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Incidence of subclinical mastitis in Deoni cattle in bidar district of Karnataka and comparison of different techniques used for its detection

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Abstract
Subclinical mastitis (SCM) causes severe economic losses in dairy cattle if left undetected. The present study was conducted on 125 lactating Deoni cows in 13 villages of Bidar district of Karnataka, part of the project "Field Performance Recording of Deoni cattle in Bidar District" under Rashtriya Gokul Mission, to compare the efficiency of different techniques in detecting SCM under field conditions. A milk sample of 50 ml was collected from each animal to conduct California Mastitis Test (CMT), Mastrip test and Electrical Conductivity test (EC). Samples positive for subclinical mastitis (SCM) by either of the tests were subjected to Somatic Cell Count. The cut-off value for the different tests for confirmation of SCM was set at 5.5mS/cm for EC and 2,00,000 cells/ml of milk for SCC, based on observations of Deoni cattle milk carried out at Livestock Research & Information Centre (Deoni), KVAFSU Bidar. The overall incidence of SCM was found to be 22.22 percent, with values ranging from 0 to 100% in different villages. The accuracy of CMT, EC and Mastrip tests with respect to the reference standard i.e. SCC was found to be 85.71, 28.57 and 100.00%, respectively. Mastrip test was found to be a rapid and efficient technique to detect SCM in Deoni cattle under field conditions.

Keywords: subclinical mastitis, California mastitis test, electrical conductivity test, Mastrip test, Deoni cattle

1. Introduction
India has 43 descript cattle breeds. As of 2018 the ICAR recognized 43 breeds that are indigenous in India (Srivastava *et al.*,) [29], Dairying & Fisheries, pure Deoni cattle population is 1,51,236, graded Deoni cattle population is 2,00,364 contributing a percentage share of 0.23 with respect to total cattle population of India.

Deoni is a dual-purpose cattle breed of India. Genetically, the Deoni breed was evolved through the crossbreeding of the Gir cattle of the Kathiawar region of Gujarat with the Dangi breed of Marathwada and local desi cattle of Nizam state from Bidar and Osmanbad (Joshi and Phillips, 1953) [11]. The home tract of Deoni breed is Udgir, Ahmedpur, Nilanga and Ausa tehsils of Latur district of Maharashtra and adjoining areas of Telangana, Bidar and Gulbarga districts of Karnataka state.

Besides causing huge economic losses to milk production, the sub-clinical mastitis remains a continuous source of infection to other herd mates. It is therefore, important to know the prevalence of sub-clinical mastitis in dairy herds and delineate the important factors responsible for it (Rabbani and Samad, 2010) [19].

The average decrease in milk yield due to clinical and subclinical mastitis was 50 and 17.5%, respectively. The economic loss were Rs. 603.87 and 483.10 crores due to subclinical mastitis and Rs. 285.64 and Rs. 234.59 crores due to clinical mastitis in cattle and buffaloes, respectively (Singh, 1994) [26]. The total economic loss due to subclinical mastitis was assessed to be in the range of Rs. 21,677 to 88,340 for a lactation period, depending on the condition of the animal (Rathod *et al.*, 2017) [21]. Keeping in view the above facts, the present study was undertaken to study the prevalence of subclinical mastitis in Deoni cattle.

2. Materials and Methods
2.1 Screening for subclinical mastitis
The present study was conducted on 125 lactating Deoni cows in 13 villages of Bidar district

of Karnataka, as part of the project "Field Performance Recording of Deoni cattle in Bidar District" under Rashtriya Gokul Mission, to compare the efficiency of different techniques in detecting subclinical mastitis (SCM) under field conditions. A milk sample of 50ml was collected from each animal to conduct California Mastitis Test (CMT), Electrical Conductivity test (EC) and (Mastrip®). Samples positive for subclinical mastitis (SCM) by either of the tests were subjected to Somatic Cell Count test for confirmation of SCM.

2.2 California Mastitis Test (CMT)

The test is based upon the amount of cellular nuclear protein present in the milk sample. CMT reflects the SCC level quite accurately (Mellenberger and Roth, 2000) [15] and is a reliable indicator of the severity of infection. The CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat (detergent dissolves fat). DNA is now released from the nuclei and together forms a stringy mass. As the number of leukocytes increase in a quarter, the amount of gel formation will increase linearly.

