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IMMUNOLOGICAL STUDIES OF TOXOPLASMOSIS IN THE SHEEP MODEL IN BAGHDAD /IRAQ

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ABSTRACT
A total of 102 Iraqi breeds mature ewes and rams (6 rams and 96 ewes') examined whether *Toxoplasma gondi* associated abortion occur in successive lambing, submitted from different points of north and central Baghdad/Iraq, were analyzed to determine the role of *T. gondii* in abortion and to study advanced techniques in diagnosis of the Toxoplasmosis infection (serology—serum electrophoresis - latex agglutination-Eliza and phagocytic activity). *We identified 96 ewes that gave birth to lambs on at least 1 successive year over and 6 males our study period, 2010–2011.* Blood samples from these animals were analyzed by latex agglutination to determine infection status with *T.gondi***. .** Phagocytic activities by ELIZA assay and serum electrophoresis during study period, 2010–2011.Results revealed that animals were highly infected with toxoplasmosis the infectivity percentage was 38% for total animals (4% males and 34% while the infectivity percentage for ewes with toxoplasmosis was 41% (11.59 rams and 29.41% ewes) highly titer of toxoplasmosis antibodies at the level of 512 were recorded 20% of ewes. While the brucella antibodies titers in other ewes were at the level of 128. Present study results reported highly infectivity percentage with toxoplasmosis in sloutring rams and ewes submitted from different points of north and central Baghdad/Iraq.

KEY WORDS: Sheep, abortion, toxoplasmosis

INTRODUCTION

Sheep are important in the epidemiology of *Toxoplasma gondii* infection; asymptomatic sheep can serve as a source of infection for humans (1). Sheep are important to the economy of many countries because they are a source of food for humans. Sheep are commonly infected with the protozoan parasite, *Toxoplasma gondii*. Infection with the parasite may cause early embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death (2 and 3). Severity of infection is associated with the stage of pregnancy at which the females becomes infected, the earlier in gestation, the more severe the consequences. Infected sheep meat is a source of *T. gondii* infection for humans and carnivorous animals (4). Most sheep acquire *T. gondii* infection after birth, and less than 4% of persistently infected sheep transmit the parasite vertically to the next generation (5). Following infection with *T. gondii*, sheep develop humoral and cell-mediated immune responses against the parasite that provides effective protection against disease in subsequent pregnancies (6). Diagnosis of toxoplasmosis is useful for human and animal health. Several techniques are employed for the diagnosis in sheep and population (7). Toxoplasmosis is a serious disease in humans and animals where it can cause abortion or congenital infection if a mature female is exposed to disease for the first time during pregnancy(8 and 9) . Infection prior to pregnancy normally results in immunity and which is capable of protecting the foetus (10). Establishment of a rapid, highly specific, and accurate method for diagnosis of Toxoplasma gondii infection is essential to control and prevent zoonotic toxoplasmosis, Laboratory diagnostics of toxoplasmosis depends primarily on serological methods detecting specific antibodies (11). Since these methods do not always enable specific and sensitive recognition of the infection and phase of toxoplasmosis, the search for new diagnostic tools continues (12 and 13). Congenital infection with *Toxoplasma gondii* is an important cause of abortion in sheep worldwide (14). The cat is the definitive host of the parasite, and infected cats may shed millions of oocysts in their feces resulting in extensive environmental contamination and an important source of infection for grazing herbivorous Toxoplasmosis, caused by the intracellular protozoan parasite *Toxoplasma gondii*, was first described in 1954 and while the incidence of ovine infection is difficult to define, it has been reported that in the UK it is responsible for between 1 and 2% of neonatal losses per annum. Recent reports have suggested that sheep persistently infected with *T. gondii* may pass infection to the fetus in subsequent pregnancies more readily than previously thought (15,16 and 17).

MATERIALS AND METHOD

Present study came out to add some understanding about the infection percentage of toxoplasmosis and immunological study in mature rams and ewes in different points of north and central Baghdad/Iraq. Experimental design is given in table (1).The latex test or buffered Toxoplasma gonadi antigen test is a rapid slide agglutination procedure developed for the direct detection of Toxoplasmosis antibodies in human and animal sera. The antigen suspension is reactive with both immunoglobulin G and immunoglobulin M antibodies being the former detected earlier (sub-clinical) and over a large period during the disease (chronic stage) than the conventional tube agglutination test. The assay is

performed by testing the buffered suspension (PH 3.6) of latex peads coated with toxoplasma gonadi antigens .The presence or absence of a visible agglutination indicates the presence or absence of antibodies in the samples tested. This experiment was carried out on coagulated blood samples for mature males and females' sheep of different region of Baghdad at period from 1-11-2010 to 31-10-2012. The animals were subdivided into five groups: Croup 1 represents healthy rams, Group 2 represents healthy ewes, Group 3 represents infected rams, Group 4 represents weak positive ewes and Group 5 represents strong positive ewes. The tests have been run following the steps outlined in the procedure as described in kit information (Omega U.S.A). Degrees of agglutination visible macroscopically represent the positive result while negative samples give smooth suspension with no visible agglutination. Phagocytic activity for polymorph cells in peripheral blood have been run following the steps outlined in the procedure as described by (18) for evaluation of oxidation burst ratio in polymorph cells. Serum electrophoresis of serum prepared from coagulated peripheral blood samples for evaluation alpha Beta and gamma regions percentage was carried out using a commercially available kit (Hellabio, Spain kit). The Hellabio Agarose Gels for protein electrophoresis is intended to be used for *in vitro* diagnosis, and they enable quantitative and qualitative estimation of proteins in serum and other biological materials. Statistical Analysis: Data were analyzed for investigated parameters and given in terms of means ± standard errors (S.E.), the mean differences were assessed by analysis of variance (ANOVA), least significant difference (LSD) and Duncan multiple rang test, using the computer program SPSS (Statistical Package of Social Sciences) version 7.5. The difference was considered significant when the probability value was equal or less than 0.05.

