Quantitative risk assessment of salmonellosis through pork consumption in Vietnam

Rakuno Gakuen University

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Sinh Dang-Xuan

Thesis

Quantitative risk assessment of salmonellosis through pork consumption in Vietnam

(ベトナムにおける豚肉喫食によるサルモネラ症リスクの定量的評価)

Veterinary Epidemiology Unit

Graduate School of Veterinary Medicine

Doctoral Program for Veterinary Epidemiology

Rakuno Gakuen University

Sinh Dang-Xuan

Supervisor: Assoc. Prof., Dr. Kohei Makita, Veterinary Epidemiology Unit, Rakuno Gakuen University

Co-supervisors: Dr. Phuc Pham-Duc, Center for Public Health and Ecosystem Research, Hanoi University of Public Health

Dr. Hung Nguyen-Viet, International Livestock Research Institute, Hanoi, Vietnam

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ABBREVIATION

ACIAR: Australian Centre for International Agricultural Research

BAM: Bacterioligical Analytical Manual

BGA: Brilliant Green Agar

BPW: Buffered Peptone Water

CFU: Conoly Forming Unit

CGIAR: Consultative Group on International Agricultural Research

CI: Confident Interval

CRP: CGIAR Research Program

EFSA: European Food Safety Authority

e.g: exempli gratia ("for example")

etc: et cetera ("and the other things")

EU: European Union

FAO: Food and Agricultural Organization

FGD: Focus Group Discussion

FMD: Foot and Mouth Disease

GSO: Genreral Statistic Office

HACCP: Hazard Analysis Critical Control Point

i.e: id est ("that is")

ILRI: International Livestock Research Institute

ISO: International Organization for Standardization

LMIC: Low and Middle Income Countries

MARD: Ministry of Agriculture and Rural Development

MOH: Ministry of Health

MPN: Most Probable Number

PRRS: Porcine Reproductive and Respiratory Syndrome

QMRA: Quantitative Microbiological Risk Assessment

RA: Risk Analysis

SD: System Dynamics

sd: standard deviation

WB: World Bank

WHO: World Health Organization

WTO: World Trade Organization

Chapter 1. General Introduction

1.1. Background

Pig production and pork consumption are important in Vietnam. Over 98% of households in Vietnam consume pork, preferring the fresh pork sold mainly in wet markets, and mostly (>70%) produced from household scale [77, 116, 147]. Small-scale pig production employs over 4 million people (two-thirds women) and brings important benefits to poor households. Farms are small and widely scattered, and smallholder producers typically keep 1-5 sows, with less than 40 finishing pigs per year, and account for 84% of all households raising pigs [126].

Pork has been described in many countries as an important source of *Salmonella* [11, 22, 23, 97, 102], with slaughterhouses and slaughtering practices being important determinants of contamination [12, 15, 64]. In Vietnam, studies on retailed pork show contamination with *Salmonella* in 33 to 40% of samples [52, 125]. Recent studies in South Vietnam described a wide range of *Salmonella* prevalence, from 5.2 to 69.9%, among pig faeces, carcasses and pork [104, 144].

Food safety issues, including pork safety, have become a priority in Vietnam over the recent decade, since there is the need of general public health improvement and increased consumer demand for food quality and safety [45]. There is also a legal requirement to produce safe food resulting from the recent integration of Vietnam into the WTO [146]. The government issued new regulations, acts and law to enhance the food safety management [88, 140]. According to the report of the Vietnam Food Administration, a total of 1,781 food poisoning outbreaks with 58,622 infected people and 412 deaths were reported in Vietnam, between 2006 and 2015 [139]. These levels of infections are much lower than the reports in countries with more developed surveillance systems, and the WHO showed that diarrhoea reported cases are greatly underestimated [151]. In Vietnam, non-typhoidal *Salmonella* have been isolated on human patients with diarrhoea [69, 144] and in various food products [52, 106].

In recent years, quantitative microbial risk assessment (QMRA) has been introduced and widely applied to assess risks in many areas of public health problems, including food safety. QMRA modeling integrates the results of experimental studies such as dose-response assessments, and observational studies such as consumption patterns and risk factors. The output is an estimate of risk as severity of health impacts and their likelihood of occurrence, which can show the number of illnesses in a population over a period of time. Risk assessment modeling also allows risk managers to develop targeted and effective intervention programs to minimize risk and reduce adverse effects on human health, economics and society. Depending on the objectives and scope of the study, QMRA can be applied at specific stage of the value chain, risk pathway or along entire pathway from “stable to table”. As a result, QMRA is one of the important elements to help policy makers to make good decisions. QMRA has been widely applied in developed countries like the USA and European countries. However, in Vietnam, QMRA is applied to limited number and extent of studies. The QMRA framework was applied in sanitation research by Khuong [74] and in food safety by Toan et al. [127]. These studies assessed the health risks of people related to water sanitation in Ha Nam, and *Salmonella* contamination in pork in Hanoi.

Although Vietnam places high emphasis on the livestock sector as an engine for pro-poor development [89], policymakers encourage development of industrial systems [35] based on their perceptions that industrialisation will improve productivity, profitability and food safety. Recent ACIAR-funded research lead by the International Livestock Research Institute (ILRI) and national partners questioned these assumptions [1]. The research found that the small-scale household pig production systems were highly competitive as a result of their use of home produced feed and household labour. Smallholder pig production generate sufficient return to household labour and retain value within traditional value chains with large numbers of poor women and men involved in transportation, slaughtering, processing and retailing.

In Vietnam, risk management and communication are as limited as risk assessment. The dominant model is command and control regulation, with food safety assurance through inspection and punishment for violations. Studies by ILRI and others show that regulation seldom has an effect in managing disease in the smallholder sector and informal markets. In fact, attempts to enforce strict regulation may on the contrary increase the food safety risks by creating larger black markets where quality attributes that are not directly verifiable. By designing interventions based on incentives, whereby market actors gain tangible benefits from changes in practice, and working in partnership rather than opposing to, value chain actors may be more successful in improving food safety. Incentives vary from a price premium for retailers for safety assured pork to adoption of improved practices in order to increase social status. Although early results are promising, such evidences are still too limited to be optimistic for large-scale adoption in informal markets of developing countries.

1.2. Research objectives and thesis layout

The overall goal of the research was to assess the extent of *Salmonella* contamination along the smallholder pork value chain and risk for salmonellosis in humans to identify the optimal risk reduction strategies. The specific objectives of this study were following:

1. To explore knowledge, perceptions, and practices regarding diseases in animals, food safety, and health risks among pork value chain actors and consumers.
2. To determine *Salmonella* prevalence and risk factors along the pork value chain.
3. To analyse cross-contamination of *Salmonella* in pork at household level.
4. To quantify salmonellosis risks at the household level using a risk model.

This thesis counts general introduction, four main research chapters, and general discussions. The four main researches (divided by chapter) were carried out from January 2013 to March 2016. This thesis focused on food safety along the smallholder pork value chain regarding *Salmonella* contamination and its implication of human health risks. Four specific objectives of this study were demonstrated according to the research topic in each of four chapters.

Chapter 2 examined the knowledge, perceptions, and practices regarding diseases in animals, food safety, and health risks among selected pork value chain actors and consumers. The study has identified risk factors associated with *Salmonella* spp. prevalence along smallholder pork value chains in study areas (Chapter 3). The third research theme was to analyse cross-contamination of *Salmonella* in pork at household level using a simulation of *Salmonella* cross-contamination in laboratory (Chapter 4). Chapter 5 quantified salmonellosis risks at the household level using a quantitative microbial risk assessment model. The overall research framework in the pork value chain of this thesis was presented in Figure 1, to show the relationship among the purposes and the studies.

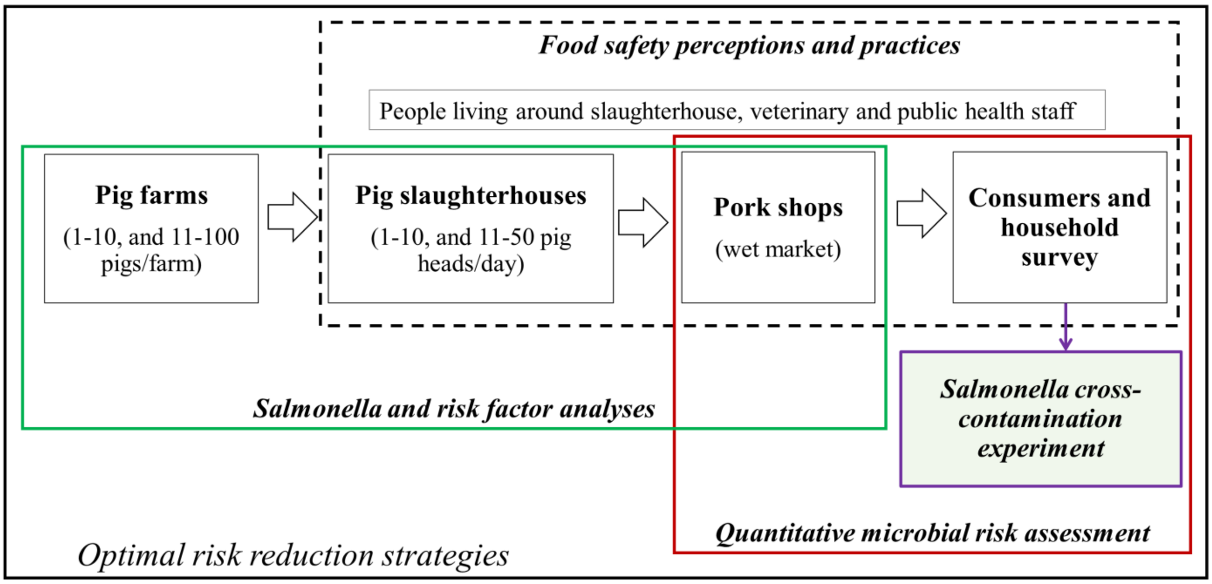

Figure 1. Research framework of studies along the pork value chain. Dashed line box was the scope of food safety perceptions and practices; green line box refered to the scope of *Salmonella* prevalence and risk factors analysis in the pork value chain; purple box refer the study on *Salmonella* cross-contamination experiment; red box was the scope of the study on the quantitative microbial risk assessment of salmonellosis.

Chapter 2. Food safety perceptions and practices among smallholder pork value chain actors in Vietnam

2. 1. Introduction

Food safety is an important public health concern worldwide, especially in emerging economies. In Vietnam, pork safety is of great concern to both consumers and policymakers and is a frequent topic in the media [5, 142, 143] and in policy discussions [61, 147]. In 2015, pork consumption (per capita) in Vietnam was 29.1 kg, among the highest in the world. Pork is the most widely consumed meat in Vietnam, making up 56% of total meat intake [101]. Up to 80% of the pork produced comes from smallholder farmers, and open wet markets are the preferred channels for purchase of pork among consumers [78]. While pork production supports food security and the livelihoods of around 4 million smallholder farmers, pork production can also lead to substantial health risks. For example, raw pork marketed in Vietnam is often contaminated with high levels of foodborne pathogens, including *Salmonella* spp. [15, 52, 104, 136], *Escherichia coli* [52, 135], *Toxoplasma gondii* (in slaughtered pigs) [63], *Taenia* spp. (in pigs) [34], and *Campylobacter* spp. [52]. Moreover, many isolates of *E. coli* and *Salmonella* spp. were resistant to one or more antibiotics [134, 136]. Contamination of pork by harmful microorganisms may occur at any stage from production to plate.

Pork value chain actors, including farmers, slaughterhouse workers, pork sellers, and consumers, along with three ministries in Vietnam (Ministry of Agriculture and Rural Development, Ministry of Health, and Ministry of Industry and Trade), have a shared responsibility for ensuring food safety (Food Safety Law No. 55/QH12/2010) [140]. The responsibility of food chain actors is also emphasized in a recent World Health Organization press release, stating that ‘‘Food producers, manufacturers and traders in Viet Nam need to take responsibility for the safety of food they produce and trade while consumers must take preventive measures and follow good food safety practices’’ [148]. While pork production systems have been described previously [65, 81], other aspects, such as how pork value chain actors perceive food safety, along with risk-mitigating behaviors, are not well understood. One study in Hanoi found that most consumers were aware of food safety risks but did not fear foodborne diseases greatly due to trust in their careful purchase and preparation of food [41]. Yet, on average from 2007 to 2015, there were 176 outbreaks and around 5,590 cases of foodborne disease reported per year in Vietnam [139]. There is a need for understanding the perceptions of pork value chain actors, especially those closer to the start of the chain, such as slaughterhouse workers, who have a greater role in ensuring food safety [99].

Given the growing concern over pork safety, the important role of smallholder value chain actors in ensuring pork safety, and the lack of understanding of food safety perceptions, the objectives of this study were to describe food safety practices among smallholder value chain actors and explore the food safety, disease, and health risk perceptions of pork value chain actors using Hung Yen Province, Vietnam (situated 60 km south of Hanoi), as a case study. A better understanding of smallholder pork value chain actors' knowledge, practices, and perceptions of food safety will inform risk communication materials and risk management strategies, leading to a reduction in pork-related foodborne diseases in Vietnam and internationally.

2.2. Materials and methods

2.2.1. Study site

This study was conducted in three districts (Van Lam, Van Giang, and My Hao) in Hung Yen Province, Vietnam (Figure 2). Hung Yen was selected because it is a peri-urban area with many livestock agriculture activities and it is in close proximity to and a major pork supplier of Hanoi, the capital city of Vietnam, where the estimated average daily pork consumption is 400 tons. As such, Hung Yen is an important area for research on pork value chain actors.

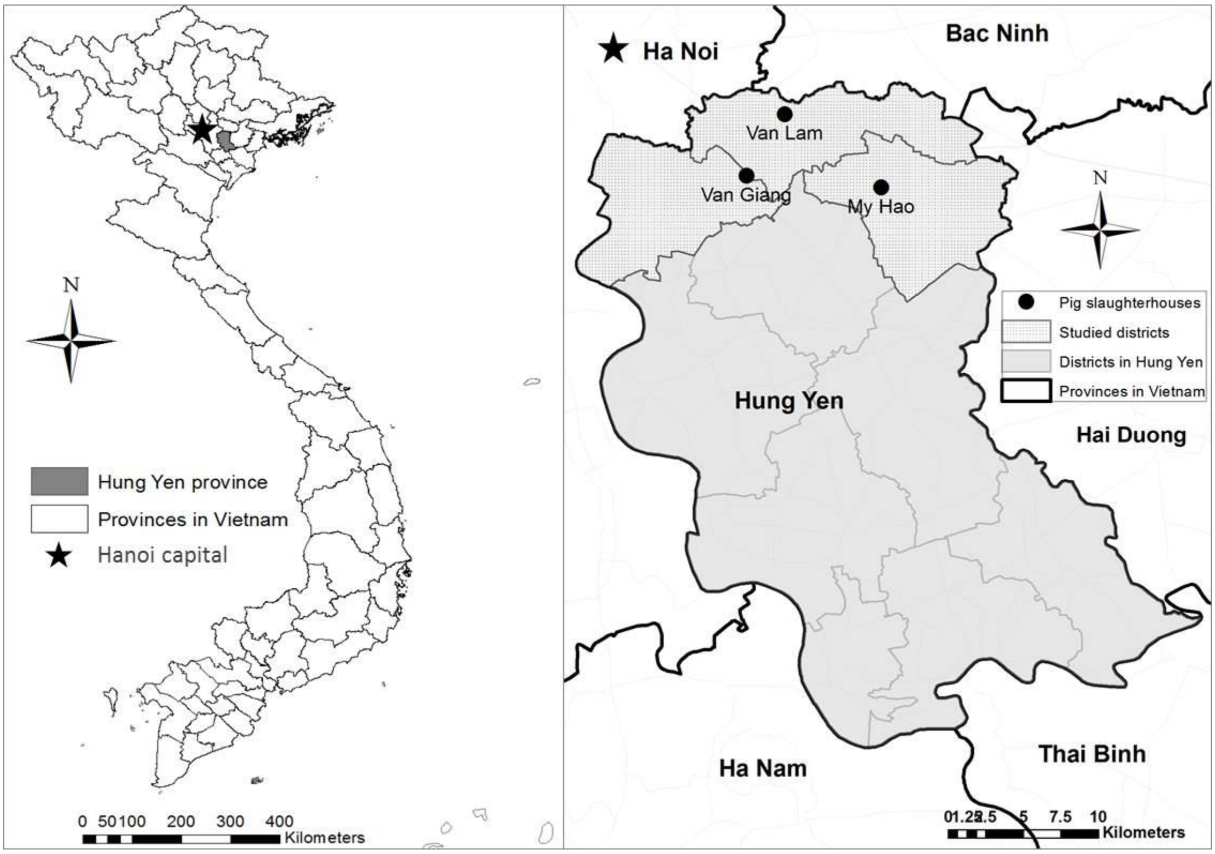

Figure 2. Study location, consisting of three selected districts and slaughterhouses in Hung Yen Province, Vietnam

2.2.3. Data collection

Both quantitative (questionnaire or checklist) and qualitative methods (interview or focus group) were used to gather information on the knowledge, perceptions and practices of selected pork value chain actors with regard to food safety during the period of January to June 2013. An overview of the value chain actors involved, data collection tools used, and types of data collected is provided in Table 1. Two teams of researchers were trained on how to use the questionnaire and checklist, as well as on conducting interviews and focus group discussions (FGDs), before the field work started. The Vietnamese language was used for data collection, and notes and transcripts were later translated into English for analysis.

