Original Article

Epidemiological Study of *Toxoplasma* Infection in Humans and Sheep Using Molecular, Serological, and Traditional Techniques in Central Iraq

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ABSTRACT

Background: The parasitic protozoan *Toxoplasma gondii* is the cause of serious diseases known as toxoplasmosis. All warm-blooded vertebrate animals are susceptible to infection. Despite its significance, recent data on the prevalence and genetic diversity of *T. gondii* in human and sheep populations in central Iraq are limited.

Objectives: This study aimed to determine the prevalence of *T. gondii* infection in humans and sheep in Diyala and Baghdad provinces, Iraq, using serological and molecular techniques, and to genetically characterize circulating strains based on the regenerating gene (Reg).

Methods: The one hundred placentas of sheep in Diyala and Baghdad (50 samples from each province) were examined. An infected sheep placenta sample was digested with the pepsin enzyme. This was intended for performing the dyeing process with Giemsa stain and extracting the genomic DNA, using the tissue genomic DNA isolation kit (buffy coat) of seropositive individuals with Geneaid, Bioteccorp, Taiwan. Polymerase chain reaction (PCR) amplification targeted a 529 bp fragment of the *REGs* gene in *T. gondii*. Human serum samples were collected from 100 human blood samples (male and female) from Diyala and Baghdad (50/50). The enzyme-linked immunosorbent assay (ELISA) results were recorded according to the manufacturer's instructions. The study period was from September 2024 to April 2025.

Results: The overall infection rate in sheep was 25% (25/100), with a higher rate in Diyala (30.3%) than in Baghdad (20%). In ewes, the highest prevalence (43.33%) was observed at 4 years of age. ELISA testing of human sera (n=100) revealed an overall seroprevalence of 23.91%. The rate was significantly higher in Diyala (30.95%) than in Baghdad (18%), and higher in females (40%) than in males (4.76%). The highest infection rate (33.33%) was found in the 25-35-year age group. "Phylogenetic analysis of the Reg sequences showed that Iraqi isolates clustered most closely with strains from Iraq, Indonesia, China, Italy, Netherlands, Pakistan, Turkey, Iran, Chile, Myanmar, Nigeria, Saudi Arabia, and India. Phylogenetic analysis of the evolutionary distribution of REGs in 20 local isolates detected in Diyala and

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Baghdad (the middle of Iraq), with 36 global isolates of *T. gondii* deposited in GenBank, indicated a diverse evolutionary lineage.

Conclusion: This study confirms that toxoplasmosis remains a highly endemic zoonosis in central Iraq, with a significant prevalence in both sheep and human populations. The high genetic diversity of *T. gondii* strains and the identified risk factors (location, gender, and age) highlight the need for targeted public health and veterinary control measures.

Keywords: Diyala, Human, Molecular, Serological, Sheep, Toxoplasmosis

Introduction

Toxoplasma gondii is a protozoan parasite that causes toxoplasmosis, a significant zoonotic disease (Al-Malki, 2021). This species belongs to the phylum Apicomplexa, a group of intracellular coccidian parasites (Delgado et al., 2022). Because of the high frequency of human transmission, the chronic stage of the illness leads to fatal consequences. Because many individuals remain unaware of their infection, toxoplasmosis is recognized as a major public health concern (Basavaraju, 2016). Although toxoplasmosis is often asymptomatic, it can become dangerous or even fatal if the infection occurs in a fetus, a neonate, or an immunocompromised individual during pregnancy (Pawełczyk et al., 2022). During the prolonged infection phase, clinical symptoms often remain subclinical and persistent (Dubey et al., 2021). Many infected individuals only discover their status because the parasite typically does not manifest pathological symptoms in hosts with robust immune systems (Melchor et al., 2019).

In contrast, immunocompromised adults may experience acute and intermittent symptoms. Severe outbreaks affect people with compromised immune systems, such as those living with HIV/AIDS, following the reactivation of bradyzoites into disseminated tachyzoites (Wang et al., 2017). During the latent stage of infection, bradyzoites reside within tissue cysts, whereas sporozoites are present in oocysts, which possess the ability to withstand environmental stressors. These oocysts are produced during the sexual stage of the parasite's life cycle. Small ruminants, such as goats and sheep, are particularly vulnerable to *T. gondii*, which can lead to various health complications in these animals (Zhao & Ewald, 2020). Transmission can occur through the consumption of raw or undercooked meat containing *Toxoplasma* cysts or oocysts (Đurković -Đakovi, 2017). Once ingested, these cysts are resistant to stomach acid and remain viable for infection (AitHamoa et al., 2021). If infection occurs during pregnancy, there is an increased risk of miscarriage, stillbirth, live births presenting with some defects, like hydrocephalus, microcephalus, brain damage, and mental disorders (Chaudhry et al., 2014).

