RISK FACTORS ASSOCIATED WITH PREVALENCE OF BRUCELLOSIS AND BACTERIAIN FERMENTED COW MILK OBTAINED FROM KAJIADO CENTRAL SUB-COUNTY IN KENYA

ABSTRACT

Cattle rearing remains to be among the most important activities practiced by Maasaipastoralists in Kajiado Central Sub-County, Kenya. However, its role has been hampered by occurrence of Brucellosis and other pathogenic conditions which has been recognized as an important public health concern. The present study assessed the prevalence and risk factors of brucellosis, and presence of pathogenic bacteria in fermented cow milk obtained from respondents inKajiado Central Sub-County. The study design was a cross-sectional where 114 fermented milk samples were sampled from all wards in the sub-county. From each household, one fermented milk sample wasobtained and assessed for brucellosis using milk ring test. The sampled milk was also transported to the laboratory wherepresence of other bacterial microorganisms was determined using standard bacteriological methods. Questionnaires were administered at farm level to assess the risk factors associated with occurrence of brucellosis. The overall prevalence of brucellosis in the fermented cattle milk was 7.89% and was reported in milk obtained from Purko (10%), Dalalekutuk (9.6%) and Matapato North (8.6%) wards of the sub-county. Herding of cattle with other livestock and use of communal bulls in breeding had a close association ($p \leq 0.05$) with the prevalence of brucellosis. There were 7 bacterial species isolated from the milk and these included *Streptococcus* spp. (43.86%), *Lactobacillus* spp. (40.35%), *Klebsiella* spp. (15.79%), Coagulase Negative *Staphylococcus* (14.91%) *Pseudomonas* spp. (14.04%), *Staphylococcus aureus* (11.4%), *Salmonella typhi* (7.89%) and *Escherichia coli* (4.39%). The mean coliform count of the fermented milk ranged from 1.61×10^1 to 5.84×10^1 CFU/Ml. In conclusion, the study showed that brucellosis, which is zoonotic in nature, is prevalent in the study area. There is a need to create awareness of occurrence of brucellosis and other pathogenic bacteria in fermented milk by addressing the observed risk factors.

Keywords: *Brucella*spp.,cows, fermented milk, pathogenic bacteria,Kajiado, Kenya

1. INTRODUCTION

Brucellosis is ranked as a top priority zoonotic disease due to the socio-economic burden and amenability to control.However, as it is common with other neglected zoonotic diseases, establishing the true morbidity and socio-economic impact of the disease is a challenge because of misdiagnosis and underreporting (1). Studies in Kenya indicate the disease is endemic in humans and livestock although this varies with geographical region and livestock production system (2, 3). In domestic animals, the prevalence from pastoral and agro-pastoral herd range from 9.9-15% (4). Identifying potential risk factors of brucellosis among the most vulnerable populations; primarily rural livestock keeping communities is important in defining control and prevention strategies (3). In the pastoralist counties where the disease is endemic, there are limited scientific studies to determine the actual prevalence of brucellosis.

On the other hand, the ingestion of viable pathogenic bacteria along with food productssuch as milk leads to food borne infection (5). The bacteria previously isolated from milk of domestic animalsamongst the pastoralists include *Salmonella* spp., *Mycobacterium bovis, Corynebacterium*spp., *Clostridium perfringens, Yersinia enterocolitica, Coxiellaburnetii, Brucella* spp., *Staphylococcus* spp., *Campylobacter jejuni, Pseudomonas* spp., *Shigella* spp.and *Escherichia coli* (6,7,8). These microorganisms are usually associated with food borne diseases and outbreaks (9). The occurrence of these pathogenic bacteria in milk is a serious public health concernespecially where unpasteurized raw milk isdrunk(10). A recent study in Garissa County (Kenya) showed that prevalence of brucellosis in camel milk was 8% while other concurrent bacteria in the milk were *Pseudomonas* spp. (32.2%), *Salmonella* spp. (30.5%), *Staphylococcus* spp. (21.2%), *Escherichia coli* (8.5%) and *Shigella* spp. (7.6%) (6).

In KajiadoCounty (Kenya), the local Maasaipastoralistsdepends on cow's milk as a major source of proteins and other nutrients, where somedrinks it raw while others ferment it using traditional gourds to improve the nutritive value (11).Where fermented milk is prepared from raw milk, there are possibilities that it will still be containing pathogenic bacteria which were present in the raw milk(12).The local veterinary and medical officers indicate that risk factors for brucellosis are common but they have not been well documented. The current study aimed at determining the prevalence, risk factors of brucellosis, and other pathogenic bacteria in fermented cow milk from Kajiado Central Sub-County.

