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Clincal, hematological and serological aspect of rams and aborted ewes with brucellosis

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ABSTRACT

The study was conducted on (60) sheep, divided into two groups: The first (healthy) group: numbered 10 ewes and were considered a control group: The second group: included 50 sheep, 40 of which were ewes recently aborted in the last two months of pregnancy, and 10 rams suspected of being infected with brucellosis, from various areas in the city of Tikrit, where the animals were examined serologically by the Rose benal and indirect ELISA tests, and blood parameters were measured by complete blood count. The study noted through the serological tests that 40% of the ewes were infected and 20% of the rams were infected through the Rose benal test. The infection rate of ewes was 30% and that of rams was 20% through the indirect ELISA test. The study also noted that there was a significant increase ($P<0.05$) in the temperature, respiratory and pulse rates of the infected animals compared to the healthy group. As for blood tests, the study noted that there was a significant decrease in Some blood values, which included the RBCs, Hb,PCV,MCV,MCH,MCHC when compared with the healthy animals, while they showed only a significant increase in ESR rate. and it also showed a significant increase TWBC and neutrophils in infected animals. The study concluded that ewes were more susceptible to infection than rams. The study showed that the impact of the disease on the abortion of pregnant ewes and the health condition of ewes and rams will lead to a major economic loss as a result of the destruction of fetuses and ewes.

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Introduction

Brucellosis is a highly zoonotic infectious disease that affects animals and humans, caused by bacteria belong to genus *Brucella.* which includes several the most important species, are *Brucella abortus, Brucella melitensis, and Brucella suis*. *Brucella melitensis* is the main cause of brucellosis in sheep, and infection rarely occurs due to *Br. abortus* [1]. Brucellosis is a widespread epidemic in the world, as the most prominent clinical signs of the disease are abortion in infected animals, which often occurs in the last months of pregnancy and retention of the placenta in ewes. In rams, it causes orchitis and epididymitis, and temporary or permanent infertility [2,3,4].

The disease is common in most developing countries [5]. In Iraq, brucellosis is the most common and endemic disease [6], which has been recorded for the first time by [7]. Brucellosis in domestic animals, particularly in sheep is a chronic infectious disease [8].

Serological tests are widely used to detect specific anti *Brucella* antibody in sera and other body fluids by a variety of techniques [9]. Rose Bengal test is a simple, rapid and an screening test[10]. IgM immunoglobins appear early in the infection and last for several weeks, while IgG immunoglobins may appear after a period of infection and last for several months and years [11]. RBPT detects IgM, IgG, and IgA antibodies, but it shows more sensitivity to IgM, the percentage of which is high at the beginning of the infection, so it is used to determine early infection in herds (Corbel, 2000). As for ELISA, it detects IgG antibodies, which are used to identify chronic infection (13).

2. Materials and Methods

The animals and Study area

The animals:

The study was conducted on 60 sheeps, the animals divided into two groups, 10 as control and infected groups their number were 50 (40 aborted ewes and 10 rams), in the period from the beginning of November/2022 to the end of April/2023 and involved the recording of Brucellosis infection with clinical and hematoloical parameters changes in aborted ewes, blood sample were collecte for serological

Serological and hematological tests:

Rose-Bengal test (RBPT): The serological tests included : Serum samples were tested using the RBPT antigen, according to (10). Briefly, 30 µL of serum and 30 µL of RBPT antigen combined on the white ceramic plate and carefully mixed. The plate was agitated for 4 min, the agglutination was considered positive. Positive and negative control sera for comparison of the results were used.

Indirect Enzyme-Linked Immunosorbent Assay (i-ELISA) test : The indirect ELISA tests were performed according to the manufacturer's protocol (SunLong Biotech, China) to measure specific antibodies generated against brucellosis. Absorbance was read at an optical density (OD) of 450 nm using the ELISA Microplate Reader (BioTek, USA). Finally, test effectiveness was determined, whereas the critical value (CUT OFF) was calculated at 0.299, and the samples were considered positive if the OD value was ≥ CUT OFF.. diagnostic kit is designed to detect antibodies directed against Brucella melitensis, Brucella abortus, and Brucella suis in ovine, bovine, caprine, and porcine sera. In addition, it minimizes the cross-reaction with other Gram-negative bacteria. (13).

