



Research article

The prevalence and factors associated with mastitis in dairy cows kept by small-scale farmers in Dodoma, Tanzania

Rosemary Peter Mramba^{*}, Mustafa Atway Mohamed

Department of Biology, The University of Dodoma, P. O. Box 338, Dodoma, Tanzania

ARTICLE INFO

Keywords:

Dairy cattle
Bovine mastitis
Milk
Small-scale farmers
And Dodoma

ABSTRACT

Bovine mastitis is among the major diseases of economic importance in the dairy industry worldwide. Hygienic conditions during milking and housing are important determinants of mastitis infections. However, arid and semi-arid areas have a scarcity of water, which is an essential component of cleanliness. Thus, the study determined the prevalence of mastitis and associated factors in Dodoma, a semi-arid region in Tanzania. Three hundred and sixty-eight lactating cows were selected randomly from 114 farms in the Dodoma urban district for mastitis determination based on clinical signs and the California Mastitis Test. About 59.8 % of the cows had mastitis, and the subclinical type dominated. Factors associated with mastitis prevalence were cleanliness (hand and udder washing before milking), farmers' awareness of mastitis, the cow's parity and lactation stage, and herd size. Milk yield was not associated with the mastitis status of the cow but was related to the breed of the cow, whether warm or cold water was used to wash the cow's udder, parity, and lactation stage. Improvement in education on the importance of hygiene is necessary, as 38.6 % of the farmers were unaware of mastitis. Further detailed studies on microbiology, such as bacteriologic culture and polymerase chain reaction, are recommended to formulate interventions.

1. Introduction

Mastitis is one of the leading causes of global economic losses in the dairy industry due to its effect on milk yield and quality [1,2]. Bovine mastitis is an inflammatory disease of the mammary gland of cows caused by a variety of pathogens and physical trauma [3]. Mastitis is caused by a wide range of bacteria, but the most important ones are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, and *Streptococcus uberis* [4–7]. Mastitis pathogens can be detected through culture methods, where milk samples are incubated on culture plates to promote bacterial growth, followed by the analysis of the bacterial colonies formed to identify the causative agent [8,9]. Additionally, a molecular diagnostic test using polymerase chain reaction is highly sensitive and specific, providing accurate identification of pathogens [8,9]. On the other hand, methods such as physical examination of the udder and milk, the California mastitis test, and somatic cell count can indicate the presence of mastitis but do not indicate the causative agent [10,11].

Based on the degree of inflammation, mastitis can be classified as clinical or subclinical [3]. Clinical mastitis is easily identified by visible abnormalities such as blood-stained milk, a red and swollen udder, and fever in the cow [12]. On the other hand, subclinical mastitis does not cause any obvious changes to the udder or milk, thus making it difficult to be recognised by farmers [1]. Economic losses due to mastitis are related to reductions in milk production by affected cows, milk discard from cows with clinical mastitis, and

^{*} Corresponding author.

E-mail addresses: mramba2008@yahoo.com, rosemary.peter@udom.ac.tz (R.P. Mramba).

medical costs [1,3]. Mastitis also affects milk quality, making it unsuitable for human consumption and processing [13,14]. Milk from a cow infected with mastitis poses health concerns for consumers [15,16]. Milk from infected cows may carry potentially harmful bacteria such as *Staphylococcus aureus*, *Streptococcus agalactiae*, or *Escherichia coli* [17,18]. The bacterial contamination increases the risk of foodborne illness if the milk is consumed without proper pasteurisation. *Staphylococcus aureus* is a major food-borne pathogen and one of the primary causes of mastitis in cattle [17]. *Staphylococcus aureus* is associated with subclinical mastitis, making it more likely to contaminate milk and other dairy products [16,17]. In addition, the use of antibiotics to treat mastitis can contribute to antibiotic resistance and antibiotic residue, which pose a public health risk [19,20].

The bacteria causing mastitis are found in the tissues of the cow's udder, where they can spread from one cow to another during milking (contagious pathogens) [2,3,21]. The bacteria are also found in the surroundings, such as walls, bedding, and the floor [2,21,22]. Based on these factors, management practices, especially those related to hygiene, are expected to influence the onset of mastitis in a cow herd. As the majority of infections occur during milking [2,5,23], hygienic practices during milking have a great role in controlling mastitis. Proper cleaning of the milking barn and milking equipment, washing hands before milking, cleaning and drying of the cow udder and teats, use of separate cleaning towels for each cow, and teat dipping are some of the basic hygienic measures to control mastitis [4,24,25]. It has been shown that a combination of pre-milking and post-milking teat dipping is more effective than post-milking teat dipping alone in combating mastitis pathogens [26,27]. Disinfectants such as iodophor solution, iodine-based gel, chlorhexidine, quaternary ammonium compounds, chlorine, and alcohol reduce contamination of the milk by bacteria from the environment and risk of new infections [28–30]. Large herd size increases the risk of mastitis because it increases the spread of pathogens from one cow to another during milking and the likelihood of injuries [2,31–33]. As a result, proper housing and stocking density are important in combating mastitis.

It has been demonstrated that animal characteristics like breed, age, parity, lactation stage, and milk output of a cow are related to the prevalence of mastitis [2,31]. Subclinical mastitis is more common in older lactating animals and at the end of lactation than at the beginning of lactation [31]. The structure of the udder and teat canals changes with age and parity because of frequent milking, increasing susceptibility to infections [3]. Furthermore, cow immunity declines with age, and continuous treatment may cause the bacteria to develop antibiotic resistance [34]. Milk yield increases with parity, and a high milk yield is positively correlated with the prevalence of mastitis [35]. Early lactation is characterised by immunosuppression caused by stress, increasing the cow's susceptibility to infections [36,37]. Immunosuppression can also be caused by nutritional stress, which happens when lactating animals do not receive enough nutrients to meet the demand for milk production [38]. Furthermore, purebred and high-yielding cows are more vulnerable to mastitis infections [36].

Tanzania has Africa's fourth-largest cattle population, with 96.5 % of the cattle being indigenous breeds grazing on natural pastures under communal grazing lands [39]. Improved breeds (crossbreeds) account for 3.5 % of the country's cattle population and are often kept in urban and peri-urban areas under intensive and semi-intensive systems [39]. Approximately 90 % of the milk in Tanzania is produced by cattle. The majority of the milk is produced in a low-input cow-meat-milk system where indigenous breeds of cattle are kept, and both milk and meat are important products [40,41]. The demand for milk and milk products in Tanzania is higher than the supply, and it is predicted to increase further in the future due to a high population, raised incomes, and urbanisation [40]. Improvements in the reproduction, feeding, marketing, and processing of milk, as well as animal health interventions, are among the government's strategies for increasing milk production [40,41]. However, successful interventions should consider regional circumstances because small-scale farmers dominate the industry [42].

Although mastitis is regarded as one of the major diseases of economic importance in the dairy industry worldwide, it is given low priority in Tanzania [43]. Both clinical and subclinical mastitis are common problems in the dairy industry in Tanzania, especially in the small-scale sector [44,45]. The majority of small-scale farmers lack knowledge of proper management practices [44,46]. In addition, about 95 % of the milk produced is sold through informal markets with little quality control, which could detect an increase in subclinical mastitis prevalence. Understanding the risk factors associated with the prevalence of mastitis and the farmer's knowledge and awareness of mastitis is important in mitigating the problem. Thus, the purpose of the study was to assess the prevalence of mastitis among small-scale dairy farmers in the Dodoma district in relation to management practices and animal factors.

