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Incidence of bovine subclinical mastitis in organized and unorganized farms based on somatic cell count

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Abstract
Mastitis, a disease of multiple etiology, had been recognized for more than a century, and still continues to be an evergreen cause of economic loss to the dairy industry and is the costliest problem all over the world where dairying is practiced. The distribution of mastitis incidence varies from country to country. Subclinical mastitis more prevalent than clinical mastitis and its prevalence varied from herd to herd and place to place. Subclinical mastitis (SCM) is most important due to its negative impact on the economy throughout the world dairy industry. Incidence of bovine SCM was studied on dry pregnant cows of organized and unorganized farms in an around Khanapara, Guwahati, Assam. Diagnosis was based on Somatic Cell Count (SCC). A total of 30 cows were examined, of which 6 cows belonged to an organized farm and rest 24 cows to unorganized farms. Four cows (13 quarters samples) from organized farm were found positive for SCM. The incidence of SCM cow-wise was 66.67 per cent and quarter-wise 54.17 per cent. On the unorganized farms, all the milk samples from 24 cows (83 quarter samples) were positive for SCM and the incidence was 100 per cent both cow- wise and quarter-wise. The overall percentage of incidence cow-wise was 93.33 and quarter-wise 90.26 per cent. High incidence of SCM on the organized farm is implicated to small sample size, whereas on the unorganized farm the management practices leading to stress may be explained as the reason of such high incidence of SCM.

Keywords: Bovine mastitis, clinical mastits, subclinical mastitis, somatic cell count

Introduction
Mastitis is one of the most prevailing diseases of high yielding dairy animals. It has been recognized for more than a century, and still continues to be an evergreen cause of economic loss [1, 2].

Mastitis is a complex disease [3] resulting from the interaction of infectious agents and poor managemental practices in dairy animals. A dairy herd without mastitis is virtually impossible under modern intensive farming condition. Subclinical mastitis (SCM) is more common than clinical mastitis (CM), but often goes undetected [4] and given less importance than CM.

The distribution of mastitis incidence varies from country to country. Economic losses due to mastitis were estimated in Norway at 200 million kroners (140 million kroners due to CM and 60 million kroners due to SCM [5]. A decreased milk production was reported extending upto 70 per cent of the total cost of mastitis as a result of damage to milk producing tissues and causing bovine mammary epithelial cell death [6]. In India, the loss due to mastitis is on the increase. An annual loss of Rs 52.9 crores was reported [7] from India. Recently it was reported that total economic loss was INR 5, 210 in native breed of cattle, INR 36, 795 in cross-bred cattle and INR 24, 175 in buffalo during a study period of one year [8]. The economic losses due to SCM estimated in the range of INR 21,677/- to INR 88,340/- for one lactation period depending on the condition of the animal [9]. Most recently, one study from India revealed a total economic loss of Rs. 7824/- in one month per cow [10].

Report from India had showed that an average loss of Rs 325.64 per cow due to reduced milk production (70%), poor quality of milk, milk discarded (7%), cost of veterinary services and medicines (8%) and labour cost, decreased market value of milk yield, high culling rate (14%) and presence of microbial agents upto 72 hours or more after treatment [11. It was estimated that on an average an affected quarter suffers a 30 per cent reduction in productivity and an affected cow losses 15 per cent of its production.

Besides the effect on the economy, mastitis is also responsible for public health hazards [12-17] which greatly affected the export of milk and milk products in the developing international trade [18].

Mastitis control is mainly focused on the use of chemical disinfection, antiseptic teat dipping and antibiotic therapy, but frequent use of antibiotics led to emergence of drug resistance in the mastitis pathogens [19]. This might lead to failure of therapy through the conventional antibiotic treatment. The presence of pathogenic bacteria and antibiotic residues in milk of mastitic cows made it unsuitable for human consumption and disseminated several diseases like tuberculosis, brucellosis, scarlet fever, gastroenteritis, food poisoning, staphylococcal toxaemia etc. Hence, the milk and its products from infected cows posed a serious threat to public's health. Consumers today want milk from healthy animals with no drug resistant bacteria and antibiotic residues in it [20].

