Seropathological Diagnosis of *Toxoplasma gondii* in Stray Cats in Baghdad Province

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Summary

The current study was performed an investigation in seropositive stray cats with *Toxoplasma gondii* and to correlate the results of pathological lesions with seropositive results of the cats. To achieve these goals, fifty blood samples and specimens from internal organs (liver, spleen, brain, kidney, intestine and lung) of stray cats were collected from different area of Baghdad Province during the period 1.10.2011-1.10.2012. Seropositive ELISA-IgG was demonstrated that 66 % of stray cats while female expressed (75 %) of seropositive ELISA-IgG were higher than male (30%) and animals with average age 2months showed high percentage of seropositive ELISA-IgG (100%) as compared with age (adult cat) that expressed(63.82 %) of seropositive. There was a significant difference (P≤0.01) among positive cases to anti-*Toxoplasma* ELISA-IgG. Severe pathological lesions were noticed in the lungs ,livers and intestines of animals that expressed high (optical density) (OD) of anti-*Toxoplasma* IgG ,in addition ,tachyzoits intracytoplasm of alveolar macrophages and hepatocytes as well as free zoites in alveolar space of the lung, were reported. Local necrosis with tachyzoites was seen in the brain of the cats, in addition to mineralization. On bases of the presence of pathological lesions in cats that expressed seropositive anti-*Toxoplasma* antibodies, it can be conclude that *T.gondii* is responsible for the appearance of inflammatory reaction in the internal organs of cats and there is a correlation between seropositive and pathological lesions of *T.gondii* infection and this parasite is highly distributed in Baghdad stray cats and it may be an important cause of abortion in the women.

Keywords: *T.gondii*, Cat, ELISA, IgG.

Introduction

Toxoplasma gondii (T.gondii) is intracellular protozoan parasite that is endemic worldwide and is a major opportunistic pathogen in immuno compromised hosts cause Toxoplasmosis, major zoonosis parasitic diseases in human beings and agricultural animals widely spread throughout the world (1). *T. gondii* infection is a global concern, and about one third of the human population has been exposed to this parasite (2). Felids are considered the only definitive hosts of *T. gondii* playing a crucial role in the transmission of the parasite (3).

Cats infected by *T. gondii* may pose a potential threat to public health, because they can shed and excrete environmentally resistant oocysts in their feces (4). Household cats are one of the most intimate companions of humans, by frequent contact with cats, people may increase their risk of acquiring *T. gondii* infection. More importantly, stray cats usually wander everywhere and play a more important role in the transmission of toxoplasmosis to other animals and humans (5). Surveys of *T. gondii* infection in stray and household cats have been reported extensively in the world (6). In Iraq, large numbers of stray cats are found roaming residential streets and increasing the risk of public health for animals and humans.

For the diagnosis of *T. gondii* infection, detection of the organism itself is confirmative but very difficult, thus, most clinical laboratories use serological tests to detect antibodies against *T. gondii* such as the latex agglutination (LA) test, ELISA and indirect fluorescent antibody test because of its high specificity and sensitivity (7), as well as polymerase chain reaction [PCR], histologic demonstration of the parasite and/or its antigens (i.e. immunoperoxidase stain), (2). However, little is known about the infection of *T. gondii* in stray cats in Iraq. The aime of this study is to determine the seroprevalence of *T. gondii* infection in stray cats in certain areas of Baghdad. In this study, it tested the efficiency

of histopathological examination of internal organs of Iraqi stray cats to detect *T.gondii* infections and whether it can be used as a confirmatory test to detect infections in cats who's showed positive serum IgG.

Materials and Methods

Sample collection: Between 1.10.2011 and1/10/ 2012, a total of 50 blood samples were obtained from clinically health stray cats from different region of Baghdad city for the serological detection of *T. gondii* infection, as well as pieces from internal organs of these cats were taken for histopathological examination. Information regarding the breed, age, gender record. Blood samples were kept at room temperature for 2 hr, centrifuged at 3, 000 rpm for 5 min, and the separated serum samples were stored at -20°C until further analysis, small specimens (1+,1+)cm from liver, spleen, brain, kidney, intestine and lung were fixed with neutral buffer formalin 10% for 3 days then, using routine procedure for preparation of histopathological section according to (8).

Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of IgG antibodies for Toxoplasmosis in serum for stray cats.(the kit /Indirect Multi-species ID VET/innovative diagnostics/ FRANCE. The test was made according to the instructions of the productive company. [ID VET/ innovative diagnostics/ France.

Statistical analysis was conducted to determine the statistical differences among the groups by using (Chi-Square) ready–made statistical design: statistical package for social science (9).

Results and Discussion

The present study showed that 33 out 50 (66%) cats seropositive had anti-Toxoplasma 1gG antibodies , and the percentage of seropositive ELISA-IgG was higher (75%) in females than in males (30%), (Table, 1).

Our result revealed a high prevalence rate of *T.gondii* infection (66%) in stray cats which may be play important role in the epidemiology of *T.gondii* among human and farm animals in Iraq and it is essential to control the number of stray cats in order to reduce the transmission of toxoplasmosis.

The present study was supported with the idea that mentioned by (10) who reported that six samples of stray cats were all positive for agglutination latex and ELISA-IgG except one case was negative to IgG, also the current study is nearly agreed with (11) in Al-Ahsa area ,Saudi Arabia, who showed that the seroprevalence anti-Toxoplasma ELISA-IgG was 90% and 20% in stray and household cat respectively and with result of (12) (63%) , also our result was higher than recorded in stray cats Iran (32.1%) (13), Guangzhou city (25.2%) (14),In Zhengzhou City (15.5%) and Beijing City (14.1%) in China (15) in Bangkok (4,8%), (16) and also our finding was higher than reported by with (17) who reported that the prevalence of *T.gondii* infection in cats in Lanzhou-China was (21.3%) also (18) reported that the antibodies to *T.gondii* were found in (35%) of cats. The differences in seroprevalences of *T. gondii* in cats are probably due to differences in ecological and geographical factors, serologic tests used and the living conditions for cats. In general *T. gondii* oocysts are more likely to survive in warm and humid environments (2).

The warm and humid climate in Iraq is favorable for the transmission of *T. gondii*. However, the high seroprevalence of *T.gondii* infection in stray cats may be due to hunting habit of stray cats that their diet includes rodents, placenta, stillborn foeti and wild birds (19). In epidemiological situations it is more advisable to examine the cat serologically for *T. gondii*-specific antibodies to find out the immune status of the cat (20). A serologically negative result suggests that the cat has not yet been exposed to *T. gondii* and is still susceptible to infection in the future. A serologically positive result suggests that the cat already has been infected with *T. gondii* in the past (21).

Table, 1: Seropositive ELISA- IgG in cats according to the sex.

Sex gender	Total examined cases	ELISA- IgG			
		+ve	- ve	% of +ve	O.D
Male	10	3	7	30%	1.239
Female	40	30	10	75%	1.1864
Total	50	33	17	66%	

Chi=79.286; df =1; significant differences between sex at P≤0.01

It reported significant (P≤0.01) higher sero positive anti-*Toxoplasma* IgG antibodies in the females (75%) than in male (30%), these may due to sex females hormonal disturbance induce by *T.gondii* infection, these result was in agreement with (16) who explained that the prevalence of *T.gondii* infection was significantly higher in females (5.6%) than in males (3.6%). Also they reported that Cats more than 5 years old had the highest infection rate (5.1%). Also it demonstrated that high optical density of anti-*Toxoplasma* IgG antibody in sreopositive animals, these result may be indicated that the stray cats suffered from severe infection and\ or repeated exposure to *T.gondii* shedding oocysts to environment.

Table (2) revealed that seropositive ELISA-IgG was high significant (P≤0.01) in young cats (100%) as compared with adult (63.82%), these observation may be indicated that *T.gondii* transmitted through the placenta to fetus or by milk through lactation. Our result was supported idea that mentioned by (16) who found that the prevalence of antibodies varied with ages, and *T. gondii* seroprevalence in older animals was generally higher than that in young animals.

