Serologic detection of *Toxoplasma gondii* infection in stray and household cats and its hematologic evaluation

Detecção sorológica da infecção por Toxoplasma gondii em gatos errantes e domiciliados e sua avaliação hematológica

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ABSTRACT

Aims: This study focused on the serologic detection of *Toxoplasma gondii* infection in two groups of cats: stray and household groups. In addition, hematologic assessment of seropositive and seronegative cats was done. **Methods:** Sixty cats were serologically tested for anti-*Toxoplasma gondii* antibodies using the latex agglutination test. Six collection sites for each group of cats were identified in the urban communities of Sta Rosa and San Pedro, Laguna, Philippines. The 60 cats collected were divided into 30 stray and 30 household cats. **Results:** Results revealed that 28 (46.67%) of the 60 cats were seropositive. There were more household cats (28.33%) which showed seropositivity compared to stray cats (18.33%), however the difference was statistically insignificant ($p>0.05$) . Hematologic tests through complete blood count showed significantly ($p<0.05$) higher number of seropositive cats with abnormalities on hemoglobin level, red blood cell count, segmenter (neutrophil) and monocyte counts compared to the control. Other parameters such as percent packed cell volume, white blood cell count, eosinophil and lymphocyte counts showed insignificant ($p>0.05$) results across seropositive cats and the control. Blood chemistry analysis showed significantly higher ($p<0.05$) potassium level irregularities in seropositive cats relative to the seronegative cats. Other parameters such as amylase, blood sugar, blood uric acid, creatinine and blood urea nitrogen were statistically insignificant ($p>0.05$). **Conclusions:** Although *Toxoplasma gondii* infection suggests possible cause of hematologic abnormalities, it is recommended that further studies on this aspect be done to provide more basic and clinical research information that would improve cat health management.

Keywords: *Toxoplasma gondii*; TOXOPLASMOSIS, ANIMAL/epidemiology; TOXOPLASMOSIS, ANIMAL/diagnosis; TOXOPLASMOSIS, ANIMAL/pathology; CATS/parasitology; CATS/blood; *Felis domesticus*; SEROLOGY; BLOOD CHEMICAL ANALYSIS/veterinary

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a coccidian apicomplexan parasite with a cosmopolitan distribution. It has a wide variety of vertebrate intermediate hosts, but only felids are its sole definitive host. Thus, cats play a vital role in the transmission of *T. gondii* in humans and other animals.[1-3]

Diseased cats manifest typical non-specific signs of toxoplasmosis. In advanced stage, however, gradually increasing severity may manifest outstanding signs in many cats, such as pneumonia, with accompanying hepatitis, diarrhea, prostration, and jaundice.[4] The presence and dissemination of *T. gondii* tachyzoites throughout the body by circulation in the blood have been observed.[1,5,6] Viable parasites can be detected in the blood 4h post-ingestion of oocysts, and 24h after bradyzoite feeding or 2 to 5 days after introducing intraperitoneally brain tissue homogenate with parasites.[7,8] This parasite circulating in the blood may potentially

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contribute to blood irregularities directly, or indirectly through its transmission in other tissues of the body.

In the Philippines, documented studies on toxoplasmosis are largely serologic in nature, in pigs,[9-11] humans,[12,13] rats,[6,14] and cats;[15,16] and few studies on histopathology in rats and cats.[7,10,17] To our knowledge, no data have been reported on studies with regard to hematologic properties of *T. gondii* seropositive cats in the local setting. Hematologic tests through blood screening help assess medical concerns and serve as baseline information for future monitoring of cats' health. Complete blood count is one of the most commonly employed blood tests in veterinary medicine as this test is designed to evaluate the red and white blood cells. On the other hand, blood chemistry tests are used to assess a wide range of conditions and the function of organs which include assessment of kidney function, blood sugar, and other substances that merit evaluation of the physiologic condition of the subjects.

