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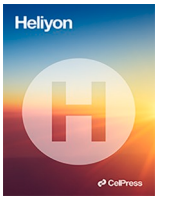

Surveillance of veterinary drug residues in food commonly consumed in Singapore and assessment of dietary exposure

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ABSTRACT

Non-judicious and indiscriminate use of veterinary drugs in animal husbandry may result in accumulation of residues in animal tissues, and consequently in food for human consumption. The abuse of veterinary drugs presents a potential risk to consumer health, especially if the residue level is higher than the health-based guidance value (HBGV) such as the acceptable daily intake (ADI). Contamination of drug residues in food also promotes the emergence of antimicrobial resistance (AMR) which poses a serious threat to public health globally. There has been limited information on the occurrence and dietary exposure to veterinary drug residues in Singapore to date. In this study, the occurrence of four classes of veterinary drugs, namely beta-agonists, coccidiostats, fluoroquinolones and macrolides, were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in food widely consumed by Singapore residents. The magnitude of dietary exposure was assessed based on the consumption profile of Singapore population. Out of 216 food samples, 9.72 % were detected positive with veterinary drug residues, where majority of the positive samples were poultry and its derived products, followed by eggs and egg products. 7 veterinary drugs, specifically ciprofloxacin, enrofloxacin, clopidol, diclazuril, lasalocid, nicarbazin and tilmicosin, were detected in the samples, with clopidol and enrofloxacin being the most frequently detected drugs. Dietary exposure was evaluated using the estimated daily intake (EDI) of the detected drugs and benchmarked against the corresponding acceptable daily intake (ADI). All the %ADI values were far less than 100 in both the average and high consumer scenarios, indicating that the health risk associated with dietary exposure to these drugs in Singapore is low.

1. Introduction

Veterinary drugs are administered to livestock, poultry and aquaculture for a plethora of reasons, including prophylaxis and treatment of diseases. Appropriate and prudent use of these drugs is critical to ensure a resilient and safe food supply. Conversely, improper and non-judicious use such as failure to comply with Good Veterinary Practices (e.g. improper withdrawal periods, inappropriate dosage and indiscriminate therapy) may result in accumulation of drug residues in animal tissue and consequently, foods of

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animal origin for human consumption [1,2]. The occurrence of veterinary drug residues in different foods of animal origin has been reported globally. For example, a livestock product post-marketing monitoring program launched by the Taiwan Food and Drug Administration from 2011 to 2015 revealed that 34 out of 1487 livestock products (edible tissues, milk) contained drug residues including banned drugs such as β-agonists and chloramphenicols [3]. In a survey of 513 poultry egg samples (hen, duck, quail and pheasant) collected from supermarkets and farm markets, 18.13 % contained at least one of the 7 antibiotic residues screened (danofloxacin, difloxacin, ofloxacin, sulfadimethoxine, sulfamonomethoxine, sulfamethoxypyridazine, sulfamethoxazole) [4]. A study of 40 pasteurised milk samples marketed in Sao Paulo, Brazil also showed the presence of monensin in 45 % of the samples at concentrations ranging from 0.1 to 0.27 μg/kg [5]. Veterinary drug residues have similarly been detected in seafood. In recent studies conducted on fishery products in South Korea and southeast China, it was found that 18 and 8 veterinary drug residues were present respectively. Common drug residues include enrofloxacin, doxycycline and sulfamethazine [6,7].

Dietary exposure to these drug residues may pose risks to human health, including toxicity and hypersensitivity [2,3]. It may also promote the emergence of antimicrobial resistance, which has been declared by World Health Organization (WHO) as one of the top 10 global public health threats facing humanity [8].

Therefore, it is pertinent to estimate the intake of various veterinary drug residues from food for risk evaluation [9]. Such exposure assessments can also aid in establishing and refining regulatory frameworks to ensure the safety of food products. A way to achieve this is via consumer-centric market monitoring, which utilizes food readily available in the market for testing to identify potential food safety risks. However, to the best of our knowledge, there has not been any study conducted so far to estimate the dietary exposure to veterinary drugs in Singapore using food commonly consumed by the population.

