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Original article

Seroepidemiological Survey of *Toxoplasma gondii* in Livestock Food Sources: Implications for Zoonotic Transmission in Al-Marj, Libya

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Abstract
Humans and animals mainly become infected by ingesting raw or meat containing cysts with bradyzoites, as well as through the ingestion of food or water contaminated with sporulated oocysts. This study aimed to determine the prevalence of zoonotic infection of *Toxoplasma gondii* in sheep and goats in the Al-Marj area. A total of 457 samples were collected from four livestock areas surrounding the city (Farzogha 96, Al-awalia 92, Taknes 100, and Botraba 90) during the period from March 1, 2024, to November 30, 2024. The samples were examined using the Enzyme-Linked Immunosorbent Assay (ELISA) to detect anti-*Toxoplasma gondii* IgG and IgM antibodies. The results revealed that 249 out of 457 examined animal samples were positive for anti-*Toxoplasma gondii* IgG and/or IgM antibodies, with an overall seroprevalence of 54.49%. A statistically significant difference in seroprevalence was observed between the two animal species, with a higher prevalence recorded in sheep (P = 0.038. Regarding age, the highest seroprevalence of *T. gondii* was observed in animals aged 1–3 years (51.40%). Furthermore, female animals show higher infection rates for both IgG and IgM antibodies. Additionally, the findings provide baseline epidemiological data that can support future research and guide policymakers in implementing effective measures to reduce the transmission of *T. gondii* in both animals and humans in the region.
Keywords: Livestock, *Toxoplasma gondii*, Enzyme-Linked Immunosorbent Assay, Seroprevalence.

Introduction

Livestock contributes to the economy of different countries; it is of particular importance when it comes to providing animal products as a basic source of human food. Therefore, the pathogens that affect this wealth are of great importance from the point of view of the study because they harm human food sources, in addition to being a secondary carrier of it. *Toxoplasma gondii* is a widespread apicomplexan parasite that causes toxoplasmosis in warm-blooded animals and humans. It is estimated that 25-30 % of the world population is seropositive for *T. gondii* [1]. Humans mainly become infected by ingesting raw or undercooked mammalian or poultry meat containing cysts with bradyzoites, as well as through ingestion of food or water contaminated with sporulated oocysts [2].

Toxoplasma gondii is a protozoan parasite that affects warm-blooded animals, including humans. The infection is usually acquired through the ingestion of oocysts, which are shed in the feces of infected cats. Oocysts can contaminate soil, water, and food, leading to transmission to other animals, including humans [3]. Many international studies, as well as studies in many neighboring countries, indicate different rates of infection among livestock species; for example, a study was conducted in 2023 in northern China. In this study, a total of 1962 blood samples from cattle (978) and sheep (984), collected from 11 administrative cities in Shanxi Province, were examined. by using the ELISA test. This study aims to survey *Toxoplasma gondii* in Livestock Food Sources and raise awareness of the dangers of spreading zoonotic disease among animals and the potential harm to humans.

Methods

This study was conducted in the city of Al-Marj, which is a city in northeastern Libya, located about 94 km east of the city of Benghazi, which is part of the Green Mountain. The total population of its district, according to the 2006 general population census, was 184,531 people.

A Total of 378 (206 sheep and 172 goats of different ages and sex, 59 (31 sheep and goats) were showing symptoms, and samples were collected from them in the veterinary pharmacy. The rest were randomly selected from among 4 Various locations in the city of Marj and its surrounding areas.

Samples were taken from sheep and goats after obtaining approval from the owners, as only veterinarians were allowed to enter the pasture and collect samples in a normal way from the animals' veins, and 3-5 ml of blood was collected in collection tubes. Samples are kept at room temperature to facilitate separation and obtaining serum. Serums are obtained using a centrifuge with a rotation capacity of 4000 revolutions per second for 4 minutes. Goats whose flocks and herds participated in the study were verbally consented to; they were interviewed using a questionnaire. The questionnaire includes the following Information: collection location, date, sex, age, type of animal, and health problems.

