Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Molecular and Serological Detection of The Presence of *Toxoplasma gondii* in Intermediate Hosts (Women and Sheep) in Al-Diwaniyah City, Iraq

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Received:2/12/2022. Accepted:1/3/2023. Published:12/9/2023

Abstract

Toxoplasma gondii (*T. gondii*) is a widespread parasite. The purpose of the present investigation was to determine the prevalence of toxoplasmosis in ewes and women in Al-Diwaniyah City, Iraq. Fifty-five blood samples were collected from women (16 non-pregnant, 20 pregnant, and 19 women with abortion), who attended the Maternity and Children Hospital, while 51 blood samples were collected from ewes (28 non-pregnant, 18 pregnant, and 5 ewes with abortion) from different areas of Al-Diwaniyah City. The latex agglutination and real-time PCR (RT-PCR) tests were employed to examine the presence of the *Toxoplasma gondii*. The results showed that *T. gondii* antibodies were detected in 40% of all tested women; however, 37.5%, 30%, and 52.63% of non-pregnant, pregnant, and women with abortion, respectively, revealed the presence of these antibodies. The outcomes revealed that there were no significant (p>0.05) differences between the group of non-pregnant women and pregnant women, while the abortion group recorded a significant (p<0.05) difference with both groups. The findings of the ewes showed that *T. gondii* antibodies were detected in 33.33% of all tested ewes; however, 35.71%, 33.33%, and 20% of non-pregnant, pregnant, and ewes with abortion, respectively, revealed the presence of these antibodies. There were no significant (p>0.05) differences between the group of non-pregnant and pregnant ewes, while the abortion group recorded significant (p<0.05) differences with both groups. The RT-PCR was significantly (p<0.05) effective in detecting the presence of *T. gondii* in the blood samples of ewes and women as 9 (17.7%) and 15 (27.3%), respectively. The present study indicates high percentages of *Toxoplasma gondii* infection in women and ewes.

Keywords: Abortion, ewes, *Toxoplasma gondii*, RT-PCR, women.

Introduction

Toxoplasmosis is considered at the highest level of importance for common parasitic illnesses that causes many health problems in both animals and humans. It leads to a zoonotic disease in humans and leads to miscarriages with congenital malformations in fetuses, while in animals it leads to abortion or death of the fetus after birth. The disease occurs individually or as an epidemic and is widely present in most parts of the world (1). It is believed that about 13% of the global people is affected by this disease, and its importance comes primarily in pregnant women, organ recipients, and people with immunodeficiency disorders. In addition to humans, sheep, goats, cattle, camels, horses, birds, mice, rats, and flies

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can be infected by *T. gondii* (1,2). This disease is caused by a protozoan called *Toxoplasma gondii*, which is an obligate organism inside the body cells of the host. This parasite is a single-celled organism that has the ability to parasitize inside the cells of a host, depending for its nutrition on the host. Felidae family is the definitive host of this protozoan, while the intermediate hosts include different types of animals such as sheep as well as humans (3,4).This disease is considered one of the important diseases that have gained medical attention in recent years due to its common presence throughout the planet. It has been confirmed that this disease has dangerous effects on pregnant women and their newborns. The parasite can induce miscarriage or stillbirth, as it goes to infect fetuses when transmitted from their mothers during pregnancy (5–7). The newborns may demonstrate risky signs, like affected retina and brain, swelling of the skull with fluids, where the head of the fetus become distorted, or the head may be smaller than normal size (8–11). As for the infection of the lymphatic system, it results in an enlarged liver and spleen and high temperature (12,13), and thus causes economic losses as a result of the loss of stillborn animals and the costs incurred as a result of mental and motor problems resulting from the congenital toxoplasmosis, with estimated losses in the United States of America 0.4-8.8 billion and 1.2-12 million in the United Kingdom. In addition to the importance of the disease in terms of public health, it is an important cause of abortion, fertility problems, financial waste in the livestock sector, and the high cost needed to establish a control and treatment program (14). The primary objective of this study was to assess the prevalence of toxoplasmosis among (ewes) and women residing in Al-Diwaniyah City, Iraq.