In view of the earlier studies suggesting the presence of *T. gondii* parasites in the blood and its wide physiologic effects on its host, and the paucity of documented data in the Philippines, this study attempted to investigate the association between the presence of *T. gondii* infection and blood irregularities on *T. gondii*-infected felines through hematological analysis. Furthermore, this study also determined which cat population (household or stray) exhibits higher *T. gondii* infection.

METHODS

Collection of cats

Collection of cats was done in the urban communities of Santa Rosa and San Pedro, Laguna, Philippines. All procedural methods concerning the collection, handling and experimentation of cats were approved by the De la Salle University-Dasmariñas (DLSU-D) Research Review Panel (RRP) prior to the conduct of the study. The consent of the cat owners to subject their cats in the study was sought prior to the collection of household cats. Those owners who have given their consent were oriented on the procedural process that the cats had to undergo.

Six collection sites (three from Santa Rosa and three from San Pedro) were identified to select household cats from among cat owners who allowed their cats to be subjected in the study. Hence, the number of cats collected was based on the number of owners who had consented in this study, which ranged from 5-7 owners from among 13-19 households with cats per area. Since five is the minimum, such number was the basis for determining the number of household cats to be subjected in the study per area. To uniformly subject the same number of stray cats, 5 were trapped in each site, with a total of 30 household and 30 stray cats subjected in the study. The basic technique on entrapment procedure for stray cat collection was done.[18]

Since stray cats were seemingly intimidating, routing feeding schedule was established for five days. Box cages with cat food were positioned on a level surface of the area where stray cats were often observed or where they usually fed. After the cages were set, a cover over the cage was placed to enclose the area since cats venture into dark, enclosed places. The trapped cats were transported by an open-windowed vehicle into the laboratory. Just like the stray cats, the selected household cats were individually placed in a cage with cover and transported by an open-windowed vehicle into the laboratory.

To determine the age of the cats, dentition of cats were examined by a certified veterinarian. The cats subjected to examination were aged 8 weeks and above. Further, there were 15 female and 15 male cats per cat classification subjected to the study.

Maintenance and care of the cats prior to experimentation

Handling of cats was done following the standard protocol on the care and use of laboratory animals.[19] Prior to laboratory tests, individually-caged cats were acclimatized for 5 days in a well-maintained and well-ventilated rearing room at room temperature. The cages and the animal facility were sanitized regularly in order to prevent build-up of dust, dirt and wastes. Cats were fed regularly with cat food and closely monitored with particular attention to their activity, behavior and general condition.

Two days after the experimentation, household cats were returned to their owners while stray cats were tagged with improvised neck tags to avoid trapping the same cats before they were released into the areas where they were earlier trapped.

Serologic assay for anti-*T.gondii* antibodies

Five mL of blood samples were extracted from each cat through venipuncture of the jugular vein.

Serum samples were assayed within 24h from the time of collection using Toxocell Latex Agglutination test (LAT) (BIOKIT Manufacturing Company, Barcelona, Spain). The test kit contained a suspension of polystyrene latex particles of uniform size coated with soluble *T. gondii* antigen. On a disposable slide containing 50 μL of the serum, one drop of the reagent was added. The last two slides served as positive control and negative control. With a stirrer, the serum was allowed to mix with the reagent, and the preparation was gently rotated for 5 min using a shaker at 60-80 rpm prior to reading the results. The latex particles allow a visual observation of the antigen-antibody reaction. In a reactive serum, the latex suspension changed its uniform appearance and a clear agglutination became evident (titre ≥15 IU/mL), while a non-reactive (=absence of Toxoplasma Ab or with a titre <15 IU/mL) serum resulted to a suspension with a homogenous appearance.

Hematologic procedure

All seropositive stray and household cats were subjected to Complete Blood Count (CBC) and blood chemistry analyses. Fifty percent (=16) of the seronegative cats were subjected to the same tests to serve as the control group.

