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Changes in haematological parameters in cattle affected with clinical mastitis

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Abstract
The current study aimed to determine the haematological changes in cattle affected with clinical mastitis. A total of 16 animals involving 23 quarters exhibiting clinical manifestations of mastitis in were included.Group-I clinical mastitis affected cattle treated with marbofloxacin @ 8mg/kg b.wt while group-II with marbofloxacin @ 2mg/kg b.wt Haematological analysis of groups I and II animals showed significant increase in TLC (10.34±0.71 and 10.59±0.54 x 10^3/µl) and neutrophils (66.25±2.11% and 65.88±2.72%) whereas as significant decrease in lymphocytes (30.38±1.9% and 30.88±2.66%) compared to healthy control group-III. In group II, post treatment values became significantly similar to healthy control group where as in group-I it was still differ. No significant change in Hb, PCV and TEC values were observed compared to healthy control group.

Keywords: Clinical mastitis, bacterial haematological changes, marbofloxacin

1. Introduction
Mastitis is defined as inflammation of parenchyma of mammary gland characterized by physical, chemical changes in milk and pathological changes in glandular tissue (Radostits *et al*., 2009) [6]. Clinical mastitis is a condition characterized by abnormalities of udder (swollen, hard and hot quarters) and milk (flakes, clots and watery appearance), which results in decrease in total milk production as well as changes in milk composition. mastitis as clinical, sub-clinical and latent mastitis. In clinical mastitis, there is presence of clinical signs characterized by changes pertaining to physical, chemical and bacteriological alterations and morphological changes in mammary gland.clinical mastitis in cattle and divided cattle into two groups such as coliform and non-coliform mastitis cattle based on clinical symptoms. Marked swelling of mammary gland, thin watery milk, systemic signs like loss of appetite and rectal temperature above 39.7 °C were seen in the first group and the second group showed local involvement of gland with moderate swelling, normal or discoloured but not watery milk and rectal temperature of 39.7 °C with normal feeding behavior (Grohn *et al.* (2004) [3]. Muhee *et al.* (2017a) [5] stated that the requirements of trace minerals like Cu, Zn, Mn and Se increased in mastitis as evidenced by their decreased levels in clinical mastitis and improved clinical response on their supplementation. Anti-oxidant trace minerals like Cu, Zn, Mn and Se significantly aid recovery in bovine mastitis and may play a significant role in prophylaxis of mastitis in lactating animals. He also concluded that Selenium is an essential micronutrient present in tissues throughout the body and is important physiologically because it is an integral element of the enzyme glutathione peroxidase. Radostits *et al*. (2007) [7] documented haematological alterations in clinical mastitis. There may be marked change in leukocyte count, packed cell volume due to severe infection of mammary gland and toxaemia. However clinical mastitis due to gram negative bacteria can cause profound leucopenia, neutropenia, lymphopenia and monocytopenia as a result of endotoxaemia. Clinical mastitis associated with gram positive bacteria in cattle result in milk leukocytosis Routine examination of blood and serum is necessary for monitoring the health status of animal.

2. Materials and methods
2.1 Selection of Animals
The presentstudy entitled “Therapeutic studies on clinical mastitis in cattle” was carried out in the animals presented to VCC, CVAS, Bikaner or from individual holding of owner during June to November 2019.

A total of 16 cattle showing signs of clinical mastitis such as inflammatory swelling of udder, pain on palpation and physical composition of milk such as color, presence of clots, flakes, pus and any other abnormalities.

2.2 Collection of blood samples

Blood samples were collected from clinical mastitis affected cattle along with apparently healthy control cattle on 0th day and 5th day after treatment for hematology. The blood samples were collected from jugular vein with all aseptic precautions in EDTA coated and non EDTA coated vials from cattle affected with clinical mastitis and healthy control animals.

The blood samples were collected for determination of Haemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC) and Differential leukocyte count (DLC). Serum was separated and stored at -20 °C for mineral estimation in clinical mastitic and healthy cattle.

