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Research Article

Study on Bovine Mastitis in Smallholder Dairy Cows in Northern State of Sudan

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ABSTRACT

A study was carried out from March 2012- September 2012 to estimate the prevalence and assess contribution of major risk factors for the occurrence of bovine mastitis in small holder dairy cows in Northern state, Sudan. Out of 400 lactating cows in 121 smallholder dairy farms were investigated. The prevalence of clinical and sub-clinical mastitis at cow level was 10.5% and 72% respectively. Risk factors such as previous exposure to mastitis (χ^2 = 158.9, P = 0.00,), type of quarter (χ^2 = 3.678, P = 0.055), sharing of milkman between farms (χ^2 = 3.518, P = 0.061) and yielding milk (χ^2 = 3.769, P = 0.052) showed statistically significant association with mastitis. However, risk factors such as age, health score, injuries in udder, presence of ticks of udder, herd size, washing of udder and teats and stage of lactation did not show statistically significant association with the occurrence of mastitis. In the multivariate analysis, previous exposure to mastitis (Exp (B) = 5.929, P= 0.00) and sanitary practice (Exp (B) = 5.979, P= 0.033) were found to be the most statistically significant risk factors.

Key words: Clinical, Mastitis, Prevalence, Risk factors, Sub-clinical

INTRODUCTION

Bovine mastitis (mast = breast; itis = inflammation), a major disease affecting dairy cattle worldwide, results from the inflammation of the mammary gland. The severity of the inflammation can be classified into sub-clinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal (Yalcin, 2000). Subclinical mastitis is the presence of an infection without apparent signs of local inflammation or systemic involvement. Although clinical mastitis is an inflammatory response to infection causing visibly abnormal milk (eg, color, fibrin clots). As the extent of the inflammation increases, changes in the udder (swelling, heat, pain, redness) may also be apparent (Cynthia *et al*., 2010). Crossbred cows were more struck by subclinical mastitis than native one, youngest cows had the most sensitivity, hot weather increased frequency, major bacterial pathogens was coagulase positive *staphylococci* and most effective antibiotics was enrofloxacin (Dar *et al.*, 2014). From the annual report of veterinary services of the Northern State, Sudan, mastitis was the second disease after pneumonia (13, 15%) of the total cases, which brought to the veterinary clinics of the state (Annual report, 2011). Causing loss in milk production in the dairy cows and this ratio may be considered only for clinical mastitis, the other types (sub-clinical and chronic) were not investigated before and the economic impact of the disease was not estimated. The keeping of dairy cows is a popular activity in many urban and peri-urban areas of the state, most of these dairy men have little knowledge of dairy husbandry and the management practices are of sub optimal standards. Laboratory records indicate that mastitis is an important problem in the state, because the dairy industry in the state is still in its infancy, it is possible that the path models of the disease may be greatly influenced by management practices and poor feeding, among other factors. The objectives of the present study are to estimate the prevalence and potential risk factors which are associated with the sub-clinical and clinical mastitis in small holders' dairy cows in Northern State of Sudan.

MATERIALS AND METHODS

Study animals and husbandry practices

The livestock population for the year 2010 was 47719 camels, 320986 cattle, 2469534 sheep, 1260841 goats and 178064 equines. The dairy cowherds range in size from one to over 30 heads, but most of herds have fewer than five dairy cattle. Local dairy cows are managed under

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traditional and extensive husbandry systems. The average daily milk production from individual cows was relatively low (average 4 to 5 liters). Crossbred dairy cows are often managed under a small-scale, semi-intensive management system. They are often provided with some supplementary diet in addition to the natural pasture and agricultural by products and are maintained usually in separate stalls a short distance from each other in a house. This type of dairy husbandry system is increasingly becoming an important source of milk supplies to households and a means of income generation in urban and peri-urban areas of the state. Manure removal is made on a daily basis. Although milking is done by hand, pre-milking and post-milking hygienic procedures, such as udder washing and drying, were not frequently practiced. Cows are allowed to dry off at late-lactation period by abrupt cessation of milking.

