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Determination of the presence of pharmacological residues in human feces by liquid chromatography-tandem mass spectrometry

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ABSTRACT

The use of veterinary drugs in animal husbandry is a normal practice to ensure animal and human health, but residues of these active substances can be present in the final food. Therefore, levels of veterinary drugs in food of animal origin are regulated within the European Union. Humans are also exposed to pharmaceuticals unintentionally through the food chain due to their presence in the environment. This article presents a study conducted on feces of 109 volunteers who did not consume pharmaceuticals within the last two months. Up to 11 different drugs were detected and their concentration ranged from 6 to 13,661 ng/g. Taking into consideration foof-frequency questionnaires and dietary records of the volunteers, results of positive and negative samples were compared but no significant difference were observed. No relationship was found between the consumption of food of animal origin or the consumption of plant-derived foods and the presence of residues of drugs in feces samples.

1. Introduction

The use of pharmaceuticals to treat diseases in humans is a common practice However, pharmaceuticals are not only used for people; they are also necessary for food-producing animals. Approximately 80 % of food-producing animals are treated with veterinary drugs (Pavlov, 2008); therefore, these substances can be present in food of animal origin including milk, honey, meat, eggs or fish. The maximum residue limit (MRL) is the highest concentration of a substance that can be present in food without causing harm to the consumer. Within the European Union MRL of pharmacologically active substances are established in Regulation 37/2010 and 124/2009. These regulations also detail the drugs that cannot be present in foods of animal origin. These values are calculated based on the estimation of a standard shopping basket, acceptable daily intake, and the amount that can be consumed daily over a lifetime without health risks. These residues in food must not endanger the health of the consumer according to various international organisations, including the European Food Safety Authority (EFSA). Similarly, the report published in February 2024, which included the results of a total 600,320 analysed samples indicated that only 0.18 % of them were declared non-compliant. Each country submits the data derived from the analysed samples to EFSA. In the case of Spain, this information is transmitted by the Ministry of Agriculture, Fisheries and Food through its National Residue Investigation Plan (PNIR). However, to date, data on samples with drug residues below the MRL are not reported to EFSA. What has been published is data from different research studies conducted in various matrices including milk (Spisso, Pereira, Ferreira, Silva, & Arisseto-Bragotto, 2022), eggs (Dasenaki and Thomaidis, 2015, Kamali, Mirlohi, Etebari, & Sepahi, 2020), chicken meat (Cetinkaya et al., 2012), pork meat (Kyriakides et al., 2020; Soares et al., 2022),and fish (Carmona, Andreu, & Picó, 2017; Pashaei, Dzingelevičienė, Abbasi, Szultka-Młyńska, & Buszewski, 2022).

In addition to veterinary treatment, antibiotics are also applied to vegetables and fruits for pest control and food preservation. In the United States, 0.5 % of the total amount of antibiotics is used in fruit and vegetables (McManus, Stockwell, Sundin, & Jones, 2002) being the more commonly employed Oxytetracycline and Streptomycin. Additionally, vegetables and fruits can also uptake antibiotics from

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contaminated soil where they have been cultivated in soils contaminated. An example of detection is the data presented by Yu et al. (2018) who measured Chlortetracycline, Oxytetracycline and Tetracycline in vegetables from Beijing, with a maximum concentration of 8.84 μg/kg.

Water can also be contaminated. When drugs are consumped they undergo different metabolic and elimination processess. Drugs are eliminated from the body as metabolite or unchanged compound mainly through urinary excretion and also in feces. The rate of excretion of a drug in feces varies depending on the drug, for example, 80 % of Dinofloxacin is eliminated in feces and 20–30 % of Diclofenac. Taken into account that sewage treatment plants are not able to completely eliminate pharmaceuticals from water they are discharged into the environment contaminating rivers, lakes and soils. Pharmaceuticals such as antibiotics, antihypertensives, anti-inflammatory agents and glucocorticoids have been reported in river water (Iglesias et al., 2014; Iglesias, Nebot, Miranda, Vazquez, & Cepeda, 2012) explaining their further detection in drinking water from Spain (Boleda, Alechaga, Moyano, Galceran, & Ventura, 2014), France (Charuaud et al., 2019), UK (Kasprzyk-Hordern, Dinsdale, & Guwy, 2008), Italy (Papagiannaki, Morgillo, Bocina, Calza, & Binetti, 2021), Germany (Burke et al., 2016), USA (Bexfield, Toccalino, Belitz, Foreman, & Furlong, 2019) and China (Ly et al., 2019). If contaminated waters are also used to irrigate vegetables and fruits, they will also absorb the drugs and therefore be ingested by consumers (Feng et al., 2018; Li et al., 2014; Tadić et al., 2021; Zhang et al., 2016).

The intake of pharmaceuticals through food (food of animal origin, vegetables or waters) at low concentrations could influence the development of bacteria with resistance genes due to the continuous exposure to low concentrations of antibiotics that will lead resistance bacteria to survive (Martinez, 2014). Nowadays, 700,000 humans die each year from bacteria resistant to antibiotics (World Health Organization, 2021) and it expects that in 2050 resistant bacteria will be the cause of 10 million of deaths every year. Other risks associated with involuntary consumption of antibiotics are the effects on gut microbiota, such as a reduction of microbiota diversity (Mani, Boelsterli, & Redinbo, 2014; Maurice, Haiser, & Turnbaugh, 2013) or alteration of metabolic activity (Lange et al., 2016; Falony et al., 2016; Zhernakova et al., 2016; Maier et al., 2018) which could cause colonization by pathogenic organisms and infection (Panda et al., 2014). Van de Leur, Vollaard, Janssen, and Dofferhoff (1997) reported elimination of Gram-Negative Bacilli in infants after exposition to low doses of Ciprofloxacin. In mouse, Roca-Saavedra et al. (2018) demonstrated that exposure to low doses (100 μg/kg) of three antibiotics (Ampicillin, Tetracycline and sulfadiazine) altered gut microbiota.

For all these reasons, it is interesting to continue investigating whether there are effects on the microbiota and its possible effect on health due to the consumption of low doses of drugs ingested involuntarily through the diet. A study conducted with mice indicated that subtherapeutic doses of antibiotics modified their microbiota, altered the lipid metabolism, and increased their obesity (Choi et al., 2013; Cox et al., 2014).

The analysis of the presence of pharmaceuticals in feces is a good indication of the involuntary intake of drugs and could help to understand their effects on human gut microbiota and possible alterations. Given that the main source of unintentional exposure to residues of veterinary pharmaceuticals is through the consumption of food of animal origin, the objective of this research was to investigate the presence of pharmaceuticals in human feces and to evaluate their possible correlation with consumption of food of animal origin by analysing the dietary records of volunteers.

2. Materials and methods

2.1. Feces samples

A total of 109 samples of feces were collected from volunteers (49 men and 60 women, between 18 and 66 years old) participating in the IBEROBDIA project, funded by CYTED (918PTE0540), and by the State Research Agency of Spain (PCI2018–093245). This project has the approval of the Ethics Committee of the Galician Healthy System (SERGAS, Xunta de Galicia), code 2018/270. It is important to highlight that one of the inclusion-exclusion criteria was that volunteers had not taken any drug in the last two months, previously to the collection of the feces samples. The two-month period without pharmacological treatment was established to ensure the absence of pharmacological residues in the feces and to allow for the subsequent study of the microbiota. The samples were collected by the volunteers at home in sterile polyethylene bottles (Anaclin) and stored in the freezer for a maximum of two days before being collected by members of the research team. The samples were then transported to the laboratory in a portable refrigerator within less than two hours. Upon arrival at the laboratory, the samples were immediately frozen until analysis. During transport, the samples were maintained in a frozen state.

