Research and Reviews: Journal of Microbiology and Biotechnology

e-ISSN: 2320-3528
p-ISSN: 2347-2286

A Study of Milk Quality of Different Localities of Kasauli Tehsil,H.P.(INDIA).

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Research Article

Received: 18/10/2021
Accepted: 01/11/2021
Published: 08/11/2021

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Keywords: Raw Milk; MBRT; Citrobacter; Staphylococcus; Antibiotic Sensitivity Test.

ABSTRACT

Raw milk is a complete food that contains protein, fat, sugars, vitamins, and minerals. Even though raw milk is sterile during secretion, contamination of milk by microorganisms can take place during milk handling, storage, and other pre-processing activities. The objective of this study was to understand the biosafety hazard in milk, its sources, and prevention. The safety of milk can ensure by comparing its quality to recommended standards. The milk quality of Kasauli Tehsil of Himachal Pradesh, India has been assessed to express the suitability of milk as a food. In the present investigation, 150 raw milk samples were collected from different localities of Kasauli Tehsil. The pH and microbiological quality of raw milk samples from Mashobra, Itawa, Nahari, Soyata, Ghat, Thauala, Sufarmaina, Kundlu, Panji, Auda, Manon, Masoolkhana, Jangeshu, Nalwa, and Ticket hatti have been assessed. The results were compared with recommended standards of milk quality. An attempt was taken to assess the initial microbial load by Methylene Blue dye Reduction Test (MBRT). The results of MBRT indicated that the values were higher in milk samples 15% lying in the good category, 25% lying in a fair category, 50% in the poor category, and 8% in the very poor category. The estimated standard plate count varied from (cfu\ml) 3 × 106 to >35 × 108 and variations of the microbial population were analyzed by biochemical identification. The isolated organisms were mainly of

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Citrobacter spp. The pH value of tested milk samples was varied from 6.4 ± 0.015 to 6.90 ± 0.113. An attempt was also taken to determine the antibiotic susceptibility test of the lactose fermenting isolate, i.e., Citrobacter sp. It was found that maximum isolates were resistant to vancomycin and sensitive to Neomycin, Tetracycline, Gentamycin, Kanamycin, and Streptomycin. The milk should be tested regularly to confirm the quality of milk from all physicochemical and microbiological points of view as in India raw milk is still consumed in different rituals.

INTRODUCTION

Milk has referred to as the "most nearly perfect" food. Milk is one of the oldest foods known to humans. People use milk due to its highly nutritious and digestible nature than in other single food. Dairy products prepared from milk preserve nutrients and are acceptable to consumers. The importance of adding milk to the human diet is because of its richness in vitamin, mineral salts, protein, carbohydrate, and fat which provide essential nutrients to the consumer, which is necessary for growth and development and provide immunological protection. The highly nutritious nature of milk serves as an excellent growth medium for microbes. Milk sterile when it is, synthesized in a healthy cow's udders. During milking, udder from the surface and storage equipment can contaminate, and the air is a possible source of contamination. There are chances of milk contamination due to udder infection or transfer from the bloodstream. The compounds present in raw milk are lactoferrin, lactoperoxidase, conglutinin which can preserve milk from spoilage, but due to mismanagement and handling of milk is contaminated by different microbes. There is a need for good quality milk which needs an effective health udder program. The microorganism can enter in milk from many sources for example Streptococcus, Propionibacterium, fungal population support dairy fermentation, Lactobacilli, and Bifidobacterium promote health while Pseudomonas, Clostridium, Bacillus, and other spore-forming bacteria cause spoilage of milk and milk product and Shigs toxin-producing *E. coli*, Salmonella, mycotoxin producing fungi cause disease. The detection of coliform and another pathogen in milk indicates contamination of bacteria from milking equipment, and water. Fresh milk drawn from the cow contains a low microbial load, i.e., less than 1000 ml-1, but bacterial load may increase up to 100 fold when stored in optimum environmental conditions. However, the bacterial load can be reduced by storing milk and milk products in a refrigerator (4˚C). Collectively the presence of the food-borne pathogen in raw milk directly or indirectly causes ingestion or transmission of food-borne disease and increases the risk of potentially harmful toxins. Proper handling can prevent the multiplication of microorganisms during milking and milk processing. The consumption of raw milk in people may cause gastrointestinal tract infection that includes typhoid fever, salmonella food poisoning, tuberculosis, Q fever, gastroenteritis, dysentery, diphtheria, and staphylococcal intoxication [1]. The initial flora of raw milk influences, microbiological quality of milk and milk products. Today, the public is demanding milk and its products for nutritional purposes without health risks and hazards, enriched nutritional values, and high biological potential.

