasma gondii* which aids in the perpetuation and spread of *Toxoplasma gondii* oocysts in the environment.

CONCLUSION

The present study provides the baseline data of the prevalence of *T.gondii* infected cats in Manila and to the best of our knowledge, the first report of the potential number of cats shedding oocyst in a metropolitan environment in the Philippines. The investigation of potential point source of infection, which in this case, oocyst shedding cats, is important especially in providing public health awareness to the community as well as establishing administrative protocols to prevent further spread of this parasitic infection to humans. The importance of hygiene and caution while playing or being in close contact with soil where cats and other small mammals defecate should be observed. Lastly, proper housing of cats in communities and in shelters may aid in reducing the risk of transmission of pathogenic oocyst to the members of the community, in particular, the pregnant and immune compromised population.

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