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Incidence of Microbiological Hazards in Organized and Peri Urban Dairy Farms and Single Animal Holdings in a Tropical Environment

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Abstract: The primary objective of this study was to determine the incidence pattern of pathogenic and spoilage organisms in organized and Peri urban dairy farms and single animal holdings in rural settings under a tropical environment. For bacteriological analysis and comparing the incidence of different microorganisms under various management systems, milk, water and swab samples were collected from all the setups. The sampling points selected were: milk from Machine Milking (MM), milk from Hand Milking (HM), Drinking Water (DW), Washing Water (WW), wet swab of Milker's Hand (MH), Animal's udder and coat (AH), teat cups and container (EH), Floor (FS) and air sampling of the inside and outside milking area. The overall microbial counts were relatively higher in Peri urban dairies and single animal holdings as compared to the organized dairies which had modernized and sophisticated production systems with more hygienic milking practices. The difference in incidence of microbial counts was found to have a direct correlation with hygiene conditions and milking practices prevalent under a particular production set-up. Therefore, dairy farmers need to be provided greater education through extension and outreach programs to adopt clean milk production and management practices. So that they not only get a good price for their milk but also meet the requirements of quality milk especially under tropical milk production conditions.

Key words: Spoilage organisms, pathogenic microbes, milking practices, organized dairies, Peri urban dairies, single animals holding, India

INTRODUCTION

Demand for liquid milk has tremendously increased in recent years. Production of milk should be economical and safe with respect to animal welfare and the environment (Anand *et al*., 2005; Dang and Anand, 2007). Milk is almost sterile from the healthy udder but contamination may occur from milking and post milking operations. The number and types of micro flora in milk serve as an index of the care taken during its production on the farm, nature of unhygienic conditions prevalent at each level and its subsequent handling. To prevent the microbial spoilage of milk and dairy products, it is important to minimize the initial contamination (Anand *et al*., 2006).

To achieve this, the nature and origin of bacterial contamination in raw milk and in particular of those bacteria with a high heat resistance, must be clearly understood. According to Oliver *et al*. (2005), the provision of a safe and nutritious food supply depends on all aspects of food production from farm to fork. India is the largest milk (102.42 million ton, 2007-08) producing country in the world (FAO, 2007). This increase in milk production has been due to better breeding, feeding and management of dairy animals. However, despite the large volume of milk produced, the quality aspects of milk production have not received adequate attention.

This has been reported to be the major obstacle in realizing the large export potential of milk and milk products. In view of this, the vital aspect of clean milk production and herd health including udder health still remains a major concern (Anand *et al*., 2006). There are virtually no reports that compare the microbial hazards that might be present in raw milk under different conditions of production and handling. Dairy farmers are aware of clean milking practices and are encouraged to follow them; there remains much to be done. Some of the current practices may also lead to increased milk somatic cell counts thereby, deteriorating the quality of milk (Mdegela *et al*., 2005; Dang and Anand, 2007). In view of this, preliminary study was conducted to evaluate the extent of microbial

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contamination of raw milk from organized dairies, Peri urban dairies and single animal holdings and to identify and compare possible sources of contamination from these three different sources.

MATERIALS AND METHODS

Samples and sampling area: For bacteriological analysis and comparing, the incidence of different microorganisms under various management systems, milk, water and swab samples were collected from an organized dairy farm, Peri urban dairy farms and single animal holdings located in villages. The experimental design was based on common points of sampling for all three management systems. The sampling points selected were: milk from Machine Milking (MM), milk from Hand Milking (HM), Drinking Water (DW), Washing Water (WW), wet swab of Milker's Hand (MH), Animal's udder and coat (AH), teat cups and container (EH), Floor (FS) and air sampling of the inside and outside milking area. During the 3 months period of experimentation (May and June) six different samples of milk, water and swabs were collected in sterile containers and dilution tubes from the all the three management set ups.

Microbiological characterization: The milk, water and swab samples were collected aseptically and analyzed as follows: Standard Plate Count (SPC) on Plate Count Agar (PCA), Coliform count on Violet Red Brilliant Green Agar (VRBGA) and further screening of *E. coli* on Eosin Methylene Blue (EMB) Agar, Salmonella on Briliant Green Agar (BGA), *Staphylococcus aureus* on Baird Parker Agar (BPA), *Bacillus cereus* on Mannitol egg-yolk Phenol-red polymyxin agar Agar (MYPA) and for hemolytic bacteria on Blood Agar (BA) on selective media. Serial dilutions of all samples in saline were plated and incubated at 37°C for 24 h. Confirmation was based on colony morphology, microscopic examination and biochemical tests using standard procedures (Marshall, 1993).