Hematological test: counted by complet blood count method, that included total count of red blood cells (RBCc), the volume of packed blood cells (PCV), the concentration of hemoglobin (Hb), as well as other blood indicators (MCV, MCH, MCHC), and the total and differential count of white blood cells (WBC) (10).

3.Results

Rose Bangal and ELISA tests

The results table (1) showed that the percentage of positive samples of brucellosis infection in aborted ewes based on Rose Bangal found 40% and 20% in rams, While indirect ELISA Test showed 30 % of brucellosis infection in aborted ewes and 20% in rams.

Table (1) Rose Bangal and ELISA tests for brucellosis diagnosis

Test	Aborted ewes No.	Ewes (n=40)	Rams (n=10)
RBPT	40	16 (40%)	2 (20%)
ELISA		12 (30%)	2 (20%)

The results table (2) showed that the Standard error ± rate of body temperature in infected ewes compared with healthy ewes was recorded (39.8 ± 0.043 and 39.1 ±

0.031) sequentially. As well respiratory rate/min in infected ewes compared with healthy ewes was recorded (30.4 ± 2.942 and 22.5 ± 2.132) sequentially. While heart rate/min in infected ewes compared with healthy ewes was recorded (86.2 ± 4.784and 77.5 ± 3.632) respecteviely While heartbeat/min in infected ewes compared with healthy ewes was recorded (86.2 ± 4.784and 77.5 ± 3.632) sequentially.

Table (1) Clinical examinatiom parameters in Healthy and infected animals

N0.	Standards	Mean ±SE	
		Infected ewes	Healthy ewes
1	body temperature	a 39.8 ± 0.043	39.1 ± 0.031 b
2	respiratory rate /min	a 30.4 ± 2.942	2.132 b 22.5 ±
3	Heart rate /min	a 86.2 ± 4.784	3.632 b 77.5 ±

Different letters horizontally refers a significant difference at the level (P<0.05).

When compared with hematological parameters in the healthy ewes and infected ewes, were found to be statistically different (p<0.05) as shown in table (3).

Table (3) hematologcal parameters in infected and healthy ewes.

NO.	**Standards**	**Mean ±SE**	
		Infected ewes	**Healthy ewes**
1	**Total number of red blood cells (× 106/µL)**	b 6.24 ± 1.141	6.94 ± 1.047 a
2	**Hemoglobin concentration (g/100ml)**	9.45 ± 0.085 b	0.180 a 10.12 ±
3	**Volume of packed blood cells (%)**	28.8 ± 1.210 b	1.135 a 32.4 ±
4	**average corpuscular volume (fl)**	b 33.10 ± 1.602	a 36.8 ± 1.450
5	**corpuscular hemoglobin (pg)**	9.08 ± 0.914 b	0.900 a 11.13 ±
6	**Average corpuscular hemoglobin concentration (%)**	29.52 ± 2.123 b	2.013 a 33.45 ±
7	**Erythrocyte sedimentation rate (mm/24 hours)**	a 11.4 ± 0.425	7.8 ± 0.250 b

Different letters horizontally refers a significant difference at the level (P<0.05).

The result in the table (4) indicate that there is a significant difference between the two groups (infected ewes and control group) at the probability level (P<0.05) according to total and differential count of white blood cells.

Table (4) Total and differential White blood cells in infected and healthy ewes.

NO.	Standards	Standard error ± rate	
		infected ewes	control group
1	Total white blood cell count (× 10^3/µL)	1.341 ± 16.6 a	0.915 9.2 ± b
2	Neutrophils (%)	3.583 50.2 ± a	2.625 ± b 43.3
3	Lymphocytes (%)	2.843 ± b 40.3	2.623 47.5 ± a
4	Eosinophils (%)	0.715 ± 3.8 a	0.631 4.3 ± a
5	Monocyte (%)	0.983 ± a 5.7	0.536 4.6 ± a
6	Basophils (%)	0.084 0.5 ± a	0.078 0.3 ± a

Different letters horizontally refers a significant difference at the level (P<0.05).

Discussion

The clinical signs that appear in aborted ewes include high temperature (fever), weight loss due to loss of appetite, increased heart rate, sore throat, and increased respiratory rate in pregnant ewes [14]. As for the disease symptoms in rams, they are characterized by inflammation of the epididymis and testicles, in addition to the occurrence of swelling in the scrotum and poor fertility. Lesions are also seen when the infection is not treated early, as they are characterized by swelling of the testicles and scrotum, which affects the quality of semen, in addition to the arthritis it causes, which is observed after the infection and for a long period[15].

