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Full Length Research Paper

Host determinants of bovine mastitis in semi-intensive production system of Khartoum state, Sudan

Nuol Aywel Madut[1], Atif Elamin Abdel Gadir[2]*, Isam Mohamed El Jalii[2]

[1]Department of Medicine, Faculty of Veterinary Medicine, University of Bahr. Elgazal. [2]Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum, Box 32, Khartoum, Sudan.

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Out of 2283 quarter milk samples, 224 (9.81%) gave positive reactivity to California mastitis test, while 600 (26.28%) and 1459 (63.9%) recorded as doubtful and negative respectively. In this study isolated *Corynebacterium* were *Corynebacterium striatum* 9 (33.3%), *Arcanobacterium pyogene* 4 (14.8%), *Corynebacterium Pseudotuberculosis* 2 (7.4%), *Corynebacterium ulcerans* 5 (18.5%), *Corynebacterium bovis* 7 (25.9%). The result showed that age, stage of lactation, teat lesion could be a risk factors for presence of bovine mastitis (OR = 1.34, 1.59 and 7.31 respectively).

Key words: *Corynebacterium* spp., California mastitis test, host determinant, semi-intensive production, Sudan.

INTRODUCTION

Livestock production systems in Africa are classified into intensive and semi-intensive systems according to husbandry practice and distribution of pasture that varies with the rainfall, season or cultivated crop (Pyne, 1976; Ruthenberg, 1976; DeBoer, 1977; Jahnke, 1977) . In Sudan, 92% of livestock population is possessed by no-mads that follow extensive system of husbandry in eas-tern, western and southern part of the Sudan (Kamal, 1983). Among the Sudanese breeds of cattle, 2 breeds namely Kenana and Butana are known to show high po-tentiality for milk production (Alim, 1960; Osman, 1970; Osman and El Amin, 1971). Recently Friesian crosses with local breeds were raised.

Mastitis remains the most common and the ambiguity disease of dairy cattle through out most of the word. It probably has been observed since man first domestic-cated the cow in the thousands of years since and in spite of all kinds of scientific progress, it remains preva-lent in most dairy herds. It is estimated that one third of all dairy cows are infected with some form of mastitis in one or more quarters (Philpot and Nickerson, 1999). Ma-stitis is often the end result of the interaction of several factors such as man, cow, environment, microorganisms and management (Blood et al., 1989).

*Corresponding author. E-mail: atifvet@yahoo.com.

Quarters infected with *Arcanobacterium pyogenes* al-ways exhibit clinical symptoms and secrete a thick, foul-smelling, greenish fluid. Infections result in a persistent form of mastitis and invariably lead to loss of the quarter and culling of the cow because treatment is ineffective. (Philpot and Nickerson, 1999). In lactating cows, infection may occur as a result of teat injuries or improper treat-ment procedure. Therefore, the objective of the study to investigate presence of the factors that affected its occur-rence of bovine mastitis.

MATERIALS AND METHODS

Study area

The study was conducted in east Nile locality - Khartoum north (Hillat Kuku dairy farms), which is considered to be the largest milk producing and marketing area in Khartoum state and regarded as semi-intensive system (small holder) of milk production. Those farms previously belonged to Hillat Kuku dairy project, which consist of 3 barns distributed in vast space each barn composed of small units contain cattle range between few number of cows to large which may reach hundreds of them. The scheme had agricultural field, factory to process the pooled milk that collected from the farms, artificial insemination center, veterinary service unite, animal production institute and banking services.

Sampling Methods

The study animals that were sampled are dairy cows mostly Frisian

Table 1. Interpretation of the (C.M.T) results (Quinn et al., 1994).

CMT score	Interpretation	Visible reaction
0	Negative	Milk fluid and normal
±	Trace	Slight precipitation
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickness with a gel formation
3	Strong positive	Viscosity greatly increased strong gel That is cohesive with a convex surface

cross (cross between Frisian and local breeds namely Kenana and Butana). Concerning sampling, one-stage sampling method was employed as described by Thrusfield (1995). Data on individual cow mainly breed, age, stage and number of lactations, previous history of mastitis, abnormalities of the udder and milk and other data related to mastitis were recorded using a special form.