2. MATERIALS AND METHODS

2.1 Study area

The study was undertaken in Kajiado Central Sub-County (Kenya) which is a semi-arid area, having an average annual temperature of 18.9°C. This area receives about 500 mm of rainfall annually, most of it falling in April. The Sub-County human population is 161,862 and has area of 4,239.50km² with number of households being 37,238 (13). The wards involved in the study were Purko, Ildamat, Dalalekutuk and Matapato North wards. The latest census shows that the number of cattle in Kajiado Central Sub County was 95,534 (13).

2.2 Study design and sampling strategy

The study design was cross-sectional. The sample size was estimated using the formula by(14). The estimated brucellosis prevalence was 8.0% based on a previous study in a pastoralist area (15). Based on the formula, a minimum of 113 respondents were to be sampled. In the current study, 114 households were randomly selected from different wards in the sub-county. A sampling frame wasdeveloped with the help of animal health officers in charge of their respective wards to ensure all areas are well represented.

2.3 Questionnaire survey

The questionnaires were administered to respondents at farm level to gather information on biodata, livestock species kept, occurrence of livestock abortion, retained placenta in the livestock, consumption of unpasteurized milk, breeding system, herd size, herding system, and farm management activities.

2.4 Milk sample collection and determination of prevalence of brucellosis in fermented cattle milk

A total of 114 traditional fermented (*Kulenaoto*in local language) milk samples (15ml) were collected into sterile universal bottles, labeled, and then put in a cool box and transported to the JKUAT Microbiology laboratory where it was kept at temperature of 4°C.

The milk ring test was carried out for detection of brucellosis (16). Briefly, the test was conducted by addition of 30µl of antigen to 2ml of fermented cowmilk. The milk/antigen mixture was incubated for an hour at 37^{o}C, and a control sample was also subjected to similar conditions. A positive reaction was indicated by the formation of a dark blue ring above the white milk column. The test was considered negative if the color of underlying milk remained homogeneously dispersed in the milk column (17).

2.5 Determination of other bacterial microorganisms present in cow milk

Each milk sample was cultured in six different agars (namely Mannitol Salt Agar (Hi media Ltd), Eosin Methylene Blue (Hi media Ltd), Salmonella Shigella Agar (Hi media Ltd), Nutrient Agar (Hi media Ltd), Pseudomonas Isolation Agar (Hi media Ltd), Man Raogosa Sharpe Agar (Hi media Ltd), Blood Agar (Hi media Ltd), Mac Conkey Agar (Hi media Ltd) and Nutrient Agar (Hi Media Ltd)) as described previously (18). Cultures were then put into an incubator for 24-48 hours at 37ºC. Bacterial identification of isolated bacteria was done based on colony morphological features and gram staining reactions on pure cultures and biochemical tests such as indole, catalase, and oxidase tests.

2.6 Determination of coliforms in fermented cattle milk

The microbiological quality of fermented milk product was further assessed by spread plate method(19). The Violet Red Bile Glucose Agar was used to detect and enumerate Enterobacteriaceae in fermented milk. Briefly, 1 ml of the serial dilution was transferred to petri dishes using two plates for each dilution. The dishes were inverted and incubated for 24 hours at 37°C for coliform growth. The colony forming units (CFU) were then enumerated.

2.7 Data analysis and management

Datacollectedwascoded and entered in MS Excel (Microsoft, USA). They were analyzed using Statistical Package for Social Sciences (SPSS, Microsoft, USA)version YY to generate descriptive statistics. The Chi-square test and ANOVA were used to determine the relationship between prevalence of pathogenic bacteria and various risk factors. .A significance level of 0.05 was used for all statistical tests.

3. RESULTS

3.1 Characteristics and knowledge of brucellosis amongst respondents

The characteristics of the sampled farmers are shown in Table 1. Most (68%) of the respondents were women and 40% ofthese respondents had not attained formal education. The majority (81%) of respondents kept a combination of cattle and other livestock species. These animals were reared in an extensive system of production. Over half (50%) of the respondents were not aware of the occurrence of brucellosis within the community. In terms of risks to transmission of brucellosis, 50% of the respondents consumed fermented milk which was prepared from raw milk. Farmers processed the milk through boiling (30%), while others (34%) undertook a combination of both boiling and fermentation. For the fermentation of milk, the respondents used the traditional gourd (*Enkunkuri*) which was smoked using traditional herbs, such as *Lantana ukambensis* and *Oleaeuropaea* for fermentation and storage of the fermented milk.