The CBC test was done by Carlos Veterinary clinic (Sucat, Paranaque City, Philippines). This analysis covered the examination of hemoglobin (Hgb) level; percentage of Packed Cell Volume (PCV), red blood cell (RBC) count and white blood cell (WBC) count. Analyses were done using Medic Drabkin's reagent Cyanmethemoglobin Method, Hayem's Solution Red Blood Cells Count, White Blood Cells Count Diluting Fluid, and Wright-Giemsa Stain Schilling's Differential Count. All reagents and kits were purchased from the Medic Diagnostic Reagents, Pasay, Philippines.

The blood chemistry of cats was analyzed by Mother Savior Polyclinic and Laboratory (San Pedro, Laguna, Philippines). Analyses were done using the Amylase Modified Caraway (A.L.S. Biochemicals, California USA); Liquid Glucose (Oxidase) Reagent test (Pointe Scientific, Inc. Michigan, USA) for Fasting Blood Sugar (FBS), Potassium Reagent Colorimetric Method (Biochem Scientific), Uric Acid (Liquid) Reagent Set, (Pointe Scientific, Inc. Michigan, USA), Creatinine Reagent Set (Chemplus Diagnostics Texas, USA); Blood Urea Nitrogen (BUN) Liquid Urea Reagent Set (Chemplus Diagnostics Texas, USA).

Results of hematologic analyses were done following the instruction in the laboratory kit used and verification from certified veterinarians; and by comparing to the standard feline hematologic data.[20] For CBC, expected values of Hgb ranged from 9.5-15 g/dl, RBC count ranged from $6x10^6$ to $10x10^6$/cumm, WBC count ranged from $5.5x10^2$ to$19.5x10^2$/cumm; and packed cell volume (PCV) with 29-45 vol% normal range. Differential counts' expected values were 35-75% for segmenters (neutrophils), 1-4% for monocytes, 20-55% for lymphocytes, 1-12% for eosinophils and 0-1% for basophils.[20] The following were the expected values for the blood chemistry tests: amylase 30-1100 mg/dL; blood sugar 70-150 mg/dL; potassium 3.7-5.8 mg/dL; blood uric acid (BUA), 0-1.0 mg/dL; creatinine 0.3-2.1 mg/dL; and blood urea nitrogen (BUN), 10-30 mg/dL.

Statistical analysis

Comparative serologic data between stray and household cats were analyzed using chi-square ($P<0.05$). Data analysis of hematologic results were compared with the cat standard values for hematologic parameters under study. Likewise, chi-square (or Fisher's exact test when required) was done to determine the hematologic results across seropositive and seronegative cats, and comparison between results of seropositive stray and household cats.

RESULTS

Serologic data

A total of 60 cats were assayed for anti-*T.gondii* antibodies. Twenty-eight (46.67%) were seropositive, of these 11 (18.33%) belonged to the stray cat group while 17 (28.33%) were household cats (Table 1). While serologic data suggested greater susceptibility of household cats relative to stray cats, statistical analysis showed no association between the rate of infection by the parasite and cat classification.

Table 1. Number of stray and household cats serologically positive to *Toxoplasma gondii* infection in the urban communities of Santa Rosa and San Pedro, Laguna, Philippines

Cat classification	Serologic Reaction		Total
	Seropositive N (%)	Seronegative N (%)	
Stray cats	11 (18.33)	19 (31.67)	30
Household cats	17 (28.33)	13 (21.67)	30
Total	28 (46.66)	32 (53.34)	60

Hematologic results

The CBC analysis showed significantly higher number of seropositive cats with abnormalities ($p<0.05$) on Hgb level, RBC count, and differential count on segmenters and monocytes compared to the control (Table 2). The levels of other parameters such as PCV, WBC, eosinophil and lymphocytes showed insignificantly different results between seropositive cats and the control.

