Prevalence of *Toxoplasma gondii* Infections in Pregnant Women and Small Ruminants: A Molecular Diagnostic Approach

Sabaa Muhsen Farhan [1*], Yasmin Ammar Adi [2], Zaid Khalid Alani [3], Jihad Talib Obead Al-Yasari [4,5], Hayder Musaad Al-Timimi [6]

Abstract

Background: Toxoplasma gondii is a globally prevalent parasite with significant health implications, particularly for pregnant women and immunocompromised individuals. Infections during pregnancy can result in serious outcomes such as miscarriage, stillbirth, or congenital disabilities. This study aimed to assess the prevalence of T. gondii infection among pregnant women and small ruminants in Al-Anbar province, Iraq, using serological and molecular diagnostic techniques. Methods: Blood samples were collectsed from 165 pregnant women and 100 small ruminants (sheep and goats) between January and May 2021. Serological screening was conducted using the Humatex-Toxo rapid test to detect antibodies against T. gondii. DNA was extracted from the blood samples, and nested polymerase chain reaction (PCR) targeting the T. gondii B1 gene was used for molecular detection. The infection rates were analyzed statistically using ANOVA and Chi-square tests, with significance set at p<0.05. Results: The serological test identified T. gondii antibodies in 32.7% of pregnant women, while the PCR detected a higher infection rate of 40%. Among the small ruminants, PCR revealed an overall infection rate of 10%, with sheep showing a higher rate (16%) compared to goats (4%). The results indicate that PCR is more sensitive than serological testing, particularly for identifying active infections. Additionally, the higher infection rate in young women may be linked to lifestyle, environmental exposure, and dietary habits, while seasonal factors likely play a role in infection distribution. Conclusion: Nested PCR is a more effective method for detecting T. gondii in pregnant women, offering a precise tool for identifying infections and potential causes of abortion. The lower infection rates in small ruminants suggest a reduced zoonotic risk in the study area, although continued monitoring is essential. Negative PCR results may be due to inhibitors in the DNA extraction process or the parasite's localization in tissues rather than blood. The findings highlight the importance of molecular diagnostics in public health strategies aimed at reducing T. gondii infections in high-risk populations.

Keywords: Toxoplasma gondii, PCR, Pregnant Women, Small Ruminants, Zoonotic Transmission.

Significance | This study emphasized the effectiveness of PCR in detecting Toxoplasma gondii infections, especially in pregnant women, for improving public health.

*Correspondence. Sabaa Muhsen Farhan, Collage of Applied Science, University of Fallujah, Al-Anbar, Iraq.
E-mail: zaidalani242@yahoo.com
Phone: 009647725066124

Editor Md Haroon Or Rashid, Ph.D., And accepted by the Editorial Board August, 12 2024 (received for review May 28, 2024)

1. Introduction

Toxoplasma gondii (*T. gondii*) is one of the most prevalent parasites affecting humans worldwide, often causing significant health concerns, especially among pregnant women and immunocompromised individuals. The immune system plays a

Author Affiliation.
[1] Collage of Applied Science, University of Fallujah, Al-Anbar, Iraq.
[2] College of Pharmacy, Al-Farahidi University, Baghdad, Iraq.
[3] College of Dentistry, Al-Bayan University, Baghdad, Iraq.
[4] College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq.
[5] Alsafwa University College, Department of Medical Laboratory Technique, Kerbala, Iraq.
[6] College of Health Medical Techniques, AL-Bayan University, Iraq.

Please Cite This:
Sabaa Muhsen Farhan, Yasmin Ammar Adi et al. (2024). "Prevalence of Toxoplasma gondii Infections in Pregnant Women and Small Ruminants: A Molecular Diagnostic Approach", Journal of Angiotherapy, 8(8), 1-6, 9855.

