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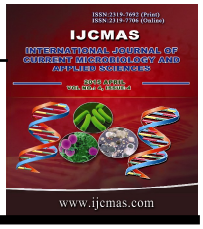

Original Research Article

Bacteriological Quality of Raw Buffalo milk from different villages in Bardoli, Gujarat, India

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A B S T R A C T

The present work was undertaken to study the influence of different factors such as feeding, housing strategies and milking practices on microbial quality of buffalo raw milk. A total of 30 raw buffalo milk samples were collected from in and around Bardoli. Based on total viable count of bacteria, 16 raw milk samples were found to be in good category followed by 8 samples were in fair category and 6 were in poor category as per Bureau of Indian standards (BIS). The samples collected from Sarbhon and Soyani villages were found to be in good category. Enumeration of coliforms revealed thatfrom 13 positive presumptive samples, 5 were positive for typical coliforms, 6 were positive for atypical coliformsand 2 were negative and higher total coliform count was found in 8 samples. Isolation and characterization of bacteria from raw milk samples showed that the dominant micro flora belongs to genus *Lactobacilli* species followed by *Staphylococcus aureus*, *Salmonella* species, *Enterobacter aerogenes* and *Escherichia coli*. Among 30 samples, 7 samples were positive for the presence of bacterial endotoxin.The present investigations indicated that unhygienic and poor sanitary practices are practiced by the village farmers for the production and handling of raw milk.

Keywords

Raw buffalo milk, Bacteriological quality, TVCB, Coliform count, Endotoxin

Introduction

In India, milk is produced mostly in non-standardized way and is usually supplied to the consumers of the urban and rural areas by milkmen (Saxena et al., 2013). For fulfilling consumer's demand production of quality milk is necessary. Quality milk means, the milk is free from pathogenic bacteria, harmful toxic substances, sediment and extraneous substances and should contain good flavour, normal composition and (Khan et al., 2008).

Microbial contamination can generally occur fromsources viz. within the udder, exterior of the udder, storage equipment, from body of the cows, litter, floor, flies, insects, rodents, water supply, milker, milk utensils, atmosphere etc. (Solomon et al., 2013; Khan et al., 2008; Rai et al., 2013). Generally in villages Buffalos are milked by hand and it might be possible that the milkers' hands and clothes are not clean and he or she is not healthy (Barbuddhe et al., 2008).

It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk. Rinsing water for milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk, which could lead to the deterioration of the milk (Torkaret al., 2008; Oliveira et al., 2012).

Milk being a major constituent of human diet, can serve as a good medium for the growth of many microorganisms especially bacterial pathogens, therefore its quality control is considered essential to the health and welfare of a community and the threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable (Edward et al., 2013). The presence of these pathogenic bacteria in milk has emerged as a major public health concern, especially for those individuals who still drink raw milk (Rai et al., 2013; Edward et al., 2013). The microbiological analysis of the milk provides useful information that reflects the conditions under which this was obtained, processed and stored (Oliveira et al., 2012).

In dairy cows, bacterial invasion and growth in the udder is most often the cause of mammary inflammation which arise from the release of endotoxin from the infecting microbes (Perkins et al., 2002).The effects of mastitis on milk yield and milk quality in cows have also been observed for buffalo milk. Mammary gland inflammation affects milk yield and quality and can lead to great economic losses for dairy farmers (Tripaldi et al., 2010). It has been reported thatfood poisoning in humans are caused by endotoxins or Lipopolysaccharide (LPS) produced by Gram-negative bacteria (Abdul Rahman et al., 2013).

Therefore the present work was carried out to analyse physical examination and assess bacteriological quality of raw buffalo milk collected from different villages of Bardoli taluka, Gujarat, India. There are so many standards regarding the range of total viable count and coliform bacteria count in different countries and regions and though the study was done in India, the standard that considered for this study is Indian standard (Chanda et al., 2008).

Materials and Methods

Sample Collection

The study was conducted from December 2013 to April 2014 to assess the bacteriological quality in raw buffalo milk in Bardoli taluka, Gujarat, India. The populated 5 villages (Ancheli, Rayam, Sarbhon, Soyani and Vankaner) of Bardoli taluka have been selected for this study. A total of 30 raw milk samples were collected from 6 different cattle barns in each village. The samples were collected in the early morning time in a sterilized screw cap tubes. The collected samples were kept in ice box and transported to the laboratory for further examinations. All the possible precautions were taken to avoid external contamination at the time of collection of samples and during transportation and storage. Samples were stored in refrigerator at 4°C before being analysed.

