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Antibiotics residue in raw milk samples from four regions of Kosovo

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Abstract

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Antibiotic residues are often present in raw milk and have a negative impact on human health. This study took place in four regions of Kosovo during four months (September, October and December 2015, and January 2016). Residues of antibiotics in milk were determined using the standard microbiological methods called Delvotest SP and SNAP test which is an enzyme-linked, receptor binding assay. Out of 1055 analysed milk samples during 2015 and 2016 in different areas of Kosovo, 106 samples (10%) were identified with antibiotic residues. The results showed large variations between weeks during the research, clearly favoring the fourth week of January 2016, with much smaller number of residues of antibiotics in fresh milk (0.4%). The highest number of residues is registered in the first week of September (2.1%). Identification of positive samples (10%) in different areas of Kosovo, is a great concern that must be taken into consideration to determine the reason for the high percentage of positive samples. Further studies are neccessary to be done.

Keywords: milk; antibiotic residues; detection method

Introduction

Milk and dairy products are extremely important in human nutrition due to nutritional and therapeutic content, but the content of antibiotics residues significantly reduces their quality. The most commonly used antimicrobials in food-producing animals are the ß-lactams, tetracyclines, aminoglycosides, lincosamides, macrolides, pleuromutilins and sulfonamides. Antimicrobials are administered to animals by injections (intravenously, intramuscularly, or subcutaneously), orally in feed or water, topically on the skin and by intramammary and intrauterine infusions (Mitchell et al., 1998). Today antimicrobial drugs are used to control, prevent and treat infection, and to enhance animal growth and feed efficiency (Tollefson & Miller, 2000). Mastitis is the most prevalent disease in cattle which requires use of antibiotics for treatment of animals. After udder treatment is performed antibiotics remain for some time depending from the withdrawal period of the used antibiotic (Delak, 1985). Antibiotic residues in milk are a rising issue in the recent years in the EU (Stolker & Brinkman, 2005). The most likely cause of violative drug residues is the failure to observe withdrawal times (Paige & Kent, 1987; Van Dresser & Wilke, 1989; Guest & Paige, 1991; Paige, 1994).

Milk is one of the basic foods consumed by the population and as such, it must respond to quality criteria. To protect consumer's health and to ensure high quality of food of animal origin, the European Union (EU) regulation 2377/90 (now amended by EU Reg. 470/2009 and 37/2010) set the procedure for establishment of the maximum permitted level of antibiotic residues in milk and meat, since then. One of the most popular world's organizations worldwide dealing with the problems of food in general and milk in particular is the Code Commission (Registration) of Food (CCF)/Codex Alimentarius Commission (CAC). This organization acts as an intergovernmental body of different countries of the world in the coordination of food standards level and international dimensions. Foodstuffs could only be marketed if they did

not contain drug residues at amounts measurable by the officially recognized methods (zero level). In 2005, the Kosovo legislation concerning the residues of veterinary drugs in foodstuffs of animal origin, was fully harmonized with the EU and the use of active ingredients, listed in Annex IV of Regulation No. 2377/90 (EC, 1990) was prohibited in farm animals (Administrative Instruction, 2005).Many countries have regulations that prohibit the sale of milk from cows treated with antibiotics, while milk routinely tested for the presence of residues of antibiotics. In this respect, effective food safety systems support the economic development of countries by providing a sound regulatory foundation for domestic and international trade in food (FAO, 2006).

Marerials and Methods

This research was carried out in four regions of Kosovo, during four months (September, October and December 2015, and January 2016). The raw milk samples were collected from four regions in Kosovo (Skenderaj, Rahovec, Suharekë and Podujevë), approximately 200 dairy farms were selected, and the total number of milk samples was 1055.

Milk samples were collected using the method Codex Alimentarius. Morning milk samples in the amount of 40-50 ml were taken at milk collection points and stored in sterile test tubes. Samples were transferred to the laboratory for analysis in mobile refrigerator at temperature 4-10°C.

