

# Microbiological quality aspects of cow's milk at a smallholder cooperative in Turrialba, Costa Rica

T. de Graaf <sup>1\*</sup> J.J. Romero Zuñiga <sup>2</sup>  
M. Caballero <sup>2</sup> R.H. Dwinger <sup>3</sup>

## Key words

Dairy cattle - California Mastitis Test - Microbiological analysis - Somatic cell count - Dairy hygiene - Quality control - Producer cooperative - Dairy industry - Milk production - Costa Rica.

## Summary

Some of the critical moments and factors influencing the hygienic quality of milk were investigated at a smallholder cooperative in Costa Rica from which all the members delivered milk to a processing plant. Twenty-two farmers provided data for this study. Milk from all lactating cows was examined with the California Mastitis Test (CMT). Apparent mastitic milk was processed for bacteriological investigation (BI). Additionally, milk samples were collected from storage containers for somatic cell count (SCC) and for BI. Out of the 22 farms 10 were selected for further study on the hygienic quality of the milk sampled at specific critical moments during handling, storage and after transportation to the processing plant. Point prevalence for CMT-positive samples was 21.8%, 36.6% and 28.4% for the months of June, August and October, respectively. The incidence rate for CMT-positive samples for this period was 27.8%. Eighty-three out of 126 milk samples taken from storage containers for SCC contained less than 400,000 cells/ml. Eight percent of the CMT-positive milk samples contained *S. aureus*. Twenty-eight percent of the milk samples from storage containers contained *S. aureus* and 79% of the samples *E. coli*. All milk samples from storage containers obtained at the processing plant had CFU counts exceeding  $2.10^6$  cells/ml. This milk was severely contaminated with bacteriological agents from environmental origin. Cooling of the milk was found to be inadequate. The udder preparation method, unclean milk equipment and the water used for cleaning purposes were the main sources of milk contamination.

## ■ INTRODUCTION

Milk and milk products play an important role in human nutrition throughout the world. Consequently, the products must be of high

hygienic quality. In less developed areas and especially in the hot tropics high quality and a safe product are most important but not easily accomplished.

Milk quality is determined by parameters of composition and hygiene. The compositional quality of milk is mainly influenced by nutritional, managerial and genetic factors. Furthermore, it is affected by proteolytic enzymes. These are either inherent to milk secretion, associated with leukocytes in mastitic milk, or synthesized by psychrotrophic bacteria that contaminate milk (10, 26). Proteolytic activity seems to be partly related to elevated somatic cell counts of raw milk (8, 23, 24). The hygienic quality of milk is influenced by pathogenic organisms, saprophytic microorganisms, residues, and other contaminants (18). Hygienic control measures are necessary in order to achieve a "clean, safe,

1. Herd Health Project, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

2. Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

3. Animal Production and Health Section, IAEA, PO Box 100, A-1400 Vienna, Austria

\* Corresponding author: Postgraduate Studies in Tropical Veterinary Medicine, Freie Universität Berlin, Luisenstrasse 56, 10117 Berlin, Germany

Tel: (49) 30 20 93 60 63; Fax: (49) 30 20 93 63 49

E-mail: tgraaf@city.vetmed.fu-berlin.de

sound and wholesome" product (12). In addition, various communicable diseases are associated with the consumption of milk and dairy products (3, 21).

In Costa Rica, apart from two larger dairy plants which are well organized and have conclusive control of the produced dairy products, there is a fair amount of small dairy product selling cooperatives whose members are smallholder dairy farmers. These cooperatives can be found in the more remote rural areas where milk collection and transport can be difficult due to a poor infrastructure. Dairy products such as cheese and cream are usually produced at the cooperative dairy processing plant and products are distributed mainly locally, but can also be transported to supermarkets in larger cities. Hygienic quality control of the end product is usually not practiced on a routine basis. Apart from these cooperatives, door-to-door milk delivery in the urban and periurban areas is practiced as well, with virtually no quality control at all.

Because of product quality inflections suffered by one of the smaller cooperatives, the present study was conducted to determine some of the factors influencing the quality of the raw milk collected and processed by the cooperative. Therefore, milk was sampled at critical moments from the cow's udder to the milk processing plant.

The objective of this descriptive study is to indicate some of the critical points in milk handling.

## ■ MATERIALS AND METHODS

### *Study area*

The study area was located near the village of Santa Cruz de Turrialba, on the slopes of the Turrialba volcano, Costa Rica. The area can be characterized as "premountain wet cloud forest" (19). The cooperative investigated had 26 members, who each had one or more lactating cows.

