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ORIGINAL ARTICLE

Exposure assessment of chemical hazards in pork meat, liver, and kidney, and health impact implication in Hung Yen and Nghe An provinces, Vietnam

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Abstract
Objectives This study assesses the risk of exposure to hazardous chemical residues in pork meat, liver, and kidney collected at wet markets in Nghe An and Hung Yen provinces and discusses health impact implication.
Methods 514 pig feed, kidney, liver, and pork samples were pooled and qualitatively and quantitatively analyzed for tetracyclines, fluoroquinolones, sulphonamide, chloramphenicol, β-agonists, and heavy metals. We compare the results with current regulations on chemical residues and discuss health implications.
Results Legal antibiotics were found in feed. Tetracycline and fluoroquinolones were not present in pork, but 11% samples were positive with sulfamethazine above maximum residue limits (MRL); 11% of packaged feed and 4% of pork pooled samples were positive for chloramphenicol, a banned substance; two feed, two liver, and one pork samples were positive for β-agonists but did not exceed current MRL; 28% of pooled samples had lead, but all were below MRL; and all samples were negative for cadmium and arsenic. Thus, the health risks due to chemical hazards in pork in Hung Yen and Nghe An seemed not as serious as what were recently communicated to the public on the mass media.
Conclusions There is potential exposure to sulphonamide, chloramphenicol, and β-agonists from pork. Risk communication needs to focus on banned chemicals, while informing the public about the minimal risks associated with heavy metals.

Keywords Antibiotic residues · Growth promoters · Heavy metals · Pork · Exposure assessment · Vietnam

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Introduction

Chemical hazards in food include environmental contaminants, natural toxins, allergens, mycotoxins, residues of pesticides, veterinary drugs and feed additives, intentional food additives, substances formed during food processing, substances derived from food contact materials, and adulterants (Andrée et al. 2010; Tuyet Hanh et al. 2015). Concern over chemical hazards is growing in Vietnam as unsafe foods are frequently reported in newspapers and on television. In Vietnam, pork makes up approximately 75% of red meat consumed (OECD 2016). Therefore, pork is a frequent target for concerns, especially those associated with chemical hazards, including veterinary drugs, heavy metals, and growth promoters. The authors undertook an internet search in January 2015 at http://www.google.com, using the Vietnamese search term "chất cấm trong chăn nuôi heo" (meaning "prohibited veterinary drugs and substances in pig husbandry") resulted in over 400,000 hits within 0.22 s.

In recent years, consumers frequently encounter stories about β-agonists as growth promoters (e.g., salbutamol and clenbuterol). For example, environmental police officers and inspectors discovered feed companies using salbutamol and other banned chemicals in pig feeds (Nguyen Van Viet 2015). In another case in 2012, the Ministry of Police collaborated with a market inspector team in Tan Binh District, Ho Chi Minh City, discovered that ONI Company Ltd. had two tonnes of pig feed which was positive for salbutamol test; the polices also recalled 4.1 tonnes of feeds with salbutamol produced by this company in 22 other provinces and cities in Vietnam. Other three large companies were also fined due to using prohibited veterinary drugs (Nguyen Van Viet 2015). In 2015, 16 out of 20 suspected feed companies were found to abuse salbutamol and/or clenbuterol in feed products. However, those violations were not seen as crimes under the Criminal Law, and being challenges to control prohibited veterinary drugs in feed and at pig farms in Vietnam (Nguyen 2015).

In addition, a conducted literature review identified chemical hazards could be problematic in pork meat and its products. This revealed that heavy metals (e.g., lead, cadmium, and arsenic), antibiotic residues (e.g., penicillin, cephalosporin, aminozid–AG, macrozid, n-lincosamid, and chloramphenicol), carcinogens (e.g., sulphamethazine, oxytetracycline, and furazolidone), growth promoters (e.g., salbutamol and clenbuterol), dioxins, persistent organic pollutants, additives (e.g., sodium nitrate, sodium nitrite, potassium nitrate, and potassium nitrite), heterocyclic aromatic amines, and polycyclic aromatic hydrocarbons were hazardous substances. They were present in pork and pork products in Vietnam and other countries worldwide (Tuyet Hanh et al. 2015). Exposure to veterinary drug residues and other chemical hazards in pork can cause acute or chronic adverse health consequences, depending on the chemical types and concentrations, and amount of pork consumed (Beyene 2016; Baynes et al. 2016; Sundlo 2014). Acute responses are usually rare, resulted from anaphylactic or pharmacologic reactions. However, outbreaks of food poisoning in some countries were caused by consuming cattle and pigs' liver contained clenbuterol. Common symptoms are palpitations, nausea, vomiting, dizziness, chest tightness, uneasiness, trembling, and instability (Sundlo 2014). Another important public health concern was consequences caused by chronic dietary exposure to drug residues at subacute doses (Sundlo 2014).

