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ASSESSMENT OF THE RISK FACTORS ASSOCIATED WITH COLIFORM MASTITIS AMONG THE PASTORAL HERDS: TOWARDS IMPROVING THE QUANTITY AND QUALITY OF COW MILK PRODUCTION IN NIGERIA

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ABSTRACT

Mastitis is a highly significant disease in dairy cattle, with significant economic implications such as decreased milk production, increased treatment, labour, and veterinary costs. While various contagious and environmental pathogens can cause mastitis, coliform bacteria are the most common cause of fatal mastitis in all types of production systems. Unlike many of the common contagious mastitis pathogens, *Escherichia coli,* genus *Klebsiella* and *Enterobacter* are opportunistic bacteria found in faeces and are present in even the most well-managed dairy environments. These coliforms can invade and cause mastitis through various routes, including hematogenous or percutaneous routes, but the most common route is through the teat canal. Therefore, it is necessary and meaningful to conduct a study to assess the risk factors associated with coliform-caused mastitis among pastoral herds in Nigeria. In this study, 147 lactating cows from 30 pastoral herds were investigated. California Mastitis Test Reagent was used for the detection of subclinical mastitis from the milk samples, while physical inspection and palpation of the udder were used for the detection of clinical mastitis among the studied cows from the pastoral herds. Structured questionnaires were used to assess the risk factors predisposing the pastoral herds to coliform-caused mastitis. The results obtained revealed that 29 (19.7%) of cows investigated were positive for subclinical mastitis and 13 (8.8%) of the mastitis-positive milk samples harboured coliform bacteria (*E. coli* and *Klebsiella pneumoniae*). The results of the risk factors analysis revealed a significant relationship (P = 0.00) between poor hygienic methods of rearing, milking cows and improper manure management, and mastitis caused by coliform bacteria among the pastoral herds. Therefore, improved hygienic methods of rearing, milking and proper manure management of the cows will drastically reduce the increasing prevalence of mastitis caused by coliforms among the pastoral herds in Nigeria.

Keywords: Risk Factors, Coliform Mastitis, Pastoral Herds, *Escherichia coli, Klebsiella pneumoniae*.

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INTRODUCTION

In Nigeria, the principal source of domestic milk originates from the pastoral herds as highlighted by Yahuza (2021). The pastoral system is predominantly executed by the Fulani ethnic group, who oversee a minimum of 95% of the cattle population. These individuals are primarily semi-nomadic, migrating to regions where seasonal water resources facilitate the availability of pasture during the dry season. While some among them lead a nomadic lifestyle, constantly in motion in pursuit of water and pasture, others maintain a sedentary existence. The Fulani manage substantial herds and depend on milk and additional dairy commodities for their sustenance. Nevertheless, there are also settled Fulani communities as documented by Sani (2006) and Makolo et al. (2019).

Milk is a white liquid secreted by the mammary glands of mammals, serving as a primary nutritional source for calves (Pehrsson et al., 2000; Makolo et al., 2019). During the initial stages of lactation, milk contains colostrum that transfers the mother's antibodies to the offspring, thus imparting immunity and lowering the susceptibility to various diseases (Pehrsson et al., 2004). Globally, over six billion individuals partake in the consumption of milk and its derivatives (Hemme & Otte, 2010).

The potential for enhancing livestock production in Nigeria can be fully actualized only when adequate measures are implemented to safeguard the animals against the negative impacts of diseases like mastitis, as suggested by Shittu et al. (2012) and Makolo et al. (2019). As indicated by Sumathi (2008), ensuring profitability in animal products necessitates the effective management of animals, given that diseases continue to pose a significant challenge to achieving optimal livestock output. Consequently, a comprehensive strategy for mastitis management is imperative to address the increasing human population and the prevailing economic challenges in Nigeria.

The grazing practices for dairy cattle in Nigeria very depending on the region, climate, and cultural context. Some common practices include pastoral grazing, Semi-pastoral grazing and confined grazing. The pastoral system of rearing cows in Nigeria can predispose them to mastitis caused by coliforms due to several factors such as poor hygiene, dirty living conditions, inadequate milking practices, lack of veterinary care, poor manure management, lack of potable water sources, stress and weakened immunity by the cows among others (Suleiman et al., 2019; Makolo et al., 2020).