Materials and Methods

Fifty-five blood samples from women (16 non-pregnant, 20 pregnant, and 19 women with abortion) were collected from patients, who attended the Maternity and Children Hospital, while 51 blood samples from ewes (28 non-pregnant, 18 pregnant, and 5 ewes with abortion) were collected from different areas of Al-Diwaniyah City. The blood samples (5-10ml/each) were placed in collecting tubes and transferred to the Laboratory of Zoonotic Diseases, Unit of Zoonotic Diseases, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq. The serum was collected from each tube by using a centrifuge at 3000rpm for 10mins. The sera were stored in a deep freezer until performing the latex agglutination test to check the presence of the *Toxoplasma gondii* antibodies. The test was done according to Al Hamada et al (15).

Real-time PCR

Extraction of DNA and RT-PCR

For the fever-based acute cases, the blood samples were used in the RT-PCR method. After collecting the blood samples with EDTA, centrifuging at 4,000g for 15mins at the ambient temperature, and the parasitic DNA was extracted by using the GenomicPrep Blood DNA isolation kit (Amersham Pharmacia Biotech, UK) with the steps from the kit was used to extract the DNA. NanoDrop was used to quantify and qualify the obtained DNA.

The B1 gene from *T. gondii* was employed for the RT-PCR method as described by Lin et al. (16). At 25µl of total reaction volume, 5µl DNA, 12.5µl (2X) master mix of RT-PCR, 2.5µl (5µM)/primer direction: F: TCCCCTCTGCTGGCGAAAAGT and R: AGCGTTCGTGGTCAACTATCGATTG, and 2.5µl (2µM) TaqMan probe (6FAM TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA) (Thermo Fischer Scientific). The conditions were 95°C for 10mins initial

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activation, 95°C for 15s and 60°C for 60s were done for 40 cycles.

Ethical approval: The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah.

Statistical analysis: The data were processed using GraphPad software 7v (California, USA). Mean and standard error of mean were employed. The *P* value was decided as less than 0.05 for potential tests.

Results

Latex-agglutination test:

The results showed that *T. gondii* antibodies were detected in 40% of all tested women; however, 37.5%, 30%, and 52.63% of non-pregnant, pregnant, and women with abortion, respectively, revealed the presence of these antibodies. The findings showed that there were no significant ($p>0.05$) differences between the group of non-pregnant women and pregnant women, while the abortion group recorded a significant ($p<0.05$) difference than other groups (Figure 1). While in the ewes showed that *T. gondii* antibodies were detected in 33.33% of all tested ewes; however, 35.71%, 33.33%, and 20% of non-pregnant, pregnant, and aborted ewes, respectively, revealed the presence of these antibodies. There were no significant ($p>0.05$) differences between the group of non-pregnant and pregnant ewes, while the abortion group recorded less significant ($p<0.05$) differences than other groups (Figure 2).