Recently, in Vietnam, scientific literatures, official communications, mass media reports, and consumer complaints have demonstrated that food safety is perceived as a major public health problem. There is emerging evidence that a relatively large share of food in Vietnam may be considered unsafe according to food safety norms and standards (World Bank 2016). Food safety was one of the two most pressing issues for people in Vietnam, even more important than education, healthcare, or governance (USAID 2015). Vietnam Food Administration recorded 373 foodborne outbreaks in 2014 and 2015 with over 10,000 cases and 66 deaths (Vietnam Food Administration 2016). However, evidence from other low—and middle—income countries suggested that these figures were greatly underestimated cases which actually have occurred in the community as only a small proportion of foodborne disease is ever recorded as outbreaks (Delia 2015). Therefore, this study aimed to assess the risks of exposure to hazardous chemicals in pig liver, kidney, and pork samples collected at Hung Yen and Nghe An provinces, and to discuss the related health impact implication in Vietnam.

Methods

To assessed the risk of exposure to chemical hazards, pig feed, liver, kidney, and pork samples were randomly selected from two provinces in Vietnam, Hung Yen and Nghe An, where pig raising activities at households were common. In each province, three districts were stratified based on typical of pig production chain and were represent for rural, peri-urban, and urban areas. This study is a component of an ongoing project titled "PigRISK" funded by ACIAR, which is assessing the health risks of chemical hazards in pork and developing incentive-based innovations to improve management of human and animal health risks in the smallholder pig value chains in Vietnam. First, we undertook hazard profiling from various potential hazardous chemicals as mentioned in introduction. Second, three groups of chemical hazards for investigation were antibiotic residues, heavy metals, and β-agonists. From April 2014 to January 2015, we conducted a comprehensive sampling strategy along the pork value chain in Van Giang, Khoai Chau, and Tien Lu districts in Hung Yen Province, and Hung Nguyen, Do Luong, and Dien Chau districts in Nghe An Province. A total of 64 samples of feed in cages and 84 samples of packaged feed were collected from pig farms. In addition, 190 fresh pork samples, 88 liver samples, and 88 kidney samples were collected at wet markets to produce 18 pooled samples of each type (representing 18 studied communes—three communes per district, Table 1). Pooled samples were formed by combining 5–15 original samples which were collected from individual pig farm or slaughtered pig or pork retailer. The use of pooled samples helped to reduce the laboratory analysis cost, while maintaining scientifically valid (Heffernan et al. 2014).

Table 1 Detailed numbers of pooled samples and chemical hazards being analyzed by different methods

Chemical	Method	Feed in cages	Feed in packs	Pork	Liver	Kidney	Total
Tetracyclines group	ELISA, LCMSMS	18	18	18	18	18	90
Fluoroquinolones group	ELISA, LCMSMS	18	18	18	18	18	90
Sulfoamides group	ELISA, LCMSMS	18	18	18	18	18	90
Chloramphenicol	ELISA, LCMSMS	18	18	18	18	18	90
Beta agonist	ELISA, LCMSMS	18	–	18	18	18	72
Lead, cadmium, arsenic	AAS	–	18	18	18	18	72

(−) samples were not analyzed for these chemicals

ELISA enzyme-linked immunosorbent assay, *LCMSMS* liquid chromatography–mass spectrometry/mass spectrometry, *AAS* atomic absorption spectroscopy

