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Prevalence of sub-clinical mastitis and its association with milking practices in an intensive dairy production region of Uganda

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ABSTRACT. A cross-sectional study was conducted to investigate the risk factors for sub-clinical mastitis (SCM) in Mbarara District, an intensive dairy production region of Uganda where hand-milking is dominant. In 30 farms, herd-level milking practices and SCM prevalence were studied. The SCM prevalences were 68.6% (417/608, 95% confidence interval (CI): 64.9–72.2%) and 39.2% (946/2,411, 37.3–41.2%) at the cow- and quarter-levels, respectively. A preventive factor for SCM was cow calmness at the end of milking (OR: 0.20, 95%CI: 0.05–0.79, *P*=0.021); a risk factor was rough teat-end (OR: 1.75, 95%CI: 1.14–2.68, *P*=0.011). Good cow hygiene was negatively associated with environmental mastitis (*P*=0.002). Appropriate hand-milking practices that avoid teat damage are expected to reduce SCM in Uganda.

KEY WORDS: dairy cattle, hand-milking, milking practice, sub-clinical mastitis, Uganda

Mastitis is one of the most prevalent production diseases in dairy herds worldwide; however, information on the economic burden of this disease is limited for developing countries [9]. Sub-clinical mastitis (SCM) is difficult to detect due to the limited clinical signs [9]. The majority of mastitis losses are from subclinical infections, which result in depressed production [4]. A high prevalence of SCM was reported in Kampala [1, 3], Kiruhura [23], Kiboga [13], and Jinja [5] Districts, Uganda. Risk factors for SCM reported in Uganda included lactation stage, breed [1], herd size, zero-grazing [3], and parity [1, 3]. Very few studies in Africa, where cows are still milked by hand, have reported a relationship between milking practices and SCM [12], and (to our knowledge) no such studies have been reported in Uganda. Mbarara District, located in southwestern Uganda, is one of the most intensive and important dairy production areas in this country [2]; however, no studies on the prevalence of SCM in this region have been reported. The objectives of this study were to investigate prevailing milk hygiene practices, as well as the related risk factors for, and prevalence of, SCM in Mbarara District, an intensive dairy production region of Uganda where hand-milking is dominant.

This study was conducted among dairy cattle herds in Mbarara District, Uganda. A cross-sectional study was carried out as a baseline survey for a three-year project; this baseline survey, designated the Japan International Cooperation Agency (JICA) Safe Milk Promotion in Mbarara (Safe Milk) Project, was conducted between October 2016 and May 2017. Thirty representative farms in Mbarara District were selected by purposive sampling by the district's principal veterinary officer, based on the following criteria: (a) herd size: 5 farms with small herds (<10 adult cows per herd, including both milking and dry cows), 20 with medium herds (between 10 and 40 adults per herd), and 5 with large herds (>40 adults per herd); (b) herd management type: 5 to 10 zero-grazing farms, and 20 to 25 grazing farms; (c) accessibility: farms accessible by the project team using a vehicle for regular

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visits; (d) distribution of farms: 2 to 5 farms per sub-county and 5 to 6 farms per milk collection center, to facilitate diffusion of techniques; and (e) commitment to continue participation in the Safe Milk Project. According to the District Veterinary Office in Mbarara, the current total cattle population and the number of dairy farms in Mbarara District are estimated to be 185,680 and 10,200, respectively (personal communication). All milking cows present in the farms at the visits were included in the study, with the exception of the largest farm, where only the first 50 cows in the milking order were evaluated.

Teat-end scoring was conducted. Teat-ends were scored according to the method described by Hulsen [11] using a scale of 1–4: 1, no observable callus ring; 2, a smooth callus ring around the teat orifice; 3, a rough callus ring; 4 a very rough callus ring. The highest quarter-level teat-end score within a cow was defined as the cow-level score.

The California Mastitis Test (CMT) was performed to detect mastitis just after the teat-end scoring. The results were classified as negative, trace, or score 1, 2, or 3, depending on the amount of gel formed [21]. A quarter was defined as CMT positive if it had a score 1 or above, and a cow was defined as CMT positive if it had at least one CMT-positive quarter. CMT-positive cows/quarters with and without clinical signs of inflammation were defined as clinical and SCM, respectively. Quarter milk samples with CMT scores 2 or higher were collected for microbiological tests.