Today antibiotics are uses to treat cattle and as well as used as a preservative for milk. Different doses of antibiotics are uses to inhibit the growth of susceptible bacteria, while resistant bacteria can grow in the presence of an antibiotic. In the present investigation, an attempt was taken to analyze the safety and quality of raw milk with recommended standards, in Kasauli Tehsil, a hilly town of District Solan, Himachal Pradesh, India.

MATERIALS AND METHODS

Study area

The study was conducted for a period of six months from February 2019 to June 2019 at selected dairy localities, Kasauli Tehsil of Himachal Pradesh. The milk sector represents the biggest contributor to revenues and the biggest job provider in agricultural holding with 10 000 dairy cows with its annual production of 860 tons. Milk is collected from the rural area and transported at ambient temperature to different marketing points of solan city and sold raw milk to consumers.

Sample Collection

Raw milk sample is to be collected directly from a cow; it was the representative of milk that will be taken for supply to the consumer. Udders were washed with hot water and proper cleaning of a teat. During milking the milk sample was collected in a sterile bottle (20 ml), holding upward direction. 150 raw milk samples were collected at a different level of collection and processed. Accordingly 10-10 samples were collected from Mashobra, Nahari Itrawa, Manon, Auda, Soyata, Masool khana, Jangeshu, Ghat, Thuala, Sufarmaina, Nalwa, Ticket hatti, kundlu and Panji. All raw milk samples were collected in an icebox and transported to the laboratory under chilled conditions within 2 hours and processed for microbiological analysis.

Physical Appearance

All raw milk samples were observed for color and pH.

Standard Plate Count

The total plate count was made by adding 1ml of raw milk sample in 10 ml of distilled water. After mixing sample was serially diluted up to 1:1010 and duplicate samples (500 µl, 1000µl) were spread in 15-20 ml standard plate count (nutrient agar plate) and then incubated at 37 ˚c for 24-48 hours. Colony count was made from plates with less than 300 but more than 30 colonies and the result was expressed, as colony forming units (cfu\ml) of the milk sample.

Isolation and Identification of Bacteria

Raw milk samples were examined following the standard procedure where a loop full bacterial colony from the master plate was streaked in the nutrient agar plate. Plates were incubated aerobically at 37˚C for 24 hours. Further, the morphologically distinct bacterial colonies were picked up and repeatedly re-streaked for further purification. Bacterial strains were identified based on morphological, microscopically, and biochemical characterization.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

Methylene Blue Dye Reduction Test (MBRT)

MBRT is used for evaluating cell viability and estimates the microbial load in milk. In this method, metabolically active organisms degrade dye and turn it colorless. The oxidation-reduction potential depends upon microbial load in raw milk. The rate of oxidation/reduction is dependent upon various factors that included the bacterial load, kind of bacteria, and their rate of metabolism. 10 ml of each raw milk sample were transferred appropriately into a labeled autoclaved test tube. 1 ml of methylene blue dye was added to each test tube containing 10ml of raw milk sample. After thoroughly mixing, the test tube was closed tightly with stoppers. The tubes were then kept in the incubator at 37˚C. The tubes were observed in the routine interval and the time of incubations was noted [2]. The experiment was continued until the sample color turns to a whitish appearance.

pH

The pH of the raw milk sample was measured in a digital pH meter. The instrument was calibrated by using buffer solution at ph 4.0 and 7.0. Each sample was taken thrice and the results represented as Mean ± SEM.

Antibiotic Sensitivity Test

Antibiotic sensitivity test was performed on *Citrobacter spp*. by antibiotic vancomycin (HIMEDIA, 30 mcg/disc), neomycin (HIMEDIA, 10 mcg/disc), gentamycin (HIMEDIA, 10 mcg/disc), kanamycin (HIMEDIA, 30 mcg/disc) and tetracycline (HIMEDIA, 10 mcg/disc). Bacterial inoculums spread on nutrient agar plate and wafer of antibiotic placed on it and incubated for 24 hr.

