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Cross-sectional study of cow comfort and management factors associated with subclinical mastitis in smallholder dairy farms in Kenya

[1]KARIUKI E., [1]VANLEEUWEN J., [2]GITAU GK., [1]MCKENNA S., [1]HEIDER L., [1]KEEFE G., [1]MUCKLE A., KATHAMBI EK.*

[1]Department of Health management, Atlantic Veterinary College, University of Prince Edward Island
[2]Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.
[3]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota

***Corresponding Author**: karisedward@gmail.com

Abstract

A number of environmental and contagious factors have been associated with subclinical mastitis (SCM), which is a common and costly problem for smallholder dairy farmers (SDF). We conducted a cross-sectional study on 118 cows in their first two months post-calving on 109 SDF in Kenya. The study objective was to investigate the relationships among various cow and farm management parameters and SCM specific to SDF.
The stall floor comfort level was assessed through knee impact and wetness tests, and cleanliness on the leg and udder were also scored. Various mastitis prevention measures were also assessed (e.g., milking protocols, and use of teat dip and dry cow therapy). Individual quarter SCM was assessed on each cow using California Mastitis Test (CMT). Univariable and multivariable logistic regression models were fit to determine management factors associated with cow-level SCM.
Farm-level, cow-level and quarter-level prevalence of SCM was 45.9% (50/109), 43.2% (51/118) and 21.9 % (103/471), respectively. The proportion of stalls scored as dirty was 33.1% while 49.1% of cows had dirty legs. Only 10.1% of farms were using either disinfectant teat dip or dry cow therapy (or both) to prevent mastitis. Low parity and poor stall hygiene were significantly associated with occurrence of SCM. At high daily milk yield, the probability of having SCM was higher in cows housed in a shed with a dirty versus clean alleyway, with no significant difference at low daily milk yield.
From the study findings, we concluded that certain cow characteristics and comfort measures were associated with SCM and need to be incorporated in education plans for farmers in SDF.

Keywords: *Cow comfort; dairy cows; sub-clinical mastitis, small-holder dairy farms; stall hygiene*

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Introduction

Mastitis is the most important cause of economic losses in the dairy industry around the world (Abebe *et al*., 2016; Bardhan, 2013) and is associated with milk reduction from infected quarters, treatment costs, milk rejection due to spoilage, and culling cows with recurring mastitis, among others. Mastitis is a painful disease that can be classified into clinical and subclinical, based on observable changes in the udder and milk, and categorized into environmental and contagious, based on the primary reservoir of the pathogens involved (Klaas & Zadoks, 2018; Smith *et al*., 1985). Subclinical mastitis (SCM) is considered the most economically important form of mastitis (Mungube *et al*., 2005) due to its higher prevalence, need for detection, and long-term effects, as compared to clinical mastitis. It accounts for more than 90% of total loss in milk production (Schepers & Dijkhuizen, 1991), and is a substantial animal welfare concern (Peters *et al*., 2015). CMT is a quick and easy qualitative screening test and is a reliable way to indirectly measure somatic cell count used widely to detect SCM (Leslie KE *et al*., 2002)).

The dairy industry is a strong pillar in the economy of many developing countries. In Kenya, 80% of the dairy cattle population is on two million smallholder farms, which contribute an estimated 60% of the country's milk supply (Bonilla *et al*., 2017; Peere & Omore, 2017), and 8% of the national gross domestic product (USAID & GoK, 2009). Previous studies have estimated cow-level prevalence of SCM in Kenya to be between 44% and 65% (Gitau *et al*., 1994; Muraya *et al*., 2018; Mureithi & Njuguna, 2016).

While rearing dairy cows on pasture is the natural environment, it is not possible on most SDF in central Kenya due to decreasing land sizes and tick-borne disease control recommendations (VanLeeuwen *et al*., 2012). For these reasons, a majority of SDF rear their cows in zero-grazing systems using confinement free-stall units, where a cow has a stall to lie down and a short alleyway to walk to a nearby feed and water trough (Gitau *et al*., 1994). If well-constructed and maintained, these structures can provide excellent comfort and welfare levels, promoting good hygiene and low mastitis incidence. Unfortunately, many SDF in developing countries have lagged in adopting practices and structures that promote optimal welfare of their dairy cows (Kawonga *et al*., 2012; Nkya *et al*., 2007) and thus, SDF continue to grapple with the associated milk production losses.

