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Prevalence of subclinical mastitis in buffaloes in Ayodhya district of Uttar Pradesh

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Abstract

Mastitis is common in buffaloes with significant impact on the rural economy. Detection of subclinical mastitis (SCM) is important for its management and control. Objective of present study was to study the prevalence of subclinical mastitis in Ayodhya district of Uttar Pradesh. Total 180 buffaloes from 3 blocks and 6 villages were screened using California Mastitis Test (CMT) and White Side Test (WST). The prevalence of sub clinical mastitis was 40% in Ayodhya district. Out of total 180 animals' samples screened for subclinical mastitis, 59 (32.77%) samples were tested as positive for *Staphylococcus aureus*. Among the samples that were positive for mastitis i.e., 72, the number of samples that were positive for *Staphylococcus aureus* was 59 (81.94%).

Keywords: Prevalence, subclinical mastitis, California mastitis test, white side test

Introduction

Mastitis is inflammation of the parenchyma of mammary glands resulting in bacteriological changes in milk, drop in milk production and pathological alteration in glandular tissues (Bansod *et al.*, 2021) [2]. It is the most widespread and economically important disease worldwide (Kovacevic *et al.*, 2021) [9]. Detection of subclinical mastitis (SCM) is important for its management and control. SCM is the most expensive and devastating disease in the dairy sector, causing significant financial losses. The annual combined cattle and buffalo milk production was 187.75 million tonnes during the year 2019 in India, which has increased by 6.5% when compared to 2018, and 394 grams per day was the per capita availability of milk (BAHS, 2019) [1]. Among all agents causing mastitis, *Staphylococcus aureus* is a major pathogen which can play vital and significant role in mastitis (Lundberg *et al.*, 2014) [12]. According to study conducted by Krishnamoorthy *et al.* (2021) [10] the bacterial species-wise analysis revealed a higher prevalence of *S. aureus* (25%) mainly related to subclinical mastitis and *S. epidermidis* (9%). There are two forms of mastitis: clinical form and subclinical form. The clinical form is characterized by local visible inflammatory changes in milk and udder tissue with or without systemic clinical signs, whereas subclinical form does not manifest clinical signs of mastitis but increased somatic cell counts with the presence of the causative agent. The diagnosis of the subclinical form of mastitis requires cow side test such as California mastitis test (CMT) or various laboratory tests including somatic cell count (SCC) and milk bacteriological culture. Early diagnosis of subclinical form of mastitis is very much essential for successful treatment and control of infection (Lakshmi, 2016) [11].

Subclinical mastitis has a negative influence on the performance of dairy cows and buffaloes. It has a significant impact on production, nearly 10-20% decrease in milk production, as it causes an undesirable effect on the milk constituents, which affects its nutritional value and renders it unfit for processing and consumption (Iraguha *et al.*, 2015) [7]. It includes reduced milk production, changes in milk quality and treatment cost. Rathod *et al.* (2017) [17] estimated the economic losses due to subclinical form of mastitis were approximately in the range of INR 21,677 to INR 88,340 per animal for a lactation period. Mastitis, specifically sub clinical type is well known for development of multiple drug resistance strains and emergence of new pathogens due to indiscriminate use of antimicrobials over long period (Raberg *et al.*, 2007) [15].

Hence it becomes important to diagnose the etiological agent of subclinical mastitis and subsequently the effective antimicrobial to prevent the indiscriminate use of antibiotics.

Thus, the present study was designed to determine the prevalence of subclinical mastitis in buffaloes of Ayodhya district.

Materials and Methods

Selection of animals

The area for study was at Ayodhya district of Uttar Pradesh. Milk samples were collected from lactating buffaloes with the history of decreased milk yield but showed no visible physical abnormality in udder and changes in milk. Most of the buffaloes under study were between four to eight years of age. On the basis of stratified random sampling technique three blocks were selected, then two villages from each block and thirty samples were collected from each village (total 180 samples). The physical examination of udder was performed for exclusion of abnormalities of udder and teat (Schalm *et al.*, 1971)^[18].

Clinical examination of udder of buffalo

Thorough examination of udder was done to find any abnormalities in the udder like presence of any lesion, pain, heat and swelling. Milk from each quarter was stripped out for detecting the abnormalities in the milk related to colour and consistency. These examinations were continued until final selection of buffaloes that had sub clinical mastitis (Radostits *et al.*, 2007)^[16].

Collection of milk samples

Collection of milk sample in each case was as follows, washing the udder and teat with clean water and mild antiseptic solution (potassium permanganate), wiping the thoroughly with dry clean cloth, discarding first 2 to 3 milking streams from each quarter and then collecting about 10 ml of milk sample in a sterile container.

Screening of buffaloes for sub clinical mastitis

Tests namely White side test and California mastitis test were used to detect sub clinical mastitis in buffaloes and those samples which showed strong positive reaction were selected for isolation of *S. aureus*. The CMT was performed to detect the presence of sub clinical infections, procedure described by Quinn *et al.* (2011)^[14]. White side test was performed using standard procedures as described by Schalm *et al.* (1971)^[18].

