



Serological Detection of *Toxoplasma Gondii* in Animals and Human in Baghdad Province, Iraq

Ali A. Shatti¹

¹Department of Microbiology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq

*Corresponding Author: Ali A. Shatti

Email: alishati@uowasit.edu.iq



Article Info

Article history:

Received 5 May 2024

Received in revised form 3 June 2024

Accepted 14 June 2024

Keywords:

Zoonotic Diseases

ELISA

Floatation

Cats

Cattle

Sheep

Abstract

This study aims to investigate the prevalence of Toxoplasma gondii in field animals (cattle and sheep) and their owners at various areas in Baghdad province (Iraq) during September (2023) and February (2024). Also, it aims to identify the role of cat in transmission of infection to humans and animals. A total of 222 venous blood samples including 84 humans and 138 animals were collected under aseptic conditions into free-anticoagulant glass tubes. In addition, a total of 112 fecal cat samples were collected from different areas in Baghdad city into plastic containers to be examined by the flotation method. The results showed that 84 blood samples from animal owners detected the infection rate were 36.90%. In addition, there is a relationship between infection and age. The study shows that the highest age for infection is 26-30 years, and the percentage was 66.66%. also, the study included examining 138 blood samples from animals divided into 68 sheep and 70 cows' infection parasite rate was also 41.30%, and through examining the collection of 120 samples of soil from different areas in the city of Baghdad, the percentage of infection with T. gondii parasite reached 32.50%, addition to the infection rate in cats was 50% by examining 112 stool samples collected from different areas in the same city. This study reported the increasing seroprevalence of T. gondii using different samples. The reason for this increase may be due to poor health conditions, the large number of stray cats, the accumulation of dirt, the low degree of health education, and ignorance of the sources of infection for the disease and the attempt to avoid them, as it was noted through the study that a large number of women raise cats in their homes. They do not know that cats are the source of the disease.

Introduction

Toxoplasma gondii causes a disease known toxoplasmosis, which is infects humans and animals at all stages of life. The rate of infection by *T. gondii* is more than 40% of the world's population (Mohammed & Al-Janabi, 2019), and the risk of infection with this parasite is infecting pregnant women during pregnancy (Majeed & Abbas, 2018), transmitted from the mother to her fetus through the placenta (Wassef & Abdel-Malek, 2019) and causes miscarriage in pregnant women, Fetal malformation, or even serious disease symptoms in the fetus (Salih et al., 2020), and the greatest danger is that toxoplasmosis is without clinical signs in most of those infected (Abdullah & Al-Ubbyde, 2012). The statistics of epidemiology on the spread of this disease vary between countries of the world (Tonouhewa et al., 2017), as an epidemiological study conducted in Saudi Arabia indicated that a percentage of 37% of pregnant women are infected with this disease (Wallander 2016). Also, the study was conducted in Qatar in 2008, which showed that the highest prevalence rate was among people \square 45 years of age, reaching 41.2% (Guo et al., 2016). As for Iraq, several studies and

epidemiological research were recorded on the spread of the disease in different areas, and the first person to record the disease in 1939 was the researcher Machattia, who isolated the parasite from the loose spleen of dogs (Shallal, 2017). Al-Kappany et al. (2018) conducted an epidemiological study in which they explained that the infection rate reached 45.3% using the indirect hematological agglutination test (IHAT). A study conducted by Al-Sanjary & Hussein (2012) found that the infection rate among women of childbearing age reached 66.3%, and the highest infection rate was in the age group 20-24 years, with a percentage of 32.8%. Saleem and Al-Samarai (2018) found that the rate of transmission of the parasite from the mother to the fetus varies during the months of pregnancy (Noori, 2021) as found that transmission occurs at a rate of (15%) in the first three months of pregnancy, (30%) in the middle three months, and (60%) in the last three months of pregnancy (Wassef & Abdel-Malek, 2019). Pregnancy meaning that the rate of parasite transmission increases as the months of pregnancy increase or advance, depending on the placental blood flow (Madjeed et al., 2022; Saleem et al., 2022).