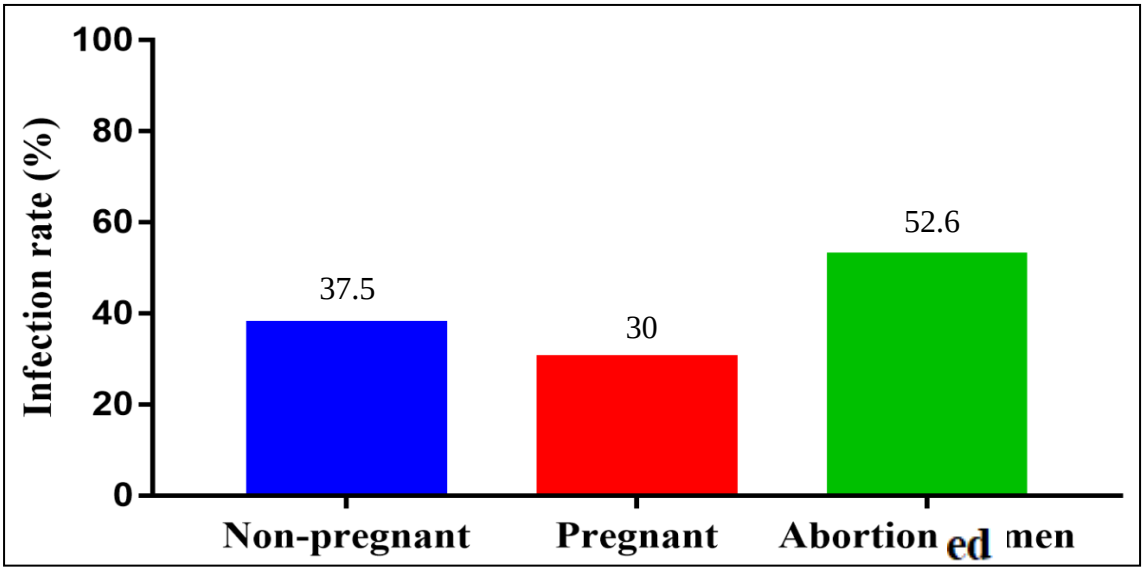

Figure 1: Infection rates of *Toxoplasma gondii* in women by latex-agglutination test.

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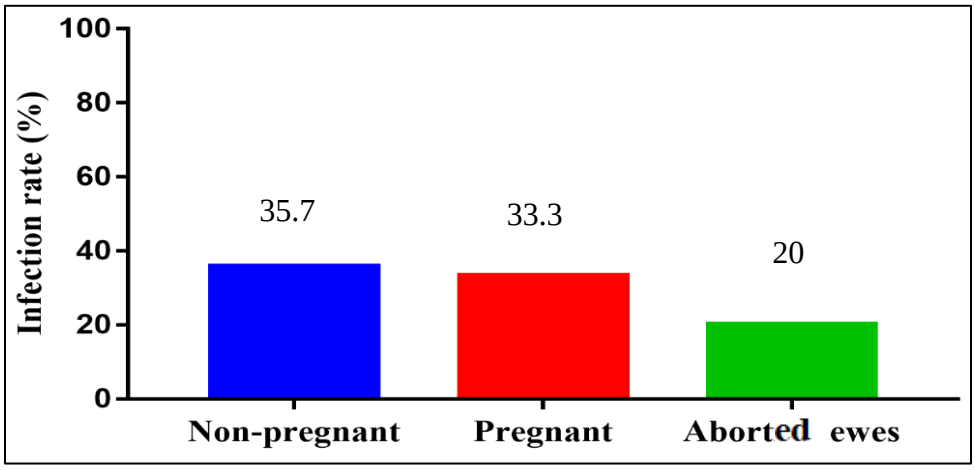

Figure 2: Infection rates of *Toxoplasma gondii* in ewes.

Real time PCR

The findings of the RT-PCR showed that the test was significantly (p<0.05) effective in detecting the presence of *T. gondii* in the blood samples of ewes and women as 9 (17.7%) and 15 (27.3%), respectively (Figure 3).

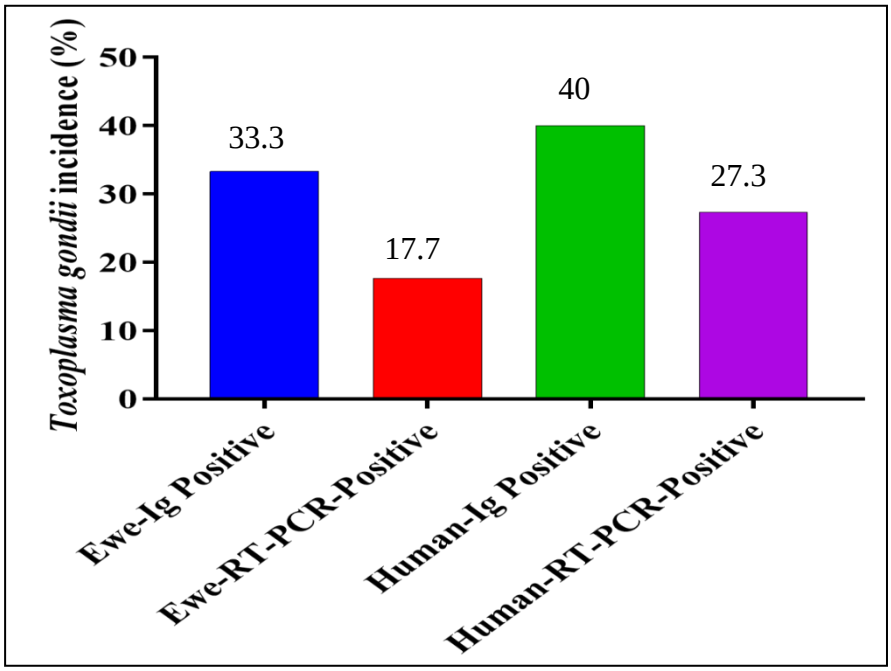

Figure 3: Infection rates of *Toxoplasma gondii* in women and ewes confirmed by real-time PCR.

