SEROPREVALENCE OF *Toxoplasma gondii* ANTIBODIES IN DOMESTIC SHORT-HAIRED CATS (*Felis catus*) IN A WILDLIFE FACILITY IN MANILA

Marco F. Reyes[1], Verman G. Guevara[2], Dianne Grace DG. San Roque[3], Marianne Leila S. Flores[1] and Emilia A. Lastica[1]

ABSTRACT

Thirty apparently healthy domestic short-haired cats (*Felis catus*) of both sexes, 3-48 months old, living in a wildlife facility in Manila were used in the study. The animals had no record of deworming or vaccination. Cats with owners were excluded in the study. Blood sera were tested for *Toxoplasma gondii* antibodies using an Enzyme-Linked Immunosorbent Assay Test kit. It was observed that 46.67% of all the animals tested had serologic evidence of exposure to *T. gondii*. Males (66.67%) were found to be more prone to the infection than females (26.67%). All animals that tested positive were adults. This study showed that male, adult domestic short-haired cats were more prone to exposure to the parasite than females and young animals.

Keywords: cat, domestic, toxoplasmosis

INTRODUCTION

Toxoplasma gondii is a protozoan parasite that is found worldwide (Aiello, 1997). It affects a wide variety of warm blooded animals (Dubey, 2005; Molina and Ridley-Dash, 2008; Thompson, 2009), including man (Lucas *et al.,* 1999; Dubey, 2005; Little, 2008; Weiss and Dubey, 2009). It was first isolated by Sabin and Olitsky in 1937 proving infections in animals are similar to human infections (De Camps *et al.,* 2008). *T. gondii* is an intracellular parasite that affects almost all nucleated cells and tissues of the body, primarily intestinal and muscle tissues, and may include the brain and liver (Quinn and McCraw, 1972; Dubey, 1986). Because of its medical and veterinary importance, *Toxoplasma gondii* is perhaps one of the most well-studied parasites.

Although it can infect a wide variety of hosts, only the members of the Felidae family are the definitive host (Lukesova and Literak, 1998; Samuel *et al.,* 2001; Bowman *et al.,* 2002; Pena *et al.,* 2006; Little, 2008). Felids, such as domestic cats, acquire infection through ingestion of any of the three infectious

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, Laguna, Philippines (email: marcofreyes@yahoo.com), [2]Chareon Pokphand Foods, San Fernando, Pampanga, Philippines; [3]Batangas Farm Incorporated, Malvar, Batangas, Philippines.

stages of *T. gondii*: tachyzoites, bradyzoites and sporozoites (Dubey, 1986). Other animal hosts become infected by ingesting food or water contaminated with oocysts shed by felids through their feces or by consuming secondary hosts harbouring encysted bradyzoites (Lucas *et al.,* 1999; Wolfe, 2003; Elmore *et al.,* 2010).

The disease may be asymptomatic in apparently healthy domestic cats (Dubey, 1986; Lappin, 2005) but may cause severe illness and clinical disease among neonates, geriatrics and immunocompromised animals (De Camps *et al.,* 2008; Elmore *et al.,* 2010). Immunosuppression may exacerbate the on-going disease condition. Symptoms may range from asymptomatic to grave clinical signs, including cerebral encephalitis, meningitis, abortion and stillbirth, depending on the stage of the infective cyst and the state of the host's immune system (Quinn and McCraw, 1972; De Camps *et al.,* 2008).

Wild felids, on the other hand, rarely encounter the disease, thus, may exhibit more grave symptoms once infected (Wolfe, 2003). Felids, being the only host able to pass environmentally resistant oocyst through the feces, are very important in disseminating the disease to other hosts, including man (Marchiondo *et al.,* 1976).

Despite being well-studied in other parts of the world, as supported by numerous studies, there are very few studies on the serologic detection of toxoplasmosis on domestic cats in the country. It is the particular interest of this study to determine the prevalence of *T. gondii* antibodies in domestic short-haired cats within a wildlife facility in Manila, Philippines.

