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Risk factors associated with seropositivity to *Toxoplasma* among sheep and goats in Northern Iraq

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ABSTRACT

Serum samples from 423 small ruminants (335 sheep and 97 goats) from 72 farms in 6 districts in the province of Dohuk, north Iraq, were tested for the presence of antibodies against *Toxoplasma gondii* using a latex agglutination test (Latex) and an indirect enzyme-linked immunosorbent assay (iELISA). When the test results were interpreted in parallel, 42.1% (95% confidence interval (CI): 36.7, 47.7) of sheep and 36.1% (95% CI: 26.6, 46.5) of goats were found to have antibodies against *Toxoplasma*. A multivariable logistic regression model was developed to determine the risk factors for *Toxoplasma* seropositivity in small ruminant flocks. Factors which increased the risk of infection included the presence of cats near the feed of animals (Odds ratio (OR) = 6.3; 95% CI 1.6, 24.6) and a history of abortions in sheep in the preceding 12 months (OR = 13.4; 95% CI 2.1; 86.7). For every ten goats aborting in the preceding 12 months the odds of seropositivity increased significantly (OR = 6.7; 95% CI 1.3; 32.9). Results of the regression model indicate that for every 1000 Iraqi Dinars (~0.85 USD) spent by the farmers on prophylactic treatment in their flocks the odds of *Toxoplasma* seropositivity decreased significantly (OR = 0.94; 95% CI 0.90, 0.98). This study contributes to the epidemiology of toxoplasmosis in small ruminants in northern Iraq.

1. Introduction

Toxoplasmosis, caused by the intracellular protozoan *Toxoplasma gondii*, affects all warm-blooded animals, including humans (Innes 2010). The definitive hosts of *T. gondii* are felines, and these play an essential role in the contamination of the environment with oocysts. Infection of sheep and goats can occur after consumption of feed or pasture contaminated with sporulated oocysts (Elmore et al. 2010). The primary source of infection in humans is the ingestion of uncooked meat containing tissue cysts, especially in countries where the meat from sheep and goats is regularly eaten (Kijlstra and Jongert 2008). In livestock, toxoplasmosis mainly affects the reproductive organs resulting in abortions, fetal mummifications, stillbirths and the birth of weak offspring and the disease has the potential to have sever negative socio-economic impacts on people, especially in low-income countries (Anastasia et al. 2013; Masala et al. 2003).

In Iraq, there are approximately 1.5–2.0 million goats and 7–8 million sheep (Bechtol et al. 2011). Small ruminants in Iraq are an essential source of meat and milk and play a valuable role in the economy and food security of the nation. The veterinary infrastructure in Iraq is weak because of many years of international sanctions and ongoing ethnic and political conflicts, and consequently, the control of endemic animal diseases is challenging. Antibodies to *Toxoplasma* have been reported in small ruminants, including ewes that aborted in Northern Iraq (Mikail and Al-Barwary 2014). However, no studies have been published elucidating the risk factors for potential infection in either humans or other animals in the country. The aims of the research outlined in this manuscript were to investigate the seropositivity towards antibodies of *Toxoplasma* in small ruminants (sheep and goats) from Dohuk Province, north Iraq, and to identify potential risk factors for seropositivity.

2. Materials and methods

2.1. Study area and population included

This study was conducted in Dohuk Province, which is located in the north of Iraq and borders Syria and Turkey. There are 1.2 million

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Table 1
Univariable analysis of categorical risk factors associated with flocks with a seropositivity to *Toxoplasma*.

