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Diagnostic studies on clinical mastitis in goats

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Abstract

During the investigation “Diagnostic studies on clinical mastitis in goats” was under taken on 260 quarters of 130 goats which were screened for clinical mastitis (CM), using different diagnostic tests out of which 54 quarters of 33 goats were found positive for clinical mastitis based on bacterial culture examination. Clinical signs recorded were inappetence/anorexia, decreased milk production, swollen and painful udder, pyrexia, warmthness of udder, pale mucous membrane, udder erythema, dehydration, asymmetry of teats, depression and hardness of udder. Vital parameters like meanrectal temperature (°F), pulse rates (perminute), respiratory rates (breath/min.) were non-significantly increased along with decreased rumen motility in mastitis affected goats as compared with healthy control. The conjunctival mucous membrane was pale. Physical examination of milk samples revealed milk colour changes, consistency along with appearance of flakes and clots. Qualitative changes of milk in CM affected goats were assessed based on MCMT, SCC and milk pH. Hematological studies of CM affected goats revealed significant reduction in mean levels of hemoglobin, TEC, PCV, eosinophils, monocytes with leukocytosis, neutrophilia and lymphopenia. The bacteria found from clinical mastitis affected quarter milk samples as single infection include coagulase negative *Staphylococcus* spp., *Escherichia coli* spp., *Klebsiella* spp., *Streptococcus* spp. and coagulase positive *Staphylococcus* spp. The bacteria identifies in mixed infection were coagulase negative *Staphylococcus* spp. in combination with *Escherichia coli*, Coagulase negative *Staphylococcus* spp. with *Klebsiella* spp., *E. coli* with *Streptococcus* spp., Coagulase negative *Staphylococcus* spp. with *Streptococcus* spp. and coagulase positive *Staphylococcus* spp., with *Escherichia coli* 7.41, 5.56, 3.70, 3.70 and 1.85 percent respectively.

Keywords: Goats, clinical mastitis, diagnosis, SCC, MCMT and cultural examination

Introduction

Mastitis is a complex and multi-etiological disease *viz.*, inflammation of the mammary gland parenchyma characterized by physical, chemical, and bacteriological changes in the milk and pathological changes in the glandular udder tissues (Islam *et al.*, 2011a) [9]. In general, Occurrence of mastitis is in two forms like clinical and sub-clinical forms. The clinical form of mastitis is again categorised as subclinical (hidden), serous-catarrhal, purulent-catarrhal and gangrenous. Clinical mastitis (CM) is manifested by redness of the udder, reduced and altered milk secretion from the affected quarters, sudden onset of swelling and pain. In addition, the milk may contain flakes or clots with watery in consistency, accompanied by anorexia, depression and fever and (Faruq and Nikolai, 2019) [5]. Clinical examination of the mastitis-affected udder and milk aids in the identification of clinical mastitis. Milk composition changes are in direct proportion to the quantity and intensity of the inflammatory process (Singh *et al.* 2018) [22]. The accuracy of diagnosis can be primarily investigated through estimation of hemato-biochemical parameters. Changes in normal hemato-biochemical indicative of ill health and help in diagnostic and evaluating the prognosis and severity of the disease condition, which is evident through accumulation of White Blood cells (WBCs) in the udder (Misialek *et al.*, 2015) [11]. Various organisms associated with mastitis was studied widely. However, more focus on research is essential, as the microorganisms of various etiological agents changes. Nearly hundred species of microorganisms are responsible for the udder inflammation (Hristov *et al.*, 2016) [8]. Bacteriological isolation and PCR identification can also be done to hasten diagnostic procedures.

Materials and Methods

The current investigation was carried out to investigate clinical mastitis among goats which were presented to the Veterinary Ambulatory Clinic and Veterinary clinical complex, College of Veterinary Science, Rajendranagar, Hyderabad. During the period under study (August, 2021 to July, 2022), a total of 2,595 goats were presented. Out of which, 260 quarter milk samples of 130 lactating goats were suspected and screened for clinical mastitis on the basis of clinical symptoms. Out of which, a total of 33 goats with 54 quarters were diagnosed with clinical mastitis based on clinical signs, qualitative changes in the milk, hematology and cultural examination. Data pertaining to age, breed, season, lactation number, stage of lactation and quarter disposition were collected in detail. Incidence of clinical mastitis was calculated taking into account the milk samples positive for bacterial growth out of total samples screened on animal and quarter basis irrespective of other tests performed. The milk samples were collected from a total of 54 quarters of 33 lactating goats to diagnose clinical mastitis.

