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RESEARCH ARTICLE

PREVALENCE AND SEVERITY OF SUB-CLINICAL MASTITIS IN LACTATING COWS: DETECTION BY SURF FIELD MASTITIS TEST.

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Abstract

A total number of one hundred cow milk samples were examined in the present study for the Sub-clinical mastitis. In our study 44% milk samples were found positive for Surf Field Mastitis Test (SFMT). Quarter wise infection rate was found to be 18.5% and 31.5% in fore and hind-quarters respectively. According to the IDF criteria, 14.17, 18.33 and 8.35% quarters had subclinical, latent and non specific mastitis, respectively. Incidence of sub-clinical mastitis was assessed in dairy cows kept under different farm management conditions in two villages of Panipat district, Haryana. The prevalence of mastitis in cows were determined by examination of changes in the udder viz., swelling, redness and hardness of udder, changes in milk colour and reduction in quality of the milk. Further, pH testing of the milk and Surf Field Mastitis Test (SFMT) was done to confirm the subclinical mastitis.

Introduction:-

Mastitis is an inflammatory condition of the udder. It is characterized by physical, chemical and microbiological changes in the milk. It poses the risk for transmission of many zoonotic diseases like tuberculosis, leptospirosis, brucellosis, streptococcal sore throat and gastroenteritis (Radostits *et al*., 1994). It has also been indicated that mastitis is an important cause of premature culling (Samiullah *et al*., 2000). Mastitis represents a serious problem to be considered due to the economic loss for which it is responsible (Ahmad, 2001). The SCM usually goes unnoticed because the milk and udder appear normal. Sub-clinically infected quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al.*, 2003). Sub-clinical mastitis causes two third loss of the total milk production due to affected quarters of animal (Radostits *et al.*, 2007). At present, it is one of the most economically important diseases worldwide (Chishty *et al*., 2007 and Hashemi *et al*., 2011) and the most important economical factor affecting the dairy animals worldwide (Ali *et al.*, 2011). This dairy scourge not only reduces the milk yield of affected animals (nearly 15 to 20%) but also renders the milk unsuitable for human consumption (Ghulam Muhammad and Imaad Rashid, 2012). The sub-clinical or hidden form of mastitis is 15-40 times more common than clinical mastitis. Clinical cases are preceded by the hidden form of mastitis. An early diagnosis of hidden mastitis is imperative to save the udder and prevent transmission of disease-producing organisms to other animals in the herd. It is also important from the public health viewpoint as the milk of animals affected with sub-clinical mastitis contains disease producing bacteria, their toxins, abnormal milk constituents as well as pus cells. Inflammation of the parenchyma of mammary gland takes place in mastitis (Souto *et al*., 2010). *Proteus spp*., *Salmonella spp*. and *Bacillus spp*. have been isolated from milk samples collected from goats suffering from mastitis (Iqbal *et al.*, 2004). *Streptococcus spp*., *Staphylococcus spp.*, *Pasteurella spp*. and *E. coli* were also reported (Contreras *et al.*, 2007).