As reported by Sharma et al. (2007), bovine mastitis refers to an inflammation of the mammary gland characterized by both physical and chemical alterations in the milk, as well as pathological changes in the udder tissues. It is further described as an inflammation of the mammary gland resulting from bacterial infection and the presence of their toxins (Sharma et al., 2007). The presence of bacteria in the milk of affected cows leads to its unsuitability for human consumption, while also serving as a vehicle for the transmission of diseases such as tuberculosis, sore throat, Q-fever, brucellosis, and leptospirosis, among others, holding significant zoonotic implications (Sharma et al., 2007).

Changes in milk that are significant in cases of bovine mastitis include the presence of clots, milk discoloration, and elevated levels of leukocytes (Makolo et al., 2019). Additionally, clinical manifestations of bovine mastitis involve observable signs such as swelling, heat, udder pain upon palpation, and the presence of fibrotic quarters according to Suleiman et al. (2019).

Bovine mastitis is typically induced by bacterial pathogens, which can be categorized into two main groups. The first group consists of contagious pathogens, including *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Mycoplasma bovis*. The second group comprises environmental pathogens, such as *Streptococcus* species (*Streptococcus uberis* and *Streptococcus dysgalactiae*) and coliforms (*Klebsiella* spp., *Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *Enterobacter faecalis*, and *Enterobacter faecium*), as well as other gram-negative bacteria, particularly Pseudomonas, Serratia, and Proteus (Makolo et al., 2019).

Mastitis has a global impact, negatively affecting animal health, milk quality, and the economics of milk production. This problem is prevalent in all countries, including developed ones, and results in significant financial losses (Rodriguez et al., 2024; Panchal et al., 2024). Various authors agree that mastitis is one of the most widespread infectious diseases in dairy cattle and, from an economic perspective, it is the most damaging (Suleiman et al., 2019; Mbuk et al., 2016; Makolo et al., 2019; Rodriquez et al., 2024).

The term Coliform bovine mastitis is often misused to refer to mammary disease caused by all Gram-negative bacteria, but the Genera classified as Coliform include Escherichia spp, Klebsiella spp, and Enterobacter spp (Hogan & Smith, 2003; Elbayoumy et al., 2024). Specifically, *E. coli* and *Klebsiella pneumoniae* are the Coliform species most frequently identified in intramammary infections and cases of clinical mastitis (Makolo et al., 2020; Nery et al., 2024). In certain regions, the incidence of Coliform mastitis is on the rise, and the management of Coliform mastitis is a pressing concern due to the emergence of multiple drug-resistant coliforms (Nery et al., 2024). As a result, inadequate hygiene standards in rearing and milking may expose the animals to cases of Coliform mastitis (Maji et al., 2024) (Plates I, II and III). Consequently, this research aims to evaluate the risk factors linked to coliform bovine mastitis among pastoral herds in Nigeria.

MATERIALS AND METHODS

Study area

The research was conducted among the pastoral herds in Kaduna State, Nigeria, situated in the Northwest Geopolitical Zone of Nigeria. It is positioned between latitude 6^o and 11^o North and longitude 7^o and 44^o East, with an elevation of 608 m above sea level. The region experiences distinct wet and dry seasons within the Guinea Savannah and part of the Sudan Savannah in Nigeria. Kaduna State comprises 23 Local Government Areas (LGAs) and covers approximately 48,473.25 square kilometers (Makolo et al., 2020) (Fig. 1).