Twenty-two members of the cooperative participated in the study and provided the data presented below. The selected farms were visited three times: in June, August and October 1994.

### *Farm characteristics*

All farms participating in the study had Jersey cattle, except one farm which had the Holstein breed. The estimated average milk yield was 10 kg/cow/day. The number of lactating cows present at the farms ranged from 1 to 54, with an average of 12.7 animals per farm. All animals were identified by eartag numbers or by name. All farms were accessible by road. The farmers did not receive technical assistance.

The following two milking procedures have to be distinguished:

1. Hand milking farmers milk directly into a bucket. When the cow is milked, the bucket is then emptied into a churn. Therefore, a churn contains milk from different cows, whereas the bucket contains milk from one identifiable cow;
2. Farmers who apply machine milking milk direct into a churn. After a cow is milked, the milking equipment is connected to another cow. The churn thus contains milk from different cows.

In both situations churns are topped up with milk from other churns, to lower the amount of churns to be transported. These topped up churns will be referred to as storage containers, since the milk is cooled in these churns and transported to the processing plant.

All farmers milked twice daily, early in the morning and late in the afternoon.

## *Sampling procedures*

### *General data sampling*

In all farms, basic farm data and data on milking technique, udder preparation, milk handling, hygiene and storage were obtained through a questionnaire.

### *Milk data sampling*

#### ■ Milk sampled at the farm

All lactating cows at the 22 participating farms were examined three times (in June, August and October 1994) with the California Mastitis Test (CMT) (29) in order to detect the presence of elevated somatic cell counts (SCC), which may be indicative of mastitis (clinical or subclinical). Scores were given in accordance with Schalm and Noorlander (29). A milk sample was taken aseptically for bacteriological investigation from the samples with apparently elevated SCC.

#### ■ Milk sampled at the processing plant

In addition, milk samples were collected from all farms' storage containers delivered at the processing plant to establish SCC microscopically and to assess the presence of bacteriological agents.

#### ■ Milk sampled at critical moments during handling, storage and after transportation

At ten selected farms, additional evening milk samples were collected at critical moments that can be regarded as critical control points; these moments can be associated with a hazard, when a measurement can be conducted and when control measures can be taken in order to reduce the hazard to an acceptable level.

The presence of bacteriological agents was assessed and a CFU count performed on pooled evening milk samples collected at random at the following critical moments:

1. Directly taken from the cows' udders from five randomly selected animals;
2. From the bucket or the churn;
3. From the storage container before cooling;
4. From the storage container after cooling;
5. From the storage container upon arrival at the plant.

These moments are graphically displayed in figure 1.

Additionally, the temperatures of the evening milk were measured at moment 2, moment 3, moment 4 and moment 5, as well as the time elapsed between measurements. Successive milk samples were collected each time from the same storage container.

The morning milk temperature was measured upon arrival at the plant.

*Figure 1: moments at which milk samples were taken.*

### Water samples

Water samples were collected at the ten selected farms, from water sources that provided water used on the farm for cleaning milk equipment and from water that remained in the churns after cleaning (rest water), in order to assess hygienic quality.

### Laboratory examinations

Milk and water samples were processed in the laboratory of the School for Veterinary Medicine within 48h after sampling. Between sampling and processing the samples were stored at approximately 4°C.

Determination of the somatic cell count of the milk sampled from the churns and storage containers was done microscopically as described by Schalm *et al.* (28). The samples were processed according to standard laboratory methods. Partial differentiation of bacterial agents took place after 24h incubation at 37°C. Twenty-five samples of *Staphylococcus* spp. and all samples of *Streptococcus* spp. were examined more intensively using API (API Staph and API 20 Strep, BioMérieux, France). The *Staphylococcus* spp. which acted coagulase-positive were presumed to be *S. aureus*.

Water samples were processed according to WHO standards (31) and examined for the presence of coliform bacteria.

### Statistical analyses

Microsoft EXCEL (Version 5.0) basic descriptive statistics was used to analyze the data.

Because of the log-normal distribution of the bacterial counts, multiple comparisons of means using ANOVA was applied after log transformation, in order to calculate statistical differences between the five moments of sampling.

## ■ RESULTS

### General data

#### Udder preparation and hygiene

All farmers used water from a hose or a basin to wet the cows and clean them from soil and dirt. The water source was either a small stream or a spring. Twelve farmers used to wash the hindquarters and the udder, whereas ten farmers cleaned only the udder. Either one towel or one brush was used for cleaning purposes for all cows. None of the udders was properly dried. Though in four cases the practice of teat dipping was mentioned, this practice was not confirmed by personal observation. The use of detergents and disinfectants for cleaning milk equipment, as recommended by the FAO (11), was not observed. Hot water, indispensable for cleaning milking equipment, was not available at the farms.