A structured questionnaire was used to gather basic information on slaughterhouses, slaughtering process, and procurement of pigs from slaughterhouse owners. The questionnaire was developed in Vietnamese and pretested in villages close to Hung Yen. After revision, the questionnaire was administered face-to-face by trained, experienced research assistants. Each interview lasted approximately 30 minutes. Furthermore, the hygienic practices of slaughterhouse workers and pork sellers were observed, and risks for microbial contamination were identified. Observational checklists based on sanitation guidelines (for slaughterhouse workers, circular no. 60/2010/TT-BNNPTNT [90], and for food handlers, circular no. 15/2012/TT-BYT [95]) were used to determine if slaughterhouse workers and pork sellers were operating according to food safety guidelines.

Key informant interviews were also conducted with three public health staff and three veterinary staff (one in each of the three districts) to determine the general responsibilities of staff for food safety management. Consumers were interviewed to determine perceptions of pork-borne diseases in pork quality and safety, while community members living around slaughterhouses were interviewed to determine perceptions surrounding slaughterhouses in general. Each interview was conducted face-to-face and lasted about 30 to 45 minutes. The interviewer and assistant took notes and recorded the conversation.

FGDs were conducted with slaughterhouse workers (2 groups) and pork sellers (3 groups), focusing on food safety in general. About five or six participants formed a group and were led by one facilitator with a note taker to capture the discussion. Participants reflected on perceptions and practices of food safety and ranked potential risk factors for contamination of pork. Each discussion was audio recorded after gaining permission from all participants, and the discussions lasted about 1.5 hours each. FGDs were conducted in each district (with the exception of slaughterhouse workers in My Hao district).

Table 1. Number of participants, data collection methods, and key topics explored surrounding food safety for each group of pork production chain actors

Pork value chain actor	**Number of participant**	**Data collection method**	**Type of data gathered**
Slaughterhouse owner	3	Questionnaire	General information on pig procurement and slaughtering process
Slaughterhouse workers	10 (in two groups)	Focus group discussion	Perception of pig diseases, food safety, and food safety practices
Community members living around slaughterhouse	9	Key informant interview	Advantages and disadvantages of slaughterhouses in area
Pork sellers	15 (in three groups)	Focus group discussion	Perception of pig diseases, food safety, and food safety practices
Consumers	9	Key informant interview	Criteria for selecting pork, perceptions on pork-borne diseases, and food safety
Veterinary staff	3	Key informant interview	Responsibilities, food safety management, and collaborations
Public health staff	3	Key informant interview	Responsibilities, food safety management, and collaborations

2.2.4. Data management and analysis

Qualitative data were carefully noted and/or tape recorded. The data were coded into topics as the research progressed. Towards the end of the fieldwork, the main themes were formed and analyzed in depth. Descriptive statistical analysis was used to describe demographic information of participants and general processes along the pork value chain. We triangulated all data to check for consistency.

2.3. Results

2.3.1. Characteristics of participants

A total of 52 participants were engaged in this study; most were between 40 and 60 years of age (63%). Most had completed education up to high school only (54%). Public health and veterinary staff, along with some consumers, were the most educated (many had completed some college or university), while most sellers and slaughterhouse workers were the least educated (had completed only secondary school or lower). Overall, there was a nearly equal gender balance (54% male and 46% female); however, more men worked in slaughterhouses, whereas more women were sellers. General demographic information about the participants in the three districts studied is provided in Table 2.

Table 2. Demographic information, including education, age, and gender, of pork value chain actors participating in this study

Demongraphic Information	**No. of participants among:**							**Total (%)**
	SH owner (n=3)	**SH worker (n=10)**	**Community members living around SH (n=9)**	**Pork seller (n=15)**	**Consumer (n=9)**	**Veterinary staff (n=3)**	**PH staff (n=3)**	
Education								
Secondary school	3	7	5	12	1			*28 (54)*
High school		2	4	3	2			*11 (21)*
College		1			4		1	*6 (12)*
University or higher					2	3	2	*7 (13)*
Gender								
Male	3	9	6	3	3	3	1	*28 (54)*
Female		1	3	12	6		2	*24 (46)*
Age (yr)								
18-30	1	2	1			1		*5 (10)*
31-40	2	4	1	3	1	2		*13 (25)*
41-50		1	4	6	3		1	*15 (29)*
51-60		3	3	6	4		2	*18 (34)*
61-70					1			*1 (2)*

SH: Slaughterhouse; PH: Public health

2.3.2. Pig slaughterhouses and pork markets

All three slaughterhouses studied bought pigs from one farm at a time, which were then slaughtered over the following days before pigs from other farms were bought and introduced. Some private butchers also came to these slaughterhouses to buy a live pig(s) and slaughter them using the workers there. The number of pigs slaughtered ranged from 10 to 40 pigs per day, and generally male workers (from 4 to 6) worked in the slaughterhouses. Slaughterhouses operated mostly in the early morning from 2 a.m. till 6 a.m., and slaughtering and processing were done on the floor with limited separation areas for bleeding, scalding, and dehairing. No abnormal clinical signs (e.g., thin or visibly sick pigs) were observed in the lairage at any time during the slaughterhouse visits. With regard to hygienic measures, one slaughterhouse had a separate entrance for pigs, but in all slaughterhouses selected, people could freely access the slaughter area. Workers frequently wore boots, but wearing uniforms or aprons were not observed.

Pork markets opened daily from around 5 a.m. to 11 a.m. The amount of pork sold at pork shops varied from 20 to 300 kg daily. Those pork shops were retail (around, e.g., 20 to 80 kg) or wholesale (e.g., 80 to 300 kg) or both retail and wholesale. Approximately half the sellers transported the carcass or pork themselves, mostly by motorbike. None of the sellers stored the pork in cooled cabinets or covered the pork. Most of the sellers did not use gloves to handle pork, but they always wore aprons. During selling, all pork sellers used cloths to wipe and clean the meat, table, or equipment but also used their bare hands to handle pork and equipment.

2.3.3. Food safety practices of value chain actors

According to slaughterhouse workers, there are no specific regulations or standard operating procedures in the slaughterhouse. However, there are ‘‘informal rules,’’ where senior workers show juniors how to operate in the slaughterhouse, and the work becomes a habit and a routine within the group. Workers have a shared understanding of hygiene and try to maintain cleanliness and coordinate the slaughter process in an organized way; as one worker stated, ‘‘both slaughterhouse workers and slaughterhouse owners need to have an awareness of maintaining cleanliness and observing hygiene during slaughtering. There are no regulations or punishment, we just remind each other when one did not do something properly.’’ Workers reported wearing gloves and masks to protect their health and prevent contamination.

Most pork sellers prefer to use wood surface tables, even if the government had helped them to build tables with enamel tiles, steel, or a granite surface. Pork sellers explained that wood is easier to clean than other surfaces and that ‘‘wood table surface can help pork stay dry and keep pork fresher’’ by absorbing water. Sellers mentioned the use of cloths to dry pork, clean equipment, and clean hands and tables, emphasizing ‘‘it is necessary to have dried cloth to wipe pork and table to avoid wetness, so the pork will be less pale and rancid.’’ For personal protective equipment, sellers reported wearing aprons and sometimes thin gloves but rarely used masks or protective hats. Masks or protective hats were avoided because they thought that consumers would think that sellers who covered their face or head had health problems that they were trying to conceal.

Retailers said that if pork was left over, they would sell it to restaurants or canteens at a relatively lower price or process it into products such as nem chao (boiled pork skin with roasted rice powder) and gio thu (mixed pork and ham) and sell them to consumers. For consumers, the most important criteria for selecting pork were ‘‘bright red, soft and sticky,’’ followed by ‘‘fresh looking and good smell.’’ Consumers also emphasized trust in pork sellers and cleanness of seller stalls as factors that strongly influenced their pork purchasing. In contrast, price and accessibility were less important.

All three public health officers interviewed stated that their responsibilities were for ‘‘cooked food,’’ while raw meat was under the veterinary authorities’ responsibilities. Indeed, public health officers inspect finished pork products, whereas veterinary staff inspect raw pork products. Public health officers are responsible for compliance with regulations on management of foodborne diseases and zoonoses, including inspecting food centers, restaurants, food processing shops and plants for compliance with regulations and guidelines on food safety, ensuring food handlers have health certification, and training food handlers and processors on food safety and hygienic practice.

Veterinary staff mentioned a gap between existing legislation and inspection practices for pork safety surrounding transportation, slaughterhouses, markets, and raw meat handling and processing. Inspection legislation mainly applied to the big or medium slaughterhouses or markets, whereas small or private butchers or retailers were not frequently inspected. At the slaughterhouse, one veterinary staff member reflected, ‘‘it cannot be 100% guaranteed that all pigs were inspected at the slaughterhouse, 80 to 90% is a good number. The government still has difficulties in taking care of this duty.’’

2.3.4. Food safety risk perceptions

During FGDs with slaughterhouse workers, risks for microbial contamination of pig carcasses were discussed and ranked in terms of risk level. Although there were varying responses between the two FGDs, both groups emphasized that feces on skin of live pigs, punctured intestines, and the water source were likely sources of contamination. In contrast, using cloths for dry wiping carcasses and transporting carcasses were ranked as not a source of contamination. One worker explained that cloth is safe ‘‘because everyone has to wash it and keep it clean every day. So there is no problem. After selling and working all day, they wash and dry it for the following day.’’ Slaughterhouse workers also perceived that swine infectious diseases impaired pork quality and safety, including foot and mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS), liver flukes, helminths, and pig diarrhea. FMD and PRRS were emphasized as main causes of poor pork quality and safety. Some of the slaughterhouse workers perceived cysticercosis and leptospirosis as uncommon; as one worker explained, ‘‘there have been cases of leptospirosis or cysticercosis, but this was observed a long time ago (4 to 5 years). Now these are fewer than before and every year there may be only 1 to 2 cases reported.’’ In general, workers perceived that pig diseases are more important to food safety than zoonotic diseases.

Pork sellers have some familiarity with the slaughtering process (e.g., from observing the process or having worked in a slaughterhouse previously), and all pork sellers mentioned that pork quality was strongly related to the slaughtered pig’s condition and the manner of slaughtering. One seller explained, ‘‘when restraining pigs for slaughtering, if we struggle with the pig for a long time, the pork would not have a good quality.’’ Sellers mentioned non-zoonotic pig diseases affecting pork quality and safety, including cysticercosis and leptospirosis (both zoonoses), PRRS and FMD, classical swine fever, and pasteurellosis. Sellers did not perceive zoonotic diseases as a major food safety concern. During FGDs, sellers ranked potential risk factors that may lead to pork contamination. Although there were some differences among the FGDs, in general, uncleanliness of table or surface, wastewater drain next to the shop, and uncleanliness of the surrounding shop area were ranked as high risk factors. Both groups ranked the clothes and shoes of sellers as low risk factors for microbial contamination in pork.

Consumers assumed that less-safe pork may originate from sick or dead pigs, explaining that less-safe pork ‘‘is less fresh, has a bad smell, rancid,’’ and the pork ‘‘is pale, has a strange smell, and has a wet feel when touched.’’ The majority of consumers mentioned at least one pig disease affecting pork safety and quality, such as cysticercosis, streptococcal infection, or pig diarrhea. One consumer explained ‘‘cysticercosis causes taenia disease in human due to eating infected cysticercosis pork. For prevention, when buying, check the pork, it should not have dots that look like white rice seed.’’ Another consumer explained “Lien cau khuan” (in Vietnamese; the name for *Streptococcus suis*) can cause illness via eating. To prevent, do not eat raw pork, blood, or not-well-cooked internal organs, and do not touch raw pork if you have a scratch on your skin or hand.’’

Community members living around slaughterhouses emphasized some advantages to having a slaughterhouse near their residence, such as providing jobs, creating business opportunities, and the convenience of buying fresh pork nearby. Community members explained ‘‘Slaughterhouse here provides pigs and pork sources for butchers and pork sellers, no need to go far. Slaughterhouse creates work for some workers.’’ Some disadvantages, such as noise, were reported; however, all respondents mentioned that they have become accustomed to the presence of the slaughterhouse and so the noise is not much of a disturbance. One community member remarked, ‘‘no disadvantage, do not know since I sleep deeply, the environment around is normal. The noise is negligible.’’ A few community members said that the presence of the slaughterhouse can result in odor, polluted water, and the spread of animal diseases. Most community members mentioned potential impacts on human health, for example, ‘‘sometimes in summer when the weather is hot and humid, the smell might spread and then get inhaled, or heavy rain could stagnate the dirty water, which may cause itchiness on people’s hands and feet.’’

The participants mentioned that information about pig disease and pork-borne diseases came from mass media, such as newspapers, the internet, local radio, or television. Veterinary or public health sources were not mentioned as sources of information. Some slaughterhouse workers gained knowledge about food safety or hygienic practices from following their fellow workers’ work habits, or ‘‘learning by doing.’’ Other slaughterhouse workers had attended some training programs on food safety which were organized by the province.

2.3.5. Diarrheal illness

Slaughterhouse workers and pork sellers reported no cases of illness or diarrhea among themselves over the last six months and were also not too worried about diseases because they perceived that the pork production process was safe and control measures were applied. In addition, workers reported wearing masks, gloves, and boots to protect their health and to limit risks in case of suspected diseases in pigs. However, based on the researchers’ own observations, the workers did not wear any masks or gloves during slaughter. For pork sellers, neither they nor their family members reported being affected with pig-related diseases or had symptoms after consuming pork within six months or even one year in the past. Consumers also stated that no cases of illness or diarrhea were observed from eating pork or pork products in their family within the last 12 months; however, one consumer mentioned that her 3-year-old daughter got diarrhea once after pork consumption, but was unsure about the cause.

2.4. Discussion

Our study captured valuable insights into practices and perceptions of pork safety and quality among smallholder pork value chain actors and consumers. Subtle but important differences in perceptions of pig diseases and food safety among smallholder pork value chain actors were noted. For instance, slaughterhouse owners knew more about pig diseases affecting food safety and quality than pork sellers and consumers, perhaps because they had more frequent and direct encounters with pigs and were motivated to learn more. However, there were considerable misperceptions surrounding zoonotic and foodborne disease among these three groups. This study also demonstrated that slaughterhouse workers and pork sellers knew about some food safety risks associated with their pork handling practices but might not be aware of the degree of risk. For instance, slaughterhouse workers often use a clean cloth to dry pig carcasses. Workers assumed there was little risk for microbial contamination of carcasses as they reported washing the cloth every day, despite the possibility for contamination of the cloth through drying multiple carcasses throughout the day. Incorporating value chain actor's perceptions surrounding safe food safety practices can better inform education and communication intervention efforts to improve food safety [119].

Several practices to prevent contamination of pig carcasses, such as avoiding puncturing the gut and washing the pig and carcass thoroughly, were observed at the slaughterhouse. Indeed, pig skin can have high rates of *Salmonella* [133], and efforts appear to be made to prevent contamination; however, the same cloth is often used to dry multiple carcasses during the day, which can be problematic if the cloth is contaminated with bacteria. Workers also stated that they used gloves and masks to protect their health and limit contamination, but these practices were not observed during visits. Our simultaneous study on *Salmonella* contamination (data not presented) showed that there is a high chance of finding *Salmonella* on workers' hands. Encouragement of the use of protective equipment such as gloves to prevent carcass contamination is recommended. Slaughterhouse workers stated that they ‘‘learn by doing’’ and follow informal rules of the slaughterhouse rather than getting trained by relevant authorities. Furthermore, the source of food safety information for slaughterhouse workers was mainly through mass media, as opposed to public health or veterinary services. And yet, training can be and is provided by public health and veterinary staff. Based on the food safety law of 2010 (No. 55/2010/QH12), a national strategy for food safety from 2011 to 2020 has been approved by the Prime Minister, with the objectives, among others, to improve the knowledge and practice of food safety for different groups and strengthen the management system for food safety [140, 141]. We suggest the organization of further training and incorporating the needs and perceptions of slaughterhouse workers in training materials to ensure that good slaughterhouse hygiene is recommended [15].

Vietnamese food safety regulations emphasize the importance of good hygienic practices through regulations and guidelines (including hazard analysis and critical control point principles); however, at small- and medium-scale pig slaughterhouses, regulations or guidelines are often not applied. For example, according to the Vietnamese regulations, slaughterhouses have to be separated from residential areas (at least 100 m) and have to use appropriate waste treatment systems [87]. All three slaughterhouses used biogas systems for slaughterhouse waste management. However, due to the limited land area and the initial start point from a traditional household-based slaughterhouse, all three slaughterhouses were not able to follow the regulation surrounding residential distance. Furthermore, limited financial capacity and land may constrain building a new and separate slaughterhouse in another location. Interestingly, however, some community members living around slaughterhouses seemed to be accustomed to the slaughterhouse presence and to feel minimally or not at all disturbed. On the other hand, some veterinary staff, public health staff, and community members expressed interest for slaughterhouses to be relocated to more suitable areas and follow existing requirements and regulations.

