JRVM
Journal of Research in Veterinary Medicine

RESEARCH ARTICLES
J Res Vet Med. 2019: 38 (1) 52-58
DOI:10.30782/uluvfd.439782

Screening of Veterinary Growth-Promoting Agent and Antibacterial Residues in Beef Cattle and Broiler Meats Consumed in Bursa, Turkey

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Received 02.07.2018 Accepted 18.02.2019

Abstract

This study was aimed to determine residues of growth-promoting agents and some antibacterials in beef cattle and broiler meats consumed in Bursa, as well as to evaluate their hazards on public health. A total of 45 meat samples consisting of 36 beef cattle meat and 9 broiler meat samples were collected from supermarkets and butchers between November and December in 2016. The analysis was carried out by biochip array-based immunoassay technique. This system is also currently used for simultaneous detection and quantitation of different anabolics consisting of β-agonists, boldenone, corticosteroids, nandrolone, ractopamine, stanozolol, stilbenes, trenbolone and zeranol, and six group of antimicrobials consisting of quinolones, cephalosporins, amphenicols, aminoglycosides, macrolides and tetracyclines. Although residues of growth-promoting agents could not be detected in any of the samples, antimicrobial residues from all groups were detected in 10 beef cattle meat samples and tetracycline residues were detected in two broiler meat samples at various levels. In conclusion, there is no risk to consumers for growth-promoter residues according to the results. The detected antibacterial levels were generally lower than hazardous concentrations of residue. However, some detected levels for quinolone, amphenicol, macrolide and tetracycline groups in beef meat samples, and detected concentrations for tetracycline group in two broiler meat samples exceeded the maximum residue limits, and could pose a risk for public health.

Keywords: Growth-promoting agents, antibacterials, biochip array-based immunoassay, beef cattle and broiler meat, residue analysis.

Introduction

Veterinary drugs are being extensively used in livestock for the treatment and prevention of diseases and to improve feed efficiency and promote growth. As a main group of veterinary drugs, antibacterials are mainly used for treatment of infectious diseases in animals. Antibacterials and hormonal substances can also be used legally or illegally for the growth promotion in food producing animals. When the veterinary drugs are being misused or abused in food-producing animals, the drug residues in edible products could lead to health hazards such as allergic reactions and the development of resistant bacterial strains. Hormonal substances may also cause cancer in humans. Hormones or their metabolites could be discharged by excretion into water with effects on environmental pollution, which can lead to hermaphroditism in wildlife.[1-3] Furthermore, some cases by consumption of lamb and bovine meat containing residues of clenbuterol resulted intoxications in humans in different countries with symptoms such as gross tremors of the extremities, tachycardia, nausea, headaches and dizziness.[4-9] In addition, the consumption of trace levels of antibacterial residues in foods of animal origin may have consequences on the indigenous human

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intestinal microflora which constitutes an essential component of human physiology. In view of all these circumstances, foods of animal origin must be monitored for the presence of drug residues.[10]

Simultaneous analysis of different groups of antimicrobials and growth promoters is a difficult task but highly desirable in diagnostic laboratories. A biochip array-based immunoassay test (Antimicrobial Array II, Growth Promoter Agents, Randox Laboratories Ltd., Crumlin, UK) can quantitatively analyse different groups of antimicrobials and growth promoters simultaneously in selected matrices. This apparatus was recently developed and has been used for analysis of foods of animal origin. The test can be used to simultaneously quantify multiple analytes from a single sample.[11,12] Biochip array-based immunoassay technique is a screening test method and was approved by Bornova Veterinary Control Institute (İzmir, Turkey) for antimicrobial array-I, II, III, IV and beta lactam kits in 2015. Many countries have regulated the use of antibacterials as well as growth-promoting agents to prevent hazardous effects on human health. The Codex Alimentarius Commission of the Food and Agriculture Organization, the World Health Organization (WHO)[13] and the European Community (EC)[14] have set maximum residue limits (MRLs) for veterinary drugs used in edible products of food-producing animals. Growth promoters use in food-producing animals has been prohibited by the European Union (EU) by Council Directive 96/22/EC and has also been prohibited in China in 2002 and in Turkey in 2003.[15-17] On the other hand The Codex Alimentarius International Food Standards[13] allows the use of naturally occurring growth promoters and synthetically derived hormones for animal production.

Some news related with beef cattle and broiler meats containing residues of growth-promoting agents and antimicrobials have appeared in visual and written media in Turkey recently. There is not enough data about beef cattle and broiler meats containing residues of growth promoters and antibacterials. Therefore, objectives of this study were to determine residues of antibacterial and growth-promoting agents in beef cattle and broiler meats consumed in Bursa and to evaluate their risks on public health.