Hence, this study aims to bridge the data gap on food safety risk assessment by determining the occurrence of 38 drug residues belonging to four veterinary drug classes for food animals, specifically beta-agonists, coccidiostats, fluoroquinolones and macrolides, in food commonly consumed by Singaporeans via liquid chromatography-tandem mass spectrometry (LC-MS/MS) and assessing the level of dietary exposure to the population.

2. Materials and Methods

2.1. Food sample collection and preparation

The list of foods to be analysed was established based on the 2010 National Nutrition Survey (NNS) and other Food Consumption Surveys (FCS) conducted in recent years among the Singapore population. The topmost consumed foods by weight, foods consumed by >15 % of consumers, foods that are consumed slightly higher by a particular ethnic group and foods consumed infrequently but with high contribution to dietary exposure were chosen for this study. Food samples currently available on the market were purchased from different sources (e.g. online platforms, supermarkets, wet markets). To ensure that they were representative, 15 samples consisting of different brands, varieties and countries of origin were purchased for each food. The individual food items were cooked, if necessary, in the experimental kitchen of a tertiary institution (Temasek Polytechnic) using recipes that are representative of the food preparation habits of the population. The processed 15 samples were then pooled, mixed and blended to obtain homogenized single composite samples and frozen until chemical analysis.

2.2. Chemicals and reagents

HPLC grade acetonitrile was purchased from Tedia Company (OH, USA) while LC-MS grade methanol was procured from Elite Advanced Materials Sdn Bhd (Selangor, Malaysia). Ultra-pure water (resistivity >18.2MΩ·cm) was obtained using ELGA PURELAB Option Q7 (ELGA LabWater, IL, USA) water purification system. Analytical grade formic acid, magnesium sulfate and ammonium acetate were from Sinopharm Chemical Reagent Co Ltd (Shanghai, China). Analytical standards of the veterinary drugs and deuterated internal standards were purchased from various suppliers. An internal standard mix solution consisting of Clenbuterol-D9, Ractopamine-D6, Decoquinate-D5, Nicarbazine-D8, Robenidine-D8, Ciprofloxacin-D8, Enrofloxacin-D5 and Erythromycin-D8, was prepared from the respective standards at a concentration of 1 mg/kg in methanol. The solution was stored at 5 °C and stable for 6 months. A standard mix solution of beta-agonists (Brombuterol, Clenbuterol, Clenpenterol, Cimaterol, Cimbuterol, Mabuterol, Mapenterol, Ractopamine, Salbutamol, Salmeterol, Terbutaline, Tulobuterol, Zilpaterol), fluoroquinolones (Ciprofloxacin, Danofloxacin, Difloxacin, Enrofloxacin, Marbofloxacin, Norfloxacin, Sarafloxacin), macrolides (Erythromycin, Josamycin, Oleandomycin, Spiramycin, Tiamulin, Tilmicosin, Tylosin) and coccidiostats (Amprolium, Clopidol, Decoquinate, Diclazuril, Lasalocid, Maduramicin, Monensin, Narasin, Nicarbazin, Robenidine, Salinomycin) was prepared at 100 μg/kg in methanol. The solution was stored at −20 °C and stable for 1 week.

2.3. Sample extraction

Five grams of the homogenized test sample in 50 mL polypropylene centrifuge tube was spiked with internal standard mix solution to attain a concentration level of 12.5 μg/kg and allowed to stand for 15 min 10 mL of 0.1 % v/v formic acid in acetonitrile was then added to the sample and shaken at about 420 rpm for at least 10 min using a linear shaker (Edmund Bühler GmbH, Bodelshausen, Germany). For milk samples, anhydrous magnesium sulfate was also added. Following which, the tube was centrifuged (Thermo Fisher Scientific, MA, USA) at 4000 rpm for 10 min at 10 °C. 1 mL of the supernatant was subsequently transferred into a 15 mL polypropylene centrifuge tube before being evaporated to dryness under a continuous stream of nitrogen at 45 °C (Caliper Life Sciences, MA, USA).

The dried extract was reconstituted with 1 mL of 2 % methanol in water for beta-agonists and fluoroquinolones analyses and 1 mL of 75 % v/v acetonitrile in 0.1 % v/v formic acid in water for macrolides and coccidiostats analyses. The sample was then filtered using a 3 kDa MWCO centrifugal filter prior to LC-MS/MS analysis.