Variables	Number of flocks	% of seropositive flocks (95% CI)	OR (95%CI)	*P*-value
Cats near feed	48	79.17 (65.0, 89.0)	4.49 (1.55, 13.00)	0.004[a]
Cats not near feed	24	45.83 (20.7, 72.6)	1.0	
Cat faeces in feed	42	83.33 (68.6, 93.0)	5.71 (1.93, 16.88)	0.001[a]
No cat faeces in feed	30	46.67 (23.7, 70.7)	1.0	
Cat faeces in water	48	58.33 (43.2, 72.4)	0.20 (0.052, 0.76)	0.012[a]
No cat faeces in water	24	87.50 (61.3, 98.5)	1.0	
Animals graze outside farm	27	70.37 (49.8, 86.2)	1.19 (0.42, 3.34)	0.741
No animals graze outside farm	45	66.7 (51.0, 80.0)	1.0	
Occurrence of abortions in the sheep flock during the last 12 months	60	75.00 (62.1, 85.3)	6.00 (1.58, 22.8)	0.012[a]
No abortions in the sheep flock during the last 12 months	12	33.33 (6.2, 72.8)	1.0	
Occurrence of abortions in the goat flock during the last 12 months	37	72.9 (55.9, 86.2)	1.60 (0.59, 4.33)	0.361
No abortions in the goat flock during the preceding 12 months	35	62.86 (39.9, 82.3)	1.0	
Animals use a common grazing area	42	73.81 (58.0, 86.1)	1.88 (0.69, 5.12)	0.223[a]
Animals do not have access to a common grazing area	30	60.00 (35.3, 81.5)	1.0	
Livestock grazed with other herds	40	77.50 (61.5, 89.2)	2.68 (0.97, 7.42)	0.052[a]
Livestock is not grazed with other herds	32	56.25 (32.6, 78.0)	1.0	
Water troughs not cleaned weekly	56	69.64 (55.9, 81.2)	1.38 (0.43, 4.41)	0.591
Water troughs cleaned at least weekly	16	62.50 (28.7, 89.1)	1.0	
Feed troughs not cleaned weekly	60	66.67 (49.3, 81.3)	1.5 (0.37, 6.16)	0.741
Feed troughs cleaned at least weekly	12	75.00 (42.8, 94.5)	1.0	
Source of water				
River only	26	69.23 (42.2,89.1)	1.8 (0.38, 8.53)	
Reticulated supply	21	71.4 (47.8, 88.7)	2.00 (0.40, 10.11)	0.861
Both rain and river	16	68.75 (41.3, 89.0)	1.76 (0.33, 9.51)	
Well only	9	55.56 (21.2, 86.3)	1.0	
Feeder trough made from				
Metal	23	69.57 (40.8, 90.3)	1.23 (0.34, 4.42)	
Tire	18	66.67 (41.0, 86.7)	1.08 (0.28, 4.13)	0.927
Wood	11	72.73 (39.0, 94.0)	1.44 (0.29, 7.21)	
Concrete	20	65.00 (34.3, 88.6)	1.0	
District number				
Amadiya	11	91.67 (52.3, 100.0)	11.0 (1.06, 114.09)	
Aqarh	7	58.33 (27.7, 84.8)	1.40 (0.28, 7.02)	
Zakho	8	66.67 (34.9, 90.1)	2.00 (0.38, 10.41)	0.341
Simele	9	75.00 (42.8, 94.5)	3.00 (0.53, 16.90)	
Shekhn	8	66.67 (34.9, 90.1)	2.00 (0.38, 10.41)	
Duhok	6	50.00 (21.1, 78.9)	1.0	

[a] *P*-value $\leq .25$, thus these variables were offered to the multivariable logistic regression model. It is recommended to use an initial screening *P*-value cut-off point of 0.25, as more traditional levels such as 0.05 can fail in identifying variables known to be important.

people and approximately 1 million sheep and goats in the province. The province is divided into seven districts, with each district containing two sub-districts and many villages (Mohammed 2013). In this study, flocks from six districts (twelve sub-districts) were sampled between February and April 2016. It was not possible to access one of the districts due to security concerns.

Dohuk has extensive areas of a pasture where sheep and goats are either grazed separately or together. The animals are usually grazed under the supervision of farmers or by employed shepherds who may supervise the grazing of stock belonging to different owners. Flocks containing > 100 animals (representing the typical number for the majority of the flocks in the region; based on personal experience of the first author) and both sheep and goats were eligible for inclusion in this study.

2.2. Sampling strategy

There was no sampling frame available from the local veterinary services to advice on the number and distribution of animals and flocks in the sampled districts. Consequently, we adopted a two-stage convenience sampling, based on a participatory approach using the local farmers' knowledge and networks. All of the twelve sub-districts of the six surveyed districts in Duhok province were included in this study. Local community leaders of the sub-district were approached at the central mosque, and were asked to voluntarily provide information about the farmers within the target sub-district who raised sheep and goats, and who met the inclusion criteria set for sampling (excluding farms with < 100 animals). From each sub-district, all farms meeting the study inclusion criteria for sampling (typically 2–3 farms), and whose owners agreed to participate in the study, were approached to request arranging a visit to their farms. From each farm, six individual animals were randomly selected for sampling; thus, a total of 432 individual blood samples were collected from 335 sheep and 97 goats that were raised in 72 mixed flocks in six districts of Dohuk.

