

Original Research Article

Seroprevalence of *Toxoplasma gondii* among stray cats using different serological techniques in Erbil City: Kurdistan Region/Iraq

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ABSTRACT

Background: The causative agent of toxoplasmosis is *Toxoplasma gondii* which is an intracellular protozoan. Felines including cats are the only definitive hosts for *T. gondii* and they play a significant role in spreading the oocysts in the environment. It has been estimated that *T. gondii* infects about one-third of the human population. This study was performed to estimate the seroprevalence rate of Anti-*T. gondii* IgG Abs in 100 strays by using different serological techniques.

Methods: Hundred stray cats of both sexes (50 males and 50 females) from different ages (one month to 48 months) were hunted by trap cage and shooting tranquilizer gun in Erbil city and used for detecting *T. gondii* antibodies.

Results: The seroprevalence rates obtained were: 22%, 20% and 11% by MAT, ELISA and LAT respectively. Male cats showed higher seroprevalence than females (13% versus 7%). According to the agreement between MAT titers and ELISA IgG (cutoff ELISA ≥ 1) in stray cat's sera, the percent of positive results in ELISA (cutoff ≥ 1) method were exactly matched with the MAT titer ($\geq 1:50$) while the overall agreement and negative percent agreement were elevated by increasing MAT titers and the positive percent agreement was stable in all titers. The overall agreement results were 96% and 100% for MAT titers $\geq 1:25$ and $\geq 1:50$, respectively and negative percent agreement results were 97.4% and 100% for MAT titers $\geq 1:25$ and $\geq 1:50$ respectively.

Conclusions: Exactly matched between MAT technique titer ($\geq 1:50$) and ELISA technique. Index (cutoff ≥ 1). Therefore, MAT could be used for detecting IgG Abs of *Toxoplasma gondii* instead of ELISA technique.

Keywords: ELISA, LAT, MAT, Stray cats, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii is one of the most successful coccidian protozoans with potential zoonotic impact among humans, other mammals and birds worldwide, capable of infecting virtually all warm-blooded animals.^{1,2} *Toxoplasma gondii*, exhibits wide host specificity by possessing a vast host range, infecting both

mammals and birds. In addition to having this broad host range, *T. gondii* also seems to be nearly ubiquitous geographically, having been isolated from a variety of climatic regions on every continent surveyed.² The life cycle of the parasite includes an asexual reproduction in the intermediate hosts (mammals and birds) and sexual reproduction in the definitive host (feline). Ingestion of sporulated oocysts by drinking water or eating unwashed vegetables, eating raw or undercooked meat containing

tissue cysts are the main transmission routes in humans and animals.^{3,4} Cats are the only definitive hosts for *T. gondii* and they play a significant role in spreading the oocysts in the environment.^{1,2,5} Seroprevalence studies in cats indicate that *T. gondii* infection increases with age and are more common in feral cats than in domestic cats.⁶ Determination of the antibodies in cat serum is the best measure to get information about the prevalence of *T. gondii* as compared to the fecal examination for oocysts because, only about 1% of cats shed oocysts at any given time.⁷ In experimental infections, it is found that cats usually stop shedding *T. gondii* oocysts by the time they were seropositive for Abs.⁸

To understand the epidemiology of *T. gondii* transmission, it is important to diagnose the infection in animal especially cats as a final host. A number of serological assays are used for this purpose, among these tests, the modified agglutination test (MAT) has been widely performed in animals due to its ease of use.² In the present study in addition to MAT, ELISA and Latex tests were also used for the evaluation of the most accurate test.

METHODS

In this study, 100 stray cats of both sexes (50 males and 50 females) from different ages (one month to 48 months) were hunted by trap cage and shooting tranquilizer gun in Erbil city from June 2015 to March 2016 and used for detecting *T. gondii* antibodies. For temporary anesthetizing the cats, blowpipe syringe tranquilizer (B11) was used which contained 0.6 mL (100 mg/ml) of ketamine chlorhydrate and 0.4 ml (8 mg/ml) of xylazine.

The cats were transferred to animal house, reared for 30 days, with good biosecurity plan. Before withdrawing the blood, the animals were sedated by I.M injection with Medetomidine (0.4 ml). Three ml of the blood was taken from the cephalic vein using a sterile disposable syringe; the blood was transferred to labeled tubes without anticoagulant. Each tube was labeled clearly and transferred to the laboratory of Erbil Directorate veterinary department for serological test, which included ELISA, MAT and LAT tests.

Serological techniques

MAT test

MAT test was performed in Biology Department College of Science, Salahaddin University using Kerafast kit (EH2001, USA), the procedure described previously. 9, 10 using 96 well U-bottom microtiter plates. The serum samples were serially diluted from 1:25 to 1:3200. Positive and negative controls were included for each microtiter plate. The cutoff for MAT titer was 1:25, titers $\geq 1:25$ is considered seropositive for *T. gondii* infection.