Statistics: Results are expressed as mean+SD. The mean was constituted by three replicates for each sample. Calculation of mean, Standard Error (SE), standard deviation and coefficient of variation [CV% = (SD/average) ×100] was performed by subjecting data to various statistical analyses as and when needed using SYSTAT 6.0.1., Statistical Software Package (SSP), 1996, SPSS, Inc., Richmond, California, USA, Microsoft R excel 2000 Software Package, Microsoft Corporation, One Microsoft Way Redmond, Washington, USA.

RESULTS AND DISCUSSION

Incidences of different biological hazards at different sampling points in organized dairy are shown in Table 1. The major microbiological hazards observed were high Plate counts, Coliforms and *E. coli* in some of the samples. The counts in these samples were significantly lower than in Peri urban and single animal holdings located in villages. This may be because dairy utensils used on the organized farms were properly cleaned with detergents, the milking area was washed with a sanitizer before and after milking and disinfected thrice a week and the animals were properly washed mainly udder and teats before milking.

Fadlelmoula *et al.* (2007) also reported that large dairy farms were subjected to some modernization in husbandry and milking systems and changes of management practices that resulted in reducing the frequency of pathogenic and spoilage organisms.

A fairly wide variation was observed in the incidence pattern of microorganisms at different sampling points in the periurban set up (Table 2). The plate counts and coliforms were higher ($p>0.5$) with incidence of *E. coli* and *S. aureus* at different sampling points. Similarly, *B. cereus* and other haemolytic bacteria were also isolated from the milking area (Iyer *et al*., 2009). In a similar study by Lingathurai and Vellathurai (2010), the microbiological quality and safety of raw milk from 60 dairy farms in Madurai was analyzed and the mean counts per mL for TPC, psychrotrophs and thermophiles were 12.5×10^6, 5×10^3 and 6.85×10^3, respectively. From the 60 milk samples tested, Coliform bacteria were present in approximately 90%, *E. coli* about 70%, *S. aureus* >61.7%, *E. coli* 0.157: H7 65% and Salmonella 13.3% of the samples. Lack of proper animal and milk handling procedures, improper washing of milking equipment and ignorance of hygienic practices may be the possible reasons for such observations (Gelsomino *et al*., 2002; Giraffa, 2002; Rodrigues *et al*., 2005; Gonzalo *et al*., 2006; Anand *et al*., 2006).

The incidence of microorganisms at different sampling points in single animal holding showed a much wider variation in counts. The plate count and coliforms were higher ($p>0.05$) with incidence of *E. coli* and *B. cereus* in prominence as compared to *S. aureus* at the various sampling points (Table 3).

In a previous study, Faith *et al.* (1996) reported that drinking water was a source of *E. coli* on farms. In another study, Hamilton *et al.* (2006) found that infections with *E. coli* were numerically more common in the organic herds whereas, the incidence of infections with *S. aureus*, *S. uberis* and *S. dysgalactiae* were of the same order as that found on conventional farms. This may be due to the use of organic manure on organized farms. In the study, we observed that factors such as unsanitary conditions prevailing in single animal milking areas, improperly

Table 1: Incidence of different microbial hazards at different sampling Points [b] in an Organized dairy[a]

Samples	SPC	Enterobacter	*E. coli*	Salmonela	*S. aureus*	*B. cereus*	Hemolytic bacteria
Water	3.41±0.2	-	-	-	-	-	-
Drinking	4.47±0.2	-	-	-	-	-	-
Washing							
Machine milk	8.0±0.1	-	-	-	-	-	-
Hand milk	8.1±0.2	-	-	-	-	-	-
Pooled milk	7.41±0.3	-	-	-	-	-	-
Milker hygiene hand swab	8.3±0.5	-	-	-	-	-	-
Equipment hygiene	8.4±0.3	5.73±0.3	2.4±0.3	-	-	-	-
Animal hygiene	7.13±0.9	-		-	-	-	-
Environment sample							
Floor swab	7.86±0.5	-	3.7±0.9	-	-	-	-
Air	2.27[c]±0.5	-					

[a]Experiments: n = 6; [b]Mean values±SD are indicated and the number of samples in triplicate n = 3; [c]Air sampling count = cfu. 5^2 $feet^{-1}$; Swabs = cfu. 900 cm^{2-1}; Count = log cfu mL^{-1}

Table 2: Incidence of different biological hazards at different sampling points[b] in a Peri urban dairy[a]

