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Prevalence of sub-clinical mastitis caused by *Staphylococcus aureus* in buffaloes: A field study from smallholder farms in Sultanpur district of Uttar Pradesh

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Abstract

Mastitis is one of the most economically significant diseases in dairy animals, especially in developing countries. Sub-clinical mastitis (SCM), often undetected due to the absence of visible symptoms, causes significant economic losses through reduced milk production, increased treatment costs, and culling of affected animals. This study aimed to determine the prevalence of sub-clinical mastitis and its association with *Staphylococcus aureus* infections in smallholder buffalo farms in Sultanpur district, Uttar Pradesh. A total of 180 non-descript lactating buffaloes were screened using the California Mastitis Test (CMT) and White Side Test (WST). Stratified random sampling was used to select three blocks, and two villages from each block, with thirty buffaloes sampled per village. The overall prevalence of SCM was 49.44%. The highest prevalence was recorded in Baldirai block (56.67%) and the lowest in Dostpur block (46.67%). Risk factors such as lactation stage, parity, udder conformation, and hygiene practices were found to influence the prevalence. Identification and isolation of *S. aureus* from milk samples confirm its major role in SCM. The study emphasizes the need for regular screening and better management practices to reduce mastitis incidence.

Keywords: Mastitis, sub-clinical, prevalence, buffalo, *Staphylococcus aureus*, risk factors

Introduction

Mastitis, an inflammation of the mammary gland, is one of the most economically devastating diseases affecting the global dairy industry. It causes substantial losses in terms of reduced milk production, increased treatment costs, culling of affected animals, decreased reproductive performance, and overall reduced farm profitability (Seegers *et al.*, 2003) [1]. The disease exists in both clinical and sub-clinical forms. While clinical mastitis presents with observable symptoms such as swelling, heat, pain in the udder, and changes in milk consistency, sub-clinical mastitis (SCM) lacks visible signs, making it more insidious and harder to detect without laboratory-based or field diagnostic tests.

SCM is especially problematic in developing countries like India, where smallholder dairy systems predominate, and awareness of udder health, hygienic practices, and disease prevention remains low. As a result, infections often go undetected and untreated for prolonged periods, leading to chronic inflammation, permanent damage to mammary tissue, and continuous economic losses. It is estimated that sub-clinical mastitis causes greater cumulative damage to herd productivity than clinical mastitis, due to its persistent and often undiagnosed nature.

In India, buffaloes contribute significantly to milk production, yet most studies on mastitis prevalence and associated risk factors focus predominantly on exotic or crossbred dairy cattle. The lack of species-specific research on buffaloes limits the development of targeted control strategies. Given the anatomical and physiological differences between cattle and buffaloes, and their varied husbandry conditions, extrapolation of cattle-based data may not yield accurate conclusions. Among the pathogens involved in mastitis, *Staphylococcus aureus* is one of the most commonly isolated organisms, particularly in sub-clinical cases. This Gram-positive bacterium colonizes the skin and teat canal and is adept at evading host

immune responses, often leading to chronic infections. Methicillin-resistant *Staphylococcus aureus* (MRSA), a more virulent and drug-resistant variant, has recently emerged as a global concern in both human and veterinary medicine. The misuse and overuse of antibiotics in veterinary practice have significantly contributed to the development of MRSA, complicating treatment regimens and reducing therapeutic options (Kamal *et al.*, 2013; Locatelli *et al.*, 2017)^[7, 8].

The risk factors for mastitis are multifactorial, including animal-related factors such as age, parity, milk yield, and udder conformation, as well as management-related factors like hygiene, milking practices, nutrition, and environmental conditions. Understanding these predisposing factors is crucial to designing effective prevention and control strategies. Therefore, the present study was designed to determine the prevalence of sub-clinical mastitis in non-descript buffaloes in the Sultanpur district of Uttar Pradesh, with a specific focus on the isolation of *Staphylococcus aureus* and identification of associated risk factors. The findings aim to provide baseline data to improve udder health management and minimize economic losses in smallholder buffalo farming systems.

Materials and Methods

Ethical Approval: The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of ANDUAT, Ayodhya, Uttar Pradesh.