Table 2. Number of seropositive cats and control showing hematologic abnormalities based on complete blood count analysis

Blood count	Abnormal blood count*	
	seropositive cats (% based on N=28)	control (% based on N=16)
Hemoglobin	25 [a] (89.3)	5 [b] (31.3)
Red blood cell (RBC)	28 [a] (100)	3 [b] (18.8)
Packed cell volume (PCV)	13 [a] (46.4)	6 [a] (37.5)
White blood cell (WBC)	17 [a] (60.7)	8 [a] (50.0)
Eosinophil	5 [a] (17.8)	3 [a] (18.7)
Lymphocytes	16 [a] (57.1)	6 [a] (37.5)
Monocytes	21 [a] (75.0)	2 [b] (12.5)
Segmenters	18 [a] (64.3)	5 [b] (31.3)

* Values per parameter with different letters were statistically significant.

In blood chemistry analysis, significantly higher number of seropositive cats show irregularities in potassium level as compared to the seronegative cats (Table 3). Other parameters such as amylase, blood sugar, BUA, creatinine and BUN were statistically insignificant in terms of the number of affected seropositive and seronegative cats manifesting abnormalities.

Significantly affected hematologic parameters were noteworthy implication of active toxoplasmosis in seropositive cats (Table 4). This study further determined the type of abnormality as to whether the value range was abnormally high or low. The result of hematologic analysis showed significantly low levels of Hgb with a value ranging from 6.8-9.0 g/dL as against the normal 9.5 to 15 g/dL, RBC count with a value range of $3.3x10^6$-$5.7x10^6/mm^3$ compared to the normal value of $6.0x10^6$-$10x10^6/mm^3$, and monocytes with no traces or very few (<1-4%) were seen. Abnormally high levels of segmenters (neutrophils) were observed with a value range of 79-96% (normal=35-75%), and potassium with a slightly higher value ranging 5.9-6.1 mg/dL (normal=3.7-5.8 mg/dL). The types of abnormalities manifested by both stray and household seropositive cats were the same. Furthermore, the number of affected household and stray seropositive cats revealed no significant differences as regards abnormalities on segmenters, monocytes, RBC, Hgb and potassium levels.

Table 3. Number of seropositive cats and control showing hematologic abnormalities based on blood chemistry analysis

Blood chemistry	Abnormal blood chemistry*	
	seropositive cats (% based on N=28)	seronegative cats (% based on N=16
Blood uric acid (BUA)	5 [a] (17.9)	1 [a] (6.3)
Fasting blood sugar (FBS)	8 [a] (28.6)	8 [a] (50.0)
Creatinine	1 [a] (3.6)	0 [a] (0.0)
Blood urea nitrogen (BUN)	7 [a] (25.0)	4 [a] (25.0)
Potassium	21 [a] (89.3)	4 [b] (25.0)
Amylase	1 [a] (3.6)	1 [a] (6.3)

* Values per parameter with different letters were statistically significant.

Table 4. Number of seropositive stray and household cats manifesting abnormally high or low values on hematologic analyses

Hematologic parameters	Abnormalities (%)*			
	Stray cats (N=11)		Household cats (N=17)	
	High range	Low range	High range	Low range
Potassium	8 [a] (100)	0 [b] (0.0)	13 [a] (100)	0 [b] (0.0)
Hemoglobin	1 [a] (10.0)	9 [b] (90.0)	2 [a] (13.3)	13 [b] (86.7)
Red blood cell (RBC)	0 [a] (0.0)	11 [b] (100)	0 [a] (0.0)	17 [b] (100)
Monocytes	0 [a] (0.0)	8 [b] (100.)	0 [a] (0.0)	13 [b] (100)
Segmenters	7 [a] (100)	0 [b] (0.0)	11 [a] (100)	0 [b] (0.0)

* Values per parameter with different letters were statistically significant across same range level/cat classification, and across different ranges within cat classification

DISCUSSION

The relatively high number of seropositive cats is consistent with earlier documented studies in domestic cats.[1, 17, 21] The high infectivity of *T. gondii* to cats can be attributed to the relatively wide range of portal of entry of the parasite to its definitive host. The ingestion of any of the three infective stages (oocysts, tachyzoites and bradyzoites) of *T. gondii through* preying on infected wild animals like rats and mice, or eating contaminated raw meat or dairy products from infected sources, is probably the most common route of *T. gondii* infection in cats.[2, 22]

The seropositivity of cats to *T. gondii* is indicative of an outcome of shedding episode of oocysts in cats.[16,23,24] This suggests that seropositive cats already posed lesser risk of exposure to infection, and thereby shedding oocysts to potentially transmit the infection to humans and other possible intermediate hosts is minimal, unless they are immunocompromised. In this study, however, the oocyst shedding episodes of cats were not recorded and thus, the likely transmission of oocysts from seropositive cats to humans and other animals during the active shedding episodes was not established.

