

STUDY REGARDING SEASONAL INCIDENCE OF FLUOROQUINOLONS IN COW RAW MILK AND THEIR RELATIONSHIP WITH SOMATIC CELLS COUNT

STUDIU PRIVIND INCIDENȚA SEZONIERĂ A FLUOROCHINOLONELOR ÎN LAPTELE CRUD DE VACĂ ȘI RELAȚIA ACESTORA CU NUMĂRUL DE CELULE SOMATICE

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ABSTRACT | REZUMAT

The aim of this study was to investigate the incidence of fluoroquinolone residues in raw cow milk and to evaluate the relationship between these residues and somatic cell count (SCC) value during summer and winter season. The study was carried out in the period May 2016 - June 2017, on 360 milk samples obtained from three dairy farms from north-western Romania. The milk samples were analysed by LC-MS/MS method. Out of all samples, 243 samples (67.50%) were positive for fluoroquinolone residues.

The examined samples presented values below the recommended maximum residue limit (MRL). Enrofloxacin and its indicator residue, i.e. ciprofloxacin were the most frequently determined.

The results of the study indicate that incidence of fluoroquinolone residues in winter season was higher than in summer. Also, SCC value was higher for milk samples with positive antibiotic residue. Our investigations denote that fluoroquinolones are frequently administered to the dairy cows despite of the EU and national recommendations.

Keywords: food safety, milk quality, veterinary drugs

Scopul acestui studiu a fost de a investiga incidența reziduurilor de fluorochinolone în laptele crud de vacă și de a evalua relația dintre aceste reziduuri și numărul de celule somatice (NCS) în timpul sezonului de vară și de iarnă. Studiul a fost efectuat în perioada mai 2016 - iunie 2017 pe 360 probe de lapte, obținute de la trei ferme de vaci din nord-vestul României. Probele de lapte au fost analizate prin metoda LC-MS/MS. Din totalul probelor 243 de eșantioane (67,50%) au fost pozitive pentru reziduurile de fluorochinolone.

Probele examinate au prezentat valori sub limita maximă recomandată pentru reziduuri (LMR). Enrofloxacin și reziduul său indicator, ciprofloxacin, au fost cele mai frecvent determinate. Rezultatele studiului arată că incidența reziduurilor de fluorochinolone în sezonul de iarnă a fost mai mare decât în perioada de vară. De asemenea, valoarea NCS a fost mai mare pentru probele de lapte cu reziduuri pozitive de antibiotice. Investigațiile noastre arată că fluorochinolonele sunt administrate frecvent la vacile de lapte, în ciuda recomandărilor UE și naționale.

Cuvinte cheie: siguranța alimentară, calitatea laptelui, medicamente de uz veterinar

The extensive and improper use of the fluoroquinolones can lead to the presence of their residues in milk, risks to human health, the development of microbial drug resistance, the spread of resistant pathogens, and a loss of industrial output and technological problems in dairy production (12, 15, 11).

Since 1976, when the first monofluoroquinolone - flumequine was developed, many fluoroquinolone representatives have been synthesised and described.

Fluoroquinolones are used for the treatment of infections caused by various bacterial agents in both - and veterinary medicine. In veterinary medicine, they are useful especially in the therapy for gastrointestinal and respiration infections (1).

Fluoroquinolones are used in intramammary preparations for the treatment of mastitis in lactating dairy cows, for dairy cows during dry periods, and also for mastitis prevention (5).

The occurrence of antibiotic residues in milk intended for human consumption is undesirable for a number of reasons such as: cause allergic reactions, incidence of bacterial resistance, disrupting of the balance of gut microflora, carcinogenesis, mutagenesis and malformation risks. In addition, the presence of antibiotic residues in milk be used in dairy industry can have adverse effects on production of fermented dairy products such as yogurt and cheese (2, 8, 9).

The objective of the present study was to investigate the presence of fluoroquinolone residues in raw cow milk and establish the relationship between milk SCC and fluoroquinolone residues value in raw milk, during winter and summer.

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METHODS AND MATERIALS

The research has been carried out between May 2016 - June 2017 on 360 bulk tank milk samples obtained from three dairy farms from north-western Romania, Satu-Mare (SM) county (Fig. 1).

In our study were involved 425 lactating cows, aged between 2.5 and 7 years, in various stages of lactation: in farm A 189 cows, in farm B a number of 112 dairy cows and 124 cows in farm C. All cows were milked in herringbone type milking parlors, twice a day, and all milk was collected in a refrigerated tank.



Fig. 1. Geographic location of the study

Each month (from May 2016 to April 2017) 10 bulk milk samples were collected from each farm included in our experiments. The raw milk samples were collected in sterile plastic bottles and sent to the laboratory on the same day, using refrigerated cooler and stacked ice-bag, stored at 4°C. All samples came from cows declared healthy by the farmers. The milk samples were analyzed by LC-MS/MS (Liquid Chromatography coupled with tandem mass spectrometry), according to a previously described protocol (6). The analytes determined were the fluoroquinolone residues: enrofloxacin, ciprofloxacin, marbofloxacin and danofloxacin. The method was validated in accordance with the procedure outlined in the Commission Decision No. 2002/657/ EC. All the investigations in the present study were performed in a laboratory fully accredited by The Accreditation Association in Romania (RENAR). SCC analysis was performed electronically with flux cytometer (Fossomatic 90).