#### Milking technique, milk cooling and transport

Fifteen farmers milked by hand and seven farmers applied machine milking. All farmers cooled the evening milk, but applied different methods:

- 15 farmers put the storage container in a basin with water at ambient temperature (about 15°C);

- 3 farmers put the storage container in a basin with mechanically cooled water (about 5°C);

- 4 farmers used a *cortina*, a surface cooler based on a counter-flow principle, to cool the milk before putting the storage container in one of the above mentioned types of basins.

The evening milk was cooled during the first hours after milking and subsequently put aside overnight at ambient temperatures ranging from about 5 to 15°C. The morning milk was delivered to the plant without cooling. The milk was transported either in plastic or aluminum storage containers to the processing plant every morning. The storage containers were put alongside the road to await transportation.

### Milk data

#### CMT results

Crude point prevalence of mastitis based on a positive CMT result for the months of June, August and October was calculated at 21.8%, 36.6% and 28.4%, respectively, for all cows. Based on the CMT results, there were 194 cows at risk in June (negative CMT), of which 54 became positive in the successive sampling periods. The incidence rate for the period June-October can therefore be calculated at 27.8%.

#### Somatic cell count of milk sampled from storage containers

A total of 126 milk samples were collected from storage containers upon delivery at the plant. In 83 samples the number of somatic cells did not exceed 400,000 somatic cells/ml. Thirty milk samples had a somatic cell count between 400,000 and 700,000 somatic cells/ml, while 13 milk samples contained more than 700,000 somatic cells/ml.

#### Bacterial agents

##### ■ Bacterial isolates of milk sampled from the udder

A total of 262 CMT positive samples were bacteriologically processed. Forty percent of the samples did not result in any growth, whereas 60% did ( $n = 157$ ). The results of the bacteriological investigation of the CMT positive milk samples are shown in table I.

Twenty-two out of the 157 bacteriologically positive samples contained *S. aureus*. API-test results on 25 coagulase-negative *Staphylococcus* spp. and API-test results on all *Streptococcus* spp. ( $n = 12$ ) are shown in table II.

Table I

Bacterial isolates from bacteriologically positive milk samples ( $n = 157$ ) taken from the udder

Bacterial agent	Number of isolates
<i>Staphylococcus</i> spp. coagulase +	22
<i>Staphylococcus</i> spp. coagulase -	88
<i>Streptococcus</i> spp.	12
<i>E. coli</i>	14
<i>Klebsiella</i> spp.	4
<i>Proteus</i> spp.	4
Non fermenting G-	3
Mixed cultures	10

Table II

API results of *Staphylococcus* spp. and *Streptococcus* spp. isolates

	Bacterial agent	Number of isolates
API Staph	<i>S. epidermidis</i>	17
	<i>S. simulans</i>	3
	<i>S. xylosus</i>	3
	<i>S. chromogenes</i>	2
API Strep 20	<i>Str. agalactiae</i>	7
	<i>Str. uberis</i>	4
	<i>Str. faecium</i>	1

Table III

Bacterial isolates of milk samples (n = 126) from storage containers upon delivery to the milk processing plant

Bacterial agent	Number of isolates	% of infected samples
<i>Staphylococcus aureus</i>	35	28
<i>Staphylococcus</i> spp.		
Coagulase-negative	102	81
<i>E. coli</i>	100	79
Non fermenting G-	37	29
<i>Klebsiella</i> spp.	24	19
<i>Streptococcus</i> spp.	23	18
<i>Proteus</i> spp.	7	6
<i>Enterobacter</i> spp.	3	2
<i>Citrobacter</i> spp.	3	2
<i>Corynebacterium</i> spp.	2	2

Table IV

Bacterial isolates of milk sampled at critical moments during handling, storage and after transportation

Farm	Moment of sampling				
	Udder	Bucket/churn	Before cooling	After cooling	Upon arrival at plant
1*	neg	EC, Sta	EC, Sta, Cit	idem	idem
2**	Sta	EC, Sta, Kleb, NF	idem	idem	idem
3**	neg	EC, Sta, Str, NF	idem	idem	idem
4**	neg	EC, Prot, Sta, Cit	idem	idem	idem
5*	neg	EC, NF	idem	idem	idem
6*	EC, Sta	idem	EC, Sta, Kleb, Cit	idem	idem
7**	neg	EC, Sta, Kleb	idem	idem	idem
8*	EC, Sta, Kleb	EC, Sta, Kleb, NF, Ent	idem	idem	idem
9**	EC, NF	EC, Sta, NF	idem	idem	idem
10*	EC, Sta	EC, Sta, NF, Ent	idem	idem	idem

\* Farm which applied handmilking \*\* Farm which applied machine milking

neg: no growth; EC: *E. coli*; Sta: *Staphylococcus* spp.; Cit: *Citrobacter* spp.; Kleb: *Klebsiella* spp.; NF: non-fermenting bacterial agents; Str: *Streptococcus* spp.; Prot: *Proteus* spp.; Ent: *Enterobacter* spp.