Microbiological assay of milk sample

Milk samples collected from buffaloes with subclinical mastitis were incubated in sterile nutrient broth at 37 °C for 24 hr to check growth of bacteria; and then bacteria were streaked on mannitol salt agar (MSA) plate and incubated in incubator, the grown bacterial colonies were stained with Gram's staining. Gram positive bacteria were transferred to nutrient agar slant.

Identification of *S. aureus*

Each sample of milk was directly inoculated onto mannitol salt agar (MSA) and incubated at 37 °C for 24 hours to identify *S. aureus*, as described by Talan *et al.* (1989)^[21]. Mannitol fermented colonies from primary cultures were purified by subculture onto nutrient agar slants and

incubated at 37 °C for 24-48 hour and stored at 4 °C until further use. Grams stained slides were examined according to Barrow and Feltham, (2003)^[3].

Biochemical characterization of *Staphylococcus aureus*

For biochemical characterization of bacteria, Methyl Red test, Voges-Proskauer test, catalase test, Slide and Tube coagulase test, Nitrate reduction test and haemolysis on blood agar were performed.

Results and Discussion

The screening of animals for detection of sub clinical mastitis was done by selecting three different blocks with two different villages in each block in Ayodhya district. Total 180 buffaloes from 3 blocks and 6 villages were screened using California Mastitis Test (CMT) and White Side Test (WST).

Among the total of 180 buffaloes screened 72 (40%) buffaloes were tested as positive for sub clinical mastitis. Amongst three blocks that were selected Milkpur block had a greater number of positive cases i.e., 28 (46.66%), followed by Harrington Ganj block with 23 (38.33%) positive cases and Khandasa block with least number of cases i.e., 21 (35%). Our findings are in agreement with findings of Hussain *et al.* (2020)^[6] which showed 43.6% prevalence of mastitis. Hussain *et al.* (2020)^[6] reported higher prevalence of subclinical mastitis in buffaloes i.e., 79.1%.

Biswas *et al.* (2020)^[4] found that the overall prevalence of SCM in buffalo was 20.0% and Sharma *et al.* (2018)^[19] also found that overall prevalence of SCM in buffaloes on animal basis was 23.85% in buffaloes, which were less than the findings of present study.

Amongst six villages selected, Nimadi village had a highest number of positive cases i.e., 16 (53.33%), followed by Deogiri 14 (46.67%), Kadanpur 12 (40%), Gadouli 11 (36.67%), Kanji 10 (33.33%) and Reona village with least number of cases i.e., 9 (30%). The prevalence findings of subclinical mastitis in villages Reona and Kanji are in agreement with findings of Sharma *et al.* (2018)^[19] and Srinivasan *et al.* (2013)^[20] which showed prevalence of subclinical mastitis as 33.76%, and 28.33% respectively.

Out of total 180 animals' samples screened for subclinical mastitis, 59 (32.77%) samples were tested as positive for *S. aureus*. Among the samples that were positive for mastitis i.e., 72, the number of samples that were positive for *S. aureus* was 59 (81.94%). Amongst three blocks that were selected Khandasa and Milkpur both had 20 (33.33%) samples positive for *S. aureus* and Harrington Ganj block had 19 (31.67%) samples positive for *S. aureus*. According to Chhabra *et al.* (2020)^[5] prevalence of *S. aureus* was 36.46% which is in accordance with the present study. Study report of Krishnamoorthy *et al.* (2021)^[10] also suggests that prevalence of *S. aureus* was 28%.

Amongst six villages selected Nimadi village had a highest number of positive cases for *S. aureus* i.e., 12 (40%), followed by Deogiri and Gadouli both with 11 (36.67%), Kanji with 9 (30%) and then Kadanpur and Reona villages with least number of cases i.e., 8 (26.67%). Maalik *et al.* (2019)^[13] suggested that 25% of the samples tested were positive for *S. aureus*. The findings of present study are lesser than findings of Hussain *et al.* (2020)^[6] who reported that the frequency of *Staphylococcus aureus* mastitis was 65% and also lesser than the 45% reported by Khan *et al.* (2005)^[8].

S. aureus was isolated from milk samples those were found positive for subclinical mastitis and isolates were subjected to biochemical tests namely methyl red test, Voges-Proskauer test, catalase test, slide coagulase test, tube coagulase test, nitrate reduction test and hemolytic activity on blood agar. Among the total of 59 samples were found positive for *S. aureus*, 54 (91.52%) were tested positive for methyl red test, 49 (83.05%) were tested positive for Voges-Proskauer test, 50 (84.74%) were tested positive for catalase test, 48

(81.35%) were tested positive for slide coagulase test, 47 (79.66%) were tested positive for tube coagulase test, 50 (84.74%) were tested positive for nitrate reduction test and 44 (74.57%) were tested positive for beta-haemolysis. Hussain *et al.* (2020) [6] found 100% samples positive for catalase test and beta-haemolysis, which is higher than that found in present study and 64% samples tested positive for coagulase test, which is lesser than that found in present study.

