

## Investigation of Sulfonamide and Florfenicol Residues in Beef collected in Burdur Province, Turkey

Aslı Aygel Ozer<sup>1a</sup>, Murat Bayezit<sup>2b\*</sup>

<sup>1</sup> Ankara Provincial Health Directorate, 112 Provincial Ambulance Service Chief Physician, Ankara, Turkey

<sup>2</sup> Burdur Mehmet Akif Ersoy University, Veterinary Faculty, Department of Pharmacology and Toxicology, Burdur, Turkey

<sup>a</sup>ORCID: 0000-0003-1305-1379; <sup>b</sup>ORCID: 0000-0002-9667-7651

\*Corresponding Author

E-mail: muratbayezit@mehmetakif.edu.tr

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### Abstract

In this study, it was aimed to determine the presence of florfenicol and sulfonamide residue in beef meat samples collected from slaughterhouses in Burdur province center. For this purpose, 100 g of meat samples were collected and florfenicol and sulfonamide residue analyzes were performed by ELISA. Each meat sample was also recorded for which the sample was taken from the particular animal. Florfenicol was detected in 3 of 88 samples (3.4%) at concentrations of 0.97, 1.35, 1.40 µg/kg (mean level: 1.24±0.23), and sulfonamide residues were detected in 3 of 86 samples (3.5%) at concentrations of 27.13, 34.50, 44.40 (mean level: 35.34±8.66). Concentrations of florfenicol and sulfonamide in the residues detected were found to be below the maximum allowed residue limits (200 µg/kg and 100 µg/kg, respectively). No residue were found in remaining 85 and 83 samples. Therefore, it was concluded that florfenicol and sulfonamide residues in samples were not at a level that would threaten the public health.

**Keywords:** Beef meat samples, ELISA, florfenicol, slaughterhouse, sulfonamide.

### INTRODUCTION

Apart from the use for the treatment of infectious diseases and for preventive purposes (Yıbar and Soyutemiz, 2013), antibiotics are also widely used for other purposes such as the growth of food-grade farm animals, increasing the efficiency of these animals and increasing the durability of meat (Weneger, 2003; Tuncer, 2007; Elaldi, 2015).

However, there are some countries that have restricted or even banned the use of antibiotics as growth factors in recent years (Tuncer, 2007).

The most commonly used antibiotics in veterinary medicine; beta-lactams (penicillins and cephalosporins), tetracyclines, macrolides, lincosamides, quinolones, aminoglycosides, sulfonamides, polymyxins (Pericás et al., 2010; Yıbar and Soyutemiz, 2013) and glycopeptides (Yıbar and Soyutemiz, 2013).

The most commonly used antimicrobials in animals raised for food are generally grouped under five groups. These are beta-lactams, tetracyclines, aminoglycosides, macrolides and sulfonamides (Avci, 2010).

Drug residues in foods are usually not found in amounts that can cause pharmacological effects. Some substances with high efficacy may cause undesirable effects in humans by consuming the nutrients containing them, if the animals are slaughtered without observing the pre-slaughter holding period. In 1990, 22 individuals who consumed the liver of clenbuterol-infused calves in France encountered such a situation (Yarsan and Tayyar, 2014).

Like other veterinary drugs, antibiotics can leave residues in milk, meat and animal products such as eggs, depending on their pharmacokinetic properties, so a part of the molecule given to the animal passes to the consumers. For this reason, some approaches are applied within the

framework of the concepts determined by the United Nations affiliated organizations (WHO, FAO) during the registration phase of veterinary drugs to be used in animals whose products are considered as human food (Şener and Yıldırım, 2002).

Antibiotic residues in foods generally cause allergic reactions, damage to some tissues and organs, development of resistance and disruption of the ecological balance in the intestinal flora (Oruç et al., 2007; Özcan et al., 2011; Yıbar and Soyutemiz, 2013; Yarsan and Tayyar, 2014). There are also some antibiotics with reported carcinogenic effects; chloramphenicol, nitrofurans, some sulfonamides and imidazole compounds (aristolochia types such as metronidazole, ronidazole) (Yarsan and Tayyar, 2014). Since antibiotics used in aquaculture can accumulate in various organs and tissues, especially in kidney, liver and muscle, they adversely affect human health, especially animal foods, and the economy of the country (Gökmen et al., 2014). The most common residues are antibiotic residues. Of the antibiotics, streptomycin, penicillin, oxytetracycline and neomycin are the ones that cause the most residual problems. Among the sulfonamides, sulfamethazine is the most problematic residue. Antibiotic residues are mostly found in beef, veal and pork (Gökçen and Atalay, 2012). Sulfonamides are widely used in human and veterinary medicine, especially in the treatment of bacterial and protozoal infectious diseases (Kayaalp, 1984; Kaya et al., 1997; Özalp Dural, 2012). Due to the wide range of uses, sulfonamide residues in animal products cause potential health risks due to their allergic properties and are in the first place in residue detection today. In the 1970s, they started to be used again in specific infections with the introduction of the

