

Toxoplasmosis in females from Al-Anbar, Iraq

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Abstract

Background: Toxoplasmosis is a common zoonotic disease affecting one-third of the world population with potentially serious health implications.

Objectives: To find the prevalence and risk factors of *Toxoplasma gondii* infection among females in Al-Anbar, Iraq.

Methods: Over an eight-month period from (1st November 2021 to 30th June 2022), 200 females visiting private veterinary clinics in Al-Anbar (92 owned cats) participated in this prospective study. Age and residency were recorded. Blood samples were collected from all females and subjected first to the Toxoplasma Latex Agglutination test (TLAT). Samples of cat feces were collected from 57 (62%) pet and 35 (38%) stray cats and subjected first to direct microscopy looking for Toxoplasma oocysts. Blood and fecal samples were then tested by nested polymerase chain reaction (n-PCR) and real-time PCR. Results were then statistically analyzed.

Results: Rates of human toxoplasmosis were (n=48, 24%), (n=60, 30%), and (n=77, 38.5%) with TLAT, n-PCR, and RT-PCR tests respectively. Likewise, rates of cat toxoplasmosis were (n=7, 7.6%), (n=55, 60%), and (n=80, 87.0%) with TLAT, n-PCR, and RT-PCR tests respectively. Results of the latex test revealed that females >40 had a high rate of infection (45.8%) compared with those aged 31-40 years (33.8%) and those aged 21-30 years (13.0%) (P≤0.01). City resident females had a lower infection rate compared to village residents (29.7% vs. 55.4%).

Conclusions: Toxoplasmosis was more prevalent in females >40 from rural areas but wasn't related to contact with cats. Real-time PCR provided a better diagnostic yield.

Keywords: Toxoplasmosis; zoonosis; pets; cats; Al-Anbar; Iraq.

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Introduction:

Toxoplasma gondii is one of the most serious parasites shared between humans and cats (1). *T. gondii*, discovered one century ago, is an intracellular obligate parasite (2) able to infect all warm-blooded animals including one third of humans world-wide (3). Toxoplasmosis is one of the human parasitic diseases that can be transferred from mother to fetus. Toxoplasmosis during pregnancy can lead to complications in 20-30% of cases such as spontaneous abortion, preterm labor, stillbirth, and fetal abnormalities (4,5). Moreover, children infected with Toxoplasmosis may have late complications such as blindness and deafness. Toxoplasmosis is the chief infection known to cause blindness throughout the world (5). Domestic and wild cats play a central role in the spread of toxoplasmosis as they are the only species capable of excreting millions of oocysts, resistant to the environment, in their feces (6,7). Hence, it is reasonable to assume that cat owners would have a higher level of anti-toxoplasma antibodies than would normal persons of a similar age. However, literature reviews show that owning a cat is not always a major risk factor for getting

this parasitic infection (8). Toxoplasmosis is mostly acquired by consumption of viable tissue cysts in the undercooked or raw meat, or by eating food or drinking water contaminated with oocysts. Blood transfusion or organ transplant from infected people are rare routes of getting toxoplasmosis (4,9). In Iraq, plenty of research has been published about toxoplasmosis of humans and cats (4,5,10–14). This study aimed to determine the prevalence and risk of toxoplasmosis in human females in Al-Anbar governorate, Iraq particularly in relation to contact with stray and pet cats and thus put forward a preventive strategy for this common and serious threat both to human and animal health.

Materials and Methods:

This prospective study was carried out over an eight-months period (1st of November 2021 to 30th of June 2022) in Al-Anbar governorate, west of Iraq. The study was approved by the Ethical Committee of the College of Veterinary Medicine/ University of Baghdad and was carried out according to the guidelines of the World Medical Association for studies involving humans and animals. Informed consents were obtained from all the participating females. A total of 200 blood samples were collected from females visiting private veterinary clinics in different parts of Al-Anbar governorate.

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Ninety-two blood samples were collected from females who had cats and 108 blood samples from females who didn't own or had contact with cats. After collection, the blood samples were tested by Toxoplasma Latex Agglutination test. Fecal samples were collected from pet and stray cats and subjected to direct light microscopy to look for Toxoplasma eggs and cysts. The oocysts of *Toxoplasma gondii* in cat stool were recognized according to the Sheather, Hoffman Pons Janer or Lutz (HPJL), Willis techniques (15–17). The blood and fecal samples were then sent to the College of Veterinary Medicine/ University of Baghdad for molecular tests, namely nested polymerase chain reaction (n-PCR) and Real-time PCR (RT-PCR) tests. All blood samples were tested for the existence of IgG and IgM anti-Toxoplasma antibodies. The results were statistically analyzed using SPSS (The Statistical Package for Social Sciences 2020 version 9.1) software. The Chi square (χ^2) test was performed to test associations between variables and P values < 0.05 were considered significant.

Results

Table 1 shows that there were 92 cat-owners and 108 non-cat owners. Thirty-Five (38%) of the first group owned stray cats and fifty-seven (62%) females owned pet cats.