Although lesser infectivity was observed in stray cats, the uninfected stray cats are still considered a potential risk of infection because these cats, being unowned, are more exposed to free environment where they have their uncontrolled activities. This makes them more exposed to *T. gondii* infection by ingesting infective oocysts or by ingesting tissues cysts from intermediate hosts, such as small rodents which they prey on.[7, 25] The rate of *T. gondii* infection in small rodents in some parts of the world was estimated up to 73%.[26] In the Philippines, high population of small rodents has been reported.[14] These rodents had been established to be one of the most available intermediate hosts of *T.gondii* because of their close proximity with cat habitation, thus, they play a significant role in infecting stray cats.

Although in some studies hematologic values usually remain unaltered during the course of uncomplicated toxoplasmosis,[27] this study implicated otherwise. In fact, significantly affected hematologic parameters were noteworthy implication of active toxoplasmosis in seropositive cats.

Cats with clinical toxoplasmosis show variety of clinocopathologic abnormalities associated with irregularities in blood components either their morphological characteristics or the number of cells present.[27] Studies had established the development of anemia in cats as shown in low percentage of PCV, Hgb concentration, and RBC as compared to the reference range.[27,28] In this study, PCV did not show critical value, but the RBC count and Hgb concentration were below the reference range, which may be suggestive of possible development of anemia in seropositive cats. Low RBC count and Hgb, among other combined factors, could cause anemia which has been recorded in toxoplasmic cats and even in humans.[29,30] These results were suggestive of *T. gondii*-infection induced effect on cats.

In totality, the presence of WBC in the blood reflects immunologic condition of seropositive cats.[27, 28] Any changes in the WBC may reflect serious abnormalities on the health condition of the cats. The differential counts on neutrophils and monocytes revealed appreciable results in this study as earlier mentioned.

Defects in neutrophil functions can be due to a reduction of neutrophil count at a critical level due to impaired immunity or high levels may indicate an active infection.[31,32] Both conditions can be present in *T.gondii* infection, depending on the underlying health conditions of the host or aggravating factors that could influence the condition of the host.[31,33] In the present study, neutrophil count was significantly higher in seropositive cats. Such result agreed with previous studies on *T. gondii* infected cats showing diseases associated with increased neutrophil which may be indicative of an active infection. On the other hand, this study showed that seropositive cats lacked or had very few monocytes. Ironically, most toxoplasmic conditions in humans were associated with monocytosis (increased monocyte counts) rather than low monocyte which when progresses, develops into monocytopenia (a form of leukopenia associated with a deficiency of monocytes).[33] The absence or low monocytes level in the blood can occur in response to the release of toxins into the blood by certain microorganisms, specially bacteria. In retrospect, leucopenia is often associated with acute and chronic forms of toxoplasmosis.[29]

Potassium levels that are too high or too low can increase the risk of an abnormal physiologic condition. Abnormal potassium level posed serious problems together with underlying factors such as increased BUN and creatinine which is a manifestation of renal dysfunction.[34] In this study the slightly high level of potassium may not implicate serious effect as other factors such as creatinine, BUA and BUN did not pose significant findings in this study. Other studies however reported that creatinine was observed to be elevated during *Toxoplasma* infection.[27] And high level of potassium has been reported to affect abnormal heartbeat in humans.

This study also showed that amylase was not affected by seropositivity of the cats. Such result is in congruence with an earlier report that amylase is unreliable determinant of *T. gondii* infection.[35,36] The fasting blood sugar analysis, which tested the carbohydrate metabolism through measurement of blood glucose levels after the cats fasted, showed insignificant association with *T. gondii* infection. Based on our knowledge, no study has directly established the association between *T.gondii* infection and abnormal blood sugar level in cats.

