

# Presence of inhibitory substances in raw milk in the area of Montenegro

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Received - Prispjelo: 20.09.2010.

Accepted - Prihvaćeno: 04.05.2011.

## Summary

The Dairy Laboratory at Biotechnical Faculty in Podgorica tested using microbiological inhibitor test - Delvotest® Accelerator, presence of inhibitory substances in raw milk produced on dairy farms of individual producers, cooperates of Montenegrin dairies. During period of six months, 6161 samples of raw milk were tested and it was determined that 478 samples or 7.84 % were positive. This is a significantly higher percentage of positive samples in comparison to European Union countries, but it is not surprising, considering that in these countries there is a decades-old regular system for antibiotic residues control in milk, while in Montenegro testing is provided on level of annual monitoring. The results of analysis indicate that in Montenegro, it is necessary to introduce regular systematic control of residues of antibiotics and other inhibitory substances in raw milk.

*Key words:* raw milk, inhibitory substances, Delvotest

## Introduction

The presence of antimicrobial drugs residues in milk is a public health issue. It is accepted that antimicrobial drugs residues may give allergic reactions in sensitive individuals and may have negative effects on the composition of the human intestinal flora. In more general terms, the overuse of antimicrobials has led to the development of multi-drug resistant animal and human pathogens (Alanis, 2005). Furthermore, milk contaminated with antimicrobial drug residues may also create problems in the production of fermented milk products because such compounds inhibit the growth of the starter cultures. Milk and dairy products with proper hygienic quality can be obtained only from healthy animals. Antibiotics, or antibiotic residues are the most present inhibitory substances in milk because of their frequent usage in prevention or treatment of various diseases of bacterial etiology, including udder inflammation, the most common disease of dairy animals in intensive milk production (Bruun et al., 2003; Petrović et al., 2008). Antibiotics are

usually present in milk after intramammary application during mastitis treatment, but also after parenterally, orally, intrauterine, intramuscular and intravenous implementation (Vuković, 2005). Antibiotic residues may be found in the milk, after feeding or watering the dairy cows with food or drinking water that is contaminated with antibiotics. The udder tissue resorbs part of the applied doses of antibiotics, and a certain amount is excreted in milk. The excretion time of antibiotics varies from animal to animal, and depends on: type of used antibiotic, quantity of given antibiotic and the way of applications, but also it depends on age, health status, lactation stage and individual features of dairy animals (Samaržija and Antunac, 2002).

The most common cause of the presence of antibiotic residues in milk is non-compliance of carena - period of antibiotic excretion from animal body (Kirbiš, 2006). Other causes may be: overdose, use of antibiotics that are banned for usage, and subsequent or deliberate addition of antibiotics in milk in order to prevent multiplication of microorganisms whose result is the deterioration of milk (McEwen et al., 1992; Vuković, 2005).

Systematic controls of antibiotic residues presence in milk are usually done at two levels: annual monitoring of the presence of inhibitory substances in milk and regular control of the presence of antibacterial substances in raw-milk on farms that cooperate with dairies (Romnee and Dehareng, 2008; Ghidini et al., 2002). Government organizations, and government agencies responsible for safety and control of food of animal origin, carried out annual monitoring of residues of various substances in milk, and they use different physical-chemical methods for confirmation and quantification of residues of antibiotics (Shitandi, 2004). Detection of antibiotic residues in raw milk produced on farms of individual producers, dairy cooperates, is done by fast and cost-effective "screening" tests, which can analyze a large number of samples for a relatively short time without the complex methods of sample preparation. For this routine control microbiological inhibitor tests, microbiological tests receptor, immune receptor tests and enzyme tests are usually used (FSAI, 2002; Navratilova, 2008). These tests have a wide range of detection, so they can determine the presence of residues of different groups of antibiotics such as  $\beta$ -lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides. Microbiological inhibitor tests that use bacterial strains of the test organisms, such as *Geobacillus stearothermophilus* var. *calidolactis*, are simple for application, fast and economical, and most commonly used as routine tests in laboratories for quality control of milk throughout the European Union (FSAI, 2002; Molina et al., 2003; Hilleron and Berry, 2004; Comunian et al., 2010).

The purpose of the paper was to test raw milk produced on farm of all cooperates of Montenegrin dairies, and to determine the percentage of positive samples, since such detailed and comprehensive testing was not implemented on area of Montenegro until now.

## Materials and methods

The latest version of Delvotest for the detection of antibiotics is Delvotest®Accelerator, which is an integrated system for incubation and reading of Delvotest ampoules or SP-NT plates (sulphur-lin no tablet). The system has a device for heating during the incubation process that maintains the temperature

at  $64 \pm 0.5$  °C, and the scanning device that monitors changes of color during the incubation period (every 60 seconds, the device scans the color of ampoules or plates). Delvotest®Accelerator scanner software (DSM Food Specialties) is installed on a PC with Windows XP operating system and connected to the HP Scanjet scanner 8300. Delvotest®Accelerator can simultaneously incubate and read 4 plates (4 x 96 samples), and characteristics of each plate is stored in the software by loading bar-code from the plates before incubation starts.

