# The hygienic status of raw and sour milk from smallholder dairy farms and local markets and potential risk for public health in The Gambia, Senegal and Guinea

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| *B. cereus* | *Bacillus cereus* |
| cfu | colony forming units |
| *Cl. perfringens* | *Clostridium perfringens* |
| *E. coli* | *Escherichia coli* |
| EHEC | enterohaemorrhagic *Escherichia coli* |
| EIEC | enteroinvasive *Escherichia coli* |
| EPEC | enteropathogenic *Escherichia coli* |
| ETEC | enterotoxigenic *Escherichia coli* |
| FAO | Food and Agriculture Organization of the United Nations |
| IDF | International Dairy Federation |
| ISO | International Standardization Organization |
| KEBS | Kenya Bureau of Standards |
| *L. monocytogenes* | *Listeria monocytogenes* |
| ml | millilitre |
| *spp* | *subspecies* |
| *Staph* | *Staphylococcus* |
| TBC | Total bacterial count |

## SUMMARY

Milk in the countries of the West-African region is usually consumed raw or fermented. There is little information about the quality either at farm level (milk already infected with pathogenic agents or contaminated because of unhygienic handling) or at market level. As milk can, under certain conditions, pose a potential health hazard, particularly when consumed raw, it is not only the quantity of milk but also its quality and safety that needs to be investigated in order to both improve the nutritional base of an increasing population in urban and peri-urban areas and the marketing of milk and derived products.
The objectives of this study are the identification and quantification of bacterial contaminants and zoonotic agents at levels of producers, traders and vendors and associated risk for the consumer. Additionally, the study intended to assess the improvement in milk quality and hygiene via pasteurisation in Senegal.

Prior to the collection of raw and sour milk samples at key stations of the commodity chain, information on milk vendors, collectors and farmers was gathered by way of structured questionnaires for the provision of information on market structures and on the prevailing marketing chains of milk and derived products.
The microbial contamination of 915 milk samples from milk producers, collectors and vendors from The Gambia, Guinea and Senegal subsequently was investigated. Samples were tested for coliform bacteria, *E. coli*, coagulase-positive *Staphylococci* spp., *Salmonella* spp., *Bacillus cereus*, *Listeria* spp. and $H_2S$- reducing *Clostridia*. All methods used for culturing and identification of microorganisms comply with the International Standardization Organization (ISO) and International Dairy Federation (IDF).

The raw milk from herds and collectors from all three countries was highly contaminated. The highest contamination rate with coliform bacteria was found in The Gambia (88.6% above $5x10^4$ cfu/ml). The samples from Guinea and Senegal also had high rates of contamination (53.6% and 52.3% respectively). A large proportion of

coliform bacteria isolated in Guinea were confirmed to be *E. coli* (49.3% above $1x10^5$ cfu/ml). Coagulase-positive *Staphylococci* spp. (above $2x10^3$ cfu/ml) were frequent in all three countries, highest in Guinea with 33.3%, followed by Senegal (32.1%) and The Gambia (29.2%). *Listeria* spp. was isolated much more frequently in Guinea (10.1%). *Bacillus cereus* was isolated in many of the samples, especially in Senegal (35.2%) and in Guinea (33.3%). $H_2S$-reducing *Clostridia* spp. was particularly frequent in samples from Guinea (39.1%).

Fermented milk samples also were showing high contamination levels. The highest counts of coliform bacteria were found in fermented milk from The Gambia. 19.0% of the samples showed counts above $1x10^6$ cfu/ml and 17.6% of Gambian samples had counts above $1.5x10^5$ cfu/ml, similar to Guinea where 17.1% of samples belonged to that group. Only 45.1% of Gambian fermented milk but 81.9% of Guinean fermented milk had counts below $5x10^4$ cfu/ml.

The samples taken from yoghurt produced in the pasteurization units in Senegal were also highly contaminated. Only 63.2% of them had coliform counts below $5x10^4$ cfu/ml while 31.6% were above $1.5x10^5$ cfu/ml.

Most of the coliform bacteria in the samples of fermented milk from Guinea were confirmed to be *E. coli* (15.1% above $1x10^5$ cfu/ml), similar to results from raw milk. 10.5% of the samples from the pasteurization units had *E. coli* counts above $6x10^4$ cfu/ml.

High counts of coagulase-positive *Staphylococci* spp. above $1x10^5$ cfu/ml were found in 2.1% of Gambian samples and 3.7% of the Guinean samples. Even in the yoghurts from Senegal counts between $1x10^4$ cfu/ml and $1x10^5$ cfu/ml were found in 5.6% and counts above $1.2x10^5$ cfu/ml in 5.6% of samples.

These results clearly indicate that consumption of milk offered at local markets as raw or fermented milk poses a health risk. Pathogenic microorganisms present in milk could be one of the causes for the frequent occurrence of diarrhoeal diseases, especially in children (45,644 recorded cases of diarrhoea in children under 5 years in The Gambia in 2002). Though a direct link between the high contamination of milk and cases of milk-borne diseases in the population at present cannot be established in

the framework of this study, it can be assumed that the consumption of such milk might lead to mild to severe symptoms of food infection and/or -intoxication.

## INTRODUCTION

The biological value of milk is second to eggs in regards to essential amino acids, energy, calcium and vitamins. In many parts of the world it contributes significantly to the wholesomeness of human diets not only but especially during childhood. The increasing demand for milk and its products also makes it one of the prime commodities for marketing and trade. A study on the suitability of cow's milk for the dietary supplementation of rural Gambian children showed that milk availability and the ability to afford it are the most important factors which regulate its use as a food for children (Erinoso *et al*., 1992). Milk is considered an attractive source of energy, proteins and calcium for infants and young children who have few alternative sources for these nutrients.

Besides its beneficial effects on nutrition, milk though can also act as a vehicle for the transmission of diseases of bacterial (like brucellosis, tuberculosis, salmonellosis, listeriosis), viral (like hepatitis, foot-and-mouth-disease), ricketsial (Q-fever) or parasitological (toxoplasmosis, giardiasis) origin. Milk is an excellent culture and protective medium for certain microorganisms, particularly bacterial pathogens, whose multiplication depends mainly on temperature and competing microorganisms and their metabolic products. Where milk is produced under poor hygienic conditions and is not cooled, the main contaminants are usually lactic acid producers which cause rapid souring. Lactic acid has an inhibitory effect on pathogenic bacteria but this cannot be depended upon to provide a safe milk product (Heeschen, 1994).

The diseases transmissible to humans through the consumption of milk like brucellosis, tuberculosis, salmonellosis, listeriosis, *E.coli* infections and many others were described extensively in 1962 by Kaplan *et al*.

Pathogenic organisms in milk can derive from the cow itself, from human handlers and from the environment. Cows suffering from mastitis discharge large numbers of pathogens into the milk, like *Staphylococcus aureus*, *E. coli* and *Clostridium perfringens*. Microorganisms from soil, litter, feed, water, faeces and other items in a

farm environment commonly contaminate the surface of the udder and teats and the hair and skins of cows. From these sources they can get into the milk during milking. Unhygienic milking procedures and equipment used for milking, filtering, cooling, storing or distributing milk is also an important source of microorganisms. This situation is aggravated if the equipment is not properly cleaned and sanitized after use. Milk residues left on equipment and utensil surfaces provide nutrients to support the growth of many microorganisms, including pathogens (Bryan, 1983).

Individuals, who either do milk animals or handle milk can contribute additional organisms to the milk.

Pasteurisation or more severe heat-treatments applied to raw milk is the only way to ensure that pathogens present are killed and that the milk is safe. It also improves the shelf life of milk by reducing the number of non-pathogenic microorganisms that would otherwise cause spoilage (Burton, 1986).

## OBJECTIVES

The objective of this study was the assessment of the hygienic quality of milk and associated public health risks that originate from the consumption of milk and milk products.

The specific objectives were to provide an overview of milk production, processing and market systems and the identification of bacterial contamination and pathogenic agents in raw and sour milk samples along stages of this chain. Furthermore, the study intended to increase information about milking hygiene and to create baseline information for improvement of milk quality and the establishment of a quality control system.

## MATERIAL AND METHODS

The study was carried out between February 2000 and June 2002. Markets with an important role for the sale of milk were selected as a starting point for the identification of milk vendors, collectors and supplying milk producers. In The

Gambia four markets (Brikama, Soma, Brikamaba, and Basse) were selected, in Guinea five (Conakry (2), Dubréka, Boké, Kolaboui) and in Senegal two (Kolda, Tambacounda). Prior to the collection of milk samples, information was gathered by way of structured questionnaires on market structures and on the existing production and marketing chains for milk and derived products. Interviewed were 259 milk producers (The Gambia: 53; Guinea: 44; Senegal: 162), 102 collectors (The Gambia: 16; Guinea: 19; Senegal: 67) and 111 market vendors (The Gambia: 54; Guinea: 49; Senegal: 8). Additionally, 6 pasteurisation centres in Senegal participated in this activity.

Based on information obtained through the questionnaires, respective marketing ways were elaborated and individual producers, collectors and vendors in these chains were identified for the sampling of fresh and sour milk.

Milk samples were repeatedly collected from 330 milk vendors (The Gambia: 127; Guinea: 193; Senegal: 10), 79 collectors (The Gambia: 48; Guinea: 6; Senegal: 25) and 443 dairy farmers (The Gambia: 203; Guinea: 69; Senegal: 171) over a period of 12 months: during three months of the rainy season, once during the early dry season and once more during the late dry season. On the spot, pH-value, temperature and visible purity were recorded. Samples were transported in a cool box to the laboratory and immediately inoculated on culturing media.

Samples collected from the pasteurization units were taken at different levels: for milk at reception, at pasteurization temperature, while cooling, for yoghurt at inoculation temperature and from the final product.

All samples were tested for total mesophilic bacteria (only raw milk), coliform bacteria, *E.coli*, coagulase-positive *Staphylococci spp., Listeria spp., Salmonella spp., Bacillus cereus,* $H_2S$-reducing *Clostridia spp.* and Yeasts and Moulds (only fermented milk). Testing for Brucella spp. was not included, as this was the objective of another study (Unger *et al*., 2003).

All methods used for culturing and identification of microorganisms complied with the International Standardisation Organisation (ISO) or the International Dairy Federation (IDF). For details see Annexes 1 to 8.

The study intended to assess a) potential risk for consumers and b) raw milk's potential for dairy processing. Therefore, results were evaluated in reference to international standards.

