Journal of Animal and Veterinary Advances 10 (21): 2779-2782, 2011
ISSN: 1680-5593
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Toxoplasmosis in Goats in Riyadh, Saudi Arabia

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Abstract: During an outbreak of caprine abortion in a farm in Riyadh, Saudi Arabia, serum samples from 57 Aardi goats with recent history of abortion and stillbirths (herd 1) along with samples from 30 control goats without abortion history (herd 2) were analyzed for anti-*Toxoplasma gondii* antibodies using indirect Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Haemagglutination (IHA) tests. Antibodies against *T. gondii* were detected by ELISA in 63.2% of the goats in herd 1 and 50% of the control goats (herd 2). About >80% of the former goats had ELISA percent optical densities (O.D.%) ≥100 while >86% of the control goats were weakly positive with ELISA O.D.'s (%) <100. IHA tests were positive in 51.7% of herd 1 versus 30% of control goats. About 78% of all IHA positive goats in herd 1 had antibody titers ranging between 1:640-1:2560 including nearly 30% with titers ranging between 1:1280-1:2560. By contrast, all IHA positive goats in herd 2 had titers ranging only between 1:80-1:160, except one goat with a titer of 1:640 and none with higher titers. IHA tests for IgM antibodies using 2-mercaptoethanol treated sera revealed IgM antibodies in 16 (28%) goats with abortion history (herd 1) and none in control goats (herd 2). Focal pathological changes were observed in the fetal brain and the placenta of some of the aborted goats. These findings strongly suggest *T. gondii* as the cause of abortion in herd 1.

Key words: *Toxoplasma gondii*, goats, ELISA, IHA, Riyadh, Saudi Arabia

INTRODUCTION

Toxoplasmosis is a worldwide protozoan zoonosis caused by a heteroxenous coccidium, *Toxoplasma gondii* which uses cats and other felids as definitive hosts and a wide range of mammals and birds as intermediate hosts (Mead *et al*., 1999). In man, the infection may be acquired congenitally or post-natally. In immunocompetent persons, post-natal infection is usually asymptomatic. However, serious manifestations including neurological disorders, encephalitis and blindness may occur in congenitally infected children while a fatal systemic disease may occur in post-natally infected immunocompromised patients (Becker *et al*., 2010).

In animals, particularly small ruminants, toxoplasmosis is a major cause of abortion, stillbirth and perinatal mortality (Dubey, 2009a, b). Human toxoplasmosis and associated clinical manifestations were reported throughout the Kingdom of Saudi Arabia. However, little is known about the prevalence of *T. gondii* in goats under field conditions in that country. A serological prevalence of caprine toxoplasmosis ranging between 14-96% has been reported in different countries worldwide (Fayer, 1981). The aim of the following study was to elucidate the prevalence of toxoplasmosis in a herd of Aardi goat in Riyadh region where an outbreak of abortions and stillbirths had occurred and to compare it with a control herd of the same breed.

MATERIALS AND METHODS

Total 87 female goats of the indigenous Aardi breed, aged 2-4 years were investigated. The animals consisted of a herd of 57 goats in which numerous abortions and stillbirths had recently occurred (herd 1). Another herd of 30 goats in a nearby location without abortion history, served as controls (herd 2). Both herds were housed in pens and fed on a ration comprising Rhodes grass (*Chloris gayana*), alfalfa (*Medicago sativa*) and commercial concentrate cubes (13% crude protein) they were vaccinated against brucellosis and other enzootic diseases in the region and were given anthelminthic medication and coccidiostats as necessary. About 5 mL blood samples were collected by jugular venipuncture from each goat into plain vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, N.J., USA) and allowed to clot at room temperature for 3 h. Serum was separated from the clotted samples by centrifugation (1,500 g for 15 min) and stored at -20°C. Samples from male goats in either herd were not submitted to the laboratory.

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Specimens of brain and placenta of five aborted fetuses from flock A were also submitted. Specimens of brain and placenta of five aborted fetuses from herd 1 were available.