#### ■ Bacterial isolates of milk sampled from storage containers

Out of a total of 126 milk samples collected from storage containers upon delivery at the milk processing plant, 2 proved to be negative on culture. In most of the positive samples mixed cultures were found with *E. coli* and negative *Staphylococcus* spp. prevailing. The isolates obtained from positive cultures are displayed in table III.

#### ■ Bacterial isolates of milk sampled at critical moments during handling, storage and after transportation

At the cow site level (moment 1), 50% of the pooled milk samples proved to be bacteriologically negative. In the positive cases most of the samples showed cow specific agents, i.e. *Staphylococcus* spp. In addition, environmental bacterial

agents, i.e. *E. coli*, *Klebsiella* and non fermenting bacterial agents were detected (table IV).

When milk was sampled at a later stage (churn - plant), the milk appeared to be severely contaminated with environmental bacterial agents, i.e. *E. coli*, *Proteus* spp., *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., and non fermenting bacterial agents.

#### *Bacterial counts*

#### ■ Bacterial counts of milk sampled at critical moments during handling, storage and after transportation

CFU counts of milk sampled at critical moments during handling, storage and after transportation are shown in table V. Almost all subsequent CFU counts showed a significant increase, however, during the cooling process the CFU count did not increase significantly.

Table V

Colony forming unit CFU counts of pooled milk sampled at critical moments during handling, storage and after transportation - counts x 1000 CFU/ml

Farm	Moment of sampling		Before cooling	After cooling	Upon arrival at plant
	Udder	Bucket/churn			
1*	0	20	160	1230	15,500
2**	126	1200	1460	3700	> 30,000
3**	0	1780	5100	9600	> 30,000
4**	0	2120	4300	10,100	> 30,000
5*	0	300	1700	4800	19,700
6*	12	28	800	7800	> 30,000
7**	0	2340	4800	12,100	> 30,000
8* <sup>1</sup>	1340	2130	3240	217	2300
9**	183	2300	19,000	30,000	> 300,000
10*	43	1230	27,000	30,000	170,000

ANOVA and Multiple Range Test results show a significant difference between all means ( $p < 0.00$ ), except for the difference of the means between the moments before and after cooling ( $p > 0.05$ )

\* Farm which applied handmilking

\*\* Farm which applied machine milking

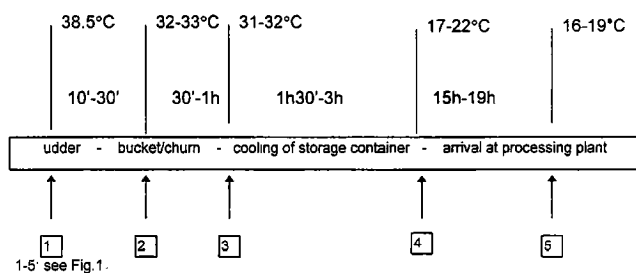
<sup>1</sup> This farm administered potassium nitrate to the milk

#### Temperature of the milk at critical moments and time elapsed between measurements

The temperature of the milk at critical moments and the time elapsed between measurements are represented in figure 2. A decrease in temperature of the milk was present on all farms during the cooling operation. The average temperature of the cooled evening milk upon arrival at the plant the next morning was 18.2°C (15.6-19.3°C), that of the non cooled morning milk 28.9°C (18.3-31.5°C). The cooling operation lasted 1h30'-3h, thereafter it took 15-19h for the milk to arrive at the processing plant, during which the temperature lowered a few degrees, due to the low ambient night temperature.

#### Water quality

One water sample proved to be of good quality and one was acceptable. The other eight samples originated from water of poor (not drinkable) quality with CFU counts of coliform bacteria exceeding 1100 cells/ml.



1-5 see Fig. 1.

Figure 2: temperature of the milk at critical moments and time elapsed between measurements.