Results were classified, where applicable, according to Standards set by the Council of the European Communities (Council Directive 92/46/EEC of 16 June 1992) and/or set by the Kenya Bureau of Standards (KEBS, Kenya standard 05-04, 1996) for unprocessed whole milk for human consumption:

| | **Council Directive 92/46/EEC** | **KEBS** |
|---|---|---|
| Plate count 30°C (per ml) | ≤ 100,000 | ≤ 2,000,000 |
| Coliform bacteria 30°C (per ml) | m= 0, M= 5, n=5, c= 2 (Guideline) | ≤ 50,000 |
| *Staphylococcus aureus* (per ml) | m= 500, M= 2,000, n= 5, c=2 | |
| *Salmonella spp.* (in 25g) | Absent, n=5, c=0 | |
| *Listeria monocytogenes* (in 1 g) | Absent, n=5, c=0 | |
| | m= threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all sample units does not exceed m;<br>M= maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more sample units is M or more;<br>n= number of sample units comprising the sample;<br>c= number of sample units where the bacterial count may be between 'm' and 'M', the sample still being considered acceptable if the bacterial count of the other sample units is 'm' or less. | |

Bacterial counts were evaluated for two criteria: For1) contamination levels: colonies which could be counted were grouped and named 'a'; results of colonies which did exceed countable numbers are expressed as "more than" and labelled 'b'. For contamination levels, subgroups were created to the $10^{th}$ exponent in ascending order. For 2) comparison with standards: Group 1 contains all samples which would have been accepted according to Kenyan standards with regards to total bacteria count and numbers of coliform bacteria and according to European standards with regards to coagulase-positive *Staphylococci spp., Salmonella spp.* and *Listeria monocytogenes.*

All samples in groups other than group 1 would have been rejected according to the two Directives.

Neither the EU Directive nor the Kenyan legislation does indicate an acceptable number of *E. coli* in fermented milk. Therefore, a number of 100,000 cfu/ml was taken in the course of this study as guideline value.

## RESULTS

### Questionnaires

*Herd health practices*

From 259 interviewed farmers, only 3 Gambians mentioned to own cattle breeds other than N'Dama. The average herd size is 72.1 heads (The Gambia: 78.7; Guinea: 79; Senegal: 58.6), adult females constitute about half of the herd (45.5% in The Gambia, 55.1% in Guinea and 46.9% in Senegal). The most important general clinical signs farmers observe in their herds are diarrhoea, conjunctivitis and weakness. Only in Guinea abortion and mastitis were additionally frequently mentioned. Prophylactic deworming is commonly practised in Guinea by 97.7% and in Senegal by 45.7% of the participating farmers. In The Gambia, only two farmers (3.8%) use prophylactic deworming to prevent helminthoses. The prophylactic use of trypanocides is more common in Senegal (63.0%) than in Guinea (22.7%) or The Gambia (22.6%). Acaricides are only used in Guinea by 47.7% of the interviewed farmers. 90.9% of the Guinean and 69.8% of the Senegalese milk producers are regularly vaccinating their herds against Black Quarter, Anthrax and/or Hemorrhagic Septicaemia. In The Gambia, only 22.6% of the herds are vaccinated.

*Milk production*

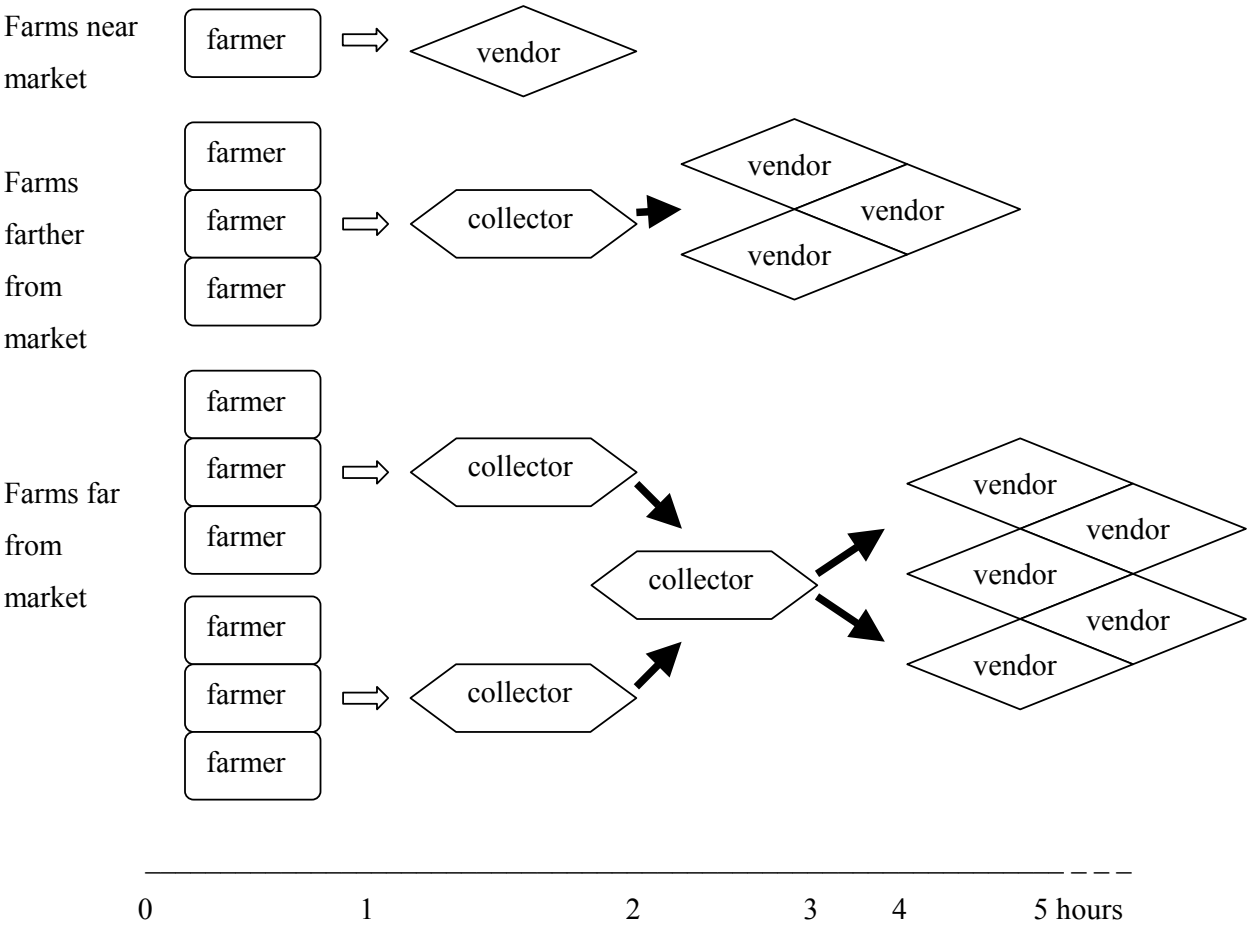
Milking in the three countries is usually done in the morning, only in Tambacounda in Senegal cattle are milked both at mornings and evenings. It is not common practice to clean the udder before milking. One exception is Kolda in Senegal, where 37.2% of the milkers are practicing it. The average milk yield per herd per day, according to

information given by the producers, is 10.2 litres in The Gambia, 7.0 litres in Senegal and 7.7 litres in Guinea.

*Milk marketing*

Milk collectors are buying milk at farm gate, transporting it to markets and then selling it in large volumes (usually 5 or 10 litres) to milk vendors. They collect milk either directly from the herds or from other collectors, depending on the distance between milk producers and markets (see also Somda *et al*., 2003 for more detailed information). Distance is also affecting certain practices like pooling milk from different producers, with larger distances leading to more producers supplying the same collector. Rural markets are usually supplied by milk producers themselves, without involvement of collectors. Milk in this case is often taken to the market and sold to consumers by (female) family members of the herd owner without adding milk from other producers. In cases where the market is far from the production areas, like Conakry in Guinea or Brikama in The Gambia, milk collectors buy milk from several producers, pool it and deliver it to the market where they sell the milk per 5 or 10 litres to market vendors, who are specialised in the sale of fermented milk. The longer the distance, the more collectors are involved in the transport. Figure 1 portrays this situation graphically.

Figure 1: Marketing chains of milk in reference to distances and time between producers and markets




The time collectors spend to transport milk to the market, varies between less than one hour and more than five hours. Depending on distance and availability, either public transport is used, or bicycles or horse/donkey carts. Shorter distances are covered by foot. Table 1 gives an overview of the time collectors spend to transport milk to markets.

Table 1: Distances from milk producers to markets (numbers of collectors per time category)

| Country | < 1 hour | 1-2 hours | 2-3 hours | 3-4 hours | 4-5 hours | > 5 hours |
|---|---|---|---|---|---|---|
| The Gambia | 8 | 4 | 3 | 0 | 0 | 1 |
| Senegal | 36 | 30 | 1 | 0 | 0 | 0 |
| Guinea | 0 | 9 | 0 | 0 | 8 | 2 |

The average quantity of milk that collectors purchase per day from producers varies greatly, depending on demand and on availability of milk. Demand is particularly high during the Muslim fasting month and availability is increased during the rainy season. Annually collectors buy 9.4 litres per day from producers in The Gambia and 18.7 litres in Senegal (Kolda: 30 litres). In Guinea milk is usually collected for 5 consecutive days before it is taken to the market as fermented milk. The mean volume sold to the vendors per operation is 121.4 litres. In Conakry it amounts to 356.7 litres (22.4 litres in the dry season and 488.9 in the rainy season).

Milk vendors at the markets, most of them women, are selling mainly fermented milk in small quantities (200ml) to consumers. The average volume of milk that they buy daily also varies depending on the availability. In The Gambia it is 14.4 litres, in Senegal 45 litres and in Guinea 191 litres (Conakry: 189.6 litres). Vendors spend about 10 hours at the market every day and need one or two days to sell the milk of the previous day as fermented milk. Fermented milk can be kept two to three days before it gets too sour, foamy and separates a lot of water. But it is usually used for home-consumption by the vendors before it gets spoiled.

The most common practice to clean milking equipment and containers in this study is washing them with cold water and soap. All milkers in The Gambia and in Senegal clean their utensils this way. Interestingly, all milkers in Guinea, in contrast, use hot water with soap. In The Gambia, all collectors (except one) and vendors wash with cold water and soap. The same practice is used in Tambacounda in Senegal. In Kolda (Senegal), all collectors (except one) and vendors use hot water and soap, and 31.8% of the collectors rinse afterwards with diluted household bleach.