2.2. Chemicals, reagents, and stock solutions

Chloramphenicol, Chlortetracycline, Ciprofloxacin, Clarithromycin, Danofloxacin, Dexamethasone, Diclofenac, Difloxacin, Doxycycline, Enrofloxacin, Florfenicol, Griseofulvin, Levofloxacin, Lincomycin, Mefenamic Acid, Norfloxacin, Oxytetracycline, Paracetamol, Propranolol, Sarafloxacin, Spectinomycin, Sulfachloropyridine, Sulfadiazine, Sulfadimethoxine, Sulfamerazine, Sulfamethoxazole, Sulfamethoxypyridazine, Sulfamethasone, Sulfapyridine, Sulfaquinoxaline, Sulfathiazole, Tetracycline and Trimethoprim with purity between 98 and 101 % were bought from Sigma-Aldrich (MO, USA). Acetonitrile (ACN) and methanol (MeOH) (HPLC grade $\geq$ 99 %) were obtained from Acros Organics (Geel, Belgium) and purified water was prepared in the laboratory with a Milli-Q system from Millipore (Redford, MA, EEUU).

2.3. Preparation of standard solutions

The standards were prepared from certified standards in which purity is described. Standard solutions were prepared as described in an accepted and published research by Míguez-Suárez et al. (2022). Ten or 20 mg of each pharmaceutical was weighed with an accuracy (±0,1 mg) with an analytic balance Ohaus GA 200 (Näkiton, Switzerland) and transferred to a 25 mL volumetric flask containing water, acetonitrile or methanol, depending on the substance and its solubility. Each individual solution was mixed to prepare a final working standard solution at 5 μg/mL for each pharmaceutical. All solutions were stored at −20 °C. All standards were prepared in accordance with ISO 17025.

2.4. Equipment

To extract and analyse the feces samples the following equipments were used: laboratory centrifuge Eppendorf 5910 R (Eppendorf, Hamburg, Germany), rotatory shaker RSLAB-9 digital rotisserie (Rogo Sampaic, Wissous, France), MS2 Minishaker vortex mixer (IKA, Staufen, Germany) and column HPLC Intensity Solo 2 C18 (100 × 2.1 mm) (Brucker, Bremen, Germany)

2.5. Extraction of pharmaceuticals

Samples were analysed using the method published by Míguez-Suárez et al. (2022). Briefly, 500 mg of feces was weighed in a 15 mL falcon tubes and the extraction solvent was added, after vortex-mixed, for 10 s, and rotary shaker, for 20 min, the mixture was centrifugated, for 15 min at 4 °C at 4500 rpm. Approximately 1 mL of supernatant was filtered through Acrodisc Syringe Filter (Waters, MA, USA) and transferred to an HPLC amber vial for analysis. With each batch of samples, control samples spiked with pharmaceuticals at concentrations of 0, 10, 25, 50, 100, 250, 500, 750, 1000, and 2000 ng/g were prepared and

extracted at the same time.

2.6. LC-MS/MS conditions

Sample extracts were analysed using HPLC-MS/MS system consisting of a Bruker Elute UHPLC coupled to a Bruker EVOQ LC-TQ triquadruple mass spectrometer (Bruker, Bremen, Germany). A total volume of 15 μL of the sample extract was injected into an Intensity solo 2 C18 (100 × 2.1 mm) HPLC column (Brucker, Bremen, Germany). The mobile phase consisted of a mixture of two solvents mixed on a gradient mode as described by Míguez-Suárez et al. (2022): solvent A was water with 0.1 % of formic acid and solvent B was acetonitrile with 0.1 % formic acid. Mass spectrometry detection was performed using positive electrospray ionization (ESI^+), except for chloramphenicol and florfenicol, which were detected using negative electrospray ionization (ESI^-). Pharmaceuticals were identified and quantified employing two multiple reaction monitoring (MRM) transition and their corresponding retention times. The following MS parameters were held constant during the analysis: spray voltage was 4800 V for positive ionization and 4500 V for negative ionization. The cone temperature was set up as 300 °C and the flow at 20 psi. The temperature of the heated probe was 500 °C, the nebulizer gas flow at 30 psi and the exhaust gas at 50 psi.

2.7. Statistical analysis

To conduct the study 109 samples were collected from volunteers and dietary record and food consumption frequency was obtained from 84 volunteers. Estimated food intake was based on the average intake of each subject across dietary records. Voluntary intakes were compared with recommendation of the Spanish Agency for Food Safety and Nutrition (2022). For comparison recommendation of each food group, we look at the number of servings per week and the serving size and divide by the 7 days of the week to estimate the daily recommendation.

Hazar ratios were calculated using with number of positive and negative samples and considering exposure when the consumption of a food group was higher than those reported in ENALIA 2 (National Food Survey among adults, seniors, and pregnant women): (Spanish Agency for Food Safety and Nutrition, 2016; AESAN, 2016). The statistical treatment of the results was done with SPSS (IBM Statistical Package for the Social Sciences) software version 28.0.

Firstly, the Normality Test was carried out to find out whether the data obtained follow a normal distribution. Statistical treatment was performed using descriptive statistics and Mann-Whitney U to observe whether there are statistically significant differences between the intake by food group of volunteers with positive and negative samples. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

Baseline characteristics of subjects were compared with the average intake of Spain with data consulted in AESAN using X^2 tests (Spanish Agency for Food Safety and Nutrition, 2016). We investigated for each group of food if there were statistical differences between the positive samples and negative samples applying X^2 tests. We calculated Hazard Ratios (HR) and 95 % confidence intervals to characterize the association between the consumption of any group of food and the presence of any pharmaceutical in feces.

Based on the feces method limit of detection (Supplemental material), MRL and drug metabolism data, the amount of food required to be consumed to detect pharmaceuticals in the feces samples was estimated. Considering that the lowest limit of detection for the drugs analysed (6 ng/g), an average fecal volume of 200 g per day, the lowest drug metabolism (10 %), three values of MRL (100, 50 or 25 ng/g), the estimated daily intake of food necessary for the detection of the pharmaceuticals would be approximately 120, 240 and 480 g, respectively.

