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Original Article

Risk Factors Analysis Associated with Seropositivity to *Toxoplasma gondii* in Sheep and Goats in Southeastern Iran Using Modified Agglutination Test (MAT)

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Abstract

Background: *Toxoplasma gondii* infection is widely prevalent in many species of warm-blooded animals including human. The aim of this study was to determine the prevalence of antibodies to *T. gondii* from slaughtered sleep and goats by modified agglutination test (MAT) in Kerman region**,** southeastern Iran.

Methods: Altogether 1340 blood samples were collected from 562 sheep and 778 goats from April to September 2005 in Kerman slaughterhouse. The sera were examined for *T. gondii* antibodies by MAT using an antibody titer of 1:20 or higher considered positive. The statistical analysis was performed by chi-square test and logistic regressions to analyze the influence of all examined factor (age, sex and type of animals) on seroprevalence of toxoplasmosis.

Results: Antibodies were found in sera of 262 out of 1340 (19.6%) samples. of 262 seropositive sera, 139 sheep (24.7%) and 123 goats (15.8%) were infected. Seropositive animals more than one year were 1.6 times more likely to be seropositive than the others were. Sheep were 1.5 times more likely to be infected than goats were (OR=1.53, 95% CI=1.15-2.04, p=0.004).

Conclusion: Serological results indicated a widespread exposure to *T. gondii* among sheep and goats slaughtered in Kerman region and suggest that consumption of raw and undercooked meat of these animals can be a probable source of human toxoplasmosis.

Keywords: Toxoplasma gondii, *Sheep, Goat, Modified agglutination test, Iran*

Introduction

Toxoplasma gondii infection is an important issue both in veterinary and in medicine as it is widely prevalent in many species of warm-blooded animals, including human and livestock, in most parts of the world (1-3). Although *T. gondii* infection in healthy adults is asymptomatic, it can be life threatening for immunocompromised individuals and newborns (4). Humans can become infected by consumption of drinking water or fresh vegetables contaminated by oocysts (5) or by ingestion of undercooked or raw meat which is the major route of transmission (6).

Sheep and goats are widely used as food animals in Iran. There have been a few studies reporting seroprevalence of *T. go*ndii in sheep and goats in some parts of Iran (7-10). Using Modified Agglutination Test (MAT) we conducted a survey on seroprevalence of *T. gondii* infection of sheep and goats in Kerman (southeastern Iran) and to investigate the possible role of these animals in transmission of human toxoplasmosis.

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Materials and Methods

Sampling

A total of 1340 sheep and goats, including 562 sheep and 778 goats coming from different neighboring regions to Kerman abattoir were selected randomly, from April to September 2005.

Study location

The city of Kerman is located in southeast of Iran and has an area of 60218 km^2, with the altitude of 1750 m above the sea level. Average temperature in July is 29 ^oC and in December is eight ^oC, while its rainfall is 100-150mm/year. Average morning humidity is 45% (range 29-68) and evening humidity as 20% (range 12-38).

Blood Collection

The blood samples were collected in tubes without anticoagulant directly from the jugular vein in the abattoir. The samples were transported to the research laboratory on the same day under cold condition and the sera were separated after centrifugation and stored at -20 ^oC until analysis.

Serological Examination

T. gondii specific IgG antibodies were examined by the modified agglutination test (MAT) as described earlier (11). The test is based on a direct agglutination of fixed tachyzoites with serum samples pre-treated with 2ME for IgM elimination. Formalin-fixed whole tachyzoites of RH strain of *T.gondii* were prepared as antigen, sera from slaughtered sheep and goats were tested at titers of 1:20 to 1:320, while positive, and negative controls were included in each test. Antibody titer of $\geq$1:20 was considered positive (12).

Statistical Analysis

The data analysis was performed by Chi-square test using SPSS 11.5. Chi-square was used to analyze the associations between seropositivity and influence of risk factors such as sex, age and type of animals. The logistic regression models were assessed using Hosmer and Lemeshow goodness-of-fit statistics. Results were presented as odds ratios (OR) with 95% confidence intervals (95% CI). The differences were considered statistically significant when $P \leq 0.05$.

Results

Overall, 1340 samples from sheep and goats were tested by MAT. Antibodies were detected in sera of 262 out of 1340 (19.6%) sheep and goats samples tested. The seropositive rates of sheep and goats were 24.7% (139/562) and 15.8% (123/778), respectively, with titers ranging from 1:20 to 1:320. The distribution of *T. gondii* antibody level in seropositive sheep and goats is shown in Fig. 1.

The results of univariate analysis of age associated with seropositivity showed an almost two-fold higher likelihood of infection in sheep more than one years old than the younger ones (P<0.001) (Table1). No significant differences in age were found in goats.

The results of univariate analysis of sex associated with seropositivity showed no significant differences in both sheep and goat (Table 1). The final logistic regression mode (Table 2) showed more than 1.6 times higher likelihood of infection in animals (sheep and goat) over one year old than the younger ones (P<0.001). The results of multivariate analysis also showed that the seropositivity is 1.5 times higher in sheep than goats (P<0.004). However, no significant difference was observed in the presence of antibodies in females as compared with males.

Table 1: *T. gondii* specific antibodies in sheep and goats in Kerman according to sex and age.

