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RESULTS FROM MONITORING THE EDIBLE ANIMAL TISSUES FOR RESIDUES OF SOME VETERINARY DRUGS

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ABSTRACT

A total of 635 muscle and kidney samples were collected during 2010 and 2011 at slaughter houses within the monitoring plan for food from animal origin in Macedonia. The tissue samples were examined for chloramphenicol, sulfonamides, quinolones and tetracyclines. Enzyme-linked immunosorbent methods were used for the determination of chloramphenicol, sulfonamides and quinolones, and high performance liquid chromatography with Diode Array detection was applied for screening of tetracyclines. The methods were validated according to the recommendations laid down by European Commission Decision 2002/657/EC. The obtained data for the method's accuracy, precision and detection capabilities confirmed that the methods were appropriate for detection of antimicrobial substances determined, at the concentration level of interest. The measured range of concentrations was 13.9-74.5 μg/kg for sulfonamides, 9.7-28.0 μg/kg for quinolones and 18.4-80.1 μg/kg for total tetracyclines, with calculated mean values 6.6 μg/kg for sulfonamides, 3.8 μg/kg for qinolones and 1.6 μg/kg for tetracyclines. No exceeding of the established maximum residue levels has been observed. Chloramphenicol has not been detected over the minimum required performance level (MPIJI) value of the screening method in none of the samples tested. The calculated estimated daily intakes for the average daily consumption of 135 g of meat, reveals levels 20 to over 1000 times lower than the values of the acceptable daily intakes fixed by World Health Organization and European Medicines Agency.

Key words: muscle, kidney, veterinary drug residues, chlorampenicol, tetracyclines, sulfonamides quinolones, ELISA, HPLC-DAD

INTRODUCTION

Antimicrobial veterinary drugs are utilized at food producing animals not only for treatment of diseases, but also sub-therapeutically, to maintain health and promote growth. The use of unauthorized antibiotics or the failure to follow the label directions for approved antibiotics could result in unsafe antibiotic residues in food products. These residues could exhibit direct toxic effects on consumers, e.g., allergic reactions in hypersensitive individuals, or they may cause problems indirectly through induction of resistant strains of bacteria. Due to harmful effects of veterinary products residues, surveillance systems are enforced in the European Union related to the requirements laid down in the Council Directive 96/23/EC [1]. According with these requirements, the Macedonian legislation was fully aligned with the EU legislation concerning residues of veterinary drugs in foodstuffs of animal origin in reference to the Council regulation 37/2010/EU [2].

In order to detect such residues in food and tissues, bioassay techniques are widely used as screening methods. Although these methods generally do not distinguish between members of a class of antibiotics, still they provide a semi-quantitative estimate of 'total' residues detected. Nevertheless, they continue to be used because of their simplicity and low-cost. However, before samples are declared to contain concentrations of antibiotics exceeding the tolerance levels, confirmation (and identification of the individual compounds) by sufficiently selective and sensitive instrumental methods such as LC–MS or GC–MS are required [3]. All methods used for that purpose have to detect antibiotics at or below their permissible limits or MRLs and also have to be validated in accordance with the Commission Decision 2002/657/EC [4].

The annual consumption of meat in Macedonia for year 2009 was around 50.5 kg per capita [5]. In the last decades there is a lack of data from investigations of veterinary drug residues in tissue samples collected from Macedonia. With an aim to monitor veterinary drug contamination in tissue samples from slaughter houses, residual concentrations of chloramphenicol, sulfonamides, qinolones and tetracyclines were examined. Sample analyses applying validated method according to the Commission Decision [4] provide accurate and reliable analytical results. Additionally, an estimation of the dietary intake of veterinary drugs residues derived from meat consumption was calculated.

MATERIALS AND METHODS

A total of 635 muscle and kidney tissue samples were collected during 2010 and 2011 from slaughter houses in Macedonia. 221 kidney samples were analyzed for residues of sulfonamides and quinolones, 221 muscle samples for residues of tetracyclines and 193 muscle samples for residues of chloramphenicol. The tissue samples were with origin from different animal species – bovine, ovine and porcine. Samples were stored at 4-8°C

if analyzed within 24 hours after sampling, or kept at less than -20°C for four weeks.