Table, 2: Seropositive of ELISA- IgG in stray cats according to the age

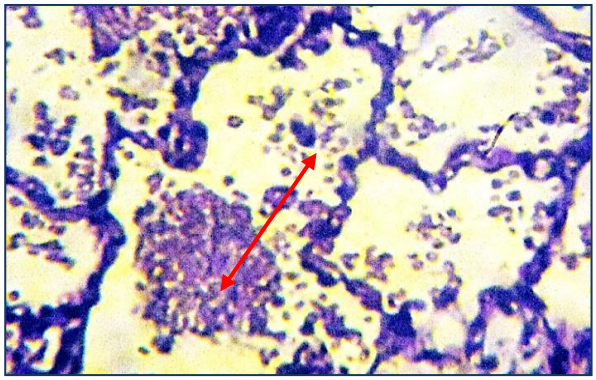
Age	Total examined cases	ELISA- IgG			
		+ve	-ve	% of +ve	O.D
2 months	3	3	-	100 %	1.190
Adult	47	30	17	63.82 %	1.1913
Total	50	33	17		

Chi=7.902; df=1; significant differences between age at P≤0.01

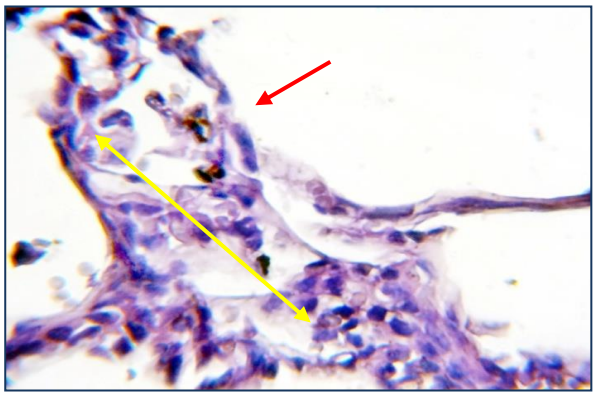
In the present survey it revealed a high seroprevalence of *T. gondii* infection in stray cats in certain area of Baghdad. Knowing that cats play an important role in the transmission of *T. gondii*, revealing thus a significant public health concern, integrated strategies with efficient management measures should be taken to prevent and control *T. gondii* infection in cats in Baghdad.

Histopathological examination for stray cats: The pathological lesions in the internal organs of the animals that express high anti-*Toxoplasma* antibodies OD showed severe pathological changes as comparing to those animals that express low values of OD of anti-*Toxoplasma* antibodies. The lesions in the lung characterized by severe hemorrhage were seen in the alveolar space with inflammatory cells particulary neutrophils and macrophages (Fig. 1). In other section,it was reported proliferation of pneumocytes type II with aggregation of mononuclear cells particulary macrophages in the alveolar space (Fig. 2). The brain showed congestion of blood vessels with neutrophils and mononuclear cells in their lumen and in perivascular area of pia mater (Fig. 3). In addition to congestion of blood vessels in brain parenchyma with wallerian degeneration of nerve fiber. The histopathological examination of the intestine revealed inflammatory cells mainly neutrophils in the lumen of mucosal glands (Fig. 4), as well as, edema, RBCs and inflammatory cells particularly neutrophils were recorded in the submucosal layer. The intestine of animal with anti-toxoplasma IgG OD.141.94%, expressed shizon in the epithelial cells surrounded by erosion lesion and these cyst also was seen in the LP of the intestine as well as inflammatory cells mainly eosinophils and neutrophils infiltration in the lamina properia, also it recorded lymphocytic cells hyperplasia in peyers patch (Fig. 5). The liver showed large necrotic area with basophilic tachyzoits intracytoplasm of swelling hepatocyte (Fig. 6), in addition to granuloma -atous lesion consisting from aggregation of macrophages and lymphocytes and zoites in sinusoids (Fig.7). It reported severe patholo -gical lesions in the lung, intestine, liver and spleen of cats that expressed high level of OD anti-*toxoplasma* IgG antibody.

