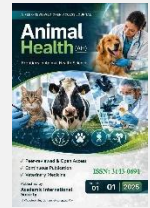




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Epidemiology and Risk Factors of Subclinical Mastitis in Smallholder Dairy Systems

Stanley N. Gathekia

College of Veterinary Medicine, Jomo Kenyatta University of Agriculture and Technology, Kiambu, Kenya

Corresponding author:

E-mail: njorogestan12@gmail.com

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Abstract

Background: Subclinical mastitis [SCM] remains one of the most economically relevant diseases of dairy cattle on the global level, characterized by elevated somatic cell counts and impaired milk quality without any obvious clinical symptoms. It is a disproportionate burden on smallholder dairy producers who usually have no access to regular diagnostic testing and preventive veterinary care. **Aim:** To determine the prevalence of subclinical mastitis at both the cow and quarter levels, and to identify the key risk factors associated with its occurrence in smallholder dairy farms. **Methods:** The cross-sectional study was done on 320 dairy cows that were lactating in 64 smallholder farms. The screen at the quarter level was the California Mastitis Test [CMT]. Bacteriological culture and identification of milk samples in CMT-positive quarters was carried out. The measurements of the management practices were obtained using a structured questionnaire. To determine the significant risk factors, the Univariable and multivariable logistic regression analyses were conducted. **Findings:** Cow-level and quarter-level prevalence rates of SCM were 43.1 and 24.4% respectively. The front quarters were not as affected as the rear quarters. The most common isolate was *Staphylococcus aureus* [31.4%], then coagulase-negative staphylococci [22.8%] and *Streptococcus agalactiae* [17.3%]. In the multivariable model, poor milking hygiene (OR = 4.92; 95% CI: 2.46–9.85), moderate milking hygiene (OR = 2.04; 95% CI: 1.18–3.53), advanced parity (OR = 3.14; 95% CI: 1.74–5.67), and absence of pre-milking teat disinfection (OR = 2.01; 95% CI: 1.29–3.13) were significantly associated with subclinical mastitis. **Conclusion:** It was found that the prevalence of subclinical mastitis was high among the smallholder dairy farms, which could be traced mainly to the management shortcomings that are rectifiable. The most effective control measures suggested are specific training in milking hygiene, frequent CMT screening, and more intensive use of dry-cow therapy.

Introduction

Mastitis, defined as inflammation of the mammary gland parenchyma, is one of the most prevalent and economically significant diseases affecting dairy cattle in both intensive and smallholder production systems [1]. It is broadly classified into clinical mastitis, which presents visible abnormalities in milk and udder, and subclinical mastitis (SCM), which occurs without apparent clinical signs despite ongoing pathological changes at the tissue level [2].

Subclinical mastitis is estimated to be four to five times more prevalent than its clinical counterpart and represents a major cause of reduced milk yield, increased antimicrobial use, premature culling, and overall economic losses in dairy production worldwide [3]. Diagnosis of SCM primarily relies on indirect assessment of somatic cell count (SCC), with values exceeding 200,000 cells/mL commonly indicating intramammary infection [4]. In resource-limited settings, the California Mastitis Test (CMT) is widely used as a rapid, cost-effective, and practical field-based proxy for SCC, allowing quarter-level assessment in the absence of laboratory infrastructure [5]. Furthermore, bacteriological culture of milk samples from CMT-positive quarters enables identification of causative pathogens, facilitating targeted control strategies.

Smallholder dairy farms, typically characterized by fewer than 20 lactating cows and household-level production systems, are exposed to a distinct set of risk factors for SCM. These include poor milking hygiene, inadequate housing conditions, limited access to veterinary services, inconsistent use of dry-cow therapy (DCT), and lack of systematic udder health monitoring [6]. In many low- and middle-income countries, the smallholder dairy sector contributes substantially to national milk production and plays a critical role in household income and food security, thereby amplifying the broader agricultural and public health implications of SCM [7].

