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Prevalence of subclinical mastitis in cattle in Bikaner

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Abstract
The present study was conducted on 472 quarter milk samples from 120 apparently healthy lactating cows to determine the quarter, parity and lactation stage-wise prevalence of sub-clinical mastitis in cattle based on the modified California mastitis test (MCMT). The animal and overall quarter-wise prevalence of subclinical mastitis were 37.16 percent and 22.03 percent, respectively. The quarter type-wise highest prevalence of SCM was found in right hind quarters (32.76%), followed by left hind quarters (23.52%), right fore quarters (19.65%) and least prevalence was recorded in left fore quarters (12.50%). The parity-wise highest prevalence of SCM was noticed in IVth parity followed by IIIrd, Vth, VIth, VIIth and above, IInd and least in Ist parity. Whereas, lactation stage-wise, the highest prevalence of SCM was recorded in early lactating stage 25 (43.86%) followed by mid-lactating stage 17(36.20%) and least in late lactating stage 03 (18.75%) by modified California mastitis test.

Keywords: Lactation stage, modified California mastitis test, parity, subclinical mastitis

Introduction
Bovine mastitis is a multiple etiological disease involving inflammation of the mammary gland parenchyma and characterized by physical, chemical, and bacteriological changes in milk (Constable *et al.*, 2017) [8]. Etiologically about 135-150 bacterial species, sub-species and serovariants have been isolated from bovine mammary glands affected with mastitis. Majority of the mastitis cases are caused by staphylococci, streptococci, and *E. coli* bacteria (Bradley, 2002) [6]. Subclinical mastitis (SCM) does not cause any visible changes in milk or udder appearance but affects milk quality and quantity causing a reduction in milk yield up to two-third losses of the total milk production (Haque, 2014; Singh *et al.*, 2015) [13, 32], altered milk composition, and the presence of inflammatory components and bacteria in milk. The diagnosis of bovine mastitis involves recognition of disease and identification of the causative pathogens (Ashraf and Imran, 2018) [2].
To screen the milk for sub-clinical mastitis various direct and indirect methods can be employed for diagnosis such as California mastitis test (CMT), from the milk samples. CMT is an animal-side test and a qualitative measurement of the somatic cells in the mastitic milk. (Duarte *et al.*, 2015; Ashraf and Imran, 2018) [32, 2].
Organism as diverse as bacteria, viruses, mycoplasma, yeast, and algae are involve in the infection. Although the majority of subclinical mastitis is of bacterial origin and *Staphylococcus* spp. *Streptococcus* spp. *Escherichia coli* are the most common bacterial pathogen isolated from sub clinical mastitis in cows by various workers (Kachhawa, 2018; Choudhary, 2018; Savita *et al.*, 2020 and Solanki, 2021) [15, 27, 34].
To manage these infections, farmers and veterinarians frequently rely on antimicrobial drugs. However, the widespread and sometimes indiscriminate use of these drugs has led to the development of antimicrobial resistance (AMR) among the pathogens responsible for mastitis (Kumar *et al.*, 2012; Raorane *et al.*, 2013; Poojitha *et al.*, 2022) [16, 22, 21]. The Bikaner region in Rajasthan, known for its arid climate and traditional dairy practices, is particularly vulnerable to this condition due to environmental stressors and management challenges. Factors such as climate, animal husbandry practices, and breed susceptibility are known to influence of subclinical mastitis (SCM) occurrence in this region, adding complexity to its detection and control (Shabaz *et al.*, 2020; Bhati *et al.*, 2021) [29, 5]. The present study was conducted to detect the prevalence of subclinical mastitis in cattle within the Bikaner region.

Materials and Methods

Source of animals

In the present study, 472 functional quarters of 120 apparently healthy lactating cows were screened for subclinical mastitis using modified California mastitis from unorganized dairy farms as well as animals of individual holdings in and around Bikaner. The cows in early lactation (<1 month post-calving) and late lactation were excluded from the study.

Ethical Approval

This study was approved by Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner with approval No. CVAS/IAEC/2024-25/17).