Even in the absence of clinical symptoms, a pregnant woman infected with *Toxoplasma* may transmit the infection to the fetus via the placenta; however, symptoms may not manifest until after birth (Peyron et al., 2019). There is a possibility that toxoplasmosis infection contributes to elevated testosterone production following birth (Borráz-León et al., 2022). Additionally, evidence from both human and animal models suggests that toxoplasmosis may alter certain physical traits to make infected males more desirable to females, which appears to be an instance of parasite manipulation. Infection can also occur by consuming meat containing sporulated oocysts.

During the acute phase of the disease, tachyzoites attack the cardiac muscle, lymphoid tissues, endothelial organs, and the central nervous system (Zhu et al., 2022). Clinical symptoms include fever, malaise, muscle discomfort, pneumonia, inflammation of the meninges, and impairments in psychomotor function (Layton et al., 2023). Toxoplasmosis is transmitted to hosts through various routes, including the ingestion of oocysts via inhalation, saliva, mucus, feces, or milk in infants (Gebremedhin et al., 2014). Due to its utility in veterinary and human medicine, as well as its suitability as a paradigm for cytological and molecular protozoan studies, *T. gondii* is a unicellular organism that remains a major subject of research (Marín-García et al., 2022). The parasite exists in several forms, including oocysts, tachyzoites, and tissue cysts (Álvarez et al., 2021).

Although it can infect any host during the asexual stage, including humans and cats, the sexual stage (gametogony) occurs exclusively within felids (Tong et al., 2021). It was initially unknown that sheep and goats could contract toxoplasmosis, a fact noted in the first case reported by Feldman and Miller (1956). Both domestic and wild animals serve as major hosts for the

pathogen (Li et al., 2016). *T. gondii* is considered one of the most significant parasites globally due to its wide range of warm-blooded mammalian hosts, which includes the potential to infect one-third of the world's human population (Mendez & Koshy, 2017). Raw or unpasteurized milk of sheep and goats has been identified as the source of human infection (Garcia et al., 2012), and it is estimated that 33% of individuals worldwide suffer from toxoplasmosis (Ai, 2020).

Polymerase chain reaction (PCR) was used to detect *T. gondii* DNA in milk by targeting its *B1* gene (Tavassoli, 2013). As a sensitive method that amplifies DNA sequences from various clinical samples, including tissues, PCR has been utilized as an alternative to serological testing (Attias et al., 2020). Fluctuations in prevalence may be attributed to the presence of cats on farms and regional climatic differences (Dubey, 2010). The traditional method for diagnosing toxoplasmosis involves detecting particular antibodies against *Toxoplasma*, such as immunoglobulin M (IgM) and immunoglobulin G (IgG) (Stelzer et al., 2019). Although statistically significant, the seroprevalence of infection was higher in adult dairy ewes and sheep from slaughterhouses than in juveniles. This supports previous research indicating that sheep exposure to toxoplasmosis increases with age (Ibrahim et al., 2017; Subedi et al., 2018).

In the Baghdad Governorate, women were more likely than men to contract toxoplasmosis (Alkubaisi & Al-Zubaidy, 2023). Using ELISA and latex agglutination tests, infection rates were 87.32% for females and 71.42% for males, respectively (Al-Ani et al., 2020).

The aim of the study was to detect and confirm optimal diagnosis methods for zoonotic toxoplasmosis in both humans and sheep using different classic and recent techniques in central Iraq, thereby evaluating the epidemiological relationship between sheep and human prevalence of toxoplasmosis.

Materials and Methods

Animals and study areas

The one hundred placentas of sheep from different areas of Diyala (50) and Baghdad (50) were examined, and samples were collected from placental tissues of different ages (ranging from 2 to 6 years) for macroscopic examination and molecular study. Human blood samples were obtained from 100 individuals of different age groups and genders (male and female) from patients arriving at hospitals in Diyala and Baghdad randomly for detection by enzyme-linked immunosorbent assay (ELISA) techniques; the study period was from September 2024 to the end of April 2025.

