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**MAIN FACTORS FOR HIGH BACTERIAL
COUNT IN RAW COW MILK
GLAVNI FAKTORI VISOKOG BROJA BAKTERIJA
U SIROVOM KRAVLJEM MLIJEKU**

Abstract

This study had two aims. The first was to describe several factors that most influence the bacterial count in raw cow milk. Among them, the greatest emphasis was on contamination of milk from the udder, contamination from the udder exterior, the impact of equipment maintenance and sanitation procedures and, finally, the temperature and duration of raw milk storage. The second aim was to evaluate the current situation regarding bacterial counts in raw cow milk in the FY Republic of Macedonia, which is one of the elements that define raw milk quality.

For the purposes of this research, 3470 milk samples were taken from milk cooling tanks in several regions of the FY Republic of Macedonia during the period from January – June 2009.

After the tests were finished, it was determined that 56.28% of the samples fulfill the criteria given in the national legislation, compared with the EU legislation, where it was noted that only 18.04% of the samples meet the given criteria.

The results obtained clearly indicate insufficient hygiene in the breeding of milk cows, and incorrect milk handling after milking as a result of the insufficient farmer knowledge regarding hygiene procedures for primary milk production.

Key words: total number of microorganisms, cow milk, milk quality

Izvod

Ovaj rad je imao dva cilja. Prvi cilj je bio opisati faktore koji najviše utiču na broj bakterija u sirovom kravljem mlijeku. Najveći naglasak je dat na izvore kontaminacije mlijeka u unutrašnjosti vimana, kontaminaciju mlijeka iz spoljne sredine, uticaj postupaka održavanja i sanitacije opreme za mužu i na kraju uticaj temperature i vremena uvanja mlijeka nakon muže. Drugi cilj je bio procijeniti trenutnu situaciju u pogledu ukupnog broja bakterija u sirovom mlijeku na području Makedonije, što je jedan od elemenata koji definišu kvalitet.

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Za potrebe istraživanja, u periodu januar-jun 2009, sa nekoliko podru ja Makedonije, iz opreme za hla enje mlijeka, uzorkovano je 3470 uzoraka sirovog kravljeg mlijeka.

Nakon odra enih analiza utvr eno je da 56,28% uzoraka ispunjava kriterijume date u nacionalnom zakonodavstvu. U pore enju sa EU zakonodavstvom, samo 18,04% ispunjava zadane kriterijume.

Dobijeni rezultati jasno ukazuju na nedovoljnu higijenu u uzgoju krava i nepravilno rukovanje mlijekom nakon muže, što je rezultat nezadovoljavaju eg stepena edukacije proizvo a a mlijeka o higijenskim postupcima u primarnoj proizvodnji mlijeka.

ključne riječi: ukupan broj mikroorganizama, kravlje mlijeko, kvalitet mlijeka

INTRODUCTION

The Book of Rules for specific requirements for safety and hygiene and the manner and procedure for performing official controls on milk and dairy products "Official Gazette of R.M. No.151/2007 (**The Book of rules for specific requirements for safety and hygiene and the manner and procedure for performing official controls on milk and dairy products, 2007**) and the EU Directive 92/46 (Council directive 92/46/EEC" laying down the health rules for the production and marketing of raw milk, heat-treated milk and milk-based products, set the basic criteria against for evaluating the hygiene and safety of raw cow's milk. The only difference between these two documents is the transitional period of four years, which is included in Macedonian regulations. This period provides a gradual reduction in the allowable number of microorganisms and somatic cells in raw cow milk. For the year 2009, the legislation allowed a maximum number of microorganisms in raw cow's milk of up to 600,000 cfu/ml. In 2008, the laboratory for testing the quality of raw milk, at the Faculty of Veterinary Medicine in Skopje, prepared a preliminary study on the number of bacteria and somatic cells in raw cow's milk (**Angelovski, Lj. et al. 2008**). This survey found that in terms of the number of bacteria in raw cow milk, 41.55% of the samples met the requirements set out in national legislation, while 10.7% of the samples met the criteria of the European legislation.