All collected animal serum samples were tested for IgG and IgM antibodies against *Toxoplasma gondii*, using the ELISA test for toxoplasmosis. (Perkin Elmer Toxoplasma IgG & IgM kits).

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Results

A total of four hundred and fifty-seven (257 sheep and 200 goats) samples tested by ELISA test (IgG and IgM) to determine the *T. gondii* infection, seventy-nine individuals out of the total exhibited symptoms and the remaining three hundred and seventy-eight was randomly collected from four regions surrounding Al-Marj city of Libya, among the tested samples, 202 from the randomly collected group and 47 from the clinically symptomatic group tested positive.

Prevalence and Animal species

The results of the table show that there are statistically significant differences between sheep and goats in terms of infection rate, with a P-value < 0.05.

Table 1. The overall prevalence of T. gondii antibodies among sheep and goats.

Examined special	No. Examined	Infected	% Prevalence the total number of infected
Sheep	257	150	60.24%
Goat	200	99	39.76%
Total	457	249	54.49%
Chi square = 4.316, d.f= 1, P-value= 0.038			

The overall Seroprevalence of T. gondii IgM antibody among sheep and goats.

In Table 2, we note that there are no statistically significant differences between sheep and goats in terms of infection rate (positive IgM). with P-value > 0.05.

Table 2. The overall Seroprevalence of T. gondii IgM antibody among sheep and goats.

Examined special	No. Examined	Positive IgM	% Prevalence the total number of infected
Sheep	257	58	58.58%
Goat	200	41	41.41%
Total	457	99	21.66%
Chi square =0.038, d.f=1		*P-value*=0.0846	

The overall Seroprevalence of T. gondii IgG antibody among sheep and goats.

From the results of Table 3 above, we note that there are no statistically significant differences between sheep and goats in terms of infection rate (positive IgG). With P-value > 0.05

Table 3. The overall Seroprevalence of T. gondii IgG antibody among sheep and goats.

Examined special	No. Examined	Positive IgG	% Prevalence the total number of infected
Sheep	257	93	62.00%
Goat	200	57	38.00%
Total	457	150	32.82%
Chi square =2.475, d.f=1, P-value=0.116			

The overall seroprevalence of T. gondii antibodies (IgG and IgM) in tested animals according to age groups

According to age groups the result revealed (Table 4) that the overall seroprevalence of *T. gondii* was higher in tested animals of 1-3 years of age 128 (51.40%) and lower in age of >3 years or above 48 (19.27%), While the ages <1 years were 73 (29.32%), we note that there are statistically significant differences between age groups in terms of infection rate (P-value = 0.048).

Table 4. The overall seroprevalence of T. gondii antibodies (IgG and IgM) in tested animals based on age group

Examined special	No. Examined	Infected	Positive IgG	Positive IgM	% Prevalence the total number of infected
<1	152	73	44 (29.3%)	29 (29.2%)	29.32%
1 - 3	215	128	80 (53.3%)	48 (48.5%)	51.40%
>3	90	48	26 (17.4%)	22 (22.3%)	19.27%
Total	457	249	150 (60.2%)	99 (39.8%)	54.49%
Chi square =6.070, d.f=2, p-value=0.048					

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The seroprevalence of *T. gondii* antibodies (IgG and IgM) among sheep and goats according to gender.
Table 5 presents the seroprevalence of *T. gondii* antibodies in male (10.44%) and female (89.56%) sheep and goats. Although females showed a higher prevalence than males, there are statistically significant differences between males and females in terms of infection rate (P-value = 0.000).