combination of trimethoprim and sulfamethoxazole (Bactrim) (Özalp Dural, 2012). Sulfonamides are generally safe substances, but when used, many undesirable and toxic effects of acute and chronic nature are encountered. The main ones are; stomach irritability, increased salivation, nausea, exile, muscle weakness, urinary tract crystallization, urinary incontinence, acute hemolytic anemia, decrease in white blood cell count, vitamin K deficiency, deterioration in the shape of eggshell, urticaria, hypersensitivity reactions (shedding on skin and mucous membranes, drug disease, drug fever, cell death in the liver) (Kaya et al., 2002). It has been reported in the literature that sulfonamides frequently cause fever and diarrhea. Also, hemolytic anemia, Stevens-Johnson syndrome and toxic epidermal necrolysis are rare side effects (Tačić et al., 2017).

In veterinary medicine, the use of florfenicol, especially in food animals, has an important place (Gökmen et al., 2014). Florfenicol is a broad spectrum bacteriostatic antimicrobial agent mostly used in the treatment of bovine respiratory disease (Lambert, 2012). Florfenicol is widely used in the treatment of diseases of animals with nutritional value, due to reasons such as its antibacterial activity is higher than chloramphenicol, it is effective not only against pathogens sensitive to chloramphenicol, but also against chloramphenicol-resistant bacteria, and has less side effects, less acute toxicity, and does not suppress the bone marrow, unlike chloramphenicol. It is known as one of the safest antibiotics widely used (Bektemuroğlu and Şireli, 2011; Ceyhan, 2017). However, the fact that the use of florfenicol in humans has not yet been approved makes it and its residue important (Wang et al., 2012). However, studies show that florfenicol may cause dose-related bone marrow suppression (Sutuli et al., 2021).

The use of various antibiotics in Turkey and the increase in the trade of live animals and animal products at the national and international level necessitate the analysis of animal foods in terms of antibiotic residues (Yüksek, 2001).

Burdur region is one of the important centers of the Mediterranean Region in terms of animal food trade (Mor et al., 2011). In commercial terms, it is important to examine the presence of antibiotic residues in meat in Burdur, where there are a total of 7 facilities in the city and district centers and that these facilities are sold throughout the country, and that they are offered for consumption in Burdur.

In this study, it was aimed to investigate the florfenicol and sulfonamide residue levels in beef collected in Burdur region and to draw attention to serious health problems that may arise as a result of unconscious use of these drugs.

## MATERIALS AND METHODS

The sample of the study consisted of 100 female beef pieces of 100 grams each collected from the slaughterhouses in the city center of Burdur.

### Sampling

Approximately 100 g samples were collected and labeled from slaughterhouses in the city center of Burdur. Collected samples were brought to Burdur Mehmet Akif

Ersoy University, Faculty of Veterinary Medicine, Pharmacology and Toxicology Department Research Laboratory under cold chain. Samples were stored at -20 °C until ELISA analysis.

### Analysis

Sulfonamide (multi-sulfonamide II Elisa - Europroxima\_510 SULMI [5]06.15) and florfenicol (LYS-10008) screenings were performed with commercial ELISA kits in the samples collected from the meat of 100 cattle born between 2000 and 2014, slaughtered in January 2016. Sulfonamide and florfenicol samples were read at 450 nm in an ELISA microplate reader (ELX-800, Bio-Tek Instruments Inc., Winooski, VT, USA). The dilution coefficient was taken as 1 in florfenicol and 12.5 in sulfonamide according to the working principle of the kit.

Which animal the samples belonged to, ear tag numbers, ages, names, surnames, business numbers and addresses of the animal owners were recorded.

The date of birth, the city center or the district from which it came, and the breed of each animal within the scope of the research are given in Table 1.

**Table 1.** Information about samples.

Birth date	Province/Town Center	Breed and number of the animal
2000	Bucak (1).	Holstein (1)
2001	Center (1).	Montofon (1)
2004	Karamanlı (1), Center (1).	Holstein (2)
2005	Bucak (1).	Holstein (1)
2006	Bucak (1), Kemer (1), Center (3), Yeşilova (2).	Holstein (7)
2007	Çeltikçi (1), Karamanlı (1), Center (4).	Holstein (5) Simental (1)
2008	Bucak (2), Center (1).	Holstein (3)
2009	Bucak (2), Çeltikçi (1), Center (6).	Holstein (9)
2010	Bucak (3), Çeltikçi (1), Kemer (2), Karamanlı (1), Tefenni (1), Yeşilova (1), Center (8).	Holstein (16) Simental (1)
2011	Bucak (5), Kemer (5), Center (7), Yeşilova (1).	Holstein (18)
2012	Center (10), Çeltikçi (1), Bucak (3).	Holstein (13) Simental (1)
2013	Bucak (5), Çeltikçi (2), Kemer (1), Karamanlı (1), Center (2), Yeşilova (1).	Holstein (8) Simental (4)
2014	Bucak (3), Center (5), Yeşilova (1).	Holstein (8) Montofon (1)

