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Research Article

Risk Factors Associated with Occurrence of Mastitis in Mathira East in Nyeri County, Kenya

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Abstract

Mastitis is a major challenge to dairy farming in many regions of the world and causes great losses which range from low milk yields, high costs of treatment to culling of affected animals. It is caused by various bacteria which include normal teat flora, opportunistic and environmental pathogens. We sought to estimate proportion of lactating dairy cows with mastitis, identify mastitis causing pathogens and determine risk factors for mastitis in Nyeri County, Kenya. We used a cross-sectional study design and randomly selected 19 villages and 169 farms from the 6 wards of Mathira Constituency. California mastitis test was used to detect positive animals whose samples were collected for culture and identification. Association between prevalence and breed, stage of lactation, floor type, udder cleanliness, milker, body condition, udder consistency, average milk production and parity were compared using Chi square tests. Farmers' knowledge of signs of mastitis was also assessed including measures taken by farmers to minimize incidents of mastitis. Prevalence of mastitis was 92% at cow level and 86.5% at quarter level. *Staphylococcus aureus* was the most common bacteria isolated (68.5%) either singly or in combination with other bacteria. Holstein/Friesian breed and poor udder hygiene were identified as major risk factors for mastitis ($p \leq 0.05$). Majority of the farmers were able to tell whether a cow has mastitis when there was a reduction in milk production (60%) or when animals produced milk with curds or which curds on boiling (59%). All farmers interviewed cleaned hands and animal udder before milking as measures to control mastitis. Disposable hand towels (4%), udder disinfectants (9) and dry cow therapy (5%) were rarely used. Good hygiene which includes dairy farm, cow and milker hygiene is key in eliminating mastitis. Udder cleanliness should be emphasized as dirty udders serve as means by which bacteria enter the teat canal. Frequent screening can help identify cases early and reduce incidences of pathological changes in the udder tissue. Farmers' education on awareness and common mastitis signs could help in detecting cases early. Use of disposable paper towels, udder disinfectants and dry cow therapy should be embraced to reduce incidences of mastitis.

Keywords: *Mastitis; Prevalence; Risk Factors; Udder Cleanliness*

Introduction

Mastitis is the most economically important disease in dairy farming globally with its occurrence either in clinical or sub-clinical forms leading to adverse losses in dairy production. Mastitis can be caused by a series of pathogens, differentiated into two broad categories: those causing contagious mastitis and those causing environmental mastitis [1]. The presence of bacteria in milk is due to factors which range from direct contact with contaminated sources in the dairy farm environment, infection or injury of the udder and poor hygiene

practices [2]. Sub-clinical mastitis is often undetected by farmers making it persist longer in the herds and has been found to be more important economically than clinical mastitis [3]. Mastitis risk factors or disease determinants can be classified into three groups: host factors which include age, immunity and parity, pathogen virulence and drug resistance and environmental determinants which could promote survival of the pathogen around the animal and the cowshed [4].

Prevalence of mastitis has been found to be high in most regions of the world with great economic losses incurred by farmers. A study done in the United Kingdom and Wales estimated clinical mastitis incidence in 2007 to be 47 cases per 100 cows per year [5]. In Africa, prevalence of mastitis has been estimated to be over 30% [6]. In Kenya, a study done in Nyeri County in 2012 estimated prevalence of mastitis to be 87.4% [7].

Losses caused by mastitis range from low milk yields, discarding of milk during treatment and withdrawal periods, cost of veterinary services and drugs and in extreme cases culling of affected animals. In North America, mastitis has been found to cause economic losses of approximately $2 billion annually with sub-clinical mastitis being responsible for 70% of cases encountered [8]. A study done by Sinha., *et al*. in India identified a loss due to mastitis of $18.5 per animal per lactation among cross-bred cattle of which 37% is due to reduction in milk production, the rest being attributed to cost of Veterinary services [9].

Various factors have been found to increase a cow's susceptibility to mastitis ranging from breed of cow, stage of lactation, average milk yield, parity, type of floor in the cowshed to milking hygiene practices. A study done by Kivaria., *et al*. on risk factors for mastitis among small holder dairy farms in Tanzania found that incidence was significantly associated with cow factors (body condition score, parity, stage of lactation, and udder consistency), housing (floor type) conditions and milking (cow and udder preparation) practices [10].

Farmers in Nyeri county and elsewhere experience losses in milk production due to either clinical or sub-clinical mastitis. This poses a big challenge in the economic viability of the dairy industry most especially because farmers are unable to detect mastitis unless there is visible inflammation of the udder tissue or change in milk consistency while others get to know about it when the milk is rejected in the dairy where they sell their milk. This study sought to investigate the proportion of lactating dairy cows with mastitis (either clinical or sub-clinical), to identify the mastitis causing pathogens and determine the risk factors associated with mastitis in Mathira constituency, Nyeri County, Kenya. We also sought to assess farmers' knowledge of signs of mastitis in dairy cows and measures taken to prevent mastitis.