RESULTS

Questionnaire Survey

This questionnaire survey was performed in Kasauli Tehsil, H.P.(India) in (Table 1).

Table 1: Dairy farm hygienic practice.

Farmers Practices	Performance	No. of Farmers	Frequency (%)
Barn cleaning	Once per day	80	0.53
	Twice per day	40	0.26
	Three time per weak	20	0.13
	Once per weak	10	0.1
Udder washing before milking	No washing	20	0.13
	Using cold type water	60	0.4
	Using tap water	20	0.13

	Warm water	40	0.26
	Cold river water	10	0.06
Washing of milking equipments	Twice per day	30	0.2
	Once per day	90	0.6
	Every other day	30	0.2
	Once per week	0	
Hand wash before milking	Yes	100	0.66
	No	50	0.33

The color of raw milk observed was white in appearance (80%) to yellow (20%). The pH ranges from 6.4 to 6.9. The microbial count was determined by standard plate count as per BIS standard and compared with a standard chart of microbiology quality of raw milk. Out of 150 raw milk samples, only 21% of raw milk was found in the category of good quality, 38% was in a fair category and 71% was in the poor, and 10% was in very poor category. Microbial characterization by gram staining, a biochemical test revealed that 60 samples were containing Citrobacter. Antibiotic sensitivity test was done by using five antibiotics, i.e., vancomycin, gentamycin, tetracycline, streptomycin, neomycin. Isolated and characterized, all Citrobacter sp. isolates 75% were sensitive to Neomycin, Gentamycin, Streptomycin, Kanamycin, and Tetracycline and resistant to vancomycin [3]. In addition, *Citrobacter sp*. was found to be resistant to neomycin (25%), whereas 8 % of isolates were sensitive to all antibiotics (Tables 2-6, Figures 1-9).

Table 2: Everage pH value of raw milk samples collected from different localities.

Sr. No.	Location	pH
1	Mashobra	6.68 ± 0.040
2	Nahari	6.45 ± 0.075
3	Itawa	6.70 ± 0.046
4	Manon	6.6 ± 0.058
5	Auda	6.40 ± 0.024
6	Soyata	6.8 ± 0.032
7	Masoolkhana	6.90 ± 0.086
8	Jangeshu	6.90 ± 0.113
9	Ghat	6.50 ± 110
10	Thaula	6.7 ± 0.058
11	Sufarmaina	6.65 ± 0.103
12	Tickethatti	6.55 ± 0.283
13	Kundlu	6.71 ± 0.080
14	Panji	6.60 ± 0.167
15	Nalwa	6.61 ± 0.114
Values showing mean ± standard error (10 samples distributed in each village)		

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Table 3: Data showed MBRT test on raw milk samples.

	MBRT values	No. of samples	Quality of grade
Total milk sample-150	>20,000,000	10	Very poor quality
	>4,000,000	70	Poor quality
	>5,00,000	40	Fair quality
	<5,00,000	30	Good quality

Table 4: Microbial examination of raw milk sample.

Bacterial isolates	Total positive sample
Citrobacter sp.	60
Staphylococcus sp.	10

Figure 1: Experiment on MBRT Test of milk samples collected from different localities.

