Comparison of seasonal effects on some hematological and biochemical parameters between ewes with subclinical mastitis and healthy ewes

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Abstract

This study was conducted to compare the effects of season on some hematological and biochemical parameters in ewes with subclinical mastitis and healthy ewes. A total of 1192 milk and blood samples were collected from ewes between February and April (791 samples in spring), August and October (401 samples in summer). California Mastitis Test (CMT), milk culture, hematologic and serum biochemical parameters were obtained from the medical records. Samples which were positive by bacterial culture and CMT as were deemed to have glands with subclinical mastitis (SCM). The prevalence of subclinical mastitis in our study was around 12% (in one year). Of 144 (18.2%) and 47 (11.72%) positive samples in CMT, 134 (16.94%) and 44 (10.97%) samples were positive for bacterial culture in spring and summer, respectively. Comparison of results of hematological and serum biochemical parameters in ewes with SCM and healthy ewes in spring and summer showed that WBC counts, total protein concentrations and plasma fibrinogen in both groups of ewes were significantly higher in spring than summer ($P<0.05$). The results of the present study indicated that when interpreting hematological and serum biochemical parameters in ewes with SCM should be consider the effect of season on these parameters.

Keywords: Biochemical parameters; hematological parameters; season; subclinical mastitis; sheep
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مقارنة التاثيرات الموسمية على بعض المعايير الدموية والكيموحيوية بين النعاج المصابة بالتهاب الضرع تحت السريري والنعاج السليمة

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الخلاصة

اجريت هذه الدراسة لمقارنة تاثيرات الموسم على بعض المعايير الدموية والكيموحيوية للنعاج المصابة بالتهاب الضرع تحت السريري والنعاج السليمة. جمعت ١١٩٢ عينة حليب ودم من النعاج مابين شهر شباط ونيسان (٧٩١ عينة خلال الربيع)، شهر آب وتشرين الاول (٤٠١ عينة خلال الصيف). اختبار كاليفورنيا، وسط الحليب، المعايير الدموية والكيموحيوية للمصل تم الحصول عليها من السجلات الطبية. العينات التي اعطت نتيجة ايجابية في الاوساط الزرعية البكتيرية واختبار كاليفورنيا اعتبرت مصابة بالهاب الضرع تحت السريري. نسبة الاصابة بالتهاب الضرع تحت السريري في دراستنا كانت بحدود ١٢% (في سنة واحدة). من مجموع ١٤٤ (١٨,٢%) و ٤٧ (١١,٧٢%) عينة كانت موجبة لاختبار كاليفورنيا، و ١٣٤ (١٦,٩٤%) و ٤٤ (١٠,٩٧%) عينة كانت موجبة للزرع البكتيري خلال الربيع والصيف، على التوالي. مقارنة النتائج للمعايير الدموية والكيموحيوية للمصل للنعاج المصابة بالتهاب الضرع نحت السريري والنعاج السليمة خلال الربيع والصيف اظهرت ان عدد كريات الدم البيضاء وتركيز البروتين الكلي والفايبرينوجين في المصل في كلا

المجموعتين من النعاج كانت مرتفعة معنويا" في الربيع عنه في الصيف ($P<0.05$). اشارت نتائج الدراسة الحالية الى اهمية الاخذ بنظر الاعتبار تاثير الموسم على قراءة المعايير الدموية والكيموحيوية للمصل في النعاج المصابة بالتهاب الضرع تحت السريري.

Introduction

There has been less work done regarding the inflammation of the mammary gland in sheep. The disease remains one of the most important causes of loss in milk yield, mortality and premature culling of ewes in many countries (1-5). Control of mastitis and somatic cell counts in dairy animals is aided by an understanding of the pathogens involved, the rate of new intramammary infection (IMI), the spontaneous cure rate and the source of infection. Calofornia Mastitis Test has been standardized for cow milk and is less accurate for sheep and goats. Furthermore, it is a subjective test and its capability to predict ovine IMI depends on the prevalence and the agents of IMI in the flock (6).

On the other, Hematological and biochemical examination is an essential diagnostic procedure that can provide significant information, additional to that resulting from the general clinical examination of the patient (7,8). Hematological and biochemical tests are an important tool for evaluation of health status of farm animals and almost indispensible in organic farming, where allowed veterinary interventions are strictly regulated and limited in scope. Hematological (9) and biochemical (10) analyses have been extensively discussed as an essential part of a clinical examination, often pointing to a specific differential diagnosis or suggesting a prognosis (10). Despite the importance of hematological and biochemical examinations, this examination should be interpreted with caution. Because many factors can influence them specially season (11,12). Therefore, this study was conducted to compare the effects of season on some hematological and biochemical parameters in ewes with subclinical mastitis and healthy ewes.

