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Prevalence of subclinical mastitis in dairy cows in the area of Bikaner district of Rajasthan

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Abstract
The objective of this study was to determine the prevalence of subclinical mastitis in dairy cows of the area of Bikaner district of Rajasthan. A total of 392 quarters milk sample of 100 HF crossbred cattle were collected for study which were subjected to physical examination and afterward screened for subclinical mastitis using four indirect tests *viz*. modified california mastitis test (MCMT), somatic cell count, electric conductivity and pH. Overall prevalence of subclinical mastitis (SCM) in 100 lactating dairy cows found in this study on the basis of MCMT, SCC, EC and pH were 53 per cent, 57 per cent, 51 per cent and 49 per cent on animal basis and 29.33 per cent, 32.14 per cent, 28.57 per cent, 27.29 per cent, respectively on quarter basis. Hind quarters were found more affected than fore quarters.

Keywords: Dairy cows, prevalence, subclinical mastitis

Introduction
Mastitis is an inflammation of the mammary gland that can be caused by various physical or chemical agents but the majority of the causes are infectious and usually caused by bacteria that invade the udder, multiply and produce several toxins that are harm to the mammary gland (Radostits *et al.*, 2007) [15]. The disease is complex and multi-factorial, and its occurrence is influenced by a host of variables, including the animal, the pathogen, and the environment. Mastitis occurs in two forms; clinical and subclinical mastitis. Subclinical mastitis is a non-observable form of mastitis, such as no visible abnormalities in udder tissues as well as in milk (Kathiriya *et al.*, 2014) [9]. Sub clinical mastitis remains a herd problem, without visible clinical signs or changes in milk grossly, which may be detected by indirect testing like the Modified California Mastitis Test (MCMT), total somatic cell count (TSCC), electrical conductivity (EC) and a cultural examination of the milk. *Staphylococcus* spp. is found to be the main etiological agents of clinical and subclinical mastitis in cows while, *Staphylococcus aureus* and *E. coli* are most frequently isolated pathogen from the clinical mastitis, coagulase negative *Staphylococci* (CNS) are the most commonly isolated pathogens from the subclinical mastitis (Contreras *et al.*, 2003) [4]. The dairy industry suffers much greater losses due to subclinical mastitis (SCM) than clinical mastitis (Kader *et al.*, 2002) [7]. Many risk factors have been identified for clinical and subclinical mastitis in the dairy animals such as breed, parity, age, stage of lactation, nutritional status, physical condition of teat, udder hygiene, genetic factor and housing system (Constable *et al.*, 2017) [3]. It is important to determine the prevalence of SCM in dairy herds and also to demarcate the main factors responsible for it.

Materials and Methods
Collection of milk samples
For the present study, 100 apparently healthy lactating cattle from private dairy farms, Livestock Research Station, Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner and animals of individual holding in and around the Bikaner were screened for subclinical mastitis. The tests performed immediately after collection of milk. Udder and teats were washed with water and air-dried and then wiped off by spirit swab. First three-four stripping of fore milk were discardedand then 10 ml of milk taken into sterilized test tubes with rubber cap.

Physical examination of milk samples
Physical examination performed immediately after collection of milk samples to determine any abnormalities in colour, odour, consistency and presence of flakes, clot, blood and any other visible abnormalities.

Modified California Mastitis test
The MCMT was carried out on milk samples using the Schalm and Noorlander method (1957) [18]. In present study, as an anionic active surface agent, Ezee was used in place of aryl sulphates (Chahar, 2001 and Savita, 2016) [2, 17].
The test was carried out with 3 ml of milk samples collected from each quarter into the respective 4 cups in CMT paddle. Then equal amount of MCMT reagent was added to each cup of paddle. The content was mixed by gentle circular motion of paddle in the horizontal plane. Depending on the degree of precipitation and formation of gel, readings of positive test were categorized as negative, trace, weak positive (+), distinct positive (++) and strong positive (+++), respectively.

Somatic Cell Count
About 0.01 ml (10 µl) milk was withdrawn with the help of micropipette and spread evenly in 1 square cm area on a grease free glass slide. The smear was allowed to dried in air. Then, a few drops of xylene poured over the milk smear. Then, the smear was air dried and fixed with 99 per cent methanol for 2 minutes and washed with distilled water. After fixing, the smear was stained with Giemsa stain for 30 minutes. Then smear was kept in phosphate buffer solution (pH 7.0) in coupling jar for 5 minutes and bloat dried. This smear was used for somatic cell count under oil immersion and the cells were counted in total 20 fields. The average number of cells (per square cm) area was calculated. For counting of cells per ml of milk the average numbers of cells per field were multiplied by microscopic factor.

Electric Conductivity
Electrical conductivity of milk samples was determined by Pen type EC-035 (ATC) Conductivity meter of ERMA instruments.

pH
Milk pH was measured by using single electrode Pen type digital pH meter immediately.