MATERIALS AND METHODS

Domestic short-haired cats, of both sexes, with age ranging approximately from three to 48 months found within a wildlife facility in Metro Manila were considered in the study. The diet of these animals included, but not limited to, table scraps from a nearby picnic area and smaller animals such as rodents and birds. The animals were randomly captured using a drop trap (Figure 1) with bait inside. The drop trap was made of light weight, sturdy wood, covered by chicken wire mesh and propped by a stick attached to a rope. The captured cats were placed in individual cages until owners claimed the captured cats and these animals were excluded in the study. Unclaimed animals were considered and allowed to acclimatize.

Chemical restraint was done using tiletamine hypochloride-zolazepam hypochloride (Zoletil® 50mg/ml Virbac Laboratories, Carros, France) given intramuscularly (IM) at a dose of 5 mg/kg body weight using a 23-gauge hypodermic needle attached to a sterile 5.0 ml disposable syringe. Once sufficient plane of sedation was achieved, routine systemic physical examination was performed. Age approximation was done by complete examination of the dental arcades. Approximately 3.0-5.0 ml of blood were collected from each animal via jugular vein (Figure 2). The collected blood samples were placed in a sterile red and lavender capped vacuum tube (Vacutainer®).

Each red-capped tube was allowed to stand for at least 30 min while waiting

Figure 1. The Drop-trap made of light weight, sturdy wood covered with a 1 in x 1 in chicken wire mesh. The dimensions of 3 ft x 3 ft x 14 in (L x W x H) propped up by a 16-in stick connected to a string. A small, sliding wooden door of 7 in x 18 in (W x H) was placed to facilitate transfer of animals into portable cages.

for clot formation. During this time, routine hematological test (PCV, tWBC, relative and absolute dWBC) and direct fecal examination as described by Coles (1986) were done to fully assess the animal's health. Once clot formation was observed, the samples were centrifuged at 2000 RPM for 10 min and serum was collected and placed in clean, labelled Eppendorf® tubes.

After physical examination, routine hematological test and direct fecal examination, 30 domestic short-haired cats (15 males and 15 females) were used in the study. Of these 30 animals, three were juveniles and 27 were mature animals. Age groups were estimated based on the animal's dentition and those animals <12 months were considered as juveniles while those animals ≥12months were considered mature. These animals had no apparent disease or infection during the study.

The sera were tested using commercially available *Toxoplasma* test kit (ImmunoComb® Feline *Toxoplasma gondii* and *Chlamydophila felis* IgG Antibody Test Kit, Biogal Laboratories, Kibbutz Galed, Israel). Interpretation of the ImmunoComb® reactions (Figure 3) was made using the BiogalCombScan® 2000 (BioGal Laboratories, Kibbutz Galed, Israel). Approximate Z-test (α=0.05) and Fischer's Exact test (α=0.05) were used for analysis.

Figure 2. Sample of the commercially available *Toxoplasma gondii* antibody ImmunoComb® card with positive and negative reactions.

RESULTS AND DISCUSSION

A total of thirty domestic short-haired cats, found within a wildlife facility in Metro Manila were tested for *Toxoplasma gondii* antibodies. These animals have been assessed and were found to be negative for parasitic and protozoan parasites in direct fecal examination. Routine blood test (PCV, tWBC, relative and absolute dWBC) results were all within normal ranges (Table 1).

The results summarized in Table 2 show that 46.67% (14/30) of the cats had serologic confirmation of exposure to *T. gondii*. These results are closely similar to the reports made by Molina and Ridley-Dash (2008) in Kabacan, Cotabato and Advincula *et al.* (2010) within urban communities of Sta. Rosa and San Pedro, Laguna, Philippines, which reported 33.3% and 46.67% of positively exposed animals, respectively. Approximate Z-test (α=0.05) showed that there was no significant proportion between seropositive and seronegative cats.

The parasite may have been acquired by these animals via ingestion of raw or undercooked oocyst-infected meat of animals, contaminated food or water or in pregnant animals, transplacentally (Fayer, 1981). Carnivorous animals in the wild and feral cats get infected by chronically feeding on infected meat, prey or carreon (Lucas *et al.,* 1999; De Craeye *et al.,* 2011) and in this case, possibly from ingestion of oocyst in rodents and birds. Furthermore, these animals may have also acquired infection by direct contact with environmentally resistant oocyst in the soil and water (Elmore *et al*., 2010), since these cats were left to roam around the facility.