The CMT provides only an indication of somatic cell count, not an exact value. The CMT was performed on the individual milk samples as per procedure of (Schalm and Noorlander, 1957) [23] employing a modified CMT reagent of (Sharma and Rajani, 1969) [25] which comprised of:

Sodium hydroxide	:	15.00 g
Teepol	:	5.00g
Bromothymol blue	:	0.10 g
Distilled water	:	1000 ml

About 3 ml of milk from a quarter was taken in a cup of the CMT paddle and an equal volume of reagent was added to it and the contents were mixed gently by horizontal rotation for 15-30 seconds and the results were recorded as:

Negative = Normal

Traces = No distinct wave, tiny lumps

+ = Thick and definitive wave like, not adhered to the bottom of cup

++ = markedly thick and adhered to the bottom of cup

+++ = Too much thickening which appeared like a ball

CMT reactions were scored according to (Radostitis *et al.*, 2007) [20] as follows

Table 1: CMT scoring system (Scandinavian scale)

Score	Interpretation	SCC range
Negative	The mixture remained liquid with no evidence of precipitation	0-2 lakh cells/ml
Trace	A slight precipitation, that tends to disappear with continued movements of the paddle	1.5-5 lakh cells/ml
+/Weak positive	A distinct precipitation was formed but no tendency toward gel formation	4-15 lakh cells/ml
++/Distinct positive	The mixture thickens immediately due to gel formation. Mixture tends to move towards the centre leaving the bottom of the outer edge during swirling motion	8-50 lakh cells/ml
+++/strong positive	A distinct gel forms that tend to adhere to bottom of the paddle and a distinct centre peak forms during swirling.	More than 50 lakh cells/ml

2.3 Electrical Conductivity (EC)

Principle: The test is based on the ionic changes which occur during intra-mammary inflammations (IMI). Since the sodium and chloride concentrations increase in milk, the electrical conductivity of milk increases which can be detected by an electrical conductivity meter.

Procedure: Electrical Conductivity of milk sample was determined by using a hand-held Ecotester (Oakion®). Initially, the instrument calibration was checked with potassium chloride solution provided by the manufacturer. Five ml of milk sample was placed into the receptacle of milk checker and instrument was switched on to read the conductivity in milli Siemens per centimetre (mS/cm). The stable readings displayed in the display window of the electrical conductivity meter were recorded promptly. The standard range for EC of normal milk is between 4.0-5.5mS/cm (Spakauskas *et al*., 2006) [28].

2.4 Impregnated pH Strip Test (Mastrip test)

The pH strip test was performed as per procedure mentioned by (Davis 1999) [4]. Mastrip is a cellulose based Bromothymol blue (BTM) strip impregnated with stabilized ion sensitive indicator for detection of mastitis.

Procedure

1. Quarter milk samples were first mixed by inverting the milk containing tubes 10-20 times to obtain a uniform distribution of cells.
2. The mixed samples were then allowed to stand for 2-5 minutes to permit air bubbles to rise and the foam to disappear.
3. One drop of milk with the help of micropipette with plastic needle was put on one strip of Mastrip and change in colour of the strip was observed within 30 seconds.
4. Separate strip was used for each quarter milk sample.

The interpretation of the changed colour was one as follows

Colour Index	Indication
Yellow	Normal
Greenish yellow	Sub-clinical mastitis
Green	Advanced sub-clinical mastitis
Blue	Clinical mastitis

2.5 Somatic Cell Count (SCC)

Somatic cell count is an indicator of the milk quality. White blood cells (leukocytes) constitute the majority of somatic cells in question. The number of somatic cells will be increased in response to pathogenic bacteria causing mastitis. The SCC is quantified as cells per ml. The procedure followed was according to the general principle advocated by Prescott and Breed as detailed by Schalm *et al*. (1971) [22].

Procedure

i. The milk samples were mixed 15-25 times to obtain a uniform distribution of cells. The samples were allowed to stand for 2-5 min to permit air bubbles to rise and foam to disappear. Identification number of the sample was written on a clean microscopic slide.

ii. A level surface was selected and the slide was placed over the template to outline four 1 sq.cm. area. Ten µl of

milk was placed exactly in the centre of the 1 sq.cm. template and was spread evenly to cover all the area delineated by the template. From each sample, two films were prepared using successive areas of the slide. The films were dried at room temperature.

iii. The slide was placed on the slide rack and the smears were flooded with Newman Modified stain (Himedia) for 1 min. The excess stain was drained off by running water and air dried.

