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Studies on prevalence of subclinical mastitis in dairy animals

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Abstract
The present investigation on prevalence of subclinical mastitis in dairy animals was carried out in Latur district of Marathwada region of Maharashtra during the year 2015 – 16. Eight villages of Latur tahsil were selecteted purposively to study the prevalence of subclinical mastitis in cows and buffaloes. For diagnosis of subclinical mastitis in dairy animals Modified California Mastitis Test was done. The incidence was found more in cows as compared to buffaloes. Hind quarters were affected more as compared to forequarters. This Study was also carried out to evaluate the effect of subclinical mastitis in the physico-chemical properties of milk (cows and buffaloes). The result of this study was revealed the average pH value of subclinical mastitis milk were increased significantly (P<0.01) as compared the normal milk. Where the fat, protein, lactose, solid not fat and total solid percentage was decreased significantly (P<0.01). The incidence of Subclinical mastitis was also observed due to the some risk factors, viz. are Age, lactation number, stage of lactation, method of milking and housing was important risk factors precipitating occurrence of subclinical mastitis.

Keywords: Prevalence, Subclinical mastitis, Physico-chemical Properties, Risk factors

Introduction
The subclinical mastitis (SCM) is a more serious and responsible for much greater loss to the dairy industry (Kader *et al.,* 2002) [3]. The subclinical mastitis (SCM) can be known only after laboratory examination**,** as there are no gross inflammatory changes in the udder tissue. Singh and Singh (1994) [8] reported more than three times losses due to SCM, as compared to clinical mastitis (CM). Besides causing huge losses to milk production, the sub clinically 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. Subclinical mastitis (SCM) is a herd problem, acts as a repository of microorganisms that leads to the spread of infection to the other animals undetectable to naked eyes. Detection of mastitis at subclinical form is also ignored owing to mixing of milk from different sources without proper checking at milk collection point in the villages. Also, the accuracy of indirect tests to detect mastitis in field varies from place to place. It is, therefore, important to know the prevalence of subclinical mastitis (SCM) in dairy herds and delineate the important factors responsible for it. The subclinical form of mastitis mostly remains undetected due to absence of apparent changes in udder or in mammary gland. It results into clinical mastitis when left untreated and becomes very difficult to cure and permanently affection of the udder resulting into stable loss of production trend. Therefore, early detection of mastitis is most important for preventing the further economic losses of the farmers.

Material and methods
Selection of animal
Total 120 dairy animals (60 cows and 60 buffaloes) from the eight villages were selected on the basis of examination of udder and grading of Modified California Mastitis Test (MCMT).

Method of collection of milk sample
Milk samples from all four quarters were collected separately at the time of milking in morning. Udder of animal was thoroughly washed with clean water prior to the collection of milk samples. The standard Modified California Mastitis Test Reagent was prepared and used for detection of subclinical mastitis on the survey field.

Procedure

The MCMT was conducted in milking shed at the start of milking of each cow. A plastic paddle with four shallow cups marked as left-fore (LF), left-hind (LH), right-fore (RF) and right-hind (RH) were used to detect the individual quarter incidence of subclinical mastitis. Approximately 2-3 ml of first striping of milk (fore milk) was taken from individual quarter in the respective cup of 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. Then the sample was observed for precipitation or gel formation. If the gel like substance formed, MCMT was said to be positive and quarter was noted as affected with subclinical mastitis. But if the solution remains watery, the MCMT was negative, indicating the quarter was not infected with SCM. The data regarding subclinical mastitis of each individual quarter was recorded on a data sheet.

Analysis of milk samples

The milk collected samples were subjected to chemical analysis viz., pH, Fat, lactose, protein, solid not fat (SNF) and total solid (TS). Analysis of milk samples were done by using Lactoscan milk analyzer at the visited spot where as the pH and total solid were analyzed by bringing the milk sample at Department of Animal Husbandry and Dairy science, college of Agriculture, Latur.

Statistical analysis

A tabular analysis of collected data was used to accomplish the objectives of study. A student 't' test used to test the level of significance between normal milk and subclinical mastitis affected milk by Snedecor and Cochran (1968) [9]. A Chi square test used to determine the level of significance among the effect of risk factors of the subclinical mastitis in dairy animal by Chandel (1998) [1]. P values less than 5 per cent was considered statistically significant.

Result and discussion

The overall prevalence of subclinical mastitis in dairy animal

The data related to the overall prevalence of SCM in dairy animals are presented in Table 1. The total 60 cows were screened against subclinical mastitis, out of which 35.00 per cent (21) cows were found positive suffering from subclinical mastitis, Where as in, 60 buffaloes 28.33 per cent (17) are found positive.

In present study, the overall prevalence of SCM was lower in buffaloes as compared to the cows. This observation is in agreement with the findings of Khan and Muhammad (2005) [4]. This lower prevalence in buffalo as compared to cow might be attributed to the tighter teat sphincter of buffaloes as compared to that of cow. (Uppal *et al.* 1994) [10].