TABLE 1: Infected percentage of ewes with Toxoplasmosis.

Groups	No. of Ewes	Latex agglutination test for toxoplasmosis				infectivity%
		1/16	1/32	1/64	1/128	
1	2	Negative	Negative	Negative	Negative	0
2	27	Negative	Negative	Negative	Negative	0
3	1	Negative	Positive	Positive	Positive	1.96
4	2	Negative	Positive	Positive	Weak Positive	3.92
5	19	Positive	Positive	Positive	Strong Positive	37.25

TABLE 2: Phagocytic activity percentage (NBT by ELIZA assay of healthy and infected ewes with Toxoplasmosis

Phagocytic activity%	No. of ewes	Groups
61.5	2	1
43	27	2
120	1	3
64	2	4
97.2	19	5

TABLE 3: Serum electrophoresis % of healthy and infected ewes with Toxoplasmosis.

Gamma % region	Beta % region	Alpha-2 % region	Alpha-1 % region	No. of ewes	Groups
14	20	7.35	1.16	2	1
14.86	23.5	6.02	2.17	27	2
16	20	3	2	1	3
15.5	29	7.6	1.25	2	4
21.5	23.3	5.5	1.6	19	5

RESULTS AND DISCUSSION

The Latex agglutination test is internationally recommended for the screening of Toxoplasmosis in small ruminants (2). An important problem affecting the sensitivity of the latex agglutination test concerns the standardization of the antigen. These standardization conditions, which seem to be suitable for the diagnosis of toxoplasmosis infection in sheep, limit the sensitivity of the test resulting in reduced performance for the diagnosis of toxoplasmosis infection in ewes (1). These phenomena have raised serious questions over the efficacy of using the latex agglutination test as an individual test in small ruminants (7). However, if the antigen is standardized differently to give a higher analytical sensitivity, the diagnostic sensitivity is much-improved (4). The results (table 1) revealed high infectivity percentage in a series of ewes sera of group 5 (37.25%) while the lowest infectivity percentage (0 %) was showed in a series of ewes sera of group 1 and 2, the highly infectivity percentage in ewes was due to the selectivity of owners to slaughtering the recurrent aborted ewes.

Results of phagocytic activity % by NBT test evaluated by ELIZA assay (table 2) had showed a significantly increased percentage ($P \leq 0.01$) in group 1, 3 and 5, (61.5% , 120%, 97.2% respectively) and at level ($P \leq 0.05$) in group 4 (64 %)as compared with group 2(healthy control). These are in favor of such agreement, and activate murine macrophages, which can destroy intracellular parasites. Nitro Blue Tetrazolium reduction by polymorph nuclear cells may require oxidative metabolism by the hexose monophophate shunt, and is impermeable to cell membrane, but it enters the cell during the process of Phagocytosis, and it is reduced by diaphoreses activity within phagosom (19). Although macrophages and monocytes possess phagocytic mechanisms in the resting state, these mechanisms can be enhanced, and new mechanisms can be expressed when they are activated (16). Activation can occur through exposure to microbial products (Toxoplasm gondi antigen), Both innate and adaptive immune responses are activated following *T. gondii* infection (7). The lowest results of phagocytic activity% in groups 1 and 2 represent the phagocytic activity percentage of phagocytic monocytes, Neutrophils and natural killer cells) mechanisms in the resting state while the high phagocytic activity% in group 3 and 5 represent the infection in male and female sheep, moderate phagocytic activity in group 4 indicate chronic or carrier state (7). Such picture is enhanced by the findings of our study and confirmed by other researches (6, 14, 20, and 61). Monocytes and Neutrophils, which are important in resistance to early stages of Toxoplasmosis infection (19). The results of gamma globulin fraction were given in table (3) for each group of sheep, revealed a significant increase in the percentage of gamma globulin fraction in group 3 (16%), and 4 (15.5%) while the highest percentage was recorded in group 5 (21.5%) as compared to group 2 (14.86%) . The results of beta fraction were given in table 3, All groups of sheep were showed increased percentage of serum beta fraction in infected groups as compared with healthy group, but a significant increased was observed in groups 4 (29 %) and 5 (23.3%), while the lowest percentage was recorded in groups 1 and 3. The results of alpha fraction (table 3) showed slightly decreased percentage of serum alpha-1 fraction in groups 3 (2%), 4 (1.25%) and 5 (1.6%) as compared to healthy group 2 (2.17 %). The results of alpha-2 fraction showed a significant decreased percentage in group 3 (3%) and 5 (5.5%), while group 4, showed significant increased percentages as compared to healthy group 2 (6.02%).

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