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Detection of Toxoplasmosis in Rat (*Rattus rattus*) in Baghdad governorate/Iraq

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Abstract

The *Toxoplasma gondii* infects human beings and wild rats (*Rattus rattus*) worldwide. Wild rats are infected with *T. gondii* due to ingestion of food or water contaminated with oocysts and may play a significant role in the transmission of *T. gondii* infection to the humans. The aim of the present study was to determine the seroprevalence of *T. gondii* among wild rats. Acute and chronic cases of toxoplasmosis in rats caught from old buildings and garbage in Baghdad city/Iraq were determined serologically. The percentage of positive rats for anti-*T. gondii* antibodies was 45%. Moreover, the higher infection rate observed in male rats. The percentages of acute and chronic infected rats were 10% and 35% respectively. The association between the presence of infection with the rat sex and age and their collection sites was insignificant ($p>0.05$). In conclusion, this study approved the presence of acute and chronic toxoplasmosis in wild rats in Baghdad city. However, the insignificant correlation between rat's sex and age and its collection sites also observed. The authors recommend another future study including large numbers of rats in extended geographical area in Baghdad governorate to determine the incidence of *Toxoplasma gondii* in wild rats that might have an impact on the public health.

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Keywords
Anti-*T. gondii* antibodies, Baghdad, *Rattus rattus*, Toxoplasmosis.

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Introduction

Toxoplasma gondii, is a zoonotic, an obligate intracellular parasite. It can infect humans and animals and has evolved several possible ways of transmission within and among different host species (Levine, 1973; Ferguson *et al.*, 1999). The most species of domestic and wild rats are chronically carriers of Toxoplasma and serve as a potential reservoir of infection to cat's population, as well as other livestock animals (Wallace, 1973; Webster and MacDonald, 1995; Battersby, 1998). *T. gondii* can be preserved by vertical transmission in rat's population with the absence of cats (Dubey, 1997; Webster *et al.*, 1994). Wastling *et al.*, (2000) pointed to the possibility of the natural life cycle of Toxoplasmosis through a cat-to-rodent-to-cat transmission, which may indiscriminately involve infection of other warm-blooded animals. *T. gondii* is mainly transmitted congenitally or by ingestion either of bradyzoites in infected tissues, or of oocysts spread in the environment. The risk of infection by ingestion for rodents is related to the complexity of environmental factors that determine the presence of other mammals, especially felids, and oocysts. Climate also conditions the dispersal, density, and survival of oocysts, since their resistance depends on humidity and temperature (Dubey and Frenkel, 1998).

The human beings exploitation of nature favors the proliferation of rodents. Most the wild rodents inhabit wild environments, some of these rats have adapted to urban conditions and can be considered synanthropic rodents. These species, especially the murid omnivores, are different from wild rodents and live close to humans, where they find water, shelter, and food (grains) to survive. In this context, these rodents can cause economic losses to people, such as damage to food for human consumption and the destruction of grains and seeds in planting areas and storage facilities (Brasil, 2004). In Iraq, previous studies revealed that laboratory rats were infected with *Hymenolepis diminuta* (Abdulkarim J and Al-Salihi, 2014), while Methicillin Resistant *Staphylococcus aureus* (MRSA) was isolated from wild rats (Khalaf *et al.*, 2015). Searching the PubMed/Medline and Google Scholar databases for relevant articles that highlighted the Toxoplasmosis in wild rats in Baghdad city/ Iraq, revealed scarce information. Therefore, this study intends to detect Toxoplasmosis in Rat (*Rattus rattus*), and its effect on liver and kidney functions.

Materials and methods

Animals

This study was conducting during 1/September/2015 to 30 / March /2016. The level of antibodies against *T. gondii* measured in 100 captured rats. The rats consisted of 54 males and 46 females, weighing between 110 gm to 340gm, 12.70 to 18.25 cm long, and 6.5 to 10 cm long of the tail. The rats caught randomly from different Baghdad region (Abu Ghraib, Mahmudiyah, Sayyidiyah, Ur Quarter, Karrada, Assalam Quarter, New Baghdad neighborhood, Bunook) from the old buildings and garbage. All rats brought alive to the unit of zoonotic disease. Traps baited with cheese, and fresh vegetable, in addition, meat

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was set in different sites (Asgari *et al.,* 2007). Each live trap with captured animal tagged locality and brought to the laboratory. It kept in big transparent polythene cage that helped to observe the movement of animals for two hours. After that, the rats were caught from tail and IP injected with 0.1 mL of the anesthesia (9:1, Ketamine +Xylasine) per 100gm rat body weight as described by (Struck *et al.,* 2011).

