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BACTERIAL ETIOLOGIES OF SUBCLINICAL MASTITIS IN COWS JOS METROPOLIS, NIGERIA

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ABSTRACT

This study was conducted to investigate the prevalence of subclinical mastitis and its contagious and environmental bacterial causes in dairy cows in Jos Metropolis of Plateau State. A total of 208 milk samples were collected aseptically from 52 lactating cows and subjected to the California Mastitis Tests (CMT). Milk samples were transferred into peptone water broth for enrichment, followed with bacteriological assays and biochemical identification of bacteria. Using R Commander version 3.6.2, data such as: breed, age, parity, lactation, and management system of cows were collected, and analysed to determine their relationship with bovine subclinical mastitis. Out of 208 quarter milk samples collected from 52 cows, 35.09 % showed subclinical mastitis. While the Cross-breed cows had 15 % subclinical mastitis recorded, the White Fulani had 78.13 %. Cows of the age 3 to 5 years had the highest prevalence of mastitis, while cows with 4 to 6 calves showed the highest prevalence in the parity category. The early lactation stage of these cows showed the highest prevalence. The environmental bacteria isolated where *E. coli, K. pneumoniae, K. oxytoca, C. freundii, K. aerogenes,* and *Proteus* sp. with *E. coli* having the highest frequency of 40.86 %, followed by *K. Pneumoniae* with 17.79 %. The contagious bacteria isolated were *S. aureus, S. agalactiae,* and *Corynebacterium* sp. with *S. aureus* occurring most with 69.71 %. This study concluded that the prevalence of subclinical mastitis is high in cows in the study area. It was also discovered that the breed of cow, age of cow, parity number, stage of lactation and husbandry systems have an impact on subclinical mastitis. *S. aureus* with the highest frequency, indicated that contagious microbes have more effect on subclinical mastitis than environmental microbes.

Key words: bovine; CMT; contagious bacteria; environmental bacteria; subclinical mastitis

INTRODUCTION

Mastitis is a multi-etiologic disease of the mammary gland, characterized by reduction in milk production and considered as an economically important disease in the diary subsector in developed and developing nations [19]. Mastitis is the most economically significant disease of dairy cattle, accounting for 38 % of the total direct costs of the common production diseases [3]. Mastitis is a global problem as it adversely affects animal health, quality of milk, and economics of milk production, and every country, including developed ones, suffer substantial financial losses [15]. It is the most critical deadly disease of dairy animals. It is responsible for heavy economic losses due to reduced milk yield (up to 70 %), milk discard after treatment (9 %), cost of veterinary services (7 %), and premature culling (14 %) [16].

Any microbe that can opportunistically invade tissues and cause infection can cause mastitis. Bacteria are the most common culprits of microbial mastitis. They are classified distinctly into two groups, viz., contagious and environmental. Contagious mastitis involves introducing pathogens during the milking processes via milking equipment (malfunctioning pulsation and vacuum controllers) or milkers' hands. Some of the more commonly listed species that exploit this transmission mode include *Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium bovis,* and *Mycoplasma* spp. Environmental mastitis is caused by pathogens found in the habitat of the cow, such as: soil, plant material, manure, bedding, or a contaminated water source. Frequently, isolated causative pathogens contributing to environmental bovine mastitis include streptococci and gram-negative bacteria, such as coliforms (*Escherichia coli, Klebsiella* spp., and *Enterobacter*) [5]. Studies have shown that coliform mastitis, apart from resulting in agalactia, can cause substantial loses to producers [12]. Cows affected with coliform causing mastitis can show clinical signs and progress to a per-acute form that can lead to the animal's death. These cases have a sudden onset, with swollen and hot quarters and yellowish, watery milk that contains clots and flakes. Severe cases occur in early lactation, particularly around calving [8]. Both contagious and environmental mastitis can result in subclinical and clinical forms with profound economic implications for the dairy industry in developing countries.