Previous studies conducted in sub-Saharan Africa, South Asia, and the Middle East have reported SCM prevalence ranging from 30% to 70% at the cow level in smallholder systems, with associated production losses estimated at 10–25% per affected quarter [8,9]. Several animal- and management-related risk factors have been consistently identified, including increasing parity, lack of teat disinfection practices, poor housing conditions, and inadequate implementation of dry-cow therapy [10,11]. However, despite the recognized burden of SCM, there remains a relative scarcity of comprehensive epidemiological studies that evaluate the independent and combined effects of these risk factors within smallholder production systems.

Therefore, the present study aimed to determine the prevalence of subclinical mastitis at both cow and quarter levels, identify the major bacterial pathogens involved, and assess animal- and management-level risk factors associated with SCM in smallholder dairy farms.

Methodology

The study design used was cross-sectional. A systematic random sampling procedure was applied to a sampling frame built upon a database of the provincial veterinary authority on the official livestock registration to enroll 64 smallholder dairy farms. The eligibility criteria included farms, which kept at least three lactating dairy cows, none of which received intramammary treatment in the past two weeks and agreed to be examined completely. All the enrolled farm visits were made once in the course of the study.

Three hundred and twenty lactating dairy cows on the 64 farms were analyzed. All four mammary quarters were screened individually, and each cow was restrained in a standing posture and the California Mastitis Test [CMT] was used in compliance with the standard protocol reported by

Schalm and Noorlander [1957]. A CMT score of 2+ or 3+ was considered a positive outcome that signifies subclinical mastitis. Data were measured at a quarter-by-quarter level, and a cow was considered to be SCM-positive when one or more quarters resulted in a CMT score of 2+ or higher. Bacteriological examination of all the CMT-positive quarters was done using aseptic milk samples. Before collecting the samples, the teat ends were wiped down with 70% isopropyl alcohol impregnated swabs. The samples were kept in ice and taken to the diagnostic laboratory within four hours of collection, and they were done in 24 hours. General bacteriological culture methods were used: the samples were streaked on blood agar and MacConkey agar plates and incubated at 37 °C overnight (24-48h). Isolates were identified based on colony morphology, microscopic examination, Gram staining, catalase test, coagulase test, and the standard biochemical tests such as IMViC [12].

The data on the potential risk factors were obtained by a structured questionnaire conducted to farm owners or managers during the farm visit. Data were gathered on the variables on the animal level, such as parity and days in milk [DIM], and management practices on the farm level, such as milking hygiene practices [measured on a three-level ordinal scale: Good, Moderate, and Poor according to preset criteria], housing type, pre-milking teat disinfection practices, post-milking teat dipping practices, dry-cow therapy application, and herd size.

The descriptive statistics of each of the variables in the study were calculated. As a binary outcome variable, the cow-level SCM status [positive/negative] was applied. To obtain unadjusted odds ratios [OR] with 95% confidence intervals [CI], univariable logistic regression was first performed on each of the putative risk factors. The variables whose P-value was equal or less than 0.2 in the univariable analysis were determined as being eligible to be included in the multivariable model. The manual backward stepwise elimination procedure was subsequently used and only those variables which were still significant at $P = 0.05$ were kept in the final multivariable model. All the tests were conducted using Stata v.17 [Stata Corp, College Station, TX, USA]. The Institutional Animal Ethics Committee gave ethical approval to the study.

Results

A total of 320 lactating dairy cows from 64 smallholder farms were included in this study. The prevalence of subclinical mastitis (SCM) at the cow level, as determined by the California Mastitis Test (CMT), was 43.1% (138/320).

At the quarter level, 312 out of 1,280 examined quarters tested positive, yielding a prevalence of 24.4%. The rear quarters were more frequently affected than the front quarters, with the highest prevalence observed in the rear-right quarter (27.8%).

At the farm level, 92.2% of the farms had at least one cow affected by SCM. A summary of these findings is presented in Table 1.