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Staphylococcus aureus, Streptococcus spp. and *E. coli* revealed in milk collected from subclinical mastitis were found to be 61, 15 and 5 percent, respectively (Aydin *et al*., 2009). *Staphylococcus aureus* had been reported as most frequent etiological agent (45.34%) in cases of dairy goat mastitis (Ali *et al*., 2010). Major organisms causing infection are *Staphylococci* spp., *Streptococci* spp. and gram-negative bacteria (Mubarack *et al*., 2012). Diagnosis in clinical as well as sub-clinical cases largely depends on the presence of significantly higher leukocytes count in the milk from affected glands. In context of milk, these leukocytes are called somatic cells. It may be mentioned here that the sub-clinical mastitis is reasonable for greater pecuniary loss to the farmers than its clinical counterpart. In Sub-clinical mastitis (SCM) there are no much visible abnormalities in udder tissues except an elevated Somatic Cell Count (SCC) (MacDougall *et al*., 2001). The effective prevention and etiological therapy of this disease requires precise bacteriological diagnosis along with sensitivity testing of microbial agents against various antimicrobials (Malinowski *et al.*, 2002). Concurrent resistance of bacterial species to antimicrobials of different classes is increasing in multitude and complicating the therapeutic management of infections (Iqbal *et al.*, 2002). Such bacterial pathogens which are not responding to more than two antibiotic classes in therapeutic dose are considered multiple drug resistant (MDR). It is one of the emerging problems of mastitis causing bacteria (Hameed *et al.*, 2007). The presence of multiple drug resistant bacteria in cow or goat mastitis milk deteriorates its quality. Use of such milk may lead to the transfer of resistance to normal flora of consumers. The Clinical Mastitis (CM) is usually accompanied by physical, chemical, pathological and bacteriological changes both in milk and glandular tissues (Samad, M.A. 2008)). Maintaining hygiene with antimicrobial therapy plays an impotant role in mastitis control by reducing the levels of herd infection (Unakal *et al*., 2010). Among different available tests, California Mastitis Test (CMT) (Contreras *et al*., 1995), White Slide Test (WST) (Kahir, M.A. 2006) and Surf Field Mastitis Test (SFMT) (Muhammad *et al*., 1995) are considered as simple, easily applicable, rapid indirect screening tests for determining SCM. Reagents of these tests contain detergents which change the structure and conductivity of cell membrane and nucleus of somatic cells, stimulate proteolytic enzymes, and increase milk viscosity (Middleton *et al*., 2004). Sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease and specificity refers ability to correctly identify those patients without the disease (Lalkhen *et al*., 2013). Haryana is one of the top-ten states in milk production and may be considered as the milk pocket of India. There was no much information on Haryana based study for SCM. Therefore, the present study was conducted to detect the prevalence and severity of sub-clinical mastitis in lactating cows using indirect screening tests i.e. SFMT.

Materials and Methods:-

Present study was carried out on bovine sub-clinical mastitis in cow, in Village Israna and Mundlana (Panipat) India. After explaining the objectives of the study, consent was taken from all participating farmers and information on age, lactation stage and previous history of mastitis was collected. Both commercial and backyard dairy cows from rural areas were selected randomly. A total number of one hundred (1^{st} to 5^{th} lactation) milk samples of cows aged between 5-10 years were obtained and considered for this study. Standard mastitis control measures such as post milking antiseptic teat dipping and dry period antibiotic therapy were not practiced in these cows.

Milk sampling:-

Quarter fore-milk samples were collected aseptically for assay as described by Haltia and Honkanen-Buzalski *et al.*, (2006). Prior to collection of milk samples, the udder was washed with water and dried with paper towel. Teat ends were disinfected with cotton swabs soaked in 70% ethanol and allowed to dry. The first streams of milk were discarded. Milk samples of the respective quarters of an animal were collected in sterile 15 ml tubes and properly marked with number of animal and position of quarter. The milk samples were then transported in an ice box to the laboratory for further testing and analysis.

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, flakes and any other visible abnormalities (Muhammad Shahid*et al.*, 2011).

Indirect screening tests:-

pH determination:-

In this study pH was used as one of the important parameter as the indicator of mastitis. Normal milk pH is 6.6 – 6.7 where as milk with higher pH indicated the positive test for mastitis (Bachaya H.A. *et al.*, 2011).

Surf field mastitis test:-
The samples were subjected to Surf Field Mastitis test (SFMT). The principle of the test is that when detergent is added into milk sample, it causes rupture of somatic cells, releases DNA and other cell contents. DNA is acidic in nature, while detergent contains alkyl-arylsulfonate, which is basic in nature. Combination of DNA and detergent resulted into a gel. Consistency of gel depends upon the number of somatic cells. Higher the number of cells, thicker gel formation and vice-versa.

Special attributes of Surf Field Mastitis Test:-
It is easy to conduct due to user friendly nature of the test and less time consuming, having good sensitivity (72.81 and 66.22 in cows and buffaloes respectively) of detection *vis-à-vis* other tests like California Mastitis Test (75.73 and 70.27 cows and buffaloes respectively (Muhammad *et al*., 2010) and the gold standard of mastitis diagnosis i.e. microbiological examination of aseptically collected milk samples. The test reagent, i.e. Surf Excel Powder (Unilever Ltd.) is easy available in almost every village.