2.3 Therapeutic trial groups

2.3.1 Slection criteria for inclusion in therapeutic trials

The cattle that were treated with antibiotics within the past 30 days not included in the present therapeutic trials. A total of 16 cattle which were affected with clinical mastitis were randomly divided in to 2 groups consisting of 8 cattle each.

Group I: In this group cattle were administered with Marbomet® @ 8mg/kg body weight intramuscular once.

Group II: In this group cattle were administered with Marbomet® @ 2mg/kg body weight intramuscular for three consecutive days.

In addition both group I and II will be supplemented with trisodium citrate and antioxidant therapy (Mammidium Powder) 50gm oral OD as electuary for 4 days along with NSAID's (Inj. Flunixin meglumine @ 1.1 mg/kg B. wt. intramuscular BD) for 3 days.

Group III: In this group 8 healthy control animals were taken.

The response to therapy was recorded based on clinical and bacterial culture examination on 0th day as pre treatment and 5th day after treatment to know *in vivo* therapeutic efficacy.

The qualitative changes in hematological parameters on 0th day and on 5th day post treatment were recorded.

3. Statistical analysis

The results obtained were subjected to statistical analysis as per the methods described by Snedecor and Cochran (1994) [12] and by using SPSS 20.0.0 version.

4. Results and Discussion

The study was carried out in the Department of Clinical Veterinary Medicine E&J, College of Veterinary and Animal Science, Bikaner from June to November 2019. Some of the blood samples were also collected from individual holdings.

Clinical mastitis was manifested by change in gross appearance of udder like swelling, pain on palpation, erythema, warmth and hardness. There was gross change in appearance of milk like change in colour (yellow), consistency (viscous and purulent), presence of flakes and clots. Change in hematological parameters of cattle were examined and recorded before and after treatment.

4.1 Haematological parameters

Haematological response to the inflammation of mammary gland was assessed by analyzing various parameters namely Haemoglobin (g/dl), Packed Cell Volume (%), Total Erythrocyte count ($\times 10^6$/µl) Total Leukocyte Count ($\times 10^3$/µl), Differential Leukocyte Count (%) before treatment and 5th day after treatment. The data pertaining to haematological analysis of mastitic cattle before and after treatment and their correlation with healthy controls are summerised in (Table-1).

4.1.2 Haemoglobin (g/dl)

The haemoglobin concentration in clinical mastitic cattle of group-I and group-II before treatment was 9.85±0.21 and 9.35±0.40 gm/dl respectively, as against 10.55±0.42 gm/dl of healthy controls (Group-III). There was no significant difference between control and clinical mastitic animal (Group-I and Group-II) values (Table1).

The post treatment haemoglobin concentration in clinical mastitic cattle of group-I and group-II was 9.93±0.21 and 10.1±0.11 gm/dl respectively, as against 10.55±0.42 gm/dl of healthy control group-III showing no significant difference.

4.1.3 Packed cell volume (PCV)

The average packed cell volume (PCV) before treatment was 28.5±0.31% and 27.84±0.78% in clinical mastitic cattle of group-I and group-II, respectively over 30.39±0.84% of healthy control group-III. There was no significant difference between control and clinical mastitic animals (Group-I and Group-II) values (Table-1).

The average packed cell volume (PCV) after treatment was 28.95±0.37% and 28.2±0.56% in clinical mastitic cattle group-I and group-II, respectively over 30.39±0.84% of healthy control group-III showing no significant difference.

4.1.4 Total erythrocyte count (TEC)

In the present study the mean ± SE value of total erythrocyte count of clinical mastitic group-I and group-II was 7.29±0.15 and 7.16±0.15 x 10^6 cells/µl before treatment and of control group-III was 7.39±0.36x10^6 cells/µl. There was no significant difference between control and clinical mastitic group values (Table-1).