2.4. Analysis of veterinary drug levels via LC-MS/MS

LC-MS/MS was performed using SCIEX ExionLC™ AD coupled to Triple Quad 6500+ system (SCIEX, Massachusetts, USA). Chromatographic separation was performed on an Agilent Zorbax SB-Phenyl column (100 mm × 3.0 mm, 3.5 μm). If a drug residue was detected, the results were confirmed by rerunning the sample using another column – Thermo Fisher HyPURITY™ C18 column (100 mm × 3.0 mm, 3.0 μm). The injection volume was 10 μL and the flow rates were 0.5 mL/min, 0.45 mL/min, 0.45 mL/min and 0.4 mL/min for beta-agonists, fluoroquinolones, macrolides and coccidiostats analyses respectively. For beta-agonists, fluroquinolones, macrolides analyses and coccidiostats analysed in positive mode, mobile phase A was 2 mM ammonium acetate and 0.1 % v/v formic acid in water while mobile phase B was 0.1 % v/v formic acid in methanol. Acetonitrile was used as mobile phase B for coccidiostats analysed in negative mode instead. The solvent gradient used was as follow for beta-agonists analysis: 2–55 % B at 0–4 min, 55–100 % B at 4–5 min, 100 % B at 5–8 min. For fluoroquinolones analysis, the solvent gradient used was as follow: 5–95 % B at 0–3 min, 95 % B at 3–9 min. The solvent gradient used was as follow for macrolides analysis: 10–85 % B at 0–1 min, 85 % B at 1–9 min. For coccidiostats analysis, the solvent gradient used was 5–95 % B at 0–2 min, 95 % B 2–9 min for positive mode and 5–75 % B at 0–1 min, 75 % B at 1–5 min for negative mode.

MS/MS acquisition was performed using both positive and negative electrospray ionization (ESI) for coccidiostats analyses and only positive ESI for beta-agonists, fluoroquinolones and macrolides analyses. Multiple reaction monitoring (MRM) was conducted for each target analyte. Three transitions were monitored for each analyte, with the most abundant transition used for quantitation. The ion source temperature was set at 550 °C (500 °C for coccidiostats), with curtain gas pressure of 25 psi, ion spray voltage of 4500 V (−4500V for negative mode), ion source gas 1 and 2 pressures of 50 psi. Nitrogen was used as collision gas. The MRM parameters (precursor ion, product ions, collision energy, dwell time, declustering potential, retention time) were optimized and set accordingly for each individual compound as summarized in Table S1. All acquisitions and data analyses were performed using the Analyst Software (AB Sciex Pte Ltd).

2.5. Exposure assessment

Dietary exposures were assessed only when a veterinary drug was detected in the food samples. The estimated daily intake (EDI) of each veterinary drug for the general population was calculated according to the equation as follow:

$$EDI = \sum \frac{C \times IR}{BW \times 1000}$$

C is the mean concentration of the veterinary drug detected in a particular food item obtained from all the evaluated samples in the study expressed in μg/kg. For other food commodities where the veterinary drug was not detected (i.e. below the limit of quantitation (LOQ)), zero and half the LOQ were used to calculate lower and upper bound intakes respectively. Based on the validation results in Table S2, the LOQ was taken to be 1.5 μg/kg. Ingestion rate (IR) represents the daily consumption of that food item for consumers in g per person per day at 50th (average scenario) and 95th (high consumer scenario) percentile and the data were obtained from Singapore Food Agency (SFA)'s 2021 Food Consumption Survey (24-h recall). BW refers to the average body weight in kg which was assumed to be 60 kg in this study. To obtain the total EDI of the veterinary drug per day, the EDIs of all the food items were then summed.

A health-based guidance value (HBGV) is the maximum oral exposure to a substance that is not expected to result in an appreciable health risk. Two HBGVs are commonly used in exposure assessment, namely tolerable daily intake (TDI) and acceptable daily intake (ADI). Joint FAO/WHO Expert Committee on Food Additives (JECFA) uses ADI for substances intentionally added to food, such as food additives, residues of pesticides and veterinary drugs. On the other hand, TDI is used for food contaminants that are generally unavoidable [9]. Similar definitions have been adopted by the European Food Safety Authority [10]. Thus for risk characterization, the estimated dietary exposures were compared with the ADI of the drug recommended by JECFA

$$\%\ ADI = \frac{EDI}{ADI} \times 100$$

If % ADI <100, the EDI of veterinary drug is lower than the ADI and the chronic health risk from drug residues via food consumption is assessed to be low.

