

Assessing the Hygienic Quality of Raw Cow Milk from Transylvanian Farms

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Abstract

The aim of this study was to assess the hygienic quality of raw cow milk based on the total bacterial count (TBC), in farms from Transylvania. There were collected and analysed 288 milk samples from 12 farms (24 samples/farm). The total bacterial counts varied greatly in the milk from the investigated farms, from 55 CFU/ml to 693×10^3 CFU/ml. Significant differences were demonstrated among the majority of the farms ($P < 0.05$). The mean values of TBC were significantly higher in summer than in winter ($P < 0.05$). In 58.33% of the studied farms the raw milk's bacterial counts exceeded the limits admitted by the European standards. The obtained results show high microbial contamination of the raw milk in more than half of the investigated farms, most probable because that the hygienic regulations are not respected.

Keywords: microbial contamination, raw cow milk, total bacterial count (TBC).

1. Introduction

A useful indicator for monitoring the sanitary conditions present during the production, collection, and handling of raw milk is the total bacterial count [1]. Milk produced under hygienic conditions from healthy animals should not contain more than 5×10^3 CFU/ml [2]. Regulation No. 853/2004 of the European Parliament and of the Council (EC) sets down the hygienic limit at $\leq 1,00 \times 10^5$ CFU/ml milk for the total bacterial count in raw cow milk.

Oliver et al. [3] reported that milk and milk products derived from milk of dairy cows can harbour a variety of microorganisms and can be important sources of food borne pathogens. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass [4]. Microorganisms, in turn, potentially proliferate in milk all the time after its synthesis, therefore its microbiological

quality at the moment of collection by the dairy farm depends directly on health status of the udder, and also on milking hygiene and milk handling after milking [5, 6].

The aim of this study was to assess the hygienic quality of raw cow milk based on the total bacterial count (TBC), in farms from Transylvania.

2. Materials and methods

The material for the study comprised 288 samples of bulk-tank milk from 12 Transylvanian dairy farms with free stalls. The milk samples were collected during one year, two samples per month from each of the farms. Sampling was done directly from the tank, with raw milk, in sterile test tubes. After collection, the samples were transported to the laboratory using ice boxes.

Serial dilutions of the milk samples were done in order to determine the total bacterial count (TBC): to obtain the 10^{-1} dilution, 1 ml of the milk was mixed with 9 ml of saline (0.9%), and then further dilutions were made: 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} .

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Using a sterile pipette, 0.1 ml was collected from each dilution and inoculated on plain agar in Petri dishes, by means of a sterile glass loop. The plates were then incubated at 30 °C for 24-48 hours (SR EN ISO 4833/2003). After incubation, the colonies were counted and the plates with 30 to 300 colonies were taken into consideration. The average number of colonies in the Petri dishes was multiplied by the dilution factor to get TBC. This was expressed as colony-forming units per ml (CFU/ml). The obtained data were statistically processed using the GraphPad InStat software. The descriptive statistical indicators (mean, standard deviation, median, minimum and maximum) were calculated for the TBC in the investigated dairy farms. The comparison of the values obtained was made with the Dunn's Multiple Comparisons Test.

3. Results and discussion

The descriptive statistics for the TBC in the studied farms (mean, standard deviation, median, minimum and maximum) are listed in Table 1. The TBC had large variations during the study with averages ranging between 182 (farm 7) and 482.69×10^3 CFU/ml (farm 5). The minimum values were between 55 and 395.41×10^3 CFU/ml, and the maximum between 288 and 693×10^3 CFU/ml. In 58.33% of the farms, the milk contained bacterial numbers that exceed the limits allowed by the EU standards ($\leq 100\,000$ CFU/ml). Total bacterial count (TBC) is used as

an indicator of the level of bacterial contamination of milk [7]. TBC does not measure the entire bacterial population, but rather the number of bacteria that grow in the presence of oxygen and at medium range temperatures. TBC in bulk tank milk is the most common way to evaluate the hygienic quality of raw milk at farm level. A high TBC can affect the quality of pasteurized milk and milk products, shelf-life and organoleptic characteristics [8].

TBC values in this study were higher than those obtained by Popescu et al. [9] in the analysis of milk collected from dairy farms with tie-stall housing systems, but similar to other reported results [10, 11]. In contrast to these results, Cempirkova [12] indicated the lowest values of TBC in the technology of loose cubicle littered housing compared to the stanchion littered technology and loose slatted floor housing without litter. Microbial contamination of raw milk is influenced by several factors: health and hygiene of the cows, hygiene of the environment in which dairy cows are housed and milked, the preparation of the udder and milking techniques, the cleaning and disinfecting of the milking equipment and milk tanks, the milker's hygiene, the cooling rate of the milk to the indicated temperature, and the storage time of the milk [13].