Design and stall maintenance have a major effect on cow comfort parameters. A cross-sectional study on 80 Kenyan SDF found skin abrasions on 85% of hocks, 75% of carpi, 61% of necks, 44% on briskets and 29% on udders and teats (Aleri *et al*., 2011; Aleri *et al*., 2012). Cows frequently lying down in dirty stalls or alleys have poor hygiene scores, and subsequently, more mastitis (Sant'Anna & Paranhos da Costa, 2011).

Numerous studies in developed countries have shown associations between cow comfort outcomes and production indices, such as mastitis and milk production. (Lombard *et al*., 2010; Sprecher *et al*., 1997; Tucker *et al*., 2004; Zurbrigg *et al*., 2005) A previous study conducted in Kenya found significant association between stall comfort parameters and cow lying time (Kathambi *et al*., 2019) but did not explore the comfort effects on mastitis.

It is important to determine the current comfort status and practices in SDF, and particularly those that affect the occurrence of SCM, in order to guide informed interventions, and recommendations towards comfort standards. This study aimed at investigating the status and impact of cow comfort and mastitis management practices, on the occurrence of SCM in SDF in central Kenya.

Materials and methods

Ethical approval

This study was approved by the Research Ethics Board and the Animal Care Committee of the University of Prince Edward Island. We received consent from the Naari and Buuri Dairy Farmers Cooperative Societies, and Farmers Helping Farmers, a partnering non-governmental organization working with the dairy societies. Written consent was also sought from individual farmers on the first farm visit.

Study population

The study was conducted in Buuri Constituency in Meru County in the central region of Kenya. Farmers in this region mainly practice mixed farming, whereby dairy farming is conducted alongside the cultivation of potatoes and other vegetables. Typical dairy units have less than 5 cows, with a majority having only one or 2 milking cows. An initial sampling frame of 1500 farms shipping milk to Naari and Buuri Dairy Farmers Cooperative Societies was provided, from which we recruited all farms that met the following criteria: 1) less than five milking cows; 2) cows reared in a zero-grazing unit; and 3) at least one cow that was less than 60 days in milk at the time of recruitment. To attain a 25% difference in SCM between factor positive and factor negative cows, with the desired power of at least 0.8 and 0.05 significance level, we needed a sample size of 116 cows. We recruited all farms that met the inclusion criteria during the two-month recruitment period and ended up with a total of 118 cows on 109 farms recruited.

Data collection

We conducted the study between August and October 2020 which is usually a drier period of the year. A questionnaire administered in-person, in the farmers' local dialect (Kimeru), was used to collect farm and animal demographic characteristics. Questions also included aspects of mastitis prevalence, cow comfort and mastitis management practices on the farm (e.g., bedding and manure management).

General health status (by routine veterinary physical examination) and body condition score (scored on 1-5 scale with ½ point increments) (Wildman *et al*., 1982) were assessed for each cow. Cow weight was estimated using a heart girth tape. Hygiene scoring of each cow's udder, flank and legs, and freestall hygiene was assessed, using a whole point scale of 1 (very clean) to 5 (very dirty) (Reneau *et al*., 2005). Injuries and lameness, scored from 1 (no injuries/ lameness) to 3 (severe injuries/lameness) were assessed, modified from a known 1-5 score (Sprecher *et al*., 1997), which combined score 2 and 3 together, as well as 4 and 5 together. Injuries were assessed at the neck, carpus and hock regions.

Each cow was tested for individual quarter subclinical mastitis using California Mastitis Test (CMT), whereby the first strip of foremilk was milked from each teat into separate wells of a CMT paddle. An equal amount of 3% CMT solution (Immucell Corporation, USA) was added to each milk sample and whirled for about 15 seconds. Color changes and consistency of the mixture were then observed and used as a diagnosis for presence and severity of SCM (Harmon, 1994). Quarter level subclinical mastitis was scored as negative (0 or trace) and positive (1, 2, or 3) with increasing severity of SCM (National Mastitis Council, 2004; Quinn *et al*., 1994).