## Laboratory Analyses

### The Gambia

A total of 378 samples were collected, 236 raw milk and 142 sour milk samples. The mean temperature at the moment of sample collection was 29.7°C (20-39) for raw milk and 28.6°C (19-33) for sour milk. The mean pH-values were 6.1 (5-7) and 4.2 (3.6-6) respectively. 84 of the raw milk and 12 of the sour milk samples were containing impurities.

Table 2 gives an overview of the numbers and percentages of samples positive for the various microorganisms.

Table 2: Prevalence of microorganisms in raw and sour milk (The Gambia)

| **Milk sample** | **coliform bacteria *** | ***E.coli*** ** | ***Staph. spp.*** *** | ***Salmonella spp.*** | ***Listeria spp.*** | ***B.cereus*** | ***Clostridia spp.*** |
|---|---|---|---|---|---|---|---|
| **Raw milk** | 209 (88.6%) | 53 (23.5%) | 69 (29.2%) | 1 (0.4%) | 5 (2.1%) | 40 (17%) | 41 (22.3%) |
| **Sour milk** | 78 (54.9%) | 32 (23.7%) | 24 (17%) | 0 | 0 | 18 (12.7%) | 17 (14.4%) |

* cfu/ml above $5x10^4$cfu/ml

** cfu/ml above $1x10^5$cfu/ml

*** cfu/ml above $2x10^3$cfu/ml

88.6% of the raw milk samples and 54.9% of the sour milk samples had coliform bacteria counts above $5x10^4$ colony forming units per millilitre (cfu/ml). The percentage of *E.coli* (counts above $1x10^5$cfu/ml) was higher in sour milk (32.7%) than in raw milk (23.5%). Coagulase-positive *Staphylococci* counts were above $2x10^3$cfu/ml in 29.2% of the raw milk samples and in 17.0% of sour milk samples. *Salmonella* spp. and *Listeria* spp. were only isolated in raw milk (0.4% and 2.1% respectively). The presence of *B.cereus* was more frequent in raw milk (17%) than in

sour milk (12.7%), the same applied to $H_2S$-reducing *Clostridia* spp. with 22.3% and 14.4% respectively.

In most of the contaminated samples only one of the investigated bacteria was isolated (40.3% in raw and 30.3% in sour milk). Combinations of two bacteria species were found in 10.2% in raw and 15.5% in sour milk, with three in 4.2% in raw milk and with even four bacteria species in 2.1% in raw milk.

*Total mesophilic bacteria counts in raw milk*

Mesophilic bacteria are bacteria with an optimal growth at temperatures between 25°C and 40°C.

Results grouped according to levels of contamination are given in Table 3.

Table 3: Total mesophilic bacteria counts in raw milk (The Gambia)

| | **cfu/ml** | **Bacterial Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<2x10^6$ | 21 (9.1%) | 21 (9.1%) |
| Group 2a | $2x10^6$-$1x10^7$ | 54 (23.5%) | 60 (26.1%) |
| Group 2b | $>3x10^6$ | 6 (2.6%) | |
| Group 3a | $1x10^7$-$1x10^8$ | 71 (30.9%) | 83 (36.1%) |
| Group 3b | $>3x10^7$ | 12 (5.2%) | |
| Group 4a | $1x10^8$-$1x10^9$ | 23 (10%) | 66 (28.7%) |
| Group 4b | $>3x10^8$ | 43 (18.7%) | |
| **Total** | | **230** | |

90.9% of the samples would not have been accepted according to Kenyan standards. Only 9.1% would have been accepted (group 1).

26.1% contained between $2x10^6$ and $1x10^7$ cfu/ml or at least $3x10^6$ cfu/ml (group 2a+b).

36.1% had a mesophilic bacteria count between $1x10^7$cfu/ml and $1x10^8$cfu/ml or at least $3x10^7$cfu/ml (group 3a+b) whilst 28.7% of the samples contained between $1x10^8$cfu/ml and $1x10^9$cfu/ml or at least $3x10^8$cfu/ml (group 4a+b).

*Coliform bacteria counts in raw and sour milk*

As for mesophilic bacteria counts, results of coliform bacteria counts were also grouped according to levels of contamination (Table 4 and Table 5).

Table 4: Coliform bacteria counts in raw milk (The Gambia)

| | **cfu/ml** | **Coliform Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<5x10^4$ | 85 (36.1%) | 85 (36.1%) |
| Group 2a | $5x10^4$-$1x10^6$ | 45 (19.1%) | 65 (27.5%) |
| Group 2b | $>1.5x10^5$ | 20 (8.5%) | |
| Group 3a | $1x10^6$-$1x10^7$ | 11 (4.6%) | 86 (36.4%) |
| Group 3b | $>1.5x10^6$ | 75 (31.8%) | |
| **Total** | | **236** | |

Of all raw milk samples collected, 64% showed coliform bacteria counts above the Kenyan acceptance limit of $5x10^4$cfu/ml. Only 36.1% would have been accepted (group 1). 27.5% of the raw milk samples contained coliform bacteria between $5x10^4$cfu/ml and $1x10^6$cfu/ml or at least $1.5x10^5$cfu/ml (group 2a+b). 36.4% of the samples had counts between $1x10^6$cfu/ml and $1x10^7$cfu/ml or at least $1.5x10^6$cfu/ml (group 3a+b).

Table 5: Coliform bacteria counts in sour milk (The Gambia)

| | **cfu/ml** | **Coliform Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<5x10^4$ | 64 (45.1%) | 64 (45.1%) |
| Group 2a | $5x10^4$-$1x10^6$ | 26 (18.3%) | 51 (35.9%) |
| Group 2b | $>1.5x10^5$ | 25 (17.6%) | |
| Group 3a | $1x10^6$-$1x10^7$ | 2 (1.4%) | 27 (19.1%) |
| Group 3b | $>1.5x10^6$ | 25 (17.6%) | |
| **Total** | | **142** | |

In the sour milk samples, coliform bacteria counts were lower than in raw milk. 45.1% had coliform bacteria counts below $5x10^4$cfu/ml (group 1). Only 19.1% of the samples showed counts between $1x10^6$cfu/ml and $1x10^7$cfu/ml or at least $1.5x10^6$cfu/ml (group 3a+b) while 35.9% of the samples contained between $5x10^4$cfu/ml and $1x10^6$cfu/ml or at least $1.5x10^5$cfu/ml (group 2a+b).

*Counts of Escherichia coli in raw and sour milk*

Coliform bacteria detected were further investigated for the presence and concentrations of *E.coli*. Table 6 and Table 7 indicate the numbers of *E. coli* detected in raw and sour milk specifically.

Table 6: Counts of *E. coli* in raw milk (The Gambia)

| | **cfu/ml** | ***E. coli* Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<1x10^5$ | 175 (77.4%) | 175 (77.4%) |
| Group 2a | $1x10^5$-$1x10^6$ | 5 (2.2%) | 35 (15.5%) |
| Group 2b | $>1.2x10^5$ | 30 (13.3%) | |
| Group 3a | $1x10^6$-$1x10^7$ | 0 | 16 (7.1%) |
| Group 3b | $>1.2x10^6$ | 16 (7.1%) | |
| **Total** | | **226** | |

*E. coli* above $1x10^5$cfu/ml could be isolated in 22.6% of the raw milk samples. The counts ranged between $1x10^5$cfu/ml and $1x10^6$cfu/ml or at least more than $1.2x10^5$ for 15.5% (group 2a+b) and above $1.2x10^6$cfu/ml for 7.1% of the samples (group 3).

Table 7: Counts of *E. coli* in sour milk (The Gambia)

| | **cfu/ml** | ***E. coli* Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<1x10^5$ | 103 (76.3%) | 103 (76.3%) |
| Group 2a | $1x10^5$-$1x10^6$ | 5 (3.7%) | 27 (20.0%) |
| Group 2b | $>1.2x10^5$ | 22 (16.3%) | |
| Group 3a | $1x10^6$-$1x10^7$ | 1 (0.7%) | 5 (3.7%) |
| Group 3b | $>1.2x10^6$ | 4 (3%) | |
| **Total** | | **135** | |

23.7% of the sour milk samples had counts of E.coli above $1x10^5$ cfu/ml. 20% of the samples had counts between $1x10^5$ and $1x10^6$ cfu/ml or at least $1.2x10^5$ cfu/ml (group 2a+b). Only 3.7% did exceed $1x10^6$ cfu/ml (group 3a+b).

*Counts of coagulase-positive Staphylococci spp. in raw and sour milk*

Both raw and sour milk samples were tested for the presence of coagulase-positive *Staphylococci* spp. The results are listed in Table 8 and Table 9.

Table 8: Counts of coagulase-positive *Staphylococci* spp. in raw milk (The Gambia)

| | **cfu/ml** | ***Staphylococci spp.* Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<2x10^3$ | 177 (75%) | 177 (75%) |
| Group 2a | $2x10^3$-$1x10^4$ | 16 (6.8%) | 19 (8.1%) |
| Group 2b | $>3x10^3$ | 3 (1.3%) | |
| Group 3a | $1x10^4$-$1x10^5$ | 16 (6.8%) | 37 (15.7%) |
| Group 3b | $>3x10^4$ | 21 (8.9%) | |
| Group 4a | $1x10^5$-$1x10^6$ | 1 (0.4%) | 3 (1.3%) |
| Group 4b | $>1.2x10^5$ | 2 (0.8%) | |
| **Total** | | **236** | |

25% of the samples were not within the limits of acceptance according to European standard. Counts above $2x10^3$cfu/ml but below $1x10^4$cfu/ml were seen in 6.8% of the raw milk samples (group 2a). High counts, between $1x10^4$cfu/ml and $1x10^5$cfu/ml or at least $1.2x10^4$, were found in 15.7% of the samples (group 3a+b). 1.3% of the milk samples had very high counts above $1x10^5$cfu/ml (group 4).

Table 9: Counts of coagulase-positive *Staphylococci* spp. in sour milk (The Gambia)

| | **cfu/ml** | ***Staphylococci spp.* Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<2x10^3$ | 117 (83%) | 117 (83%) |
| Group 2a | $2x10^3$-$1x10^4$ | 5 (3.6%) | 6 (4.3%) |
| Group 2b | $>3x10^3$ | 1 (0.7%) | |
| Group 3a | $1x10^4$-$1x10^5$ | 6 (4.3%) | 15 (10.6%) |
| Group 3b | $>3x10^4$ | 9 (6.4%) | |
| Group 4a | $1x10^5$-$1x10^6$ | 1 (0.7%) | 3 (2.1%) |
| Group 4b | $>1.2x10^5$ | 2 (1.4%) | |
| **Total** | | **141** | |

17.1% of the sour milk samples were above European standards for the production of raw milk products. 4.3% of the samples showed counts between $1x10^3$cfu/ml and $1x10^4$cfu/ml or at least $3x10^3$cfu/ml (group 2a+b). Moreover, 10.6% of the samples

contained between $1x10^4$cfu/ml and $1x10^5$cfu/ml or at least $3x10^4$ cfu/ml (group 3a+b). Very high counts above $1x10^5$ cfu/ml were seen in 2.1% of the sour milk samples (group 4a+b).