Table 1: The overall prevalence of subclinical mastitis in dairy animal

Animal species	Total no. of animal tested	No. of animal positive for SCM	Prevalence percentage of SCM animal	No. of animal without SCM	Percentage of animal without SCM
Cow	60	21	35.00	39	65.00
Buffalo	60	17	28.33	43	71.67

Quarter wise prevalence of SCM in dairy animal

In present survey quarter wise milk samples from cows and buffaloes were collected and screened against SCM. The data presented in table 2 shows that out of 240 quarters of cows, 45 quarters (18.25 per cent) and out of 240 quarters of buffaloes, 35 quarters (14.58 per cent) showed positive reaction tested by Modified California Mastitis Test (MCMT). In cows out of 45 affected quarters, 11 (24.44 per cent) right fore, 13 (29.54 per cent) right hind, 9 (20.45 per cent), left fore and 12 (27.27 per cent) left hind quarters were found positive, where as in the buffaloes out of 35 quarters, 7 (20.00 per cent) right fore, 11 (31.42 per cent) right hind, 7 (20.00 per cent) left fore and 10 (28.57 per cent) left hind quarters were found positive for subclinical mastitis (Table 2).

Thus, there was higher incidence found in hindquarters of buffaloes than cows and, right hind quarters were found to be more susceptible in both species. In case of forequarters, there was higher incidence in cows than buffaloes and right forequarters were found to be more susceptible.

Similar result also found by Khan and Muhammad (2005) [4] who reported that total number of quarters affected with subclinical mastitis were 54(27.00 per cent) out of 200 in buffaloes. Among these, 8(14.8 per cent) were right fore, 16(29.60 per cent) right hind, 10(18.50 per cent) left fore and 20(37.00 per cent) left hind quarters. In cows, 72 out of 200 (36.00 per cent) quarters were infected. This included 14 (19.40 per cent) right fore, 20 (27.80 per cent) right hind, 13(18.10 per cent) left fore and 25(34.70 per cent) left hind quarters.

Table 2: Quarter wise prevalence of SCM in dairy animal

Species	No. of animal		Quarter (per cent)					
	Tested	Positive	Tested	Positive	RF	RH	LF	LH
Cow	60	21	240	45	11	13	9	12
Percentage		(35.00)		(18.25)	(24.44)	(29.54)	(20.45)	(27.27)
Buffalo	60	17	240	35	7	11	7	10
Percentage		(28.33)		(14.58)	(20.00)	(31.42)	(20.00)	(28.57)

Effect of subclinical mastitis on physico-chemical properties of milk

The physico-chemical properties of normal milk and subclinical mastitis affected milk of cows and buffaloes are presented in table 3. The pH of cow milk increased from 6.57 ± 0.021 normal milk to 6.88 ± 0.015 SCM affected milk, also in buffaloes from 6.68±0.016 normal milk to 6.91 ± 0.021 SCM affected milk. Increased in pH of affected milk in the study could be due to increased permeability of the gland tissue to blood components results in higher values in milk. This might also be partially due to increased movement of bicarbonate ions into milk, since the lactose production decreased and the alkaline salts from the blood enters, the milk become more alkaline showing pH above 7.0 as

indicated by Rao (1990) [5]. Whereas fat percentage in cow milk decreased from 4.24 ± 0.059 normal milk to 3.45 ± 0.030 in SCM affected milk and in buffalo decrease in fat per cent from 6.63 ± 0.049 to 4.91 ± 0.074 in the normal milk to SCM affected milk, respectively. Decrease in fat content in milk due to impaired synthetic and secretary activity of the udder epithelial cell, (Schultz, 1977) [6]. The protein percentage was decreased in cow from 4.59 ± 0.067 in normal milk to 4.08 ± 0.061 in SCM affected milk and in buffalo milk decreased protein percentage from 4.23 ± 0.070 to 3.75 ± 0.064 in SCM affected milk. Decrease in protein content in milk from infected animal milk due to high increase in the activity a proteolyticine enzyme (plasma) that cause extensive destroyed for milk protein in udder before milk removal. Shreekumar *et al.* (1975) [7] also noticed that the total protein in subclinical mastitis milk was decreased.

Lactose percentage was decreased in cow from 3.95 ± 0.093 normal milk to 3.24 ± 0.098 in SCM affected milk, also found decreased lactose percentage in buffaloes from 5.07 ± 0.104 normal milk to 4.18 ± 0.098 in SCM affected milk. Lactose is synthesized in the gland cells of the udder from glucose and galactose during inflammatory, reduced secretary activity at those mammary cells due to destruction of the epithelial cells by the leukocytes, these changes were linked with many factors such as breed, feeding, environmental conditions and age.

The changes in milk composition i.e. SNF and total solid was also observed. SNF content reduced in cow from 9.74 ± 0.040 normal milk to 8.12 ± 0.069 SCM affected milk and also in buffaloes from 10.02±0.051 in normal milk to 8.73 ± 0.060 in SCM milk. Lactose and protein were major components of SNF, it appeared that drop in SNF was mainly due to decreased lactose content in mastitis milk reported by Rao (1990) [5]. The total solid content was lowered in the SCM milk, in cow it is decreased from 13.99 ± 0.070 to 11.57 ± 0.068, in buffaloes same trend was seen 16.64 ± 0.071 to 13.64±0.092.