Study period and Location

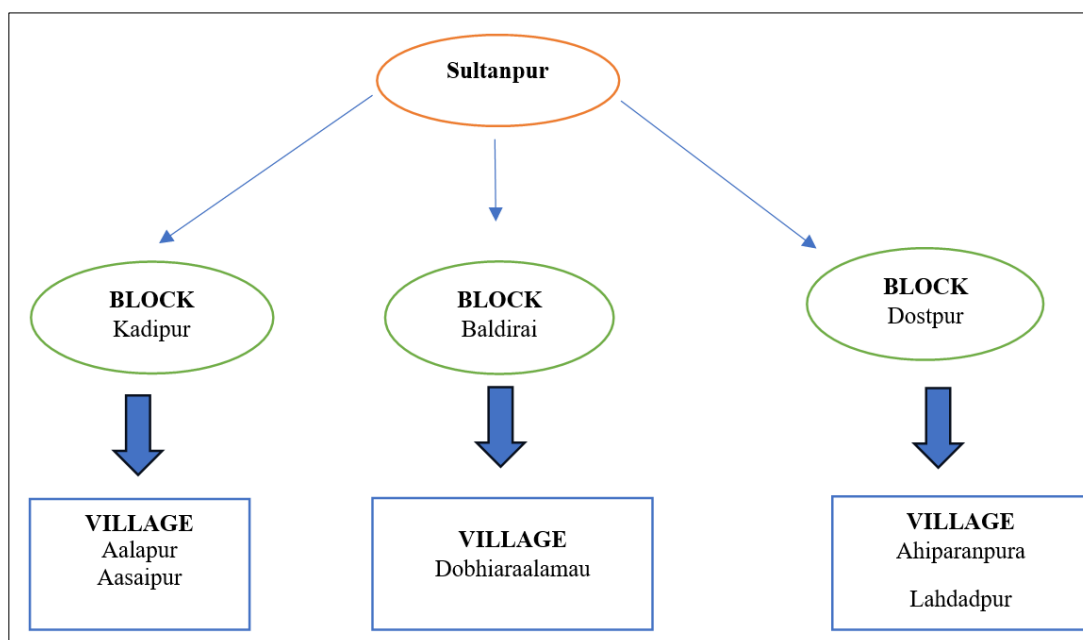
Ethical Approval

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of ANDUAT, Ayodhya, Uttar Pradesh.

Study Period and Location

The study was conducted between December 2021 and June 2022 across three blocks (Kadipur, Baldirai, and Dostpur) of Sultanpur district, U.P. Two villages from each block were selected using stratified random sampling. A total of 180 lactating buffaloes, aged 3-8 years, were screened for sub-clinical mastitis.

Sampling Procedure: From each of the six villages, 30 buffaloes were selected. Milk samples were collected from buffaloes showing no visible signs of clinical mastitis but had a history of decreased milk yield.



Diagnosis with Mastitis

Field tests namely White side test (WST) and California mastitis test (CMT) were used to detect sub clinical mastitis in buffaloes and those samples which showed strong positive reaction were selected for isolation of *S. aureus*. 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 it 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. Field 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*.

Inquisition and Exploration: Data on hygiene, housing, milking procedures, and animal characteristics such breed,

milk output, parity, and lactation stage were gathered using structured interrogation. Data on drug usage, buffalo sickness history, and social-demographic characteristics of the households were also collected via the questionnaire. To learn more about the buffaloshed's state, a visual inspection was conducted. Regulations and rules that were pertinent to the study were followed. Every participant was asked for their informed consent before any personal information was shared.

Data Interpretation

The screening of animals for detection of mastitis was done on the basis of stratified random sampling technique 3 blocks were selected from Sultanpur district followed by 2 villages from each block and 30 samples were collected from each village. Total 180 buffaloes from 3 blocks and 6 villages were screened using California Mastitis Test (CMT) and White Side Test (WST).