Factor (n)	Prevalence % (95% CI)	OR 95% CI	*P*-value
Goat			
Gender	15.3 (12.57-18.43)	0.849 (0.538-1.342)	0.484
Female (613)	17.6 (12.10-24.26)	1.00	
Male (165)			
Goat			
Age group	15.1 (12.38-18.20)	1.266 (0.805-1.992)	0.308
<1 year (615)	18.4 (12.78-25.22)	1.00	
>1 year (163)			
Total (778)	15.8 (13.32-18.57)		
Sheep			
Gender	26.1 (21.91-30.63)	1.324 (0.815-2.014)	0.283
Female (410)	21.1 (14.87-28.40)	1.00	
Male (152)			
Sheep			
Age group	18.7 (14.35-23.77)	1.934 (1.293-2.836)	<0.001
<1 year (283)	30.8 (25.46-36.61)	1.00	
>1 year (279)			
Total (562)	24.7 (21.22-28.52)		

Results of univariate analysis presented as odds ratio (OR) and 95% confidence intervals (CI).

Table 2: Risk factors for *T.gondii* infection in sheep and goat in Kerman.

Factor	OR	95% CI	*p*-value
Type of animal			
Goat	1		
Sheep	1.5	1.15-2.04	0.004
Sex			
Male	1		
Female	1.08	0.79-1.50	0.60
Age			
Under one year	1		
Over one year	1.6	1.21-2.16	0.001

Final logistic regression model. Results presented as odds ratio (OR) and 95% confidence intervals (CI).

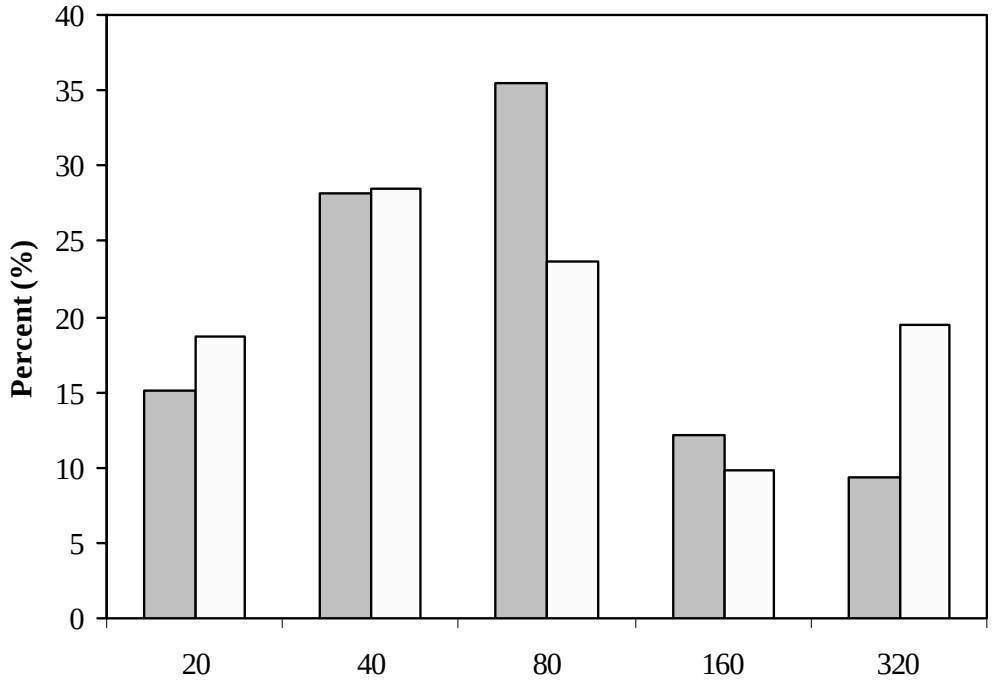

Fig. 1: Distribution of *Toxoplasma gondii* antibody level in sheep (in gray) and goats (in white).

Discussion

In the present study, the overall prevalence of *T. gondii* in sheep and goats slaughtered in Kerman was 19.6%. In addition, these results indicated 24.7% and 15.8% prevalence of *T. gondii* in sheep and goats, respectively. Hashemi-Fesharki (9) showed that seropositivity in sheep and goats were 24.5% and 19.25%, respectively. In northern Iran, a survey by Ghorbani *et al*. (7) showed a seroprevalence rate of 32.5% and 17.7% in sheep and goats, respectively. Hoghooghi-Rad and Afraa (8) showed seropositivity of 13.8% and 13.1% in sheep and goats, respectively in Ahwaz (southwest Iran). Ghazaei (10) showed seropositivity of 31% in sheep and 17% in goats in north-west of Iran. The results of our study are near the world's average (seropositivity of toxoplasmosis in sheep and goats) which is estimated 31% (13). Comparing the results of present study with those of earlier studies from other parts of the world showed more or less similarities/differences (14-18). Discrepancies in the rates might be attributed to climatic variations from one region to another (13), different serological methods used and farm management as well.

The higher infection rate in sheep than the goats is a uniform finding in many seroprevalence studies in the world (19, 20). Lower infection rates in goats compared with those in sheep may be attributed to the differences in susceptibility to *T. gondii* and in the feeding habits of the animals.

Our data also indicated that sheep >1 years old were significantly more infected than <1 year old (P=0.0001). The higher rate of infection in older animals has also been shown in previous surveys (21-23). An age-related difference in *T. gondii* infection is expected because older animals are exposed to *T. gondii* oocysts for longer periods.

In conclusion, as the goats and sheep are the main sources of meat in Kerman and regarding relatively sunny and dry conditions in the region, results of the present study suggest that consumption of raw or undercooked meat of these animals may be a probable source in the transmission of human toxoplasmosis.

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The authors declare that they have no Conflict of Interests.

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