Questioner design

Farm data on knowledge related to level of education and occupation of the owner, breed, management, animal health, hygiene status were recorded. The questionnaire survey was done in the study area based on the willingness of the owners. (Non probability sampling method, Thursfield, 1995).

Collection of milk samples

Before the collection of quarter milk samples from the tested cows, the udder was thoroughly cleaned with soap and water, rubbed dried and the teats were disinfected with cotton wool moistened with 70% ethyl alcohol, which is been allowed to be air dried. The first few squirts of milk were discarded. 5 - 20 ml of milk was collected in a sterile universal bottle. The quarter milk samples were kept in ice container and transported as soon as possible to the laboratory at the faculty of veterinary medicine, Khartoum university, (Shambat).

California mastitis test (CMT)

All collected milk samples were examined for mastitis using California mastitis test. **(**CMT) was carried out using the method described by (Quinn et al*.,* 1994). Briefly, equal volumes (5 ml) of commercial CMT reagent (avatar rapid mastitis test Kit-Alvetera Gmbh-Germany) and quarter milk were mixed and the changes in milk fluidity and viscosity were observed. The interpretation of the result was done according to the method described by Quinn et al. (1994). Negative (0) and trace (+/-) were considered as negative and different intensities of positive (1, 2 and 3) were considered as positive (Table 1).

CULTURING METHODS

The bacteriological culture was performed following the standard microbiological technique (Quinn et al., 1994). One loop full of milk was streaked on 5% sheep blood agar and MacConkey agar to detect bacteria that could grow on this medium. The plates were incubated aerobically at 37°C for 24 - 48 h. The plates were examined for growth, morphologic features of the colonies and hemolytic characteristic. Presumptive identification of bacteria on pure culture was done on the basis of colony morphology, heamolytic characteristics, Gram-stain and biochemical tests such as, coagulase test, heamolyses, pigment production, fermentation of maltose (purple agar +1% maltose). Presence of *Streptococcus* spp. and *Enterococcus* spp. was determined according to CAMP reaction, type of heamolyses, growth characteristic on Edward's medium and sugar fermentation.

Corynebacterium spp. and *Bacillus* spp. were identified based on heamolytic characteristics, catalase test, growth on 9% NaCl, CAMP reaction and sugar fermentation tests. Gram-negative isolates were identified based on growth on MacConkey agar, catalase test, oxidase reaction, triple sugar iron agar (TSI), IMVIC test, urease and sugar fermentation tests. The differentiation of microbial isolates was carried out as summarized in Table 1. Identification of the isolated Corynbacterium spp. to species level was done using commercial identification kit (API Coryne BIOMERIEUX, FRANCE)**.**

Determination of clinical and sub- clinical mastitis

Clinical mastitis was recognized by abnormal milk and signs of udder infection (abnormalities of the udder). While sub-clinical mastitis refers to the existence of inflammation of the udder in the absence of gross signs, this was established by California mastitis test and bacteriological examinations.

Statistical analysis

Microsoft excel, 2003 and Stata 6.0 for windows 98/95/NT were used for data analysis. Descriptive statistics were used for all the variables. Chi-square (x 2) was used for assessing the statistical associations of various factors with mastitis. The logistic regression model was employed to obtain the odds ratio (OR) only for those factors which gave statistical significant ($P < 0.05$) with regard to mastitis for Instance, the factor could be a risk factor when the OR > 1.

RESULTS

Questionnaire survey

The results showed that mastitis is regarded as one of the common diseases in dairy farms in Kuku area (33.3%, n = 10). However, most of the dairy farms have access to veterinary services as (100%, n = 30). Generally, the house condition was mostly bad (63.3 %, n = 19) although in some of them was good (36.7%, n = 11). Concerning hygienic status in the dairy farms in the study site, 30 owners did not clean the udder or wash their hands before and between milking or practiced teat disinfection (100%). All the responses to the questionnaire survey are summarized in (Table 2).

Table 2. Summary of the questionnaire survey responses by thirty owners of the diary farms in Kuku area.

Unit	Frequency (%)
Common disease	
mastitis	10 (33.3)
tick borne diseases	5 (16.7)
other diseases	15 (50.0)
Veterinary services	
yes	30(100)
no	0(00.0)
Clean teat and udder	
yes	0(00.0)
no	30(100)
Water hands before milking	
yes	0(00.0)
no	30(100)
Level of hygiene	
excellent	1 (3.3)
good	14(46.7)
poor	15(50)
Housing condition	
excellent	0(00.0)
good	11(36.7)
poor	19(63.3)

Table 3. The prevalence of clinical and sub-clinical mastitis based on CMT in examined farms at cow level.

