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Epidemiological factors associated with anti-toxoplasma seropositivity in aborted Ewes in Nineveh Governorate, Iraq

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ABSTRACT

The objective of the current study was to examine the epidemiological factors associated with anti-toxoplasma seropositivity in aborted ewes in the Nineveh governorate in Iraq. Serum collected from a total of 213 aborted ewes was examined using the Latex agglutination test, and the titer of 1/16 was set as a cut-off titer for the seropositivity. Logistic regression was applied to examine the association of different epidemiological factors with the seropositivity. The prevalence of anti-toxoplasma seropositivity in aborted ewes was 61.5%. The seropositivity was greater in 3 and 4 years-old ewes compared to those ≤ 2-years-old, and higher in flocks > 200 head compared to smaller flocks. The prevalence of potential recent infection was 26%. Recent infection was not identified in ewes with the last stages of gestation. The odds of recent infection were higher in aborted ewes during summer-autumn season than those aborted in winter-spring, and greater in flocks < 100 head compared to flocks > 200 head. The study concluded that *Toxoplasma gondii* infection is not negligible and could be considered a crucial pathogenic cause of abortion in ewes in the Nineveh governorate. Finally, flock management system is an important factor that can influence the prevalence of *T. gondii* in the governorate.

Introduction

Toxoplasma gondii is a protozoan parasite that infects a wide range of animals as well as humans. As a definitive host, cats shed large number of oocysts that contaminate the environment, which subsequently sporulate within days and form sporozoites, the main infective stage to farm animals (Ortega-Mora *et al*., 2007). Sheep are considered highly susceptible to *T. gondii* infection (Stelzer *et al*., 2019). As an intermediate host, sheep become infected via ingestion of sporulated oocysts from the environment (Ortega-Mora *et al*., 2007). Parasitemia occurs within 5 days after the infection, where the invasive tachyzoites are formed and rapidly replicate several rounds and then form intracellular tissue cysts that contain bradyzoites (Dubey, 2010; Constable *et al*., 2017). The immunity is developed within 2 to 3 weeks after the infection (Trees *et al*., 1989).

The main clinical manifestation of *T. gondii* infection is abortion. However, abortion occurs only if the infection occurred during the pregnancy (Constable *et al*., 2017). As a function of parasitemia, tachyzoites invade the uterus and placenta causing fetal mortality due to placental insufficiency as a result of placentitis (Dubey, 2010). Abortion happens if pregnant ewes are infected during the first and middle stages of gestation, although stillbirth occurred when some fetuses survive from the middle stage of gestation infection, whereas infected and immunized lambs are born if pregnant ewes are infected during the last stage of gestation (Constable *et al*., 2017). Finally, ewes develop an effective immune response as a function of the infection, and usually do not suffer from subsequent abortion (Ortega-Mora *et al*., 2007).

Locally, a recent meta-analysis indicated that the seroprevalence of anti-toxoplasma antibodies in sheep was estimated at 51.43% (Alameen and Dahl, 2022). However, epidemiological factors associated with anti-toxoplasma seropositivity have been poorly examined in sheep in Nineveh. For instance, the seropositivity varied according to the geographical location in the governorate (Al-Sim'ani, 2000; Altaee, 2002). Nevertheless, the real effect of the geographical location is most likely attributed to underlying factors such as flock management system and climate (Stelzer *et al*., 2019). On the other hand, age was reported as an effector in only one study (Al-Sim'ani, 2000). Other epidemiological effectors have been overlooked. The objective of the current study was to examine the epidemiological factors affecting anti-toxoplasma seropositivity in aborted ewes in the Nineveh governorate in Iraq. Aborted ewes were targeted because abortion is considered the main clinical manifestation of toxoplasmosis.

Materials and methods

Ethical Approval

The current study was approved by the scientific committee at the Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul. Blood samples have been collected from study ewes according to the standard procedure for blood sampling.