All collected samples were pre-washed with clean tap water to remove any surface dirt. Samples were then separately packed in labeled polypropylene bags, kept in ice box, and transported to the Laboratory of the Center for Veterinary Hygiene and Inspection No.1 in Hanoi. Samples were stored in the freezers at −20 °C prior to analysis. Samples were first screened using the enzyme-linked immunosorbent assay (ELISA) method. Positive samples from screened ELISA were quantitatively assessed using liquid chromatography–mass spectrometry/mass spectrometry (LCMS/MS) or atomic absorption spectroscopy (AAS) according to specific hazards. Detection limits in each analytical method and for each specific chemical are presented in Tables 1 and 2. Researchers, policy makers, local leaders, veterinarians, pig farmers, traders, and slaughterhouse workers were involved in meetings and discussions. Moreover, chemical hazards in pork and their toxicity also gathered from literatures to assess as evidences for hazard assessment. For chemical hazards with concentrations exceeding standard levels, estimated daily intakes were calculated for exposure assessment, based on chemical concentrations in pork and amount of pork consumed per day. An average amount of pork consumed daily was derived from a study of Toan et al. (2013). Ethical clearance and approval were obtained from the Hanoi University of Public Health's Institutional Review Board (Number 148/2012/YTCC-HD3).

Results

Qualitatively screening chemical hazards in pig feed, kidney, liver, and pork using the ELISA test

Screening found antibiotics were commonly presence in feed (Fig. 1). Tetracyclines, fluoroquinolones, and sulfonamides groups detected in packed feed samples were 13/18 (72%), 8/18 (44%), and 11/18 (61%), respectively. While feed samples taken in cages positive for tetracycline, fluoroquinolone, and sulfonamide groups were 10/18 (55%), 12/18 (66%), and 5/18 (27.8%), respectively.

All liver, kidney, and pork samples were negative for tetracyclines. Only one kidney sample was positive for fluoroquinolones and 24% of liver, kidney, and pork samples were positive for sulfonamides. Two packed feed and three pork samples were positive for chloramphenicol. Two cage feed, two liver, and one pork samples were positive for β-agonists. For heavy metal groups, arsenic was not found in all tested samples. Two packed feed and 41% of food samples (liver 56%; kidney 39%; pork 28%) were positive for lead. In all, 17 packaged feed samples, all 18 liver samples, and 18 kidney samples were positive for cadmium, while all 18 pork samples were negative (Fig. 2).

Concentrations of chemical hazards in pig feed, kidney, liver, and pork

Results of chemical hazards concentration in feed, kidney, liver, and pork samples are presented in Table 2. Large proportions (from 27.8 to 72%) of feed samples taken from packs and cages were positive with different antibiotics. However, several screened positive samples were not detected, because the concentrations might be lower the limited point of detection. For example, in fluoroquinolones group, although 44 and 67% of feed samples taken from packs and pig cages were positive for fluoroquinolone when screened by ELISA, only one sample of feed in cage was detected with flumequine (Table 2). In tetracyclines group, five samples of feed in packs had an average tetracycline concentration of 3690.7 μg/kg (ranged from 100.7 to 15046.7 μg/kg), while only one feed sample taken from pig cage detected this antibiotic with the level of 178.97 μg/kg.

For sulfonamides group, an average concentration of 10 feed samples taken from the packs was 21468.7 μg/kg (ranged from 67.8 to 131874.9 μg/kg) and that in feed samples taken from cages was 3080.5 μg/kg (ranged from

Table 2 Results of screening chemicals in feed samples taken in packs and cages using ELISA and quantitatively assessed using LC–MS/MS or AAS methods