Total number of animal examined	Prevalence (%)	
	Clinical	Sub-clinical
585	(21)3.58	(118)20.17

Clinical mastitis based on detection of udder and milk
Sub-clinical mastitis based on (CMT)
Cut off level of CMT: +++, ++, + + ve ±, - - ve

California mastitis test

Out of 2283 quarter milk samples, 224 (9.81%) gave positive reactivity to California mastitis test, while 600 (26.28%) and 1459 (63.9%) recorded as doubtful and negative respectively.

Clinical and sub-clinical mastitis

Prevalence (9.81%) of sub-clinical mastitis according to CMT was obtained from farms examined while low prevalence (3.58%) of clinical mastitis was reported (Table 3).

Bacteriological examination

205 bacterial isolates were recovered from milk samples examined. The isolated bacteria were *Staphylococcus spp*. 107 (52.5%), *Streptococcus* spp. 25 (12.3%), *Enterobacterium* spp. 4 (2%), *Lactobacillus* spp. 4 (2%), *Coryneform* bacteria 27 (13.2%), *Micrococcus* spp. 10 (4.9%), *Pseudomonas* spp., 11 (5.9%), *Bacillus* spp., 10 (4.9%) and *Aercoccus* spp*.,* (Table 4). In this study isolated *Corynebacterium* were *Corynebacterium striatum* 9 (33.3%), *Arcanobacterium pyogene* 4 (14.8%), *Corynebacterium pseudotuberculosis* 2 (7.4%), *Corynebacterium ulcerans* 5 (18.5%), *Corynebacterium bovis* 7 (25.9%).

Risk factors analysis

Factors such age, stage of lactation, tick infestation, confirmation of udder, teat lesion and previous history of mastitis were found statistically significance with regard to occurrence of bovine mastitis in Kuku area (Table 5). To quantify these relationships, the logistic regression model was adopted. The result showed that age, stage of lactation, teat lesion could be a risk factors for presence of bovine mastitis (OR = 1.34, 1.59 and 7.31 respectively) (Table 6). On the other hand, strong relationship was found between milk production and occurrence of bovine mastitis. (t - test = 51.32, $P < 0.01$).

DISCUSSION

Mastitis can be defined as an inflammation of the mammary glands caused by physical or chemical agent, but the majority of the infections are usually caused by bacteria (Quinn et al., 1994). Bovine mastitis is of great economic importance to diary industry world wide (Miller et al., 1984). Those farms previously belonged to Hillat kuku dairy project. The area was chosen in accordance to the result that obtain from the Khartoum state ministry of agriculture and animal resources (2003) which conducted survey on milk hygiene in Kuku area at the farm level, bulk milk and venders. The survey proved that Kuku area is the mostly bad in this concern.

The questionnaires survey revealed that, most of the owners were poorly managed their farms because they didn't know the basic of farm production management and also they have not consulted professionals to help them on managing their farms. This resulted in a poor performance of dairy production. (Saluiemi, 1980) stated that current knowledge on the impact of the production environment on udder health is considerable. Moreover, practical experience of mastitis control has confirmed the importance of the stand structures, ventilation, milking machine, management practices, milking technique in particular and hygiene on udder health. Also Abdullah (2002) claimed that good management is the key factor on controlling the environment for protection and hence mastitis occurrence.

According to our findings, mastitis is one of the common diseases in dairy farms (33%), although most of the farmers had access to veterinary services. (Miller et al*.,* 1984) reported that mastitis is the most common disease

Table 4. Gram positive and negative bacteria isolated.

Isolates	Clinical No (%)	Sub-clinical No (%)	Total No (%)
Staphylococcus spp.	8 (38.1)	99(53.8)	107 (52.5)
Streptococcus spp.	4(19)	21(11.4)	25(12.3)
Enterbacterium spp.	---	4(2.2)	4(2)
Lactobacillus spp.	---	4(2.2)	4(2)
Coryneform bacterium	4(19)	23(12.5)	27(13.2)
Micrococcus spp.	1(4.8)	9(4.9)	10(4.9)
Pseudomonas spp.	3(14.3)	8(4.3)	11(5.9)
Bacillus spp.	1(4.8)	9(4.9)	10(4.9)
Aercoccus spp.	----	7(3.8)	7(3.2)
Total	21(100.0)	184(100.0)	205(100.0)

Table 5. The association between the occurrence of mastitis and various factors.

