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Research Article

The Residues and Risk Assessment of Sulfonamides in Animal Products

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Objective. To investigate and assess the risk of sulfonamide residues in livestock and poultry products in Shijiazhuang and determine the risk level of the dietary intake of sulfonamides, in order to provide the basis for the safety production, consumption, and safety supervision of livestock products. *Methods*. Totally, 1200 samples of livestock products were collected, and the samples were detected by high-performance liquid chromatography-tandem mass spectrometry. Combined with the data of Chinese residents' dietary survey in 2015, a nonparametric probability assessment model was constructed to assess the risk of sulfonamides in the livestock and poultry products of Shijiazhuang residents by using the professional risk assessment software @Risk. Risk assessment of the consumption of sulfonamide veterinary drugs in livestock and poultry products of Shijiazhuang residents was conducted. *Results*. Of the 1200 main livestock products tested, 8 were found to have sulfonamide residues, which were mainly sulfadiazine residue, sulfadiazine, and sulfadimethoxy, with the detection rates of 0.17%, 0.25%, and 0.25%, respectively. The average residual concentrations were 0.66, 0.50, and 0.50 g/kg, respectively, which were lower than the national residue limit of China (100 μg/kg). The food safety index was 2.95×10^{-4}, which was far less than 1. *Conclusion*. The risk of residual exposure to sulfonamides in livestock and poultry meat in Shijiazhuang is very low and is at a very safe level. However, it is still necessary to strengthen the supervision of animal products in order to reduce the residues of veterinary drugs in the human body.

1. Introduction

In recent years, livestock and poultry products, such as meat, eggs, and milk, are taking an increasing proportion in the diet of Chinese consumers, and food quality has received extensive concern throughout the society. Among others, sulfonamide residue is a critical influencing factor of livestock and poultry product quality. Sulfonamides are a type of synthetic drugs mainly used in livestock production and veterinarian clinical practices and are very effective to cure and prevent bacterial diseases and parasitic diseases [1]. Sulfonamides have made remarkable contribution to the development of stock farming industries ever since their extensive application, are especially featured by affordable cost, easy application, wide antimicrobial spectrum, and stable properties, and provide improved antimicrobial activity and bactericidal effect when used together with synergists [2]. Sulfonamides have a peculiar p-aminobenzenesulfonamide structure and compete with para-aminobenzoic acid for dihydrofolate synthetase in the body metabolism, preventing the synthesis of folic acid in the bacteria [3]. However, sulfonamides have a slow metabolism and long stay in the body, which result in excess residues in the livestock and poultry. Long-term intake of the animal products with excess sulfonamide residues may cause the building up of sulfonamide residues in the human body and cause a variety of toxic effects, such as hemopoietic system disorder, allergy, and cancer [4]. In the US and EU, laws and standards require that the maximum residue limit of sulfonamides in animal source foods is 100 μg/kg [4, 5], and in China, the maximum residue limit of sulfonamides in the animal muscle and liver is also 100 μg/kg (MOA Announcement No. 235) [6].

Currently, many research studies are focused on the risk assessment of pesticide residues in vegetables and fruits [7, 8], but the research on animal-based livestock and poultry products is mainly focused on the detection methods. There are also many research studies on the livestock and poultry product quality assurance and tracing systems as well as the detection methods [9, 10]; however, only a small number of the research studies are aimed to report and study the risk assessment of sulfonamide residues in livestock and poultry products. This research studies the sulfonamide residues and risk levels in livestock and poultry products available and marketed in Shijiazhuang, by taking 1,200 random samples of pork, beef, mutton, and chicken in Shijiazhuang, on the basis of livestock and poultry product quality monitoring findings in Shijiazhuang between 2015 and 2017 and by taking the 2015 Report on Chinese Resident's Chronic Disease and Nutrition and dietary guidelines into overall consideration and aims to provide data reference and scientific basis for resident's consumption and scientific supervision.

2. Materials and Methods

2.1. Sampling. Totally, 1,200 samples, in 600 g each, of pork, beef, mutton, and chicken products were collected from the markets of Shijiazhuang. The samples collected are products available in various markets: supermarkets, open fair, and slaughter companies, throughout the 18 counties and 6 districts in Shijiazhuang. After samples were collected, they are homogenized and stored in a refrigerator at −20°C. All samples were collected in accordance with *Sampling Criterion for the Monitoring of Veterinary Drug Residues in Animals and Animal Products* (NY/T 1897–2010) [11].