Materials and Methods

This study was undertaken in the months of June and July 2018.

Study area

The study area was Mathira constituency of Nyeri County, in Central Kenya which has 2 sub-counties, Mathira east and west. In East Africa, Kenya is the leading producer of milk, producing an estimated 3.2 billion liters per year by approximately 600,000 smallholder farmers [11]. In Nyeri County, which is a highly productive area, dairy cattle are reared on small holder basis. The rainfall average lies between 500 mm and 1500 mm during the short and long rains periods making it conducive for its diverse agricultural activity.

Study design and sample size determination

The study was a cross-sectional study. Sample size for the study was calculated using Epitools 99% CI, 87% Prevalence [7], Power of 0.8 to get a sample size of n = 301. Approximately 3 villages from each of the 6 wards were selected randomly and farms within the two villages were also selected randomly. In farms which had up-to two lactating cows, both animals were sampled, but where there were

more than two, two were randomly selected from the herd and sampled. During sampling, data on the breed, stage of lactation, floor type (earthen/cemented), udder cleanliness (clean/dirty), milker (owner/employed), body condition (good/poor or fair), udder consistency (normal/abnormal), milk production and parity were collected using a standard data collection tool. The udder was considered dirty if there was any visible dirt or mud on the teats or udder tissue. Udder consistency was scored to be abnormal if by observation any wounds, pimples or warts were found on udder tissue and if there were abnormalities in consistency of the udder tissue on palpation. Body condition was considered poor if there were marked protrusions of the ribs, backbone and/or hip bone [12]. Details of farmers' knowledge of common signs of mastitis was obtained from 5 common signs: swelling and redness of the udder, change in colour or consistency of milk, cow exhibiting pain on milking, reduction in milk production or curding of the milk. On measures taken to prevent mastitis we assessed 4 major practices: cleaning of animal udder and milker's hands, use of cloth hand towels against use of disposable paper towels, use of udder washing disinfectants and use of dry cow therapy.

California mastitis test (CMT)

The California Mastitis Test (CMT) was done at the farm following the guidelines of the National Mastitis Council (1999) [13]. Udder was cleaned using soap and water and each teat cleaned using a disposable paper towel. Three streams of milk from each teat were discarded before each teat was cleaned with a cotton ball soaked in alcohol. Approximately two milliliters of milk was drawn from each quarter into a plastic paddle (CMT plate). The CMT reagent of an equal amount was added to the milk and the paddle rotated to mix reagent and milk. The score was read and results were recorded as 0 (negative/trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive) based on the thickness of the gel formed by CMT reagent-milk mixture. Cows with at least one CMT-positive quarter were defined as CMT-positive. Milk from cows whose CMT results were read as positive was collected in universal bottles and transported in ice to the Regional Veterinary Investigation laboratory, Karatina for bacteriological analysis.

Bacteriology culture procedures

Milk samples were examined following the protocol described by Gitau., *et al* [14]. An aliquot of each milk sample was streaked onto blood agar and Mac-Conkey agar plates and incubated overnight at 37°C. Plates were examined for growth after 24 hours and those without growth were further incubated. Gram staining and examination under a microscope was performed to identify gram-positive and gram-negative organisms according to standard microbiological methods. Biochemical tests were performed to further identify the micro-organisms according to the Clinical Laboratory Standards Institute [15].

Data analysis

Proportions and percentages were used to determine prevalence of mastitis. The association of the breed of the cow, udder cleanliness, udder consistency, stage of lactation, milk production, body condition, parity, and floor type with the CMT positivity was determined by Chi-square test using EPI INFO Version 7 at 95% confidence interval. Proportions were calculated to determine farmers' knowledge of common mastitis signs and measures taken to reduce incidences of mastitis.

Results

Milk was collected from 314 dairy cows and 1250 teats (6 teats were blocked and produced no milk) in 19 villages and 169 homesteads. Two hundred and eighty nine cows (289/314 = 92%) had positive California mastitis test results. Mastitis quarter prevalence was 86.5% (1080/1250). Milk from 265 out of the 289 (91.7%) cows with positive CMT results had bacterial cultural growth. *Staphylococcus aureus* was the most common isolate either singly (69.2 %) or in combination with other bacteria (83.7 %). One isolate was identified as fungal growth (*Candida* species). Table 1 below shows the results of the cultural isolates obtained.