Subclinical mastitis being the most common form of mastitis is 15 to 40 times more prevalent than CM and its prevalence varied from herd to herd and place to place. The incidence of SCM was higher in cows than in buffaloes and higher in cross bred than in the native breeds. Clinical mastitis is problem of an individual cow that can be diagnosed easily with the presence of inflammation in the udder and changes in milk where as SCM is a herd problem and difficult to detect as no gross signs of inflammation and changes in milk composition are observed [21, 22]. Subclinical mastitis is most important due to its negative impact on the economy throughout the world. While assessing the economic effects of mastitis at the herd level it was stressed that analysis of the loss included detailed managemental accounts including both fixed cost (annual depreciation of equipments, labour costs, building rental) and cost that vary according to the presence of diseases, veterinary services, feeds, replacement of animals and the types of disease management programmes adopted which influenced the milk quality and an annual reduction in somatic cell count [23].

There are several screening tests for detection of bovine subclinical mastitis, viz. Strip cup test, Bromothymol Blue Tests (BTBT), Chloride Test, Modified Whiteside Test (MWT), Modified California Mastitis Test (MCMT), Modified Aulendorfer Mastitis Probe (MAMP), Electrical Conductivity (EC), Somatic Cell Count (SCC), Bacterial Culture etc.

Somatic cell count is a measure of the white blood cells count in milk. Somatic cells are indicators of both resistance and susceptibility of cows to mastitis and can be used to monitor the level or occurrence of subclinical mastitis in herds or individual cows. Somatic cell count is a useful predictor of intra-mammary infection (IMI), and therefore, an important component of milk in assessment of aspects of quality, hygiene and mastitis control. Yet many producers fail to completely understand the implications of SCC for udder health or how high SCC can affect milk production and quality. Somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (leukocytes) that have entered the mammary gland in response to injury or infection [24]. The milk somatic cells include 75 per cent leucocytes, i.e. neutrophils, macrophages, lymphocytes, erythrocytes, and 25 per cent epithelial cells. Studies identifying cell types in milk have shown that epithelial cells or the cells which produce milk are infrequently found in udder secretions, including the dry gland, at levels ranging from 0 to 7 per cent of the cell population [25]. The epithelial cells of the glands are normally shed and get renewed, however, during infection and injuries the numbers increase. The white blood cells serve as a defense mechanism to fight infection and assist in the repair of damaged tissue. During inflammation (mastitis) the major increase in SCC is due to the influx of neutrophils into the milk to fight infection and estimated at over 90 per cent [26, 27] and the measurement of SCC in milk is known as a somatic cell count. The normal composition of milk somatic cells varies with the type of secretion or lactation cycle (Table 1). Normally, in milk from a healthy mammary gland, the SCC is lower than 1×10^5 cells/ml, while bacterial infection can cause it to increase to above 1×10^6 cells/ml [28].

Various diagnostic tests for detection of subclinical mastitis had been compared by several workers and reported highest sensitivity of SCC [29, 30].

Therefore, keeping the points in view the importance of subclinical mastitis with its negative impact on dairy industry and sensitivity of somatic cell count, the present study was carried out to detect subclinical mastitis by somatic cell count and to study the incidence of bovine subclinical mastitis in organized and unorganized farms.

Materials and Methods

1. Ethical Approval

The research work was duly permitted by the Institutional Animal Ethics Committee. All samples were collected as per standard procedure without harming or laying stress to the animals.

2. Source Of Animals

The study was carried out during the period October, 2008 to June, 2009 on the Instructional Livestock Farm, College of Veterinary Science, Khanapara and on the 17 private dairy units located on the hillock nearby the Khanapara campus of Assam Agricultural University.

A total of 30 dry pregnant cows (irrespective of their parity from private dairy units henceforth referred to as unorganized farms) on the hillock nearby the Khanapara campus of Assam Agricultural University and the Instructional Livestock Farm, College of Veterinary Science Khanapara were included in the present study.

3. Milking Practice

Milking was done manually on all the farms by dry fist method. However, towards the end of the milking, strip milking was also observed to be carried out by the milkers. At the time of milking, teats were massaged with mustard oil.

Milking was mostly done by the owners, in all the farms.

Milking was done twice daily on all the farms. In the morning, milking started in between 4.30 am to 5 am and in the afternoon milking was started from 3 pm onwards.

4. Detection of Subclinical Mastitis

1. Disinfection of Teats: Proper aseptic measures were adopted during collection of milk.

A piece of cotton soaked with 70 per cent alcohol large enough to permit scrubbing the teats was used for thorough cleaning of the teat ends. Individual piece of cotton was used for each teat for complete disinfection of the teats.