Physical examination of raw milk Samples

All milk samples were tested for pH, specific gravityand organoleptic by visually, nasally and lingually to determine colour, flavour and texture by taking 10 different individuals and mean values were taken**.**

Total viable count of bacteria from raw buffalo milk

Thirty raw milk samples were serially diluted up to 10^{-7} using normal saline. After that 0.1ml of each dilution was inoculated in to SCDA (Soyabean casein digest agar) using sterile pipette and spreaded by using a sterile glass spreader for each sample. The plates were then incubated at 37ºC for 24-48 hrs. After incubation, for accuracy of results, the plates which contain colonies between 30-300 were counted. Final Colony Forming Units/ml (CFUs/ml) were calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution and the reciprocal of the volume plated.

Test for coliforms

To detect coliforms, a three stage procedure was carried out in systematic order according to the results of each step(Cappuccino et al., 2013; Cara et al., 2002).

1. Presumptive test: Raw milk samples were inoculated as [5 Mac Conkey's lactose bile broth (MLBB) double strength tubes with 10 ml sample, 1 MLBB single strength tube with 1.0 ml sample, 1 MLBB single strength tube with 0.1 ml sample] along with control tube. All the tubes were incubated at 37ºC for 24-48 hours. After 48 hours presence or absence of acid and gas was recorded at each examination.

2. Confirmed test: A loopful of suspension from positive tube was streaked on (Eosin Methylene Blue (EMB) agar plate and incubated at 37ºC for 24 hrs. After incubation results were recorded and interpreted as typical, atypical and negative colonies.

3. Completed test: Half of the well isolated typical/atypical colony from EMB agar plate was transferred to Brilliant Green Lactose Bile Broth (BGLB) and remaining half of the same colony was streaked over the surface of nutrient agar slant. Slant and broth were incubated at 37ºC for 24hrs. After incubation lactose broth was checked for the presence of acid and gas and Gram staining of the growth from the agar slant. Results were recorded and interpreted.

Enumeration of coliforms from raw milk samples

For the enumeration of coliforms MacConkey's lactose bile broth (MLBB) was used. Briefly, one tube consisting of MLBB 50 ml (2X) with 50 ml raw milk, followed by 5 tubes of MLBB 10ml (2X) with 10ml of sample , 5 tubes of MLBB 5ml (X) with 1ml of sample , and 5 tubes of MLBB 5ml (X) with 0.1ml of each sample respectively along with one control tube. All the tubes were incubated at 37ºC for 24 hrs. After incubation period the tubes were examined for acid and gas. If the tube showed no acid and gas then all the tubes were reincubated for another 24hrs. After incubationperiod by taking combination of positive tube in each set, the results were interpreted by using McCrady's Most Probable Number (MPN) table. (Jamie et al., 1996).

Isolation and characterization of bacteria from raw milk sample

The isolation of bacteria from the raw milk samples were carried out using selective

media such as Eosin Methylene Blue agar (*Escherichia coli*), Mannitol Salt Agar (*Staphylococcus aureus*), *Salmonella Shigella* agar (*Salmonella*spp), Tomato juice agar (aerobic lactobacilli) and deMan, Rogosa and Sharpe agar (anaerobic lactobacilli) along with controls. The plates were incubated at ambient temperature for 24-72 hrs. After incubation period the obtained bacterial colonies were examined macroscopically for colonial characteristics and microscopically by Gram's staining. Acid fast staining were performed for microscopic observation of *Mycobacterium*speices.

Detection of endotoxin in raw milk

The raw milk samples were also tested for the presence of endotoxin by using Limulus amoebocyte Lysate Gel clot assay along with positive and negative control (Mulayet al., 2011; USP, 2011). All the tubes were incubated at 37ºC for 60 minutes and results were observed.

Results and Discussion

The data of physical examination of collected milk samples has been shown in Table 1which includes pH, Specific gravity & Mean values of organoleptic test. As per BIS standard(Barbuddheet al., 2008), the total bacterial count indicated that out of 30 samples, 16 were lying in Good category, 8 were found in fair category and 6 were found in poorcategory.

