

Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda

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Abstract It is widely recognized that subclinical mastitis (SCM) is an extensive problem in the dairy industry worldwide. It is of particular concern in developing countries. The aim of this study was to establish the prevalence of SCM in dairy cattle in the urban and peri-urban areas of Kampala, Uganda and to gain information about pathogens and antibiotic resistance patterns. The study was conducted as a field study in 18 smallholder dairy farms in peri-urban Kampala, Uganda. All cows at the farms were physically examined, and cows with signs of clinical mastitis were excluded. Cows ($n=195$) were tested with California Mastitis Test (CMT), and udder quarters with CMT score ≥ 3 (scale 1–5) were milk sampled for bacteriological analysis. To allow further sub-analysis of the results, the stage of lactation, parity, milk production, production type, udder hygiene, and cow breed were recorded. Results indicate that 86.2 % ($n=168$) of the tested cows had SCM in one or more quarters. The most common bacteriological outcome was infection with coagulase-negative staphylococci (54.7 %), followed by negative growth (24.9 %) and streptococci (16.2 %); all of which ($n=34$) were sensitive to penicillin. Of the tested staphylococci ($n=17$), the majority (58.9 %) were positive for penicillinase production. Factors with significant impact on the prevalence of SCM at cow level were the stage of

lactation, parity, and production type. The results suggest that the prevalence of SCM in Uganda is substantially higher than reported in previous studies and in other comparable developing countries. This implies that SCM deserves more attention and that improvement in dairy cow husbandry in terms of hygiene and management is necessary.

Keywords Udder health · Smallholder dairy farming · Holstein-Friesian cows · East Africa

Introduction

Several recent reports indicate that the production of meat and milk in the developing world has doubled in recent decades, as a result of increasing demands (Food and Agriculture Organization of the United Nations (FAO) 2011a). This so-called livestock revolution provides income, employment, and high-quality nutrition, and the trend is expected to continue. Milk and other dairy products represent a very important energy source for many people and contribute to a substantial portion of the total energy intake. Infectious diseases, like mastitis, among cattle however represent a serious potential constraint to further the development of smallholder dairy cow production in developing countries and might drive rural smallholders into chronic poverty and/or starvation. In the example Uganda, the agriculture sector is the most important sector in terms of labor capacity utilization rate (FAO 2011a). The country has a population of almost 34 million people, and out of these, almost 74 % work in the agricultural sector (Uganda Bureau of Statistics, report 2008). Despite the fact that Uganda is, in most parts, a very fertile country, about 21 % of Ugandans suffer from undernourishment (FAO 2011a). This underlines the need for increased agricultural production in the country, in which milk production plays a key role (FAO 2011c).

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Several different studies point out that subclinical mastitis (SCM) is more economically important than clinical mastitis (CM) (Godkin et al. 1990; Kader et al. 2003; Joshi and Gokhale 2006; Seegers et al. 2003; Singh and Sing 1994). This is explained by the fact that SCM is more difficult to diagnose and therefore usually persists longer in the herds, causing production losses. As any infectious disease, SCM can have many different consequences, of which two have been pointed out: (1) reduced herd population by death or culling and (2) reduced production and income (FAO 2011c). According to Hogan (2005), there might also be potential food safety risks indirectly associated with high SCC, such as ingestion of potentially pathogenic microorganisms (especially if the milk is not pasteurized), bacterial toxins, and/or antibiotic residues. The lower quality and the diminished sustainability of milk with a high SCC both constitute a potential health risk and also affect the possibility of producing other dairy products, e.g., cheese and yoghurt (Andersson et al. 2011). Hence, the prevalence, diagnosis, and treatment of SCM are very important fields both in terms of veterinary medicine and economy of developing countries.

According to previous studies in Uganda, the prevalence of SCM in Ugandan dairy cattle is substantial (Okello-Uma and Gibson 1976; Nakavuma et al. 1994; Kintu et al. 2000; Byarugaba et al. 2008). For example, Byarugaba et al. (2008) reported an overall cow level prevalence of SCM at 37.2 % in the Jinja Province in Uganda. Studies of prevalence and incidence of dairy cow SCM performed in other African developing countries also show a considerable prevalence. For example, Bitew et al. (2010) and Mungube et al. (2005) reported an overall prevalence of SCM at 25.2 and 52.3 % at cow level and 12.3 and 32.4 % at quarter level in Ethiopian Holstein crossbred cows and indigenous breeds, respectively. Harouna et al. (2009), in peri-urban Hamdallaye, Niger, showed that the prevalence rate of SCM ranged between 27.1 to 55.2 % ($p < 0.05$) between dry and rainy seasons. In Tanzania, Mdegela et al. (2009) noted prevalence rates in the range of 51.6 % at cow level and 30.0 % at quarter level for both CM and SCM among smallholder cows.

