The effect of Brucellosis in some blood Parameters of Sheep

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ABSTRACT

This study was carried out to estimate the seroprevalence of brucellosis in sheep and their contact humans in addition the appearance of signs of undulant fever among some contact humans. Also, to identify the risk factors for brucellosis seropositivity at human and animal level in different regions of the Baghdad province. The study involved randomly collection of forty blood samples from 40 herds of sheep and from each blood samples, 40 (37 ewes and 3 rams) were tested for Rose-Bengal plate test (RBPT) then confirmed this diagnosis of the positive and negative samples of RBPT. The results showed there were differences in the infection rates of sheep brucellosis according to gender, four (4) rams' sera were tested,4(100%) were positive ,36 ewes sera were tested ,14(36.8%) were positive with Rose Bengal test. According to age, the results showed that a high sera prevalence rate (70%) was at 3 years old comparison with low sera prevalence rate (40%) was mention at 4 years. Conclusions: *Brucella* infection in male is higher than females due to a smaller number of male companions to female, while females recorded higher infection rate. *Brucella* infection was recorded at age of 3years in addition 5 years. Neutrophils were higher than other mononuclear cells in WBCs count of *Brucella* in infected sheep .

Keywords: ovine brucellosis; seroprevalence; *Brucella meltiness*; zoonosis; Rose-Bengal plate test(RBPT); abortion; Sheep.

Introduction: -

Human Brucellosis is one of the most common zoonotic diseases worldwide. Disease transmission often occurs through the handling of domestic livestock, as well as ingestion of unpasteurized milk and cheese, but can have enhanced infectivity if aerosolized (1).

Brucellosis is one of the most frequently encountered bacterial zoonosis globally, The disease affects domesticated animals and wildlife as well as humans, causing substantial economic losses in the livestock industry due to abortion, reproductive failure, sterility and drops in milk production, and significant public health problems
(2,3).

Despite the disease being notorious in veterinary medicine, its importance, diagnosis, and control have attracted little attention in human medicine. The World Health Organization (WHO) considered brucellosis a neglected disease (4). While brucellosis affects sheep and results in late abortions, stillbirths, reduced fertility and decreased milk production that result in significant economic losses, it affects humans and has a wide range of clinical symptoms like undulant fever,

malaise, insomnia, arthralgia, sexual impotence, nervousness and depression (5). The disease is endemic in the Middle East, Mediterranean countries, and the Arabian Gulf area among humans and animals, and prevalences in small ruminant populations are among the highest worldwide (6). Brucella melitensis is the primary cause of brucellosis in sheep and goats. Sheep and goats are also significant reservoirs for maintenance, spread and transmission (7). Due to grazing regimes, ways of rearing and management systems in place, these animals maintain the infection and shed pathogenic agents into the environment (8). The course of the disease and clinical picture in certain breeds of sheep is similar to that in goats.

Ethical approval

This study was approved by the Research Ethics Committee at Baghdad University, Veterinary College. All blood samples were collected following standard procedures without any animal harm with the acceptance of owners.

Materials and Methods:-

Rose Bengal test was done according to Croma test Kit, spain; in addition Geiemza stain Syrbio, Syria.

Fourty (40) blood samples of sheep collected randomly about 5 ml. Each sample was divided into two parts the first part was to prepare thin blood smear for differential WBCs count according to Coles (13), and second part to use Rose Bengal plate test (14).

1- **Serum preparation:-**

5ml of blood sample were collected from the jugular vein of sheep, cleaning and disinfecting of the dragging site collected in a test tube until clotting, then kept in refrigerator overnight in stand position, then centrifuged at 1500rpm/10 minutes stored freezed at -20C(15).

Brucella melitensis is also the member of the genus Brucella with the highest zoonotic risk, and most human cases are caused by B. melitensis worldwide (9). Although many developing countries in the Middle East have implemented highly restrictive control programs, the disease is still endemic, resulting in significant public health problems in most of the Middle Eastern countries (6,9). Brucellosis is caused by a Gram-negative bacterium in the genus *Brucella*. These bacteria are facultatively anaerobic, non-motile, and intracellular coccobacilli. Brucellosis affects a wide range of mammals, including man, sheep, camels, cattle, goats, swine, and wildlife (10,11-12).

