Seroprevalence of acute and chronic *Toxoplasma gondii* infections in sheep

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ARTICLE INFO	ABSTRACT
	Toxoplasma gondii, causing toxoplasmosis, is a ubiquitous intracellular parasite of worldwide importance which infects a wide-range of wild and domestic animals as well humans resulting in severe economic losses and public health concerns. This study was conducted to investigate the prevalence rate and risk of acute and chronic *T. gondii* infections in sheep, and identifying the association of positivity to animal risk factors. An overall 368 sheep were selected randomly from a number of rural areas located in Al-Qadisiyah province (Iraq) during April to June (2024). After sampling of venous blood, the serum samples were obtained and tested by specific *T. gondii* IgM and IgG kits to detection the acute and chronic infections respectively. Age and sex of study animals were considered as potential risk factors to seropositivity. Totally, 10.05% and 41.85% of study sheep were shown acute and chronic *T. gondii* infections, respectively; with a level of ODs was 0.36 ± 0.0093 and 0.375 ± 0.0079, respectively. According to levels of positivity, the mild, moderate and severe infections were identified respectively in 45.95%, 48.65%, and 5.41% of acute infection; and in 46.75%, 31.82%, and 21.43% of chronic infection. In association to risk factors, the findings of acute and chronic infections were increased apparently in sheep aged 1-<3 years (16.959% and 57.31%, respectively) when compared to other age groups; < 1 (5.932% and 33.05%, respectively), 3- <5 (1.923% and 26.92%, respectively), and ≥ 5 (0% and 11.11%, respectively) years. In addition, the risk of both infections was elevated in sheep aged 1-<3 in comparison to other age groups. Relation to sex, females were showed higher rates of positivity and risk to acute and chronic *T. gondii* infection than males. In conclusion, this represents the first study in Al-Qadisiyah province (Iraq) which detect serologically both the acute and chronic *T. gondii* infections in sheep by ELISA. However, the obtained data demonstrates that toxoplasmosis continue to negatively impact sheep and compromising food security in Iraq suggesting the importance of extensive prevention and controlling strategies. Also, application of serology and molecular diagnostic methods could provide more accurate data about the rate of prevalence and the risk of toxoplasmosis on domestic animals and humans.

1. Introduction

Toxoplasma gondii is a single-celled protozoan, which first discovered in 1908 by Nicolle and Manceaux in a hamster-like rodent, the gundi, and believed to be a piroplasm, then *Leishmania*, but soon released as new organism (Ander, 2019). The parasite is a member of the Apicomplexa phylum, a group of intracellular parasites characterized by their unique apical complex organelles that play a crucial role in host cell invasion (Cova et al., 2022; Razooqi et al., 2022). Traditionally, *T. gondii* was classified into three major genotypes, known as type I, type II and type III, based on differences on their genetic markers and phenotypic characteristics (Fernández-Escobar et al., 2022). Beyond the traditional genotypes, researchers have identified a large number of atypical strains that exhibit a wide range of genetic diversity, virulence and host preference, which all underscoring the complexity the complexity structure of *T. gondii* (Dardé et al., 2020; Ghenciu et al., 2024). Like many other Apicomplexa parasites, *T. gondii* has a complex lifecycle that includes both asexually reproducing forms and sexual stages. Because these stages are timely formed through unique differentiation processes for tachyzoite and bradyzoite during bi-directional conversion in different hosts, the lifecycle of *T. gondii* is described as heteroxenous (Attias et al., 2020; Pavlou,

2020; Delgado et al., 2022; Majidiani et al., 2024). However, the hosts in which the parasite can sexually reproduce is known as definitive including only the felids, while those supporting only asexual reproduction is named as intermediates including all the other warm-blooded animals (Tomasina and Francia, 2020; Nayeri et al., 2021). Therefore, the epidemiology of toxoplasmosis is complicated with various risk factors and transmission routes contributing to its global distribution (Pleyer et al., 2019). One of the primary risk factors is exposure to domestic infected cats that can shed millions of infectious oocysts in their feces, and contamination of the environment resulting epidemic or endemic occurrence of toxoplasmosis after their ingestion by the intermediate hosts (Shapiro et al., 2019; Zhu et al., 2022; Dixit et al., 2024).