The Chloramphenicol ELISA kit (type AB630) and quinolones (enrofloxacin, ciprofloxacin, norfloxacin, danofloxacin, oxolinic acid, flumequine) ELISA kit (type AB630) were purchased by TECNA (Trieste, Italy). The Multi-sulfonamides (sulfadimidine, sulfamerazine, sulfachloropyridazine, sulfisoxazole, sulfadiazine, sulfachloropyridazine, sulfametoxazole) ELISA kit (type 5101SULMp) was supplied from Europroxima (Arnhem, The Netherlands). Ethyl acetate, citric acid, disodium hydrogen phosphate and oxalic acid were with p.a. purity, supplied from Merck (Darmstadt, Germany). Sodium EDTA was supplied by Sigma (St. Louis, USA). Methanol, acetonitrile and water were with HPLC grade, and were supplied from Sigma (St. Louis, USA).

The certified standards of sulfadimidine, sulfisoxazole, oxytetracycline hydrochloride, tetracycline hydrochloride, chlorotetracycline hydrochloride, doxycycline hyclate, enrofloxacin and chloramphenicol, with a purity over 97 %, were supplied from Sigma-Aldrich (St. Louis, USA). From these substances standard solutions were prepared and used for spiking blank tissue samples at different levels. Following fortification, samples were allowed to equilibrate for some time before extraction.

For sample preparation vortex model Relax Top by Heidolph (Schwabach, Germany), centrifuge model 2K15 by Sigma (St. Louis, USA), evaporator model DriBlock DB-3D by TECHNE (Staffordshire, UK) were used. The optical density at 450 nm for the EIA tests was measured by microplate reader BDSL Immunoscan (Labsystem, Switzerland). Tetracyclines were determined by HPLC-DAD, employing isocratic elution on reverse-phase C_8 Ascentis column (Supelco, Belafonte, USA), with a mixture of 0,01 mol/dm^3 oxalic acid, methanol and acetnitrile in ratio 70:20:10 (V/V/V). Detection of tetracyclines was performed on Perkin Elmer LC 235C diode array instrument (Norwalk, USA) at 365 nm.

The whole procedure for testing chloramphenicol, sulfonamides and quinolones, was according to the manufacturer's instructions. Tetracyclines (oxytetracycline, tetracycline, chlorotetracycline and doxycycline) were determined by modified and optimized screening reverse-phase High-performance liquid chromatography/diode array detector (HPLC-DAD) method [6]. Previously the samples were undergone through extraction with McIlvaine buffer and purification of the sample extracts with OASIS HLB solid-phase extraction cartridges (Waters, Milford, USA). The residues were finally dissolved in 0.01 mol/dm^3 oxalic acid buffer, filtered through 0.45 μm syringe filters and injected into the HLPC system. The dilution factor was 0.2.

The estimated daily intake (EDI) was calculated by the data for the mean detected concentrations for the analyzed residues and average daily consumption based on 60 kg body weight.

Results and discussion

The objective of this study was to screen the residual levels of chloramphenicol as a prohibited substance, and tetracyclines, quinolones and sulfonamides throughout two years monitoring of antimicrobials in Macedonia, to reveal if the abuse of prohibited substance is present, or the maximum residue limits have been exceeded. According to the literature data, this is the first study to evaluate the contamination of edible animal tissues with antimicrobial drug residues and the dietary exposure to these substances in Macedonia.