Bacterial isolate	Number (N = 289)	Percentage
Staphylococcus aureus	198	68.5%
Staphylococcus aureus + Corynebacterium spp.	25	8.7%
Staphylococcus aureus + Streptococcus spp.	5	1.7%
Staphylococcus aureus + Klebsiella spp.	3	1%
Staphylococcus aureus + Escherichia coli	3	1%
Staphylococcus aureus + Streptococcus spp.	5	1.7%
Staphylococcus aureus + Klebsiella spp.	3	1%
Staphylococcus aureus + Escherichia coli	3	1%
Staphylococcus aureus + Enterobacter spp	2	0.7%
Staphylococcus aureus + Pseudomonas spp.	1	0.35%
Staphylococcus aureus + Listeria spp.	1	0.35%
Staphylococcus aureus + Corynebacterium spp. + Listeria spp.	1	0.35%
Staphylococcus aureus + Candida spp	1	0.35%
Corynebacterium spp.	13	4.5%
Pseudomonas spp.	5	1.7%
Listeria spp.	2	0.7%
Escherichia coli	2	0.7%
Streptococcus spp	1	0.35%
Enterobacter spp	1	0.35%
Corynebacterium spp. + Listeria spp	1	0.35%
No isolates	24	8%
Total	289	100%

Table 1: *Bacteriological cultural profile of milk samples collected in Mathira east and west, 2018.*

On comparison of CMT positivity against risk factors, Friesian breed and poor udder hygiene were identified as risk factors for mastitis (Table 2).

Variables	Categories	Positive	Negative	x^2	P Value
Breed	Friesian	215	12	8.003	0.0047
	Non Friesian	74	13		
Stage of lactation	Early (< 6 months)	83	8	4.767	0.092
	Mid (6 - 12 months)	24	5		
	Late (over 1 year) Not known	178 5	11		
Floor type	Earthen	99	10	0.335	0.563
	Cemented	190	15		
Udder hygiene	Clean	245	16		
	Dirty	44	9	7.078	0.0078
Milker	Owner	205	21	1.95	0.163
	Employed	84	4		
Body condition	Good	230	18	0.797	0.371
	Poor/Fair	59	7		
Udder consistency	Normal	253	22	0.0044	0.947
	Abnormal	26	3		
Milk production	< 10 LT	221	18	0.511	0.774
	10 LT-20 LT	62	6		
	> 20 LT	6	1		
Parity	≤ 3 > 3 Not known	179 99 11	17 7 1	0.403	0.525

Table 2: *Comparison of risk factors for mastitis against CMT positivity.*

169 questionnaires were administered to determine farmers' knowledge of common mastitis signs and measures put in place to control mastitis. Seven questionnaires were incomplete and were removed from the analysis. Majority of the farmers interviewed were able to tell whether a cow has mastitis mostly when there is a reduction in milk production (60%) or when animals produces milk with curds or which curds on boiling (59%). All farmers interviewed took measures of cleaning hands and udder before milking to prevent their animals from developing mastitis. Disposable hand towels (4%), udder disinfectants (9) and dry cow therapy (5%) were rarely used. The results on farmers' knowledge of common signs of mastitis and measures taken to prevent mastitis from 162 farmers interviewed are as shown in the table 3 below.

Parameter	Number	Percentage (%)
Reduction in milk production	96	60
Swelling of the udder	90	56
Change in colour/consistency of milk	92	57
Milk clotting/ sometimes when boiled	95	59
Animal exhibiting pain during milking	72	45
Cleaning of udder	161	100
Cleaning of milker's hands	161	100
Use of disposable paper towels	6	3.73
Use of udder disinfectants	14	8.7
Use of dry cow therapy	8	5

Table 3: *Farmers' knowledge of common mastitis signs and measures used to control mastitis.*

Discussion

This study reveals a high prevalence of mastitis (92% at cow level and 86.5% at quarter level). These results were higher than those in previous reports by Biru (1989), who reported prevalence of mastitis in dairy cows in Addis Ababa, Ethiopia at 67.4% [15]. In Thika, Kenya, Mureithi., *et al*. (2016) obtained a 64% prevalence of sub-clinical mastitis [17]. The results were slightly higher than those reported by Abrahmsén., *et al*. (2014) in Uganda in which a prevalence of 86.3% was obtained [18]. The results of this study concur with results in a study done by Nkoroi (2014) in Mathira east in which a prevalence of 87% was obtained at quarter level [7]. This study therefore shows that mastitis either in clinical and sub-clinical forms is a common occurrence in dairy cattle and most times goes unnoticed by farmers who are not aware of common signs of mastitis. There was no bacterial isolates recovered from 24 (8%) of the isolates and these were attributed to animals which were currently on intra-mammary therapies. Presence of antibiotics in a milk sample could prevent growth of micro-organisms. The host's natural defenses have been found to clear the bacterial infection completely or to a level below the detection limit of the culture method prior to sample collection. Historically, Gram-negative infections have been reported to have high self-cure rates, prompting a recommendation of no antibiotic treatment in uncomplicated cases [19].

