MICROBIAL QUALITY AND HEALTH RISKS ASSOCIATED WITH CONSUMPTION OF RAW MILK IN THE RURAL AREA OF KARNATAKA

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ABSTRACT

Background: *Risks of milk-borne zoonoses posed by the informal market are amplified by poor handling procedures in the market, the lack of quality standards and the fact that most consumers prefer raw milk over pasteurized milk.*
Objective: *To evaluate microbial quality and health risks associated with consumption of raw milk.*
Methods: *A community based cross sectional study was done among 250 households to assess knowledge regarding health risks associated with consumption of raw milk. Microbial quality assessment was undertaken by evaluating 10% of households (25) by collecting 50mL of raw milk, in rural area of Karnataka. Milk quality was assessed using a combination of tests in order to quantify the occurrence of Brucellosis (milk ring test, BMRT), Coliform bacteria (coliform test, CT). Methylene blue reduction test (MBRT) and Specific gravity was used as an indicator of adulteration.*
Results: *None of the participants had knowledge that milk can transmit the diseases. But one third of them could name some reasons for milk contamination as addition of water, unclean handling, utensil or animals. Among randomly selected 25 samples, 13 milk samples were from own house animals and 12 were from vendors. Mean specific gravity was 1.0250 ± 0.007. Nine samples (36%) showed positive results for CT and MBRT; whereas 2 samples (8%) were positive for BMRT.*
Conclusion: *It can be concluded that raw market milk in the study area is of poor bacteriological quality and hazardous for human consumption. This highlights the need to implement good hygiene practices and effective monitoring from production through the delivery chain to the consumer. Further studies are needed for detection of standard plate count for E.coli, toxins that are produced by E. coli, other pathogenic spore forming bacteria (Bacillus spp& Clostridium spp) and other harmful microorganisms.*

Key words: *Raw milk, Milk borne hazard, Brucella milk ring test, Health risks.*

BACKGROUND

Being a nutritional, balanced foodstuff, milk is a well-known medium that favours the growth of several microorganisms [1]. However, milk is a natural food that has no protection from external contamination and can be contaminated easily, when it is separated from the cow [2].

India keeps over three times the number of cattle as the USA. In addition, 94 million buffaloes contribute to milk production in India.It is estimated that around 15% of the milk produced in India is marketed through formal channels, while the remaining 85% is informally handled [3]. Risks of milk-borne zoonoses posed by the informal market are amplified by poor handling procedures in the market, the lack of quality standards and the fact that most consumers prefer raw milk over pasteurized milk [1].

Although milk is produced mostly in rural areas, its demand is high in urban areas. Milk preservation prior to distribution and sale is a major problem in tropical climate of India [4]. There is limited data existing on raw milk consumption and corresponding risks of milk borne illnesses. It is a highly perishable commodity and poor handling can exert both a public health and economic toll, thus requiring hygienic vigilance throughout the production to consumer chain [5].

It is impossible to produce sterile milk. Sources of contamination include commensals or pathogenic flora of the udder or teat canal, the animal's skin, fecal soiling of the udder, contaminated milking equipment, and water used to clean the milking equipment and milk storage containers [6].

However, there is limited information available on the microbial load contained in raw market milk. This paper reports on an assessment of the microbial quality of raw milk.

OBJECTIVE

To evaluate microbial quality and health risks associated with consumption of raw milk.

MATERIALS & METHODS

A community based cross sectional study was done in one of rural area in Karnataka, South India. A total of 20 % (250 households) of households were selected by systematic random sampling to collect data by predesigned and pretested questionnaire, to assess knowledge regarding health risks associated with consumption of raw milk. Microbial quality assessment was undertaken in 10 % of the selected households, i.e. 25 houses, using informally marketed milk by simple random sampling with collection of 50mL raw milk under aseptic precautions. Written informed consent was obtained. Institutional Ethics Committee clearance was obtained.

The information thus collected was computerized and analyzed by using Statistical Package for Social Science (SPSS 10.0) software program for Windows. Data was expressed in terms of rates, ratios and percentages. Laboratory reports were analyzed separately. Statistical analysis was done using Chi Square test and Fisher exact test. A probability value (p value) of less than 0.05 was considered as significant.

