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Original Article

Influence of *Staphylococcus aureus* Mastitis on Milk Composition of Different Dairy Breeds of Cattle in Khartoum State, Sudan

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ABSTRACT

The purpose of the present study was to investigate the effect of *S. aureus* mastitis on the compositional quality of milk. Sixty milk samples were collected from 19 Frisian, 16 cross and 25 local cows suffering from subclinical (14) or clinical (46) mastitis from different farms in Khartoum State, Sudan. Total bacterial count, *S. aureus* count and some compositional quality were estimated. Non-significant ($P \geq 0.05$) differences were recorded between the milk obtained from the three groups of dairy cattle for total bacterial count. However, significantly ($P \leq 0.05$) higher *S. aureus* count was obtained ($2.7\times 10^3 \pm 0.7$ cfu/ ml) for milk samples from cross- bred cows. Chemical analysis revealed no significant ($P \geq 0.05$) differences in total solids (11.87± 1.7 and 11.1± 1.6%), protein (4.3± 2.8 and 3.6± 0.5%), fat (3.1± 0.7% and 3.10± 0.5), lactose (2.3± 0.5 and 2.1± 0.7%) and acidity (0.211± 0.14 and 0.44± 0.36 %) for milk obtained from cows with subclinical and clinical mastitis, respectively. However, significantly higher ash content was found in milk samples collected from clinical mastitis (0.68± 0.12%) compared to subclinical ones (0.6 ± 0.15%). Milk constituents of infected milk by *S. aureus* revealed non-significant ($P \geq 0.05$) differences expect for lactose ($P < 0.01$), which showed variations between breeds. Total bacterial count showed significantly ($P \leq 0.05$) negative correlation (r= -0.037) when compared with lactose content of mastitic milk. It could be concluded that mastitis caused by *S. aureus* would resulted in the decrease the nutritive content of milk.

KEY WORDS: *Staphylococcus aureus*, Mastitis, Milk Composition, Dairy Breeds, Sudan

INTRODUCTION

Mastitis caused by *Staphylococcus aureus* is recognized worldwide in dairy cows as subclinical and clinical intramammary infection (Akineden et al., 2001); it is of considerable importance in Sudan (Mohamed et al., 1997; El Zubeir et al., 2006). The infection is spread at milking time when *S. aureus* contaminated milk from an infected gland comes in contact with an uninfected gland, and the bacteria penetrate the teat canal. One reason for the failure to eradicate *S. aureus* intramammary infection may be the control measures for mastitis do little to control this disease in prepartum period in heifers (Roberson et al., 1998). Moreover Ebliny et al. (2001) reported that *S. aureus* causes infection of longer duration.

Recently, it was reported that 3% of all animals are infected with *S. aureus* (Schukken, et al., 2009). Tenhagen et al. (2009) added that *S. aureus* infection represents 10 to 12 % of clinical mastitis cases. Moreover, in a study conducted in Egypt, it was found that 16% of all mastitis cases are caused by *S. aureus* (Hameed et al., 2006). In Ethiopia, examination of 300 lactating cows (140) 46.7% mastitis cases; 10.0% and 36.7% for clinical and subclinical infections, respectively (Abera et al., 2010). Moreover, with severe clinical mastitis, abnormalities of milk are easily observed and such milk is discarded by the producer. However, milk of cows with subclinical mastitis, (with no visible changes) is accidentally mixed into bulk milk; it enters food chain and can be dangerous to humans (Hameed et al., 2006). Contagious mastitis, transmitted from cow to cow during milking is mainly caused by *S. aureus* (Fox and Gay, 1993).

The onset of udder infection (mastitis) is known to influence the chemical composition of milk constituents (Mohamed et al., 1997). *Staphylococcus aureus* causes severe tissue damage that result in excretion of cells into milk (Kitchen, 1981; (Petersson-Wolfe et al., 2010). Clinical mastitis infection detected by California Mastitis Test (CMT) has a significant effect on the total solid of milk (Mohamed et al., 1999), Bovine *S. aureus* strains express many

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virulence factors, which may enable this organism to cause chronic infection (Sutra and Poutrel, 1994). Further Middleton (2002) reported that *S. aureus* caused a significant (P<0.01) decrease in the infected quarter compared with the uninfected quarters in the same cow. Hence the objectives of the study were isolation, enumeration of *S. aureus* and assessment of its influence on composition of milk with clinical and subclinical mastitis along with breed dependent differences in the innate immune response in different cow breeds to *S. aureus* intramammary infection

MATERIALS AND METHODS

Animal

In the present study, Friesian, local breeds (Kenana and Butana) and cross breed (local cows and Friesian) from farms in Khartoum State were examined for presence of clinical and subclinical mastitis. Mastitis milk samples were collected from Shambat (n=18), Tayba farm (n=4), Azaheer Company farm (n=8), El Radwan Project (n=9), Western Elgrif area (n=5), El Ailafoon (n=6), Eid Babikr (n=6) and Hamza farm (n=4).