iv. Stained films were examined under oil immersion objective and the number of cells in 20 fields was counted. The fields were selected by moving the slide horizontally from one edge of the film through the centre to the opposite edge and then, repeated in a vertical direction. The average number of cells per field was multiplied by the microscopic factor.

v. The diameter of the microscopic field seen through oil immersion objective was measured using a stage micrometer slide ruled in 0.1and 0.01mm. The diameter of the field was measured up to two decimal points and the area of the field was calculated using the formula Πr^2.

$$\text{Microscopic factor (MF)} = \frac{\text{Area of the smear (in mm}^2\text{)}}{\text{Area of the microscopic field}}$$

The diameter was 0.16, then r = 0.08 so,

$$\text{Microscopic factor (MF)} = \frac{100}{3.14\text{x}0.08\text{x}0.08}$$

Microscopic factor (MF) = 4972≈5000

Since the milk sample taken on the slide was 0.01 ml, the total number of cells per ml of milk was calculated using formula:
Cell count per ml of milk = Average number of cells per field×MF×100.
In present study the SCC of more than 2,00,000 per ml of milk was considered as positive for subclinical mastitis (International Dairy Federation, 1997) [8] (Hillerton, 1999) [7]. (Guha and Guha, 2012) [6].

3. Results and Discussion

3.1 Prevalence of Subclinical Mastitis in Deoni cattle

3.1.1 California Mastitis Test

In the present investigation, California Mastitis Test was carried out in 125 Deoni cows. Out of these, 23 were CMT positive. Table 2 represents the prevalence of SCM in different villages of Bidar district using CMT in Deoni cattle. Overall prevalence of SCM using CMT was found to be 18.40%.
The village-wise prevalence of SCM ranged from 66.66% in Dongapur to 0.00% in Kaknal and Kalgapur. Village Ladha had second highest prevalence of 50%. Low prevalence of 7.14% was observed in village Shivni with highest number of milk samples.

3.1.2 Electrical Conductivity

Prevalence of SCM using EC in Deoni cattle is represented in Table 3. Overall mean for prevalence of SCM using EC test was found to be 4.80%. Out of 125 samples only 6 milk samples were found positive for SCM using EC, which was least among all other tests used in this study.
The highest mean of 5.13 ± 0.131 mS/cm was observed in Lakhangaon village and least of 4.15 ± 0.0278mS/cm in Saigaon. The village wise prevalence of SCM ranged from 22.22% in Dongapur to 0.00% in Kaknal, Kalgapur, Ladha, Mehkar, Saigaon, Shivni, and Sompur.

3.1.3 Mastrip Test

Prevalence of SCM using Mastrip test in Deoni cattle is presented in Table 4. Out of 125 milk samples tested for SCM using Mastrip test, 27 were found positive. Overall prevalence of SCM using Mastrip test was found to be 21.60%.The village wise prevalence of SCM ranged from 77.77% in Dongapur to 0.00% in Kaknal and Kalgapur. Village Shivni with highest number of samples tested 10.71% positive. Village Atterga had 42.85% prevalence, village Ladha had 50% prevalence of SCM.

3.1.4 Somatic Cell Count

In the present study, prevalence of SCM using SCC in Deoni cattle is presented in Table 5. Overall prevalence of SCM was found to be 21.60%. Out of 125 milk samples tested for SCM using SCC test, 27 were found to be positive.The village wise prevalence of SCM ranged from 77.77% in Dongapur to 0.00% in Kaknal and Kalgapur. Village Shivni with highest samples tested 10.71% positive. Village Ladha had second highest prevalence of 50% for SCM.