Material and Methods

Sample Collection

A total of 45 samples consisting of 36 beef cattle meat samples (18 from different supermarkets and 18 from butchers) and 9 broiler meat samples (9 from different supermarkets) were randomly collected as customer in November and December of 2016 in Bursa. Each sample was 100 to 200 grams and was brought to the laboratory for analysis in cold chain. Samples were kept frozen at -20 °C until nalysis.

Growth Promoter Multiple Matrix Screen (GPMMS)

Biochip array analysis steps were carried out according to the GPMMS EV 3726 manufacturer's instructions.[18] GPMMS kit quantitatively analyse different groups of growth-promoting agents including β-agonists (clenbuterol, carbuterol, brombuterol, salbutamol, methly-clenbuterol, cimbuterol, terbutaline etc.), boldenone (17β-boldenone, 1,4-androstadiene-3, 17-dione and 17α-boldenone), corticosteroids (dexamethasone, flumethasone, betamethasone, dexamethasone 21 acetate and betamethasone 21 acetate), nandrolone (19-nortestosterone (17β), trenbolone, trenbolone acetate, 19-nor-4-androstene,3,17-dione, 19-nortestosterone (17α) and 19-nortestosterone (17β) sulphate), ractopamine (ractopamine and ractopamine hydrocloride), stanozolol (stanozolol and 16β-hydroxystanozolol), stilbenes (hexestrol, diethylstilbestrol and dienestrol), trenbolone (trenbolone (17β) and trenbolone (17α)) and zeranol (zeranol and α-zearalenone) individually or in groups in selected matrices. Immunoaffinity column, column wash buffer, assay diluent, working strength conjugate, working strength reagent were supplied by the manufacturer of the kits. Assay ranges were between 0-5 µg/kg for β-agonists, boldenone, corticosteroids, ractopamine, stanozolol, stilbenes, zeranol and 0-4.5 µg/kg for trenbolone, 0-7 µg/kg for nandrolone and 0-11.5 µg/kg according to GPMMS EV 3726.

Antimicrobial Array-II (AM-II)

Biochip array analysis steps were carried out according to the AM-II EV 3524 manufacturer's instructions.[19] AM-II kit quantitatively analyse different groups of antibacterials including quinolones (norfloxacin, enrofloxacin, ciprofloxacin, oflaxacin, enoxacin and danofloxacin, etc.), ceftiofur, amphenicols (florfenicol and tiamphenicol), streptomycin (streptomycin and dihydrostreptomycin), macrolides (tylosin and tilmicosin) and tetracyclines (tetracycline, 4-epitetracycline, rolitetracycline, 4-epioxytetracycline, oxytetracycline, chlortetracycline, demeclocycline, doxycycline and 4-epichlortetracycline) individually or in groups, simultaneously. Assay diluent, working strength conjugate, working strength reagent were supplied by the manufacturer of the kits. Assay ranges were between 0-7 µg/kg for ceftiofur, 0-5 µg/kg for florfenicol and tylosin, 0-75 µg/kg for streptomycin, and 0-2.5 µg/kg for tetracycline according to AM-II EV 3524.

Three steps immunoaffinity column procedure are available for growth-promoting agents' extraction, consisting of pre-column, column and post-column. In the pre-column step the sample is run through a series of extraction steps to prepare for the column procedure. Column is the

step where the sample is free of impurities and the eluent is obtained. Post-column is the last step where the sample is prepared for the biochip array-based immuonoassay test procedure.[18]

AM-II Extraction Procedure

Nine ml of working strength wash buffer was added to one g of homogenised tissue sample by ultrathorax (Janke and Kunkel Ika-werke, Germany) and mixed by vortex (Boeco Vortex, V1 plus, Germany) for 30 seconds and centrifuged (Sigma, 2-16K, Germany) for 10 minutes at 400 rpm at room temperature. Two hundred µl of supernatant was collected and diluted with 200 µl of working strength wash buffer. Then, the extract was ready for biochip array test procedure.[19]

Biochip Array-Based Immunoassay Test Procedure

Biochip array analysis steps were the same for GPMMS and AM-II. Biochips were equilibrated to room temperature for approximately 30 minutes. After extraction, 100 µl of "assay diluent" was pipetted into the biochip wells. One hundred µl of calibrator or sample was pipetted into the wells. Biochips were incubated at 25°C and 370 rpm for 30 minutes in a thermoshaker (Randox Laboratories Ltd., Crumlin, UK). One hundred µl of working strength conjugate was pipetted into the wells. Biochip wells were incubated at 25°C and 370 rpm for 60 minutes in the thermoshaker. Reagents were discarded to the waste container. Two quick wash cycles were immediately carried out with "diluted wash buffer". Four additional wash cycles were used, then biochips were left to soak in wash buffer for 2 minutes. After the final wash, 250 µl "working signal reagent-EV 805" was added to each well and covered to protect from light in the thermoshaker. After two minutes, the carrier was placed into the Evidence Investigator (Randox Laboratories Ltd., Crumlin, UK). Captures of images were automatically initiated as defined by the dedicated software. Dilution factor was 2.5 for GPMMS and 20 for AM-II.