3. Results and discussions

3.1. Detected pharmaceuticals in human feces

A total of 28 feces samples contained residues of 11 pharmaceuticals, representing 25,68 % of the feces samples monitored. Pharmaceuticals detected included Chloramphenicol, Chlortetracycline, Ciprofloxacin, Danofloxacin, Diclofenac, Difloxacin, Doxycycline, Levofloxacin, Mefenamic Acid, Sulfaquinoxaline, and Tetracycline (Fig. 1). None of the samples contained more than one drug. Drug concentrations ranged from 6 to 13,661 ng/g (Table 1). It is remarkable that diclofenac was the drug most frequently detected (42 % of the positive samples) and at the highest concentration (13,661 ng/g). The result of highest doses of diclofenac may be due to the drug intake and the volunteer forgot to mention, with the exception of this case, the detection of Dicloneac in the other samples can assume that they are due to unintentional intake. Figs. 2 and 3 show the two transitions corresponding to a blank sample, a sample spiked at 100 ng/g, and a sample that tested positive for Diclofenac and Sulfachloropyridine. Fig. 4 shows the TIC chromatograms of the same samples. Diclofenac has a high rate of excretion (up to 75 %, in urine and 20–30 % in feces) and it is very stable in the environment (Quintana, Weiss, & Reemtsma, 2005), these two factors could explain its widespread occurrence in the environment (Tiedeken, Tahar, McHugh, & Rowan, 2017). On the other hand, Chlortetracycline, Difloxacin, Doxycycline, Levofloxacin, Mefenamic acid and Tetracycline were found in one or two samples, 4–7 % (Fig. 1), these drugs were measured at concentrations between 6 ng/g (Mefenamic Acid) and 456 ng/g (Chlortetracycline) and (Table1). Regarding the drugs measured in the feces samples six (Chlortetracycline, Ciprofloxacin, Diclofenac, Difloxacin, Doxycycline, Tetracycline) could be used at the time of the study in human and veterinary medicine. Five positive samples contained measurable level of drugs that cannot be present in the food of animal origin. The presence of Chloramphenicol, Levofloxacin and Mefenamic Acid in these samples could be from food of non-animal origin such as fruit, vegetables, water, nuts…

Regarding therapeutic classes, 50 % of the detections belonged to the group of antibiotics (Amphenicol, Tetracyclines, Quinolones and Sulphonamides) and 50 % to the anti-inflammatory drugs group (Diclofenac and Mefenamic Acid). This high frequency of detection of antibiotics and anti-inflammatory drugs could be due to their high prescription, especially in Spain where in the last ten years the consumption of analgesic drugs has increased by 50 % (Spanish Medicines Agency, 2021). Diclofenac is the fourth anti-inflammatory agent more used in Spain, behind Ibuprofen, Naproxen and Etoricoxib (Spanish Medicines Agency, 2017) and Levofloxacin is the second drug more used in Spain in veterinary medicine (Spanish Medicines Agency, 2020). The high prescription of these drugs, in both humans and animals, together with their

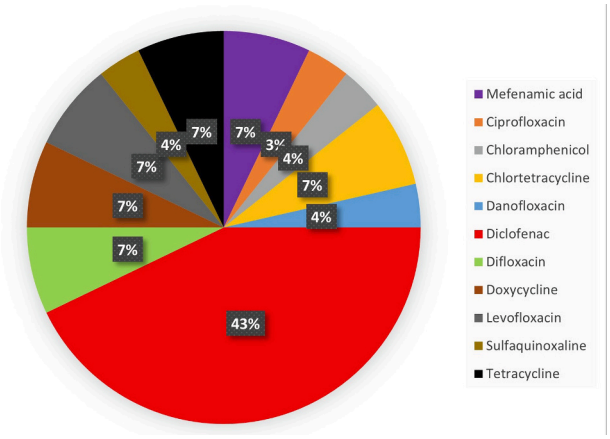

Fig. 1. Detection of pharmaceuticals in feces samples.

Table 1
Drugs detected, their used, and the number of detections and maximum concentration detected.

	Pharmaceutical authorization	Number of Detections	Maximum (ng/g)
Chloramphenicol	Human	1	266
Chlortetracycline	Human and Veterinary	2	456
Ciprofloxacin	Human and Veterinary	1	698
Danofloxacin	Veterinary	1	31
Diclofenac	Human and Veterinary	12	13,661
Difloxacin	Human and Veterinary	2	117
Doxycycline	Human and Veterinary	2	133
Levofloxacin	Human	2	110
Mefenamic acid	Human	2	7
Sulfaquinoxaline	Veterinary	1	71
Tetracycline	Human and Veterinary	2	73

extraction rates and low removal efficiency from sewage water could favours the appearance of residues of these substances in the environment and consequently could enter the food chain.

Similar research was conducted in China, Wang et al. (2020) analysed feces sample of the Chinese population and without disease and no drugs exposure for three months. While in the present study 26 % of the Spanish samples were positive to at least one pharmaceutical, the rate of positive sample in China was double, 50 % of positive samples (Wang et al., 2020), with lower number of compounds investigated (19 antibiotics). This may be due to the level of contamination of the food consumed by population evaluated. Although Europe and China have similar MRLs, in Europe the number of non-compliant food is normally low (0.18 %) (European Food Safety Authority, 2024), while in China this number, only in Chicken meat, is much higher (2.94 %) (Fei et al., 2023). An increase in the presence of residues of drugs in in Chinese food has also been highlighted (Niu, Yan, Yao, & Dou, 2023; Shao, Pan, & Han, 2024). In the Chinese samples sulphonamides was the group of antibiotics most frequently detected, 90 % of samples were positive for Sulfadimidine, while only 3.6 % of the Spanish samples were positive for this group.

The study conducted by Zhang, Lv, Li, et al. (2023) is the only other research which investigated the presence of pharmaceuticals in feces samples. Zhang et al. (2023) also collected samples from Chinese population, in particular, elderly individuals and investigated the presence of 78 different antibiotics, volunteers fulfilled the requirements of not taking drugs for 3 months prior to the study. The positivity rate, again, was higher than in the Spanish research, present work, 52.8 % of samples were positive, and 25 samples (17.9 %) contained more than one antibiotic. These differences may be due to the high level of environmental contamination in China as opposed to Spain mentioned earlier. In this study, Chinese second study, sulphonamides were not the most prevalent antibiotic, which may be due to the fact that the samples in this study are obtained exclusively from one city and not from the countryside as Wang et al. (2020) did. It is remarkable that Zhang et al. (2023) also analysed food to investigate the presence of residues of drug. They detected their presence in pork, chicken, eggs and fish, and in some cases the levels were higher than the MRL. An example is Doxycycline in pork measured at 133 μg/kg (33 % higher than the MRL).

3.2. Relation between food intake and positive samples

To evaluate the possible relationship between food intake and the presence of pharmaceuticals in the feces samples (positive and negative samples) the diet of the volunteers was classified in food groups: water, eggs, pork, milk and milk products, beef, rabbit, fish, honey and food of non-animal origin. Firstly, the Normality Test was carried out to determine whether the data obtained followed a normal distribution, and it was observed that the data obtained from the dietary records did not follow a normal distribution. To determine whether there were significant differences between volunteers with positive and negative samples, based on the number of samples and the non-normal distribution of the data, the Mann-Whitney *U* test was applied. The overall mean amount of food consumed per day by volunteers with negative samples was 4.04 % higher than those consumed by volunteers with positive samples. Similar results were observed for food of non-animal origin (fruit, vegetables, chickpeas…), where the consumption by volunteers with negative samples was 17 % higher than for volunteers with positive samples. Therefore, it could be speculated that positive samples of