Table 1: Prevalence of sub clinical mastitis in buffaloes

Name of district	Name of block	Name of Villages	No. of Buffaloes screened	No. of Buffaloes positive for SCM	No. of samples positive for <i>S. aureus</i>
Ayodhya	Harrington Ganj	Reona	30	9 (30%)	8 (88.89%)
		Deogiri	30	14 (46.67%)	11 (78.57%)
	Khandasa	Gadouli	30	11 (36.67%)	11 (100%)
		Kanji	30	10 (33.33%)	9 (90%)
	Milkipur	Kadanpur	30	12 (40%)	8 (66.67%)
		Nimadi	30	16 (53.33%)	12 (75%)
	Total		180	72 (40%)	59 (81.94%)

References

- BAHS. Basic animal husbandry statistics. New Delhi: Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying; 2019.
- Bansod A, Masand R, Jadhao A, Bhardwaj A, Singh S, Gaikwad V. An overview of subclinical mastitis in dairy cattle. Indian J Anim Health. 2021. doi:10.36062/ijah.2021.04521.
- Barrow GI, Feltham RKA. Cowan and Steel's manual for the identification of medical bacteria. 2nd ed. Cambridge: Cambridge University Press; 2003.
- Biswas D, Hanif SM, Rana EA, Anower AM. Study on udder health management practices, reproductive disorders and subclinical mastitis in buffalo herds in coastal region of Bangladesh. Turk J Agric Food Sci Technol. 2020;8(8):1662-1667.
- Chhabra R, Shrinet G, Yadav R, Talukda SJ. Prevalence of subclinical mastitis in an organized buffalo dairy farm along with antibiogram. Int J Curr Microbiol Appl Sci. 2020;9(1):1605-1612.
- Hussain A, Ahmad M, Mushtaq MH, Chaudhry M, Nisar M, Khan MS, *et al.* Prevalence and sensitivity of *Staphylococcus aureus* causing mastitis in buffaloes around Lahore, Pakistan. Buffalo Bull. 2020;39(1):1.
- Iraguha B, Hamudikuwanda H, Mushonga B. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. J S Afr Vet Assoc. 2015;86(1):1228.
- Khan AZ, Muhammad G, Ahmed T, Khan A. Prevalence of mastitis in crossbred cows and buffaloes and antibiotic sensitivity profiles of isolates. Pak Vet J. 2005;26:74-76.
- Kovacevic Z, Radenkovic M, Cabarkapa I, Kladar N, Bozin B. Natural agents against bovine mastitis pathogens. Antibiotics. 2021;10:205.
- Krishnamoorthy P, Suresh KP, Jayamma KS, Shome BR, Patil SS, Amachawadi RG. An understanding of the global status of major bacterial pathogens of milk concerning bovine mastitis: A systematic review and meta-analysis (scientometrics). Pathogens. 2021;10:545.
- Lakshmi R. A review on mastitis. Int J Med Res. 2016;1:118-123.
- Lundberg A, Nyman A, Unnerstad HE, Waller KP. Prevalence of bacterial genotypes and outcome of bovine clinical mastitis due to *Streptococcus dysgalactiae* and *Streptococcus uberis*. Acta Vet Scand. 2014;56:80.
- Maalik A, Ali S, Iftikhar A, Rizwan M, Ahmad H, Khan I. Prevalence and antibiotic resistance of *Staphylococcus aureus* and risk factors for bovine subclinical mastitis in District Kasur, Punjab, Pakistan. Pak J Zool. 2019;51(3):1123-1130.
- Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan PJ. Veterinary microbiology and microbial disease. 2nd ed. West Sussex: Wiley-Blackwell; 2011. p. 912.
- Raberg L, Sim D, Read AF. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science. 2007;318:812-814.
- Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. London: Elsevier Saunders; 2007. p. 563-618, 966-994.
- Rathod P, Shivamurthy V, Desai AR. Economic losses due to subclinical mastitis in dairy animals: A study in Bidar district of Karnataka. Indian J Vet Sci Biotechnol. 2017;13(1):37-41.
- Schalm OW, Carroll EJ, Jain NC. Bovine mastitis. Philadelphia: Lea and Febiger; 1971. p. 360.
- Sharma N, Huma Z, Singh SG, Navjot, Sharma S, Gupta SK, *et al.* Prevalence of clinical and subclinical mastitis in buffaloes of Jammu region. Int J Agric Environ Biotechnol. 2018;11(2):415-420.
- Srinivasan P, Jagadeswaran D, Manoharan R, Giri T, Balasubramaniam GA, Balachandran P. Prevalence and etiology of subclinical mastitis among buffaloes (*Bubalus bubalus*) in Namakkal, India. Pak J Biol Sci. 2013;16:1776-1780.
- Talan DA, Staats D, Staats A, Goldstein EJC, Singer K, Oerturf GD. *Staphylococcus intermedius* in canine gingival and canine infected human wound infections: Laboratory characterisation of a newly recognised zoonotic pathogen. J Clin Microbiol. 1989;27:78-81.