Total length (front wall to rear curb), body length (neck rail to rear curb) and width of each stall were measured in centimeters and categorized as 1 (adequate), 2 (marginal), or 3 (inappropriate) based on recommendations relating to the weight of the cow lying in the stall (Cook, 2009). Based on its height from the floor of the stall and distance from the rear curb, neck rails and brisket boards were reported as: 1) present and well-positioned, 2) present but wrongly positioned, or 3) absent. Forward/side lunge space and leg space were reported as: 1) sufficient, 2) marginal, or 3) inappropriate, based on recommendations (Cook, 2009)). Knee impact and wetness tests (McFarland, 1991) were used to assess the condition of the stall floor. Presence and type of bedding in the stall was also recorded. Alleyway hygiene was scored, based on the amount of manure present, as clean (no manure), fairly clean (small amount of manure that can be easily avoided while walking) and muddy (large amount of manure that could not be avoided while walking). The roof of the cow shed was examined for holes and for appropriate coverage of the cow stall. It was also recorded whether surface water was able to flow into the stalls or diverted around the stalls.

Statistical analysis

Information on the questionnaires was entered into Microsoft Excel (Microsoft lnc., Sacramento, California, USA) where it was cross-checked for accuracy and coded, and later imported into Stata 16.1 (Stata Corp LLC, College Station, Texas, USA) for statistical analysis. Injury scores were dichotomized as either 0 (no lesions) or 1 (scores 1 and above), since scores above 1 were few, and were reported as injury prevalence. Hygiene scores were also dichotomized, with scores 1 and 2 classified as 0 (clean) and scores 3, 4 and 5 (dirty) classified as 1. Cow-level prevalence of SCM was reported based on a cow having at least one quarter positive (score 1 and above) on CMT. Farm-level prevalence was reported based on farms that had at least one cow with at least one quarter positive on CMT. Quarter-level prevalence was reported as the proportion of all quarters sampled that tested positive on CMT.

Logistic regression modeling was utilized to determine associations between predictor variables and cow-level SCM (outcome variable). Parity was recategorized for improved model fit, where parities 2 and 3 were grouped together, and parities 4 and above were grouped together. Current daily milk yield was categorized into three groups: having milk yield between 1 and 8, 9 and 15, and above 15 liters per day, guided by a Lowess smoother plot of linearity between probability of having SCM and current daily milk yield. Predictor variables were initially assessed for univariable associations, and those with p-values equal to or below 0.25 were retained for multivariable regression. Correlation among variables that met this cut-off were determined using either Pearson correlation coefficient (continuous variables) or Chi-square test (categorical variables). Backward stepwise elimination was used to systematically remove variables with no significant association with SCM from the model. The causal diagram in Figure 1 was utilized to guide the model building. Each of the removed variables were individually fitted back into the final model to assess for uncontrolled confounding. Interaction was also assessed for all pairs of final model variables. Goodness of fit for the model was assessed using the Hosmer-Lemeshow test, and influential observations were assessed by evaluation of standardized Pearson residuals, leverage and delta beta. A mixed effect model was not explored since only 4 farms had more than one cow with three having 2 cows and one having 3.