Table 1: Prevalence of Subclinical Mastitis at the Cow, Quarter, and Farm Level

Parameter	Examined (n)	Positive (n)	Prevalence (%)
Cow-level (CMT)	320	138	43.1
Quarter-level (CMT)	1,280	312	24.4
Quarter Position			
Front Left [FL]	320	67	20.9
Front Right [FR]	320	71	22.2
Rear Left [RL]	320	85	26.6

Rear Right [RR]	320	89	27.8
Farm-level			
Farms with ≥ 1 SCM case	64	59	92.2

Milk culture Bacteriological milk culture of CMT-positive quarters produced isolates in 298 of 312 samples [95.5%]. The rest of 14 samples [4.5%] had culture-negative samples, which were in line with sterile inflammation or technical culture failure. The characterization of a total of 312 isolates was done, table 2. *Staphylococcus aureus* (31.4 percent), coagulase-negative staphylococci [CNS; 22.8 percent], and *Streptococcus agalactiae* (17.3 percent) were the most commonly identified [as shown in Table 3]. Gram-negative organisms that were identified include *Escherichia coli* [11.5%] and *Klebsiella* spp. [5.8%] but the other flora was mixed or other.

Table 2: Phenotypic and Biochemical Characteristics of Bacterial Isolates Used for Identification

Bacterial Isolate	Microscopic and colony Shape	Hemolysis	Catalase Test	IMViC Pattern
<i>Staphylococcus aureus</i>	Gram-positive cocci in clusters- Golden yellow, round, smooth colonies	β -hemolysis	Positive	-
<i>Coagulase-negative Staphylococci</i> [CNS]	Gram-positive cocci in clusters- White to gray, small, smooth	Usually, non-hemolytic	Positive	-
<i>Streptococcus agalactiae</i>	Gram-positive cocci in chains- Small, translucent colonies	β -hemolysis (narrow zone) positive for CAMP test	Negative	-
<i>Streptococcus dysgalactiae</i>	Gram-positive cocci in chains- small, grayish colonies	α or β -hemolysis	Negative	-
<i>Escherichia coli</i>	Gram-negative rods- Large, smooth, moist, pink on MacConkey	Usually β -hemolysis or none	Negative	++--
<i>Klebsiella</i> spp.	Gram-negative rods- Large, mucoid, pink on MacConkey	Non-hemolytic (γ)	Negative	--++

Table 3: Bacteriological Profile of Milk Isolates from CMT-Positive Quarters

Bacterial Isolate	Isolates (n)	Proportion (%)	Gram Reaction
Gram-Positive Cocci			
<i>Staphylococcus aureus</i>	98	31.4	Gram +ve
Coagulase-negative Staphylococci [CNS]	71	22.8	Gram +ve
<i>Streptococcus agalactiae</i>	54	17.3	Gram +ve
<i>Streptococcus dysgalactiae</i>	22	7.1	Gram +ve
Gram-Negative Rods			
<i>Escherichia coli</i>	36	11.5	Gram -ve
<i>Klebsiella</i> spp.	18	5.8	Gram -ve
Other / Mixed flora	13	4.2	—
Total	312	100	

Figure 1 presents a schematic representation of the distribution of positive CMT results across the four quarter positions. The rear quarters collectively accounted for 55.8% of all positive quarter results, while the front quarters contributed 44.2%. This difference was statistically significant [$\chi^2 = 5.42$; $P = 0.020$].