*Counts of Yeasts and Moulds in sour milk*

Only sour milk samples were analysed for the presence and concentration of yeasts and moulds. These micro-organisms are very common as source for spoilage in fermented milk products. Results are shown in Table 10.

Table 10: Counts of Yeasts and Moulds in sour milk (The Gambia)

| | **cfu/ml** | **Yeasts and Moulds Counts Distribution** |
|---|---|---|
| Group 1 | $<1x10^5$ | 3 (3%) |
| Group 2a | $1x10^5$-$1x10^6$ | 28 (27.7%) |
| Group 3a | $1x10^6$-$1x10^7$ | 59 (58.4%) |
| Group 4b | $>1x10^7$ | 11 (10.9%) |
| **Total** | | **101** |

More than half of the sour milk samples (58.4%) had counts between $1x10^6$cfu/ml and $1x10^7$cfu/ml (group 3). 27.7% of the samples contained between $1x10^5$cfu/ml and $1x10^6$cfu/ml (group 2). High counts above $1x10^7$cfu/ml were seen in 10.9% of the samples.

## Guinea

A total of 268 samples were collected, 69 were raw milk and 199 sour milk samples. The mean temperature at the moment of sample collection was 30.0°C (26-35) for raw milk and 29.2°C (23-35) for sour milk. The mean pH-values were 6.2 (5-7) and 4.1 (3.3-4.5) respectively. 25 of the raw milk and 125 of the sour milk samples were containing impurities.

Table 11 gives an overview of the numbers and percentages of samples positive for the various microorganisms.

Table 11: Prevalence of microorganisms in raw and sour milk (Guinea)

| Milk sample | Coliform Bacteria* | *E. coli*** | *Staph. spp.**** | *Salmonella spp.* | *Listeria spp.* | *B.cereus* | *Clostridia* spp. |
|---|---|---|---|---|---|---|---|
| **Raw** | 37 (53.6%) | 34 (49.3%) | 21 (33.3%) | 0 | 7 (10.1%) | 23 (33.3%) | 27 (39.1%) |
| **Sour** | 36 (18.1%) | 30 (15.1%) | 11 (6.8%) | 0 | 12 (6.5%) | 96 (48.2%) | 89 (44.7%) |

* cfu/ml above $5x10^4$cfu/ml

**cfu/ml above $1x10^5$cfu/ml

**cfu/ml above $2x10^3$cfu/ml

Coliform counts above $5x10^4$cfu/ml were found in 53.6% of the raw milk and in 18.1% of the sour milk samples. *E. coli* was also more frequent in raw (49.3%) than in sour milk (15.1%).

Coagulase-positive *Staphylococci* spp. could also be isolated in many samples. 33.3% of the raw milk samples and 6.8% of the sour milk samples contained more than $2x10^3$cfu/ml coagulase-positive *Staphylococci* spp. No *Salmonella* spp. could be isolated, neither in raw nor in sour milk. *Listeria* spp., in contrast, was found in both raw milk (10.1%) and sour milk (6.5%). *Bacillus cereus* and $H_2S$-reducing *Clostridia* spp. were more frequent in sour milk (48.2% resp. 44.7%) than in raw milk (33.3% resp. 39.1%).

Contamination with one of the investigated micro-organisms was seen in 34.8% of the raw and 38.7% of the sour milk samples. Many of the samples were contaminated with more than one bacteria species. In raw milk combinations of two (34,8%), three (11.6%), four (2.9%) and even five bacteria species (2-9%) were found. In sour milk, respective proportions were 33.2%, 4% and 0.5%.

*Total mesophilic bacteria counts in raw milk*

Total mesophilic bacteria counts were investigated only in raw milk. Table 12 summarizes the results.

Table 12: Total mesophilic bacteria counts in raw milk (Guinea)

| | **cfu/ml** | **Bacterial Counts Distribution** |
|---|---|---|
| Group 1 | $<2x10^6$ | 6 (8.7%) |
| Group 2a | $2x10^6$-$1x10^7$ | 25 (36.2%) |
| Group 3a | $1x10^7$-$1x10^8$ | 26 (37.7%) |
| Group 4a | $1x10^8$-$1x10^9$ | 12 (17.4%) |
| **Total** | | **69** |

91.3% of all raw milk samples had total bacterial counts above $2x10^6$cfu/ml and therefore would have been rejected according to Kenyan standards.

36.2% of the samples showed total bacteria counts between $2x10^6$cfu/ml and $1x10^7$cfu/ml (group 2) and 37.7% between $1x10^7$cfu/ml and $1x10^8$cfu/ml (group 3). 17.4% contained very high numbers of mesophilic bacteria between $1x10^8$cfu/ml and $1x10^9$cfu/ml (group 4).

*Coliform bacteria counts in raw and sour milk*

The concentration of coliform bacteria was analysed in raw and sour milk. Results are given in Table 13 and Table 14.

Table 13: Counts of coliform bacteria in raw milk (Guinea)

| | **cfu/ml** | **Coliform Counts Distribution** |
|---|---|---|
| Group 1 | $<5x10^4$ | 32 (46.4%) |
| Group 2a | $5x10^4$-$1x10^6$ | 0 |
| Group 2b | $>1.5x10^5$ | 37 (53.6%) |
| **Total** | | **69** |

53.6% of the raw milk samples did exceed the Kenyan limit of acceptance for coliform bacteria in raw milk, which is $5x10^4$cfu/ml. 46.4% were clearly below this limit.

Table 14: Counts of coliform bacteria in sour milk (Guinea)

| | **cfu/ml** | **Coliform Counts Distribution** |
|---|---|---|
| Group 1 | $<5x10^4$ | 163 (81.9%) |
| Group 2a | $5x10^4$-$1x10^6$ | 2 (1%) |
| Group 2b | $>1.5x10^5$ | 34 (17.1%) |
| **Total** | | **199** |

The number of coliform bacteria was much lower in sour milk than in raw milk. 81.9% of the sour milk samples were below $5x10^4$cfu/ml, while counts above $1.5x10^5$cfu/ml were found in 17.1% of the sour milk samples.

*Counts of Escherichia coli in raw and sour milk*

The concentrations of specifically *E. coli* within the group of coliform bacteria are contained in Table 15 and Table 16.

Table 15: Counts of *E. coli* in raw milk (Guinea)

| | **cfu/ml** | ***E. coli* Counts Distribution** |
|---|---|---|
| Group 1 | $<1x10^5$ | 35 (50.7%) |
| Group 2a | $1x10^5$$1x10^6$ | 0 |
| Group 2b | $>1.2x10^5$ | 34 (49.3%) |
| **Total** | | **69** |

49.3% of samples had counts above $1x10^5$ cfu/ml. 50.7% of the samples were below that concentration.

Table 16: Counts of *E. coli* in sour milk (Guinea)

| | **cfu/ml** | ***E. coli* Counts Distribution** |
|---|---|---|

| | | | |
|---|---|---|---|
| Group 1 | <1x10$^5$ | 169 (84.9%) | 169 (84.9%) |
| Group 2a | 1x10$^5$1x10$^6$ | 1 (0.5%) | 30 (15.1%) |
| Group 2b | >1.2x10$^5$ | 29 (14.6%) | |
| **Total** | | **199** | |

15.1% of the samples contained more than 1x10$^5$cfu/ml. The majority of the sour milk samples (84.9%) showed counts of *E.coli* less than 5x10$^4$cfu/ml.

*Counts of coagulase-positive Staphylococci spp. in raw and sour milk*

In both raw and sour milk, coagulase-positive *Staphylococci* were quantified. Results are summarized in Table 17 and Table 18.

Table 17: Counts of coagulase-positive *Staphylococci spp.* in raw milk (Guinea)

| | **cfu/ml** | ***Staph. spp.* Counts Distribution** | |
|---|---|---|---|
| Group 1 | <2x10$^3$ | 42 (66.7%) | 42 (66.7%) |
| Group 2a | 2x10$^3$-1x10$^4$ | 3 (4.8%) | 9 (14.3%) |
| Group 2b | >2x10$^3$ | 6 (9.5%) | |
| Group 3a | 1x10$^4$-1x10$^5$ | 5 (7.9%) | 6 (9.5%) |
| Group 3b | >9x10$^4$ | 1 (1.6%) | |
| Group 4a | 1x10$^5$-1x10$^6$ | 6 (9.5%) | 6 (9.5%) |
| Group 4b | >2x10$^5$ | 0 | |
| **Total** | | **63** | |

33.3% of the raw milk samples showed counts of coagulase-positive *Staphylococci spp*. above 2x10$^3$cfu/ml, this milk would not have been accepted by European standards for the production of raw milk products. Counts between 1x10$^4$cfu/ml and 1x10$^5$cfu/ml or at least 9x10$^4$cfu/ml were found in 9.5% of the samples (group 3a+b). For 9.5% of the raw milk samples extreme counts between 1x10$^5$cfu/ml and 1x10$^6$cfu/ml were recorded (group 4a).

Table 18: Counts of coagulase-positive *Staphylococci spp*. in sour milk (Guinea)

| | **Cfu/ml** | ***Staph. spp.* Counts Distribution** | |
|---|---|---|---|
| Group 1 | $<2x10^3$ | 152 (93.3%) | 152 (93.3%) |
| Group 2a | $2x10^3$-$1x10^4$ | 3 (1.8%) | 3 (1.8%) |
| Group 2b | $>2x10^3$ | 0 | |
| Group 3a | $1x10^4$-$1x10^5$ | 2 (1.2%) | 2 (1.2%) |
| Group 3b | $>9x10^4$ | 0 | |
| Group 4a | $1x10^5$-$1x10^6$ | 5 (3.1%) | 6 (3.7%) |
| Group 4b | $>2x10^5$ | 1 (0.6%) | |
| **Total** | | **163** | |

Sour milk contained less coagulase-positive *Staphylococci* spp. than raw milk. 93.3% of the samples showed counts below $2x10^3$cfu/ml. Very high counts between $1x10^5$cfu/ml and $1x10^6$cfu/ml, in contrast, were seen in 3.1% of the sour milk samples.

*Counts of Yeasts and Moulds in sour milk*

The concentration of yeasts and moulds was determined only in sour milk. Results are listed in Table 19.

