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Full Length Research Paper

Prevalence and risk factors associated with subclinical mastitis in lactating dairy cows under smallholder dairy farming in North East Tanzania

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A cross-sectional survey was carried out between October 2016 and May 2017 to determine the prevalence of subclinical mastitis (SCM), associated bacterial pathogens and risk factors under smallholdersmall holder dairy farms in North East Tanzania. The study involved 195 cross breed dairy cows from 130 dairy farms. Data were collected based on questionnaire interview, direct observation, screening using California Mastitis Test (CMT) and culture of bacteria. The overall prevalence of SCM based on California Mastitis Test (CMT) was 70.8 and 66.4% and bacteria isolation recorded at 56.4 and 38.4% at cow and quarter levels, respectively. Prevalence defined by CMT was significantly ($p < 0.0001$) associated with wet-dirty bedding material (OR=11.61) and poor udder (OR =6.67). Increased culture-positivity at quarter level was significantly associated with CMT-positive cows (OR= 20.59), teat injuries (OR=23.56), wooden floor (OR=2.02) and poor udder hygiene (OR =2.16). Stripping method of hand milking and first and second parity were significantly associated with lower prevalence of CMT-positive cows and culture positive quarters ($p < 0.05$). Major bacteria species isolated included *Staphylococcus aureus* (55.4%), *Staphylococcus epidermidis* (10.8%), *Escherichia coli* (7.9%) and *Streptococcus agalactiae* (5.9%). This study demonstrated SCM is a major health constraint of dairy cattle in North Eastern Tanzania.

Key words: Prevalence, Subclinical -mastitis, California Mastitis Test, bacteria, risk factors.

INTRODUCTION

Subclinical mastitis (SCM) occurs when both milk and mammary gland appear normal but somatic cell counts (SCC) is elevated to a level above 200,000 cells/mL, and this result in reductions in the amount and quality of milk

(Seegers et al., 2003). In developed countries where bulk tank somatic cell counting is carried out routinely, high levels of somatic cells in milk as a result of SCM also contribute to the economic loss, due to the penalties

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imposed by dairy companies (Yalcin et al., 1999). A study done in the USA and India reported that mastitis caused an annual loss in the dairy industry of about 2 billion dollars where 70% of that loss was due to SCM (Varshney and Naresh, 2004). Further, studies elsewhere revealed that SCM is economically important than clinical mastitis (CM) (Kader et al., 2003; Seegers et al., 2003; Joshi and Gokhale, 2006).

In East Africa, the prevalence of SCM defined by CMT, has been reported to be 28.6% in Tanzania (Suleiman et al., 2017), 50.4% in Rwanda (Mpatwenumugabo et al., 2017), 86.2% (Abrahmsén et al., 2013) and 32.4% (Gitau et al., 2014) in Kenya. In Tanzania, prevalence of SCM by a bacteriological culture at a cow and the quarter level was reported to be 43.8 and 24.7% (Karimuribo et al., 2008) and 70.9 and 42.9%, respectively (Suleiman et al., 2017). Major isolates frequently encountered from apparently healthy udders were staphylococci, streptococci, and coliforms (Mdegela et al., 2004; Karimuribo et al., 2008; Mdegela et al., 2009; Hosseinzadeh and Saei, 2014). Risk factors that were reported to be associated with an increased risk of culture-positive quarter and udder include bought in-cows, CMT positive cows and older cows (Karimuribo et al., 2008). Also, Kivaria et al. (2004) reported; water scarcity, ban size, residual suckling, single udder towel, and dairy labourers as being the most substantial risk indicators of SCM. The current study in Lushoto and Korogwe is speculating whether previous dairy training through various projects had an influence on the prevalence of SCM. Therefore, the objective of this study was to estimate the prevalence of subclinical mastitis, isolation of bacterial pathogens and identify major risk factors attributed to the occurrence of subclinical mastitis in the area and suggest interventional approaches; for reducing the prevalence and increase milk production.

