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Hematology and serum biochemistry analyses in Awassi sheep affected with clinical and subclinical mastitis caused by *Staphylococcus aureus* and antimicrobial sensitivity patterns of the isolated bacterial strains

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Abstract. The aims of this study were to evaluate the hematology and serum biochemical changes associated with *Staphylococcus aureus* mastitis in Awassi sheep and to determine the antimicrobial sensitivity patterns of the isolated bacteria. *S. aureus* was isolated from 24 mastitic udder halves. There were 18 udder halves affected with subclinical mastitis (normal looking udders and milk SCC more than 250,000 cells/mL) and 6 udder halves affected with clinical mastitis (abnormal looking udders and milk SCC more than 250,000 cells/mL). In the hematology and serum biochemical analyses, there were no statistically significant changes in any of the parameters between mastitic and normal sheep and between those affected with clinical or subclinical mastitis. The antibacterial sensitivity patterns of the isolated *S. aureus* against 11 different antibacterial agents revealed *in-vitro* sensitivity to enrofloxacin, ciprofluxacin and gentamicin only. Results of this study show an increased incidence of multidrug resistant *S. aureus* mastitis in Awassi sheep.
Key Words: Subclinical mastitis, clinical mastitis, Awassi ewes, multidrug resistant bacteria.

Introduction. Sheep are a major source of wool, milk and meat in many countries in the world. Sheep are sturdy animals and can adopt to live in harsh environments with little food supply and minimal care. Sheep keeping is considered in many communities a main source of livelihood income especially in low income regions of the world. Here in Jordan, there has been an increased demand for various sheep products in recent years (Alekish et al 2014; Hawari et al 2014).

Mastitis is an inflammation of the mammary gland mainly of bacterial origin (Contreras et al 2007; Scott 2007; Blowey & Edmondson 2010). In dairy farms, the health of the udder remains one of the key concerns throughout the world (Contreras et al 2007; Blowey & Edmondson 2010). Because mastitis can result in significant economic losses, it is considered as one of the most important diseases in dairy animals (Contreras et al 2007; Blowey & Edmondson 2010). This economic loss are mainly due to decreased milk production, usage of antimicrobial therapy, increased culling rate, discarded milk and labor cost (Contreras et al 2007; Blowey & Edmondson 2010).

The prevalence of clinical and subclinical mastitis in sheep has been reported as 30% 35%, 39%, and 14% in Jordan, Spain Iran, and Australia respectively (White 2007; Sampimon et al 2008; Alekish et al 2014; Hawari et al 2014). *Staphylococcus aureus* is considered one of the most important contagious pathogens causing mastitis in dairy animals. This microorganism is associated with increased economic losses because of the chronic nature of the disease and difficulties hindering successful treatment (Contreras et al 2007). It is known that *S. aureus* causes microabscessation of the udder tissue making local and systemic treatment ineffective during the lactation period (Contreras et al 2007). Furthermore, the bacteria rapidly develop drug resistance further complicating the

situation (Contreras et al 2007). Therefore, the aims of this study were to evaluate the hematology and serum biochemical changes associated with *S. aureus* mastitis in Awassi sheep and to determine the antimicrobial sensitivity patterns of the isolated bacteria.

Material and Method. All experiments were performed at The Veterinary Health Center, Faculty of Veterinary Medicine, Jordan University of Science and Technology.

All experimental protocols performed in this study were reviewed and approved by the Jordan University of Science and technology Animal Care and Use Committee (JUST-ACUC).

This study was performed using 12 mastitic and 10 normal Awassi sheep. The ewes were approximately 2-5 years of age and weighing 50-70 kg. All the ewes were in their first month of lactation and were routinely milked manually twice per day. The ewes were presented to the veterinary Health Center at Jordan University of Science and Technology with a primary complaint of mastitis or other udder related problems.

All sheep were subjected to a complete physical examination including body temperature, pulse rate and respiration rate. The udder and teat were palpated for any abnormalities. A whole blood sample was collected from all sheep via vein puncture for hematology and blood biochemistry evaluation using routine laboratory methods. Milk samples were collected from each half individually and submitted to the laboratory for bacterial culture and antibacterial sensitivity testing. Somatic cell counts were determined for each sample manually. Whole blood and milk samples were also collected from 10 normal sheep from the same herds with similar management and stage of lactation and used as control.

Whole blood samples were collected via vein puncture from the jugular vein from each animal and placed in plain and EDTA containing blood tubes. Hematology and serum biochemical analyses were determined using previously described methods (Thrall et al 2004). Parameters investigated were: total white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets count. Serum was analyzed to determine total protein, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), calcium, sodium, chloride, magnesium and potassium using commercially available kits according to manufacturer's recommendations. Fibrinogen concentrations were also determined.