Table 5. The overall seroprevalence of T. gondii in tested animals based on sex

Examined special	No. Examined	Infected	Positive IgG	Positive IgM	% Prevalence the total number of infected
Male	53	26	20 (13.3%)	6(6.0%)	10.44%
Female	404	223	130 (86.7%)	93 (94.0%)	89.56%
Total	457	249	150 (60.2%)	99 (39.8%)	64.21%
Chi square =17.299, d.f=1, P-value=0.000					

Discussion

It is essential to know that this study represents the first academic investigation into the prevalence of *Toxoplasma gondii* infection in sheep and goats in the city of Al Marj and its surrounding villages. The study focused on the infection rate and its association with species, gender, and age.
The findings, based on the examination of 457 serum samples from sheep and goats using the enzyme-linked immunosorbent assay (ELISA) to detect IgG and IgM antibodies, revealed that 249 samples tested positive for *T. gondii*. This corresponds to an overall seroprevalence rate of 54.49%. From the results, we note that there are statistically significant differences between sheep and goats in terms of infection rate, with a P-value < 0.05.
The high overall seroprevalence (54.49%) recorded in this study is consistent with results reported by many authors from different parts of the world [4-6]. These authors recorded an overall seroprevalence of 67%, 52%, and 83.33% in small ruminants from Zimbabwe, Sudan, and South Africa, respectively. In the meantime, lower prevalence rates than the result of this study were recorded from many countries, including 17.68% from Ethiopia [7], 26.7% from Egypt [8], 35.1% from Jordan, 31% from Turkey [9], 37% from Tunisia [10], 28.3% from Gabon [11], and 35% from Nigeria [12].
The result revealed that the prevalence of *T. gondii* IgM in the sheep was 58 (58.5%) samples tested positive out of 99 samples, and as for the goats, 41 (41.4%) of 99 samples. There are no statistically significant differences between sheep and goats in terms of infection rate (positive IgM). With a P> 0.05, these results are consistent with several studies from Brazil (44%) [13] and Iraq (18.7%) [14]. And differ from this study because the percentage was low in Bulgaria (3%) [15].
IgG results were in sheep: 93 (62.00%) samples tested positive. As for the goats, 57 (38.00%) samples tested positive out of 150 samples. There are no statistically significant differences between sheep and goats in terms of infection rate (positive IgG). With P> 0.05, these results are consistent with several studies: IgG rates in Bulgaria (Sheep 48.2% - Goats 52.8%) [15], Iraq (Sheep 29% - Goats 28%) [14], South Africa (Sheep 64.5% - Goats 53.9%) [6], and 40.7% in Libya [16].
The differences in seroprevalence in these studies could be related to the risk factors, which include species, age, sex, location, and season, found in many studies around the world [14,15,17-21].
The observed statistically significant difference in *T. gondii* seroprevalence between animal species, with sheep showing a higher prevalence than goats (p=0.038), aligns with findings reported in several previous studies [10,14,15,22]. However, other studies have reported a higher *T. gondii* prevalence in goats compared to sheep [10,14,15,22]. The difference between seroprevalences of *T. gondii* in sheep and goats could be attributed to the susceptibility of the species, feeding behavior, and sample size.
The prevalence of infection was higher in females (89.65%) than in males (10.35%). Out of 223 positives in females (IgG 86.7%) (IgM 94.0%) and 26 positives in males (IgG 13.3%) (IgM 6.0%), there are statistically significant differences between males and females in terms of infection rate (p=0.000) [10,19,20,23]. In addition to the difference in sample size, this may be partly attributed to the additional physiological stress experienced by females, such as pregnancy and lactation, which could reduce their resistance to *T. gondii* infection [24].
The present findings showed that the seroprevalence in female sheep was slightly higher compared to goats. In sheep, 111 positive (93.28%) were seropositive, with IgG (67) and IgM (44) antibodies detected. In goats, 77 (92.77%) were positive for IgG (43) and IgM (34). This higher proportion of seropositivity in sheep may indicate a greater susceptibility or longer exposure period compared to goats. However, the difference between species was relatively small, suggesting that management and environmental factors likely played a more significant role in exposure risk than host species alone. This finding is in agreement with several previous studies [7,25]. In contrast, some studies reported a higher prevalence of infection in males compared to females [2,7]
The results across different age categories suggested a higher seroprevalence of *T. gondii* in animals 1-3 years old (51.40%); there are statistically significant differences between age groups in terms of infection

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rate (p= 0.048) [26-28]. The difference was not statistically significant, aligning with previous studies that found no correlation between age and infection [22-29].