Study Ewes

Aborted ewes from different flocks located in Nineveh governorate, Iraq were considered for inclusion in this study. Ewes were raised in flocks with rams and lambs, kept usually in the open, and grazed mainly on natural grassland grown throughout the year. Study ewes had two principal pregnancy seasons: summer-autumn (June to November) and winter-spring (December to April), with a gestation period of approximate-

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ly 150 days. A sample size of 197 to 218 aborted ewes was enough to achieve estimation with 5% precision and 95% confidence based on the following assumptions: expected prevalence of toxoplasmosis in aborted ewes is 50% and about 400 to 500 aborted ewes are accessible to be sampled (https://www.openepi.com/SampleSize/SSPropor.htm). In this study, only ewes aborted within two weeks from the abortion event to the time of sampling were included. Blood samples from a total of 213 aborted ewes from 54 different flocks were collected.

Evidence of Anti-toxoplasma Seropositivity

Latex agglutination test (LAT) was used to detect *T. gondii* antibodies in the collected sera as a rapid test used in epidemiological investigations as a screening test can give a relatively accurate detection of anti-toxoplasma antibodies in ewes' sera (Trees *et al*., 1989). Both qualitative and semi-quatitative methods were performed using Plasmatec Toxoplasmosis latex kit (Lab21 Healthcare Ltd, UK) according to the manufacturer's instructions. The qualitative LAT method, in brief, was performed as the following: a drop of undiluted serum was mixed with 25 µL of the latex reagent on a slide using a stirrer. The slide was gently tilted backwards and forwards for four minutes. Presence of an agglutination reaction within the four minutes indicated an existence of antibodies and interpreted as a positive result. The semi-quantitative LAT method, on the other hand, was performed to quantify antibody titers in sera tested positive with the qualitative LAT method. In brief, a U-shaped 96-well microtiter plate were used to dilute positive sera with 0.9% normal saline two-fold serially dilutions to achieve the following dilutions: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, and 1/1024, taking into consideration that each well contained 50 µL diluted serum, and serum was mixed with the saline thoroughly inside each well before an amount of 50 µL was transformed to the next well for the next dilution. Later on, 5 µL from each dilution was mixed with 5 µL of Latex reagent on a slide as previously described. The anti-toxoplasma titer was the reciprocal of the last dilution of sera (highest dilution) where agglutination reaction appeared. In the current study, the cut-off titer was set at 1/16 for an infected ewe, because antibody titer less than that could have been produced as a function of non-specific reactions (Trees *et al*., 1989; Dubey, 2009; Dempster *et al*., 2011). In addition, to discriminate the activity of IgM antibodies from IgG, sera tested positive with LAT were treated with 2-Mercaptoethanol (2ME) because it inactivates IgM (Capel *et al*., 1980), and then LAT was repeated on these sera. The 2-ME solution was manually prepared as the following: 8.5 g sodium chloride was dissolved in 7.14 mL of concentrated 2-ME (both provided by Scharlab S. L., Spain), and the volume was completed by distilled water to 1,000 mL (Alton *et al*., 1975). To complete the test, 0.5 mL of sera were mixed with 0.5 mL 2-ME solution inside wells of U-shaped 96-well microtiter plate and incubated in 37°C for 30 minutes. Later on, 5 µL from each sample was mixed with 5 µL of Latex reagent (Plasmatec, UK) on a slide as previously described with LAT. Presence of an agglutination reaction was considered a function of IgG activity in the sample. On the other hand, absence of the agglutination was considered a function of IgM activity in the sample resulted from potential recent *T. gondii* infection as IgM produced by the body can be detected within three weeks after the infection (Trees *et al*., 1989), and sera in our study were collected within two weeks of abortion event.

Data collection

The following data were collected for each study ewe: number of the ewe in the study, age of the aborted ewe, stage of gestation when the abortion occurred (first, middle, last), gestation number (first, second, or later), pregnancy season (summer-autumn; June to November, winter-spring; December to April), geographical location of the flock (east, west; relative to Tigris River), flock management system as: flock size (<100, 100 to 200, >200 head per a flock), movement of the flock during the gestation period (yes, no), raising goats in the flock (sheep only, sheep and goats), and type of rearing (outdoor, mixed), as well as results of laboratory tests as qualitative LAT (positive, negative), quantitative LAT (titers from 1/2 to 1/1024), 2ME (positive, negative). In this study, the negative result for 2ME was considered a potential recent infection, because the sera had already tested positive with LAT, i.e., the positive result in LAT was due to IgM, and the abortion was most likely due to *T. gondii* infection.