Milk samples were subjected to following tests

Physical test:

- Specific gravity of milk by lactometer

This was measured at the point of collection by the investigator using lactometer. Specific gravity of milk was measured using lactometer of Amber Company to detect the change in density of adulterated milk with water. Milk sample was gently poured into a measuring cylinder (50 mL). The lactometer was left to sink slowly into the milk. Measurement was read and recorded to the last Lactometer degree (°L) (30) just above the surface of the milk. For the calculations, lactometer degrees were used, and for the conversion to density 1.0 was written in front of the true lactometer reading, that is, 1.030 g/mL. The average specific gravities considered were;

- Cow Milk - 1.028 to 1.030
- Buffalo Milk - 1.030 to 1.032
- Goat Milk - 1.028 to 1.030 [5]

Microbiological tests: [7]

- Methylene blue reduction test to test presence of bacteria (adulteration)

  10ml of milk and 1ml of methylene blue solution was added to the 20ml of sterilized test tubes. Then tubes were closed with sterile rubber stopper, slowly tubes were inverted once or twice and then kept in water bath. Test was considered positive when whole column of milk was decolorized within 30minutes.

- Coliform test to detect faecal contamination of milk
  Varying amounts of milk were added to tubes of bile salt lactose medium. For unknown quality of milk the following series was suggested (1 ml of milk in 9 ml of MacConkey broth)
  1.0 ml of a 1 in 10 dilution of milk
  1.0 ml of a 1 in 100 dilution of milk
  1.0 ml of a 1 in 1,000 dilution of milk
  1.0 ml of a 1 in 10,000 dilution of milk

The smallest amount that yields acid and gas was ascertained. Under the Scottish regulations, for standard milk, these tubes were inoculated each with 1 ml of 1 in 1000 dilution. The milk sample was taken to have passed the test if acid and gas were absent from two of the three tubes. Samples were considered positive for coli form test, if

showed more than 10^5 bacteria per ml of milk.

- Brucella milk ring test

  The milk was mixed thoroughly and poured into a test tube sufficient to give a column of milk about 1 in high. One drop of stained antigen was added and mixed thoroughly by shaking. Frothing was avoided which could interfere with reading of the test. It was incubated at 37^0 C water bath for about 40 to 50 minutes, which was sufficient time of the cream to rise.

  In milk containing brucellaagglutins the bacteria were agglutinated and raised with the cream forming a blue cream line, having the skin milk white in samples, in which there were no agglutinins. There was a white cream line and the rest of the milk remained blue.

  The results were interpreted as positive (+++). Cream layer formed a deep blue ring on top of a completely white column of milk. This indicated a high concentration of agglutinins. The white cream layer and milk column blue were considered as negative.

RESULTS

This one year community based cross-sectional study surveyed 250 households consuming informally marketed milk about knowledge of health risks associated with raw milk and raw milk samples were collected from 10% of households (25 houses) for testing microbial quality.

Most (69%) of the households were from joint family and majority (77.2%) of the responsible household member for handling milk were illiterates, with mean age ± SD being 44.30 ± 14.60 years and median 45 years. According to Modified B.G.Prasad's classification, majority (90.4%) belong to Class III and Class IV socio economic status [8].

None of the responsible household member (participant) had knowledge that milk can transmit disease, but one third of them could name some reasons for milk contamination as addition of water, unclean handling of milk, utensils or animals. The knowledge regarding milk contamination with that of practice of addition of water to milk and covering milk utensils with lid was poor in the study participants, which showed statistically significant results (p=0.000). (Table 1)

Table 1: Association between knowledge and practice (Milk contamination and washing hands and utensils before milk collection)

Knowledge and practice		Milk contamination			
		Rural (n=250)			
		Yes		No	
		No	%	No	%
Washing hands before collection	Yes	102	40.8	16	6.4
	No	117	46.8	15	6
	Total	**219**	**87.6**	**31**	**12.4**
		x^2=0.877		p=0.349	
Washing utensil before collection	Yes	103	41.2	15	6
	No	117	46.8	15	6
	Total	**220**	**88**	**30**	**12**
		x^2=5.572		p=0.018	
Addition of water to milk after collection	Yes	78	31.2	138	55.2
	No	82	32.8	50	20
	Total	**160**	**64**	**188**	**75.2**
		x^2=22.316		p=0.000	
Cover milk utensil with lid after collection	Yes	99	39.6	19	7.6
	No	111	44.4	21	8.4
	Total	**210**	**84**	**40**	**16**
		x^2=36.570		p=0.000	