2. Materials and methods

2.1. Study area

The Dodoma region is located in central Tanzania. Its climate is primarily semi-arid, with a single rainy season between November and April. Annual rainfall ranges from 500 mm to 800 mm, with significant geographical, seasonal, and annual fluctuations [47]. Temperatures range from about 15 °C in July to 30 °C in October [47]. Livestock production and farming are the main economic activities of the native Gogo tribe of the region, where cattle are the dominant livestock species [48,49]. The Gogo are pastoralists who keep the local breeds of cattle in rural areas [50]. The cattle are primarily kept for milk and meat production under the low-input grazing system. Due to seasonal variations in rainfall, which restrict feed availability, milk production is largely for subsistence consumption [51]. According to the 2004 livestock census, local breeds of cattle account for 99.4 % of the cattle population in the region, which is primarily found in the Dodoma rural district, while dairy cattle are mainly kept in urban and peri-urban areas.

2.2. Study design and sampling

A cross-sectional study was conducted in March and April 2022 in the Dodoma urban district to determine the prevalence of

mastitis and associated factors. The study's population consisted of lactating dairy cows in the Dodoma urban district. The Dodoma urban district was selected among the seven districts of the Dodoma region because it contains a large number of dairy cattle farmers. Based on the information from district livestock officers on the availability of dairy farms, we purposefully selected seven wards for this study, namely Msalato, Iyumbu, Miyuji, Dodoma Makulu, Nzuguni, Nkuhungu, and Mkonze (Fig. 1). The sample of cows was determined by using formula expressed in equation (1):

$$N = \frac{1.96^2 \times Pexp(1 - Pexp)}{d^2} \quad (1)$$

where Pexp = expected prevalence.

d = desired absolute precision.

N = the total sample size.

Based on the previous studies in the region [32,40], we expected the prevalence of mastitis to be 60 %. With a CI of 95 % and 5 % absolute precision, the computed sample size for the study was 368 lactating cows. The proportional allocation method was used to determine the number of cows required in each ward. A simple random sampling method was used to obtain the required farms. Each farm was assigned a unique number by using a random number generator to select numbers corresponding to individuals for inclusion in the sample. We expected to sample 20 % of lactating cows on each farm to get the required number of cattle, but the majority of the farms had 2-5 lactating cows, and the maximum number was 8 lactating cows per farm. Thus, we randomly sampled 50 % of lactating cows on each farm for mastitis tests. A simple random sampling method was also used to obtain the cows from each farm using the lottery method. To get the required number of lactating cows, 114 farms were visited.

2.3. Diagnosis with mastitis

Mastitis was determined based on clinical signs and the California Mastitis Test (CMT). Clinical mastitis was identified based on clinical signs of mastitis, such as udder abnormalities including swelling, hardness, heat, redness, or pain, and abnormalities in the appearance of the milk produced, such as watery appearance, clots, flakes, and blood stains. Sub-clinical mastitis was defined as any positive CMT from cows without the clinical signs of mastitis. The cow's udder was observed for any abnormalities in size and shape and for blind teats. A careful palpation of the udder was done to identify signs of inflammation, swelling, and hardness. The udder and teats were then thoroughly cleaned with water, sprayed with an iodine-based disinfectant, and dried with a clean towel. Milk samples from each mammary quarter were drawn into a clean cup after the first milk was removed. The appearance of the milk sample was observed to determine whether it appeared watery or contained clots, flakes, and blood.

Milk samples were collected where the sampled cow didn't show signs of clinical mastitis to determine whether they were positive for subclinical mastitis by using CMT. Squirts of milk containing approximately 2 mL were drawn from each teat into a separate compartment of the CMT. An equal amount of Kepro CMT solution was added to each compartment of the CMT paddle. The reagent and the milk sample were mixed by gently rotating the paddle in a horizontal plane, swirling the mixture for 15 s. The mixture was observed, graded, and interpreted as shown in Table 1.

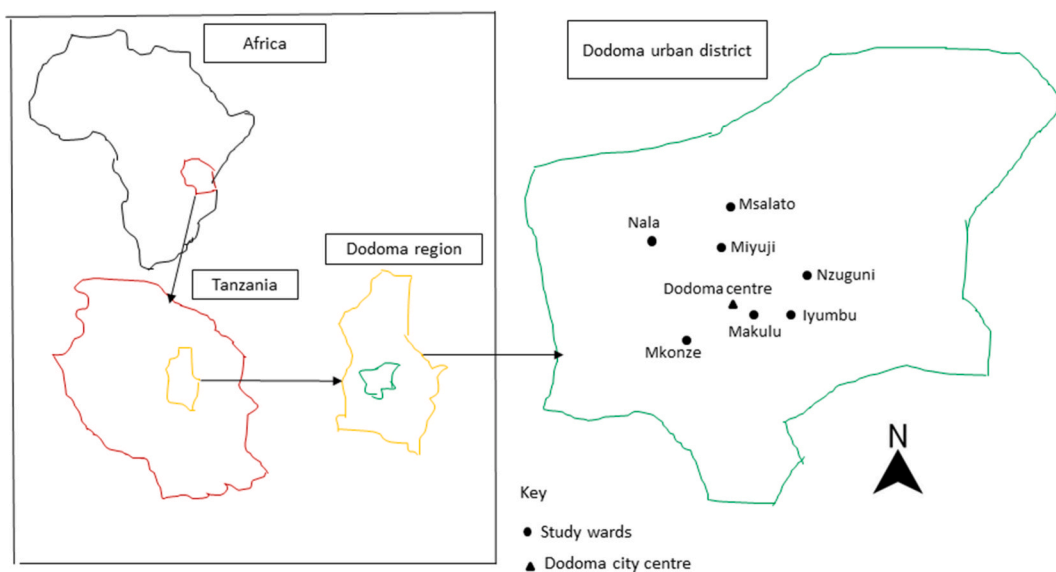


Fig. 1. Map of the study area showing the study wards.

2.4. Interview using a questionnaire and a check list

Structured questionnaires were used to collect information about milking practices, housing, cleanliness, and cattle information such as breed, milk yield, parity, and lactation stage. The questionnaire also gathered information about social-demographic aspects of the households, the history of cow illness, and drug use. A visual observation was made to gather information about the condition of the cowshed. The study was carried out in accordance with relevant guidelines and regulations. The study sought informed consent from all participants, and personal information was disclosed. The University of Dodoma issued an ethical approval for conducting the study with reference number MA.84/261/02.

2.5. Data analysis

The data was initially stored in a Microsoft Excel spreadsheet for viewing and cleaning. Then, the data were coded in R version 4.2.0 for further analysis. Logistic regression was used to determine the associations between the likelihood of mastitis occurrence and animal- and farm-level factors. The animal-level factors included breed, parity, lactation length, milk yield, and mastitis history, while farm-level factors were herd size, sanitation practices, and mastitis awareness. Animal factors such as milk yield, parity, and lactation stage are interrelated. For example, high milk yield is linked with a high prevalence of mastitis, while milk yield increases with parity and lactation stage. However, these relationships are not always linear, and milk yield peaks at a certain point in the lactation period and parity. Thus, we also expect the prevalence of mastitis to follow a similar trend. Based on this assumption, we used a logistic generalised additive model (GAM) to determine the relationship between positive and negative mastitis tests and animal and management factors. Initially, we tested each independent variable separately, then fitted a multivariable model to understand the relationships among variables. GAM models are flexible and use smooth functions to fit response variables into explanatory variables for both continuous numeric and binary outcomes [52,53]. Furthermore, we incorporated random effects that model independence among observations from the same site by using generalised additive mixed-effect models (GAMM) [54].