Statistical Analysis

In this study, the prevalence of anti-toxoplasma seropositivity in aborted ewes constituted the proportion of ewes that tested positive with LAT and had anti-toxoplasma titer ≥ 16 among all study ewes. Prevalence of potential recent infection of *T. gondii* in aborted ewes constituted the proportion of ewes that tested negative with 2-ME compared to aborted ewes tested negative with LAT. The difference of the prevalence in each side in the governorate was examined by the use of Chi-square test (2-tailed). Logistic regression was used to examine the relationship between different epidemiological factors and (i) the prevalence of anti-toxoplasma seropositivity, and (ii) potential recent infection. In this analysis, geographical location of the flock and stage of gestation were not included in the modeling process because the true effect of geographical location can be attributed to other factors such as flock management system (Stelzer *et al*., 2019), and ewes can get infected with toxoplasma regardless to the pregnancy status (Abdelbaset *et al*., 2020). The initial screening was performed using univariable analysis. Variables with a value of P ≤ 0.20 were further examined in the multivariable analysis. The association between variables was examined, and when a pair of variables was associated by the use of a chi-square test (two-tailed), the exposure variable judged as most biologically plausible was further examined. In the multivariable analysis, variables selection method was used as a model-building strategy, and a manual forward selection technique was used by adding one variable at a time (the lowest P-value was added first) and assessing the model goodness-of-fit using the likelihood ratio test statistic (Hosmer and Lemeshow, 2000). Exposure factors retained in final models were examined for confounding by adding each of the variables to the model, and assessing changes in odds ratios (i.e., ≥ 20%) of the remaining variables in the model (Szklo and Nieto, 2007). In the final models, variables with a value of P ≤ 0.05 (two-tailed) were considered significant, and the adjusted odds ratio (OR) with 95% CI were reported. Finally, statistical analyses were performed using STATA 13.0 (StataCorp., College Station, TX, USA).

Results

The prevalence of *T. gondii* in aborted ewes was 61.5% (95% CI = 54.7, 67.8). Seventy five percent of reported ewes had anti-toxoplasma titer ≤ 256 (min = 16, median = 128, and max 1,024; Fig. 1). The prevalence of *T. gondii* was higher in aborted ewes located in the east side compared to those in the west side of Nineveh governorate (P = 0.04; Fig. 2), with same trend of anti-toxoplasma titer in each side. The univariable analysis revealed that anti-toxoplasma seropositivity was associated with age of ewes and flock size (i.e., P ≤ 0.01; Table 1). In the multivariable analysis, the odds of the seropositivity were greater in ewes with 3- and 4-years-old compared to those 2-years or younger (OR = 2.02; 95% CI = 0.91-4.52, and 3.40; 95% CI = 1.23-9.37, respectively), after controlling for flock size (Table 2). However, the effect of age (3-years-old) was confounded by the effect of flock size (adjusted OR decreased by > 20%). In our data, large flocks (> 200 head) had 34% of ewes with 3-years-old. On the other hand, the odds of seropositivity were 4.06 times and 2.16 times greater in ewes from flocks with < 100 head and 100-200 head, respectively, compared to those with > 200 head (Table 2), after controlling for age. The effect of flock size was not confounded by age (adjusted OR changed

6-7% only). Finally, variables for season and raising goats with sheep did not improve the model goodness-of-fit; therefore, they were removed from the model.

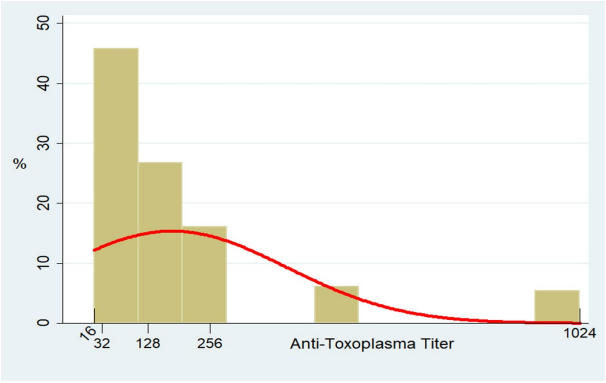

Fig. 1. Distribution of anti-toxoplasma titers in aborted ewes judged as positive using LAT; min = 16, 1st quartile = 32, median = 128, 3rd quartile = 256, and max = 1,024.