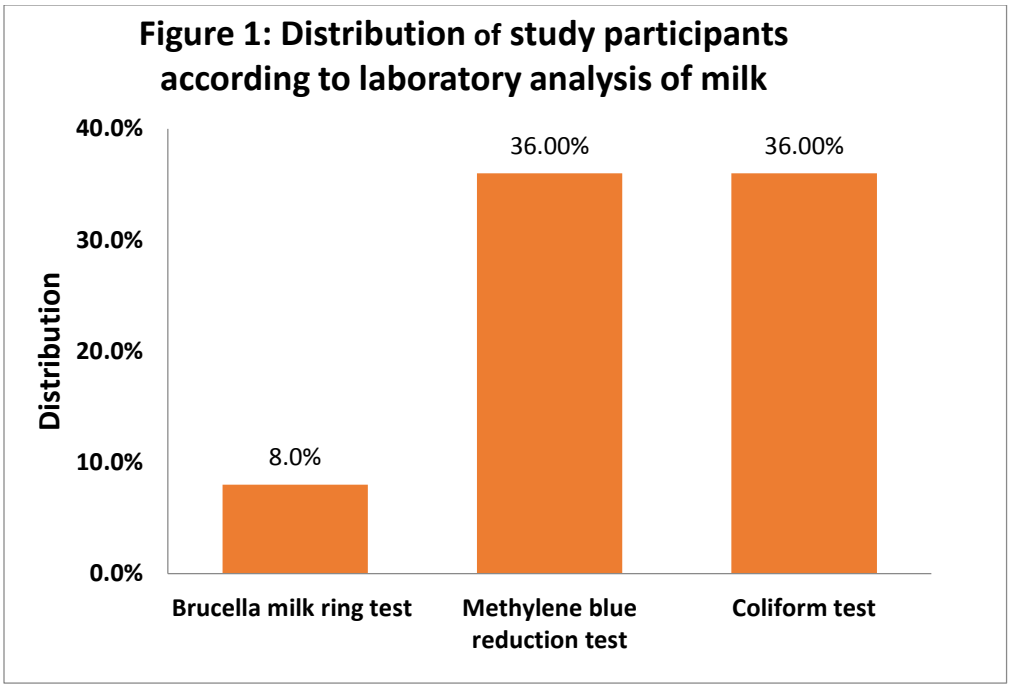
None of the households possessed refrigerator facility to store milk, and almost 80% of them used to consume milk next day of collection. 70% of the participants told that they used to add water to milk before consumption. Among them, 43.6% added 50mL of water, and remaining added more than 50mL. Most (62.4%) of them used to consume raw milk and main reason stated is raw milk is being very healthy and convenient to consume. It was found that there was no relation between the literacy levels and consumption of raw milk or reasons to consume raw milk ($p > 0.05$).

118 out of 250 households (47.2%) had their own milk producing animals, among them three fourth possessed buffalos as main milk source animal. The practices of washing animals, udder, and utensils are shown in Table 2. Majority (95.7%) of households had no facility for separate shed for the milk producing animals.

Table 2: Distribution of study participants according to their practices of washing animals and cleaning the udder, washing utensil before milking.

Practices		Frequency	Rural (n=250)	
			Number	**Percent**
Washing animals	Yes	Daily	80	32
		Once in 2 days	16	6.4
		Once in 3 days	2	0.8
		> 3 days	20	8
		Total	**118**	**47.2**
	No		132	52.8
	Total		**250**	**100**
Cleaning udder	**Not applicable**		132	52.8
	Yes		103	41.2
	No		15	6
	Total		**250**	**100**
Washing utensils before milking	**Not applicable**		132	52.8
	Yes		118	47.2
	No		0	0
	Total		**250**	**100**

A total of 25 raw milk samples were collected, out of which 13 were from own house animals and 12 selected from milk vendors randomly. The mean specific gravity was 1.025 ±0.007. Fifty milk samples showed normal range i.e. 1.028 to 1.030 of specific gravity, no adulteration with water. Two out of 25 (8%) milk samples showed positive result for Brucella milk ring test (Figure 1). Nine out of 25 (36%) milk samples showed contamination with E.coli i.e. more than 10^5 bacteria per mL of milk, detected as positive test results for Methylene blue reduction test and E.coli test.



Graph1: Distribution of study participants according to laboratory analysis of animal milk samples collected

DISCUSSION

This study has shown that the responsible household member (participant) among 250 households surveyed, had nil knowledge about transmission of any disease through milk, but one third of them could name some reasons for milk contamination as addition of water, unclean handling of milk, utensils or animals. Various studies showed that 23% to 68.5% of the study participants were aware of diseases transmitted from milk [9, 10].