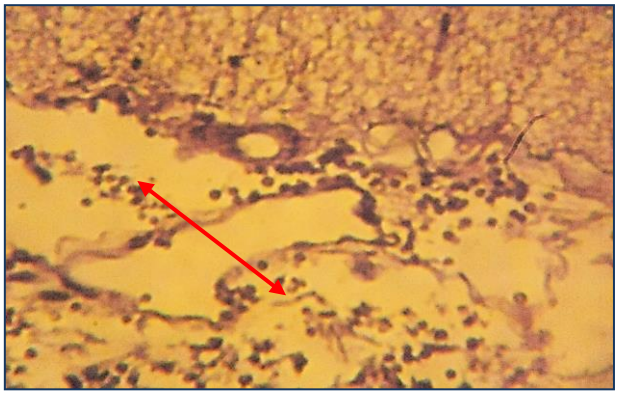
These observations may indicat that the animals were exposed to acute toxoplasmosis, these results were in consistent with (22) who reported necrosis in many organs of the body during acute toxoplasmosis . We reported large area of hepatocytes necrosis with intracellular tachyzoites , these result was agreement with (23) who showed generalized necrosis of the hepatocytes and some of hepatocytes filled with a large group of parasites and the nuclei of hepatocytes cells expressed karyorrhesis and karyolysis also (24) revealed multiple foci of acute coagulation necrosis with infiltration of small amounts of neutrophilic granulocytes and macrophages in lymph nodes, liver, and lung.



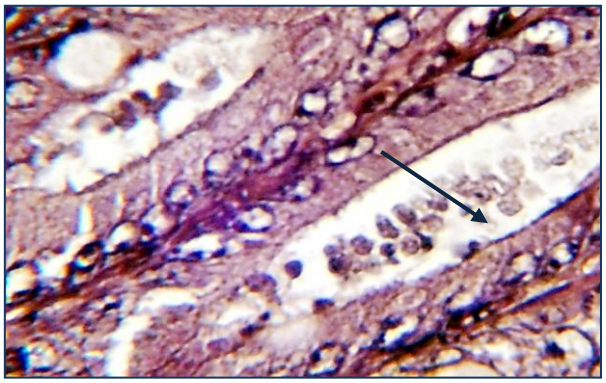
Fig, 1: Histopathological section in the lung of cat shows RBCs, neutrophils in alevolar lumina with accumilation of neutrophils around endospor (H & E stain 40X).



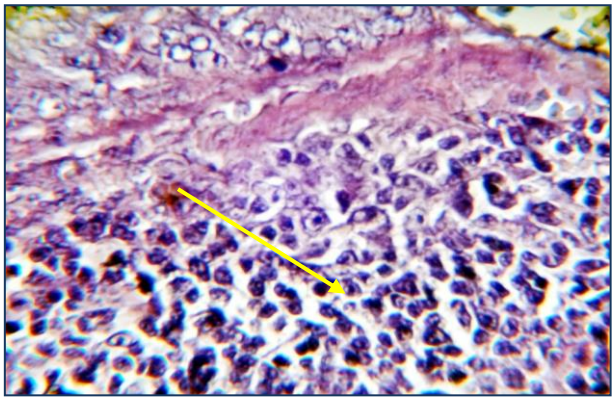
Fig, 2: Histopathological section in the lung of cat showing proliferation of pneumocytes type II with macrophages engulf tachyzoites in the alveolar lumina ⟷ (H&Estain 40X).



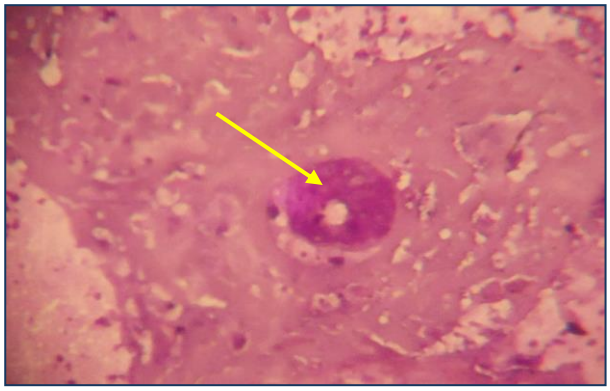
Fig, 3: Histopathological section in the brain of cat showing congestion of blood vessels of meninges with neutrophils in their lumen in pia mater ⟷ (H&Estain 40X).