Figure 2: Gram staining of *Citrobacter sp.*

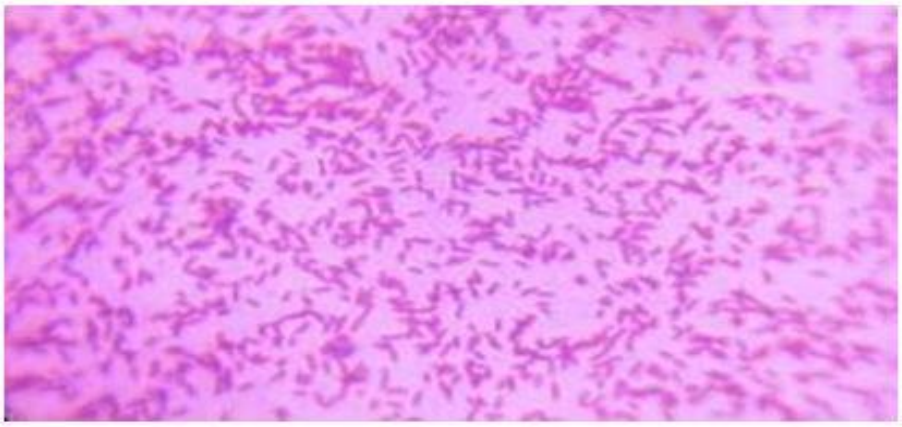

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Figure 3: Gram staining of *Staphylococcus sp.*

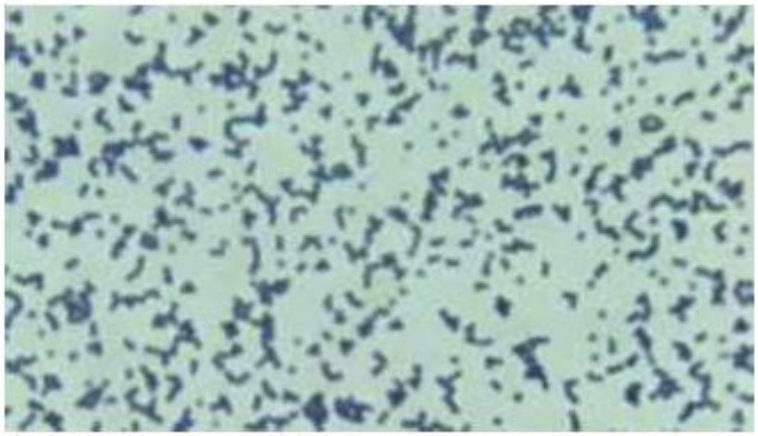

Figure 4: Lactose fermenting colonies of Citrobaceter on MacConkey agar.

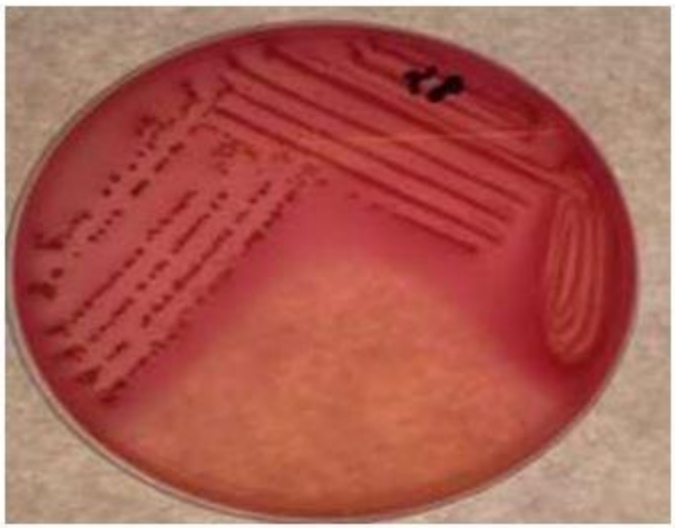

Figure 5: Colonies of *Citrobacter sp*. on nutrient agar.

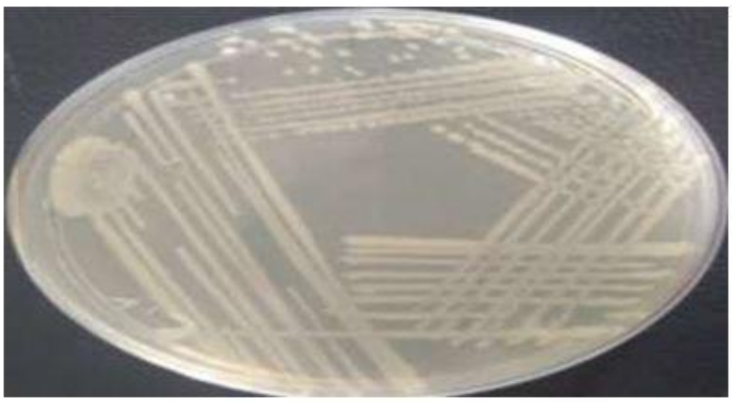

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Figure 6: Colonies of Staphylococcus sp. on nutrient agar.