In sheep and goats, the 1–3-year age group showed the highest seroprevalence of *T. gondii* (sheep: 47.90%, IgG: 31/IgM: 26) (goats: 45.78%, IgG: 22/IgM: 16). This may be due to increased exposure through grazing and environmental contact. While age appears to influence risk, previous studies indicate that management and environmental factors also play a major role [22,30], and some studies reported no significant association between age and infection [22,30].

Out of a total of 51 (IgG 20 / IgM 12) sheep samples, 32 tested positives, while 15 out of 28 goat samples were positive. The highest infection rate in sheep was observed in the 1–3-year age group (71.88%), and the prevalence was higher in females than in males (87.50%). Age was found to be statistically significant, whereas sex did not show a significant association with infection; these findings are consistent with previous studies reporting higher susceptibility in sheep and young adult small ruminants, while sex has a minor effect on infection risk [17,26]. Furthermore, some studies have reported differing results regarding the influence of sex and age on *Toxoplasma gondii* infection in sheep [31-43].

In goats, the infection rate was 53.57%, with the highest prevalence in the 1–3-year age group (80.00%) and a higher rate in females (≈80.00%). These findings suggest that both age and sex influence *Toxoplasma gondii* infection, as younger adults are more exposed during their productive period and females may be more susceptible due to physiological stress from pregnancy and lactation. Similar trends have been reported in continental studies [3,35]. However, other investigations did not observe significant associations with age or sex, likely due to differences in management systems, environmental conditions, and sample size variations across regions [16].

The Taknes area showed the highest *Toxoplasma gondii* infection rate (31.19%), followed by Boutraba, Farzogha, and Al-Awillia. This pattern aligns with studies reporting geographic differences in prevalence [30], likely due to environmental factors such as climate, cat density, and pasture contamination. However, other studies found no significant differences between locations, suggesting that host factors, age, sex, breed, and management practices may play a stronger role than geography alone. These results indicate that *T. gondii* infection is influenced by multiple interacting factors, and preventive measures should combine environmental management with farm hygiene and cat control.

The seroprevalence of *Toxoplasma gondii* was higher in the spring (58.91%) compared to both autumn and summer; the probability value (P=0.00) is less than the level of statistical significance (.05), and therefore there is a statistically significant relationship between the seasons and incidence of the parasite. This may be related to seasonal differences in transmission, as infection rates tend to increase during the wet months when the climate favors the survival of oocysts, leading to higher seroprevalence in the subsequent dry season. Oocysts are spread in the environment through wind, rain, surface water, and contaminated harvested feeds such as hay, straw, and grain [38]. These factors could explain the variation in *T. gondii* seroprevalence across different locations and seasons.

Conclusion

This study revealed a high seroprevalence of *Toxoplasma gondii* antibodies (IgG and IgM) in sheep and goats from the city of Al Marj and its surrounding areas, supporting the findings of previous studies conducted in Libya [15,34,35]. The results suggest that the consumption of undercooked meat from these animals may represent a potential risk of infection to humans. Therefore, raising awareness among government authorities, public health sectors, and the community regarding toxoplasmosis is essential, especially since these animals constitute the primary source of meat in Al Marj. *T. gondii* is widely prevalent in the city of Al Marj. Although the exact source of infection remains unclear, sheep and goat meat cannot be excluded as a possible route of transmission. Consequently, public education on transmission pathways and preventive measures should be strengthened, and further studies are recommended to assess the impact of the disease on food animal production.

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Conflict of interests

The authors report no conflicts of interest**.**

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