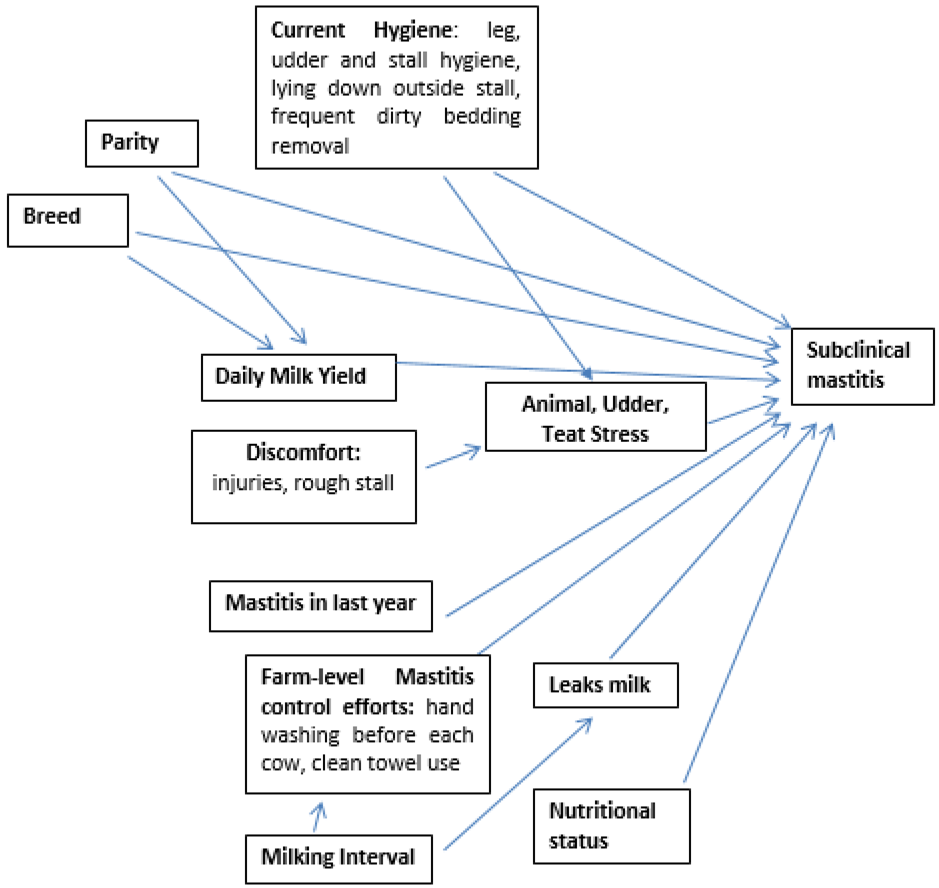

Figure 1. Causal diagram for factors associated with occurrence of subclinical mastitis in smallholder dairy farms in Kenya.

Results

Descriptive statistics

The study population consisted of 118 cows from 109 SDF. The breeds consisted predominantly of *Bos taurus* dairy breeds (Friesians, Ayrshires, Guernseys and Jerseys; 87.3%) and their various crosses (11.0%) and a small portion of *Bos indicus* cows (1.7%). The body condition of the cows ranged from 1 to 3.5, with 65.3% having a body condition score of 2.5 and above. They weighed on average, 350 kg (±69.5 s.d.), ranging from 230 to 698 kg, and had an average daily milk yield of 10.7 (±4.28) liters, ranging from 1.5 to 28 liters. Only 11 of the farms (10.1%) used dry cow treatment and disinfectant teat dip, while 29 (26.6%) were familiar with California Mastitis Test (CMT) by virtue of having a cow on their farm previously checked for and diagnosed with subclinical mastitis.

Farm-level SCM prevalence was 45.9% (50/109). Cow-level prevalence of SCM was 43.2% (51/118). One cow had the right-fore quarter completely dried off due to a previous mastitis problem, leaving 471 quarters from the 118 cows. Quarter-level prevalence of SCM was 21.9% (103/471). Out of the 109 farms recruited, 35 (32.1%) had experienced a case of mastitis in at least one of their cows within the last one year.

The study cows were all reared in zero-grazing units, and 87.2% of farms (95/109) had freestalls with partitions between stalls. Among the 95 farms with separate stalls for individual cows, 38 (40%) had a neck rail, of which 18 were well-positioned and 20 were inappropriately positioned. Of these 95 farms with separate stalls, 11 (11.6%) had a brisket board, of which 3 and 8 farms had poorly and well-positioned brisket boards, respectively. Only 14 of the 109 farms (12.8%) lacked an appropriate cow shed roof (too short or holes), while surface water was observed leaking into the stalls in 19 (16.2%) of the sheds.

For the 118 cows, a majority of their stalls (111, 94.1%) had a dirt floor, while 5 (4.2%) had concrete floors and 2 (1.7%) had a wooden stall floor. Two-thirds of the 118 stalls (67.8%) had bedding in the stall. Regarding bedding types for the 118 cows, 31.4% had crop waste, 31.4% had wood shavings or saw dust, 2.5% had additional loose soil, and 0.8% was straw. The remaining 40 cows had no bedding, although rubber mats were used for two of these cows.