A herd-level checklist was used to evaluate the milking practice and the level of hygiene. The contents of the checklist included cow hygiene, cow comfort (if the cows showed stepping, kicking, or restive behavior), and the other parameters as shown in Table 1. A hand-milking technique, holding a teat in the palm and removing the milk by squeezing the teat with all five fingers [6, 12] was assumed appropriate. Cow hygiene was classified into two levels, good or not, by referring to a udder hygiene score chart [22]. The original hygiene score [22] had four levels (score 1 to 4), but the score was simplified as good (corresponding to score 1 in the original rating system) and not good (score >1 in the original rating system). The milking cows' comfort was checked at three different times (before, beginning of, and end of milking) to understand cows' behaviors when the animals were close to workers (an indicator of stress from humans [11]), when their teats were touched by workers (an indicator of a pre-existing teat or udder problem), and when the milk flow was low (an indicator of over-milking [18]).

The farm-level information collected included herd size and conduct of grazing, and the cow-level information, breed, age, and parity.

The microbiological testing of milk samples followed the conventional biochemical tests recommended by the National Mastitis Council of the United States of America [16]. Immediately upon collection, milk samples were stored in an ice-cooler box with cold packs, then transferred to the laboratory at Mbarara District Veterinary Office and kept at 4°C in a refrigerator. Within 24 hr after collection, a 10-μl aliquot of each sample was inoculated to a 5% sheep blood agar plate using an inoculating loop; the plates were then incubated aerobically at 37°C for 24 to 48 hr. The diagnostic tests were performed according to the method mentioned above [16]. Milk samples that yielded more than two different microbial species on a plate were considered contaminated, unless *Staphylococcus aureus* was present, in which case *S. aureus* was defined as the dominant pathogen.

Descriptive statistical analyses were performed for all of the collected data. For numeric variables, the mean, median, and range were calculated; for categorical variables, the response counts and proportions were calculated. The overall prevalences of SCM were calculated at the cow- and quarter-levels. Pathogens that are known to cause mastitis, that tend to live on the cow's udder and teat skin, and that are transferred cow/teat to cow/teat during milking were defined as contagious [4]; the microbes isolated by plating were classified into two groups of pathogens: contagious or environmental. The proportion of contagious mastitis out of total mastitis cases from which microbes were isolated was calculated.

Table 1. Milking hygiene practices in the 29 studied dairy farms in Mbarara, Uganda

Variables	Number of farms	Percentage
Mastitic cows are milked last (NA=1)	1	3.6
Milkers' hands		
Wearing gloves	0	0
Hands are disinfected	5	17.2
Presence of buckets to rinse and disinfect hands	6	20.7
Hands are dry before touching teats (NA=1)	2	7.1
Forestrip 4–5 times	4	13.8
Calf suckling before milking	18	62.1
Try to detect abnormal milk	3	10.3
Apply proper hand-milking technique	5	17.2
Apply pre-dipping	0	0
Wipe and dry teat completely with towel before milking	7	24.1
Wipe teat-end before milking	5	17.2
Use of one towel per cow	5	17.2
Start milking 60–90 sec after the first teat stimulation	14	48.3
Finish milking within 5 min after first teat stimulation (NA=2)	17	63.0
Apply post-dipping (NA=1)	3	10.7
Teat coverage rate of >75% in post-dipping (NA=1)	2	7.1

NA: not available, because the checklist conductor failed to observe.

In risk factor analysis, first, univariable analyses between SCM, and each farm and cow attribute, milking practice, and teat-end score were performed at the cow-level using generalized linear mixed-effects models (GLMMs) with binomial errors, selecting SCM positivity as a response variable, and herd as a random effect. Cows for which values were missing were excluded from the univariable analyses. Second, in order to understand the relationships between milking practices and mastitis, and to identify confounders and/or intervening variables of the relationships in multivariable analyses, a causal diagram [7] was generated (Fig. 1) using the DAGitty software [24]. In the diagram, SCM positivity and milking practices with *p*-values less than 0.2 in the univariable analyses, as well as other potential confounders for the relationship between the milking practices and SCM on a priori basis, were included. Third, multivariable GLMMs were built for each milking practice variable whose *P*-values were <0.2 in the univariable analyses with potential confounders defined by the causal web as explanatory variables to estimate the total effect of each milking practice [7]. An integrated prediction GLMM for SCM also was built with all the milking practice variables in the diagram and potential confounders and their interaction terms. The variables with multi-collinearity and less biological plausibility were excluded from the model, and backward stepwise model simplification was performed. In the multivariable analyses, two-level multiple imputation [25] was employed for the missing data using the MICE package in the R statistical software. In order to evaluate the effect of clustering at the herd level, intraclass correlation coefficients (ICCs) were calculated [8].

