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Prevalence and haematological evaluation in clinical and subclinical mastitis in cattle in Bikaner district of Rajasthan

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Abstract
The study was conducted on 270 dairy cows. The milk samples of these cows were screened by California Mastitis Test (CMT) to find out prevalence. A total of 50 cows were taken for further study and divided into subclinical mastitis group (give only positive CMT reaction and no clinical findings) and control(cows show negative CMT reaction and absence of clinical findings).25 animals were taken in clinical mastitis group on the basis of clinical examination and California Mastitis Test (CMT) from various places. The cows showing negative reaction were considered as healthy control. Blood samples of these cows were taken for haematological studies. There was 16.29% animal wise prevalence of subclinical mastitis in dairy cattle of Bikaner. A significant increase (p<.05) in neutrophil and significant decrease (p<.05) in haemoglobin, total erythrocyte count, packed cell volume were observed. No significant difference were observed in monocyte, lymphocyte and total leukocyte count in cows with mastitis groups.

Keywords: Milk, mastitis, prevalence, haematological

Introduction
India is an agricultural country with animal husbandry being an integral part of agriculture. Cattle are the most common type of large domesticated animals. Cattle are raised as dairy animals for milk and other dairy products. Rearing of cattle is an enterprise, which enables poor and landless farmers to earn income using common-property resources and land that has no other sustainable agricultural use.

Milk is one of the most important foods of human beings. It is universally recognized as a complete diet due to its essential components. It contains a wide range of dietary components of vital importance like water, proteins, lactose, minerals and vitamin. The composition of milk varies with species and breed of the animal, feeding regims, stage of lactation, parity and udder health.

As per the 19th livestock census (2012), India posseses 1,90,904 million cattle population. India the largest producer of milk in the world, is set to produce over 133 million tonnes milk during 2012-13. India contributed 38% of total milk production in the world (National Dairy Research Institute, Karnal).The dairy industry in India has been on a sturdy path of progression since Indian independence. The milk production of India has grown from 17 million tonnes (1951) to 127 million tonnes (2012) and expected to increase upto 190 million tonnes, worths 0.05 lakh. Billion by the year 2015.

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature (International Diary Federation, 1987) [4]. It is characterized by physical, chemical and usually bacteriological changes in the milk and by pathological changes in the glandular udder tissue. It results from the introduction and multiplication of pathogenic microorganisms in the mammary gland that is a complex series of events leading to reduced synthetic activity, compositional changes, and elevated somatic cell count (SCC).

Etiological agents of mastitis can be infectious or non-infectious (Watts, 1988, Radostits *et al*., 1994) [12, 8]. Organisms as diverse as bacteria, viruses, mycoplasma, yeasts and algae have been implicated as causes of the mastitis. Usually, the majority of the infections is caused by the contagious pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and by the environmental pathogens *Streptococcus uberis*, *disgalactiae*, *Coliforms*, *Pseudomonas*,

Prototheca, yeasts. These pathogens infect the udder generally through the ductus papillaris, which is the only opening of the udder to the outside world.

Staphylococcus aureus is the most problematic and significant pathogen in contagious mastitis due to the occurrence of pathogen strains particularly resistant to antibiotics. It is most commonly isolated from subclinical mastitis and one of the most important worldwide causes of mastitis in cattle.It is responsible for major financial losses in dairy farming and for the culling of many cows (Awale *et al*., 2012) [1]. Once cows become infected they posses significant problems. When infections have become established, they are difficult to remove and infected cows are a potential source of infection to other cows.

Mastitis is classified as acute, subacute, chronic, subclinical mastitis. Clinical acute mastitis start suddenly, usually accompanied by systemic effects as swollen and painful quarters, fever, inappetence, dehydration, a remarkable decrease in milk production and evident milk alterations. Sub-acute mastitis is characterized by lack of symptomatology, few udder clinical signs and some milk alterations. Subclinical mastitis is observed more frequently, characterized by normal gland and milk appearance and increase of somatic cell count. They are often not diagnosed and consequently their alterations are only detected by using screening tests, such as bacterial agent detection, California Mastitis Test, electrical, conductivity test etc. Chronic mastitis show lack of symptomatology, no milk production and udder healings conductivity (Fagiolo and Lai, 2010) [3].

Diagnosis of clinical mastitis is based on the local and systemic reactions and changes in milk (e.g. off-color, watery, bloody appearance and presence of flakes, clots and pus) both in dairy buffalo and cow. The amount udder swelling, severity of pain and the overall appearance of the animal will indicate the severity of infection and serve as a guide for the course of treatment (Sharma *et al*., 2011) [10].