Residues of antibiotics in milk were determined using the standard microbiological methods called Delvotest SP (DSM Food Specialities, Dairy Ingredients, Delft. The Netherlands) and SNAP test (Idexx Laboratories Inc., Westbrook, ME, USA) which is an enzyme-linked, receptor binding assay. The screening tests were carried out at the Lab of Food Safety Control in Kosovo Food and Veterinary Agency (KVFA). Both tests were carried out according to the instructions of the manufacturer.

SNAP new beta lactam test (Idexx Laboratories Inc., Westbrook, ME, USA) is an enzyme-linked, receptor binding assay in which β-lactams are captured by a binding protein on a solid support adsorbent matrix housed in a moulded plastic unit. Using this test, penicillin can be detected in the amount of 4 μg/kg, ampicillin or amoxicillin in the amounts of 10 μg/kg, cephapirin 8 μg/kg, and ceftiofur 50 μg/kg.

The values obtained were processed statistically by JMP-starter business unit of SAS program (SAS Institute Inc., 2004).

Results

Samples of milk with residues of antibiotics during 2015-2016 were higher in September and October 2015 and lower in December 2015 and January 2016. Statistical data of milk samples included in the experiment, for different months of the year, are presented in Table 1.

Results obtained from laboratory tests shows no statis-

Table 1
Impact of the month on the presence of residues in fresh milk (%)

Sampling period	N	X±SE	STDEV
September – October 2015	570	1.8 ± 0.03 [a]	1.4
December 2015 – January 2016	485	0.7 ± 0.02 [b]	0.5
Analysis of variance	*df*	Pr > F	
Month	1	<.0001	

ticaly significant major variables between the four months during the survey (P <0.0001), clearly favoring January 2016. The highest number of antibiotics residues was recorded in September (2.1 ± 0.02) and October (1.4 ± 0.01). In 2015 there were found significant seasonal trends of antibiotic residue, although the frequency was slightly higher in September (2.1%) and lowest in January (0.4%) of 2016. Results and impact of the locality in the presence of residues of antibiotics is shown in Table 2. Suharekë and Rahovec had a smaller number of antibiotic residues in fresh milk (0.9 ± 0.01). The highest number of registered residue was in Podujevë (1.7 ± 0.02) and Skenderaj (1.7 ± 0.02).

Table 2
Impact of the locality on the presence of residues in fresh milk (%)

Location of sampling	N	X ± SE	STDEV
Podujevë	267	1.7 ± 0.02 [a]	1.3
Rahovec	340	0.9 ± 0.01 [b]	0.6
Skenderaj	255	1.7 ± 0.02 [a]	1.2
Suharekë	193	0.9 ± 0.01 [b]	0.8
Analysis of variance	*df*	Pr > F	
Month	3	0.0001	

Based on statistical data impact of a week during the sampling are presented in Table 3. The obtained results showed large variations between weeks during the survey (P <0.0001), clearly favoring the fourth week of January 2016, with less antibiotic residues in fresh milk (0.4 ± 0.01). The highest number of registered residue was first week of September 2015 (2.1 ± 0:02), followed by the second and third week of September (1.8 ± 0.01) and (1.7 ± 0.01), the fourth week of September on the % of antibiotics residue in milk (1.4 ± 0.01), followed by a decrease of residue in December 2015 and January 2016 (1.2 ± 0.1), (0.8 ± 0.01), (0.4 ± 0.0), (0.5 ± 0.01) respectively.

Table 3
Impact of the week (sampling period) on the presence of residues in fresh milk (%)

Period of sampling	N	X ± SE	STDEV
September – October 2015, Week 1	167	2.1 ± 0.02 [a]	1.4
September – October 2015, Week II	126	1.8 ± 0.01 [ab]	0.7
September – October 2015 Week III	143	1.7 ± 0.01 [ab]	1.3
September – October 2015, Week IV	143	1.4 ± 0.01 [b]	0.7
December 2015 – January 2016, Week I	125	1.2 ± 0.01 [a]	0.8
December 2015 – January 2016, Week II	112	0.8 ± 0.01 [bc]	0.3
December 2015 – January 2016, Week III	111	0.5 ± 0.01 [c]	0.2
December 2015 – January 2016, Week IV	128	0.4 ± 0.01 [c]	0.3
Analysis of variance	*df*	Pr > F	
Month	3	0.0001	

Discussion

In this study, results for presence of antibiotic residues in milk showed that milk samples collected from 4 different collection points in Kosovo were contaminated with antibiotic residues. Out of 1055 analysed milk samples during 2015 and 2016 in different areas of Kosovo, 106 samples (10%) were identified with antibiotic residues. Results of the sampling during 2015/2016 gave an overview of the contamination of milk with antibiotics.

