



The hematological changes associated with ovine subclinical mastitis.

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Abstract

The aim of the present study was to investigate ovine subclinical mastitis and associated with the hematological changes were studied in awassi sheep flock consists of ninety ovine lactating (58 normal and 32 ewes subclinical mastitis) both groups aged 1.5-4 years in AL-Najaf province /Iraq. Milk samples were collected for California mastitis test (CMT) and blood samples were collected from jugular vein during the period February 2016 till April 2016.

The results showed that the means \pm SE in normal lactating and ewes subclinical mastitis were as follows; Packed cell volume (PCV) 31.9 ± 0.4 % and 25 ± 0.3 %, hemoglobin (Hb) 10.3 ± 0.16 g/dl and 7.3 ± 0.12 g/dl, red blood cell count (RBC) $10.2 \pm 0.13 \times 10^6/\mu\text{L}$ and $8.9 \pm 0.28 \times 10^6/\mu\text{L}$, mean cell volume (MCV) 31.3 ± 0.36 fl and 30.9 ± 0.62 fl, mean cell hemoglobin (MCH) 10.1 ± 0.12 pg and 9.7 ± 0.21 pg, mean cell hemoglobin concentration (MCHC) 32.2 ± 0.18 g/dl and 31.2 ± 0.36 g/dl, erythrocyte sedimentation rate (ESR) 9.9 ± 0.46 mm/24 hrs and 17.9 ± 2.08 mm/24 hrs, Platelets $283.8 \pm 16.6 \times 10^3/\mu\text{L}$ and $184.5 \pm 8.3 \times 10^3/\mu\text{L}$, white blood cell count (WBC) $10784 \pm 268.3 \times 10^3/\mu\text{L}$ and $13956 \pm 578.4 \times 10^3/\mu\text{L}$, lymphocytes 54.3 ± 1.36 and $50.9 \pm 2.51/\mu\text{L}$, neutrophils 40.7 ± 1.41 and $52.8 \pm 1.84/\mu\text{L}$, monocytes 1.83 ± 0.16 and $3.0 \pm 0.27/\mu\text{L}$, eosinophil's 2.84 ± 0.31 and $5.81 \pm 0.76/\mu\text{L}$ and basophils 0.28 ± 0.05 and $0.78 \pm 0.07/\mu\text{L}$ respectively.

The PCV, Hb, RBC and platelets were significantly higher ($P < 0.05$) in normal lactating compared to that of ovine subclinical mastitis. The MCV, MCH and MCHC showed no significant differences between normal and ewes with subclinical mastitis. While ESR and WBC count were significantly lower in normal lactating.

Key words: Hematological parameters, clinically lactating normal, subclinical mastitis awassi sheep.

التغيرات الدموية المرتبطة بالتهاب الضرع تحت السريري في الاغنام.

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الخلاصة:

الهدف من الدراسة هو لبحث التهاب الضرع تحت السريري في الاغنام العواسية ومقارنتها بالتغيرات الدموية, يتكون العدد الكلي لحيوانات الدراسة من تسعين نعجة حلوبة (58 نعجة سليمة و32 مصابة بالتهاب الضرع تحت السريري), تتراوح اعمار المجموعتين من 1.5-4 سنوات. عينات الحليب جمعت لإجراء فحص (سي ام تي) وعينات الدم جمعت من الوريد الوداجي لقياس المعايير الدمية خلال الفترة من شهر شباط الى شهر نيسان من عام 2016 في محافظة النجف الاشرف.