MATERIALS AND METHODS

Study area

This study was carried out in two districts (Lushoto and Korogwe) in Tanga region whereas Lushoto is situated in the northern part; lies between 4°25'–4°55'S and 30°10'–38°35'E. It has an altitude of 1000–2100 m above the sea level with an average annual temperature of 17.3°C and 1074 mm of rainfall. Korogwe is located at 4°15'–5°15'S and 38°0'–38°45'E with an annual average temperature of 26°C and rainfall of 1051 mm. Selection of sites (villages) was based on the availability of the adequate number of improved dairy breeds kept under zero grazing management system (Figure 1).

Study design

The study was a cross-sectional survey with cows selected randomly in the two areas and the total number of cows included in the study was 195 kept on 130 smallholder farms practicing dairy production. The sample size was determined by the formula given by Thrusfield (2005). The number of cows involved in the study

was determined by using the formula, $n = Z^2PQ/L^2$, where n = sample size, P = prevalence in the previous study, $Q = 1-P$, L = required precision. We used previous prevalence 42.1% ($P = 0.421$) as per a study that was done in the same environment in Tanzania (Karimuribo et al., 2008) with a precision of 5% ($L = 0.05$) and confidence level 95% ($Z = 1.96$). The sample size calculated was $n = 272$ cows. However, the selected farms had a total number of 195 lactating cows.

Data collection procedure

The cattle management system in the area is a small-scale dairy system, mainly zero grazing; which is a cut and carrier system. Data was collected using a semi-structured questionnaire with the objective of elucidating the multifactorial background of mastitis. Data collected includes age, parity number, lactation stage, milk yield, teat injuries, body condition score, dry cow therapy, milking procedures, fodder source, bedding material, calf feeding, and cowshed design.

Cow-side and bacterial culture

California Mastitis Test (CMT) was used to screen lactating cows for SCM and the cow-side test using CMT reagent (BOVAVET®, Kruuse, Denmark) and was carried out according to the method described by Schuppel and Schwöpe (1998). According to Schuppel and Schwöpe (1998) and as per manufacturer's instructions, the reaction result was classified as; 0 (negative or trace), 1+ (slightly positive/weak positive), 2+ (positive) and 3+ (highly positive) depending on the amount of gel that formed. The samples for bacteria isolation was collected from CMT positive and negative quarters and was placed in an icebox and transported to the laboratory at Sokoine University of Agriculture (SUA) for processing and culturing. Bacteria isolation and identification were done based on the identification key set by Quinn et al. (1994).

Data processing and analysis

Cow, quarter and farm-level data were entered into a spreadsheet program, Microsoft Excel and transferred into Statistical package of Software (IBM SPSS 21.0) to estimate the strength and statistical significance of associations between predictor and outcome variables ($P < 0.05$) using regression model analysis. The outcome variable in this study was the prevalence of SCM as defined by CMT or bacteria culture at cow and quarter levels. A cow's quarter was considered positive for CMT if the score was a positive one and above while a cow was regarded positive if at least one quarter was CMT positive (Doherr et al., 2007). Based on culture results, a quarter was considered culture positive if bacteria were isolated from the sample collected and a cow was considered culture positive if bacteria were isolated from at least one quarter. Then the prevalence of SCM was calculated as the percentage of mastitis-affected cows or quarters out of the total lactating cows or quarters. Initially, data (17 and 15 risk factors as defined for CMT and bacteria isolation respectively) were organized for bivariable analysis to find out the effect of the individual risk factor on SCM defined as CMT and culture positive. The variables that resulted at $p < 0.05$ in the bivariable analysis were included in the final model of risk factors that significantly associated with SCM.

Ethical consideration

School of Life Science and Bioengineering of Nelson Mandela Institution of Science and Technology (NM-AIST) reviewed the

Table 1. Prevalence of subclinical mastitis at cow and quarter level as defined by CMT and culture.

Level	CMT			Culture		
	Total no. tested	No. affected	% Positive	Total no. tested	No. affected	% Positive
Cow level	195	138	70.8	195	110	56.4
Quarter level	764	507	66.4	745	286	38.4

Table 2. Quarter prevalence of subclinical mastitis using the California mastitis test.