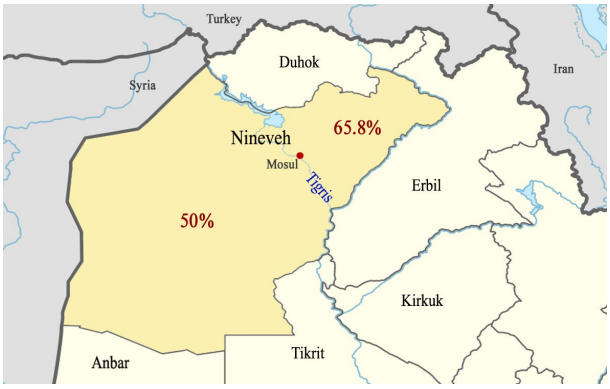

Fig. 2. Prevalence of *T. gondii* in aborted ewes in each side of Nineveh governorate, Iraq.

The proportion of aborted ewes with potential recent infection was 13.6% among all tested ewes (Fig. 3), which constituted 22.1% of ewes judged as anti-toxoplasma seropositive. The prevalence of potential recent infection among tested ewes was 26% (95% CI = 18.7, 35.2), and it

Table 1. Univariable logistic regression analysis for the odds of exposure factors associated anti-toxoplasma seropositivity in aborted ewes in Nineveh governorate, Iraq using LAT.

Variables	Anti-toxoplasma (LAT)		Odds Ratio	95% CI	*P*-value
	Positive (n=131)	Negative (n=82)			
Age (years)					
≤ Two	21	24	1	Reference	NA
Three	64	28	2.61	1.25, 5.44	0.01
Four	25	8	3.57	1.32, 9.59	0.01
≥ Five	21	22	1.09	0.47, 2.51	0.83
Pregnancy Season					
Summer-Autumn	66	50	1	Reference	NA
Winter-Spring	65	32	1.53	0.87, 2.69	0.13
Flock size					
< 100 head	40	10	4.33	1.95, 9.60	< 0.01
100 – 200 head	43	20	2.32	1.20, 4.50	0.01
> 200 head	48	52	1	Reference	NA
Movement of the flock					
No	80	43	1.42	0.81, 2.48	0.21
Yes	51	39	1	Reference	NA
Mixed animals					
Sheep only	40	18	1.56	0.82, 2.96	0.17
Sheep and Goats	91	64	1	Reference	NA
Type of rearing					
Outdoor	103	65	1	Reference	NA
Mixed (indoor/outdoor)	28	17	1.03	0.52, 2.04	0.91

Table 2. Multivariable conditional logistic regression analysis for the odds of exposure factors associated anti-toxoplasma seropositivity in aborted ewes in Nineveh governorate, Iraq using LAT.

Variables	Adjusted Odds Ratio	95% CI	*P*-value
Age (years)			
≤ Two	-	-	-
Three	2.02	0.91, 4.52	0.08
Four	3.4	1.23, 9.37	0.02
≥ Five	0.95	0.40, 2.28	0.92
Flock size			
< 100 head	4.06	1.76, 9.32	< 0.01
100 – 200 head	2.16	1.09, 4.28	0.02
> 200 head	-	-	-

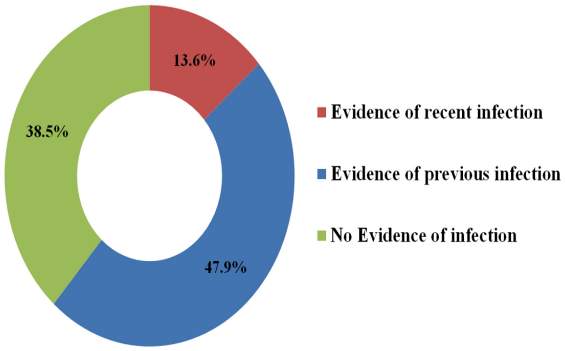

Fig. 3. Proportion of recent, previous, and no evidence of *T. gondii* infection in aborted ewes judged by MAT using 2-mercaptoethanol with latex reagent.