The mean ± SE value of total erythrocyte count of clinical mastitic group-I and group-II was 7.32±0.13 and 7.22±0.20x10^6 cells/µl after treatment over 7.39±0.36x10^6 cells/µl of healthy control group-III showing no significant difference.

4.1.5 Total leukocyte count (TLC)

In the present study the mean ± SE value of total leukocyte count of control group was 6.82±0.33 x10^3 cells/µl and of clinical mastitic animals of group-I and group-II before treatment was 10.34±0.71 and 10.59±0.54 x10^3 cells/µl, respectively. The mean ± SE value of total leukocyte count in clinical mastitic animals was significantly higher than healthy control group-III values (Table-18).

The post treatment mean ± SE value of total leukocyte count in group I and group II (8.25±0.41 and 7.28±0.38x10^3 cells/µl) were significantly decreased than their pre-treatment values but mean±SE value of group I was still significantly higher than control group III while the post treatment mean±SE value of total leukocyte count in group II was differ non significantly with mean value of control group III.

4.1.6 Differential leukocyte count (DLC)

4.1.6.1 Neutrophils

In the present study the mean ± SE value of neutrophil of control group was 30.38±1.06% and of clinical mastitic group-I and group-II before therapy was 66.25±2.11% and 65.88±2.72% respectively. The mean ± SE value of neutrophil in clinical mastitic animals was significantly higher than healthy control group-III (Table-1).

The post treatment mean ± SE value of neutrophils in group I and group II (44.88±1.52 and 33.38±1.74%) were significantly decreased than their pre-treatment values but post treatment mean±SE value of neutrophils in group I was still significantly higher than control group III while the post treatment mean±SE value of neutrophils in group II was differ non significantly with mean value of control group III.

4.1.6.2 Lymphocytes

In the present study the mean ± SE value of lymphocytes of control group was 65.88±1.26% and of clinical mastitic animals of group-I and group-II before therapy was 30.38±1.9% and 30.88±2.66%, respectively. The mean±SE value of lymphocytes in clinical mastitic animals was significant lower than healthy control group-III (Table-1).

The post treatment mean±SE value of lymphocytes in group I and group II (52.13±1.43 and 62.88±1.86%) were significantly increased than their pre-treatment values but post treatment mean±SE value of group I was still significantly lower than control group III while the post treatment mean±SE value of lymphocytes in group II was differ non significantly with mean value of control group III.

4.1.6.3 Monocytes

In the present study the mean±SE value of monocytes of control group was 2.13±0.39% and of clinical mastitic animals of group-I and group-II before treatment was 1.75±0.31% and 1.50±0.18%, respectively. There was no significant difference between control and clinical mastitic animal values (Table-1).

The mean±SE value of monocytes of clinical mastitic animals (Group-I and Group-II) after treatment was 1.5±0.26% and 1.75±0.25%, respectively with no significant difference compared to healthy control group.

4.1.6.4 Eosinophils

In the present study the mean±SE value of eosinophils of control group was 1.38±0.18% and of clinical mastitic animals (Group-I and Group-II) before therapy was 1.38±0.18 and 1.63±0.18%, respectively. There was no significant difference between control and clinical mastitic animal values (Table-1).

The mean±SE value of eosinophils of clinical mastitic animals (Group-I and Group-II) after treatment was 1.38±0.18% and 1.75±0.16%, respectively with no significant difference compared to healthy control group.

4.1.6.5 Basophils

In the present study the mean±SE value of basophils of control group was 0.25±0.16% and of clinical mastitic animals (Group-I and Group-II) before therapy was 0.25±0.16% and 0.13±0.12%, respectively. There was no significant difference between control and clinical mastitic animal values (Table-18 and Figure 1).

The mean±SE value of basophils of clinical mastitic animals (Group-I and Group-II) after treatment was 0.13±0.12 and 0.25±0.16% respectively with no significant difference compared to healthy control group.

