



## PREVALENCE OF SUBCLINICAL MASTITIS IN DAIRY COWS IN RAJKOT DISTRICT OF GUJARAT

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### ABSTRACT

This research was carried out to determine the prevalence of subclinical mastitis in lactating Dairy Cow of rural areas of Rajkot district of Gujarat during the period of July 20012 to April 2013. A total of 200 milk samples (40 from organized farm and 160 from rural are of Rajkot district) were collected for this study which were subjected to physical examination and subsequently screened for subclinical mastitis using three indirect tests viz. White Side Test (WST), California Mastitis Test (CMT), and Surf Field Mastitis Test (SFMT). Overall prevalence of subclinical mastitis (SCM) in lactating dairy cows found in this study was 29%. Cows were infected with SCM 29.5%, 27.5% and 25.5% detection by CMT, WST and SFMT respectively. Higher prevalence of SCM was detected in milch crossbred cows (36.36%) in comparison to local bred cows (24.61%) maintained under extensive management system in rural area of Rajkot district. The prevalence of SCM was recorded in 31.58%, 30.76% and 68.75% in cows of local area of Rajkot district, and 25.0%, 40.0% and 71.42% in cows of organized farm during the early, mid and late stages of lactation respectively. The highest prevalence of SCM was recorded during the early lactation stage in both the local breed cows (30.0%) and cows of organized farm (45.83%) in comparison to their respective mid and late stages of lactation. The prevalence of SCM was highest in lactating cows having third lactation and high yielding (cows produced >10 liter milk per day) both in local breed and crossbred cows.

**KEY WORDS:** Subclinical mastitis, prevalence, dairy cows.

### INTRODUCTION

Mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue<sup>[1]</sup>. The disease is usually classified as subclinical, acute, subacute and chronic based on etio-pathological findings and observations<sup>[2]</sup>. Mastitis, the inflammation of mammary gland remains a serious problem for dairy animals industry and has very serious ramifications for dairy industry worldwide. The subclinical form of mastitis in dairy cows is important because this form is (a) 15 to 40 times more prevalent than the clinical form, (b) it usually precedes the clinical form, (c) it is of long duration, (d) it is difficult to detect, (e) It reduces milk production, and (f) it adversely affects milk quality<sup>[3]</sup>. Several causative agents and predisposing factors have been implicated in mastitis in dairy cows including bacterial, mycoplasmal and yeast pathogens<sup>[4]</sup>. *Staphylococcus* sp. is the main etiological agents of clinical and subclinical mastitis in cows while, *S. aureus* and *Escherichia coli* are most commonly isolated pathogen from the clinical mastitis, coagulase negative *Staphylococci* (CNS) are the most frequently isolated pathogens from the subclinical cows mastitis<sup>[5]</sup>. Predisposing factors such as poor management and hygiene, teat injuries and faulty milking machines are known to hasten the entry of infectious agents and the course of the disease<sup>[6]</sup>.

It is now a well known fact that the subclinical mastitis (SCM) is more serious and is responsible for much greater loss to the dairy industry<sup>[7]</sup>. The SCM can be known only after laboratory examination, as there are no gross

inflammatory changes in the udder tissue. Singh and Singh (1982) reported more than three times losses due to SCM, as compared to clinical mastitis (CM). Besides causing huge losses to milk production, the subclinically affected animals remain a continuous source of infection to other herd mates. If the infection persists for longer periods, then it may form a fibrous tissue barrier between the organisms and the antibiotic preparations, thus, limiting their efficacy. It is, therefore, important to know the prevalence of SCM in dairy herds and delineate the important factors responsible for it. Therefore, the present research work was undertaken with the aim to detect the prevalence and risk factors of subclinical mastitis (SCM) in lactating cows in organized farm and rural area of Rajkot district.

### MATERIALS & METHODS

#### Study area and period

The present research was carried out in dairy cows of organized farm and rural areas of Rajkot district. All the tests were performed at field condition immediately after collection of milk during the period from July 20012 to April 2013.

#### Husbandry and management system

Crossbred dairy cows used in this study were managed under semi intensive husbandry systems at organized farm. They are provided with green grass in addition to natural pasture and concentrate diet and are kept together in common shed but at peri-parturient period (advanced pregnancy and early lactating stage) they are maintained in

separate stalls. Local breed cow at rural areas Rajkot district reared under extensive husbandry system with ground muddy floor and without concentrated feed.

#### Survey design and sampling

A cross-sectional observational study was conducted in organized farm and rural area of Rajkot district. Milk samples were collected by owner and by the farm attendants instructing them to soak the teat with 70% ethanol and drying off by tissue paper, one to two drops of milk were discarded and then 10 ml of milk taken into numbered sterilized test tubes with rubber cap to perform other tests.