2.2. Reagents and Instruments

2.2.1. Reagents and Consumables. Methyl alcohol and acetonitrile (chromatographically pure, Fisher Scientific); n-hexane and n-propyl alcohol (analytically pure, Tianjin No.1 Chemical Reagent Factory), methanoic acid, acetic acid, ethyl acetate, sodium dihydrogen phosphate, and anhydrous sodium sulfate (analytically pure, Shenyang No. 1 Reagent Factory); solid-phase column extractor (Alumina-B SPE extractor, Waters); etc. were used. Experiment water is the ultrapure water produced by the Milli ultrapure water system.

2.2.2. Reference Material. The reference materials in this research are sulfonamides, which mainly and specifically include sulfadiazine (SD), sulfamerazine (SM1), sulfamethazine (SM2), sulfamonomethoxine (SMM), sulfadimethoxine (SDM), sulfamoxol, sulfamethoxazole (SMZ), sulfisoxazole (SIZ), sulfaquinoxaline (SQX), and sulfachloropyridazine (SCP) (purity ≥99.0%, China Institute of Veterinary Drug Control).

2.2.3. Instruments. A 5982–9110 solid-phase extraction device, 1290–6410B high-performance liquid chromatography-tandem mass spectrum analyzer (Agilent); 3K30 high-speed freezing centrifuge (SIGMA); RE-52AA rotary evaporator (Shanghai Yarong), N-EVAP112 Termovap sample concentrator (Organomation); ultrapure water system (Millipore); SK-1 vortex mixer (Jiangsu Jintan); and BSA2202S electronic balance (Sartorius) were used.

2.3. Testing Method. Samples are tested in accordance with *Determination of Sulfonamide Residues in Edible Tissues of Animal, Liquid Chromatography-Tandem Mass Spectrometry* [12] (MOA Announcement No. 1025-23-2008) and reference resources of *Rapid analysis of fifteen sulfonamide residues in pork and fish samples by automated online solid phase extraction coupled to liquid chromatography-tandem mass spectrometry* [13] for the sulfonamide levels, and the results obtained from the tests are evaluated in accordance with the methods provided in the standard.

For all results reported as nondetectable, the data are processed in accordance with the principles provided in Session 2 of GEMS/FOOD regarding "reliable evaluation of contamination in food": data of the nondetectable samples should be substituted by the Limit of Detection (LOD) when more than 60% data are "not detected" or substituted by 1/2LOD when 60% and less data are "not detected" [14–16].

2.4. Average Intake of Livestock and Poultry Products by Consumer Groups in Shijiazhuang. As for the intake of animal source food, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is currently using a very conservative artificial estimation method against reference food, which assumes that a person of 60 kg body weight consumes 300 g animal/poultry muscle, equivalent to the upper limit of daily intake of animal tissue and products [17]. However, in this research, as shown in Table 1, the mean intake of animal products by consumers in Shijiazhuang is based on the *2015 Report on Chinese Resident's Chronic Disease and Nutrition* [18], according to which the mean daily intake of animal/poultry food by each Chinese resident is 89.7 g, including 64.3 g pork, 8.2 g other animal products, 2.5 g internal organs, and 14.7 g poultry food.

2.5. Mean Body Weight of Populations in Shijiazhuang. The mean body weight of populations in Shijiazhuang is based on the *2015 Report on Chinese Resident's Chronic Disease and Nutrition*, and this research only studies and evaluates the sulfonamide residues in animal/poultry food of adult consumers aged 18 or above and groups the research objects by age, place (urban or rural), and gender, on the basis of the reference intake of Chinese residents and food consumption models [19]. The body weight data of these target consumers are shown in Table 2.

2.6. Risk Assessment Methods. In accordance with the definition and procedures provided by the Codex Alimentarius Commission (CAC) for risk assessment of contaminants in foods, this research assesses the safety of animal/poultry products using the mean value of Index of Food Safety (IFS) [20]. The research is conducted on the basis of the Acceptable Daily Intake (ADI, in μg/(kg·bw·d)) provided by the Ministry

Table 1: Main food intake of Chinese residents in 2012 (g/d•person).

