PUBLIC HEALTH SAFETY IN RELATION TO MICROBIOLOGICAL QUALITY OF FRESHLY DRAWN COW'S MILK IN BANGLADESH

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ABSTRACT

The aim of the present study was to determine the microbial quality of freshly drawn cow's milk belonging to the arsenic affected and non-affected areas. In the present work 25 milk sample were collected from five different arsenic affected and non-affected areas. The analysis comprised enumeration of total viable count (TVC), total coliform count (TCC) and total staphylococcal count (TSC) for the determination of sanitary quality. The highest total viable count, total coliform count and total staphylococcus count were log 5.894 ± 0.221, log 2.832 ± 0.129 and log 2.898 ± 0.162 respectively. *Staphylococcus spp, Escherichia coli, Pseudomonas spp* and *Bacillus spp* were isolated from the milk samples. Among them *Escherichia coli* were isolated from the maximum milk samples. It is found that the level of microbial quality in terms of TVC, TCC and TSC were high in arsenic affected areas than arsenic non-affected area. It also found that TVC and TCC were positively correlated with each other. This survey indicates that most of the raw milk samples were not satisfactory in course of public health standard as some pathogenic bacteria were detected from these samples and it is necessary to improve the hygienic practices required for the handling and processing of milk.

Key words: Microbial quality, milk, total viable count, total coliform count, total staphylococcal count

INTRODUCTION

Milk and milk products are ideal foods for all age groups in both rural and urban people all around the world (U.S. Public Health Services, 1965). Milk constituents include water (87.20%), protein (3.50%), fat (3.70%), milk sugar or lactose (4.90%), ash (0.70%) and dry matter (12.80%) (Byron *et al*., 1974). The milk and milk products derived from milk of dairy cows can harbor a variety of microorganisms and can be important sources of food-borne pathogens. The presence of food-borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal (Oliver *et al.*, 2005). Illness caused by the consumption of contaminated foods has a detrimental impact on the economy and the public health worldwide (Mead *et al*., 1999). Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (Tolle, 1980). Beyond this stage of milk production, microbial contamination can generally occur from three main sources (Bramley and McKinnon, 1990) from the udder, from the exterior of the udder and from the surface of milk handling and storage equipment. Bacterial contamination of freshly drawn cow's milk can also originate from different sources: air, milking equipment, feed, soil, feces and grass. Public health problems associated with the consumption of unpasteurized cow milk and raw milk products has been well documented (Cody *et al*., 1999; Baird-Parker and Bruce Tomkin, 2000; De Valk *et al*., 2000; De Buyser *et al*., 2001; Harrington *et al*., 2002). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits, 2008). High coliform counts of a food indicate potential public health hazards related to food. Normally Staphylococcus is found in the nasal cavity and skin of man and animal. It is important to note that the presence of Staphylococcus in food represents human health hazard as well as associated with food-borne diseases of man. This organism also causes intramammary infection and was found to be highly resistant to antibiotics (Kyozaire *et al.*, 2005). The quality and safety of milk and milk products were determined by the presence of indicator bacteria and other bacteria in lesser number.

Water is the most important nutrient in animal feeds and if drinking water was contaminated with arsenic then animal takes a large amount of arsenic per day. In Bangladesh arsenic contaminated rice straw and husk were widely used as feed for cattle (Jahiruddin, 2002). Arsenic uptake and accumulation in plant were greatly affected with increasing water and soil arsenic levels (Marin *et al.*, 1992 and Abedin *et al.*, 2002). It is found that the level of microbial quality in milk are high in arsenic affected areas than arsenic non-affected area. Arsenic enters into human through food chain. Moreover, dairy milk and beef are also reported to contain arsenic (Awal, 2003). In Nazirpur thana of Pirujpur district of Bangladesh, few cattle died of arsenic poisoning by drinking arsenic contaminated water and milk of affected dairy cows contained arsenic (Awal, 2001). Supply of safe and sound food to consumer is a major responsibility for veterinarian. According to European Union Standards for Raw Milk Production, the bacterial count should be less than 10^5cfu/ml. So, microbiological assessments have an important role to play in the dairy industry to protect the public health and can reduce economic losses by the early detection of inadequate processing, packaging or refrigeration. So its needs immediately detection of micro-organisms from cow that have significant contribution in food chain by milk. Under these circumstances, the present research was undertaken with a view to determine the microbial quality of freshly drown cow's milk inhabiting arsenic affected and non-affected areas and taking arsenic contaminated water.