Table 19: Counts of yeasts and moulds in sour milk (Guinea)

| | **Cfu/ml** | **Yeasts and Moulds Counts Distribution** |
|---|---|---|
| Group 1 | $<1x10^5$ | 0 |
| Group 2a | $1x10^5$-$1x10^6$ | 4 (2.1%) |
| Group 3a | $1x10^6$-$1x10^7$ | 99 (51.6%) |
| Group 4b | $>1x10^7$ | 89 (46.4%) |

| | | |
|---|---|---|
| **Total** | | **192** |

Yeasts and moulds were frequently isolated in sour milk samples. 51.6% of the samples contained between $1x10^6$cfu/ml and $1x10^7$cfu/ml. Excessive values above $1x10^7$cfu/ml were found in 46.4% of the samples.

**Senegal**

In Senegal, samples were taken from milk producers (n=171) and collectors (n=25), who are supplying small scale pasteurization units. Consequently, samples were collected at the pasteurisation units both during the pasteurization process (n=44) and from the final product (n=19). Additionally, sour milk samples from the market (n=10) were added to the study. In total, 240 raw milk samples and 29 sour milk samples were collected and analysed.

The mean temperature of the raw milk was 30.4°C (22-38°C) when it reached the pasteurisation unit. It was 31.7°C (24-34°C) in sour milk at the moment of sample collection at the market. The mean pH-value in raw milk was 6.0 (5.4-6.2) and in sour milk 4.6 (4.1-6.4). 36 of the raw milk samples and none of the sour milk samples had impurities.

The pasteurisation temperature used by the pasteurisation centres in Senegal is 85°C. This temperature is maintained until foam develops and then the milk is cooled down to around 45°C before it is inoculated with starter cultures. The milk is then left over night at ambient temperatures (around 25-30°C) for fermentation into yoghurt. Sugar and flavours are added and the yoghurt then is packaged into plastic bags, which are sealed.

Table 20 gives the overview of the numbers and percentages of samples positive for the various microorganisms.

Table 20: Prevalence of microorganisms in raw and sour milk and after pasteurization (Senegal)

| | **Coliform Bacteria*** | ***E.coli**** | ***Staph. spp.***** | ***Salmonella spp.*** | ***Listeria spp.*** | ***B.cereus*** | ***Clostridia spp.*** |
|---|---|---|---|---|---|---|---|
| **Raw Milk total** | 102 (52.3%) | 26 (13.3%) | 63 (32.1%) | 3 (1.5%) | 1 (0.5%) | 69 (35.2%) | 22 (11.2%) |
| **Raw milk at farm level** | 77 (45.3%) | 17 (9.9%) | 47 (27.5%) | 3 (1.8%) | 1 (0.6%) | 57 (33.3%) | 20 (11.7%) |
| **Raw milk at collector level** | 25 (100%) | 9 (36%) | 16 (64%) | 0 | 0 | 12 (48%) | 2 (8%) |
| **Pasteurisation Unit** | 16 (25.4%) | 3 (7.9%) | 5 (8.1%) | 0 | 0 | 24 (38.1%) | 7 (11.1%) |
| **Sour milk** | 1 (10%) | 0 | 2 (20%) | 1 (10%) | 0 | 3 (30%) | 3 (30%) |

* cfu/ml above $5x10^4$cfu/ml

**cfu/ml above $1x10^5$cfu/ml

***cfu/ml above $2x10^3$cfu/ml

In general, contamination in raw milk is higher than in sour milk, except for *Salmonella* spp. and $H_2S$-reducing *Clostridia* spp. Comparing the different levels of the production-consumption chain, contamination is highest at collector's level. All raw milk samples from collectors had coliform bacteria counts above $5x10^4$cfu/ml. The same applies to the number of *E. coli* 36% of the samples from collectors contained more than $1x10^5$cfu/ml *E. coli*. Even more striking are the counts of coagulase-positive *Staphylococci* spp. 64% of the samples from collectors showed counts above $2x10^3$cfu/ml.

In contrast, counts of coliform bacteria were much lower in sour milk than in raw milk. Only 10% of the sour milk samples had counts above $5x10^4$cfu/ml. However, sour milk contained a high number of coagulase-positive *Staphylococci* spp. (20%

above $2x10^3$cfu/ml) and *Salmonella* spp. (10%), whose growth is not so much affected by the low pH-value of sour milk. $H_2S$-reducing *Clostridia* spp. were also more frequent in sour milk (30%) than in raw milk (11.2%). *Bacillus cereus* was isolated from all levels of the chain at high rates, 35.2% in raw milk and 30% in sour milk.

In 45.4% of the raw and in 35.7% of the sour milk samples only one investigated bacteria species was isolated. Combinations of two bacteria species were found in 15% and 14.3% respectively. Combinations of three and four bacteria species were only seen in raw milk (4.2% and 0.4% respectively).

Pasteurization did reduce bacterial load considerably. Compared to milk prior to pasteurization, the number of coliform bacteria in pasteurized milk is reduced by the factor 6.4. The number of *E. coli* is reduced by the factor 8.7. The highest pasteurisation effect is seen in coagulase-positive *Staphylococci* spp., which are reduced by the factor 12.6.
Pasteurized milk still did contain *B. cereus* (38.1%) and $H_2S$-reducing *Clostridia* spp. (11.1%), which can be explained by the fact that these are spore-forming bacteria resisting heat-treatment.

*Total mesophilic bacteria counts in raw milk and pasteurized milk*

Total mesophilic bacteria were considerably reduced after pasteurization. Results are shown in Table 21.

Table 21 : Total mesophilic bacteria counts in raw and pasteurized milk (Senegal)

| | **cfu/ml** | **Raw milk** | | **Pasteurized milk** |
|---|---|---|---|---|
| Group 1 | $<2x10^6$ | 44 (22.5%) | 44 (22.5%) | 30 (68.2%) |
| Group 2a | $2x10^6$-$1x10^7$ | 43 (21.9%) | 43 (21.9%) | 6 (13.6%) |
| Group 3a | $1x10^7$-$1x10^8$ | 41 (20.9%) | 43 (21.9%) | 3 (6.8%) |
| Group 3b | $1.5x10^7$ | 2 (1%) | | 0 |
| Group 4a | $1x10^8$-$1x10^9$ | 21 (10.7%) | 66 (33.7%) | 0 |
| Group 4b | $>3x10^8$ | 45 (23%) | | 5 (11.4%) |
| **Total** | | **196** | | **44** |

77.6% of total raw milk samples and 31.8% of the pasteurized milk samples would not have been accepted according to European standards. Particular high counts above $1x10^8$cfu/ml were seen in 33.7% of the raw milk but only in 11.4% of the pasteurized milk. This comparison shows clearly the positive effect of pasteurisation on bacterial load but also clearly points to the risk of post-pasteurization contamination.

*Coliform bacteria counts in raw and pasteurized milk*

The concentration of coliform bacteria was determined in both raw and pasteurized milk. Results are listed in Table 22.

Table 22: Coliform bacteria counts in raw and pasteurized milk (Senegal)

| | **cfu/ml** | **Raw milk** | | **Pasteurized milk** | |
|---|---|---|---|---|---|
| Group 1 | $<5x10^4$ | 93 (47.7%) | 93 (47.7%) | 47 (74.6%) | 47 (74.6%) |
| Group 2a | $5x10^4$-$1x10^6$ | 31 (15.9%) | 47 (24.1%) | 4 (6.4%) | 14 (22.2%) |
| Group 2b | $>1.5x10^5$ | 16 (8.2%) | | 10 (15.9%) | |
| Group 3a | $1x10^6$-$1x10^7$ | 8 (4.1%) | 55 (28.2%) | 0 | 2 (3.2%) |
| Group 3b | $1.5x10^6$ | 47 (24.1%) | | 2 (3.2%) | |
| **Total** | | **195** | | **63** | |

Pasteurization did reduce markedly the concentration of coliform bacteria. 52.3% of the raw milk samples had counts above $5x10^4$cfu/ml, this number decreased to 25.4% of the pasteurized milk samples. 28.2% of the raw milk showed counts above $1x10^6$cfu/ml, in pasteurized milk only 3.2% samples had such high counts.

**Comparison of the three countries**

*Raw milk*

In general, all raw milk tested from The Gambia, Guinea and Senegal was highly contaminated. The highest contamination rate with coliform bacteria was found in The Gambia with 88.6% of all samples having contamination levels above $5x10^4$ cfu/ml, followed by Guinea and Senegal with respective rates of 53.6% and 52.3%. *E. coli* was particularly more frequently isolated in Guinea, where 49.3% of the samples contained

more than $1x10^5$ cfu/ml. Coagulase-positive *Staphylococci* spp. (above $2x10^3$ cfu/ml) were frequent in all three countries, highest in Guinea with 33.3%, followed by Senegal (32.1%) and The Gambia (29.2%). *Listeria* spp. was isolated more frequently in Guinea (10.1%). *Bacillus cereus* was isolated in about one third of samples, especially in Senegal (35.2%) and in Guinea (33.3%). $H_2S$-reducing *Clostridia* spp. were particularly frequent in samples from Guinea (39.1%).

*Fermented milk*

The highest counts of coliform bacteria were found in fermented milk from The Gambia. 54.9% of the samples had counts above $5x10^4$ cfu/ml. The sour milk samples from Guinea and Senegal were much less contaminated with coliform bacteria, with 18.1% (Guinea) and 10% (Senegal) of samples containing more than $5x10^4$ cfu/ml.

*E. coli* was found in concentrations above $1x10^5$ cfu/ml in 23.7% of the Gambian samples and in 15.1% of the Guinean samples, whereas none of the Senegalese samples exceeded this concentration. High counts of coagulase-positive *Staphylococci* spp. above $2x10^3$ cfu/ml were found in Senegalese (20%) and Gambian (17%) samples. This concentration was less frequent in Guinea (6.8%). *Salmonella spp*. was only isolated in Senegal (10%) and *Listeria spp.* only in Guinea (6.5%). The occurrence of *B. cereus* and $H_2S$-red. *Clostridia spp.* in sour milk was very high throughout, especially in Guinea (48.2% resp. 44.7%) and Senegal (both 30%), less in The Gambia (12.7% resp. 14.4%). Table 23 summarizes results from the three countries.