## RESULTS

In the study, the presence of florfenicol in 88 and sulfonamide residues in 86 of beef pieces of 100 grams collected from slaughterhouses in Burdur city center were investigated by ELISA method. ELISA test results regarding the presence of florfenicol and sulfonamide residues in samples taken from meat are given in Table 2.

**Table 2.** Occurrence of florfenicol and sulfonamide in meat samples.

Samples	Antibiotic	Tested (n)	Positive n (%)	Average Amount ( $\mu\text{g}/\text{kg}$ ) Contamination	
				Ranges	Mean $\pm$ SD <sup>b</sup>
Meat	Florfenicol	88	3 (3.4)	1.35 1.40 0.97	1.24 $\pm$ 0.23
	Sulfonamide	86	3 (3.5)	44.40 34.50 27.13	35.34 $\pm$ 8.66

a The Turkish limit for Florfenicol is 200  $\mu\text{g}/\text{kg}$  in meat samples.

a.The Turkish limit for Sulfonamide is 100  $\mu\text{g}/\text{kg}$  in meat samples.

b SD: Standart deviation

In this study, florfenicol was detected between 1.35  $\mu\text{g}/\text{kg}$  and 1.40  $\mu\text{g}/\text{kg}$ , 0.97  $\mu\text{g}/\text{kg}$  (mean 1.24  $\mu\text{g}/\text{kg}$ ) levels in 3 (3.4%) of 88 bovine muscle tissue samples.

In this study, sulfonamides were detected at the levels of 44.40  $\mu\text{g}/\text{kg}$ , 34.50  $\mu\text{g}/\text{kg}$ , 27.13  $\mu\text{g}/\text{kg}$  (mean 35.34  $\mu\text{g}/\text{kg}$ ) in 3 (3.5%) of 86 bovine muscle tissue samples.

As a result, from bovine muscle tissue samples; florfenicol was detected in 3 (3.5%) of 88 and sulfonamide in 3 (3.4%) of 86 samples. No residue was found in the other 85 florfenicol samples and 83 sulfonamide samples examined.

## DISCUSSION AND CONCLUSION

Antibiotics used in cattle industry for various reasons such as curing diseases and increasing live weight could be dangerous for health (Mor et al., 2011).

In this study, it was aimed to determine whether the residue levels of sulfonamides and florfenicol which are frequently used in veterinary medicine, in beef samples collected from slaughterhouses in the center of Burdur province, pose a risk to human health. In addition, it is aimed to make a contribution to the literature since other studies on sulfonamides and florfenicol antibiotic residues are very few.

As a result of study, florfenicol was detected between 1.35  $\mu\text{g}/\text{kg}$  and 1.40  $\mu\text{g}/\text{kg}$ , 0.97  $\mu\text{g}/\text{kg}$  in 3 (3.4%) of 88 bovine muscle tissue samples.

Gökmen et al. (2014) studied the antibiotic residues in the skin, muscle and liver tissue of 50 trout which were from 25 pre-harvest and 25 during harvest periods from five different trout farms in Elazığ. Florfenicol was reported to be detected in 44% of skin tissue, 32% of muscle tissue and 16% of liver tissue in preharvest trout, and 40% of skin tissue, 36% of muscle tissue and 32% of liver tissue of the trout during harvest period. In this study, sulfonamides were detected at the levels of 44.40  $\mu\text{g}/\text{kg}$ , 34.50  $\mu\text{g}/\text{kg}$ , and 27.13  $\mu\text{g}/\text{kg}$  in 3 (3.5%) of 86 bovine muscle tissue samples.

Oruç et al. (2007) found streptomycin residues at levels between 25.2  $\mu\text{g}/\text{kg}$  and 31.4  $\mu\text{g}$  in 4 of 60 beef samples collected between 2005 and 2006, and sulfamethazine residues at the level of 12  $\mu\text{g}/\text{kg}$  in one of 60 samples in a study they conducted with ELISA. It was concluded that sulfamethazine and streptomycin residues detected in this study could not pose a risk to consumer health. In this study, the percentage of sulfonamide values found in meats (3.5%) Oruç et al. (2007) found that it is less than the percentage of their values (Streptomycin 6%, sulfamethazine 1.5%).