MATERIALS AND METHODS

To conduct this research microbial content of arsenic affected areas were selected based on prior study and the arsenic surveillance report published by Department of Public Health Engineering (DPHE). The areas from where samples were collected are:
  Area 1: Bangladesh Agricultural University dairy farm of Mymensingh district
  Area 2: Sujanagar thana of Pabna district
  Area 3: Faridganj thana of Chandpur district
  Area 4: Kulaura thana of Moulavibazar district
  Area 5: Sadar thana of Bogra district
Area 1 comprises non arsenic affected area and taken as control and remaining 4 were arsenic affected area.

Collection of samples

The apparently healthy lactating cows were selected as the source of sample. All of these cows received drinking water from tube-well and without providing any arsenical feed supplements. From the selected areas milk samples were collected directly from the udder. Prior to collection the udder was washed with fresh water and the udder surface was dried by using a towel. A quantity of 25 ml milk was drawn from each cow and collected aseptically. For milk collection sterile screw capped tubes were used. From each area 5 milk samples were collected and brought from the experimental site as early as possible. Milk samples were transferred in a large wide mouthed thermo flask containing good quality ice thus maintaining the temperature to about 4^0C.

Preparation and examination of milk samples for the determination of sanitary quality

To determine the sanitary quality from each group only five representative milk samples were subjected to microbial quality examination. All steps of preparation of milk samples and determination of their sanitary quality are carried out using sterile sampling equipment and procedure as per recommendation of International Commission for the Microbiological Specification of Foods (1986).

Isolation of microorganisms from milk samples

Serial dilutions of samples were made up to 10^7 in sterile normal saline.

Enumeration of total viable count (TVC)

The total viable bacterial count was carried out by the spread plate technique. This enables the number of living organisms or clumps of organisms (i.e. colony forming units) in a form sample to be counted, subject to the appropriate medium and incubation conditions being used. For the determination of total viable counts, 0.1 ml of each ten folds dilution was transferred and spread on PCA agar using a sterile pipette for each dilution. The diluents samples were spread as quickly as possible on the surfaces of the plate with a sterile glass spreader. The plates were kept in an incubator at 37°C for 24 to 48 hours and then count colonies. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total

viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/gm) of milk sample.

Enumeration of total coliform count (TCC)

For the determination of total coliform count the procedures of sampling, dilution and streaking were similar to those followed in total viable bacterial count. For the determination of total coliform count, o.1 ml of each ten-fold dilution was transferred and spread on MacConkey agar. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. The plates were kept in an incubator at 37^0C for 48 hours and then count colonies. The results were expressed as the number of organism or colony forming units per gram (CFU/gm) of milk sample.

Enumeration of Total Staphylococcal Count (TSC)

For the determination of TSC the procedures of sampling, dilution and streaking were similar to those followed in total viable bacterial count. Only in case of staphylococcal count, Mannitol salt agar was used. The calculation for TSC was similar to that of total viable count.

Morphological characterization by Gram stain

Respective bacteria colonies were characterized microscopically using Gram's stain as per recommendation of (Merchant and Packer, 1967).

Use of statistical calculation for processing of data

Data on the total viable count, coliform count and staphylococcal count/ml of milk sample were analyzed statistically using one way analysis of variance and correlation with the help of the SPSS software to find out the level of significance and determine any significant correlation between the variable factors.