Factors associated with milk yield were also determined. Normality tests for the independent variable (milk yield) were done using histograms and the Shapiro–Wilk Test, while the relationship between milk yield and the independent variables was visualised using scatter graphs. The tests showed that the data was not normally distributed, and scatter graphs showed that the associations between milk yield and the independent numerical variables were not linear. Multi-collinearity between numerical variables was determined by using the variance inflation factor (vif). The data did not fit a linear model, so we opted for a non-linear model. Thus, we fitted a GAMM model to determine the associations between milk yield and animal and farm factors as independent variables and farm ID as a random variable.

The models were evaluated by using the `gam.check` function in R. The functions run diagnostic tests to determine whether the basis-dimension choices are adequate. The diagnostic information produced is four residual plots, the degree of freedom (edf), the k-index, and the p-values for each variable. A low p-value indicates that the k-index is too low (edf is close to k). We did not have significantly low p-values, indicating that the basis dimension choices were adequate.

3. Results

3.1. Characteristics of the households

About 46 % of the respondents were female. The respondents' ages ranged from 22 to 60 years old. Regarding education, the respondents had primary education (17.5 %), secondary education (38.6 %), and college education (43.9 %). Household size ranged from 2 to 8 people. The farmers kept Ayrshire crossbred (40.4 %) and Friesian crossbred (59.6 %) cows. The majority of the farmers (82 %) did not use bedding material on the cow's pen. Manure was washed away every morning or twice per week. The number of lactating cows per household ranged from 1 to 8 cows, with 25 % of the farms having 3 cows or fewer, 50 % having 4 cows or less, and 75 % of the farmers having 5 cows or fewer. Milking was done twice per day, in the morning and in the evening, and all farmers practiced hand milking. Milk yield per cow ranged from 3 to 20 L per day, with 25 % of cows producing 5 L or less, 50 % of the cows producing 8 L or less, and 75 % of the cows producing 10 L or less.

3.2. Management practices implemented by the households

All the households visited used the indoor system of cattle production. All cowsheds had a concrete floor, and none of the farmers

Table 1
Scoring of California mastitis test results and the number of cattle in each score.

Milk sample appearance on the CMT	Crosses	Interpretation	Number of cattle
The mixture remained liquid, homogeneous, and without signs of clotting	0	Negative	148
A slight thickening of the mixture at the bottom of the cup	+-	Trace reaction	42
The mixture formed a distinct thickening but did not form a gel	+	Weak positive	33
The mixture thickens immediately and breaks into small masses	++	Distinct positive	51
The mixture formed a gelatinous mass that clung together	+++	Strong positive	94

used bedding materials on the floor of the cowshed. Milking was done two times a day, in the morning and in the evening, by all farmers, and hand milking was a dominant practice. The majority of the farmers cleaned the cowshed every day (Table 2). All the farmers said they wash the cow's teats before milking by using either warm water or cold water (Table 2). However, only 22.8 % washed their hands before milking. The majority of the respondents were aware of mastitis and had experienced it. Further details are shown in Table 2.

3.3. Factors associated with the prevalence of mastitis

The overall prevalence of mastitis was 59.8 %, with the prevalence of clinical mastitis accounting for 24.2 % and subclinical mastitis accounting for 75.8 %. Several management practices and animal factors were associated with mastitis prevalence (Table 3). Mastitis prevalence decreased when warm water was used to wash the cow's udder (Fig. 2a) and when farmers washed their hands with soap before milking (Fig. 2b). In addition, mastitis prevalence tended to decrease when farmers were aware of the disease (Table 3). Animal factors associated with mastitis prevalence included parity, stage of lactation, and herd size (Table 3). Mastitis prevalence increased with parity (Fig. 3a) and decreased with lactation length (Fig. 3b). Conversely, mastitis prevalence increased with the number of lactating cows on the farm until approximately 4 cows, after which it decreased (Fig. 3c).

3.4. Factors associated with milk yield

Both management and animal factors were associated with milk yield (Table 4). There was a positive association between milk yield and Holstein-Friesian breed, the use of warm water to wash cows' teats during milking, and a negative association with the introduction of a calf during milking to stimulate milk let-down (Table 4). The milk yield increased with parity until the third calving, then decreased thereafter (Fig. 4a). In addition, the milk yield decreased with lactation length (Fig. 4b). The mastitis status of a cow did not have a significant association with milk yield (Table 4).

4. Discussion

The study was conducted in the Dodoma urban district to determine the prevalence of mastitis in dairy cows kept by small-scale farmers and its association with management practices and animal factors. The results show that the prevalence of mastitis was 59.8 %, with subclinical mastitis constituting 75.8 %. The prevalence was comparable to related studies in Ethiopia [24,55]. Other studies reported higher prevalence, e.g., 74.5 % [44], 74.7 % [2], 80 % [56], and 70.8 % [45]. The high prevalence in this study has been associated with poor management practices, especially those related to hygiene. A study by Mdegela et al. [44] in the same area found a prevalence of 61.2 % and was associated with poor milking practices, poor hygiene of the cow shed, and disease control. Cleaning the concrete floor of a cowshed and milking area requires the use of water, which is scarce in arid and semi-arid regions. For example, a study in Zanzibar by Suleiman et al. [57] found an association between the prevalence of mastitis and the availability of water. In addition, the availability of bedding materials in urban areas is limited. As a result, all farmers did not use bedding material on the

Table 2
Different management practised implemented by the farmers.

Variable	Parameter	Frequency	Percentage
Cleaning frequency per week (n = 114)	Everyday	72	63.2
	Two times	22	19.3
	Three times	20	17.5
Condition of the cow shed (n = 114)	Clean and dry	60	52.6
	Dirty	54	47.4
Washing hands before milking (n = 114)	Yes	26	22.8
	No	88	77.2
Teat washing (n = 114)	Yes with warm water	60	52.6
	Yes with cold water	50	47.4
Drying teats (n = 114)	Yes	54	47.4
	No	60	52.6
Introduce a calf during milking (n = 114)	Yes	20	17.5
	No	94	82.5
Use lubricants during milking (n = 114)	Yes	96	84.2
	No	18	15.8
Mastitis awareness (n = 114)	Yes	70	61.4
	No	44	38.6
Experienced mastitis before? (n = 114)	Yes	54	47.4
	No	60	52.6
Mastitis test (n = 368)	Positive	220	59.8
	Negative	148	40.1
Type of mastitis (n = 368)	Sub-clinical	279	75.8
	Clinical	89	24.2
Milk appearance (n = 368)	Clear white	323	87.7
	Blood stained	45	12.3

Table 3

Multivariable logistic regression analysis of the associations between the likelihood of positive mastitis and different factors.