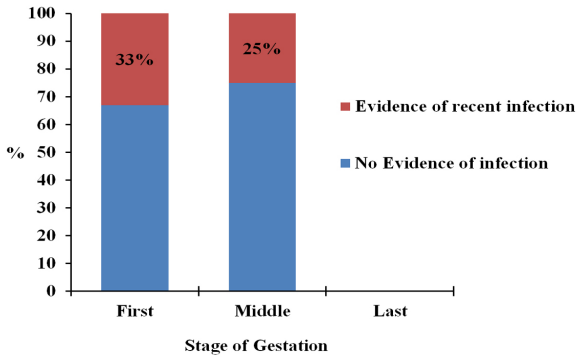

Fig. 4. The proportion of recent *T. gondii* infection in ewes aborted during different stages of gestation.

was not different between ewes located in the east side (23%) and those in the west side (31%) of Nineveh governorate (P = 0.36). The proportion of ewes had recent infection during the first and middle stages of gestation were 33% and 25%, respectively (Fig. 4). Recent infection was not recorded in the last stage of gestation (Fig 4). The univariable analysis revealed that the variables for pregnancy season and flock size had values of P < 0.20, and they were further examined in the multivariable analysis (Table 3). In the multivariable analysis, aborted ewes during summer-autumn had higher odds of recent *T. gondii* infection compared to those aborted in winter-spring (adjusted OR = 3.79; 95% CI = 1.23, 11.70), after

Table 3. Univariable logistic regression analysis for the odds of exposure factors associated recent *T. gondii* infection in aborted ewes in Nineveh governorate, Iraq using MAT.

Variables	Anti-toxoplasma (MAT)		Odds Ratio	95% CI	*P*-value
	Positive (n=29)	Negative (n=82)			
Age (years)					
≤ Two	8	24	1	Reference	NA
Three	10	28	1.07	0.36, 3.14	0.9
Four	5	8	1.87	0.47, 7.41	0.37
≥ Five	6	22	0.81	0.24, 2.73	0.74
Pregnancy Season					
Winter-Spring	6	32	1	Reference	NA
Summer-Autumn	23	50	2.45	0.90, 6.68	0.08
Flock size					
< 100 head	10	10	4.33	1.47, 12.73	< 0.01
100 – 200 head	7	20	1.51	0.52, 4.40	0.44
> 200 head	12	52	1	Reference	NA
Movement of the flock					
No	13	43	1	Reference	NA
Yes	16	39	1.35	0.58, 3.17	0.48
Mixed animals					
Sheep only	5	18	1.35	0.45, 4.04	0.59
Sheep and Goats	24	64	1	Reference	NA
Type of rearing					
Outdoor	20	65	1	Reference	NA
Mixed(indoor/outdoor)	9	17	1.72	0.66, 4.45	0.26

Table 4. Multivariable logistic regression analysis for the odds of exposure factors associated recent *T. gondii* infection in aborted ewes in Nineveh governorate, Iraq using MAT.

Variables	Adjusted Odds Ratio	95% CI	*P*-value
Pregnancy Season			
Winter-Spring	-	-	-
Summer-Autumn	3.79	1.23, 11.70	0.02
Flock size			
< 100 head	6.55	1.97, 21.82	< 0.01
100 – 200 head	1.82	0.60, 5.50	0.28
> 200 head	-	-	-

controlling for flock size (Table 4). On the other hand, aborted ewes from flocks with < 100 head had greater odds of recent *T. gondii* infection compared to those from flocks of > 200 head (adjusted OR = 6.55; 95% CI = 1.97, 21.82), after controlling for pregnancy season (Table 4). The multivariable analysis revealed that the effects of pregnancy season and flock size on recent *T. gondii* infection confounded each other (adjusted OR changed > 20%).

Discussion

The study conducted here revealed that the prevalence of toxoplasmosis in Nineveh governorate, Iraq is relatively high, particularly in the east side of the governorate, and it is increasing with the age of the ewes. However, most of ewes had anti-toxoplasma titer ≤ 256. Twenty six percent of abortion events could have been attributed to recent *T. gondii* infection, which was mostly evident during the middle stage of gestation. Flock management system had significant impact on increasing the odds of infection. Our study is considered the first local study modeled the odds of *T. gondii* infection as a function of different exposure factors related to the animal, environment and management. Our multivariable analysis procedure enabled us to control for potential confounding effects of the factors that can modify the anti-toxoplasma seropositivity in aborted ewes.

