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Evaluation of Immune Response to Brucella Infection in Sheep.

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ABSTRACT

Brucellosis in sheep and goats is a zoonotic infection (excluding Brucella ovis) with important effects on both public health and animal health. The disease is widespread in many areas of the world, particularly in some Mediterranean and Middle Eastern countries. the present study aims to find out the prevalence of brucellosis in sheep in one of the areas of An Najaf El-Ashraf city, and study of the immune response by measuring the levels of and interleukin-10 (IL-10). Current study conducted in the Najaf province (Al-Barakyia) during the period between September 2013- March 2014, fulfilled by using Rose Bengal and Enzyme Immunosorbent Assay (ELISA) tests for estimation of brucella antibodies. A total number of 48 serum samples were collected from sheep and stored at -20°C. The frequency of positive sera by Rose Bengal test (RBT) was 20% and by ELISA technique was 25%. The level of interleukin 10 (IL-10) was estimated in all surveyed animals, 12 sera samples were showed an elevated IL-10 levels, all were positive with ELISA serological test. The detection of significant concentration of IL-10 among the seropositive cases may be indicated an active infection of brucella with type 2 cytokine production. The evaluation of immune response of vaccinated sheep with brucella REV1 vaccine, depending on the estimation of IFN-γ and IL-10 ratio that will reflect the cytokines induced during vaccination and consequently, the effective or not immune response.

Keywords: Brucellosis, sheep, Rose Bengal, ELISA, Interleukin 10

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INTRODUCTION

Brucellosis in sheep and goats is a zoonotic infection (excluding *Brucella ovis*) with important effects on both public health and animal health. The disease is widespread in many areas of the world, particularly in some Mediterranean and Middle Eastern countries [1,2].

Brucellosis in small ruminants is caused mainly by *Brucella melitensis*, which was the first species in the genus *Brucella* described. It is the most virulent one and most widely encountered of all the species [3]. Brucella melitensis infection may cause abortion in pregnant animals or orchitis and epididymitis in adult males of sheep, goat and cow which may result in infertility [4]. The number of aborted animals is progressively increased in Middle East countries including Iraq during the last decades [5].

In Iraq, the prevalence of brucellosis in sheep was 0.9% in 1979, while several seroprevalence studies conducted in the recent decades showed that the prevalence of brucellosis in sheep and goat was increased markedly, in 2005 the prevalence was 8.59% [6].

Brucella spp. are facultative intracellular pathogens which resist killing by neutrophils, replicate inside macrophages and in "non-professional" phagocytes and maintain a long lasting interaction with the host cells. Therefore, host control of infection requires a set of cells and factors like CD4+ and CD8+ T lymphocytes, T-helper 1(Th1) type cytokines such as (IFNγ) and TNFα, and activated macrophages and dendritic cells (DC) which together promote a complex response against *Brucella*. It has been postulated that Th1 cytokines contribute to control brucella infection [7,8]. Interleukin-12, chiefly a product of antigen-presenting cells (APC), is usually critical for the development of Th1 responses [9,10]. IL-10 is an anti-inflammatory cytokine secreted by T cells and macrophages. It interacts with the IL-10 receptor and like IFN-γ, signals through the Jack/Stat signaling pathway [11]. It is known to downregulate Th1 response during Bucellosis [12].

The present study aims to find out the prevalence of brucellosis in sheep in one of the areas of An Najaf El-Ashraf city, and study of the immune response by measuring the levels of and interleukin-10 (IL-10) by ELISA. IL-10 level can be used as a criterion to determine the ability of immune response in controlling the disease.

MATERIAL AND METHODS

Forty eight blood samples were collected from unvaccinated three flocks of sheep in Al-Barakyia, Najaf province; a venipuncture of jugular vein was done under sterile condition to collect 10 ml of peripheral blood sample. Samples were conveyed to the laboratory in Najaf Veterinary Hospital for centrifugation to obtain the sera. The tubes were centrifuged at 3000 r.p.m., for 5 minutes, then the sera were collected in a small vials (eppendorf tubes) and stored in freezer at -20 till the time of use. Serum specimens were analyzed in two phases, In the first phase, all specimens were screened by the Rose Bengal test (RBT) for brucella anibodies, second phase all specimens were screened by indirect ELISA test to detect brucella lipopolysaccharide (LPS) antibodies, and estimation of level of IL-10 by ELISA.