Factors	^{2}	P - value
Age	6.50	0.039*
Stage of lactation	12.58	0.002**
Tick infestation	89.95	0.000**
Conformation of udder	79.87	0.000**
Teat lesion	70.91	0.000**
Previous history of mastitis	175.58	0.000**

^{2} Chi square P - *value = probability.*
* The difference was significant ($p < 0.05$).
** the difference was high significant ($p < 0.01$).

Table6. The logistic regression module to demonstrate the association between the presence of bovine mastitis and some factors.

Factor	SE	OR	95% CI
Age	0.19	1.34*	(1.02 - 1.76)
Stage of lactation	0.22	1.59 *	(1.22 - 2.08)
Tick infestation	0.03	0.15	(0.10-0.23)
Conformation	0.03	0.08	(0.04-0.15)
Teat lesion	1.90	7.31*	(4.39-12.17)
Previous history of mastitis	0.01	0.06	(0.04-0.10)

SE Standard Error OR odd ratio CI Confidence Interval.
* indicated OR > 1 and could be considered a risk factor.

that affects adult dairy cows.

In the farms surveyed, the housing condition were mostly bad (63.3%), this means that they do not even adopt to minimal standard in hygiene and all of the farms ground surface were clay (100%) that reflects on the hygiene status. Also ventilation, the space allows for the cows and wetness in the farm reflected in poor production. Saluiemi (1980) reported that in loose housing, the movements of the cows getting up and down are less restricted than when cows are confined, the lying area is usually contaminated with ample layer bedding and is therefore soft. In all farms surveyed, milkers didn't clean udders and teats before and after milking which may lead to milk contamination and udder infection. Saluiemi (1980) reported that if there is mastitis problem with cows in a loose house the cause is often poor milking hygiene or a faulty milking machine. Muddy outside pen or faulty ventilation, often combined with wet cubicles, which lead to mastitis problem caused by environmental pathogens (Radostits et al., 2000).

60% of the owners explained the absence of recording system in their farms, although records are an important part in monitoring the incidence of any disease. Mastitis is one of the few diseases where detailed analysis of the data can be used to help in the control of infection, that according to (Philpot et al., 1991). At the same time, they did not adopt culling chronically infected cows; Culling is used in mastitis control because infected udders are sources of new infection (Radostits et al., (2000).

Milk hygiene could also be evaluated by bacterial isolation from the milk samples. In the present study, the bacteria isolated from the milk were predominated, by *Staphylococcus* species and *Streptococcus* species, these bacteria may originate from udder infection or contamination due to mismanagement practices such as poor milk system (hand milking). Earlier studies showed that this method does not only reflect infection but also the possible contamination of milk in its passage through the milking process. (Nyaga et al., 1982 and Gonzalez et al., 1988) . Comparable results were reported by (Elliot et al., 1976; El Tayeb and Habiballa, 1978; Hinckely et al., 1988; Gonzalez et al., 1988; Zingeser et al., 1991).

The predominant bacteria isolated in the present study were *Staphyloccus* and *Streptococcus. Staphylococcus* species are known to cause sub clinical mastitis among dairy cattle (Shalalli et al., 1982; Zingeser et al., 1991; Aydin et al., 1995). *Staphylococcus* species may cause high incidence of sub clinical mastitis as well as out breaks of clinical mastitis among dairy cows (Keskuntepe et al., 1992). However the frequent isolation of this organism may be due to surface contamination of milk (Ku-

mar et al., 1974). Radostits et al. (2000) reported that, coagulase negative *Staphylococcus* (CNS) are commonly isolated from normal milk samples, teats canals and teats skin. He also explained that *streptococcus agalactia* is a highly contagious obligate microorganism of bovine and a major cause of udder infections. While, Todhunter et al. (1995) reported that the proportion of intra- mammary infections caused by environmental *Streptococcus* such as *Streptococcus uberis* has markedly increased. In addition, these authors indicated that this pathogen is the leading cause of both sub clinical and clinical mastitis in dairy cattle worldwide. The frequency of isolation of other organisms such as *Enterobacterium, lactobacbacilli*, was less significant than *Staphylococcus* species.

