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Prevalence of subclinical mastitis in Cows in and around Jabalpur, Madhya Pradesh

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Abstract
Subclinical mastitis is defined as inflammation of udder without any visible changes in the udder and milk. In this study, a total of 412 lactating cows were screened by california mastitis test (MCMT), somatic cell count and milk pH. The overall prevalence of infected animal was found to be 31.55 percent on animal basis and 20.18 percent on quarter basis. The age wise prevalence was found to be highest in 5-7 years of age group (38.50%). The highest prevalence of SCM was observed in 4th parity (44.12%), in early lactation stage (56.25%) and in cross bred (35.06%) animals. Prevalence in organized and unorganized dairy farms is 29.82% and 41.66% respectively. Apparently healthy cattle had a mean value of SCC 1.97 ± 3.11 X 10^5 cells/ml. The mean SCC in SCM (11.80 ± 4.18 X 10^5 cells/ml) was significantly ($p \leq 0.05$) increased as compared to control. Apparently healthy cattle had a mean value of milk pH 6.20 ± 0.08 and in SCM cow milk pH was 6.68 ± 0.09.

Keywords: Mastitis, subclinical mastitis, somatic cell count, pH, CMT

Introduction
India is the largest dairy producer in the world. Its considerable growth can be attributed to the value creation and addition that has been characteristic of this industry. The Indian dairy industry is predictable to grow at a compound annual growth rate of 15% annual to reach **C** 9.4 trillion by 2020. According to the 20th Livestock Census, India has 302.79 million bovine population containing 192.49 million cattle, in which female cattle is 145.12 million, increased by 18% over the previous census. Mastitis (Greek, Mastos = breast + itis = inflammation) is a multi etiological complex disease, defined as inflammation of parenchyma of mammary glands and characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Constable *et al.*, 2017) [7]. Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000) [4]. Mastitis can be either clinical (CM) or subclinical (SCM). Clinical mastitis gives rise to visible signs in udder, teat and milk. Mild CM causes flakes or clots in the milk, whereas severe cases are associated with heat, swelling of the udder and abnormal secretion or discolouration of milk and severe CM show systemic signs of pyrexia and inappetence. Subclinical mastitis is defined as the absence of physical signs of inflammation to the udder and milk and it is the most common and prevalent form of mastitis (Orlandini, 2011) [16]. Subclinical Mastitis (SCM) is 15 to 40 times more prevalent than clinical mastitis (Seeger *et al.*, 2003) [24]. Ruegg (2017) [21] reported that infected cows become source of infection for healthier cows. So the present study was conducted to determine the prevalence of mastitis in cattle in and around Jabalpur, Madhya Pradesh.

Materials and Methods
The work was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Livestock Farm, Adhartal, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh and different dairy farms in nearby areas of Jabalpur, Madhya Pradesh (M.P.). For this study, a total of 412 lactating cattle belonging to Sahiwal, Gir, Malwi, Crossbred and Nondescript were screened during study period. The cattle belong to the dairy farms in and around Jabalpur *viz.* Livestock Farm, Adhartal, N.D.V.S.U. and different private dairy farms of Jabalpur, (M.P.).

Different parameters about individual animals i.e. breed, age, number of calf born, lactation number, stage of lactation, herd size and number of quarters affected were recorded.

Clinical examination of the udder and teat

Cow udder and teat were examined by manual palpation of each, individual teat for atrophy, consistency or variation in the size of teat, udder indurations and asymmetry. Teat ends were observed for alterations such as scars, wounds, patent teat orifice and ease of milking. The udder was also being examined to ascertain any sort of abnormality (unilateral or bilateral). The condition of the teat orifices were scored (table 01), according to Petzer (2004) [17].