Statistical analysis
Statistical analysis of the collected observations was done on the basis of the methods described by Snedecor and Cochran (1989) [23] and by using SPSS 20.0.0 version.

Results and Discussion
In the present study, a total number of 100 HF crossbred cows were screened on the basis of MCMT, SCC, EC and pH for subclinical mastitis. Out of 400 quarters of 100 cows 392 functional quarters were examined during the study. Based on MCMT prevalence of sub-clinical mastitis was 53 per cent (53/100) on animal basis and 29.33 per cent (115/392) on quarter basis (Table 1). Prevalence of present study was accordance to the study of Tanwar *et al.* (2001) [26], Kumar (2010) [10], Marwaha (2018) [11] and Kachhawa (2019) [6] wherein they reported animal-wise prevalence as 54 per cent, 54.09 per cent, 49.78 per cent and 48 per cent, respectively and Kumar (2010) [10], Saidi *et al*. (2013) [16], Mir *et al*. (2014) [13], Savita (2016) [17] and Kachhawa (2019) [6] reported quarter-wise prevalence 31.02 per cent, 29.20 per cent, 30.73 per cent, 29.50 per cent and 27.31 per cent, respectively.
Prevalence of subclinical mastitis on basis of somatic cell (SCC) count was recorded as 57 per cent (57/100) on cow basis and 32.14 per cent (126/392) on quarter basis (Table 1). SCC is reported to be the most reliable test as it is the closest to bacteriological results by Sharma *et al*. (2010) [20]. As SCC rises in the first few days of lactation (Atakan, 2008) [1] and may continue to rise up to the end of the first month, it is considered physiological that the level increases towards lactation's end.
On the basis of electric conductivity, prevalence of subclinical mastitis was 51 per cent (51/100) and 28.57 per cent (112/392) animal-wise and quarter-wise, respectively (Table 1). The almost similar results were reported by Savita (2016) [17] and Marwaha *et al*. (2018) [11] which was 56 per cent and 52 per cent, respectively. According to Kasikci *et al.* (2012) in the detection of subclinical mastitis, EC showed comparable results with both CMT and SCC; its reliability would increase further when combined with the other methods.
According to pH, the prevalence of subclinical mastitis was recorded as 49 per cent (49/100) and 27.29 per cent (107/392) animal-wise and quarter-wise, respectively (Table 1). The prevalence of SCM in present study was similar to the study of Shahid *et al*. (2011) [19] and Sunder *et al*. (2013) [25] who reported prevalence 40.8 per cent and 48 per cent, respectively. During mastitis, the pH in the milk is higher because of increased permeability of the udder tissue to blood components like bicarbonate ions.

Table 1: Results of various diagnostic tests used for detection of subclinical mastitis (cow-wise and quarter-wise)

Diagnostic tests	Subclinical mastitis affected cows (out of 100 cows)	Percentage (%)	Subclinical mastitis affected quarters (out of 392 quarters)	Percentage (%)
Modified California Mastitis Test	53	53	115	29.33
Somatic Cell Count	57	57	126	32.14
Electric Conductivity	51	51	112	28.57
pH	49	49	107	27.29

The prevalence of SCM affected hindquarters was 32.98 per cent which was higher than the forequarters (27.77 per cent). The prevalence of right-side quarters were more than the left side quarters which 36.47 per cent (71/195) and 24.37 per cent (48/197), respectively (Table 2). Sudhan *et al.* (2005) [24], Joshi *et al*. (2006) [5] and Sharma *et al*. (2012) [21] also reported that hind quarters were affected more than fore-quarters. The higher prevalence of subclinical mastitis in hindquarters than in forequarters may be due to their higher production capacity (Radostits *et al*., 2000) and greater chances of hind quarters being soiled with urine or dung from the tail; thus, increasing their chance to get infected (Singh, 2015) [22].

Table 2: Quarter-wise prevalence of subclinical mastitis in cattle

Quarters affected	No. of affected quarters/no. of screened quarters	Percentage (%)
Right Fore	32/99	32.32
Right Hind	39/96	40.62
Left Fore	23/99	23.23
Left Hind	25/98	25.51

In the recent study, a high prevalence might be explained by the absence of dry cow therapy and post milking teat dipping, invariable hold milking practice, low culling rates of chronically ill cows, poor hygiene standards of the dairy environment, coupled with a large proportion of cows confined to zero-grazing production systems. Other factors that could influence the occurrence of SCM could be due to genetic variation in disease resistance amongst the age, breeds, immune responses, milk production capacity, udder conformation, teat structure, use of different methods of diagnosing of subclinical mastitis and the definition of infection (Mdegela *et al.*, 2005) [12].

Conclusions

The present study concluded that prevalence of subclinical mastitis in area of Bikaner was found to be high. There may be differences in prevalence rates of SCM among different dairy herds as a result of different management and hygienic practices associated with several animal factors such as population size, breed, climatic conditions, lactation stage, milk yield, udder morphology and sanitation.

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