

Sero-Epidemiological Study of Toxoplasmosis in Sheep and Goats in Dammam City, Kingdom of Saudi Arabia

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Abstract

Toxoplasma gondii is intracellular protozoan parasite that are distributed worldwide and of major economic importance in the livestock industry especially sheep and goats. Sheep and goats are thought to be biological indicators of environmental contamination with *T. gondii* oocysts. In addition, in developing countries such as Saudi Arabia, where sheep and goat meat is commonly consumed, *T. gondii* infection in small ruminants may also affect public health risks. So that we estimate the prevalence of *T. gondii* infections in small ruminants, by using indirect enzyme-linked immunosorbent assay (ELISA) to detect the antibodies to assess the seroprevalence in 130 sheep and 130 goats from Dammam city. A total of 130 sheep were sampled, of which 35 (26.9%) were positive for *T. gondii*, out of 130 tested goats 31 (23.8%) were positive for *T. gondii*. Our study also recorded 27 out 52 from the aborted ewes (51.9%) and 22 out 45 from the aborted does (48.8%) were seropositive for anti-*T. gondii* antibody. Significant differences (p value < 0.0001) were observed among previously aborted females when evaluated as risk factors for *T. gondii* infection in both ewes and does. In addition, the results revealed that the age in sheep more than 3 years give 31.1% but in goat 23%. Significant differences (p value < 0.0001) were observed among previously ages, sex and the farming system, from the results of this study showed a broad distribution for protozoan parasite (*Toxoplasma gondii*) in examined sheep and goat flocks. By using ELISA *Toxoplasma gondii* antibody test kit that provides a rapid, simple, sensitive and specific method for diagnosis of Toxoplasmosis.

Keywords: *Toxoplasma gondii*; Sheep; Goats; Saudi Arabia; Elisa

Abbreviations

ELISA: Enzyme Linked Immunosorbent Assay

Introduction

Toxoplasmosis is protozoan disease that are distributed globally and responsible for great economic losses in farm animals. Toxoplasmosis is an important zoonosis caused by an obligate intracellular parasitic protozoan, the disease can affect all warm-

blooded vertebrates, including humans. [1,2]. Toxoplasmosis is a cosmopolitan zoonotic disease caused by a protozoan parasite, *Toxoplasma gondii* (*T. gondii*) and it is of great concern for both animals and public health [3]. Subsequently, *T. gondii* and is closely related apicomplexan parasites, sharing many morphological and biological features, [1] and are associated with reproductive problems in livestock [4]. Domestic cats and other felids are definitive hosts of *T. gondii*, a large variety of mammals and birds that serve as intermediate hosts [5,6]. Sheep and goats are considered the highest susceptible herbivorous animals for infections with *T. gondii*

[7,8] causing significant reproductive disorders such as embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and weakness in newborn animals [6,3]. In Saudi Arabia, small ruminants as sheep and goats represent the major source of red meat for consumers and lambs and kids are fattened for special religious and social. Animals are raised under an extensive system, in which animals are reared in herds/flocks and kept grazing natural pastures, raised under an intensive farming system for production of fattened lambs/kids, or reared under semi-intensive farms. [9,10]. In the former semi-intensive farming system, small ruminants (sheep and/or goats) are reared with large ruminants (cattle and/or buffalo) together with domestic poultry [11]. This is the most common system for the traditional Saudian small-scale farmers and is widely scattered throughout the country. Usually, in these small farms in the farmers' houses animals are contacted with dogs and cats resulting in a high risk for occurrence of toxoplasmosis between individual animals in that farm.

Up to date regarding the eastern regional area in Saudi Arabia, a little is known about the sero-prevalence of toxoplasmosis among

small ruminants. Furthermore, therefore, this work aimed to investigate the presence of *T. gondii* infections in abortive cases in small ruminants using the ELISA technique from different farms in the Dammam city.

Materials and Methods

Farms and animals

A total number of 260 heads of small ruminants were investigated in this study during the period from Juli 2020 to Juli 2021. Sheep (n = 130) and goats (n = 130) were sampled from different farms located in Dammam city Eastern region of Saudi Arabia. These animals were classified aged less than 3 years and more than 3 years, also included both sexes. The samples obtained randomly from all the examined farms (semi-intensive and intensive farms) with a special recording of numbers of ewes and does that suffered from previous abortion. The detailed information of the various groups of tested animals according to their species, ages and sexes is shown in table 1.

Sample area	Farming system ¹	Tested animals ²				Total
		Sheep		Goats		
		Ewes ³	Rams	Does ³	Bucks	
Dammam	Semi-intensive farms	30	4	20	3	57
Dammam	Intensive farms	85	11	95	12	203
Total		115	15	115	15	260

Table 1: Localities, species, age and sex of tested animals.

¹Farming system in the visited farms in Dammam comprised extensive farming system named as Semi-intensive in which animals reared in small groups (10 - 30 heads), and intensive farming system in which animals reared in large groups (above 50 heads).

²All tested animals' groups in our approach aged from less 3 years and over 3 years.