The analysis of veterinary drugs residues were performed applying in-house validated screening methods according to the criteria laid down in Commission Decision 2002/657/EC [4]. Performance characteristics of EIA methods and the screening HPLC-DAD method were determined as prescribed for qualitative screening methods in Commission Decision 2002/657/EC [4]. The limit of detection (LOD) and limit of quantification (LOQ) were obtained by adding 3 and 10 times the standard deviation of 20 blank samples to the mean blank value. The detection capability (CCβ) for tetracyclines, quinolones and sulfonamides was determined by spiking of 20 blank samples of tissues at the one half of the MRL values, while for chloramphenicol the spiking level was below the established MRPL value of 0,3 μg/kg. Recovery was assessed by performing the experiments where fortified tissue samples were analyzed in ten replicates, at the respective maximum residue level (MRL) or minimum required performance level (MRPL) values for the substances being analyzed. From the recovery experiments the method precision was obtained, as well. The calculations were performed by the formula provided in the EU Commission Decision [4].

Validation data corresponding to the screening meth-

Table 1. *Method validation data for the screening methods of the antibiotics analyzed in tissue samples*

Analyte	Target tissue	LOD (μg/kg)	LOQ (μg/kg)	CCβ (μg/kg)	Recovery %	Precision %
Sulfonamides	kidney	13.9	44.6	62.2	96.7	7.4
Quinolones (Enrofloxacine)	kidney	9.7	32.0	132.8	90.6	21.8
Oxytetracycline	muscle	6.7	22.5	70.5	94.2	12.5
Tetracycline	muscle	10.5	31.8	67.9	89.2	10.9
Chlorotetracycline	muscle	17.4	58.0	62.3	96.8	7.5
Doxycycline	muscle	18.2	60.8	63.8	92.9	8.4
Chloramphenicol	muscle	0.034	0.112	0.18	82.7	19.3

od performance (LOD, LOQ, CCβ, recovery and precision) are presented in Table 1. All screening methods used obtained CCβ values less than the fixed MRL or MRPL values and recoveries higher than 70 %, in accordance with the regulations set by Commission Decision 2002/657/EC. Moreover, the precision values were lower than the recommended maximum by the Horwitz equation [4]. The validation data have justified that the applied methods were appropriate for the detection of residues of veterinary drugs measured.

A total of 635 tissue samples were subjected to the screening methods. The determined antimicrobial's concentrations and MRLs for tetracyclines, quinolones and sulfonamides, as well as the MRPL value for chloramphenicol are summarized in Table 2. All concentrations found for chloramphenicol were lower than the critical MRPL value established by Commission Decision 2003/181/EC [7]. Residues of tetracyclines over the LODs of the method applied have been found in 23 samples (10.4 %), with an average amount of 1.6 μg/kg. Total sulfonamides and quinolones were found in 15 (6.8 %) and 8 samples (3.6 %), respectively, and the determined average concentrations were significantly lower than the LOD values. None of the samples examined contained residues of quinolones, tetracyclines and sulfonamides over the established MRL by Commission Regulation (EU) 37/2010.

Table 2. *Veterinary drug residues (range and mean) in tissue samples collected in the period 2010 and 20111 and critical values regulated by EU and Macedonian legislation*

Analytes	2010			2011			MRL (μg/kg)
	n	Range (μg/kg)	Mean (μg/kg)	n	Range (μg/kg)	Mean (μg/kg)	
Sulfonamides	118	13.9-39.3	7.4	103	14.5-74.5	5.8	100
Quinolones	118	10.5-28.0	3.7	103	9.7-22.0	3.9	200
Oxytetracycline	118	18.4-79.5	2.3	103	38.0-80.8	2.0	100
Tetracycline	118	27.5-55.6	1.2	103	43.2-59.8	1.5	100
Chlorotetracycline	118	35.9-60.6	1.3	103	30.6-77.6	1.4	100
Doxycycline	118	>18.2	-	103	>18.2	-	100
Total tetracyclines	118	18.4-79.5	1.6	103	30.6-80.8	1.6	100
Chloramphenicol*	95	0.006 -0.145	0.052	98	0.003-0.142	0.035	0.3

* Chloramphenicol is not authorized for use in food producing animals in the European Union and in Macedonia (in MRL column the indicated value is MRPL)