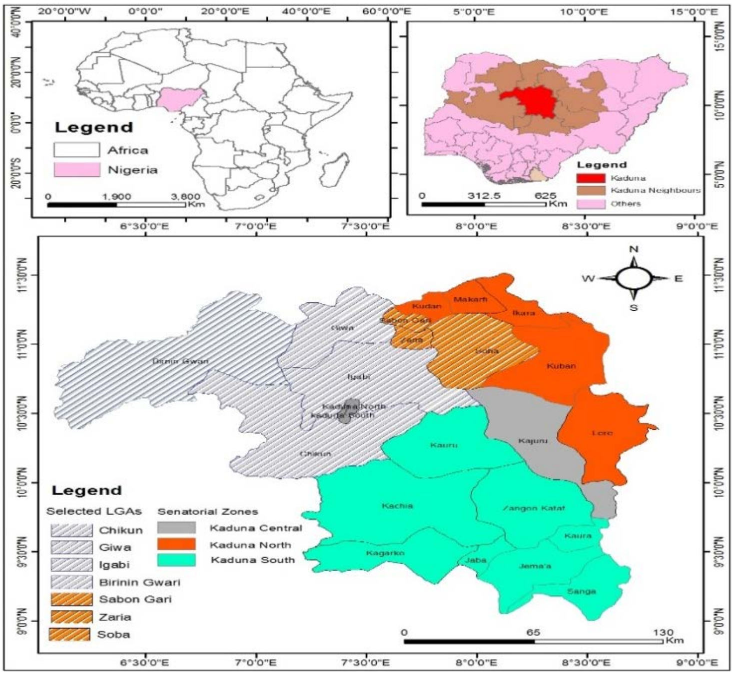

Figure 1. The map of Africa, Nigeria and Kaduna State showing the study area
[Source: Makolo et al. (2019)]

Kaduna State is divided into three Senatorial Zones: Kaduna North, Kaduna Central, and Kaduna South. It shares borders with Niger State to the West, Zamfara, Katsina, and Kano States to the North, Bauchi and Plateau States to the East, and FCT Abuja and Nasarawa State to the South. Agriculture serves as the main pillar of Kaduna State's economy, with more than 80% of the population actively involved in farming. Additionally, animal husbandry is a significant occupation, with cattle, sheep, goats, and pigs being the main animals reared (Makolo et al., 2019).

Study design

This study was conducted on thirty herds of cows in various Local Government Areas of Kaduna State, Nigeria, from December 2018 to June 2019. The sampling time spanned a period of six months (January 2019 to June 2019). The selection of the Local Government Areas and herds was purposeful, considering factors such as the presence of lactating cows not undergoing treatment, the farmers' willingness to take part in the study, and the accessibility of the location for sample transportation to the laboratory. Reference letters were assigned to the herds to maintain anonymity.

Determination of sample size

The prevalence of 10.3% reported in a study conducted in Kaduna State, Nigeria, by Mbuk et al. (2016) was used to determine the sample size. The formula reported by Sarmukaddam and Gerald (2004) was employed to calculate the sample size as follows:

$$n = \frac{Z^2 p(1-p)}{L^2}$$

Where: n = is the number of samples; Z = is the standard normal distribution at 95% confidence interval = 1.96; p = is the prevalence of previous study = 10.3% = 0.103; 1 – p = 1 – 0.103 = 0.897; L = is the allowable error, which is taken at 5% = 0.05.

$$n = \frac{1.96^2 \times 0.103 \times 0.897}{0.05^2} = 142$$

The determined sample size was one hundred and forty-two (142), which is the minimum sample size to be used. For this study, one hundred and forty-seven (147) lactating bovines in parts of Kaduna State were enrolled, and the corresponding number of raw milk samples were collected from them at the cow level.

Proportionate distribution of samples

Table 1. Proportional distribution of samples across the study population

S/N	Local Government Area/Settlements	No. of Herds	No. of Lactating Cows	No. of Cows Examined
1	Giwa (Settlement A)	5	50	24
2	Igabi (Settlement B)	3	30	15
3	Chikun (Settlement C)	4	39	19
4	Soba (Settlement D)	3	27	12
5	Zaria (Settlement E)	5	55	26
6	Sabongari (Settlement F)	4	40	19
7	Birnin Gwari (Settlement G)	6	68	32
	Total	30	309	147

The sample size of one hundred and forty-seven (147) cows was proportionately distributed across thirty herds studied, as shown in Table 1. This ensured that in herds with a higher number of lactating cows, more samples were collected, while in herds with a lower number of lactating cows, fewer samples were collected.