Procedure:-
3% Surf solution (pH = 10.3) was prepared by dissolving 3 grams of commonly available Surf Excel (Uniliver, India) in 100 ml of Distilled water. This test solution is stable for six months at room temperature. Equal quantity of 3% reagent and milk were mixed in a sterile conical flask. The mixture is swirled for about 1 minute and then poured into a petri plate. After a few seconds, visual examination for the presence of floccules or gel formation indicated the positive results for intra mammary infection. While absence of any floccules or gel formation in the sample, was considered negative.

The changes in consistency of milk indicated mastitis, while no change in consistency of milk indicated healthy samples. The mastitis was graded into further four categories based on the severity of disease from lower to higher intensity as, +(moderate), ++(severe), +++(more severe), ++++(very severe) (Muhammad *et al.,* 1995; Fazal-ur-Rehman, 1995). The percentage of prevalence was calculated by using the following formula as- Prevalence (%) = No. of samples positive /No. of samples tested x 100

Microbial Culture:-
Collected milk samples were primarily cultured on blood agar using 'spread out technique' as described by Lafi and Hailat (1998). The plates were incubated for 24 hrs at 37°C. Bacterial growth was purified by streaking. Gram's staining was performed to study the morphology of bacteria. Pure bacterial cultures were identified on the basis of cultural, morphological and biochemical characteristics (John, 2000).

Results:-

Mastitis in cows were determined by examination of changes in the udder viz., swelling, redness and hardness of udder, changes in milk colour and reduction in quality of the milk. An increase in the pH of milk was observed which indicated the subclinical mastitis. Further, Surf Field Mastitis Test (SFMT) of milk samples was performed to confirm the subclinical mastitis. In our study on cow milk samples, 44 out of 100 i.e. (44%) quarters were found to be positive for SFMT. Thus the prevalence of SCM by SFMT was 44% (n=100). Among these infected quarters, 19% were right fore, 25% right hind, 18% left fore and 38% left hind quarters. Mean value of sub-clinical mastitis was higher (31.5%) in hindquarters of cows in comparison to (18.5%) in fore-quarters. Thus, there was higher incidence in hindquarters. Among hindquarters, left hindquarters were found to be slightly more susceptible (38%). In case of fore-quarters, right fore-quarters were found to be more susceptible (19%), as also reported by Saini *et al*. (1994). Incidence of sub-clinical mastitis was found to be highest in the early stage of lactation (52%) followed by mid (31%) and late stage of lactations (17%). In present study, *Staphylococcus aureus* was isolated as top ranking pathogen from cases positive for mastitis. In previous studies, it was also reported as major pathogen (Kapur *et al*., 1992; Allore, 1993; Rabello *et al*,. 2005; Arshad *et al.,* 2006; Ebrahimi *et al*., 2007; Ali *et al.,* 2008; Botrel *et al.,* 2009). According to a report 70-80 % of all the clinical and subclinical mastitis cases were either infected with *Staphylococcus aureus* or *Streptococcus agalactiae* in cows and buffaloes (Memon *et al.,* 1999).

Table and Graph-1:-Prevalence of Sub-Clinical Mastitis in Cows Detected by Surf Field Mastitis Test

		Grading of gel					
Testing Method Used	**Total No. of Milk Samples**	**(+) ve %**	**(++) ve %**	**(+++) ve %**	**(++++)ve %**	**(-) ve %**	**Overall Prevalence (SCM) %**