Factors that affect the total number of bacteria in raw cow's milk

Milk is synthesized in specialised cells in the mammary gland, and at the time of secretion in the alveoli, the udder milk is sterile (**Tolle, A. 1980**). After this phase of milk production, microorganism contamination can occur from three main sources (**Bramley, A.J. and McKinnon, C.H. 1990; McKinnon, C.H. et al. 1990**) as follows: from the mammary gland, from the outside of the udder, and from milk handling and the equipment for milk storage. The health and hygiene of the animal, the environment in which animals are kept and exploited, and the procedures used for cleaning and sanitation of the equipment are key factors in contamination with microorganisms in raw milk. Equally

important are the temperature and time of storage that would not allow the microorganisms to multiply and increase their number. All these factors affect the total number and type of microorganisms in the bulk raw cow milk.

Contamination with microorganisms from the udder

The raw milk from healthy cows normally contains low numbers of microorganisms and generally contains less than 1000 bacteria/ml (**Kurweil, R. and Busse, M. 1973**). In contrast to this, where the udder contributes only slightly to the number of microorganisms, in milk cows with mastitis the udder has the potential to secrete huge numbers of microorganisms in the milk. The influence of mastitis on the total number of microorganisms in milk depends on the strain of the pathogen microorganisms, the stage of infection and the percentage of infected cows in the herd. Infected cows have the potential to excrete over 10^7 bacteria/ml (**Bramley, A.J. and McKinnon, C.H. 1990**).

From the group of microorganisms that cause mastitis, it is known that the greatest impact on the total number of bacteria is from *Streptococcus spp.* and the most important of these are: *Streptococcus agalactiae* and *Streptococcus uberis*. It should be noted that *Staphylococcus aureus* is not considered one of the strongest factors, although up to 60,000 ml of bacteria were detected in infection with this microorganism (**Gonzalez, R.N. et al. 1986**). The detection of pathogenic microorganisms in milk does not necessarily indicate that they originated from animals with mastitis. The potential causes of mastitis in milk may come from other sources, e.g., unclean animals, unhygienic equipment or poorly performed milk cooling. Increasing numbers of somatic cells in milk can serve as evidence of the presence of mastitis-causing bacteria, which also cause an increased number of bacteria in milk. This seems to be more reliable for *Streptococcus spp.* than for *Staphylococcus aureus*, which is secreted in the milk in smaller numbers (**Fenlon, D.R. et al. 1995**). However, *Streptococcus agalactiae* and *Staphylococcus aureus* do not multiply significantly on the milking equipment and therefore their presence in bulk milk is considered strong evidence that they originate from infected animals (**Bramley, A.J. and McKinnon, C.H., 1990; Gonzalez, R.N. et al. 1986**).

The bacteria that cause environmental mastitis (including here, the coliforms, streptococci and certain – coagulase – negative staphylococci) are naturally related to the environment in which the animals reside and can affect the number of bacteria in bulk milk (**Bramley, A.J. 1982; Zehner, M.M. et al. 1986**).

Microorganisms that are naturally associated with the skin of the animal or the environment in which animals are kept and exploited may inhabit the exterior of the udder. The direct impact of these natural inhabitants is considered a weak factor in the contamination of raw milk because these microorganisms do not multiply significantly in the milk. Of greater significance are those microorganisms that grow and multiply on the udder contaminated with mud, feces, food or bedding.