On a scale of 1 (very clean) to 5 (very dirty), 16 (13.6%), 63 (53.4%), 31 (26.3%), 7 (5.9%) and 1 (0.9%) stalls were scored as 1, 2, 3, 4 and 5, respectively. When recategorized on a dichotomous scale (Table 1), 79 (66.9%) stalls were scored as clean (scores 1 and 2) on the day of the visit while 39 (33.1%) stalls were categorized as having a dirty floor surface (score 3, 4 or 5). In terms of hygiene, close to half of all cows had dirty legs (Table 1), while a fifth of udders were dirty. Alleyways were largely categorized as clean. The proportion of cows with neck lesions was substantially higher than for carpal and hock lesions (Table 1). Lameness was rare.

Table 1. Prevalence of poor hygiene and injuries observed on 118 cows on 109 smallholder dairy farms in Kenya, August to September 2020.

Outcome	**No. of cows**	**Prevalence(%)**	**95% CI**
Stall hygiene[A]	39	33.1	24.6 – 42.3
Leg hygiene[A]	58	49.1	39.3 – 58.5
Udder hygiene[A]	23	19.5	12.8 – 27.8
Alleyway hygiene[A]	97	82.2	74.1 – 88.6
Neck injuries	22	18.6	12.1 – 26.8
Carpal injuries	4	3.39	0.93 – 8.45
Hock injuries	3	2.54	0.52 – 7.19
Lameness	1	0.85	0.02 – 4.63

[A]Number/ prevalence of hygiene categorized as dirty (≥ 3 on a 1-5 scale)

Farmers were applying a number of mastitis control protocols (Table 2). All farms were milking all their cows by hand. Most of the farmers were giving fresh feed after milking (87.0%), washing hands between milking different cows (80.7%), milking mastitic teats last (91.7%), and milking mastitic cows last (65.9%), where applicable. However, other mastitis control measures were infrequently employed, such as using different towels to wash each cow udder (49.5%), using dry cow therapy (10.1%), using disinfectant teat dip (10.1%), and stripping the first milk to assess physical changes suggestive of mastitis (21.1%).

Table 2. Prevalence of farms that practiced various mastitis management protocols among 109 smallholder dairy farms in Kenya.

Outcome	No. farms responded	No of farms applying	Prevalence (%)	95% CI
Give fresh feed after milking	108	94	87.0	79.2 – 92.7
Wash hands between cows[A]	89 [A]	68	76.4	66.1 – 84.8
Mastitic teat milked last[B]	48 [B]	41	85.4	72.2 – 93.9
Mastitic cow milked last[C]	39 [C]	30	76.9	60.7 – 88.9
Different udder towels[D]	80 [D]	54	49.5	39.8 – 59.3
Disinfectant teat dip	109	11	10.1	5.15 – 17.3
Dry cow therapy (antibiotic)	109	11	10.1	5.15 – 17.3
Strip out first milk	109	23	21.1	13.9 – 30.0

[A] only includes farms that had multiple milking cows.

[B] only includes farms that had encountered mastitis.

[C] only includes farms that had more than one milking cow and had experienced mastitis in the past.

[D] only includes farms that had multiple milking cows.

Factors associated with occurrence of cow-level subclinical mastitis

Out of the factors assessed for unconditional association, parity, alleyway hygiene, stripping out first milk, having mastitis in the previous year, breed, body condition score, knee impact, current daily milk yield, and stall hygiene met the cutoff to be included in the multivariable regression model (Table 3). In the multivariable model, parity, alleyway hygiene, stall hygiene, current daily milk yield, and an interaction between alleyway hygiene and current daily milk yield had significant associations with occurrence of subclinical mastitis (Table 4). No removed variables were re-introduced to account for uncontrolled confounding

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Table 3. Factors associated with occurrence of subclinical mastitis in univariable analyses at p<=0.25 among 118 cows on 109 smallholder dairy farms in Kenya.

