Raw milk for sale in Pisa province: biosecurity of dairy farms and hygienic evaluation of milk

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Abstract Selling raw milk by automatic dispenser on the farm is a good way to increase income. The aim of the present research is to evaluate both the biosecurity of dairy farms in the Pisa province and the hygienic quality of raw milk. Two farms, chosen because of previously analyzed results carried out on animals and milk, were monitored for 9 months according to the regional law DGR 381/2007. The results obtained showed that these farms presented good cattle health status. The raw milk tested was of a satisfactory hygienic quality, with great variability among milk samples in certain parameters, such as somatic cell count. This study confirmed the importance of consistent, ongoing control of safety conditions on dairy farms.

Keywords Raw milk · Total bacterial count · Somatic cells

Abbreviations

CFU	colony forming units
DGR	decreto gazzetta regionale
SCC	somatic cell count
TBC	total mesophilic bacterial count
AGID	agar gel immuno diffusion
MAT	micro agglutination test

Introduction

In recent years, the number of dairy cow breedings in Pisa province was significantly reduced, because of the low price of milk paid to farmers and the current lack of interest in this type of activity; thus, many farms have closed down. Actually, there are six farms in Pisa province with a small number of animals, approximately forty breeding cows each, and

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the type of farming is semi-intensive with a feed plan based on maize silage. All farms have mechanical milking and a milking parlor trench. Produced milk is taken to the milk plant in Florence. DGR of Tuscany No. 381 of May 28, 2007 allows direct sale of raw milk from a farm. We have been commissioned by the Provincial Farmers Association of Pisa to evaluate the suitability of quality and hygienic care at the six farms involved in the sale of raw milk. We have been facilitated by the fact that all farms are officially free of brucellosis, tuberculosis, and bovine leucosis; Tuscany, in fact, has been officially free of these infectious diseases for several years. To carry out our evaluation, we considered several objective parameters. We performed a retrospective survey based on the data provided by the Provincial Farmers Association of Pisa about the values of the percentages of fat, protein, and lactose, as well as values of somatic cell count (SCC), total mesophilic bacterial count (TBC), and presence of inhibitory substances in milk. We evaluated production in 2006 and 2007. Only two farms (A and B) showed acceptable parameters: high percentages of protein, fat, and lactose; absence of inhibitory substances; and values of TBC and SCC always below the threshold.

Farm A, with 60 Italian Friesian cows, showed the following values: 3.18% protein, 3.48% fat, 4.80% lactose, TBC of 46360 CFU/ml, SCC of 296548, and absence of inhibitory substances. Farm B, with 30 Italian Friesian cows, showed the following values: 3.15% protein, 3.53% fat, 4.72% lactose, TBC of 58145 CFU/ml, SCC of 210652, and absence of inhibitory substances. Milk must come from herds free of infectious diseases transmissible or potentially transmissible to humans through milk or dairy products. For this reason, serological tests were carried out to detect antibodies to: *Leptospira interrogans* (serovars *pomona*, *hardjo*, *grippotyphosa*, *icterohaemorrhagiae*, *bratislava*) (MAT; positivity $\geq 1:100$), *Coxiella burnetii* (CF; positivity $\geq 1:10$), *Chlamydomphila abortus* (CF; positivity $\geq 1:32$), *Mycobacterium avium paratuberculosis* (AGID) (Andreani et al. 1986, 1994; Cerri et al. 2002, 2003). Serological tests were repeated 1 month after the first sampling. Milk samples from each lactating cow were collected and analyzed for the presence of the bacterial agents mastitis (Carter and Cole 1990) and *Listeria monocytogenes* (ISO 11290 - 1). Samples of feces from each animal were taken to detect *Salmonella* spp (BSOP ID 24i2), *Escherichia coli* O:157 (BSOP ID 22i2), and thermotolerant *Campylobacter* (POS F 21i2.1).

All serological tests and bacteriological investigations carried out on feces and milk for the detection of *L. monocytogenes* were negative. Bacteriological analysis on milk samples taken from each cow allowed us to isolate *S. aureus* from two animals from farm B. Subsequently, bacteriological analysis were carried out on milk samples taken from individual quarters; after identifying the infected quarters, the cows were properly treated on the basis of the *in vitro* sensitivity test results. Two weeks from the end of the therapy, bacteriological examinations of the two cows showed negative results. At this point, 20 animals from farm A and 16 from farm B were chosen for the production of raw milk, also considering the number of lactations, between two and six.