Blood sample collection and serum preparation

Blood samples (5 ml) collected from the heart of the anesthetic rat by heart puncture methods using a 5ml disposable syringe. The needle inserted into the lateral thoracic region of the area of maximal heart palpitation between ribs of the left side after disinfecting the site of collection by ethanol. The blood samples kept in sterile tubes without anticoagulant. The tubes allowed to stand and coagulate at room temperature. Later on, serum was separated by centrifugation at 3000 rpm for 10 minutes. The serum samples dispensed into Eppendorf-tubes by using micropipettes and stored in a freezer at -20C˚ until further analysis.

Serological assay

Serum samples were tested for *T. gondii* antibodies by the following methods:

A. Modified Enzyme Linked Immuno Sorbent Assay (ELISA) used to detect *T. gondii* antibodies in the rat serum samples. The coated microwells with the p30 antigen of *Toxoplasma gondii* used in this study. The Rat serum samples added to the wells, in addition, positive and negative control were also added. The procedures of ELISA was done according to the instructions. Later on, a multi-species peroxidase (po) conjugate added to the wells, that fixe to the antibodies and formed an antigen-antibody-conjugate-po complex.

B. Rat Tox IgM ELISA Kit

A quantitative sandwich Tox IgM ELISA immunoassay was also used in this study. The provided microtiter plates coated with a Tox IgM specific antibody. Captured Tox IgM was quantitatively detected horseradish peroxidase (HRP)-conjugated anti Tox IgM incubated with HRP substrates (solutions A and B). The binding of the HRP-conjugated antibody visualized by the production of colorimetric reaction products that quantitatively measured by absorbance at 450nm.

Statistical analysis

Means compared by unpaired t-test to assess significant differences. Chi-square compared proportions. $P < 0.05$ was considered statistically significant.

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Results

1. Detection of *Toxoplasma gondii* infection in captured rats according to ELISA-IgM and –IgG

The percentages of positive samples for IgM and IgG were 10% and 35% respectively out of a total 100 rats serum samples that collected randomly from 6 regions in Baghdad city. These results revealed that chronic infection (IgG) was a higher percentage than the acute infection (IgM) in rats (Table.1).

Table.1. Shows the total infection rate of *Toxoplasma gondii* in wild rats

	Healthy	Infected	Rate
ELISA IgG	65	35	35%
ELISA IgM	90	10	10%
total			
Chi square value	17.92	45	45%
P	<0.0001		

2. The infection rate according to the age of the rats

No significant effect ($p < 0.0001$) was observed between the age of rats and rate of *Toxoplasma gondii* infection. However, the percentages of infection rates in adult (10.71 and 39.28 %) and sub-adult (10.41 and 37.5 %) age group was higher than juvenile age groups (8.33 and 25 %) in the ELISA IgM, IgG respectively (Table. 2) .

Table. 2: relationship between +ve cases of acute and chronic toxoplasmosis with age group in rats

age	No. of sample examined	IgM +ve sample	Percentage (%)	IgG +ve sample	Percentage (%)
Adult	28	3	10.71	11	39.28
Sub adult	48	5	10.41	18	37.5
juvinle	24	2	8.33	6	25
total	100	10	10	35	35
Chi- square	26.16				
p	< 0.0001				

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3. The infection according to the sex of rats

A slightly higher toxoplasmosis infection rate (11 and 35%) observed in the males than females (8 and 34%) by ELISA (IgM and IgG respectively without a significant difference (P > 0.05)) (Table.3).

Table.3. Shows the infection rate of *Toxoplasma gondii* according to the sex of the animals

sex	ELISA IgM			ELISA IgG		
	Healthy	Infected	Rate	Healthy	Infected	Rate
Male	48	6	11%	35	19	35%
Female	42	4	8%	30	16	34%
total	90	10	19%	65	35	69%
Chi square value			0.161			0.0018
P			0.68			0.96

4. Prevalence of *T. gondii* infection among wild rats captured in different seasons and regions of Baghdad city, Iraq as determined by using ELISA IgM

A variations appeared between the infection rates of toxoplasmosis that estimated in different regions and seasons but without significant difference. The higher infection rate appear in autumn and winter than spring according to the ELISA IgM (Table.4&5).

Table.4. Shows total seropositive cases in captured rats infected by *Toxoplasma gondii* according to the region and season

Region	No. of the sample examined	Spring Positive	Autumn Positive	Winter Positive
Abu Ghraib	17	1	1	o
Mahmudiyah	25	0	3	2
New Baghdad neighborhood	15	1	0	0
Assalam Quarter	15	1	1	0
Karrada	18	0	0	0
Ur Quarter	10	0	0	0

Table.5. Shows the prevalence of *Toxoplasma gondii* infection among captured rats that captured in different seasons and regions of Baghdad city/Iraq as determined by using ELISA IgM

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Region	Total no.	Spring	Autumn	Winter
Abu Ghraib	17	3	4	1
Mahmudiyah	25	2	5	3
New Baghdad neighborhood	15	2	2	3
Assalam Quarter	15	0	0	0
karrada	18	0	1	5
Ur Quarter	10	4	0	1

Discussion

Wild rat's populations consider as a probably an important host reservoir for the transmission of different zoonotic diseases such as *T. gondii*. The prevalence of *T. gondii* antibodies in the wild rat's population approved in previous studies with variation in the percentages of infection (1-30%). These variations were depending on the method, number of animals studied and the geographic area (Zhang *et al.*, 2009).