2207-872X/© 2024 ANGIOTHERPY, a publication of Eman Research, USA.
This is an open access article under the CC BY-NC-ND license.
(http.//creativecommons.org/licenses/by-nc-nd/4.0/).
(https./publishing.emanresearch.org).

crucial role in controlling the initial infection, as cell-mediated immunity helps to clear *T. gondii* from the body in most cases (Ali et al., 2020; Raissi et al., 2020). However, in individuals with compromised immune systems or during pregnancy, the parasite poses a more significant threat, leading to severe health outcomes.

Pregnant women who contract toxoplasmosis are particularly at risk, as the infection can be transmitted vertically from mother to fetus. *T. gondii* infections during pregnancy are especially dangerous, with potential consequences such as miscarriage, stillbirth, and congenital disabilities. According to studies by Pinto-Ferreira, the risk of transmission to the fetus is highest during the third trimester, although infections can occur at any stage of pregnancy. More than 211 million births occur globally each year, making this a critical public health issue (Dubey, 2009; Elamin et al., 2012). The transmission of *T. gondii* often occurs through the ingestion of oocysts, which are shed in the feces of infected cats. These oocysts can contaminate soil, water, and food, increasing the risk of infection in humans who come into contact with these sources.

In immunocompromised individuals, *T. gondii* can have particularly severe consequences. Once the parasite enters the host, it has the ability to encyst in various organs, including the brain, heart, liver, kidneys, and skeletal muscles. The parasite's strong affinity for nerve cells makes it particularly dangerous, as it can form cysts in the brain, leading to neurological complications. In cases of acute infection, particularly in pregnant women, *T. gondii* can cross the placenta and cause severe outcomes, including stillbirths, neonatal mortality, and congenital abnormalities (Oyeyemi et al., 2020). In more severe cases, toxoplasmosis may lead to systemic infections, coma, brain damage, pneumonia, and even death, especially in those with weakened immune defenses.

Despite being a global health concern, there is still limited information regarding the prevalence of *T. gondii* in certain regions, such as the World Health Organization (WHO) Eastern Mediterranean Region (Dasa et al., 2021). This highlights the need for further research and surveillance in these areas to better understand the extent of the problem. Additionally, while it is widely known that cats and humans serve as reservoirs for the disease, more attention is needed to address the environmental contamination caused by *T. gondii* oocysts. These oocysts are highly resistant to common chemicals and disinfectants, making contaminated water sources a significant cause of outbreaks in both animals and humans (Khan and Noordin, 2020).

Given the risks posed by toxoplasmosis, pregnant women are advised to take precautionary measures, such as avoiding contact with cat litter, soil, and raw or undercooked meat, all of which may contain the parasite. The current study aimed to use molecular techniques to identify *T. gondii* infections in pregnant women and small ruminants, focusing on the heightened susceptibility of these groups compared to conventional detection methods. This research holds potential to improve prevention strategies and treatment protocols for toxoplasmosis.

2. Materials and Methods

2.1. Ethical Approval

This study was approved by the Animals and Humans Ethics Committee of the College of Applied Science, University of Fallujah, Al-Anbar, Iraq (Approval No. 01.12.2020). The research adhered to all ethical guidelines and principles regarding human and animal subject research.

2.2. Sample Collection

Between January 2021 and May 2021, a total of 165 blood samples were collected from pregnant women attending two healthcare facilities in Al-Anbar province: the Al-Ramadi Gynecology and Pediatrics Educational Hospital and the Al-Fallujah Educational Hospital of Gynecology and Pediatrics. The sampling was conducted randomly. Additionally, 100 blood samples from small ruminants (sheep and goats) were collected from local slaughterhouses during the same period. All samples were transported under optimal conditions to the laboratory for further testing.

2.3. Serological Diagnosis

Initial screening for *T. gondii* infection was performed using a rapid serological test, Humatex-Toxo, following the manufacturer's protocol. This test was used to detect anti-*T. gondii* antibodies in both human and animal blood samples as a preliminary diagnostic method.