A large number (70%) of respondents had experienced cases of retained placenta amongst their livestock. The placenta was either thrown away to the bush (30%), buried (14%) or given to the dogs (70%) for consumption. The respondents associated brucellosis in their herds as causing infertility (18%), abortions (30%), retained afterbirth (40%) and still births (26%).

Table 1: Characteristics and knowledge ofoccurrence of brucellosis amongst respondents from Kajiado Central Sub County(Kenya)

Attributes	Category	Frequency (N)	Percentage (%)
Gender	Male	46	40
	Female	68	60
Level of education	Never gone to school	40	35
	Primary	30	26
	Secondary	33	27
	Tertiary	11	12
Method of processing fresh milk	Boiling	30	26
	Fermentation of raw milk	50	50
	Boiling, Fermentation	34	28
Knowledge on brucellosis	Yes	64	56
	No	50	44
Cases of retained afterbirth in cattle	Yes	80	70
	No	34	30
Disposal of placenta	Throw away in the bush	30	26
	Bury	14	12

	Give to dogs	70	62
Effects of brucellosis in the animals	Infertility	18	16
	storm abortions	30	26
	Retained afterbirth	40	35
	Stillbirths	26	23

3.2 Prevalence of brucellosis

Out of the 114 cattle fermented milk examined, 9(7.89%) samples were positive forbrucellosis (Table 2). For the 9 positive cattle milk samples, 3 (10.0%) originated from Purko Ward, 1 (9.6%) from Dalalekutuk Ward and 5 (8.6%) from Matapato North Ward (Table 2).

Table 2: Prevalence of brucellosis as categorized by wards.

Ward	Total sampled	No. Positive	Prevalence (%)
Purko	30	3	10.0
Illdamat	15	0	0.0
Dalalekutuk	11	1	9.6
Matapato North	58	5	8.6
Total	**114**	**9**	**7.9**

The prevalence of brucellosis was the highest (14%) in milk from cows from farms where the communal bull was used for breeding. Lower prevalence (3.12%) was found in milk from cattle where individual bull was used. The prevalence of brucellosis was higher (8.5%) in milk from cows where the herd size was above 100 than those below 100 (4.16%). Herd size was found to be insignificant ($P \geq 0.05$) contributor of prevalence of brucellosis. The prevalence of brucellosis was found to be higher ($p < 0.05$) in milk from cows where cattle were herded with other livestock species (8.7%) as compared to those of not herded with other livestock (4.5%).

3.3 Bacteria isolated from fermented cattle milk

There were 7 bacterial species isolated namely *Streptococcu*s spp. (43.86%), *Lactobacillus* spp. (40.35%), *Klebsiella* spp. (15.79%), Coagulase Negative *Staphylococcus* (14.91%) *Pseudomonas* spp. (14.04%), *Staphylococcus aureus*(11.4%), *Salmonella typhi* (7.89%) and *Escherichia coli* (4.39%) (Table 3).

Table 3: Proportion of bacteria isolated from fermented cattlemilk (n = 114)

Isolate	Number of positive samples	Frequency (%)

Streptococcus spp.	50	43.86
Lactobacillus spp.	46	40.35
Klebsiella spp.	18	15.79
Coagulase Negative Staphylococcus	17	14.91
Pseudomonas spp.	16	14.04
Staphylococcus aureus	13	11.40
Salmonella typhi	9	7.89
Escherichia coli	5	4.39

3.4 Total coliform count isolated of locally collected fermented milk

The total coliform counts are shown in Table 4. The mean coliform count ranged from 1.61×10^1 to 5.84×10^1 CFU/Ml. Milk from Dalalekutukward had higher (3.55×10^1) coliform counts than those from the other wards.

Table 4: Total coliform count isolated from locally collected fermented milk as categorized from each Ward.

Location (Ward)	Number of Samples	Mean TCC(Cfu/ml)
Purko	30	5.84×10^1
IIdamat	15	1.61×10^1
Dalalekutuk	11	3.55×10^1
Matapato North	58	2.61×10^1

TCC:Total Coliform Count;Cfu/ml: Coliform Forming Unit by milliliter

4. DISCUSSION

The current study evaluated the prevalence of brucellosis and other pathogenic bacteria isolated from fermented cattle milk consumed by households in Kajiado Central Sub-County. The study showed the prevalence of brucellosis was 7.9% and varied according to the wards of origin. This rate is lower than those found previously byNdarathi&Waghela(4) in Kenya. Theyshowed that seroprevalenceof brucellosis in cattle from pastoral and agro-pastoral herd to range from 9.9-15%. Using the milk ring test, a study done by Chotaet al. (20) in West Pokot Kenya, reported the cattle milk to have a prevalence of between 21.9% and 21.2%. Other studies have been reported lower values of brucellosis prevalence in other parts of Kenya with different climatic conditions (21). The present study demonstrated considerable levels of exposure to*Brucella*spp. in lactating herds, a finding that shows the public health risk posed by the disease in the study areas.A recent study in camelsin Kenya showed the prevalence of brucellosis of 8% (5). The occurrence of brucellosis is influenced by various factors including tests used in diagnosis, livestock production systems, and variation of risk factors in different countries. In this study, the farmers reported thatinfertility, abortion, and reduced milk production was common amongst their cattle. These clinical signs have been associated with brucellosis in pastoral systems (22,23, 24).