Collection of milk sample

Approximately 30 ml of milk from respective quarters were collected aseptically into sterile test tubes and marked as right fore, right hind, left fore and left hind. Milk samples were immediately transported to the laboratory on ice and maintained at 4 ºC until further testing.

Modified California mastitis test

The California mastitis test was developed by Schalm and Noorlander (1957) [28]. The MCMT is a simple, rapid, highly sensitive and inexpensive test that accurately predicts the inflammatory cell counts and infection in milk from individual quarters or pooled milk samples.In the present study, Ezee was used in place of pure laryl sulphates or sulphonates of sodium or potassium as an anionic surface active agentand Bromo thymol blue was replaced by bromocresol purple (Savita *et al.*, 2020) [27].

Procedure

The test was carried out with 3 ml of milk from each quarter into the respective 4 cups in plastic paddle. An equal amount of the above test reagent was added in each cup and gently mixed by circular movement of the paddle in horizontal plane. The total cell count is reflected by the degree of precipitation or gel formation that occurs. The pH change associated with abnormal milk is indicated by a color reaction with bromocresol purple. California mastitis test reactions were scored according to Radostits *et al.* (2007) [23] as follows.

Results and Discussion

In the present study, 472 functional quarters of 120 apparently healthy lactating cattle were screened for subclinical mastitis by the modified California mastitis test (MCMT)

Animal-wise prevalence of subclinical mastitis

In the present study, out of 120 cattle, 45 (37.16%) cattle were found positive for subclinical mastitis based on MCMT. The prevalence obtained in the current study was almost in line with studies by Savita *et al.* (2020) [27] Gupta (2021) [12] and Sain (2022) [25]. Who reported, animal-wise prevalence was 49.78 percent, 38 percent and 38.87 percent, respectively.

Overall quarter-wise prevalence of subclinical mastitis

In the current study, out of 472 quarters, 104 (22.03%) quarters were found positive for subclinical mastitis based on MCMT. In the present investigation, overall quarter-wise prevalence of subclinical mastitis was recorded 17.99. percent, 26.18 percent, 16.30 percent and 28.06 percent in left side quarters, right side quarters, fore quarters and hind quarters, respectively.

Data of the present study revealed that right-side quarters showed the highest prevalence as compared to left-side quarters.

Quarter wise prevalence of subclinical mastitis

The quarter wise prevalence of subclinical mastitis was recorded as 12.50 percent, 23.52 percent, 19.65 percent and 32.76 percent in left fore, left hind, right fore and right hind quarters, respectively based on MCMT. In the current study, it was observed that the prevalence of subclinical mastitis was higher in the hindquarters as compared to the fore quarters. It was also noticed that the highest prevalence of SCM was recorded in right hindquarters, followed by left hind quarters, right fore quarters and least in left fore quarters.

Findings of the present study are in agreement with the observations of Gupta (2021) [12], Sain (2022) [25] and Gautam (2022) [11] who also observed the highest quarter-wise prevalence of subclinical mastitis in right hind quarters followed by left hind quarters, right fore quarters and least in left fore quarters detected by MCMT.

Kachhawa (2018) [15], Choudhary (2018) [7] and Solanki (2021) [34], Kiran (2023) [17] and Sharmila (2023) [30] also revealed that the prevalence of subclinical mastitis was higher in the hind quarters than in the fore quarters. Right hind quarters were more affected than the left fore quarters Gautam *et al.*, 2022 [11] Dangi *et al.* 2025 [9] also obsereved that right hind quarters were most frequently affected (16.66%) in cattle.

However, Barmendra *et al.* (2011) [4], Mustafa *et al.* (2011) [20], Shittu *et al.* (2012) [31] and Srinivasan *et al.* (2013) [35] found higher prevalence in fore quarter than the hind quarter. Due to increased exposure to feces, milkmen's custom of approaching from the side when milking high urine and production capacity, large mass and capacity, increased susceptibility to direct trauma and comparatively closer proximity to the floor than in the forequarters, the higher prevalence of subclinical mastitis was observed in the hind quarters (Akhtar *et al.* 2012) [1].

