

Toxoplasma gondii in Beja Sheep in The Red Sea State, Sudan

Awadia A.A/ Hafiz, Osman, A.Y. and Manal Y. Ishag
Veterinary Research Institute, P. O. Box 8067 (El Amarat), Khartoum, Sudan.

ملخص البحث

أجريت هذه الدراسة في ولاية البحر الاحمر، السودان، لتحديد الإيجابية المصلية لطيفلى المقوسة القوندية فى الضأن البجاوى وذلك بإستخدام إختبار تراص اللاتكس (LAT). جمعت 700 عينة دم من الضأن المذبوح بمسلخى بورتسودان وطوكر خلال الفترة من فبراير الى أغسطس 2010. وكانت الحيوانات من أماكن و جنس و أعمار و فصائل ونظم تربية مختلفة. أظهر الفحص المصلي أن 318 عينة مصل (45.4%) تحتوى على أضداد طيفلى المقوسة القوندية. كانت نسبة إنتشار أضداد المقوسة القوندية مختلفة معنويا ($P < 0.05$) فى الضأن ذى النمط البيئى ايتباوى (Etbawi). لم يكن لعوامل المكان و الجنس و العمر ونظم الإدارة تأثير معنوى على نسبة الإيجابية المصلية. كما كانت أعداد عينات المصل الموجبة لإختبار LAT: 14 عينة عند تخفيف 8:1، 67 عند 16:1، 52 عند 32:1، 142 عند 64:1، 35 عند 128:1 و 8 عند 256:1.

Summary

This study was carried out in Red Sea State, Sudan, to determine seropositivity of *Toxoplasma gondii* in different sheep eco-types (Beja sheep) using Latex agglutination test (LAT). Seven hundred blood samples were collected from Beja sheep slaughtered at Port Sudan and Tokar slaughterhouses during February-August 2010. The sheep were from different locations and of different sex, ages, eco-types and management systems. Three hundred and eighteen (45.4%) Beja sheep were seropositive for *T. gondii* antigens. Among these sera, the numbers of seropositive samples at different dilution were as follows: 14 at 1:8, 67 at 1:16, 52 at 1:32, 142 at 1:64, 35 at 1:128 and 8 at 1:256. The prevalence of *Toxoplasma* antibodies was significantly high ($P < 0.05$) in Etbawi eco-type sheep. No significant difference was observed in prevalence rate with regard to location, sex, age and management systems. In conclusion, serological results of sheep slaughtered at Port Sudan and Tokar, indicate their widespread exposure to *T. gondii*. The study suggests that consumption of raw and undercooked sheep meat can be problem source of human toxoplasmosis.

Introduction

Toxoplasmosis is an important zoonotic disease caused by the intracellular protozoan parasite *Toxoplasma gondii* (Fayer, 1981). It is capable of infecting all warm-blooded animals, including mammals, birds and humans (Fayer, 1981). The domestic cat and wild felids are the definitive hosts (Dubey and Beattie, 1988). Under favourable environmental conditions, the oocysts which are excreted by the definitive hosts, sporulate and infect intermediate hosts, mammals including animals and humans, and birds (Dubey, 1995). The disease is worldwide distributed (Dubey, 1990) and is one of the major causes of reproductive failure (Freyre *et al*, 1999). Transmission occurs following ingestion of food, feed and water contaminated with sporulated oocysts, and even by un-pasteurized milk containing tachyzoites, or by bradyzoites cysts present in tissues of food producing animals. It also occurs by blood transfusion and transplacentally (Sukthana, 2006). The main clinical signs of the disease in sheep and goats are abortion and foetal death, resorption or mummification (Dubey, 2009). Meagre data were recorded on the prevalence of ovine toxoplasmosis in Sudan (EL Bedawi *et al*, 1984; Zein Eldin *et al*, 1985; Khalil and Intisar, 2011). There was no systemic study on prevalence of *T. gondii* infection in sheep in Red Sea State; however, seroprevalence of human toxoplasmosis was found to range from 68% to 78 % (Anon, 2006 to 2010; Musa, 2008).