The chemicals utilised in this investigation were procured from M/s Qualigens (Mumbai) and SD Fine Chemicals (Mumbai). All the bio-chemical test reagents were obtained from M/s Himedia (Mumbai). The following chemicals and biochemical reagents were used for this study. 4% sodium hydroxide, 4% potassium hydroxide, Kovac's Indole reagent, 5%-Naphthol reagent, Methyl Red indicator, Eko milk dyes, California mastitis test reagent, 10% potassium chromate solution and 0.1345% Silver nitrate reagent solution. The following equipments were used for analysis of milk and blood and for bacterial isolation. Hot air oven, Autoclave, Laminar air flow, ABXMicros ESV 60 blood analyzer, Eko milk scan somatic cells analyzer, Incubator, Digital pH meter.

Modified CMT was performed as per the method described by Pandit and Mehra (1969) [15]. Reagents were prepared by adding 2 ml. Stock Solution B (Bromocresolpurple Reagent) in order to make volume 100 ml by adding remaining volume of Stock-solution A (Sodium lauryl Sulphate Reagent). Total somatic cell count was estimated using Eko milk scan somatic cells analyzer. The values obtained were expressed as $\times 10^5$ cells/ml of milk. The milk samples containing SCC range of $<7 \times 10^5$ cells/ml (Radostits *et al.*, 2007) was considered healthy. Milk pH was estimated using Digital pH Meter. Blood samples were collected from the mastitis affected goats aseptically from the Jugular vein in a sterile tube containing EDTA for hematological examination. 1ml of blood sample from Jugular vein of MCMT positive animals were collected for analysis by ABX Micros ESV 60 blood analyzer supplied by Horiba India PVT. LTD as per the manufacturer's instructions. Blood sample was analyzed for hemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), differential leucocyte count (DLC). Milk samples from the affected quarters of goats were collected in sterile vials with aseptic precautions and then submitted to bacteriological investigation for etiological agents isolation. The data obtained was compiled and statistically analyzed using paired-T test and student T test as per Snedecor & Cochran (1994) [24].

Results and Discussion

During the present study, out of 260 quarter milk samples of 130 lactating goats, 54 quarters from 33 goats were

diagnosed with clinical mastitis based on a cultural examination, MCMT and other diagnostic techniques. Clinical signs recorded in goats affected with clinical mastitis were inappetence/anorexia (100%), decreased milk production (93.94%), swollen and painful udder (81.82%), pyrexia (78.79%), warmth of udder (78.79%), pale mucous membrane (72.73%), udder erythema (63.64%), dehydration (57.58%), asymmetry of teats (45.45%), depression (39.40%) and hardness of udder (27.27%) (Fig.1). The present findings were in agreement with Ved and Siddiqua (2009) [28]; Singh *et al.* (2018) [22]; Mohanty *et al.* (2019) [13] and Biswas *et al.* (2021) [4] who observed similar clinical signs in their study, such as udder redness, abnormal milk, firmness and other systemic signs like pyrexia nasal discharges, pneumonic signs, anemia, agalactia and inappetence/ anorexia.

In the present investigation, the mean Rectal temperature ($^{\circ}$ F), pulse rate (per minute) and respiratory rate (breath/min.) in Mastitis-affected goats were (104.09 ± 1.52), (94.21 ± 0.10) and (27.75 ± 0.11) which were non-significantly increased. While, the rumen motility (2.24 ± 0.04) was decreased in mastitis-affected goats compared to healthy goats (Table 1). The present findings are in agreement with Tariq *et al.* (2014) [26] who reported high temperature (104.2° F), increased heart rate (110 beats/min), and purulent discharge from the teats and the affected teat was sloughed off in a 3-year-old Beetal goat affected with gangrenous mastitis. Similarly, Shinozuka *et al.* (2018) [20] reported, an increased body temperature, respiratory rate and pulse rates. In another study, Younis *et al.* (2003) [29] reported systemic signs like increased heart rate (70-80 beats/min), tachypnea (85-110 breaths/min), increases rectal temperature with reduced rumen activity (1 to 2 contractions per minute).

In the present investigation, the color of milk samples collected from mastitis-affected goats was red (11.11%) and white (88.89%) (Fig.2). The consistency of the milk samples was watery (57.41%), viscous (22.22%) and purulent (20.37%). Presence of flakes (35.19%) and clots (12.96%) were also observed. The present findings are in agreement with Anderson *et al.* (2002) [3]; Tariq *et al.* (2014) [26] and Rizwan *et al.* (2016) [17] who observed changes in colour, presence of clots, elevated levels of leukocytes in milk and purulent discharge from the teats. Fluids, blood clotting components, and antibodies go to the infection site. Either a moderate or severe infection of the udder parenchyma could result in clinical mastitis (Singh *et al.*, 2003) [21].