Decrease in total solid was observed in present study due to decrease in fat and solids not fat percentage in affected quarters. Similar trend was found by Hassan (2013) [2] he reported that the Fat, SNF, Protein, Lactose of SCM milk was decreased where as pH of SCM milk was increased.

Table 3: The overall physicochemical properties of normal and SCM milk of dairy animal

Physico-chemical properties	Cow (n=60)		Buffalo (n=60)	
	Normal	SCM Affected	normal	SCM Affected
pH	6.57 ± 0.021	6.88 ± 0.015	6.68 ± 0.016	6.91 ± 0.021
Fat	4.24 ± 0.059	3.45 ± 0.030	6.63 ± 0.049	4.91 ± 0.074
Protein	4.59 ± 0.067	4.08 ± 0.061	4.23 ± 0.070	3.75 ± 0.064
Lactose	3.95 ± 0.093	3.24 ± 0.098	5.07 ± 0.104	4.18 ± 0.098
SNF	9.74 ± 0.040	8.12 ± 0.069	10.02 ± 0.051	8.73 ± 0.060
Total solid	13.99 ± 0.070	11.57 ± 0.068	16.64 ± 0.071	13.64 ± 0.092

Association between some of factors with occurrence of subclinical mastitis in cows and buffaloes

In present investigation the various risk factors and their effect on prevalence of SCM in dairy animal was studied. The prevalence of subclinical mastitis was observed higher in adult age group of animal in both cows and buffaloes i.e. 40.54 per cent and 35.89, respectively. In cows the effect of lactation number on prevalence of SCM was observed higher in 7-9 lactation number i.e. 75.00 per cent followed by 4-6 lactation number, 25.80 per cent while in buffalo the higher prevalence was observed in 7-9 lactation number i.e. 50.00 per cent followed by 4-6 lactation number. In prevalence of subclinical mastitis was observed in the mid lactation stage i.e. 40.90 per cent, late lactation stage i.e. 31.81 per cent in cows and buffaloes respectively. The subclinical mastitis was observed more in the knuckling method of milking in cows and buffaloes followed by the full hand. The nature of housing had higher prevalence in kutcha type 54.94 per cent and 27.90 for puccka type was observed in cows, while 31.81 per cent in kutcha and 26.31 puccka type were seen in buffaloes. The result of statistical analysis revealed no significant difference observed in infection among the stage of lactation, method of milking and housing of the animal. Whereas in cow age group and lactation number were found statistically significant ($P<0.05$).

Table 4: Association between some risk factors with occurrence of subclinical mastitis in cows and buffaloes

Risk factor		Cow		Prevalence (per cent)	X^2	P value	Buffalo		Prevalence (per cent)	X^2	P value
		Total	SCM Affected				Total	SCM Affected			
Age	Young	23	06	26.08	4.85	3.84	21	03	14.28	3.15	3.84
	Adult	37	15	40.54			39	14	35.89		
Lactation no.	1-3	17	04	23.52	10.59	5.99	17	03	17.64	2.80	5.99
	4-6	31	08	25.80			35	10	28.57		
	7-9	12	09	75.00			08	04	50.00		
Stage of Lactation	Early	21	7	33.33	0.59	5.99	20	06	30.00	0.49	5.99
	Mid	22	9	40.90			18	04	22.22		
	Late	17	5	29.41			22	07	31.81		
Method of milking	Full Hand	09	03	33.33	0.16	5.99	11	02	18.18	3.69	5.99
	Knuckling	41	15	36.58			48	15	31.25		
	Machine	10	03	30.00			01	00	00.00		
Housing	Kutcha	17	09	54.94	3.32	3.84	22	07	31.81	0.19	3.84
	Puccka	43	12	27.90			38	10	26.31		

(Young: < 6 years, adult: ≥ 6 year. Early lactation: 1 to 120 days, Mid lactation: 120 to 240 days, Late lactation: >240 days of lactation)

Conclusions

1. The forgoing result and discussion lead to conclude that, the prevalence of subclinical mastitis in cow was 35.00 per cent and in buffaloes it was 28.33 per cent. It means that the incidence of subclinical mastitis was more in cows as compared to the buffaloes. The animal wise (6.67 per cent) and quarter wise (3.67 per cent) incidence was found more in cows as compared to buffaloes.
2. It was observed that prevalence of subclinical mastitis was found more in hind quarter of buffaloes and more in fore quarter of cows.
3. The hind quarters are found more affected than the fore quarters, in both the species.
4. The pH in subclinical mastitis affected milk increased whereas fat, protein, lactose, total solids and solids not fat gets decreased significantly which hampered the milk quality.
5. Age, lactation number, stage of lactation, method of milking and housing was major risk factors precipitating occurrence of subclinical mastitis.

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