With regards to gender, males were found to have higher frequency at 66.67% (10/15) than females with 26.67% (4/15). This may be due to its behavioural habits of roaming and territoriality which increases the possible contact of these

Table 1. Hematologic values of domestic short haired cats.

Parameters	Mean±SD	Reference values*
PCV (%)	32.382 ± 4.51	27.86 - 36.94
Total WBC (cells/µl)	17,020 ± 2.44	10,648 - 19,648
Absolute differential WBC (cells/µl)		
Banded neutrophils	239 ± 7	0 - 250
Segmented neutrophils	10,671 ± 268	10,268 – 10,974
Lymphocytes	3,914.6 ± 185	1439 - 7007
Monocytes	510 ± 216	57 - 982
Eosinophils	887 ± 27	245 - 915
Basophils	None observed	None observed

*Peralta AM and Bisa MTB. 2000. *Hematologic Profile of Apparently Healthy Domestic Shorthair Cats (Felis domesticus) in Los Baños.* (Unpublished data).

Table 2. Frequency distribution of domestic short haired cats (n=30) with serologic evidence of exposure to *T. gondii* by sex.

Interpretation	Males%	Females %	Total (%)
Seronegative (≤1:16)	5/15 (33.33)	11/15 (73.33)	16 (53.33)
Seropositive (≥1:32)	10/15 (66.67)	4/15 (26.67)	14 (46.67)
Total	15	15	30 (100)

animals to oocysts contaminated soil and water. This finding is similar to those reported by Lucas *et al.* (1999), Pena *et al.* (2006) and Advincula *et al.* (2010).

As for age, all the domestic short-haired cats with serologic evidence of exposure to *T. gondii* were mature (Table 3). None of the juvenile animals tested was found to be positive. Among the mature domestic short haired cats, 51.85% (14/27) had serologic evidence of *T. gondii* infection. Most feline infections occur post-natally through ingestion of infected tissue cysts (Elmore *et al.,* 2010). Domestic and wild cats become infected after weaning, when these animals start to hunt for small rodents and birds (Lucas *et al.,* 1999). This may account for the

Table 3. Frequency distribution of domestic short-haired cats (n=30) with serologic evidence of exposure to *T. gondii* by age.

Age	Total (%)
Mature (≥ 12 months)	14/27 (51.85)
Juvenile (<12 months)	0/3 (0.00)
Total	14/30 (46.67)

higher frequency in adult animals as these animals have greater tendency to roam and hunt smaller prey or ingest oocysts infected carreon or be in contact with oocysts contaminated soil and water. Fischer's Exact test (α=0.05) showed no significant correlation between the occurrence of *T. gondii* antibodies to sex and age.

The study shows that there is relatively high serologic evidence that the domestic short-haired cats found within a wildlife facility in Metro Manila, are exposed to *Toxoplasma gondii*. These values were similar to those previously reported and, thus, may show a possible trend in the seroprevalence of *T. gondii* in the country. However, further studies in other locations or on a national level may be needed to prove this.

Kawashima *et al.* (2000) reported an overall 11% seropositivity in humans in Metro Manila, which confirms the presence of the parasite in human population. Although this value is lower than what was reported in this study, nonetheless, it opens the possibility of an active cycle of *T. gondii* in an area where cats and humans co-exist.

REFERENCES

Aiello SE (ed). 1997. *The Merck Veterinary Manual.* 8th ed. Rahway, New Jersey: Merck and Company.

Advincula JKDC, Iewida SYP and Salibay CC. 2010. Serologic detection of *Toxoplasma gondii* infection in stray and household cats and its hematologic evaluation. *Scientia Medica (PotoAlegre)* (20) 1: 76-82.

Bowman DD, Hendrix CM, Lindsay DS and Barr SC. 2002. *Feline Clinical Parasitology.* Ames, Iowa: Iowa State University Press.

Chandler EA, Gaskell CJ and Gaskell RM. 1994*. Feline Medicine and Therapeutics.* 2nd ed. Ames, Iowa: Blackwell Scientific Publication.

Coles, EH. 1986. *Veterinary Clinical Pathology.* 4th ed. Philadelphia: W.B. Saunders Company.

De Camps S, Dubey JP and Saville WJA. 2008. Seroepidemiology of *Toxoplasma gondii* in zoo animals in selected zoo in the Midwestern United States. *J*

Parasitol 94 (3): 648-653.