This study revealed, the percentage of positive samples of brucellosis infection in aborted ewes based on Rose Bangal found 40%. While ELISA test showed 30 % of brucellosis infection in aborted ewes Because of the ELISA examination, it is more accurate than the Rose Bengal examination, which has an incoming error rate. this study showed similar results between RBT and ELISA, which were consistent with [16]. This results of the RBT decreased from the study [17], which amounted to 66.7% when conducting the RBT for aborted sheep serum, and the results recorded by [18], which showed 39 samples out of 50 samples gave a positive result and rate was 78%. The reason for this was attributed to the difference in the age of the animal and the nature of the samples.

The results of this study showed that there were differences in the rates of infection with brucellosis in sheep according to sex. The percentage of ewes infected more than the rams that gave positive results for the RBT was 40% in the ewes and 20% in the rams.. As mentioned (19), that ewes are more infected than rams. The study (20) stated that males are less likely to be infected with brucellosis than females due to the absence of erythritol.

Erythritol is known as a substance produced by the fetus, which is naturally present in the placenta and which is responsible for the presence of Brucella, as it works to stimulate B. abortus and invade the uterine tissue and cause uterine ulceration [14]. The study done by [21] found that males are less infected than females and attributed the reason to the fact that erythritol is not present in the reproductive organs of males.

This is consistent with what was indicated by [22] and that blood parameters in sheep infected with Brucella may decrease from their normal numbers, which is likely to be caused by oxidative stress, which increases in sheep infected with Brucella and thus affects the bone marrow. Other study [23] showed a decrease in the total number of red blood cells, the size of packed blood cells, and the hemoglobin concentration of aborted ewes infected with Brucella.

The decrease in red blood cells and hemoglobin concentration is due to a decrease in the formation of red blood cells resulting from insufficient production of the hormone erythropoietin, which is responsible for the formation of red blood cells. In addition to normochromic anemia, which is known as a chronic disease associated with an increase in inflammatory cytokines such as TNF-α and IL6, which directly affect erythrocyte cells and also inhibit the production and effectiveness of erythropoietin. The size of the packed blood cells is inversely proportional to the rate of sedimentation of red blood cells, as the more the anemia reaches its peak, the rate of sedimentation speed increases [24].

Other study (22) observed a significant decrease in the values of neutrophils, monocytes, and lymphocytes in sheep infected with brucellosis, which suggested that the reason for the increase in the number of neutrophils was related to oxidative stress that leads to tissue damage. Low values of lymphocytes and mononuclear cells could be due to poor immune response of cattle infected with brucellosis. A few studies have reported that mononucleosis is mainly caused by tissue damage in the reproductive and urinary tract of Brucella animals [25, 26].

Conclusion

The study concluded that ewes were more susceptible to infection than rams. The study showed that the impact of the disease on the abortion of pregnant ewes and the health condition of ewes and rams will lead to a major economic loss as a result of the destruction of fetuses and ewes.

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التشخيص المصلي لمرض الحمى المالطا وعلاقته مع التغيرات الدموية في النعاج المجهضة

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الملخص

أجريت الدراسة على (60) من الضأن، تم تقسيمها إلى مجموعتين حسب الأعراض السريرية الظاهرة على الحيوان. وكالتي: المجموعة الاصحاء: تم اعتبار عدد (10) أغنام مجموعة ضابطة، واغنام المصابة بلغت العدد (50), (40) نعجة مجهضة و 10 كبشاً). أظهرت نتائج الدراسة أن نسبة الإصابة بمرض البروسيلا في النعاج المجهضة ، بناءً على اختبارات Rose Bangal و ELISA ، كانت (40٪ ، 30٪) على التوالي. بينما كانت نسبة الإجهاض في النعاج 40٪ والإصابة في الكباش 20٪ وذلك بناءً على فحص RBT. اظهرت النتائج الدموية والعدد الكلي والتفاضلي لخلايا الدم البيضاء ذات فروقات معنوية إحصائيا ($P < 0.05$).

الاستنتاج: أشارت الدراسة إلى أن النعاج كانت أكثر عرضة للإصابة من الكبش. وأوضحت الدراسة تأثير مرض حمى المالطا على الحمل وعلى الحالة الصحية للنعاج مما يؤدي إلى خسارة اقتصادية كبيرة نتيجة وفاة الأجنة ووفاة الأمهات.