Elevated microbial contamination of the milk in the 7 investigated farms had a clear link to the housing and milking hygiene deficiencies. Failure of hygiene in dairy cattle housing and milking is reflected in the values of total number of bacteria in milk [14, 15].

Table 1. Total bacterial counts ($\times 10^3$ CFU/ml) in bulk milk samples in the examined farms

| Farm | n | Mean | Standard deviation | Median | Minimum | Maximum |
|------|----|--------|--------------------|--------|---------|---------|
| 1 | 24 | 167.58 | 76.19 | 151.70 | 11.00 | 301.00 |
| 2 | 24 | 309.01 | 27.81 | 302.11 | 268.11 | 380.52 |
| 3 | 24 | 162.11 | 13.73 | 162.22 | 139.00 | 183.31 |
| 4 | 24 | 190.52 | 60.26 | 173.67 | 19.30 | 297.00 |
| 5 | 24 | 482.69 | 97.06 | 438.56 | 395.41 | 693.00 |
| 6 | 24 | 40.48 | 17.35 | 32.13 | 23.00 | 81.32 |
| 7 | 24 | 0.182 | 0.063 | 0.190 | 0.055 | 0.288 |
| 8 | 24 | 42.67 | 13.29 | 38.43 | 28.30 | 72.10 |
| 9 | 24 | 0.50 | 0.23 | 0.53 | 0.17 | 0.81 |
| 10 | 24 | 21.12 | 6.61 | 19.00 | 13.50 | 38.55 |
| 11 | 24 | 174.57 | 12.89 | 173.10 | 149.71 | 197.10 |
| 12 | 24 | 192.52 | 10.65 | 192.78 | 171.89 | 208.35 |

Holm et al. [16] analyzed the dominant microflora in lower quality milk (TBC $> 3.0 \times 10^4$ /ml) and showed that 64% of the samples

contained microorganisms associated with poor hygiene, 28% of the samples were contaminated with bacteria that grow at low temperatures

(psychrophilic bacteria), also in relation with poor hygiene and only 8% of the samples had evidence of contamination with bacteria related to mastitis.

The lowest values of milk TBC were recorded in the farms with adequate hygienic conditions. There were significant differences between the studied farms in terms of the TBC (Table 2).

Table 2. Statistical differences in the TBC in the studied farms

| Farm | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|------|----|----|----|---|----|----|----|----|----|----|----|
| 1 | ns | ns | ns | * | ns | * | ns | * | * | ns | ns |
| 2 | | * | ns | * | * | * | * | * | * | ns | ns |
| 3 | | | ns | * | ns | * | ns | * | * | ns | ns |
| 4 | | | | * | * | * | * | * | * | ns | ns |
| 5 | | | | | * | * | * | * | * | * | ns |
| 6 | | | | | | ns | ns | ns | ns | * | * |
| 7 | | | | | | | * | ns | ns | * | * |
| 8 | | | | | | | | ns | ns | ns | * |
| 9 | | | | | | | | | ns | * | * |
| 10 | | | | | | | | | | * | * |
| 11 | | | | | | | | | | | ns |

* = p<0.05 significant; ns = p>0.05 not significant

The values of TBC in different seasons (winter, spring, summer and autumn) are listed in Table 3. The highest average value was recorded in summer and the lowest in winter, the difference

being statistically significant (P<0.05). The differences between the values obtained in spring and autumn are not statistically significant (P>0.05).

Table 3. The effect of season on the total number of bacteria (x 10³ CFU/ml) in raw milk

| | Winter | Spring | Summer | Autumn |
|--------------------|----------|--------|----------|--------|
| n | 72 | 72 | 72 | 72 |
| Mean | 126.05 * | 134.07 | 182.36 * | 152.18 |
| Standard deviation | 122.13 | 129.96 | 176.18 | 134.60 |
| Median | 135.50 | 134.15 | 180.60 | 167.85 |
| Minimum | 0.055 | 0.180 | 0.241 | 0.240 |
| Maximum | 437.13 | 479.20 | 693.00 | 49.96 |

* = P <0.05 significant

Data obtained from pervious studies also indicated higher microbial counts during summer [17-19]. Commercial milk was reported to have three times more bacteria in the warm seasons (spring and summer) than in the cold seasons (autumn and winter) [19].

4. Conclusions

The obtained results show high microbial contamination of the raw milk in more than half of the investigated farms, most probable because the hygienic regulations are not respected. The implementation and application of good hygiene practices is very important beginning with the first step of raw cow milk processing, especially during the warm season when the bacterial load of this

animal product is even higher than in the cold season.

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