2.4. DNA Extraction

DNA was extracted from whole blood samples using a commercially available DNA extraction kit (Bioneer, Korea). The procedure was carried out according to the manufacturer's instructions. After extraction, the DNA samples were stored at -20°C until further molecular analysis.

2.5. Detection of T. gondii DNA by PCR Assay

Polymerase chain reaction (PCR) was employed to detect the presence of *T. gondii* DNA in the blood samples of both pregnant women and small ruminants. Two sets of specific primers targeting the B1 gene of *T. gondii* were used to amplify DNA fragments of 580 bp and 531 bp, respectively (Al-Masoudi, 2015). The primer sequences used for amplification were as follows:

Forward primer 1: 5'-TGT TCT GTC CTA TCG CAA CG-3'
Reverse primer 1: 5'-ACG GAT GCA GTT CCT TTC TG-3'
Forward primer 2: 5'-TCT TCC CAG ACG TGG ATT TC-3'
Reverse primer 2: 5'-CTC GAC AAT ACG CTG CTT GA-3'

PCR reactions were carried out in a total volume of 24 µL, containing 12.5 µL of green master mix (Promega, USA), 5 µL of genomic DNA, 1 µL of each primer, and 5.5 µL of nuclease-free water. The PCR protocol consisted of an initial denaturation at

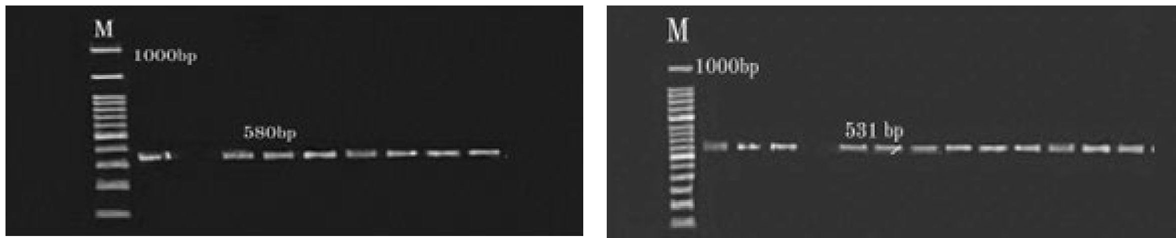

Figure 1. A. The PCR result displayed a 580 bp fragment amplification DNA ladder of 1000 bp 1.5% agarose gel electrophoresis for detection. B. For PCR product detection, a 1.5% agarose gel electrophoresis revealed a 531 bp fragment amplification DNA ladder of 1000 bp.

Table 1. *T. gondii* distribution among age groups based on rapid test

Age	**Positive**	**%**	**Negative**	**%**	**Total**
18-25 years	18	32.7	37	67.2	55
25-35 years	12	21.8	43	78.1	55
35-40 years	6	10.9	49	89	55
Chi-Square (χ^2)			**($P \leq 0.01$) Significant different**		

Table 2. The PCR data revealed the spread of *T. gondii* in Sheep and Goat

Host	**Examination No.**	**Positive No.**	**%**
Sheep	50	8	16
Goat	50	2	4
Total	100	10	10

94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 5 minutes.

Post-amplification, 5 μL of each PCR product was subjected to electrophoresis on a 1.5% agarose gel stained with ethidium bromide. DNA bands were visualized under a UV transilluminator to confirm the presence of *T. gondii* DNA.

2.6. Statistical Analysis

Data analysis was performed using SPSS software. The mean differences between groups were analyzed using Analysis of Variance (ANOVA), while the Chi-square test was employed for categorical variables. Statistical significance was set at a p-value of less than 0.05 (Dani and Al Quraan, 2023).

3. Results and Discussion

The results of this study highlight the prevalence of *Toxoplasma gondii* infections in both pregnant women and small ruminants, with significant findings obtained using serological and molecular diagnostic methods. In pregnant women, the infection rate using the rapid serological test was found to be 32.7%, while the rate detected using polymerase chain reaction (PCR) was higher at 40%. These findings underscore the sensitivity of PCR compared to serological tests, particularly in detecting active infections. Among the ruminants tested, the overall infection rate was 10%, with sheep showing a higher infection rate (16%) compared to goats (4%) (Figure 1).