Materials and Methods

1. Animals: The study population was 270 dairy cattle in Bikaner. The samples were taken from various places (Tilaknagar, Shivbari, Patelnagar, Murli manohar gaushala, khaturia colony, Jassusar gate, Udayramsar areas) of Bikaner. The Breeds of cows were Gir, Rathi, Jersy, Holestein Freisen, American and nondescript. The cows were 2.5-8 years old, yielding 3- 30 litres of milk per day and between 1 to 9 months of lactation in the first to sixth parity. The study was conducted within a period of 7 month from March2015 to September2015. There was little quantity of green fodder was available to the animals. Dry fodder included wheat straw, bajra kadbi, pala, groundnut bhusa and dry grasses of sewen (*lasiurus scindicus*) and bhurut (*cenchrus biflorus*) were usually provided ad libitum to all the categories of dairy cattle. There was no variation in the type of dry fodder fed to the dairy cattle in milk shed and non-milkshed areas. The concentrate mixture included crushed bajra, groundnut cake, mustard cake, guar churi, moth churi and mung churi according to the milk yield. The cows had free access to feed and water all the time.

After the clinical examination of cows, the milk samples were aseptically collected into sterilized glass tubes. The milk samples were taken for California mastitis test (CMT) to find out the prevalence of subclinical mastitis. Test. For further study 50 animals were included from this group (270) and divided into subclinical mastitis group and control group.25 animals were taken in clinical mastitis group on the basis of clinical examination and California Mastitis Test (CMT) from various places. The cows belonging to the group clinical mastitis group(CM) has positive CMT reaction and clinical findings (gross abnormality in milk, inflammation of udder and hardness in udder) whereas the group subclinical mastitis(SCM) give only positive CMT reaction. There was no clinical findings. The healthy cows show negative CMT reaction and there was absence of clinical findings.

2. Collection of milk samples

To collect the milk aseptically, udder and teat were washed with water and dried. Then each teat was wiped off by spirit swab. Two-three strippings of fore milk were discarded. Approximately 30 ml of fore milk from each teat was collected in the sterilized test tubes. Care was taken to avoid any type of contamination in the milk. All the samples of milk were brought to the laboratory and analyzed immediately.

3. Modified California mastitis test

California Mastitis Test is the most commonly employed screening test for the diagnosis of Somatic cell count and can be conveniently used in the field conditions.

The California Mastitis Test has been developed by Schalm and Moorlander (1957) [9]. The CMT is a simple method to estimate the DNA content in milk. It is based on the use of an anionic detergent, sodium-lauryl sulphate (SDS), which dissolves cell membranes and nuclei. Consequently DNA is released and it forms a transient gel with the detergent, which the viscosity is proportional to the DNA content of the sample. Besides, a pH indicator, the bromcresol purple, is included in the reagent and it gives a purple colour in the presence of alkaline mastitic milk. According to the colour change and viscosity of the formed gel, the CMT score is considered as negative (-), trace (T) or positive (1+ to 3+).

In present study Ezee (a Godrej detergent) was used in place of pure alkyl aryl Sulphates or sulphonates of sodium or potassium as an anionic surface-active agent. Cresol red was replaced by Bromocresol purple.

The final composition of test reagent was as follows

Ezee = 2.0 ml
Sodium hydroxide (AR) = 4.5 g
Bromocresol purple = 10.0 mg
Distilled water to make 1000 ml.

Procedure

The test was carried out with 3 ml of milk from each quarter in the 2 cups of plastic paddle. An equal amount of the above test reagent was added in both cups and gently mixed by circular movements 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 colour reaction with bromocresol purple. This test has specificity for leukocytes in the milk.

CMT reaction was scored as follows

Negative (-) Mixture remained liquid with no evidence of formation of a precipitate.

Trace (T) A slight precipitate, which tends to disappear with continued movements of the paddle.

1+ A distinct precipitate but no tendency towards gel formation

2+ The mixture thickens immediately with a suggestion of gel formation. As the mixture is caused to swirl, it tends to move towards the centre, leaving the bottom of the outer edge of the cup exposed. When the motion is stopped, the mixture levels out again covering the bottom of the cup.
3+ A distinct gel forms that tends to adhere to the bottom of the paddle, and during swirling a distinct central peak forms.

4. Haematological evaluations
The blood samples were collected in heparinised tubes by puncture from the jugular vein of each animal (control, CM and SCM) for haematological evaluations Blood with anticoagulant (EDTA) was used for determining haemoglobin (Hb) content, packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), total platelet count (TPC) and differential leucocyte count (DLC) as per method described by Jain, 1986 [5].