Sulfonamides and tetracyclines, together with beta-lactams are considered to be the most frequently utilized antimicrobial substances. Sulfonamides play important role as effective chemotherapeutics of bacterial and protozoan diseases and as growth promoters in veterinary medicine. The Commission regulation has established the MRL as a sum of all substances belonging to the sulfonamide group, which for tissues should not exceed 100 μg/kg [2]. According to World Health Organization (WHO) [8] the acceptable daily intake (ADI) for sulfadimidine was established at 3 mg for 60 kg BW. The highest determined total sulfonamide's concentration was 74.5 μg/kg. Tetracyclines are globally used as broad spectrum antibiotics in veterinary medicine against a wide range of Gram-positive and Gram-negative aerobics and anaerobic bacteria. In the present study, the highest tetracycline's level detected was below the MRL value and it was 80.80 μg/kg for oxytetracycline. The mean tetracyclines concentration was more approximately eight times lower than the assigned MRL. Quinolones are a group of relatively new antimicrobials synthesized from 3-quinolone carboxylic acid. The established MRL for these substances are 200 μg/kg [2] In the present study, the mean quinolones concentration (expressed as enrofloxacin) showed levels more than ten times below the established MRLs.

Due to the potential risk to human health, the use of chloramphenicol is prohibited in food-producing animals in the European Union [2]. The European Union introduced the concept of the minimum required performance limit (MRPL) of 0.3 μg/kg, the highest concentration level at which the screening and confirmatory method shall demonstrate satisfactory performances regarding the sensitivity, accuracy and precision [4]. In this investigation, the measured chloramphenicol mean concentration of 35 ng/kg was substantially lower than the MRPL value, and practically it was the signal obtained from the blank.

According to the Commission Decision 2002/657/EC [4] it is mandatory to confirm the obtained positive samples by the screening with confirmatory analysis, applying methods that provides unambiguous identification and quantification of the concerned analyte.

For the purpose of evaluation of dietary exposure with veterinary drugs residues through the intake of animal tissues controlled in the present study, the EDIs for consumers were estimated. Table 3 presents the EDIs of veterinary drug residues based on the concentrations

found in the present work, calculated with presumed average daily meat consumption for an adult of 135 g [5]. Residue values of all MRL drugs measured, ranged from 0.032 to 0.213 mg/kg BW/day, and were 20 to over 1000 times lower than the set values of ADIs by the European Medicinal Agency (EMEA) [9,10]. However, ADIs values have not been established for chloramphenicol.

Table 3. *Estimation of daily intakes (EDIs) of veterinary drug residues through tissues consumption based on the mean concentrations found in the period 2010-2011*

Analyte	EDI	ADI
	µg/kg BW/day	µg/kg BW/day
Sulfonamides	0.168	50
Enrofloxacin	0.213	372
Oxytetracycline	0.054	1
Tetracycline	0.032	1
Chlorotetracycline	0.035	1
Doxycycline	/	NE**
Total tetracyclines	0.121	3
Chloramphenicol*	0.92*	NE**
Total acceptable daily intake	0.503	428

*EDI for chloramphenicol was expressed as ng/kg BW/day
**NE: Not yet have been established

The highest calculation for EDI was obtained for total tetracyclines, which is approximately 5 % of the ADI value. The total EDI value obtained was 0.503 mg/kg BW/day, substantially lower than the total acceptable daily intake. Therefore, the toxicological risk associated with the consumption of analyzed tissue samples, could not be considered as a public health issue with regards to the analyzed veterinary antimicrobial substances.

CONCLUSION

The methods used for determination of veterinary drugs residues in edible animal tissue samples were validated according to Commission Decision 2002/657/EC and proved to be rapid, simple and reliable, exhibiting good accuracy and repeatability. The obtained recoveries were over 70% with precision values fulfilling the requirements of the Horwitz equation. In the two years period of monitoring, the residues measured for sulfonamides, quinolones and tetracyclines were below the maximum residue limits (MRLs) set by the legislation. None of the samples exceeded the MRPL value for chloramphenicol. The EDIs calculated indicates that the contribution of edible animal tissues to dietary intake of the investigated antibiotics was from 20 to over 1000 times below the ADIs proposed by EMEA and WHO. This indicates that the edible tissues in Macedonia, in average, contain low levels of veterinary drugs, and therefore, they could be considered as safe for human consumption.