Most important predisposing factor increasing the chance of mastitis is poor hygienic management and unhygienic housing systems. The more the presence of bacteria in areas where cows are kept, the higher the chances of infection. Just after milking it takes about 20 minutes before the teat canal is fully closed, so lack of clean floors and resting places increases the chance of contracting mastitis. Another risk factor is milk yield; cows with a high milk yield will develop mastitis more easily [6]. Their udder tissue is more active and has longer milking time. This makes the teat canal open for a longer period of time. High milk production is also connected with more energy demand which possibly decreases the resistance against infections. Poor milking technique and unhygienic milking procedures are risk factors [6]; force-milking can cause injury to the teat and teat canal making the entrance of bacteria to the udder easier. Lack of cleaning utensils, the hand of the milker and the udder, as well as, incomplete milking are other factors which may cause the multiplication of bacteria.

Therapy is given on the premise that productivity gains will outweigh treatment costs after the elimination of infections. In the case of contagious pathogens, elimination may also decrease the reservoir of infection for previously noninfected cows. By intra-mammary administration, antibacterial agents are used in the treatment of mastitis [10]. For instance, in *Streptococcus agalactiae*, all four quarters of infected cows should be treated to eliminate the pathogen and prevent possible cross-infection of a noninfected quarter. Cure rates can often be 75–90 %. The labelled use of commercial intramammary products that contain amoxicillin, penicillin, and erythromycin are as efficacious as procaine penicillin G infusions derived from multiple-dose vials [10]. Most other streptococci also display *in vitro* susceptibility to numerous antibacterial agents, especially β-lactam drugs.

The objective of this study was to determine the prevalence of subclinical mastitis and its contagious and environmental causes in dairy cows in Jos Metropolis.

MATERIALS AND METHODS

Study area

The research was carried out in Jos Metropolis covering three Local Government Areas in Plateau State viz., Jos East, Jos North, and Jos South from June 2019 to May 2020. It has a total area of 1,821 km^2 and a population of 821,618 people. Its headquarters is located in the city of Jos having coordinates of 09°55′00″N 08°53′25″E. At an altitude of 1,217 m (3,993 ft) above sea level, Jos' climate is closer to temperate than that of the vast majority of Nigeria. Jos receives about 1400 millimetres of rainfall annually. Due to security issues bordering on herders/farmers clashes, the dairy farms involved in this study were selected by convenience.

Study population and sample collection

The three farms that were selected for this study from Jos Metropolis had 54, 63 and 68 dairy cows respectively. Two types of dairy cows were sampled, namely White Fulani and cross-bred cows (Fig. 1 and 2). White Fulani cows are medium sized animal with long horns. They are easily characterized by their long and lyre-shaped horns. They weigh about 250–380 kg while the cross-bred weigh 450 kg on the average. A White Fulani cow produces about 6 litres of milk per day while the cross-bred cows can produce 30 litres per day. At each farm, cows included in this study were selected by a blind random sampling technique. A total of 208 milk samples were collected aseptically from 52 lactating cows based on the minimum sample size according to Anueyiagu et. al. [1]. During the farm visits for sample collection, 52 structured questionnaires were administered to collect information concerning the herds such as: breed, age, parity, lactation stage, blind-udder, previous history of mastitis, and husbandry system.

Ethical clearance

In line with the guidelines of the Animal Use and Care Committee (AUCC) of National Veterinary Research Institute (NVRI), Vom, Nigeria, the procedure involved in the milk sampling was non-invasive and does not inflict pain or discomfort to the animals, Therefore, no ethical approval was required.

Fig. 1. White Fulani cow

Detection of subclinical mastitis

To eliminate debris and bacteria from the environment on the udder and teat, cows were restrained and each udder was rinsed clean with water and swabbed with 70 % ethanol. The presence of subclinical mastitis was determined by the California Mastitis Test (CMT) and the severity of the infection by the nature of coagulation and viscosity of the mixture (milk and CMT reagent) [14]. Briefly, a squirt of milk sample from each quarter of the udder was deposited in each of the cups on the CMT paddle, and an equal amount of 3 % CMT reagent was added to each cup and thoroughly mixed. Negative reactions were reported as 0 and trace, whereas positive reactions were graded as +1, +2 [2].

Bacteriological isolation and identification

The milk positive for the California Mastitis Test (CMT) samples were bacteriologically examined according to the procedures given by Anueyiagu et al. [2]. One ml of each milk sample was inoculated into 9 ml peptone water broth for enrichment and incubated at 37 °C

Fig. 2. Cross-bred cow

overnight. Thereafter, a loopful of the broth culture was streaked on blood agar (BA), mannitol salt agar (MSA), MacConkey agar (MCA) and eosin methylene blue agar (EMB).