Factors	Categories	No. cows	Odds ratio	Odd ratio 95% CI	P- value
Parity	Reference	39			0.065[A]
	2 and 3	49	0.58	0.25 – 1.35	0.208
	>=4	29	0.29	0.10 – 0.83	0.020
Alleyway hygiene	Clean	21			
	Dirty	97	3.48	1.15 – 10.5	0.054
Strips out first milk	No	93			
	Yes	25	0.43	0.16 – 1.13	0.089
Mastitis in last year	No	81			
	Yes	37	2.63	1.18 – 5.48	0.047
Breed	Other breeds	46			
	Friesian	72	2.07	0.96 – 4.46	0.065
Body condition score	>=2.5	77			
	<2.5	41	0.48	0.21 – 1.05	0.068

Knee impact	Reference	46			0.250[A]
	2	61	0.52	0.24 – 1.13	0.097
	3	11	0.76	0.20 – 2.86	0.689
Current daily milk yield (L)	<8	28			<0.001[A]
	8-15	70	0.79	0.32 – 1.92	0.600
	>15	19	2.89	0.41 – 9.81	0.080
Stall hygiene	<=2	79			
	>2	39	1.63	0.75 – 3.53	0.216

[A]Global p-value for categorical variable

[B]Outcomes not reported for the interaction since interpretation relies on the main effects.

[A]Global p-value for categorical variable

The Hosmer-Lemeshow goodness-of-fit test showed good fit of the model to the data (p=0.387). There was only one standardized Pearson residual greater than 3 (3.18) and none less than -3. The greatest leverage value was 0.27.

Table 4. Factors associated with occurrence of subclinical mastitis and their significance in a multivariable logistic regression model among 109 smallholder dairy farms in Kenya.

Factor	**Categories**	**No. cows**	**Odds ratio**	**Odds ratio 95% CI**	**P-value**
Parity	1	39	Reference		0.005[A]
	2 & 3	49	0.29	0.10 – 0.82	0.020
	> 3	29	0.12	0.03 – 0.45	0.002
Stall hygiene	1&2	78	Reference		
	>2	39	3.99	1.36 – 11.7	0.012
Alleyway hygiene	1&2	20	Reference		
	>2	97	0.16	0.013 – 1.86	0.143
Current daily milk yield (L)	1-8	28	Reference		<0.001[A]
	8-15	70	0.02	<0.01 – 0.34	0.008
	>15	19	21.3	3.96 – 114.2	<0.001
Alleyway hygiene * Current daily milk yield Interaction term	B	B	B	B	0.003

Interpreting the coefficients in Table 4, cows in the second and third lactations were 3.4 times (i.e.,1/0.29) less likely to have SCM compared to cows in their first lactation, while those in their fourth and subsequent lactations were 8.3 times (i.e., 1/0.12) less likely to have SCM compared to cows in their first lactation. Cows that had a dirtier stall were 4 times more likely to have SCM. The coefficients of the dirty alleyway variable and daily milk production variable cannot be interpreted independently without considering the other variable, and Figure 2 demonstrates this dependency in the interaction variable involving these two variables. At low milk production, there was no difference in the odds of SCM by alleyway hygiene score; however, at higher milk production levels, the odds of SCM were lower with a clean alleyway score (score = 1 or 2) and higher with a dirty alleyway score (score = 3).

Table 5: Pairwise correlation matrix for variables that had significant unconditional association with subclinical mastitis

	Parity	**Alleyway hygiene**	**Strips out first milk**	**Mastitis in the last year**	**Breed**	**Body condition score**	**Knee impact**	**Current daily milk yield**	**Stall hygiene**
Parity	1.00								
Alleyway hygiene	0.028	1.00							
Strips out first milk	-0.074	-0.030	1.00						
Mastitis in the last year	-0.167	0.019	0.069	1.00					
Breed	-0.208	0.084	-0.053	0.057	1.00				
Body condition score	0.055	0.013	-0.074	0.019	-0.074	1.00			
Knee impact	-0.018	-0.184	0.113	-0.091	-0.156	-0.024	1.00		
Current daily milk yield	0.184	0.052	-0.102	0.057	0.322	-0.120	-0.123	1.00	
Stall hygiene	0.088	-0.272	0.112	0.153	-0.031	-0.110	0.034	-0.159	1.00

There was considerable negative correlation (-0.37) between alleyway hygiene and stall hygiene, indicating that clean stalls were sometimes observed in sheds with dirty alleyways, and vice versa, Table 5.