Therefore, this study aims to detect specific antibodies by ELISA in the serum of field animals (cattle and sheep) as well as in their owners to find the relationship between both in transmission of toxoplasmosis. As well as studying the effect of some factors that might play a role in increasing sensitivity to infection.

Methods

Human blood samples

Eighty-four blood samples were collected from owners of animals suspected of being infected with toxoplasmosis, ages ranging between (16-45 years) as mentioned in table 2, in different seasons of the 2023 year in various areas of Baghdad. At the same time, to record information about as the age, place of residence, type of animal raised, and the type of breeding used for the animal, where a blood sample was taken intravenously (4 ml) using a sterile syringe, and the sample drawn (blood) from the owners was placed in special test tubes to leave for 10-30 minutes. Immediately after extraction, the samples are placed in the centrifuge for 5 minutes to isolate the serum. A kit produced by the Spanish company Bio Kit-Sa, called Toxo-Cell-late to detect the presence of specialized antibodies against the *T. gondii* parasite in the patient's serum. The compound 2-mercaptoethanol (2ME) was used for positive samples in the latex agglutination test to diagnose the type of infection, acute or chronic. The ASO test (Anti-Streptolysin-O) was used, which depends on the interaction of antibodies present in the patient's serum with the antigen called Streptolysin-O, which covers the latex granules in the latex reagent for the test.

Animal blood samples

Blood samples of 68 sheep and 70 cows were drained from the jugular vein at a rate of (5 ml). The serum was isolated in the same manner mentioned above, and a direct agglutination test was performed on to detect animals infected with the *Toxoplasma* parasite.

Fecal cat samples

A total of 112 fecal samples of cat were collected from different areas in Baghdad city into plastic containers with tight lids to contain potassium dichromate solution 5% was placed in them as a preservative and examined in the laboratory using a flotation method using zinc sulfate (331 grams of 1 milligram of sulfate salt were dissolved Zinc in a liter of distilled water) by adding 1 gram of feces to 10 milliliters of warm water in test tubes suitable for placing in the centrifuge for minutes. Then, the sediment was taken and the process of suspending it in clean water was repeated, ignoring the suspended part. Finally, add 2 milliliters of zinc sulfate,

to purify the precipitate, The tube was filled to the upper end with the solution and covered with a slide cover. Another deposition process was performed at the same speed. Then the slide cover was transferred to a slide containing a drop of local iodine dye and was examined microscopically at two powers (10×) and (40×), noting that three smears were prepared.

Statistical analysis

The results were analyzed statistically using standard methods to calculate the average, standard deviation, number, ratio, and percentage to find differences between the groups. The results were considered significant at a probability level of 0.05 or less (Gharban et al., 2023).

Results and Discussion

This study shows the rate of infection by *Toxoplasma* among owners of animals of all types, ages, and locations, which is 36.90% through 84 blood samples, and the infection rate among animals combined as a whole reached 41.30% of the total number of 138 animals (Table 1). The statistical analysis also shows significant differences between blood samples of owners and animals. This high percentage of infections among animal owners includes active and latent cases of the disease, and if this percentage is compared to the infection rates found by Abdullah researcher (Abdullah & Al-Ubbyde, 2012) in a study on the prevalence of toxoplasmosis in animal owners reached (43.6%), as detected by Abdullah (2001) who gave an infection rate of 56.6%. Rufash & Yousif (2018) indicated that the infection rate in females (66%) and in males 51% in animal owners' patients in Mosul, so we find the incidence of this disease is constantly increasing year after year,

Table 1. Total results for seroprevalence of *T. gondii* among study population

Sample type	No. of sample	No. of Infection	Infection %
Human	84	31	36.90
Animals	138	57	41.30

The findings show the relationship of the infection rate with the age of the animal owner, where the highest infection rate was 66.66% in the age group 26-30 years, and the lowest infection rate was 25% in the age group 36-40 years (Table 2, Figure 1). The statistical analysis showed that there were significant differences with age, $p < 0.05$. This is consistent with the saleem study and the Abbas study in the Babylon and Salahal-Din governorates, and the Fatohi study (1985) in the Anbar governorate as the highest infection rate of 43.47% was recorded in the age group 33-26 years. The reason is that this age is one of the appropriate stages for childbirth, and this leads to the danger of the disease because causes miscarriage due to the infection being transmitted through the umbilical cord to the fetus or causing birth defects if the disease occurs in the last stages of pregnancy from animals.