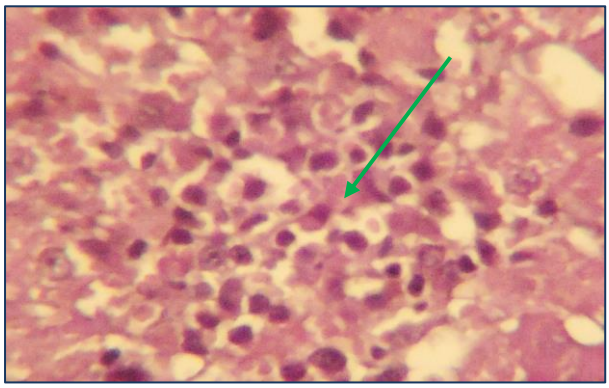
Fig, 4: Histopathological section in the intestine of the cat showing cellular debress in the lumen of mucosal glands (H&Estain 40X).



Fig, 5: Histological section in the intestine of cat showing hyperplasia of lymphoid tissues in peyers patch (H&Estain 40X)



Fig, 6: Histopathological section in the liver of cat showing large necrotic area with intracellular tachyzoits (Giemsa stain 40X).



Fig, 7: Histopathological section in liver of cat showing granulomatous lesion consisting from aggregation macrophages and lymphocytes and zoites in sinusoids → (H&E stain 40X).

The current study revealed granulomatous lesion in the liver, these result may be indicate that the body attempts to limit the parasitic infection by aggregation macrophages around the pathogens and these macrophages, which were stimulated by *T.gonddi* antigens destroyed the parasite. Cell mediated immune response play essential in role eradication of *T. gondii* may be due to these parasite is intracellular pathogen, this investigation was in agreement with (25).

The cytokines involve in the immune process against *T.gondii* are IL-2, IFN-y,TNF-a and IL-6 but the IFN-y and TNF-a are the critical mediators in the cell mediated immune response against *T.gondii* infection(26) also,in mice (27) reported that the activation of macrophages by IFN-y in the presence of C-signals such as TNF-a is necessary to trigger the cytotoxic activity of the macrophages against *T.gondii*.

It found congestion of blood vessels with neutrophils in pia mater and perivascular mononuclear cells cuffing in the brain parenchyma , these lesion may be indicated that the animals were exposed to chronic toxoplasmosis, these investigation confirm the results of (28) who explained that chronic *Toxoplasma* infection lesions occur more often in muscle, eye and brain than in visceral tissues, also (29) recorded that the pathological lesions associated with chronic *Toxoplasma* in brain include congestion of the meninges with numerous mononuclear cells invading the meninges and some cuffing of mononuclear cells around blood vessels.

References

1. Smith, J.E. and Reduck, N.R. (2000). *Toxoplasma gondii* strain variation and pathogenicity. In: Cary, J.W., Linz, J.E.and Bhatnagar, B. (Eds). Microbial Foodborne Diseases: Mechanisms of Pathogenesis and Toxin Synthesis. Technnomic Publishing, Lancaster, PA, Pp:405- 431.
2. Dubey, J.P. (2010). Toxoplasmosis of Animals and humans. CRC Press Inc., Boca Raton, New York; 2nd Ed. Pp:1-313.
3. Montoya, J.G. and Liesenfeld, O. (2004). Toxoplasmosis. Lancet, 363:7-8
4. Cenci- Goga , B.T.; Rossitto , P.V.; Sechi , P.; McCrindle, C.M. and Cullor ,J. S. (2011). Toxoplasma in animals, food, and humans: an old parasite of new concern. Foodborne Pathog Dis., 8:751-762.
5. Elmore, S.A.; Jones, J.L.; Conrad,P.A.; Patton, S.; Lindsay, D.S. and Dubey, J.P. (2010). *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends Parasitol., 26:190-196.
6. Lee, W.J. and Nam, H.W. (2010). Prevalence of *Toxoplasma gondii* infection in stray and household cats in regions of Seoul, Korea. Korean .J. Parasitol., 48:267-270.
7. Cook, A.J.; Gilbert, R.E.; Buffolano, W.; Zufferey, J.; Petersen, E.; Jenum, P.A.; Foulon, W.; Semprini, A.E.and Dunn , D.T. (2000). Sources of Toxoplasma infection in pregnant women: European

multi center case-control study. Br. Med. J., 312:142-147.