#### DISCUSSION

The results from the questionnaire show that no warm water was used for milk equipment cleaning. Insufficient milk equipment cleaning is a major cause of milk contamination. Furthermore, no detergents were used at the farms involved in this study. Detergents are chemical agents that assist in the cleaning process by dissolving the deposited dirt, making its removal easier. However, prior to using detergents the equipment has to be washed with cold water to remove as much milk and dirt as possible, then with warm water to remove fatty deposits. After using detergents the equipment has to be washed again with warm water. The equipment has to be stored afterwards in a clean, dry and dustfree area.

Subsequently, premilking udder preparations play an important part in the contamination of milk during milking as reported by Galton *et al.* (13, 14, 15). All farmers in the present study cleaned the cows' udders and teats with water from a hose or basin in order to remove soil and dirt. Drying the udders and teats was not or insufficiently practiced. Preparing the udders by wetting both udder surfaces and teats had a higher standard plate count in milk compared with methods that wetted teats only (15). Procedures that allowed water laden with bacteria to drain into teat cups during milking resulted also in elevated numbers of bacteria on the standard plate count. Manual drying of teats was found essential as part of any procedure in order to achieve the greatest reduction in bacterial counts (14). Udder surfaces should be dry and teats should be clean and dry at machine attachment (13).

The CMT positive results, demonstrating elevated SCC, are indicative of clinical or sub-clinical mastitis. The prevalence of mastitis found in this study is comparatively similar to the prevalence of mastitis found in other Costarican dairy farms by de Graaf and Dwinger (17).

In this study about one third of the milk samples taken from the storage containers at the moment of delivery to the plant had

somatic cell counts that exceeded 400,000 cells/ml. Somatic cell counts above 500,000 cells/ml will reduce cheese yield (9), causing economic loss. Furthermore, protein deterioration in milk starts at a low somatic cell count, as low as 250,000 cells/ml (23).

The compositional quality of milk is affected by proteolytic enzymes, the activity of which is partly related to a somatic cell count (16, 23, 24). Proteolytic enzymes cause time- and temperature-dependent breakdown of casein, the major milk protein (27). Another consequence of the presence of proteolytic enzymes such as heat resistant proteinase is a reduced shelf-life of milk following pasteurization or UHT treatment (6, 30).

The hygienic quality of milk is influenced by the type and number of bacteria present in the milk. About 8% of the CMT positive samples contained *S. aureus*. This may be of concern for human health since some strains of *S. aureus* are capable of producing heat stable enterotoxins. Another potential hazard may be the fact that 28% of the milk samples obtained from the storage containers at the plant contained *S. aureus*. However, compared with data obtained from a similar study in Trinidad, where 95% of the milk sampled at the processing plant were contaminated with *S. aureus*, the number found in the present study is relatively low (1).

Fifty percent of the pooled milk samples collected directly from the udders were agent free. A few samples contained *E. coli*, non-fermenting coliform bacteria and *Klebsiella* spp. However, all samples collected from churns or buckets contained bacterial agents. They were consistently in larger numbers than those collected from the udders. High bacterial counts are indicative of an elevated number of psychrotrophic bacteria (*Pseudomonas* spp., *Flavobacterium*, *Achromobacter*) which in turn are responsible for an elevated production of proteinase and lipase (2). Although most emphasis was placed on the detection of udder pathogens, one might assume, based on the high bacterial counts, that psychrotrophic bacteria were present in high numbers. Psychrotrophic bacteria are important because, although mostly not thermophilic, many of them produce extracellular thermostable proteolytic and lipolytic enzymes which can survive pasteurization (6), thus affecting the shelf-life and quality of the dairy product.

The result from the sampling at the critical control points showed significant increases in CFU count at the moments 2, 3 and 5. The first contamination occurs upon collection of the milk into the bucket or churn, followed by further contamination due to additional handling of the milk such as topping up of the buckets or churns in storage containers. The high CFU count at moments 2 and 3 is assumed not to be caused by bacterial growth, because of the relatively short time elapsed between these moments and the foregoing sampling moment and because of the existence of bacteriostatic compounds in fresh raw milk. However, the high CFU count at moment 5 is assumed to be caused by bacterial growth supported by the long storage time of the milk at moderate temperatures. Under tropical circumstances, high CFU counts of milk delivered at milk processing plants are more often encountered in smallholder dairy cooperatives (20). Even then the CFU count observed in this study has to be considered too high.

When sampled directly from the udder, the milk was either bacteriologically negative or contaminated with less bacterial agents than the milk sampled from the bucket or churn, thus indicating possible contamination from outside the udder. This was confirmed by the bacteriological cultures indicating bacteria from environmental origin. Environmental bacterial contamination of milk can be airborne or depend on udder preparation, teat

dipping and the cleaning procedures of milking equipment (7). In the case of hand milking, milk contamination might be caused by water droplets falling into the bucket when the hindquarters have been washed but not sufficiently dried, as well as when neighboring cows defecate.