	Tested						
SFMT	100	31	9	3	1	56	44

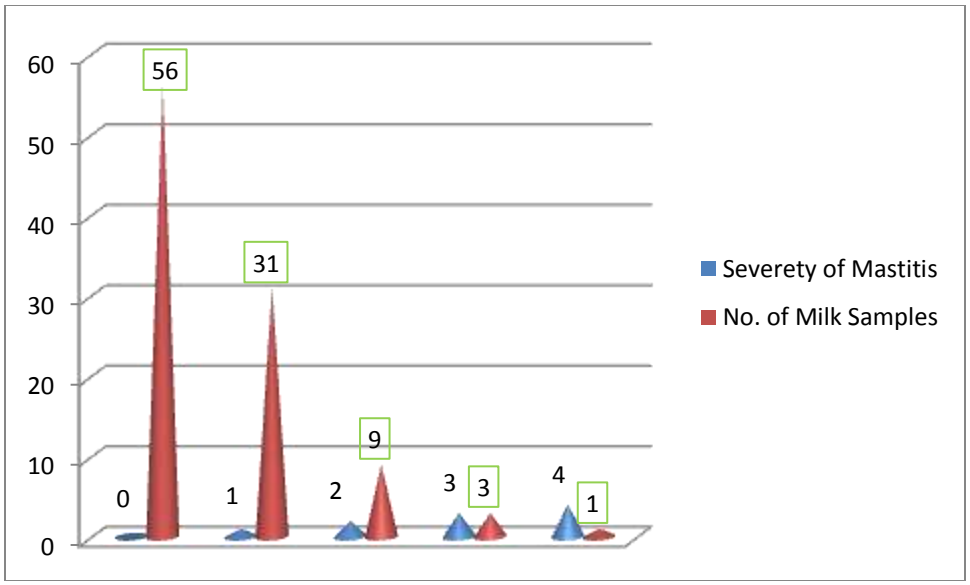


Table and Graph-2:-Mastitis Incidence (Percentages) in Cows in Different Stages of Lactation

S.N.	Lactation Stage	Mastitis Incidence (%)
1	Early	52
2	Mid	31
3	Late	17

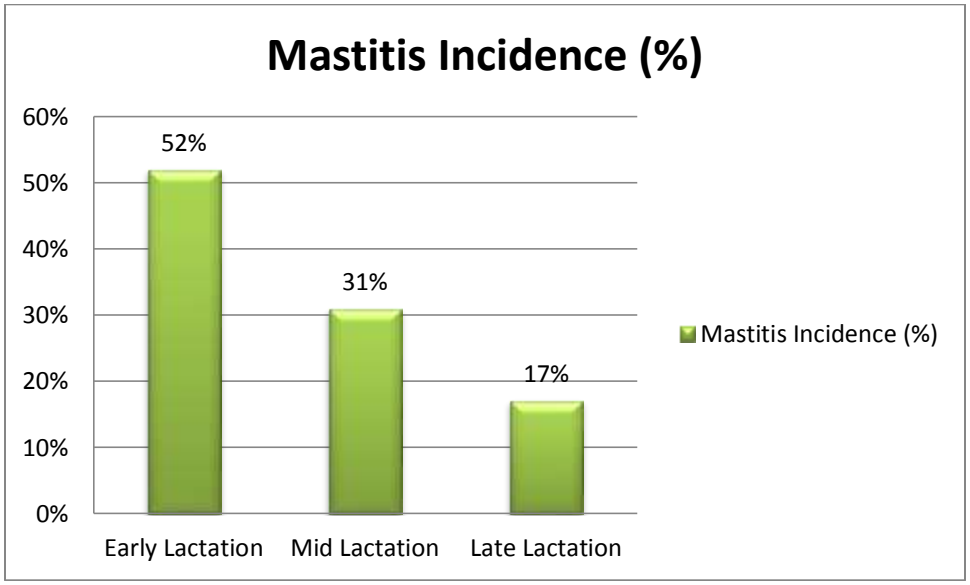


Table and Graph-3:-Quarterwise Mastitis Incidence (Percentages) in Cows

S. N.	Quarters	Mastitis Incidence (Percentages)
1.	Right Fore Quarters	19%
2.	Right Hind Quarters	25%
3.	Left Fore Quarters	18%
4.	Left Hind Quarters	38%