Samples	SPC	Enterobacter	*E. coli*	Salmonela	*S. aureus*	*B. cereus*	Hemolytic bacteria
Water	4.47±0.8	-	-	-	-	4.17±0.1	-
Drinking	3.97±0.5	3.75±0.2	-	-	1.6±0.6	-	-
Washing							
Machine milk	7.2±0.2	8.47±0.7	8.4±0.3	-	-	6.3±0.2	4.4±0.4
Hand milk	8.47±0.2	-	-	-	-	-	-
Pooled milk	8.46±0.5	7.39±0.9	8.4±0.5	-	-	6.9±0.3	5.3±0.5
Milker hygiene hand swab	5.95±0.4	5.95±0.1	-	-	-	5.3±0.3	6.1±0.1
Equipment hygiene	7.43±0.5	7.43±0.5	7.4±0.8	-	-	5.6±0.5	6.4±0.3
Animal hygiene	7.43±0.2	6.79±0.5	8.0±0.2	-	-	5.0±0.1	4.3±0.5
Environment							
Floor swab	7.51±0.1	6.43±0.3	-	-	-	5.9±0.3	6.0±0.3
Air	2.26c±0.9	--					

[a]Experiments: n = 6; [b]Mean values±SD are indicated and the number of samples in triplicate n = 3; [c]Air sampling count = cfu. 5^2 $feet^{-1}$; Swabs = cfu. 900 cm^{2-1}; Count = log cfu mL^{-1}

Table 3: Incidence of different biological hazards at different sampling points[b] in a single animal dairy[a]

Samples	SPC	Enterobacter	*E. coli*	Salmonela	*S. aureus*	*B. cereus*	Hemolytic bacteria
Water	4.47±0.3	4.47±0.7	2.7±0.9	-	-	-	-
Drinking	4.47±0.8	4.47±0.2	2.7±0.4	-	-	-	-
Washing							
Hand milk	7.76±0.4	-	-	-	-	-	-
Pooled milk	6.47±0.2	2.47±0.2	-	-	-	-	-
Milker hygiene hand swab	7.65±0.4	7.95±0.8	-	-	-	-	-
Equipment hygiene	8.43±0.2	8.43±0.5	6.9±0.6	-	-	-	-
Animal hygiene	8.43±0.5	-	2.4±0.3	-	6.25±0.7	5.0±0.3	-
Environment sample							
Floor swab	7.95±0.8	-	7.9±0.5	-	-	-	-
Air (outside/inside)	2.47c±0.6	1.2[c]±1	-	-	-		

[a]Experiments: n = 6; [b]Mean values±SD are indicated and the number of samples in triplicate n = 3; [c]Air sampling count = cfu. 5^2 $feet^{-1}$; Swabs = cfu. 900 cm^{2-1}; Count = log cfu mL^{-1}

cleaned utensils, lack of proper facilities and poor knowledge of sanitary principles were largely responsible for the exceptionally higher bacterial counts in these samples. The screening of the pathogenic micro organisms in the samples of milk, water and swab of environment, personnel, milking equipment and animal showed the incidence of different organisms at different sampling points in different farms. The ubiquitous nature of aerobic pathogenic bacteria leads to numerous points of potential entry into raw milk (McKinnon and Pettipher, 1983; Sohrab, 2005; Thakar, 2005). Soiling of the udder and teats is considered one of the most important factors in the contamination of raw milk by spores (Waes, 1976). High levels of aerobic spores, ranging from 10 to $>10^5$ cfu g^{-1} were found in silage (Slaghuis *et al*., 1997) and levels of 10^3-10^6 cfu g^{-1} spores was e found in feed concentrate (Vaerewijck *et al*., 2001). When animals consume feed contaminated with spore-forming bacteria, large quantities of spores can be present in their feces which in turn can contaminate the udders and teats. The microbiological quality of milk is affected by general health of animals and milk handling practices subsequent to milk handling. In addition, inadequately cleaned milking equipment, pipelines and farm bulk tanks may be important sources of contamination (Phillips and Griffiths, 1986). Incidence of microbial counts in the organized (Fig. 1), Peri urban (Fig. 2) and single animal dairy holdings located in villages (Fig. 3) and their effect on milk quality have been depicted by the frequency chart of the different bacteria

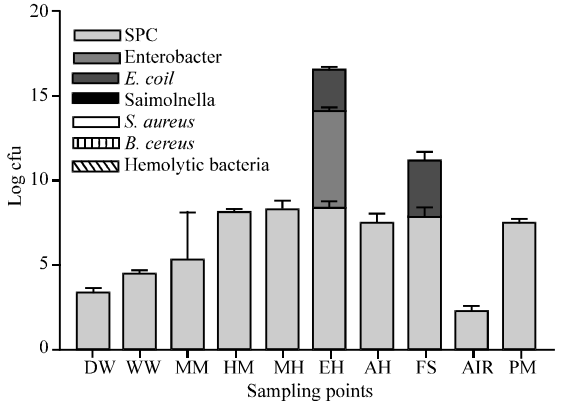

Fig. 1: Frequency distribution of different bacteria at various sampling points viz; DW: Drinking Water; WW: Washing Water; HM: Hand Milk; AH: Animal Hygiene; MH: Milker's Hygiene; EH: Equipment Hygiene; FS: Floor Swab; AIR: Air Sampling; PM: Pooled Milk in Organized dairy farm