The udder of the cows can be contaminated when they lie down or when they are kept in a muddy outlet. The liner of the udder contains a large number of microorganisms. The total number of bacteria usually ranges through 10^8 - 10^{10} /g. (**Bramley, A.J. 1982; Bramley, A.J. and McKinnon, C.H. 1990; Hogan, J.S. et al. 1989; Zehner, M.M. et al. 1986**). Microorganisms originating from the bed and contaminating the udder are usually: streptococci, staphylococci, coliforms, spore-forming bacteria and other Gram-negative microorganisms. Regularly found on the udder are thermophilic (bacteria that survive pasteurization) and psychrophilic strains of bacteria (bacteria that grow at low temperatures), indicating that contamination from the outside of the udder can have enormous impact on the total number of bacteria in raw cow milk (**Bramley, A.J. and McKinnon, C.H. 1990**).

The impact of maintenance of equipment and procedures for sanitation

The level of hygiene of the milking system affects the total number of bacteria more than all of the other listed factors (**Olson, J.C. and Mocquat, G. 1980; Palmer, J. 1980**). The residues of milk on the contact surfaces of equipment support the growth of a large number of microorganisms. These surfaces are ideal for the development of microorganisms originating from the environment (from bedding, food, garbage). Irregular procedures for cleaning and sanitation can affect the degree of bacterial growth on surfaces of the milking system. This occurs in a defective milking system in which microorganisms are multiplying or establishing the conditions that develop only certain groups of microorganisms. More resistant and/or thermophilic bacteria can survive on a smaller number of equipment surfaces that are considered to be well cleaned with warm water. Old and cracked rubber parts of the equipment are considered as a good source of these thermophilic bacteria. Inefficient sanitation and cleaning procedures, using lower temperatures and/or not using a chemical agent for this purpose leads to increased growth of the less resistant, fast-growing Gram-negative microorganisms (coliforms and *Pseudomonas spp.*) and streptococci (**Olson, J.C. and Mocquat, G. 1980**).

Temperature and time of storage of milk

Maintaining a low temperature, which prevents the growth of other microorganisms, at the same time promotes the psychrophilic bacteria that can be found in milk originating from unclean animals, the environment, and poorly maintained equipment. Minimizing the level of contamination of milk from these sources will reduce the number of psychrophilic bacteria and their high participation in the total number of bacteria. This type of bacteria is sensitive to thermal treatment and does not survive pasteurization.

In situations where the milk cooling was performed at temperatures higher than 6 °C, conditions are set for the development of other microorganisms besides the psychrophilic. Although such incidents rarely occur, more attention is needed in the transport of milk in cans. Streptococci are often associated with

poor cooling of milk. These bacteria also increase the acidity of milk. Certain strains are responsible for the occurrence of unpleasant odor in milk. Milk storage above 15 °C supports the development of these bacteria (**Gehringer, G. 1980**). However, the type of bacteria that will develop and prevail in the total bacterial population mostly depends on the raw milk's initial micro flora (**Bramley, A.J. and McKinnon, C.H. 1990**).

MATERIALS AND METHOD

For determination of the number of microorganisms, 3470 samples were analyzed in the period from January to June 2009. The samples were taken from milk producers in different regions of the FY Republic of Macedonia. These samples were taken and submitted in sterile plastic containers in quantities of 50 ml with previously added preservative "Azidiol" (0.25 ml). Samples were transported at a temperature of 4 °C to the laboratory for testing the quality of raw milk at the Faculty of Veterinary Medicine in Skopje.

All samples were analysed with accredited laboratory methods in accordance with ISO/IEC 17025:2005 with references to IDF Standard 161A: 1995 Milk-Quantitative determination of bacteriological quality. The instrument used for the tests was a Bactoscan 8000S (Foss Electric, Denmark). This device works on the principle of staining bacteria with fluorescent dye. After coloring the bacteria, a thin film of milk sample is applied on a rotating disk that passes under the lens of a fluorescence microscope. This microscope counts the colored bacteria as pulses of light, which are converted electronically and presented as numerical results.