Results and Discussion
In the present study milk samples from 270 dairy cows were taken from various places of Bikaner. The prevalence of subclinical mastitis was recorded on the basis of California mastitis test. Test. For further study 50 animals were included from this group (270) and divided into subclinical mastitis group (give only positive CMT reaction and no clinical findings) and control(cows show negative CMT reaction and absence of clinical findings).25 animals were taken in clinical mastitis group on the basis of clinical examination and California Mastitis Test (CMT) from various places. The haematological analysis was performed at TVCC (Teaching Veterinary Clinical Complex) Bikaner.

1. Prevalence: The result of CMT used for detection of subclinical mastitis are presented in table 1.

Table 1: Results of California mastitis test (CMT) used for detection of subclinical mastitis in dairy cows

Diagnostic test	No of animals	Positive animals	Percentage (%)
California mastitis test (CMT)	270	44	16.29

Milk samples of 270 cows were examined for subclinical mastitis on the basis of California mastitis test (CMT).Out of these 16.29% (44/270) were found positive for subclinical mastitis. Chahar *et al*. (2005) [2] reported 51.28 (40/78) per cent animal-wise prevalence of sub-clinical mastitis (SCM) in cows and on the basis of California mastitis test (CMT).

2. Haematological parameters
Haemoglobin (g/dl)
The mean ± SE values of haemoglobin in clinical mastitis, subclinical mastitis and control group are presented in table 2.

Table 2: Mean ± SE value of Haemoglobin (Hb) of clinical mastitis, subclinical mastitis and control group

Groups	Hb (g/dl)	Range(g/dl)
Control(n=25)	9.44±0.13 [a]	8.4-10.6
Subclinical (n=25)	8.88±0.16 [b]	7.6-10.8
Clinical (n=25)	8.54±0.20 [b]	7-10.6

a, b Different superscript differ significantly ($P< 0.05$)

The Mean ± SE values of Hb of clinical mastitis, subclinical mastitis and control group were 8.54±0.20, 8.88±0.16 and 9.44±0.13 g/dl with range of 7-10.6, 7.6-10.8 and 8.4-10.6 g/dl, respectively.
In the present study the mean value of Hb in clinical and subclinical groups was found significantly low (P≤0.05) as compared to the mean value recorded in control groups. This finding was in agreement with the finding of Zaki *et al*. (2007), Padhya *et al*. (2014) [7] and Siddiqe *et al*. (2015) [11] in mastitis.

Total erythrocyte count (million/cumm): The mean ± SE values of total erythrocyte count of control, subclinical and clinical mastitis group have been presented in Table 3.

Table 3: Mean ± SE value of total erythrocyte count of control, subclinical and clinical mastitis group

Group	Mean ± SE	Range
Control	7.57± 0.43[a]	5.29– 8.4
Subclinical	7.22± 0.08 [b]	5.74 -8
Clinical	7.10± 0.11[b]	5.6- 7.6

a, b Different superscript differ significantly (P≤0.05)

The mean ± SE value of total erythrocyte count of clinical mastitis, subclinical mastitis and control group were 7.10± 0.11, 7.27± 0.08 and 7.57± 0.43 million/cumm with range of 5.6-7.6, 5.74-8 and 5.29– 8.4 million/cumm, respectively. In the present study the mean value of TEC in clinical and subclinical was found significantly low (P≤0.05) as compared to the mean value recorded in control group. The finding was in conformity with the finding of Zaki *et al*. (2007), Padhya *et al*. (2014) [7] and Siddiqe *et al*. (2015) [11] in mastitis.

Packed cell volume (%): The mean ± SE values of pack cell volume of control, subclinical and clinical mastitis group have been presented in Table 4.

Table 4: Mean ± SE value of packed cell volume value of control, subclinical and clinical mastitis group

Group	Mean ± SE (%)	Range (%)
Control	33.71± 0.54 [a]	26.7- 36.1
Subclinical	33.28 ± 0.082 [b]	27.6- 38.9
Clinical	31.10 ± 0.55[b]	25.9- 36.3

a, b Different superscript differ significantly (P≤0.05)

The mean ± SE values of PCV of clinical mastitis, subclinical mastitis and control group were 31.10 ± 0.55, 33.28 ± 0.082, 33.71± 0.54% with range of 25.9- 36.3, 27.6- 38.9 and 26.7- 36.1 percent, respectively. In the present study the mean value of TEC in clinical and subclinical group was found significantly low ($P\leq$ 0.05) as compared to the mean value recorded in control group. The finding was in conformity with the finding of Zaki *et al*. (2007), Padhya *et al*. (2014) [7] and Siddiqe *et al*. (2015) [11] in mastitis.