There are many obstacles in the diagnosis of toxoplasmosis depending on the clinical signs, and exhibition of the causative agents in tissues or body fluids (Buxton *et al.*, 2007), therefore, the detection of Ab response by different serological tests is under the spotlight for all researchers. In this study, ELISA technique was used to determine the prevalence of toxoplasmosis in wild rats (*Rattus rattus*) to understand its role in the transmission of toxoplasmosis. It is well known that IgM Abs appear early as two weeks after the first infection and last for three months, while IgG Abs appear from two months of infection and last for two years (Aiello, 2000; Radostits *et al.*, 2007). Both antibodies determined in this study. ELISA has excellent sensitivity, quality, objectivity, although it needs a refinement in the procedures (Gamble *et al.*, 2005; Shaapan *et al.*, 2008). Also, it can be used successfully in screening and epidemiological survey (Dubey, 2009).

No previous studies found in the literature regarding *Toxoplasma gondii* in the wild rat (*Rattus rattus*) in Baghdad city. So, this study is the first prevalence study that intend to determine the toxoplasmosis in wild rats in Baghdad. The results of this study showed that 45% of the rats were infected with toxoplasmosis and approved the incidence of toxoplasmosis in wild rats in Baghdad city. These infected rats might play significant roles in the transmission of toxoplasmosis to the cat's population and other livestock. The percentage of toxoplasmosis infection that reported in the present study are higher than previous study (Franti *et al.*, 1976) in the State of California, USA. Franti *et al.*, 1976 observed a seroprevalence of 4% in 160 urban rats, and of 38% in 47 cats. Another previous study also reported a prevalence of 1.96% in 766 domestics and peri-domestic rodents in Niamey, Niger (Mercier *et al.*, 2013). Some studies reported lower prevalence rate in urban sinantropics rodents; in Umuarama, Paraná State in Brazil. None of 24 *R. rattus* and 19 *Mus musculus* showed positive results in the serological tests Araújo *et al.*, (2010). However, in São Paulo, Muradian *et al.*, (2012) established the prevalence of 0.46% in 217 captured rodents using bioassays. The results of this study were also in agreement with previous studies that reported in Costa Rica (30.4% *Rattus rattus*),Panama (23.3% *R. norvegicus*), England (59% *M. domesticus*) and Pakistan (58.57%

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R. Rattus; 36.66% *M. musculus*) (Chinchilla , 1978; Marshall *et al.*, 2004; Frenkel *et al.*, 1995; Ahmad *et al.*, 2012).

The results of this study observed a higher sero-prevalence in male than female wild rats; without significant (P > 0.05) difference. This result is incompatible with the study that conducted by Yin *et al.*, (2010) in China that showed that all of the infected rats (3.2%; 7 out of 217) were female. Moreover, no *T. gondii* antibodies were detected in male rats and these results were in agreement with study by Salibay and Claveria, (2005) in the Philippines that showed higher *T. gondii* infection rate in male than in female rats, and those caught in the commercial site that had 100% seropositivity. Webster, (1994) also showed no significant differences between age, sex and the site of rats populations with the *T. gondii* prevalence irrespective of habitat type or presence of the cat. In the present study, the association between the presence of infection and gender and collection site was also insignificant (P > 0.05). Other studies have also shown that gender is to be a determining factor for *T. gondii* prevalence. The seasonal variations in the prevalence of Toxoplasma and seasonal effects on the infection rate might reflect both climatic changes, which affect the parasite, as well as changes in the photoperiod, which influence the host's physiology (Dubey, 1998). In conclusion, this study approved that 10 and 35 % of wild rats expressed seropositive to anti-*Toxoplasma gondii* ELISA- IgM and anti-*Toxoplasma gondii* ELISA-IgG respectively. Moreover, the male *Rattus rattus* showed higher toxoplasmosis infection rate than female, in addition, the prevalence of chronic toxoplasmosis was higher than acute. These results explain that wild rats might act as a suitable reservoir for transmitting infection to the cats through the tissue cyst.

The authors recommend another epidemiological study regarding the distributions of *Toxoplasma gondii* in rats in the different governorates of Iraq. Moreover, community education program should be targeted cat's owners relating to the impact of cat toxoplasmosis on the public health.

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