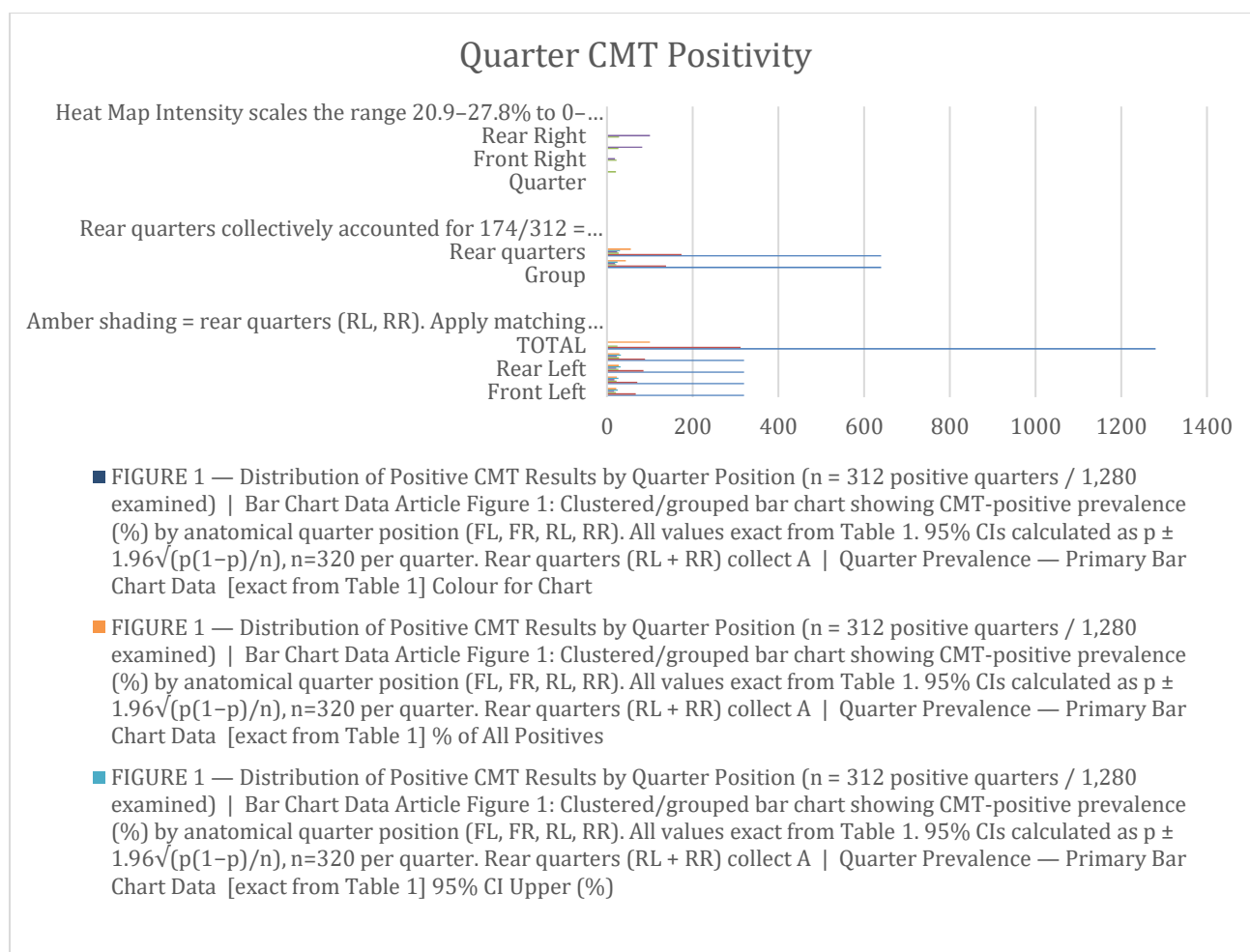


Figure 1. Allocation of positive quarters of CMT-by anatomical location (n=312 positive quarters of 1,280 positive quarters compared). FL front left FR front right RL rear left RR rear right.

Table 4 shows the findings of the univariable logistic regression analysis of the candidate risk factors. Parity [$P < 0.001$], late stage of lactation [$P = 0.009$], milking hygiene score [P less than 0.003], lack of pre-milking teat disinfection [P less than 0.001], and lack of dry-cow therapy were found to have statistically significant relations [P less than 0.05].

Table 4: Univariable Logistic Regression Analysis of Risk Factors for Subclinical Mastitis

Risk Factor	Examined	Positive	Prevalence (%)	OR	95% CI / P-value
Parity					
1–2 (Reference)	102	28	27.5	1.00	—
3–4	121	54	44.6	2.13	1.22–3.72; 0.008
≥ 5	97	56	57.7	3.68	2.01–6.74; <0.001
Stage of Lactation					
Early [1–90 DIM]	88	31	35.2	1.00	—
Mid [91–200 DIM]	134	54	40.3	1.24	0.71–2.16; 0.452
Late [> 200 DIM]	98	53	54.1	2.18	1.21–3.92; 0.009
Milking Hygiene Score					