In the present investigation, goats were screened for Modified CMT and it was scored accordingly 3+ (distinct slime formation which adheres to the bottom of the paddle), 2+ (slime formation) 1+ (distinct precipitate but no tendency towards gel formation and negative (no evidence of a formation of precipitation) was observed in 51.85, 38.89, 9.26 and 0 percent (Table 2). The present findings were in agreement with Mohanty *et al.* (2019) [13] screened goats for mastitis by Modified Californian Mastitis Test (MCMT) and revealed Modified CMT score 3+ (Distinct Slime Formation adhering to the paddle bottom) seen with increased Somatic Cell Count. The test depends on disrupting the membranes of somatic cells by the reagent (Sodium Lauryl Sulfate) and the reaction of DNA and proteins contained in the cells and the subsequent formation of gel which relates to the number of somatic cells in the milk (Moni and Samad, 2020) [14]. The mean somatic cell count (18.09 ± 0.71) values of mastitis-affected goats increased significantly ($p < 0.01$) as

compared to healthy control (7.04 ± 0.14) (Table 3). The findings were in accordance with Smistad *et al.* (2021) [23] who documented that the SCC was significantly higher in mastitis infected goats as compared to non-infected goats. Milk Somatic cell count increases in animals infected with mastitis and reported significant ($p < 0.05$) increase in total leukocytes and neutrophils, while significantly reduced concentration of lymphocytes and macrophages population Krishnappa *et al.* (2016) [10] & Sarvesha *et al.* (2017) [18]. The mean pH (7.12 ± 0.06) values of mastitis affected goats increased significantly ($p < 0.01$) as compared to healthy control (6.81 ± 0.12) (Table 3). These findings were in accordance with Tomar *et al.* (2018) [27] and Mohamed *et al.* (2014) [12] who reported increased milk pH in mastitis-affected goats. Increased pH in clinical mastitis milk samples was due to increased udder inflammation and responsible for an increase in the mammary gland permeability to the blood components like bicarbonates and other alkaline salts into the milk, along with reduced production of lactose by the gland so that the milk pH became above 7.0 (Mohamed *et al.*, 2014) [12].

In the present investigation, the mean haemoglobin (g/dl), total erythrocyte count ($\times 10^6/\mu\text{l}$), packed cell volume (%), total leucocyte count ($\times 10^3/\mu\text{l}$), Neutrophils (%), Lymphocytes (%), Eosinophil's (%) and Monocytes (%) values were 10.37 ± 0.56 , 9.36 ± 0.61 , 21.01 ± 2.26 , 17.12 ± 1.46 , 60.38 ± 0.21 , 39.05 ± 0.03 , 0.36 ± 0.08 and 0.21 ± 0.08 respectively. There was a significant decline in Haemoglobin, Total Erythrocyte Count, Packed Cell Volume, while elevated levels in Total Leucocyte count and Neutrophils with significant decrease in lymphocyte count and a non-significant reduction in eosinophils and monocyte value was observed in clinical mastitis affected goats as compared to healthy control (Table 4). These findings were in agreement with Abba *et al.* (2013) [1] & Singh *et al.* (2018) [22] who observed decreased Hb levels, leukocytosis, neutrophilia and lymphopenia in mastitis affected goats.

Significant decline in Hb, TEC, and PCV values suggests anemia. Elevated total leucocyte count, neutrophilia and lymphopenia may contribute to infection in animals with mastitis (Sarvesha *et al.*, 2017) [18]. In infectious conditions, inflammatory process will be activated to kill the infectious agents, during which the inflammatory cells (neutrophils, monocytes and lymphocytes) count will be increased (Ajuwape *et al.*, 2005) [2].