Another concern raised by veterinary and public health staff was the limited capacity for inspecting slaughterhouses and markets due to lack of human resources. Inspections are mainly implemented for large- or medium-scale slaughterhouses (more than 10 to 20 pigs per day), and are not frequent for small-scale slaughterhouses, private butchers, or retailers. There is a need to strengthen the capacity for inspections [83].

Our findings demonstrated that pork sellers use wooden tables because of the perception that wood makes meat look fresh for longer periods of time than do other surface types; the preference for a fresh appearance is driven by consumer demand. The seller groups preferred a wooden table surface over other surfaces, such as enamel, granite, or steel. They explained that the water absorption capacity of wood seems better than that of enamel, granite, or steel, so it makes pork look drier and less rancid. However, in terms of food safety, the wood surface is more prone to bacterial contamination. For example, when comparing materials to reduce *E. coli* contamination (e.g., laminate, wood, tile, and granite), granite has been shown to perform the best in reducing *E. coli* contamination, with less of the bacteria remaining after simply cleaning with soap, while wood performed quite poorly [115]. Finding a suitable material that is affordable, prevents contamination, and makes the pork appear appealing to consumers is challenging but needed.

Sellers at almost all pork shops used cloths to wipe pork dry or to clean hands or equipment, a practice similar to that of slaughterhouse workers. The cloths may be possible carriers of contaminants, and yet, they are perceived to be low risk by the sellers. This misperception should be specifically addressed in future training activities. In addition, the use of masks, gloves, or hats by sellers gave some consumers the impression that the seller may have been concealing health problems (such as skin or respiratory disease), and thus, buyers are hesitant to buy pork from them. Sellers also mentioned that pork quality was related to the manner of restraining and slaughtering pigs, which can lead to pale, soft, and exudative meat. Research has shown that pale, soft, and exudative meat has a strong correlation with preslaughter animal handling, stunning, dehairing, and carcass chilling [80]. As such, it appears that sellers are more knowledgeable and concerned about aspects relating to food quality rather than food safety.

The consumer groups focused on sensorial or physical characteristics of pork (perceived freshness, color, smell, or texture) as indicators for pork purchasing behavior, pork quality, and safe pork [50]. In our study, the color and smell of pork were determined to be the most important selection criteria when purchasing pork, while price was least important. For consumers, ‘‘wet’’ looking pork was an indicator for low quality of pork, hence the sellers' practice of continuously drying the meat using cloth. Accessibility of pork was less important for consumer preference, since the mobile shops or vendors sell pork widely at villages or communes. Moreover, trust in sellers, butchers, and the pork production process was also mentioned as an important criterion in selecting pork. And yet, consumers and other value chain actors emphasized trust in their own food safety practices rather than the practices of others along the pork value chain. Integrated efforts, along with traceability along the pork value chain, are recommended.

A cross-cutting synthesis of our findings related to zoonoses revealed that slaughterhouse workers and pork sellers have little knowledge and some misperceptions about zoonoses. Limited knowledge on zoonoses may be due to concern over other issues, such as severe and contagious pig diseases (FMD and PRRS), consistent with a consumer study that found that pig diseases, along with growth promoter residues, were the main concerns [62]. Information on pig diseases is usually related to urgent or reemerging zoonoses, such as avian influenza and *S. suis*. This information was also gathered from discussions and interviews in our study. Participants' mention of zoonotic diseases, including leptospirosis and cysticercosis, could be because they remembered cases of cysticercosis that occurred a few decades ago in Vietnam [138]. And yet, respondents viewed zoonotic diseases as less of concern for food safety than pig diseases. Clarifying the degrees of risk for food safety should be emphasized in education initiatives.

There are some limitations inherent in this study. First, our sample size per research group was small; as such, quantitative analytical methods are not appropriate. Furthermore, our sample came from three districts in Hung Yen and, thus, might not be representative of other regions in Vietnam. However, this study provides important insights into perspectives on food safety among slaughterhouse owners, workers, sellers, consumers, and authorities responsible for pork safety in the area (veterinary staff and public health staff). Second, our study did not explore the food safety perceptions and practices of other actors along the pork value chain (e.g., farmers and traders), who also have a role in ensuring food safety. Future research may consider the roles and perceptions of such actors.

In this study, the food safety practices and perceptions of pork value chain actors were explored. Pig slaughtering practices, along with pork handling practices by sellers, are often performed without using adequate protective equipment. Sellers prefer to use wood tables over other materials to maintain perceived ‘‘freshness’’ of pork, despite the high risk of wood tables for microbial contamination of pork. Both slaughterhouse workers and pork sellers often use the same cloths for cleaning equipment and drying meat, presenting risks for cross-contamination. Misperceptions of slaughterhouse workers and pork sellers surrounding the risks of zoonoses for pork safety were observed. These findings suggest that more education and training interventions to promote appropriate food safety practices are needed. They also suggest that incorporating the perceptions and actual practices of pork value chain actors into the training should be a priority. Furthermore, an emphasis on pig disease, food safety risks, and zoonoses in food safety interventions is warranted. However, training alone is unlikely to change behavior unless there are some additional motivations in place. Finally, integrated efforts among all pork value chain actors, along with traceability in the chain, are needed to ensure pork safety. Future research to substantiate the findings could include examining the risk perceptions and risky practices of pork value chain actors in other communities (in Vietnam and other countries), exploring interventions to improve risk perceptions and risk communication resulting in reducing risky practices, or determining those for other meat categories, e.g., chicken or beef.

2.5. Summary of Chapter 2

Pork safety is an important public health concern in Vietnam and is a shared responsibility among many actors along the pork value chain. We examined the knowledge, perceptions, and practices regarding food safety, disease, and health risk among select pork value chain actors (slaughterhouse owners and workers, people living around slaughterhouses, pork sellers, consumers, and veterinary and public health staff) in three districts in Hung Yen Province, Vietnam. We randomly selected 52 pork value chain actors to be surveyed through questionnaires, observation checklists, key informant interviews, and focus group discussions. Most slaughterhouse workers acquired knowledge and experience of food safety through ‘‘learning by doing’’ rather than from training by a veterinary or public health professional. Both slaughterhouse worker and pork seller groups had some accurate perceptions about pig diseases and foodborne diseases; however, misperceptions of risk and, especially, of zoonoses were present. Furthermore, while workers and sellers often use cloths to dry the meat and clean equipment, they did not think this was a risk for meat contamination. Moreover, when sellers wear protective equipment, such as gloves, masks, or hats, consumers perceive that the sellers may have health issues they are trying to conceal and so consumers avoid buying from them. The perceived freshness of pork, along with trust in the seller and in the pork production process, were strong indicators of consumer preference. And yet, pork value chain actors tend to trust their own individual food safety practices more, rather than the practices of other actors along the chain. Veterinary and public health staff emphasized the gap between regulations and food safety practices. Education and training on food safety risks and proper handling are priorities, along with integrated and intensive efforts to improve food safety among pork value chain actors.

Chapter 3. Risk factors associated with *Salmonella* spp. prevalence along smallholder pork value chains in Vietnam

3.1. Introduction

Salmonella is an important foodborne pathogen worldwide. There are an estimated 22.8 million human salmonellosis cases in the South East Asia region each year [84], approximately 40,000 cases in 2009 in the United States [20] and 94,000 cases in the European Union in 2015 [38]. Pork has been implicated as one of the most important sources of *Salmonella* (together with egg and poultry) in several countries [29, 37, 55, 105]. The estimated annual cost of human *Salmonella* infections from all sources was about € 608 million in the European Union [40] and about $3.4 billion in the US [131]. This economic burden of *Salmonella* infection is significant in both developing and developed countries implying the need for enhanced monitoring and reporting systems, improved food safety and greater consumer awareness [112]. However, intervention programs to control *Salmonella* in pork production are costly, requiring investment in biosecurity facilities and trainings on hygiene practices in farms, slaughterhouses, processing plants, and retail outlets. Therefore, understanding risk factors greatly helps targeting effective intervention points and reducing these costs.

Salmonella prevalence and related risk factors in the pork value chain have been well characterized in the United States, Australia, and Canada, as well as European Union countries. *Salmonella* contamination of finished carcasses can be linked to farm level *Salmonella* infection in pigs destined for slaughter [10]. At the slaughterhouse, cross-contamination has been shown to significantly affect the occurrence of *Salmonella* on pig carcasses [36]. At distribution level, *Salmonella* contamination has been found to be related to the type of retail outlet [54]. As a result of contamination of pork, it has been estimated that

15-20% of *Salmonella* infections in humans were caused by consumption of contaminated pork or processed pork in the Netherlands and Germany [10, 117].

Pork consumption in Vietnam is relatively high compared to other countries with similar GDP (29.1 kg pork per capita yearly) [101]; most pork (80%) is produced by small-scale producers and sold by small-scale retailers [78]. Recent studies in the Mekong Delta showed poor hygiene in small-scale pig farms: 8.2% of drinking water for pigs sourced from local rivers or ponds was contaminated with *Salmonella* [128]. Pig abattoirs in Hanoi processing 10-30 pigs/day, had *Salmonella* prevalences of 52.1%, 62.5%, and 95.7% for caecal content, tank water, and carcass swab samples, respectively [79]. In Hue province in central Vietnam, similar results were found after sampling various surfaces in slaughterhouses, such as floors (47.4%), weighing bowls (38.1%), and cooking boards (28.6%) [120]. As regards retail, most pork is sold in wet informal markets - the open-air markets which can be categorised as central markets, village markets, and roadside vendors, with as many as 20 pork stalls or as few as 1-2 in Vietnam [28]. Studies in northern Vietnam found prevalences of *Salmonella* in wet market pork of 39.6% [123] and 25% [153], and 69.9% in southern Vietnam [104]. These studies illustrate that *Salmonella* prevalence varies widely in different settings along the pig value chain, but is generally high in the Vietnamese pork value chain.

This study was conducted as a part of the project, entitled “Reducing disease risks and improving food safety in smallholder pig value chains in Vietnam (PigRISK)”, aiming to assess impacts of pork-borne diseases on human health and to understand best-bet risk management. The aim of this study was to investigate the prevalence of, and risk factors for, *Salmonella* contamination along the smallholder pork value chain in northern Vietnam.

3.2. Materials and methods

3.2.1. Study sites and target population

The study designs comprised a repeated cross-sectional for the farm and pork shops, and a longitudinal for slaughterhouses. The study was carried out in Hung Yen and Nghe An provinces between April 2014 and February 2015. Three districts were selected from each province to represent different value chain pathways: rural to rural, rural to peri-urban, and peri-urban to urban, according to a set of criteria developed by the PigRisk project which had identified these types of value chains as different domains for analysis and intervention [67]. Three communes were randomly selected from each of these selected districts, making in total 18 communes (nine out of 161 communes in Hung Yen, and nine out of 469 in Nghe An). Hung Yen province is located northeast of the Red River Delta and Nghe An province is in the northwest of central Vietnam (Figure 3). The scope of the research was the smallholder pork value chain (i.e., pig farm, slaughterhouse, and market), and slaughtering process, illustrated in Figure 4. Therefore, farms, slaughterhouses, and markets in this study were selected to represent both small- to medium-scale farms (i.e., $\leq$10, 11-100 pigs), small- to medium-scale slaughterhouses (i.e., 1-10 pigs/day, 11-50 pigs/day) and wet markets [28].

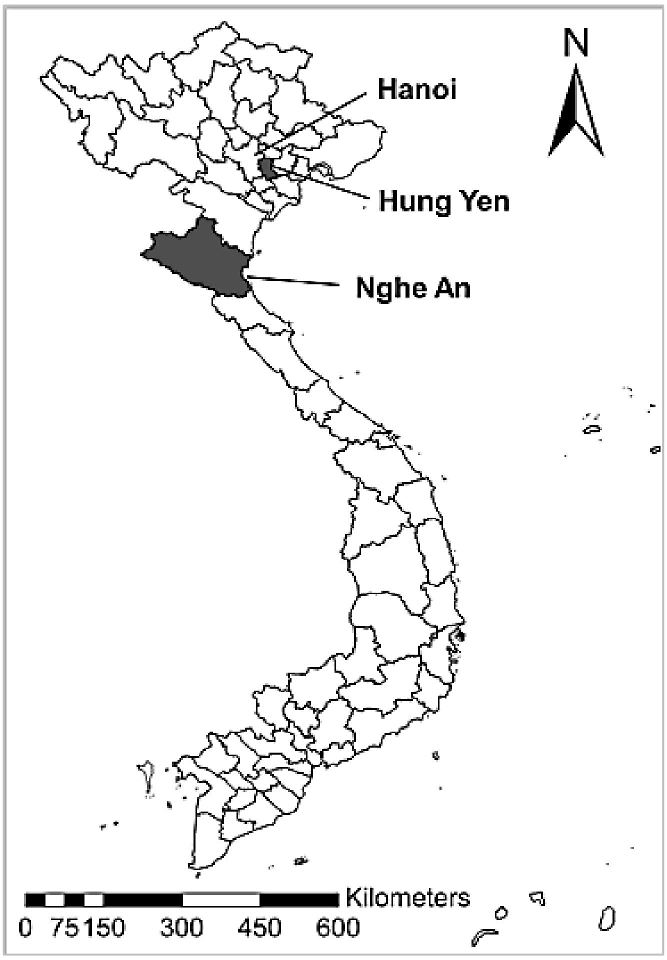

Figure 3. Location of the two studied, Hung Yen and Nghe An, provinces in Vietnam

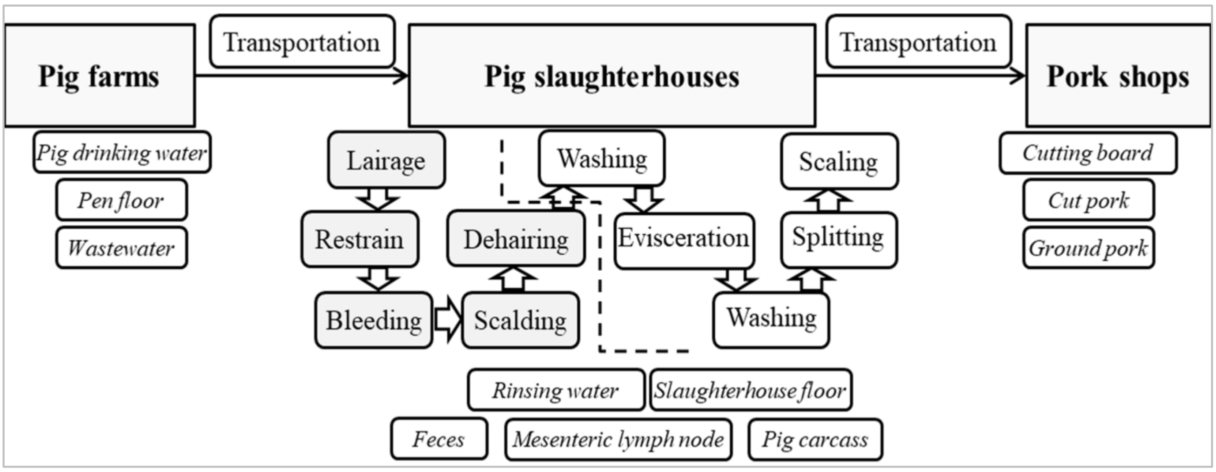

Figure 4. The smallholder pork value chain (i.e., pig farm, slaughterhouse, and market) and slaughtering process (dashed line illustrates areas between dirty and clean zones)

3.2.2. Study design

Sampling size

Sample sizes were based on a comparison of two proportions with precision minimal detectable difference of 10% at a confidence level of 95% and power of 80%. Considering potential medium level of confounding for multivariable analysis, the calculated sample size was increased by 20% [33].

For the sample size at the farm level, the expected *Salmonella* prevalence on the pig pen floor was set as 25%, which was around the middle of reported prevalences (8.2% [128] and 49.4% [144]), and the difference in prevalence between exposed and non-exposed groups to detect was set as 15%. The expected *Salmonella* prevalences on slaughtered pig carcass and retailed cut pork were determined to be 34.9% [26] and 32.8% [120], respectively as previously described and the difference of prevalences between exposed and non-exposed groups to detect was set as 10% both for carcass and cut pork. Conseqently, the minimum required sample sizes were 60 farms, 146 pig carcasses, and 143 pork shops.

Power calculations

This study was part of a larger multi-faceted research program and the sample size available for this study was dictated by other needs of the program and resource constraints. As such, it was only feasible to collect data from 72 farms, 149 carcasses from 13 abbatoirs and 217 pork samples from 145 shops. We used the following assumptions to estimate the power of each phase (farm, slaughterhouse and shop) of the study: alpha=0.1; prevalence in unexposed units (e.g., farm) was 30%, intra-cluster correlation coefficient for samples from the same slaughterhouse or shop (across all visits) was 0.1 and the exposures of interest were present in approximately 50% of the population (i.e., equal split between exposed and no-exposed). With these assumptions, the farm and slaughterhouse (carcass swabs) phases of the study had approximately 80% power to detect effects with an odds ratio (OR) of 3.5 or greater. For pork samples, the minimum OR for 80% power was 2.2 (details of calculations available on request).