Detection of mastitis

The California Mastitis Reagent was used for the detection of mastitis according to the manufacturer's guidelines. In summary, 2 mL of combined milk from every quarter of the udder was combined with 2 mL of CMT reagent in the test paddle, gently agitated, and then monitored for coagulation or gel formation. The outcome was classified following the criteria outlined by Makolo et al. (2019) and Gayathri et al. (2024).

Isolation and genotypic identification of coliforms from mastitis positive milk samples

The coliform isolates were identified and characterized by both phenotypic and genotypic methods following standard bacteriological procedures as previously reported by Makolo et al. (2019) and Makolo et al. (2010).

Administration of questionnaires

Figure 2. Cows and their manure leaving together and no separate paddock for them

Figure 3. Poor manure management and environmental hygiene in one of the pastoral Herds investigated

Structured questionnaires were used to obtain information on the herds and mastitis, environment (Figs. 2, 3, 4), rearing methods and milking techniques that could influence the prevalence of coliform bovine mastitis among animals by observation and interviewing different herds' attendants and owners. The questionnaires were also used to obtain information on the common diseases affecting them as a result of consuming raw milk from their bovines. Nomadic Education Expert from National Commission for Nomadic Education (NCNE), Kaduna State Headquarters, who is proficient in writing and speaking English, Hausa and Fulani languages, assisted in the administration of questionnaires. In order to avoid inter-interviewer variations, one person permanently administered the questionnaire. The Key Informant Interview (Oral interview) method was used to obtain the needed information.

Figure 4. An uncovered open well as the major source of water in one of the pastoral herdsstudied

Analysis of data

The Microsoft Excel package was used for data management, computation of descriptive statistics and drawing graphs. The statistical analysis was carried out using Strata version 13.1 statistical software. Descriptive statistics utilized in this study involved simple frequency and percentages. The presence of a relationship between risk factors and the prevalence of subclinical mastitis, along with the presence of coliforms, was evaluated through Student's T-test at a 95% level of significance. A p-value of ≤ 0.05 was deemed statistically significant in all analyses.

RESULTS

Assessment of the breed and udder health management

Assessment of the breed and udder health management among the study population is presented in Table 2. In this study, all the cows investigated were Bunaji (White Fulani Cow) breeds. All 147 (100%) of the sampled cows were apparently healthy and without any symptoms of clinical mastitis. Furthermore, across the thirty herds of cows under this study, 25 (83.3%) of the pastoralists regularly checked the udder health, while 5 (16.7%) did not check regularly. When any of the animals fall sick among the herds investigated, 7 (23.3%) of the pastoralists report to a veterinarian immediately, 20 (66.6%) of the pastoralists report to a veterinarian after failed traditional methods of treatment, and 3 (10.0%) use traditional methods of treatment only. More so, this research revealed that when any of their animals is discovered to have mastitis, none of the herds will treat and keep the animal, 9 (30.0%) of the herds cull the animal after treatment, 21 (70.0%) of the herds cull the animal without treatment, and none of the herds kills the infected animal for consumption.

Table 2. Assessment of the breed and udder health management among the study population

Parameter (n = 147)	Frequency	Percentage (%)
Breed of the animals		
Bunaji (White Fulani)	147	100
Rahaji (Red Bororo or Abore)	0	0.0
Sokoto Gudali	0	0.0
Azawak (Tagama)	0	0.0
Wadara (Shuwa or Choa)	0	0.0
Others	0	0.0
State of health of the bovine		
Apparently healthy	147	100
Not healthy	0	0.0
Symptom of Clinical mastitis		
Apparently healthy	147	100
Not healthy	0	0.00
Regular check of udder health		
Yes	25	83.33
No	5	16.67
Action taken when animal falls sick		
Report to a veterinarian immediately	7	23.33
Report to a veterinarian after failed traditional method	20	66.66
Use traditional method only	3	10.00
Action taken when animal has mastitis		
Treat and keep the animal	0	0.00
Cull the animal after treatment	9	30.00
Cull the animal without treatment	21	70.00
Kill the animal	0	0.00