Quarter	No examined	Positive	Frequency (%)	No. of blind teats
LF(Left front)	192	124	64.6	3
LH(Left hind)	190	127	66.8	5
RH(Right hind)	191	130	68.1	4
RF(Right front)	191	126	66.0	4
Total/Average	764	507	66.4	16

Table 3. Distribution of mastitis pathogen isolates to California mastitis test scores.

Bacterial isolates	CMT score					% Isolates (n=286)	% Sample (n=745)
	-	1+	2+	3+	Total		
<i>Staphylococcus aureus</i>	1	8	48	112	169	59.1	22.7
<i>Staphylococcus epidermidis</i>	0	7	9	17	33	11.5	4.4
<i>Escherichia coli</i>	5	1	8	10	24	8.4	3.2
<i>Streptococcus agalactiae</i>	0	3	12	3	18	6.3	2.4
Other <i>Staphylococcus</i> species	0	8	3	1	12	4.2	1.6
<i>Streptococcus species</i>	0	4	5	2	11	3.8	1.5
<i>Corynebacterium species</i>	4	2	2	3	11	3.8	1.5
<i>Bacillus species</i>	1	2	4	1	8	2.8	1.1
Total number of Isolates	11	36	102	156	286	99.9	38.4

Increased culture positive quarters were significantly associated with CMT positive cows (OR 20.585, $p < 0.0001$).

Risk factors for increased CMT-positive cows and culture positive quarters

A total of 17 of both animal and management or environmental risk factors were tested in the bivariable analysis of which eight factors qualified for multivariable analysis $p < 0.05$ (Table 4).

Out of eight risk factors tested in the multivariable analysis, four risk factors were significantly associated with increased CMT positive cows (poor udder hygiene and wet and dirty bedding material) and decreased CMT positive cows (stripping milking technique and parity of one and two) (Table 5).

Bivariable analysis for risk factors for increased culture positive quarters involved 15 animal and management risk

factors of which 11 factors qualified for multivariable analysis (Table 6).

However, teats injuries, poor udder hygiene and wooden emerged as risk factors while stripping technique and parity of one calf were protective factors (Table 5).

DISCUSSION

The prevalence of SCM as described by CMT at cow and quarter level were higher (70.8 and 66.4%) compared to culture (56.4 and 38.4%), respectively. The high SCM prevalence in Tanzania was reported by Kivaria et al. (2004), Mdegela et al. (2004), Karimuribo et al. (2008) and Suleiman et al. (2017). In other East African countries, the high prevalence (86.2%) was reported by Abrahmsén et al. (2013) and (87.4%) reported by Nkoroi and Maitho (2014). The reason for the high prevalence in this study might be attributed to poor milking hygiene and other management factors including dirty cowshed and

Table 4. Bivariable regression model analysis of potential risk factors for subclinical mastitis by CMT test.

Risk factors	Subclinical mastitis		OR (95% CI)	P value
	Present (n=138)	Absent (n=57)		
	[No. (%)]			
Age				
2-4 years	26 (44.8)	32 (55.2)	0.072 (0.027-0.191)	<0.0001
>4-8 years	44 (69.8)	19 (30.2)	0.204 (0.076-0.552)	0.002
>8 years	68 (91.9)	6 (8.1)	Reference*	
BCS				
Poor	27 (93.1)	2 (6.9)	14.318 (3.154-65.009)	0.001
Medium	78 (79.6)	20 (20.4)	4.136 (2.088-8.195)	<0.0001
Good	33 (48.5)	35 (51.5)	Reference*	
Bedding materials				
Do not provide	63 (69.2)	28 (30.8)	7.500 (2.718-20.698)	<0.0001
Wet dirty	69 (85.5)	9 (11.5)	25.556 (8.119-80.440)	<0.0001
Dry clean	6 (23.1)	20 (76.9)	Reference*	
Cowshed type				
Tie stall	89 (85.6)	15 (14.4)	5.086 (2.564-10.088)	<0.0001
Free stall	49 (53.8)	42 (46.2)	Reference*	
Milking techniques				
Stripping	54 (50.9)	52 (49.1)	0.062 (0.023-0.165)	<0.0001
Five finger squeezing	84 (94.4)	5 (5.6)	Reference*	
Teat Lubricants				
No	90 (89.1)	11 (10.9)	7.841 (3.721-16.521)	<0.0001
Yes	48 (51.1)	46 (48.9)	Reference*	
Udder hygiene				
Poor	78 (94.0)	5 (6.0)	13.520 (5.087-35.932)	<0.0001
Good	60 (53.6)	52 (46.4)	Reference*	

Table 5. Final models for analysis of potential risk factors for increased CMT-positive cows.