The mean bacterial count for good, fair andpoor milk samples were found to be 8.9×10^5, 39.2×10^5, and52.6×10^5CFU/ml respectively (Table 2). The total bacterial counts ranged from 8, 90,000 to 52, 60,000. The variation in Total viable count of bacteria of the milk is indicative of hygienic and sanitary practice of milking followed by farmers. The results of this experiment is correlated with the findings of Khan et al., (2008). The researchers reported that the total bacterial counts ranged from 8, 33, 333 to 13,40,500 per ml of milk depending on milking techniques, housing environment, and cleanliness that was maintained during milking.

The coliform test for the 30 raw milk samples showed,13 samples were positive for presumptive test (Table 3). From 13 positive presumptive samples, 5 were positive for typical colonies (small nucleated with or without greenish metallic sheen), 6 were positive for atypical colonies (large, opaque, pink, non-nucleated and mucoid which tend to merge with each other) and 2 were positive for negative colonies (all other types of colonies developing on the plate).

Typical coliforms are commensal of the intestine and derived almost exclusively from this habitat and atypical coliforms are also commensal of intestine but beside that they grow in soil and on vegetation, so they can be present in milk which is not fecaly contaminated**(**Cara et al., 2002).The acceptable limits of Coliform counts in milk should be less than 100 cell/ml according to BIS standard (Baskaran et al., 2002; Barbuddhe et al., 2008). Higher total coliform count was found in 8 samples.So according to (Saxena et al., 2013)higher count of coliforms may be due to Poor hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment can lead to higher coliform counts in raw milk.

Bacterial isolation and characterization of raw milk samples showed high prevalence of *Lactobacillus* species followed *Staphylococcusaureus* and presence of *Salmonella* species in few samples (Table 5).

Table.1 Physical examination of raw milk samples

Village name	Sample name	Colour	pH	Flavour	Texture	Specific Gravity
	R_1	Creamy white	6.6	Sweet aroma	Creamy	1.028
	R_2	White	6.5	Normal	Creamy	1.029
Ancheli	R_3	Opaque White	6.7	Normal	Creamy	1.028
	R_4	White	6.5	Flat	Normal	1.031
	R_5	White	6.6	Normal	Creamy	1.032
	R_6	White	6.6	Normal	Creamy	1.030
	R_7	Creamy White	6.7	Sweet aroma	Creamy	1.033
	R_8	White	6.7	Normal	Thin	1.029
Rayam	R_9	Off White	6.5	Flat	Creamy	1.031
	R_{10}	White	6.6	Normal	Creamy	1.031
	R_{11}	Creamy White	6.5	Normal	Creamy	1.032
	R_{12}	Off White	6.7	Normal	Normal	1.030
	R_{13}	White	6.6	Normal	Creamy	1.030
	R_{14}	White	6.5	Sweet aroma	Watery	1.028
Sarbhon	R_{15}	White	6.5	Normal	Creamy	1.030
	R_{16}	White	6.5	Normal	Creamy	1.029
	R_{17}	White	6.6	Normal	Creamy	1.032
	R_{18}	White	6.6	Normal	Creamy	1.030
	R_{19}	Off White	6.7	Normal	Thin	1.031
	R_{20}	White	6.6	Normal	Creamy	1.029
Soyani	R_{21}	White	6.6	Normal	Creamy	1.033
	R_{22}	White	6.7	Normal	Normal	1.031
	R_{23}	Creamy White	6.5	Normal	Creamy	1.032
	R_{24}	White	6.7	Normal	Creamy	1.029
	R_{25}	White	6.5	Sweet aroma	Creamy	1.029
	R_{26}	White	6.5	Normal	Creamy	1.033
Vankaner	R_{27}	Creamy White	6.5	Normal	Creamy	1.028
	R_{28}	White	6.7	Normal	Watery	1.030
	R_{29}	Opaque White	6.6	Flat	Creamy	1.032
	R_{30}	White	6.5	Normal	Creamy	1.028