Assessment of the hygienic condition of the herds' environment

Assessment of the hygienic condition of the herds' environment and proper management of manure is depicted in Table 3. This result revealed that 9 (30.0%) of the herds use river water, 21 (70.0%) use well water, and none use borehole water for their domestic and animal rearing needs. It was observed in this study that all the herds were housed on the earth floor without bedding; feeding and sleeping in the same place. More so, none of the herds regularly removes the manure either daily or weekly, 3(10.0%) remove the manure monthly, and 27(90.0%) of the herds remove the manure annually, especially before the rainy season and use it for agricultural purposes or sell it to the farmers. None of herds practices wearing hand gloves before manure removal. However, 27 (90.0%) of the herds practice hand washing immediately after manure removal, while 3 (10.0%) of the herds do not practise immediate hand washing after manure removal. Furthermore, 3 (10.0%) of the herds had a separate calving paddock, while 27 (90.0%) did not. In 18 (60.0%) of the herds, feeding of the animals after milking is practised, while 12 (40.0%) of the herds do not practice such. This study confirmed that 9 (30.0%) of the herds were involved in the practice of feeding the animals in a feed pad, while 21 (70.0%) were fed in the paddock.

Table 3. Assessment of the hygienic condition of the herds' environment and proper management of manure among the study population

Parameter (n = 30)	Respondents	Percentage (%)
Water Source		
River	9	30.0
Well	21	70.0
Borehole	0	0.00
Type of bedding materials used		
Sawdust	0	0.00
Soil/sand	30	100
Grass	0	0.00
Others	0	0.00
Frequency of manure removal		
Daily	0	0.00
Weekly	0	0.00
Monthly	3	10.00
Yearly	0	0.00
Seasonally	27	90.0
Never	0	0.00
Wearing of hand gloves before manure removal		
Yes	0	0.00
No	30	100
Hand washing after manure removal		
Yes	27	90.00
No	3	10.00
Separate calving paddock for the animals		
Yes	3	10.00
No	27	90.00
Feeding of bovines after milking		
Yes	18	60.00
No	12	40.00
Fed in a feed pad or paddock		
Feed pad	9	30.00
Paddock	21	70.00

Assessment on the hygienic level of milking practice

Table 4 shows the assessment on the hygienic level of milking practice among the study population. Findings in this study revealed that all the herds studied practices the hand milking method. Furthermore, 11 (36.7%) of the herds practice regular hand washing before milking, while in 19 (63.3%), regular hand washing before milking was not practised. All the herds did not wash and dry the teats before milking. In all the herds, teat dipping/spraying before milking was not practised, and the milking utensils were not properly washed regularly. Within the study population, 21 (70.0%) herds replace the milking utensils annually, while 9 (30.0%) replace only when the existing ones are missing or broken.

Table 4. Assessment of the hygienic level of milking practices among the study population

Parameters (n = 30)	Respondents	Percentage (%)
Milking Method		
Hand	30	100
Machine	0	0.00
Hand washing before milking		
Yes	11	36.67
No	19	63.33
Teat cleaning before milking		
Yes	0	0.00
No	30	100
Teat dipping/spraying before milking		
Yes	0	0.00
No	30	100
Regular washing of milking utensils		
Daily	30	100
Weekly	0	0.00
Monthly	0	0.00
Yearly	0	0.00
Never	0	0.00
Regular Replacement of milking utensils		
Monthly	0	0.0
Annually	21	70.0
Conditionally (Only when broken or missing)	9	30.0

Assessment of the prevalence of subclinical mastitis and associated coliforms in relation to stage of lactation

The prevalence of subclinical mastitis and associated coliforms in relation to stage of lactation among the investigated cows is presented in Table 5. The result revealed that cows within the lactation stage of 0–5 months recorded the highest prevalence of subclinical mastitis (10.9%) and were more associated with coliforms (6.1%), while the lactation stage of 10 months and above had the lowest prevalence of subclinical mastitis (2.7%) and lowest isolates of coliforms (0.7%). Therefore, coliform bovine mastitis increased with the early stage of lactation (1–5 months).