8. Luna, L.G. (1968). Manual of histologic staining methods of the armed force institute of pathology 3rd Ed. McGraw-Hill, New York. USA.
9. SPSS, (2008). Statistical Package for the Social Sciences, Version 17 (Win/ Mac/ Linux) Users guides SPSS Inc., Chiaqo III, USA Web site http://WWW.SPSS.com.
10. Dawood, K. A.; Abdulrazaq, A. M. and Naamah, A. J. (2010). Detection of Toxoplasmosis in human and cats immunologically. Kufa J. For Veterinary Medical Sciences, 1:65-71.
11. Mohammad, H.I.; Amin, T.T.; Balaha, M.H. and Moghannum, M.S. (2010). Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: Seroprevalence and possible risk factors. Ann. Trop. Med. Parasitol., 104:493-504.
12. Miro, G.; Montoya, A.; Jimenez, S.; Frisuelos, C.; Mateo, M. and Fuentes, I. (2004). Prevalence of antibodies to *Toxoplasma gondii* and intestinal parasites in stray,farm and household cats in Spain.Vet. Parasitol., 126:249-255.
13. Akhtardanesh, B.; Ziaali, N.; Sharifi, H. and Rezaei, S. (2010). Feline immunodeficiency virus, feline leukemia virus and *Toxoplasma gondii* in stray and household cats in Kerman-Iran: Seroprevalence and correlation with clinical and laboratory findings. Res. Vet. Sci., 89:306-310.
14. Dubey, J.P.; Zhu, X.Q.; Sundar, N.; Zhang, H.; Kwok, O.C. and Su, C. (2007). Genetic and biologic characterization of *Toxoplasma gondii* isolates of cats from China. Vet. Parasitol., 145:352–356.
15. Zhang, H.; Zhou, D.H.; Chen, Y.Z.; Lin, R.Q.; Yuan, Z.G.; Song, H.Q.; Li, S.J. and Zhu, X.Q. (2010). Antibodies to *Toxoplasma gondii* in stray and household dogs in Guangzhou, China. J. Parasitol, 96:671–672.
16. Jittapalapong, S.; Sangvaranond, A.; Pinyopanuwat, N.; Chimnoi, W.; Khachaeram, W.; Koizumi, S. and Maruyama, S. (2005). Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Satun Province, Thailand. Vet. Parasitol., 127: 17-22.
17. Song-Ming, W.; Xing-Quan, Z.; Dong-Hui, Z.; Bao-Quan, F.; Jia, Chen; Jian-Fa, Y.; Hui-Qun, S. ; Ya-Biao, W. and De-He, Y. (2011). Seroprevalence of *Toxoplasma gondii* infection in household and stray cats in Lanzhou, northwest China Parasit Vectors., 4: 214.
18. Asthana, S. P.; Macpherson, C. N.; Weiss, S. H.; Stephens, R.; Denny, T. N.; Sharma, R. N. and Dubey, J. P. (2006). Seroprevalence of *Toxoplasma gondii* in pregnant women and cats in Grenada, West Indies. J. Parasitol., 92: 644–645.
19. Mineo, T.W.P.; Silva, D.A.O. and Costa, G.H.N. (2001). Detection of IgG antibodies to *Neospora caninum* and *Toxoplasma gondii* in dogs examined in a veterinary hospital from Brazil. Vet. Parasitol., 98:239-245,.
20. Lappin, M.R. (1996). Feline toxoplasmosis: interpretation of diagnostic test results. Semin. Vet. Med. Surg, (Small Anim), 11:154–60.
21. Dubey, J.P.; Lappin, M.R. and Thulliez, P. (1995). Long-term antibody responses of cats fed Toxoplasma *gondii* tissue cysts. J. Parasitol., 81:887–93.
22. Dubey, J.P. (1996). Infectivity and pathogenicity of *Toxoplasma gondii* oocysts for cats. J. Parasitol., 82:957–961.
23. Dubey, J.P.; Garner, M.W.; Willette, M.M.; Batey, K.L. and Gardiner, C.H. (2001). Disseminated toxoplasmosis in magpie geese (*Anseranas semipalmata*) with large numbers of tissue cysts in livers. J. Parasitol., 87: 219-223.
24. Spycher, A.; Geigy, C.; Howard, J.; Posthaus, H.; Gendron, K.; Gottstein, B.; Debache, K.; Herrmann, D. C.; Schares ,G. and Caroline F. (2011). Frey Isolation and genotyping of *Toxoplasma gondii* causing fatal systemic toxoplasmosis in animmunocompetent 10-year-old cat J. Vet. Diagn. Invest, 23:104–108.
25. AL-Kennany, E. R. and Al-Saidya, A. M. A. (2006). Pathological and histochemical