The economic and public health importance of toxoplasmosis in livestock particularly in sheep has been well-documented (Stelzer et al., 2019; Al-Hamada, 2021). It was estimated that the annual cost of *T. gondii* infections in livestock could reach 650-3,100 million USD in 2010 due to losses in sheep production due to abortions, stillbirths and neonatal mortality (Ben-Harari and Connolly, 2019). Because of clinical signs of almost *T. gondii* infections are nonspecific and are not sufficiently characteristics, diagnosis is generally done either indirectly by serologic and molecular testing or by postmortem diagnosis via histologic and bioassay methods (Liu et al., 2015; de Haan et al., 2021; Uddin et al., 2021). In last decades, several serological tools have been developed and modified for detection of *Toxoplasma*-specific IgG or IgM antibodies such as Sabin-Feldman dye test, indirect fluorescent antibody test (IFAT), ELISA, agglutination test and avidity test (Rostami et al., 2018; Chorawala et al., 2024). The sensitivity and specificity has been validated by comparing serologic data from naturally and experimentally infected animals (Huertas-López et al., 2024). In Iraq, several serological studies have been performed to identify chronic *T. gondii* infection in sheep or to compare serology with clinical or molecular data (Mikaeel and Al-Saeed, 2020; Aghwan et al., 2021; Al-abodi, 2021; Kareem, 2023; Shatti, 2024). Hence, this study was conducted to investigate the prevalence rate and risk of acute and chronic *T. gondii* infections in sheep, and identifying the association of positivity to animal risk factors.

2. Methodology

Ethical Approval
This study was licensed by the Scientific Committee of the Animal Production Department in the College of Agriculture (University of Al-Qadisiyah).

Samples
A total of 368 sheep of various ages and both sexes were selected randomly from a number of rural areas located in Al-Qadisiyah province (Iraq) during April to June (2024). Under aseptic conditions, each study animal was subjected to sampling 5ml of venous blood into free-anticoagulant glass-gel tube that transported in vertical position in cooled plastic ice-bag. At laboratory, all blood samples were centrifuged at 5000rpm for 5min, and the obtained sera were transferred into labeled 1.5ml Eppendorf tubes that kept frozen at -4°C until be tested by ELISA. Data concerning the risk factors (age and sex) were documented to evaluate their associations to acute and chronic infections (Razooqi et al., 2022).

Serology
Sheep Anti-*Toxoplasma* IgG Antibody (Catalogue No: SL00141Sp) and Sheep Anti-Toxoplasma IgM Antibody (Catalogue No: SL00140Sp) ELISA Kits were used in current study. Following the manufacturer instructions (SunLong Biotech, China), solutions of each kit in addition to the serum samples of study animals were prepared at room temperature, processed step-by-step; and finally, the optical density (OD) was read at an absorbance of 450nm using the Automated Microplate Reader (Agilent Technologies, USA). The test effectiveness and critical value (Cutoff) of each kit was determined, and the samples were considered positive if their ODs ≥ Cutoff. Also, the positive ODs were divided into three levels according to their severity (Gharban et al., 2022; Mohammad et al., 2022). The Cutoff values of IgG and IgM antibodies were identified at 0.210 and 0.272, respectively.

Statistical analysis
The *t*-test, Two-Way ANOVA, Confidence Interval 95% (CI), Odds Ratio (OR) and Relative Risk (RR) in the GraphPad Prism Software (*version 8.0.1*) were served to evaluate the link between the acute and chronic *T. gondii* infections to animals' risk factors (age and sex). Values were reported as either percentage (%) or Mean ± Standard Error (M±SE), and difference between values is considered significant at $p < 0.05$ (Gharban and Yousif, 2021; Al-Taee et al., 2023).

3. Results
The findings revealed that 10.05% (37/368) of study sheep are infected with acute *T. gondii* infection; while, 89.95% (331/368) were negatives (Figure 1). Subsequently, statistical analysis of ODs of acute infection showed that the value (M±SE) was 0.36 ± 0.0093 with a range 0.272-0.509 and CI at 0.01891.