Table 5. Assessment of the prevalence of subclinical mastitis and associated coliforms in relation to stage of lactation among the investigated cows

Stage of Lactation (n = 147)	No. (%) Positive for Subclinical Mastitis	No. (%) Negative for Subclinical Mastitis	No. (%) Positive for Coliforms	No. (%) Negative for Coliforms
1–5 Months	16 (10.9)	43 (29.3)	9 (6.1)	50 (34.0)
6–10 Months	9 (6.1)	43 (29.3	3 (2.0)	49 (33.3)
Above 10 Months	4 (2.7)	32 (21.8)	1 (0.7)	35 (23.8)
Total	29 (19.7)	118 (80.3)	13 (8.8)	134 (91.2)

Assessment of the prevalence of subclinical mastitis and associated coliforms in relation to age

Assessment of the prevalence of subclinical mastitis and associated coliforms in relation to age among the investigated cows is shown in Table 6. This result indicates that the investigated bovines within the age group of 6–8 and above 9 years had the highest subclinical mastitis prevalence of 13 (8.8%) each. Bovines within the age group of 9 years and above had the highest coliform bacteria prevalence of 6 (4.1%). Therefore, it was observed in this study that coliform bovine mastitis increases with increased age among the study population.

Table 6. Assessment of the prevalence of subclinical mastitis and associated coliforms in relation to age among the investigated cows

Age of cows (n = 147)	No. (%) Positive for subclinical mastitis	No. (%) Negative for subclinical mastitis	No. (%) Positive for coliforms	No. (%) Negative for coliforms
3–5 Years	3 (2.0)	24 (16.2)	2 (1.4)	25 (17.0)
6–8 Years	13 (8.8)	77 (52.4)	5 (3.4))	85 (57.8)
9 Years and above	13 (8.8)	17 (11.6)	6(4.1)	24 (16.3)
Total	29 (19.7)	118 (80.3)	13 (8.8)	134 (91.2)

Assessment of the common diseases affecting the pastoralists due to the consumption of raw milk

Table 7 shows the assessment of the common diseases affecting the pastoralists due to the consumption of raw milk among the study population. The result revealed that all the respondents admitted that they consume raw milk without either being boiled or pasteurized. Consequently, 13 (43.3) of herds had experienced illness after consuming raw milk, and diarrhoea was suffered by 17 (56.7%) of the herds as the major and common disease ever experienced in association with milk consumption.

The degree of relationship between the selected potential predisposing risk factors and the prevalence of subclinical mastitis

The degree of relationship between the selected potential predisposing risk factors and prevalence of subclinical mastitis is shown on Table 8. The results showed that all the risk factors examined were significantly associated with the prevalence of subclinical mastitis ($p \leq 0.0001$) among the pastoral herds studied. However, lack of thorough and regular hand washing after manure removal had the highest level of association with the prevalence of subclinical mastitis among the study population (OR = 2.285714, 95% CI =

0.6029294–8.665176), followed by lack of a separate paddock for the bovines (OR = 2.117647, 95% CI = 0.1958399–22.89844). Regular check of udder health showed the lowest degree of association with the prevalence of subclinical mastitis among the study population (OR = 0.8024845, 95% CI = 0.827917–7.778333).

Table 7. Assessment of the common diseases affecting the pastoralists among the study population

Parameter (n = 30)	Frequency	Percentage
Regular consumption of milk from bovines		
Yes	23	76.6
No	7	23.3
State of milk consumed		
Boiled/Pasteurized	0	0.0
Not boiled/Not pasteurized	30	100
Illness after raw milk consumption by any family member		
Yes	17	56.7
No	13	43.3
Diseases experienced		
Sore throat	0	0.0
Thrush	0	0.0
Tuberculosis	0	0.0
Typhoid	0	0.0
Diarrhoea	17	56.7
No disease experienced	13	43.3

Table 8. Predisposing risk factors associated with the prevalence of subclinical mastitis among the cows investigated

