ROLE OF TOXOPLASMA GONDII IN ABORTION OF EWES IN AHVAZ (SOUTH-WEST IRAN)

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Abstract

To investigate the role of Toxoplasma gondii in abortion of ewes in Ahvaz, South-West Iran, a commercial ELISA kit and a modified agglutination test (MAT) were used for the detection of antibodies to Toxoplasma gondii in ewes. Out of 150 examined ewes, 100 of them had recently aborted and 50 did not have any abortion history. Toxoplasma gondii antibodies were detected in 109 (72.6%) ewes by ELISA and in 104 (69.3%) ewes by MAT. Seropositivity in the group with abortion history was 85% by ELISA and 80% by MAT and in the group with no abortion history was 58%, by both ELISA and MAT. Good correlation was seen between the ELISA and MAT results. The results suggested that the ELISA and MAT were good tools for epidemiological studies of Toxoplasma gondii infection in sheep. Toxoplasma gondii may play an important role in ewe’s abortion in Ahvaz.

Key words: ewes, Toxoplasma gondii, abortion, immunodiagnosis, Iran.

Toxoplasma gondii is a widespread protozoan parasite that can determine serious disease in humans, small ruminants, and many other warm-blooded mammals (19). The disease is caused by an obligate intracellular, protozoan parasite Toxoplasma gondii (3). Toxoplasmosis is a common infection of sheep worldwide (7, 10) and it is recognised as one of the major causes of infectious reproductive failure in many countries (1, 9, 13, 18). Toxoplasmosis causes foetal resorption, abortion at any stage of pregnancy, foetal mummification, stillbirth, or birth of live but weak offspring in sheep. The incidence of abortion is very high in Iran with inadequate diagnostic data and prophylaxis. The economic losses due to lamb mortality and missed lactation are probably very high.

The diagnosis of T. gondii abortion is based on the detection of specific antibodies in the adult population by serological tests and the visualisation of characteristic histological lesions in the placenta and brain of the aborted foetuses (2, 3, 8).

Since there is little information on the prevalence of infection in South-West Iran, the aim of the presented study was to estimate the prevalence of the T. gondii antibodies in ewes, and subsequently to estimate the role of this parasite in the abortion of sheep in Ahvaz, Iran. Furthermore, to determine the agreement and correlation between commercial ELISA and a modified direct agglutination test for the detection of T. gondii antibodies.

Material and Methods

Flocks, in which abortions had been reported, were investigated. Totally, 150 blood samples were collected from 100 ewes, which recently aborted and from 50 ewes without abortion history, which served as a control group. The samples were centrifuged at 1 000 x g and the supernatants were frozen at −20°C until the examinations were performed. The presence of antibodies to T. gondii were tested by an indirect ELISA and a modified agglutination test (MAT), as described below.

The ELISA was performed by a commercial kit (Institute Pourquier, France) according to the manufacturer’s instructions. The samples were tested in single wells unless there was a discrepancy between qualitative results of the MAT. The results were expressed as the percentage of the mean absorbance values of the sample (S) to the mean absorbance value of the positive (P) control sample provided with the diagnostic kit. The resultant S–P ratio was expressed as a percentage (S/P%). According to manufacturer’s recommendation, sera with S/P% ≤40% should be regarded as negative, between 40% and 50% as suspicious, and ≥50% as positive. The sera were also tested for the presence of T. gondii antibodies using the MAT based on the direct agglutination of fixed parasites with sera pre-treated with 2-mercaptoethanol to prevent non-specific IgM agglutination, as described by Desmonet and Remington (5) and Dubey and Desmonets.

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The detection of antibodies between the MAT and the indirect ELISA for the κ-agreement based on Kappa statistic (κ) was used to test the level of agreement between the two tests after taking into account the correlation between ELISA and MAT for detecting T. gondii antibodies. Kappa and its 95% CI, was used further to measure the degree of agreement between the two tests after taking into account the probability of agreement by chance alone. Strength of agreement based on κ was judged according to the following guidelines: <0.2=slight; 0.2–0.4=fair; 0.4–0.6=moderate; 0.6–0.8=good; >0.8=very good (6).