#### Questionnaire-based data collection and processing

Data from each animal and herd were collected using a questionnaire. Parameters studied were age, breed, number of parity, lactation stage and per day milk production. Age, parity, lactation stage were determined by asking owner and farm attendant and also from the farm records where available. The statistical analysis of collected data was performed by using SPSS version 11.5.

#### Physical examination of milk sample

Immediately after collection, milk samples were subjected to physical examination with naked eyes to detect any abnormalities in colour, odour, consistency and presence of clot, blood, lumps and any other visible abnormalities.

#### White Side Test (WST)

The WST was performed as per procedure described by Kahir *et al.*, [8], in brief, after thorough mixing avoiding violent shaking, 50 µl (five drops) of milk were placed on a glass slide with a dark background by micropipette. Subsequently 20 µl of WST reagent (4% NaOH) were added to the milk sample and the mixture was stirred rapidly with a toothpick for 20-25 seconds. A breaking up of milk in flakes, shreds and viscid mass was indicative of positive reaction. On the other hand, milky and opaque and entirely free of precipitant was indicative of negative reaction. The grading of positive WST scores was shown in Table 1.

**TABLE 1.** Grading of the White Side Test (WST) scores

Sr. No.	Visible reaction	Interpretation	Symbol
1.	Mixture is milky and opaque and entirely free of precipitant.	Negative	-
2.	The background is less opaque but still somewhat milky, with larger particles of coagulated materials being present and thickly scattered through the area. A slight degree of clumping is observed.	Weak positive	1+
3.	The background is more watery and large clumps of coagulated materials are present. If the stirring has been rapid, fine threads or strings may be present.	Distinct	2+
4.	The background is very watery and whey-like, with large masses of coagulated material forming into strings and shreds.	Strong positive	3+

#### Surf Field Mastitis Test (SFMT)

This test was performed and scored following the method described by Muhammad *et al.* [9] in brief, about 2 ml milk was drawn from bottle into test cup and an estimated 2 ml reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and immediately scored as 1+, 2+ and 3+.

#### California Mastitis Test (CMT)

The procedure of CMT was followed in this study as per manufacturer's instruction (Leukocyst®, Synbiotics

Corporation, France). In brief, about 2 ml milk was drawn from bottle into the cup and an estimated equal volume of CMT reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored. The CMT reaction/ results were read immediately as per manufacturer's recommendation and were scored for each half gland (teat) depending on the amount and thickness of gel formed as described by Ikram<sup>[10]</sup> as shown in Table 2.

**TABLE 2:** Scoring of California mastitis test results

Sr. No.	Reading Aspect	Score		Interpretation of Infection	Related with the average cellular numeration (x 10 <sup>3</sup> /ml)
		Value	Cross		
1.	Consistency normal or Gray color	0	(0)	Absent	100
2.	Light gel disappearing after stirring or Purplish gray color	1	(+/-)	Infection risk by minor pathogenic	300
3.	Light persistent gel-crumbly filaments or Purple gray	2	(+)	Sub clinical mastitis	900
4.	Immediate thickening viscous cluster at the bottom of the well	3	(++)	Sub clinical mastitis	2700
5.	Thick gel consistency of egg white color dark purple	4	(+++)	Subclinical mastitis near the clinical expression	8100

## RESULTS & DISCUSSION

Of 200 samples collected from organized farm and rural areas of Rajkot district and were examined by CMT, SFMT and WST, of which yielded an overall prevalence of SCM as 29% (n=58), 25.5% (n=55) and 27.5% (n=51). Findings of the present study are supported by earlier findings of Kader *et al.*<sup>[7]</sup> who reported 46.6% SCM in Bangladesh in cows on bacteriological examination and a higher prevalence (54.0%) of SCM in cows in India were reported by Singh and Baxi<sup>[11]</sup>.

### Breed related prevalence

All the cows of organized farm were cross breed and show 37.5% positive reaction whereas local and cross breed of Rajkot district show 24.6% and 36.66% positive reaction. Of 70 crossbred cows screened for SCM showed 37.5% and 36.66% in rural area of Rajkot district and in organized farm whereas local breed cows showed 24.61% positive reaction for SCM (Table 3). The results of this study is higher than that of Rabbani<sup>[12]</sup> who reported 20.31% in Holstein Friesian cross cows and 13.24% in Red Chittagong cows and of Prodhan *et al.*<sup>[13]</sup> who reported 16.52% and 15.77% incidence of SCM with WST and CMT at Sirajganj.