Collection of milk samples

The udder was first cleaned with soap then dried with clean towels. The teats were swabbed with 70% alcohol. Visual observations and (CMT) were done before the collection of samples to group the cows into clinical and subclinical cases. Then, approximately 100 ml milk from each cow was taken into sterile Mc-Cartney bottles.

Microbiological examination

Total bacterial count was done on plate count agar (Merck, Darmstadt, Germany) by pour plating methods following the manufactures instructions (Marshall, 1992). The appropriate dilution of milk samples was selected and the plates were incubated at 32º C for 48 hours. For the determination of *S. aureus*, Mannitol salt agar medium (Oxoid, Hampshire, England) was used followed by confirmation by biochemical test. The plate counting 25- 250 colonies were selected as described by Houghtby et al. (1992). All counts were done in duplicates, multiplied by the reciprocal of the dilution and reported as colony forming unit per milliliter (cfu/ ml).

The presence of *S. aureus*

Staphylococcus aureus isolates were Gram stained and subjected to motility and catalase test, acid production and oxidation fermentation (OF) test. Moreover, fermentation of sugars, methyl red (MR), Voges-Proskauer (VP) and tube coagulase test were done as secondary confirmatory tests according to the criteria outlined by Barrow and Feltham (1993).

Chemical analysis

The chemical constituents including total solids, fat (Gerber's method), protein (Kjeldahl method) and ash of milk samples were done according to AOAC (1990). The lactose was determined by anthrone method according to Richards (1959). The acidity of milk samples was also determined using the titration methods according to AOAC (1990).

Statistical analysis

Data were arranged into computer coding format to facilitate the statistical analysis. The SPSS (Statistical Package for Social Science) was used by using randomized complete block design to differentiate between the intensity of infection and different breeds of cows. Also the graphs was plotted using excel program.

RESULTS AND DISCUSSION

The numbers of mastitic cows detected from Friesian, local and cross breed in Khartoum State are shown in Figure 1. The isolation of *S. aureus* from subclinical and clinical mastitic cows indicated that *S. aureus* is one of important pathogens causing mastitis. This result was in line with Tenhagen et al. (2009). Moreover, in dairy cows, *S. aureus* is considered a contagious organism, which easily spread between cows during milking (Paape et al., 2000). *Staphylococcus aureus* was isolated in Sudan from medical laboratories environment by El Mansouri (1997), from mastitic cows' milk by Mohamed et al. (1997) and El Zubeir et al. (2006) and from raw camels' milk by Shuiep et al. (2009).

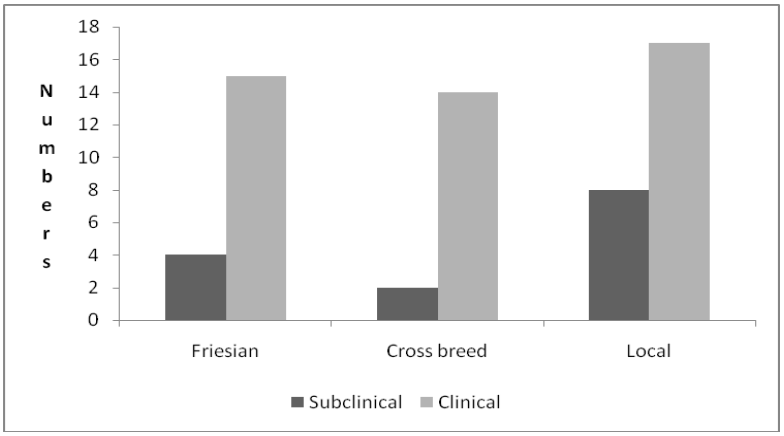

Figure1. Comparison of prevalence of *S. aureus* mastitis in different cows at dairy farms in Khartoum State

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Differences were reported between the three cattle breeds for the total bacterial count (Table 1). Milk from Friesian cows showed less *S. aureus* count than local cows. This result supported Bannerman et al. (2008) who reported that *S. aureus* intramammary infection showed differences in the innate immune response in different cow breeds. Those could be attributed to resistance of local cows to bacterial infection (El Deeb and Hassan, 1987). On the other hands, Berry et al. (2007) reported that Jersey cows have lower prevalence of mastitis than Friesian cows. In all 3 types of cows the total bacterial count was more compared to *S. aureus* count (Table 1).