Risk Factor (n = 30)	P-Value	Odds Ratio	Confidence Interval at 95%
Milking method	0.0001	1.0	
Hand washing before milking	0.0001	1.22807	0.2506517–6.016941
Aseptic teat cleaning before milking	0.0001	1.0	
Thorough washing of milking utensils	0.0001	1.0	
Regular replacement of milking utensils	0.0001	1.345833	0.152546–11.87358
Separate paddock for the animals	0.0001	2.117647	0.1958399–22.89844
Heifers and animals having the same paddock	0.0001	1.0	
Bedding materials used	0.0001	1.0	
Regular check of udder health	0.0001	0.8024845	0.0827917–7.778333
Appropriate action taken when bovines fall sick	0.0001	1.0	
Source of water	0.0001	0.9074852	0.3783593–2.17658
Regular removal of manure	0.0001	1.0	
Wearing hand gloves during manure removal	0.0001	1.0	
Thorough hand washing after manure removal	0.0001	2.285714	0.6029294–8.665176
Hand washing with antiseptics after manure removal	0.0001	1.0	

Results obtained revealed that all the examined risk factors were statistically significant ($p \leq 0.0001$). However, lack of separate paddock for the cows showed the highest degree of association with the prevalence of coliforms (OR = 2.117647, 95% CI = 0.1958399–22.89845), followed by source of water used (OR = 1.680336, 95% CI = 0.5153267–5.479105) while regular check of udders showed least degrees of association (OR = 0.1498397, 95% CI = 0.0049188–4.564563) with the prevalence of coliforms in the mastitis milk of the animals studied.

Assessment of the relationship between the selected potential predisposing risk factors and prevalence of coliforms associated with bovine mastitis

Table 9 establishes the degree of relationship between the selected potential predisposing risk factors and prevalence of coliforms associated with bovine mastitis. Results obtained revealed that all the examined risk factors were statistically significant ($p \leq 0.0001$). However, lack of separate paddock for the cows showed the highest degree of association with the prevalence of coliforms (OR = 2.117647, 95% CI = 0.1958399–22.89845), followed by source of water used (OR = 1.680336, 95% CI = 0.5153267–5.479105) while regular check of udders showed least degrees of association (OR = 0.1498397, 95% CI = 0.0049188–4.564563) with the prevalence of coliforms in the mastitis milk of the animals studied.

Table 9. Predisposing risk factors associated with the prevalence of coliforms in the milk samples of cows investigated

Risk Factors (n = 30)	P-Value	Odds Ratio	Confidence Interval at 95%
Milking method	0.0001	1.0	
Hand washing before milking	0.0001	1.454545	0.1211602–17.46203
Aseptic teat cleaning before milking	0.0001	1.0	
Thorough washing of milking utensils	0.0001	1.0	
Regular replacement of milking utensils	0.0001	0.4174107	
Separate paddock for the animals	0.0001	2.117647	0.1958399–22.89845
Heifers and animals having the same paddock	0.0001	1.0	
Bedding materials used	0.0001	1.0	
Regular check of udder health	0.0001	0.1498397	
Appropriate action taken when bovines fall sick	0.0001	1.0	
Source of water	0.0001	1.680336	0.5153267–5.479105
Regular removal of manure	0.0001	1.0	
Wearing hand gloves during manure removal	0.0001	1.0	
Thorough hand washing after manure removal	0.0001	1.435897	0.2134205–9.660748
Hand washing with antiseptics after manure removal	0.0001	1.0	

DISCUSSION

Statistical analysis of data obtained from the assessment of risk factors in this study revealed that all the herds investigated practised hand milking methods, which was the major predisposing factor responsible for the prevalence of coliform bovine mastitis recorded. Poor rearing and milking hygiene were evident among the study population, which contributed greatly to the presence of coliforms in the samples. This study revealed that 63.3% of herds do not practice hand washing before milking, all the herds do not properly clean the teat before milking, all the herds do not practice teat dipping/spraying before milking, all the herds do not replace milking utensils regularly, animals are left on the earth floor without bedding material in all the herds, 90.0% of the herds do not remove the manure regularly, and all the herds do not have potable water sources. This exposes the cows' teat ends to constant contact with manure and contaminated earth floor that could facilitate the entry of coliforms and other pathogenic organisms into the mammary gland through the teat canal.

The results are consistent with the research conducted by Maji et al. (2024), which indicated that poor hygienic methods of milking and rearing the animals are the major risk factors associated with mastitis among the pastoral herds. Similarly, Abdurrahman (2006) found in a study conducted in Ethiopia that the nomadic system of cattle rearing and inadequate hygiene during the milking process were identified as risk factors for the development of coliform mastitis.