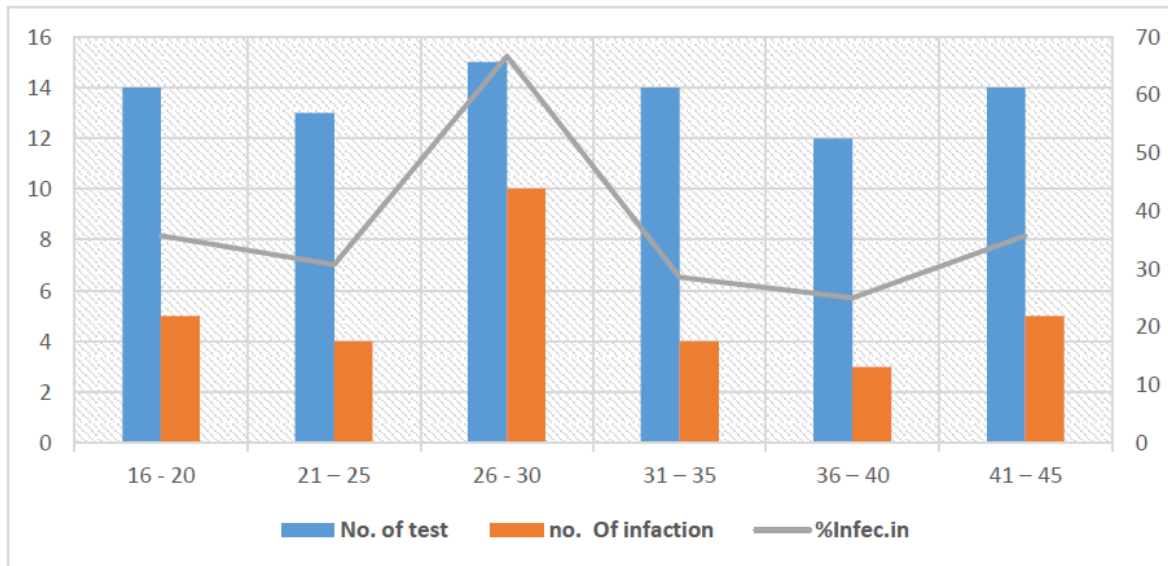


Figure 1. Distribution of *T. gondii* infection among animal owners and association to age groups

Table 2. Prevalence of *T. gondii* infection among animal owners and association to age groups

Age (Years)	Total No.	Positive	
		No.	%
16 - 20	14	5	35.71
21 - 25	13	4	30.76
26 - 30	15	10	66.66
31 - 35	14	4	28.57
36 - 40	12	3	25
41 - 45	14	5	35.71

The results of the study showed a total infection in animals of 41.30% with *T. gondii* through examining 138 samples from all animals (Table 3). The infection rate in sheep was 38.57% lower compared with higher cows in the rate of 44.11%. These results agree with Wilson's study, in the city of Tikrit (Wilson et al., 2017). The high infection rate in cows and sheep is due to the large presence of cats in breeding fields and pastures that shed egg sacs and animals ingest them. Also, relying on fodder stored by sheep and cow owners helps the spread of infection as a result of this fodder being exposed to rot and the presence of mycotoxins that work to suppress the immune system in these animals (Jawetz, 2007)

Table 3. Total results for detection of *T. gondii* in study animals

Animal	Total No.	Positive	
		No.	%
Sheep	70	27	38.57
Cow	68	30	44.11
Total	138	57	41.30

Also, our findings showed that the examining 120 soil samples from different areas in Baghdad Governorate, the total infection rate was 32.50%, and the highest infection rate, was 57.14% in the AL-Dora region, while the Al-Mashtal region the lowest infection rate (11.11%), (Table 4).