In this study, isolated *Corynebacterium* were *Corynebacterium striatum* 9 (33.3%), *Arcanobacterium pyogene* 4(14.8%), *Corynebacterium pseudotuberculosis* 2 (7.4%), *Corynebacterium ulcerans* 5(18.5%), *Corynebacterium bovis 7 (25.9%).*

Corynebacterium species isolated from bovine udder include *Coryebacterium pyogenes*, *Corynebacterium bovis*, *Corynebacterium mursiseptium*, *Corynebacterium xerosus*, *Corynebacterium alcerance* and *Corynebacterium striatum*. (Higgs et al*.*, 1967). *Corynebacterium* spp. alone was found to be associated with clinical mastitis (Kurek, 1973; Jha et al., 1994). And in some areas they were incriminated as the second most common bacteria isolated from bovine mastitis (Shalalli et al*.,* 1982).

Although *Corynebacterium bovis* is usually thought to be saprophytic, it has been incriminated as disease producing organism that is associated with mild udder infection (Bourland et al., 1967; Timms and Schultz, 1987). Moreover, Costa et al. (1998) isolated *Corynebacterium bovis* alone or mixed with *streptococcus* spp. from clinical and sub clinical bovine mastitis. The main reservoir is the infected gland or teats duct (Radostits et al. (2000). They also reported that in contrast to contagious mastitis, environmental mastitis caused by coli forms bacteria for example *Escherichia Coli*, is primarily associated with clinical mastitis, rather than sub-clinical mastitis.

The present study also revealed a close positive relationship between isolation of bacteria from mastitic milk samples and California mastitis test. As all milk samples that positive to CMT, specific bacteria was isolated. This means that CMT was a good diagnostic tool in the detection of sub-clinical mastitis hence it could be most reliable test to be conducted to investigate sub-clinical mastitis in the dairy farms of the Sudan. On the other hand the culture method may be used to confirm and aid for proper treatment (Sharma and Rajani, 1969; Adlan et al., 1980; Shuklaad Sypekar, 1982), these observation are similar to that of (Motie et al*.,* 1985; Bekele and Molla, 2001). Who reported a strong positive correlation between the CMT scores and bacteriological results?

Prevalence of 20.17% of sub-clinical mastitis was obtained, where as 3.58% were clinical mastitis, this based on the California mastitis test.

Risk factors such as age difference, stage of lactation, tick infestation, confirmation of the udder, teat lesion and previous history of mastitis were highly significant in the mastitis prevalence ($p < 0.01$), this result found to be agreed with Saluiemi (1980), who stated that the concentration of antibacterial factors in udder secretion are under genetic control and depend on the lactation stage and udder health. Moreover the teat canal represent a physiccal barrier to the penetration of bacteria when dilated, the risk of ascending infection is high. The teat canal remains open after milking for approximately 2 h, in that time the cow may lye down during this critical period. Tick infestation and teat lesions findings were in contrast with the observation of Bekele and Molla (2001), who suggested that heavy tick infestation and teat lesions might be responsible for udder infection and also lead to udder abnormallities and deformities and blind in teats.

The relationship between occurrence of mastitis and milk production was found significant (P - value 0.00) when the milk production increase also the risk of the infection increase and the animal been very susceptible to the disease, similar result were found by Gröhn (2000) contracted the disease, compared to their health and general lower yield herd. In contrast to our findings, Eberhart and Guss, (1970) reported that the rate of new intra mammary infections is significantly high in the dry period than during lactation period, also Bush and Oliver (1987) mentioned that the greatest increase in susceptibility is during the first 3 weeks of the dry period in which the new infection rate is higher than during the preceding lactation and the second period of heightened susceptibility occurs just prior to calving and in the immediate post partum period.

Conclusions

i.) The prevalence of mastitis found to be high (20.17%) for the sub-clinical mastitis while low prevalence (3.58%) for clinical mastitis in farms examined.
ii) Poor farms management affect occurrence of mastitis in the study farms.
iii) California mastitis test is a good for epidemiological survey of sub-clinical mastitis.
iv) Different *Corynebacterium* species were isolated from the clinical cases of mastitis.

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