3. Results

3.1. Types of food samples analysed

A total of 103 food items common in the Singapore diet corresponding to 216 individual samples were analysed for 38 veterinary drugs belonging to the specified 4 drug classes, namely beta-agonists, coccidiostats, fluoroquinolones and macrolides. The samples covered 15 food categories in total (beverages, bakery products, confectionery, eggs and egg products, fats and oil, fish and seafood, fruits and fruit products, grains and grain-based products, meat and meat products, nuts and seeds (including spices), milk and dairy

products, sauces and condiments, vegetables, composite foods and others e.g. infant food, vegetable protein, RTE savouries). A visual representation of the sample breakdown is shown in Fig. 1. Majority of the samples were fish and seafood products (42.6 %). This was followed by meat and meat products (25.5 %) then milk and dairy products (7.9 %).

3.2. Occurrence of veterinary drug residues in food samples analysed

The analytical method was validated, and the results have been summarized in Table S2. From a total of 216 food samples analysed, 21 samples (9.72 %) contained at least one veterinary drug residue at levels above the method's LOQ. Of these 21 samples, 12 contained only 1 drug residue, 2 contained 2 residues, 3 contained 3 residues while 4 samples contained 4 residues (Fig. 2). Majority of the positive samples were poultry and its derived products (chicken, duck, chicken nugget and ham) followed by eggs and egg products (century egg, salted duck egg and egg tofu). Table 1 presents the detection frequencies and concentration ranges of the veterinary drug residues detected in the food samples. 7 veterinary drugs were detected in the samples, namely ciprofloxacin, enrofloxacin, clopidol, diclazuril, lasalocid, nicarbazin and tilmicosin. Clopidol was the most detected (29.3 %), followed by enrofloxacin (22.0 %) then nicarbazin (17.1 %) and lasalocid (14.6 %). The maximum contamination was 41.2 μg/kg (enrofloxacin in egg tofu). For co-occurrence of two antibiotics and above, the most prevalent combination was coccidiostats and fluoroquinolones followed by combination of coccidiostats.

3.3. Calculation of veterinary drug dietary exposure

Based on the mean concentrations of the veterinary drug residues detected in the samples and the daily consumption (mean and 95th percentile) of each food item from SFA's 2021 Food Consumption Survey (24-h recall) (g/kg bw), the dietary exposures to the detected veterinary drugs were calculated. The results are summarized in Table 2. The calculated EDIs were well below the respective ADIs recommended by JECFA for all veterinary drugs in both scenarios for both lower and upper bound intakes and under average and high consumer scenarios, suggesting no appreciable health risks.

Due to the lack of ADI set by JECFA, risk characterization of clopidol was not possible. The ADI of enrofloxacin was used for exposure assessment of enrofloxacin and its metabolite, ciprofloxacin. Ciprofloxacin and enrofloxacin had the highest %ADI (Average: 1.88 %, High Consumer: 3.56 % for lower bound intake; Average: 8.13 %, High Consumer: 16.4 % for upper bound intake) while nicarbazin had the lowest %ADI (Average: 0.00208 %, High Consumer: 0.00420 % for lower bound intake; Average: 0.0177 %, High Consumer: 0.0362 % for upper bound intake).