These findings demonstrate the importance of using both serological and molecular techniques for *T. gondii* detection. While serological tests can identify individuals who have been exposed to the parasite, PCR is more effective in detecting active infections, especially when targeting specific genes like the B1 gene, which is frequently used as a diagnostic marker for *T. gondii* (Al-Masoudi, 2015).

The observed seroprevalence in pregnant women (32.7%) aligns with the range of rates reported in similar studies across various regions. For instance, in a study conducted in Bénin, the seroprevalence of toxoplasmosis using a rapid test was 48.9% in older women (Ogouyèmi-Hounto et al., 2014), while in Nigeria, the seroprevalence based on rapid tests for IgG and IgM antibodies was 28.1% (Table 1). These variations in infection rates can be attributed to differences in age groups, diagnostic methods, and regional factors such as dietary habits and environmental exposure to *T. gondii* oocysts.

Comparing the PCR results in this study to other research reveals some discrepancies. The 40% PCR positivity rate in pregnant women is higher than what was reported in Babylon Province, where 16.9% of women tested positive for *T. gondii* using nested PCR (Al-Masoudi, 2015). Similarly, the PCR detection rate in this study differs from findings in other countries. For example, in Iran, the PCR detection rate based on the SAG1 gene was 97.3% (Aly et al., 2023), while in India, 8.6% of patients tested positive for *T. gondii* using a B1 gene-based PCR (Datta et al., 2024). In Egypt, a study found a 57.3% infection rate in aborted women (Elaadli et al., 2023). These differences could be explained by several factors, including the type of sample collected, the stage of infection (acute vs. chronic), and environmental or lifestyle factors that influence exposure to the parasite.

In small ruminants, the overall infection rate of 10% detected via PCR is comparable to studies conducted in other regions, although the infection rates in sheep (16%) and goats (4%) vary from previous reports (Table 2). In a study conducted in India, sheep tested positive for *T. gondii* at a rate of 3.67%, while the infection rate in goats was 3.50% (Satbige et al., 2016). In Iran, the infection rate was found to be 5.9% in small ruminants (Amroabadi et al., 2021). Regional factors such as climate, animal management practices, and the prevalence of infected cats, which are the primary hosts for *T. gondii*, likely influence the variability in infection rates. The differences in diagnostic techniques, such as the use of different primers or PCR protocols, may also account for variations in the detection rates of *T. gondii*.

The higher infection rate in sheep compared to goats observed in this study is consistent with other research findings. For instance, a study conducted in Duhok, Iraq, reported a 21.6% infection rate in ewes (Mikaeel and Al-Saeed, 2020), while in Baghdad, 13.92% of goats tested positive for *T. gondii* using PCR (Al-abodi, 2021). In Al-Qadisiyah province, a similar study found 9% of sheep infected with *T. gondii* using RT-PCR (Al-abodi, 2021). The relatively lower infection rate in goats may be due to differences in grazing behavior or exposure to contaminated environments, as goats typically browse on higher vegetation, reducing their contact with *T. gondii* oocysts present in soil.

One notable limitation of PCR detection in this study is that the parasite may not always be present in the blood at the time of sample collection. *T. gondii* often encysts in tissues such as the brain, muscles, or organs like the liver and heart, making it challenging to detect during the chronic phase of infection when the parasite has localized within tissue cysts. The low PCR detection rate in some studies may also be attributed to the acute phase of infection passing before blood samples were taken, leading to reduced detection sensitivity (Boothroyd, 2009). Additionally, environmental factors and sample handling conditions could influence PCR results, potentially leading to variations in infection rates across studies (Al-Abodi, 2018).