According to level of positive infection, the mild, moderate and severe infections were identified in 45.95% (17/37), 48.65% (18/37), and 5.41% (2/37), respectively at a significance of p≤0.014 and CI of 26.84 to 93.51 (Figure 2); whereas, the values

(M±SE) of positive ODs in these groups were 0.309 ± 0.0058, 0.395 ± 0.0056, and 0.482 ± 0.0275, respectively at a significance of p≤0.0156 and CI of 0.1805 to 0.610 (Figure 3).
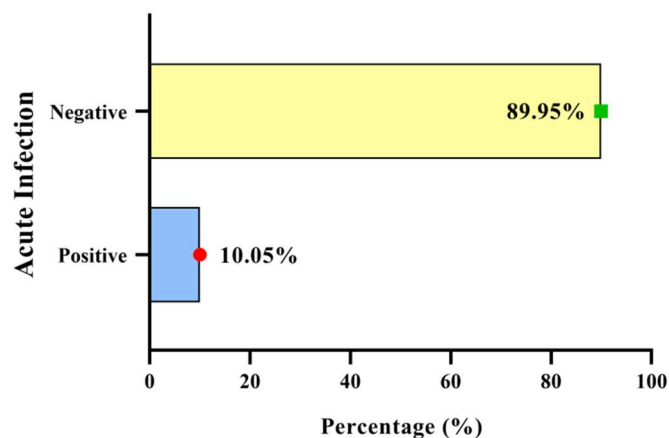

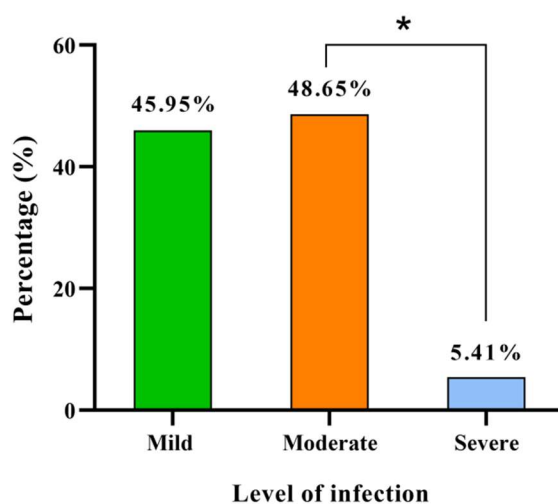*Figure 1. Prevalence of acute T. gondii infection identified by ELISA among totally 368 study sheep.*

Figure 2. Prevalence rate of positivity among the mild, moderate and severe acute T. gondii infections (Total No=37) detected by ELISA.
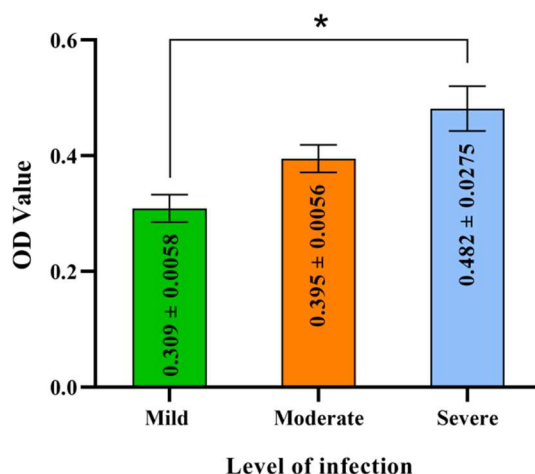
89

Figure 3. Levels of 37 positive ODs comprises the mild, moderate and severe acute T. gondii infections detected by ELISA.

Concerning chronic *T. gondii* infection, 41.85% (154/368) of study sheep were detected to be positives while 58.15% (214/368) were negatives (Figure 4). Relation to ODs of positive samples, the value (M±SE), range and CI were 0.375 ± 0.0079, 0.210-0.690, and 0.01558, respectively.
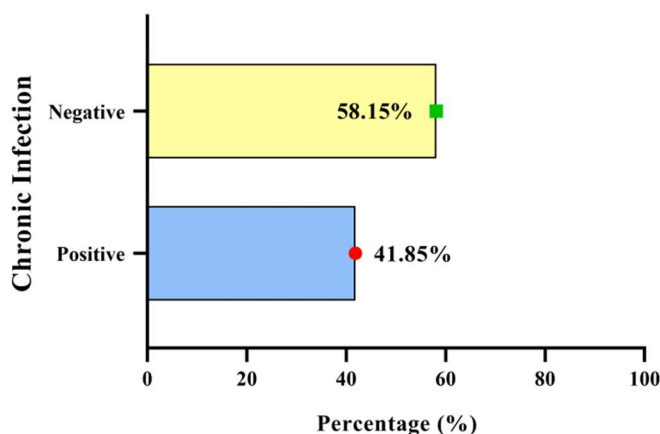