Sample collection

Placental samples of slaughtered sheep kept in an Icebox from the different areas in Baghdad and Diyala were transmitted to the laboratories of College of Veterinary Medicine, University of Diyala, while the blood serum was kept in an 8 ˚C refrigerator until examination (Asproudis et al., 2013).

Macroscopic examination

Placental samples were examined grossly by the naked eye to detect tissue cysts (Faraj, 2012; Ahmed et al., 2016).

Digestion method

A placental specimen from an infected sheep was digested with pepsin enzyme. The pepsin was formulated by dissolving 1.3 g of pepsin, 2.5 g of NaCl, and 3.5 mL of HCL in 500 mL of sterile distilled water to create the digested medium. Each placenta sample was placed in a flask with 100 mL of digested medium for 12 to 18 hours at room temperature (25 ˚C). Sterile double-layer gauze was used to filter the materials, and they were all centrifuged for 5 minutes at 2800 rpm. Lastly, the sediment was transferred to 1.5 mL Eppendorf tubes and stored at -20 ˚C until molecular analysis. Additionally, the slides made from the sediment drop were stained with Giemsa to identify bradyzoites when viewed at 100x magnification (Hamidinejat et al., 2015).

Staining method

The slides were counterstained in a staining jar filled with a 1:10 dilution of Giemsa stain (10 mL of Giemsa stain (stock) plus 90 mL of buffer solution, pH 7.0) for 20 minutes. The stain was poured off and rinsed multiple times with distilled water. The slides were then allowed to dry on the staining rack and examined at ×100 magnification with oil immersion using a light microscope (Waheeb, 2018).

Preparing human serum samples

After blood collection, the blood was left alone at room temperature for 10 to 20 minutes to clot, and then centrifuged for 20 minutes at 2000 to 3000 rpm to remove the blood clot. If precipitates formed during the suspension, the sample should be centrifuged once more. Ad-

ditionally, the test was conducted in accordance with the manufacturer's guidelines.

Serological examination

The human serum sample was collected from 100 human blood samples (male and female) from Diyala and Baghdad (50\50). The serological examination (ELISA) used the Human Anti-*T. gondii* IgG ELISA Kit from Sunlong Biotech Co., Ltd., China, which is an indirect ELISA for the qualitative detection of IgG class antibodies against *T. gondii* in human plasma and serum samples.

Molecular technique

DNA extraction

Genomic DNA was extracted from the buffy coat of seropositive individuals using a tissue genomic DNA isolation kit (Geneaid, Bioteccorp, Taiwan). PCR amplification targeted a 529 bp fragment using two specific primers targeted a 529 bp fragment of the *REG*s gene in *T. gondii* (Table 1).

Polymerase chain reaction

The Polymerase chain reaction (PCR) reaction was performed in a 25 µL mixture containing 1.25 units of Taq DNA polymerase, 1 µL of extracted DNA, 1.5 mM of $MgCl_2$, 10 pmol of each primer, 0.2 mM of dNTPs, and 1× PCR buffer. The thermal cycling conditions included an initial denaturation at 94 ˚C for 5 minutes, followed by 35 cycles of denaturation at 94 ˚C for 35 seconds, annealing at 56 ˚C for 1 minute, and extension at 72 ˚C for 1 minute, with a final extension step at 72 ˚C for 10 minutes. PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide.

Phylogenetic analysis

The amplified products (amplicons) were sent to Korea for sequencing, and the resulting sequences were analyzed using BLASTn. The sequences were then submitted to the NCBI database. Finally, a phylogenetic tree was constructed using MEGA software.

Statistical analysis:

The significance of the results was determined using the Statistical Analysis System (SAS, 2018). This program was used to detect the different factors that affect the study percentage. The chi-square test was used to compare percentages (0.05 and 0.01).

Results

The total infection rate of *Toxoplasma* infection in sheep was 25% (25\100), depending on traditional and molecular diagnosis. Diyala recorded the highest rate, 30.3% (15\50), while in Baghdad, the rate was 20% (10\50) without significant differences between the provinces.

Among ewes that experienced abortions, the *Toxoplasma* infection rate was highest in the 4-year-old age group, at 43.33% (13\30), while the other age groups (3, 5, and 6) showed percentages less than 16, 25, and 30%, respectively. The lowest rate recorded in the 2-year age group was 0% (0\15), with a highly significant ($P \leq 0.01$) difference among sheep age groups (Table 2).