2- **Serological test**

A- Rose Bengal plate test (RBPT):-

This test was use as described by Oie (15) as following:

1- Serum samples and antigen brought at room temperature (22±4C).
2- Each serum (3ml) was placed on a white tile.
3- The antigen bottle was shaking well, gently and placed an equal volume of antigen near each serum spot.
4- Immediately after the last drop of antigen has been added to the plate, mix the serum and antigen thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter.
5- The mixture is agitated gently for 4 minutes at ambient temperature on a rocker. The agglutination was read immediately after the 4 minute period was completed. Any visible reaction was considered positive as shown below:

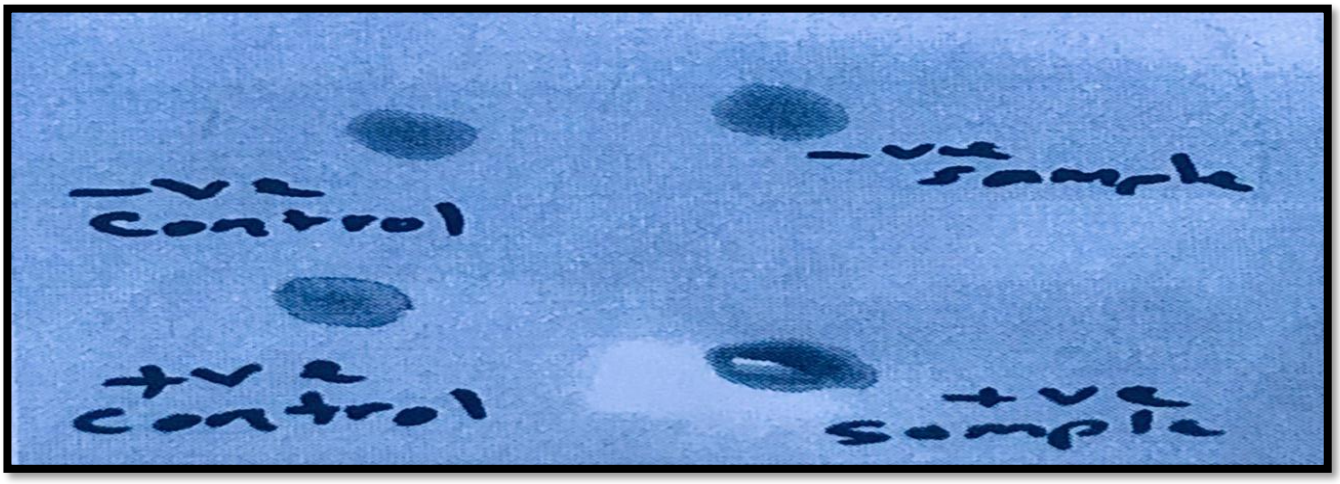

Figure -1: show Rose Bengal Plate Test (RBPT) the results on blood sample of sheep.

B- Differential WBCs count:-

It is measured according to (15).Smears were air-dried,fixed with methanol stained with Giemsa stain and carefully examined under the oil immersion objective to estimate the white blood differential count and percentage of cell was calculated in 100 cells.