Overall, the results of this study underscore the importance of using molecular diagnostic techniques like PCR to complement serological methods in detecting *T. gondii* infections, especially in high-risk populations like pregnant women and animals. The

findings highlight regional variations in infection rates, influenced by factors such as sample type, infection stage, and environmental conditions, which must be considered when comparing data across different studies. Further research is needed to explore these factors in greater detail, particularly in regions where *T. gondii* prevalence is poorly understood.

5. Conclusion

This study concludes that nested PCR proved to be a more sensitive and effective method for detecting the B1 gene of *Toxoplasma gondii*, allowing for the precise identification of the pathogen responsible for infections and potential abortions in pregnant women. The results highlight a higher rate of infection among young women, which may be influenced by various factors, including lifestyle, dietary habits, environmental exposure, and residency. Seasonal variations likely also play a role in the distribution and transmission of the parasite. The lower infection rates observed in small ruminants, compared to other research, suggest that the risk of zoonotic transmission from these animals may be lower in the study area. However, continued monitoring and testing are crucial for understanding the full scope of toxoplasmosis transmission. Additionally, negative PCR results in some samples may be attributed to potential inhibitors affecting the DNA extraction process or the parasite being present in tissues rather than blood. These findings underscore the importance of molecular techniques in improving diagnostic accuracy and informing public health strategies to reduce the risk of *T. gondii* infections in both humans and animals.

Author contributions

S.M.F led the conceptualization, fieldwork, data analysis, and drafting of the original manuscript. Y.A.A and ZKA contributed to research design, methodology validation, data analysis, and manuscript editing. J.T.O.A.Y was responsible for investigation, supervision, and manuscript review, while HMA-T assisted in methodology validation, visualization, and data interpretation. All authors, including S.M.F, Y.A.A, Z.K.A, J.T.O.A.Y, and HMA-T, collaboratively reviewed and approved the final version of the manuscript.

Acknowledgment

The authors were grateful to the College of Dentistry, Al-Bayan University as well as the field technicians who helped with the research.

Competing financial interests

The authors have no conflict of interest.

References

Al-abodi, H. R. (2021, May). Diagnostic study to detect toxoplasmosis in some Iraqi sheep. Journal of Physics: Conference Series, 1879(2), 022050. https://doi.org/10.1088/1742-6596/1879/2/022050

Al-Abodi, H. R. J. (2018). Suspicion in the form of infection is the basis for selecting the appropriate method for examining the toxoplasmosis disease of bends that have no symptoms from patients. International Journal of Advanced Research, 6(9), 655-662. https://doi.org/10.21474/IJAR01/7743

Al-Gharibawi, Y. K., & Alwaaly, A. B. (2021). Pregnant women with toxoplasmosis in Al-Hai City, Wasit Governorate, Iraq. Natural Volatiles & Essential Oils (NVEO), 8(6), 5431-5440.

Ali, S., Amjad, Z., Khan, T. M., Maalik, A., Iftikhar, A., Khan, I., & Ahmed, H. (2020). Occurrence of Toxoplasma gondii antibodies and associated risk factors in women in selected districts of Punjab province, Pakistan. Parasitology, 147(10), 1133-1139. https://doi.org/10.1017/S0031182020001192

Alkanaq, M. N., Shallal, Z. S., Alrammah, H. S. A. A., & Alhadi, H. (2020). Detection of Toxoplasma gondii in blood and milk of infected goats and pregnant women by rapid test cassette and conventional-PCR methods in AL-Qadisiyah province, Iraq. International Journal of Pharmaceutical Research, 12(2).

Allamy, S. S., & Alsaady, H. A. M. (2023). Using antibody rapid test, RT-qPCR, and RFLP/PCR for detecting Toxoplasma gondii in the placenta of abortion women and determining the genotype in Maysan province, Iraq. Journal of Wasit for Science and Medicine, 16(3), 28-43.

Al-Masoudi, H. K. (2015). Utilization of molecular and serological methods to investigate Toxoplasma gondii in healthy apparently students in Babylon Province. Medical Journal of Babylon, 12(4), 934-942.