Serological tests: Tests for antibodies against *T. gondii* were performed using an indirect ELISA immunoassay designed to screen IgG antibodies against toxoplasmosis in ruminants (Chekit-Toxotest, IDEXX laboratories, Bommeli Diagnostics, AG, Bern, Switzerland). A horseradish peroxidase-labeled monoclonal anti-ruminant IgG conjugate was used and the test was performed in microtiter plates according to manufacturer's procedure. Known positive and negative goat sera were included in each test plate. The tests were performed in duplicates and the optical density was determined at 450 nm using a microtiter plate reader. The percent Optical Density (OD%) of the samples was expressed according to the following equation:

$$\text{OD (\%) of the test sample} = \frac{100\ (S - N)}{(P - N)}$$

where, S, N and P are the O.D. values of the test, negative and positive sera, respectively. Samples giving percent optical density (O.D.%) of ≥30-99% were considered weak positive. Samples with O.D.≥100% were considered positive (O.D. 100-149%) or strong positive (O.D.≥150%). The test was validated based on the positive and negative control O.D. (%) values. The same samples were also tested for specific anti-toxoplasmosis antibodies using an Indirect Hemagglutination (IHA) test sensitive for both IgG and IgM antibodies (Toxo-HAI Fumouze; Fumouze Diagnostics; Cedex, France).

The test was performed in microtiter plates following manufacturer's recommended procedure and control positive and negative sera, provided by the manufacturer were included in each test plate. Test samples showing reactivity at 1:80 dilutions were considered positive and were further diluted to the end-point titer. For determination of IgM antibodies, the sera were treated with 0.1 M 2-mercaptoethanol solution and a decrease of ≥2 titers were taken to indicate the presence of IgM in the sample.

Histopathology: Pieces of fetal brains and placenta were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μ thickness and stained with hematoxylin and eosin.

Statistical analysis: Results were statistically analyzed using SAS 8.1 program for Windows. Chi-square (χ^2) test was used to compare prevalence in the two herds. Significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The prevalence of anti-*T. gondii* antibodies as determined by indirect ELISA and IHA tests in goats is shown in Table 1. Anti-*T. gondii* antibodies were detected by ELISA in 63.2% of goats with recent history of abortion including stillbirths (herd 1) as compared to 50% of the control goats (herd 2). The difference in prevalence between the two herds was statistically significant ($p<0.05$). More importantly, the O.D. (%) reached or exceeded 100 in over 80% of ELISA positive samples in herd 1 goats with 10 (~28%) of these animals giving O.D. (%) ranging between 150-200. Conversely, >86% of control goats (herd 2) were only weakly positive, i.e. with O.D. (%) <100 while only two animals (13.3%) had O.D. (%) >100 (namely, 117 and 126). On the other hand, IHA test was positive for antibodies against toxoplasmosis in 51.7% of the goats ub herd 1 versus 30% of control (herd 2) goats ($p<0.0005$).

As observed in ELISA results, the vast majority (78%) of IHA positive goats in herd 1 had antibody titers ranging between 1:640-1:2560 whereas all IHA positive goats in the control herd had low titers ranging between 1:80-1:160 with the exception of one goat with a titer of 1:640 and none with higher titer. All ELISA-positive animals in herd 1 except three were also positive by IHA. Statistical analysis showed significant agreement between ELISA and IHA results ($p<0.0005$). Test for IgM antibodies using 2-mercaptoethanol treated sera revealed specific IgM antibodies against *T. gondii* in 16 (28%) goats in herd 1 and none in the control herd. Fetal brain samples showed no gross changes while the placental cotyledons were edematous, congested and speckled with whitish spots.

Histopathological examination revealed focal areas of mononuclear aggregates and microgliosis in the fetal brain cortex while congestion and multiple foci of necrosis, mononuclear cellular infiltration and calcification were observed in the placenta. A single extracellular *T. gondii* tachyzoite was seen in sections of the placenta of one animal.