Study design

The study involved a cross-sectional observation in a multistage sampling technique. Four localities; Dongola, Aldaba, Algolid and Alborgiag were randomly selected from the seven localities of the state. Then from each locality, two administration units were selected and five villages were selected from each unit. Finally, animals were selected by using cluster random sampling from each farm in the village.

Sample size determination

The sample size was calculated by the formula

$$N = \frac{4xPxQ}{L2}$$

N= sample size
P= expected prevalence
L= desired absolute precision
Q= (1-P). (Martin *et al.*, 1987)
The expected prevalence in this formula was 44.1% (Girma, 2010).

$$N = \frac{4x(0.441)x(0.559)}{(0.0025)} = 394 \text{ animals}$$

Clinical inspection of udder

The udder was first examined visually and then through palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue, and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Mammary quarters often became blind when there were repeated infections and little or no treatment was provided. Information related to the previous health history of the mammary quarters and causes of blindness of teats was obtained from interviews with owners of the farm. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder was also inspected for the presence of any grossly visible injury and ticks. Injuries caused by ticks and vigorous calf suckling were described based on location, size, and nature. Injuries caused by ticks were identified as indurate necrotic lesions following detachment of the parasites; these could be with or without abscess formation. Injuries caused by vigorous calf suckling were identified as circumscribed lesions around the teats.

Detection of Sub-clinical mastitis

Sub-clinical mastitis was diagnosed according to California Mastitis Test (CMT) results and the nature of coagulation and viscosity of the mixture, which show the presence and severity of the infection, respectively, (Quinn *et al*., 1994). A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 sec. The reaction was interpreted according to Quinn *et al*. (1994). The result was based on the gel formation and categorize as negative if there is no gel formation, or positive if there is gel formation, ranging from +1 to +3. If at least one quarter is positive by the CMT then the cow is considered positive. Therefore, a cow was considered as having mastitis if one or more quarters are CMT positive with or without isolation of microorganisms.

Milk sample collection

Milk samples were collected according to the National Mastitis Council (NMC, 1990). First the whole udder, then the quarter was washed with tap water and dried (in cases when there was a considerable amount of dirt to be removed) the teat end swabbed with cotton soaked in 70% ethyl alcohol. Approximately 10 ml of milk was collected aseptically from clinical and sub clinical (CMT positive) mastitis cows into sterile universal bottles after discarding the first three milking streams.

Data collection

A pre-tested structured questionnaire with the primary objective of elucidating the multi factorial background of clinical and sub-clinical mastitis was conducted in an interactive manner at every farm. Five herds at least visited in each village and all the dairy cows in the farm were examined and filled out the questionnaire by asking the owner about the cow attributes, the farm attributes and the general management factors.

Statistical analysis

All data collected were entered into Microsoft excel spreadsheet. For analysis of the data SPSS version 16 and EpiInfo 2006, software was used. Data were analyzed descriptively in the first step, using the frequency table and cross tabulation. Then the association of the different variables with the prevalence of bovine mastitis at the cow level was analyzed using a Chi-squared test. The level of significance was set at $P<0.25$. For the investigation of the association between the probabilities of occurrence of mastitis in response to potential individual, management and hygienic risk factors, multivariate analysis was performed in which logistic regression model was used. The strength of association between the risk factors and the prevalence of bovine mastitis was analyzed using the odds ratio and the level of significance was set at $P<0.05$.

RESULTS

Prevalence

Out of 400 lactating cows (172 local and 228 cross) in smallholder dairy farms investigated for bovine mastitis, the prevalence of clinical mastitis at cow level was 10.5% (42). The prevalence of sub-clinical mastitis at cow level was 72% (288). As shown in table 1 and table 2 within localities (4 localities), the highest prevalence of clinical mastitis was reported in Algolid locality (11.9%) and the highest prevalence of sub-clinical mastitis in Aldaba locality (81.4%).While Dongola locality showed the lowest prevalence of clinical and sub -clinical mastitis (8.9%) and (57.4%) respectively. The prevalence of clinical mastitis at the unit level was high in Argo unit (16.3%), and the prevalence of sub-clinical mastitis at the unit level was high in Aldaba unit (82.4%). However, the prevalence of clinical and sub -clinical mastitis was low in Dongola unit (4.1%) and (55.1%) respectively.