Cultural examination was conducted to know the specific etiological agent and 54 quarter milk samples were positive for pathogenic bacteria. The bacteria identified as single infections from clinical mastitis-affected quarter milk samples include coagulase-negative *Staphylococcus* spp., *Escherichia coli* spp., *Klebsiella* spp., *Streptococcus* spp. and coagulase-positive *Staphylococcus* spp., isolated in 29.63, 20.37, 11.11, 9.26 and 7.41 percent respectively. While, the bacteria identified in mixed infection included coagulase-negative *Staphylococcus* spp. along with *Escherichia coli*, Coagulase-negative *Staphylococcus* spp. With *Klebsiella* spp., *E.coli* with *Streptococcus* spp., Coagulase-negative *Staphylococcus* spp. with *Streptococcus* spp. & coagulase-positive *Staphylococcus* spp., with *Escherichia coli* 7.41, 5.56, 3.70, 3.70 and 1.85 percent (Table 5). The present findings were in almost agreement with Ferdous *et al.* (2018) [6]; Gabli *et al.* (2019) [7] and Savita *et al.* (2020) [19] reported increased occurrence of *Staphylococcus* spp. and it was considered to be the most prevalent organism in the quarter milk samples affected with CM. On contrary, Radostits *et al.* (2007) [16] opined that *Streptococcus* spp. was the predominant bacteria along with *Staphylococcus* spp. due to infected udder and poor managemental dairy practice methods. Increased occurrence of *Staphylococcus* spp. was because of their ubiquitous nature and adaption to survive in the udder following establishment of mild Sub Clinical Mstitis of prolonged period (Srinivasan *et al.*, 2013) [25].

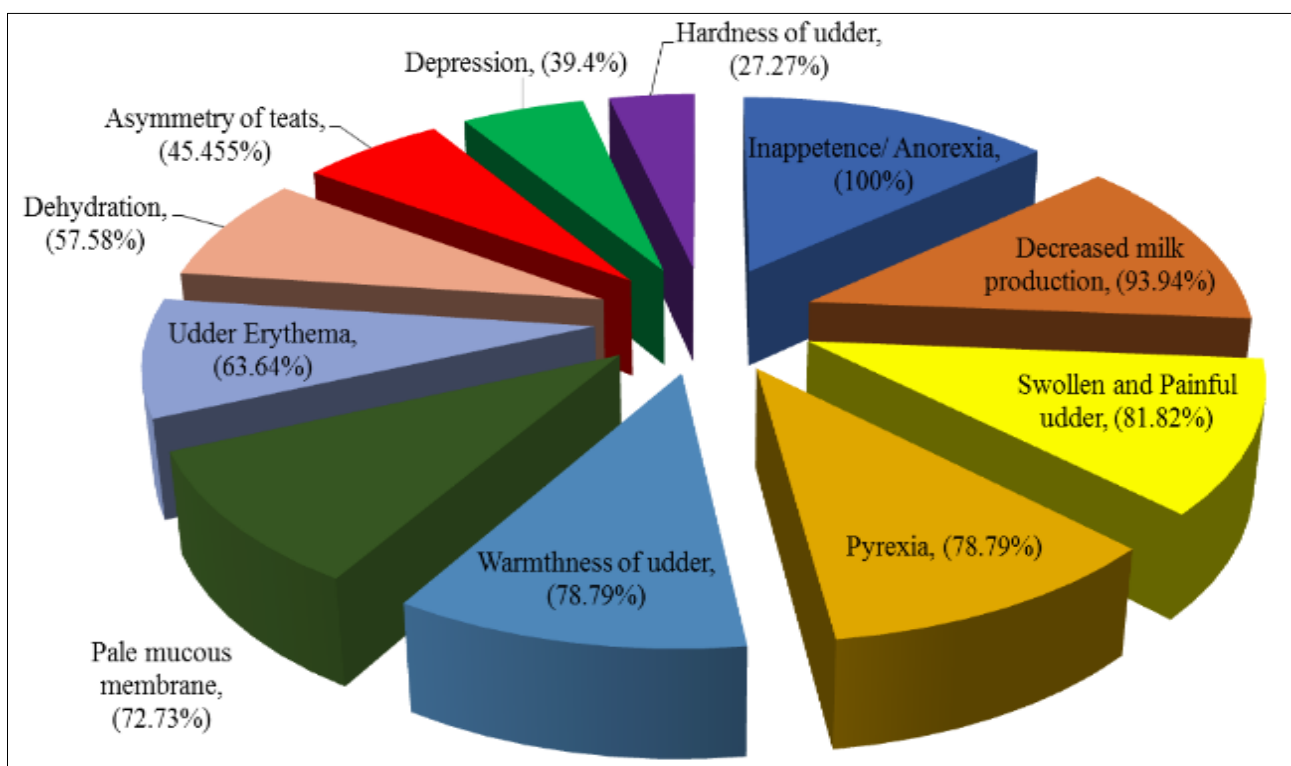


Fig 1: Clinical signs in goats affected with clinical mastitis.