Figure 4. Prevalence of chronic T. gondii infection identified by ELISA among totally 368 study sheep..

According to levels of infection, the findings of chronic *T. gondii* infection revealed that the prevalence rate of mild infection [46.75% (72/154)] was higher than moderate [31.82% (49/154)] and severe [21.43% (33/154)] infections at a significance of p≤0.0453 and CI of 1.716 to 64.95 (Figure 5). Also, the levels of positive ODs for the mild, moderate and severe chronic infections were 0.293 ± 0.0037, 0.394 ± 0.0034, and 0.525 ± 0.0107, respectively at a significance of p≤0.0265 and CI of 0.1150 to 0.6930 (Figure 6).
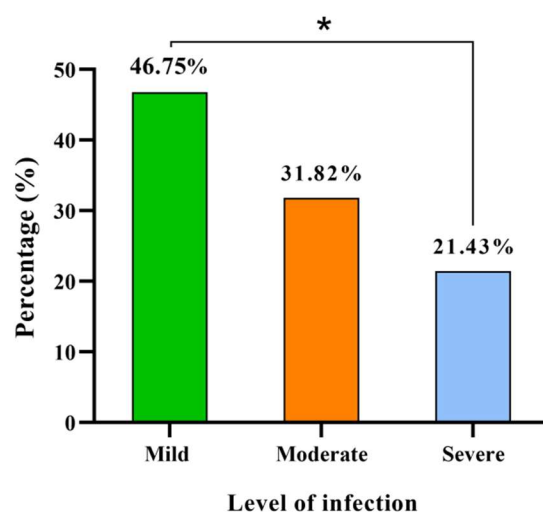
Figure 5: Prevalence rate of positivity among the mild, moderate and severe chronic T. gondii infections (Total No=154) detected by ELISA.
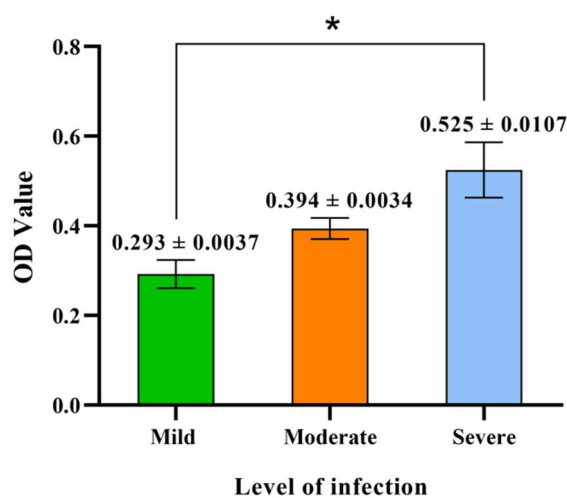
Figure 6: Levels of 154 positive ODs comprises the mild, moderate and severe chronic T. gondii infections detected by ELISA.