Table 1: Teat score on the basis of teat orifice

Teat opening score	Description
1	The surface around the teat opening is smooth (Normal)
2	A raised ring is visible around teat opening
3	Small cracks are visible in a ring around the teat opening
4	Large cracks and teat canal eversion are visible
5	Mechanical damage to the teat tip, or presence of ulceration, or wart

Collection of Milk sample

The each udder of cow was thoroughly washed with potassium permanganate (0.01%) and wiped with clean cloth. Later on, 15 ml of milk was collected aseptically during mid stream from each teat in sterilized vials and brought for further examination.

Modified California Mastitis Test (MCMT)

MCMT is the cow side test designed for use in the dairy barn on milk drawn directly from the individual mammary quarters. A white plastic paddle containing four shallow cups is used. Draw foremilk from each quarter into separate cups of a four-cup plastic paddle add equal quantity of test reagent to each cup. Rotate the paddle to mix and observe changes in color and gel formation within 10 to 15 seconds after mixing. Milk from a normal quarter remains liquid and flows freely. In a moderate reaction, the gel that forms is fragile and breaks into small masses or clumps. Milk samples that react strongly form a gelatinous mass that clings together as the paddle is rotated. MCMT was scored as described by Constable *et al.* (2017) [7].

Somatic Cell Count (SCC)

The somatic cell count in the subclinical mastitis milk was performed to assess the degree of infection in the respective quarter(s). The SCC was performed with the modified Newman's stain.

Preparation of milk smear: The smear of milk for SCC was prepared within one hour of it's collection to minimize disintegration of leukocyte. Each milk sample was uniformly mixed by gentle shaking of the vials and the milk (0.01 ml) was spread with sterilized bacteriological loop, over one cm rectangular area on a clean micro slide. The milk smear from the test sample was stained by modified Newman's stain. A total of 30 fields were counted under oil immersion lens and average number of cells per field was worked out. The average number of cells was multiplied by the multiplication factor of the microscope to obtain the number of cells per ml of the milk.

Microscopic factor determination

The diameter of the field of 10x eye piece was determined by using stage micrometer. The lowest division of micrometer scale was 0.01 mm. accordingly the diameter was measured and obtained as 0.016 cm. The area of the microscopic field was determined by the formula πr^2. The calculations were made as under:

Diameter = 0.016 cm.
Radius = 0.008 cm
Area = 3.14 x $(0.008)^2$
= 0.02
Microscopic factor = 100 x (1/Area)
= 100 x (1/0.02)
= 5000 mm^2/ 0.1 ml of milk
= 500000 mm^2/ 1 ml of milk

For counting minimum we need to count 50 microscopic fields.

So, each field = 500000/50
= 10000 (working factor)

Milk pH

Estimation of the pH was done by the digital pH meter by dipping the bulb of pH meter in the milk sample and record the data of screen.

Results and Discussion

Modified California mastitis test (MCMT)

In the present study, screening of cattle for SCM was performed by MCMT. It is simple, cheap, quick, reliable cow side test. The above findings were similar to the Bastan *et al.* (2008) [2], Sharma *et al.* (2010) [26], Ayano *et al.* (2013) [1], Maheshwari *et al.* (2016) [11] and Kandeel *et al.* (2019) [10], who reported that MCMT performed much better as a clinical test. It was accurate and reliable diagnostic test in the field conditions.

In the present study 412 lactating cattle were screened for SCM and 31.55 percent (130 out of 412 cows) found positive by MCMT.

MCMT grading

MCMT score in cattle with SCM are given in table 02. A score of 1+, 2+ and 3+ in MCMT was noticed in 58.18 percent (192 out of 330 quarters), 29.09 percent (96 out of 330 quarters) and 12.73 percent (42 out of 330 quarters) in cattle affected with SCM, respectively. Nearly Similar finding was reported by Maheshwari *et al.* (2016) [11] who reported the 57.55 percent (122 out of 212 quarters), 31.60 percent (67 out of 212 quarters) and 10.85 percent (23 out of 212 quarters) occurrence respectively on the basis of MCMT grading in cattle affected with SCM.