The increase in CFU count between the samples taken before and after cooling indicates that the cooling operation is not very efficient, the differences between the sample means is, however, not significant, which may be due to the short cooling period.

All the water samples showed the presence of coliform bacteria, which are particularly undesirable in water. Eight out of the 10 water samples were of undrinkable quality according to WHO standards (30). The presence of coliform bacteria may indicate pollution of water by sewage and the possible presence of pathogenic bacteria (25). The bacterial flora of most water supplies in the tropics consists mainly of Gram negative rods. Many of these may be proteolytic and lipolytic and will cause spoilage of milk and milk products if processing and storage conditions are not correct. Water of good bacteriological quality is important to protect human health and to avoid milk contamination.

At farm 8, the administration to the milk of potassium nitrate acting as a bacteriostatic agent resulted in a considerable decrease in CFU count. An alternative method for raw milk preservation has been advocated, i.e. the lactoperoxidase antibacterial system (4, 5, 22).

## ■ CONCLUSION

Udder health was not a major constraint for good quality milk. Results from sampling at critical control points clearly showed that severe contamination started from the first moment the milk left the udder. The differences between CFU counts and bacterial isolates at critical control points clearly demonstrated exogenous sources of milk bacterial contamination. This occurred primarily at the very initial phase of the milking procedure and was most probably due to the use of contaminated water, improper milking technique and unclean milk equipment. Adequate udder preparation, especially drying of the udder, and a hygienic milking technique, together with the use of disinfected milk equipment could considerably improve the milk hygienic quality. The desired permanent storage milk temperature of 4°C was never achieved. The cooling is inefficient and inadequate, resulting in clearly measurable increases in CFU counts throughout the process at the farm until delivery at the milk processing plant. Based on the high CFU counts found in the milk upon delivery at the plant, one may suppose that this milk may pose a public health risk.

Efficient milk cooling is required not only at the farm but also during transportation. Since there is a long time interval between milking and delivery at the plant, the use of additional milk preservation methods such as the lactoperoxidase system may be considered. Improving farmers' knowledge by the implementation of technical assistance may also lead to a good quality product.

The cooperative investigated in the present study is one of many existing and similarly operating cooperatives in Costa Rica. The conclusions drawn based on this study may also be applicable to similar cooperatives in other parts of mountainous Costa Rica.

## Acknowledgements

Financial support was obtained from the Dutch Ministry of Foreign Affairs through the interuniversity collaborative project between the Universidad Nacional in Costa Rica and the University of Utrecht, The Netherlands. We are grateful for the logistical support provided by the Proyecto Salud de Hato.