Total leucocyte count (thousand/cumm)
The mean ± SE values of total leucocyte count of control, subclinical and clinical mastitis group have been presented in Table 5.

Table 5: Mean ± SE value of total leucocyte count (thousand/cumm) of control, subclinical and clinical mastitis group

Group	Mean ± SE (thousand/cumm)	Range(thousand/cumm)
Control	9.707± 0.19	7.38 – 11.1
Subclinical	9.708 ± 0.44	8-12.05
Clinical	9.722±0.06	6.5 -12.4

The mean ± SE values of TLC in clinical mastitis, subclinical mastitis and control group were 9.722 ±0.06, 9.708± 0.44 and 9.707± 0.19thousand/cumm with range of 6.5 -12.4, 8-12.05 and 7.38 – 11.1thousand/cumm, respectively. The mean value of TLC in clinical mastitis and subclinical mastitis group were not show significant ($P \leq 0.05$) difference from the mean value recorded in control group.

Differential leucocyte count (%)

The mean ± SE values of differential leucocyte count of control, subclinical and clinical mastitis group have been presented in Table 6.

Table 6: Mean ± SE value of differential leucocytes count (DLC) of control, subclinical and clinical mastitis group

Group	N%	L%	M%	E%	B%
Control	31.46±1.17[a]	64±1.69	5.533±0.21	1.43±0.59	0.11±0.03
Subclinical	35.66±1.64[b]	64.12±1.11	5.535±0.14	1.70±0.69	0.13±0.05
Clinical	37.28±2.10[b]	64.69±2.08	5.537±0.12	1.84±0.67	0.2 ± 0.04

a, b Different superscript differ significantly ($P \leq 0.05$)

Neutrophils

The mean ± SE values of neutrophils of clinical mastitis, subclinical mastitis and control group were 37.28 ± 2.10, 35.66 ± 1.64 and 31.46±1.17 percent with range of 24.7-56.8, 22-54 and 22.3–42.9 percent, respectively. Increased in absolute number of neutrophils in mastitis were reported by Khan *et al*. (1997) Zaki *et al*. (2007) and Siddiqe *et al*. (2015) [11].

Lymphocytes

The mean ± SE values of lymphocytes of clinical mastitis, subclinical mastitis and control group were 64.69±2.08(39.7-77.4), 64.12±1.11(56.3-72.4) and 64±1.69(51.1-78.4) percent, respectively. The mean values of clinical and subclinical mastitis group cows were not found significantly ($P \leq 0.05$) different when compared to mean value of apparently healthy cows.

Monocytes

The mean ± SE values of monocytes of clinical mastitis, subclinical mastitis and control group were 5.537±0.12(4.6-7.1), 5.535±0.14 (4.1-7.6) and 5.533±0.21(3.6-7.6) percent, respectively. No significant difference were found between mean values of monocytes of clinical and subclinical mastitis group when compared to mean ± SE value of control group.

Eosinophils

The mean ± SE values of eosinophils of clinical mastitis, subclinical mastitis and control group were 1.84± 0.67 (0-9.1), 1.70 ± 0.69 (0-9.3) 1.43± 0.59 (0-8.5) percent, respectively. The mean values of eosinophil of clinical and subclinical mastitis group were not found significantly different when compared to mean value of healthy cows.

Basophils

The mean ± SE values of basophils of clinical mastitis, subclinical mastitis and control group were 0.2 ± 0.04(0-0.7) and 0.13 ± 0.05 (0-0.5), 0.11 ± 0.03 (0-0.5) percent, respectively. No significant difference were found in the values of basophil of clinical and subclinical mastitis group when compared to mean value of apparently healthy cows.

Haematological values determined for clinical and subclinical mastitis groups show a significant increase in neutrophils and a significant decrease in haemoglobin, packed cell volume and total erythrocyte counts. The significant changes observed between mastitis groups and control group might be due to the degree of inflammatory responses at systemic levels.

Conclusion

The findings of present investigation concluded that animal wise prevalence of subclinical mastitis in cows were 16.29(44/270) percent in dairy cattle of Bikaner.

Haematological studies in mastitis infected cows revealed significant decrease in haemoglobin, total erythrocyte counts and packed cell volume, significant increase in neutrophils counts and no significant difference in total leucocyte counts, lymphocytes, monocytes in mastitis groups when compared with apparently healthy cows.

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