RESULTS AND DISCUSSION

In view of the above perspectives, the present study reflects an approach to the recognition of potential public health hazard microbial content in cow's milk in different arsenic affected and arsenic non affected areas.

The total viable bacterial count is the number of bacteria in a sample that can grow and form countable colonies on Nutrient agar after being held at 37^0c for 24 hours. The data presented in the Table 1 reflects the value of TVC per ml of milk sample collected from different arsenic affected and non-affected areas. According to the Table 1 it is clear that the bacterial count was not uniform and there prevails clear fluctuation. The highest total viable bacterial count (log 5.894 ± 0.221) was found in area 4 that may results from poor sanitary condition of dairy yard, increase contamination of body surface by feces and soil, lack of washing of animal and unsanitary milking utensils and the lowest total viable count (log 5.546 ± 0.221) was found in area 1, it may results from the relatively better hygienic status due to organized dairy farm production system, better quality of water, and short period of transportation and preparation of sample. This finding strongly supports with the findings of Sandu and Man (2006). The present study results strongly supports with the findings of Lee *et al.* (1983) and Muehlherr *et al.* (2003)) who founded that the total bacterial count in milk ranged from 4 x 10^6 to 2.7 x 10^7 per ml. In present study elevated TVC was found than the findings of (Reichart, 1982) who found 0.3 million bacteria/ ml in raw milk from high quality dairy farm. Which may results from poor hygienic condition of dairy farm and its atmosphere, bacterial count in stable air, use of unhygienic water for washing of udder, unsanitary milking utensil. Stable air influences the hygienic quality of fresh milk (Matkovic, 2006).

Results of this study partially supports the findings of Godefay and Molla (2000) who founded 1.1 x 10^5 cfu /ml in bucket milk and 4 x 10^6 cfu /ml in storage container before cooling. Present study show increase TVC than his bucket milk count, it may results from relatively poor hygienic condition or increase time in sample transportation and preparation process. Standard plate count (SPC) increased with increasing the storage period Zeng *et al.* (2007). Present study strongly differ from the findings of Pandey *et al.* (1996), who founded standard plate count of raw milk log 7.66 to log 9.15 per ml in area where sanitary quality of in approximately 45% raw milk was not acceptable. The lowest TVC in present study may results from better sanitary condition of present study area than the previous study. It also indicates the sanitary quality of present study areas are not in unacceptable category. Total bacterial count reflects sanitary quality of food and degree of freshness Dickens *et al.* (1992) and Stern *et al.* (1995) and high counts reflects possible adverse effects on public health Sharma *et al.* (2005).

Table 1. Microorganisms (TVC, TCC and TSC) in milk samples collected from different experimental areas

Areas	Samples	TVC value obtained	TVC value obtained	TVC value obtained	Usual range of TVC, TCC and TSC/ ml		Bacterial counts/ml in log value (Mean±SD		
					Min.	Max.	TVC	TCC	TSC
Area 1	I	5.63	2.50	2.97	5.28,	5.85,	5.546±	2.526±	2.782±
	II	5.85	2.62	2.62	2.36,	2.74,	0.221	0.155	0.154
	III	5.28	2.41	2.76	2.62	2.97			
	IV	5.58	2.74	2.91					
	V	5.39	2.36	2.65					
Area 2	I	5.42	2.59	2.74			5.688±	2.668±	2.792±
	II	5.83	2.74	2.68	5.42,	5.91,	0.194	0.147	0.154
	III	5.59	2.68	2.94	2.47,	2.86,			
	IV	5.69	2.86	2.63	2.63	2.97			
	V	5.91	2.47	2.97					
Area 3	I	5.87	2.77	2.94			5.716±	2.632±	2.796±
	II	5.65	2.64	2.64	5.47,	5.98,	0.206	0.104	0.128
	III	5.47	2.52	2.84	2.52,	2.77,			
	IV	5.98	2.54	2.82	2.64	2.94			
	V	5.61	2.69	2.79					
Area 4	I	5.64	2.64	2.79			5.894±	2.83±	2.898±
	II	6.19	2.96	2.95	5.64,	6.19,	0.221	0.129	0.162
	III	6.03	2.91	2.68	2.64,	2.96,			
	IV	5.74	2.88	3.09	2.68	3.09			
	V	5.87	2.76	2.98					
Area 5	I	5.68	2.66	2.79			5.768±	2.668±	2.876±
	II	5.74	2.67	2.98	5.56,	6.23,	0.266	0.147	0.141
	III	6.23	2.86	3.04	2.47,	2.86,			
	IV	5.63	2.41	2.69	2.69	3.04			
	V	5.56	2.54	2.88					