ELISA IgG

ELISA test was performed in the Laboratory Department of Erbil Veterinary Directorate of Kurdistan Region/Iraq. The commercial ELISA indirect multi-species IgG kit (ID. Vet, France) was used. Serum samples were processed following manufactures instruction. The results were interpreted as positive, equivocal or negative by determining the immunoglobulin index. IgG index values <0.90 , $0.90-0.99$ and ≥ 1.0 were considered negative, equivocal and positive, respectively.

Latex IgG

LAT test was performed in the Laboratory Department of Erbil Veterinary Directorate, Kurdistan Region/Iraq. The commercial LAT kit for Anti-Toxoplasma IgG (ID. Vet, France) was used. The samples were tested following the manufacturers instruction. The results were read under high intensity lamp. Appearance of agglutination indicates positive reaction, whereas, lack of agglutinations indicates negative reaction.

Data analysis

The comparison between MAT, LAT, and ELISA was analyzed using Cohen’s Kappa by Assessing agreement equations between two methods without references (Table 1). 11 and Pearson Correlation Coefficient for figures using a computer statistics program, GraphPad Prism (GraphPad Software, Inc. USA).¹²

Ethical approval

The study was approved by the Institutional Ethics Committee of Erbil Health Technical College Board before the study was set and written informed agreement was obtained from the study participants.

RESULTS

The seroprevalence of *T. gondii* among 100 stray cats (50 males and 50 females) from different ages tested by MAT, ELISA and LAT tests is summarized in Table 2.

Table 1: Assessing agreement equations between two methods without references (controls).

		Test		
		Positive	Negative	Total
Test	Positive	a	b	a+b
	Negative	c	d	c+d
	Total	a+c	b+d	a+b+c+d

Overall percent agreement = $(a+d)/(a+b+c+d)$; Positive percent agreement = $a/(a+c)$; Negative percent agreement = $d/(b+d)$.

The highest IgG Abs seroprevalence rates for these tests were 22% by MAT test ($\geq 1:25$), 20% by ELISA and 11% by LAT. With regard to age, the age group 37-48 months

showed the highest (16%) seroprevalence rate of *T. gondii* IgG Ab and statistically these differences were significant ($p < 0.01$) as shown in Table 3.

With respect to cat sex, male cats showed higher (13%) seropositivity than females (7%) also, these differences were statistically significant ($p < 0.01$) (Table 4).

Table 2: Seropositivity of anti-*T. gondii* IgG by MAT, ELISA and latex tests in serum of cats (n=100).

Type of test	Positive	Percentage
MAT $\geq 1:25$	22	22
ELISA IgG	20	20
LAT IgG	11	11

Table 3: Seropositivity of anti-*T. gondii* IgG Abs by ELISA in stray cats according to sex.

Sex	Tested	Seropositive	%
Male	50	13	13
Female	50	7	7

P value < 0.01.

Table 4: Seropositivity of anti-*T. gondii* IgG Abs using ELISA in stray cats according to age.

Age groups (months)	Tested	Seropositive	%
01-12	1	0	0
13-24	3	0	0
25-36	22	4	4
37-48	74	16	16

P value < 0.01

The comparison of MAT with ELISA for the detection of *T. gondii* IgG Abs in stray cats sera was statistically significant based on the Pearson correlation when the MAT titer was 1:25 and ELISA cutoff for IgG was ≥ 1 (Figure.1).

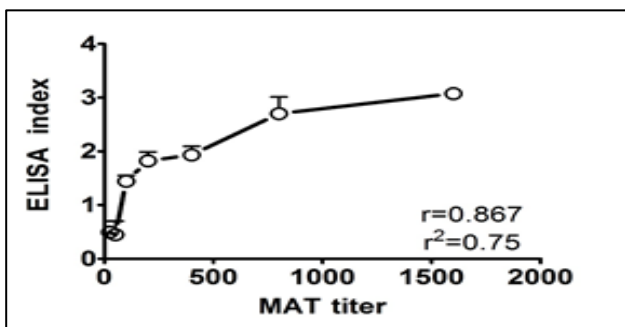


Figure 1: Correlation between ELISA and MAT IgG titers in sera of cats based on the Pearson correlation analysis. Significant positive correlation was observed between ELISA index and MAT titers ($p \leq 0.05$).

In the current findings, the percent of positive results in ELISA (cutoff ≥ 1) method were exactly matched with the

MAT titer ($\geq 1:50$) (Table 6) while the overall agreement and negative percent agreement were elevated by increasing MAT titers and the positive percent agreement was stable in all titers. The overall agreement results were 96% and 100% for MAT titers $\geq 1:25$ and $\geq 1:50$, respectively and negative percent agreement results were 97.4% and 100% for MAT titers $\geq 1:25$ and $\geq 1:50$ respectively (Table 5, Table 6).