Since the most common cause of mastitis in dairy cattle is bacterial infection, antibiotic therapy is an important tool in the control of mastitis. However, misuse of antibiotics poses great risk of spreading antibiotic resistance among bacteria. Hence, antibiotic treatment of SCM is generally recommended only during drying off and not during lactation (SVS 2011), albeit other policies exist. For mastitis, as for most other illnesses, resistance is not due to mutations as much as the spread of resistant bacteria and resistance genes—yet another reason to keep the prevalence of SCM and CM low. Given this, the main purpose of this study was to investigate the prevalence of SCM in dairy cattle in the urban and peri-urban areas of Kampala, based on California Mastitis Test (CMT), and furthermore to gain information about pathogens,

antibiotic resistance patterns, different risk factors, and possibilities of future prevention.

Materials and methods

Study design

The study has been approved and performed in accordance with the ethical standards of the policy of the Institutional Review Board (ref. no. VAB/REC/11/115) of the School of Veterinary Medicine and Animal Resources, Makerere University. It was conducted within a 20-km radius of Kampala during October 2011. The study populations were lactating cows of all parities on 18 smallholder farms. All cows were hand milked twice daily. The herd size of the farms ranged from 1 to 34 cows. A total of 195 lactating cows were included in the study. The distribution of breeds included in the study was 101 Holstein-Friesian cows, 70 Holstein-Friesian/local crossbred cows, 18 Jersey/Guernsey cows, and 6 other mixed breed cows.

Each farm was visited once and the cows were examined at cow and quarter level to rule out clinical mastitis (temperature ≤ 39.5 °C, no signs of illness and/or inflammatory signs of the udder, and normal milk upon ocular inspection). The cows were tested with the CMT according to Schalm and Noordlander (1957) and Mellenberger and Roth (2000) to detect the prevalence of subclinical mastitis. To allow further analysis of the results, the stage of lactation (<day 60, days 60–120, >day 120), parity (primiparous/multiparous), daily milk production (<7 l, 7–15 l, >15 l; according to information from the farmer), grazing system (zero grazing/open grazing), udder hygiene (good/poor), and cow breed (Holstein-Friesian/other breeds) were also recorded.

The CMT test result was scored from 1 to 5 according to the Scandinavian scoring system, where 1 is negative result (no gel formation), 2 is traceable (possible infection), and 3 or above indicates a positive result, where 5 has the most gel formation (Saloniemi 1995). All quarters with CMT ≥ 3 were milk sampled during ongoing milking, for further bacterial examination. The sampling was carried out according to the method recommended by the National Mastitis Council (NMC 2004). After collection, the milk samples were transported in an ice-cooled box and later transferred into a fridge at 4 °C. Milk samples were then processed within 24 h after their collection and cultured.

The milk samples were analyzed at the microbiology laboratory, Faculty of Veterinary Medicine, Makerere University, Kampala. Ten microliters of each milk sample was spread on blood agar plates (5 % bovine blood). The plates were then put into aerobic incubators at 37 °C for 16–24 h, where plates with dubious growth were allowed an extra 24 h before final examination. To be classified as a positive bacterial growth,

at least one colony-forming unit (CFU) was needed for *Staphylococcus aureus* and *Streptococcus agalactiae* and at least three CFUs for the other genera.

The bacteria on each plate were then analyzed and categorized according to colony morphology and α -, β -, or double hemolysis. Depending on genera, the cultures were then categorized based on potassium hydroxide test reaction, catalase test reaction, and β -glucuronidase test reaction. *S. aureus* isolates were tested for penicillinase production, using the cefinase test. To determine the amount of bacterial growth, the amount of colonies in each sample was quantified and then put into three categories: mild growth (<10 colonies), moderate growth (10–50 colonies), and severe growth (>50 colonies). If the plate had a growth of ≥ 3 diverse bacterial agents, it was classified as mixed growth.