The serological diagnosis of human toxoplasmosis was done based on the ELISA method, which showed an overall human serum infection rate of 23.91% (22/92). Humans (males and females) in Diyala had a higher infection rate of 30.95% (13/42), whereas the rate in Baghdad was 18% (9/50), with a significant difference ($P \leq 0.01$) between the study areas (Table 3).

The gender-specific results in humans showed a higher infection rate of 40% (20/50) among females, whereas males had the lowest infection rate of 4.76% (2/42). A significant difference ($P \leq 0.01$) was observed between the genders in both Diyala and Baghdad provinces (Table 4).

According to age group, human toxoplasmosis showed the highest infection rate in the 25–35-year-old group (33.33%), followed by the 35–45-year-old group (28%). The lowest infection rate occurred in the 15–25-year-old group (10%). A highly significant difference was observed among the different age groups (Table 5).

Table 1. Specific primers used in PCR amplification to target a 529 bp fragment

Gene		Primer sequence 5' to 3"	Product Size	Annealing	Ref.
TOXO	F	(CAGGGAGGAAGACGAAAGTTG)	529 bp	58	NCBI- Blast
	R	(CAGACACAGTGCATCTGGATT).			

Table 2. Toxoplasmosis in Ewes according to age group

Age (y)	Examined Sheep	Infected Sheep	Percentage (%)
2	15	0	0
3	25	4	16
4	30	13	43.33
5	20	5	25
6	10	3	30
Total	100	25	25
Chi-square: χ^2 (P)	--	--	11.976 ** (0.0001)

**P≤0.01.

Table 3. Total *Toxoplasma* infection rate in humans

Area	Examined Cases	Positive Samples	Percentage (%)
Diyala	42	13	30.95
Baghdad	50	9	18
Total	92	22	23.91
Chi-square: χ^2 (P)	--	--	5.285* (0.0417)

*P≤0.05.

Molecular results of *T. gondii* from sheep

The molecular detection of *T. gondii* isolated from sheep placentas was performed via PCR using specific primers. A partial fragment (529 bp) of the repetitive DNA sequence of *T. gondii* was successfully amplified from the twenty strains (Figure 1). The DNA sequences of these 20 parasite strains were analyzed using the BLASTn tool against the core nucleotide database (core_nt). The BLASTn output revealed that all queried nucleotide sequences were affiliated with *T. gondii* repetitive DNA sequences, with 100% similarity and 100% query coverage. Moreover, the nucleotide sequences of the 20 *T. gondii* strains isolated in this study were deposited in the GenBank database under the following accession numbers: PV766898.1, PV766899.1, PV766900.1, PV766901.1, PV766902.1, PV766903.1, PV766904.1, PV766905.1, PV766906.1, PV766907.1, PV766908.1, PV766909.1, PV766910.1, PV766911.1, PV766912.1, PV766913.1, PV766914.1, PV766915.1, PV766916.1, and PV766917.1.

To determine the evolutionary relationships among the twenty *T. gondii* strains, multiple sequence alignment (MSA) was performed using CLC Sequence Viewer 8.0, comparing these twenty repetitive DNA sequences

Table 4. Infection rate in humans by Gender

Gender	Total Cases	Infected Cases	Percentage (%)
Male	42	2	4.76
Female	50	20	40
Total	92	22	23.91
Chi-square: χ^2 (P)	--	--	14.727** (0.0001)

**P≤0.01.

Table 5. Human *Toxoplasma* infection rates among different age groups

Age Group (Y)	Total Cases	Positive Sample	Percentage (%)
15-25	20	2	10
25-35	30	10	33.33
35-45	25	7	28
45-55	17	3	17.64
Total	92	22	23.91
Chi-square: χ^2 (P)	--	--	8.227** (0.0069)

**P≤0.01.

with other closely related *T. gondii* sequences identified via BLASTn (Figure 2). As shown in Figure 2, there are regions showing 100% conservation among all *T. gondii* repetitive nucleotide sequences subjected to MSA. In stark contrast, other regions showed a partial degree of conservation. For example, though not exclusively, the nucleotide positions 159, 163, 168, 169, and 172-175 demonstrated full conservation (100%) among the aligned sequences. Conversely, the region spanning from 50 to 59 nucleotides showed only partial conservation among all sequences analyzed. Finally, the remaining seventeen strains exhibited close relatedness among the Iraqi isolates.