Group	Nationwide	City	Countryside
Pork	64.3	68.8	59.9
Cattle/sheep	8.2	10.5	6.0
Gut	2.5	2.9	2.2
Chicken	14.7	16.3	13.1
Sum	89.7	98.5	81.2

Table 2: Average weight of Chinese residents (kg).

Group (age)	Nationwide			City			Countryside		
	Male + female	Male	Female	Male + female	Male	Female	Male + female	Male	Female
18~44	62.00	67.00	56.70	63.10	68.70	57.20	61.00	65.30	56.30
45~59	63.10	66.60	59.50	64.50	68.50	60.40	61.50	64.40	58.40
≥60	58.90	62.40	55.60	61.00	61.00	57.40	56.70	60.00	53.60
Sum	61.80	66.20	57.30	63.20	68.00	58.20	60.40	64.30	56.30

of Agriculture and Rural Affairs for sulfonamides, adopts the internationally adopted probability assessment methods, i.e., a Monte Carlo simulation-based @Risk7.5 risk assessment software system in random simulations, by which the daily sulfonamide intake is obtained as the product of the daily intake of animal/poultry products multiplied by the sulfonamide residues, and estimates the exposure of main population groups in Shijiazhuang, both urban and rural, to the veterinary antibiotics through diet [21].

The evaluation and calculation formula is [22]

$$\mathrm{EDI}_c = \sum (R_i \times F_i),$$
$$\mathrm{IFS}_c = \sum \frac{R_i \times F_i \times f}{\mathrm{SI}_c \times bw}, \quad (1)$$

where IFS_C is the food safety index of sulfonamide C; EDI_C is the estimated daily intake of sulfonamide C; i is the type of livestock and poultry product; R_i is the residue level of sulfonamide C in product i, expressed as μg/kg; F_i is the per-capita daily intake of livestock and poultry product i, expressed as kg/person·day; bw is the body weight, expressed as kg; and f is a correction factor. $f = 1$, if the ADI, RFD, and PTDI are adopted as the safe intake data. SI_C is the safe intake and obtained from the ADI, PTWI, or RFD data depending on the chemicals concerned. In this research, the evaluation is based on the Acceptable Daily Intake (ADI, in μg/(kg·bw·d)) provided by the Ministry of Agriculture and Rural Affairs for veterinary drugs [6, 23]. According to related standards, the ADI for sulfonamides is, respectively, 10, 20, and 50 μg/(kg·d), among which the ADI for sulfadimidine is 50 μg/(kg·d). Some internationally adopted ADI values are also taken into consideration; specifically, the ADI for sulfadimethoxine is set as 6 μg/(kg·d) by referring to the internationally adopted 6 μg/(kg·d) ADI of sulfamonomethoxine.

The IFS value, if smaller than 1, means that the sulfonamides detected may cause insignificant harm to human health and the risks are acceptable, and if the IFS value is larger than 1, it means that the harm of sulfonamides detected to human health is unacceptable and the risk control procedures should be activated [24].

3. Analysis and Results

3.1. Sulfonamide Residues in Livestock and Poultry Products. As shown in the Table 3, sulfonamide residues were detected in 8 out of 1,200 livestock and poultry meat samples collected from markets in Shijiazhuang, and the residues mainly include sulfamethazine, sulfamonomethoxine, and sulfadimethoxine, with detection rate being 0.17%, 0.25%, and 0.25%, and on the other hand, sulfadiazine, sulfamerazine, sulfamoxol, sulfamethoxazole, sulfisoxazole, sulfaquinoxaline, and sulfachloropyridazine were not detected. The mean residue concentration of sulfadimidine, sulfamonomethoxine, and sulfadimethoxine detected in the animal/poultry products is 0.66, 0.50, and 0.50 μg/kg, respectively, which are much lower than the maximum residue limit of 100 μg/kg as stipulated by national standards.