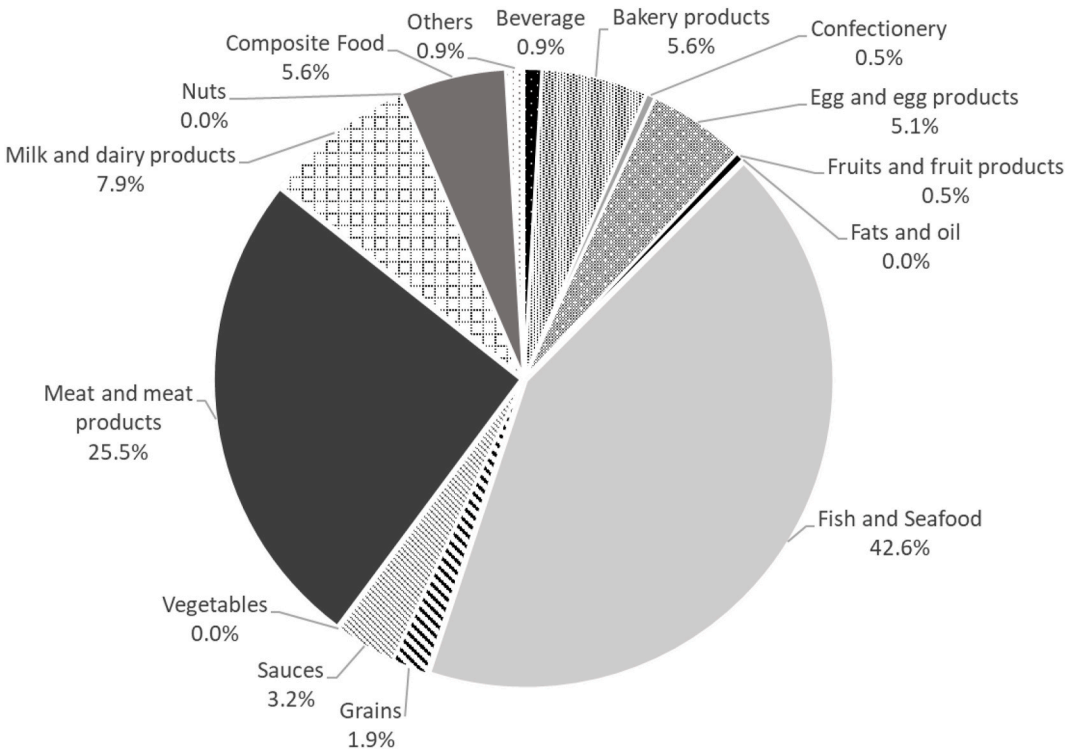

Fig. 1. Breakdown of food samples analysed for the presence of veterinary drugs.

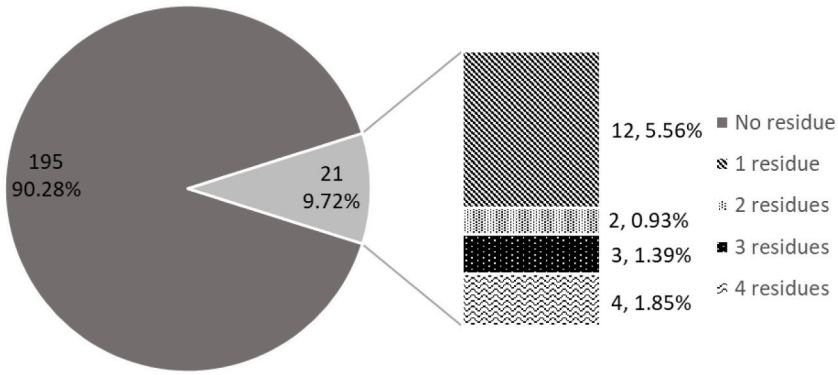

Fig. 2. Number of veterinary drug residues detected in food samples.

Table 1
Occurrence and residue levels of the detected veterinary drugs.

Veterinary drug	Sample(s) detected	Number of positive samples (%)	Min (μg/kg)	Max (μg/kg)	Mean (μg/kg)
Ciprofloxacin	Egg tofu	2 (0.93)	1.8	2.5	2.15
Enrofloxacin	Egg tofu (2), shrimp ball (1), chicken (6)	9 (4.17)	3.2	41.2	17.23
Clopidol	Chicken ham (1), chicken nugget (2), chicken (5), duck (3), salted duck egg (1)	12 (5.56)	1.9	11.5	6.35
Diclazuril	Chicken	3 (1.39)	5.6	29.6	15.93
Lasalocid	Chicken	6 (2.78)	2.0	5.7	3.53
Nicarbazin	Egg tofu (2), chicken sausage (2), century egg (2), salted duck egg (1)	7 (3.24)	1.7	9.7	4.09
Tilmicosin	Egg tofu	2 (0.93)	6.8	10.2	8.5

Table 2
Estimated daily intake (EDI) in μg/kg body weight (bw)/day and % acceptable daily intake (ADI) of veterinary drugs for lower and upper bound intakes, under average and high consumer scenarios.