Table 1: The distribution of studied females according to housing cats

Housing of Cats	No. (%)	Total
Females having cats	35 (38%)	92
Stray Cat	57 (62%)	
Females without cats	108 (100%)	108

The rate of infection with toxoplasmosis among females in the study group using the latex agglutination test is shown in table 2. Almost one quarter (24%) of all females were infected with toxoplasmosis and they were almost equally divided between cat-owners (25%) and non-cat owners (23.2%), with no statistically significant association.

Table 2: The rate of toxoplasmosis among females according to the latex agglutination test

Cat ownership	Number (%)	Positive latex agglutination test (%)
Women having cats	92 (46.0%)	23 (25.0%)
Women having no cats	108 (54.0%)	25 (23.2%)
Total	200 (100.0%)	48 (24.0%)
Statistics	$\chi^2= 0.083$, P value= 0.773 (non-signifiant)	

Using the nested PCR test, the total rate of toxoplasmosis infection among females was 30% (60/200) and it was even higher (38.5%) when Real-time PCR test was used (77/200), Table-3.

Table 3: Rates of females *T gondii* infection according to three diagnostic methods

Cat exposure	Number	Positive Results – Number (%)			Chi-square value	P value
		Latex test	Nested PCR	Real-time PCR		
Yes	92	23 (25%)	27 (29.3%)	35 (48.1%)	10.08	0.07
No	108	25 (23.1%)	33 (30.6%)	42 (38.9%)		
Total	200	48 (24%)	60 (30%)	77 (38.5%)		

Results of the latex test revealed that females above the age of 40 had a higher rate of infection (45.8%) compared to those 31-40 years (33.8%) and those 21-30 years (13.0%), (P<0.01). The age distribution of the studied females by test positivity is shown in Table 4. Regarding the geographical locations, a lower infection rate was found among city residents compared to those living in rural areas (29.7% vs. 55.4%) using the real-time PCR test.

Table 4: The age distribution of females with positive and negative samples

Age group (years)	Number	Negative	Positive
21-30	108	94 (87.0%)	14 (13.0%)
31-40	68	45 (66.2%)	23 (33.8%)
>40	24	13 (54.2%)	11 (45.8%)
Total	200	152	48
Chi-square		65.697	4.875
P value		0.000**	0.087ns

Results of microscopy of fecal samples of pet and stray cats are shown in Table 5.

Table 5: Microscopic results of cats' fecal samples

Type of Cats	Positive – No. (%)	Negative – No. (%)	Total (100%)
Stray	5 (14.3%)	30 (85.7%)	35
Pet	2 (3.5%)	55 (96.5%)	57
Total	7 (7.6%)	85 (92.4%)	92

Testing of cats' feces revealed *Toxoplasma gondii* infection in 7 (7.6%) of cats. The majority of the infected cats were stray cats, while the majority of pet cats were non-infected. Using the nested PCR test, the total rate of toxoplasmosis infection among cats was 60% (55/92) and it was even higher (86.95%) when Real-time PCR test was used (80/92).

Discussion:

Like other studies on toxoplasmosis, and due to the non-specific clinical features of the disease (6), we used laboratory methods to confirm the diagnosis of *Toxoplasma gondii* infection. The easily prepared latex serological examination test was used as a screening test whose sensitivity and specificity can be compared with other diagnostic methods. Serological tests of *Toxoplasma gondii* detect the antibodies in the serum. IgM antibodies are the first category of antibodies

discovered after getting primary *T gondii* infection and decrease quicker than IgG antibodies (6). The total rate of infection with Toxoplasma in the blood of females recorded in the present study was close to infection rates previously reported from Iraq including Almosawi and Algezi from Thiqr (21.9% and 23%) respectively (11, 18). Razan and Hamad (12) reported lower rates (16.1%) in Kirkuk, while Qazaz (14) reported a much higher rate (78.3%) in Baghdad. These variations can be attributed to differences in the numbers of collected samples, type of serological tests used, cultural and socioeconomic habits of the community and contact with cats.

According to the findings of the present study, the infection rates among cats varied with the diagnostic technique used. The least accurate diagnostic method was the direct microscopy which revealed infection rates among pet and stray cats of 3.5% and 14.3% respectively. While the most sensitive and accurate technique was the real-time PCR, which revealed 79.0% and 100% infection rates among pet and stray cats respectively. Similarly, *T. Gondii* human infection rates differed according to the diagnostic method; being low (24%) with latex agglutination test and high (38.5%) when a Real-time PCR test was used. Our results coincide with the results of Burg *et al* (19) who showed that even a single *T. gondii* parasite could be distinguished by PCR. Another explanation of the high diagnostic yield of PCR is due to the intensification of B1 gene so that the parasite DNA would be detected rather than a live pathogen (20). Wastling *et al* showed that (100%) of placental sections were proved positive *T. gondii* by PCR test in their study (20).