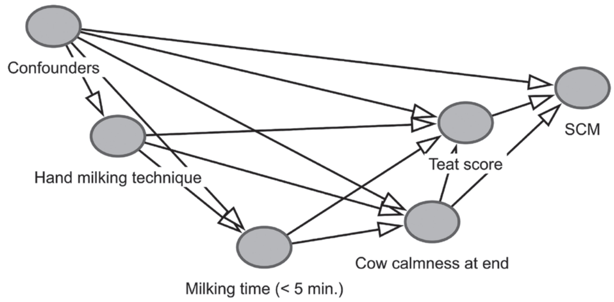

Fig. 1. Causal diagram showing relationships between SCM and milking practices. The variables of milking practices that yielded *P*<0.2 in univariable analyses, and the observed variables associated with the milking practices and SCM on an a priori basis, are included in the diagram.

In addition to the risk factor analyses, the relationships between infection with contagious pathogens and milking practices/the level of cow hygiene were analyzed using the data from quarters that yielded microbes. Univariable analyses were performed based on whether the isolated microbes were contagious or not as the response variable in GLMMs; the non-contagious isolates corresponded to environmental microbes. Quarters from which two microbes were isolated were classified as belonging to the contagious category if at least one contagious pathogen was detected. Milking practices that may facilitate infection with contagious pathogens, and the level of cow hygiene that could be a cause of environmental mastitis, were selected as explanatory variables. All of these statistical analyses were performed using the R statistical software, version 3.6.0 [20].

Table 1 shows the milking hygiene practices observed in the studied farms. On the majority of the studied farms, cows were clean (89.7%, 26/29), and were calm before (89.7%, 26/29), at the beginning of (89.7%, 26/29), and at the end of (82.8%, 24/29) milking.

The 30 herds evaluated in the present study comprised a total of 608 cows, of which three (0.5%, 95%CI: 0.1–1.4) cows had clinical mastitis, and 417 (68.6%, 95%CI: 64.9–72.2) had SCM in at least one quarter. A total of 2,411 quarters were checked for mastitis; the prevalence of clinical mastitis was 0.2% (6/2,411, 95%CI: 0.0–0.4) and that of SCM was 39.2% (946/2,411, 95%CI: 37.3–41.2). In the teat-end scoring, the majority (1,679/2,062, 81.4%) was normal (score 1), 342 (16.6%) were smooth (score 2), and 41 (2.0%) were rough (score 3). Very rough teat-ends (score 4) were not observed. Microbiological test results are shown in Table 2. Contagious bacteria consisted of *Corynebacterium bovis*, *S. aureus*, and *Streptococcus agalactiae*; the remaining isolates

Table 2. Microbiological test results from 576 quarter milk samples of which California Mastitis Test scores were 2 or more in studied dairy farms in Mbarara, Uganda

Pathogen isolated	Number of samples	Percentage
Corynebacterium bovis	88	15.3
Staphylococcus aureus	65	11.3
Coagulase-negative staphylococci	56	9.7
Other streptococci (unidentified)	29	5.0
Trueperella pyogenes	24	4.2
Streptococcus agalactiae	5	0.9
Coliforms	4	0.7
Prototheca species	1	0.2
Yeast	1	0.2
Others (unidentified)	10	1.7
No growth	278	48.3
Two different species[a)]	6	1.0
Contaminated[b)]	9	1.6

a) Pairs of the listed microbes. b) Milk samples with more than two different species isolated on a plate were considered contaminated, unless one of the species was *S. aureus*, in which case *S. aureus* was defined as the dominant pathogen.

were classified as environmental microbes. The proportion of contagious mastitis quarters among those from which microbes were isolated was 55.7% (161/289, 95%CI: 49.8–61.5).

Mean herd size for the 30 participating farms was 35.5 cattle (median: 28.5; range: 4–250). Twenty-eight (93.3%) of 30 farms employed grazing systems. The mean parity was 2.7 (median: 2, range: 1–11, *n*=293) and the mean daily milk yield (*l*/day) was 9.5 (median: 8, range: 1–32, *n*=503). The majority of cows (184/214, 86%) consisted of exotic breeds such as Holstein Friesian, while 14% (30/214) were cross breeds between indigenous Ankole and exotic breeds.