Significantly, the prevalence rate of chronic infection (41.85%) was higher than the positive result of acute infection (10.05%) at p≤0.035 and CI of 8.529 (Figure 7). However, insignificant differences were detected in values of positive ODs of acute (0.36 ± 0.0093) and chronic (0.375 ± 0.0079) infections (Figure 8).
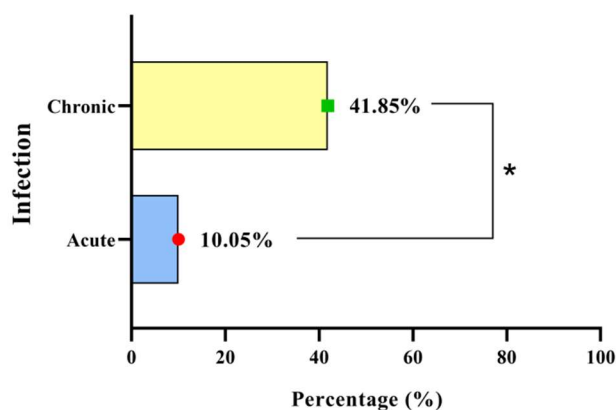
Figure 7: Prevalence rate of acute and chronic T. gondii infections detected by ELISAs among 368 study sheep.
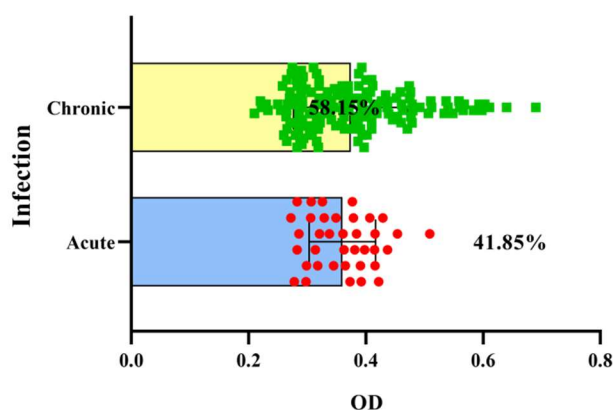

Figure 8. Levels of positive ODs for acute and chronic T. gondii infections detected by ELISAs among 368 study sheep.

The positive results of acute *T. gondii* infection were showed a significant variation (p<0.05) in their distributions (Table 1). For age, the prevalence rate was increased significantly (p≤0.0399) in sheep aged 1-<3 (16.959%) years when compared to other age groups; < 1 (5.932%), 3-<5 (1.923%), and ≥ 5 (0%) years. In addition, values of OR and RR were increased significantly (p≤0.0001) in sheep aged 1-<3 (4.823 and 4.146, respectively) years when compared to other age groups; < 1 (0.463 and 0.494, respectively), 3-<5 (0.152 and 0.167, respectively), and ≥ 5 (0 and o, respectively) years.

Relation to sex, values of positivity, OR and RR were elevated significantly (p≤0.0437, p≤0.0001, and p≤0.0001, respectively) in females (11.314%, 1.882, and 1.771, respectively) when compared to males (6.383%, 0.531, and 0.565, respectively).

Table 1. Association of acute T. gondii infection to animal risk factors (age and sex).

Factor	Group	Total No.	Positive No.	Positive %	OR	RR
Age (Year)	< 1	118	7	5.932	0.463	0.494
	1-<3	171	29	16.959 *	4.823 ****	4.146 ****
	3- <5	52	1	1.923	0.152	0.167
	≥ 5	27	0	0	0	0
p-value				0.0399	0.0001	0.0001
CI				5.865 to 18.27	2.327 to 5.046	1.939 to 4.342
Sex	Female	274	31	11.314 *	1.882 ****	1.771 ****
	Male	94	6	6.383	0.531	0.565
p-value				0.0437	0.0001	0.0001
CI				22.48 to 40.18	7.377 to 9.790	6.494 to 8.830

Relationship between the seropositive chronic *T. gondii* infection and risk factors demonstrated the presence differences (p<0.05) among the groups of each age and sex factors (Table 2). Regarding age, elevation in values of seropositivity, OR and RR were reported in sheep of 1-<3 (57.31%, 3.38, and 2.018, respectively) when compared to < 1 (33.05%, 0.58, and 0.72, respectively), 3- <5 (26.92%, 0.463, and 0.607, respectively) and ≥ 5 (11.11%, 0.157, and 0.251, respectively) years.

Table 2. Association of chronic T. gondii infection to animal risk factors (age and sex).