Parametric coefficients							
Response variable	Predictors	Levels of predictors	Estimate	Std. error	z-value	p-value	
Mastitis status (positive = 1, negative = 0)	Intercept		1.915	0.960	1.993	0.046	
	Breed	Ayrshire	0				
		Holstein Friesian	-0.349	0.602	-0.579	0.562	
	Hand washing before milking	No	0				
		Yes	-1.636	0.822	-1.989	0.046	
	Teat washing	With cold water	0				
		With warm water	-1.934	0.594	-2.141	0.011	
	Teat lubricant	No	0				
		Yes	0.296	0.083	0.354	0.723	
	Cleaning frequency	Every day	0				
		2 times week ⁻¹	1.102	0.930	1.186	0.235	
		4 times week ⁻¹	0.756	-0.950	-1.257	0.208	
	Mastitis awareness	No	0				
		Yes	-0.946	0.695	-1.462	0.043	
	Milk yield		0.155	0.112	1.378	0.168	
	Approximate significance of smooth terms			edf	Ref. df	Chi.sq	p-value
		Parity		1.701	2.156	7.774	0.025
		Lactation length (mos)		1.863	1.979	8.878	0.012
		Herd size		1.909	1.991	11.179	0.005

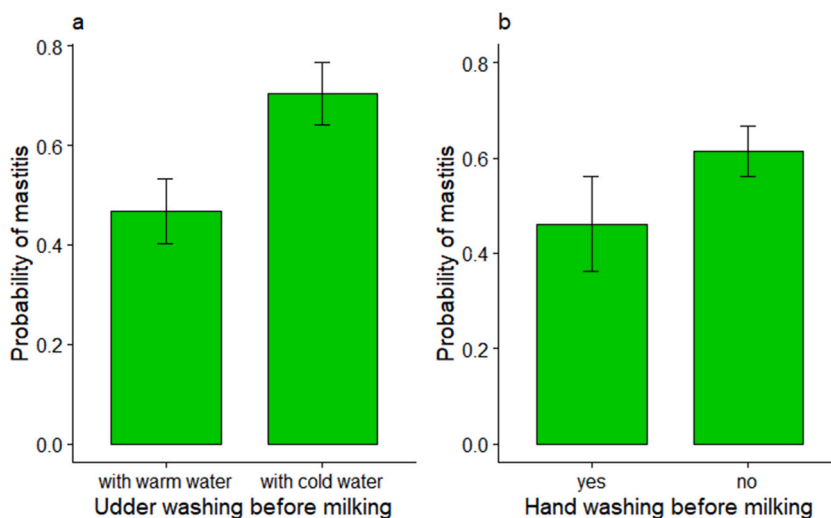


Fig. 2. The relationship between the likelihood of a positive mastitis test and whether (a) cold or warm water was used for washing the cow teats and (b) the farmer washed hands before milking. The error bars indicate the standard deviation of the mean.

cowshed, so cows stayed in dirty areas when the house was not cleaned every day.

A recent rise in human population and urbanisation has increased milk demand and dairy cattle farming under the zero-grazing system in the Dodoma urban district [47]. However, the arid nature and short growing season of the region limit the supply of forage and water needed for sanitation, increasing the risk of mastitis [58]. The Dodoma region has potential for improvement in dairy cattle production because of the presence of key livestock actors such as the Tanzania Livestock Research Institute, the Kongwa Ranch, and the Mpwapwa Livestock Training Agency. The government has set aside an area for the construction of a milk processing industry in the Dodoma urban district and milk collection centres in all other districts, which will control milk quality [47]. Thus, training farmers on proper management and hygienic milking practices is necessary to avoid losses through milk discard.

Some of the management practices were associated with the prevalence of mastitis. Cleaning hands before milking and using warm water to wash the cow's teats during milking were associated with a lower prevalence of mastitis. Hand washing is an important hygienic practice during milking, as the hands of the milker can carry pathogens to the teats and between cows [55,59]. About 77 % of farmers in this study said they don't wash their hands before milking. This could be due to a lack of understanding about the significance of washing hands before milking.

More than half of the farmers used warm water to wash the cow's teat during milking. The use of warm water appears to be a common practice among small-scale dairy farmers. For example, a study by Tekliye and Gizaw [60] in Ethiopia and Karimuribo et al.

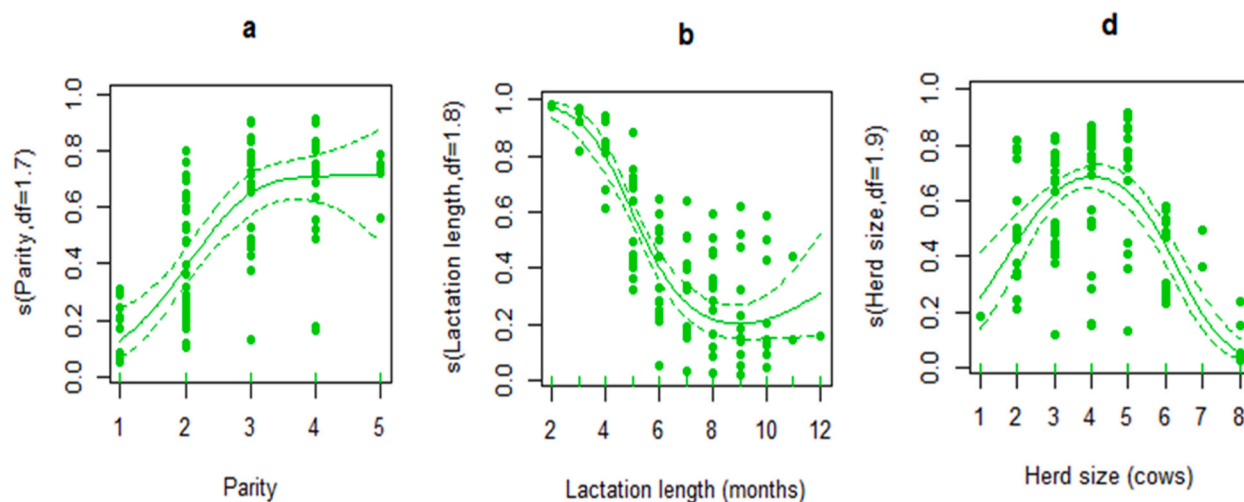


Fig. 3. The relationships between the likelihood of a positive mastitis test and (a) parity, (b) lactation length, and (c) herd size.

Table 4

Parameter estimates for the generalised additive model between milk yield and a set of explanatory variables.