Discussion

This study was carried out in central Iraq to detect *Toxoplasma* infection as a zoonotic disease in sheep. Using both traditional and molecular diagnostic methods, the total infection rate of toxoplasmosis in sheep was 25% (25/100). Diyala recorded the highest rate at 30.3% (15/50), while the rate in Baghdad was 20% (10/50), with no significant difference observed between the provinces. These results are consistent with previous studies in Iraq and Iran (Al-Ethawi et al., 2013; Bahadori, 2025), which reported infection rates of 23.9% and 23%, respectively. Discrepancies between these findings and higher prevalence rates reported elsewhere—such as 51.7% in Sulaimania or the 33% prevalence in naturally aborted ewes out of 92 examined in Duhok Province—may be attributed to various factors. In Diyala, ovine toxoplasmosis infection may be influenced by diversified factors, such as the host-parasite relationship, the pathogenicity of *T. gondii* strains, the immune status of the infected ewes, management practices, geographical location, the duration of exposure to parasitic infection, and the biological behavior of *Toxoplasma*. Additionally, the breeding system is considered a significant factor affecting the epidemiology of toxoplasmosis (AbouZeid et al., 2010).

The infection of *Toxoplasma* in ewes, whether suffering from abortion or not, showed the highest rate at 4 years of age, 43.33 (13/30), while the percentages in the other age groups (3, 5, and 6 years) were less than 16, 25, and 30%, respectively, among sheep age groups. These

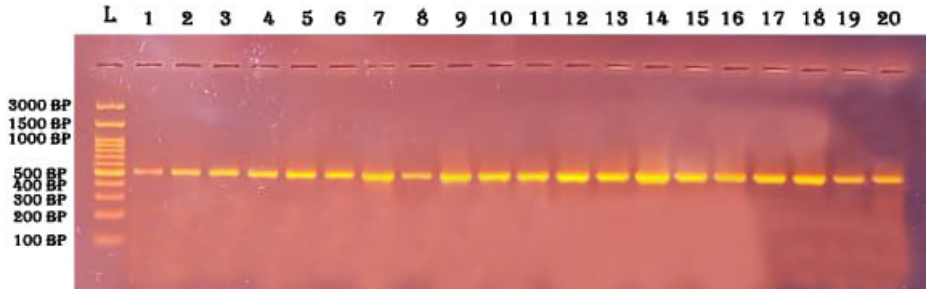

Figure 1. Agarose gel electrophoresis (1%) showing the PCR product amplified from twenty *T. gondii* strains isolated from humans and sheep in this study

Note: A partial fragment of 529 bp was amplified using a *T. gondii*-specific primer set.