Veterinary drug	Average Scenario				High Consumer Scenario (95th percentile)			
	Lower bound		Upper bound		Lower bound		Upper bound	
	EDI (μg/kg bw/day)	% ADI	EDI (μg/kg bw/day)	% ADI	EDI (μg/kg bw/day)	% ADI	EDI (μg/kg bw/day)	% ADI
Ciprofloxacin	0.00130	–	0.0644	–	0.00234	–	0.132	–
Enrofloxacin	0.0362	–	0.0982	–	0.0689	–	0.196	–
Sum of Ciprofloxacin and Enrofloxacin	0.0375	1.88	0.163	8.13	0.0712	3.56	0.328	16.4
Clopidol	0.0190	–	0.0912	–	0.0401	–	0.164	–
Diclazuril	0.0171	0.0569	0.0798	0.266	0.0353	0.118	0.164	0.546
Lasalocid	0.00379	0.0758	0.0665	1.33	0.00782	0.156	0.136	2.73
Nicarbazin	0.00831	0.00208	0.0707	0.0177	0.0168	0.00420	0.145	0.0362
Tilmicosin	0.00512	0.0128	0.0682	0.170	0.00927	0.0232	0.139	0.347

4. Discussion

4.1. Occurrence of veterinary drug residues in food samples analysed

Monogastric animals (e.g. pig, poultry) are more efficient in terms of total resource use than ruminants (e.g. cattle, sheep, goat) and are hence reared in intensive systems. Intensive poultry production systems are more widespread than pig systems and it has been estimated that more than 70 % of poultry meat is now produced in intensive systems globally [11]. Due to the high stock densities in poultry houses, it provides ideal conditions for the manifestation and transmission of diseases such as coccidiosis, typhoid, *E. coli* infections and Samonellosis [12,13]. Farmers may thus, require high doses of anti-coccidiosis and antimicrobial agents to prevent and treat these diseases. Consequently, veterinary drug residues are more likely to be detected in poultry compared to other food producing animals. The presence of various veterinary drug residues in poultry meat has been reported in numerous studies [12,14]. This concurs with what was observed in this study, where most of the positive samples were poultry and its derived products.

Due to the concern of veterinary drug residues transferring into eggs and it is not economically viable to place withdrawal period

for layer hens since the contaminated eggs must be discarded, relatively few drugs have been approved for use in laying hens. Despite this, detection of multiple drug residues in eggs and egg products have still been reported globally. For example, sulfonamides, macrolides, tetracycline and beta-lactam residues were detected in a survey of 184 table eggs pooled from layer farms, shopping malls and supermarkets [15]. In more recent studies, enrofloxacin was detected in 8 of 111 samples at concentrations of 2.4–1485 μg/kg, with ciprofloxacin detected concurrently in 5 of the 8 enrofloxacin-positive samples. Other drugs such as tetracyclines, beta-lactams, aminoglycosides, and macrolides (e.g. tilmicosin) were also detected [16]. Cornejo et al. also reported the presence of tetracyclines, beta-lactams, aminoglycosides and macrolides in 73 % of eggs collected from backyard poultry production systems in the central region of Chile [17]. Although veterinary drug residues were not detected in chicken eggs in this study, they were detected in egg tofu. As egg tofu is made from soy milk and eggs, any veterinary drug residue present in the raw eggs would have been transferred to the final processed product.

Century egg and salted duck egg are preserved egg products widely consumed in Southeast Asian countries including Singapore. In a previous study on antibiotic analysis in ready-to-eat duck eggs (salted and century), Liu et al. reported the presence of enrofloxacin residues in 2.94 % of salted duck eggs as well as chloramphenicol. Similarly, Zhu et al. detected florfenicol in salted duck eggs at a concentration of 1.44 μg/kg. Ji et al. also reported the detection of 5 sulfonamides and 6 quinolones in 136 salted duck eggs, with ciprofloxacin being the most prevalent followed by enrofloxacin. In century eggs, sulfamethazine, enrofloxacin and ofloxacin residues were detected [18]. Results obtained in this study differ from those reported in literature, where clopidol and nicarbazin were detected in salted duck egg and nicarbazin in century eggs instead. This may be attributed to different farms utilizing different veterinary drugs depending on the type of diseases prevalent. In addition, the samples were not screened for sulfonamides in this study which may be included in future.