Some countries have a lower rate of *T. gondii* in cat feces due to greater access of domestic cats to human food which carries a low risk of infection. In contrast, the high prevalence noted in other countries could be attributed to feeding the domestic cats with undercooked meat by their owners or hunting and the way of living of the final host of the parasite (20).

Our results revealed that there was no significant relationship between having or not having cats and toxoplasma infection of females detected by rapid test, nested PCR test, and Real-time PCR test. In other words, there was no relationship between housing a cat and getting toxoplasmosis but other ways of transmission of infection such as contact with soil, and poor hygienic conditions should be blamed.

The finding that older females had the highest toxoplasmosis infection rate contradicts the results of a study from Ethiopia, where Tammam *et al* recorded a higher infection rate in the age group <25 old and a very low infection rate (1%) in the >40 years age group (21). The high infection rate in the older females in our study can be attributed to longer exposure to the causative agent (10). Al-Sadoon *et al* from Basra, Iraq found that neither age of the person nor contact with animals were associated with Toxoplasmosis infection. Rather, contact with soil (gardening) had a positive association

(13). Rigorous hand hygiene after gardening or soil contact is thus highly recommended (22).

The higher rate of *Toxoplasma gondii* infection among females living in the rural areas compared to those living in cities was also reported by other authors (7, 23) and was attributed to the lack of health education and poor hygienic conditions in rural areas. It may also be due to the direct contact with soil contaminated with parasite oocyst expelled with the feces of infected cats during the plowing and cultivation process, as the soil is considered the most important source of infection (7).

Conclusions

This study revealed a high rate of toxoplasmosis among females above 40 years and from rural areas of Al-Anbar governorate. The new diagnostic tests such as real-time PCR had higher diagnostic yields compared with toxoplasma agglutination test. Neither contact with cats nor the type of cat (pet vs. stray) had a significant association with human toxoplasmosis.

Authors' declaration: We hereby confirm that all the tables in the manuscript are ours. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in the University of Baghdad/College of Veterinary Medicine according to the code number (297in 5th Feb 2023).

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داء المقوسات بين النساء في الأنبار، العراق

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الخلفية: داء المقوسات هو مرض حيواني المنشأ شائع يصيب ثلث سكان العالم مع آثار صحية خطيرة محتملة. **هدف الدراسة:** تحديد مدى انتشار وعوامل الخطورة للإصابة بمرض التوكسوبلازما بين النساء في محافظة الأنبار، العراق. **المرضى والطرق:** على مدى 8 أشهر من الأول من تشرين الثاني 2021 إلى 30 يونيو 2022، شاركت 200 سيدة في زيارة عيادات بيطرية خاصة في الأنبار (92 منهن كان لديهن ققط) في هذه الدراسة المستقبلية. تم تسجيل عمر ومكان إقامة النساء المشاركات في الدراسة. تم جمع عينات الدم من جميع النساء وخضعت العينات أولاً لاختبار تلازن اللاتكس السريع (TLAT). جمعت عينات البراز من 57 (62%) قطة أليفة و 35 (38%) قطة ضالة وخضعت عينات البراز أولاً للفحص المجهرى المباشر بحثاً عن بيوض التوكسوبلازما. ثم تم اختبار عينات الدم والبراز عن طريق تفاعل البلمرة المتسلسل (n-PCR) وتفاعل البلمرة المتسلسل في الوقت الحقيقي (RT-PCR) ثم تم تحليل النتائج إحصائياً.

النتائج: كانت معدلات داء المقوسات البشري (ن = 48، 24%)، (ن = 60، 30%)، و (ن = 77، 38.5%) باختبارات TLAT، n-PCR، و RT-PCR على التوالي. وبالمثل، كانت معدلات داء المقوسات في الققط (ن = 7، 7.61%)، (ن = 55، 60%)، و (ن = 80، 87.0%) باختبارات TLAT، n-PCR، و RT-PCR على التوالي. أظهرت نتائج اختبار اللاتكس أن النساء فوق ال 40 سنة كان لديهن معدل إصابة مرتفع (45.8%) مقارنة باللواتي تتراوح أعمارهن بين 31-40 سنة (33.8%) واللواتي تتراوح أعمارهن بين 21-30 سنة (13.0%) ($P \leq 0.01$). كان معدل إصابة النساء في المدينة أقل مقارنة بالقرية (29.7% مقابل 55.4%).

الاستنتاجات: وجدنا من خلال هذه الدراسة ان داء المقوسات أكثر انتشاراً بين النساء فوق ال 40 سنة وفي المناطق الريفية ولكنه لم يكن مرتبطاً بملامسة الققط. وفر تفاعل البلمرة المتسلسل في الوقت الحقيقي (RT-PCR) عائداً تشخيصياً أفضل. حتى يتم اكتشاف لقاح، تظل النظافة أفضل إجراء وقائي لداء المقوسات البشري.

الكلمات الدالة: داء المقوسات، الأمراض الحيوانية المنشأ، الحيوانات الأليفة، الققط، الأنبار، العراق