Risk factors	Odds Ratio	95% CI	P value
Parity			
1 calf	0.015	0.001-0.165	0.015
2 calves	0.188	0.040-0.873	0.033
≥3 calves	Reference*		
Milking techniques			
Stripping	0.113	0.027-0.467	0.003
Five finger	Reference*		
Bedding materials			
No beddings	3.804	0.877-16.510	0.074
Wet-dirty	11.612	1.707-79.003	0.012
Dry-clean	Reference*		
Udder hygiene			
Poor	6.673	1.650-26.996	0.008
Good	Reference*		

Table 6. Bivariable regression model analysis of potential risk factors for increased culture-positive quarters.

Risk factors	Subclinical mastitis		OR (95% CI)	P value
	Present (n=286)	Absent (n=459)		
	[No. (%)]			
Age (years)				
2-4 years	44 (19.6)	181 (80.4)	0.166 (0.110-0.250)	<0.0001
>4-8 years	81 (32.5)	168(67.5)	0.329(0.230-0.472)	<0.0001
>8 year	161 (59.4)	110(40.6)	Reference*	
Bedding materials				
No beddings	133 (38.2)	215 (61.8)	3.057 (1.738-5.374)	<0.0001
Wet-dirty	136(45.9)	160 (54.1)	4.200(2,377-7.421)	<0.0001
Dry-Clean	17(16.8)	84(83.2)	Reference*	
Cow stall type				
Tie stall	199 (50.4)	196 (49.6)	3.069 (2.245-4.196)	<0.0001
Free stall	87 (24.9)	263 (75.1)	Reference*	
Floor type				
Soil/mud	140 (50.9)	135 (49.1)	1.828(1.272-2.625)	<0.0001
Wooden	66(26.5)	183 (73.5)	0.636(0.429-0.942)	<0.0001
Concrete	80(36.2)	141(63.8)	Reference*	
Dung removal				
Once per day	117 (34.5)	222(65.5)	0.394(0.280-0.555)	<0.0001
Twice per day	34 (20.0)	136 (80.0)	0.187 (0.119-0.295)	<0.0001
Once weekly	135 (57.2)	101 (42.8)	Reference*	
Lactation stage				
<60 days	37(22.6)	127 (77.4)	0.199(0.127-0.314)	<0.0001
60-120 days	116(32.5)	241(67.5)	0.329(0.233-0.466)	<0.0001
>120 days	133 (59.4)	91(40.6)	Reference*	
Milking techniques				
Stripping	91 (22.5)	314(77.5)	0.215 (0.157-0.296)	<0.0001
Five finger squeezing	195(57.4)	145(42.6)	Reference*	
Parity (calves)				
1 calf	31 (15.5)	169 (84.5)	0.157 (0.102-0.242)	<0.0001
2 calves	45 (29.0)	110(71.0)	0.351 (0.235-0.523)	<0.0001
≥ 3 calves	210(53.8)	180(46.2)	Reference*	
Teat injuries				
Yes	162(85.7)	27 (14.3)	20.903(13.279-32.905)	<0.0001
No	124(22.3)	432(77.7)	Reference*	
Udder hygiene				
Poor	178(57.6)	131 (42.4)	4.127(3.017-5.645)	<0.0001
Good	108 (24.8)	328 (75.2)	Reference*	

beddings which were the reciprocal of zero grazing, due to the fact that restricting animals to stay in confined areas eventually causes higher infection pressure and associated risk increase of infection (Taponen et al., 2016). According to Radostits et al. (2007), mastitis is a multi-factorial disease which involves an interaction of

microorganism with host and management factors; hence its prevalence varies from place to place, farm to farm and between breeds.