The statistical evaluation involved some basic statistical - Mean, Standard Deviation, Minimum, Maximum, Sum, Confidence Level (95,0%).

Data were subjected to analysis of variance

(ANOVA). Results were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSIONS

In our study, 243 milk samples (67.50%) were positive for fluoroquinolone residues. From fluoroquinolones class the following residues were identified: enrofloxacin, ciprofloxacin, enrofloxacin + ciprofloxacin, marbofloxacin and danofloxacin. Enrofloxacin and its indicator residue, i.e. ciprofloxacin were the most frequently determined (67.50%). Enrofloxacin was determined in 8 samples (2.22%) while the presence of ciprofloxacin was identified in 11 samples (3.05%).

Marbofloxacin residue was determined in 41 samples (11.94%). The examined samples presented values below the recommended maximum residue limit (MRL). MRL levels established by EU legislation for fluoroquinolones in milk vary between the individual compounds: 75 µg/kg for marbofloxacin and 100 µg/kg for the sum of enrofloxacin and ciprofloxacin residues, 100 µg/kg for both enrofloxacin and (17). The results of the study indicate that fluoroquinolone residues in winter season were higher than in summer. The mean values are presented in Table 1.

In the present study, SCC value was significantly higher ($p < 0.05$) for milk samples with positive antibiotic residues. Also, SCC data were substantially high during winter season compared with summer samples ($p < 0.05$) (Table 2). The mean SCC value in summer was 121.47 ± 21.2 for negative samples and 354.41 ± 65.7 for positive samples, while in winter the mean SCC value was 114.11 ± 7.6 for negative and 427.6 ± 27.5 for positive samples. Only 8 samples (2,22%) revealed concentrations higher than 400.000 cells/ml, maximum limit admitted by EU legislation.

The main metabolites of enrofloxacin residues eliminated from the body are enrofloxacin amide and ciprofloxacin, and, to a lesser degree, other metabolites. If enrofloxacin is administered intravenously to lactating cows, ciprofloxacin may continue to be detected at relatively high concentrations over a longer period than enrofloxacin itself (1). Marbofloxacin is used to treat bovine respiratory diseases. Same authors showed that 73–89% of the total sum of marbofloxacin residues in lactating dairy cows milk is made up of the original substance. Another study regarding the fluoroquinolone residues in raw cow's milk reported the presence of the following fluoroquinolone residues: danofloxacin, marbofloxacin, enrofloxacin, ciprofloxacin and sum of enrofloxacin and ciprofloxacin residues (10).

Table 1

Concentration of fluoroquinolone residues determined from milk samples according to the season

	summer		winter		MRL ($\mu\text{g}/\text{kg}$)
	Mean \pm SD ($\mu\text{g}/\text{kg}$)	Min - Max ($\mu\text{g}/\text{kg}$)	Mean \pm SD ($\mu\text{g}/\text{kg}$)	Min - Max ($\mu\text{g}/\text{kg}$)	
Enrofloxacin	12.34 \pm 1.32 ^a	1.07- 31.60	23.34 \pm 1.42 ^b	7.23-57.90	100
Ciprofloxacin	7.30 \pm 2.44 ^a	1.70-14.40	26.89 \pm 2.,25 ^b	8.45-61.60	100
Enrofloxacin + Ciprofloxacin	16.70 \pm 4.47 ^a	1.88-38.40	34 \pm 3.69 ^b	9.68-69.80	100
Marbofloxacin	6.01 \pm 0.28 ^a	2.40-6.00	16.00 \pm 2.54 ^b	6.78-28.54	75

SD -standard deviation; **Min**-minimum; **Max**-maximum; **MRL**- maximum residue limit

Table 2

The correlation between SCC in milk samples and season

	summer			winter		
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
Negative samples	121.47 \pm 21.2 ^c	65.34	158.90	114.11 \pm 7.6 ^a	59.30	145.10
Positive samples	354.41 \pm 65.7 ^b	98.60	404.50	427.6 \pm 27.5 ^b	312.35	457.54

The data from this study indicates a higher percentage of fluoroquinolone residues than those indicated by us (87.3% vs 67.5%). But, like in our research, the values obtained did not exceed the MRL value in any of the samples.

The SCC can be high in health udder in some conditions such as: flock management, colostral period, and the end of lactation period, cow age, season and stress (3). Some previous studies described that higher bulk milk SCC levels are associated with antibiotic residues violations (13, 12, 14, 16). Other authors (7) documented that SCC values were higher for milk samples with positive antibiotic residues and the data recorded in winter were considerably higher. Other studies (4) found out that the milk contamination with antibiotics was associated with increased SCC and TBC values. They revealed the presence of others antimicrobial residues: betalactams, gentamicin /neomycin, gentamicin /streptomycin, macrolides and tetracyclines.

CONCLUSIONS

The results of the study indicate that fluoroquinolones are frequently administered to dairy cows in spite of the European and national recommendations.

Data analyses indicated that the fluoroquinolone residues were associated with high SCC levels during winter season.

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