**TABLE 3:** Breed wise prevalence of Subclinical mastitis (SCM) in local and crossbred cows

Sr. No.	Aspect	Organized farm		Rajkot district	
		Local breed	Cross breed	Local breed	Cross breed
1.	No. of samples tested	0	40	130	30
2.	Positive cases	0	15	32	11
3.	Prevalence	0	37.5%	24.61%	36.66%

### Age wise prevalence

The prevalence of SCM was recorded as 22.22%, 27.94%, 21.21% and 18.18%, respectively at the age group of 3 years to 5 years, >5 years to 8 years, >8 years to 12 years and >12 years in local breed cows. The prevalence of

SCM was recorded 33.33%, 40.90%, 28.57% and 0.00% respectively at the age group of 3 years to 5 years, >5 years to 8 years, >8 years to 12 years and >12 years in cross breed cows (Table 4).

**TABLE 4:** Age wise prevalence of Subclinical mastitis (SCM) in dairy cows

Sr. No.	Age groups (Years)	Local breed		Cross breed	
		Sample tested	Positive (%)	Sample tested	Positive (%)
1.	3 to 5	18	4 (22.22)	18	6 (33.33)
2.	>5 to 8	68	19 (27.94)	44	18 (40.90)
3.	>8 to 12	33	7 (21.21)	7	2 (28.57)
4.	>12	11	2 (18.18)	1	0 (0.00)

### Parity related prevalence

The prevalence of SCM in local breed cows was recorded in 18.75%, 22.22%, 29.55%, 26.66%, 18.18% and 23.52% during the parity numbers 1, 2, 3, 4, 5 and 6, respectively. The parity wise prevalence of SCM in cross breed cows were 28.57%, 36.84%, 47.05%, 36.36%, 33.33% and 33.33% during the parity numbers 1, 2, 3, 4, 5 and 6, respectively (Fig. 1).

It indicates that the prevalence of SCM was found highest at 3<sup>rd</sup> (29.55%) and (47.05%) parity in local and cross breed cows respectively in comparison to 1<sup>st</sup> and 2<sup>nd</sup> parity but increasing tendency was recorded with increase of parity. This observation supports with the reports of Rasool *et al.*<sup>[14]</sup> and Devi *et al.*<sup>[15]</sup> who reported an increasing prevalence of SCM with advancing parity.

### Lactation stage related prevalence

It appears from the Table 5 that, all three stages of lactation in both the local cows and cross breed cows affected with SCM. The prevalence of SCM was recorded in 30.00%, 17.14% and 25.45% in local breed cows, and 45.83%, 31.58% and 33.33% in cross breed cows during the early, mid and late stages of lactation, respectively. The highest prevalence of SCM was recorded during the early lactation stage in both the local breed cows (30.0%) and cross breed cows (45.83%) in comparison to their respective mid and early stages of lactation. The overall prevalence of SCM on the basis of lactation stage in cows showed that all the three lactation stages had SCM but there was a tendency to decrease the prevalence of SCM from early (45.83%) to mid (31.58%) but more in late stage than mid (33.33%) stage.

**TABLE 5:** Lactation stage related prevalence

Sr. No.	Stage of lactation	Local breed		Cross breed	
		Sample tested	Positive (%)	Sample tested	Positive (%)
1.	Early (60-90 days)	40	12 (30.00)	24	11 (45.83)
2.	Mid (91-180 days)	35	6 (17.14)	19	6 (31.58)
3.	Late (>180 days)	55	14 (25.45)	27	9 (33.33)

Stage of lactation Local breed Cross breed Sample tested Positive (%) Sample tested Positive (%) Early (60-90 days) 40 12(30.00) 24 11(45.83) Mid (91-180 days) 35 6 (17.14) 19 6 (31.58) Late (> 180 days) 55 14(25.45) 27

9 (33.33) The prevalence of SCM in the early, mid and late stage lactation in local breed cows was 30%, 17.14% and 25.45%, respectively. Similarly, it was recorded as 45.83%, 31.58% and 33.33% in the early (mid) and (late)

stages of lactations in cross breed cows. It indicates that the prevalence of SCM was found highest at early stage in both the local (30%) and cross breed (45.83%) cows in comparison to mid and late stages of lactations. These results contradicts the earlier finding of Rahman *et al.*,<sup>[16]</sup> who reported highest prevalence of SCM during the 3<sup>rd</sup> months (34%) of lactation. However, these results agree with Pal and Verma<sup>[17]</sup> who reported lower prevalence of SCM in stages of lactation above five months. Review of literature revealed that the higher incidence of CM usually occurred during early stage of lactation due to high yielding enlarged status of udder which is particularly prone to infection and inflammatory process became of physiological stress strain of heavy milk production, but the prevalence of SCM could be high at late stage of lactation.

#### Prevalence of SCM on the basis of milk production

The local breed cows were grouped into five and cross breed cows were grouped into five on the basis of milk production and the highest prevalence of SCM was recorded in cows with low milk production in both the local breed cows and cross breed cows. The prevalence of SCM in local breed cows was recorded in 13.04%, 20.75% and 30.95%, 37.5% and 50%, respectively, 0.5-1 lit, >1-2, >2-5, >5-10 lit milk production per day, whereas the prevalence of SCM in cross breed cows were 28.57%, 35.48%, 40.00% and 42.85%, respectively >1-2 lit >2-5 lit, >5-10, and >10 lit milk production per day (Fig 2.).