Given that only a few veterinary drugs are permitted in layers, the detection of drug residues in egg and egg products in this study suggests the illegal administration of medicated feed/water or unintended cross-contamination to non-target feed. The carryover of veterinary drugs from feed to food of animal origin may sometimes be unavoidable even if best practices (e.g. Codex Code of Practice on Good Animal Feeding, Good Manufacturing Practices, Hazard Analysis and Critical Control Points) have been followed. This has been recognized as a potential source of human health risks and trade issues by regulatory agencies. For example, the EU Commission has set maximum levels for coccidiostats in non-target feed following unavoidable carryover (EU Regulation No 574/2011). The Codex Alimentarius Committee on Residues of Veterinary Drugs in Food (CCRVDF) has also established a working group to set action levels for veterinary drug residues in food products from non-target animals due to unintended and unavoidable carryover from feed [19].

Out of the four veterinary drug classes screened in this study (beta-agonists, coccidiostats, fluoroquinolones and macrolides), coccidiostats were most frequently detected among the positive samples. Coccidiosis is a contagious protozoan infection that affects the health and productivity of numerous animals, especially poultry and rabbits [19]. Intensive farming, poor hygiene practices and failure to isolate infected animals further encourage proliferation of the disease. Thus, the use of coccidiostats is deemed necessary to maintain animal health and welfare as well as prevent economic losses due to infection. In contrast to most antimicrobials, coccidiostats are administered as a feed additive throughout the life of the animal for prophylactic rather than therapeutic purposes [19,20]. As such, there is widespread use of coccidiostats in poultry production. The high number of poultry and poultry-derived positive samples may thus explain coccidiostats as the most detected drug in this study. In addition, some coccidiostats are strongly electrostatic and the use of powder formulation further increases the risk of unintended carryover from medicated feed to subsequent batches of non-target feed during production and feeding [19]. The detection of coccidiostat residues in edible tissues and eggs have also been reported in other studies. In a survey of 202 muscle samples from bovine, swine, poultry, ovine and rabbit in Italy between 2012 and 2017, the mean frequency of positive samples was 33.7 %. Synthetic coccidiostats were more frequently detected than ionophores, with nicarbazin and diclazuril being the most detected. The most common ionophores were narasin and lasalocid [21]. In another study of 74 animal muscle tissue (poultry, porcine, bovine, ovine and rabbit) and 8 egg samples, coccidiostat residues were present in 25 samples. Majority of these positive samples were related to poultry production [22]. A survey of nine ionophore and synthetic coccidiostats in 101 chicken and turkey muscle samples in Portugal also revealed a positive rate of 20.8 % [23]. While comparison with other studies is difficult due to differing methodologies, the present study had a positive rate of 1.39–5.56 % which is significantly lower than those reported in literature and the levels detected were also lower. As Singapore imports more than 90 % of food, this suggests the effectiveness of our regulatory framework in ensuring absence of drug residues in imported food products. This includes pre-import control measures such as inspection of farms and slaughter houses of source countries, requirements for export certification and pre-import testing as well as stringent import control like regulatory sampling and testing of high risk food commodities.

Besides coccidiostats, fluoroquinolones were also frequently detected in the samples. This represents a potential area of concern, given that this class of antimicrobials is also used exhaustively in human medicine and has been identified by WHO as Critically Important Antimicrobials [24]. As such, the prudent use of antimicrobials should be strongly promoted to prevent the development of resistance and negative human health impact.

4.2. Dietary exposure assessment

Exposure assessments are essential to quantify the dietary risk of chemical residues present in food. In this study, dietary exposure assessment was performed using a deterministic model based on food consumption data and mean concentrations of veterinary drug residues in the food samples analysed. The resulting dietary exposure estimate was then compared with the ADI of the drug for risk characterization. In general, the calculated EDIs were significantly lower than the respective ADIs recommended by JECFA for lower and upper bound intakes in both average and high consumers (%ADI 0.00208–8.13 % for average consumer, 0.00420–16.4 % for high consumer), indicating very low risk level. The use of tilmicosin and fluoroquinolones such as ciprofloxacin and enrofloxacin in animals