Table 1. Chemical composition and microbial counts of milk from mastitic dairy cows in Khartoum State

Measurements	Friesian (n=19)	Cross breed (n=16)	Local breed (n=25)	Sig. level (P-value)
Acidity	0.28±0.3	0.33±0.3	0.35±0.3	0.86 ^{NS}
Total solids	11.3±1.6	10.95±1.8	11.66±1.5	0.50 ^{NS}
Ash	0.68±0.13	0.7±0.14	0.62±0.12	0.23 ^{NS}
Fat	3.2±0.41	2.99±0.6	3.1±0.68	0.61 ^{NS}
Protein	3.4±0.55	3.66±0.35	4.3±0.2	0.26 ^{NS}
Lactose	2.42±0.6	1.67±0.7	2.3±0.6	0.009 **
S. aureus count (cfu/ml)	$5.9\times10^{2}\pm0.9$	$2.7\times10^{3}\pm0.7$	$1.5\times10^{3}\pm0.5$	0.05*
Total bacterial counts (cfu/ml)	$1.04\times10^{4}\pm0.6$	$1.9\times10^{5}\pm0.9$	$1.04\times10^{3}\pm0.12$	0.47 ^{NS}

N.S = Non Significant; * = Significant ($P \leq 0.05$); ** = Significant ($P \leq 0.01$).

Table 2. Comparison between chemical composition and microbial counts of subclinical and clinical mastitic milk of dairy cow in Khartoum State

Measurements	Subclinical	Clinical	Sig. level (P-value)
Acidity (%)	0.211±0.14	0.44±0.36	0.2 ^{NS}
Total solids (%)	11.87±1.7	11.1±0.6	0.19 ^{NS}
Ash (%)	0.6±0.15	0.68±0.12	0.05 *
Fat (%)	3.1±0.7	3.1±0.54	0.93 ^{NS}
Protein (%)	4.3±2.8	3.6±0.5	0.16 ^{NS}
Lactose (%)	2.3±0.5	2.1±0.7	0.38 ^{NS}
S. aureus count (cfu/ml)	$6.3\times10^{2}\pm0.9$	$1.9\times10^{3}\pm0.7$	0.21 ^{NS}
Total bacterial counts (cfu/ml)	$7.9\times10^{4}\pm0.2$	$1.4\times10^{5}\pm0.2$	0.4 ^{NS}

N.S = Non Significant; * = Significant ($P \leq 0.05$); ** = Significant ($P \leq 0.01$).

The present study indicated variations in means of bacterial counts and milk constituents for subclinical and clinical mastitic milk samples (Table 2). The mean bacterial count for clinical mastitis milk ($1.9\times 10^{3}\pm 0.7$ cfu/ ml) was higher than that of subclinical mastitis milk ($6.3\times10^{2}\pm0.9$ cfu/ ml). This was in accordance with the finding of El Deeb and Hassan (1987) who reported that total bacterial count increased when milk tested positive for mastitis. Also Mohammed et al. (1997) reported high log bacterial count for clinical followed by subclinical mastitis milk compared to those from healthy cows. It also agrees with the findings of Bautista et al. (1986) who reported a level of 10^{4}– 10^{5} cfu/ ml *S. aureus* in milk from subclinical infected ewes. The levels of total protein in this study (Table 1 and Table 2) were found to be similar to those reported by Kitchen (1981) who concluded that total protein content in mastitic milk does not change with increasing the level of infections in the mammary gland.

Table 2 showed that the mean acidity of clinical mastitic milk is higher than that of subclinical mastitis milk. Mastitic milk from Friesian, cross and local cows revealed mean acidity of 0.28± 0.3%, 0.33± 0.3% and 0.35±0.30%, respectively (Table 1). The lower values of titratable acidity obtained during the present study is in agreement with those of Schulz et al. (1988) who reported significant association between mastitis and low milk acidity, which might be due to more alkaline constituents of the blood that passing into the milk. The lactose value of the present study showed lower values when compared with those of healthy cows, which supports Mohamed et al. (1997). Moreover, mastitic milk was reported to have lower lactose contents (Gunha, 1989), especially in the staphylococcal infection (Mohamed et al., 1997).

The relatively high significant level ($P<0.01$) of ash observed during the present study when comparing subclinical cases to clinical ones was in agreement with the finding of El Deeb and Hassan (1987). The fat content from mastitis milk was found to be lower than those from healthy cows. The reduction of fat may be due to the increase rate of lipolysis during infection (Murphy et al., 1989).

It is concluded that the compositional content of milk from mastitic animals showed variations that influences by the breed and the degree of infection. Moreover in subclincal mastitis, when the farmers were unable to recognize the disease public health hazards might occur due to consumption of infected milk that contains pathogenic bacteria or their toxins.

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