The prevalence of subclinical mastitis as determined by WST, SFMT and CMT in dairy cows was very high an indicative of a health and production problems in the small holder dairy cows farmer in the selected areas. Consistent with other factors recorded to be associated with mastitis, udder and teat lesions were identified and quantified as number of parity, age, lactation stage of milk production and breed of cows.

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#### REFERENCES

- [1]. Samad, M.A. (2008) Animal Husbandry and Veterinary Science, volume II, LEP pub no.11, Bangladesh Agricultural University campus, Mymensingh.
- [2]. Triptahi, B.V. and Chaltopadhyay, S.K. (1993) Caprine mastitis: Clinico morphological and aetipathological findings in spontaneously occurring cases in India goats. *International Journal of Animal Science*. 8:101-111.
- [3]. Seegers, H., Fourichon, C. and Beaudeau, F. (2003) Production effects related to mastitis and mastitis economics in dairy cattle herds> *Veterinary Research* 34: 475-491.
- [4]. Egwu, G.O., Zaria, L.T., Onyeyili, P.A., Ambali, A.G., Adamu, S.S. and Birdling, M. (1994) Studies on the microbiological flora of caprine mastitis and antibiotic inhibitory concentration in Nigeria. *Small Ruminant Research* 14: 233-239.
- [5]. Contreras, A., Luengo, C., Sanchez, A. and Corrales, J.C. (2003) The role of intramammary pathogens in dairy goats. *Livestock Production Science* 79: 273-283.
- [6]. Majic, B., Jovanovic, B.V., Ljubic, Z. and Kukovics, S. (1993) Typical problems encountered in Croatia in the operation of goats milking machines. Proceedings of the 5<sup>th</sup> International symposium on machine milking of small ruminants. *Budapest, Hungary*. pp. 377-379.
- [7]. Kader, M. A., Samad, M.A., Saha, S. and Taleb, M.A. (2002) Prevalence and aetiology of sub-clinical mastitis with antibiotic sensitivity to isolated organisms among milch cows in Bangladesh. *Indian Journal of Dairy Science* 55:218-223.
- [8]. Kahir, M.A., Islam, M.A., Rahman, AKMA, Nahar A, Rahman, M.S. and Song, H.J. (2008) Prevalence and risk factors of subclinical bovine mastitis in some dairy farms of Sylhet district of Bangladesh. *Korean Journal of Veterinary Services* 31(4): 497-504.
- [9]. Muhammad, G., Athar, M., Shakoor, A., Khan, M.Z., Fazal-ur-Rehman and Ahmad, M.T. (1995) Surf Field Mastitis Test: An inexpensive new tool for evaluations of wholesomeness of fresh milk. *Pakistan Journal of Food Science* 5:91-93.
- [10]. Ikram, M. (1997) Diagnostic microbiology. In: W Paul and V M D Pratt (editors), *Laboratory Procedures for Veterinary Technicians*. R R Donnelley and Sons Company. St. Louis, Missouri. 159-160 pp.
- [11]. Singh, K.B. and Baxi, K.K. (1988) Studies on the incidence and diagnosis of sub-clinical mastitis in milch animals. *Indian Veterinary Journal* 47:723-729.
- [12]. Rabbani, A.F.G. (2008) Studies on Bovine Subclinical Mastitis by Using California Mastitis Test. M.S thesis in the Department of Medicine, Bangladesh Agricultural University, Mymensingh.
- [13]. Prodhan, M.A.M., Kamal, A.H.M. and Mahbub- E-Elahi, A.T.M. (1996) Prevalence of sub-clinical mastitis in cows of Baghabari Milkshed area. *Bangladesh Veterinary Journal* 30: 59-60.
- [14]. Rasool, G., Jabbar, M.A., Kazmi, S.E. and Ahmed, A. (1985) Incidence of sub-clinical mastitis in Nilli-Ravi buffaloes and Sahiwal cows. *Pakistan Veterinary Journal* 5: 76-78.
- [15]. Devi, B.K., Shukla, P.C. and Bagherwal, R.K. (1997) Incidence of sub-clinical mastitis in cows. *Indian Journal Dairy Science* 50: 477-478.
- [16]. Rahman, M.S., Nooruddin, M. and Rahman, M.M. (1997) Prevalence and distribution of mastitis in cross-bred and exotic dairy cows. *Bangladesh Veterinary Journal* 14 : 1-4
- [17]. Pal, P. and Verma, B.B. (1988) Some observation on efficacy of indirect test for detection of sub-clinical mastitis. *Indian Journal Veterinary Medicine* 8: 40-41.