Table 4. Total results for testing the soil samples in study areas of Baghdad province

Area	Total No.	Positive	
		No.	%
Al-Mashtal	18	2	11.11
AL-Kadhimiya	20	4	20.00
AL-Dora	21	12	57.14
Baghdad Al Jadeeda	19	6	31.57
AL-Ameria	24	12	50.00
Sadr City	18	3	16.66
Total	130	39	32.50

While, the percentage of Infection among cats by examining 112 fecal samples from different areas of Baghdad province appearing the highest infection rate was 60.71% in the Al-Dora area also, and the lowest in the Al-Mashtal area, at a rate of 16.66%, with statistically significant differences $P < 0.05$. The total infection rate among the examined cats reached 50% (Table 5).

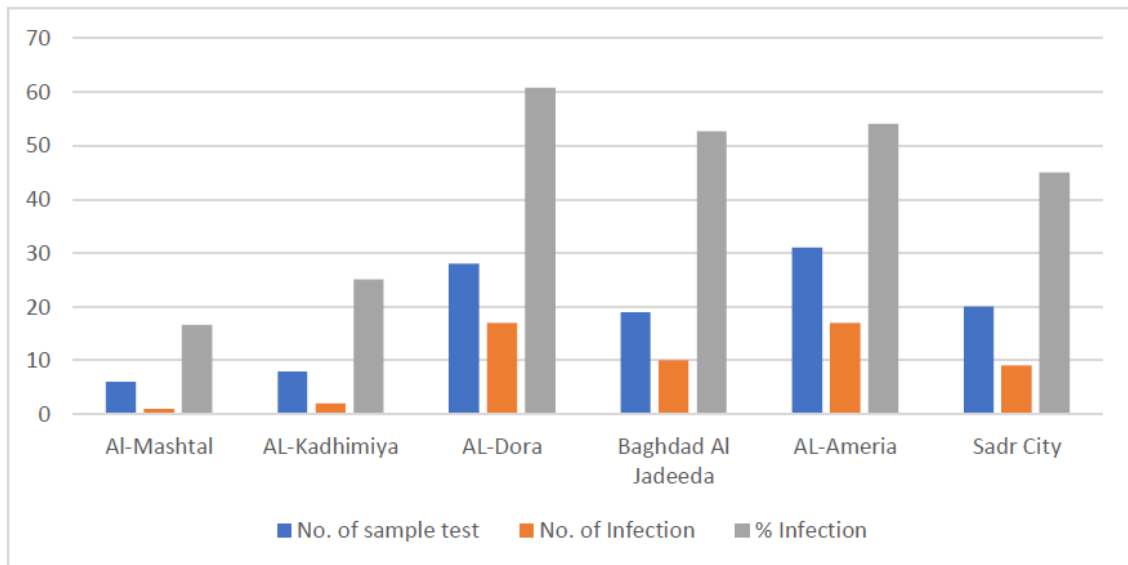


Figure 2. Fecal samples of examined and infected cats and the percentage of infection with the parasite *T. gondii*

As for the relationship of infection to the location of animals and animal owners, appears that animal owners are more susceptible to infection in one place and less in another. This is consistent with the Al-Bayati et al. (2021) in Anbar Governorate. This is attributed to the lack of health awareness, lack of interest in individual and collective hygiene, and ignorance of public health rules (Al-Bayati et al., 2021).