Material and methods

Since January 2008, Pisa ASL No. 5, after verifying the validity of the data obtained by a private laboratory and performing two official samples taken 15 days apart, both with values below the threshold, has authorized two farms to install a raw milk automatic distributor. After the beginning of the raw milk sale, simultaneously with the private laboratory responsible for the controls, we carried out the examinations provided by DGR No. 381 of May 28, 2007; this decree complies with Regulation CE 853/2004 and

Table 1 Values of the total mesophilic bacterial count (TBC) in CFU/ml

Period	TBC (CFU/ml)	
	Farm A	Farm A
January-February	13904.107	39190.39
February-March	13298.8	41496.64
March-April	22771.525	63517.2
April-May	28291.179	75982.45
May-June	24961.006	84935.13
June-July	35737.287	57883.24
July-August	37101.741	81958.34
August-September	23114.739	56705.36

implements the agreement between the Government, the Regions, and Autonomous Provinces of Trento and Bolzano of January 25, 2007 and required for farms to adopt a system of hygiene standards. Particularly, we determined the total bacterial count (geometric media over a period of 2 months with at least two samples per month) (ISO 4833), and using the same samples, we also determined the presence of inhibitory substances (Delvotest®), values of somatic cells (geometric media over a period of 3 months with at least one sample per month) (fluor-opto-electronic method), and the presence of M1 aflatoxin (Immunoscreen AFLA M1 - Tecna srl. - Trieste). Furthermore, as required by law, the following microorganisms were researched: *S. aureus* (ISO 6888-2 and biochemical identification with API Staph BioMérieux), *L. monocytogenes* (ISO 11290-1), *Salmonella* spp (ISO 6579), thermotolerant *Campylobacter* (POS F 21i2.1), and *E. coli* O:157 (BSOP ID 22i2) in samples of bulk tank milk, starting with one sample per month for 2 months, followed by one sample every 2 months. Finally, samples of feces from each cow were tested every 2 months for the detection of *E. coli* O:157 (BSOP ID 22i2).

Results

The results about the TBC and the SCC are reported in Tables 1 and 2. TBC was always lower than the threshold [100,000 colony forming units (CFU)/ml]. In farm A, an

Table 2 Values of somatic cells count (SCC) in No./ml

Period	SCC (No./ml)	
	Farm A	Farm A
January-February-March	241459.28	156251.4
February-March-April	387860.94	134905.3
March-April-May	286343.3	166975.3
April-May-June	302271.16	236626.1
May-June-July	316344.77	265925.3
June-July-August	390923.13	295064.9
July-August-September	325801.22	240162.4

increasing in SCC to near the threshold (400,000 CFU/ml) was observed from February to April (387,860.94/ml) and from June to August (390,923.13/ml). In the first period, the single SCC values were always under the threshold, whereas in the second period and particularly in July, the three values were always over 500,000/ml.

Inhibitory substances and M1 aflatoxin were never found. With respect to *L. monocytogenes*, *Salmonella* spp, thermotolerant *Campylobacter*, and *E. coli* O:157, bulk tank milk samples were always negative. The values of *S. aureus* were always lower than legal limits. *E. coli* O:157 was never isolated from fecal samples.

Discussion

On the basis of our results, we can affirm that the two chosen farms satisfy regulations for animal health and the hygienic quality of milk. Our data differ from those reported by other authors in other regions (Giaccone et al. 2007; Vallone et al. 2008). However, the SCC values from farm A at some times of the year should be noted. In fact, this parameter, even if always lower than legal limits, shows the highest values in July, without a corresponding TBC increasing. Increased SCC may be due to several managerial or physiological causes. Our results are encouraging, but also demonstrate the necessity of a constant regulation of farms, with consideration of the microbiological and qualitative delicacy of raw milk.

Recent O.M. of October 12, 2009, which introduced the requirement for boiling before milk consumption due to some cases of hemolytic uremic syndrome likely associated to the consumption of raw milk, confirms that raw milk is a potentially dangerous food especially for some categories of consumers. However, the direct sale of raw milk represents a good opportunity for development of small farms such as those present in Pisa province.

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