اظهرت النتائج للمعدلات \pm الخطأ القياسي في النعاج الحلوبة الطبيعية والنعاج المصابة بالتهاب الضرع تحت السريري كما يلي: حجم الخلايا المرصوص 0.4 ± 31.9 و 0.3 ± 25 % , خضاب الدم 0.16 ± 10.3 و 7.3 ± 0.12 غم/دسي لتر, العد الكلي لكريات الدم الحمراء $0.13 \pm 10.2 \times 10^6$ μ I و $0.28 \pm 8.9 \times 10^6$ μ I , معدل الحجم الكاريبي 0.36 ± 31.3 فمتولتر و 0.62 ± 30.9 فمتولتر , معدل خضاب الدم الكاريبي 10.1 ± 0.12 بيكوغرام و 0.21 ± 9.7 بيكوغرام , معدل تركيز خضاب الدم الكاريبي 0.18 ± 32.2 غم/دسي لتر و 0.36 ± 31.2 غم/دسي لتر, معدل تنقل كريات الدم الحمراء 0.46 ± 9.9 مليمتتر / 24 ساعة و 2.08 ± 17.9 مليمتتر / 24 ساعة , العد الكلي للصفائح الدموية $16.6 \pm 283.8 \times 10^3$ /ميكرولتتر و 8.3 ± 84.5 , العد الكلي لكريات الدم البيضاء 268.3 ± 10784 /ميكرولتتر و 578.4 ± 13956 /مايكرولتتر, الخلايا اللمفية 54.3 ± 1.36 /مايكرولتتر و 2.51 ± 50.9 /مايكرولتتر, الخلايا المتعادلة 1.41 ± 40.7 /مايكرولتتر و 1.84 ± 52.8 /مايكرولتتر, الخلايا وحيدة النواة 0.16 ± 1.83 /مايكرولتتر و 0.27 ± 3.0 /مايكرولتتر, الخلايا الحمضية 0.31 ± 2.84 /مايكرولتتر و 0.76 ± 5.81 /مايكرولتتر, الخلايا القاعدية 0.05 ± 0.28 /مايكرولتتر و 0.78 ± 0.07 /مايكرولتتر على التوالي.

واظهرت النتائج ان حجم الخلايا المرصوص, خضاب الدم, العد الكلي لكريات الدم الحمراء والصفائح الدموية زيادة معنوية ($P > 0.05$) في النعاج الطبيعية مقارنة بالنعاج المصابة بالتهاب الضرع تحت السريري. لا توجد فروق معنوية بين النعاج الطبيعية والمصابة بالتهاب الضرع تحت السريري في معدل الحجم الكاريبي, معدل خضاب الدم الكاريبي, معدل تركيز خضاب الدم الكاريبي بينما لوحظ انخفاضاً معنوياً ($P > 0.05$) في معدل تنقل كريات الدم الحمراء و العد الكلي لكريات الدم البيضاء, الخلايا المتعادلة, الخلايا وحيدة النواة, الخلايا الحمضية, الخلايا القاعدية, مع عدم وجود فرق معنوي بنسبة الخلايا اللمفية.

الكلمات المفتاحية: المعايير الدمية, النعاج الحلوبة الطبيعية, المصابة بالتهاب الضرع تحت السريري, الاغنام العواسية

Introduction:

Subclinical mastitis is a worldwide problem and its economic importance attributed mainly to its higher prevalence and effect on quality and quantity of the produced milk (1). Also, subclinical mastitis has adverse effects on the hygienic quality and physicochemical properties of milk (2).

On the other hand, subclinical mastitis is the most prevalent type of mastitis characterized by no detectable changes in the udder and no visual abnormalities in milk (3). However, (4) reporting the absence of clinical signs specific to subclinical mastitis in sheep.

Diagnosis of such cases mainly depends upon bacterial culture results and/or indirect methods as CMT and SCC. Somatic cell count (SCC) considered a very helpful diagnostic tool in diagnosis of subclinical mastitis in dairy ewes (5). The limits of somatic cell counts of sheep milk, have not yet been definitely established, although it has been suggested that a threshold

level for subclinical mastitis in sheep should be close to 1,500 000 cell mL, much higher than that set for cows ($<500,000$ cell mL) (6).

Materials and Methods

The California Mastitis Test CMT (Jorgensen laboratories, Inc. Loveland, Colorado) was applied to all samples collected using the method of (7). According to the reactions obtained, the results were classified as: (negative), (traces), 1, 2 and 3, recorded as (-), (+), (++) and (+++), respectively.

On the other hand, blood samples were collected into EDTA tubes (EDTA.K3 Italy) from the jugular vein of 90 ovine lactating (58 normal and 32 ewes with subclinical mastitis) during the period from February 2016 till April 2016 both aged 1.5-4 years in Al-Najaf governorate.