Table 5. Prevalence of *T. gondii* infection in cats and their association to study areas

Area	Total No.	Positive	
		No.	%
Al-Mashtal	6	1	16.66
AL-Kadhimiya	8	2	25
AL-Dora	28	17	60.71
Baghdad Al Jadeeda	19	10	52.63
AL-Ameria	31	17	54
Sadr City	20	9	45

As well as indifference in their daily dealings with the sources of infection surrounding them, and the infection rate among animal keepers in areas where there is less cultural awareness is more than in civilized areas, and this is agreed with a study by Saleem in a different area from Baghdad city and a study by Peretz (1994). The cause directly concerns tissues and infected animals as high infection rates in cats and other ruminants (Saleem et al., 2021). Also, owners in uninhabited areas work in activities related to agriculture with jobs that require contact with hosts. As mentioned by Alobaidy et al. (2022). Therefore, for animal owners who work cleaning animal pens, cats, and ruminants, and direct exposure to soil in agriculture, exposure to the disease is more dangerous through the egg sac parasites.

Conclusion

This study reported the increasing seroprevalence of *T. gondii* using different samples. The reason for this increase may be due to poor health conditions, the large number of stray cats, the accumulation of dirt, the low degree of health education, and ignorance of the sources of infection for the disease and the attempt to avoid them, as it was noted through the study that a large number of women raise cats in their homes. They do not know that cats are the source of the disease.

References

- Abdulla, B. A. (2001). Toxoplasmosis in high risk pregnancies in Mosul. *Iraq. Raf. J. Sci*, 12(2), 1-4.
- Abdullah, B. A., & Al-Ubbyde, G. T. (2012). Toxoplasmosis in high risk pregnant women and it's relation with some serological parameters in Nineveh governorate. *Iraqi Journal of Veterinary Sciences*, 26, 283-288. <https://doi.org/10.33899/ijvs.2012.168738>
- Al-Bayati, L. H., Razooqi, M. A., & Saleem, H. D. (2021). Serological detection of *Coxiella burnetii* in raw milk of goats in Baghdad, Iraq. *Biochemical & Cellular Archives*, 21(2).
- Al-Kappany, Y. M., Abbas, I. E., Devleeschauwer, B., Dorny, P., Jennes, M., & Cox, E. (2018). Seroprevalence of anti-Toxoplasma gondii antibodies in Egyptian sheep and goats. *BMC veterinary research*, 14, 1-5.
- Alobaidy, H., Saleem, H. D., & Al-Dhalimy, A. M. B. (2022). Comparative physiological aspects of plasma hemostasis of some commercial fish species. *Journal of Water and Land Development*, (54).
- Al-Sanjary, R. A., & Hussein, T. H. (2012). Using species-specific PCR technique to detect *Toxoplasma gondii* in broiler chickens. *Iraqi Journal of Veterinary Sciences*, 26(2), 53-56.
- Fatohi, F. A. M. (1985). *Detection of toxoplasmosis among different groups of populations in Mousl city by using IFAT and CFT* (Doctoral dissertation, M. Sc. Thesis, College of Medicine, University of Mousl).
- Gharban, H. A., Al-Ghuraibawi, H. N., Al-Rubaye, Z. A., Jahlol, H. A., Al-Zergany, A. A., & Al-Abedi, G. J. (2023). Most Clinically Detected Viral Diseases in Field Animals of Wasit Province, Iraq. *Annals of the Romanian Society for Cell Biology*, 27(01), 154-168.
- Guo, M., Mishra, A., Buchanan, R. L., Dubey, J. P., Hill, D. E., Gamble, H. R., & Pradhan, A. K. (2016). A systematic meta-analysis of *Toxoplasma gondii* prevalence in food animals in the United States. *Foodborne pathogens and disease*, 13(3), 109-118.