Al-Omer, N. S. N. K., & Al-Marsoomy, A. H. A. M. (2021). Molecular and serological detection of Toxoplasma gondii in random samples of pregnant and aborted women of Nineveh. College of Basic Education Research Journal, 17(2), 1848-1864.

Aly, N. S., Kim, H. S., Marei, Y. M., Elhamshary, A. S., Bayoumi, I. R., Omar, R. E., Mohammed, D. A., Miyoshi, S. I., & Rashed, G. A. (2023). Diagnosis of toxoplasmosis using surface antigen grade 1 detection by ELISA, nano-gold ELISA, and PCR in pregnant women. International Journal of Nanomedicine, 18, 1335-1345. https://doi.org/10.2147/IJN.S387105

Amroabadi, M. A., Rahimi, E., & Shakerian, A. (2021). Study of the seasonal and geographical prevalence of Toxoplasma gondii in the milk of ruminants by nested-PCR. Veterinary Parasitology, 291, 109376. https://doi.org/10.1016/j.vetpar.2021.109376

Boothroyd, J. C. (2009). Toxoplasma gondii: 25 years and 25 major advances for the field. International Journal for Parasitology, 39(8), 935-946. https://doi.org/10.1016/j.ijpara.2009.02.003

Cerutti, A., Blanchard, N., & Besteiro, S. (2020). The bradyzoite: A key developmental stage for the persistence and pathogenesis of toxoplasmosis. Pathogens, 9(3), 234. https://doi.org/10.3390/pathogens9030234

Dani, A., & Al Quraan, E. (2023). Investigating research students' perceptions about statistics and its impact on their choice of research approach. Heliyon, 9(10), e20427. https://doi.org/10.1016/j.heliyon.2023.e20427

Dasa, T. T., Geta, T. G., Yalew, A. Z., Abebe, R. M., & Kele, H. U. (2021). Toxoplasmosis infection among pregnant women in Africa: A systematic review and meta-

analysis. PLOS ONE, 16(7), e0254209. https://doi.org/10.1371/journal.pone.0254209

Datta, P., Garg, P., Rattan, D., Bagga, R., Rohilla, M., Khurana, S., & Sehgal, R. (2024). Comparison of B1 and RE529 gene targets by real-time PCR and LAMP assay for diagnosis of toxoplasmosis in pregnant females. Indian Journal of Medical Microbiology, 47, 100481. https://doi.org/10.1016/j.ijmmb.2024.100481

Dentico, P., Volpe, A., Putoto, G., Ramadani, N., Bertinato, L., Berisha, M., Schinaia, N., Quaglio, G., & Maggi, P. (2011). Toxoplasmosis in Kosovo pregnant women. Microbiologica-Quarterly Journal of Microbiological Sciences, 34(2), 203.

Dubey, J. P. (2009). History of the discovery of the life cycle of Toxoplasma gondii. International Journal for Parasitology, 39(8), 877-882. https://doi.org/10.1016/j.ijpara.2009.01.005

Edward, I. U., Ibrahim, G., Kabiru, M., Ikeh, E. I., Shallangwa, I. B., & Edward, D. S. (2024). Detection of B1 gene in toxoplasmosis using PCR among pregnant women attending antenatal clinic in Kaduna State, Northwest Nigeria. South Asian Journal of Parasitology, 7(1), 1-11.

Elaadli, H., Abo El-Makarem, H., Abd Elrahma, A. H., Shaapan, R. M., & Bessat, M. (2023). Prevalence and associated risk factors of Toxoplasma gondii infection in sheep and aborted women in Egypt. Iraqi Journal of Veterinary Sciences, 37(2), 437-445.