Good (Reference)	74	16	21.6	1.00	—
Moderate	148	62	41.9	2.62	1.40–4.91; 0.003
Poor	98	60	61.2	5.77	2.89–11.52; <0.001
Housing Type					
Concrete floor	193	75	38.9	1.00	—
Earthen/open floor	127	63	49.6	1.56	0.99–2.46; 0.056
Pre-milking Teat Disinfection					
Practiced	109	33	30.3	1.00	—
Not practiced	211	105	49.8	2.29	1.40–3.75; <0.001
Dry-Cow Therapy [DCT]					
Applied	84	22	26.2	1.00	—
Not applied	236	116	49.2	2.74	1.59–4.73; <0.001

After the backward stepwise elimination of the multivariate model, eight variables remained independent predictors of SCM [Table 5]. The best animal-level predictor [adjusted OR 3.14; 95% CI 1.745.67; P less than 0.001] was advanced parity [5 or more calvings]. The strongest risk factor among the management factors was poor milking hygiene score [adjusted OR 4.92; 95% CI 2.46985; P < 0.001], then omission of dry-cow therapy [adjusted OR 2.43; 95% CI 1.46985; P = 0.002], late stage of lactation [adjusted OR 2.04; 95% CI 1.183.53; P = 0.011]. Another independent predictor [adjusted OR 1.62; 95% CI 1.032.55; P = 0.038] was larger herd size [> 10 cows].

Table 5: Multivariable Logistic Regression Model — Independent Predictors of Subclinical Mastitis

Independent Variable	Adjusted OR	P-value	95% CI
Parity ≥ 5 vs. 1–2	3.14	< 0.001	1.74–5.67
Parity 3–4 vs. 1–2	1.88	0.012	1.15–3.08
Late lactation [> 200 DIM]	2.04	0.011	1.18–3.53
Poor milking hygiene score	4.92	< 0.001	2.46–9.85
Moderate milking hygiene score	2.37	0.004	1.32–4.24
No pre-milking teat disinfection	2.01	0.002	1.29–3.13
No dry-cow therapy	2.43	< 0.001	1.46–4.05
Herd size > 10 cows	1.62	0.038	1.03–2.55