Rose Bengal test (RBT)

RBT was carried out according to [13] with brucella abortus S99 antigen (Spinreact SA, Girona, Spain). Briefly, 30 of µL antigen was mixed on a white glossy ceramic tile, with an equal volume of sheep serum. The tile was then rocked at room temperature for 4 minutes and any visible agglutination and/or the appearance of a typical rim was taken as a positive result.

Indirect ELISA

The SERELISA®Brucella OCB Ab Mono indirect kit (SYNBIOTICS EUROPE SAS, Cedex, France) was used to test the serum samples for antibodies to B. abortus and B. melitensis according to the manufacturer's instructions. The optical density (OD) values for each of the controls provided in the kit and serum samples in the wells were read at 450 nm using a microplate photometer (Universal Microplate Reader, Bio-Tek Instruments, Inc.).

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IL-10 detection by ELISA

Serum levels of IL-10 was measured using ELISA Kit supplied by (USBiological, USA). The assay was done according to protocol provided by the manufacturer.

RESULTS

Out of 48 serum samples collected from sheep revealed 10 (20.86%) were positive with Rose Bengal test, while 12 (25%) samples were positive indirect ELISA test.

Plotting standard curve for IL-10

Standard preparation is necessary for immunoassay. Using a standard preparation, we draw a standard curve from graded reaction results of various standard concentrations, and by comparison of a sample reaction result with the standard curve, we get assay value of the sample as in Figure 1.

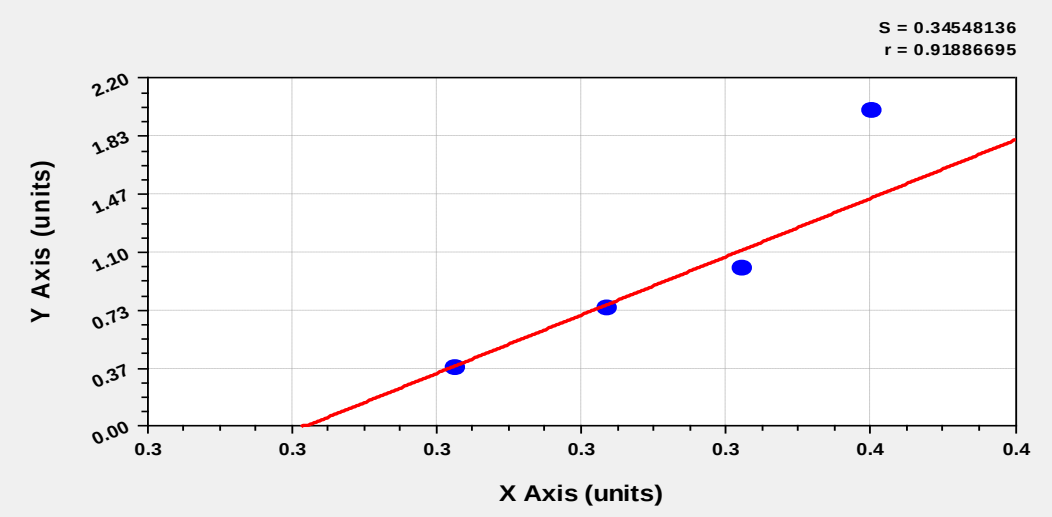

Figure 1: Standard curve for various standard concentrations

As shown in table 1, 12 out of 48 sera samples showed detectable levels of IL-10, all were ELISA positive.

Table 1: Serume IL-10 levels in study groups.