Table 2: MCMT grading in SCM

S. No.	Score	No. of positive quarters for SCM(n= 330)	Percent (%)
1.	1+	192	58.18
2.	2+	96	29.09
3.	3+	42	12.73

Somatic cell count (SCC)

Apparently healthy cattle had a mean value of SCC 1.97 ± 3.11 X 10^5 cells/ml. The mean SCC in SCM (11.80 ± 4.18 X 10^5 cells/ml) was significantly increased as compared to control (Table 03).

Table 3: Milk profile in SCM

S. No.	Parameters	Apparently healthy control (n= 8)	Subclinical mastitis (n=130)
1.	SCC (10^3 cells/ml)	228.75 ± 4.79	1180.12 ± 4.18**
2.	pH	6.20 ± 0.02	6.68 ± 0.02**

**- Differ significantly ($P < 0.05$)

Milk pH

Apparently healthy cattle had a mean value of milk pH 6.20 ± 0.02 whereas in SCM milk pH was 6.68 ± 0.02.

Prevalence of subclinical mastitis

The overall prevalence of infected cattle was found to be 31.55 percent (130/412) on animal basis and 20.18 percent (330/1635) on quarter basis (table 04).

These findings are closely similar with Maheshwari *et al.* (2016) [11], Swami *et al.* (2017) [28], Shaikh *et al.* (2019) [25] and Constable *et al.* (2019) [6] who have recorded 27.81 percent, 35 percent, 31.21 percent and 33 percent respectively. However, higher prevalence was reported by Mir *et al.* (2014) [13], Mekonnen *et al.* (2017) [12] and Ndahetuye *et al.* (2019) [15] as 57.80 percent, 37.2 percent, 62 percent and 76.25 percent respectively. The variation in the present prevalence of SCM and also reported earlier studies may be attributed to various factors including the selection of animals, season, management practices of the farm, prevention of mastitis strategies adopted in particular farm. Ergun *et al*., (2004) [8] stated factors like herd size, milking method practice, agro climatic conditions of the region, variations in socio-cultural practices, milk marketing, literacy level of the animal owner and feeding practices were affecting the prevalence of subclinical mastitis.

Table 4: Prevalence of SCM in Cows

S.No.	Particulars	Number Screened	Number positive	Prevalence (%)
1	Total number of animals	412	130	31.55
2	Total number of quarters	1635 (13 blind teats)	330	20.18
	$\chi2 = 24.418$ df = 01 P < 0.00001			

Individual quarter wise

The prevalence of SCM in different quarters of udder in cattle is illustrated in table 05. The prevalence was highest in right fore quarter i.e. 22.14 percent (91 out of 411 quarters) followed by 21.08 percent (86 out of 408 quarters) in right hind quarter, 19.32 percent (79 out of 409 quarters) in left fore quarter and lowest prevalence of 18.18 percent (74 out of 407 quarters) in left hind quarter was found in cattle with SCM. The prevalence was higher in forequarters i.e. 20.73 percent (170 out of 820 quarters) as compared to hind quarters of 19.63 percent (160 out of 815 quarters), left side of 18.75 percent (153 out of 816 quarters) as compared to right side of 21.61 percent (177 out of 819 quarters). The quarter wise prevalence revealed a non-significant variation.

Similar finding were reported by of Qadri (2015) [18] and Maheshwari *et al.* (2016) [11] i.e. higher prevalence in right and left side quarters i.e.11.23%, 9.03%, and 32.25%, 27.25%, respectively. Milking practices adopted by milkman and the high chance of getting faecal and environmental contamination (Bansal *et al.*, 1995) [2] furthermore, the high production capacity of hind quarters could be attributed to these findings (Radostits *et al*., 2010) [19].