Sampling framework

Sampling was performed in four periods over a year (2014 April-June, July-September, October-November, and December-2015 February), collecting from farms and wet markets in all 18 communes, and from slaughterhouses in 13 communes. No samples were collected in March. During each sampling visit, researchers visited the slaughterhouses from 2 a.m. to 6 a.m., the market from 6.30 a.m. to 9 a.m., and the farms between 9 a.m. and 11 a.m.

For farm, one purposively selected farm was visited in each commune, and thus 18 farms were sampled in each sampling period, for a total of 72 over the year of study. The farms were selected by local veterinarians based on the scale criteria of this study as mentioned above. At every farm visit, one sample each from pig drinking water source, pen floor, as well as farm wastewater was aseptically collected. Different farms were selected at each sampling period, and no farm was sampled twice.

Up to two slaughterhouses were visited per district during a sampling period (except for the initial visit when maximum three were visited), so in total 13 slaughterhouses were visited, of which seven were in Hung Yen and six in Nghe An. Samples were collected from mesenteric lymph nodes (MLN), faeces, pig carcass of the same pigs, the slaughterhouse splitting floor, and carcass rinse water. These slaughterhouses were repeatedly visited (four times) during the study period.

For markets, nine pork shops were purposively selected in each district in each sampling period, except for one district in the third period when ten shops were collected. At each pork shop, one piece of cut pork and a cutting board surface swab were sampled. In addition, one sample of ground pork was collected from shops equipped with pork grinders. During the year of study, because of the limited number of pork shops in selected communes, forty-one shops were visited more than once. In addition, to understand the attribution of contamination of finished carcasses at slaughterhouse to the contamination status of cut pork at market, a sub-set with 63 carcasses was traced to sample both these carcasses and their cut pork.

Sampling methods

At each farm, four 25 cm^2 representative positions of pen floor were swabbed for a total sample surface area of 100 cm^2 using sterile pre-moistened gauze (with Buffered Peptone Water, BPW; Merck-Germany), forceps and a 5 x 5 cm steel frame. One litter of drinking water for pigs and 0.1 litter of wastewater (from pig pens wastewater tank or biogas effluent tank) samples were also collected aseptically into sterile bottles.

In slaughterhouses, immediately after evisceration, a rectal faecal sample (approximately 50 grams) was collected using sterile forceps and a wooden stick, and a MLN sample (approximately 30 grams) using sterile forceps and scalpel. Immediately after the final washing step, each split carcass was systematically swabbed at four positions (4 x 100 cm^2) along the medial carcass surface (i.e., lower part of neck, mid-back, abdomen, hind limb), using the non-destructive technique described above, for a total sample surface area of 400 cm^2 [71]. At the middle of the slaughtering operation, a 25 cm^2 section of slaughterhouse floor where carcass splitting was carried out was swabbed, and one litter of carcass rinsing water was collected using the same procedures used on farms (described above).

At the market, approximately 400 grams of cut pork and/or ground pork were purchased. These samples were collected by the shop owners using their own equipment and transferred into sterile plastic bags provided. A 25 cm^2 section of the cutting board surface in the pork shop was swabbed using the same procedure as the pen floor (described above).

All surface samples were placed in sterile bags containing approximately 20 ml BPW. All collected samples were stored in an insulated container with ice packs and transported to the laboratory for analysis within 10 hours. The laboratory tests were performed at the National Institute of Veterinary Research, Hanoi, Vietnam.

3.2.3. *Salmonella* microbiological analysis

All surface samples were diluted in the original sterile plastic bags with BPW up to 100 ml. Ten ml of farm wastewater were pipetted into a sterile plastic bag containing 90 ml BPW. A 100 ml aliquot of the pig drinking water or carcass rinse water was filtered through a membrane (0.45 µm pore size; Millipore, USA), and each membrane placed in a sterile plastic bag containing 100 ml BPW. Ten grams of rectal faeces or MLN were added to 90 ml BPW in a sterile plastic bag. A 25 grams portion of cut or ground pork was added to 225 ml BPW in a sterile plastic bag.

Salmonella isolation followed the ISO procedure [70]. The BPW homogenate was incubated for 16-20 hours at 37 °C as a pre-enrichment step prior to inoculation of selective media. Muller Kauffmann Tetrathionate (TT; Merck, Germany) broth was inoculated with a 1ml aliquot, and Modified Semisolid Rappaport-Vassiliadis (MSRV; Merck, Germany) agar plate was inoculated with three pipette drops (approximately 50 µl). Both were incubated for 16-20 hours at 37 °C. This selection step was repeated, using one loop (approximately 10 µl)

TT and MSRV, to inoculate Xylose Lysine Tergitol 4 (XLT4; Merck, Germany) and Ramback (Merck, Germany) agar plate selective media. One to two typical *Salmonella* colonies per plate were used to biochemically confirm *Salmonella* (e.g., Lactose, Indol, Lysine, H_2S, Urease) and another one to two colonies to inoculate nutrition agar (NA; Merck, Germany) to grow *Salmonella* for the final serological confirmation, using Antiserum *Salmonella* Polyvalent-O (Bio-Rad, France). *Salmonella* enumeration was done only for the pork (cut and ground) samples using a 3-tube MPN (Most Probable Number) method [103], and the calculation table was used to determine the MPN [31].

3.2.4. Data collection

Observation checklists were used to collect information on management, facilities, equipment and hygienic practices at farm, slaughterhouse and market (Tables 5-7). Information on live pig management during transportation to slaughterhouse was obtained from a questionnaire. The observational checklists were based on the Vietnamese sanitation guidelines for farmers and slaughterhouse workers (Circular No. 60/2010/TT-BNNPTNT), and food handlers (Circular No. 15/2012/TT-BYT), and were used to determine if farmers, slaughterhouse workers, and pork sellers were operating according to requirements. Checklists and questionnaires were developed in the Vietnamese language and pre-tested in Hung Yen province. Checklist data came from direct observation on farm, in slaughterhouses and market operations by experienced researchers, whereas the questionnaire was administered face-to-face with slaughterhouse owners during each sampling visit.

3.2.5. Data management and statistical analysis

Checklist and questionnaire data and laboratory results were recorded and processed in Microsoft Excel 2010 spreadsheets. Data from checklists and questionnaire was screened to eliminate the variables which were considered as redundancy or low variation. Descriptive statistics were performed and statistical computing was interpreted with *p*-value of 0.1. Statistical software R version 3.3.1 [110], was used for data analysis. For the characterization of pig farming and slaughterhouses in Hung Yen and Nghe An, Chi-squared tests were used to compare general information (e.g., the proportions of farms keeping cross breed pigs), and Wilcoxon Rank Sum tests were performed to compare the average weight of live pigs and the number of pigs slaughtered between two provinces.

Univariable analysis was used to investigate the relationship between *Salmonella* positive samples and management, facilities and practices at the farm, slaughterhouse, and market levels using generalized linear mixed-effects models (GLMMs) in lme4 package [9] in R. For the farm level model, the primary outcome variable of interest was *Salmonella* contamination status (positive or negative) on the pen floor, and commune was set as random effect. For the slaughterhouse level, *Salmonella* status on the finished carcass was used as the outcome variable, and sampling visit and identification of slaughterhouses were set as random effects. For the market level, *Salmonella* contamination status on cut pork samples was used as outcome variable and commune was set as random effect for the analysis. The explanatory variables were extracted from the questionnaires and/or observation checklist data.

In multivariable analyses, causal diagrams (http://www.dagitty.net/dags.html) were used to identify exposure variables of interest, intervening variables, as well as potential confounders related to the outcome variables of interest (*Salmonella* contamination status on pig pen floor, finished carcass at a slaughterhouse, and cut pork at shop, Figure 5). Season and scale of farm, slaughterhouse or shop were considered as confounders. Intervening variables were other *Salmonella* contamination measures (e.g., *Salmonella* in drinking or rinsing water) as indicated in the diagrams. These were excluded from all multivariable models. Separate GLMMs were prepared for farm, slaughterhouse, and market data. The explanatory variables were selected from the univariable analysis results based on a *p*-value of ≤ 0.2. Season and farm/slaughterhouse/shop scale variables were forced into all models to control confounding bias. Backward stepwise model simplification was performed to determine the risk or preventive factors.

In addition, for the comparisons of MPN/g on cut and ground pork sold in markets, Wilcoxon Rank Sum tests were used on MPN/g, and Fisher's exact test was used on the proportions above 30 MPN/g. For the attribution of contamination of cut pork at the market to the contamination of carcasses at slaughterhouses, attributable risk percent [33] was also calculated.

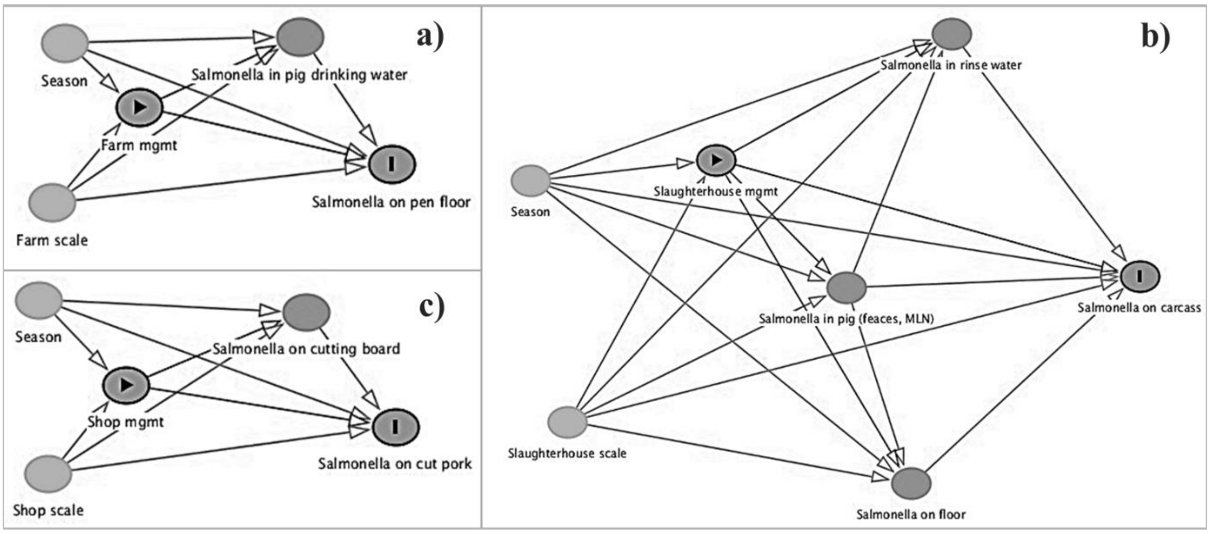

Figure 5. Causal diagrams, for farm (a), slaughterhouse (b) and market (c), shows exposure variables of interest (dark circle with a triangle), intervening variables (dark grey circle), as well as potential confounders (light grey circle) related to the outcome variables (dark circle with a vertical bar, adapted from: http://www.dagitty.net/dags.html). *Farm mgm*t includes variables regarding to farm management, facitilies and biosecurity; *Slaughterhouse mgmt* includes variables related to live pig management during transportation, slaughterhouse management, facilities and practices; *Shop mgmt* includes variables regarding to management, equipment and hygiene practices at shop; MLN: mesenteric lymph nodes

3.2.6. Ethical consideration

During sampling and data collection, the research team provided potential participants with information about the questionnaire and checklist and a time estimate for their involvement. Participants were informed that they could freely end their involvement at any time without adverse consequences. Written consent was obtained from participants before conducting interviews. This study was reviewed and approved by the ethical committee at the Hanoi University of Public Health (No.148/2012/YTCC-HD3).

3.3. Results

3.3.1. Smallholder pork value chains

In both studied provinces, the 72 farms had an average of 17 fattening pigs (range: 4-84), and exotic pigs dominated (68.1%, 49 farms), and significantly higher in Hung Yen compared to that in Nghe An (Table 3). Out of 26 variables in the farm checklist, we have subjectively excluded six redundance and five low variability variables. There were 13 variables were included for the univariabe analysis, and other two variables was used for description.

Out of the 13 slaughterhouses involved in the study, six slaughterhouses had the capacity to slaughter 11-50 pigs/day, and seven slaughterhouses slaughtered ≤10 pigs/day. In total, slaughterhouses were visited 49 times, and 149 pigs were sampled. We aimed to sample three pigs per slaughterhouse in each sampling period over the year. However, sometimes fewer pigs were sampled due to the availability of pigs, and occasionally up to five pigs were sampled per slaughterhouse to catch up with the schedule. Even in the same slaughterhouse, information on the origin of pigs and slaughter practices was not always available, and here summary results in Table 3 are presented based on the number of visits. Out of 60 variables (17 from questionnaire, and 43 from checklist) from slaughterhouses, we have subjectively excluded 18 redundance and 19 low variability variables. There were 23 variables at slaughterhouse were included for general description and the univariabe analysis.

The pork shops surveyed in the both provinces were all informal wet markets, as described above. Most markets had areas for pork retailers separate from other foods (for example, vegetables, dried foods, poultry, and fish). Out of 43 variables from checklist at shops, we have excluded 16 redundance and 13 low variability variables. There were 14 variables at shop were remained for general description and the univariabe analysis.

3.3.2. *Salmonella* prevalence

Table 4 shows the *Salmonella* prevalence along smallholder pork value chain, and in general prevalence was high at all stages (farm, slaughterhouse, and market). In pig farms, the prevalence on pen floor (36.1%) and wastewater (38.9%) were significantly higher than of drinking water (19.4%, $\chi^2 = 4.2$, df = 1, $p = 0.04$; and $\chi^2 = 5.7$, df = 1, $p = 0.02$, respectively).

In slaughterhouses, the prevalence on pig carcasses (38.9%) was not significantly different from rectal faeces (33.6%, $\chi^2 = 0.7$, df = 1, $p = 0.40$). Swabs from floors where carcasses were split were often contaminated with *Salmonella* (22.5%), as was the water used to rinse carcasses (20.4%; this water was used for washing hands and equipment). The McNemar test showed no significant agreement between the *Salmonella* infection status in faeces and MLN ($\chi^2 = 0.08$, df = 1, $p = 0.77$).

At pork markets, the prevalence of *Salmonella* in cut pork (44.7%) and in ground pork (41.3%) were significantly higher than that on cutting board surfaces (25.3%, $\chi^2 = 17.0$, df = 1, $p < 0.01$, and $\chi^2 = 6.3$, df = 1, $p = 0.01$, respectively, Table 4). The *Salmonella* prevalence in cut pork and on cutting boards showed a high level of agreement in McNemar test ($\chi^2 = 25.5$, df = 1, $p < 0.01$).

Table 3. General information and descriptive statistics to compare between the two provinces

Information	Hung Yen	Nghe An	Overall (Range, or %)
Pig farm (72 farms, 72 visits)			
Number of farms sampled	36	36	72
Number of fattening pigs per farm (median, range)	25 (6-84)	12 (4-25)	17 (4-84)**
Number of farms with cross bred pigs, exotic	3, 33	20, 16	23, 49**
Pig slaughterhouse (13 slaughterhouses, 49 visits)			
Slaughterhouses with capacity <10,11-50 pigs/day	4, 3	3, 3	7, 6
Number of visits, and number of pig carcass samples	25, 72	24, 77	49, 149
Live weight (kg) of slaughtered pigs (median, range)	100 (89-150)	60 (40-95)	95 (40-150)**
Number of pigs slaughtered per day (median, range)	12 (1-45)	10 (2-34)	11 (1-45)
Small- or medium-, large scale farms originated (visit)	12, 13	21, 3	33, 16
Pork shop (145 shops, 217 visits)			
Number of shops, number of visits/samples	69, 108	76, 109	145, 217
Pork selling area was separate from other foods	73	53	126 (58.1)**
Number of visit when use of a table taller than 60 cm was observed	105	89	194 (89.4)**

Note: (*) and (**) statistic significance at levels of 0.05 and 0.01, respectively.