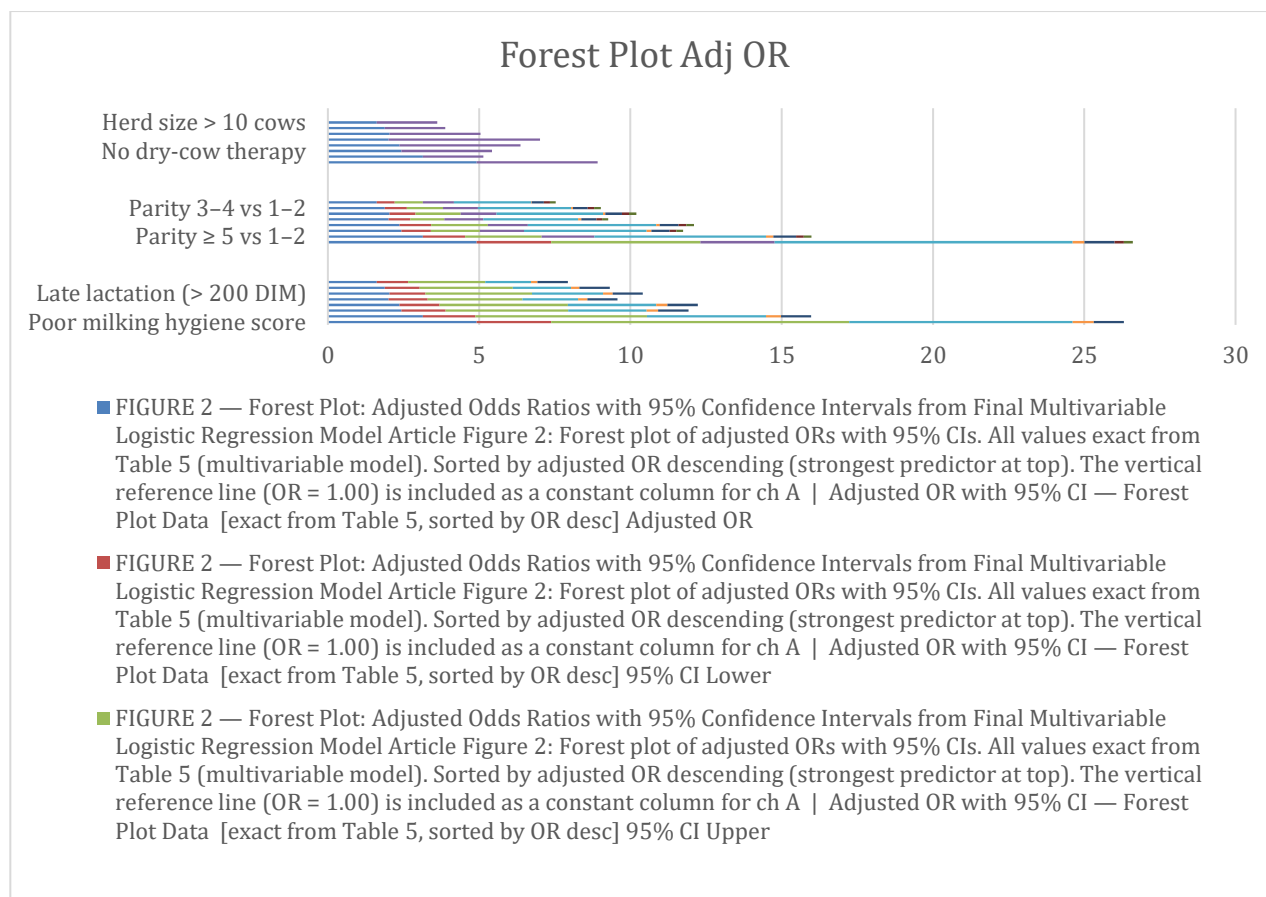


Figure 2. Forest plot of adjusted odds ratios with 95% confidence intervals from the final multivariable logistic regression model. DCT = dry-cow therapy; DIM = days in milk.

Discussion

The current study had a cow-level SCM prevalence of 43.1 which is within the range which was reported in smallholder systems in similar environments. As per Mungube et al., the cow-level prevalence in smallholder dairy farms in peri-urban Kenya is 44.5% [9], and Abebe et al. found a prevalence of 47.3% in Ethiopian smallholder dairy herds [13]. South Asian smallholder studies have also reported prevalences of between 38-52% [14]. The prevalence levels, evident in all these geographically varied settings, all boil down to the same conclusion, namely that SCM is not being properly controlled in smallholder systems, as at least part of it is due to the lack of management excellence found in the current risk factor analysis.

Its prevalence of 24.4 per quarter with a higher prevalence in rear quarters than in front quarters is not unexpected given epidemiological data on bovine mastitis. Rear quarters also generate a relatively higher amount of milk and are anatomically more vulnerable to the effects of environmental contamination, which makes them more vulnerable to ascending intramammary infection [15]. Several past studies have reported this trend and underlined the practical use of quarter-level CMT data as opposed to cow-level diagnosis only [16].

The preponderance of *Staphylococcus aureus* as the principal aetiological agent [31.4%] is consistent with the available literature on the subject of bovine SCM in smallholder systems. *S. aureus* is regarded as a prototype of contagious mastitis pathogen, which is spread mainly during milking in contact with dirty hands, milking cloths, and teat ends [17]. Its intracellular persistence ability and biofilm developing capability make it immune to antibiotic therapy and highlight the significant need to prevent diseases instead of cure them in the resource-limiting conditions of smallholder

environments. The comparatively high ratio of coagulase-negative staphylococci [22.8%] is also in line with the current reports, where CNS are becoming more of an important opportunistic pathogen instead of a contaminant [18]. Streptococcal galactiae [17.3%] is one of the contagious agents that are of particular concern to the smallholder context because of its ability to spread among the herd at the time of poor milking hygiene.