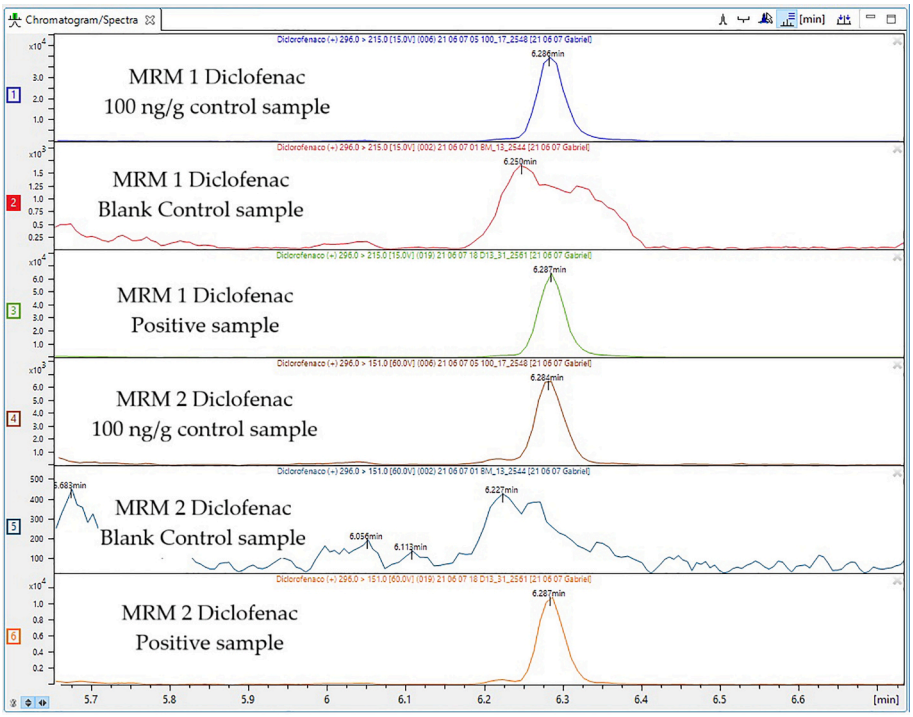

Fig. 2. Chromatogram of the two transitions corresponding to a blank sample, a sample spiked at 100 ng/g, and a sample that tested positive for Diclofenac.

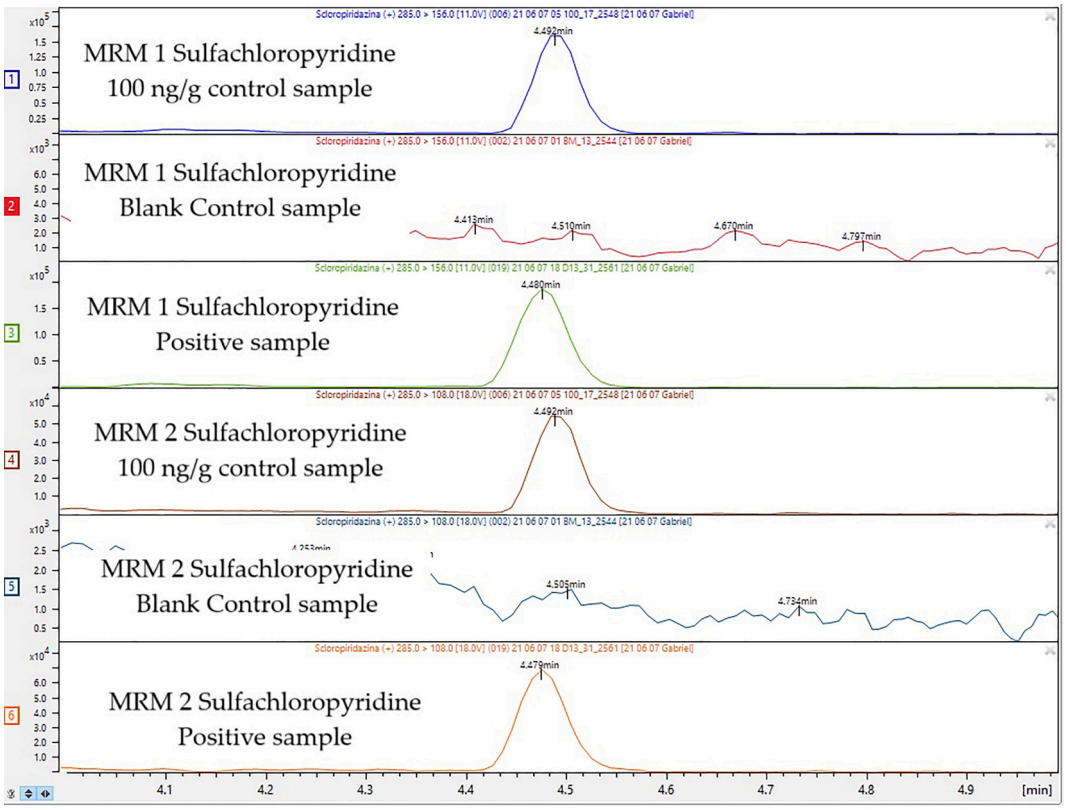

Fig. 3. Chromatogram of the two transitions corresponding to a blank sample, a sample spiked at 100 ng/g, and a sample that tested positive for Sulfachloropyridine.

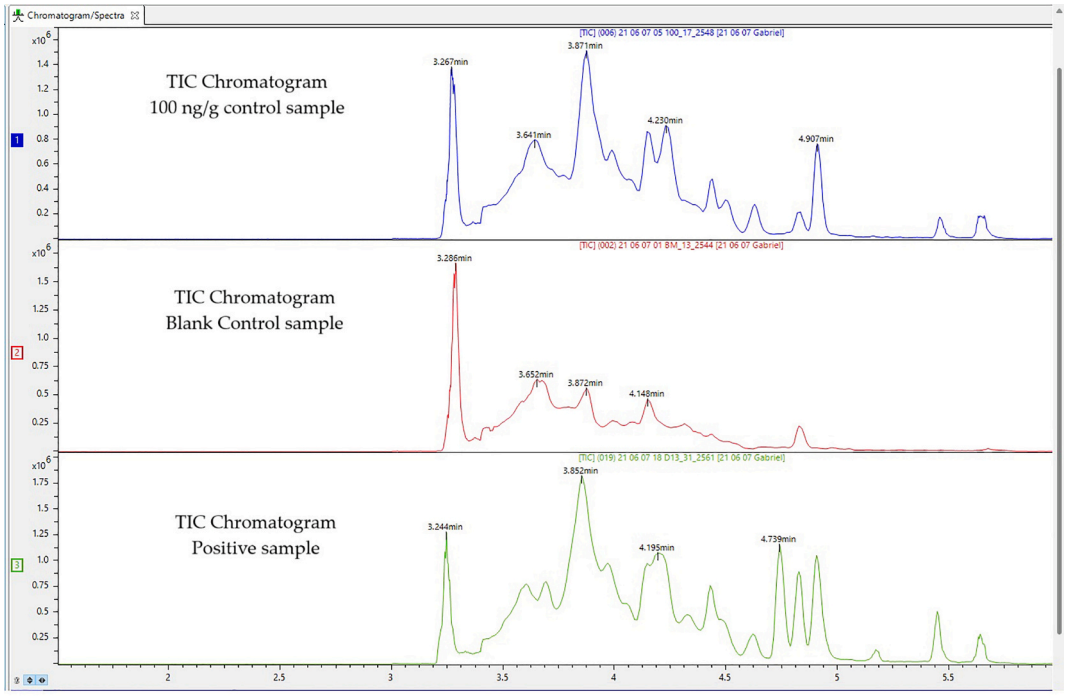

Fig. 4. TIC chromatograms corresponding to a blank sample, a sample spiked at 100 ng/g, and a sample confirmed positive.

volunteers with a low consumption of foods of animal origin may be due to the consumption of vegetables, fruit and water contaminated with residues of these compounds. González García et al. (2018) demonstrated that lettuce can absorb Diclofenac from the soil reaching a concentration of 82.28 ng/g and Tadić et al. (2021) also demonstrated the uptaken of antibiotics such as Quinolones and Sulphonamides in vegetables (lettuce, tomato, cauliflower and broad beans) with concentration ranging between 0.09 and 3.61 ng/g.