The isolates were identified using, colony morphology, Gram staining and biochemical tests. The confirmatory screening was conducted on presumptive Gram negative bacteria using Oxiod™ Microbact™ GNB 24E. Coagulase test and CAMP tests were used on Gram positive bacteria.

Statistical analysis

Data collected were analysed using R Commander Software (Version 3.6.2) to conduct Chi-squared test (χ^2), and p values $P < 0.05$ were considered statistically significant.

RESULTS

Out of the total of 208 milk samples collected aseptically from lactating cows, 35.09 % showed subclinical mastitis (Table 1). Clinical mastitis was not observed in this work. The back left and back right quarters of cows sampled showed higher prevalence than the front quarters (Table 2). There was statistical significance in the quarter-wise prevalence of the subclinical mastitis, with p values less than 0.05. If not appropriately managed, the cows with these positive results might develop into either stronger CMT positive or clinical mastitis.

Table 1. Prevalence of clinical and subclinical mastitis of cows

Forms of mastitis	No. of positive Cow level	Prevalence [%] (n = 52)	No. of positive Quarter level	Prevalence [%] (n = 208)
Clinical	0	0	0	0
Subclinical	28	53.84	73	35.09
Total	**28**	**53.84**	**73**	**35.09**

Data from: breed, age, parity, lactation, floor type, and husbandry system were studied from the questionnaires collected. While the cross-breed cows had 15 % subclinical mastitis recorded, the White Fulani had 78.13 % (Table 3). The age 3 to 5 had the highest prevalence of mastitis, while cows with 4 to 6 lactations showed the highest prevalence in the parity category (Table 3). The early lactation stage of these cows showed the highest prevalence. The nomadic system of rearing showed a lesser prevalence of mastitis than the intensive system (Table 3).

The environmental bacteria isolated were: *E. coli, K. pneumoniae, K. oxytoca, C. freundii, K. aerogenes*, and *Proteus* sp. with *E. coli* having the highest frequency of 40.86 %, followed by *K. Pneumoniae* with 17.79 % (Table 4). The contagious bacteria isolated were *S. aureus, S. agalactiae*, and *Corynebacterium* sp. with *S. aureus* occurring the most. There was statistical significance in subclinical mastitis in both groups of bacteria with P values less than 0.05 (Table 4).

DISCUSSION

In this study, the overall prevalence of subclinical mastitis was 35.09 % and was corroborated by the results pre-

Table 2. Quarter-wise prevalence of subclinical mastitis in lactating cows

Quarters	Number tested	CMT positive [%]			χ^2	P-value
		Trace	1+	2+		
FR	52	12(23.08)	2(3.85)	6(11.54)	29.443	0.000***
FL	52	9(17.31)	4(7.69)	6(11.54)		
BR	52	13(25)	18(34.62)	11(21.15)		
BL	52	9(17.31)	20(38.46)	6(11.54)		
Total	**208**	**43(20.67)**	**44(21.15)**	**29(13.94)**	–	–

FR – Front right quarter; FL – Front left quarter; BR – Back right quarter; BL – Back left quarter;
Trace – 200,000–400,000 SCC; 1+ – 400,000–1,200,000 SCC; 2+ – 1,200,000–5,000,000 SCC

Table 3. Risk factors associated with bovine mastitis in Jos

Risk factors	Category	No. of cows examined	No. of cows affected	Prevalence [%]	χ^2	P-value
Breed	White Fulani	32	25	78.13	19.734	0.000***
	Cross	20	3	15.00		
Age	3–5 years	7	5	71.43	2.079	0.354
	5–9 years	19	8	42.11		
	10–14 years	26	15	57.69		
Parity	1–3 calves	23	10	43.48	3.079	0.214
	4–6 calves	20	11	55.00		
	7–12 calves	9	7	77.78		
Lactation stage	1–3 months	25	14	56.00	3.166	0.205
	4–7 months	21	9	42.86		
	8–12 months	6	5	83.33		
Husbandry system	Nomadic	37	19	51.35	0.321	0.571
	Intensive	15	9	60.00		