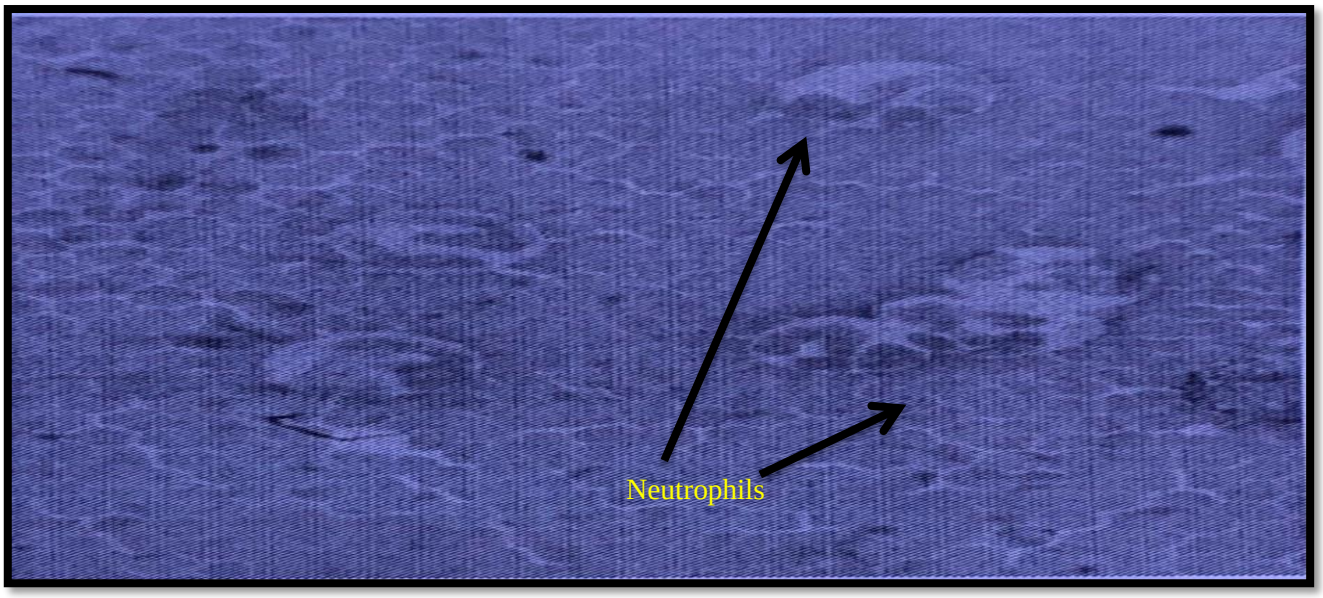

Figure-2: show Neutrophils examined by Olympus light microscope(Hubei-China) under magnification 100x (oil emersions).

Results and Discussion:-

1- Rose Bengal Agglutination test:-

a- According to gender

The results showed there were differences in the infection rates of sheep brucellosis according to gender, three (3) rams sera were tested,3(100%) were positive ,37 ewes sera were tested ,14(37.8%) were positive with Rose Bengal test. The higher percentage in rams in comparison with ewes indicated to insertion of few numbers of rams to fixed large numbers of ewes during management of natural breeding leading to raise the infection percentage (Table – 1).

Table-1 percentage of sheep brucellosis by using RBT according to gender

Gender	No. of sera tested	Positive sera	Percentage (100%)
Ram	**3**	**3**	**100%**
Ewes	**37**	**14**	**37.8%**

Total	**40**	**17**	**42.5%**

b- According to age:-

The results showed that a high sero prevalence rate (60%) was at 2 years old comparison with low sero prevalence rate (36.3%) was mention at 4 years (Table_2).

Table-2 prevalence of sheep brucellosis by using RBT according to age

Age of group	No. of sera tested	No. of sheep sera positive			Percentage%
		Rams	Ewes	Sum	
3	10	1	5	6	60%
4	-	0	8	8	42.1%
5	11	2	2	4	36.3 %
Total	40	3	15	18	45%

This result was disagreement with Al - A bdaly (16) who referred the sero prevalence rates of rams were higher thn ewes 7.4% and 6.5% respectively.

Saleem(17) referred that seroprevalence rates that the sero prevalence rates of rams were much higher than ewes 65.6% and 10.6% respectively, also the high sero prevalence rates was at 2-3 years age ,this results were found are agreed with results founded by Mustafa(18) who reported that high sero prevalence rate at 2-3 years may be due to the differences in the age of group.

2- Differential Leukocyte count:-

The results in table -3 showed that percentage of different count of WBCs in sheep infected with brucellosis and control.

Table_3 White blood cells count in brucellosis and control sheep

Groups	Neutrophi	Lymphocyte	Monocyte	Eosinophil	Basophil
Infected	37.36	71.24	9.22	3.39	0.28
Control (un infected)	48.36	39.62	9.26	4.65	0.14

The results were conformed to previous reports of Rotrosen and Gallin (19). As they referred that phagocytosis system is the earliest non-specific defuse mechanism against microbes with neutrophils, monocyte in addition eosinophils , responsible for killing in addition ingestion bacteria.IL-1acts on bone marrow to stimulate release of neutrophils in the circulation and causes a neutrophilia thus attracts to the sites of bactericidal activity stimulating oxidative metabolism. I L-1enhances inflammation by degranulation basophils in addition mast cell by activating neutrophils and eosinophils therefore they release their lysosomal enzyme. The lymppocytes have receptors on their surface according to their function that recognized antigen(20).

Conclusions and Recommendations:

Rose Bengal test is diagnostic, screening in addition very rapid test. Therefore, confirmed by other confirmatory tests to avoid the false positive in addition false negative results of Rose Bengal test.

The propagation of Brucella infection in male is higher than females due to a smaller number of male companions to female, while females recorded higher infection rate.

Brucella infection was recorded at age of 3years in addition 5 years.

Neutrophils were higher than other mononuclear cells in WBCs count of Brucella in infected sheep.

Recommendations: -

Skin test should be done to all sheep flocks to recognize infected, non-infected sheep in addition vaccinated sheep.

Brucella infections disclose to continue vaccination procedure with a view to reach the lowest incidence rate in addition starting eradication roles.

Diagnosing Brucellosis by using other modern tests ,for instance PCR in animals, because is a sensitive and time-saving test for brucellosis diagnosis.

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