³From all of the visited farms in Dammam some females (ewes and does) were suffering from previous abortion cases.

Blood sampling

Blood samples were collected from sheep (n = 130) and goats (n = 130) for the serological investigations of *T. gondii*. About 5ml blood without anticoagulant was collected via jugular venipuncture under aseptic conditions [12], then transferred to plain vacutainer tubes and centrifuged for 15 minutes at 3000 rpm for separation of serum. Collected sera were stored at -20°C until tested.

Detection of *T. gondii* antibodies by ELISA

Sheep (n = 130) and goats (n = 130) from different farms in Dammam city were serologically investigated for infection by *T. gondii* via indirect ELISA assay, using commercial ELISA kits. All 260 serum samples were tested for the presence of anti-*T. gondii* antibodies by using IDEXX Toxotest kit for detecting antibodies against *Toxoplasma gondii* in serum of small ruminants (IDEXX

Laboratories, Inc. One IDEXX Drive Westbrook, Maine, USA). Steps for ELISA protocol done according to the manufacturer’s instructions. The optical density (OD) was measured at 450 nm. The S/P percentage of each sample calculated as follows:

ODnc: mean OD values of negative control, ODpc: mean OD values of positive control, and samples with an S/P value ≥ 30% considered positive.

Statistical analysis

The infection rates of toxoplasmosis among sheep and goats were determined by direct counting. The significance of the differences in the incidence rate of the disease and risk factors was

determined with a chi-square (χ²) test. A P value of < 0.05 considered statistically significant. Odds ratios and the 95% confidence intervals were calculated using www.vassarstats.net.

Results

Serum samples were collected from 260 animals (Sheep and goats), in Dammam city (Table 1). Out of 130 heads of sheep investigated here, 35 (26.9%) in sheep. From 130 goats examined here 31 (23.8%) were seropositive for *T. gondii* (Table 2). In our investigations, samples were collected from both genders, but limited numbers of rams and bucks were available from the visited farms. Indeed, three rams out of 15 tested were positive for *T. gondii* antibodies and two of the 15 bucks tested positive for toxoplasmosis (Table 3, 4).

Localities	Farms ID ¹	Occurrence of Toxoplasmosis			
		Sheep		Goat	
		No. tested	No. positive (%) <i>T. gondii</i>	No. tested	No. positive (%) <i>T. gondii</i>
Total		130	35 (26.9)	130	31 (23.8)

Table 2: Infection rates of Toxoplasmosis in small ruminants in some Dammam farms that determined by ELISA.

¹In all of the visited farms in Dammam sheep and goats were reared together in one group.

Analyzed risk factor	No. tested	No. positive (%)	OR (95% CI) ¹	P-value ²
Age				
Less 3 years	40	7 (17.5)	0.82 (0.32 - 2.08)	0.68
More 3 years	90	28 (31.1)		
Sex				
Male	15	3 (20.0)	0.99 (0.15 - 6.23)	-
Female	115	32 (27.8)		
History-of abortion				
Aborted ewes	52	27 (51.9)	11.33 (3.75 - 34.24)	< 0.0001
Non-aborted ewes	63	5 (7.9)		
Farming system				
Semi-intensive	34	12 (35.2)	1.1 (0.36 - 1.95)	0.69
Intensive farms	96	23 (23.9)		

Table 3: Risk factors associated with *T. gondii* infection among tested sheep.

¹OR= Odds Ratio, CI = Confidence Interval.

²chi-square (χ²) test. A P value of < 0.05 considered statistically significant

^{1, 2}calculated using www.vassarstats.net.

Analyzed risk factor	No. tested	No. positive (%)	OR (95% CI) ¹	P-value ²
Age				
Less 3 years	30	8 (26.6)	1.81 (0.68 - 4.84)	0.22
More 3 years	100	23 (23.0)		
Sex				
Male	15	2 (13.3)	0.93 (0.16 - 5.39)	-
Female	115	29 (25.2)		
History of abortion				
Aborted does	45	22 (48.8)	22.50 (5.97 - 84.75)	< 0.0001
Non-aborted does	70	7 (10)		
Farming system				
Semi-intensive farms	23	13 (56.6)	1.07(0.45 - 2.55)	0.86
Intensive farms	117	18 (15.3)		

Table 4: Risk factors associated with *T. gondii* infection among tested goats.

¹OR= Odds Ratio, CI = Confidence Interval.

² chi-square (χ^2) test. A P value of < 0.05 considered statistically significant

^{1, 2}calculated using www.vassarstats.net.

Out of 115 ewes and 115 does 52 and 45 females had a history of abortion, respectively. Among them, 27 ewes from 52 aborted ones were tested positive for toxoplasmosis with 51.9 percentage. However, from 63 non-aborted ewes five cases (7.9%) were seropositive for anti-*T. gondii* antibody (Table 3). Similarly, out of 45 aborted does 22 (48.8%) were positive for *T. gondii* and only 7 females were tested positive from 70 non-aborted ones (Table 4).