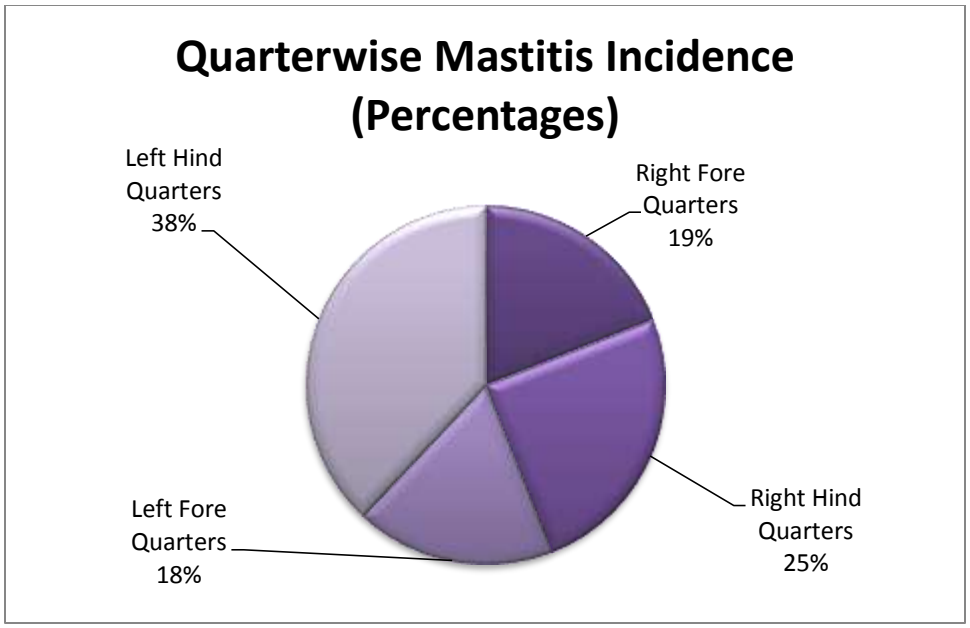
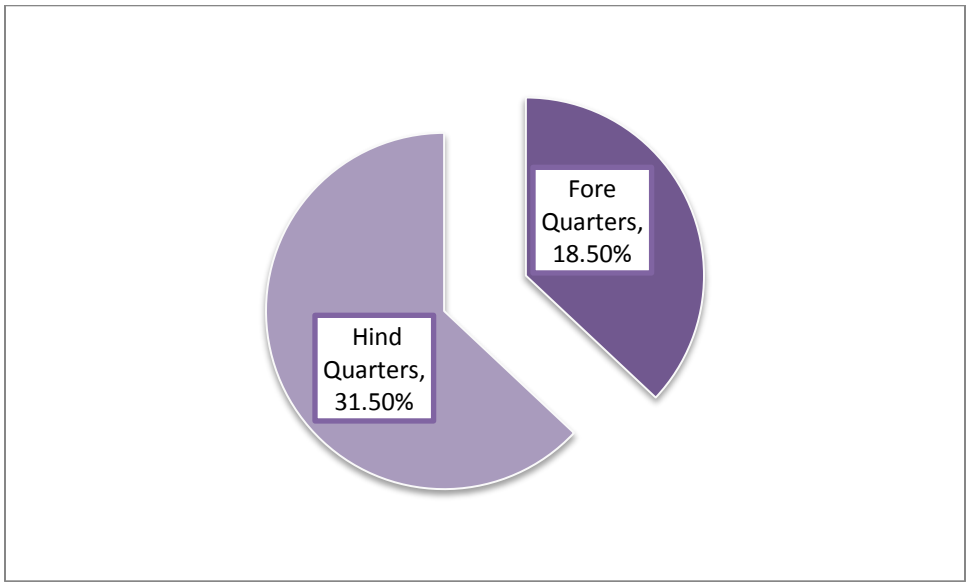


Table and Graph-4:-Mean Value of Sub-Clinical Mastitis Incidence (%) in Fore and Hind Quarters of Cows

Quarters	Quarterwise Mastitis Incidence (Percentages)
Fore Quarters	18.50%
Hind Quarters	31.50%



Interestingly, Mean value of sub-clinical mastitis was higher (31.5%) in hindquarters of cows in comparison to (18.5%) in fore-quarters.