Individual milk samples from the right and left udder halves were aseptically collected from each ewe and submitted for bacterial culture and sensitivity testing as described previously (Ajuwape et al 2005; Alekish et al 2013). Bacterial sensitivity was determined against 11 different antibacterial agents including ampicillin (AMP, 10µg), penicillin G (PEN) (10 IU), streptomycin (STR) (10 µg), gentamycin (GEN) (10 µg), erythromycin (ERY) (15 µg), ciprofloxacin (CIP) (5 µg), oxytetracycline (OXTE) (30 µg), trimethoprim/sulphamethoxazole (Trim/SULPHA) (25 µg), enrofloxacin (ENR) (5 µg), doxycyclin (DOX) (30 µg) and neomycin (NEO) (30 µg).

Somatic cell count was performed manually as described previously (Alekish et al 2013). Briefly, milk samples were gently shaken before a 0.01 mL of milk was smeared on one centimeter square on a glass slide, dried, fixed, and stained with Newman-Lambert stain. Thirty fields were then counted under light microscope at 100X oil immersion lens. The following equation was used to determine the total somatic cells per mL: Somatic cells/mL = microscopic factor (MF) * (WBCs/field)*100

Data were analyzed using SPSS statistical package (IBM, SPSS version 20, 2011). Descriptive statistics were performed using frequency as well as means and standard error of the means (SEM) to describe the numeric data. Student t-test and one way ANOVA for independent samples were used to compare means of the normally distributed numeric variables.

Results. Twenty two Awassi ewes (12 mastitic and 10 normal) in their first month of lactation were used in this study. Only animals from which *S. aureus* (24 udder halves) was isolated were included in the study. Individual udder halves were further classified

according to udder health and SCC into normal (n=20 udder halves) (normal looking udder and milk SCC less than 250,000 cells/mL), subclinical mastitis (n=18 udder halves) (normal looking udders and milk SCC more than 25,000 cells/mL) and clinical mastitis (n=6 udder halves) (abnormal looking udders and milk SCC more than 250,000 cells/mL).

Tables 1 and 2 show the Means ± SE of various hematology and serum biochemical parameters in Awassi sheep affected with clinical and subclinical mastitis caused by *S. aureus* as compared to normal sheep. There were no statistically significant differences in any of the parameters.

Table 1

Mean ± SE of various hematology parameters in Awassi sheep with clinical and subclinical mastitis caused by *Staphylococcus aureus*

Parameters	*Clinical mastitis*	*Subclinical mastitis*	*Normal udders*	*P value*
WBC ($\times 10^3$ cells/µL)	13±1.1	11±1.2	12±1.7	0.4
RBC ($x10^6$ cells/µL)	8±0.4	9±0.8	9±0.5	0.7
HB (g/dL)	8±0.3	8±0.6	9±0.5	0.5
PCV (%)	25±1.2	26±2.8	26±1.1	0.7
MCV (fl)	29±0.3	30±0.4	29±0.5	0.4
MCHC (g/dL)	35±1.0	31±0.8	34±0.9	0.07
Platelets ($\times 10^3$ cells/µL)	710±64	740±107	608±154	0.7

WBC- white blood cell count, RBC- red blood cell count, Hb- hemoglobin concentration, PCV- packed cell volume, MCV- mean corpuscular volume, MCHC- mean corpuscular hemoglobin concentration.

Table 2

Mean ± SE of various biochemistry parameters in Awassi sheep with clinical and subclinical mastitis caused by *Staphylococcus aureus*

Parameters	*Clinical mastitis*	*Subclinical mastitis*	*Normal udders*	*P value*
BUN (mg/dL)	15±3.0	12±2.0	12±3.0	0.34
Creatinine (mg/dL)	1.1±0.15	0.8±0.06	0.8±0.07	0.27
Total protein (g/L)	71±2.7	79±2.0	79±2.6	0.6
AST (IU/L)	59±6.7	45±10.5	41±12	0.15
ALT (IU/L)	17±2.2	22±5.5	24±8.2	0.47
ALP (IU/L)	106±32	204±34	226±57	0.06
Fibrinogen (mg/dL)	546±22	444±42	460±40	0.06
Calcium (mg/dL)	8.0±0.6	8.0±0.5	10±0.9	0.09
Magnesium (mg/dL)	2.0±0.17	2.5±0.1	2.6±0.18	0.42
Sodium (mmol/L)	151±3.0	153±1.4	149±1.8	0.76
Potassium (mmol/L)	4.6±0.4	5.4±0.25	5.4±0.16	0.27
Chloride (mmol/L)	103±8	112±0.58	112±0.6	0.77

BUN- blood urea nitrogen, AST- aspartate aminotransferase, ALT- alanine aminotransferase, ALP- alkaline phosphatase.