Antibiotic susceptibility testing

All streptococci and some staphylococci bacterial isolates were saved in agar tubes and brought to the National Veterinary Institute (SVA) in Uppsala, Sweden. Streptococci isolates were subtyped using CAMP test and 12 biochemical reactions to distinguish among *S. agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and other *Streptococcus* spp. Staphylococci isolates were tested for penicillinase production, using the penicillinase test (Sensititre method) (Franklin and Wierup 1982), to distinguish if they were sensitive to benzylpenicillin or not.

Isolates of *S. agalactiae*, *S. dysgalactiae*, and *S. uberis* were tested for antimicrobial susceptibility by determination of minimum inhibitory concentration (MIC), using a micro dilution method. The testing was performed according to recommendations from the Clinical and Laboratory Standards Institute using VetMIC™ panels (National Veterinary Institute, Uppsala, Sweden) and cation adjusted Mueller-Hinton broth (Becton Dickinson, Cockeysville, USA) (Clinical and Laboratory Standards Institute 2007). The streptococci isolates were classified as susceptible or resistant based on species-specific epidemiological cutoff values for each type of antibiotic, issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Statistical analyses

Analyses of the frequency of positive cows (at least one quarter with CMT ≥ 3) were first performed. The results were hereafter first analyzed by χ^2 test for each individual factor (stage of lactation, parity, milk production, production system, udder hygiene, and cow breed). The effects of close to significant factors from the first χ^2 analysis were further analyzed together in a multivariable analysis while using logistic models (PROC LOGISTIC, SAS version 9.2).

Results

Descriptive and statistic data

The results of the CMT screening indicate that 86.2 % ($n=195$) of the tested cows had SCM in one or more quarters. At quarter level, the prevalence of SCM was 55.4 % ($n=760$). No correlation was found between the prevalence of SCM and milk production and cow breed. The factors significantly linked to the prevalence are presented below.

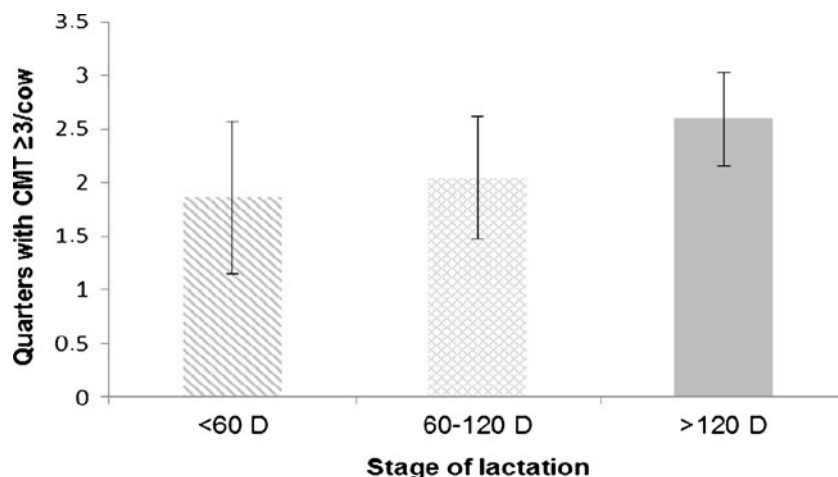
The prevalence of SCM was analyzed on basis of stage of lactation (Table 1). The figures from the cow level results in the first χ^2 test, in which the individual factors/marker of the frequency of positive cows (at least one quarter with CMT ≥ 3), were analyzed and showed that there is a close-to-significant positive correlation for SCM and stage of lactation ($p<0.06$). When these results then were analyzed in the second multivariable χ^2 test, in which correlation for the effects of other factors/markers was analyzed, it resulted in a significant ($p<0.02$) difference between the cows, where cows with less than 60 days elapsed from last calving had a lower prevalence of SCM than cows with more than 120 days from last calving. The number of quarters with CMT scores ≥ 3 /cow in each subgroup of stage of lactation (Fig. 1) points in the same direction: cows with <60 days had 1.86 affected quarters/cow and cows with

Table 1 Factors related to the prevalence of subclinical mastitis at cow and quarter level