Table 23: Comparison of contamination rates of raw and sour milk in The Gambia, Guinea and Senegal

| **Country** | **Milk product** | **Coliform bacteria*** | ***E.coli*** ** | **Coag.-pos. *Staph. spp.*** *** | ***Salmonella spp.*** | ***Listeria spp.*** | ***B.cereus*** | **$H_2S$-red. *Clostridia spp.*** |
|---|---|---|---|---|---|---|---|---|
| The Gambia | Raw milk (n=236) | 88.6% | 23.5% | 29.2% | 0.4% | 2.1% | 17% | 22.3% |
| Guinea | Raw milk (n=69) | 53.6% | 49.3% | 33.3% | 0 | 10.1% | 33.3% | 39.1% |
| Senegal | Raw milk (n=196) | 52.3% | 13.3% | 32.1% | 1.5% | 0.5% | 35.2% | 11.2% |
| The Gambia | Sour milk (n=142) | 54.9% | 23.7% | 17% | 0 | 0 | 12.7% | 14.4% |
| Guinea | Sour milk (n=199) | 18.1% | 15.1% | 6.8% | 0 | 6.5% | 48.2% | 44.7% |
| Senegal | Sour milk (n=10) | 10% | 0 | 20% | 10% | 0 | 30% | 30% |
| Senegal | Past. Milk (n=63) | 25.4% | 7.9% | 8.1% | 0 | 0 | 38.1% | 11.1% |

* cfu/ml above $5x10^4$cfu/ml

**cfu/ml above $1x10^5$cfu/ml

***cfu/ml above $2x10^3$cfu/ml

## DISCUSSION

Whereas total bacterial counts does mainly reflect the time which elapsed since milking and the prevailing ambient temperature (if milk is not chilled), especially coliform counts are associated with milking hygiene since they are mainly of faecal origin. Under ambient temperatures that prevail in the tropics, a bacterial cell in milk will multiply to 2,000,000 cells in a typical generation time of 20 minutes within 7 hours. This value is set by the Kenyan Bureau of Standards for total bacterial plate counts in raw milk. However, if the generation time is reduced to only two hours by lowering the temperature of the milk to below 10°C, the same bacterial cell would only multiply to 32 cells within the same period (FAO, 1979). With an additional

higher initial load of bacterial cells due to unhygienic milking, the time taken to reach upper threshold levels is reduced considerably.

Poor hygiene often arises from poor handling at the farm, at collection centres, during transportation and at retail points. Common sources of bacterial contamination, especially coliforms, are faeces (of animal or human origin), personnel, water and containers. A high bacterial count reduces the shelf life of milk and enhances the risk of milk-borne bacterial infections and intoxications if the milk is not properly heated or if thermal injured pathogens recover under suitable temperatures (Andrew and Russel, 1984; Kayihura *et al*., 1987).

*Coliform bacteria*

Coliform bacteria are usually used as marker organisms in the examination of pasteurized milk and ice cream. According to regulations of the European Union (Directive 92/46/EEC), coliform bacteria in pasteurized milk should not exceed 5 cfu/ml. Though it has been suggested by many authors to replace coliform bacteria by Enterobacteriacea as indicator organisms, their use is still common practice in many laboratories.

In the Kenyan legislation, raw milk has to contain less than $5x10^4$ cfu/ml coliform bacteria.

In this survey 59.2% of all raw milk samples and 32.76% of all sour milk samples did exceed this limit. This high contamination rate is due to very poor hygienic conditions during milking, handling and transportation of milk and the way it is offered for sale.

Milking in all three countries is done by hand. The cows are usually milked at the same spot where they were tied up for the night. Udders of the animals consequently come in contact with faeces and the udders are usually not washed before milking. It neither is not common practice among milkers to wash their hands with soap before milking. When handling the animals, like tying their hind legs prior to milking, milkers' hands and clothes become further contaminated. Bacteria then are transferred to the milk during milking. Many milkers even are dipping their fingers into the milk to moisten them which adds dirt and bacteria to the milk.

The equipment used for milking, filtering and storing the milk is also an important factor contributing to high contamination. Cleaning of equipment is usually done with cold water and soap, as evaluated by questionnaires administered to farmers prior to milk sampling. Only milk producers and collectors in Kolda, Senegal, are aware of the importance of udder cleaning and use of hot water and disinfectant for cleaning of equipment. According to questionnaire results, 72.7% of interviewed milk collectors in Kolda are using hot water to clean their equipment and 31% rinse additionally with household bleach. 37.5% of milkers in Kolda are cleaning the udders prior to milking. This practise though is not reflected in the microbiological results as still 67% of raw milk from collectors and farmers from Kolda contain more than $2x10^6$ mesophilic bacteria per ml and 48.9% more than $1x10^5$ coliform bacteria per ml.

A further contributing factor to contamination is flies. Wherever there is milk, there are also flies and it is very difficult to prevent flies from falling into the milk and by that way contaminating the milk.

The high initial contamination of milk already at herd level is further increasing with time along the marketing chain, as bacteria multiply quickly, especially without cooling.

Counts of coliform bacteria were lower in fermented than in fresh milk which can be explained with the low pH-value in fermented milk. Especially fermented milk from Guinea had a very low pH, due to the fact that milk is usually collected during six consecutive days before it eventually is transported as fermented milk to the markets. Samples of fermented milk collected from the central market in Conakry and Kolda had surprisingly low counts (below $1x10^2$ cfu/ml and $1x10^3$ cfu/ml respectively). Further investigations revealed that such milk sold at markets was actually reconstituted milk.

*Escherichia coli*

*E. coli* strains are a common part of the normal facultative anaerobic microflora of the intestinal tracts of humans and warm-blooded animals. Most *E. coli* strains are harmless commensals; however, some strains are pathogenic and cause diarrhoeal disease.

Presently, four main types of pathogenic *E.coli* have been associated with food-borne disease: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic *E.coli* (*E.coli* O157:H7; EHEC).

In developing countries ETEC without doubt are an important cause of diarrhoea in all people's age groups due to poor hygiene; in the tropics, isolation rates of ETEC between 10 and 70% have been reported from children and adults suffering from diarrhoea (Gross and Rowe, 1985). ETEC are a major cause of infantile diarrhoea in such less developed countries, where investigations on infants, prospectively followed up by frequent household surveillance, suggest that children during the first 2-3 years of life may suffer from as many as two to three clinical infections with ETEC per child per year (Black et al., 1982).

In this study, no attempt was made to differentiate strains of isolated *E. coli*. A high contamination rate with *E.coli* in general does not necessarily implicate a public health risk, but is an indication for potential risk.

Contamination of fresh and fermented milk with *E. coli* was high. 24.6% of the raw milk samples and 20.9% of the sour milk samples showed counts above $5x10^4$ cfu/ml. Counts of *E. coli* were lower in fermented than in fresh milk. The low pH in fermented milk inhibits growth of pathogenic *E. coli*. In a study carried out by Frank and Marth in 1977, pathogenic *E. coli* in fermented milk with pH 4.5 did decrease by 0.7-1.3/ 7 d (log/time of strain *2 TOX*) and by 1.4-2.3/ 7 d (log/ time of strain *2 Inv*).

*Coagulase-positive Staphylococci spp.*

Enterotoxin-producing staphylococcal species, *Staphylococcus aureus* in particular, are the leading cause of food-borne illness throughout the world. Sickness results from the ingestion of one or more preformed staphylococcal enterotoxins in staphylococcus-contaminated food. Milk and milk products can become contaminated unless good hygiene (including mastitis control) occurs on farms, the milk is adequately pasteurized, and precautions are taken to prevent contamination and subsequent growth of *staphylococci* during the manufacturing processes and in the finished products. The pathogenicity of *Staphylococcus aureus* has been recognized for many years, it may cause mastitis and/or skin diseases in milk-producing animals or lead to

food-borne intoxications in consumers of milk and milk products (Bolstridge and Roth, 1985). Other species like *S. intermedius* and *S. hyicus*, which are likewise found in milk, also have the potential to produce enterotoxin that causes gastroenteritis. Production of coagulase is a characteristic used to distinguish pathogenic from non-pathogenic strains of *S. aureus* (Baird-Parker, 1963). This characteristic is shared with other enterotoxin-producing *Staphylococci* (*S .intermedius* and *S. hyicus*) and therefore, the analysis of this study did not focus on *S. aureus* alone but on coagulase-positive *Staphylococci*.

According to European legislation (Directive 92/46/EEC), raw cow's milk for human consumption must not contain more than $5x10^2$ cfu/ml (*S. aureus*). 29.8% of the raw milk samples collected during this study did exceed this limit. The acceptable limit was exceeded by 14.6% of the fermented milk. Contamination with coagulase-positive *Staphylococci* was particularly high in raw milk from Guinea (42.9% above $5x10^2$ cfu/ml). The source of this contamination is difficult to trace due to the ubiquitous nature of *staphylococci*. *S. aureus* is carried in the nose of some 30% of persons, who also tend to be skin carriers, and it is frequent in a number of animals (Olsvic *et al*., 1982).

Pasteurisation did reduce coagulase-positive *Staphylococci* significantly (4.84% above $5x10^2$ cfu/ml) but could not eliminate them. If recommended pasteurization temperature and time (63°C for 30 minutes or 72°C for 15 seconds) are not respected, *S.aureus* is able to survive heat treatment, as studies revealed. Bhatt and Bennett (1964) heated a mixture of 171 strains of *S. aureus* in milk. They found 1.5% to have survived after 30 minutes at 62°C and no survivors after 45 minutes. Unlike the vegetative germs, the enterotoxins of *staphylococci* are remarkably resistant to heat. Baird-Parker (1990) states the temperature conditions for destruction of *Staphylococcus aureus* to be: 0.43 – 8 minutes at 60°C compared to 3 – 8 minutes at 121°C for enterotoxin.

If the initial contamination with enterotoxin-producing *Staphylococci* is high ($10^5$ cfu/ml), enough enterotoxin could be produced which will withstand pasteurization. This certainly implies a public health risk. Such risk would have been exerted by 6.2% of all raw milk samples and 5% of the sour milk samples in this study. In Senegal

alone, where raw milk undergoes pasteurization, 14.3% of the raw milk samples had respective high counts prior to pasteurization.

*Salmonella spp.*

Food-borne disease outbreaks associated with *Salmonella* have been known for a long time and continue to be a problem in both developed and developing countries (Bean *et al*., 1990). Most outbreaks have implicated foods containing eggs or poultry products. Nevertheless, there have been several outbreaks of salmonellosis for which milk or milk products were responsible. Contamination of raw milk usually takes place by *salmonellae* from external sources as they are rarely shed into the milk. Sources can be faeces, the farmer or his family, polluted water, dust etc. Healthy cows can also regularly excrete *salmonellae* in their dung.