Hindquarters were much affected than forequarters; this is in agreement with the report done by Zeryehun et al. (2013) and Duguma et al. (2014). The variation in

quarter prevalence in the present study could be due to the fact that hindquarters have more milk production capacity and anatomical position prone to contamination compared to forequarters (Radostits et al., 2007). Wet and dirty bedding materials reported from this study could have increased dirtiness of hindquarters thereby increasing the chance of infection as it was also reported by Sori et al. (2005).

Findings of this study indicated that it was more likely to isolate bacteria from CMT positive cases of bovine subclinical mastitis compared to CMT negative (OR = 20.59). A similar result was reported by Karimuribo et al. (2008) who reported CMT positive results without bacteria isolation. This may be due to the presence of other micro-organisms rather than bacteria that trigger cow immunological response and hence increased number of somatic cell counts in the milk samples, pathogens like fungi, algae, mycoplasma, mycobacteria, etc. that require special culturing media and methods. Also, the occurrence of infections of short-term nature that was already cleared during samples collection may explain such false positive CMT results as reported elsewhere by Abdel-Rady and Sayed (2009). Therefore, special culturing media and methods are suggested in order to avoid the false positives observed under CMT.

Isolation of bacteria in CMT-negative samples is similar to what was reported by Almaw et al. (2008), who reported the isolation of *S. aureus* and *Streptococcus agalactiae* in CMT-negative quarters in Ethiopia. CMT negative samples that harboured bacteria may be explained by the presence of bacteria that do not trigger the immunological response of the infected quarter resulting in a limited increase in the somatic cell count (Gitau et al., 2014). This is what can be identified as intra-mammary infection (IMI) where bacteria were isolated without somatic cell count change of the infected quarter (Mdegela et al., 2009). Likewise, contaminants occurring in the milk samples despite hygienic precautions may lead to false positive detections. Teat injuries increased the risk of quarters to be infected with SCM by about twenty-three times (OR = 23.56). It is known that presence of wounds near the teat orifice may be accompanied with the presence of opportunistic bacteria nearby that may invade the teat canal during or immediately after milking, resulting into the isolation of bacteria in CMT negative (Madut et al., 2009).

The predominant bacteria species isolated from this study were *S. aureus* constituting about 59.1% of the bacteria isolated. Similar results are also found in previous studies done by Yohannis and Molla (2013), Zenebe et al. (2014), Tekle and Berihe (2016) and Suleiman et al. (2017). *S. aureus* was reported to be the chief etiological agent of mastitis in cattle and buffaloes in Asian countries (Sharma et al., 2007; Rahman et al., 2010; Ali et al., 2011). It is known that *S. aureus* is adapted to survive in the udder and usually establishes mild subclinical infection of long duration from which it is

shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process (Radostits et al., 1994). The predominance of the bacteria species in this study was found to be associated with teat injuries and poor udder hygiene (Table 6). Poor udder hygiene and failure of farmers to use dry cow therapy and pre and post-milking dips might have influenced the predominance of *Staphylococcus*. Otherwise, other bacteria species isolated in this study were also described in other studies conducted in Tanzania or other East African countries (Karimuribo et al., 2008; Mpatwenumugabo et al., 2017; Suleiman et al., 2017).

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Increased CMT-positive cows in this study were significantly associated with wet-dirty bedding and poor udder hygiene. Moisture in bedding has been reported as one of most difficult factor to control in compost bedded pack systems (Lobeck et al., 2011), because it can be influenced by bedding management and weather conditions. Wetness of the bedding material in this study

Table 7. Final model for analysis of potential risk factors for increased culture positive quarters.

Risk factors	Odds ratio	95% CI	P value
Teat injuries			
Yes	23.556	13.618-40.747	0.000
No		Reference	
Udder hygiene			
Poor	2.158	1.287-3.618	0.004
Good		Reference	
Milk techniques			
Stripping	0.226	0.129-0.396	0.000
Five finger squeezing		Reference	
Floor type			
Earth/mud	1.210	0.679-2.036	0.615
Wooden	2.023	1.029-3.979	0.041
Concrete		Reference	
Parity			
One calf	0.272	0.101-0.729	0.010
Two calves	1.003	0.547-1.841	0.992
>3 Calves		Reference	