Parametric coefficients							
Response variable	Predictors	Levels of predictors	Estimate	Std. error	t-value	p-value	
Milk yield (l)	Intercept		7.470	0.701	10.665	<0.001	
	Breed	Ayrshire	0				
		Holstein Friesian	1.813	0.578	2.653	0.028	
	Mastitis status	Sub-clinical	0				
		Clinical	-0.848	0.550	-0.965	0.142	
	Previous experience of mastitis	Negative	0.375	0.694	0.540	0.590	
		No	0				
	Teat washing	Yes	0.2093	0.548	0.381	0.703	
		Cold water	0				
	Introduce a calf during milking	Warm water	1.307	0.571	2.287	0.024	
		No	0				
	Approximate significance of smooth terms	Yes	-1.772	0.755	-2.346	0.021	
		No	0				
	Variables			edf	Ref.df	F-test	p-value
		Parity		1.952	1.997	7.565	0.001
		Lactation length (mos)		2.606	3.124	26.062	<0.001

[61] in Tanzania found that about 66 % and 86 % of farmers, respectively, used warm water to wash cows' teats. It is not clear what motivates the farmers to use warm water, yet milk yield was higher where cow teats were washed with warm water (Table 3). This could imply that the warm water facilitated milk letdown by the cow. Incomplete milking is associated with a high prevalence of clinical mastitis [62,63]. Apart from removing dirt and pathogens, stimulation through wiping or washing the cow's teat increases milk letdown by aiding the release of the oxytocin hormone [62–64]. Warm water may feel more comfortable to the animal compared to cold water. However, Dzidic et al. [65] found that both cold and warm water were effective in inducing milk ejection in cows. The stimulation provided by touch is more important in machine milking.

The prevalence of mastitis increased with the parity of the cow and decreased with lactation length. These results are supported by numerous previous studies, such as Zhang et al. [66], Sinha et al. [35], Lean et al. [67], etc. Milk yield, parity, and lactation stage are interrelated and influence overall udder health [68,69]. In this study, milk yield increased with parity, reaching maximum yield in the third lactation, while it decreased with lactation length. Parity influences the size of the udder and changes in teat structure through milk production [69–71]. In addition, teat trauma increases with parity [67]. Large teat canals and impaired teat sphincter function cause milk leakage, increasing the risk of infection [71]. Furthermore, body condition loss and metabolic disorders increase with parity, reducing the animal's ability to resist infections as well as milk production [72]. On the other hand, the prevalence of mastitis increased with herd sizes up to 5 cows per household, then decreased thereafter. The reason for this observation is not clearly understood, as other studies have shown a linear increase in mastitis prevalence with herd size because of the transmission of pathogens between cows [33,73]. However, the maximum herd size per household in this study was 8 cows. Having many cows means more investment; thus, farmers may be more careful with hygiene and other management practices, decreasing mastitis prevalence in the largest herds.

The presence of a calf during milking has been linked with higher milk yields through the stimulation of the cow to release milk [74,

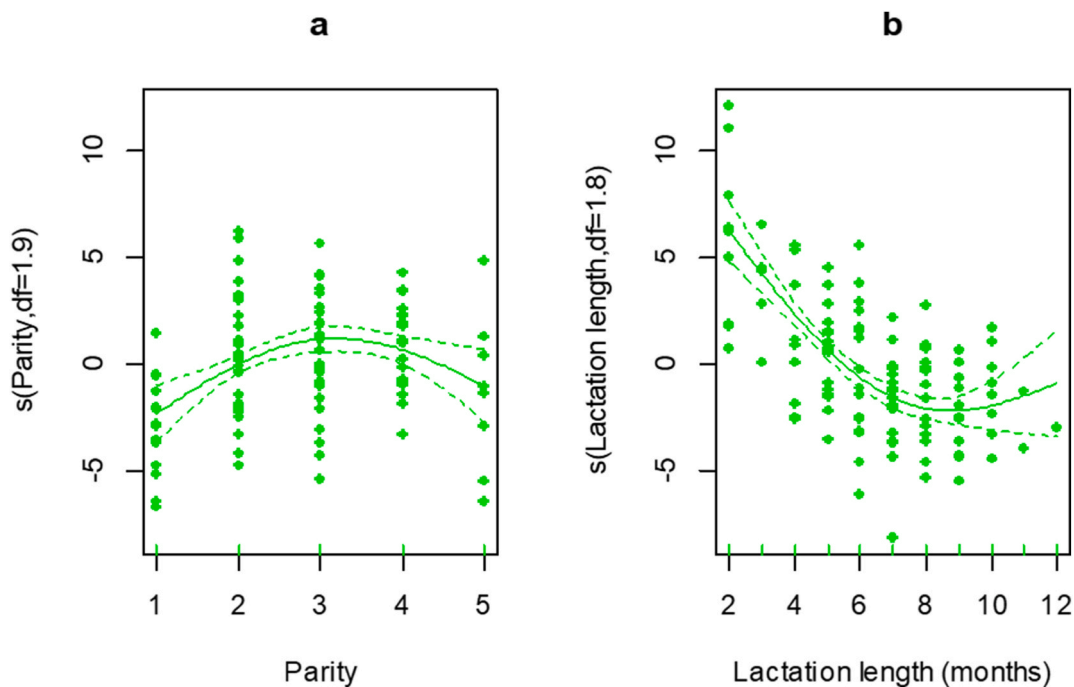


Fig. 4. The associations between milk yield and (a) parity and (b) lactation length.

75]. This may involve a visual presence or allowing the calf to suckle for a short time before milking [74,75]. In this study, 17.5 % of farmers allowed the calf to suckle before milking. However, these farmers reported a lower milk yield, probably because the calves sucked up a large amount of milk or the cows were disturbed when their young were removed. The study did not consider a control group of non-exposed cattle in sample size determination. Thus, this might have affected the interpretation of the risk factors associated with mastitis.

5. Conclusion

The study shows that mastitis, especially the subclinical type, is a prevalent health problem affecting small-scale dairy production in Dodoma. Hygiene is one of the factors associated with the prevalence of mastitis. Because water is scarce in the study area, the use of bedding materials is important to keep the cowshed clean and dry. Increasing awareness among farmers through extension services will improve management practices and reduce mastitis prevalence. Since the study was based only on clinical signs and CTM, we cannot tell which pathogen caused the infections, whether they were from the environment or the cow udder.

Thus, further studies on microbiology, such as bacteriologic culture and polymerase chain reaction, are recommended in order to develop control measures.

Funding

This study did not receive grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

CRedit authorship contribution statement

Rosemary Peter Mramba: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Mustafa Atway Mohamed:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the staff at the Tanzania Veterinary Laboratory Agency in Dodoma for their assistance and guidance. We thank the livestock officers, local leaders, and the participating households in the Dodoma urban district. We are grateful for the support from the University of Dodoma.