The haematological findings of present study are similar with the findings observed by Sarvesha *et al*. (2017) [8], Das *et al.*, (2018) [2], Tripathy *et al*., (2018) [13] and Singh *et al*. (2014) [10] who showed a significant (P< 0.05) increase in neutrophil and total leukocyte count in crossbred cattle affected with clinical mastitis.

Table-1: Pre and post-treatment mean ± SE value of haematological parameters of clinical mastitic (Group-I and Group-II) and healthy control (Group-III) animals

S. No.	Parameters	Group-I		Group-II		Group-III
		Pre treatment	Post treatment	Pre treatment	Post treatment	Control
1.	Hb (g/dl)	9.85 ± 0.21	9.93±0.21	9.35 ± 0.40	10.1±0.11	10.55 ± 0.42
2.	PCV (%)	28.5 ± 0.31	28.95± 0.37	27.84 ± 0.78	28.2±0.56	30.39 ± 0.84
3.	TEC(x10^6 cells/µl)	7.29 ± 0.15	7.32±0.13	7.16 ± 0.17	7.22±0.20	7.39 ± 0.36
4.	TLC(x10^3 cells/µl)	10.34 ± 0.71^b	8.25±0.41^c	10.59 ± 0.54^b	7.28±0.38^a	6.82 ± 0.33^a
5.	Neutrophils (%)	66.25 ± 2.11^c	44.88±1.52^b	65.88 ± 2.72^c	33.38±1.74^a	30.38 ± 1.06^a
6.	Lymphocytes (%)	30.38 ± 1.9^a	52.13±1.43^b	30.88 ± 2.66^a	62.88±1.86^c	65.88 ± 1.26^c
7.	Monocytes (%)	1.75 ± 0.31	1.5±0.26	1.50 ± 0.18	1.75±0.25	2.13 ± 0.39
8.	Eosinophils (%)	1.38 ± 0.18	1.38±0.18	1.63 ± 0.18	1.75±0.16	1.38 ± 0.18
9.	Basophils (%)	0.25±0.16	0.13±0.12	0.13±0.12	0.25±0.16	0.25±0.16

Mean having different superscript (a,b,c) in a row differ significantly p<0.05

Haemoglobin and PCV level of mastitic cattle showed non significant decrease compared with healthy animals. Finding corroborates with the observation of Sischo *et al.*, (1997) [11] Sarvesha *et al.*, (2017) [8], and Singh *et al.*, (2014) [10] who observed that PCV and Hb did not exhibit any specific trend in the animals suffering from mastitis however, Zaki *et al.* (2010) [14], Das *et al.*, (2018) [2] reported that anaemia in mastitic cattle was due to decrease in Hb, RBC, and PCV levels.

Similar findings were reported by Khan *et al*. (1997) [4] as increased TLC and increase in absolute number of monocytes, eosinophils and neutrophils in mastitis. These findings could be due to persistent infection noticed in clinical mastitis (Cebra *et al*., 1996; Zaki *et al*., 2008) [1, 15].

5. Conclusion

Haematological analysis of groups I and II animals showed significant increase in TLC (10.34±0.71 and 10.59±0.54 x 10^3/µl) and neutrophils (66.25±2.11% and 65.88±2.72%) whereas as significant decrease in lymphocytes (30.38±1.9% and 30.88±2.66%) compared to healthy control group-III. In group II, post treatment values became significantly similar to healthy control group where as in group-I it was still differ. No significant change in Hb, PCV and TEC values were

observed compared to healthy control group.
The bacteriological cure rate on fifth day of post treatment was observed quarter-wise as 58.33% and 81.81% in Group I and II, respectively.
In the present study, therapeutic efficacy on the basis of improvement in clinical findings, haematological parameters and bacterial cure rate of group-II was better compared to group-I.

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8. Authors Contribution: Part of M.V.Sc research work carried out by Dinesh Kumar Saharan under the guidance of Assistant Professor Sunita Choudhary.

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