SD = Standard deviation [*]All the counts are expressed in logarithm

Presence of coliform bacteria, such as *E. coli*, in milk is a common indicator of fecal contamination. *E. coli* was isolated from maximum milk samples. The highest coliform bacterial count (log 2.83 ± 0.129) was found in area 4 indicates poor hygienic status of dairy farm in that area, which may results from irregular bathing of animal, feeding of animal in low land, muddy cow yard, unsanitary milking utensil and contamination of body surface by feces. The lowest total coliform count (log 2.526 ± 0.155) was found in area 1 might be due to relatively better hygienic condition due to regular bathing, good drainage system, washing of floor and use of relatively better milking utensil (Table 1). Higher prevalence of *E. coli* was reported by many authors. In Egypt, Aly and Galal (2002) should the presence of *E. coli* in raw milk and the number reduced in the heat treated one. In India, the raw milk and products were heavily contaminated by *E. coli* Soomro *et al*. (2002). Present study findings strongly supports with the findings of Saharia *et al.* (1997), Saitanu *et al.* (1996) and Zeng *et al.* (2007) who founded average coliform count of milk within 375 to 500 cfu/ml. In present study relatively lower TCC was found than Hempen (2006), Godefay and Molla (2000) and Uddin *et al*. (2011) who found coliform counts above 1×10^4 cfu/ml. It may results from increase use of tube-well water for livestock feeding and management, and washing of udder before milking. This study finding differs from the Alam (1990) who found coliform 130.45 ± 60.12 and 92.30 ± 31.91 cfu/ml in milk of traditionally and hygienically managed dairy farm. In present study higher count was found than previous it may results from higher coliform counts in farm water, presence of fecal materials on skin surface, poor farm atmosphere, contamination of milk by milkers and utensils.

Coagulase positive *Staphylococcus* may cause human disease through the production of toxin. The formation of effective levels of toxin requires a high number of microorganisms (approximately 10^5–10^6 microorganisms

per ml of food) IDA (1994). In this experiment, *Staphylococcus spp were* isolated from the milk samples. In this present study, the highest total *staphylococcus* count (log 2.898 ± 0.162) was found in area 4 which may results from poor hygienic condition of farms of study areas, presence of subclinical mastitis or contamination from respiratory system and body surface. *Staphylococcus spp* were the most common cause of intramammary infection (Kyozaire *et al.* 2005). The lowest *staphylococcus* count (log 2.782 ± 0.154) was found in area 1 (Table 1) due to good hygienic condition of farms of study areas, absence of subclinical mastitis or contamination free from respiratory system and body surface. Present study supports the findings of Muehlherr *et al.* (2003) who reported that 90.4% of raw milk sampled from cows was contaminated by *Staphylococcus sp*.

From the present study, it can be concluded that the microbiological quality of most of the raw milk samples collected from different arsenic affected and non-affected areas were not satisfactory as some pathogenic bacteria such as *Escherichia coli*, *Staphylococcus spp* and *Bacillus spp* were detected from the samples. The presence of these organisms will render milk unfit for human consumption, since sufficient number of these organisms will cause infection and intoxication. Raw milk should be pasteurized, so that milk remains free from pathogenic microbes. Proper refrigeration temperature should be maintained.

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