Various serological tests are available for screening goats for antibodies against *T. gondii* among these, ELISA and IHA tests are widely used due to their reliability and easiness (Hashemi-Fesharki, 1996; Vitor *et al*., 1999; Bisson *et al*., 2000; Figueiredo *et al*., 2001; Prelezov *et al*., 2008; Sharma *et al*., 2008; Carneiro *et al*., 2009). In the present study, both of these tests were used which detected anti-*T. gondii* antibodies in a significant proportion of the tested goats. The study not only showed higher prevalence but also higher titers of antibodies against *T. gondii* in goats with recent

Table 1: Serological prevalence of Anti-*Toxoplasma gondii* antibodies in female Aardi goats in Saudi Arabia using ELISA and IHAT

					O.D. (%)					
Test	Herd	Total	Negative (%)	Positive (%)	30-99		100-149	150-199		≥200
ELISA	1	57	21 (36.8)	36 (63.2)	7 (19.4%)		19 (52.8%)	9 (25.0%)		1 (2.8%)
	2	30	15 (50.0)	15 (50.0)	13 (86.7%)		2 (13.3%)	0		0
					Antibody titers					
					1:80	1:160	1:320	1:640	1:1280	1:2560
IHA	1	57	16 (48.3)	41 (51.7)	1 (2.4%)	3 (7.3%)	5 (12%)	20 (48.8%)	11 (26.8%)	1 (2.4%)
	2	30	21 (70.0)	9 (30.0)	5 (55.6%)	3 (33.3%)	0	1 (11.1%)	0	0

history of abortion as compared to control goats. Nearly 30% of all IHA-positive goats in which abortion occurred (herd 1) had IHA titers exceeding 1:2000 while none of the control goats had IHA titer beyond 1:640. In fact, 8/9 (89%) goats in the control herd had titers of 1:80-1:160 and only one with a titer of 1:640. Similarly, around 80% of all ELISA-positive goats with abortion history had O.D. (%) of 100 or more compared to only 13% of the control goats. The study further revealed specific IgM antibodies only in the sera of aborted goats indicating active or recent infection (Figueiredo *et al*., 2001; Prelezov *et al*., 2008). These results, coupled with histopathological findings, strongly suggest that *T. gondii* was responsible for abortion in these animals.

Association between caprine toxoplasmosis and reproductive problems is well known (Leite-Browning, 2006). These animals usually become infected with the parasite after ingesting feed or water contaminated by infected cat's feces. The infection then passes from the goat's small intestine to the blood stream which carries it to different parts of the body. It may penetrate the placenta of pregnant does causing various reproductive problems such as fetal resorption, abortion or stillbirth. The parasite may also become encysted in the goat's brain, muscles and other organs where it may remain viable for years.

In Saudi Arabia, the population of goats is around 3.5 million belonging to different breeds of which the Aardi breed is the most popular. These medium-sized goats are adapted to arid conditions and are highly valued by Saudi citizens for their milk and meat. Most of them are still reared by traditional methods in rural areas but small numbers of Aardi goat herds are kept in farms around main cities for milk production. A few goats are also kept at home by some individuals.

Toxoplasmosis can be an important infection in these animals causing significant economic loss to producers and can also be transmitted from goats to humans through the consumption of goat milk or undercooked meat (Pepin *et al*., 1997; Figueiredo *et al*., 2001). It is therefore, highly imperative to undertake detailed investigations to evaluate the present situation of caprine toxoplasmosis in Saudi Arabia, from both the veterinary and public health standpoints.

Comprehensive studies should thus be conducted to elucidate the prevalence and distribution of caprine toxoplasmosis in different parts of the kingdom and to investigate its epizootiological features, economic consequences and public health impact. Studies are also needed to isolate infective stages of the parasite in goat milk and meat intended for human consumption (Dubey, 2009a).

CONCLUSION

Antibodies against *T. gondii* are common among goats in Riyadh region, Saudi Arabia, Serological tests indicate that this protozoan can be an important cause of caprine abortion in the region as evidenced by high antibody titers and detection of IgM antibodies against *T. gondii* in recently aborted goats.

ACKNOWLEDGEMENTS

The researchers are grateful to the Deanship of Scientific Research and the Agricultural Research Center of the College of Food Sciences and Agriculture, King Saud University, for financial support. Thanks are also extended to Messrs Arabian Agricultural Services Company (ARASCO) for additional support and to Mr. Masoud N. AlOtabbi for technical assistance.

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