The current study judged 61.5% of aborted ewes as seropositive for anti-toxoplasma antibody. The study conducted here overcame overestimation of the seroprevalence by assigning a cut-off titer (1/16) for infected ewes (Trees *et al*., 1989; Dubey, 2009). In a previous study, anti-toxoplasma seropositivity was detected in 79% of aborted ewes using LAT (Altaee, 2002), the same test used in the current study. However, the former study did not assign a cut-off titer for an infected ewe, which potentially included ewes with antibody non-specific to *T. gondii*, i.e., those with titer 1/16 or less. Number of aborted ewes tested positive with LAT was lower previous years; 29.5% (Al-Sim'ani, 2000). One reason is that the infection was spread between sheep due to poor control measures with presence of cats around sheep. In addition, anti-toxoplasma seropositivity in aborted ewes was reported at 46.8% (Al-Sim'ani, 2000) and 45.78% (Hassan *et al*., 2005). Both reports were almost identical, but lower than our report. Potential reason is that they both used indirect hemagglutinating antibody test (IHAT), whereas we used LAT. Although the sensitivity and specificity of LAT compared to Sabin-Feldman Dye Test (SFDT) was estimated at 78.57% and 61.90 %, respectively (Oncel *et al*., 2005), they were estimated compared to PCR at 90.7% and 84.5%, respectively (Shaapan *et al*., 2015). Finally, 75% of ewes in our study had anti-toxoplasma titer ≤ 256, which could be evidence of a previous infection, reflecting an endemic status of the disease in the governorate. Predominance of aborted ewes with relatively low titers was previously evident, too (Al-Sim'ani, 2000; Hassan *et al*., 2005).

In this study, aborted ewes located in the east side of the governorate showed higher prevalence of anti-toxoplasma seropositivity compared to those in the west side. Similar evidence in the governorate was previously revealed (Al-Sim'ani, 2000; Altaee, 2002). The most possible reason is the management system of sheep flocks in each side. That is, sheep flocks in the east side are usually smaller than those in the west part. Small sheep flocks usually don't move and raise goats with sheep, with usually existence of cats. Therefore, this situation of management increases the probability of spread of the infection due to potential overcrowding as. Such management can easily expose cats to uterine and vaginal discharges as well as aborted fetus and fetal membranes of aborted ewes, and later contaminate the flock environment with the infective stage. In contrast, large size flock usually move in the open without accompany of cats, particularly in the west part of Nineveh. The current investigation is the first local investigation addressed effect of flock management on anti-toxoplasma seropositivity in local ewes. In this analysis, the odds of seropositivity were higher in flocks < 100 head and 100 – 200 head compared to those > 200. In our study, effect of flock management on anti-toxoplasma seropositivity was in line with other different studies worldwide which indicated that intensive or semi-intensive systems (in our study constituted flocks with size < 100 or 100 – 200 heads that don't move and raise goats with sheep) had higher toxoplasmosis prevalence compared to extensive system (Skjerve *et al*., 1998; Samra *et al*., 2007; Sechi *et al*., 2013).

In the current study, ewes with 3- and 4-years-old had higher odds of seropositivity compared to younger ewes. This result indicates that ewes acquire the infection with the age as a result of environmental contamination with infective stage. There is extensive evidence that prevalence of toxoplasmosis increases with the age of the animals (Olsen *et al*., 2019; Stelzer *et al*., 2019). A similar result has been previously revealed in the governorate (Al-Sim'ani, 2000). However, data of the current study indicated that ewes with age ≥ 5-years-old had low anti-toxoplasma seropositivity. One possible explanation is that owners usually don't keep old ewes, particularly those with previous history of abortion. The current result is identical to the former study by Al-Sim'ani (2000) who found a decline in anti-toxoplasma seropositivity in ewes with age ≥ 5-years-old. Finally, the analysis revealed that adjusted OR for the age group of 3-years-old was decreased by > 20% compared to the crude OR after controlling for flock size, which is considered a confounding effect. The most possible reason to this effect is that the highest percentage of age groups in large flocks was the age group of 3-years-old. Consequently, the odds of anti-toxoplasma seropositivity in this group decreased when the variable for age was adjusted for flock size.