References

- [1] P. Guzmán-Luna, R. Nag, I. Martínez, M. Mauricio-Iglesias, A. Hospido, E. Cummins, Quantifying current and future raw milk losses due to bovine mastitis in European dairy farms under climate change scenarios, *Sci. Total Environ.* 833 (2022) 155149, <https://doi.org/10.1016/j.scitotenv.2022.155149>.
- [2] R. Abebe, H. Hatiya, M. Abera, B. Megersa, K. Asmare, Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia, *BMC Vet. Res.* 12 (1) (2016) 1–11, <https://doi.org/10.1186/s12917-016-0905-3>.
- [3] W.N. Cheng, S.G. Han, Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments—a review, *Asian Australas. J. Anim. Sci.* 33 (11) (2020) 1699–1713, <https://doi.org/10.5713/ajas.20.0156>.
- [4] F. Zigo, M. Vasil, S. Ondrašovičová, J. Vyrostkova, J. Bujok, E. Pecka-Kielb, Maintaining optimal mammary gland health and prevention of mastitis, *Front. Vet. Sci.* 8 (2021) 607311, <https://doi.org/10.3389/fvets.2021.607311>.
- [5] M. Cobirka, V. Tancin, P. Slama, Epidemiology and classification of mastitis, *Animals* 10 (12) (2020) 2212, <https://doi.org/10.3390/ani10122212>.
- [6] P. Slama, Z. Sladek, D. Rysanek, T. Langrova, Effect of *Staphylococcus aureus* and *Streptococcus uberis* on apoptosis of bovine mammary gland lymphocytes, *Res. Vet. Sci.* 87 (2) (2009) 233–238, <https://doi.org/10.1016/j.rvsc.2009.03.005>.
- [7] A. Girma, D. Tamir, Prevalence of bovine mastitis and its associated risk factors among dairy cows in Ethiopia during 2005–2022: a systematic review and meta-analysis, *Vet. Med. Int.* 2022 (2022), <https://doi.org/10.1155/2022/7775197>.
- [8] S.A. Martins, V.C. Martins, F.A. Cardoso, J. Germano, M. Rodrigues, C. Duarte, R. Bexiga, S. Cardoso, P.P. Freitas, Biosensors for on-farm diagnosis of mastitis, *Front. Bioeng. Biotechnol.* 7 (2019) 186, <https://doi.org/10.3389/fbioe.2019.00186>.
- [9] P.R.F. Adkins, J.R. Middleton, Methods for diagnosing mastitis, *Veterinary Clinics: Food Animal Practice* 34 (3) (2018) 479–491, <https://doi.org/10.1016/j.cvfa.2018.07.003>.
- [10] G.D. Dahesa, T. Taju, I.A. Kebede, A.D. Ahmed, Review on current diagnostic methods of bovine mastitis, *Vet. Med. Open J.* 8 (2) (2023) 56–65, <https://doi.org/10.17140/VMOJ-8-17>.
- [11] S. Chakraborty, K. Dhama, R. Tiwari, M. Iqbal Yattoo, S.K. Khurana, R. Khandia, A. Munjal, P. Munuswamy, M.A. Kumar, M. Singh, Technological interventions and advances in the diagnosis of intramammary infections in animals with emphasis on bovine population—a review, *Vet. Q.* 39 (1) (2019) 76–94, <https://doi.org/10.1080/01652176.2019.1642546>.
- [12] R. Giannechini, C. Concha, R. Rivero, I. Delucci, J.M. López, Occurrence of clinical and sub-clinical mastitis in dairy herds in the West Littoral Region in Uruguay, *Acta Vet. Scand.* 43 (4) (2002) 221–230, <https://doi.org/10.1186/1751-0147-43-221>.
- [13] A. Marti-De Olives, C. Peris, M.P. Molina, Effect of subclinical mastitis on the yield and cheese-making properties of Ewe's milk, *Small Rumin. Res.* 184 (2020) 106044, <https://doi.org/10.1016/j.smallrumres.2019.106044>.
- [14] A. Neculai-Valeanu, A.-M. Ariton, Udder health monitoring for prevention of bovine mastitis and improvement of milk quality, *Bioengineering* 9 (11) (2022) 608, <https://doi.org/10.3390/bioengineering9110608>.
- [15] K.G.A. Hameed, G. Sender, A. Korwin-Kossakowska, Public health hazard due to mastitis in dairy cows, *Anim. Sci. Pap. Rep.* 25 (2) (2007) 73–85.
- [16] H. Fagundes, L. Barchesi, A. Nader Filho, L.M. Ferreira, C.A.F. Oliveira, Occurrence of *Staphylococcus aureus* in raw milk produced in dairy farms in São Paulo state, Brazil, *Braz. J. Microbiol.* 41 (2010) 376–380.
- [17] G.A. Abril, T.G. Villa, J. Barros-Velázquez, B. Cañas, A. Sánchez-Pérez, P. Calo-Mata, M. Carrera, *Staphylococcus aureus* exotoxins and their detection in the dairy industry and mastitis, *Toxins* 12 (9) (2020) 537, <https://doi.org/10.3390/toxins12090537>.
- [18] M. Garvey, Chronic bovine mastitis: a food safety issue and public health hazard, *Nutr. Food Sci. Int. J.* 8 (2019) 1–6.
- [19] R. Paramasivam, D.R. Gopal, R. Dhandapani, R. Subbarayalu, M.P. Elangovan, B. Prabhu, V. Veerappan, A. Nandheeswaran, S. Paramasivam, S. Muthupandian, Is AMR in dairy products a threat to human health? An updated Review on the origin, prevention, treatment, and economic impacts of subclinical mastitis, *Infect. Drug Resist.* 16 (2023) 155–178, <https://doi.org/10.2147/IDR.S384776>, 2023.
- [20] R.D. Abdi, B.E. Gillespie, S. Ivey, G.M. Pighetti, R.A. Almeida, O. Kerro Dego, Antimicrobial resistance of major bacterial pathogens from dairy cows with high somatic cell count and clinical mastitis, *Animals* 11 (1) (2021) 131, <https://doi.org/10.3390/ani11010131>.
- [21] S. Dufour, J. Labrie, M. Jacques, The mastitis pathogens culture collection, *Microbiol. Resour. Announc.* 8 (15) (2019) 79–82, <https://doi.org/10.1128/MRA.00133-19>.
- [22] M. Vargová, F. Zigo, J. Výrostková, Z. Farkašová, I.F. Rehan, Biofilm-producing ability of *Staphylococcus aureus* obtained from surfaces and milk of mastitic cows, *Vet. Sci.* 10 (6) (2023) 386, <https://doi.org/10.3390/vetsci10060386>.
- [23] F. Zigo, J. Elečko, Z. Farkašová, M. Zígová, M. Vasil, S. Ondrašovičová, K. Lenka, Preventive methods in reduction of mastitis pathogens in dairy cows, *J. Microbiol. Biotechnol. Food Sci.* 9 (1) (2019), <https://doi.org/10.15414/jmbfs.2019.9.1.121-126>.
- [24] M. Tezera, E. Aman Ali, Prevalence and associated risk factors of Bovine mastitis in dairy cows in and around Assosa town, Benishangul-Gumuz Regional State, Western Ethiopia, *Vet. Med. Sci.* 7 (4) (2021) 1280–1286, <https://doi.org/10.1002/vms3.454>.
- [25] E.O. Mungube, B.-A. Tenhagen, T. Kassa, F. Regassa, M.N. Kyule, M. Greiner, M.P.O. Baumann, Risk factors for dairy cow mastitis in the central highlands of Ethiopia, *Trop. Anim. Health Prod.* 36 (2004) 463–472, <https://doi.org/10.1023/B:TROP.0000034999.08368.f3>.
- [26] R.M. Kamal, M.A. Bayoumi, Efficacy of premilking and postmilking teat dipping as a control of subclinical mastitis in Egyptian Dairy cattle, *Int. Food Res. J.* 22 (3) (2015).
- [27] B. Rohit, M.L. Kamboj, Effect of pre and post milking teat dipping on udder health and milk quality of Murrah buffaloes, *Indian Vet. J.* 87 (3) (2010) 264–266.
- [28] K. Wattenburger, R. Schmidt, L. Placheta, J.R. Middleton, P.R.F. Adkins, Evaluation of 4 different teat disinfection methods prior to collection of milk samples for bacterial culture in dairy cattle, *J. Dairy Sci.* 103 (5) (2020) 4579–4587, <https://doi.org/10.3168/jds.2019-17338>.
- [29] L. Özsavári, D. Ivanyos, The use of teat disinfectants and milking machine cleaning products in commercial Holstein-Friesian farms, *Front. Vet. Sci.* 9 (2022) 956843, <https://doi.org/10.3389/fvets.2022.956843>.
- [30] W. Suriyasathaporn, V. Chupia, Reduction in numbers of bacteria after pre-milking teat dipping in milking dairy cows, *J. Nat. Sci.* 10 (2) (2011) 301–306.
- [31] R. Rahularaj, R.M.C. Deshpriya, R.M.S.B.K. Ranasinghe, Influence of bovine sub-clinical mastitis and associated risk factors on calving interval in a population of crossed lactating cows in Sri Lanka, *Trop. Anim. Health Prod.* 51 (8) (2019) 2413–2419, <https://doi.org/10.1007/s11250-019-01957-4>.
- [32] R.A. Nicholas, L.K. Fox, I. Lysnyansky, *Mycoplasma mastitis* in cattle: to cull or not to cull, *Vet. J.* 216 (2016) 142–147, <https://doi.org/10.5897/AJMR2017.8529>.