Salmonellosis is caused by the ingestion of living bacteria of the *salmonella* group. In contrast to staphylococcal food poisoning, the ingestion of viable cells is necessary for salmonellosis. The number of cells which have to be ingested to cause disease varies according to the type of strain, the type of food consumed and the consumer. Numbers varying from one cell of *S. typhi* to several millions of, for example *S. derby* or *S. anatum,* are mentioned (D'Aoust, 1989). Infants as well as very young and aged people are especially sensitive and a smaller dose can result in disease.

In regards to pathogenesis, two main disease groups can be distinguished: (1) typhoid and paratyphoid fevers, and (2) gastro-enteric infections. The latter, also indicated as "enterocolitis", are the most frequent detected. The symptoms of this disease-complex are caused by diarrhoeagenic toxins produced during the invasive action of *salmonellae*.

In the present study, *Salmonellae* were rarely isolated from the milk samples. Only 0.4% of the raw milk samples in The Gambia, none in Guinea and 1.5% in Senegal also contained *Salmonellae*. One out of ten sour milk samples collected from markets in Senegal contained *Salmonella* spp., not surprising as the reported minimum pH for growth is 3.8 (Chung and Goepfert, 1970). None of the pasteurized milk samples contained *Salmonella spp*. *Salmonellae* are sensitive to heat treatment and are readily destroyed at milk pasteurization temperatures.

*Listeria spp.*

Listeriosis has emerged as one of the major foodborne disease during the last decade. It is an atypical food-borne disease of major public health concern because of the severity and the non-enteric nature of the disease: meningitis, septicaemia and abortion, a high case-fatality rate (around 20-30% of cases), a frequently long incubation time, and a predisposition for individuals who have underlying conditions leading to the impairment of T-cell-mediated immunity. *Listeria monocytogenes* differs in many respects from most other foodborne pathogens: it is ubiquitous, is resistant to diverse environmental conditions such as low pH and high NaCl concentrations, and is microaerobic and psychrotrophic. The various ways in which the bacterium can enter into food processing plants, its ability to survive for long periods of time in the environment (soil, plants and water) and on foods, and its ability to grow at very low temperatures (2-4°C) have made *L. monocytogenes* a major concern for the agrifood industry during the last decade (Rocourt and Cossart, 1997).

*L. monocytogenes* may be excreted into the milk from a cow suffering from mastitis (Gitter *et al.*, 1980). The by far most common source is through contamination from the environment. Husu (1990) comments that faecal contamination may be an important source of contamination for raw milk. The proportion of cattle excreting *Listeria* spp. has been reported to be as high as 52% and Husu suggests that this may result in the contamination of the teat surface and hence contamination of the raw milk. Cattle feeds have also been reported to be an important source of *Listeria*, especially silage of poor quality (Gray, 1960). Direct contamination from milking equipment is furthermore a source of contamination.

Very few milk samples collected in the course of this study did contain *Listeria* spp. The bacterium could be isolated only in 2.1% of raw milk samples in The Gambia, in 0.5% of raw milk samples in Senegal but in 10.1% of raw milk samples in Guinea (6.5% of sour milk samples in Guinea).

It is very difficult to determine where exactly contamination has taken place because *Listeria* spp. is ubiquitous and able to survive for long periods of time in the environment. It seems very likely though that contamination takes place while milking, as udders are frequently covered with faeces which might contain *Listeria* spp.

*Bacillus cereus*

*Bacillus cereus* causes two different types of food poisoning: the diarrhoeal type and the emetic type. The first type is caused by an enterotoxin produced during the vegetative growth of *B. cereus* in the small intestine, while emetic toxin is produced by cells growing in the food. For both types of foodborne illness, the food involved is usually been heat-treated, and surviving spores are the source of the illness. *B. cereus* is not a competitive microorganism but grows well after cooking and cooling (<48°C). Heat treatment will cause spore germination, and in the absence of competing flora, *B. cereus* grows well. *B.cereus* is widespread in nature, being frequently isolated from soil and growing plants. Contamination of milk and milk products with *B.cereus* is caused by spread of the bacterium from soil and grass to the udders of cows and into the raw milk. Through sporulation, *B. cereus* spores survive pasteurization, and after germination, the cells are free from competition from other vegetative cells (Anderson *et al*., 1995).

This ability to withstand pasteurization is also reflected in the results from this study. *B. cereus* was found very frequently in both raw (28.5%) and sour milk (30.3%). In pasteurized milk, the percentage was even higher (38.1%).

*$H_2S$-reducing Clostridia spp.*

The most important bacterium among the group of $H_2S$-reducing *Clostridia* is *Clostridium perfringens*. *Cl. perfringens* type A food poisoning annually ranks among the most common foodborne diseases in the United States and Europe. However, since most cases of this disease go undetected, official statistics significantly understate the true prevalence and impact of *Cl. perfringens* type A food poisoning. The disease is characterized by diarrhoea and abdominal cramps. The symptoms result from the release of enterotoxins by cells undergoing sporulation in the lower gastrointestinal tract.

*Cl. perfringens* is widely distributed in soil, dust, vegetation and raw, dehydrated and cooked food; it is part of the normal flora of the intestinal tract of man and animals. The presence of spore populations is likely to be the result of faecal contamination.

The most common food vehicles for *Cl. perfringens* type A food poisoning are meat and poultry (Bean and Griffin, 1990) but dairy products can be contaminated as well. The common factor in all outbreaks is survival of only spores in cooked food. After cooking, slow cooling at temperatures between 55°C and about 35°C allows the heat-activated spores of *Cl. perfringens* to germinate and multiply very rapidly in the absence of competition from other organisms. Consequently, the infective level of microorganisms necessary to survive passage through the stomach and reach the lower intestinal tract in sufficient numbers to permit growth and sporulation is achieved after a few hours.

$H_2S$-reducing *Clostridia* were isolated in many milk samples collected during this study. 31.6% of the raw milk and 34.5% of the sour milk samples did contain $H_2S$-reducing *Clostridia*. It was also isolated in 11.1% of the pasteurized milk samples.

It is assumed that faecal contamination of raw milk is the source of contamination as *Cl. perfringens* is present in the intestinal tract of cattle. *Cl. perfringens* does not grow well in fermented foods as the minimal pH for growth is reported to be 5.5-5.8. The mean pH of local sour milk is 4.3. Processing of raw milk into sour milk (fermentation at ambient temperatures at 24°C) gives the vegetative cells though sufficient time to multiply before the low pH takes effect on their growth.

*Yeasts and Moulds*

The spoilage of yoghurt by yeasts is well documented (Davis *et al*., 1971; Arnott *et al*., 1971). The presence of yeasts as primary contaminants of yoghurt is encouraged by high acidity, sugar content, low storage temperature and the types of preservatives used. The control of yeast spoilage has become one of the main concerns of yoghurt manufacturers. Davis et al. (1971) recommended that yoghurts should be held at 5°C or below and consumed within 10 days.

Sour milk samples collected during this study had very high counts of yeasts and moulds. 83.6% of all samples showed counts above $10^6$ cfu/ml, almost all of them above $10^5$ cfu/ml. Dublin Green and Ibe (1987) analysed yoghurts produced commercially in Lagos, Nigeria and reported total counts of yeast in the range of $10^5$

and $10^6$ cfu/ml in about 40% of the samples. On the whole, 73% of the 100 samples they analysed had counts above $10^3$ cfu/ml.

The extend of contamination observed in this study suggests both a high initial contamination level during manufacture and poor storage conditions, leading to growth of the yeast contaminants. Davis (1971) suggested a standard in which yoghurt samples with less than 10 yeast cfu/g were satisfactory while those with more than 100 yeast cfu/g and more than 10 mould cfu/g were unsatisfactory. On this basis, almost all of the samples analysed would be considered unsatisfactory due to yeast and mould contamination.

## CONCLUSION

The results of this study clearly indicate that milk, which is locally produced and consumed as raw or fermented milk, poses a public health risk for consumers. A sizeable number of samples was highly contaminated with coliform bacteria as well as with potentially pathogenic bacteria.

The sources of contamination are manifold. Starting from the milking procedure, bacteria enter into the milk from the cow's udder or the milker's hands. It is not common to clean the udder or wash the hands thoroughly before milking. Also very important is further contamination through flies falling into the milk. Another important factor throughout the commodity chain is the milking and transport/storage equipment. Commonly used are calabashes for milking and plastic buckets with a lid for milk storage. Large volumes of milk are usually transported for practical reasons (no spillage) in discarded vegetable oil jerry cans with a volume of 20 litres. The problem with those jerry cans is the difficulty to clean them properly as the opening is too small to enter with hands or cleaning tools. Some milkers and milk traders use a cloth to strain out the flies and dirt, but those cloths though are not washed adequately. The milk vendors at the markets use spoons or cups as measuring tool, which also are not cleaned properly during the day. They are mostly just wiped with some piece of cloth. Some vendors would even hand out a spoon of sour milk to a sceptical customer for testing in order to boost their product. By this way, the milk vendors are increasing the already high bacterial load of their products throughout the day. All materials used

to collect or handle milk are usually only washed with cold water and soap and dried in the sun. The use of disinfectants is not practiced at all.

Additional to these sources of contamination are high ambient temperatures around 30°C and the lack of cooling facilities adding to favourable conditions for bacteria to consequently multiply at high rates. Therefore, fresh milk does ferment very quickly in only two to three hours and pathogenic bacteria quickly reach infective doses.

This general situation is slightly improved in Kolda, where pasteurization has been introduced and milk producers and collectors have been trained on hygienic principles. Results from samples collected from the pasteurisation units clearly demonstrated a marked positive effect of pasteurisation on total bacteriological count (reduction from average values of $10^7$ to $10^4$ cfu/ml), count of coliform bacteria (reduction from average values of $10^5$ to $10^2$ cfu/ml), *E. coli* (reduction from average values of $10^2$ to $10^1$ cfu/ml) and coagulase-positive Staphylococci (reduction from average values of $10^2$ to $10^1$ cfu/ml). However, contamination with *B. cereus* on the other hand was even increased with pasteurisation caused by the ability of the bacterium to sporulate and survive pasteurization. Though milk hygiene is improved somewhat by pasteurisation, it has to be noted that the bacteriological results for pasteurized milk still are not satisfying. Pasteurization is not done according to recommendations concerning temperature and time (63°C for 30 minutes), which results in the persistence of even heat-sensitive bacteria. A second important factor to high bacteria counts is post-pasteurization contamination. A crucial point is cooling after pasteurization, which is done differently in each pasteurization centre. Most of them leave the milk to cool down for up to four hours without covering it, which increases the risk of contamination through flies and dust. At the time of sampling, only one pasteurization centre used a cold water bath to reduce the time for cooling to one hour.