Table 4. Frequency of environmental and contagious aetiologies

Bacterial species	No positive [%] (n = 208)	$\chi2$	P-Value
	Environmental bacteria		
E. coli	85 (40.86)	253.41	0.000***
K. pnuemonie	37 (17.79)		
K. oxytoca	15 (7.21)		
C. freundii	2 (0.96)		
K. aerogenes	3 (1.44)		
***Proteus* sp.**	2 (0.96)		
Total	**144**	–	–
	Contagious bacteria		
S. aureus	145 (69.71)	364.81	0.000***
S. agalactiae	2 (0.96)		
***Corynebacterium* sp.**	1 (0.48)		
Total	**148**	–	–

sented in a systematic review of 14 articles by A n u e y i- a g u et al. [1] which recorded a prevalence range between 26.9 and 57.8 % for subclinical mastitis. This high prevalence in this work necessitates prompt action by policy makers and farmers to reduce economic downturn in the dairy industry.

Statistically, the prevalence of mastitis among the breed types of ruminants was significant. This may be due to a better adaptive ability of the White Fulani breeds to environmental factors compared to cross breeds that may have traits of foreign breeds imported into the nation from temperate environments. The findings in this study agreed with reports by other authors that stated that exotic breeds like Friesian breeds were more susceptible to bovine mastitis than the indigenous breeds [7].

Cows between the age of 3 and 5 years showed a higher prevalence of subclinical mastitis than older ones. The report of C o m p t o n et al. [7] disagreed with the results of this study. This may be due to the low immunity in younger animals compared to the older ones. However, the result of this study was in agreement with the report of S h i t t u et al. [17], who claimed to have younger cows more susceptible to mastitis. Cows that have had more calves, in this study, showed a higher prevalence than those with lesser number of calves [13]. Early lactation stages and nomadic husbandry system showed higher prevalence as well [2, 18].

This study showed that from 208 samples, environmental udder pathogens in 144 cases of Sub-clinical Mastitis (SCM) were identified as: *E. coli, K. pneumoniae, K. oxytoca, C. fruendii, K. aerogenes*, and *Proteus* sp. This was in close agreement with K a l l a et al. [11], who reported: *Escherichia coli, Klebsiella spp., Enterobacter* spp., *Citrobacter, Serratia* and *Proteus* as the major mastitogens [9]. These bacteria are generally found in high concentrations in organic matter, such as bedding and manure (environment). Therefore, from an epidemiologic standpoint, the primary source of infection for some pathogens in this study was environmental. These bacteria invade the udder through the teat sphincter when teat ends come in contact with coliform bacteria. Once they have entered the mammary gland, they either multiply rapidly or remain dormant. However, 148 contagious bacteria such as *S. aureus, S. agalactiae*, and *Corynebacterium* sp. were identified with *S. aureus* having the highest prevalence (69.71 %). This was in line with the work of B i r h a n u et al. [4] where *S. aureus* had the highest prevalence of 44.9 % followed by *Streptococcus* sp. with 25.3 %.

CONCLUSIONS

This study concluded that the prevalence of subclinical mastitis was high in cows in the study area. It was also discovered that: the breed of cow, age of cow, parity number, stage of lactation and husbandry systems have impact on subclinical mastitis. *S. aureus* with the highest frequency indicated that contagious microbes had more effect on subclinical mastitis than environmental microbes.