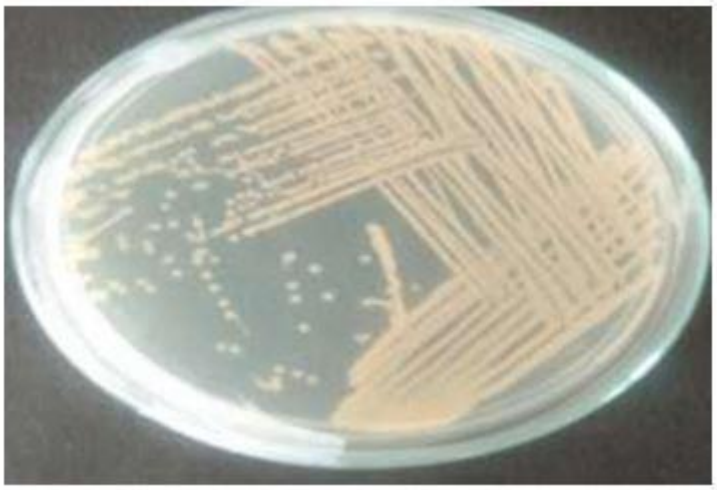

Table 5: Biochemical Analysis of *Citrobacter spp.*

S.no.	Biochemicals	*Citrobacter sp.*	*Staphylococcus sp.*
1	Catalase	+ve	+ve
2	Oxidase	-ve	-ve
3	Indole	+ve	-ve
4	MR	+ve	+ve
5	VP	-ve	+ve
6	Citrate	+ve	+ve
7	TSI	K/A H2S GAS	K/A,G
8	Urease	+ve	+ve
9	NR	+ve	+ve
10	Sugars	-	-
11	Glucose	+ve	+ve
12	Lactose	+ve	+ve
13	Sucrose	+ve	+ve
14	Maltose	+ve	+ve
15	Amino Acids	-	-
16	Lysine	-ve	-ve
17	Arginine	-ve	+ve
18	Ornithine	-ve	-ve