Staphylococcus aureus species of bacteria was found to be the predominant mastitis causing agent (83.7%) either singly or in combination with other bacteria. In his study, Nkoroi also identified a higher prevalence of *Staphylococcus aureus* species in 98.5% of the isolates. Our finding was slightly higher than that by Gitau., *et al*. (2014) where *Staphylococcus aureus* species was isolated in 72.9% of samples [14]. *Corynebacterium* species of bacteria also had a high prevalence at 13% singly or in combination with *Staphylococcus aureus* or with other bacteria.

Bacteria are spread during milking by the milker from one animal to another or from the environment to the animal especially where the floor of the cowshed may not be clean. Various factors have been attributed to increase a cow's risk of mastitis: among them are breed,

parity, stage of lactation, level of milk production, floor type, body condition of the animal and udder health. Our study identified being of Hosetin-Friesian breed being at a higher risk factor for mastitis. The Holstein-Friesian is a high producing breed usually kept in intensive farming conditions and has been found to be more susceptible to udder inflammation than other exotic breeds or crosses probably due to its high milk yielding capacity. Dirty udder was also identified as a major risk factor for mastitis ($p \leq 0.05$). Bacteria penetrate the udder usually by entry through the teat canal. Infections are transmitted to the teat skin by milking machine liners, milkers' hands, washing cloth and any source that can act as a carrier for germs. These together with the Cow's environment particularly bedding have been found to be potential sources of mastitis causing pathogens. Poor udder health and consistency have also been identified as major risk factor in udder infections [20]. Though this study did not identify these as significant risk factors, wounds, tick bites, pimples and warts on udder were found in some of the animals sampled and these serve as mechanisms by which infectious organisms could enter into the teat canal [21]. Higher level of productivity and a higher parity have also been identified as major risk factors for mastitis [22]. Cows with higher parity are usually older and more likely to be immune-compromised and could also have developed a chronic infection. The defense mechanism in older cows is likely to be weaker than in younger cows due to less active polymorphonuclear leukocyte function in multiparous cows [23].

Most farmers lacked knowledge of common mastitis signs. Awareness of common signs of mastitis like udder swelling and redness, change in colour and consistency of milk, reduction in milk production could help identify mastitis early in order to avoid losses. Milk production reduction could easily go unnoticed as it could be associated with other factors. On common measures taken to prevent mastitis, farmers used cleaning of the animal's udder and milkers' hand as major control measures. Use of cloth towels to clean udder before milking was common compared to use of disposable paper towels with only 4% using disposable towels. Cloth towels could act as a medium within which bacteria thrive and could be transferred from dirty udder environment and from cow to cow when towels are shared. Whenever possible, use of disposable paper towels should be encouraged. Udder disinfectants were used by a small percentage of farmers (9%), with few using dry cow therapy when drying animals after lactation (5%). Most farmers practiced intermittent milking prior to drying with others stopping milking the animals suddenly. Farmers should be encouraged to frequently use udder and teat dips which help destroy bacteria present in the udder which could easily enter the teat canal and cause mastitis. They should also be made aware of dry cow antibiotics which help in destroying bacteria present in the udder after stoppage of milking thus preventing an animal from developing mastitis during the next calving season.

Conclusion and Recommendations

Good hygiene still remains the key weapon against mastitis in dairy herds. Housing animals in clean sheds which are easy to clean reduces risk of cattle getting in contact with mastitis causing pathogens. Several practices lead to high prevalence of sub-clinical mastitis in dairy cattle. These include dirty environment, lack of post milking teat dipping, use of same towel to wipe dry the udder of different animals in a herd leading to spread of bacteria. Keeping animals in a clean environment, use of paper towels or use of individual towels for each cow can help reduce transmission of bacteria between herds. Milkers' should ensure hand hygiene by cleaning hands before milking and when moving from cow to cow between milking to avoid transfer of germs from cow to cow. Use to teat dips helps reduce bacteria present in animals' skin and teat surface from entering the teat canal. Early treatment of wounds or injuries on teats could reduce chances of bacterial entry into the teat canal. Frequent screening for mastitis using strip cups and California mastitis test can help eliminate sub-clinical and clinical cases of mastitis therefore getting rid of bacteria before they spread further to the environment and to other cattle within the same herd. Sub-clinical mastitis can persist in cattle for a long time causing great losses due to lack of awareness by the farmer. Mastitis control programs usually rely on the farmers' ability to detect mastitis in a timely and accurate manner. Farmers' awareness of mastitis, especially subclinical should be enhanced through training in identification of early signs of mastitis to ensure quick diagnosis. Treatment for those found to have mastitis should be initiated as early as possible to reduce potential losses in milk production, treatment of chronic cases which is very expensive and helps reduce chances of growth of resistant bacteria. Practicing dry cow therapy when drying cattle should also be encouraged.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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