## REFERENCES

1. ADESIYUN A.A., WEBB L., RAHAMAN S., 1995. Microbiological quality of raw cow's milk at collection centers in Trinidad. *J. Food Prot.*, **58**: 139-146.
2. BACHMAN M.R., 1987. Milk collection and raw milk hygiene: possibilities and limitations. In: Dairy development in East Africa. Proc. IDF seminar on appropriate dairy technology transfer for social and economic development in East Africa, Nairobi, Kenya, March 9-13, 1987. Brussels, Belgium, IDF, p. 69-71. (Bulletin)
3. BARRETT N.J., 1986. Communicable disease associated with milk and dairy products in England and Wales: 1983-1984. *J. Infect.*, **12**: 265-272.
4. BJÖRCK L., 1979. Enzymatic stabilization of milk - Utilization of the milk peroxidase for the preservation of raw milk. In: Proc. IDF Annual Sessions, Montreux, Switzerland, September 9-14, 1979.
5. BJÖRCK L., ROSEN C.G., MARSHALL V., REITER B., 1975. The antibacterial activity of the lactoperoxidase system in milk against *Pseudomonas* and other Gram-negative bacteria. *Appl. Microbiol.*, **30**: 199.
6. COLLINS S.J., BESTER B.H., MCGILL A.E., 1993. Influence of psychrotrophic bacterial growth in raw milk on the sensory acceptance of UHT skim milk. *J. Food Prot.*, **56**: 418-425.
7. COUSINS C.M., 1978. Milking techniques and the microbial flora of milk. In: XX International Dairy Congress, Paris, France, June 26-30, 1978, sci. & tech. Sessions, Part 60.
8. DE RHAM O., ANDREWS A.T., 1982. Qualitative and quantitative determination of proteolysis in mastitic milk. *J. Dairy Res.*, **49**: 587-596.
9. EVERSON T.C., 1984. Concerns and problems of processing and manufacturing in super plants. *J. Dairy Sci.*, **67**: 2095-2099.
10. FAIRBAIRN D.J., LAW B.A., 1986. Proteinases of psychrotrophic bacteria: their production, properties, effects and control. *J. Dairy Res.*, **53**: 139-177.
11. FAO, 1989. Milking, milk production hygiene and udder health. Rome, Italy, FAO. (Animal production health paper No. 78)
12. FAO, WHO, 1992. Codex Alimentarius Commission: "Revised general principles of food hygiene". Rome, Italy, FAO, Geneva, Switzerland, WHO. (Unpublished document CL/1992/30-FH)
13. GALTON D.M., ADKINSON R.W., THOMAS C.V., SMITH T.W., 1982. Effects of premilking udder preparation on environmental bacterial contamination of milk. *J. Dairy Sci.*, **65**: 1540-1543.
14. GALTON D.M., PETERSSON L.G., MERRIL W.G., 1986. Effects of premilking udder preparation practices on bacterial counts in milk and on teats. *J. Dairy Sci.*, **69**: 260-266.
15. GALTON D.M., PETERSSON L.G., MERRIL W.G., BANDLER D.K., SHUSTER D.E., 1984. Effects of premilking udder preparation on bacterial population, sediment, and iodine residue in milk. *J. Dairy Sci.*, **67**: 2580-2589.
16. GILLIS W.T., CARTLEDGE M.F., RODRIGUEZ I.R., SUAREZ E.J., 1985. Effect of raw milk quality on ultra-high temperature processed milk. *J. Dairy Sci.*, **68**: 2875-2879.
17. GRAAF T. de, DWINGER R.H., 1996. Estimation of milk production losses due to sub-clinical mastitis in dairy cattle in Costa Rica. *Prev. vet. Med.*, **26**: 215-222.
18. HEESCHEN W., REICHMUTH J., 1995. Mastitis: Influence on qualitative and hygienic properties of milk. In: IDF Proc. 3rd International mastitis seminar, Tel Aviv, Israel, May 28-June 1, 1995.
19. HOLDRIDGE L.R., 1967. Live zone ecology. San José, Costa Rica, Tropical Science Centre, 206 p.
20. IDF, 1986. Milk collection in developing countries. Brussels, Belgium, IDF. (Bulletin No. 205)
21. JOHNSTON A.M., 1990. Veterinary sources of foodborne illness. *Lancet*, October: 856-858.
22. KORHONEN K., 1980. A new method for preserving raw milk - the lactoperoxidase antibacterial system. *World Anim. Rev.*, **35**: 23-29.
23. LE ROUX Y., COLIN O., LAURENT F., 1995. Proteolysis in samples of quarter milk with varying somatic cell counts. 1. Comparison of some indicators of endogenous proteolysis in milk. *J. Dairy Sci.*, **78**: 1289-1297.
24. LE ROUX Y., GIRARDET J.M., HUMBERT G., LAURENT F., LINDEN G., 1995. Proteolysis in samples of quarter milk with varying somatic cell counts. 2. Component PP3 and -Casein-1P f29-105 and f29-107 of the proteose-peptone fraction. *J. Dairy Sci.*, **78**: 1298-1305.
25. O'CONNOR C.B., 1994. Rural dairy technology. Addis Ababa, Ethiopia, ILRI, 119 p. (Training manual 1)
26. POLITIS I., NG KWAI HANG K.F., GIROUX R.N., 1989. Environmental factors affecting plasmin activity in milk. *J. Dairy Sci.*, **72**: 1713-1718.
27. SAEMAN A.I., VERDI R.J., GALTON D.M., BARBANO D.M., 1988. Effect of mastitis on proteolytic activity in bovine milk. *J. Dairy Sci.*, **71**: 505-512.
28. SCHALM O.W., CAROLL E.J., JAIN N.C., 1971. Bovine Mastitis. Philadelphia, USA, Lea & Febiger.
29. SCHALM O.W., NOORLANDER D.O., 1957. Experiments and observations leading to the development of the California Mastitis Test. *JAVMA*, **130**: 199-207.
30. SHELLY A.W., DEETH H.C., MACRAE I.C., 1986. Growth of lipolytic psychrotrophic *Pseudomonas* in raw and ultra-heat-treated milk. *J. Appl. Bact.*, **61**: 395-400.
31. WHO, 1972. International standards for drinking water, 3rd ed. Geneva, Switzerland, WHO.