Regarding water consumption, the mean water consumption of volunteers with positive samples was 15.8 % higher (Table 2) than in

those volunteers with negative samples. The descriptive statistic indicated a positive asymmetry ($p = 1.252$, Table 5), most volunteers consumed more water than the mean value, the kurtosis was also positive, the curve was steepened upwards ($p = 1.882$, Table 5), and the Mann-Whitney U test did not show a significant difference between volunteers with positive samples and those with negative samples ($p = 0.250$, Table 4). In this research, Diclofenac was the most detected pharmaceutical in fecal samples. Based on findings reported by other researchers on the detection of Diclofenac in river water (Boleda et al., 2014; Hernando, Heath, Petrovic, & Barceló, 2006; Iglesias et al., 2014; Rodil et al., 2012; Valcárcel et al., 2011) and in drinking water (Carmona, Andreu, & Picó, 2014), it could suggest that diclofenac was consumed by voluntaries though water consumption, explain the high frequency of detection of this drug in feces samples. Chloramphenicol was also measured in one feces sample; the suggestion is that it may have been consumed through water as this drug is prohibited in animal food production and it should not be present in food of animal origin. This speculation can be made taking into consideration that this drug has been reported in river waters (Osorio, Marcé, Pérez, et al., 2012), that rivers are drinking water catchment sites, and that drinking water treatment does not eliminate efficiently pharmacological residues.

Quinolones (six samples) and sulphonamides (one sample) were detected in feces samples during the present research, and their presence was also reported in drinking water in Spain (Cabeza, Candela, Ronen, & Teijon, 2012; Galán, Cruz, & Barceló, 2010; Gros, Mozaz, & Barceló, 2012; Serna, Pérez, Ginebreda, et al., 2010). When the were evaluated, no relationship between water consumption and frequency of detection was found.

It should be noted that studies analysing drug residues in milk detected the presence of Quinolones in Spain (Junza et al., 2014) and Greece (Dasenaki and Thomaidis, 2015) at concentrations below the MRL. This could suggest that higher presence of the drugs in feces samples could be related to volunteers with higher milk consumption. However, results showed the opposite, volunteers with negative samples consumed 40 g/day more milk than those with positive samples (13.6 %). Furthermore, statistical analysis showed no significant difference between milk and dairy products consumption and positive samples ($p = -0.165$, Table 4). Applying descriptive statistics, we can see as positive skewness that the curve is shifted to the right ($p = 1.247$, Table 5), and a positive kurtosis ($p = 2.419$, Table 5).

Several studies also demonstrated the presence of Quinolones, Tetracyclines and Sulfamides in eggs (Gaudin, Rault, Hedou, Soumet, & Verdon, 2017; Jiménez, Rubies, Centrich, Companyó, & Guiteras, 2011) and honey (Bonvehí and Gutiérrez, 2009; Vidal, Aguilera-Luiz, Romero-González, & Frenich, 2009). After evaluating the consumption of eggs ($p = 0.897$, Table 4) and honey ($p = 0.978$, Table 4) by volunteers no statistically significant differences in consumption were observed regarding volunteers with positive and negative samples. The average consumption of eggs hardly varies by 11.1 % and honey consumption only varied by a few grams per day (121.4 %). From the descriptive statistics a positive skewness was obtained, the curve for egg consumption is shifted to the right ($p = 2.040$, Table 5), and a positive kurtosis ($p = 6.185$, Table 5). For honey, the skewness and kurtosis were positive, so that the curve is shifted to the right ($p = 4.219$, Table 5) and bulging upwards ($p = 22.573$, Table 5). This means that there are more low values than high values in the sample. Positive kurtosis implies that the distribution has more very high and very low values than a normal distribution.

An unexpected result was observed for fish and shellfish, mean intake was greater in volunteers with positive samples than in those volunteers with negative samples. While volunteers with positive samples consumed an average 82 g/day of fish and shellfish (3.92 %), those with negative samples consumed 59.8 g/day (2.74 %). The difference was 37.29 % more, when U-Mann Whitney test was applied to these values and no statistically significant differences were obtained ($p = 0.122$, Table 4). With respect to the types of fish and shellfish consumed, volunteers with negative samples consumed white fish such as hake and codfish, while volunteers with positive samples consumed more blue fish like salmon and sardine. The frequency of fish consumption was very similar between the two groups. The majority of volunteer lived in Galicia, and fish is a food that is very present in the diet. Spain is one of the world's largest fish consumers (Food and Agriculture Organization,

Table 3
Results of Hazard Risk, Chi of Pearson, U of Mann and 95 % CI of volunteers' consumption data.

Food Group	U of Mann	Chi of Pearson	Hazard Risk	Confidence Intervale 95 %
Origin animal food	0.976	0.492	0.761	0.358–1.620
Water	0.298	0.875	0.939	0.428–2.061
Eggs	0.765	0.712	1.15	0.542–2.440
Milk and dairy products	0.283	0.233	0.846	0.429–1.669
Pig and pork products	0.566	0.933	1.036	0.449–2.391
Beef and beef products	0.523	0.835	1.075	0.546–2.117
Chicken	0.746	0.624	1.185	0.603–2.328
Rabbit	0.321	0.382	1.571	0.620–3.982
Fish	0.284	0.885	1.055	0.513–2.166
Honey	0.708	0.606	0.788	0.310–2.002
Food not origin animal	0.078	0.028	0.445	0.232–0.854
Total Intake	0.699	0.832	1.078	0.535–2.174

Table 2
Comparison of consumption by food group for volunteers and ENALIA 2.

Group of food	Recommendation AESAN (g)	Average consumption ENALIA 2 (g)	Average of volunteers with positive samples (g)	Average of volunteers with negative samples (g)	Mann-Whitney, Significance level above the above the Spanish average
Food of animal origin	524.39	542.31	599.9 ± 244	599.1 ± 212	−0.498
Water	2000.00	625	491.5 ± 331.6	424.5 ± 355.2	−0.038
Eggs	33.14	17.21	37.9 ± 40.5	42.1 ± 48.7	−0.160
Milk	378.75	319.69	305.9 ± 185.3	347.6 ± 195.6	−0.697
Pig	8.03	28.7	78.6 ± 77.2	78.8 ± 58.5	−0.445
Beef	8.03	24.01	41.0 ± 50.5	31.0 ± 51.0	−0.050
Chicken	16.05	37.00	39.2 ± 51.7	34.9 ± 44.3	−0.824
Rabbit	16.05	0.60	11.1 ± 30.7	3.5 ± 14.4	−0.630
Fish	64.28	46.18	82.1 ± 68.9	59.8 ± 46.4	−0.645
Honey	–	0.96	3.1 ± 8.1	1.4 ± 3.5	−0.268
Intake Total	4773.61	1830.96	2093.6 ± 442.5	2178.2 ± 396.8	−0.670
No animal food	2152.85	663.65	1003.0 ± 588.4	1154.5 ± 486.9	−2.454

Table 4
Mann-Whitney U statistic values.