The blood were used directly for hematological parameters investigations. PCV was measured by

using micro hematocrit centrifuge according to (8), the hemoglobin was determined by acid hematin method (9). Red blood cells and white blood cell counts were measured by using hemocytometer (10). The MCV, MCH and MCHC were calculated according to the following formulas ; $MCV = PCV/RBC \times 10\text{fl}$, $MCH = Hb/RBC \times 10\text{pg}$ and $MCHC = Hb/PCV \times 100\text{g/dl}$ (9). ESR was measured using westergren tubes according to (11).

A general guideline for estimating platelet numbers on a blood smear is to determine the average number of platelets in 10 oil immersion fields using a 100 objective and multiply the average by 15×10^3 to obtain the estimated number of platelets per microliter (12). While the differential leukocyte count was carried on 200 WBC in giemsa stained blood film according to the method of (9).

Statistic

Data were analyzed using SPSS version 20. The least significant differences test (LSD) was used to determine differences among groups. Data were subjected to analysis of variance statistically using one-way ANOVA and the Duncan range test (Statistic a).

Results and Discussion

In general, diagnosis of subclinical mastitis mainly depends upon bacterial culture results and/or

indirect methods as CMT and SCC. (13) as they stated that the CMT is very useful technique in diagnosis of subclinical mastitis in lactating ewes. According to the results of CMT method.

Table (1) illustrated The hematological parameters for normal lactating and ovine subclinical mastitis. The ranges and means \pm SE of hematological parameters in normal lactating and ovine subclinical mastitis were as follows : PCV 28 - 40% and 31.9 ± 0.4 %, 20 - 27 and 25 ± 0.3 , Hb 8.6 - 13.5 and 10.3 ± 0.16 g/dl, 5.6 - 8.1 and 7.3 ± 0.12 g/dl, RBC $8.8 - 13.3$ and $10.2 \pm 0.13 \times 10^6/\mu\text{L}$, $5.3 - 11.4$ and $8.9 \pm 0.28 \times 10^6/\mu\text{L}$, MCV 27.5 - 37.1 and 31.3 ± 0.36 fl, 23.4 - 37.4 and 30.9 ± 0.62 fl, MCH 8.6 - 12 and 10.1 ± 0.12 pg, 8.1 - 12.1 and 9.7 ± 0.21 pg, MCHC 30.5 - 35.7 and 32.2 ± 0.18 g/dl, 28.1 - 35.6 and 31.2 ± 0.36 g/dl, ESR 3 - 17 and 9.9 ± 0.46 mm/24 hrs, 8 - 63 and 17.9 ± 2.08 mm/24 hrs, Platelets 122 - 723 and $283.8 \pm 16.6 \times 10^3/\mu\text{L}$, 103 - 280 and $184.5 \pm 8.3 \times 10^3/\mu\text{L}$ respectively.

The means of PCV, Hb, RBCs and platelets were significantly high ($P < 0.05$), there was no significant differences in MCV, MCH and MCHC, while ESR showed a significant decrease ($P < 0.05$) in normal lactating compared to that of ewes with subclinical mastitis (table1).

Table (1). The hematological parameters for normal lactating and ovine subclinical mastitis; ranges and means \pm SE.

parameters	groups	
	Normal lactating N=58	Subclinical mastitis N=32
PCV (%)	28-40	20-27

	31.9±0.4 A	25±0.3 B
Hb(g/dL)	8.6-13.5 10.3±0.16 A	5.6-8.1 7.3±0.12 B
RBC(×10 ⁶ /μ L)	8.8-13.3 10.2±0.13 A	5.3-11.4 8.9±0.28 B
MCV(fL)	27.5-37.1 31.3±0.36 A	23.4-37.4 30.9±0.62 A
MCH(pg)	8.6-12 10.1±0.12 A	8.1-12.1 9.7±0.21 A
MCHC(g/dL)	30.5-35.7 32.2±0.18 A	28.1-35.6 31.2±0.36 A
ESR mm/24h	3-17 9.9±0.46 B	8-63 17.9±2.08 A
platelets(×10 ³ /μL)	122-723 283.8±16.6 A	103-280 184.5±8.3 B
WBC(/ μ L)	3950-12950 10784±268.3 B	8150-23550 13956±578.4 A
L%	31-72.5 54.3±1.36 A	13.5-76.5 50.9±2.51 A
N%	22-67.5 40.7±1.41 B	33.5-73.0 52.8±1.84 A
M%	0.5-6.0 1.83±0.16 B	0.5-6.5 3.0±0.27 A
E%	0-9.5 2.84±0.31 B	0-20.0 5.81±0.76 A
B%	0-1.5 0.28±0.05 B	0-1.5 0.78±0.07 A

The differences in capital letters horizontally refer to the presence of significant value at (<0.05).