2. Collection of Milk Samples: Screw capped flat bottomed plastic milk vials of 20 ml capacity were used for collection of milk samples. These were sterilized by autoclaving at 15 lb pressure for 15 minutes. On the body of the vials respective teats were marked as RF (Right Fore), RH (Right Hind), LF (Left Fore) and LH (Left Hind) along with the cow numbers. About 20 ml of milk was collected after discarding the first three strippings from each quarter and cap was replaced

tightly. The milk samples were brought to the laboratory as soon as possible for further processing.

3. Somatic Cell Count: The somatic cell count was carried out on all the milk samples as per the standard method to detect subclinical mastitis [31].
The stain used in this study was the Modified Newman-Lampert Stain.

Composition of Modified Newman-Lampert Staining reagent was as follows
(For 100ml)

Methylene Blue Powder	1gm
95% Ethyle Alcohol	54ml
Tetrachlorethane	40ml
Glacial Acetic Acid	6ml

Range of somatic cell count: [32]
<5,00,000 cell/ml of milk – normal
>5,00,000 cell/ml of milk – positive for mastitis

Procedure
The milk samples were thoroughly mixed by shaking the vials and 0.01ml of milk was taken with the platinum loop on the redrawn one square centimeter area over a grease free glass microslide which was then uniformly smeared. The smears were dried and stained them with Modified Newman-Lampert Stain. The counting of cells in 10 fields was carried out under oil immersion lens (100X). The total number of cells in the milk was estimated by multiplying total number of cells in 10 fields to the working factor of the microscope and was expressed per ml of milk sample.

$$\text{Working factor} = \frac{\text{Microscopic factor}}{10}$$

Working factor for 10 number of fields = 50,000
Somatic cell count = Total number of cells X 50,000 cells/ml of milk.

Results and Discussion

1. Detection of subclinical mastitis by somatic cell count
In the present study, subclinical mastitis was detected by somatic cell count as it could be considered as a primary indicator of mastitis and milk quality of dairy herd. Besides several workers evaluated the comparative efficacy of different diagnostic tests for detection of SCM and reported SCC to be the most sensitive test [29, 30, 33, 34]

2. Incidence Of Subclinical Mastitis
Incidence of bovine mastitis of SCM on different farms are shown in Table 1 based on the result of somatic cell count (SCC) of the milk samples collected from the cows on the verge of lactation and before drying off.
A total of 30 cows were examined; out of which 28 cows were found positive for SCM (organized farm n=4/6; unorganized farms n=24/24)). The incidence of SCM 66.67 per cent (n=4) on cow level and 54.17 per cent (n=13) quarter level on organized farms. The incidence SCM in 24 cows from various private dairy units was 100 per cent both cow level and quarter level, over all being 93.33 per cent cow-wise and 90.26 per cent quarter-wise.
The present findings were in agreement with the findings of [35-37]. The high incidence may be due to a number of factors viz. poor or no mastitis control programmes, overcrowding of cows unhygienic milking practice and variation in the location of farms. On the organized farm the findings of 66.67 per cent incidence cow-wise and 54.17 per cent quarter-wise is comparable to previous reports [38-40]. The incidence of 100 per cent SCM both cow-wise and quarter-wise may be attributed to the factors discussed above.
The overall incidence of mastitis at cow level in this study (93.33%) was found to be much higher than the results reported from different parts of the world i.e. 19.14 per cent in Egypt, 23.18 per cent in Eastern part of Ethiopia, 66.0 per cent and 51.3 per cent in India [41-44].
Again, the quarter level incidence of mastitis (90.26 %) in this study is much higher than several previous studies i.e. 60.0, 66.0, 73.85 and 47.21 per cent. [43, 45-47].

Table 1: Incidence of subclinical mastitis (SCM).

Groups	No. of cows examined	No of cows + ve for SCM	No of quarters examined	No of quarters + ve for SCM	Incidence rate (%)	
					Cow-wise	Quarter-wise
Organized	6	4	24	13	66.67	54.17
Unorganized	24	24	89*	89	100	100
Total	30	28	113	102	93.33	90.26

*7 quarters were excluded due to deformities in the teats

Conclusion
The present study was carried out to observe the incidence of bovine subclinical mastitis on organized and unorganized farms. Subclinical mastitis was diagnosed with somatic cell count (SCC). The incidence of SCM was 66.67 per cent (cow-wise) and 54.17 per cent (quarter-wise) on the organized farm whereas on the unorganized farm the incidence was 100 per cent both cow-wise and quarter-wise. The overall percentage of incidence cow-wise was 93.33 and quarter-wise 90.26 per cent.
Incidence of SCM was highest among the cows from unorganized farm than the cows from the organized farm

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Competing Interests
The authors declare that they have no competing interests.

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