- [33] K.E. Kemal, S. Tesfaye, S. Ashanafi, A.F. Muhammadhussien, Prevalence, risk factors and multidrug resistance profile of *Staphylococcus aureus* isolated from bovine mastitis in selected dairy farms in and around Asella town, Arsi Zone, South Eastern Ethiopia, *Afr. J. Microbiol. Res.* 11 (45) (2017) 1632–1642, <https://doi.org/10.5897/AJMR2017.8529>.
- [34] M.M. Kurjogi, B.B. Kaliwal, Epidemiology of bovine mastitis in cows of Dharwad district, *Int. Sch. Res. Notices* 2014 (2014) 1–9, <https://doi.org/10.1155/2014/968076>.
- [35] R. Sinha, B. Sinha, R. Kumari, V. Mr, A. Verma, I.D. Gupta, Effect of season, stage of lactation, parity and level of milk production on incidence of clinical mastitis in Karan Fries and Sahiwal cows, *Biol. Rhythm. Res.* 52 (4) (2021) 593–602, <https://doi.org/10.1080/09291016.2019.1621064>.
- [36] M. Shaheem, H. Tantary, S.U. Nabi, A Treatise on bovine mastitis: disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic management, prevention and control strategy, *Adv Dairy Res* 4 (2016) 1–10, <https://doi.org/10.4172/2329-888X.1000150>, 1-10, *Advances in dairy Research* 4 (2016).
- [37] N. Sharma, N.K. Singh, O.P. Singh, V. Pandey, P.K. Verma, Oxidative stress and antioxidant status during transition period in dairy cows, *Asian Australas. J. Anim. Sci.* 24 (4) (2011) 479–484.
- [38] F. Zigo, Z. Farkasova, M. Lapin, M. Chripkova, A. Czernski, Effect of parenteral administration of Selenium and vitamin E on health status of mammary gland and on selected antioxidant indexes in blood of dairy cows, *Pol. J. Vet. Sci.* 17 (2) (2014) 217–223, <https://doi.org/10.2478/pjvs-2014-0031>.
- [39] United Republic of Tanzania, National Sample Census of Agriculture 2019/20. Key Findings Report, National Bureau of Statistics, Dar es Salaam, Tanzania, 2021.
- [40] United Republic of Tanzania, Tanzania Livestock Master Plan (2017/2018 – 2021/2022), Ministry of Livestock and Fisheries, Dar es Salaam, Tanzania, 2017.
- [41] J.M.K. Ojango, C.B. Wasike, D.K. Enahoro, A.M. Okeyo, Dairy production systems and the adoption of genetic and breeding technologies in Tanzania, Kenya, India and Nicaragua, *Anim. Genet. Resour.* 59 (2016) 81–95, <https://doi.org/10.1017/S2078633616000096>.
- [42] E.S. Swai, E.D. Karimuribo, Smallholder dairy farming in Tanzania: current profiles and prospects for development, *Outlook Agric.* 40 (1) (2011) 21–27, <https://doi.org/10.5367/oa.2011.0034>.
- [43] G. Msalya, Contamination levels and identification of bacteria in milk sampled from three regions of Tanzania: evidence from literature and laboratory analyses, *Vet. Med. Int.* 2017 (2017) 1–10, <https://doi.org/10.1155/2017/9096149>.
- [44] R.H. Mdegela, L.J.M. Kusiluka, A. Kapaga, E.D. Karimuribo, F.M. Turuka, A. Bundala, F. Kivaria, B. Kabula, A. Manjurano, T. Loken, Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in Eastern Tanzania, *J. Vet. Med.* 51 (3) (2004) 123–128, <https://doi.org/10.1111/j.1439-0450.2004.00735.x>.
- [45] K. Damian, M. Robinson, K. Lughano, S. Gabriel, Prevalence and risk factors associated with subclinical mastitis in lactating dairy cows under smallholder dairy farming in North East Tanzania, *J. Vet. Med. Anim. Health* 13 (1) (2021) 55–64, <https://doi.org/10.5897/JVMAH2019.0775>.
- [46] E. Blackmore, A. Guarin, C. Kinyua, W. Vorley, D. Grace, S. Alonso, The governance of quality and safety in Tanzania's informal milk markets, *Front. Sustain. Food Syst.* 6 (2022) 971961, <https://doi.org/10.3389/fsufs.2022.971961>.
- [47] United Republic of Tanzania, Dodoma Region Investment Guide Dodoma, Tanzania, Dodoma Tanzania, President's Office Regional Administration and Local Government, 2019.
- [48] N. Bundala, J. Kinabo, T. Jumbe, C. Rybak, S. Sieber, Does homestead livestock production and ownership contribute to consumption of animal source foods? A pre-intervention assessment of rural farming communities in Tanzania, *Sci. Afr.* 7 (2020) e00252, <https://doi.org/10.1186/s13570-022-00254-4>.
- [49] K. Ngongolo, M. Kilonzo, Communities' awareness of afforestation and its contribution to the conservation of lizards in Dodoma, Tanzania, *Sci. Rep.* 12 (1) (2022) 22592, <https://doi.org/10.1038/s41598-022-27268-7>.
- [50] United Republic of Tanzania, National Sample Census of Agriculture 2002/2003 Regional Report: Dodoma Region, National Bureau of Statistics, Dar es Salaam, Tanzania, 2006.
- [51] L.R. Kurwijila, A.O. Omore, D. Grace, Tanzania Dairy Industry Overview 2012, Morogoro, Tanzania, Sokoine University of Agriculture, 2012.
- [52] A. Guisan, T.C. Edwards Jr., T. Hastie, Generalized linear and generalized additive models in studies of species distributions: setting the scene, *Ecol. Model.* 157 (2–3) (2002) 89–100, [https://doi.org/10.1016/S0304-3800\(02\)00204-1](https://doi.org/10.1016/S0304-3800(02)00204-1).
- [53] S.N. Wood, Stable and efficient multiple smoothing parameter estimation for generalized additive models, *J. Am. Stat. Assoc.* 99 (467) (2004) 673–686, <https://doi.org/10.1198/016214504000000980>.
- [54] A. Groll, G. Tutz, Regularization for generalized additive mixed models by likelihood-based boosting, *Methods Inf. Med.* 51 (2) (2012) 168–177.
- [55] N. Belay, N. Mohammed, W. Seyoum, Bovine mastitis: prevalence, risk factors, and bacterial pathogens isolated in lactating cows in Gamo zone, southern Ethiopia, *Vet. Med. Res. Rep.* 13 (2022) 9–19, <https://doi.org/10.2147/VMRR.S344024>.
- [56] C.M. Mbindyo, G.C. Gitao, C.M. Mulei, Prevalence, etiology, and risk factors of mastitis in dairy cattle in Embu and Kajijado Counties, Kenya, *Vet. Med. Int.* 2020 (2020), <https://doi.org/10.1155/2020/8831172>.
- [57] T. Suleiman, E. Karimuribo, R. Mdegela, Prevalence of bovine subclinical mastitis and antibiotic susceptibility patterns of major mastitis pathogens isolated in Unguja island of Zanzibar, Tanzania, *Trop. Anim. Health Prod.* 50 (2018) 259–266, <https://doi.org/10.1007/s11250-017-1424-3>.
- [58] M.V. Mdemu, Community's vulnerability to drought-driven Water scarcity and food insecurity in central and northern semi-arid areas of Tanzania, *Front. Clim.* 3 (2021) 737655, <https://doi.org/10.3389/fclim.2021.737655>.
- [59] J.B. Ndahutuye, Y. Persson, A.-K. Nyman, M. Tukei, M.P. Ongol, R. Båge, Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda, *Trop. Anim. Health Prod.* 51 (2019) 2037–2044, <https://doi.org/10.1007/s11250-019-01905-2>.
- [60] M. Tekliye, M. Gizaw, Handling practices, evaluation of adulteration and microbial quality of raw cow milk around Bahir Dar, Ethiopia, *Front. Bioeng. Biotechnol.* 61 (2017) 1–9.
- [61] E.D. Karimuribo, J.L. Fitzpatrick, E.S. Swai, C. Bell, M.J. Bryant, N.H. Ogden, D.M. Kambarage, N.P. French, Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania, *Vet. Rec.* 163 (1) (2008) 16–21, <https://doi.org/10.1093/jas/skz113>.
- [62] J.F. Penry, E.L. Endres, B. De Bruijn, A. Kleinhans, P. Crump, D.J. Reinemann, L.L. Hernandez, Effect of incomplete milking on milk production rate and composition with 2 daily milkings, *J. Dairy Sci.* 100 (2) (2017) 1535–1540, <https://doi.org/10.3168/jds.2016-11935>.
- [63] J.M. Kuehn, M.K. Connelly, A. Dzidic, M. Lauber, H.P. Fricke, M. Klister, E. Olstad, M. Balbach, E. Timlin, V. Psczolkowski, The effects of incomplete milking and increased milking frequency on milk production rate and milk composition, *J. Anim. Sci.* 97 (6) (2019) 2424–2432, <https://doi.org/10.1093/jas/skz113>.
- [64] J. Penry, E. Endres, B. De Bruijn, A. Kleinhans, P. Crump, D.J. Reinemann, L. Hernandez, Effect of incomplete milking on milk production rate and composition with 2 daily milkings, *J. Dairy Sci.* 100 (2) (2017) 1535–1540.
- [65] A. Dzidic, J. Macuhova, R.M. Bruckmaier, Effects of cleaning duration and water temperature on oxytocin release and milk removal in an automatic milking system, *J. Dairy Sci.* 87 (12) (2004) 4163–4169, [https://doi.org/10.3168/jds.S0022-0302\(04\)73559-6](https://doi.org/10.3168/jds.S0022-0302(04)73559-6).
- [66] Z. Zhang, X.P. Li, F. Yang, J.Y. Luo, X.R. Wang, L.H. Liu, H.S. Li, Influences of season, parity, lactation, udder area, milk yield, and clinical symptoms on intramammary infection in dairy cows, *J. Dairy Sci.* 99 (8) (2016) 6484–6493, <https://doi.org/10.3168/jds.2016-10932>.
- [67] I.J. Lean, S.J. LeBlanc, D.B. Sheedy, T. Duffield, J.E.P. Santos, H.M. Golder, Associations of parity with health disorders and blood metabolite concentrations in Holstein cows in different production systems, *J. Dairy Sci.* 106 (1) (2023) 500–518, <https://doi.org/10.3168/jds.2021-21673>.
- [68] A. Sabek, C. Li, C. Du, L. Nan, J. Ni, E. Elgazzar, Y. Ma, A.Z.M. Salem, S. Zhang, Effects of parity and days in milk on milk composition in correlation with β -hydroxybutyrate in tropic dairy cows, *Trop. Anim. Health Prod.* 53 (2) (2021) 1–8, <https://doi.org/10.1007/s11250-021-02690-7>.
- [69] I. Klaas, C. Enevoldsen, M. Vaarst, H. Houe, Systematic clinical examinations for identification of latent udder health types in Danish dairy herds, *J. Dairy Sci.* 87 (5) (2004) 1217–1228, [https://doi.org/10.3168/jds.S0022-0302\(04\)73272-5](https://doi.org/10.3168/jds.S0022-0302(04)73272-5).
- [70] J.F. Guarin, P.L. Ruegg, Pre- and postmilking anatomical characteristics of teats and their associations with risk of clinical mastitis in dairy cows, *J. Dairy Sci.* 99 (10) (2016) 8323–8329, <https://doi.org/10.3168/jds.2015-10093>.
- [71] I.C. Klaas, C. Enevoldsen, A.K. Ersbøll, U. Tölle, Cow-related risk factors for milk leakage, *J. Dairy Sci.* 88 (1) (2005) 128–136, [https://doi.org/10.3168/jds.S0022-0302\(05\)72670-9](https://doi.org/10.3168/jds.S0022-0302(05)72670-9).