Results

Biochip array-based immunoassay technique is a screening test and the detected growth promoter and antibacterial levels are different for each substance due to sensitivity differences (%) of the substances according to the method. The analysis system detect the results for a substance for each group that has 100% sensitivity as shown as bold in Table 1. Therefore, equals of a detected other active substance levels for sensitivity of % and positive samples results are presented in Table 1. As a result, we can calculate substance concentrations in a group for positive samples depends on the percent (%) sensitivity and used active substance in veterinary medicine in Turkey. Therefore, some positive results were determined for main molecule by the instrument, and suspicious results should confirm by Liquid Cromatography systems as much as possible.

Growth-promoting agents' residues were not detected in any of the samples. However, antibacterial residues were detected in 10 of 36 beef cattle meat samples (27.7%) and in two of nine broiler meat samples (22.2%). Six antibacterial group residues were detected in 10 beef cattle meat samples and tetracycline residues were only detected in two broiler meat samples. Nine samples were from supermarkets and three samples were from butchers. In beef cattle meat samples, seven positive samples of 18 samples (38.8%) were from markets and three positive samples of 18 samples (16.6%) were from butchers (Table 1).

Discussion

Food safety is important for public health due to outbreaks of meat, liver and offal poisonings with growth promoter residues.[4-9,20-22] and presence of anabolic and antibacterial agents' residues.[23-27]

The use of veterinary medicinal products within the EC is governed by directives and regulations that describe the requirements for application, safety, quality, and efficacy of these products. In Turkey, regulations for veterinary medicinal products are harmonious with European Community directives. The residue of veterinary drugs in meat and related products should be monitored by government authorities and related sections of universities in Turkey.

Any residues of growth-promoting agents were not detected either in beef cattle or broiler meat samples analysed in this study. These results are totally safe for public health for the anabolic agents' residues in beef cattle and broiler meats sold in Bursa. In Turkey, growth promoter substances are forbidden in food producing animals for the growth promotion of livestock, and the detected results can be due to consciousness of veterinarians and management of farms for drug residues and related regulations, and sensitivity of the consumer in Turkey. However, although clenbuterol use as a growth-promoting agent is forbidden, there have been various intoxications caused by the ingestion of liver, meat and offal containing clenbuterol residues in Spain[4,7], France[5], Italy[6,8,21], China[22] and Portuga[l9]. In previous studies in Turkey, although Akkaya et al.24 found higher levels of diethylstilbestrol (DES) (e.g. 1500 ng/kg), zeranol (e.g. 2500 ng/kg), ostrediol (e.g. 1500 ng/kg) and clenbuterol (e.g. 2500 ng/kg) residues in broiler meats, Oruç et al.[25], Mor et al.[28], and Sever et al.[29] detected lower concentrations of zeranol, DES and trenbolen residues in beef cattle meats. Quinolon group was detected a beef cattle meat sample

Table 1. Analysis results of positive samples (µg/kg).

AM-II Groups	Number of Positive Samples		% Sensitivity of Antimicrobial Substances		Positive Sample Results				MRL for Cattle and Chicken Muscle	
					Beef		Broiler			
	(M)	(B)			(M)	(B)	(M)	(B)	Turkey and EU	Codex
QNL	1	-	Norfloxacin	**%100**	**M1: 78.5**	-	-	-	-	-
			Enrofloxacin	%76	M1: 103,3	-	-	-	100	-
			Danofloxacin	%20	M1: 392,8	-	-	-	200	200
CEF	2	2	Ceftiofur	**%100**	**M1: 64.8 M2: 66.0**	**B1: 65.7 B2: 65.0**	-	-	1000	1000
TAF	1	-	Florfenicol	**%100**	**M1: >92**	-	-	-	200	-
			Tiamphenicol	%53	M1: >173,5	-		-	50	-
STR	1	-	Streptomycin	**%100**	**M1: 251.7**	-	-	-	500	600
			Dihydrostreptomycin	%182	M1: 138.3	-	-	-	500	600
TYL	1	-	Tylosin	**%100**	**M1: 72.5**	-	-	-	100	100
			Tilmicosin	%37	M1: 196.1	-	-	-	75	100
TCN	3	1	Tetracycline	**%100**	**M1: >57.6**	**B1: 52.2**	**M2: 49.8 M3: 50.9**	-	100	200
			Oxytetracycline	%52	M1: >110,7	B1: 100.4	M2: 95.8 M3: 97.9	-		
			4-Epioxytetracycline	%52	M1: >110,7	B1: 100.4	M2: 95.8 M3: 97.9	-		
			Doxycycline	%23	M1: >250,4	B1: 227.0	M2: 216.6 M3: 221.3	-		