The results of the present study revealed that, 69% of household belonged to joint family and 77.2% responsible milk handling member were illiterate with mean age ± SD being 44.30 ± 14.60 years. 90.4% of the houses had belonged to class III and class IV groups of socioeconomic status.

In the present study, none of the households possessed refrigerator facility to store milk, and almost 80% of them used to consume milk next day of collection. This may cause growth of bacteria like E.coli, because of improper maintenance of temperature. Various studies reported, 23% to 61% respondent's stored milk in refrigerator [11, 12, 13].

Present study reported that, 70% of the participants used to add water to milk before consumption; shows adulteration is more common among rural people. Among them, 43.6% added 50mL of water, and remaining added more than 50mL. Various studies reported that 20% to 83% of milk samples were adulterated with water [5,11,12,13 & 14]. Among the participants, it was also found that as knowledge regarding milk contamination was very poor, their practices towards milk hygiene like, washing hands, utensils, addition of water showed poor results.

Most (62.4%) of them used to consume raw milk and main reason stated is raw milk is being very healthy and convenient to consume. Similar study done in USA in 2006, 42.3% of dairy producers surveyed reported the taste and convenience as primary reason [9].

Present study found that, there was no statistical association between the literacy level and consumption of raw milk. It shows that, may be raw milk consumption was blind practice for rural people.

Our study revealed about the animal sources of milk from the households

selected, 47.2% had their own milk producing animals and of which, one third had buffaloes as main source of milk. And majority (95.7%) of households had no facility for separate shed to keep the milk producing animals. A study done in Ghana and Tanzania in 2003 showed, 68% and 14% of the households had milk sourced from own animals [12].

Present study showed, 32% of study participants reported to wash animals daily once, whereas 8% of participants washed animals at more than 3 days interval period. It may be because of difficulties in procuring sufficient of water.

In the present study, 41.2% of the participants reported to clean udder before milking every time, whereas all the participants (47.2%) washed utensils / washed hands before milking. A study in India in 1962 reported, rarely animals were washed before milking and utensils were not washed properly.[15] Another study in India in 2006 revealed, 92 out of 100 farmers used to wash their hands before and after milking each cow [13].

All the participants having milk producing animals (47.2%) sought treatment for illness of animals from the veterinary doctor in the year preceding the survey.

Laboratory analysis of milk samples:

The microbiological quality and safety of milk is of utmost importance in the field of public health. Present study showed mean specific gravity for collected milk samples was 1.025 ± 0.007. Various studies showed, specific gravity of milk samples ranged from 1.027 to 1.030 [5, 12]. Overall 50% of the milk samples had specific gravity below 1.028, indicative of adulteration by adding water (either intentional or accidentally), which was also likely to be of poor bacteriological quality. The practice of adulteration of milk by adding water is more common during the dry season when milk is scarce and market demand is high. Verification of this observation could not be ascertained in the present study because sampling was carried out only once during all season.

In the present study, 2 (8%) milk samples were positive for brucella milk ring test and 9 (36%) were positive for methylene blue reduction test as well as coliform test i.e. more than 10^5 bacteria per mL of milk. The higher number of microflora in raw milk might be due to contamination from the animal and unhygienic milking procedures or equipment leading to entry of pathogens from dairy utensils and milk contact surfaces. The cleaning of milking utensils with detergents and good quality water helps remove the milk remains including microorganisms. Various studies reported, 37% to 56% samples were positive for Brucella milk ring test [5,12].

Various studies reported 20% to 100% samples were positive for E coli bacteria by coliform test [16, 17, 18, 19 & 20]. A study done in north-east India in 2006 showed, 70% of milk samples were positive for methylene blue reduction test [21].

CONCLUSION

This study provides important information regarding the presence of pathogenic microorganisms in the vendor's milk. It is a serious public health concern, especially for the vulnerable population such as infants, growing children, pregnant women, and elderly people.

The current study clearly underscores the attention required to improve the pre and post pasteurization processes including hygienic conditions at milk processing units. Efforts should also be made to maintain the cold chain of milk from suppliers to end users. Milk vendors should be educated adequately to implement the hygienic mulching practices. It is recommended that simple household steps like good personal hygiene, use of clean utensils, practice of boiling the milk before consumption and refrigeration for storage should be undertaken to improve the microbiological as well as keeping quality of the vendor's milk.

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