Table 4. *Salmonella* prevalence by sample type in smallholder pork value chain in Vietnam

Sample type	No. of positive/No. of samples	*Salmonella* prevalence (95% CI)[*]
Pig farm		
Pig drinking water	14/72	19.4 (12.0 - 30.0)[a]
Pig pen floor	26/72	36.1 (26.0 - 47.7)[b]
Pig pen wastewater	28/72	38.9 (28.5 - 50.4)[b]
Pig slaughterhouse		
Rinse water	10/49	20.4 (11.5 - 33.6)[a]
Splitting floor	11/49	22.5 (13.0 - 35.9)[a]
Rectal faeces	50/149	33.6 (26.5 - 41.5)[b]
Mesenteric lymph node	53/149	35.6 (28.3 - 43.5)[b]
Pig carcass	58/149	38.9 (31.5 - 46.9)[b]
Pork market		
Cutting board	55/217	25.3 (20.0 - 31.5)[a]
Ground pork	33/80	41.3 (31.1 - 52.2)[b]
Cut pork	97/217	44.7 (38.2 - 51.4)[b]

CI: Confidence interval; [(*)] Prevalences with different letters were significantly different ($p < 0.05$)

3.3.3. Univariable analyses

Table 5, 6 and 7 show the univariable GLMMs analyses results for pig farms, slaughterhouses, and markets, respectively. At farms, there were two significant factor related with *Salmonella* prevalence on pig pen floors: medium scale of pigs farm (estimate = 1.967, p = 0.026), and freely enter the farm by visitors (estimate = 1.489, p = 0.043). At slaughterhouses, winter season (sampling from December to February) was associated with decreased risk of *Salmonella* positivity (estimate = -2.483, p = 0.008). At markets, the significant factor associated with reduction of *Salmonella* positive cut pork was winter season (estimate = -1.457, p = 0.001), while presence of fly or insect on pork or table was risk factor for *Salmonella* positive cut pork (estimate = 0.576, p = 0.045).

3.3.4. Multivariable analyses

At farms, there were two risk factors associated with the contamination of a pen floor with *Salmonella,* having pig pens located next to households (estimate = 2.371, p = 0.055, Table 8), and visitor can freely enter farm (estimate = 1.867, p = 0.061). When farm scale was removed from the model, the estimates for having pig pens located next to households and freely enter the farm by visitors changed by 4.3% (estimate = 2.473, p = 0.051) and 13.5% (estimate = 1.913, , p = 0.026), respectively, suggesting medium level of confounding with the factor removed (data not shown).

Table 5. Univariable GLMMs results at farm

Variables	*Salmonella* positive	*Salmonella* negative	Pre (%)	Estimate	SE	*p*-value
Pig farm level						
Sampling season						0.455
April-June	5	13	27.8	Ref	-	-
July-September	8	10	44.4	0.876	0.780	0.261
October-November	8	10	44.4	0.888	0.784	0.257
December-February	5	13	27.8	-0.013	0.803	0.987
Farm management						
Scale of fattening pigs farm						
Small (1-10 pigs)	2	16	11.1	-1.967	0.895	**0.026**
Medium (11-100 pigs)	24	30	44.4			
Keeping sows in farm for breeding						
Yes	20	31	39.2	0.478	0.635	0.452
No	6	15	28.6			
Keeping pigs and other animals in the same area						
Yes	7	10	41.2	0.322	0.665	0.628
No	19	36	34.5			
Mix pig batches in the same pen						
Yes	2	1	66.7	1.567	1.523	0.304
No	24	45	34.8			
Using biogas system to treat waste from pens						
Yes	18	30	30.8	0.261	0.639	0.683
No	8	16	33.3			
Farm facility and biosecurity						
Pig pens located next to household						
Yes	24	34	41.4	1.552	0.930	**0.095**
No	2	12	14.3			
Store pig drinking water in open tank						
Yes	15	20	42.9	0.667	0.597	0.264
No	11	26	29.7			

Table 5. Univariable GLMMs results at farm *(continue)*

Variables	***Salmonella* positive**	***Salmonella* negative**	**Pre (%)**	**Estimate**	**SE**	***p*-value**
Visitors can freely enter farm						
Yes	15	16	48.4	1.489	0.730	**0.042**
No	11	30	26.8			
Having boot disinfection bath at farm						
Yes	8	15	34.8	-0.271	0.645	0.674
No	18	31	36.7			
Worker wear uniform and boots						
Yes	8	15	34.8	0.483	1.165	0.678
No	18	31	36.7			
Farm facilities are clean						
Yes	16	29	35.6	0.022	0.608	0.971
No	10	17	37.0			
Presence of insect at farm						
Yes	20	33	37.7	0.426	0.668	0.523
No	6	13	31.6			

Pre: prevalence; SE: standard error; *p*-value in bold: selected variables (with $p \leq 0.2$) for multivariable analysis

Table 6. Univariable GLMMs results at slaughterhouse

Variables	*Salmonella* positive	*Salmonella* negative	Pre (%)	Estimate	SE	*p*-value
Sampling season						**0.017**
April-June	19	18	51.4			
July-September	16	23	41.0	-0.735	0.754	0.329
October-November	17	19	47.2	-0.454	0.754	0.547
December-February	6	31	16.2	-2.483	0.932	0.008
Live pig management during transportation						
Live pig farm scale						
Small (<10 pigs)	12	34	26.1	1.017	0.617	**0.100**
Medium (11-100 pigs)	46	57				
Breed of pig						
Indigenous	16	34	32.0	-0.498	0.599	0.405
Exotic	42	57	42.4			
Clean and disinfection transport vehicle						
Yes	40	57	41.2	0.224	0.585	0.701
No	18	34	34.6			
Time of transport pig from farm to slaughterhouse						
Afternoon (of a previous day)	14	20	41.2	0.191	0.655	0.771
Morning (of a previous day)	44	71	38.3			
Duration of transportation live pigs						
More than 1 hours	4	5	44.4	0.444	1.154	0.700
Less than 1 hour	54	86	38.6			
Slaughterhouse management						
Scale of slaughterhouse						
Small (<10 pigs/day)	16	38	29.6	-0.766	0.572	**0.181**
Medium (11-100 pigs/day)	42	53	44.2			
Slaughterhouse in the same house's compartment						
Yes	31	53	41.5	-0.242	0.585	0.679
No	27	38	36.9			

Table 6. Univariable GLMMs results at slaughterhouse *(continue)*

Variables	***Salmonella* positive**	***Salmonella* negative**	**Pre (%)**	**Estimate**	**SE**	***p*-value**
Keep more than 1 pig per m^2 in lairage						
Yes	40	66	41.9	0.450	0.646	0.486
No	18	25	37.8			
Slaughter area closes to lairage without hygienic measures						
Yes	53	70	43.1	1.611	0.871	**0.064**
No	5	21	19.2			
Presence of fly, blue fly or rat in slaughter areas						
Yes	23	30	43.4	0.380	0.626	0.544
No	35	61	36.5			
Slaughterhouse facilities						
Slaughtering is processed on table or shelf						
Yes	3	2	60.0	1.405	1.429	0.326
No	55	89	38.2			
Using tank water for washing carcass and floor						
Yes	43	68	38.7	-0.365	0.656	0.577
No	15	23	39.5			
Use scalding vat water in slaughtering						
Yes	23	23	50.0	0.808	0.620	**0.192**
No	35	68	34.0			
Slaughtering practices						
Wash live pig before slaughtering						
Yes	12	29	29.3	-0.840	0.653	**0.199**
No	46	62	42.6			
Separate internal organs and carcass						
Yes	19	32	37.3	-0.221	0.602	0.714
No	39	59	39.8			

Table 6. Univariable GLMMs results at slaughterhouse *(continue)*

Variables	***Salmonella* positive**	***Salmonella* negative**	**Pre (%)**	**Estimate**	**SE**	***p*-value**
Wash floor after slaughtering each pigs						
Yes	38	71	34.9	-0.892	0.657	**0.174**
No	20	20	50.0			
Wash tools, hands after each pig						
Yes	18	38	32.1	-0.657	0.594	0.269
No	40	53	43.0			
Wash tools, hand in rinse water tank						
Yes	46	59	43.8	0.867	0.627	**0.167**
No	12	32	27.3			
Use of cloth to wipe carcasses						
Yes	23	33	41.1	-0.031	0.582	0.957
No	35	58	37.6			

Pre: prevalence, SE: standard error, *p*-value in bold: selected variables (with $p \leq 0.2$) for multivariable analysis

Table 7. Univariable GLMMs results at market

Variables	*Salmonella* positive	*Salmonella* negative	Pre (%)	Estimate	SE	*p*-value
Sampling season						**0.001**
April-June	31	23	57.4	Ref	-	-
July-September	30	24	55.6	-0.073	0.390	0.852
October-November	23	32	41.8	-0.633	0.390	0.105
December-February	13	41	24.1	-1.457	0.426	0.001
Shop management						
Market scale						0.378
Commune market	36	40	47.4	Ref	-	-
Central market	53	63	45.7	-0.067	0.296	0.820
Roadside vendor	8	17	32.0	-0.648	0.486	0.183
Shop located in the area for selling pork						
Yes	60	66	47.6	0.283	0.278	0.309
No	37	54	40.7			
Shop is next to the sewerage or stagnant water						
Yes	12	7	63.2	0.824	0.497	**0.097**
No	85	113	42.9			
Presence of fly or insect on pork, table						
Yes	41	35	53.9	0.576	0.287	**0.045**
No	56	85	39.7			
Equipment and hygiene practices at shop						
Shop uses a pork grinder						
Yes	23	32	41.8	-0.157	0.316	0.619
No	74	88	45.7			
Use tap water at shop						
Yes	43	63	40.6	-0.328	0.274	0.232
No	54	57	48.6			
Table is higher than 60 cm						
Yes	85	109	52.2	-0.336	0.442	0.447
No	12	11	43.8			

Table 7. Univariable GLMMs results at market *(continue)*

Variables	***Salmonella* positive**	***Salmonella* negative**	**Pre (%)**	**Estimate**	**SE**	***p*-value**
Type of table surface material						0.793
Carton	34	46	42.5	Ref	-	-
Granite or steel	29	37	43.9	0.059	0.336	0.861
Wood	34	37	47.9	0.218	0.328	0.507
Using cutting board for cutting pork						
Yes	75	91	45.2	0.083	0.323	0.797
No	22	29	43.1			
Pork is put close or next to raw internal organs						
Yes	26	22	54.2	0.489	0.329	**0.137**
No	71	98	42.0			
Using cloth for wiping pork, hands and equipment at shop						
Yes	88	98	47.3	0.786	0.422	**0.063**
No	9	22	29.0			

Pre: prevalence, SE: standard error, *p*-value in bold: selected variables (with $p \leq 0.2$) for multivariable analysis

At slaughterhouses, the risk factors for *Salmonella* contamination on finished carcasses was slaughter area closes to lairage without hygienic measures (estimate = 1.723, $p = 0.031$, Table 8). At markets, there were two risk factors for *Salmonella* contamination on cut pork: presence of fly or insect on pork or table (estimate = 0.835, $p = 0.021$), and the use of cloth to wipe pork, hands and equipment at the shop (estimate = 1.040, $p = 0.023$). Winter season (December to February) was associated with decreased risk of *Salmonella* positivity in both carcass at slaughterhouse (estimate = -2.483, $p = 0.004$) and cut pork at market (estimate = -1.696, $p < 0.001$).

3.3.5. *Salmonella* concentration in pork and attribution of contamination

Salmonella concentration on most cut pork samples (77.3%, 75/97) was less than 3.0 MPN/g. Considering highly contaminated samples, that is the proportions of *Salmonella* concentration above 30 MPN/g, there were no significant differences between cut and ground pork samples (10/97, 10.3% versus 5/33, 15.2%, $p = 0.5$, Fisher's Exact test). The overall *Salmonella* concentration was also not significantly different between cut and ground pork samples (W = 1859, $p = 0.15$, Wilcoxon Rank Sum test). Between two provinces, the proportions of cut pork samples with above 30 MPN/g were not significantly different: Nghe An was 16.3% (8/49) and Hung Yen was 4.2% (2/48, $p = 0.09$, Fisher's Exact test), although the *p*-value was marginal (Table 9).

Out of the 63 carcasses traced, 25 carcasses were contaminated with *Salmonella* (39.7%), of which 16 cut pork samples were positive (16/25, 64%). Out of 38 negative carcasses, 14 cut pork samples were positive (14/38, 36.8%). Attributable risk percent was 42.4%, suggesting 42.4% contaminated pork at shops was attributable to the contamination of carcasses at the slaughterhouse (data not shown).

Table 8. Multivariable GLMMs results at farm, slaughterhouse and market

Factors	**Estimate**	**SE**	***p*-value**
Pig farm (pen's floor)			
Pig pens located next to household	2.371	1.237	**0.055**
Visitors can freely enter farm	1.867	0.901	**0.061**
Pig slaughterhouse (pig carcass)			
Slaughter area closes to lairage without hygienic measures	1.723	0.798	**0.031**
Market (cut pork)			
Presence of fly or insect on pork or table	0.835	0.361	**0.021**
Using cloth for wiping pork, hands and equipment at shop	1.040	0.457	**0.023**

SE: Standard error, *p*-value in bold: statistically significance at level of $p \leq 0.1$

Table 9. *Salmonella* concentration in cut and ground pork at markets by province

Province	**Sample type**	**No. of *Salmonella* positive/n**	**Frequencies of *Salmonella* MPN/g ranges**				
			<0.3	**0.3-3.0**	**3.1-30.0**	**30.1-110**	**>110**
Hung Yen	Cut pork	48/108	18	22	6	1	1
	Ground pork	21/56	7	7	5	2	0
Nghe An	Cut pork	49/109	22	13	6	4	4
	Ground pork	12/24	3	5	1	1	2
Overall	Cut pork	97/217	40	35	12	5	5
	Ground pork	33/80	10	12	6	3	2

MPN: Most probable number

3.4. Discussion

This study elucidated the prevalence of *Salmonella*, hygiene practice, and risk factors for contamination with *Salmonella* along the informal pork value chain in wet markets in northern Vietnam. As described in the introduction, this informal pork value chain dominates the domestic pork supply, and thus the information provided by this study is very important for food safety in Vietnam.

In terms of pig breeds in farms and the facilities in shops in markets, Hung Yen showed evidence of more agri-food system transformation than Nghe An, but the capacity of slaughterhouse and level of contamination at market were not different. At all sampling season of slaughterhouse, and market, lower *Salmonella* prevalence in colder seasons was observed in this study, which was also reported in a study among 12 European pig slaughterhouses [53].

In the small-scale pig farms studied, bio-security measures were generally not adequate to keep farming environment hygienic, and contamination with *Salmonella* could occur easily both from outside and within farms. It is reported that poor biosecurity measures are important risk factors for *Salmonella* prevalence at farm [3]. Our study also indicated the importance of improvement of bio-security measures in the small scale pig farms, particularly on the farm location and access.

The *Salmonella* prevalence on finished carcasses in this study was comparable to the reports from Belgium (37%) [15], and Thailand (28%) [102], but much higher than on chilled carcasses in the United States (2 to 4%) examined between 2001 and 2009 [43]. The multivariable risk factor analysis for the contamination of finished carcasses in slaughterhouses found the risk factor related to slaughter area closed to lairage without hygienic measures. This finding has an implication of cross-contamination of finished carcasses with unclean slaughtering areas where high risk materials (e.g., faeces, wastewater) from lairage can be either contaminated pig carcass directly or dispersed by workers, equipment to slaughterhouse surface. This contamination risk is common to swine processing worldwide because the intestinal tract carrying *Salmonella* can be incidentally lacerated during processing, and the carcass may be cross-contaminated [6, 11, 14].

The prevalence on MLN (35.6%) in our study was higher than the reports from European and North American countries (10.9%) [42]. *Salmonella* positive MLN is considered a proxy for the sub-clinical level of *Salmonella* infection in apparently healthy pigs [44], as infected pigs can asymptomatically carry *Salmonella* in the tonsils, intestines and the gut associated lymphoid tissue [16, 109, 152]. In our study, prevalences of *Salmonella* in faeces and MLN showed poor agreement. In the latent state, faecal samples from *Salmonella* infected pigs may produce negative results, but excretion can be reactivated [10, 145], which may explain our finding. Moreover, considering the reactivation of *Salmonella* excretion may occur due to stress, higher prevalence among pigs from larger farms observed might be due to the stress under intensive farming environment. Therefore, raising and transporting pigs in a low-stress environment may be one of the manageable options for swine salmonellosis [16, 145].

Although they did not remain in the final model, several problematic slaughtering practices were observed, and they were not found to be significant because there was very little variability (if no one has good practices, it is impossible to identify the risk factor). First, the proportion of slaughterhouses washing live pigs was low. Second, splitting carcasses was exclusively conducted on the floor that was commonly contaminated with *Salmonella*, where no segregation between clean and dirty zones was practiced. Third, slaughterhouse workers washed tools and hands in a water tank from which the water is used for rinsing carcasses. Fourth, it is also reasonable to assume pig carcasses will contaminate the scalding vat water; for example the skin of pigs in lairage are commonly contaminated with pig feces, and the (optional) pre-scalding water rinse step cannot remove all *Salmonella*. The previous report from Vietnam also described that these practices were common during processing at the conventional slaughterhouses [27]. Cleaning and disinfection procedures along the slaughter line are beneficial [4, 30], and hygiene in slaughtering must be continuously improved in Vietnam, regardless the results of risk factor analysis.