study on cats and mice experimentally infected with *Toxoplasma gondii*. Iraqi J. of Vet. Sci., 20:249-263.

26. Fung, H.B. and Kirschenbaum, H.L. (1996). Treatment regimens for patients with toxoplasmic encephalitis. Clin. Ther., 18:1037-1056.
27. Fillisetti, D. and Candolfi, E. (2004). Immune response to *Toxoplasma gondii*. Ann. Ist. Super sanita., 40(1): 71-80.
28. Dubey, J. P. and Beatti, C. P. (1988). Toxoplasmosis of animals and man. CRC Press, Boca Raton, Florida, USA. Pp: 1–220.
29. Ferrira, M. S. and Borges, A. S. (2002). Some aspects of protozoan infections in immunocompromised patients. Mem. Inst. Oswaldo. Cruz., 97: 443-45.

التشخيص السيرولوجي والامراضي لطفيلي المقوسة القندية في القطط السائبة في محافظة بغداد

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الخلاصة

اجريت الدراسة الحالية للتحقق في مدى انتشار داء المقوسات في القطط السائبة وعلاقة نتائج الاختبار السيرولوجي الموجبة مع التغيرات المرضية في القطط .ولانجاز هذه الدراسة اخذت ٥٠عينة دم ونماذج من الاعضاء الداخلية للقطط (الكبد، الطحال، الدماغ، الكلية، الامعاء، الرئة) وقد اخذت القطط من مناطق مختلفة من مدينة بغداد اثناء الفترة ٢٠١١\١٠\١ الى ٢٠١٢\١٠\١. وجد بأن ٦٦% من هذه القطط كانت موجبة لاختبار ارتباط الانزيم للادمصاص المناعي غير المباشر (الالايزا) نوع IgG وقد ابدت الاناث مستوى اعلى (٧٥%) من الذكور .أما القطط ذات العمر الذي يتراوح بين شهرين فقد اظهرت نسبة اعلى (١٠٠%) لفحص (الالايزا) نوع IgG كمقارنة بالعمر (القطط البالغة) والتي اظهرت (٦٣,٨٢%) وكان هناك اختلاف هام ($P\leq0.01$) في الحالات الايجابية لفحص (الالايزا) نوع IgG . الافات الامراضية الشديدة لوحظت في الرئة ،الكبد والامعاء للحيوانات التي اظهرت اعلى نسبة (الكثافة البصرية) للمستضدات المناعية نوع IgG anti-Toxoplasma بالاضافة الى الطور اللاجنسي من الطفيلي في هيولي البلاعم السنخية ،والخلايا العملاقة في الحيز السنخي من الرئة والكبد ،بالاضافة الى الاطوار الحرة من الطفيلي في الحيز السنخي من الرئة، ولقد لوحظ نخر موضعي مع حصول التمعدن في دماغ القطط. وعلى أساس وجود هذه الافات الامراضية في القطط والتي اظهرت نتائج سيرولوجية موجبة للاضداد المناعية IgG فقد استنتجنا ان داء المقوسات هو المسؤول عن ظهور هذه التفاعلات الالتهابية في الاعضاء الداخلية للقطط وهنالك ارتباط واضح بين النتائج السيرولوجية الموجبة والافات المرضية في داء المقوسات ،وهذا الطفيلي منتشر بشكل كبير في القطط السائبة،والذي قد يكون من الاسباب المهمة التي تسبب الاجهاض في النساء.

الكلمات مفتاحية : داء المقوسات، القطط ، فحص (الاليزا)، المستضد المناعية.