	Water	Egg	Milk derivates	Pork	Beef	Chicken	Rabbit	Fish	Honey	Total Food	Total non-animal	Total Animal
U of Mann-Whitney	649.500	756.000	624.500	738.00	736.000	667.000	732.000	608.500	767.500	754.000	609.000	762.000
W of Wilcoxon	2302.500	1134.000	1002.500	1116.000	2389.000	2320.000	2385.000	2261.500	1145.500	1132.000	987.000	1140.000
Z	−1.150	−0.130	−1.389	−0.302	−0.328	−1.035	−0.749	−1.545	−0.027	−0.148	−1.537	−0.072
Sig. asin. (bilateral)	0.250	0.897	−0.165	0.763	0.743	0.301	0.454	0.122	0.978	0.882	0.124	0.943

2022). There were also no statistically significant differences in fish consumption, but skewness and kurtosis were positive (p = 3.057, Table 5), so the curve is shifted to the right and bulged upwards (p = 1.356, Table 5). This means that there are more low values than high values in the sample. Positive kurtosis implies that the distribution has more very high and very low values than a normal distribution.

The presence of antibiotics has been reported in animal tissues at concentrations below the MRL (Azzouz, Souhail, & Ballesteros, 2011; Aguilera-Luiz et al., 2012; Lopes, Reyes, Romero-González, Frenich, & Vidal, 2012; Serrano et al., 2022) and their intake could increase the possibility of detecting drugs in feces samples. However, no difference was observed overall. Mean consumption of chicken and turkey in volunteers with negative and positive samples was similar; volunteers ate chicken or turkey once a week and the daily consumption was 37.05 g/day. Applying U-Mann test, no statistically significant differences were found in chicken and meat consumption and the groups of volunteers with positive and negative samples (p = 0.301, Table 4). Positive skewness and positive kurtosis (p = 2.100, Table 5) indicate that the curve is shifted to the right and bulged upward (p = 1.492, Table 5). This means that there are more low values than high values in the sample. Positive kurtosis implies that the distribution has more very high and very low values than a normal distribution. On the other hand, pig and beef are the most consumed meats in the area (Galicia) and a difference was expected but pork consumption was similar between both groups. In contrast, volunteers with positive samples consumed 10 g/day more beef than those with negative samples (32.26 %).

Many drugs tend to be stored in body fat, so the most concentration of some pharmaceuticals are more in fat than in muscle or other tissue. For example, diclofenac has a log Kow = 4.5, which means that it is a very fat-soluble drug and can be stored in fat (Morissette, Vo Duy, Arp, & Sauvé, 2015), and it was detected in bovine meat in Spain (Casado, Morante-Zarcero, Pérez-Quintanilla, & Sierra, 2016). Based on this consideration, meat evaluation was conducted considering lean meat and fatty meat, referring to fatty meat pork or beef which cuts are accompanied by high amount of fat, such as steaks, cutlets, ham, bacon or salami. We observed that volunteers with positive samples consumed 15 g/day (38.00 %) more fatty meat than those with negative samples but without significant statistical difference, and neither were differences found for consumption of pork and beef. The skewness and kurtosis were positive, so that the curve is shifted to the right (p = 1.544, Table 5) and bulging upwards (p = 4.360, Table 5). This means that there are more low values than high values in the sample. Positive kurtosis implies that the distribution has more very high and very low values than a normal distribution.

No similar studies have been found that examined the relationship between diet and the detection of drug residues in human feces or urine. Further research and work are needed to understand more precisely how much we are exposed to and how much the presence of drugs in the food chain can affect human health.

3.3. Statistical analysis of food intake

3.3.1. Dietary recommendations and Spanish average national consumption

The results of positive and negative samples were also compared with National dietary recommendations from the Spanish agency AESAN and the average national consumption reported in the in the ENALIA 2 report were employed. Was employed ENALIA 2 is a food survey that includes the adult population between 18 and 75 years of age in all the Autonomous Communities. The Mann Whitney U test was applied to the consumption of each food group and positive or negative feces samples, in general no statistically significant differences were obtained. However, when food of non-animal origin was joined together into a group called “non-vegetable origin” statistically significant difference was observed (p = 0.014, Table 6).

We observed that there is a higher consumption of foods of animal origin in our volunteers than the AESAN recommendations (14.32 %) recommendations and the data obtained in ENALIA 2 (10.54 %).

Considering the lowest concentration detected, the maximum residue limit and the excretion rate of the drug, we calculated the approximate amount of food to be ingested to detect those concentrations in feces. If we consider 120, 240 and 480 g of food and take into account the MRL (25-100 ng/g), applying statistics, no further statistically significant differences were observed between volunteers considering the different intake values depending on the MRL. (Table 6, 7, 8 and 9). It was only observed for volunteers with a fish consumption of more than 120 g per day (Z = −2.086, p = 0.037, Table 7).

The following figures (Fig. 5, 6 and 7) represent the volunteers' consumption in grams of the different food groups. In this way we can observe the differences in food consumption of volunteers with positive samples and volunteers with negative samples. Each point represents each volunteer's consumption of that food group. The solid black line marks the average consumption value for positive and negative volunteers. The dashed line represents the value of the AESAN recommendations. The dotted line represents the average consumption of the Spanish population in the ENALIA 2 study. Regarding water, we can see that the average consumption of volunteers is approximately 75 % lower than the AESAN recommendation (Spanish Agency for Food Safety and Nutrition, 2022.) and lower than the average water consumption in Spain according to the ENALIA 2 report (Spanish Agency for Food Safety and Nutrition, 2016).

The volunteers consumed more animal foods than recommended. Red meat consumption was higher than the AESAN recommendations by 45.01 %. The volunteers only consumed 22.90 % of the daily water recommended by AESAN (2 Litres/day). The intake of foods of non-animal origin is also very low compared to what is recommended (50.11 % lower). The daily intake of milk and dairy products was 13.73 % lower than the AESAN recommendation (378.75 g). Overall, the consumption of white meat is 34.08 % higher than recommended (16.05 g), but if we look at the individual level, the consumption of rabbit was 54.78 % lower than this recommendation.

The average consumption data for each group of food was consulted in the ENALIA 2 report carried out by AESAN and are presented in the

Table 5
descriptive statistics for dietary records.