- Jawetz, M. (2007). *Adelberg's Medical Microbiology. Antibacterial and Antifungal chemotherapy (Prentice-Hall International Inc).*
- Madjeed Haddao, K., Dawood Saleem, H., Hameed, N. M., Mahdi Rheima, A., Alkhafaje, W. K., Salaam Abood, E., & Balasim Al-Dahy, L. (2022). Investigation of in vitro Cytotoxicity of Chelidonium majus against Leishmania Major. *Archives of Razi Institute*, 77(3), 1211-1214.
- Majeed, B. A., & Abbas, W. H. (2018). Serological and molecular detection of Toxoplasma gondii in meat and minced meat in Basra City. *Basrah Journal of Veterinary Research*, 17(3).
- Mohammed, L. J., and Al-Janabi, M. S. (2019). Seroprevalence of toxoplasmosis in aborted women in Babylon Province, Iraq. *Medical Journal of Babylon*, 16(3), 188-191.
- Noori, A. (2021). Comparisons of Toxoplasma gondii Prevalence in Rural and Urban Areas of Al-Najaf Province of Iraq Using Serological Methods. *Archives of Razi Institute*, 76(6), 1695. <https://doi.org/10.22092/ari.2021.356315.1822>
- Peretz, D. (1994). *The Middle East Today*. Bloomsbury Publishing USA.
- Rufash, H. N., & Yousif, J. J. (2018). Effect of genotypes of Toxoplasma gondii parasite on the level of some cytokines and markers in aborted women in Najaf governorate. *Biochemical and Cellular Archives*, 18(1).
- Saleem, H. D., & Al-Samarai, F. R. (2018). Plasma superoxide dismutase, malondialdehyde and lymphocyte DNA damage in horses with Theileria equi detected by qPCR. *Online Journal of Veterinary Research*, 22(6), 457-466.
- Saleem, H. D., Al-Obaidi, A. H., & Al-Tmemy, W. B. (2021). Histopathological Changes Due To Toxic Effect Of Aflatoxin B1 On Liver, Kidney And Therapeutic/Preventive Role Of Camel Milk. *Biochemical and Cellular Archives*, 21(1).
- Saleem, H. D., Hamza, T. A., Izzat, S. E., Hamad, D. A., Abdulhasan, M. J., & Adhab, A. H. (2022). Role Silver and Bimetallic Nano Particles Synthesized by Green Chemical Methods for their Therapeutic Potential for Cancer: A Review. *Journal of Pharmaceutical Quality Assurance*, 13(2), 222-226.
- Salih, J. M., Mero, W. M. M., & Eassa, S. (2020). Seroprevalence and some demographic factors associated with Toxoplasma gondii infection among male population in Duhok Province/Iraq. *Baghdad Science Journal*, 17(2), 0431-0431.
- Shallal Shahatha, S. (2017). Prevalence Of Toxoplasma Gondii Parasite And Their Causes In Al-Ramadi City Al-Anbar Governorate. *Iraqi Journal of Desert Studies*, 7(2), 109-116.
- Tonouhewa, A. B. N., Akpo, Y., Sessou, P., Adoligbe, C., Yessinou, E., Hounmanou, Y. G., & Farougou, S. (2017). Toxoplasma gondii infection in meat animals from Africa: systematic review and meta-analysis of sero-epidemiological studies. *Veterinary World*, 10(2), 194.
- Wallander, C. (2016). Toxoplasma gondii in wild boars and domestic pigs in Sweden. *Acta Universitatis Agriculturae Sueciae*, (2016: 12).
- Wassef, R., & Abdel-Malek, R. (2019). Validity of a new immunochromatographic test in detection of Toxoplasma gondii in cancer patients. *Journal of parasitic diseases*, 43, 83-86.

- Wassef, R., and Abdel-Malek, R. (2019). Validity of a new immunochromatographic test in detection of *Toxoplasma gondii* in cancer patients. *Journal of parasitic diseases*, 43, 83-86.
- Wilson, W., Taubert, K. A., Gewitz, M., Lockhart, P. B., Baddour, L. M., Levison, M., & Durack, D. T. (2017). Prevention of infective endocarditis: guidelines from the American heart association: a guideline from the American heart association rheumatic fever, endocarditis, and Kawasaki disease committee, council on cardiovascular disease in the young, and the council on clinical cardiology, council on cardiovascular surgery and anesthesia, and the quality of care and outcomes research interdisciplinary working group. *Circulation*, 116(15), 1736-1754. <https://doi.org/10.1161/CIRCULATIONAHA.106.183095>