Table 1: Mean values of vital parameters in clinical mastitis affected goats.

| S. No. | Parameters | Healthy control | Mastitis affected goats (n=33) |
|--------|--------------------------------|-----------------|--------------------------------|
| 1 | Rectal Temperature (°F) | 102.78±0.11 | 104.09±1.52 |
| 2 | Pulse rate (Per-minute) | 76.45±0.10 | 94.21±0.10 |
| 3 | Respiratory Rate (Breath/min.) | 24.05±0.01 | 27.75±0.11 |
| 4 | Rumen Motility (/3min.) | 3.01±0.05 | 2.24±0.04 |

Table 2: MCMT scores in milk samples from goats affected with clinical mastitis.

| Modified California Mastitis Test (MCMT) Reaction | | | | | |
|---------------------------------------------------|----------|-------------------|------------------------|-----------------------|-------|
| | Negative | Weak positive (+) | Distinct positive (++) | Strong positive (+++) | Total |
| No. of samples | 0 | 5 | 21 | 28 | 54 |
| Percentage | 0 | 9.26 | 38.89 | 51.85 | 100 |

**Fig 2:** Color of milk samples in mastitis affected goats.**Fig 3:** Presence of flakes and clots in mastitis affected milk samples.**Table 3:** Mean values of SCC and milk pH in healthy and mastitis affected goats.

| S. No. | Parameter | Healthy control (n=6) | Mastitis affected goats (n=6) |
|--------|--------------------------------|-----------------------|-------------------------------|
| 1 | SCC(x10 ⁵ cells/ml) | 7.04±0.14 | 18.09±0.71** |
| 2 | Milk pH | 6.81±0.12 | 7.12±0.06** |

**Significant at ($p>0.01$)

Table 4: Mean \pm SE values of haematological findings of healthy control and mastitis Affected goats.

| S. No. | Parameter | Healthy control(n=6) | Mastitis affected goats (n=33) |
|--------|---------------------------|----------------------|--------------------------------|
| 1 | Haemoglobin (g/dl) | 11.28 \pm 0.01 | 10.37 \pm 0.56** |
| 2 | TEC(x10 ⁶ /ul) | 13.09 \pm 0.09 | 9.36 \pm 0.61** |
| 3 | PCV (%) | 27.95 \pm 0.08 | 21.01 \pm 2.26* |
| 4 | TLC(x10 ³ /ul) | 9.08 \pm 0.06 | 17.12 \pm 1.46* |
| 5 | Neutrophils (%) | 39.91 \pm 0.22 | 60.38 \pm 0.21** |
| 6 | Lymphocytes (%) | 58.89 \pm 0.43 | 39.05 \pm 0.03* |
| 7 | Eosinophil's (%) | 0.66 \pm 0.18 | 0.36 \pm 0.08 |
| 8 | Monocytes (%) | 0.54 \pm 0.03 | 0.21 \pm 0.08 |

Significant at ($p < 0.01$)*Significant at ($p < 0.05$)Table 5:** Sample wise occurrence of bacterial isolates in clinical mastitis affected quarter milk samples.

| S. No. | Bacteria isolates | Total no. of samples (n= 54) | Percentage (%) |
|--------|-----------------------------------------------------------------------------|------------------------------|----------------|
| 1 | Coagulase-negative <i>Staphylococcus</i> spp. | 16 | 29.63 |
| 2 | <i>Escherichia coli</i> | 11 | 20.37 |
| 3 | <i>Klebsiella</i> spp. | 6 | 11.11 |
| 4 | <i>Streptococcus</i> spp. | 5 | 9.26 |
| 5 | Coagulase-positive <i>Staphylococcus</i> spp. | 4 | 7.41 |
| 6 | Coagulase-negative <i>Staphylococcus</i> spp. and <i>E.coli</i> | 4 | 7.41 |
| 7 | Coagulase-negative <i>Staphylococcus</i> spp. and <i>Klebsiella</i> spp. | 3 | 5.56 |
| 8 | <i>E.coli</i> and <i>Streptococcus</i> spp. | 2 | 3.70 |
| 9 | Coagulase-negative <i>Staphylococcus</i> spp. and <i>Streptococcus</i> spp. | 2 | 3.70 |
| 10 | Coagulase-positive <i>Staphylococcus</i> spp. and <i>E.coli</i> spp. | 1 | 1.85 |

Conclusion

In the investigation, it can be concluded that, Out of 260 quarters of 130 goats screened for clinical mastitis (CM), using different diagnostic tests, 54 quarters of 33 goats were found positive for clinical mastitis based on bacterial culture examination. Diagnosis of CM in goats was based on clinical signs, milk abnormalities, MCMT score, milk qualitative changes, hematology along with cultural examination of mastitis affected milk samples.

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