Material and methods

Animals

153 milk and blood samples were collected, from ewes with sub clinical mastitis. While 992 milk and blood samples were collected from normal lactating ewe without subclinical mastitis to serve as control (non-mastitic) ewes. Sampling started in August and October 2013 (summer season) and continued till February and April 2014 (spring).

Milk sampling

Ewes were restrained in a sitting position and the teat end of half udder was scrubbed thoroughly using cotton wool soaked in 70% ethyl alcohol. The first three streams were discarded, the teat orifice was disinfected again as described and 5 ml milk samples were taken in a sterile tube held horizontally. All samples were kept cold during transportation and delivered to the laboratory for examination within 2 h after collection. All milk samples of ewes were tested in mid-lactation (2^{nd} week after lambing until 10^{th} week postpartum). No clinical signs of mycoplasma infection were observed in the flocks and sheep were not checked for the presence of mycoplasmosis.

The California Mastitis Test (CMT) was applied to all samples collected using the method of (13). According to the reactions obtained, the results were classified as: negative, traces, 1, 2 and 3, recorded as -, ±, +, ++ and +++, respectively.

Blood sampling

From healthy ewes and affected ewes with subclinical mastitis, two separate blood samples were collected. For haematological studies and determination of Fibrinogen in plasma, one sample was taken in an EDTA-K2-containing vacutainer tube. To evaluate serum total protein, the second blood sample was taken in plain tube. The blood samples were centrifuged at 3 000 r/min for 10 min and the serum was separated and stored in Eppendorf tubes at −20 °C Until the analysis of biochemical parameters.

Bacteriology

All milk samples requiring bacterial culture were mixed well and a standard loopful (0.01 ml) from each milk sample was inoculated on the surface of blood agar (Bacto-Agar, Difco laboratory) containing 5% of washed sheep red blood cells and MacConkey agar plates. All plates were incubated aerobically at 37°C and examined for growth at 24 h. If there was no growth, the plates were reincubated and the final assessment was made at 48 h. The presence of six or more bacterial colonies of the same type on the medium was considered to be significant and the samples were recorded as positive. Bacteria were identified by using colony morphology, hemolytic pattern on blood agar media and further microscopic examination (Gram staining), standard biochemical methods (catalase, haemolysis, coagulase test with rabbit plasma) described by (14).

Hematology and biochemistry

Red Bloood Cell (RBC) count, Packed Cell Volume (PCV) value and Wight Blood Cell (WBC) counts were measured by routine procedures. Serum total protein concentrations were determined by Biuret method and spectrophotometer (Biochrom WPA Biowave II) and using commercial kits (Ziest Chemi Diagnostics, Tehran, Iran). The refractometry measurements of plasma fibrinogen were

obtained with an Atago (SPR-T2 Model, Fischer Bioblock, France) non temperature- compensated refractometer. The instrument was blanked with distilled water before each series of measurement. All readings were made at room temperature (approximately 20°C).

Definition

Mammary glands without clinical abnormalities and with apparently normal milk that were bacteriologically positive and with positive CMT were considered to have subclinical mastitis.

Statistical analysis

Data were organized in excel worksheets and then the data statistically were analyzed by SAS package software (SAS Inst. Inc., Cary NC ver. 9.1-2005). Two sample t-Test method were used for analyzing data in T-TEST procedure. The means were considered as significant when the p values were less than 0.05.

Results

The estimated prevalence of subclinical mastitis in the flocks sampled is shown in Table 1. The average prevalence of subclinical mastitis detected in this study in spring was 14.79%. This rate represents SCM cases means the presence of both a bacteriologically positive and CMT positive results. Of 791 milk samples collected in the spring, 144 (18.2%) samples with CMT and 134 (16.94%) samples were positive with bacterial culture. During the summer, 401 milk samples were collected. CMT and bacteriological results were compared in all samples and are shown in Table 1. As prevalence of SCM (based of SCM definition) was 8.9%. Of 401 milk samples collected in the summer, 47 (11.72%) samples with CMT and 44 (10.97%) samples were positive with bacterial culture.