QNL: Quinolones, CEFT: Ceftiofur, TAF: Thiamphenicol, STR: Streptomycin, TYL: Tylosin, TCN: Tetracycline, (-): Negative, M: Market, B: Butcher.

obtained from market. Among the detected quinolone antibacterial residues, enrofloxacin and danofloxacin are used for cattle in Turkey and both of their levels (103.3 µg/kg and 392.8 µg/kg, respectively) exceed MRL of Turkish, EU (100 µg/kg and 200 µg/kg, respectively) and Codex Alimentarius MRL (Table 1). Especially, detected level of danofloxacin may be a problem for public health. Ceftiofur residues were detected in four samples, but none of them

exceeded the MRL (Table 1). For amphenicols, there was a positive sample and the detected level (>92 µg/kg) was above the assay range of the system. This result may exceed the MRL of 200 µg/kg for florfenicol and could have risk for public health (Table 1). Tiamphenicol evaluation was not done as this substance is not used systemically in beef cattle in Turkey. Detected streptomycin (251.7 µg/kg) and dihydrostreptomycin (138.3 µg/kg) concentrations did not exceed the MRL of 500 µg/kg. Tylosin and tilmicosin from macrolides, were detected in a sample. Tylosin level (72.5 µg/kg) did not exceed the MRL of 100 µg/kg, although tilmicosin level (196.1 µg/kg) exceeded the MRL of 75 µg/kg. Tilmicosin residue level could include risk for public health (Table 1). The detected tetracycline group level (52.2 µg/kg) in a positive sample collected from a butcher exceeded the MRL of 100 µg/kg for oxytetracycline (100.4 µg/kg) and 4-epioxytetracycline (100.4 µg/kg) and doxycycline (227.0 µg/kg). However, concentration of another positive sample (>57.6 µg/kg) supplied from market was above the assay range of the system. This result may exceed the MRL of 100 µg/kg for oxytetracycline (>110.7 µg/kg) and 4-epioxytetracycline (>110.7 µg/kg), and would exceed for doxycycline (250.4 µg/kg). These results could have a risk for public health.

Two broiler meat samples were positive from nine samples and tetracycline residues were the only detected residues. The detected tetracycline group concentrations were 49.8 and 50.9 µg/kg in positive samples collected from markets and did not exceed the MRL of 100 µg/kg for oxytetracycline (95.8 µg/kg and 97,9 µg/kg) and 4-epioxytetracycline (95.8 µg/kg and 97,9 µg/kg). However, doxycycline levels (216.6 and 221.1 µg/kg) exceed the MRL of 100 µg/kg (Table 1). Doxycycline is extensively used in broilers for antibacterial therapy in Turkey. If this broilers treated with doxycycline, the detected levels could have risk for public health. Two doxycycline results in this study could exceed the MRL of Codex Alimentarius and similarly MRL of EU (Table 1). In Turkey, detected positive antibacterials are commonly used in beef cattle and broilers and determination of antibacterial residues is ordinary. However, when the food producing animals are treated with antibacterials and the withdrawal time of the used drug is not considered, drug residues can be found in meats above the MRL. Higher concentrations above the MRL observed in this study may be due not considering the withdrawal time or applying higher doses of antibacterials in beef cattle and broiler. In previous studies[27,30-31], antibacterial residue levels were generally lower than MRL in Turkey. However, some residue results in cattle and sheep meats could exceed MRL[26], such as our results. In previous studies in Oman[32] and Bangladesh[33] in broiler and other meats, antimictobial residues were detected under the MRL. When the results from the market and butchers were considered, although the number of beef cattle meat samples from markets and butchers were same (n=18) positive samples (38.6%) from markets were higher than positive samples from butchers (16.6%).

In conclusion, growth-promoting agents' residues were not detected in any of the samples. Therefore, these beef cattle and broiler meats are safe for public health. However, the detected antibacterial levels including danofloxacin or enrofloxacin residue in a sample, florfenicol residue in a sample, tilmicosin residue in a sample, oxytetracycline or doxycycline residue in two samples in beef cattle meats, and doxycycline residue in two samples in broiler meats exceeded the MRL of Turkey and EU, and Codex Alimentarius and these levels could have risk for public health. For this reason, foods of animal origin should be closely monitored by the Turkish Government authorities for the presence of drug residues especially antibacterials in Turkey.

Acknowledgement

The project was supported by The Commission of Scientific Research Project of Uludag University (KUAP-(V) 2017/4).

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