Pasteurization and cooling procedures have to be optimized in order to achieve better quality. Pasteurization temperature should always be confirmed with a thermometer and this temperature should be kept for 30 minutes. The time for cooling further has to be reduced considerably using a cold water bath. For the production of fermented milk it is also advisable to package the milk right after inoculation with starter cultures, instead of packaging after fermentation. It is expected that doing so will further reduce post-contamination.

In order to reduce the initial bacterial load in raw milk at farm level, it is necessary to introduce appropriate hygienic milking practices. Udders should be washed with chlorine solution and wiped dry before milking and milkers should also wash their hands prior to milking. More attention should also be given to clean clothes and a clean milking place. All equipment that gets in contact with milk should be washed with soap and be disinfected (e.g. with household bleach) afterwards.

Efforts should also be put into reducing time to transport milk from the farms to the markets and in the cooling of milk throughout the marketing chain. Where producers live far from markets they could organize themselves in a way that milk is collected from one or more collectors who will take the milk from several farms to the market using a bicycle, motorbike or car (public transport). The establishment of collection points, where producers can bring their milk to, would facilitate such a collection system. In places, where cooling can't be done using refrigerators, cold water tanks could be used alternatively to reduce the temperature of the milk.

All these changes of common practices can only be achieved through practical training of all those who are involved in the production, collection, processing and sale of milk. Extension materials have to be produced and training courses will have to be organized. Milk producers and processors have to be given assistance in order to improve the quality of their products.

The public awareness has to be made aware concerning the importance of good quality milk and of the risk arising from contaminated milk and milk products. Therefore, a media campaign should be launched to address this issue.

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# Annex 1

**Flow diagram: Colony count technique**

Reference: ISO 4833 (1991), IDF 100B:1991
IDF 153:1991
Pour plate method

1. **Sample**

2. **Homogenisation**
   Inoculation: 1 ml sample in 9 ml Maximum Recovery Diluent
   Homogenisation: Vortex 30 seconds.

3. **Decimal dilution**
   Inoculum: 1 ml of initial suspension and following dilutions in 9 ml Maximum Recovery Diluent

4. **Inoculation**
   (Double test)
   Inoculum: 1 ml dilution
   Medium: Plate count agar
   Incubation: 37°C, 48h

5. **Evaluation**
   10-300 cfu/plate

## Annex 2

**Flow diagram: Coliform bacteria/ E.coli**

Reference: IDF 73B

Milk and Milk Products- Enumeration of Coliforms

Part 1: Colony Count Technique at 30°C without resuscitation

1. **Sample**
2. **Homogenisation**

   Inoculum: 1 ml sample in 9 ml Maximum Recovery Diluent

   Homogenisation: Vortex 30 seconds
3. **Decimal dilution**

   Inoculum: 1 ml of initial suspension and following dilutions in

   9 ml Maximum Recovery Diluent
4. **Inoculation**

   (Double test)

   Inoculum: 1 ml dilution

   Medium: McConkey (instead of VRBL)

   After complete solidification, about 4ml of medium are poured on the surface of the inoculated medium

   Incubation: 30°C, 24h
5. **Evaluation**

   10-150 cfu/plate
6. **Confirmation**

   **Gas production in selective medium**

   Inoculate five colonies of each doubtful type

   Inoculum: One loop in 10 ml lactose-bile-brilliant-green- broth

   Incubation: 30°C, 24 h

   **Indol formation in tryptone water**

   Confirmation of each tube showing gas formation

   Inoculum: One loop in 10 ml tryptone water

   Incubation: 45°C (water bath), 24 h

   Add 0.5 ml indol reagent to each tube, mix well and examine the tubes for a red colour in the alcoholic phase after 1 minute

## Annex 3

**Flow diagram: Coagulase- positive Staphylococci**

Reference: ISO/DIS 6888-1 (1997)

Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase- positive Staphylococci (Staphylococcus aureus and other species)

Part 1: Technique including confirmation of colonies

1. **Sample**
2. **Homogenisation**

   Inoculation: 1 ml sample in 9 ml Maximum Recovery Diluent

   Homogenisation: Vortex: 30 seconds
3. **Decimal dilution**

   Inoculation: 1 ml of initial suspension and following dilutions in 9 ml Maximum Recovery Diluent
4. **Inoculation** (Double test)

   4.1. Transfer 0,1 ml of initial suspension and following dilutions to the surface of Baird Parker-agar

   4.2. Spread the inoculum as quickly as possible over the surface of the agar plate

   4.3. Leave the plate in a horizontal position, approximately 15 minutes, until the inoculum is dry

   4.4. Incubation: 37°C, 48 h

   4.5. Enumeration : Plates, which contain a maximum of 150 typical and/ or atypical colonies in two successive dilution
5. **Confirmation**

   Select five typical and atypical colonies from each plate

**Pure culture**

Inoculate selected colonies into 5 ml brain heart infusion broth, each by means of a loop

Incubation: 37°C, 24 h

**Coagulase test**

Add 0.1 ml brain heart infusion broth in 3 ml human plasma (instead of rabbit plasma)

Incubation: 37°C, 12 h

Evaluation: Consider the coagulase test to be positive if the volume of the clot occupies more than three quarters of the original volume of the liquid

## Annex 4

**Flow diagram: Salmonella**

Reference: ISO 6579 : 1993 (E)

General guidance on methods for the detection of Salmonella

**1. Sample**

**2. Pre-enrichment**

Inoculation: 1 ml sample in 9 ml buffered peptone water; Incubation:37°C, 16 - 20 h

**3. Selective enrichment**

**First liquid selective enrichment**

Inoculation: 0.1 ml pre-enrichment to 10 ml Rappaport-Vassiliadis medium

Incubation: 42°C, 24 h

**Second liquid selective enrichment**

Inoculation: 1 ml pre-enrichment to 10 ml tetrathionate broth

Incubation: 37°C, 24 h

**4. Isolation**

**Solid medium with brillant-green**

Inoculum: Loop strike from each selective enrichment media on BPLS- agar

Incubation: 37°C, 24 h

**Solid medium without brilliant-green**

Inoculum: Loop strike from each selective enrichment media on XLD-agar

Incubation: 37°C, 24 h

**5. Confirmation**

Select five caraczeristic colonies from each selective solid media

**Pure culture**

Inoculation: Loop strike on nutrient agar of the selected colonies

Incubation: 7°C, 24 h

**Biochemical confirmation**

1. Glucose (+ gas formation) : positive
2. Lactose : negative
3. Sucrose : negative
4. Hydrogen sulfide : positive
5. Urea splitting : negative

## Annex 5

**Flow diagram: Listeria monocytogenes**

Reference: ISO / DIS 11290-1
Horizontal method for the detection and enumeration of
Listeria monocytogenes
Part 1: Detection method

1. **Sample**
2. **Primary enrichment**

Inoculum: 1 ml sample in 9 ml half Fraser broth

Incubation: 30°C, 24 h

3. **Secondary enrichment**

Inoculum: 0.1 ml primary enrichment in 10 ml Fraser broth

Incubation: 37°C, 48 h

4. **Plating out**

| **First selective solid medium** | **Second selectiv solid medium** |
|---|---|
| Inoculum: Loop strike from enrichment broth on Oxford-agar | Inoculum: Loop strike from enrichment broth on PALCAM-agar |
| Incubation: 37°C, 48 h | Incubation: 37°C, 48 h |

5. **Confirmation**

Select five characteristic colonies from each selective solid media

**Pure culture**

Inoculum: Loop strike on tryptone soya yeast extract agar of the selected colonies

Incubation: 37°C, 24 h

**Tests:**

1. Henry illumination test : typical
2. Catalase reaction : positive
3. Gram staining : positive
4. Mobility test : positive

# Annex 6

**Flow diagram: Bacillus cereus**

Reference: ISO 7932: 1993 (E)
Microbiology- General guidance for the enumeration of Bacillus cereus
Colony count technique at 30°C

**1. Sample**

**2. Homogenisation**

Inoculation: 1 ml sample in 9 ml Maximum Recovery Diluent

Homogenisation : Vortex 30 seconds

**3. Decimal dilution**

Inoculation: 1 ml of initial suspension and following dilutions in 9 ml Maximum Recovery Diluent

**4. Inoculation (double test)**

1. Transfer 0,1 ml of initial suspension and following dilutions to the surface of MYP-agar
2. Spread the inoculum as quickly as possible over the surface of the agar plate
3. Leave the plates in a horizontal position, approximately 15 minutes, until the inoculum is dry
4. Incubation: 30°C, 24 h

## Annex 7

**Flow diagram: Clostridium perfringens**

Reference: ISO 7937 - 1985

Microbiology- General guidance for enumeration of Clostridium perfringens

Colony count technique

1. **Sample**
2. **Homogenisation**

Inoculation: 1 ml sample in 9 ml Maximum Recovery Diluant

Homogenisation: Vortex 30 seconds

3. **Decimal dilution**

Inoculation: 1 ml of initial suspension and following dilutions in 9 ml Maximum Recovery Diluent

4. **Isolation (Double test)**

1. Transfer 1 ml of each dilution step to the centre of an empty Petri dish
2. Pour 15 ml of egg-yolk-free tryptose-sulfite-cycloserine agar (TSC agar) into the dish and mix well with the inoculum by gently rotating each dish
3. When the inoculum has solidified, add an overlayer of 10 ml of the same agar
4. Leave the plates in a horizontal position until the agar has solidified
5. Incubation: 37°C, 20 h, anaerobic conditions

5. **Confirmation**

Select five caracteristic colonies

**TSC base agar**

Inoculation: Inoculate TSC base agar plates each by means of a loop and add an overlayer of 10 ml of the base agar

Incubation: 37°C, 24 h, anaerobic conditions

# Annex 8

**Flow diagram: Yeasts and Moulds**

References: ISO 13681:1995
Meat and meat products - Enumeration of yeasts and moulds - Colony count technique
IDF 94B:1990
Enumeration of yeasts and moulds
Colony count technique at 25°C

**1. Sample**

**2. Homogenisation**

Inoculum: 1 ml sample in 9 ml Maximum Recovery Diluent

Homogenisation: Vortex 30 seconds

**3. Decimal dilution**

Inoculum: 1 ml of initial suspension and following dilutions in 9 ml Maximum Recovery Diluent

**4. Isolation (Double test)**

1. Transfer 1 ml of each dilution step to the center of an empty Petri dish
2. Pour 15 ml of the yeast extract-dextrose-chloramphenicol-agar into the dish and mix well with the inoculum by gently rotating each dish
3. Leave the plates in a horizontal position until the agar has solidified
4. Incubation: 30°C, 48 h

**5. Evaluation**

Evaluation:10 - 150 cfu/plate