Duration of the test is 90 to 120 minutes and the system, by itself, determines the end of the measurements. Software results are recorded numerically (as "z" values - numerical values of colour intensity for multiplate cell). Values higher than -8.00 are positive and less than -8.00 are negative. The end of incubation and scanning process is achieved when 10 negative samples reach the z-value -12.00. In addition to numerical values, the software indicates each sample as "positive" or "negative" (DSM Food Specialties, 2008).

## Results and discussion

In Montenegro, so far, the systematic control of antibiotic residues in raw milk is not accompanied and there is insufficient number of data about their presence. Veterinary Administration, since 2007, began to implement the monitoring of antibiotic substances in milk and dairy products, according to the Regulations on monitoring residues in animals, foodstuffs of animal origin and food (Sl. list RCG 46/06). During the 2007, 26 samples of raw milk were tested for the presence of chloramphenicol, of which 1 sample was positive, but subsequent testing proved it as negative. Also, presence of  $\beta$ -lactams, tetracycline, chinolons and sulphonamids was tested in 32 samples of raw milk and all of them were negative (VUCG, 2008).

Investigation of the frequency of antibiotic residues and other inhibitory substances occurrence in raw milk, during six-month period, was carried out in Dairy Laboratory, one of the departments of Biotechnical Faculty in Podgorica. From April 15<sup>th</sup> to October 15<sup>th</sup> 2008, 6161 samples of raw milk were tested and in this test individual producers, cooperates of Montenegrin dairies, were included. The results of investigations are presented in Table 1.

Table 1. The frequency of occurrence of antibiotic residues and other inhibitory substances in raw milk

Dairy	Number of tested samples	Number of positive samples
“Srna” Nikšić	637	15
“San Nikola” Ulcinj	962	71
“Šimšić Montmilk” Lazine	977	53
“Žir” Bijelo Polje	244	26
“Zora” Berane	901	87
“Krisma” Bijelo Polje	713	119
“Nika” Nikšić	430	39
“Vulaš” Čevo	5	0
“Mljekara” Podgorica	65	2
“Arena Milk” Ulcinj	49	4
“RM Komerc” Pljevlja	291	26
“Cijevna” Podgorica	21	0
“Mljekara Pljevlja” Pljevlja	743	39
“Čevo” Čevo	57	0
“Ital Produkt” Podgorica	66	2
Total	6161	483

Out of 6161 analyzed samples, 483 or 7.84 % were positive (Table 1). Percentage of positive samples by dairies varied from 0 to 16.69% as it is shown in Figure 1.

In most EU countries, the percentage of positive samples of raw milk for the presence of antibiotics is low, and less than 0.5 % (Hillerton and Berry, 2004). In Belgium and Denmark the percentage of positive samples was 0.1 % (FSA, 2006). During 2006, in Belgium, more than 800000 samples of raw milk were tested, and only 782 samples were positive (Romnee and Dehareng, 2008). The percentage of positive samples of raw milk on antibiotic residues in UK was 0.16 % and Spain 0.18 % (FSA, 2001; Garcia et al., 2001). In Czech Republic antibacterial substances were detected in 0.5 % of analyzed samples of raw milk, and in Lithuania 0.8 % (EC, 2000; Žvirdauskiene et al., 2004). Such low percentages of raw milk samples positive on inhibitory substances presence are result of multi-decade systematic control of residues in most countries of the European Union, for example, regular milk inspection from

farms in England and Wales for the presence of antibiotic residues is performed since 1965 (Booth and Harding, 1986). During 2003, in the United States, 665 627 samples of raw milk from dairy farms were tested, and 1009 or 0.15 % of samples were positive (FDA, 2003). Delvotest method was used for detection of antibacterial substances in raw milk in Croatia. Total number of tested samples in 2007, was 291 411 and 0.40 % of those samples were positive (SLKM, 2008). In countries that do not have well regulated system of antibiotic residues detection in raw milk, the percentage of positive samples varied from 8 to 15 %, and more (Ruegg, 2005). In Kenya, 14.9 % of raw milk samples were positive on presence of inhibitory substances (Shitandi, 2004). Tests conducted in China during 2002 and 2003, showed that 37 % of samples of raw milk and 17.24 % UHT milk were contaminated by antibiotic residues (Deng et al., 2004). In Pakistan, 36.5 % of raw milk that was delivered on market in 2006 was positive on  $\beta$ -lactam presence (Khaskheli et al., 2008). Investigations done in Brazil showed that approximately 50 % of