2.3. Serological analyses

Five ml of blood was collected from the jugular vein of each selected animal. Blood samples were allowed to clot at room temperature and then stored on ice and dispatched to the Veterinary Diagnostic Laboratory in Dohuk on the same day of collection. Serum was extracted from whole blood by centrifugation at 3000 rpm for 10 min and then stored at −20 °C until testing. Each serum sample was tested for anti-*Toxoplasma* antibodies with a Toxoplasmosis Latex Test (Plasmatec, UK) and a commercial ELISA (NovaTec, Germany) according to the manufacturer's instructions. For Latex test, negative and positive controls from sheep reference sera were used for each run (collected during earlier screening and validated with both Modified agglutination test (MAT) and ELISA). The naked-eye visible of any degree of agglutination indicated the positive reaction, while smooth suspension with no visible agglutination regarded as negative reaction. The ELISA kit included plates coated with cell culture derived *T. gondii*

Table 2
Univariable analysis of continuous risk factors associated with flocks with seropositivity to *Toxoplasma*.

Variable	Median		*P*-value[a]
	Flocks < median prevalence	Flocks ≥ median prevalence	
Total number of sheep (×100)	0.22	0.41	< 0.001
Total number of goats (×100)	0.2	0.51	< 0.001
Number of sheep aborting in last 12 months (×10)	1.4	4.9	< 0.001
Number of goats aborting in last 12 months (×10)	2.1	5.6	< 0.001
Money spent (in 1000 Iraqi Dinars) on the prophylactic treatment in the flock in the preceding 12 months	0.62	0.27	< 0.001

[a] *P*-value ≤.25, thus these variables were offered to the multivariable logistic regression model. It is recommended to use an initial screening *P*-value cut-off point of 0.25, as more traditional levels such as 0.05 can fail in identifying variables known to be important.

tachyzoite-antigen, a peroxidase-labelled anti-small ruminant secondary antibody, tetramethyl benzidine (TMB) as a chromogenic substrate, control sera and buffer solutions. Testing was carried out in a nationally accredited commercial laboratory in Dohuk. The serological status of individual serum (animals) was assessed by interpreting the test results in parallel where an animal was classified as serologically positive if one or both of the tests were positive.

2.4. Questionnaire implementation

At the time of sampling, a questionnaire was administered to the visited farmers using a face-to-face format. The questionnaire was initially developed in English, then translated into the local Kurdish language and administered to the farmers by a native speaking Kurd. For all interviewed farmers, the questionnaires were administered by the same person to avoid any bias and to ensure consistency over time. Information was gathered about the structure of the flock, management and husbandry practices adopted, the presence of cat faeces in animal feed and water, and history of abortions in the flock (Tables 1 and 2).

2.5. Statistical analyses

The data from serology testings and from questionnaires were entered and analysed using STATA, Version 15, Software (Stata Corp LP, College Station, Texas, USA) and SPSS (Version 24, IBM). The questionnaire answers provided by farmers were described either as categorical or continuous variables (Tables 1 and 2). Initially, the association between putative management, husbandry factors and flock positivity were determined with a Chi-Square test. Data that were of a continuous nature were analysed with a Kruskal-Wallis ANOVA. Factors with a *P*-value ≤.25 were then offered to a multivariable logistic regression model to assess the association between seropositivity to *Toxoplasma* and predictor factors. It is recommended to use an initial screening P-value cut-off point of 0.25, as more traditional levels such as 0.05 can fail in identifying variables known to be important (Bursac et al. 2008). A backward stepwise selection approach was used and factors with a P-value < .05 were retained in the final model. All pairwise interactions between the variables in the final model were examined for significance. Goodness-of-fit of the final model was assessed using the Hosmer–Lemeshow test. The associations between seropositivity and the risk factors were assessed with odds ratios (ORs) and 95% confidence intervals (CI).

2.6. Ethics approval

The study had been approved by the animal and human ethics committees of Murdoch University. All procedures were explained to the farmers, and informed verbal consent was obtained from all participants before sampling and administering the questionnaire.