In this study, the prevalence of brucellosis was found to be higher in farms using community bull than those using individual bull. Previous studies have shown that brucellosis was more prevalent on farms where communal bulls were used for breeding (25,26). Bull exchange for breeding and introduction of new animals to the herd are major risk factors for occurrence of brucellosis (27,28). Thecurrent study also showed that milk from households herding cattle and other livestock species had more brucellosis cases than those keeping cattle only. It is possible that herding of these animals together increases the chance of cross-species transmission of *Brucella* organisms (29).

In the present study,*Streptococcu*s spp., *Lactobacillus* spp., *Klebsiella* spp., Coagulase Negative Staphylococcus, *Pseudomonas* spp., *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*wereisolated from the traditional fermented Maasai milk. Apart from *Lactobacillus* spp., which is a commensal in milk and facilitates the process of fermentation, the rest of the bacteria could be pathogenic. A similar range of pathogenic bacteria were observed in studies carried out in Egypt (Wigdan et al., 2006) and Sudan (Abdelgadir et al.,1998) and the authors attributed the presence of these bacteria to absence of hygienic conditions during milking and cleaning of utensils.The prevalence of *Streptococcus* spp. in this study was close to that reported by Mathaara et al. (2004) (25%) in the same study area in Kenya. However, the prevalence of *Lactobacillus* spp. here was lower than that reported by the investigators in the same study (56.1%)(Mathaara et al., 2004).

The findings of this study indicate that the fermented milk harbored more coagulase negative Staphylococci as compared to *Staphylococcus aureus*. The occurrence of *Staphylococcus* spp. was high compared to those reported by others (30) in indigenous fermented milk from cattle in other parts of Kenya. Indigenous fermented milk often has high bacterial load due to low hygiene and sanitation practices during handling and processing (31,32). Other studies have reported the occurrence of low hygiene practices in African milk value chains (33, 34). The presence of *Staphylococcus* spp. in milk could also have originated from the use of milk from animals having clinical or subclinical mastitis. In Kajiado County, the prevalence of cattle mastitis is quite high and caused by a wide spectrum of pathogens including *Staphylococcus aureus*, *Streptococcus agalactae*, *E.coli*, *Klebsiella* spp.,*Pseudomonas* spp. (Ngotho et al., 2022, Mbidyo et al., 2022). Thus, it is possible that these bacteria could be found in fermented milk, if the latter is not well handled and processed.

In previous studies, the occurrence of *E. coli*in milk has been associated with fecal contamination and this often leads to milk spoilage. In many African countries, indigenous fermented milk is processed, transported and stored in reusable containers made from locally available materials such as wood fiber, clay pots and plastic containers (35).According to Mathara et al. (36), amongst the Maasais, sources of contaminations in *kulenaoto* traditional milk include starter cultures, the cow udder and the utensils used in preparation.

In our study, the mean coliform count ranged from 1.61×10^{1} to 5.84×10^{1} CFU/Ml which is close to that reported by Cissé et al. (37). From this study, the overall mean of the total

coliforms was below the reference point of 100 cell/ml which could be the effect of fermentation process where the level of lactic acid bacteria increases the acidity of the milk that makes growth conditions of microorganisms other than lactic acid bacteria increasingly unfavorable.

5. CONCLUSION

The overall prevalence of brucellosis in the study area was found to be close to that reported amongst other pastoralists in Kenya. Thus, in the study area, brucellosis could be affecting the animals significantly andrisks getting transmitted to humans through traditional fermented milk. The latter is preparedfrom raw milk and thus the high risks not only for brucellosis but also for pathogenic bacteria such as *Streptococcu*s spp., *Klebsiella* spp., *Staphylococcus* spp., *Pseudomonas* spp., *S. typhi* and *E. coli*which were observed in the study area. Public health education on milk hygiene and occurrences of milk-borne disease should be conducted in the study area with an aim of improving hygiene, milk preparation and modern breeding of cattle. Future studies on prevalence of brucellosis in humans in the study area should be done to determine the endemicity of the disease in the locality.

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