Parity-wise prevalence of subclinical mastitis

In the present study 120 cattle were screened. Out of these, 12, 13, 20, 35, 14, 16 and 10 cattle were in first (I^{st}), second (II^{nd}), third (III^{rd}), forth (IV^{th}), fifth (V^{th}), sixth (VI^{th}) and seventh (VII^{th}) and above parity, respectively In the present study based on positive in MCMT, 16.66 percent, 23.80 percent, 40.0 percent, 54.14 percent, 42 percent, 31.25 percent and 20 percent cattle found positive for SCM were in their I^{st}, II^{nd}, III^{rd} , IV^{th} , V th, VI^{th} and VII^{th} and above parity, respectively. Parity wise highest prevalence of SCM was observed in IV^{th} parity, followed by V^{th}, III^{rd}, VI^{th}, VII^{th}, II^{nd} and least in I^{st} parity.

Data analysis revealed that the prevalence of subclinical mastitis in cattle was lowest in the first parity, then increased until the IV^{th} parity, after which it remain almost steady. In agreement with Gupta (2021) [12] and Sain (2022) [25], Gautam (2022) [11] Sharmila (2023) [30] and Kiran (2023) [17] all reported similar findings of a higher prevalence of subclinical mastitis in IV^{th} parity.

Devi *et al.* (1997) [10] reported lowest prevalence of SCM during first parity than increased with subsequent parities which are also in accordance to the present study. This could be due to lowered resistance of the animals as lactation number increased and improper functioning of the teat sphincter results of increased incidence of new intramammary infection (Singh and Baxi, 1980, Devi *et al.* 1997 and Sampimon *et al.*, 2009) [33, 10, 26].

Lactation stage-wise prevalence of subclinical mastitis

In current study, out of 120 screened cows 57, 47 and 16 were in the early, mid and late lactation stage, respectively. The cows in early lactation (less than one month after calving) or late lactation (more than nine months after calving) were not included in the study. On the basis of MCMT, out of 120 cattle, 25(43.86%), 17(36.20%) and 3(18.75%) were found positive from early, mid and late lactation stage, respectively. Highest prevalence of subclinical mastitis was observed in early lactating stage followed by mid lactating stage and least in late lactation stage.

The findings of current study are consistent with those of Choudhary (2018) [7], Mourya *et al.* (2020) [19] and Gupta (2021) [12], Sain (2022) [25], Gautam (2022) [11], Kiran (2023) [17] who also found that the majority of subclinical mastitis occurred in the early stage of lactation as compared to the mid and late stages. The maximum prevalence of SCM was recorded as 30.0% and 47.46%, during the early lactation period by Islam *et al.* (2011) [14] and Maheshwari *et al.* (2016) [18], respectively. Badiuzzamann *et al.* (2015) [3], found that the early lactation stage had the highest prevalence of subclinical mastitis, followed by the mid and late lactation stages.

Negative energy balance, the physiological stress of lactation, changes in homeostasis mechanisms and postpartum rapid physiological changes in the mammary tissue that result in lower or reduced udder resistance could all be contributing factors to the higher prevalence of subclinical mastitis in the early lactation stage, (Rasool *et al.*, 1985 and Mourya *et al.*, 2020) [24, 19]. stated that a cow's body undergoes numerous physiological changes in the early stages of lactation, which results in ongoing stress and weakened immunity.

Conclusions

In the present experiment, animal-wise prevalence of SCM was observed as 37.16 percent and quarter-wise prevalence of SCM was recorded as 22.03 percent by modified California mastitis test. The prevalence of SCM in fore quarters was noted as 16.30 percent and in hindquarters was recorded as 28.06 percent. Right hindquarters were more affected than the left fore quarters. Parity wise highest prevalence of SCM was observed in IVth parity. Lactation stage wise highest prevalence of subclinical mastitis was found in the early lactation stage followed by mid-lactation and least in the late lactation stage.

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