Discussion

This study is the first to simultaneously explore and demonstrate the relationship between cow comfort and mastitis control parameters and occurrence of SCM in SDF in developing countries. Cow-level (43.2%) and quarter-level (21.9%) SCM prevalences were lower than reported in previous studies in Kenya. Others (Mureithi & Njuguna, 2016) found cow-level prevalence of 64% and quarter-level prevalence of 55.8%. Cow-level prevalences of 56% and 65% were found in two other districts in Kenya (Bundi *et al.*, 2014). Our lower prevalence could be seasonal variation (the sampling time was at the end of the dry season), or it could be attributed to the farmer assistance and education program offered by Farmers Helping Farmers.

The proportion of farms with a partitioned stall (87.3%) is consistent with other studies conducted in Kenya (Aleri *et al.*, 2012; Kathambi *et al.*, 2019) which found 83% and 87.4%. The proportion of stalls reported as dirty (33.1%) and use of bedding in the stalls (68.4%) was consistent with other findings from studies done previously in the same region (Kathambi *et al.*, 2019), which found 35% dirty stalls and 72% of farms using bedding on the stall floor. Our study found that cows that were lying down in stalls with poor hygiene were associated with more mastitis, an important association. We postulated an association between bedding and injuries and stall hygiene. This study reported low prevalence of injuries; therefore, we didn't model factors associated with injuries. Theoretically, stalls with good dry bedding would likely have good stall hygiene and fewer hock and knee injuries.

For the interaction between alleyway hygiene and current daily milk yield, at low milk production, there was no difference in the odds of SCM by alleyway hygiene score; however, at higher milk production levels, the odds of SCM were lower with a clean alleyway score. This finding demonstrates the importance of a clean-living environment for dairy cows, not just the stall. Cows will carry manure and mud on their feet from the alleyway into their stalls if the alleyway is left uncleaned, leading to udder exposure to manure and mud in the stall.

The negative correlation observed between alleyway and stall hygiene levels could be explained by farmers not cleaning the alleyway as often as the stall, or not cleaning the stall as often as the alleyway. This negative correlation could also suggest that some cows were not using their stalls and preferentially lying down in the alleyway due to a dirty or lumpy stall or a stall with inappropriate dimensions for the various rails used for the stall (e.g., short stall length). Cows not lying down in the stall means that they don't pass manure or urine in the stall, so the stall remains clean. Cows lying down on a dirty alleyway have been found to be more likely to have higher incidence of mastitis (Kathambi *et al.*, 2019; Kerro & Tareke, 2003; Lakew *et al.*, 2009; Mungube *et al.*, 2005) compared to those lying down in a clean stall. This result also greatly underpins the important interplay between proper stall design and management, potential animal welfare indicators and various mastitis control protocols in the effective management of udder infections in these farms.

The substantially more neck injuries observed in the study was noted to be contributed not only from the neck rail in the stall but also from the poles on top of the feed bunk preventing cows from entering it. Design of the feed bunk was noted to be an important contributor of injuries when the poles were placed too low and rubbing on the cow's neck. Therefore, feed bunk design should be given due consideration while designing zero-grazing units. Injuries on other body regions and lameness were minimal and substantially less than what was reported in previous studies (Aleri *et al.*, 2011; Aleri *et al.*, 2012). This disparity could be attributed to difference in scoring used in the two studies or due to actual differences in prevalence of injuries.

Our study found the average daily milk yield (10.7 kg/day) to be substantially higher than in a study conducted in the same locality previously (Kathambi *et al.*, 2018) which reported 6.6 kg/day, and slightly higher than the 9.3 kg/day reported in a study done in the Mukurweini district of Kenya (Richards *et al*., 2019). The latter study recruited recently calved cows, which was similar to our study population. Conversely, the former study recruited at the herd-level, and they were not specific about recruiting cows in a specific lactation stage (Kathambi *et al*., 2019), while we recruited cows in their first 2 months post-calving, which is associated with a peak in milk production. Also, the difference in milk production between our study and the former study could be attributed to a continuing education program working with dairy farmers in the region to equip them with knowledge on better husbandry, feeding and breeding protocols.