3.2 Comparison of Different Techniques for Detection of SCM in Deoni cattle

In the present study 125 lactating Deoni cattle were studied for prevalence of SCM. The results indicated that 18.40% positive based on CMT, 4.80% positive based on EC values, 21.60% based on Mastrip test and 21.60% positive based on SCC.
The comparison of different techniques for detection of SCM in Deoni cattle is represented in Table 6. Accuracy in comparison with SCC was highest (100%) in Mastrip test followed by CMT (85.18%) and EC (25.92%). SCC above 2lakhs cell/ml and EC above 5.5mS/cm were taken as positive for SCM. All samples showing positive results by Mastrip were also positive by SCC.
The present results are in agreement with Sharma *et al*., 2010 [24] (CMT 67.76%, SCC 53.73%)Supriya *et al*., 2010 [29] (SCC 35%), Mustafa *et al*., 2012 [17] (30.63%), Kamal *et al*., 2014 [12] (CMT 73%), Kathiriya *et al*., 2014 [13] (CMT 29%), Mir *et al*., 2014 [16] (57.80%), Bangar *et al*., 2015 [2] (46.35%), Jena *et al*., 2015 [10] (67.27 and 74.55% by CMT and SCC respectively), Singh 2015 [25] (CMT 66%), Preethirani *et al*., 2015 [18] (48.4, 40.0 and 45.8% by SCC, EC and CMT respectively), Kushwaha 2016 [14] (modified CMT 16.29%) and Dasohari *et al*., 2017 [3] (66.18%, 59.56% and 55.15% using CMT, White Side Test and Surf Field Mastitis Test, respectively). These results were obtained in organised crossbred dairy farms and crossbred animals maintained by farmers. The lower prevalence of SCM in the population under study can be attributed to the natural disease resistance in indigenous cattle over crossbred cattle.
The values reported by Jagadeesh *et al*., 2016 [9] were lower for prevalence of SCM of 12% using CMT and Mastrip.
The accuracy of different techniques in detection of SCM using CMT, EC and Mastrip with respect to SCC were 85.18%, 25.92% and 100% respectively. The results were found in comparison with Guha and Gera, 2011 [5]. (CMT 84.7 and SCC 86.3%), Badiuzzaman *et al*., 2015 [1] (CMT

75.68% and SCC 91.22%).

The high incidence of sub-clinical mastitis (21.60%) without corresponding number of cases of clinical mastitis in Deoni cattle under field conditions suggests that indigenous cattle have some level of innate immunity to many of the microbes causing mastitis. In view of the accuracy, ease of testing and low cost, Mastrip test was found to be effective for detection of sub-clinical mastitis in Deoni cattle under field conditions as compared to California Mastitis test and Electrical Conductivity test. It is suggested that in order to prevent losses to farmers, routine screening of Deoni cattle should be carried out to detect sub-clinical mastitis and treat.

Table 2: Prevalence of SCM in Deoni cattle using California Mastitis Test

Sl.	Village	N	Positive score				Negatives	% Positive
			Traces	1	2	3		
1.	Atterga	7	3	-	-	-	4	42.85
2.	Dongapur	9	3	3	-	-	3	66.66
3.	Kaknal	18	-	-	-	-	18	0.00
4.	Kalgapur	4	-	-	-	-	4	0.00
5.	Ladha	6	3	-	-	-	3	50.00
6.	Lakhangaon	6	2	-	-	-	4	33.33
7.	Manikeshwar	8	2	-	-	-	6	25.00
8.	Mehkar	6	1	-	-	-	5	16.66
9.	Saigaon	7	1	-	-	-	6	14.28
10.	Shivni	28	2	-	-	-	26	7.14
11.	Sompur	7	1	-	-	-	6	14.28
12.	Talwad	19	2	-	-	-	17	10.52
	Overall	125	20	3	0	0	102	18.40

Table 3: Prevalence of SCM in Deoni cattle using Electrical Conductivity test

Sl.	Village	n	Mean ± SE	Range	+ve	%
1	Atterga	7	4.86 ± 0.189	4.3-5.6	1	14.28
2	Dongapur	9	5.06 ± 0.244	4.6-6.8	2	22.22
3	Kaknal	18	4.16 ± 0.186	2.5-5.4	0	0.00
4	Kalgapur	4	4.83 ± 0.131	4.6-5.1	0	0.00
5	Ladha	6	4.85 ± 0.106	4.5-5.2	0	0.00
6	Lakhangaon	6	5.13 ± 0.131	4.7-5.6	1	16.66
7	Manikeshwar	8	4.70 ± 0.167	4.2-5.7	1	0.13
8	Mehkar	6	4.72 ± 0.130	4.3-5.1	0	0.00
9	Saigaon	7	4.15 ± 0.278	3.2-5.6	0	0.00
10	Shivni	28	4.43 ± 0.072	3.5-5.1	0	0.00
11	Sompur	7	4.76 ± 0.115	4.3-5.3	0	0.00
12	Talwad	19	4.87 ± 0.092	4.4-5.8	1	5.26
	Overall	125	4.64 ± 0.052	2.5-6.8	6	4.80