RESULTS AND DISCUSSION

From the obtained results (Table 1) it can be concluded that in accordance with national regulations, 56.28% of the samples meet the specified criteria. This is a significant improvement in the relatively short period since our study last year in which only 41.55% of the samples met the specified criteria. The number of analysed samples that meet the criteria for total number of bacteria, set by the European Regulation (Council Directive 92/46 EEC), is 626 samples, or 18.04%. This result can be characterized as low, but there is improvement in this category since the year 2008, when the participation of the samples containing up to 100.000 cfu/ml was 10.7%. This means that the domestic milk producers are adopting modern standards and methods in terms of hygiene, milking, nutrition and keeping of the cows.

Percentage share of samples that meet the criteria set out in domestic legislation was 56.28%. Regarding the European legislation, this share is 18.04%. There is improvement in this category compared to last year's study in 2008 (**Angelovski, Lj. et al. 2008**) where the ratio was 41.55% or 10.7%. Contamination with microorganisms in raw milk may be of different types of bacteria from many sources. Therefore, determining the cause of a high number of bacteria is not always easy.

Table 1: The total number of microorganisms classified according to the Book of Rules No. 151 (classified according to the Appendix 3)

Tabela 1: Ukupni broj mikororganizama klasifikovanom prema Pravilniku (the Book of Rules) No. 151 (klasifikovani prema Aneksu 3)

Year ²	Allowed total number of bacteria	No. of samples that meet the given criteria	%
Out of class.	< 600.001 cfu/ml	1517	43.72
2009	> 600.000 cfu/ml	299	8.61
2010	> 400.000 cfu/ml	543	15.65
2011	> 200.000 cfu/ml	485	13.98
2012	> 100.000 cfu/ml	626	18.04
Total:		3470	100.0

Table 2: The total number of microorganisms classified under Council Directive 92/46 EEC

Tabela 1: Rezultati ukupnog broja mikororganizama klasifikovanom prema Direktivi Savjeta (Council Directive 92/46 EEC)

Category	Total number of bacteria	%
Samples that meet the criteria laid down in Council Directive 92/46 EEC	0-100,000	18.04
Samples that do not meet the criteria set out in Council Directive 92/46 EEC	100,001 or more	81.96

However, there is often one important source of the high number of bacteria in the milk, which may be a result of a combination of several factors (e.g., dirty equipment and poor cooling).

CONCLUSION

After testing the samples and tabulating the obtained results, the conclusion is that hygiene standards in primary production of cow's milk are still low. With the exception of a small number of farmers, most of them do not consistently follow the procedures for hygiene in the storage, use, and feeding of milk cows.

This certainly highlights the need for further engagement of people involved in the entire process of milk production including farmers, doctors of veterinary medicine who are working in field practice, the scientific staff of educational institutions, and persons responsible for this issue at the dairies.

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² Transitional period of four years, which is included in Macedonian regulations. Gradual reduction: for the year 2009, the legislation allowed a maximum number of microorganisms in raw cow's milk up to 600.000 cfu/ml. for 2012 up to 100.000 cfu/ml.

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Summary

This study had two aims. The first one was to describe several factors that influence the most the bacterial count in the raw cow milk. Amongst them, biggest accent was made on contamination of the milk from the udder, the contamination from the udder exterior, the impact of the equipment maintenance and sanitation procedures and at the end on the temperature and the time of raw milk storage. The second aim was to evaluate the current situation with the bacterial count of the raw cow milk in R. of Macedonia, which is one of the elements that define the raw milk quality.

For the purposes of this research 3470 milk samples, were taken from milk cooling tanks in several regions in R. of Macedonia in the period from January - June.

After the tests were finished it was identified that 56.20% of the samples fulfill the criteria given in the national legislative. Compared with the EU legislative it was noted that only 18.04% of the samples meet the given criteria.

The results obtained clearly indicate at the insufficient hygiene in the breeding of the milk cows and incorrect milk handling after the milking as a result of the unsatisfactory farmers knowledge for the hygiene procedures in the primary milk production.