Table.2 Total viable count of bacteria from raw buffalo milk

Total Samples	**No. of samples**	**Samples**	**Mean Bacterial Count (CFU/ml)**	**Quality grade of Milk**
30	16	R_3, R_5, R_6, R_9, R_{10}, R_{14}, R_{15} - R_{17}, R_{19} -R_{21}, R_{23}, R_{24}, R_{26}, R_{30}	8.9×10^5	Good
	8	R_4, R_7, R_8, R_{12}, R_{13}, R_{22}, R_{27} R_{28},	39.2×10^5	Fair
	6	R_1, R_2, R_{11}, R_{18}, R_{25}, R_{29}	52.6×10^5	Poor

CFU= Colony forming unit

Table.3 Test for coliforms

Village name	Sample	Test for Coliform			
		Presumptive Test	Confirmed Test		
			Typical	Atypical	Negative
Ancheli	R_1	+	+	-	-
	R_2	+	+	-	-
	R_3	-	NA	NA	NA
	R_4	+	-	+	-
	R_5	-	NA	NA	NA
	R_6	-	NA	NA	NA
Rayam	R_7	+	-	+	-
	R_8	-	NA	NA	NA
	R_9	-	NA	NA	NA
	R_{10}	-	NA	NA	NA
	R_{11}	+	+	-	-
	R_{12}	+	-	+	-
Sarbhon	R_{13}	+	-	-	+
	R_{14}	-	NA	NA	NA
	R_{15}	-	NA	NA	NA
	R_{16}	-	NA	NA	NA
	R_{17}	-	NA	NA	NA
	R_{18}	+	+	-	-
Soyani	R_{19}	-	NA	NA	NA
	R_{20}	-	NA	NA	NA
	R_{21}	-	NA	NA	NA
	R_{22}	+	-	+	-
	R_{23}	-	NA	NA	NA
	R_{24}	-	NA	NA	NA
Vankaner	R_{25}	+	+	-	-
	R_{26}	-	NA	NA	NA
	R_{27}	+	-	+	-
	R_{28}	+	-	-	+
	R_{29}	+	-	+	-
	R_{30}	-	NA	NA	NA

+ = Positive, - = Negative, NA= Not Applicable

Table.4 Enumeration of coliforms

Location (Bardoli taluka)	Sample	Combination of Positives	MPN index per 100ml	95% Confidence limit	
				Upper	**Lower**
	R_1	1-1-1	6	2.0	18
	R_2	2-0-0	4	1.0	17
Ancheli	R_3	1-1-0	4	1.0	15
	R_4	2-3-0	12	5.0	29
	R_5	1-1-0	4	1.0	15
	R_6	0-1-0	2	1.0	10
	R_7	3-2-1	17	7.0	40
	R_8	0-0-1	2.0	1.0	10
Rayam	R_9	2-0-0	4.0	1.0	17
	R_{10}	0-0-0	<2	-	-
	R_{11}	4-1-2	26	12.0	63
	R_{12}	1-2-0	6	2.0	18
	R_{13}	2-0-1	7	2.0	20
	R_{14}	2-0-0	4	1.0	17
Sarbhon	R_{15}	1-0-0	2	1.0	11
	R_{16}	1-0-1	4.0	1.0	15
	R_{17}	0-0-0	<2	-	-
	R_{18}	4-1-1	21	9.0	55
	R_{19}	1-1-0	4	1.0	15
	R_{20}	2-0-0	4	1.0	17
	R_{21}	0-0-0	<2	-	-
Soyani	R_{22}	2-3-0	12	5.0	29
	R_{23}	1-0-0	2.0	1.0	11
	R_{24}	1-1-0	4.0	1.0	15
	R_{25}	5-1-1	50	20	150
	R_{26}	0-1-0	2.0	1.0	10
Vankaner	R_{27}	4-1-1	21	9.0	55
	R_{28}	3-0-1	11	4.0	29
	R_{29}	1-2-0	6	2.0	18
	R_{30}	2-0-0	4	1.0	17