The central practical importance is given to the identification of the poor milking hygiene as the most potent modifiable risk factor [adjusted OR 4.92]. Milking hygiene is a complex of measures such as preparation of the udder before milking, separation of individual towels or gloves, preventing cross contamination of animals, disinfection of teats after milking and post milking. Hygiene in smallholder environments, where the education on optimal practices might be limited, and material resources are constrained by the economy, often remain poor [6]. The strength of the relationship observed here is also commensurate with those reported by Almaw et al. in Ethiopia [adjusted OR 5.12; 95% CI 2.47-10.59] [19] and lends support to the fact that the prioritization of the targeted training and behavior change communication as a core control intervention.

The independent protective effect of dry-cow therapy [adjusted OR 2.43 omission] is consistent with decades of evidence of the effectiveness of DCT as an effective measure of eliminating existing intramammary infections at drying off and new infections during the dry period [20]. This inefficient adoption of DCT in the current farms is in tandem with trends recorded in other smallholder environments, where cost, low access of veterinary services and ignorance are the leading impediments [21]. Special subsidy programmers and veterinary extension services have proved to be effective in enhancing DCT uptake in similar environments and should be considered as an option of policy.

High-order parity [5+ calvings] as an independent variable [adjusted OR 3.14] is biologically sound and in line with the literature. The degeneration of teat sphincter, the cumulative exposure to intramammary pathogens, and the decreased immune competence of the cows in old age all contribute to the susceptibility of SCM [22]. These results justify the exploration of selective culling policies to address older high-parity animals with recurrent SCM as the component of the overall strategy of herd health management, but the cost-effectiveness of such choices in smallholder environments with limited resources needs to be calculated.

Later lactation [> 200 DIM] was also found to predict SCM independent of stage of lactation [adjusted OR 2.04] which is a progressive immunosuppression and changes in the milk composition typical of pre-dry-off stage [23]. This observation, together with the protective DCT effect, further highlights the significance of controlling the transition period as an imperative period of intervention in terms of udder health.

Limitations of the Study.

The cross-sectional nature of the present study does not allow determining the causality of the outlined risk factors and the presence of SCM over time. Although the CMT is a proven and popular screening instrument, it has a certain level of operator inconsistency and fails to give any quantitative data on SCC. One visit to the farm was made, and seasonal change in the prevalence of SCM was not observed. Moreover, it was beyond the scope of the current study to provide molecular typing of the isolates such as methicillin resistance in staphylococci and capsular typing in streptococci. Further longitudinal research with somatic measuring of cell count, molecular characterization of isolates and economic analysis are justified.

Conclusion

Staphylococcus aureus was identified as the predominant etiological agent, consistent with its well-established role in contagion-mediated transmission under suboptimal milking hygiene conditions. Poor milking hygiene, absence of dry-cow therapy, lack of pre-milking teat disinfection, and late stage of lactation were independently associated with subclinical mastitis.

These findings indicate that the high burden of subclinical mastitis in smallholder dairy systems is largely driven by modifiable management practices. Therefore, improving milking hygiene, promoting routine CMT-based screening, and encouraging the widespread adoption of dry-cow therapy should be prioritized as practical and cost-effective interventions to reduce disease prevalence and improve udder health in these systems.

Recommendations

Routine screening of CMT must become the standard protocol of the udder health monitoring of all the smallholder dairy farms where the screening should be done at least once a lactation at the quarter level. It should administer targeted training programmers on milking hygiene, in particular, including pre-milking teat preparation, individual towel use, hand and post-milking teat dipping, to smallholder producers through veterinary extension services.

The concept of dry-cow therapy must be applied universally by all cows when lactation is stopped and national and regional governments must investigate the concept of subsidy or voucher system to enhance its availability in resource constrained environments.

Determination of high-parity cows to undergo selective culling or premature drying-off should be part of the plan of herd management based on regular CMT and SCC monitoring.

Antimicrobial resistance national surveillance programmers on the use of the antimicrobials against bovine mastitis pathogens, especially methicillin-resistant *Staphylococcus aureus* [MRSA], should be developed to ensure responsible use of antimicrobials and to protect animal and human health.

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