Table 5: The agreement between MAT ($\geq 1:25$) and ELISA IgG (cutoff ELISA ≥ 1) in the sera of stray cats.

	ELISA IgG			
	Positive	Negative	Total	
MAT	Positive	22	2	24
	Negative	0	76	76
Total		22	78	100

Overall percent agreement = $(22+74)/100 \times 100 = 96\%$; Positive percent agreement = $22/22 \times 100 = 100\%$; Negative percent agreement = $76/(76+2) \times 100 = 97.4\%$

Table 6: The agreement between MAT ($\geq 1:50$) and ELISA IgG (cutoff ELISA ≥ 1) in the sera of stray cats.

	ELISA IgG			
	Positive	Negative	Total	
MAT	Positive	20	0	20
	Negative	0	80	80
Total		20	80	100

Overall percent agreement = $(20+80)/100 \times 100 = 100\%$; Positive percent agreement = $20/20 \times 100 = 100\%$; Negative percent agreement = $80/(80+0) \times 100 = 100\%$.

DISCUSSION

Cats are essential in the life cycle of *T. gondii*, because they are the only hosts in nature that can excrete the environmentally resistant oocysts.² In this study the highest number of seropositive cats for Anti-Toxoplasma IgG Abs obtained by MAT test, titer $\geq 1:25$ which was 22%. The same seropositive cats were further tested by ELISA and Latex tests which gave lower rates of seropositivity (20% and 11%, respectively) for IgG Abs.

Modified agglutination test (MAT) was described by Dubey et al and was used for the first time in this study in Kurdistan Region /Iraq for detecting Anti -*T. gondii* IgG Abs in the sera of stray urban cats using two-fold serial dilutions (1:25 to 1:3, 200).¹⁰

The seroprevalence rate of Anti-*T. gondii* IgG Abs in the present study is more or less similar to that of in Lyon/ France who reported a seroprevalence of 18.6% of Anti-*T. gondii* IgG Abs in urban population of domestic cats.¹³ A higher seroprevalence rate (30.4%) for Anti-*T. gondii* IgG Abs was reported in Baghdad /Iraq, while in Romania much higher rate (47 %) for anti- Toxoplasma IgG was observed by ELISA.^{14,15} The variation in the seroprevalence rate of *T. gondii* may be due to the

method of calculation or to variation of infection with *T. gondii* among different countries, within different areas of the same country, and even within the same city.¹⁶

Regarding the age, cats aged 37-48 months showed the highest (16%) seroprevalence of Anti-*T. gondii* IgG Abs. The increase in the rate of seropositivity with the age indicates postnatal transmission of *T. gondii*.¹³ Similarly, in Netherland recorded a seroprevalence of 18.2 % for *T. gondii* among young age cats, which increased with age up to 20-30% in cats at the age of 4 years. In other parts of the world, some researchers reported similar relation between rate of infection with *T. gondii* and cat age in Sri Lanka in China and in Iran.¹⁷⁻²⁰

Considering the sex of cats, male cats showed higher (13%) seropositivity than females (7%). This is in agreement with studies performed in Northwest/ Iran and China who also reported a slightly higher rate among male than female cats.^{19,21} Modified agglutination test has been widely used for determining *T. gondii* infection in animals.² The whole-cell antigen of *T. gondii* RH strain was used in MAT test for human sera, while in the present study the whole -cell antigen of *T. gondii* from Kera fast, company was used for testing cat sera.²²

Considering the comparison of MAT with ELISA, in the current study, the positive results of ELISA (cutoff ≥ 1) method exactly matched with MAT titers ($\geq 1:50$). This study is in support to the study in Iran which confirmed the efficacy of ELISA and MAT for the detection of Anti- *T. gondii* Abs in cats, and in pigs in the United States of America who found good correlation between MAT titers and ELISA test.^{23,24} Furthermore, MAT test showed perfect agreement with IFAT in detecting positive and negative results in cat sera.²⁵ This study is in agreement with the study in pigs who also observed high agreement between ELISA and MAT using the same agreement.²⁶ On the other hand, it contradicts with the study performed in detecting Anti-*T. gondii* IgG abs in swine sera in which higher sensitivity for ELISA than MAT. The present study can be considered as a first step in Erbil Province to investigate the prevalence of Toxoplasmosis among Cats and their relation to sex and age of Cats by using different immunological techniques include ELISA, MAT and LAT techniques. Detecting of *Toxoplasma gondii* IgG abs shows by MAT and ELISA it was same qualifications while MAT is easier and cheaper than ELISA and used for the first time in Iraq for detecting IgG Abs in Cats sera. The current outcomes indicate that Toxoplasmosis has high prevalent among males of Cats then females and the age group 37-48 months showed the highest in the current findings, the percent of positive results in ELISA (cutoff ≥ 1) method were exactly matched with the MAT titer ($\geq 1:50$).

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