- [72] J.-Y. Lee, I.-H. Kim, Advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds, *J. Vet. Sci.* 7 (2) (2006) 161–166, <https://doi.org/10.4142/jvs.2006.7.2.161>.
- [73] A. Hussain, M.H. Mushtaq, M. Chaudhry, M.S. Khan, M. Reichel, T. Hussain, A. Khan, M. Nisar, I.A. Khan, Prevalence of overall and teatwise mastitis and effect of herd size in dairy buffaloes, *Pak. J. Zool.* 50 (3) (2018) 1107–1112, <https://doi.org/10.17582/journal.pjz/2018.50.3.1107.1112>.
- [74] A. Cozma, B. Martin, C. Cirie, I. Verdier-Metz, J. Agabriel, A. Ferlay, Influence of the calf presence during milking on dairy performance, milk fatty acid composition, lipolysis and cheese composition in Salers cows during winter and grazing seasons, *J. Anim. Physiol. Anim. Nutr.* 101 (5) (2017) 949–963, <https://doi.org/10.1111/jpn.12530>.
- [75] J. Combellas, M. Tesorero, L. Gabaldón, Effect of calf stimulation during milking on milk yield and fat content of *Bos indicus* × *Bos taurus* cows, *Livest. Prod. Sci.* 79 (2–3) (2003) 227–232, [https://doi.org/10.1016/S0301-6226\(02\)00153-7](https://doi.org/10.1016/S0301-6226(02)00153-7).