Sheep population in The Red Sea State is estimated to be 661 thousands; most of them are thin-tailed desert sheep, Beja sheep. The climate in Red Sea State varies from dry-hot during May to September to cool-rainy during October to April. The average temperature, relative humidity and the rainfall during the study period were 28.5 °C, 55% and 9.7mm, respectively. The objective of this study was to determine the sero-prevalence of *T. gondii* in Beja sheep in Port Sudan and Tokar, Red Sea State.

Materials and Methods

Sample collection

Seven hundred blood samples were collected from Beja sheep slaughtered at Port Sudan and Tokar abattoirs, ice kept in boxes and transported to Port Sudan veterinary laboratory. They were taken from sheep of different age, sex, management systems and ecotypes (Table 1). The sera were separated by centrifugation at 400 rpm for ten minutes and kept at -20 °C till tested.

Table 1: Blood sample collected from Beja sheep in Red Sea State according to site, eco-type, sex, age, and management system during February to August, 2010.

Variable		No. examined
Location	Tokar	400
	Port Sudan	300
Eco-types	Etbawi	100
	Geradawi	300
	Mixed	300
Sex	Female	474
	Male	226
Age (year)	< 1	36
	1-2	301
	< 2	363
Management system	Semi-intensive	400
	Extensive	300
Total		700

Latex agglutination test (LAT)

All sera were screened for *T. gondii* antibodies presence by Latex agglutination test (Toxo-Latex kit, Montgal-Barcelona, Spain). The test was carried out according to the manufacturer's instructions. Serial two-fold dilutions of the positive samples in saline solution (9g/L) were done; sera were initially screened at 1:8 to 1:256 dilutions.

Statistical analysis

The seroprevalence of *T. gondii* was calculated in percentage by dividing the total number of animal examined by the number of seropositive animals. Chi-square or Fisher Exact test was applied to test the significant of the risk factors with two levels such as age and eco-type. Risk factors with more than two categorical levels such as location, sex and management were examined using univariate analysis. Binary logistic regression was performed to test the significance revealed by the univariate analysis using backward method. All statistical tests were conducted using SPSS version 18 (SPSS Inc., Chicago) at $\alpha = 0.05$ significance level.

Results

The diagnostic sensitivity and specificity were 96.1% and 89.6%, respectively: 318 (45.4%) out of 700 Beja sheep sera were positive for *T. gondii* antibodies, at dilutions of 1:8 to 1:256 (Table 2). The prevalence was a significantly ($P < 0.05$) high in Etbawi eco-type sheep. Nevertheless, no significant differences were found between the other risk factors (locations, sex, age and management systems) and seroprevalence (Table 3).

Table 2: The prevalence rate of *T. gondii* antibodies in Beja sheep in Red Sea State at different dilutions of sera collected during February to August 2010.

Serum dilutions	No. Positive	Prevalence rate (%)
1:8	14	04.4
1:16	67	21.1
1:32	52	16.4
1:64	142	44.6
1:128	35	11.0
1:256	08	02.5
Total	318	100

Table 3: Univariate analysis for the association of the risk factors with *T. gondii* seroprevalence

Risk factors	No examined	Positive No (%)	Chi-square values	P-value
Location			1.283	0.257
Port Sudan	400	189(47.30)		
Tokar	300	129 (43.00)		
Eco-types			13.746	0.001
Etbawi	100	63 (63.00)		
Geradawi	300	130 (43.30)		
Mixed	300	125 (41.70)		
Sex			0.847	0.357
Female	474	221 (46.60)		
Male	226	97 (42.90)		
Age(year)			7.739	0.021
< 1	36	10 (27.80)		
1-2	301	129 (42.90)		
> 2	363	179 (49.30)		
Management systems			1.099	0.294
Semi-intensive	400	189 (47.30)		
Extensive	300	129 (43.00)		

Discussion

Toxoplasmosis is common in sheep world-wide, and has been reported with different prevalence ranging from 0 to 100% (Tenter *et al*, 2000). Seroprevalence of *T. gondii* in slaughtered sheep varied from 3% in Pakistan (Zaki, 1995) to 92% in France (Cabannes *et al*, 1997).