Milk culture and antibiotics sensitivity test results are shown in Table 3. *S. aureus* was isolated from 24 milk samples. In-vitro sensitivity patterns of the isolated *S. aureus* that the majority of these bacteria were sensitive to enrofluxacin and ciprofluxacin (20 isolates each) and gentamicin (21 isolates).

Table 3

Antibacterial sensitivity patterns of *Staphylococcus aureus* isolated from mastitic milk of Awassi sheep

Number of samples	*Antimicrobial*	*Number and percentage of sensitive isolates*
24	Enrofloxacin	20/24 (83%)
	Penicillin	4/24 (17%)
	Ciprofloxacin	20/24 (83%)
	Gentamicin	21/24 (88%)
	Ampicillin	3/24 (13%)
	Oxytetracyclin	6/24 (26%)

Discussion. Somatic cell count (SCC) in healthy udders of sheep can vary greatly and a cut point has not been established yet. Normal milk in sheep can have SCC of over than 10^6 cells per milliliter (Menzies 2000; Gonzalo et al 2002; Schukken et al 2008). However, many researchers suggested that a 250,000 cells per milliliter as an upper limit for SCC in healthy sheep udder (Menzies 2000; Schukken et al 2008; Alekish et al 2014). In this study, we used a cut off limit 250,000 cells per milliliter SCC to detect mastitis as suggested previously.

The prevalence of mastitis including both clinical and subclinical cases in Awassi sheep in Jordan has been reported to be around 30% (Lafi et al 1998; Al-Majali & Jawabreh 2003; Alekish et al 2014). The type of microorganisms found in milk can vary from one sheep flock to another and may depend on udder hygiene during milking, milking routine and environmental hygiene. In this study, *S. aureus* was isolated from 24 udder halves affected with either clinician or subclinical mastitis. All sheep were milked manually and that may explain the high prevalence of this pathogen in the sheep used in this study. It has been reported that the main route of transmission being during milking process via contaminated milker's hands.

In recent years, there have been an increased evidence of the emergence of antimicrobial resistance by most bacteria causing mastitis making successful treatment more difficult (Lafi et al 1998; Al-Majali & Jawabreh 2003; Alekish et al 2014). Our results are in agreement with previously reported data from Jordan regarding antimicrobial resistance of this particular microorganism.

Clinically, the sheep in this study were either affected with clinical or subclinical mastitis. In clinical mastitis group, the sheep appeared mildly or not at all systemically affected as indicated by normal heart rate, respiration rate and rectal temperature. Signs of inflammation were limited to the affected gland which was detected to be swollen and hot in most cases with abnormal milk secretion. These results are similar to previous reports of clinical and subclinical mastitis in sheep (Menzies 2000; Scott 2007; Schukken et al 2008).

Furthermore, the hematology analyses performed in this study revealed normal values for total WBC, RBC count, Hb and PCV in both clinical and subclinical mastitis groups. In addition all RBC indices were within normal limits. Complete blood count (CBC) including the leukon and eythron evaluation is commonly used to assess the systemic status of sick animals (Radostits et al 2007). However, it has been reported that changes in the hematology analyses in cases of mastitis were limited to those caused by gram-negative bacteria but not gram-positive bacteria (Smith et al 2001; Radostits et al 2007; Bani Ismail & Dickinson 2010). Lack of endotoxemia in cases of clinical mastitis caused by gram-positive bacteria was suggested as an explanation for this difference (Smith et al 2001). This also explains the normal vital signs reported in this study.

In the contrary, some researchers reported significantly increased or decreased PCV and RBC in some clinical mastitis cases (Ajuwape et al 2005). Decreased PCV and RBC count in some cases of mastitis may indicate chronic disease not related to mastitis such internal parasitism, blood loss anemia or malnutrition. In on the other hand, increased PCV and RBC may indicate dehydration and hemoconcentration in severe

clinical cases (Ajuwape et al 2005). In the serum biochemical analysis, our results show that there were no significant differences in the clinical and subclinical mastitis groups when compared to the normal sheep. These results are similar to previously reported data in sheep and dairy cows (Smith et al 2001; Radostits et al 2007; Bani Ismail & Dickinson 2010). Serum electrolytes including calcium, potassium, sodium, magnesium and chloride were also in the normal limits for sheep in this study. These results are contrary to some other previous reports who found these electrolytes imbalances due to anorexia, rumen stasis, illius or diarrhea (Kaneko et al 1997).

Conclusions. The study revealed that the most common cause of clinical and subclinical mastitis in Awassi sheep in Jordan is the infection with the pathological agent *S. aureus*. In most cases the infection does not appear to significantly affect the general health of the affected ewes. Most importantly and also disquieting, the results of this study show an increased incidence of multidrug resistant *S. aureus* mastitis in Awassi sheep.

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