Chemical hazards	Limit of detection (µg/kg)	Feed in pack		Feed in cage	
		No. positive/*n* (%)	Residue level [mean (min–max)] µg/kg	No. positive/*n* (%)	Residue level [mean (min–max)] µg/kg
Tetracyclines		**13/18 (72)**		**10/18 (55)**	
Oxytetracycline	50	1	403	0	
Tetracycline	50	5	3,691 (101–15,047)	1	179
Chlortetracycline	50	5	68448.5 (883.9–252,119)	1	5035
Fluoroquinolones		**8/18 (44)**		**12/18 (67)**	
Enrofloxacine	30	0	–	1	60
Norfloxacine	30	0	–	0	–
Flumequine	30	1	254	0	–
Sulfonamides		**11/18 (61)**		**5/18 (28)**	
Sulfamethazine	30	10	21,469 (68–13,1875)	4	3080 (222–10,100)
Sulfaquinoxalin	30	1	116.06	0	–
Chloramphenicol	0.75	2	2.10 (1.76–2.43)	0	–
β-agonists		–		**2/18 (11)**	
Salbutamol	3	–	–	**2**	127 (53–201)
Clenbuterol	3	–	–	0	–
Heavy metals					
Lead	70	2/18 (11)	367 (118–617)	–	–
Cadmium	10	17/18 (99)	20 (11–99)	–	–
Arsenic	50	0	–	–	–

Bold reflects the name of group of chemicals

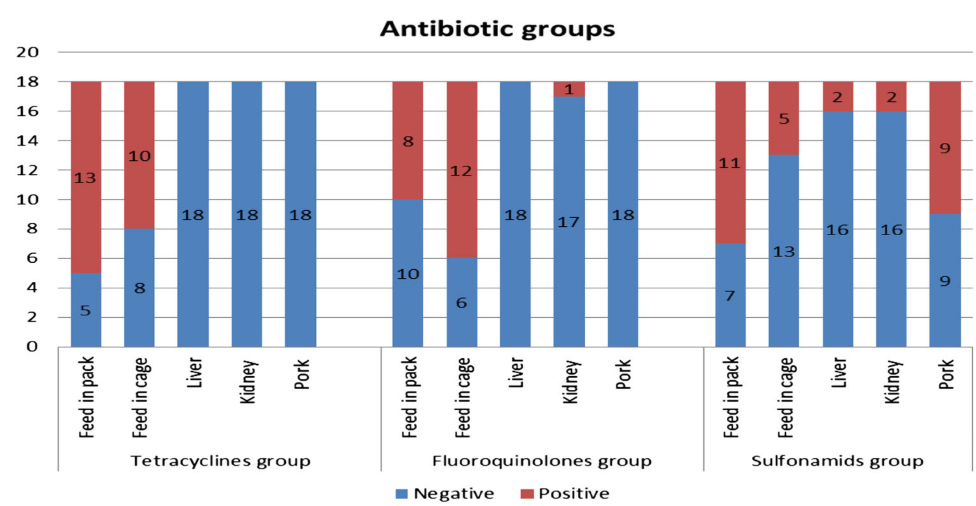

Fig. 1 Result of screening pooled feed, liver, kidney, and pork samples for antibiotic residues with ELISA

222.3 to 10100.2 µg/kg) (Table 2). Regarding β-agonists, no packed feed samples were detected with this banned chemical, while two feed samples taken from cages were contaminated with salbutamol at the concentration of 53.4 and 201.1 µg/kg. No feeds in cages were detected with heavy metals, while two feed samples taken from packs were positive with lead at the concentration of 117.8 and 616.6 µg/kg. 17/18 (99%) feed samples taken from packs were detected with cadmium at an average level of 29.9 µg/kg (ranged from 11.42 to 99.05 µg/kg).

The detailed results of the chemical hazards quantification in pig liver, kidney, and pork are presented in Table 3. Tetracyclines, fluoroquinolones groups, and clenbuterol were not quantified in food (liver, kidney, and pork) samples. Nonetheless, sulfamethazine, chloramphenicol, and salbutamol were found in those samples.