	N		Statistical		Average		Standard Deviation	Statistical	Asymmetry		Kurtosis	
	Statistical	Statistical Range	Minimum	Maximum	Statistical	Statistical Range	Statistical	Variance	Statistical	Standard Error	Statistical	Standard Error
Water	84	1583	0	1583	444.470	37.942	347.743	120,925.460	1.252	0.263	1.882	0.520
Egg	84	267	0	267	40.840	5.040	46.192	2133.667	2.040	0.263	6.185	0.520
Milk	84	980	40	1020	335.180	20.998	192.450	37,036.830	1.247	0.263	2.419	0.520
Pork	84	376	0	376	78.770	6.994	64.102	4109.064	1.544	0.263	4.360	0.520
Beef	84	250	0	250	34.000	5.538	50.578	2576.325	2.076	0.263	4.757	0.520
Chicken	84	200	0	200	36.190	5.060	46.375	2150.670	1.492	0.263	2.100	0.520
Rabbit	84	100	0	100	5.720	2.260	20.717	429.200	3.622	0.263	12.004	0.520
Fish	84	298	0	298	66.490	5.958	54.605	2981.753	1.356	0.263	3.057	0.520
Honey	84	37	0	37	1.930	0.580	5.312	28.219	4.219	0.263	22.573	0.520
Total Food	84	3158	788	3946	2152.980	56.405	516.963	267,250.806	0.481	0.263	1.370	0.520
Total non-animal	84	2011	208	2219	1109.390	45.185	414.127	171,501.317	0.255	0.263	−0.448	0.520
Total animal	84	1095	224	1318	599.060	24.091	220.795	48,750.532	0.819	0.263	1.344	0.520

Table 6
Mann-Whitney U statistic values higher than the Spanish average. Mann-Whitney. Significance level higher than the Spanish average.

	Water	Egg	Milk	Pork	Beef	Chicken	Rabbit	Fish	Honey	Total Food	Total no-animal	Total Animal	Water
U of Mann-Whitney	766.500	756.000	706.500	736.500	765.000	696.000	738.000	714.000	750.000	717.000	609.000	726.000	766.500
W of Wilcoxon	1144.500	1134.000	1084.500	2389.500	2418.000	2349.000	2391.000	2367.000	1128.000	1095.000	987.000	1104.000	1144.500
Z	−0.038	−0.160	−0.697	−0.445	−0.050	−0.824	−0.630	−0.645	−0.268	−0.670	−2.454	−0.498	−0.038
Sig. Asin. (Bilateral)	0.970	0.873	0.484	0.657	0.960	0.410	0.529	0.519	0.788	0.503	0.014	0.618	0.970

Table 7
Mann-Whitney U statistic values higher than 120 g (MRL = 100 ng/g). **Mann-Whitney, Significance level higher than 120 g (MRL = 100 ng/g).**

	Water	Egg	Milk	Pork	Beef	Chicken	Rabbit	Fish	Honey	Total Food	Total non-animal	Total Animal
U of Mann-Whitney	706.500	768.000	709.500	681.000	744.000	711.000	769.500	637.500	769.500	769.500	769.500	769.500
W of Wilcoxon	2359.500	2421.000	1087.500	1059.000	1122.000	2364.000	2422.500	2290.500	2422.500	2422.500	2422.500	2422.500
Z	−0.935	−0.045	−1.130	−1.170	−0.596	−1.367	0.000	−2.086	0.000	0.000	0.000	0.000
Sig. Asin. (Bilateral)	0.350	0.964	0.258	0.242	0.551	0.172	1.000	0.037	1.000	1.000	1.000	1.000

Table 8
Mann-Whitney U statistic values higher than 480 g (MRL = 25 ng/g). **Mann-Whitney, Significance level higher than 480 g (MRL = 25 ng/g).**

	Water	Egg	Milk derivates	Pork	Beef	Chicken	Rabbit	Fish	Honey	Total Food	Total non-animal	Total Animal
U of Mann-Whitney	754.500	769.500	763.500	769.500	769.500	769.500	769.500	769.500	769.500	769.500	726.000	714.000
W of Wilcoxon	2407.500	2422.500	2416.500	2422.500	2422.500	2422.500	2422.500	2422.500	2422.500	2422.500	1104.000	1092.000
Z	−0.173	0.000	−0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	−1.296	−0.657
Sig. asin (bilateral)	0.863	1.000	0.924	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.195	0.511

Table 9
Mann-Whitney U statistic values higher than 240 g (MRL = 50 ng/g). **Mann-Whitney, Significance level higher than 240 g (MRL = 50 ng/g).**

	Water	Egg	Milk derivates	Pork	Beef	Chicken	Rabbit	Fish	Honey	Total Food	Total non-animal	Total Animal
U of Mann-Whitney	615.000	756.000	600.000	741.000	756.000	769.500	769.500	741.000	769.500	769.500	741.000	754.500
W of Wilcoxon	2268.000	1134.000	978.000	2394.000	1134.000	2422.500	2422.500	2394.000	2422.500	2422.500	1119.000	1132.500
Z	−1.829	−0.688	−1.942	−1.453	−0.688	0.000	0.000	−1.453	0.000	0.000	−1.453	−0.544
Sig. asin (bilateral)	0.067	0.491	0.052	0.146	0.491	1.000	1.000	0.146	1.000	1.000	0.146	0.586

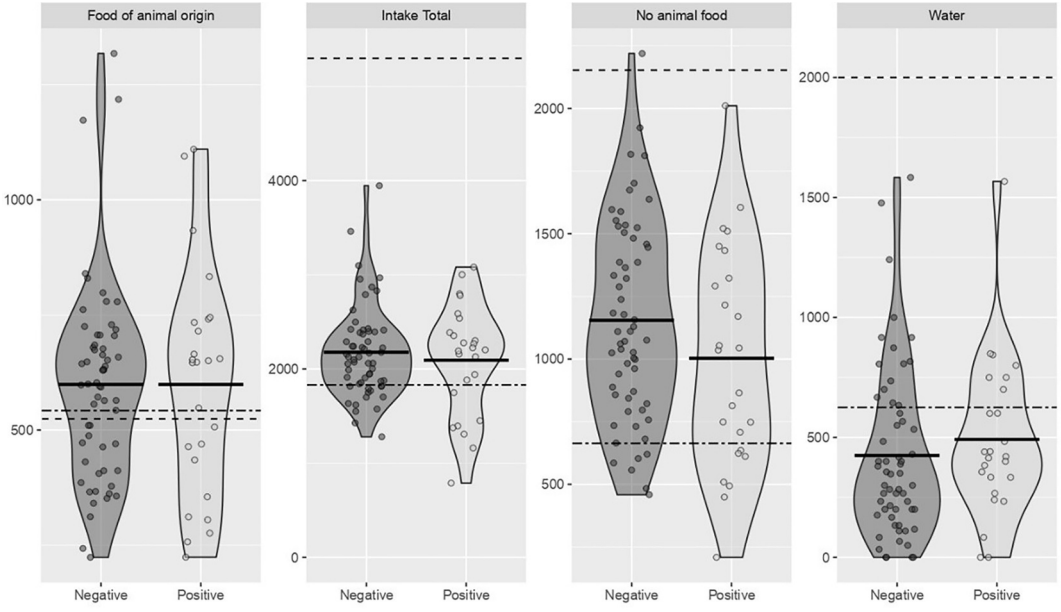

Figure 5: Graphical data on total food consumption, consumption of food of animal origin, food of non-animal origin and water consumption in volunteers.

Fig. 5. Graphical data on total food consumption, consumption of food of animal origin, food of non-animal origin and water consumption in volunteers.