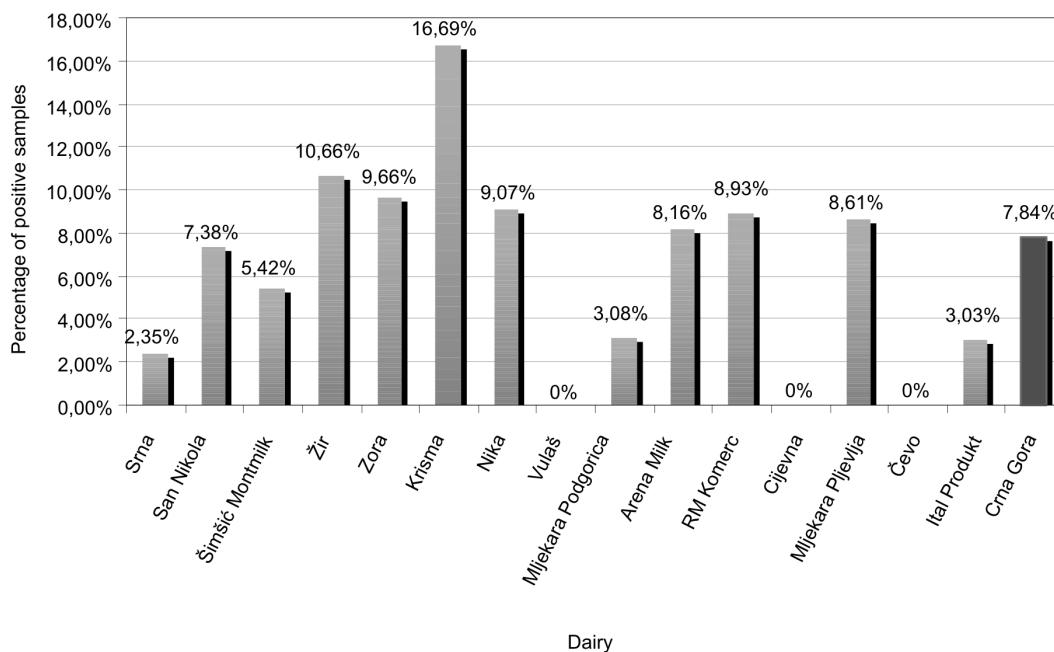


Figure 1. Percentage of positive samples of raw milk by dairies

pasteurized milk delivered on market contained antibiotic residues (Nascimento et al., 2001).

## Conclusions

Preliminary results of testing the antibiotic and other inhibitory substances presence in raw milk, produced on area of Montenegro, indicate that 7.84 % of samples were positive. Achieved percentage is significantly higher than the percentages established in the European Union countries, where the percentage of samples in which antibacterial substances were detected is less than 0.5 %.

The results of investigation indicate that it is urgent to establish regular system for control of antibiotic residues and other inhibitory substances presence in raw milk in Montenegro. Existing Decree for criteria and way of raw milk paying (Sl. list RCG, 62/03), beside analysis of fat and protein content, and detection of somatic cells and total bacteria count, should be expanded by introduction of one more obligatory parameter - detection of antibiotic residues and other inhibitory substances. It should not be a big effort, because Dairy Laboratory at Biotechnical faculty in Podgorica, according to its capacity, equipment, trained staff and well organized system of sample collection, could provide testing of

raw milk on above mentioned parameter for all cooperates of Montenegrin dairies, at least once a month.

## *Prisutnost inhibitornih tvari u sirovom mlijeku na području Crne Gore*

### Sažetak

Laboratorij za mljekarstvo Biotehničkog fakulteta iz Podgorice korištenjem mikrobiološkog inhibitor testa - Delvotest Accelerator, testirao je prisustvo inhibitornih tvari u sirovom mlijeku proizvedenom na farmama individualnih proizvođača, kooperanata mljekara u Crnoj Gori. Tijekom šestomjesečnog razdoblja testiran je 6161 uzorak sirovog mlijeka, a pritom je utvrđeno kako je 478 ili 7,84 % uzoraka bilo pozitivno. U usporedbi sa zemljama Europske Unije, ovo je značajno veći postotak pozitivnih uzoraka, što ne iznenađuje obzirom na to da u zemljama EU-a već desetljećima postoji redoviti sustav za kontrolu rezidua u mlijeku, dok se testiranje u Crnoj Gori provodi na razini godišnjeg monitoringa. Rezultati analiza upozoravaju da je u Crnoj Gori nužno uvesti redoviti sustavni nadzor rezidua antibiotika i drugih inhibitornih tvari u sirovom mlijeku.

*Ključne riječi:* sirovo mlijeko, inhibitorne tvari, Delvotest

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