Associated risk factors

The association between the occurrence of mastitis (both clinical and sub-clinical) in the selected dairy cows and different potential risk factors was shown in table 1 and table 2. Accordingly, clinical mastitis prevalence showed statistically significant association with parity (χ^2 = 2.996, P = 0.224), previous exposure to mastitis (χ^2 = 158.9, P = 0.00,), breed (χ^2 = 1.789, p = 0.181), type of quarter (χ^2 = 3.678, P = 0.055), sharing of milkman between farms (χ^2 = 3.518, P = 0.061), education of farmers (χ^2 = 1.308, P = 0.253) and milk yield (χ^2 = 3.769, P = 0.052,). However, risk factors like age, health score, injuries in udder, presence of udder ticks, herd size, sanitary practice, washing of hands, udder and teats, stage of lactation, bedding and frequency of bedding removal did not show statistically significant association with the occurrence of clinical mastitis (P>0.25). In comparison to sub-clinical mastitis, also some other risk factors showed significant association like locality (χ^2 = 18.425, P = 0.00), unit (χ^2 = 20.095, P = 0.005), barn size (χ^2 = 3.234, P = 0.072), bedding (χ^2 = 5.169, P = 0.023), frequency removing bedding (χ^2 = 5.169, P = 0.023), sanitary practice (χ^2 = 11.224, P = 0.001), washing hands before milking (χ^2 = 3.307, P = 0.069), source of water (χ^2 = 2.361, P = 0.124), immediate dung removal (χ^2 = 3.200, P = 0.074), cow restrain for milking (χ^2 = 4.405, P = 0.036) and mastitis cow milking last (χ^2 = 7.351, P = 0.007). While, health score, parity, age, injuries in udder, presence of ticks, milk yield , herd size, washing udder, washing teat, type of fencing, sharing milkman between farms, milking technique, education and stage of lactation did not show significant statistical association with sub-clinical mastitis. In the multivariate analysis using logistic regression in table 3, only one risk factor had statistically significant association with the occurrence of clinical mastitis. Previous exposure to mastitis (Exp (B) = 58.323, P=0.00). In the multivariate analysis using logistic regression in table 4, only two risk factors had statistically significant association with the occurrence of sub-clinical mastitis. Previous exposure to mastitis (Exp (B) = 5.929, P = 0.00) and sanitary practice (Exp (B) = 5.979, P = 0.033).