Factors	Type	CMT-positive cows (%)	CMT-positive quarters (%)
Stage of lactation	<60 days	25/31 (80.6)	55/122 (45.1)
	60–120 days	27/36 (75.0)	64/138 (46.4)
	>120 days	107/119 (89.9)	282/464 (60.1)
	Total	159/186 (85.5)	401/714 (56.2)
Parity	Primiparous	38/48 (79.2)	91/190 (47.9)
	Multiparous	129/146 (88.4)	327/566 (57.8)
	Total	167/194 (86.1)	418/756 (55.3)
Milk production	>15 l	22/27 (81.5)	62/108 (57.4)
	7–15 l	126/143 (88.1)	301/553 (54.4)
	<7 l	20/25 (80.0)	58/99 (58.6)
	Total	168/195 (86.2)	421/760 (55.4)
Cow breed	Holstein	88/101 (87.1)	235/396 (59.3)
	Other breeds	80/94 (85.1)	186/364 (51.1)
	Total	168/195 (86.2)	421/760 (55.4)
Udder hygiene	Good	45/55 (81.8)	103/211 (48.8)
	Poor	123/140 (87.9)	318/549 (57.9)
	Total	168/195 (86.2)	421/760 (55.4)
Grazing system	Zero grazing	22/23 (95.7)	61/83 (73.5)
	Grazing	119/142 (83.8)	301/560 (53.8)
	Total	141/165 (85.5)	362/643 (56.3)

CMT California Mastitis Test

Fig. 1 Quarters with California Mastitis Test score ≥ 3 /cow in the subgroups of stage of lactation. The columns describe the mean number of quarters with California Mastitis Test (CMT) score ≥ 3 /cow of all cows in each subgroup of stage of lactation with error bars showing ± 2 standard deviations. D = days



>120 days had 2.59 affected quarters/cow, which is close to significance ($p < 0.06$).

The prevalence of SCM was also analyzed on the basis of parity (Table 1). When the results were analyzed in the second multivariable χ^2 test, it showed a strong significance ($p < 0.02$), where primiparous cows had a lower prevalence of SCM than multiparous cows.

Looking at the number of quarters with CMT ≥ 3 /cow, analyzing the udder hygiene of just Holstein-Friesian (HF) cows in each subgroup, HF with good hygiene had 1.63 quarters/cow and cows with poor hygiene had 2.72 quarters/cow. This is also a significant correlation ($p < 0.003$). There was no such significant correlation for the group other breeds.

The last factor analyzed was SCM on the basis of production system (Table 1). When analyzing the figures from the cow level result in the first χ^2 test, there was a tendency of significant correlation ($p = 0.13$) between SCM and cow production system, where grazing cows had a lower prevalence.

Distribution of udder pathogens

The most common bacteriological outcome was infection with coagulase-negative staphylococci (54.7 %), followed by negative growth (24.9 %), streptococci (16.2 %), mixed growth (1.6 %), *Escherichia coli* (1.3 %), and *S. aureus* (0.9 %). Of the 73 strains of streptococci, 48 were subtyped. Out of this, the distribution was 15 *S. agalactiae*, 12 *S. dysgalactiae*, 7 *S. uberis*, and 14 other streptococci species.

Antimicrobial susceptibility testing

The results from the streptococci susceptibility test are shown in Table 2. Final concentrations of antibiotics ranged from ≤ 0.03 to 64 mg/l. One hundred percent of the tested streptococci ($n = 34$) were sensitive to penicillin (Table 2). Of the tested staphylococci, six out of nine CNS were positive for penicillinase production in the penicillinase test carried out at SVA and four out of four *S.*

aureus were tested positive. Of the four cefinase-tested *S. aureus* in Uganda, all four were negative for penicillinase production.

Discussion

The overall prevalence of SCM is unexpectedly high. This applies especially to the prevalence at cow level, where Byarugaba et al. (2008) reported an overall cow level prevalence of SCM at 37.2 % in their study from the Jinja Province in Uganda. Studies from other countries using similar criteria to characterize a cow as SCM positive or not (Bitew et al. 2010; Giannechini et al. 2002; Harouna et al. 2009; Joshi and Gokhale 2006; Mdegela et al. 2009; Mungube et al. 2005; Rabbani and Samad 2010) reported prevalence rates ranging from 25.2 to 55.2 % at cow level. A possible explanation to the high prevalence of SCM found around Kampala could be that risk factors contributing to SCM were more predominant, e.g., a high proportion of cow confinement in zero grazing production system, a high proportion of poor udder hygiene practices, and a higher proportion of cows in late parity and in late stages of lactation.