De Craeye S, Speybroeck N, Ajzenberg D, Darde ML, Collinet F, Tavernier P, Van Gucht S, Dorny P and Dierick K. 2011. *Toxoplasma gondii* and *Neospora caninum* in wildlife: common parasites in Belgian foxes and Cervidae. *Vet Parasitol* 178: 64-69.

Dubey JP. 1986. Toxoplasmosis in cats. *Feline Practice*. 16(4): 12-26.

Dubey JP. 2005. *Toxoplasmosis* in cats and dogs. *Proc World Small Anim Vet Assoc Mexico City, Mexico*.

Dubey JP. 2009. History of the discovery of the life cycle of *Toxoplasma gondii*. *Int J Parasitol* 39: 877-882.

Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS and Dubey JP. 2010. *Toxoplasma gondii*: epidemiology, feline aspects, and prevention. *Trends Parasitol* 26 (4): 190-196.

Fayer R. 1981. Toxoplasma update and public health implications. *Can Vet J* 22: 344-352.

Gonzales P, Carbonell E, Urios V and Rozhnov VV. 2007. Coprology of *Panthera tigris altaica* and *Felis bengalensis euptilurus* from the Russian Far East. *J Parasitol* 93(4): 229-231.

Kawashima T, Khin-Sane-Win, Kawabata M, Bargaza N, Matsuda H and Konishi E. 2000. Prevalence of antibodies to *Toxoplasma gondii* among urban and rural residents in the Philippines. *Southeast Asian J Trop Med Public Health* 31 (4): 742-746.

Lappin MR (ed). 2005. Protozoal and miscellaneous infections. *Textbook of Veterinary Internal Medicine*. 6th Ed. Philadelphia, PA: Elsevier Saunders.

Little S. 2008. *Cats and Toxoplasmosis*. Accessed 01 March 2011. http:// www.winnfelinehealth.org.

Lucas SRR, Hagiwara MK, Loureiro V, Ikesaki JYH and Birgel EH. 1999. *Toxoplasma gondii* infection in Brazilian domestic outpatient cats. *Rev Inst Med Trop S Paulo* 41 (4): 221-224.

Lukesova D and Literak L. 1998. Shedding of *Toxoplasma gondii* oocysts by felidae in zoos in the Czech Republic. *Vet Parasitol* 74: 1-7.

Marchiondo AA, Duszynski DW and Maupin GO. 1976. Prevalence of *Toxoplasma gondii* in wild and domestic animals of New Mexico, Arizona and Colorado. *J Wildl Dis* 12: 225-232.

Molina EC and Ridley-Dash LS. 2008. *Toxoplasmosis* in cats at Kabacan, Cotabato, Philippines. *USM R & D J* 16 (1): 53-55.

Pena HFJ, Soares RM, Amaku N, Dubey JP and Gennari SM. 2006. *Toxoplasma gondii* infection in cats from Sao Paulo state, Brazil: seroprevalence, oocyst shedding, isolation, in mice, and biologic and molecular characterization. *Res Vet Sci* 81: 58-67.

Quinn PJ and McCraw BM. 1972. Current status of *Toxoplasma* and toxoplasmosis: a review. *Can Vet J* 13 (11): 247-262.

Samuel WM, Pybus MJ and Kocan AA. 2001. *Parasitic Diseases of Wild Mammals*. (2nd ed.). Ames, IA: Iowa State University Press.

Silva JCR, Ogassarawa S, Adania CH, Ferreira F, Gennari SM, Dubey JP and Ferreira-Neto JS. 2001. Seroprevalence of *Toxoplasma gondii* in captive neotropical felids from Brazil. *Vet Parasitol* 102: 217-224.

Thompson ARC, Kutz SJ and Smith A. 2009. Parasite zoonoses and wildlife: emerging issues. *Int J Environ Res Public Health* 6: 678-693.

Weiss LM and Dubey JP. 2009. Toxoplasmosis: a history of clinical observations. *Int J Parasitol* 39(8):895-901.

Wolfe BA. 2003. Toxoplasmosis. In: Fowler ME and Miller RE (eds.) (5th ed.). *Zoo and Wild Animal Medicine*. Philadelphia: W.B. Saunders Company.