In the study conducted here, the proportion of aborted ewes with potential recent infection was 26%. This proportion is considered relatively high, and it is most likely a function of poor control measures to the source of infection such as stray cats that are easily exposed to uterine and vaginal discharge of aborted ewes due to unhygienic disposal to the aborted fetuses (Constable *et al*., 2017; Stelzer *et al*., 2019). The evidence of recent infection in aborted ewes can infer that the infection caused the abortion; particularly this evidence was detected during the first and middle stages of gestation, as the infection during the first or middle stage of gestation can cause fetal death and subsequently abortion (Constable *et al*., 2017). In the current analysis, anti-toxoplasma evidence was not detected during the last stage of gestation. Infection in this stage does not cause abortion and might produce clinically normal lamb (Ortega-Mora *et al*., 2007). Finally, the impact of recent infection can be extended from the abortion in affected pregnant ewes to persistence of the infection and increase of the prevalence of the disease (Dubey, 2009).

The current analysis revealed that a recent infection with *T. gondii* in aborted ewes was related to higher odds of abortion during summer-autumn compared to winter-spring. The odds of infection increase in the humid and moist seasons (Ortega-Mora *et al*., 2007; Stelzer *et al*., 2019). Local climate is hot and dry during summer, whereas cold and rainy during winter. The most possible time with moist and warm climate would be end of March to April when lambing season starts for winter-spring pregnancy season, and potentially September; the middle to last gestation period for summer-autumn pregnancy season. A possible explanation in the increase the odds of recent *T. gondii* infection in ewes aborted during summer-autumn season compared to winter-spring in our study is that the local climate and environment at the end of spring and start of summer is suitable for oocyst survival which can produce recent infection in summer-autumn pregnancy season; particularly during the first stage of gestation. Another potential explanation is that the local rainfall was very poor during winter 2020-2021; thus, the season was cold and almost dry which affect oocyst survival (Dubey, 2010), and could contribute in the decrease the odds of recent toxoplasma infection among pregnant ewes during winter-spring pregnancy season. Additional possible reason is that pregnancy season of summer-autumn was investigated before winter-spring in the current study, and the decrease in the odds of recent *T. gondii* infection in ewes aborted in winter-spring pregnancy season was a function of the previous infection. A similar observation has been previously revealed in Texas, USA (Edwards and Dubey, 2013). More studies are important, however, to confirm this observation.

In this analysis, aborted ewes from small flocks showed higher odds of recent *T. gondii* infection compared to those from larger flocks. It has been mentioned that small flocks in this study usually don't move and raise goats with sheep, which can create a situation of overcrowding that increases the chance of new infections due to environmental contamination that can easily expose cats to uterine and vaginal discharges as well as aborted fetus and fetal membranes. Finally, effects of pregnancy season and flock size on recent *T. gondii* infection confounded each other; however, the direction and the significance of the magnitude did not change. Indeed, the magnitude of the effects has increased after the adjustment. One explanation is that the impact of season (summer-autumn) is exacerbated by flock management system that leads to overcrowding situation and created a stressful environment, which increase the odds of new infection in the flock.

This study has limitations. One limitation is the use of LAT and MAT only without additional serological tests, such as ELISA, to confirm the result. Nevertheless, a standard serological test for anti-toxoplasma antibodies with 100% sensitivity and 100% specificity in sheep is not available (Olsen *et al*., 2019). Indeed, LAT can give a relatively accurate detection of anti-toxoplasma antibodies in ewes sera (Trees *et al*., 1989), with sensitivity of 90.7% and specificity of 84.5% compared to polymerase chain reaction (PCR) method (Shaapan *et al*., 2015). Another possible limitation is that the study did not investigate evidence of toxoplasma in aborted fetuses. However, it was difficult to achieve it as owners did not immediately contact us after abortion event; thus, aborted fetuses usually were missed.

Conclusion

The current study confirmed the endemic status of toxoplasmosis in Nineveh governorate. *Toxoplasma gondii* is potentially responsible for 20-30% of abortion events in pregnant ewes in each pregnancy season. In addition, flock management system is considered an important factor that can influence the prevalence of *T. gondii* in the governorate. Policy options for owners of sheep might include follow good flocking practices such as avoiding overcrowding and stressful environment, separating aborted ewes, and practicing hygienic flock's measures in disposal of aborted fetuses and fetal membranes. Finally, agricultural and veterinary authorities should take serious steps toward controlling the spread of *T. gondii* infection among sheep flocks.

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Conflict of interest

The authors declare that there is no conflict of interest in this research.

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