3. Results

Antibodies to *T. gondii* were detected in animals from all the six districts that were involved in the study and the test results (Latex and iELISA) were interpreted in parallel. Overall, there was no significant difference (*P*-value = .09) between the proportion of seropositivity for *Toxoplasma* in sheep (42.1%; 95% CI: 36.7, 47.7) compared to goats (36.1%; 95%CI: 26.6, 46.5). Added to that, there was no significant difference (*P* = .19) in the seropositivity across the different districts. The mean number of seropositive animals per farm was 2.43 ± standard deviation (SD) of 1.84; the high value of SD is indicative to a wide margin of variability in the within-farm seropositivity status between animals. Overall the farm level prevalence (farms containing one or more seropositive animals) was 36.1% (26/72) (95% CI: 24.4, 50.7).

The questionnaire answers provided by farmers were described either as categorical (e.g. choice from multiple answers) or continuous (e.g. providing numerical answers on the number of animals or the value of money spent). In Tables 1 and 2, the results are summarized for the univariable analyses of the categorical and continuous variables, respectively. Of the categorical variables (Table 1), the presence of cats near the feed of the animals, the presence of cat faeces in feed or water, the occurrence of abortions in sheep in the preceding 12-month period, and using a common grazing area and grazing with other animals had a *P*-value ≤.25; these variables were offered to the multivariable logistic regression model. For the continuous variables (Table 2), the total number of sheep (in hundreds owned) and the number of goats which aborted (in tens) in the flock in the preceding 12-month period, and the amount of money (in 1000 Dinars) spent on the prophylactic treatment had a P-value ≤.25 and were offered to the multivariable logistic regression model.

In the final multivariable logistic regression model (Table 3) the odds of seropositivity were significantly higher in flocks that: had cats near the feed of the animals (OR = 6.34; 95% CI: 1.63, 24.58); and had a history of abortions in sheep in the preceding 12 months (OR = 13.41; 95% CI: 2.076, 86.71) (Table 3). For every ten goats which aborted on a farm in the 12 months preceding the survey, the odds of seropositivity increased by 6.65 (95% CI 1.34; 32.86). For every 1000 Iraqi Dinars (~0.85 US Dollar) that farmers reported that they spent on the prophylactic treatment of *Toxoplasma* in the 12 months preceding the survey, the odds of seropositivity decreased significantly (OR = 0.94; 95% CI: 0.90, 0.98) (Table 3).

None of the two-way interactions of the final reduced subset model were statistically significant (all *P* values > .05). The Hosmer–Lemeshow value supported a good fit of the data in the final model (7.94; *P* = .43).

4. Discussion

The current study highlights the endemic nature of *T. gondii* in small ruminants in the north of Iraq with seropositivity rates of 42.1% (95% CI: 36.7, 47.7) and 36.1% (95% CI: 26.6, 46.5) in sheep and goats,

Table 3
Multivariable logistic regression model of herd-level risk factors associated with toxoplasmosis seropositivity among small ruminants from Duhok, north Iraq.

Variables	Category	OR (95% CI)	S.E.	*P*-value
Cats near feed	Yes	6.34 (1.63,24.58)	4.38	0.008
	No	1.0	_	_
Abortion among sheep on farm in the preceding 12 months	Yes	13.41 (2.08,86.71)	12.77	0.006
	No	1.0	_	_
Number of goats aborting in last 12 months (×10)	Yes	6.66 (1.34,32.86)	5.42	0.02
Money spent (in 1000 Iraqi Dinars) on the prophylactic treatment in the flock in the preceding 12 months		0.94 (0.90, 0.98)	0.022	0.015
Constant		–	0.057	0.007

OR, odds ratio; CI, confidence interval; S.E, standard error.

respectively. Compared to our results an abattoir based study in Saudi Arabia reported an overall seropositivity of 36.0% in small ruminants (Alanazi 2013), while a recent study in Sudan reported higher seroprevalence of *T. gondii* as 52.0% in sheep and goats (Atail et al. 2017). A study in Iran reported a slightly lower prevalence compared to our results, with *Toxoplasma* infection among sheep was found to be 35.94% and 34.32% based on serological and molecular method, respectively (Armand et al. 2016). In Syria, a survey conducted in nine provinces, on clinically healthy Awassi sheep, concluded that the mean of sero-prevalence was 44.56% (El-Moukdad 2002), which is closely related to results from the present survey in the north of Iraq.