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p-ISSN: 2347-2286

Figure 7: Biochemical characterization of bacterial isolate *Citrobacter sp.*

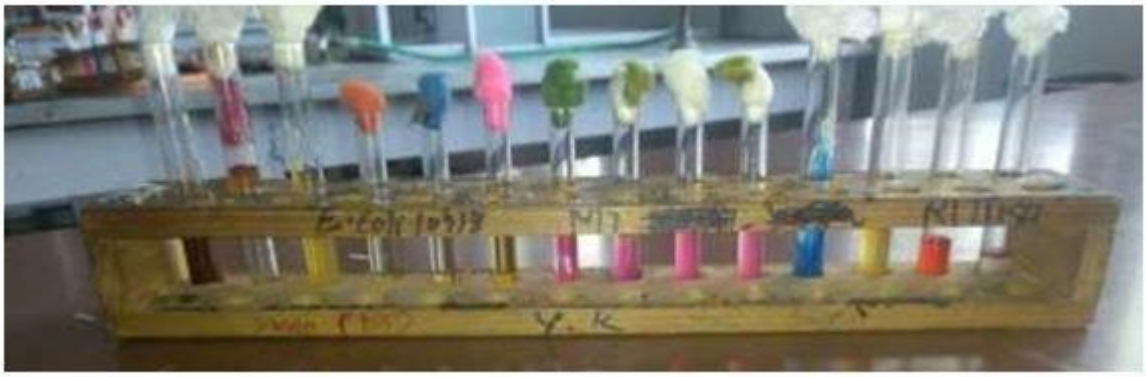


Figure 8: Biochemical characterization of bacterial isolate *Staphylococcus sp.*

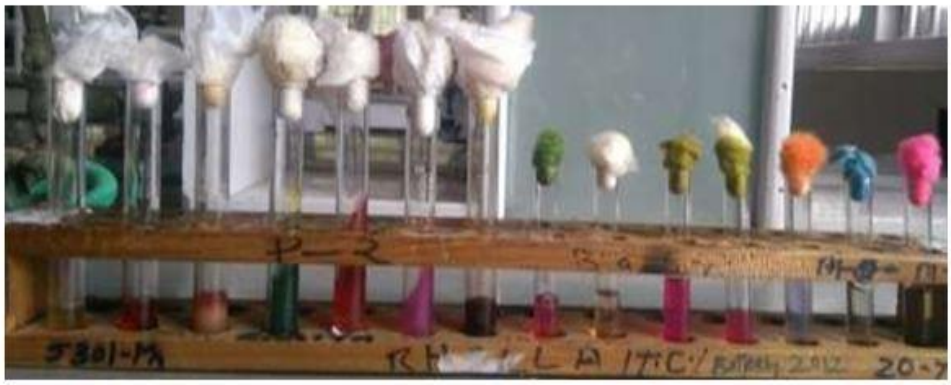
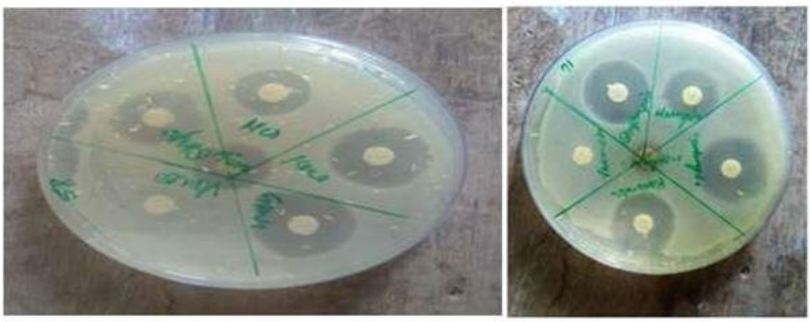


Table 6: Antibiotic Sensitivity Test.

Citrobacter sp.	Antibiotics					
	Neomycin	Vancomycin	Gentamycin	Streptomycin	Kanamycin	Tetracyline
40	S	R	S	S	S	S
15	R	R	S	S	S	S
5	S	S	S	S	S	S
R- Resistent, S-sensitive						

Figure 9: Image showing result of Antibiotic Sensitivity Test.



e-ISSN: 2320-3528
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DISCUSSION

The color of raw milk observed was white in appearance (80%) to yellow (20%). The pH ranges from 6.4 to 6.9. The microbial count was determined by standard plate count as per BIS standard and compared with a standard chart of microbiology quality of raw milk [4]. Out of 150 raw milk samples, only 21% of raw milk was found in the category of good quality, 38% was in a fair category and 71% was in the poor, and 10% was in the very poor category. Microbial characterization by gram staining, a biochemical test revealed that 60 samples were containing *Citrobacter*. Antibiotic sensitivity test was done by using five antibiotics, i.e., vancomycin, gentamycin, tetracycline, streptomycin, neomycin. Isolated and characterized, all *Citrobacter* sp.isolates 75% were sensitive to Neomycin, Gentamycin, Streptomycin, Kanamycin, and Tetracycline and resistant to vancomycin. In addition, *Citrobacter* sp. was found to be resistant to neomycin (25%), whereas 8 % of isolates were sensitive to all antibiotics [5].

CONCLUSION

Based on obtained data in the present study, the conclusion may be drawn that microbial load and antibiotic resistance in raw milk distributed in Kasauli Tehsil, H.P. is increasing very fast. The main cause of contamination of milk is due to milking from the infected udder of a cow, unclean equipment, and unhygienic milking practices, and improper storage conditions. Lack of awareness and negligence in this area was observed. Raw cow milk has always presented risks to public health because of the presence of pathogenic bacteria when consumed without pasteurization. In this village, some people use raw milk without pasteurization and also in Indian rituals. There is a need for awareness among people to use pasteurized milk and storage of milk at ambient temperature. The most important part of milk quality analysis is a microbiological analysis of milk as this quality is directly related to human and other animal health issues. Primarily from a bacteriological point of view, some evaluated milk samples were found to have contaminated with fecal/non-fecal origin. In the interest of public health, the milk should be tested regularly to confirm the quality of milk from a physicochemical and microbiological point of view. It is not possible to detect all the contaminants frequently but reliance must be placed on testing for total microbiological load, presence of pathogenic bacteria, and any suspected pH changes.

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