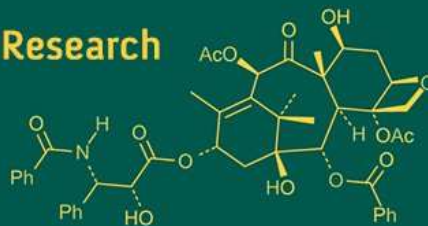
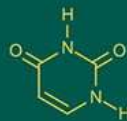
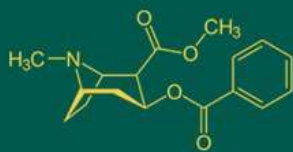


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Prevalence and identification of *Staphylococcal* mastitis in cattle of the Rewa, Vindhya region, India

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Abstract

The purpose of this study was to investigate the prevalence of *Staphylococcus aureus* associated clinical mastitis cases in organized and unorganized dairy farms and. The study was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry (COVSc & A.H), Rewa, Vindhya region, Madhya Pradesh. during a period of six months (August 2025-January 2026). A total of 240 lactating crossbred cows (960 quarters) were screened for clinical mastitis. Samples positive for clinical mastitis were subjected for isolation and biochemical characterization. Isolates that were confirmed for *S. aureus* were subjected for *S. aureus* specific bacteriophage susceptibility.

The study revealed an overall prevalence of clinical mastitis as 23.75% (57/240), while that of staphylococcal mastitis as 12.91% (31/240). Higher prevalence was observed in organized farms, in stall-fed animals, cattle in age group 6-10 years. Animals with fourth parity and above, and during early lactation were more susceptible. Overall quarter wise prevalence was 6.43% (61/948), with highest recorded in right hind quarter.

Keywords: Clinical mastitis, *Staphylococcus aureus*, dairy cows, prevalence

Introduction

Bovine mastitis is one of the most challenging disease and has stood just next to Foot and Mouth disease (FMD) in high yielding cross bred dairy animals in India (Joshi and Gokhale, 2006; Sharma, 2008; Sailo and Chakraborty, 2012) [5, 14, 13]. The prevalence of this disease in many countries have shown to be approximately 50% (Radostits *et al.*, 2010) [10]. Economic losses associated with mastitis arise primarily from reduced milk yield, deterioration in milk quality, and milk discarded before and during antibiotic treatment (Petrovski *et al.*, 2006) [9]. In monetary terms, mastitis-related losses have been estimated at approximately INR 1,390 per animal per lactation, with nearly 49% attributed to reduced milk value and about 37% to veterinary expenses, amounting to INR 509 (Sinha, 2014) [17]. Since bovine mastitis may be responsible for zoonosis and food toxin infections, it is a threat to human health (Gomes *et al.*, 2016) [4].

Mastitis is a multifactorial disease with infectious and non-infectious etiological agents. Among all, bacterial infection remains the major cause of mastitis (Petrovski *et al.*, 2006; Bogni *et al.*, 2011) [9, 3] with *Staphylococcus species* of bacteria considered the major and most common mastitis pathogens. Its ability to persist in a herd in the form of undetected, subclinical infections and low cure rate to most of the antibiotics makes it the most resistant pathogen. Its prevalence ranging from 16.36% to 37.5% (Maheshwari *et al.*, 2016; Singh *et al.*, 2023; Rawat *et al.*, 2024; Yadav *et al.*, 2024) [7, 16, 11, 9]. The present study was undertaken to investigate the incidence of *Staphylococcus aureus* associated clinical mastitis in dairy cattle.

Materials and Methods

The study was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry (COVSc & A.H), Rewa, Madhya Pradesh. during a period of six months (August 2025-January 2026).

For this study, 240 cattle were screened by California Mastitis Test (CMT) from those presented at the Veterinary Clinical Complex (VCC), COVSc & A.H., Rewa, as well as from organized and unorganized farms in and around Rewa district, Madhya Pradesh as mentioned in table 01.

Table 1: Cattle screened for clinical mastitis from different places from in and around Rewa-

S. No.	Farm	No. of cattle screened
1.	MTR	48
2.	CKR	36
3.	AUR	26
4.	KC	38
5.	HA	30
6.	UR	19
7.	APR	6
8.	CNR	17
9.	AR	20
Total		240

(MTR = Mahajan Tola Rewa, CKR = Chirahula colony Rewa, AUR = APS University Road, KC = Krishna Colony, HA = Hardi, UR = Uprahiti Rewa, APR = Anant Pur Rewa, CNR = Chhatrapati Nagar Rewa, AR = Amahiya Rewa)

All the animals from the above-mentioned places showing positive CMT test results were included for epidemiological study. Different parameters of individual animals were recorded such as age, parity, stage of lactation, days in lactation, udder palpation score and quarters affected. Clinical Examination for mastitis was carried out by the udder Palpation Scoring and Milk Quality Assessment (CMT and pH).