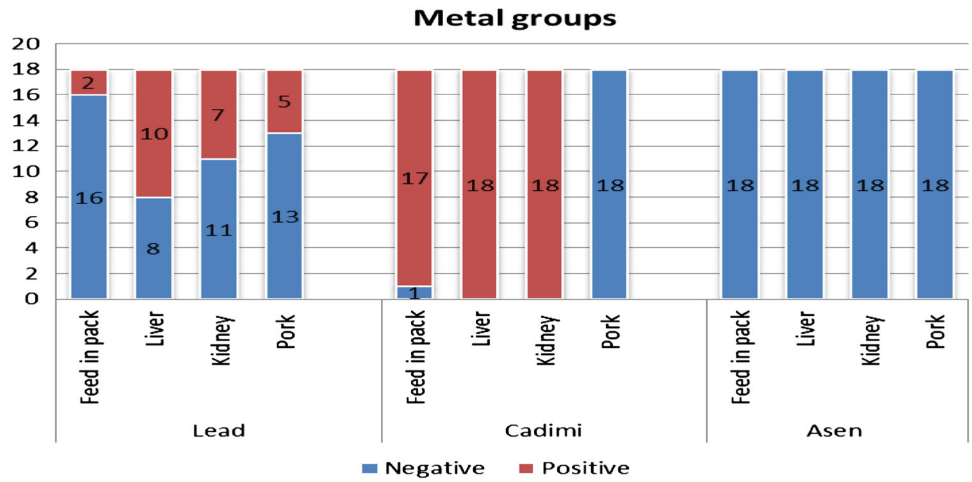

Fig. 2 Result of screening pooled feed, liver, kidney, and pork samples for lead, cadmium, and arsenic residues with ELISA

Table 3 Results of screening chemicals in liver, kidney, and pork samples using the ELISA method and quantitatively assessed using the LC–MS/MS or AAS methods

Chemical hazards	Limit of detection (µg/kg)	Liver		Kidney		Meat	
		No. positive/ *n* (%)	Residue level [mean (min–max)] µg/kg	No. positive/ *n* (%)	Residue level [mean (min–max)] µg/kg	No. positive/ *n* (%)	Residue level [mean (min–max)] µg/kg
Tetracyclines	50	0/18	–	0/18	–	0/18	–
Fluoroquinolones	30	0/18	–	1/18	–	0/18	–
Sulfonamides		**2/18 (11)**		**2/18 (11)**		**9/18(50)**	
Sulfamethazine	15	2	68 (45–91)	1	87	5	155.5 (36–263)
Sulfaquinoxalin	15	0	–	0	–	0	–
Chloramphenicol	0.15	0	–	0	–	**3/18 (17)**	0.54 (0.34–0.76)
β-agonists		**2/18(11)**		**0/18**		**1/18 (5)**	
Salbutamol	3	2	4.24 (2.77–5.71)	0	–	1	1.09
Clenbuterol	3	0	–	0	–	0	–
Heavy metals		**18/18 (100)**		**18/18 (100)**		**5/18 (28)**	
Lead	70	10/18 (55)	117 (71–303)	7/18 (39)	128 (71–208)	5	74 (70–79)
Cadmium	10	18/18 (100)	17.5 (10.4–31.6)	18/18 (100)	223 (126–383)	0	–
Arsenic	50	0	–	0	–	0	–

Bold reflects the name of group of chemicals

Two liver samples had sulfamethazine at concentration of 44.7 and 90.8 µg/kg, and one kidney sample had sulfamethazine at concentration of 86.93 µg/kg, whereas five meat samples had sulfamethazine with an average concentration of 155.5 µg/kg (ranged from 35.6 to 263.2 µg/kg). For chloramphenicol, neither liver nor kidney samples were detected with this antibiotic, but surprisingly three meat samples were quantified with an average concentration of 0.54 µg/kg (ranged from 0.34 to 0.76 µg/kg). Level of salbutamol detected in two liver and one pork samples was 2.77 and 5.71, and 1.09 µg/kg, respectively.

For heavy metals group, an average concentration of lead in liver and kidney samples was 117.4 µg/kg (ranged from 71.3 to 302.7 µg/kg) and 127.9 µg/kg (ranged from 70.5 to 208.1 µg/kg), respectively. Cadmium was not detected in pork, but it was detected in all liver and kidney samples. Arsenic was not detected from any liver, kidney, or pork samples. For salbutamol, we also conducted an exposure assessment. We assumed people consume 86.1 g of pork meat per day (Toan et al. 2013), then their potential daily intake of salbutamol from pork would be 0.094 µg per day. To determine salbutamol daily intake per kg body weight, we used the average body weight for an adult (both genders), at ages of 20 and above of approximately 50 kg (Vietnam National Institute of Nutrition & Unicef 2010). Thus, the average daily intake of salbutamol from pork would be approximately 0.0019 µg/kg/day.