REFERENCES

1. Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC OJ L 125, 23.5.1996, p. 10–32.
2. Commission Regulation (EU) 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, OJ L 15/1, 20.01.2010, p. 1–2 (ANNEX I).
3. A.A.M. Stolker, U.A.Th. Brinkman (2005). Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals—a review, J. of Chromatogr. A, 1067, 15-53.
4. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results OJ L 221, 17.8.2002, p. 8–36.
5. Worldwide Animal Meat Consumption per Capita (www.ChartsBin/wiew/bhy, 09.06.20120).
6. Cinquina, A. L., Longo, F., Anastasi, G., Gianetti, L., Cozzaani, R. (2003): Validation of high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlorotetracycline and doxycycline in bovine milk and muscule, J. Cromatogr. A, **987**: 227–223.
7. Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results OJ L 221, 17.8.2002, p. 8–36.
8. JECFA (1995): Joint FAO/WHO expert Committee on food additives. WHO technical report series 851, forty second meeting of JECFA.
9. EMEA (1995): Oxytetracycline, Tetracycline, Chlortetracycline, summary report (3) Committee for veterinary

medicinal products, EMEA, EMEA/MRL/023/95.
10. EMEA (2002): Enrofloxacin, summary report (5) Committee for veterinary medicinal products, EMEA, EMEA/MRL820/02 final, January 2002.

РЕЗУЛТАТИ ОД МОНИТОРИНГ НА ЖИВОТИНСКИ ТКИВА КОИ СЕ КОНСУМИРААТ ЗА РЕЗИДУИ ОД НЕКОИ ВЕТЕРИНАРНИ ЛЕКОВИ

Димитриеска-Стојковиќ Елизабета[1], Арсова Гордана[1], Хајрулаи-Муслиу Зехра[1], Стојановска-Димзоска Билјана[1], Узунов Ристо[1]

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АПСТРАКТ
Вкуп 635 примероци од мускул и бубрег, во рамките на мониторинг планот за храна од животинско потекло во Македонија, беа собрани од кланици во текот на 2010 и 2011. Примероците од ткива беа анализирани за присуство на хлорамфеникол, сулфонамиди, хинолони и тетрациклини. Ензимски-врзаните имуносорбентни методи беа користени за оределување на хлорамфеникол, сулфонамиди и хинолони, а високо-ефикасната течна хроматографија со детектор со низа од диоди беше применета за скрининг на тетрациклини. Методите беа валидирани согласно препораките пропишани во Одлуката на Европската Комисија 2002/657/ЕС. Добиените податоци за точноста, прецизноста и можноста за детекција потврдија дека методите се соодветни за определување на селектираните антимикробни супстанци, на нивото на концентрации кое е од интерес. Измерениот опсег на концентрации за сулфонамиди беше 13,9-74,5 µg/kg, за хинолони 9,7-28,0 µg/kg и 18,4-80,1 µg/kg за вкупни тетрациклини. Во ниту еден случај не е утврдено надминување на воспоставеното максимално ниво на резидуи. Хлорамфениколот не е детектиран над минималното потребно ниво на перформанси (MRPL) на скрининг методот во ниту еден анализиран примерок. Пресметаните проценки за дневниот внос при просечна дневна консумација од 135 g месо, утврдија нивоа кои беа за 20 до над 1000 пати помали од вредностите за прифатлив дневен внос определени од Светската здравствена организација и Европската агенција за лекови.
Клучни зборови: мускул, бубрег, резидуи на ветеринарни лекови, хлорамфеникол, тетрациклини, сулфонамиди, хинолони, ELISA, HPLC-DAD