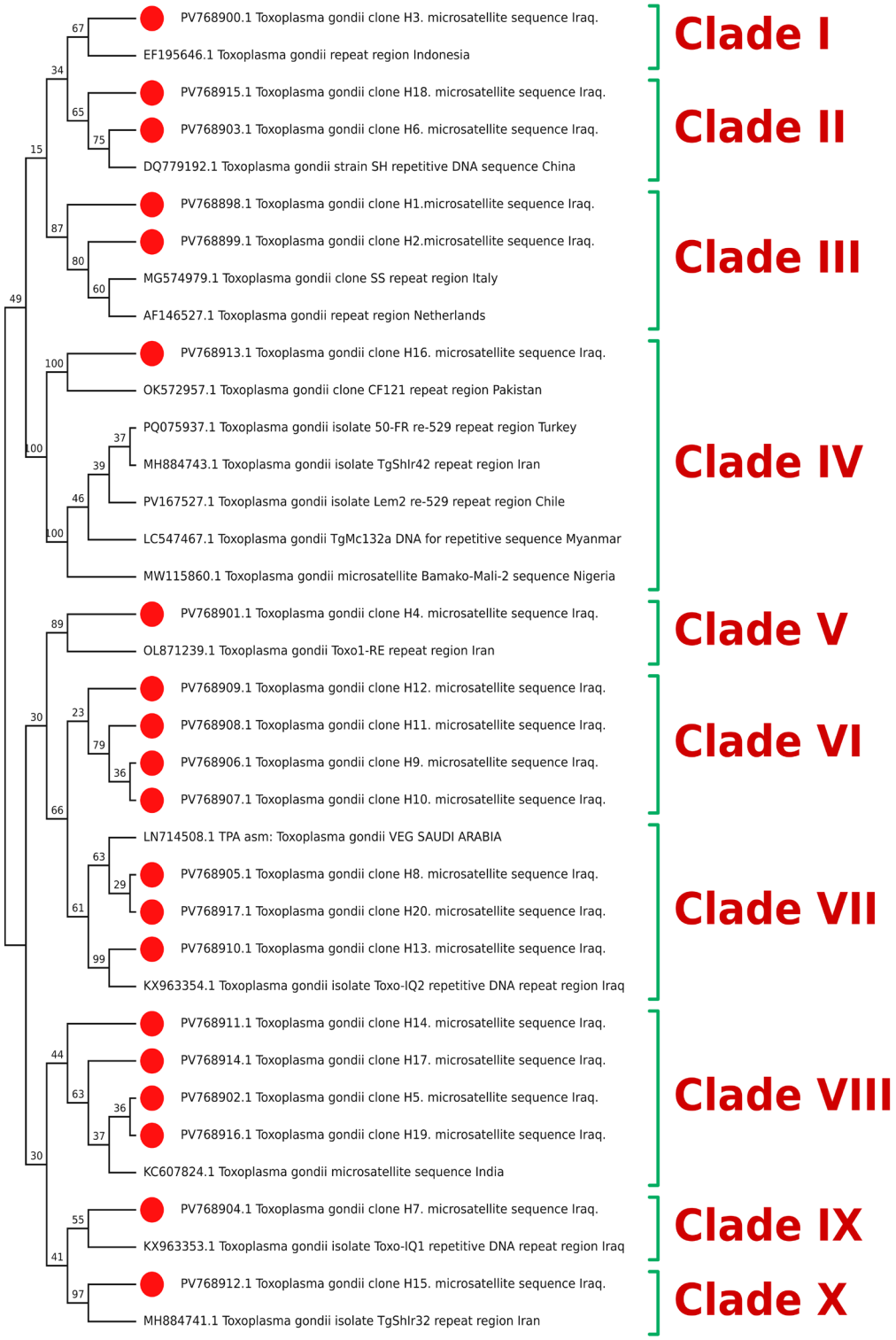

Figure 2. Phylogenetic tree based on partial nucleotide sequences (530 bp) of the ITS1 rDNA gene for *T. gondii* detected in Diyala and Baghdad (middle of Iraq), along with other isolates deposited in GenBank

Note: A phylogenetic tree was constructed using MEGA software. The host (sheep), country of origin (Iraq), and GenBank accession numbers are provided in parentheses. Sequences analyzed using BLASTn obtained in the present study are indicated with a red square.

findings are in agreement with other studies (Al-Ethawi et al., 2013; Ibrahim et al., 2017; Subedi et al., 2018), which showed an increased infection rate (30%) in the (3–6 years old) age group, compared with the second group (2 years age group), which recorded the lowest rate, 0% (0/15). Age is an important factor; *Toxoplasma* infection is more common in older sheep than in younger ones (Hassanain et al., 2011). The findings of the current study are supported by Lashari and Tasawar (2010) in Pakistan, who discovered that, in 518 sheep assessed for the relation between age of sheep and their infection with toxoplasmosis, the highest rate was recorded in the age group of 1–2 years (38.88%), and the lowest in the age group of 5–6 years (8.51%).

Serological diagnosis of human toxoplasmosis was done via ELISA, which revealed a total human serum infection rate of 23.91% (22\92). In Diyala, humans (both male and female) recorded a higher infection rate of 30.95% (13/42), while the rate in Baghdad was 18% (9/50), representing a significant difference (P≤0.01) among the study areas. This was in agreement with the majority of research in other nations (Bahadori & Eslami, 2021; Moghaddam et al., 2022), reporting that the frequency of infection varies greatly across the globe and is more prevalent in warm climates and mountainous regions. Garcia et al. (2006) and AitHamou et al. (2021) proposed that *Toxoplasma* infection varies from place to place and year to year based on factors, like feeding, movement, and grazing type, and Zhu et al. (2022) demonstrated notable variations in seroprevalence between locations. Domestic cats and other members of the Felidae family may eventually pollute the surrounding environment with sporulated *T. gondii* oocysts (Montazeri et al., 2020), factors which contribute to the high prevalence and long-term resistance of these oocysts.