At the markets, *Salmonella* prevalence on cut pork in our study was comparable to the other reports in Vietnam: 32.8% [120], 39.6% [123], 28.6% [153], and 44.4% [28]. The risk factor analysis for markets control options: applied fly control and discouragement of using a cloth to wipe pork, hands and equipment. Affordable and practical methods to allow effective cleaning and disinfection of shop equipment (e.g., table surface, cutting boards, knives, and weight scale) are needed. A study in Uganda has shown the benefit of a wooden frame covered with a net on which deltamethrin applied on the window of pork shops in reducing the number of flies [57]. In Vietnam, informal pork shops operate business in an open environment, but public health authorities should bear in mind such applicable and effective control options. Another recommendation is to focus on the effect of temperature on *Salmonella* prevalence shown in this study. Limiting pork sale during the cooler morning hours may reduce both prevalence and bacteria load on pork.

Our study has some inherent limitations. The biggest limitation of this study was the limited power to detect effects of factors influencing the prevalence of *Salmonella*. We embarked upon the project realizing that power was limited and this was one reason for using an alpha of 0.1. Still, the study was only able to detect, and find significant, factors that were strongly associated with *Salmonella* prevalence and which were relatively common in the population. In addition, the sampling plan did not include those points in the value chain that link farms to slaughterhouses and slaughterhouses to markets, especially transportation, lairage, and distribution, which themselves are opportunities for *Salmonella* cross-contamination [53, 82]. Samples were taken from a limited number of available sample sites at each value chain location.

This study provided useful information in planning applicable and effective intervention programs at each step of the value chain. However, planning interventions requires several more considerations, as smallholder pork value chains have complex relationships (characterized by many-to-many interactions among actors). First, the form of intervention may be needed to consider, for example, a single project or a collaborative trans-disciplinary program. Second, risk managers and policy makers should decide which points in the value chain are targeted to achieve the best outcome. Third, they also need to create a balance between intervention costs (development, implementation, and—most importantly—monitoring and evaluation of compliance) and the subsequent risk reduction in terms of both the number of illness or death avoided, and the public health cost saved. Moreover, economic impacts on the livelihood of smallholder actors due to the cost of compliance should be considered [48], as they may be vulnerable to the change even for better public health.

3.5. Summary of Chapter 3

The objective of this study was to investigate the prevalence of, and risk factors for, *Salmonella* contamination along the smallholder pork value chain in northern Vietnam. A repeated cross-sectional study was carried out in Hung Yen and Nghe An provinces in four sampling periods over a year (April 2014 to February 2015). In total, 72 pig farms and 217 pork shops were visited during the period, and 13 slaughterhouses were visited four times. Information on management and hygiene practices was collected using checklists and questionnaires, and risk factor analyses were performed using generalized mixed-effects models at the farm, slaughterhouse, and pork shop levels, respectively.

Salmonella prevalence was 36.1% (26/72), 38.9% (58/149), and 44.7% (97/217) on pig pen floors, pig carcasses in slaughterhouses, and cut pork in pork shops, respectively. The risk factor for *Salmonella* prevalence on pig pen floors were having pig pen next to household (p = 0.055) and freely access farm by visitor ((p = 0.061). Our slaughterhouse model found a single risk factor for carcass contamination: slaughter area closes to lairage without hygienic measures (p = 0.031). For pork shops, presence of fly or insect on pork at shop (p = 0.021), and use of a cloth at pork shop (p = 0.023) were risk factors. The *Salmonella* prevalence on pig carcass and cut pork was significantly lower in winter season compared to that in other seasons. Our study results will contribute to the better planning of effective and affordable control options for human salmonellosis by improving pork hygiene along the informal pork value chain in northern Vietnam.

Chapter 4. A simulation of *Salmonella* cross-contamination on boiled but originally contaminated pork through cooking at home kitchen in Vietnam

4.1. Introduction

Foodborne diseases (FBD) are a common health problem and an important cause of reduced economic productivity [48, 149]. The first global assessment found the health burden of FBD was comparable to that of malaria, tuberculosis or HIV/AIDs [149]. In Vietnam, 1,781 food poisoning outbreaks with 58,622 infected people and 412 deaths were reported between 2006 and 2015 [139]. The actual number of cases is likely far higher as under-reporting of FBD is common [55]. For example, in Australia around one in five people fall ill from FBD each year [75]. Pork and pork products are the most commonly consumed meats in Vietnam [101]. However, they are also an important source of *Salmonella*, second to eggs and poultry meat [37, 55, 105].

In Vietnam, most pork (80%) is produced by smallholders and mainly processed and sold through the informal wet value chain [78]. Moreover, pigs commonly harbor *Salmonella* without showing clinical signs [16, 109, 152], and such carrier pigs are one of the main reservoirs of human salmonellosis. Several studies in Vietnam reported *Salmonella* in feces of apparently healthy pigs; one study reporting a prevalence of 5.2% of pigs at farms [128]. Other studies report prevalences of 38.9% to 49.4% in feces collected at slaughterhouses [28, 144]. Reported prevalences of *Salmonella* on finished carcasses in slaughterhouses in Vietnam vary from 15.5% to 95.7% [28, 79, 120, 153]. The prevalence of *Salmonella* on pork in the wet markets in Vietnam ranges from 32.8% to 69.9% [28, 104, 120, 123]. The general increase in prevalence along the value chain suggests cross-contamination occurs and one paper suggested 46.7% of cross-contamination occurs at transportation to and at the market phases [153].

Information on *Salmonella* prevalence in pork in households in Vietnam is not available, partly because of ethical and practical challenges in collecting data. During food handling and preparation at home, microorganisms on raw foods can be transferred indirectly via air or directly via contact from hands to food or various surfaces, e.g., equipment and utensils, to food [32]. Household preparation may be more risky than commercial food establishments [108].

Microbiological food safety risk assessment is a powerful tool in understanding the magnitude of the health risk from pathogenic bacteria not only in developed countries, but also in developing countries [47, 85]. As part of this, transfer experiments can provide data allowing risk assessment steps to be better described and modeled [94]. This study was designed to fill the knowledge gap on the level of *Salmonella* cross-contamination at home kitchen to conduct the risk assessment for salmonellosis in Vietnam. The information generated in this study has been utilized in a risk assessment published recently [28], and here the cross-contamination module is described in detail.

4.2. Materials and methods

4.2.1. Household survey on hygiene management in cooking boiled pork

In 2013, a total of 416 households in both Hung Yen and Nghe An provinces (208 in each province) were visited and interviews were conducted which covered cooking methods (i.e., hygiene management in cooking boiled pork) using a structured questionnaire. Three consumer areas represented for rural, peri-urban and urban were selected in two cities, 6 towns, and 6 rural communes from these two provinces, respectively. Within each study area, households were randomly selected and one representative adult member (aged above 18) per household, usually the housewife, participated in the interviews, as previously described by Nga et al. (2015) [98]. This interview information was a part of a consumer household survey on diets, food accessing and practices, food consumption and food safety knowledge and practices under the pork safety project, namely PigRISK [2].

4.2.2. Study design for cross-contamination experiment

This study was designed to quantify the potential of cross-contamination of *Salmonella* from raw to boiled pork, in home kitchens via hands, knives, and cutting boards. Raw pork was artificially inoculated with *Salmonella* strains. After washing contaminated pork with mineral *Salmonella*-free water (Lavie Ltd., Nestlé Water, Vietnam) in a basin twice, pork was cut into smaller pieces, boiled in a cooking pot with 2 - 2.5 liters of water for 15 minutes, and sliced. Four ways of preparation (scenarios) were investigated based on information about cooking practices from the household survey. The numbers of *Salmonella* remaining in raw washed pork and occurrence of cross-contamination with *Salmonella* on hands, knives, and cutting boards were tested (Table 10). The contamination status and the number of

Salmonella were examined on the boiled pork as well. The experiment was triplicated and also repeated three times, making nine trials in total for each scenario.

4.2.3. Scenarios to examine

According to the household survey, all studied households washed pork, hands, knife, and cutting board, but there were differences in the use of separate knives and/or cutting boards for raw and boiled pork. Scenario 1 examined the degree of cross-contamination with no separate use of knives and cutting boards. This represents the way pork is commonly prepared. Scenarios 2, 3, and 4 used different combinations of separate equipment and hand washing were examined in order to investigate risk mitigation options (Table 10). In detail, in Scenario 1, after the raw pork was washed and cut, the knife, cutting board and hands were washed with dish-washing detergent and clean water in a basin using a dish cloth. Then the washed knife and cutting board were reused to slice boiled pork by the same person. In Scenario 2, after the washed raw pork was cut, hands were washed with dish-washing detergent and clean water using a dish cloth, and a new cutting board and knife were used to slice boiled pork by the same person. In Scenario 3, after washed raw pork was cut, the knife was washed with dish-washing detergent and clean water using a dish cloth, and hands were disinfected. Then a separate new cutting board, and the knife washed were used to slice boiled pork. In Scenario 4, after the washed raw pork was cut, the cutting board was washed with dish detergent and clean water using a dish cloth, and hands were disinfected using alcohol and instant hand sanitizer. Boiled pork was sliced on the washed cutting board using new knife.

Table 10. Types of samples in *Salmonella* cross-contamination experiments

Sampling points	Sample type	Data
Measuring how many *Salmonella* remained after the contaminated pork was washed twice		
Water used for washing	Water for washing recovered	Qualitative
Raw pork after washed twice	Pork piece	MPN
Testing whether cross-contamination with *Salmonella* occurs after handling raw pork		
Hands after cutting raw pork	Surface swab	Qualitative
Knife after cutting raw pork	Surface swab	Qualitative
Cutting board after raw pork block was cut into large pieces	Surface swab	Qualitative
Ensuring *Salmonella* was inactivated		
Boiled pork right after boiling	Pork piece	Qualitative
Measuring the degree of cross-contamination with *Salmonella* after boiling pork in different scenarios		
Scenario 1: Washing hands, the knife, and the cutting board		
Hands before slicing boiled pork	Surface swab	Qualitative
Knife before slicing boiled pork	Surface swab	Qualitative
Cutting board before slicing boiled pork	Surface swab	Qualitative
Boiled pork after slicing	Pork slice	MPN
Scenario 2: Using a new knife and a new cutting board, and washing hands		
Hands before slicing boiled pork	Surface swab	Qualitative

Table 10. Types of samples in *Salmonella* cross-contamination experiments *(continue)*

Sampling points	**Sample type**	**Data**
Boiled pork after slicing	Pork slice	MPN
Scenario 3: Using a new cutting board, disinfecting hands, and washing the knife		
Knife before slicing boiled pork	Surface swab	Qualitative
Boiled pork after slicing	Pork slice	MPN
Scenario 4: Using a new knife, disinfecting hands, and washing the cutting board		
Cutting board before slicing boiled pork	Surface swab	Qualitative
Boiled pork after slicing	Pork slice	MPN

MPN: Most Probable Number

When hands, knives, and cutting boards were washed, washing was conducted once with *Salmonella*-free water and dish-washing detergent using a dish cloth; equipment was dried at ambient temperature, for about 75 minutes, without touching anything till the next step, slicing boiled pork. The thickness of the pork slice was about two millimeter, with about 2.5 - 5 cm width and length. The time taken for slicing was approximately 2 minutes.

4.2.4. Participants

Twelve volunteers consisting of ten females and two males, from the National Institute of Veterinary Research (NIVR, Vietnam), Hanoi University of Public Health, and Vietnam National University of Agriculture, participated in this study. All participants reported that they regularly prepare and cooked boiled pork dishes at home. The twelve volunteers were divided into three parallel experiment groups (four persons per group) in three different experiment days. Each of the three groups simulated four scenarios as described above. Two other volunteers (the first author, and a bacteriologist at NIVR) prepared and inoculated *Salmonella* on pork, and supervised, assisted, and took samples during experiment. Participants were informed of the purpose of the study, and instructed in the biosafety, clean and disinfection procedures used in the experiment. The volunteers were instructed to wash thoroughly their hands with soap and water, disinfected by both 70% ethanol and hand sanitizer before and after the experiment. Prior to the experiment, one practice trial was conducted without *Salmonella* inoculation.

4.2.5. Preparation of *Salmonella* culture

As previous studies showed, *S*. Typhimurium, *S*. Derby, and *S*. London were the most common strains in pig carcass and retailed cut pork [26, 128, 144]. These strains, isolated from pig carcass and retailed cut pork in a recent investigation by the first author [26], were used to create an inoculated medium. First, each strain was recovered and then amplified separately in a 150 ml-conical flask contained 50 ml Buffer Peptone Water (BPW, Merck-Germany) at 37^{o}C overnight (without shaking). The following day, the plate count technique was used to determine *Salmonella* concentration using Xylose Lysine Desoxycholate (XLD-Merck-Germany) agar for each cultured media. Duplicate plates made by spreading 0.1 ml of cultured BPW which was diluted at 10-fold with Maximum Recovery Diluent (MRD, Merck-Germany) on XLD were incubated at 37^{o}C for 20-24 hours. After the *Salmonella* concentration was determined, the *Salmonella* culture was appropriately diluted to have 10^5 CFU/ml. To prepare a *Salmonella* culture to inoculate pork, three sets of 5 ml medium containing each incubated strain of *Salmonella* with 10^5 CFU ml^{-1} were mixed, and 15 ml of cocktailed medium was prepared. Then, MRD (90 ml) was added into 10 ml of the 10^5 CFU ml^{-1} cocktailed medium to make 10^4 CFU ml^{-1} medium (100 ml), which was ready for pork inoculation.

4.2.6. Pork preparation

Fresh cut pork was purchased immediately after splitting and deboning at a slaughterhouse in the early morning, when pig carcasses were ready for transportation to the market. The sirloin and/or shoulders (contains both lean and fat parts) were selected. To minimize *Salmonella* contamination, sterile knives and gloves were used to cut pork, at a slaughterhouse, removing the outer part, or any surface of the carcass of selected part. Twelve pork pieces of 500-600 gram were cut and placed into separate sterile sealable plastic bags. Pork bags were kept in a cool box and transferred to the laboratory within 3 hours to perform experiment. At the laboratory, each pork piece was weighed and placed onto a sterile autoclaved tray to be prepared for *Salmonella* inoculation.

4.2.7. Inoculation of pork

Based on the weight (gram) of the pork piece, the *Salmonella* culture (concentration of 10^4 CFU ml^{-1}) was measured at a ratio of 1/1000 volume, and was inoculated on pork pieces, which made 10 *S. enterica* per gram, that referred from a *Salmonella* contamination range in market pork in previous study [26]. For example, 500 µl of the culture was inoculated on 500 g of pork piece. The culture was gently dropped (approximately 20 µl each) onto the surface of the pork piece using a 10-100 µl filter tip and pipette (Thermo Scientific, USA), coving the whole surface. An inoculated pork piece was kept on table at ambient temperature for 30 minutes to stabilize and allow cells attachment before starting the experiment.

4.2.8. Sampling in the experiment

The surface of hands, palms, fingers, webbing between the fingers of both hands, 25 cm^2 of both sides of the knife, and 25 cm^2 of the cutting board surface were swabbed using sterile pre-moisten gauze. The surface samples of hands, knives, and cutting boards were collected immediately after washing raw pork twice and just before slicing boiled pork. Wash water samples (approximately 30-40 ml per sample) were aseptically collected after washing raw pork twice. Both raw and boiled pork samples were collected using sterile scalpels and forceps. Raw pork was sampled just before placing into a boiling pot. Boiled pork was sampled directly after boiling. Boiled pork slice was sampled immediately after slicing.

4.2.9. Microbiological tests

Salmonella detection was carried out according to the ISO-6579 [70] procedure. In the pre-enrichment step, swabbed or 10 ml water samples were added up to 100 ml BPW for homogenization, while a 25 grams pork samples were homogenized in 225 ml BPW. For *Salmonella* enumeration in pork samples, a 3 tube-Most Probable Number (MPN) method was used following the ISO:21528-1 [72]. In pre-enrichment step of MPN, series of three tubes per dilution of 1-0.1-0.01 g and 10-1-0.1 g were prepared for the incubation of raw and boiled pork, respectively. Further steps of *Salmonella* detection and enumeration were previously described elsewhere [26].

4.2.10. Data analysis and modeling

All the data was digitized in Microsoft Excel 2010 spreadsheets. Descriptive statistics were performed using Chi-squared test and Fisher's exact test to compare the proportions of samples contaminated with *Salmonella* using R version 3.3.2 [110].

To estimate the distributions of *Salmonella* concentration on pork samples, parametric bootstrapping was used. Bacterial concentration follows Log-Normal distribution with the mean CFU g^{-1} as MPN g^{-1}, and the standard deviation (*sd*) can be modelled as Equation 1 [25, 86].

$$sd = 0.55\sqrt{\log_{10}\alpha} \quad \text{(Equation 1)}$$

where α is dilution ratio, ten.

In each scenario, a distribution of a MPN result was randomly selected at equal probability of selection among the MPN results of *Salmonella* positive samples for the type of the sample of interest. A value was randomly sampled from the distribution selected. For the mean MPN g^{-1} less than 0.03, a value was randomly selected from non-informative uniform distribution between natural logarithm of 0.01 MPN g^{-1} and 0.03 MPN g^{-1}, and exponential of the value was calculated. This process was iterated for 5000 times using R. The median, 2.5th and 97.5th values of the stored 5000 samples were obtained, and Log-Normal distribution was fit to the simulated sample data using maximum likelihood method in fitdist() function in the fitdistrplus package [91] to obtain the mean and standard deviation. For the presentation of the distributions, kernel density was calculated using the simulated sample data, and plotted using R.