Elamin, M. H., Al-Olayan, E. M., Omer, S. A., Alagaili, A. N., & Mohammed, O. B. (2012). Molecular detection and prevalence of Toxoplasma gondii in pregnant women in Sudan. African Journal of Microbiology Research, 6(2), 308-311. https://doi.org/10.5897/AJMR11.1882

Ghazzay, M. H., Al-Kelaby, K. K. A., Al-Hilli, E. S. A. L. I., & Hadi, N. R. (2020). Diagnosis of toxoplasmosis in pregnant women using enzyme immunoassay and nested real-time PCR in Al-Najaf city, Iraq. International Journal of Pharmaceutical Research, 12(2), 140-147.

Kareem, H. I. (2023). Molecular and serological detection of the presence of Toxoplasma gondii in intermediate hosts (women and sheep) in Al-Diwaniyah City, Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 22(1, Supplement I).

Khan, A. H., & Noordin, R. (2020). Serological and molecular rapid diagnostic tests for Toxoplasma infection in humans and animals. European Journal of Clinical Microbiology & Infectious Diseases, 39(1), 19-30. https://doi.org/10.1007/s10096-019-03719-2

Mikaeel, F. B., & Al-Saeed, A. T. M. (2020). Serological and molecular diagnosis of Toxoplasma gondii among ewes and horses in Duhok Province, Iraq. The Iraqi Journal of Agricultural Science, 51(4), 1212-1219.

Ogouyèmi-Hounto, A., Agbayahoun-Chokki, F., Sissinto Savi de Tove, Y., Biokou Bankole, B., Adinsi de Souza, V., Assogba, M., Kinde-Gazard, D., & Massougbodji, A. (2014). Evaluation of a rapid diagnostic test in the diagnosis of toxoplasmosis in pregnant women in Cotonou (Bénin). Bulletin de la Société de Pathologie Exotique, 107, 85-89. https://doi.org/10.1007/s13149-013-0323-7

Oyeyemi, O. T., Oyeyemi, I. T., Adesina, I. A., Tiamiyu, A. M., Oluwafemi, Y. D., Nwuba, R. I., & Grenfell, R. F. (2020). Toxoplasmosis in pregnancy: A neglected bane but a serious threat in Nigeria. Parasitology, 147(2), 127-134. https://doi.org/10.1017/S0031182019001115

Raissi, V., Taghipour, A., Navi, Z., Etemadi, S., Sohrabi, Z., Sohrabi, N., Getso, M., Shamsaei, S., Fasihi Karami, M., & Raiesi, O. (2020). Seroprevalence of Toxoplasma gondii and Toxocara spp. infections among pregnant women with and without previous abortions in the west of Iran. Journal of Obstetrics and Gynaecology Research, 46(3), 382-388. https://doi.org/10.1111/jog.14187

Rostami, A., Riahi, S. M., Gamble, H. R., Fakhri, Y., Shiadeh, M. N., Danesh, M., Behniafar, H., Paktinat, S., Foroutan, M., Mokdad, A. H., & Hotez, P. J. (2020). Global prevalence of latent toxoplasmosis in pregnant women: A systematic review and meta-analysis. Clinical Microbiology and Infection, 26(6), 673-683. https://doi.org/10.1016/j.cmi.2019.11.021

Saki, J., Zamanpour, M., Najafian, M., Mohammadpour, N., & Foroutan, M. (2021). Detection of acute and chronic Toxoplasma gondii infection among women with history of abortion in the Southwest of Iran. Journal of Parasitology Research, 2021, 6693070. https://doi.org/10.1155/2021/6693070

Satbige, A. S., Vijaya Bharathi, M., Ganesan, P. I., Sreekumar, C., & Rajendran, C. (2016). Detection of Toxoplasma gondii in small ruminants in Chennai using PCR and modified direct agglutination test. Journal of Parasitic Diseases, 40, 1466-1469. https://doi.org/10.1007/s12639-015-0722-z

Tenter, A. M. (2009). Toxoplasma gondii in animals used for human consumption. Memórias do Instituto Oswaldo Cruz, 104, 364-369. https://doi.org/10.1590/S0074-02762009000200035