Husbandry and management system

The dairy cattle included in the present study were maintained under organized and unorganized farming systems in and around Rewa district, Madhya Pradesh. Noticeable differences in housing, feeding, and management practices were observed, which influenced mastitis occurrence. In organized farms, animals were kept under intensive or semi-intensive systems with stall feeding and permanent housing structures. In contrast, unorganized farms followed traditional systems with smaller herd sizes and access to grazing. Feeding practices also varied, with concentrate-rich diets in organized farms, whereas grazing-based feeding in unorganized systems.

Sampling

A total of 240 lactating crossbred females were tested for clinical mastitis i.e (960 quarters) were tested. Milk samples from each quarter were collected aseptically from apparently healthy lactating crossbred females. Teats were washed thoroughly with fresh water and dried. The first few streams of milk from each teat were discarded. The teat end and orifice was sanitized with cotton swabs soaked in 70% ethanol.

Clinical mastitis detection by California mastitis test

California mastitis test (CMT) was carried out as described by Ruegg and Reinemann, 2002^[12]. About 2 mL of milk samples from all the quarters (N = 960) was collected in each shallow cup in the CMT paddle. An equal amount of CMT reagent was added. The milk reagent mixture was swirled in a circular motion and presence of gel or slime was recorded for each quarter.

Culture and identification of microorganisms

The samples were inoculated on nutrient agar and blood agar at 37°C for 24 to 48 hours. Identification of isolates was done on basis of colony morphology, Gram's staining, catalase test, Oxidase test, Methyl Red test. The colonies confirmed as *Staphylococcus aureus* were further tested *S. aureus* specific bacteriophage susceptibility.

Results and Discussion

In the present study, a total of 240 lactating cattle from organized and unorganized dairy farms in and around Rewa district of Vindhya region, Madhya Pradesh were screened for mastitis using the California Mastitis Test (CMT) as a cow-side diagnostic test. Animals showing clinical signs such as reduced milk yield, abnormal milk consistency, discoloration, udder swelling, and pain were evaluated. The findings revealed an overall prevalence of mastitis of 23.75% (57/240). The relatively high prevalence may be attributed to poor hygienic practices, inadequate milking management, and lack of routine screening for subclinical mastitis. Similar findings have been reported earlier by Radostits *et al.*, (2010)^[10], Sharma (2008)^[14] and Joshi and Gokhale (2006)^[5], who emphasized the importance of environmental and managerial factors in mastitis occurrence.

Out of the total animals screened, 31 cattle were found positive for staphylococcal mastitis, indicating an overall prevalence of 12.91% (31/240). These findings are in agreement with the results of Abera *et al.* (2010)^[1], Sharma *et al.* (2012)^[14], Kumar *et al.* (2015)^[6], Mekonnen *et al.* (2017)^[8] and Tiwari *et al.* (2019)^[18]

The predominance of *Staphylococcus* spp. as mastitis pathogens may be due to their ability to persist within the mammary gland, form biofilms, and develop resistance to commonly used antibiotics. These characteristics enable the bacteria to evade host immune responses and therapeutic interventions, thereby leading to chronic infections.

Housing

Noticeable differences in housing, feeding, and management practices were observed, which influenced mastitis occurrence. The prevalence of staphylococcal mastitis was higher in organized dairy farms (11.82%; 24/240) as compared to unorganized farms (2.91%; 7/240). This difference may be attributed to intensive or semi-intensive management practices with stall feeding, higher stocking density and increased exposure to contagious pathogens in organized systems. Inadequate ventilation, poor drainage and suboptimal hygiene practices during milking all of which increased the risk of intramammary infections. Improper udder sanitation and lack of post-milking teat disinfection further predisposed animals to mastitis. In contrast, unorganized farms followed traditional systems with smaller herd sizes, access to grazing, better natural ventilation, lower animal density, all contributed to improved udder health.

Feeding systems

In the present study a higher prevalence of staphylococcal mastitis was observed in stall-fed animals in both organized and unorganized farms. Among the 31 positive cattle, 24 animals belonged to organized farms, where the prevalence was highest in stall-fed animals (38.70%), followed by mixed feeding (22.58%) and grazing systems (16.12%). In

contrast, out of the remaining 7 cattle from unorganized farms, a similar trend was observed, with prevalence rates of 12.90% in stall-fed animals, 6.45% in mixed feeding, and 3.2% in grazing animals.

Higher incidence of staphylococcal mastitis in stall-fed cattle in organized and unorganized farm may be attributed to limited exercise, repeated exposure to contaminated equipment, use of common udder cloths, absence of post-milking teat disinfection. Favorable conditions for bacterial survival in moist, poorly ventilated housing also contribute to the cause. Continuous confinement exposes them to high concentrations of toxic gases such as ammonia, carbon dioxide, nitrous oxide, hydrogen sulphide and methane, which adversely affect respiratory health and overall immunity. Poor air circulation in stalls facilitate the spread of infectious agents within the herd and contributes to chronic inflammation, ultimately impairing both the reproductive and productive performance of the animals. Animals under mixed feeding systems show intermediate prevalence due to partial outdoor exposure, although inconsistent hygiene practices still permit pathogen transmission.