DISCUSSION

Studies with repeated measurement of intra mammary statuses are necessary to identify factors associated with the risk of clinical and sub-clinical intra mammary infection. Identification of these factors will improve understanding of how smallholder dairy herds in Northern State might control clinical and sub-clinical intra mammary infections. The current study indicated a prevalence of bovine mastitis (72% sub-clinical and 10.5% clinical) at cow level. The prevalence of clinical mastitis reported in the present study is in close agreement with the results of various researchers (Demelash *et al*., 2005; Abera *et al*., 2010; Girma, 2010) in different regions of Ethiopia. However, the prevalence of sub-clinical mastitis in the present study was found to be higher than those reported in Ethiopia (Benta *et al*., 2011; Nibert *et al*., 2011), Iran (Hashemi *et al*., 2011) and India (Dar *et al*., 2014). In contrast, our findings of the sub-clinical mastitis are lower than the previous findings of Kivaria *et al*., (2004) in Tanzania. The high prevalence (72%) of sub-clinical mastitis and low prevalence (10.5%) of clinical mastitis observed agrees with reported observations that sub-clinical mastitis is more prevalent than clinical mastitis on most farms (Schukken *et al.*, 1989 and Kivaria *et al*., 2004). The difference in prevalence of bovine mastitis in the present study and other previous studies could be probably due to differences in farm management practices, breed, geographic location, and level of production, study methods and instruments employed by the investigators (Radostits *et al*., 2000). In the present study, the locality and unit as risk factors have highly significant statistical association with sub-clinical mastitis (P<0.25), while they did not show statistically significant association with clinical mastitis (P>0.25) and this result was in close agreement with the results reported by Demelash *et al*. (2005) in southern Ethiopia. The observed risk factors presented in this study which significantly associated with clinical mastitis are in agreement with the results described by many other studies (Girma 2010, Nibret *et al*., 2011, Tigre *et al*., 2011). Although, Tigre *et al*. (2011) found that the breed of the animals was not significantly associated with mastitis. However risk factors like age, parity, health score did not show significant statistically association with mastitis. These results were also described by Benta *et al*. (2011). Although the risk factors were statistically significant, they are not necessarily causally related, and they should be interpreted in light of the causal criteria that have been proposed to transpose an observed association between a risk factor and a disease into a causal relation (Thrusfield, 1995). As a result of the paucity of dairy extension services in Northern state, the small holder dairy sector is characterized by two major features; poorly planned dairy infrastructures and the lack of dairy knowledge and skills among the producers. Consequently, dairy cows are housed under conditions of sub-optimal hygiene. Sub-optimal housing hygiene has been associated with high prevalence of clinical and sub-clinical mastitis. The observed shortcomings in management practices and milking hygiene encourage a rapid within-herd multiplication, spread and maintenance of both environmental and contagious mastitis pathogens,

Table 1: Univariate analysis of the association of different potential risk factors with the occurrence of clinical mastitis

Risk factor	T. No	No. +	percent %	df	χ^2	p-value
Locality				3	0.427	0.935
Dongola	101	9	8.9%			
Alboragiag	94	10	10.6%			
Algolid	103	12	11.7%			
Aldaba	102	11	10.8%			
Unit				7	6.693	0.462
Dongola	49	2	4.1%			
Mashow	52	7	13.5%			
Karema	51	3	5.9%			
Arego	43	7	16.3%			
Algolid	50	5	10%			
Algadar	53	7	13.2%			
Aldaba	51	4	7.8%			
Algaba	51	7	13.7			
Breed				1	1.789	0.181
Local	172	14	8.1%			
Cross	228	28	12.3%			
Health score				1	0.411	0.521
Good	278	31	11.2%			
Bad	122	11	9%			
Milk yield				1	3.769	0.052
High	209	16	7.7%			
Low	191	26	13.6%			
Injuries in udder				1	0.035	0.852
present	8	1	2.4%			
Absent	392	41	97.6%			
Parity				2	2.996	0.224
1-4	244	24	9.8%			
5-8	139	18	12.9%			
>8	17	0	0%			
Presence of ticks				1	0.831	0.362
Yes	41	6	14.6%			
No	359	36	10%			
Age				2	2.215	0.330
1-6 years	212	19	9%			
7-12 years	149	20	13.4%			
13-18 years	39	3	7.7%			
Previous exposure to mastitis				1	158.9	0.00
Yes	57	33	57.9%			
No	343	9	2.6%			
Type of quarter				1	3.678	0.055
Pendulous	49	9	18.4%			
Non pendulous	351	33	9.4%			
Frequency of bedding removal				1	0.236	0.627
Yes	2	0	0%			
No	398	42	10.6%			
Herd size				1	0.202	0.653
Large	168	19	11.3%			
Small	232	23	9.9%			
Barn size				1	0.001	0.975
Adequate	161	17	10.6%			
Not adequate	239	25	10.5%			
Bedding				1	0.236	0.627
Yes	2	0	0%			
No	398	42	10.6%			
Sanitary practice				1	0.024	0.877
Good	11	1	9.1%			
Bad	389	41	10.5%			
Washing hands				1	0.156	0.693
Yes	221	22	10%			
No	179	20	11.2%			
Washing udder				1	0.024	0.877
Yes	11	1	9.1%			
No	389	41	10.5%			
Washing teat only				1	0.836	0.361
Yes	7	0	0%			
No	393	42	10.7%			
Source of water				1	0.001	0.972
Pipeline	77	8	10.4%			
Wells	323	34	10.5%			
Immediate dung removal				1	0.017	0.895
Yes	308	32	10.4%			
No	92	10	10.9%			
Cow restrain for milking				1	0.00	0.983
Yes	153	16	10.5%			
No	247	26	10.5%			
Milk technique				1	0.076	0.783
Stripping	367	39	10.6%			
Five fingers	33	3	9.1%			
Mastitis cow milking last				1	1.094	0.296
Yes	47	7	14.9%			
No	353	35	9.9%			
Sharing of milkman between farms				1	3.518	0.061
Yes	320	29	9.1%			
No	80	13	16.2%			
Type of fencing				1	0.035	0.852
Wire	8	1	12.5%			
Wood	392	41	10.5%			
Education				1	1.308	0.253
Primary school	224	27	8.5%			
High school	176	15	12.1%			
Stage of lactation				1	0.247	0.619
Early	282	31	11%			
Late	118	11	9.3%			