MPN = Most Probable Number

Table.5 Isolation and Characterization of Bacteria from raw milk sample

Village name	sample	Name of Organism					
		E. coli	*Enterobacter aerogenes*	*Salmonella species*	*S. aureus*	*Lactobacilli*	*Mycobacterium species*
Ancheli	R_1	+	-	-	-	+	-
	R_2	+	-	+	+	+	-
	R_3	-	-	-	+	-	-
	R_4	-	+	-	-	+	-
	R_5	-	-	-	-	+	-
	R_6	-	-	-	+	-	-
	R_7	-	+	-	+	+	-
	R_8	-	-	-	+	-	-
Rayam	R_9	-	-	-	-	+	-
	R_{10}	-	-	-	-	+	-
	R_{11}	+	-	+	+	+	-
	R_{12}	-	+	-	-	-	-
	R_{13}	-	-	-	+	-	-
	R_{14}	-	-	-	+	+	-
Sarbhon	R_{15}	-	-	-	-	+	-
	R_{16}	-	-	-	-	+	-
	R_{17}	-	-	-	+	-	-
	R_{18}	+	-	+	+	+	-
	R_{19}	-	-	-	-	-	-
	R_{20}	-	-	-	-	+	-
Soyani	R_{21}	-	-	-	+	+	-
	R_{22}	-	+	+	+	-	-
	R_{23}	-	-	-	-	+	-
	R_{24}	-	-	-	-	+	-
	R_{25}	+	-	+	+	+	-
	R_{26}	-	-	-	-	+	-
Vankaner	R_{27}	-	+	+	-	+	-
	R_{28}	-	-	-	-	-	-
	R_{29}	-	+	+	+	+	-
	R_{30}	-	-	-	-	+	-

+ = Positive, - = Negative

Table.6 Detection of Endotoxin in Raw milk (Gel clot method)(USP, 2011)

Village name	Sample	Results		
		NWC	PPC	Test
Ancheli	R_1	+	+	-
	R_2	+	+	-
	R_3	+	+	+
	R_4	+	+	-
	R_5	+	+	+
	R_6	+	+	-
	R_7	+	+	-
Rayam	R_8	+	+	-
	R_9	+	+	-
	R_{10}	+	+	-
	R_{11}	+	+	+
	R_{12}	+	+	-
Sarbhon	R_{13}	+	+	-
	R_{14}	+	+	-
	R_{15}	+	+	-
	R_{16}	+	+	-
	R_{17}	+	+	-
	R_{18}	+	+	-
Soyani	R_{19}	+	+	+
	R_{20}	+	+	-
	R_{21}	+	+	-
	R_{22}	+	+	+
	R_{23}	+	+	-
	R_{24}	+	+	-
Vankaner	R_{25}	+	+	-
	R_{26}	+	+	-
	R_{27}	+	+	+
	R_{28}	+	+	-
	R_{29}	+	+	+
	R_{30}	+	+	-

+ = Positive, - = Negative

NWC= Negative Water Control, PPC= Positive Product control

The presence of *S. aureus* and *Salmonella* species indicates that poor handling practices, sewage contamination or the buffalo might be infected with mastitis problem (Yuen et al., 2012). The presence of these pathogens may pose a threat to consumers. However, all the milk samples showed negative for the presence of *Mycobacterium* spp.

Out of 30 samples, 7 samples (R_3, R_5, R_{11}, R_{19}, R_{22}, R_{27}, and R_{29}) were positive for the presence of endotoxin (Table 6). Determination of endotoxin concentration gives significance about the presence of metabolites or end products produced by specific bacteria. It is an indication of endotoxin induced mastitis in buffalo. LAL test has also been used to assess food

spoilage. Here this was done to assess milk spoilage and water quality used to wash utensils during milking. According to Bishop et al., 1986) compared to conventional method of bacterial count, this method has more significant in milk sanitary practice and shelf life.In India, the production of milk is always confined to rural area while demand is mostly in urban areas and there is always seen the inadequate practice of milking and handling, poor washing practice and use of unclean equipments. As, milk is highly perishable in nature, there is a need for good hygienic practices. The current investigations showed the high TVBC, presence of typical and atypical coliforms and several pathogenic bacteria which was attributed to unsatisfactory practice and poses a safety issue to consumer. So, the concern authorities and co-operative organization of producer's groups in respective villages should monitor and facilitate improved practices of milking, handling and transportation.

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References

Abdul Rahman S.,MukhopadhyayS. C., Pak-Lam Y.,Goicoechea J., Matias I. R., Chinthaka P., Kosel G. J., 2013. Detection of bacterial endotoxin in food: New planar inter digital sensors based approach. J. Food Eng. 114(3), 346-360.