Table 5: Individual quarter afflicted with SCM in cattle

Quarter's position		No. screened	No. positive	Prevalence (%)
Right	Fore	411	91	22.14
	Hind	408	86	21.08
	Total	819	177	21.61
	$\chi2 = .1365$ df = 01 P = 0.7117			
Left	Fore	409	79	19.32
	Hind	407	74	18.18
	Total	816	153	18.75
	$\chi2 = .1721$ df = 01 P = 0.6782			

Breed wise

The breed wise prevalence study of SCM in lactating cattle revealed a highest prevalence of 35.06 percent (88 out of 251 cattle) in cross bred cattle followed by prevalence of 33.33 percent in malwi cattle (1 out of 3 cattle), in sahiwal 31.82 percent (7 out of 22), lowest prevalence in Gir 25 percent (6 out of 24) and in N.D. 25 percent (28 out of 112) cattle. Breed wise prevalence revealed the significant ($P<0.05$) effect of different breeds on prevalence of SCM (table 06).

Maheshwarai *et al.* (2016) [11] and Shaikh *et al.* (2019) [25] detected similar finding of higher prevalence of SCM in crossbred cows as 38.80 percent, 22.48 percent and 39.01 percent. The significant difference between the breeds may be associated with their high milk yielding of the cattle, which makes them more prone to mastitis (Radostits *et al.*, 2010) [19] as compared to native breed which have higher resistance.

Table 6: Breed wise prevalence of SCM

S.No.	Breed	Number screened	Number positive	Prevalence (%)
1	Crossbred	251	88	35.06
2	Malwi	3	1	33.33
3	Sahiwal	22	7	31.82
4	Gir	24	6	25.00
5	Nondescript	112	28	25.00
	$\chi2 = 4.1384$ df = 04 P = 0.3876			

Age wise

The age wise prevalence of SCM in lactating cattle revealed highest prevalence i.e. 38.50 percent (72 out of 187 cattle) in the cattle of 5-7 years of age followed by 33.03 percent (36 out of 109 cattle) prevalence in cattle of 7 years and above age and lowest prevalence of 18.97 percent in cattle of 3 to 4 years of age (22 out of 116 cattle). The age wise prevalence revealed a significant variation (P < 0.05) among various age groups. The details are outlined in table 07.

Similar to these findings Maheshwari *et al.* (2016) [11] and Shaikh *et al.* (2019) [25] also showed that 5-7 years age group of cows were more susceptible to SCM as compared to other age groups. Defense mechanism of younger animals is efficient than older animals which make them less susceptible to infection. This might be due to increase cellular response to intra-mammary infection.

Table 7: Age wise prevalence of SCM in cattle

S.No.	Age group	Number screened	Number positive	Prevalence (%)
1.	3-4 years	116	22	18.97
2.	5-7 years	187	72	38.50
3.	7 years and above	109	36	33.03
	$\chi2 = 12.8018$ df = 02 P = 0.00166			

Parity wise

Parity wise prevalence of SCM was also recorded. The parity number was taken from 1st to 7th and more parity numbers. The parity wise prevalence revealed a significant variation ($P< 0.05$) among various parity groups. The highest prevalence of SCM was observed in 4th parity i.e. 44.12 percent (45 out of 102 cattle) followed by 36.92 percent in 3rd parity (24 out of 65cattle), 36.11 percent in 5th parity (26 out of 72 cattle), 22.73 percent in 6th parity (10 out of 44 cattle), 21.15 percent in7th and more parity (10 out of 52 cattle), while, lowest prevalence was observed in 1st and 2nd parity i.e. 16 percent (4 out of 25) and 19.23 percent (10 out of 52 cattle) respectively. The results are outlined in table 08.

Similar finding of higher prevalence in 4th parity were recorded by Islam *et al.* (2011) [9] and Maheshwari *et al.* (2016) [11]. Whereas, Nauriyal and Verma (2009) [14] recorded highest prevalence of SCM in 2nd parity. Increased prevalence may be the result of increased incidence of new intramammary infection (IMI) or increased duration of SCM infection (Sampimon *et al*., 2009) [22].