Table 1: Showing Data for Mastitis Prevalence

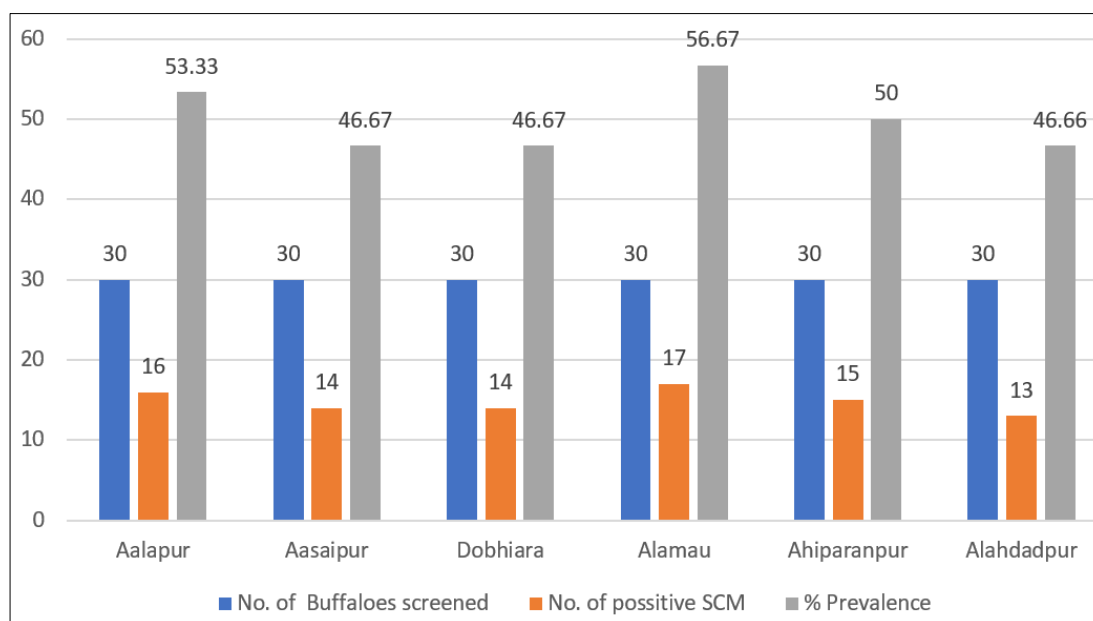
Name of district	Name of block	Name of Villages	No. of Buffaloes screened	No. of positive for SCM	% Prevalence
Sultanpur	Kadipur	Aalapur	30	16	53.33
		Aasaipur	30	14	46.67
	Baldirai	Dobhiara	30	14	46.67
		Alamau	30	17	56.67
	Dostpur	Ahiparanpur	30	15	50.00
		Alahdadpur	30	13	43.33
	Total		180	89	

Result and Discussion

The overall prevalence of mastitis was 49.44% which was recorded in Sultanpur district. Amongst different villages the prevalence of mastitis was highest at Alamau (56.67%) of Baldirai block following villages of Kadipur block Aalapur (53.33%), Ahiparanpur (50%) of Dostpur block, Aasaipur and Dobhiara villages have equal prevalence

(46.67) and lowest in Alahdadpur (43.33%). Highest prevalence was recorded in Baldirai block (56.67%, Table. 1) followed by Kadipur block (50%) and Dostpur block (46.67%).

Graphical View: Prevalence of Mastitis as Discussed

**Fig 1:** Prevalence of Mastitis

Factors associated with the prevalence of Mastitis

The overall prevalence for mastitis was 49.44% ensues as an outcome of interaction between various managerial, environmental and host agent factors. Other factors such as stage of lactation, parity, breed, milk yield, body weight, anatomical abnormality of the udder, and some managerial aspects including nutrition. High stocking density, contaminated floor, wet bedding, poor ventilation, and hot and humid climate can promote growth of mastitis pathogens and increased exposure of cows, resulting in higher occurrence of mastitis.

Almost higher prevalence in different blocks of Sultanpur district can be attributed to almost identical socio-economic factors and similar managerial practices and hygienic conditions adopted by the animal owners. Efforts should be made to create awareness of the farmers of this district regarding good managerial hygienic practices so that prevalence of mastitis could be kept at lower level. Seegers *et al.* (2003) [11] also suggested that prevalence of mastitis varies from place to place depending on managerial condition and other predisposing factors which affects the prevalence of mastitis among blocks and villages were observed.

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