Discussion

The results show that a large proportion of feed samples taken from packs and cages (from 28 to 72%) screened positive for tetracyclines, fluoroquinolones, and sulfonamides. However, tetracyclines and fluoroquinolones groups were not quantitatively detected at any liver samples, kidney samples, and pork samples (although one kidney sample was positively screened with ELISA), which indicates that the farmers did not use these antibiotics within a short period before slaughtering. In other studies, tetracycline was detected in approximately 5.5 and 10% of pork samples (Kim et al. 2013; Nhiem et al. 2006). Sulfonimides in liver and kidney were below the maximum residue level (MRL) of 100 μg/kg; however, the concentrations in two meat samples were approximately 1.9–2.6 times higher than the MRL according to the Circular Number 24 in 2013 by the Ministry of Health (2013). A recent study in Ho Chi Minh found sulfamethazine residues in 23% of marketed pork sampled (Do et al. 2016). Sulfamethazine is possibly carcinogenic to mice, carcinogenic to rates, and the toxic side-effects of sulfamethazine in humans include disorders of the hematopoietic system and hypersensitivity reactions (Kevin Woodward 2013). Thus, the inspection of this antibiotic use at pig farms should be strengthened. Several samples which were positive by screened ELISA, but were negative in quantitative analysis. This may be the result of within sample heterogeneity, or hazards below the limit of detection, or false positives.

Chloramphenicol was present in two pooled feed and three pooled pork samples, although at low concentrations. Chloramphenicol is a banned veterinary drug according to the Circular Number 15 in 2009 by the Ministry of Agriculture and Rural Development and, therefore, should not be detected at any levels (Ministry of Agriculture and Rural Development 2009a). According to JECFA CAC37 (2014), there is no safe level of residues of chloramphenicol or its metabolites in food that represents an acceptable risk to consumers; thus, competent authorities should prevent residues of chloramphenicol in food. This can be accomplished by not using chloramphenicol in food producing animals.

We found β-agonists in two feed samples and three food samples. These are illegally used as pig growth promoters to increase the lean meat-to-fat ratio and to improve feed conversion efficiency (Strydom et al. 2008). A meta-analysis review concluded that medical use of β-agonists increases the risk for adverse cardiovascular events. Beta agonists may precipitate ischemia, congestive heart failure, arrhythmias, and even sudden death (Salpeter et al. 2004). Therefore, β-agonists has been banned in most countries including Vietnam for growth-promoting purposes in farm animals. However, one report found that only 10 kg (0.2%) out of totally 6 tones salbutamol sold in the market in 2015 was with the correct medical purposes, while the rest were sold to be used in pig feeds at very high prices: 1 kg salbutamol imported to Vietnam for medical purpose has a price of $US 70–80 per kg while illegally sold to the pig feed producing companies, the price is as high as $US 750 (Van Duan et al. 2016).

Our study found the concentrations of β-agonists (Salbutamol) in two liver samples of 4.24 μg/kg and in one meat sample at the level of 1.09 μg/kg. These levels were lower than level of 5 μg/kg which was defined as quantitatively positive liver and meat samples according to the Circular number 01/2016/TT-BNNPTNT in 2016 by the Ministry of Agriculture and Rural Development (Ministry of Agriculture and Rural Development 2016). However, according to the previous Circular number 57/2012/TT-BNNPTNT, positive liver, kidney, and meat samples were those with the concentration of Salbutamol above 2 μg/kg, which the two liver samples in our study exceeded.

Our exposure assessment found that the average daily intake of salbutamol from pork would be approximately 0.0019 μg/kg/day. The recommended daily dose of salbutamol for oral administration to provide short acting bronchodilation in reversible airways obstruction for adult is 4 mg, 3 or 4 times per day, which may be increased to a maximum of 8 mg, 3 or 4 times per day. In elderly patients and patients who are unusually sensitive to this class of medicine, treatment may be initiated with 2 mg, 3 or 4 times per day (EMC 2015). The residues found in this study were quite low, which may present minimal health risks to the customers. Larger scale assessments of the residues of β-agonists in pork should be undertaken to scientifically inform risk communication activities in Vietnam, so that customers will be correctly informed about the actual risk of this hazard, and preventing economical lost and the overwhelming reaction over the risk, which in fact may be minimal. Overall, approximately 7.5% of our pooled food samples contained at least one prohibited veterinary drugs or antibiotics over the MRL. Because the 54 pooled samples represent 366 individual samples, this could correspond to approximately 7.5% of marketed pork unacceptable. According to government records, from the early 2015 to February 2016, monitoring and inspection activities identified 106 samples of meat and meat products with elevated levels of prohibited veterinary drugs and antibiotics out of the total 5,433 samples being analyzed, which accounted for approximately 2% (Van Duan et al. 2016).