Reçu le 24.12.96, accepté le 21.5.97

**Résumé**

**de Graaf T., Romero Zuñiga J.J., Caballero M., Dwinger R.H.**  
Aspects de la qualité microbiologique de lait de vache dans une coopérative de petits éleveurs à Turrialba au Costa Rica

Des facteurs et des moments critiques influant sur la qualité hygiénique du lait ont été examinés au Costa Rica dans une coopérative de petits éleveurs laitiers qui livraient tous leur lait à une laiterie. Vingt-deux éleveurs ont participé à cette étude. Le lait de toutes les vaches en lactation a été examiné avec le CMT (California Mastitis Test). Le lait positif au CMT a subi un examen bactériologique (EB). En outre, des échantillons de lait ont été prélevés dans les cuves de stockage pour le comptage des cellules somatique (CCS) et EB. Parmi ces 22 élevages, 10 ont été choisis pour subir un examen plus approfondi de la qualité hygiénique du lait à des stades spécifiques critiques lors de manipulations diverses, du stockage, ainsi qu'après transport à la laiterie. La prévalence d'échantillons positifs au CMT a été de 21,8 p. 100, 36,6 p. 100 et 28,4 p. 100, respectivement pour les mois de juin, août et octobre. Le taux d'incidence de ces échantillons a été de 27,8 p. 100 pour cette période. Quatre-vingt-trois échantillons sur 126 prélevés dans les cuves de stockage pour CCS contenaient moins de 400.000 cellules/ml. Huit pourcent des échantillons positifs au CMT ont révélé la présence de *S. aureus*. Vingt-huit pourcent des échantillons prélevés dans les cuves de stockage contenaient *S. aureus* et 79 p. 100 des échantillons contenaient *E. coli*. Tous les échantillons pris dans les cuves de stockage de la laiterie avaient un nombre de CFU supérieur à  $2.10^6$  cellules/ml. Ce lait était fortement contaminé par des agents bactériologiques provenant de l'environnement. Le refroidissement du lait était inadéquat. La méthode de préparation des mamelles, les appareils de traite insuffisamment aseptisés et l'eau de nettoyage étaient les principales sources de contamination du lait.

**Mots-clés :** Bovin laitier - California Mastitis Test - Analyse microbiologique - Numération cellulaire somatique - Hygiène du lait - Contrôle de qualité - Coopérative de producteurs - Industrie laitière - Production laitière - Costa Rica.

**Resumen**

**de Graaf T., Romero Zuñiga J.J., Caballero M., Dwinger R.H.**  
Aspectos de la calidad microbiológica de la leche bovina en una cooperativa de pequeños productores de Turrialba en Costa Rica

Algunos de los factores y los momentos críticos que influyen en la calidad higiénica-sanitaria de la leche fueron investigados en una cooperativa de pequeños productores en Costa Rica, en la que todos los miembros entregan leche a una planta procesadora. Veintidos miembros de dicha cooperativa proveyeron los datos para este trabajo. Todas las vacas en producción fueron examinadas con el California Mastitis Test (CMT). Aquellas muestras con conteos celulares elevados fueron procesadas para posterior chequeo bacteriológico (CB). Además fueron recolectadas muestras de leche de los tarros para determinar la cantidad de células somáticas (CCS) y para un CB. De estos 22 miembros se seleccionaron 10, para un estudio adicional sobre la calidad higiénica de la leche colectada en momentos específicos durante el manipuleo, almacenamiento y después del transporte a la usina láctea. La prevalencia de muestras positivas al CMT fue destacada a 21,8%, 36,6% y 28,4% en Junio, Agosto y Octubre, respectivamente. La incidencia de muestras positivas al CMT en este período fue 27,8%. Ochenta y tres de las 126 muestras de leche tomadas de los tarros para obtener el conteo de células somáticas (CCS) conteneron menos de 400.000 células/ml. Ocho por ciento de las muestras positivas al CMT fue contaminado con *S. aureus*. Veintiocho por ciento de las muestras obtenidas de los tarros fue contaminado con *S. aureus* y un 79% con *E. coli*. Todas las muestras colectadas de los tarros en la usina láctea tuvo un conteo bacteriológico más de  $2.10^6$  células/ml. Este leche fue contaminada severo con agentes bacteriológicos del origen del medio ambiente. El enfriado de la leche fue encontrado inadecuado. Los resultados indican que los métodos de preparación de la ubre, el equipo de leche sucio y el agua utilizada para la limpieza fueron tomadas las principales fuentes de contaminación de la leche.

**Palabras clave:** Ganado de leche - Prueba California - Análisis microbiológico - Conteo de células somáticas - Higiene de la leche - Control de calidad - Cooperativa de productores - Industria lechera - Producción lechera - Costa Rica.