Factor	Group	Total No.	Positive		OR	RR
			No.	%		
Age (Year)	< 1	118	39	33.05	0.58	0.72
	1-<3	171	98	57.31 *	3.38 ****	2.018 ****
	3- <5	52	14	26.92	0.463	0.607
	≥ 5	27	3	11.11	0.157	0.251
p-value				0.0314	0.0001	0.0001
CI				8.287 to 90.02	1243 to 3.533	0.3299 to 2.128
Sex	Female	274	126	45.985 *	4.16 ****	2.597 ****
	Male	94	28	29.787	0.24	0.385
p-value				0.0134	0.0001	0.0001
CI				65.02 to 140.8	22.70 to 27.10	12.56 to 15.54

3. Discussion

This study found that the acute and chronic *T. gondii* infection in sheep of Al-Qadisiyah province is found in 10.05% and 41.85%, respectively. Subsequently, statistical analysis of our data revealed that mild infection was more prevalent than the moderate and strong infections. Worldwide, the epidemiology of *T. gondii* in sheep has been the subject of numerous studies which detected that the prevalence rate of toxoplasmosis nationally was 17.35% in four southern provinces (Al-Muthanna, Basra, Missan and Thi-Qar), (Khadi et al., 2009), 51.11-60.84% in Basra (Al-Saad and Al-Husseiny, 2010), 32.8% in Nineveh (Al-Dabagh et al., 2014), 28.9% in Duhok (Mikaeel and Al-Saeed, 2020), 45-51.5% in Kirkuk (Ahmad et al., 2022), 33.33% in ewes of Al-Diwaniyah city (Kareem, 2023), and 14-18% in Diyala (Bawi and Al-Zubaidi, 2024); while internationally, it was 8.3-35.3% in Algeria (Dechicha et al., 2015), 11.9% in Bangladesh (Sah et al., 2018), 42.5% in Brazil (Sakata et al., 2012), 15.4% in China (Yan et al., 2020), 62.6% in Egypt (Elaadli et al., 2023), 50% in India (Singh et al., 2015), 35.9% in Iran (Armand et al., 2016), and 17.8% in Mexico (Cruz-Vázquez et al., 2020). The widely variations in prevalence rate of *T. gondii* in sheep might be attributed to differences in test methods, stage of infection, criteria of sampling, geographic location, climate, as well as farm management practices and grazing area (Dubey et al., 2020; Ahaduzzaman and Hasan, 2022).

The findings of age and sex detected that sheep of 1-<3 years old are more susceptible to be infected with acute and chronic toxoplasmosis that other age groups. Our findings were in agreement with that detected by a number of researchers in Iraq (Ahmad et al., 2022) as well as in other countries such as USA (Hutchinson et al., 2011), Mexico (Alvarado-Esquivel et al., 2013), and Brazil (Cosendey-KezenLeite et al., 2014). A long-term previous study in a Swedish sheep flock found that the infection rate ranged from 20-35% in lambs under one year old, with most ewes being slaughtered at the age of five (Lundén et al., 1994). Other studies found that toxoplasmosis was not differed between age groups as detected in Turkey (Çiçek et al., 2011; Çakmak and Karatepe, 2017), China (Wu et al., 2011; Yin et al., 2015), Algeria (Dahmani et al., 2018), Iran (Gharekhani et al., 2018), and Italy (Gazzonis et al., 2020); while in other studies, the obtained findings have detected that lambs are more susceptible to infection than adult as recorded in Mongolia (Pagmadulam et al., 2020).

In the current study, females were showed a significant higher rate of infection and risk than males, which similar with that reported nationally by Al Hamada et al. (2018) as well as internationally in Ethiopia (Tegegne et al., 2016), Pakistan (Ahmed et al., 2016; Ullah et al., 2018), and Lebanon (Khalife et al., 2022). In contrast, other studies reported the absence of significant differences between females and males (Williams et al., 2005) or males having a more rate of susceptibility than females (Chegini et al., 2001). However, the association of *T. gondii* infection to females might be related to pregnancy-associated immune-suppressions, and hormonal fluctuations.

4. Conclusion

This represents the first study in Al-Qadisiyah province (Iraq) which detect serologically both the acute and chronic *T. gondii* infections in sheep by ELISA. However, the obtained data demonstrates that toxoplasmosis continue to negatively impact sheep and compromising food security in Iraq suggesting the importance of extensive prevention and controlling strategies. Also, application of serology and molecular diagnostic methods could provide more accurate data about the rate of prevalence and the risk of toxoplasmosis on domestic animals and humans.

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