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Research Article

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Utilizing C-Reactive Protein as a Predictor for Subclinical Mastitis

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ABSTRACT

Mastitis stands out as the most economically significant ailment in dairy cows due to its adverse effects on milk quality and reproductive performance, often leading to the involuntary culling of affected cows. Subclinical mastitis (SCM) can be considered a precursor to severe mastitis. This current study sheds light on the role of C-reactive protein (CRP) as a potential indicator for the presence of bovine SCM. The research involved the analysis of milk samples from a total of 49 cows, encompassing crossbred and native breeds. These samples were evaluated for their CRP levels and then compared with the absolute somatic cell count. To identify SCM, a somatic cell count within the range of 5.00-7.5 lakhs/ml was used as a threshold. The results revealed elevated CRP concentrations in the milk of cows afflicted with clinical mastitis (11.72 ± 0.75 µg/mL) as well as those with subclinical mastitis (10.9 ± 1.2 µg/mL), in stark contrast to the levels observed in healthy cows (0.33 ± 0.02 µg/mL). This research suggests that CRP may serve as a valuable tool for the ongoing surveillance of bovine mastitis, ultimately contributing to improved milk production performance.

Keywords: C-Reactive Protein, Milk, Bovine, Subclinical, Mastitis

Introduction

Bovine mastitis is an inflammatory condition affecting the mammary glands, often characterized by milk stagnation in the glandular tissue. This condition is primarily observed during the lactation period, especially in high-yielding breeds. It typically leads to an ascending infection within the lactating gland, spreading through the bloodstream. The onset of mastitis can be categorized as clinical or subclinical, depending on the presence of clinical symptoms. It is a matter of concern due to its potential to progress to sepsis, septic shock, and fibrosis in later stages [1]. Therefore, there is a pressing need for early and accurate diagnosis of mastitis in bovines.

Presently, clinical bovine mastitis is primarily diagnosed through physical examination and additional tests, including pH measurement, conductivity assessment, milk microbial analysis, and somatic cell counts [2]. However, more sensitive and non-invasive biomarkers are required to enhance early diagnosis and facilitate effective therapy monitoring. The assessment of acute-phase proteins (APPs) in milk has been proposed as the most sensitive and non-invasive method for detecting bovine mastitis [3].

Despite this, the use of C-reactive protein (CRP) as a predictive marker for mastitis has not been extensively explored. CRP is a valuable indicator of inflammation and treatment progress, as its serum levels rapidly rise following tissue damage and decrease with successful therapy [4]. Therefore, we hypothesized that CRP could be measured in bovine milk samples and might exhibit elevated levels in cases of mastitis. To test this hypothesis, we estimated the CRP concentrations in random milk samples and categorized the milk as subclinical or mastitis based on

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the somatic cell counts within. Additionally, we explored the correlation between milk CRP concentrations and somatic cell counts in both healthy and diseased lactating animals.

Materials and Methods

Random milk samples (n=49) were collected directly from the udder of milking cows from Goh sala and various private farms in and around Puducherry. To count the somatic cells in the milk, the glass slides with milk smears were placed on the slide rack and were flooded with modified Newman-Lampert stain (Himedia) for 2 min. The excess stain was drained off by standing the slices on absorbent paper; slides were rinsed thrice in tap water and air-dried. The slides were examined under oil immersion objective and the number of cells in 20 fields was counted and the total number of cells per ml of milk was calculated [5]. Milk CRP was determined using a commercial assay kit (Regenix lab, Chennai). Before analysis, the milk samples were centrifuged (10000 × g, 30 min, 4 °C) to remove fat and the milk samples were diluted 1:200 in assay buffer (Tris–HCl buffered NaCl solution (pH 7.8), containing <0.1% NaN3, Tween 40, and diethylene-triaminepentaaceticacid. The assay was performed as per the manufacturer's instructions.

Results and Discussion

The findings indicated that within the bovine milk samples, C-reactive protein (CRP) concentrations ranged from 0.2 to 0.42 µg/mL in healthy animals, and from 0.55 to 15.0 µg/mL in diseased ones ($P<0.01$). Please refer to Figure 1 for a graphical representation of the milk CRP data in healthy cows and those with clinical and subclinical mastitis. Notably, cows affected by clinical mastitis exhibited significantly higher milk CRP concentrations at 11.72 ± 0.75 µg/mL, while those with subclinical mastitis had concentrations of 10.9 ± 1.2 µg/mL when compared to the 0.33 ± 0.02 µg/mL in healthy cows. Importantly, there was no statistically significant difference in milk CRP concentrations between cows with clinical and subclinical mastitis.

The early detection of bovine mastitis holds paramount clinical significance as it helps mitigate poor reproductive performance and economic losses. CRP serves as a non-specific acute-phase protein, with elevated levels indicating the presence of inflammation of any origin. In the context of bovine mastitis, increased milk CRP levels signify ongoing inflammation in the mammary gland [6]. However, it's worth noting that the diversity of pathologies involved in clinical mastitis could explain the observed high data variability. Conversely, reports have suggested that early diagnosis in clinically active cases might not detect increased CRP concentrations, as CRP levels are known to reach their peak concentrations 24 hours after tissue damage [7]. Consequently, based on findings from experimental subclinical mastitis in cows [3], it can be anticipated that increased CRP levels would be evident in severe subclinical mastitis cases. Nevertheless, further studies involving a larger number of animals are warranted to validate these observations.

Conclusions

The data from the current study reveals that milk C-reactive protein (CRP) levels were elevated in affected bovines, signifying the presence of both local and systemic inflammation in mastitis cases. Furthermore, a positive correlation was observed between CRP concentrations and the somatic cell count. However, it's important to note that there were no statistically significant differences in milk CRP concentrations between clinical and subclinical mastitis.

One of the limitations of this research pertains to the relatively small sample size, which precluded the derivation of comprehensive conclusions. Additionally, due to the random nature of many of the sampled milking sessions, the exact timing of illness onset could not be recorded and analyzed in this study. Nevertheless, the results provide a genuine clinical representation, offering promising data that warrants further investigation into this subject matter.

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Conflicts of Interest

There are no conflicts of interest.

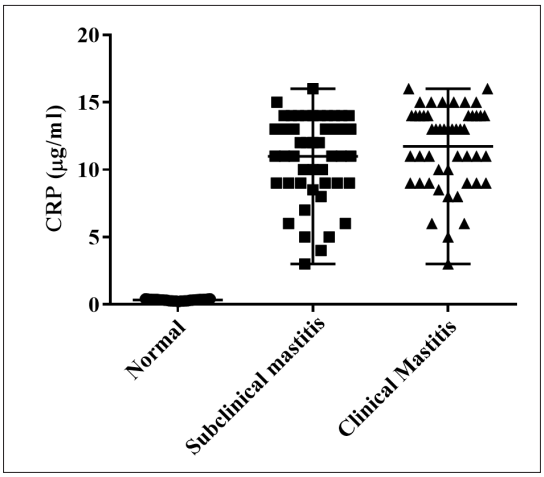

Figure 1: The level of CRP in milk samples of normal, subclinical and clinical mastitis cows.

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