Causal relationships were hypothesized for SCM and milking practices, and for calmness at the end of milking and teat score, as shown in Fig. 1. Breed [1] and parity [1, 3] have been reported to show an association with SCM on an a priori basis, while milk yield also has an association with SCM in a biological context. Therefore these variables (breed, parity, and milk yield) were regarded as confounders. The hand-milking technique also was considered as a confounder on an a priori basis because this technique might be associated with the other milking practices and teat-end score. The variable of the cow's calmness at the beginning of milking (an indicator of a pre-existing teat or udder problem) was excluded from the causal diagram; although the *P*-value was <0.2 in the univariable analysis, this factor was presumed to be an effect (rather than a cause) of mastitis. To estimate the total effect of milking practices on SCM, three multivariable GLMMs: were constructed at the cow-level while incorporating the confounders. Specifically, these GLMMs assessed the effects of a) cow calmness at the end of milking, b) milking time, and c) teat-end score (Table 3). These analyses revealed that in model a), calmness of cows at the end of milking was a preventive factor for CMT positivity (OR: 0.20, 95%CI: 0.05–0.79, *P*=0.021), while in model c), higher teat-end score was a risk factor for CMT positivity (OR: 1.75, 95%CI: 1.14–2.68, *P*=0.011). In model b), milking time did not exhibit a significant effect on SCM (*P*=0.203). The final integrated prediction model included two significant predictors: loss of cow calmness at the end of milking (OR: 0.17, 95%CI: 0.05–0.64, *P*=0.009) and adverse teat-end score (OR: 1.75, 95%CI: 1.14–2.68, *P*=0.011). No collinearity with a correlation coefficient >0.9 was found among variables in the models. No interaction terms between explanatory variables remained in the final prediction model.

Table 4 shows the relationships between milking practices and the level of cow hygiene, and the proportions of contagious mastitis quarters as a function of quarters from which microbes were isolated. Good cow hygiene was positively associated with infection by contagious bacteria (OR: 3.71, 95%CI: 1.64–8.39, *P*=0.002), indicating that good cow hygiene was negatively associated with infection by environmental pathogens.

High prevalences of SCM were seen in the study population at both the cow- (68.6%) and quarter-levels (39.2%). These prevalences were comparable to those observed in other studies in different regions of Uganda, which yielded cow-level prevalence of 60.7–90% [1, 3, 5, 13, 23]. The prevalence of clinical mastitis was low in this study; however, the burden should be measured as an incidence rate in future studies, given that most cases occur at the early lactation stage [15].

The multivariable risk factor analyses for SCM detected a preventive factor, cow calmness at the end of milking, and a risk factor, adverse teat score. Teat-end scoring originally was developed in response to the increased occurrence of damaged teats observed due to the mechanical forces of vacuum and collapsing liners following the introduction of machine milking systems [17]. However, our study identified damaged teats as a risk factor for SCM even among the hand-milking dairy farms. In the study areas, cross-breed cows have smaller teats than exotic breeds such as Friesian, and traditional milkers pull teats strongly using two fingers (middle finger and forefinger) or the combination of forefinger and thumb, especially at the end of milking when milk flow decreases (consensus observations by the authors and participating farmers). Such ways of milking might damage teats and upset the cows during milking time, either or both of which could predispose the cows to mastitis; this inference also would explain the preventive factor, cow calmness at the end of milking. On the other hand, one study reported that a stripping technique of removing milk from the teat by moving the thumb and forefinger distally along the teat is a preventive factor for SCM [12]. In the present study, the hand-milking technique itself did not have an association with SCM and the milking practice checklist was applied and recorded only for the first milker at each farm. To assess milking techniques, more detailed assessments of milking techniques may be required.

Based on the microbiological cultures, the most prevalent pathogen in the study population was *C. bovis*, followed by *S. aureus* and coagulase-negative staphylococci (CNS). Previous studies also found that staphylococci [1, 3, 5, 13, 23] and *Corynebacterium* spp. [13, 23] were the major isolates. In the present study, contagious microbes were isolated at a higher proportion than were environmental microbes. Key control options to counteract contagious bacterial transmission are post-milking teat dipping, dry cow therapy, milking hygiene, and culling [4, 10, 14, 16, 19]. Poor cow hygiene was positively associated with environmental mastitis. In the studied farms, hygiene practices were not performed consistently during milking, which might facilitate the transmission of contagious bacteria. Previous studies reported that the level of knowledge of SCM was low in Uganda [5, 13]. Such low awareness of SCM may be a cause of the poor compliance with recommended hygiene practices. Bacterial culture in this study was performed only for milk samples with CMT 2 and higher, which might underestimate the prevalence of pathogens that cause mild mastitis.