The normal reference range of PCV, Hb, RBC, WBC and platelets obtained in the present study agreed with the range of healthy sheep by (12, 14, 15), although some hematological parameters were different from the reference range of these studies, and this may be due to one or the

following; nutritional, geographical, physiologic status and genetic factors (16). On the other hand, in normal lactating the hematological parameters; MCV, MCH, MCHC, platelets and ESR of this study were no significant difference with the values reported (17).

Table (1) Results showed the ranges and means \pm SE of WBCs and differential leukocytes in normal lactating and ovine subclinical mastitis were as follows ;WBC 3950 - 12950 and $10784 \pm 268.3 \times 10^3/\mu\text{L}$, 8150 - 23550 and $13956 \pm 578.4 \times 10^3/\mu\text{L}$, lymphocytes 31 - 72.5 and 54.3 ± 1.36 , 13.5 - 76.5 and $50.9 \pm 2.51 /\mu\text{L}$, neutrophils 22 - 67.5 and 40.7 ± 1.41 , 33.5 - 73.0 and $52.8 \pm 1.84 /\mu\text{L}$, monocytes 0.5 - 6.0 and 1.83 ± 0.16 , 0.5 - 6.5 and $3.0 \pm 0.27 /\mu\text{L}$, eosinophil's 0 - 9.5 and 2.84 ± 0.31 , 0 - 20.0 and $5.81 \pm 0.76 /\mu\text{L}$ and basophils 0 - 1.5 and 0.28 ± 0.05 , 0 - 1.5 and $0.78 \pm 0.07 /\mu\text{L}$ respectively.

There was a significant increase ($p < 0.05$) in WBC count of ovine subclinical mastitis compared to that of normal lactating. However in differential leukocyte counts neutrophils, monocytes, eosinophil's and basophils were significantly higher ($p < 0.05$) in ewes with subclinical mastitis compared to normal lactating. On the other hand, the lymphocytes showed no significant difference in normal and ovine subclinical mastitis, the differences may be attributed to immune status of the host at time of infection and the pathogenicity of the causative bacteria (18).

The lymphocytes, neutrophil, monocyte, eosinophil and basophil reference range of (12, 14, 15) were as follows; Lymphocytes 2000-9000 $/\mu\text{L}$, neutrophils 700-6000 $/\mu\text{L}$, monocytes 0-750 $/\mu\text{L}$, eosinophils 0-1000 $/\mu\text{L}$ and basophil 0 – 300 $/\mu\text{L}$. These references range were similar in monocytes and eosinophils counts, narrower range in lymphocytes and neutrophils counts and a wider range in basophils counts compared to that of present data. This may be attributed to type of feed or genetic.

The values of WBC, lymphocyte, neutrophil, monocyte and eosinophil showed a significant decreased, while, basophil revealed no significant difference with (19). The WBC, neutrophils, lymphocyte, monocyte, eosinophil and basophil counts in the present study was agreement compared to that of (17). There was a non-significant increase of lymphocytes counts in normal lactating ewes compared with subclinical mastitis ewes, this is agree with (20,21).

However, this result could be due to the fact that lactating ewes were more susceptible to systemic and local infection through the udder, also serum lactic dehydrogenase (LDH) levels increased in lactating ewes (22). Moreover, the lactic dehydrogenase (LDH) may produce leukocytosis (23). Also, infection by bacteria can cause bone marrow suppression, resulting in thrombocytopenia and anemia (24, 25).

Conclusions

The data presents reference range and mean \pm SE of hematological parameters in normal lactating ewes. However, in normal lactating a significant increase ($p < 0.05$) in values of PCV, Hb, RBC and platelets and a significant decrease ($p < 0.05$) in ESR and WBC in comparison with ovine subclinical mastitis.

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