The prevalence of toxoplasmosis reported in the current study (45.4%) is similar to that reported in Morocco, 45.5% (Sawadogo *et al*, 2005) and in Bulgaria, 45.7% (Prelezov *et al*, 2008). It is higher than the world's average which is estimated to be 31% (Fayer, 1981) and than those of different African countries which ranged from 5 to 39% including Sudan (ZainEldin *et al*, 1985; Deconinck *et al*, 1996 and Nada *et al*, 2007). However, it is lower than those reported for sheep in south-west Iran, 71% (Hosseini *et al*, 2008) and Sudan, 57% (Khalil and Intisar, 2011). The worldwide difference in seroprevalence of *T. gondii* infection may be attributed to the difference in serological tests used, management systems, environmental differences between geographical areas and different topographical features that affect oocysts development and survival (Fayer, 1981).

The current study shows that some risk factors may affect infection rates. The prevalence rates of *T. gondii* infection in Port Sudan and Tokar are high (47.3% and 43%, respectively) however, there is no significant difference ($P > 0.05$). This high seroprevalence of *T. gondii* antibodies in sheep may be associated with the presence of domestic and wild cats, in almost

every farm sampled, that resulted in environmental contamination by oocysts excreted in their faeces.

Etbawi eco-type sheep (63%) had significantly higher prevalence rate ($P < 0.05$) than those of mixed and Geradawi eco-types. Van Der Puije *et al* (2000) also found significant breed differences in susceptibility to *T. gondii* infection; they related the differences to the fact that certain breeds are more resistant.

Sex did not show any significant effect ($P > 0.05$) on prevalence of *T. gondii* infection. This finding agrees with Bonyadian *et al* (2007). Moreover, this study shows that sheep of 1-year-old has significantly lower prevalence rate ($P < 0.05$) than that of more than 1-year-old. The high rate of infection in elder animals has also been reported by Ragozo *et al* (2008). The age-related difference in *T. gondii* infection is expected because old animals are continuously exposed to *T. gondii* oocysts for long periods and, therefore, horizontal transmission by ingestion of sporulated oocysts takes place.

Seroprevalence of *T. gondii* antibodies in Beja sheep kept under semi-intensive and extensive management systems show insignificant difference ($P > 0.05$). This confirms the findings of Savio and Nieto (1995) in Uruguay and Ciamak Ghazaei (2005) in Iran, which could be related to contamination of pastures by oocysts excreted by cats. In the current study, this situation may be attributed to the fact that cats are found throughout the Red Sea State (around sheep farms). Similarly, in South Africa, Nada *et al* (2007) related high prevalence of *T. gondii* infection in sheep kept under intensive management system to the presence of domestic cats living near feed stores, for controlling rodents. This situation exposed sheep to *T. gondii* oocysts shed by cats. Antibody titres reported in this study, suggest that the majority of sheep were probably chronically infected.

It may be concluded that toxoplasmosis is prevalent in Beja sheep in Red Sea State, particularly in Etbawi eco-type. The high infection rate in sheep (45.4%) may have local implications as mutton is preferable as undercooked meat (Salat and Gurar). Therefore, it could be an important potential source of human toxoplasmosis.

The data presented in this study suggest that it is possible to reduce the risk of *T. gondii* infection in sheep by preventing their direct contact with cats using intensive management systems. However, countrywide studies are needed to assess the rate of abortion caused by this parasite in sheep and other animal species, using serological tests or molecular techniques.

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