An epidemiological study was conducted as risk factors to establish the effect of age, sex, history of abortion and farming system (Semi-intensive and intensive farming system) on the prevalence of *T. gondii* infections in sheep. There were 7 (17.5%) positive samples for the age group less than 3 years, 28 (31.1%) for age group more than 3 years. The result obtained was subjected to a Chi-squared test. The result showed that seroprevalence of *T. gondii* antibody in various age groups was statistically non-significant (p value 0.68), odd ratio [OR] was (0.82). Aborted ewes 51.9% compared with non-aborted ewes 7.9% (odd ratio 11.33, P value < 0.0001). Other risk factors as sex and farming system were statistically non-significant (Table 3).

The effect of age, sex, history of abortion and farming system (intensive farming system) on the prevalence of *T. gondii* infections

in goats were evaluated. In contrast to sheep, the seroprevalence in goats was higher in younger age less than 3 years (26.6%) than in older ones (23%). The result of aborted does and non-aborted were 48.8% and 10%, respectively (OR 22.50, P value < 0.0001). Moreover, other risk factors comprising age, sex, and farming system were statically non-significant (Table 4).

Discussion

Sheep and goats are widely distributed and consider the main components of the agricultural sector. They are the main sources of animal protein and play a critical role in maintaining the food supplies and economic security of Saudi Arabia and developing countries. To date, few studies have investigated the serological prevalence of *T. gondii* infection among sheep and goats in Eastern region of Saudi Arabia. We demonstrated a higher infection rate of toxoplasmosis. *T. gondii* considers the primary parasite responsible for both sheep and goat abortion [13]. In the present study, the seroprevalence of *T. gondii* in sheep and goats was 26.9% and 23.8% by using ELISA, respectively. The seroprevalence of *T. gondii* was 86.0% among tested sheep that was higher than we identified and 87.7% in goats that was a markedly higher prevalence than we recorded [14]. Such differences in the prevalence of toxoplasmo-

sis may be attributed to farming system condition, immune status, the timing of infection, the genetic composition of the host and the organism [15,16] and diagnostic techniques, the ELISA test considers the more suitable test in the diagnosis of toxoplasmosis in ruminants [17]. Our results revealed that the *T. gondii* parasite is widely spread because they tend to wander more freely in the urban area of Saudi Arabia than other ruminants and thus have direct contact with domestic cat feces [18]. The higher prevalence rates of toxoplasmosis in ruminants in our study due to a warm and moist environment leads to long viability of *T. gondii* oocysts in the environments and the presence of a stray cat in our rural areas which increase contamination of the environment with shedding oocysts.

In this result, showed 51.9% in aborted ewes which is lower than other results as [19] (53.3%) by PCR, and our results showed higher results as [19], (40.0%) by Latex agglutination test, and our results showed higher than results as [20] 31.0% by indirect haemagglutination (IHA). Regarding to gender in the present study, the infection rate by ELISA was 27.8% in ewes and 20% in rams, which has no statistically significant difference between infection rate and gender; some research did not show a significant correlation between males and females similar to our findings [21,22]. The seroprevalence of *T. gondii* was higher in does (25.2%) than in bucks (13.3%) which has no statistically significant difference. Regarding to aborted does in the present study, the seroprevalence of *T. gondii* was 48.8% in our results higher than 35.4% by using (IHA-IgG) [7,23] and 17.1% by IHA-IgM (20). Furthermore, our study was lower than other reported in aborted does 80.0% by using Direct Antiglobulin test (DAT) [24]. *T. gondii* consider zoonotic disease as they introduce infective oocysts to the environment. Humans obtained toxoplasmosis via the eating of undercooked meat and mutton [13,25]. Infection with *T. gondii* in humans looked like flu fatigue, while immune depressed patients especially (AIDs patients) are most vulnerable to infection complicated to headache, encephalitis, convulsions, drowsiness and blindness. Females infected with *T. gondii* during pregnancy develop spontaneous abortion or give child suffer from hydrocephalus, mental retardation, blindness and deafness [10,26]. Based on the recommendation of the Centers for Disease Control and Prevention, U.S.A. (<https://www.cdc.gov/parasites/toxoplasmosis/prevent.html>) to avoid such zoonotic diseases, individuals should follow these three main precautions: reduce risk from food (thoroughly cook foods and washing well of fruits), reduce risk from the environment and avoid direct contact with the cat in pregnant women.

Conclusion

The results of this study provide new insights into the occurrence of toxoplasmosis in small ruminants in the Dammam city. The seroprevalence rate of *T. gondii* infections observed in sheep and goats in this study were relatively high. This may indicate an important role of goat and sheep in the transmission of human toxoplasmosis, given the habit of eating undercooked grilled mutton. More studies needed to understand the high rates of these parasitic infections in Saudi Arabia. This requires the application of more effective strategies for resisting these types of infections on farms in Saudi Arabia.

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Not applicable.

Conflict of Interest Statement

The author declare that this article does not have any financial or non-financial conflict of interest.

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