Increasing parity was associated with decreasing odds of having mastitis. This was contrary to studies conducted elsewhere (Islam, Rahman *et al*., 2012; Islam, Islam *et al*., 2012; Joshi & Gokhale, 2006; Kerro & Tareke, 2003; Nibret & Tekle, 2012). An increase of mastitis incidence with parity was attributed to an increased immunologic reaction of teats to infections and increased degree and frequency of previous exposure (Lakew *et al*., 2009). Our conflicting findings could have been driven by other factors, such as level of mastitis management and control measures being applied on different farms. Farms that were implementing more mastitis control protocols are likely to be more informed about general husbandry; and thus, possibly taking better care of their animals. Consequently, they are likely to keep their cows longer compared to farms with mastitis problems, among other production-related challenges, which may have led to culling their mastitic cows at an earlier age. Previous cultures of mastitis in Kenya have shown substantial infections with *S. aureus*, which is often refractory to treatment, making culling an option to consider (Bundi *et al*., 2014).

We expected to find significantly fewer cases of SCM in cows that were using dry cow therapy and teat dip disinfectant. However, the farms that applied these protocols were few, and so we could not detect significant associations to these factors. The low use of these management tools can be attributed to low knowledge levels, since a majority of the farmers that were not using these products reported that they were not aware that such products existed.

Regarding study limitations, some measures, such as stall and alleyway hygiene, were done subjectively by the principal investigator and an assistant. The study utilized a scoring system described by Reneau (Reneau *et al*., 2005) which outlined a scoring system for cow body hygiene but modified the scoring to score stalls and alleys, although there were no particular cut points to reference. The validity of this scoring system was enhanced by regular cross-checking between the principal investigator and the assistant to ensure consistency in the assessments.

Being cross-sectional in nature, results from the study are not reliable to make a causal inference about the outcome from the predictors, as there is no element of temporality between the two. The model predictor variables were assessed at the same time as the outcome, making it impossible to confirm that they existed prior to the outcome. The study was also prone to recall bias, with farms that had their cows treated for mastitis previously likely being more conversant with routine mastitis control protocols that were advised by the veterinarian that treated their cows. Since most of the farmers had low-to-moderate knowledge levels in mastitis control, such farmers with a previous encounter with mastitis could still be practicing most of the protocols advised by the veterinarian, compared to farmers that had not had a cow treated for mastitis on their farms.

Since a harmonized scoring system for overall cow comfort does not exist, we used different components of cow comfort to build the model. As such it was not possible to precisely assess the relationship between overall comfort of each cow and subclinical mastitis. There is need for a harmonized scoring system for cow comfort to be used as a standard in studies such as this one that seeks to relate cow comfort to other factors impacting on the wellbeing of dairy cows. Lessons can be borrowed from the body condition scoring of dairy cows (Wildman *et al*., 1982) which utilizes the status of different regions of the body of the cow. It would be of great benefit if a cohort or randomized controlled trial could be conducted to validate the outcomes of this study and establish a causal relationship between the factors incriminated in the occurrence of SCM in this study. For example, there is need to further explore the relationship between stall hygiene, alleyway hygiene and occurrence of SCM on SDF in Kenya, and the different factors that may influence these relationships.

Conclusion

Subclinical mastitis remains highly prevalent among Kenyan SDFs. There is relatively low uptake of some important routine mastitis control measures. Poor hygiene of the alleyway and stall were important factors associated with SCM and are highly dependent on stall design and management. Low knowledge levels appear to account for much of why these recommended practices have been poorly adopted, therefore more education on best management practices around cow housing and routine mastitis management protocols is needed.

Authors' contributions

EKN, JAV and GGK contributed to the study design, data collection, data analysis and writing of this paper. LK, GK and SM contributed to the study design, data analysis and writing of the manuscript. EKK contributed to data collection and analysis.

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