Figure 1:- Milk sample showing moderate intensity of sub-clinical mastitis

Figure 2:- Milk sample showing high intensity of sub-clinical mastitis

Discussion:-

Overall prevalence of SFMT was 44% (Table-1). The prevalence was almost nearer to the findings of (Kader *et al*., 2003 and Islam, *et al*., 2010) who had reported 46.6% and 36.46% in lactating dairy cows. Higher prevalence was reported by (Motice, *et al*., 1985) and lower prevalence was reported by (Rahman, *et al*., 2009). Difference in prevalence of SCM might be due to climatic factors (humidity, temperature, seasonal variations), geographical locations, difference of breed, management systems and other associated conditions. Several studies have reported increased mastitis with the advancing parity (Prodhan, *et al*., 1996). It has been shown that high-yielding dairy cows are more prone to mastitis as the glandular tissues are more susceptible to infection (Radostits, *et al*., 2000). Interestingly, high parity cows are more productive, and it is likely that cows with advancing parity are prone to mastitis. It is reported that the prevalence of mastitis is often high in last stage of pregnancy followed by a marked decline after parturition (Radostits, *et al*., 2000). Our results are also in agreement to these findings. There are some multiple drug resistant (MDR) pathogens responsible for causing mastitis in dairy goats. Milk consumed from these goats suffering from clinical or sub-clinical mastitis may transmit antibiotic resistance genes to micro flora of human beings. This situation may be a biggest human beings health hazard (M. F. Najeeb *et al*., 2013).

Quaterwise infection rate was found to be 16% and 28%. In a similar study, Saluja *et al.* (2004) reported infection rate of 31.3% in a dairy herd. In an earlier study, the percentages of the cows (and quarters) with SCM were found to be 43.8% (24.3%) when assessed by cultural examination (Karimuribo *et al.*, 2008). Out of 5707 quarter milk samples, 1070 (18.74%) and 1878 (32.90%) samples were found positive for clinical and subclinical mastitis, respectively (Sharma and Sindhu, 2007). The high prevalence in the present study could be attributed to a group of

factors such as poor habitat, lack of hygiene, unavailability of balanced food and climatic conditions. These factors might have played a role in rendering the udder more prone and susceptible to intra mammary infections (Ghazi and Niar, 2006).

It had been found earlier that the sensitivity of the different tests viz. CMT, SCC, WST and SFMT were 80.08%, 86.60%, 60.54% and 57.47%; specificity 69.40%, 97.81%, 63.38% and 60.66%. Percentage accuracy of these tests was 75.68%, 91.22%, 61.71% and 58.78. Quarter-wise sub-clinical mastitis (SCM) was detected in 59.68%, 51.80%, 50.68% and 50% samples by CMT, SCC, WST and SFMT, respectively, while animal-wise SCM was recorded in 72.07%, 66.67%, 64.86% and 61.26% samples by CMT, SCC, WST and SFMT, respectively. Moreover, the right hind quarters were found most significantly susceptible to SCM than other quarters. Cows with 3rd and 4th parity and at their early lactation stage had significantly higher ($p<0.001$) SCM than others (M. Badiuzzaman *et al.*, 2015).

Only cells possessing nuclei interact with superficial active agents. During interaction between detergents and nuclei of somatic cells DNA is released, which increases milk viscosity (Sargeant et al., 2001, Ruegg and Reinemann, 2002 and Middleton et al., 2004). The tests work best in a weakly alkaline medium, where changes in mixture are maintained for more than 15 minutes. This may explain the linear relationship between mastitis test results and somatic cell count in milk. Higher leukocytes count was detected in milk of affected animals (Radostits *et al*., 2006).

According to Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, GOI, milk production in India is increasing every year for last fifteen years. Thus it may be hoped that milk production will improve many folds in coming years by timely detection and treatment of SCM.

Conclusion:-

Mastitis is a common problem of dairy industries. Reduction in milk production and an irreparable damage to the udder associated with the disease are the common causes of culling of dairy cows. Milk from infected animals is not suitable for human consumption and for making different milk products. So it has a major economic importance in dairy cattle. The prevalence of sub-clinical mastitis increases in cows with a history of periparturient disease, cows without dry cow therapy, high milk producing cows and cows with the advancing age. This study recommends that regular screening of sub-clinical mastitis will reduce the prevalence of sub-clinical mastitis. The most effective way to control sub-clinical mastitis is to take preventive measures such as milkman's cleanliness, regular cleaning of the udder and floor, dry cow therapy especially in high yielding dairy cows. SFMT are easily applicable and cost effective tests for regular screening of sub-clinical mastitis. As there is no vaccine for mastitis, hygiene should be maintained at every aspect of dairy farms. Care and management should be improved. Infected milk should be properly disposed by adding 5% phenol. All the equipments and containers should be cleaned and washed properly. The SCM usually goes unnoticed, it is important to screen a particular dairy herd for this disease at regular intervals so as to adopt an effective treatment. This would also prevent us from serious economic loss.

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