Furthermore, the association between poor manure management among the herds in this study and the prevalence of coliforms mediated mastitis was statistically significant ($p = 0.0001$). This might not be unconnected with the presence of *E. coli* in the bovine milk, which inhabits the intestine of warm-blooded animals and can lead to faecal contamination as they could be transferred from manure and contaminated soil to the mammary gland through the teat canal, that are in continuous contact with the contaminated environment or milkers' hands.

Also, the association between the earth floor being used without proper bedding materials and the prevalence of coliforms mediated mastitis among the study population was statistically significant ($p = 0.0001$). Hence, the dominance of *K. pneumoniae* over other coliforms in this study could be traceable to the use of earth floor as bedding material in all the herds studied. This finding conforms to the report of Makolo et al. (2019) and Tabassum et al. (2024) that *K. pneumoniae* are natural inhabitants of the soil, while *E. coli* are found in the intestine of warm-blooded animals, which can be passed out along with their faeces.

In this study, cows within the lactation stage of 1–5 months recorded the highest prevalence of subclinical mastitis (10.9%) and also harboured the highest number of coliform isolates (6.1%), while bovines at the late stage of lactation had the lowest prevalence of subclinical mastitis (2.7%) and coliform isolates (0.7%). Hence, this finding revealed that the prevalence of coliform bovine mastitis was highest at early lactation and decreased with increasing stage of lactation. This might be due to decreased neutrophil functions and delayed migration of neutrophils to the mammary gland at early lactation, as reported by Mehrzad et al. (2002). This disagrees with the studies of Chauhan et al. (2024), who reported that cows at the late stage of lactation are more susceptible to mastitis.

This study also revealed that the age group of 6 years and above recorded the highest subclinical mastitis prevalence of 8.8% and 4.1% for coliform bacteria, while cows within the ages of 3–5 years had the lower rate of 2.0% and 1.4% for subclinical mastitis and coliform bacteria, respectively. This finding agrees with the previous studies by Bessembayeva et al. (2024), who established that increasing age keeps the teat canal permanently dilated due to repeated milking and lactations, thus facilitating the entry of pathogens into the teat canal and causing subclinical intramammary infections.

In this study, 56.7% of the studied herds reported diarrhoea as the common disease ever experienced. The herdsmen and other consumers of raw bovine milk believe that it is healthier and tastes better, but a public health threat exists, as evident in this study. The findings of Nayak et al. (2024) confirmed that consuming raw milk can be unsafe due to the presence of pathogens that can cause illnesses such as diarrhoea, tuberculosis, diphtheria, typhoid, and streptococcal infections. The results of the Microgen Identification System in this study further support these concerns, revealing that all coliform bacteria isolated were of human origin.

Furthermore, research conducted in Kaduna State over the last ten years (Mbuk et al., 2016; Umoh et al., 2015) have shown a decreasing prevalence of coliform mediated mastitis among the pastoral herds. This might not be unconnected to the increasing education and awareness programmes by the National Commission for Nomadic Education (NCNE). This commission has established schools in almost all the herdsmen settlements in Kaduna State, studied with trained teachers. This is greatly increasing their knowledge of good hygienic practices and improved living conditions.

CONCLUSION

According to the findings obtained in this research, lack of regular and thorough hand washing before milking, lack of aseptic teat cleaning, lack of thorough and regular washing of milking utensils, irregular replacement of milking utensils, lack of separate paddock for the bovines, heifers and bovines sharing the same paddock, poor manure management and exposed sources of water to environmental contamination were the significant risk factors associated with the prevalence of subclinical mastitis and presence of coliforms in the milk of pastoral herds studied. However, among the risk factors significantly associated with the prevalence of subclinical mastitis and presence of coliforms, lack of thorough and regular washing of hands after manure removal and lack of separate paddock for the animals showed the highest association, while regular check of udder health showed the lowest association, respectively. This study also revealed that the prevalence of coliform bovine mastitis was higher at the early stage of lactation and decreased with increasing lactation stage. Also, older bovines had a higher prevalence of coliform mastitis than the younger ones.

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