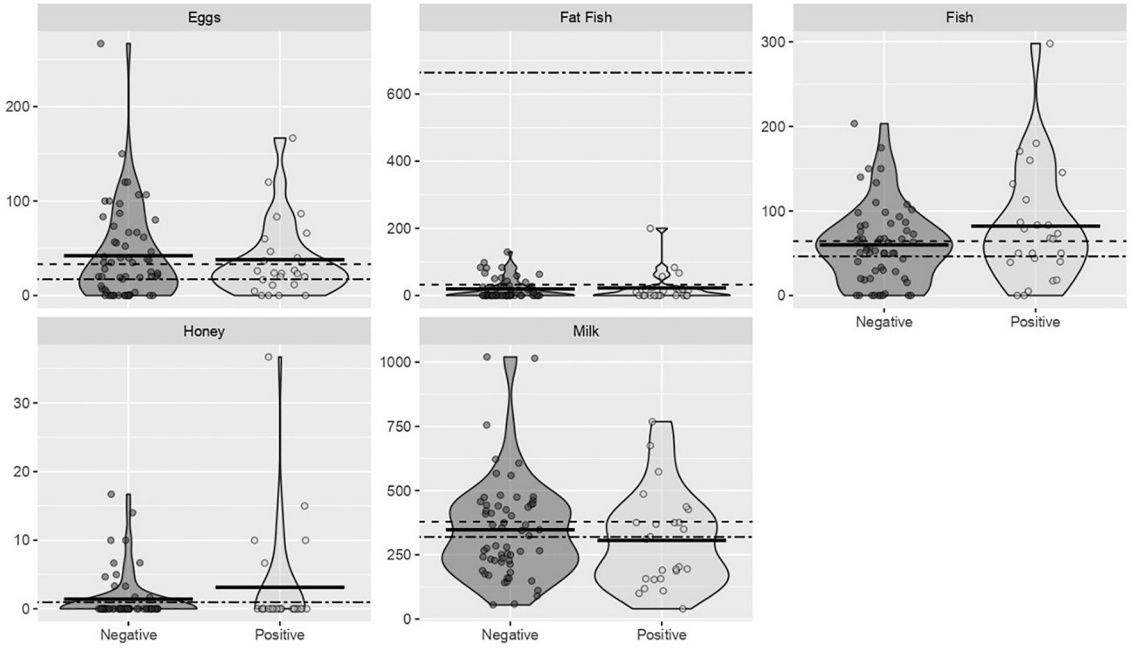

Fig. 6. Graphical data on Eggs, Fat Fish, Fish, Honey and Milk consumption data in volunteers.

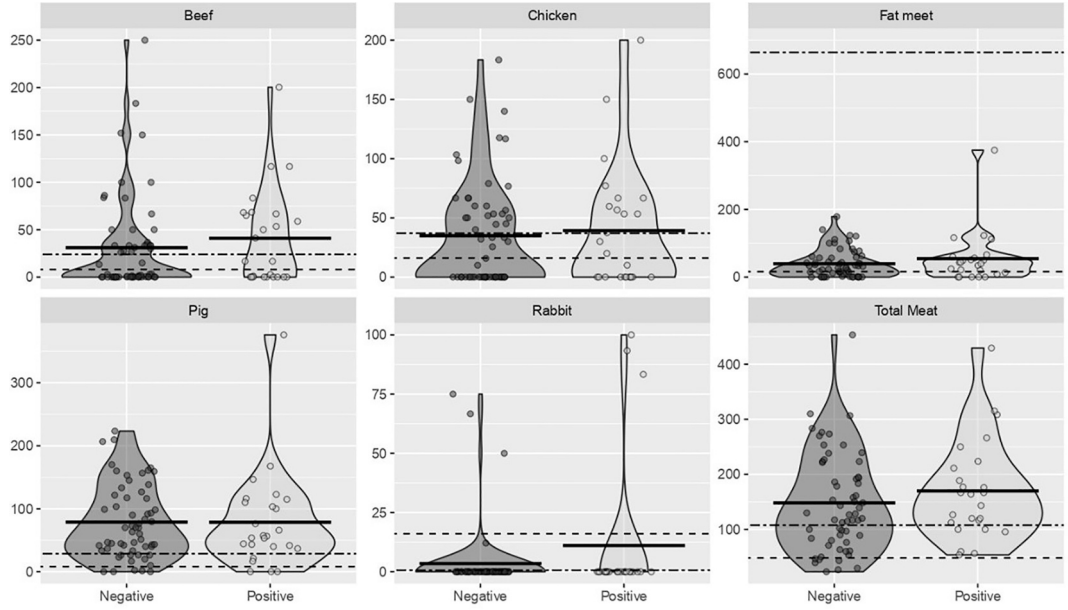

Fig. 7. Graphical data Beef, Chicken, Fat Meet, Pig, Rabbit and Total Meat consumption data in volunteers.

following table (Table 2). We compared these values with those obtained in the dietary records of the volunteers in the study, in order to find out whether the dietary habits of the volunteers align with the AESAN recommendations, and to be able to establish a relationship between the consumption of some food group and the possibility of detecting positive samples.

The statistical analysis carried out to investigate the differences between the positive and negative groups in the consumption of the different food groups did not show significant differences. The first test performed was to determine whether the data obtained followed a normal distribution or not in order to know which test to perform in case of a normal distribution. It was observed that the data did not follow a normal distribution, so to determine if there was a statistically significant difference between the positive and negative groups of each food group, the Mann-Whitney *U* test was performed. With the Mann U test, we can observe that no statistically significant difference was found between the positive and negative groups.

With these statistical results, we determined Hazard Risk (HR) to establish a relationship between the different groups of food and a sample positive to pharmaceuticals. These results show an increased risk of testing positive for drugs in those exposed to eggs, pig, beef, chicken, rabbit, fish and total intake (HR $>$ 1), but these were not statistically significant differences.

However, there is one group that has a result that needs to be analysed. The HR for foods of non-animal origin has an HR below one and a confidence interval between 0.232 and 0.854 (Table 3). This means that a higher consumption of foods of non-animal origin is associated with lower likelihood of detecting drug residues in the feces.

4. Conclusion

The study demonstrated the presence of 11 pharmaceuticals in 28 fecal samples out of 109. Volunteers reported that they had not taken any pharmaceuticals within the two months prior to sample collection. Although no significant relationship was found between the consumption of food of animal origin and the presence of pharmaceuticals in the samples, the consumption of non-animal foods above 542 g/day was associated with a higher probability of having negative samples to pharmaceuticals.

This study is among the few that have detected drug residues in human fecal samples at low concentrations. The persistent presence of antibiotic residues may promote the emergence of bacteria harboring antibiotic resistance genes. Furthermore, other potential health implications associated with the presence of pharmaceutical residues, including allergic responses, should also be taken into account.

CRediT authorship contribution statement

Gabriel Míguez-Suárez: Writing – original draft, Methodology, Investigation, Formal analysis. **Alejandra Cardelle-Cobas:** Investigation. **Laura Sinisterra-Loaiza:** Investigation. **Carlos Fernández-Lozano:** Software. **Alberto Cepeda:** Investigation. **Carolina Nebot:** Writing – review & editing, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

dietary records and frequency questionnaires are private and cannot be shared, but general data can be shared and are in the manuscript.

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