Table 1: Two-way contingency table to investigate agreement between bacteriological and California Mastitis Test results for milk samples

Season		Culture +	Culture -	Total
Spring	CMT +	117	27	144
	CMT -	17	630	647
	Total	134	657	791
Summer	CMT +	36	11	47
	CMT -	8	346	354
	Total	44	357	401

Proportion positive by CMT: 18.2% and 11.72% in spring and summer, respectively; proportion positive by culture: 16.94% and 10.97% in spring and summer respectively.

Compartment of hematological and biochemical values between healthy and SCM ewes in two seasons was reported in Table 2. Our result showed that there was no significant difference in RBC and PCV between two seasons in SCM and Healthy ewes. Comparison of results of hematological and serum biochemical parameters in ewes with SCM and healthy ewes in spring and summer showed that WBC counts, total protein concentrations and plasma fibrinogen in both groups of ewes were significantly higher in spring than summer ($P<0.05$).

Table 2: Hematological and biochemical values between healthy and SCM ewes in two seasons

Season	SCM ewes	No	RBC	WBC	PCV	T pr.	Fib
Spring		117	6450486	4910	29.83	8.66	0.47
Summer		36	6348285	4637	27.74	7.54	0.36
	Significancy (0.05%)		Ns	*	Ns	*	*
	Healthy ewes						
Spring		647	6301012	4637	29.71	8.47	0.41
summer		345	6283012	4440	28.68	7.50	0.32
	Significancy (0.05%)		Ns	*	Ns	*	*

RBC: Red Blood Cell, WBC: Wight Blood Cell, PCV: Packed Cell Volume, T pr: Total protein, Fib: Fibrinogen. Ns: Non Significant, *: Significant.

Discussion

Hematology and biochemistry tests are an important tool that is widely used to evaluate of health status and diagnosis of disease in farm animals. Hematological (9) and biochemical (10) analyses are the essential elements of clinical examinations which often refer to a specific differential diagnosis or prognosis suggests (10). For diagnosis, prognosis and monitoring of the diseases in several animal species can be used of acute phase proteins (APPs) such as total protein and fibrinogen. Also to identification of animals with subclinical diseases, the use of (APPs) has been recommended. The influence of various factors such as breed (15), age, lactation, pregnancy and

season (16) on blood parameters has been described in healthy sheep.

Subclinical intramammary infection is considered as a major source of economic loss in the dairy sheep industry. Because it leads to a decline in the quality and quantity of milk produced. Previous studies on subclinical mastitis, have demonstrated that various noninfectious factors were associated with an increased of somatic cell counts in milk of sheep. The most important of these factors include parity, stage of lactation, seasons and diurnal variation (17). With regard to noninfectious factors influencing blood parameters in healthy sheep and sheep with subclinical mastitis, we find that some of these factors such as stage of lactation and seasons between these two groups are common.

The results of Table 1 revealed the agreement between bacteriological and CMT results for milk samples. Infection rate in milk samples collected from sheep in this study by bacterial culture and CMT was higher in spring (16.94% and 18.2%) than summer (10.97% and 11.72%), respectively.

This study shows some hematological and biochemical parameters in both healthy sheep and sheep with subclinical mastitis were significant differences in spring and summer (Table 2). However, in this study we evaluated the effect of two seasons; the results are not representative of all seasons, but at least it shows how to interpret these parameters. In this study, all hematologic and biochemical parameters evaluated on infected sheep with subclinical mastitis and healthy sheep in spring higher than summer, such that WBC counts, serum total protein and plasma fibrinogen in both groups were significantly higher ($P<0.05$) in spring than summer. This is in accordance with Baumgartner and Pernthaner (16) who showed that hematological parameters and total protein in healthy sheep in summer more than winter. Other researchers (18) were found a seasonal rhythm for serum albumin, alpha-2 globulins and albumin/globulins ratio for sheep. They attributed these changes to the changes in light and temperature throughout the year. In another study that was conducted by Mohammed et al. (19), the WBC and Differentia counts of sheep in summer were higher than winter.

Considering that most the literatures have focused on hematological and biochemical differences between summer and winter, as well as the most of the studies on subclinical mastitis in sheep, would not consider the effect of seasons, so this study was conducted to determine the effect of seasons on mentioned parameters, apart from the effect of inflammation on these parameters in sheep.

Based on the results of this study, it can be stated that season has significant effects on hematology and biochemical parameters in healthy sheep. Therefore, when interpreting hematological and serum biochemical parameters in ewes with SCM should be consider the effect of season on these parameters.

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