The development of toxoplasmosis may be quiescent or produce many signs and symptoms resulting from the parasite's invasiveness of a wide-range of tissues. The reports documented through this study on the hematologic changes in cats for both stray and household cats supported the fact that toxoplasmosis is a multi-systemic disease which affects almost all major organs of the body, manifesting a wide range of symptoms and abnormalities. Because of this, the importance of cats as the only definitive hosts of *T.gondii* parasite must be underscored. It is therefore recommended that further studies on the chronicity of infection through serologic, hematologic and histopathologic investigations be done. Hence, these will provide more basic and clinical research information that would improve cat health management.

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REFERENCES

1. Dubey JP, Zhu XQ, Sundar N, et al. Genetic and biologic characterization of *Toxoplasma gondii* isolates of cats from China. Vet Parasitol. 2007;145:352-6.
2. Omata Y, Oikawa H, Kanda M, et al. Transfer of antibodies to kittens from mother cats chronically infected with Toxoplasma gondii. Vet Parasitol. 1994;52:211-8.
3. Frenkel JK. Pathology and pathogenesis of congenital toxoplasmosis. Bull NY Acad Med. 1974;50:182-91.
4. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton: CRC Press; 1988.
5. Unno A, Suzuki K, Xuan X, et al. Dissemination of extracellular and intracellular *Toxoplasma gondii* tachyzoites in the blood flow. Parasitol Int. 2008,57:515-18.
6. Cabanacan-Salibay C, Claveria FG. *Toxoplasma gondii* infection in Philippine *Rattus* spp. confirmed through bioassay in Mus musculus. Vet arh. 2006;76:351-61.
7. Salibay CC, Claveria FG. Serologically confirmed *Toxoplasma gondii* infection in Philippine *Rattus* spp. and its histopathology in Mus musculus. Phil J Sci. 2005;134: 95-104.
8. Kikuchi T, Furuta T, Kojima S. Kinetics of the nucleotide Triphosphate hydrolase of Toxoplasma gondii in mice with acute and chronic toxoplasmosis. Ann Trop Med Parasitol. 2002;96:35-41.
9. Manuel MF. Prevalence of *Toxoplasma gondii* antibodies in swine in the Philippines. Phil J Vet Med. 1982;20:71-7.
10. Marbella CO. *Toxoplasma gondii*: antibody titer determination, histopathologic and isolation studies from naturally infected swine [thesis]. Philippines, Los Baños,Laguna: University of the Philippines; 1980.
11. Mendoza CB. A serological survey on the incidence of toxoplasmosis in swine. [thesis]. Philippines, Los Baños, Laguna: University of the Philippines; 1974.
12. Kawashima T, Khin-sane-win, Kawabata M, et al. Prevalence of antibodies to *Toxoplasma gondii* among urban and rural residents in the Philippines. Southeast Asian J Trop Med Public Health. 2000;31:742-6.
13. Salibay CC, Dungca JZ, Claveria FG. Serological survey of *Toxoplasma gondii* infection among Urban (Manila) and Suburban (Dasmariñas, Cavite) Residents, Philippines. J Protozool Res. 2008;18:26-33.
14. Salibay CC, Claveria FG. Serologic detection of *Toxoplasma gondii* infection in *Rattus* spp. collected from three different sites in Dasmariñas, Cavite, Philippines. Southeast Asian J Trop Med Public Health. 2005;36:46-9.
15. Dans BK. A study on the presence of *Toxoplasma gondii* antibodies in the blood serum of stray cats in the Ninoy Aquino Parks and Wildlife Nature Center using enzyme-linked immunosorbent assay (ELISA) [thesis]. Philippines, Los Baños, Laguna: University of the Philippines; 2002.
16. Minervini N. A study on the presence of *Toxoplasma gondii* antibodies in the Philippines raised cats using the enzyme-linked immunosorbent assay test [thesis]. Philippines, Los Baños, Laguna: University of the Philippines; 1985.
17. Molina EC, Ridley-Dash LS. Toxoplasmosis in cats at Kabacan, Cotabato, Philippines. USM Res Dev J. 2008; 16:53-5.
18. Feral Cat Coalition. Humane Trapping Instruction. [homepage on the Internet] San Diego: Feral Cat Coalition [cited 2009 Nov 30]. Available from: http://www.tomahawklivetrap.au/ Humane Trapping Instructions.htm.
19. Philippine Association for Laboratory Animal Science (PALAS). Code of practice for the care and use of laboratory animals in the Philippines. Philippines: Committee on PALAS; 2002.
20. Tilley LP, Smith FWK Jr. editors. The 5 minute veterinary consult: canine and feline. Maryland: Lipincott Williams & Wilkins; 2000.
21. Dubey P, Su C, Cortés JA, et al. Prevalence of *Toxoplasma gondii* in cats from Colombia, South America and genetic characterization of *T. gondii* isolates. Vet Parasitol. 2006;141:42-7.
22. Frenkel JK. Biology of *Toxoplasma gondii*. In: Ambroise-Thomas P, Petersen E. Congenital toxoplasmosis: scientific background, clinical management and control. Paris: Springer; 2000. p. 9-25.
23. Summer B, Ackland ML. *Toxoplasma gondii* antibody in domestic cats in Melbourne. Australian Vet J. 1999; 77:447-9.