Barbuddhe S B, Swain B K, 2008. Hygienic Production of Milk, ICAR Research Complex for Goa (Indian Council of Agricultural Research). Technical Bulletin No: 11.

Baskaran D., Subramanian V.S., Singh D. B., 2002. Handbook on Quality Control in Milk and Milk Products, Chennai.

Bishop J. R. and Bodine A. B., 1986. Quality Assessment of Pasteurized fluid milk as Related to Lipopolysaccharide Content. J. Dairy Sci. 69(12), 3002-3004.

Cappuccino J. G., Sherman N., 2013. Microbiology: A Laboratory Manual, Pearson Education- Benjamin Cummings, 10th Edition.

Cara G. Nick G., 2002. The Coliform Index and Waterborne Disease: Problems of microbial drinking water assessment. CRC press. 60-67.

Chanda G. C., Noor Uddin G. M., Deb A., Bilkis T., Chowdhury S., Basir Uddin M., 2008. Microbiological Profile of the Traditionally Collected Industrial Raw Milk from the Milk Pocket Zones of Bangladesh. Bangladesh J Microbiol. 25(1), 17-20.

Edward K.C. and InyaI.M.,2013. The Microbial Quality of Raw Milk from four locations in Abia State, Nigeria. IOSR-JPBS. 5(3), 30-33.

Jamie B. and Richard B., 1996. Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies, United Nations Environment Programme and the World Health Organization.

Khan M. T. G., Zinnah M. A., Siddique M. P., Rashid M. H. A., Islam M. A., Choudhury K. A., 2008. Physical And Microbial Qualities Of Raw Milk Collected From Bangladesh Agricultural University Dairy Farm And The Surrounding Villages. Bangl. J. Vet. Med. 6 (2), 217–221.

Mulay S., Khale A., 2011. An Overview of Limulus Amoebocyte Lysate (LAL) Test. IRJP. 2(4), 67-71.

Oliveira L. P., Barros L. S. S., Silva V.C., Cirqueira M. G., 2012. Microbiological Quality and Detection of Antibiotic Residue in Raw and Pasteurized Milk Consumed in the Reconcavo Area of the State of Bahia, Brazil. J. Food Process. Technol. 3(1).

Pant R., Nirwal S. and Rai N., 2013. Prevalence Of Antibiotic Resistant Bacteria And Analysis Of Microbial Quality Of Raw Milk Samples Collected From Different Regions Of Dehradun. Int.J.PharmTech Res. 5(2), 804-810.

Perkins K. H., Vandehaar M. J., Burton J. L., Liesman J. S., Erskine R. J., and Elsasser T. H., 2002. Clinical Responses to Intramammary Endotoxin Infusion in Dairy Cows Subjected to Feed Restriction. J. Dairy Sci. ADSA. 85, 1724–1731.

Saxena M., Rai P., 2013. Microbiological and Chemical Analysis of Raw, Pasteurized and UHT Milk during Preservation in India. Int. J. ChemTech Res. 5(6), 2804-2809.

Solomon M., Mulisa M., Yibeltal M., Desalegn G., Simenew K., 2013. Bacteriological quality of bovine raw milk at selected dairy farms in DebreZeit town, Ethiopia. Comprehensive Journal of Food Sciences and Technology. 1(1), 1 – 8.

The United States Pharmacopeial Convention, 2011, Chapter 85 - Bacterial Endotoxins Test.

Torkar K. G., Teger S. G., 2008. The Microbiological Quality of Raw Milk after introducing the two Day's Milk Collecting System. Actaagriculturaeslovenica. 92(1), 61–74.

Tripaldi C., Palocci G., Miarelli M., Catta M., Orlandini S, Amatiste S., Di Bernardini R. and Catillo G., 2010. Effects of Mastitis on Buffalo Milk Quality. Asian-Aust. J. Anim. Sci. 23(10), 1319 – 1324.

Yuen S. K., Yee C. F. and Yin F. H., 2012. Microbiological Quality and the Impact of Hygienic Practices on the Raw Milk Obtained from the Small-scale Dairy Farmers in Sabah, Malaysia. International Journal of Agricultural and Food Science. 2(2), 55-59.