Ramatla et al. (2017) investigated antibiotic residue in beef, chicken and pork, liver and kidneys, using three different methods (ELISA, TLC and HPLC) in a study they conducted. They determined 18%, 92.5% and 88.8% sulfonilamide residues in a total of 150 meats in their study by ELISA, TLC and HPLC methods. Sulfanilamide residue concentrations were determined by ELISA and HPLC methods at 19.8-92.8  $\mu\text{g}/\text{kg}$  and 20.7-82.1  $\mu\text{g}/\text{kg}$ , respectively. In this study, sulfanilamide was determined in 1 (6.6%) of 15 cattle muscles, 5 (29.4%) of 17 livers and 5 (27%) of 18 kidneys; Sulfanilamide residues in 3 (12%) of 25 chicken muscles and 7 (28%) of 25 chicken livers; While it was not detected in 16 pork meats, sulfanilamide residues were detected in 4 (36.4%) of 11 pig livers and 2 (8.2%) of 23 pig kidneys.

Heat treatments applied to foods, fermentation, cold storage and other food processing methods affect the antibiotic residue percentage of the food. It has been determined that the degradation rates of sulfonamides in eggs and milk range from zero to 99% (Tian et al., 2017).

In various studies on meats, it has been determined that heat treatments reduce the sulfonamide levels at certain rates (O'Brien et al., 1981; Alfredsson and Ohlsson, 1998; Van Egmond et al., 2000; Furusawa and Hanabusa, 2002; Javadi et al., 2011; Elbagory et al., 2017).

Amfenicols is a class of broad-spectrum antibiotics that includes chloramphenicol, florfenicol, and thiamphenicols (Tian et al., 2017).

Likewise, in other studies on meat, it was determined that antibiotic residues decreased but could not be completely eliminated. As a result of the study conducted in Bulgaria to examine the effect of cold storage on the residues of tobramycin in chicken meat, it was observed that tobramycin residues in broiler tissues (thigh, breast meat and liver) were significantly reduced by keeping them at -18oC for 60 days (Pavlov et al., 2005). When the effect of heat treatments on various antibiotic residues in pork was examined, it was observed that the residues of penicillin, amoxicillin, ampicillin, cloxacillin, oxytetracycline, doxycycline, colistin, dihydrostreptomycin and sulfamethoxazole were not completely destroyed after sterilization at 134oC for 20 minutes under 3 atmospheres, and approximately 10%. It was found that the flour remained in the meat (Van Egmond et al., 2000).

As a result of the study, 88 beef samples collected from slaughterhouses in the city center of Burdur were examined for the presence of florfenicol residues and florfenicol antibiotic residues were detected in 3 beef. No florfenicol residues were found in the other 85 beef

samples examined in the study. In the same way, 86 beef meats collected from slaughterhouses in the city center of Burdur were examined for the presence of sulfonamide residues, and sulfonamide antibiotic residues were detected in 3 beef meats. No sulfonamide residues were found in the other 83 sulfonamide beef samples examined in the study.

The maximum concentration of pharmacological active substance residue allowed in animal foods refers to the Maximum Residue Limit (Official Gazette, 2017). According to the regulation of the Turkish food codex on the classification of pharmacological active substances that can be found in animal foods and the maximum residue limits, the legal maximum limit for meat in Turkey is 100 µg/kg for sulfanamide and 200 µg/kg for florfenicol (Official Gazette, 2017). In this study, both sulfonamide and florfenicol remain below the maximum residue levels determined for our country and are considered to pose no risk.

Access to quality food is one of the most fundamental problems of our time. Various measures have been taken to solve this problem, national strategies have been developed, but these solutions have brought various problems together. Foodborne diseases are increasing rapidly in many countries (Bekar, 2013).

Alfredsson and Ohlsson (1998) injected five different sulfonamides into bovine and pig muscle samples and kept them frozen for 3 months. It was determined that the drug levels did not change for 24 hours at room temperature and 1 week at -20°C, but after 1 month of frozen storage, the levels were significantly reduced in bovine and porcine muscle. After three months, the average reduction was 35% in cattle and 55% in pork muscle.

Cooking time and temperature are the two main factors affecting antibiotic residues in meat; In cooking procedures, adequate heating temperature and time can reduce several antibacterial drug residues. However, it generally does not provide consumers with additional security (Heshmati, 2015).

In order to prevent the senseless use of antibiotics by animal owners, the legislation of the Ministry should be expanded. Awareness raising can be done by giving breeder trainings in villages and cities in cooperation with both the personnel of the Ministry of Food, Agriculture and Livestock and the University. At the same time, residue problems in veterinary medicine can be prevented by taking the prohibition of the sale of antibiotics without prescription, which was enacted by the Ministry of Health. In this study, it could be concluded that florfenicol and sulfonamide amounts do not pose a risk to consumer health since they are below the maximum residual levels.

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#### Conflict of Interest

The authors declared that there is no conflict of interest.

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