The reduction rate in *Salmonella* CFU g^{-1} was modelled by dividing the value (CFU g^{-1}) sampled as above by 10 CFU g^{-1}, which is the initial *Salmonella* concentration inoculated on raw pork. The calculation of reduction rate was iterated for 5000 times to have distributions. The distribution of reduction rate was presented using histogram.

As there were two MPN values which resulted as 11 MPN g^{-1} in scenario 1 and 4, exceeding the inoculation level, above simulations on CFU g^{-1} and reduction rate distributions were performed without these MPN values, and with these values as worst case scenarios.

4.2.11. Ethical statement

The experiment and *Salmonella* analysis were carried out at the Department of Veterinary Hygiene of NIVR (Hanoi, Vietnam). All volunteers signed informed consent forms for their participation in the study. This research was a part of the PigRISK and the Taskforce for food safety risk assessment in Vietnam, funded by ACIAR. Ethical approval of this study (No.148/2012/YTCC-HD3) was obtained from the ethical committee of the Hanoi University of Public Health.

4.3. Results

4.3.1. Household survey results

All households reported that they washed hands and equipment after handling raw pork during cooking boiled pork slices (Table 11). The most common practice was using the same knife and cutting board for both raw and boiled pork (71.4%, 297/416) in both provinces. Use of separate knives and cutting boards for raw and boiled pork was less common (16.1%, 67/416).

4.3.2. Effect of washing twice in the prevalence and *Salmonella* concentration on raw pork

After washing raw pork twice, *Salmonella* was isolated from all the nine samples, and no reduction was observed in the prevalence. Table 12 shows the *Salmonella* concentrations of these raw pork samples (*Salmonella* concentration on boiled pork will be explained in the later section). The simulated CFU g^{-1} of *Salmonella* on raw pork after washing twice was 0.47 (median 0.43; 95%CI: 0.09 - 4.52, Figure 6).

Figure 7 shows the reduction rate of *Salmonella* concentration on washed raw pork. The mean reduction rate of raw pork by washing twice in *Salmonella* CFU g^{-1} was 91.9% (median 95.7%; 95%CI: 53.8% - 99.1%).

Table 11. Handling practices in boiled pork slice

Handing practices	Households in Hung Yen	Proportion (%)	Households in Nghe An	Proportion (%)
No separate use of knife or cutting board between raw and boiled pork	141	67.8	156	75.0
Separate use of both knives and cutting boards between raw and boiled pork	36	17.3	31	14.9
Separate use of cutting boards between raw and boiled pork, but same knife is used	18	8.6	18	8.6
Separate use of knives between raw and boiled pork, but same cutting board is used	11	5.3	2	1.0
Answer not provided	2	1.0	1	0.5
Total	**208**	**100**	**208**	**100**

Table 12. *Salmonella* concentration on washed raw pork and boiled pork slice

Scenario	**Number of samples fallen in *Salmonella* MPN g^{-1} categories**				**Mean CFU g^{-1} (median)**	**logSD**	**95% CI**
	<0.03	***0.03-0.30***	***0.31-3.0***	***>3.0***			
Raw pork after washing twice	0	1	8	0	0.47 (0.43)	0.94	0.09 - 4.52
Scenario 1	1	4	1	0	0.40 (0.12)	1.40	0.01 - 2.62
*Worst case scenario	1	4	1	1	2.15 (0.14)	2.01	0.01 - 17.58
Scenario 3	1	1	0	0	0.05 (0.04)	0.86	0.01 - 0.18
Scenario 4	0	3	2	0	1.17 (0.42)	1.61	0.02 - 5.34
*Worst case scenario	0	3	2	1	3.13 (0.75)	1.96	0.02 - 19.23

Worst case scenarios refered to the simulations on CFU g^{-1} and reduction rate distributions that were performed with two MPN values, which resulted as 11 MPN g^{-1} in scenario 1 and 4, exceeding the inoculation level.

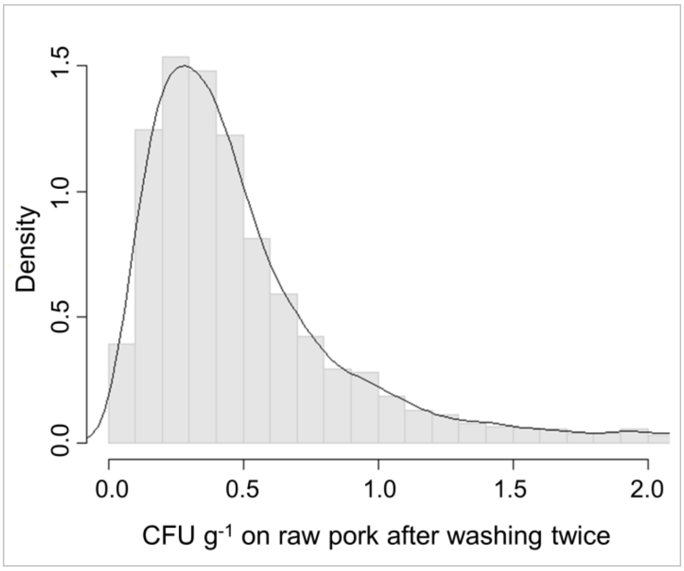

Figure 6. Distribution of *Salmonella* concentration (CFU g^{-1}) on raw pork after washing twice

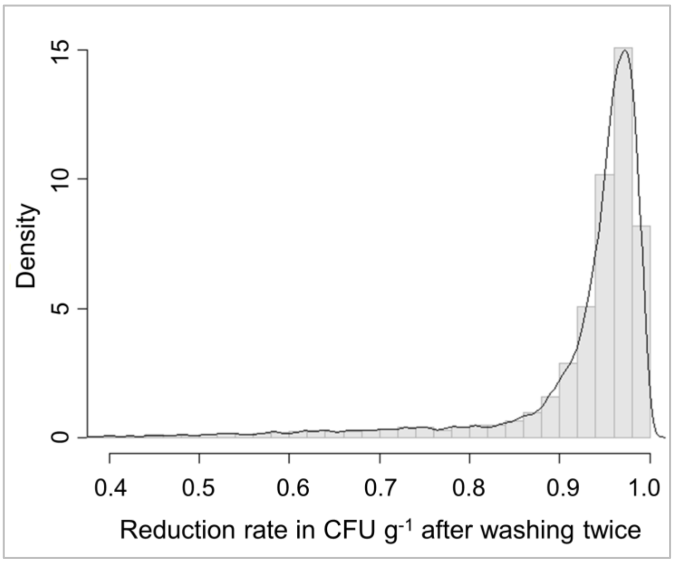

Figure 7. Reduction rate of *Salmonella* concentration in CFU g^{-1} after washing raw pork twice

4.3.3. Cross-contamination of *Salmonella* from raw pork to equipment and hands

Table 13 shows the proportions of sliced boiled pork, equipment, and hands for which pork was transferred from raw to boiled pork (cross-contamination). Although raw pork was washed twice, cross-contamination with *Salmonella* from pork to hands, knives and cutting boards was common (78%, 78%, and 100%, respectively). Eight out of nine wash water samples were positive for *Salmonella* (data not shown).

Table 13. Proportions of boiled pork slice, hands, and equipment on which cross-contamination with *Salmonella* occurred during the experiments

Type of sample	Samples cross-contaminated (n = 9)	Proportion contaminated (%)	95% CI
After contacting raw pork			
Hands	7	77.8	40.2 - 96.1
Knife	7	77.8	40.2 - 96.1
Cutting board	9	100	62.9 - 100
Scenario 1			
Boiled pork slice	7	77.8	40.2 – 96.1
Hands	3	33.3	9.0 - 69.1
Knife	4	44.4	15.3 - 77.3
Cutting board	5	55.6	22.7 - 84.7
Scenario 2			
Boiled pork slice	0	0.0	0.0 - 37.1
Hands	3	33.3	9.0 - 69.1
Scenario 3			
Boiled pork slice	2	22.2	3.9 - 59.8
Knife	0	0.0	0.0 - 37.1
Scenario 4			
Boiled pork slice	6	66.7	30.9 - 90.9
Cutting board	6	66.7	30.9 - 90.9

4.3.4. Effect of boiling pork in the prevalence of *Salmonella*

After boiling, *Salmonella* was not isolated from the nine pork samples.

4.3.5. Re-contamination on boiled pork slice with *Salmonella* by equipment and hands

After *Salmonella* was eliminated from pork by boiling, re-contamination of boiled pork occurred in scenarios 1, 3, and 4 (Table 13). In scenario 2, new equipment (knife and cutting board) was used and re-contamination did not occur. The probability of re-contamination was highest in scenario 1, which did not involve either separate use of equipment or disinfection of hands (7/9, 77.8%). There was no significant difference in the proportion of re-contamination between the scenarios re-used cutting board after washing (scenarios 1 and 4, $p = 1$, Fisher's exact test, Table 13). When the scenarios involving re-use of the same cutting board were combined (1 and 4), the probability of re-contamination (72.2%) was higher than scenarios which used a new cutting board (2 and 3) and where re-contamination was 11.1%. The difference of these proportions was significant: $x^2 = 11.4$, df = 1, $p < 0.01$).

In the scenario 4, the *Salmonella* concentration on boiled pork was highest (mean CFU $g^{-1} = 1.17$, Table 12, Figure 8c), with the lowest reduction rate of *Salmonella* concentration (mean = 88.3%, Table 14, Figure 9c). Scenario 3, which represented the risk of re-contamination through re-use of knife, showed low probability of re-contamination (mean = 22.2%, Table 13), and higher reduction rate of *Salmonella* concentration (mean = 99.6%, Table 14, Figure 9b) compared to scenario 1 and 4.

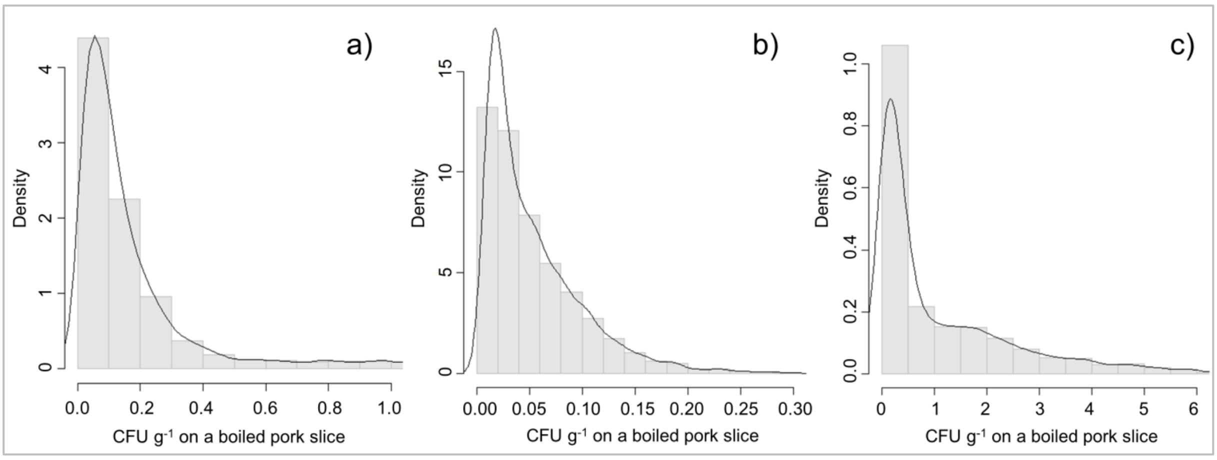

Figure 8. *Salmonella* CFU g^{-1} on a pork slice due to cross-contamination (a: Scenario 1, b: Scenario 3, c: Scenario 4)

In the two worst case scenarios, the mean of *Salmonella* concentration re-contaminated on boiled pork in scenarios 1 (Figure 10a) and 4 (Figure 10b) were 2.15 CFU g^{-1} and 3.13 CFU g^{-1} (Table 12), respectively. The probability of cross-contamination in the worst case of scenarios 1 (Figure 11a) and 4 (Figure 11b) were almost 20% higher compared to their initial scenarios (Table 14). The probabilities of exceeding initial CFU g^{-1} in both scenarios 1 and 4 were 8.5% and 9.5%, respectively (Table 14).

4.3.6. Vehicles causing re-contamination of boiled pork slice with *Salmonella*

In terms of the *Salmonella* prevalence on equipment and hands, the prevalence of *Salmonella* just before slicing boiled pork was highest on the cutting board (scenarios 1 and 4, Table 13). *Salmonella* was isolated from hands as well in the similar probabilities (scenarios 1 and 2, 33.3%). When equipment and hands were washed and re-used, the prevalences on them were similar (Table 13).

Table 14. Reduction rate of *Salmonella* concentration

Scenarios	Mean reduction rate (%)	Median (%)	Lower limit (%)	Upper limit (%)	Exceeded initial CFU g^{-1} (%)
Scenario 1	96.0	98.8	73.8	99.9	0
*Worst case scenario	78.5	98.6	-75.9	99.9	8.5
Scenario 3	99.5	99.6	98.2	99.9	0
Scenario 4	88.3	95.8	46.5	99.8	0.3
*Worst case scenario	69.0	92.3	-94.4	99.8	9.5

Worst case scenarios refered to the simulations on CFU g^{-1} and reduction rate distributions that were performed with two MPN values, which resulted as 11 MPN g^{-1} in scenario 1 and 4, exceeding the inoculation level.

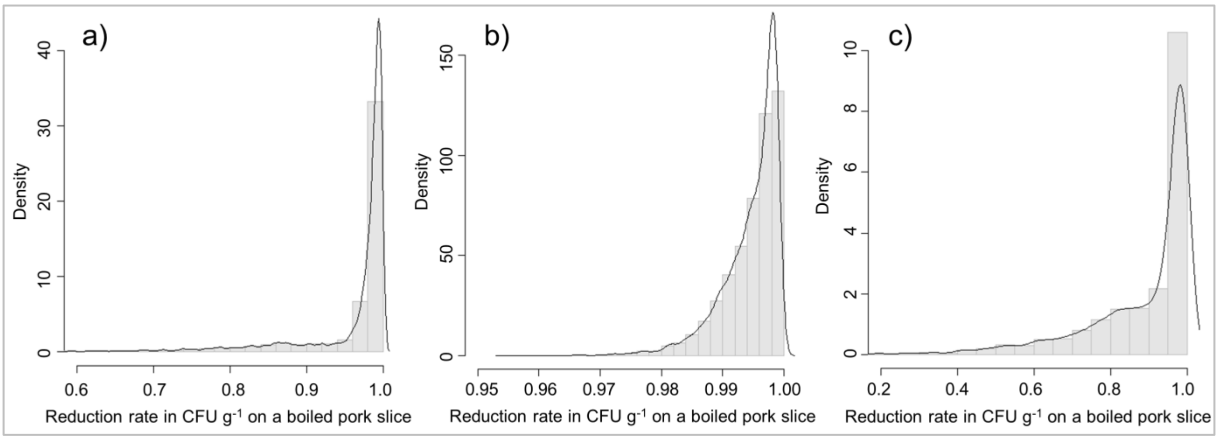

Figure 9. Reduction rates after *Salmonella* cross-contamination to boiled pork slice (a: Scenario 1, b: Scenario 3, c: Scenario 4)

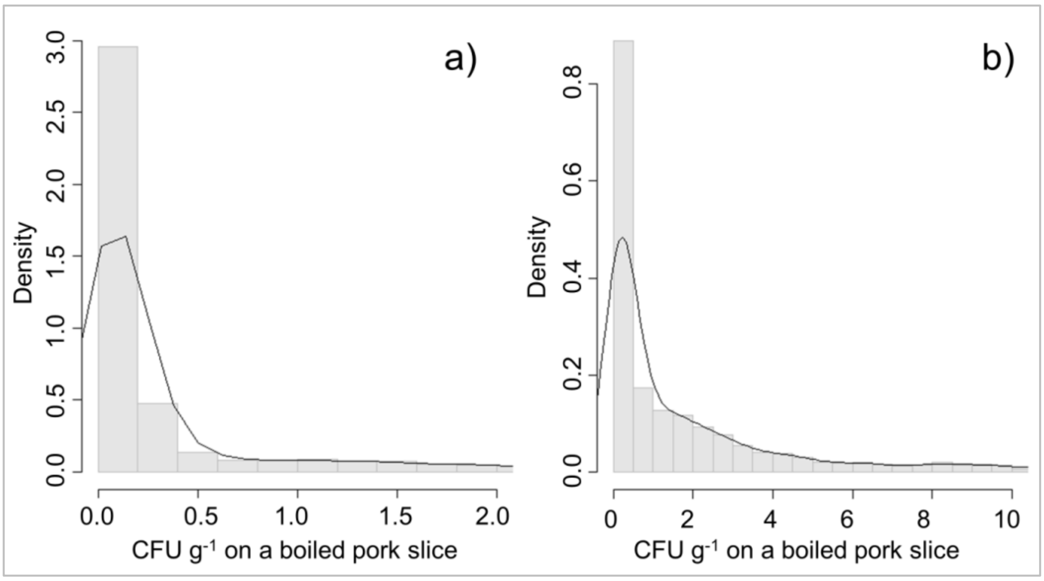

Figure 10. Worst case scenarios of *Salmonella* CFU g^{-1} on a pork slice due to cross-contamination (a: Scenario 1, b: Scenario 4). Worst case scenarios refered to the simulations on CFU g^{-1} and reduction rate distributions that were performed with two MPN values, which resulted as 11 MPN g^{-1} in scenario 1 and 4, exceeding the inoculation level.