a situation that translates itself into the observed high risk of clinical mastitis. Clinical and laboratory examination of the animals and the subsequent farm inspections were the basis for this study. Furthermore, the results of the questionnaire were used together with the results of inspection of farm records. Interpretation of this data depended directly on the validity of the information given by the farmers. Such information is more or less subjective and might therefore be biased. However, there was no other feasible method to describe farmers' practices that might influence the prevalence of clinical and sub-clinical mastitis. Validation of the farmers' information was thus, limited to plausibility verification. The observed higher prevalence of mastitis during early lactation as compared to late lactation stages was in line with the reports by Abera *et al*., (2010) who also reported the same findings in Adama town, Ethiopia. This may be due to an absence of dry period therapy and birth related influences. Radostits *et al*. (2000) suggested that, the mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which in turn may have increased the presence of potential pathogens on the skin of the teat. The finding of a high prevalence of sub-clinical mastitis in houses with muddy floors when compared with concrete floor types ($P<0.05$) shows that mastitis is strongly associated with the housing (bedding) type or condition of the farm. In this study the prevalence of clinical and sub-clinical mastitis was higher in heifers compared to older cows and this result is in agreement with reports described by other studies

Table 2: Univariate analysis of the association of different potential risk factors with the occurrence of sub-clinical mastitis