In this study, the two tests (Latex and iELISA) used in parallel deemed suitable for field screening due to their high sensitivity, however none of could be considered as a gold standard test; they can produce false-positive results through cross-reactions with antibodies to *Anaplasma marginale* or *Neospora caninum* (Gondim et al. 2017; Rodgers et al. 1998). The Latex test detects IgG1, and IgM produced during acute cases of toxoplasmosis while the iELISA detects IgG which is the dominant immunoglobulin in chronic cases (Györke et al. 2011; Jiang et al. 2008). Consequently using these two tests in parallel is recommended in order to enhance the likelihood of detecting antibodies in both acute and chronic cases, especially in study areas where the endemic status of *T. gondii* is poorly understood (Ahmed 2010; Mouhamed et al. 2018; Parkhouse 2013).

The final regression model (Table 3) indicated that the presence of cats near the feed of the flocks was identified as a significant risk factor for seropositivity. Other studies have similarly shown the important role of cats for the maintenance and transmission of *T. gondii* in sheep and goats flocks (Cenci-Goga et al. 2013; Cenci-Goga et al. 2011; Rêgo et al. 2016). Inspection of the feed sheds/stores in the current study found the presence of a large quantity of faeces of cats on bags and in the loose feed, most likely due to cats hunting rodents present in these locations. The faeces of cats can contain millions of oocysts resulting in a severely contaminated environment (Dubey 1998). Consequently, it would be expected that more intensive flock management involving feeding animals may increase the likelihood of infected small ruminants. It is most likely that other factors favoring the contamination of feed and drinking water, such as access of cats to the shed for housing animals, water source, and feedstuff, as well as the close contact between small ruminants and the excretions of cats (Skjerve et al. 1998). Worth noting, it has been documented that goats are more susceptible to infection with *Toxoplasma* than sheep (García-Vázquez et al. 1990); this is in line with our finding that the number of abortions in goats on the farm in the preceding 12 months was a risk factor for seropositivity (Table 3).

Sheep in persistently high endemic areas could acquire the infection before their first pregnancy; thus very likely to be immune at conception. This is not surprising given that toxoplasmosis commonly results in abortion; leading to environmental contamination from cysts present in the placenta and aborted foetus of affected animals (Unzaga et al. 2014). Incorrect disposal of placenta or aborted foetuses can increase the opportunity for cats to be infected through consumption of this product, continuing the lifecycle and subsequently shedding oocysts (Hamilton et al. 2014) which may survive in the environment for many months (Dubey 1998).

Our results revealed that for every 1000 Iraqi Dinar (~0.85 US Dollar) spent per farmer per year on prophylactic treatment the odds of *Toxoplasma* seropositivity decreased (OR = 0.94). Very little is known about the use of antibiotics in small ruminant flocks in Iraq. The use of such drugs can have a profound impact on infected animals, farmer income, and public health. In livestock production, antimicrobials are often administered on a regular basis by the farmer (Dunlop et al. 1998). A decreased incidence of abortions has been reported in farms using prophylactic antibiotic treatment (Pereyra et al. 2015). There is a need for economic analysis to be undertaken to determine the benefits of controlling the disease and the costs in achieving this control. Control of toxoplasmosis could result in reduced morbidity and mortality of small ruminants (Buxton et al. 1988), as well as reducing the risk of disease in humans (Buxton et al. 1991). It is recommended that to prevent infection in small ruminants cats should be prevented from accessing feed provided to sheep and goats, they should be prevented from entering areas where sheep/goats are housed and hygiene, particularly during parturition, should be improved including burial or burning of foetal membranes and aborted lambs/kids.

5. Conclusion

Both sheep and goats showed a considerable serological prevalence of *T. gondii* using the evaluated techniques. It is recommended that farmers/shepherds adopt control measures such as preventing access of cats to placental and aborted materials of their small ruminant flocks. Abu-Dalbouh et al. (Abu-Dalbouh et al. 2012) reported that the proper disposal (incineration or burying) of foetuses significantly decreased the risk of seropositivity to *Toxoplasma* in sheep and goats. Farmers could use traps or rodenticides to control rodents, in addition to keep cats out of feed-storage facilities (Cruz-Vazquez et al. 1992).

Our findings, which are based on an extensive number of blood samples, add important epidemiological information pertinent to small ruminant production in the Middle East countries. The present survey confirmed the considerable prevalence of *Toxoplasma* in the sheep and goats population in north of Iraq. Identifying the putative risk factors can help in the development of control programs to reduce the impact of infection with *Toxoplasma*.

Conflict of interest

None of the authors of this work has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this manuscript.

Ethics statement

The study had been approved by the animal and human ethics committees of Murdoch University. All procedures were explained to

the farmers, and informed verbal consent was obtained from all participants before sampling and administering the questionnaire.

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