24. Haddadzadeh HR, Khazraiinia P, Aslani M, Rezaeian M, Jamshidi S, Taheri M, Bahonar A. Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Tehran. Vet Parasitol. 2006;138:211-6.
25. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. Australian Soc Parasitol. 2000;3:34-46.
26. Jackson MH, Hutchison WM. The prevalence and source of *Toxoplasma* infection in the environment. Adv Parasitol. 1989;28:55-105.
27. Lappin MR. Feline toxoplasmosis: interpretation of diagnostic results. Semin Vet Med Surg.1996;11:154-60.
28. Okewole EA, Akpan MO. Clinical feline toxoplasmosis: parasitological, haematological and serological findings in retroviral infected and uninfected cats. Vet Arhiv. 2002; 72:67-79.
29. Smart ME, Downey RS, Stockdale PHG. Toxoplasmosis in a cat associated with cholangitis and progressive pancreatitis. Can Vet J. 1973;14:313-6.
30. Alford CA Jr, Stagno S, Reynolds DW. Congenital toxoplasmosis: clinical laboratory, and therapeutic considerations, wih special reference to subclinical disease. Bull N YAcad Med. 1974;50:160-81.
31. Bliss SK, Gavrilescu LC, Alcaraz A, et al. Neutrophil depletion during *Toxoplasma gondii* infection leads to impaired immunity and lethal systemic pathology. Infect Immunol. 2001;69:4898-905.
32. Stites DP, Stobo JD, Fudenberg HH, editors. Basic and clinical immunology. 5th ed. California: Lange Medical Publications; 1984.
33. Rajantie J, Siimes MA, Taskinen W, et al. White blood cells in infants with congenital toxoplasmosis: transient appearance of cALL antigen on reactive marrow lymphocytes. Scan J Infect Dis. 1992;24:227-32.
34. Chrisman CL. Acute weakness in the cat [Internet]. Florida: Waltham. The world's leading authority on pet care and nutrition [cited 2009 Nov 30]. Available from: http://www.walthamusa.com/articles/CHRISMAN.pdf.
35. Barr SC. Toxoplasmosis [Internet]. Westbrook: IDEXX Laboratories [cited 2009 Nov 30]. Available from: https://www.vetconnect.com.au/5min/data/11101111.htm.
36. Dubey JP, Greene CE, Lappin MR. Toxoplasmosis and neosporosis. In: Greene, CE, editor. Infectious diseases of the dog and cat. Philadelphia: WB Saunders; 1990. p.830-5.