Results obtained showed large variations between weeks during the research, clearly favoring the fourth week of January 2016, with much smaller number of antibiotic residues in fresh milk (0.4%). The highest number of antibiotic residue was registered in the first week of September 2015 (2.1%).

Identification of positive samples (10%) in different areas of Kosovo, is a concern that must be taken into consideration to determine the reason for the high percentage of positive samples. Results are in line with the findings of other developing countries such as Brazil as noted by Gonzales et al. (2009). In this study, out of the total 103 samples of milk, antibiotic residues were detected in 11 samples (10.68%). Moreover, Folly and Machado (2001) reported that among the 300 milk samples collected in the region of Rio de Janeiro, 13 were positive. In Pakistan, Khaskheli et al. (2006) showed that of all the analyzed samples of raw milk, 36.5% were contaminated with beta-lactam residues.

Rama et al. (2016) reported the qualitative detection of antibiotic residues by the Delvotest SP screening test applied to 1734 raw milk samples collected over 2 years (2009–2010) led to the identification of 106 positive samples (6.11%), and 1628 negative samples (93.9%) in Kosovo. In 2009, 52 out of 1015 samples were drug-positive (5.12%), and in 2010, 54 out of 719 samples were positive (7.51%). Nikolić et al. (2011) analyzed 6161 raw milk samples in Montenegro, 7.84% of which were drug positive. In Romania, Pogurschi et al. (2015) showed that out of the 210 analyzed samples, 66 sample (31.42%) contained antibiotic residues. In Iran the results obtained from Movassagh and Karami (2010) showed that beta-lactam residues in raw milk have been 5%.

In Turkey, in a study by Ceyhan & Bozkurt (1987), from a total 200 milk samples collected from Ankara region, the 5.5% was positive for antibiotic residues. Sanders et al. (1991) investigated about 3000 milk samples and detected the presence of sulfonamide in 1.1% of them. Kress et al. (2007) under the program for identification of positive milk samples in 1.6% of cases confirmed the presence of sulfonamide residues.

Abjean et al. (2000) shows that the application of the screening method of bovine milk in 1100 samples, 9 of them or 0.81% were contaminated with sulfonamide residues unlike the above data, which showed a relatively increased prevalence of sulfonamide. Reybroeck et al. (2010) confirmed the presence of sulfonamide in 0.05% of the total number of analyzed milk samples.

Dimitrieska-Stojković et al. (2011) in Macedonia, out of 915 analyzed samples of cow raw milk, in 1.42% of them detected the presence of sulfonamide residue.

If no control measures are taken against the occurrence of antibiotic residues in animal products, its projected that there will be over 65% increase in antibiotic residue contamination worldwide in animal products between 2010 and 2030 (Van Boeckel et al., 2015).

As widely seen by treated literature, it turns out that the problem of residue of beta-lactam and sulfonamide in milk is also present in other countries. It is necessary to conduct surveys and periodic assessments of the situation and take concrete measures to reduce and eliminate such milk, in order to protect public health.

Conclusions

Results indicate that antibiotic residues can be found in milk produced for consumption in our country. There were a number of positive samples of antibiotic residue in milk from 4 different milk collection points, dedicated for human consumption and processing.

Kosovo should aply more rigorous enforcement of EU legislation, which deals with food safety, especially products with animal origin.

Given the significant levels of antibiotic residue detected in raw milk and disturbing consequences for human health promotes a number of recommendations that should be addressed to public authorities, veterinarians, livestock producers and consumers.

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