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Discussion

The latex test is considered a good choice in serological and epidemiological studies for diagnosing infection with toxoplasma parasite in terms of cost and speed of completion. It could be close to ELISA and PCR; however, in acute or false-negative infections, RT-PCR could be the test of choice for successful detection of this protozoan (17).One of the most common protozoans in the world is *T. gondii* (18). *T. gondii* is thought to be present in up to 30% of world people. *T. gondii* infection has a seroprevalence ranging from 20% to 90% in various nations. The vast majority of adult illnesses are silent. Testing for antibodies against *T. gondii*, as well as avidity for *T. gondii*-specific IgG antibodies, may help determine if an infection is latent during pregnancy if there are no specific signs or symptoms of acute or current infection, such as fever or enlargement of lymph nodes (19).In this study, a latex test was used to determine the infection in samples of women and ewes. The outcomes of the current study confirmed the importance of the disease in the city of Al-Diwaniyah City, Iraq, where the infection rate in women was 40%. Babol and Ghaemshare cities in Iran's north had the greatest occurrence that reached up to 64%. The infection became more common as people became older. The frequency of *T. gondii* in women of reproductive age varies greatly across nations, according to epidemiological research. Prevalence of *T. gondii* in pregnant women ranges from 9 to 67% in European nations (19). When it comes to pregnant women in Asian nations, the prevalence of the parasite *T. gondii* ranges from 0.8% to 11.2% in Asia, while the prevalence might be as high as 42-55% (in an India, Malaysia, and Nepal). Turkish researchers reported that the infection of *T. gondii* was 1.3% for IgM and 25% for IgG in their investigation (19).The RT-PCR revealed that it was effective in the definitive diagnosis of *T. gondii* in ewe and woman blood samples. The test can identify the reality of presence of the infectious agent even in low amount. The use of RT-PCR has revolutionized early medical diagnosis. Bin Dajem *et al* (20) found that 41% (56/137) of women in the Saudi Arabia tested positive for the infectious agent using PCR. Parasite DNA may be detected in blood samples taken from women either before or during pregnancy, according to previous research using PCR. It is certainly clinically relevant that the existence of Toxoplasma DNA in female blood implies a newest infection or apparent parasitemia (21).Multiple PCR-derived techniques have been designed to identify T gondii DNA containing B1 gene sequences, which has been shown to be a more effective target than others. One possible explanation is that B1 is a highly repeated DNA sequence compared, which only exists in a single copy. In recent years, the AF146527 sequence has been employed; this sequence is present in around 200–300 copies in the T gondii genome. Multiple independent labs have utilized the original gene with positive results. This technique has been recognized for its sensitivity and specificity (22).Our study results show the occurrence of anti-*T. gondii* antibodies in the tested ewes. This agrees with the fact that there are evidences of the of *T. gondii* occurrence in ewes from different countries, such as from Africa at 26.1 to 29.1% in sheep. Shahighi et al (23) demonstrated that *T. gondii* was detected in 95.2% of their samples from ewes. In Najaf-Iraq, Jaber and Noori (24) conducted a study between September, 2020 to April, 2021 and found that 80 (42.1%) samples were IgG-positive with no IgM confirmed cases. In another study in Baghdad-Iraq, Harith and Ban (25) detected a 31.70%- IgG cases of pregnant women.

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Conclusion

The present study indicates high percentages of *Toxoplasma gondii* in pregnant and aborted women and ewes. RT-PCR is a sensitive and specific test for the confirmation of diagnosis of *T. gondii* in the blood samples from women and ewes.

Conflict of Interest: there is no conflict of interest.

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