Intra-herd correlations with values <0.2 are common for infectious diseases of animals [8]. The models constructed in the present study had high ICCs (>0.2), indicating that the farms exhibited a moderate clustering effect. Herd-level management might affect the prevalence of SCM, and should be the target of interventions. To deal with the clustering effect, GLMMs were used in the present study, and this statistical approach appeared adequate. However, there may have been a selection bias, given that the studied farms were participants in the Safe Milk Project and so may already have had good relationships with the District Veterinary Office. Such farms may have greater knowledge of diseases than the general population in Mbarara District, in which case our results might underestimate the actual prevalence of mastitis.

In conclusion, the prevalence of SCM, particularly due to contagious pathogens, was high in Mbarara District. Formation of

Table 3. Multivariable risk factor analysis results for calmness, milking-practice, teat-end score, and integrated effects on California Mastitis Test positivity

Variables	Odds ratio	95%CI	*P*-value
a) Effect of calmness at end of the milking			
Fixed effect variables			
Cows calm at end of the milking	0.20	0.05–0.79	0.021
Milking time (<5 min)	0.53	0.14–1.96	0.331
Hand-milking technique	0.61	0.15–2.49	0.489
Parity	1.10	0.93–1.30	0.233
Milk yield	0.97	0.92–1.03	0.346
Breed	1.20	0.40–3.55	0.730
Random effect variables	Variance	Standard deviation	ICC[a)]
Herd	1.00	1.00	0.23
b) Effect of milking time			
Fixed effect variables			
Milking time (<5 min)	0.41	0.10–1.63	0.203
Hand-milking technique	0.55	0.12–2.51	0.433
Parity	1.10	0.93–1.30	0.253
Milk yield	0.98	0.92–1.04	0.462
Breed	1.16	0.28–4.80	0.820
Random effect variables	Variance	Standard deviation	ICC[a)]
Herd	1.33	1.15	0.29
c) Effect of teat-end score			
Fixed effect variables			
Teat-end score	1.75	1.14–2.68	0.011
Cows calm at end of the milking	0.19	0.05–0.72	0.015
Milking time (<5 min)	0.53	0.15–1.89	0.317
Hand-milking technique	0.54	0.13–2.15	0.374
Parity	1.10	0.93–1.29	0.247
Milk yield	0.97	0.91–1.03	0.297
Breed	1.18	0.36–3.86	0.766
Random effect variables	Variance	Standard deviation	ICC[a)]
Herd	0.96	0.98	0.23
d) Integrated effects			
Fixed effect variables			
Cows calm at end of the milking	0.17	0.05–0.64	0.009
Teat-end score	1.75	1.14–2.68	0.011
Hand-milking technique	0.69	0.20–2.39	0.555
Parity	1.10	0.93–1.29	0.248
Milk yield	0.98	0.92–1.03	0.421
Breed	1.25	0.38–4.09	0.697
Random effect variables	Variance	Standard deviation	ICC[a)]
Herd	1.00	1.00	0.23

a) Intraclass correlation coefficient.

Table 4. Relationship between contagious mastitis and milking practices/level of cow hygiene at quarter level, among quarters from which bacteria were isolated

Variables	Percentage of contagious mastitis	Contagious/bacteria isolated quarters	Odds ratio	95%CI	*P*-value
Disinfecting hands before milking	67.6	23/34	2.01	0.69–5.86	0.201
Not conducted	54.1	138/255	Reference		
Presence of buckets to rinse and disinfect hands	68.6	35/51	2.20	0.90–5.35	0.084
Not present	52.9	126/238	Reference		
Drying hands after washing and before milking	46.2	6/13	0.53	0.11–2.66	0.441
Not conducted	57.6	151/262	Reference		
One towel per cow use for teat wiping	44.7	34/76	0.51	0.23–1.12	0.093
Not used	59.6	127/213	Reference		
Cow hygiene appeared good	60.7	142/234	3.71	1.64–8.39	0.002
Not good	34.5	19/55	Reference		

The numbers of observations were 23/176/289 at herd/cow/quarter levels (respectively), other than drying hands before milking (22/167/275).

a callosity ring around the teat orifice was a risk factor, and comfortable milking may prevent SCM; the maintenance of a clean udder environment would be expected to reduce the prevalence of environmental mastitis.

Ethical Considerations. This study was performed as the baseline survey of JICA Safe Milk Promotion in Mbarara Project, based on a bilateral agreement between JICA and the Mbarara District local government. Animals in this study were properly handled using a cattle holding frame during sampling to reduce their stress and avoid injury. Informed consent was obtained from all of the farms included in this study.

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