3.2. Risk Assessment on Sulfonamide Residues in Livestock and Poultry Products. The risks of exposure to sulfonamides by residents in Shijiazhuang are assessed in accordance with the data of mean body weight and intake of main food by Chinese residents in 2012 as provided in the *2015 Report on Chinese Resident's Chronic Disease and Nutrition*. As shown in the Table 4, it is found through calculation that based on the intake of main food by Chinese residents, the per-capita daily exposure of Shijiazhuang residents to sulfonamide residues in food is 0.4459 μg, specifically 0.4897 μg for urban residents and 0.4037 μg for rural residents; and on the other hand, if calculated on the basis of per-capita daily consumption of 300 g animal/poultry muscle as provided by JECFA, the per-capita daily exposure of Shijiazhuang residents to sulfonamide residues in animal/poultry products is 1.4915 μg. The results obtained by both methods are far below the maximum acceptable daily intake of 30 μg. According to the second calculation method, the IFS is 2.95×10^{-4} and 1.02×10^{-3}, respectively, which are much lower than 1. The results show that the sulfonamide residues in animal/poultry products available in Shijiazhuang market are at very low level and are fairly safe. The index of food safety of sulfonamides in animal products of Shijiazhuang is shown in Table 5.

Table 3: Monitoring result of sulfonamide residues in animal products.

Sulfonamides	Sample numbers	Detectable numbers	Detectable rate/%	Excessive numbers	Excessive rate/%	Min/ (μg/kg)	Max/ (μg/kg)	Average/ (μg/kg)	SD/ (μg/kg)
Sulfamethazine	1200	2	0.17	0	0	ND	26.30	0.66	0.40
Sulfamonomethoxine	1200	3	0.25	0	0	ND	4.65	0.50	0.12
Sulfadimethoxine	1200	3	0.25	0	0	ND	3.93	0.50	0.10

Table 4: The exposure of sulfonamides in animal products (μg/d▪person).

Group	Nationwide	City	Countryside
Min	0.0897	0.0985	0.0812
Middle	0.4459	0.4897	0.4037
Max	7.2146	7.9224	6.5309
JECFA		1.4915	

Table 5: The index of food safety of sulfonamides in animal products of Shijiazhuang.

Group	Age	Nationwide			City			Countryside		
		Male + female	Male	Female	Male + female	Male	Female	Male + female	Male	Female
Min	18~44	1.35×10^{-4}	1.26×10^{-4}	1.46×10^{-4}	1.45×10^{-4}	1.35×10^{-4}	1.58×10^{-4}	1.25×10^{-4}	1.18×10^{-4}	1.35×10^{-4}
	45~59	1.35×10^{-4}	1.25×10^{-4}	1.48×10^{-4}	1.46×10^{-4}	1.34×10^{-4}	1.61×10^{-4}	1.24×10^{-4}	1.16×10^{-4}	1.35×10^{-4}
	≥60	1.33×10^{-4}	1.26×10^{-4}	1.41×10^{-4}	1.43×10^{-4}	1.34×10^{-4}	1.52×10^{-4}	1.23×10^{-4}	1.18×10^{-4}	1.30×10^{-4}
	Sum	1.42×10^{-4}	1.34×10^{-4}	1.51×10^{-4}	1.51×10^{-4}	1.42×10^{-4}	1.60×10^{-4}	1.34×10^{-4}	1.26×10^{-4}	1.41×10^{-4}
	JECFA					1.40×10^{-4}				
Middle	18~44	2.94×10^{-4}	2.72×10^{-4}	3.21×10^{-4}	3.17×10^{-4}	2.91×10^{-4}	3.50×10^{-4}	2.70×10^{-4}	2.53×10^{-4}	2.93×10^{-4}
	45~59	2.89×10^{-4}	2.74×10^{-4}	3.06×10^{-4}	3.10×10^{-4}	2.92×10^{-4}	3.31×10^{-4}	2.68×10^{-4}	2.56×10^{-4}	2.83×10^{-4}
	≥60	3.09×10^{-4}	2.92×10^{-4}	3.28×10^{-4}	3.28×10^{-4}	3.28×10^{-4}	3.49×10^{-4}	2.91×10^{-4}	2.75×10^{-4}	30.8×10^{-4}
	Sum	2.95×10^{-4}	2.75×10^{-4}	3.18×10^{-4}	3.17×10^{-4}	2.94×10^{-4}	3.44×10^{-4}	2.73×10^{-4}	2.57×10^{-4}	2.93×10^{-4}
	JECFA					1.02×10^{-3}				
Max	18~44	3.31×10^{-3}	3.07×10^{-3}	3.62×10^{-3}	3.58×10^{-3}	3.28×10^{-3}	3.94×10^{-3}	3.05×10^{-3}	2.85×10^{-3}	3.30×10^{-3}
	45~59	3.26×10^{-3}	3.09×10^{-3}	3.45×10^{-3}	3.50×10^{-3}	3.29×10^{-3}	3.74×10^{-3}	3.02×10^{-3}	2.89×10^{-3}	3.18×10^{-3}
	≥60	3.49×10^{-3}	3.29×10^{-3}	3.70×10^{-3}	3.70×10^{-3}	3.49×10^{-3}	3.93×10^{-3}	3.28×10^{-3}	3.10×10^{-3}	3.47×10^{-3}
	Sum	3.32×10^{-3}	3.10×10^{-3}	3.59×10^{-3}	3.57×10^{-3}	3.32×10^{-3}	3.88×10^{-3}	3.08×10^{-3}	2.89×10^{-3}	3.30×10^{-3}
	JECFA					3.42×10^{-3}				