According to the recommendations of FAO and WHO, maximum levels of heavy metals should be 3 mg/kg for arsenic, and 2 mg/kg for lead, while the maximum concentrations of cadmium and mercury vary according to different types of foods (FAO and WHO 2002). In

Vietnam, the maximum allowable concentrations of arsenic, lead, and cadmium in feeds are 2000, 1000, and 5000 µg/kg, respectively (Ministry of Agriculture and Rural Development 2009b). In this study, we did not detect arsenic in any samples. The Vietnam MRL for lead concentration in pork is 100 µg/kg and in other parts 500 µg/kg, while the MRL for cadmium concentration in pork is 50 µg/kg, in liver is 500 µg/kg, and in kidney is 1000 µg/kg. In this study, all samples had less than the MRL. Cadmium was not detected in meat, while liver samples were lower than the MRL of 500 µg/kg and kidney samples were lower than the standard level of 1000 µg/kg.

Limitations, uncertainties, and implication for risk communication

There were some limitations inherent in this exposure assessment activity. These included the uncertainties of basing the exposure assessment on the analysis of 18 pooled samples of each type (packaged feed, feed in cages, liver, kidney, and pork meat) versus over 514 individual samples due to the high cost of chemical analysis. Although, the use of pooled samples is a scientifically valid and cost effective method of analysis (Heffernan et al. 2014), it does not allow a precise estimate of prevalence. Thus, if more funding was available, the analysis of large sample size of individual samples would provide a more comprehensive assessment. In addition, the scale of this study was limited to only two provinces in Vietnam. If a detailed and comprehensive assessment of chemical hazards in pork in different regions throughout Vietnam was undertaken, the results would be more useful to inform risk communication and risk management of chemical hazards in pork in the country at different levels. This is critical input for risk communication to re-gain the trust from the customers who are currently very worried about the safety of the pork and pork products available on the markets in Vietnam and food safety was identified as a “hot Public health issue” in the country, in which food safety risk communication is a prioritized issue to be tackled in the coming years (World Bank 2016).

Conclusions and recommendations

The results highlight the potential risks of exposure to sulphonamide, chloramphenicol, and salbutamol through pork consumption but not of tetracyclines or fluoroquinolones. Lead and cadmium detected in different samples were lower than the maximum allowable levels, while arsenic was not detected. This suggests that risk communication needs to pay attention to banned chemicals, while informing the public about the minimal risks associated with heavy metals and other hazards. The control of prohibited veterinary drugs should be undertaken at importation and feed manufacture, not just by the pig farms and marketed pork.

In recent years, the public has considered that the risks associated with chemicals hazards in foods in Vietnam are extremely high. Studies at national level would be useful to provide scientific data on health risk assessment of chemical hazards in pork in Vietnam, to inform the risk communication activities. If the results were similar to those reported in this study, then the situation may be less negative than perceived by the general public. In fact, biological hazards in pork may cause more morbidity and mortality burdens than chemical hazards. Risk assessment of chemical hazards in foods in general and in pork particular is, therefore, crucial to provide scientific information on the actual risk and to inform risk communication activities. Currently, risk communication on food safety issues has not been integrated into the recommended risk-based food safety management system as specified in the Food Safety Law 2010 in Vietnam; building national capacity in risk communication can help bridge the divide between expert risk assessment on one side and public reaction and action on the other.

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Compliance with ethical standards

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Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical clearance and approval was obtained from the Hanoi University of Public Health’s Institutional Review Board (Number 148/2012/YTCC-HD3).

Conflict of interest The authors declare no conflict of interest.

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