Table 4: Prevalence of SCM in Deoni cattle using Mastrip test

Sl.	Village	N	+ve	%
1	Atterga	7	3	42.85
2	Dongapur	9	7	77.77
3	Kaknal	18	0	0.00
4	Kalgapur	4	0	0.00
5	Ladha	6	3	50.00
6	Lakhangaon	6	2	33.33
7	Manikeshwar	8	2	25.00
8	Mehkar	6	1	16.66
9	Saigaon	7	1	14.28
10	Shivni	28	3	10.71
11	Sompur	7	1	14.28
12	Talwad	19	4	21.05
	Overall	125	27	21.60

Table 5: Prevalence of SCM in Deoni cattle using Somatic Cell Count

Sl.	Village	n	(Mean ± SE) x 10^6	(Range)x10^6	+ve	%
1	Atterga	7	10.53 ± 0.356	9.85-11.05	3	42.85
2	Dongapur	9	8.75 ± 0.942	6.50-12.50	7	77.77
3	Kaknal	18	----	----	0	0.00
4	Kalgapur	4	----	----	0	0.00
5	Ladha	6	7.73 ± 1.258	6.45-10.25	3	50.00
6	Lakhangaon	6	7.75 ± 0.25	7.50-8.00	2	33.33
7	Manikeshwar	8	10.88 ± 0.125	10.75-11.00	2	25.00
8	Mehkar	6	9.25	9.25	1	16.66
9	Saigaon	7	9.25	9.25	1	14.28
10	Shivni	28	5.95 ± 1.225	3.50-7.20	3	10.71
11	Sompur	7	8.00	8.00	1	14.28
12	Talwad	19	7.88 ± 0.554	6.5-9.00	4	21.05
	Overall	125	8.49 ± 0.396	3.50-12.50	27	21.60

Table 6: Comparison of different techniques for detection of SCM in Deoni cattle

Sl.	Cow No.	SCC	CMT	EC	MT
1.	2123	P	P	N	P
2.	3024	P	P	N	P
3.	3480	P	P	N	P
4.	3627	P	P	N	P
5.	3126	P	P	N	P
6.	1277	P	P	N	P
7.	2123	P	N	N	P
8.	2704	P	P	N	P
9.	3365	P	P	N	P
10.	3640	P	N	P	P
11.	1621	P	N	P	P
12.	1517	P	P	P	P
13.	4483	P	P	N	P
14.	1357	P	P	N	P
15.	1882	P	N	N	P
16.	1893	P	P	N	P
17.	3387	P	P	N	P
18.	0662	P	P	N	P
19.	4062	P	P	N	P
20.	0640	P	P	N	P
21.	3343	P	P	N	P
22.	4938	P	P	P	P
23.	0720	P	P	P	P
24.	0285	P	P	N	P
25.	4450	P	P	N	P
26.	3673	P	P	N	P
27.	3423	P	P	P	P
	Total	27	23	06	27

Fig 1: CMT test for subclinical mastitis

Fig 2: Electrical conductivity reading of the sample

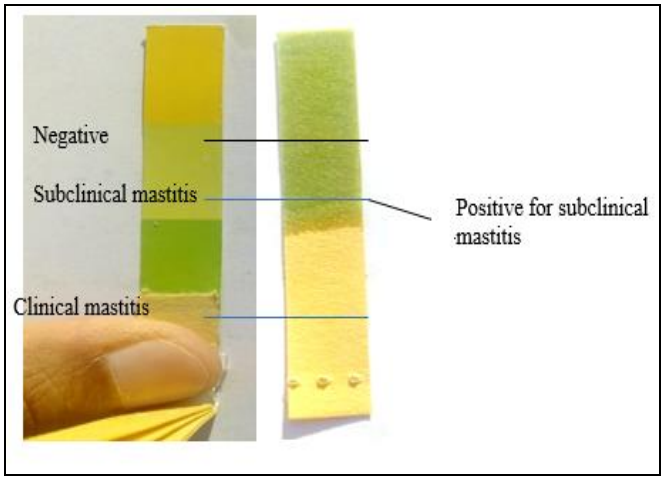

Fig 3: Mastrip showing colour change in positive sample

4. Conclusion

Incidence of sub-clinical mastitis (21.60%) without corresponding number of cases of clinical mastitis in Deoni cattle under field conditions suggests that indigenous cattle have some level of innate immunity to many of the microbes causing mastitis. In view of the accuracy, ease of testing and low cost, Mastrip test was found to be effective for detection of sub-clinical mastitis in Deoni cattle under field conditions as compared to California Mastitis test and Electrical Conductivity test. It is suggested that in order to prevent losses to farmers, routine screening of Deoni cattle should be carried out to detect sub-clinical mastitis and treat affected cows.

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