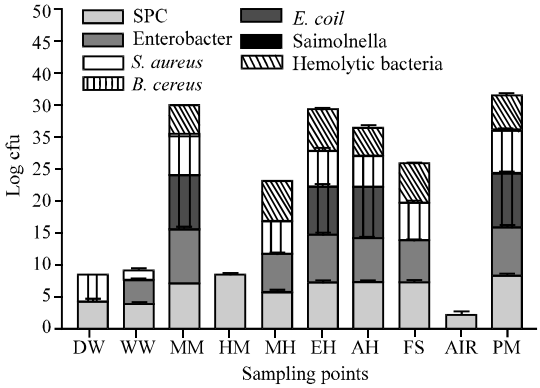

Fig. 2: Frequency distribution of different bacteria at various sampling points viz; DW: Drinking Water; WW: Washing Water; HM: Hand Milk; AH: Animal Hygiene; MH: Milker's Hygiene; EH: Equipment Hygiene; FS: Floor Swab; AIR: Air sampling; PM: Pooled Milk in Peri urban dairy farms

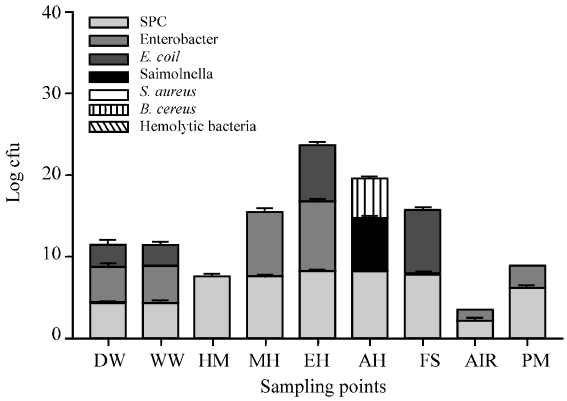

Fig. 3: Frequency distribution of different bacteria at various sampling points viz; DW: Drinking Water; WW: Washing Water; HM: Hand Milk; AH: Animal Hygiene; MH: Milker's Hygiene; EH: Equipment Hygiene; FS: Floor Swab; AIR: Air Sampling; PM: Pooled Milk in single animal holding dairy farm

at the various sampling points. Comparison of the frequency charts under the three management systems reveals a marked difference between the incidence of the plate counts and the counts of different pathogenic bacteria in organized, Peri urban and single animal dairy farms. The operations of the organized dairy being a large scale commercial unit with proper management systems, produced a quality product. In the case of Peri urban dairies, there was lack of a systematic approach of animal and milk handling. The operators of single animal holdings lacked knowledge and awareness of hygiene and handling regarding clean milk production. In addition to this, there was lack of resources due to economically weaker conditions. Bitew *et al.* (2010) showed that mastitis was the common problem of dairy cows in the Bahir Dar town and its environs and the major bacterial isolates *S. aureus* (20.3%), *S. agalactiae* (8.8%), *Corynebacterium bovis* (0.75%), *Bacillus* sp. (0.75%), *Micrococcus* sp. (3.8%), *Actinomyces pyogenes* (2.5%) were contagious pathogens therefore, hygienic milking practice, culling of chronically infected cows and hygienic practices in the environment should be followed. This resulted in a high incidence of microorganisms in single animal holding dairy farms as compared with the organized or Peri urban dairy farms. The frequency analysis of the different bacterial counts at the various sampling points in the three different management systems indicated a correlation between the counts. There was less correlation in counts of different microorganism in the Organized dairy farms while it was found to be highly significant ($p<0.001$) in the case of Peri urban dairy farms and single animal holdings. In the Organized dairy farm, there was a very low incidence of pathogenic bacteria whereas, the other two management systems had considerably high *E. coli* and *B. cereus* count leading to unclean and unsafe milk production.

CONCLUSION

This study further indicated that the quality of raw milk produced under rural conditions was largely unsatisfactory. Therefore, there is an urgent need to implement HACCP based principles for dairy farms, especially Peri urban dairies and single animal holdings as

previously reported by Anand *et al*. (2005). As consumers are becoming more health conscious and general awareness to produce clean milk is increasing, there is a need to educate the dairy farmers on proper management practices and the hygienic production of milk so that the initial bacterial load of milk is reduced with the absence of pathogenic microorganisms. With an increasing scale of farming, there is more room for investments in hygienic practices. The cost of clean milk production should not exceed the benefit of the farmers. Milk payments should be an incentive to improve the hygiene and clean milk production should be financially rewarded.

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