from which eggs are produced for human consumption is prohibited. Thus, no MRLs have been established by JECFA as well as European Union (EU) for eggs. However, the %ADIs for ciprofloxacin and enrofloxacin ranged from 1.88 to 16.4 % and tilmicosin from 0.0128 to 0.347 %. These values were much lower than 100 %, indicating low health risk. The dietary exposure assessment results obtained were comparable to those reported in literature, where dietary exposure to veterinary drugs did not pose a significant health risk [5,18,21,23,25]. Similar results were obtained in the 25th Australian Total Diet Study, where a large proportion of food samples contained no detectable anthelmintics and beta-lactam residues [26]. Estimated dietary exposures were well below the relevant ADIs, indicating no public health and safety concern on veterinary drugs via food consumption in Singapore.

4.3. Limitations

The food samples were only screened for four veterinary drug classes in this study namely beta-agonists, coccidiostats, fluoroquinolones and macrolides. Selection of these drugs was based on our past surveillance records. Therefore, the possibility of other veterinary drug residues being present in the food samples cannot be excluded. Other drugs commonly used in farming such as tetracyclines, penicillin and sulfonamides may be included in future work to provide a more comprehensive and accurate assessment of dietary exposure to veterinary drug residues in Singapore.

While foods of animal origin are not routinely screened for pesticides, there are certain pesticides such as cyfluthrin, lufenuron and emamectin benzoate which may also be used as veterinary drugs. Therefore, these dual use compounds may be present in animal commodities due to carryover when treated crops are used as animal feed as well as their use as a veterinary drug. JECFA and Joint FAO/WHO Expert Meeting on Pesticide Residues (JMPR) are currently working towards establishing single, harmonized ADI values as well as maximum residue limits (MRLs) for such dual-use compounds. These compounds should also be included in future dietary exposure studies to evaluate their food safety risk.

In a similar vein, plant products such as fruits, vegetables and grains were not analysed in this study due to the low likelihood of contamination with veterinary drug residues. With increasing awareness of circular economy, biowastes such as livestock manure have been increasingly utilized as natural fertilizers for crop production. Numerous studies have reported the presence of antibiotic residues in animal manure as well as soil amended with animal manure in various countries such as Canada, China and Malaysia. Tetracyclines have been identified as the most frequently detected antibiotics in agricultural soils fertilized with animal manure [27–29]. Consequently, this presents a risk of veterinary drug residues being introduced into food crops due to uptake from the contaminated soil. Therefore, screening of crops for the presence of veterinary drug residues may be a potential area of work in future to enable a more comprehensive dietary exposure assessment.

5. Conclusions

In this study, the occurrence of 38 veterinary drug residues belonging to 4 classes (beta-agonists, coccidiostats, fluoroquinolones and macrolides) were evaluated in 216 food samples via LC-MS/MS. It was found that fluoroquinolones (ciprofloxacin, enrofloxacin), coccidiostats (clopidol, diclazuril, lasalocid, nicarbazin) and macrolide (tilmicosin) were present in some food samples, with clopidol and enrofloxacin being the major contaminants. 9 samples also contained more than one drug residue. However, the calculated EDIs of the veterinary drugs detected in the food samples were significantly lower than the ADIs set by JECFA. Therefore, the results suggest that ingestion of trace amounts of veterinary drugs from daily food consumption poses negligible health risk to the general public in Singapore.

To the best of our knowledge, this study is the first to evaluate the level of dietary exposure to beta-agonists, coccidiostats, fluoroquinolones and macrolides in Singapore. Results from this study can serve as a useful reference to initiate more targeted work in future such as further finetuning of food safety monitoring programs and development of food safety standards.

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CRediT authorship contribution statement

Jia En Valerie Sin: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Ping Shen:** Conceptualization, Writing – original draft, Writing – review & editing. **Guat Shing Teo:** Investigation. **Lay Peng Neo:** Investigation. **Lifei Huang:** Investigation. **Patricia Chua:** Investigation. **Mui Woon Tan:** Investigation. **Yuansheng Wu:** Writing – review & editing. **Angela Li:** Supervision. **Jun Cheng Er:** Conceptualization, Methodology, Project administration. **Sheot Harn Chan:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21160.

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