The bacterial findings, however, resembled, but were not identical to the findings of earlier studies reported from Uganda (Byarugaba et al. 2008) and other developing countries (Bitew et al. 2010; Rabbani and Samad 2010; Harouna et al. 2009).

Analyzing the antibiotic resistance pattern of this study, it seems that the tested streptococci spp. required a higher MIC of tetracycline and trimethoprim, but required similar MIC of penicillin in comparison to the results of a Swedish study (Persson et al. 2011). However, this study considered only *S. dysgalactiae* and *S. uberis*, making it difficult to compare the resistance pattern of *S. agalactiae*. For some bacteria/antibiotic combinations, there are no EUCAST epidemiological cutoff values, i.e., the bacteria have not been classified as susceptible or resistant to certain antibiotics. There are other imaginable ways to determine the antibiotic susceptibility for these bacteria, e.g.,

Table 2 Resistance and distribution of minimum inhibitory concentration (MIC) for *Streptococcus agalactiae* (n=15), *Streptococcus dysgalactiae* (n=7), and *Streptococcus uberis* (n=12)

Substance	Species	Resistance (%)	Distribution (number of isolates) of MICs (mg/l)											
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>64
Cefalothin	<i>S. agal.</i>	0		3	6	6	 							
	<i>S. dysg.</i>	–			6	7								
	<i>S. uberis</i>	–			9	2		1						
Clindamycin	<i>S. agal.</i>	0				15	 							
	<i>S. dysg.</i>	0				7								
	<i>S. uberis</i>	–				12								
Ciprofloxacin	<i>S. agal.</i>	0					3	12	 					
	<i>S. dysg.</i>	–					3	4						
	<i>S. uberis</i>	–					7	5						
Chloramphenicol	<i>S. agal.</i>	–								11	4			
	<i>S. dysg.</i>	–							1	3	3			
	<i>S. uberis</i>	–								2	10			
Erythromycin	<i>S. agal.</i>	0				15								
	<i>S. dysg.</i>	–				7								
	<i>S. uberis</i>	–				12								
Fusidine	<i>S. agal.</i>	0									15		 	
	<i>S. dysg.</i>	–						1	3	1	2			
	<i>S. uberis</i>	–									12			
Gentamicin	<i>S. agal.</i>	–						1	5	5	4			
	<i>S. dysg.</i>	–						2	4	1				
	<i>S. uberis</i>	–							2	5	5			
Kanamycin	<i>S. agal.</i>	–									3	4	8	
	<i>S. dysg.</i>	–									2	4	1	
	<i>S. uberis</i>	–									1	5	6	
Oxacillin	<i>S. agal.</i>	–			2	6	6	1						
	<i>S. dysg.</i>	–			7									
	<i>S. uberis</i>	–			4	8								
Benzylpenicillin	<i>S. agal.</i>	0	3	7	5	 								
	<i>S. dysg.</i>	0	7											
	<i>S. uberis</i>	–	7	4		1								
Tetracycline	<i>S. agal.</i>	100							 		1	1	4	9
	<i>S. dysg.</i>	–									1	2	1	3
	<i>S. uberis</i>	–					12							
Trimethoprim	<i>S. agal.</i>	–								1	4	5	4	1
	<i>S. dysg.</i>	–						2	4	1				
	<i>S. uberis</i>	–							7	3	2			

MICs minimum inhibitory concentrations, *S. agal.* *Streptococcus agalactiae*, *S. dysg.* *Streptococcus dysgalactiae*, *S. uberis* *Streptococcus uberis*

White fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration. Bold vertical lines indicate the European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cutoff values. When no cutoff value is available, isolates are not classified as susceptible or resistant

cutoff values from other sources and/or clinical evaluation of antibiotic treatment. However, since the main reason of this study was not to provide recommendations of antibiotic treatment, nor evaluate the effect of such, this will not be further discussed.