Table 8: Parity wise prevalence of SCM in cow

S. No.	Parity number	Number screened	Number positive	Prevalence (%)
1.	1st	25	4	16
2.	2nd	52	10	19.23
3.	3rd	65	24	36.92
4.	4th	102	45	44.12
5.	5th	72	26	36.11
6.	6th	44	10	22.73
7.	7th and more	52	11	21.15
$\chi2 = 19.663$ df = 06 P = 0.00317				

Lactation stage wise

The lactation stage was divided in 3 classes i.e. early lactation, 1 to 3 months, mid lactation, 3 to 6 months and late lactation, 6 months and onwards till drying off. The overall prevalence of SCM according to lactation stage was observed as 56.25 percent (63 out of 112 cattle), 26.37 percent (48 out of 182 cattle) and 17.80 percent (21 out of 118 cattle) in early, mid and late lactation stage, respectively (table 09). Islam *et al.* (2011) [9] and Maheshwari *et al.* (2016) [11] also reported highest prevalence of SCM during the early lactation stage as 30.0% and 47.46% respectively. Negative energy balance and post partum rapid physiological change in the mammary tissue causes low / reduced udder resistance and higher prevalence of SCM.

Table 9: Lactation stage wise prevalence of SCM in cattle

S. No.	Lactation stage	Number screened	Number positive	Prevalence (%)
1.	Early (1-3 months)	112	63	56.25
2.	Mid (3-6 months)	182	48	26.37
3.	Late (> 6 months)	118	21	17.80
$\chi2 = 43.827$ df = 02 P = 0.00001				

Organized and unorganized dairy farms wise

No significant variation was noticed in the prevalence with respect to rearing pattern of cows in dairy farms, however, prevalence of SCM in unorganized dairy farms was observed higher i.e. 41.66 percent (25 out of 60 cattle) than the prevalence in organized sector of dairy farms i.e. 29.82 percent (105 out of 352 cattle). Results are represented in table 10 .Similar finding of higher prevalence of SCM were reported by Tiwari *et al.* (2000) [29] and Maheshwari *et al.* (2016) [11] as 26.68% 20.36% and 36.92%, 26.59% in unorganized and organized sectors respectively. Poor hygiene, management practice, awareness, milking method, bedding and types of housing were responsible for the higher prevalence of SCM in unorganized sectors as compared to organized dairy sectors.

Table 10: Prevalence of SCM in organized and unorganized dairy farms

S. No.	Sector/ Rearing Pattern	Number screened	Number positive	Prevalence (%)
1.	Organized dairy farms	352	105	29.82
2.	Unorganized dairy farms	60	25	41.66
$\chi2 = 1.3965$ df = 01 P = 0.237311				

Clinical examination of the udder/ teat/milk

Clinical examination of udder and teat were performed by manual palpation and found apparently normal consistency in cows with SCM. Similar finding were reported by Reddy *et al.* (2001) [20], Chakrabarti (2004) [5], Radostits *et al.* (2010) [19] and Maheshwari *et al.* (2016) [11], who have reported that no clinical signs associated with SCM as well as no physical abnormalities were found in the milk.

Examination of milk revealed that colour, consistency and odour of milk samples from all positive cases of SCM were found to be apparently normal. However, slight reduction in milk yield was recorded in SCM. These observations were correlated by the findings of Saravanan *et al.* (2009) [23], Suresh *et al.* (2010) [27] and Maheshwari *et al.* (2016) [11].

The teat opening score for teat orifices were recorded and found to be normal i.e. one (1) means the surface of the teat opening is smooth in all the cases of SCM (Petzer, 2004) [17].

Conclusion

The prevalence of subclinical mastitis was found to be 31.55 percent in cow on animal basis and 20.18 percent on quarter basis, in and around Jabalpur, Madhya Pradesh. On the basis of SCC, MCMT and milk pH the highest prevalence was recorded in 5-7 years of age group (38.50%), 4th parity (44.12%), early lactation stage (56.25%) and in cross bred (35.06%) animals. Prevalence in organized and unorganized dairy farms is 29.82% and 41.66% respectively. The somatic cell count, MCMT are found as the effective tool for diagnosing the subclinical mastitis in cows.

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