Regarding gender, the human results showed a significantly higher infection rate in females at 40% (20/50) compared to males, who recorded the lowest rate of 4.76% (2/42), with a significant difference (P≤0.01) across both Diyala and Baghdad provinces. This coincides with the findings of Al-Ani et al. (2020), who recorded high rates via ELISA in females (87.32%) and males (71.42%), although in Baghdad, the prevalence of toxoplasmosis infection was higher in females than in males. In a study (Al-Ethawi et al., 2013), the ELISA infection rate was 45.45% in males and 64.1% in females. The differences in positive seroprevalence results between the present study and previous studies may be due to weather and climatic conditions. Furthermore, the results suggest that these females were exposed to infection at some point in their lives and have now acquired strong immunity to the latent infection, a finding confirmed by Wang et al. (2017).

Regarding age groups, human toxoplasmosis showed the highest infection rate in the 25–35-year-old group at 33.33% (10/30), followed by the 35–45-year-old group at 28%. The lowest rate was recorded in the 15–25-year-old group at 10% of the total infection rate across different age groups, with a highly significant difference between the groups. The results regarding human age groups provide a noticeable identification. Studies carried out in a number of countries, such as Gebremedhin et al. (2014), have demonstrated that seroprevalence rises with age due to prolonged exposure to the infection. This exposure leads to the stimulation of specialized immune cells against *T. gondii*, resulting in enhanced antibody titers in the bloodstream, which promotes the immune response and contributes to the elimination of the *T. gondii* infection (Jones et al., 2001; Xiao et al., 2010; Daryani et al., 2014; Bahadori et al., 2025; Friesema et al., 2025).

The antibody detection results by patient age were displayed, stating that the most common age group was those aged 25 to 35, which accounted for 62.5%. In Iraq and the majority of the Arab world, women are most likely to marry and reach parity between the ages of 25 and 35. This particular outcome was consistent with other studies (Ghoneim et al., 2010; Arefkhah et al., 2019; McCall et al., 2022).

A 594 bp sequence alignment from NCBI was utilized to align the *T. gondii* REGs domain. The most similar isolates were identified from Iraq, Indonesia, China, Italy, the Netherlands, Pakistan, Turkey, Iran, Chile, Myanmar, Nigeria, Saudi Arabia, and India. It seems that these regions are associated with other conserved sequences of the *T. gondii REG*s gene, which supports the importance of these motifs (Arefkhah et al., 2019). A phylogenetic tree was also generated from the alignment, illustrating the evolutionary distribution of the Reg across 36 *T. gondii* isolates. The tree reveals approximately 5–8 main clades and 20 nodes within the phylogeny. Also, the phylogenetic tree is sub subdivided into 34 terminal branches that represent common ancestors that explain the evolutionary lineage of the Reg in *T. gondii*. Each branch represents a group of *T. gondii* sequences that are more closely related to each other than to sequences in other branches.

These results are specific and unique to *T. gondii* in the central regions of Iraq when compared with similar studies in other Iraqi provinces (Ibrahim et al., 2017; Subedi

et al., 2018). These findings confirm the presence of a regional circulation pattern and suggest possible shared sources of infection with neighboring countries. One limitation of this study is its focus on the intermediate host of *T. gondii* without including the definitive host (cats). Expanding research to include cats would provide a more comprehensive epidemiological understanding of the disease and its distribution in central Iraq. Therefore, we recommend conducting broader investigations on both domestic and stray cats to evaluate the prevalence of *Toxoplasma* and clarify the role of the definitive host in the spread of the disease. In addition, assessing drug efficacy in Iraq is an essential step toward effective disease control.

Conclusion

T. gondii remains an endemic zoonotic disease in central Iraq. In Diyala and Baghdad, domestic sheep are frequently affected by toxoplasmosis, which shows considerable prevalence among both sheep and humans. The findings of this study confirm the presence of a regional circulation pattern and indicate potential shared sources of infection with neighboring countries. The marked genetic diversity of *T. gondii* strains, along with risk factors, such as location, gender, and age, underscores the importance of implementing targeted public health and veterinary control strategies.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Diyala, Iraq (Approval No.: Vet Medicine (210).

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Authors' contributions

Study design and writing: Haleem Hamza Hussain Al- Zubaidei; Samples collection, data analysis, experiments, and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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