Table 8. Risk factors associated with bacterial species isolated in milk samples from the study sites

Bacteria isolated	Risk factors for bacteria isolation							
	Teat injuries		Poor udder hygiene		Wooden floor		Stripping technique	
	Odds ratio	p value	Odds ratio	p value	odds ratio	P value	Odds ratio	p value
<i>S. aureus</i>	29.067	0.000	4.92	0.000	0.790	0.343	0.248	0.000
<i>S.epidermidis</i>	11.789	0.000	5.008	0.000	0.605	0.230	0.148	0.000
<i>E. coli</i>	22.400	0.000	1.031	0.947	0.220	0.009	0.19	0.000
<i>Str.agalactiae</i>	12.800	0.000	42.565	0.000	3.082	0.316	0.178	0.001
<i>Staphylococcus sp</i>	22.400	0.000	12.519	0.001	0.770	0.854	0.092	0.002
<i>Streptococcus sp</i>	19.200	0.000	2.087	0.231	2.311	0.470	0.264	0.036
<i>Corynebacterium sp</i>	1.600	0.660	0.250	0.189	0.257	0.099	0.385	0.120
<i>Bacillus species</i>	9.600	0.003	2.504	0.199	1.3E-09	-	0.066	0.011

was influenced by porous roofs, type of cowsheds floor “without ditches” and the irregular change of bedding material. It was observed in this study that, most of the farmers did not change bedding material on daily basis; instead they added dry and clean bedding material on the top of wet-dirty ones, hence it continued soaking urine, faeces and other wastes from and around the animals, this created conducive environment for the proliferation of mastitis microbes; therefore increased exposure of infection. This finding is in line with Abera et al. (2012) who reported mastitis being associated with soil floor and the use of hay and straw bedding material.

In this study occurrence of culture positive quarter was 23 times in quarters with teat injuries than in quarter without teat injuries (Table 7). Increase of subclinical mastitis due to teat injuries has been reported by

Hailemariam and Eticha. (2017), Lakew et al. (2009), and Mekibib et al. (2010) in Ethiopia. Teat canal is the main route of entry of mastitis causing organism, hence teat injuries is most important risk factor of intra-mammary infection. Changes to teat tissue, particularly the skin of the barrel, teat end and teat canal may favor penetration of bacteria into the udder and increase the risk of new mastitis infection (Hamann et al., 1994). Some lactating cows in this study was observed to have teat injuries/ lesion on one or more teats, however the underlying cause was not established and further study is needed to discern the causes.

Milking by stripping was associated with lower odds of CMT positive cows, culture positive quarters and isolation of mastitis causing pathogens (Table 5 and 8). Similar results were reported by Suleiman et al. (2017) and

Karimuribo et al. (2008) in Tanzania. In contrast, Tolosa et al. (2013) and Kivaria et al. (2004) reported a higher likelihood of SCM in cows milked by stripping in Tanzania and Ethiopia respectively. As we do not know the underlying biological mechanisms, more work is needed to come to evidence-based advice on the optimal milking technique in tropical circumstances. Not only in tropics but also in temperate countries as it was reported by Turner. (2001) in United Kingdom, that milking by stripping was used in treating mastitis cases in the organic dairying system.

Parity in this study was associated with decrease in SCM by CMT and culture. First and second parity was recorded as protective factors for SCM by CMT test (OR=0.02–0.188) and by culture (OR=0.272) (Table 5 and 8). It was found that prevalence defined by CMT increased with increase in parity number; prevalence in first parity (35.3%), second parity (61.5%) and parity of three and above (91.4%). A similar finding has been reported by Hailemariam and Eticha. (2017) in Ethiopia and Mureithi and Njuguna (2016) in Kenya. Reason for the increase of mastitis with increasing parity in this study is not completely understood, but one reason could be impairment of leucocytes functions in older cows (Mehrzhad et al., 2006).

Conclusion

Without laboratory assistance, subclinical mastitis can be noticed by farmers, hence it acts as silent killer to farmers income who depends on milk as source of livelihood. Since subclinical mastitis was associated with wet-dirty environment and poor udder hygiene, this calls for farmers to have effective cleaning and disinfection of animal houses and animal udder.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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