Risk factor	T No	No. positive	percent %	df	χ^2	p-value
Locality				3	18.425	0.00
Dongola	101	58	57.4%			
Alboragiag	94	65	69.1%			
Algolid	103	82	79.6%			
Aldaba	102	83	81.4%			
Unit				7	20.095	0.005
Dongola	49	27	55.1%			
Mashow	52	31	59.6%			
Karama	51	33	64.7%			
Arago	43	32	74.4%			
Algolid	50	41	82%			
Algadar	53	41	77.4%			
Aldaba	51	42	82.4%			
Algaba	51	41	80.4%			
Breed				1	52.23	0.022
local	172	134	77.9%			
cross	228	154	67.5%			
Health score				1	1.012	0.314
Good	278	196	70.5%			
Bad	122	94	75.4%			
Injuries in udder				1	0.973	0.324
present	8	7	87.5			
Absent	392	281	71.7%			
Age				1	1.706	0.426
1-6	212	157	74.1%			
7-12	149	106	71.1%			
13-18	39	25	64.1%			
Parity				2	7.301	0.421
1-4	244	179	73.4%			
5-8	139	99	72.1%			
>8	17	10	58.8%			
Type of quarter				1	0.146	0.055
Pendulous	49	31	63.3%			
Non pendulous	351	257	73.2%			
Yielding milk				1	0.616	0.433
High	209	154	73.7%			
Low	191	134	70.2%			
Herd size				1	0.446	0.504
Large	168	118	70.2%			
Small	232	170	73.3%			
Barn size				1	3.234	0.072
Adequate	161	108	67.1%			
Not adequate	239	180	75.3%			
Bedding				1	5.169	0.023
Yes	2	0	0%			
No	398	288	72%			
Frequency of bedding removal				1	5.169	0.023
Yes	2	0	0%			
No	398	288	72%			
Sanitary practice				1	11.224	0.001
Good	11	3	27.3%			
Bad	389	285	73.3%			
Washing hands				1	3.307	0.069
Yes	221	151	68.3%			
No	179	137	76.5			
Washing udder				1	0.003	0.957
Yes	11	8	72.7%			
No	389	280	72%			
Washing teat only				1	0.001	0.973
Yes	7	5	71.4%			
No	393	283	72%			
Source of water				1	2.361	0.124
Pipeline	77	55	64.9%			
Wells	323	238	73.7%			
Immediate dung removal				1	3.200	0.074
Yes	301	215	69.8%			
No	92	73	79.3%			
Type of fencing				1	0.365	0.545
Wire	8	5	62.5%			
Wood	392	283	72.2%			
Cow restrain for milk				1	4.405	0.036
Yes	153	101	66%			
No	247	187	75.7%			
Milk technique				1	0.507	0.476
Stripping	367	266	72.5%			
Five fingers	33	22	66.7%			
Mastitis cow milking last				1	7.351	0.007
Yes	47	26	55.3%			
No	353	262	74.2%			
Sharing of milk man between farms				1	0.028	0.867
Yes	320	231	72.2%			
No	80	57	71.2%			
Education				1	0.149	0.700
Primary school	224	163	72.8%			
High school	176	125	71%			
Stage of lactation				1	0.248	0.618
Early	282	201	71.3%			
Late	118	87	73.7%			

Table 3: Multivariate analysis of the association of different potential risk factors with the occurrence of clinical mastitis in small holders in Northern State of Sudan

Risk factor	No. +ve (%)	Exp (B)	95% confidence interval	P-value
Previous exposure to mastitis				
Yes	57.9%	Ref.		
No	2.5%	58.323	22.952 – 148.203	0.0001
Type of quarter				
Pendulous	18.4%	Ref.		
Non pendulous	9.4%	2.392	0.674 – 8.486	0.177
Education				
Primary school	12.1%	Ref.		
High school	8.5%	2.028	0.815 – 5.049	0.129

Table 4: Multivariate analysis of the association of different potential risk factors with the occurrence of sub-clinical mastitis in small holders in Northern State of Sudan

Risk factor	No. +ve (%)	Exp (B)	95%confidence interval	P-value
Sanitary practice				
Good	27.3%	Ref.		
Bad	73.3%	5.979	1.160 – 30.818	0.033
Type of quarter				
Pendulous	63.3%	Ref.		
Non pendulous	73.2%	1.183	0.494 – 2.835	0.706
Previous exposure to mastitis				
Yes	40.4%	Ref.		
No	77.3%	5.929	3.116 – 11.284	0.0001

(Sampimon *et al*., 2008, Oliver *et al*., 2005 and Salvador *et al.*, 2012). Intra mammary infections in unbred and pregnant dairy heifers were once thought to be very infrequent. However, during the last 2 decades, several studies have shown that Intra mammary infections in heifers occur frequently during the prepartum and peripartum periods. Many of these infections can persist for long periods of time, may be associated with elevated

somatic cell counts (SCC), and may impair mammary development and affect milk production after calving (Oliver *et al*., 2005). A total of 6 risk factors showed significant statistical association with clinical mastitis in the univariate analysis were entered in the multivariate analysis. Only one risk factor (previous exposure to mastitis) has a significant statistical association with the disease (Exp (B) = 58.328, P= 0.00). While 13 risk factors showed significant statistical association with sub-clinical mastitis in the univariate analysis were entered in the multivariate analysis, from which only two risk factors (sanitary practice (Exp (B) = 5.979, P=0.033) and previous exposure to mastitis (Exp (B) = 5.929, P = 0.00) have significant statistical association with the disease.

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