Results

Out of 150 serum samples tested, 109 (72.6%) samples tested by ELISA were positive for T. gondii antibodies, whereas 104 (69.3%) samples were positive by MAT using a positive cut-off of an 80 titre. MAT titres ranged from 16 to 2,560. Serological results showed that out of 100 ewes, which aborted 85 (85%) were positive by ELISA and 80 (80%) were positive by MAT whereas in ewes without any abortion history, the positive results were demonstrated in 29 (58%) samples out of 50 samples tested by ELISA and MAT. Statistical analyses of the results showed a significant difference between positive results obtained in both tests (P<0.05) in the examination of the sera from ewes which aborted and which did not abort. McNemar’s test showed a correlation between positive results of the two applied methods and there was a good agreement between MAT titres and optical density of ELISA by Kappa test (κ = 0.88).

Discussion

T. gondii is a protozoan that infects mainly the cat as a definitive host and the sheep as a very important intermediate host. In ewes, this parasite is now considered to be the major cause of abortion worldwide (9). In Iran, however, there was no any data on the role of toxoplasmosis in abortion of ewes, so this study was carried out for this purpose and also to estimate the agreement and correlation between MAT and ELISA for serodiagnosis of toxoplasmosis.

Since direct observation of cysts in tissues is not a suitable diagnostic method to be carried out on live animals, the serological techniques appear to be the methods of choice. In veterinary laboratories, ELISA is very useful to diagnose pathogens like T. gondii, which infect various animals, because its format allows testing many species. On the other hand, several other serological tests are available and considered by several authors as reference tests for the diagnosis of toxoplasmosis. A modified agglutination test and indirect fluorescent antibody test (IFAT) are the major recommended tests for this purpose. The MAT and ELISA were compared for the detection of antibodies to T. gondii in naturally infected market-aged pigs by Gamble et al., (14). Their study showed 88.6% sensitivity for ELISA, 85.7% sensitivity for MAT, and 98.0% specificity for ELISA and 94.6% specificity for MAT. They also reported good correlation between MAT and ELISA.

The present study is the first report comparing the seroprevalence of T. gondii infection in ewes, which aborted and did not aborted in Ahvaz, South-West Iran. In Northern Iran the seroprevalence of T. gondii in sheep (35%) and goats (30%) was found by Sharif et al. (17). Additionally, Hashemi-Fesharki (15) examined serum samples from sheep, goats, and cows from Iran for T. gondii antibodies by the use of the LAT and indirect haemagglutination test and found the antibodies in 24.5% of sheep and 19.25% of goats. However, no antibodies were detected in cow sera diluted 1:8 and 1:64, and T. gondii was not found in tissues of 300 aborted foetuses from cows by direct microscopy and bioassay in mice (15).

Our study showed that prevalence of T. gondii in ewes was high, but in ewes that aborted the presence of T. gondii antibodies was significantly higher than in ewes with no abortion history by using both ELISA and MAT (P<0.05). Thus, T. gondii may be one of the important agents resulting in abortion in ewes of Ahvaz, Iran, and the consequent risk for humans of acquiring toxoplasmosis from consumption of sheep meat may be greater in this region. In Europe, up to 63% of human infections are attributable to the consumption of undercooked or cured meat products (4). The decline of the incidence of human toxoplasmosis is thus directly related to the reduction of the infection in animals, which may be achieved through suitable control strategies.

In our study, the high seroprevalence of T. gondii antibodies in sheep may be due to the fact that cats were extensively distributed throughout the province. A high prevalence of toxoplasmosis within hot and humid environments compared to cold and dry ones is attributed to the longer viability of T. gondii oocysts under humid conditions (11, 12). For this reason, the higher T. gondii prevalence in South-West Iran could be attributed to the high relative environmental conditions such as humidity that exists in this province. Our results also showed a higher prevalence of T. gondii in Iran (72.6% by ELISA and 69.3% by MAT) as compared to the world average, which is estimated at 31% (11). The differences observed could be due to the diagnostic techniques used in the different regions, frequency of felines on the farms, age of the animals, and the climatic variations from one region to another (10, 16). On the other hand, the reason of this difference may be due to the poor management conditions in related sheep flocks.

Furthermore, in this study we found a good correlation between ELISA and MAT for detecting T. gondii antibodies and we believe that MAT as ELISA is
a very useful, easy, and non-expensive method for the diagnosis of toxoplasmosis.

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References