Comparatively, the penicillin resistance of CNS and *S. aureus* seems to be higher in Uganda than the one obtained in Sweden: 6/9 resistance test for CNS and 4/8 resistance tests for *S. aureus* were positive, respectively. Still, this comparison has limitations, since the number of tested CNS and *S. aureus* is limited in this study. In addition, all (three) of the *S. aureus* cultures tested in Uganda showed to be penicillinase negative, whereas four out eight of the samples tested in Sweden were resistant to penicillin—an observation that might question the accuracy of the cefinase test. Yet, the high resistance pattern of staphylococci observed in this study is still cause for worry. In their Ugandan study, Byarugaba et al. (2008) also showed a high penicillin resistance among staphylococci, namely 86.6 %.

A plausible reason of the high antibiotic bacterial resistance in Uganda could be due to that the dairy farms do not consistently test cows to identify which ones to cull if found penicillinase positive. This could in turn explain the prevalence of udder infections with resistant bacteria of newly arrived cows. Another reason could be that farmers are able to obtain antibiotics over the counter without prescription. This laxity increases the risk of antibiotic misuse, which in turn may contribute to the development of antibiotic resistance (Byarugaba 2004; Byarugaba et al. 2008). Nonetheless, SCM in Uganda still seems to be treatable with penicillin during the dry period in most cases—a fact illustrated by the observation that 100 % of the examined streptococci were sensitive to benzylpenicillin.

The explicit significance regarding the stage of lactation, where cows with less than 60 days from the last calving date have a lower prevalence and a lower number of udder parts with $CMT \geq 3/cow$ than cows with more than 120 days past from last calving, is somewhat as expected. The udder is most sensitive to acute CM and SCM during the period after the calving, whereas chronic mastitis, most often subclinical, is more frequent later during the lactation. At the visited farms, the use of dry period was almost non-existing. Hence, some cows could be milking for a very long time, up to several years, increasing the risk of SCM. The tradition of not using dry periods might be one explanation to the figures implying that cows in late lactation were more susceptible to SCM.

In terms of parity, primiparous cows had a lower prevalence and a lower number of quarters with $CMT \geq 3/cow$ than multiparous cows. This is as expected. Joshi and Gokhale (2006) and Byarugaba et al. (2008) also found that the prevalence of SCM increased with increasing parity, and Rabbani and Samad (2010) presented similar results in their Bangladesian study. Older cows are more susceptible to SCM (Biaffa et al. 2005 in Neelesh et al. 2012) because of the breakdown of the streak canal barriers and udder tissue with progressive ageing process (Schalm et al. 1971).

Also, the clear significance regarding udder hygiene was expected. HF cows with poor udder hygiene had a higher amount of $CMT \geq 3/cow$, compared to HF cows with good udder hygiene. A dirty udder is more susceptible to SCM. Grazing cows on pasture, which have better conditions to maintain good udder hygiene, have a tendency of lower prevalence than zero grazing cows. This has also been shown in an earlier Ugandan study, where poor udder hygiene, western breed, and zero grazing were particularly pointed out as contributing factors for a high prevalence of SCM (Byarugaba et al. 2008).

The reason to this high prevalence of SCM is multifaceted, but there are a few anticipatory mechanisms deserving particular elucidation. Looking at the impact of certain factors, such as zero grazing, late stage of lactation, high parity, and poor udder hygiene, all seem to increase the risk of getting SCM.

Even though we did not perform any statistics on it, a reflection is that the overall hygiene and especially the hygiene routines around milking time are the main reasons of the high prevalence. Also, we did not observe the use of grouping the cows or milking them in a predetermined order, according to their udder status.

The results of this study provide new information and will hopefully contribute to a possibly lower prevalence of SCM in the future. The results also suggest that it is important to work with preventive work in the farms, in order to lower the prevalence of SCM. Previous studies show that the best educational results were obtained when farmers visited each other and, together with experts, discussed ways of improving their milking technique (Vaarst et al. 2007; Byarugaba et al. 2008).

This study also noted that well-functioning and healthy herds existed in the same livelihood as less healthy herds, which could serve as icons of success to use as role models for poorly functioning herds. However, further research is needed to investigate the prevalence of clinical mastitis in Uganda in order to obtain a more comprehensive picture of the current mastitis situation.

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