REFERENCES

1. **Anueyiagu, K. N., Shalangwa, I. B., Ayabimpe, G., 2019:** Prevalence of coliform bacteria in bovine mastitis in Africa: A systematic review. *Cross Current Int. J. Agri. Vet. Sci.*, 6, 2, 96–105. DOI: 10.36344/CCIJAVS.
2. **Anueyiagu, K. N., Ayanbimpe, G., Ikeh, E., 2020:** Bovine mastitis due to coliform bacteria, and susceptibility to antibiotics, Nigeria. *Int. J. Vet. Sci. Anim. Husb.*, 6, 1, 054–061.
3. **Azooz, M. F., El-Wakeel, S. A., Yousef, H. M., 2020:** Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms. *Vet. World.*, 13, 9, 1750–1759. DOI: 10.14202/vetworld.2020.1750-1759.
4. **Birhanu, M., Leta, S., Mamo, G., Tesfaye, S., 2017:** Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu town, Ethiopia. *BMC Res. Notes*, 10, 1, 767. DOI: 10.1186/s13104-017-3100-0.
5. **Carrillo-Casas, E. M., Miranda-Morales, R. E., 2012:** Bovine mastitis pathogens: Prevalence and effects on somatic cell count. In **Chaiyabutr, N.:** *Milk Production–An Up-to-date Overview of Animal Nutrition, Management and Health*. INTECH, NY, 359–374.
6. **Cheng, W. N., Han, S. G., 2020:** Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatments–A review. *Asian-Australas. J. Anim. Sci.*, 33, 11, 1699–1713. DOI: 10.5713/ajas.20.0156.
7. **Compton, C. W. R., Heurer, C., Parker, K., McDougall, S., 2007:** Risk factors for peripartum mastitis in pasture-grazed heifers. *J. Dairy Sci.*, 90, 4171–4180.
8. **Garcia, A., 2004:** *Contagious vs. Environmental Mastitis*. Extension extra; College of Agriculture and Biological Sciences, South Dakota State University, USDA. Accessed 19th July, 2021 on http://www.dairyweb.ca/Resources/US WebDocs/MastitisPathogens.pdf.
9. **Garedew, L., Berhanu, A., Mengesha, D., Tsegay, G., 2012:** Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. *Biomedical Central Public Health*, 12, 950.
10. **Hossain, M., Paul, S., Hossain, M., Islam, M., Alam, M., 2017:** Bovine mastitis and its therapeutic strategy doing antibiotic sensitivity test. *Austin J. Vet. Sci. Anim. Husb.*, 4, 1030.
11. **Kalla, D. J. U., Butswat, I. S. R., Mbap, S. T., Abdussamad, A. M., Ahmed, M. S., Okonkwo, I., 2008:** Microbio-

logical examination of camel (*Camelus dromedarius*) milk and sensitivity of milk micro-flora to commonly available antibiotics in Kano, Nigeria. *Savannah J. Agric.*, 3, 1–8.

12. **National Mastitis Council (NMC), 2000:** *Recommended Mastitis Control Program*. National mastitis Council Madison, Wisconsin, USA, 45–60.
13. **Sarba, E. J., Tola, G. K., 2017:** Cross-sectional study on bovine mastitis and its associated risk factors in Ambo district of West Shewa zone, Oromia, Ethiopia. *Vet. World*, 10, 4, 398–402. DOI: 10.14202/vetworld.2017.398-402.
14. **Schukken, Y. H., Gunther, J., Fitzpatrick, J., et al., 2011:** Host-response patterns of intramammary infections in dairy cows. *Vet. Immunol. Immunopathol.*, 144, 270–289.
15. **Sharma, N., 2007:** Alternative approach to control intramammary infection in dairy cows: A review. *Asian J. Anim. Vet. Adv.*, 2, 50–62.
16. **Sharma, N., Rho, G. J., Hong, Y. H., Kang, T. Y., Lee, H. K., Hur, T. Y., et al., 2012:** Bovine mastitis: An Asian perspective. *Asian J. Anim. Vet. Adv.*, 7: 454–476. Accessed 19th July, 2021, on https://scialert.net/abstract/?doi=ajava. 2012. 454.476.
17. **Shittu, A., Abdullahi, J., Jibril, A., Mohamed, A. A., Fasina, F. O., 2012:** Sub-clinical mastitis and associated risk factors on lactating cows in the Savannah region of Nigeria. *BMC Vet. Res.*, 8, 134.
18. **Wafa, I. A. B., Ibtisam, E. M. E., 2014:** Impact of husbandry, stages of lactation and parity number on milk yield and chemical composition of dromedary camel milk. *Emir. J. Food Agric.*, 26, 4, 333–341. DOI: 10.9755/26i4.17664.
19. **Zeryehun, T., Abera, G., 2017:** Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of eastern Harrarghe zone, Eastern Ethiopia. *J. Vet. Med.*, 6498618. DOI: 10.1155/2017/6498618.

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