Grazing animals, in contrast, exhibit lower prevalence of infection owing to exposure to sunlight, better ventilation, reduced stocking density, resulting in cleaner teat conditions, which limit the persistence and spread of pathogens. During grazing, animals consume local herbs and forage, which are rich in flavonoids, potent antioxidants, polyphenols, trace minerals, microminerals and vitamins. These compounds possess anti-inflammatory, antimicrobial and immunomodulatory properties, supporting overall health and enhancing the body's ability to resist infections, including mastitis. Grazing also reduces the contact time of the udder with environmental contaminants, improves blood circulation and promotes udder health, thereby decreasing the risk of mastitis. Additionally, physical activity during grazing provides exercise, which relieves stress, reduces inflammation and further lowers susceptibility to infection thus enhances overall health.

Overall quarter wise

In the present study, out of 960 quarters from 240 cattle, 12 teats were non-functional or blind, leaving 948 functional quarters. Among these 948 quarters, 61 quarters were positive for staphylococcal mastitis, showing an overall quarter-wise prevalence of 6.43% (61/948).

Right hind quarters were most frequently affected (29.51%; 18/61), followed by the left hind quarters (26.22%; 16/61) and the right fore quarter (22.96%; 14/61). The lowest prevalence was observed in the left fore quarter (21.31%; 13/61).

The increased incidence of infection in the hind quarters in the present study can be attributed to their comparatively lower anatomical position, which places them closer to the floor even when the animal is standing. This increases their exposure to environmental and faecal contamination (Bansal *et al.*, 2011). Additionally, hind quarters generally have higher milk production than forequarters rendering them more susceptible to infection.

Age-wise

In the present study the age-wise prevalence of staphylococcal mastitis observed in the present study, revealed the highest prevalence was recorded in the 6 to 10

years age group, accounting for 54.84% (17 out of 31 cattle) followed by cattle aged 4 to 6 years, with a prevalence of 35.48% (11 out of 31 cattle) and the lowest prevalence (9.68%) was observed in cattle aged 10 years and above age group (3 out of 31 cattle). The high prevalence of clinical mastitis observed in the mid-age group in the present study may be attributed to the fact that animals in this age group are at the peak of lactation and have undergone repeated lactations, resulting in cumulative exposure to mastitis-causing pathogens. Animals in this group may also have depleted body reserves of energy, vitamins and minerals due to previous parturitions and lactation cycles, leading to reduced body condition and compromised immunity. Furthermore, multiple lactations result in cumulative udder and teat canal damage, weakening of teat canal musculature, delayed closure of teat opening and prolonged exposure to environmental pathogens. These factors, combined with high milk yield during this phase of lactation, increases susceptibility to infection and contribute to the higher incidence of mastitis observed in this age group.

Parity-wise

The results revealed in the present study, the highest prevalence in cattle of 4th parity and above (51.62%; 16/31), followed by 3rd parity (22.59%; 7/31) and 2nd parity (16.12%; 5/31). The lowest prevalence was observed in 1st parity animals (9.67%; 3/31).

The risk of clinical mastitis increases with advancing parity. The higher incidence of clinical mastitis in animals of greater parity may be attributed to structural and physiological changes of the teat, including elongated teat shape, wider teat canal and loosening of teat sphincters, which facilitate the entry of environmental microorganisms into the mammary gland. In addition, animals in higher parity are often at or near peak milk production, creating favourable conditions for microbial proliferation due to increased milk availability and physiological stress on the udder.

Lactation stage wise

The lactation period was divided in 3 stages i.e. early lactation (0-90 days), mid lactation (91-180 days) and late lactation (above 180 days). The results of lactation stage wise prevalence in this present study were 51.61% (16 out of 31 cattle), 32.26% (10 out of 31 cattle) and 16.13% (5 out of 31 cattle) in early, mid and late lactation stage, respectively.

New intramammary infection (NIMI's) are of major concern in causing mastitis. NIMI's in dairy animals, often bacterial infections are costly diseases caused by pathogens like *Staphylococcus spp.*, *Streptococcus spp.* and *E. coli*. These infections settle in the udder during dry period and show up as clinical or subclinical infections during lactation period. Managing these NIMI's require controlling transmission and implementing strategies during dry period and early lactation to prevent bacterial entry and proliferation. Rapid physiological changes in the mammary tissues after parturition leading to decreased udder resistance may also be the cause of the high frequency of udder infection and mastitis in the early stages of lactation. A negative energy balance predisposes the animals to many metabolic diseases and disorders which may be the cause of an increased mastitis incidence during the early or peak lactation period.

Impacts

The present investigation demonstrates the presence of *S. aureus* in high percentage in clinically positive mastitis milk samples. The findings highlight that both managerial and physiological factors play a critical role in mastitis occurrence. The study underscores the need for targeted preventive strategies, including improved hygiene, optimized housing, and regular screening, to effectively control mastitis in dairy herds.

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