4. Discussion

It is found in the research that the toxic reaction of chemical contaminants is highly related to the absolute amount into the human body [25], and therefore, the actual intake of certain chemical contaminants into the body and its comparison with the safe intake amount is used to assess whether the food is safe or not, the Index of Food Safety (IFS) is used to describe whether the chemical contaminants taken into human body from food can cause harm to the human body, and the calculated results are used to assess the safety influence after consuming the food, so that the results are used to guide the risk control practice and actions are taken accordingly to lower the risks [26]. IFS takes the food consumption into overall consideration and reflects the level of food contamination and the harm to consumers by the contaminated food. Through the residue monitoring and dietary exposure assessment, we can effectively assess the level of harm to human health by certain contaminants in the food.

In this research, the IFS is used to compare the EDI of sulfonamide residues in animal/poultry products with the ADI values provided by the Chinese Ministry of Agriculture and Rural Affairs for sulfonamides [27], in order to assess the potential risk level and food safety. In this research, there are some uncertainties in the calculation of exposure levels of sulfonamides; for example, a small amount of plucks are also included as animal/poultry meat in the diet of Chinese residents, besides the detection is mainly targeted at the sulfonamides as required by the testing method, while no detection and analysis are made for other sulfonamide-type veterinary drugs available in the market. The research mainly studies and assesses the exposure and risks of adults aged 18 and above; however, infants, children, and teenagers are not considered in the research. Given that the Chinese regulations on ADI of sulfamonomethoxine and sulfadimethoxine are not yet available, the evaluation is based on the internationally adopted ADI [23] of 6 μg/(kg·d), in order to ensure the stringency of the risk assessment.

Guan et al. [2] reported that, in the risk assessment on sulfonamide residues in pork in Heilongjiang, sulfamethazine residue is detected in only 1 of the 100 samples, with a detection rate of 1%, but the result is still below the maximum residue limit allowed by related national standards. Zhang et al. [3] reported that, in the studies on cumulative exposure to sulfonamides in chicken products by 10 typical populations in a Guangdong city, residues of sulfaquinoxaline, sulfamethazine, sulfamonomethoxine, and

sulfadiazine were detected in 9 out of 100 chicken samples; however, none of the residues detected are above the maximum allowed limit and that the IFS is far lower than 1. The abovementioned research shows that sulfonamide veterinary drugs are still widely used in the Chinese livestock and poultry farming industry and the use of sulfonamide veterinary drugs varies from region to region. This research finds that no animal/poultry products were detected with sulfonamide residues higher than the national maximum limit in recent years, besides the IFS obtained, respectively, on the basis of either the main food intake data and body weight data of Chinese residents and on the basis of the JECFA standard food consumption data is much smaller than 1, which indicates that the sulfonamide residues in animal/poultry products in Shijiazhuang will not cause harm to human health and are acceptable. However, it is found in the research that sulfamethazine, sulfamonomethoxine, and sulfadimethoxine residues were detected in the animal/poultry products, despite that the residue concentration remain much lower than the national maximum limit and will not cause any harm to human health, and these residues remain potential risks that may influence the food products from pork. As such, the governmental authorities should pay sufficient attention to such potential risks and strengthen the food safety supervision, to ensure the quality and safety of the animal/poultry products.

Data Availability

The data used to support the findings of this study are currently under embargo, while the research findings are commercialized. Requests for data, 12 months after publication of this article, will be considered by the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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