Sample number	O.D.	Concentration (Pg/ml)
Standard	0.368	2
Standard	0.348	1
Standard	0.321	0.75
Standard	0.317	0.375
Standard	0.267	0
1	0.247	0
2	0.401	2.22801
3	0.328	0.89442
4	0.484	3.74429
5	0.443	2.99528
6	0.402	2.22803
7	0.480	3.70015
8	0.404	2.22901
9	0.301	0.88242
10	0.309	0.88541
11	0.492	3.82272
12	0.490	3.81163

13	0.320	0.8890
14	0.114	0
15	0.211	0
16	0.122	0
17	0.212	0
18	0.112	0
19	0.211	0
20	0.115	0
21	0.186	o
22	0.211	0
23	0.118	0
24	0.112	0
25	0.162	0
26	0.117	0
27	0.192	0
28	0.187	0
29	0.114	0
30	0.193	0
31	0.161	0
32	0.217	0
33	0.114	0
34	0.115	0
35	0.201	0
36	0.115	0
37	0.113	0
38	0.212	0
39	0.147	0
40	0.265	0
41	0.157	0
42	0.242	0
43	0.276	0
44	0.211	0
45	0.165	0
46	0.123	0
47	0.122	0
48	0.112	0

Table 2 revealed that concentration of IL10 is significantly higher among those with positive test for brucellosis than those with negative test for brucellosis.

Table 2: Concentration of IL10 among those with or without brucellosis

	Number (%)	Mean Conc. Of IL-10 (Pg/ml)
Brucellosis (+ve)*	12 (25%)	2.359198
Brucellosis (-ve)	36 (75%)	0

P value ≤ 0.05

*Brucella (+ve) = ELISA positive cases.

DISCUSSION

Brucella melitensis is primarily responsible for brucellosis in sheep and goats, and the most important zoonotic agent among *Brucella* species [14]. Small ruminant brucellosis remains a problem in some of

industurized countries as well as in all developing countries. Essentially, brucellosisis almost always present where small ruminants are kept [15].

Rose Bengal test is internationally recommended for the screening of brucellosis in small ruminants [16]. The standardization condition of the antigen limit the sensitivity of the test resulting in reduced performance for the diagnosis of *Brucella melitensis* infection in sheep [17, 18].

Present study revealed that the prevalence rate of brucellosis among sheep was higher than a previous report conducted in Iraq by [19, 20] in Jordan. However, many other researchers in the Middle East and Mediterranean countries (have reported the seroprevalence rate to be between 26.66%- 27.1% in sheep and 27.7% in goat flocks [21-23], the present results indicating that the brucellosis is an endemic health problem of sheep the increased seroprevalence of brucellosis in Najaf could be due to illegal movement and trade of flocks of sheep (small ruminant) that regarded as a reservoir for brucella infection. however, the small sample size and selection of one region in Najaf province could not be resenting the whole epidemiological picture in the city. Al-Hankawe and Rhaymah [24] mentioned that the total prevalence of brucellosis in Ninewah province was 11.8% using Rose Bengal test but the values varied markedly according to the areas, the highest prevalence was in Al-Shekan (22.7%) among seven districts of the province.

The difference between percentage of seropositivity by ELISA and Rose Bengal could be due to the high sensitivity and specificity of ELISA test [13, 2].

IL-10 is a cytokine that regulates the balance between pathogen clearance and immunopathology. Previous findings demonstrate that IL-10 modulates the proinflammatory immune response to B. abortus infection and the lack of IL-10 increases resistance to Brucella infection [25].

It was obvious in the present findings that there was an increase in IL-10 level in the sera of animals having ELISA positive results.

CONCLUSION

The detection of significant concentration of IL-10 among the seropositive cases may be indicated an active infection of brucella with type 2 cytokine production and could be down-regulation of type 1 cytokine function.

RECOMMENDATIONS

The present study recommends the evaluation of immune response of vaccinated sheep with brucella REV1 vaccine, depending on the estimation of IFN-γ and IL-10 ratio that will reflect the cytokines induced during vaccination and consequently, the effective or not immune response.

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