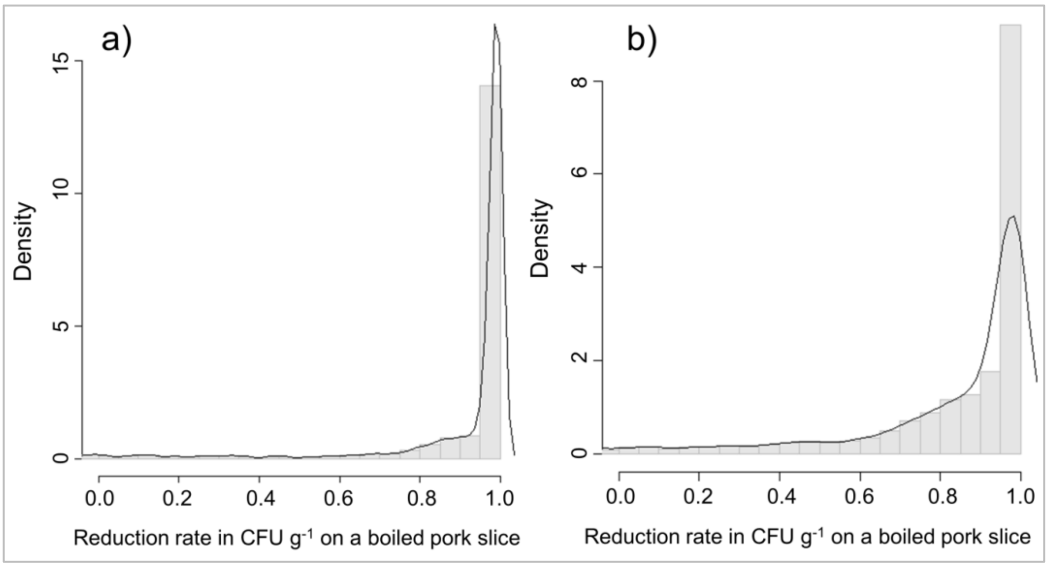

Figure 11. Reduction rate in CFU g^{-1} on a boiled pork slide in worst case scenarios (a: Scenario 1, b: Scenario 4). Worst case scenarios refered to the simulations on CFU g^{-1} and reduction rate distributions that were performed with two MPN values, which resulted as 11 MPN g^{-1} in scenario 1 and 4, exceeding the inoculation level.

4.4. Discussion

In this study, cross-contamination experiments investigated *Salmonella* transmission under four different scenarios of kitchen practices. The practices commonly used, supported by our field survey, were found to cause cross-contamination. These findings showed that the level of *Salmonella* cross-contamination at the household kitchen is depending on the re-use of kitchen utensils between raw and cooked pork. In this experiment, cross-contamination mainly occurred through use of same cutting board (scenarios 1 and 4). Remarkably, in the two worst case scenarios, a higher *Salmonella* concentration than the initial inoculum was obtained, indicating microbial growth rather than reduction. This may be the result of a wooden cutting board which can absorb moisture and where bacteria attach, maintain, and multiply inside outer surface. Some studies have showed that *Salmonella* can survive in deep cuts on wooden cutting boards [73, 154], and that wood is one of the most difficult surface to clean [116]. Although washing cutting boards was a common practice in the study areas in Vietnam, this experiment showed that bacteria can remain and be source of the cross-contamination. This finding has been reported in the previous studies as well [24, 121]. Therefore, future food safety intervention programs should focus on the risk of cross-contamination on cutting boards in home kitchens.

Moreover, the other equipment such as knives, cutlery and hands are known to play an important role in bacterial cross-contamination during cooking at home kitchen [13]. The step of washing equipment such as cutting boards and knives, and hands generally helps to reduce bacterial contamination [8]. The previous study on the use of detergent in washing equipment and hands also showed reduction in transmission of diarrhea causing pathogens [17]. However in our study, washing of these equipment and hands, even using dish detergent, was not enough to remove *Salmonella* (Scenario 1 and 4). Washing using detergent including soap was reported to be more effective than using just water in reducing the presence of coliform bacteria on hands [60]. Frequent and careful washing, as well as use of disinfecting agents such as hypochlorite [116], may further reduce the chance of transmission of not only *Salmonella* but also other diarrhea causing pathogens.

This study used a Bayesian approach, to present uncertainty and variability of *Salmonella* concentrations and reduction rate as probability distributions, using limited numbers of samples. This information, together with the probability of cross-contamination in different hygiene procedures, is particularly useful in the exposure assessment step in risk assessment which usually lacks the data [32, 76].

The study has limitations. First, we took swabs from only 25 cm^2 of each side of the knife and cooking board which may underestimate the chance of cross-contamination from them. Second, we assumed no growth of *Salmonella* during the experiment. As this experiment included the time to dry hands, which people preparing food may not do, the bacterial concentration presented in this study may be over-estimated. Third, the pork was not washed before inoculating *Salmonella* on it. However, cut pork sampling at a slaughterhouse took place aseptically, and there might not be a chance of heavy contamination with *Salmonella* to affect the results. Forth, the sample size was relatively small and repeated study would reduce the uncertainties in distributions. Moreover, the conditions of time and temperature may be better to be explored more in future studies.

This study provided the first information both on the possible occurrence and magnitude of cross-contamination which was used for quantitative risk assessment of *Salmonella* from household pork consumption in Vietnam [28]. The levels of cross-contamination in different scenarios will allow us to estimate the potential effects of possible risk-mitigating strategies. These findings therefore can help advocate for improving food handling practices at household, as well as support risk communication and food safety education for consumers [19, 93], to minimize adverse health risk consequences. The findings can help counter the common misperception that if pork is boiled well before eating, it does not present a risk. The presence of *Salmonella* in ready-to-eat or cooked food due to cross-contamination have been reported in several studies [19, 46], and the findings in our study can be used for assessing the risks in these foods as well. Further research needs for intervention include studies on behavior-change theories, and trial intervention in the target population using validated instruments [114]. Together with such information, intervention planning will become feasible and realistic to reduce the burden of salmonellosis in Vietnam and other parts of the world.

4.5. Summary of Chapter 4

The aim of this chapter was to assess the potential for cross-contamination of *Salmonella* from raw to boiled pork in households. Three repeated experiments were conducted to simulate cross-contamination. Surfaces (hands, knives and cutting boards), water used for washing (wash-water), and pork were sampled to identify the presence and concentration of *Salmonella*. Parametric bootstrapping was applied to simulate transfer rate and variation in *Salmonella* cross-contamination. Surveys in two provinces were conducted to understand the hygiene practices related to pork cooking in 416 households. In these households, most people (71%) used the same knife and cutting board for both raw and boiled pork, and almost all people washed hand and equipment in between handling raw and boiled pork. Simulation experiments indicated that hands, wash-water, knives and cutting boards exposed to raw contaminated pork were the main source of spread to boiled pork. There was a high risk of cross-contamination to boiled pork when the same hands, knife and cutting board were used for raw and boiled pork (78% of boiled pork samples were contaminated with *Salmonella*). Using the same cutting board resulted 67% of boiled pork samples becoming contaminated with *Salmonella*. Understanding cross-contamination was part of microbial risk assessment, and in addition provides insights leading to better food safety management, communication, and education.

Chapter 5. Quantitative risk assessment of human salmonellosis in the smallholder pig value chains in urban of Vietnam

5.1. Introduction

Food safety is a major public health anxiety worldwide, particularly in developing countries where demand for safe and nutritious food supplies is increasing rapidly [49]. In Vietnam, food safety and especially pork safety are of major matter to both consumers and policy makers; it is frequently reported in the media and is the subject of high level policy discussions [49, 61]. Pork safety is important because Vietnam's per capita pork consumption of 29.1 kg per year is among the highest in the world, and pork is the most popular consumed meat in Vietnam accounts for the 56% of total meat intake [101]. In Vietnam, up to 80% of pork is produced by smallholder farmers, and most pork is sold in wet markets [78].

Nontyphoidal *Salmonella* spp. are one of the most important causes of foodborne disease [56]. Previous studies in Vietnam have found prevalences of *Salmonella* in cut pork at market ranging from 37 up to 69% [58, 104, 123, 136]. However, the extent to which this hazard translates into human health risk depends on consumer behaviors especially those relating to cooking and consumption. Quantitative risk assessment consists of hazard identification, hazard characterization, exposure assessment and risk characterization (CAC/GL 30, 1999) [18] and provides an estimation of the probability and severity of illness in a given population from eating contaminated food. As such it supports information that is more useful to policy makers and risk managers than results of prevalence surveys [47].

Risk assessments have been used very successfully in developed countries to help address issues of food safety. In recent years, this approach has also been used to assess microbial pathogens in informal markets [85]. Nonetheless, this has not been widely applied to food safety in Vietnam, although a small number of studies have assessed health risk related to *Salmonella* and dioxin contaminated foods [127, 129]. The aim of this paper is to present a QMRA model for the smallholder pork value chains in Vietnam and an estimate of salmonellosis risk in humans. Development and implementation of this model will help scientists and policy makers involved in food safety by providing insight into the risks present in the value chain and help identify potential areas for successful mitigation of the risk.

5.2. Materials and methods

5.2.1. Study sites

This study was carried out in three out of ten districts, Tien Lu, Khoai Chau and Van Giang, in Hung Yen Province. Hung Yen is located in the Red River delta and is a neighbor province to Hanoi, the capital city of Vietnam. In 2014, the population of Hung Yen was 1.2 million. The province has a hot and cold season with average temperatures of 29.0°C in the summer (May to October) and 20.1°C in the winter (November to April) [51]. The three selected districts represent three different pork production-consumption pathways: from peri-urban to urban (Tien Lu), rural to rural (Khoai Chau) and rural to urban (Van Giang). In each districts, we randomly selected three communes for sampling.

5.2.2. Study design

We applied a cross-sectional design for sample collection along the smallholder pork value chain between April 2014 and February 2015. In this study, household based pig producers were scales of small (keeping 10 or fewer fattening pigs), medium (from 11 to 30 pigs) and large (more than 30 fattening pigs). For slaughterhouses, they were categorized as small and medium scales if they slaughtered 1-10 pigs/day and from 11-50 pigs/days, respectively. At retail level, we distinguished between roadside vendors as 1-2 stalls, commune markets as 3-20 stalls and central markets as over 20 stalls. In addition, selected study sites were traditional slaughterhouses (i.e. not modern slaughterhouse with hanging system), and retailers did not have a supermarket in the area. At consumer level, households in Hung Yen were defined as urban, peri-urban and rural areas.

5.2.3. Sample collection

In four sampling stages, a total of 36 pig farms were randomly selected by sampling one farm in each of nine recruited communes. A 100 cm^2 surface of pen floor sample represented by four different sites (25 cm^2 per site) have been swabbed using a pre-moistened cotton swab. At slaughterhouse, one to five pigs, which depends on the number of slaughtered pigs of the slaughterhouse, have been randomly selected from 25 slaughterhouses to collect carcass swab (n=72) and rectal content (n=72) samples. This sample size was calculated based on recent *Salmonella* prevalence on pig carcass (25%) reported in a study in 2014 from Hung Yen by Yokozawa et al. (2016) [153], with an absolute precision at 10% and confidence interval of 95%. To sample carcasses, done at final washing steps at slaughterhouses, a 400 cm^2 surface of carcass from 4 different sites (hind limb-medial, abdomen-medial, mid-back and lower part of neck) was swabbed (following ISO17604:2003) and pooled to one sample, while feces samples were taken from rectal content using sterilized forceps and sticks right after evisceration. At market, three pork shops in each of the selected communes were randomly selected for sampling. After four sampling visits at markets, a total of 108 cut pork samples had been collected by purchasing 400-500 grams of lean meat from three to four parts of a carcass [66]. Swab samples were stored in 20 ml Buffered Peptone Water (BPW; Merck, Darmstadt, Germany) medium and pork was placed in sterilized bag. All samples were kept in a cool box and transported to laboratory for analysis within 8-10 hours of sampling.

5.2.4. *Salmonella* analysis

Salmonella qualitative and quantitative tests were done following ISO 6579:2002 and 3-tube MPN method, respectively. In brief, 25 g pork and 10 g feces or swab samples were added up by 225 ml and 100 ml BPW (Merck, Darmstadt, Germany), respectively, for homogenization. All homogenates were incubated at 37°C for 16-20 h. After incubation, *Salmonella* was cultured consecutively in the first selective media (Muller-Kauffmann Tetrathionate-Novobiocin broth and Semisolid Modification Rappaport-Vassiliadis agar) and in the second selective media (Xylose-Lysine-Tergitol 4 agar and Rambach agar) (Merck, Darmstadt Germany). After culturing of suspected colonies in nutrition agar (Merck, Darmstadt Germany), biochemical tests were conducted to confirm typical profiles of *Salmonella* in Triple Sugar Iron Agar, Urea broth and Motility-indole-lysine agar (Merck, Darmstadt, Germany). Each *Salmonella* isolate was further serotyped using *Salmonella* polyvalent O antiserum (Bio-Rad, London, UK) according to Kaulfmann-White scheme. *Salmonella* enumeration was performed for retail pork using the 3-tube MPN method [31, 72]. All samples were processed and analyzed at the laboratory of the National Institute of Veterinary Research, Hanoi, Vietnam.

5.2.5. Consumer survey

A structured questionnaire was used for face-to-face interviews with 30 urban consumer households. This household survey was a subsample of a larger consumer survey carried out in Hung Yen province. In addition, eight participants represented to consumer households in Hung Yen urban were gathered in a focus group discussion (FGD) to explore pork cooking practices and consumption behavior. The questionnaire was developed in English, translated into Vietnamese and pre-tested with 5 consumers in Hung Yen prior to actual field survey. The FGD was led by one facilitator and one note-taker using audio recording and lasted about 1.5 hours. Written consent was obtained before conducting the interview and discussion group. The interview and FGD were conducted by the first author and four trained and experienced research assistants.

5.2.6. Quantitative microbial risk assessments

Codex Alimentarius Commission quantitative microbial risk assessment (QMRA) [18] consisting of hazard identification, hazard characterization, exposure assessment and risk characterization was applied. Hazard identification was described in the Introduction. For hazard characterization, bacteria growth and dose–response relationship models were obtained from the literatures [122, 137].

Exposure assessment was done using surveys data described above. Reduction and cross-contamination of *Salmonella* in term of probability and concentration during cooking was modelled based on an experiment, which was described in Chapter 4 and published elsewhere [113]. In this study, four different scenarios for cooking procedures were considered with different possibilities of cross-contamination by equipment and hand from raw to boiled pork slices. Briefly, *Scenario 1* presented a practice of after cutting raw pork, the knife, cutting board and hands used for it were washed once with soap and clean water. That same knife and cutting board were used to slice boiled pork without disinfection of hands. The probability of cross contamination of scenario 1 was 72.7% and *Salmonella* concentration reduced from 10 CFU/g to 0.34 CFU/g). *Scenario 2* presented after cutting raw pork, the hands were washed once with soap and clean water. A new knife and a new cutting board were used for cutting boiled pork, but without hand disinfection. The probability of cross contamination of this scenario was 9.1% and *Salmonella* concentration reduced from 10 CFU/g to 0.047 CFU/g). *Scenario 3* implied after cutting raw pork, the knife used for it was washed once with soap and clean water. Hands were washed and disinfected. Boiled pork was sliced using the same knife used for raw pork, but on a new cutting board. The probability of cross contamination of scenario 3 was 27.3% and *Salmonella* concentration reduced from 10

CFU/g to 0.047 CFU/g). *Scenarios 4* indicated after cutting raw pork, the knife used was washed once with soap and clean water. Hands were washed and disinfected. Boiled pork was sliced using the same cutting board used for raw pork, but with a new knife. The probability of cross contamination of scenario 4 was 63.6% and *Salmonella* concentration reduced from 10 CFU/g to 1.0 CFU/g).

Risk characterization was carried out by combining the dose–response and exposure assessment by developing a risk model. The developed risk model comprised of four parts: (i) prevalence and concentration of *Salmonella* on pork from different types of markets (central, commune markets and roadside vendors), (ii) *Salmonella* growth model between purchasing and cooking, (iii) cross-contamination of *Salmonella* in after boiling pork, and (iv) boiled pork consumption patterns (frequency and quantity) in different gender and age groups (less than 5 years old, male and female adults, and elders over 60 years old, Figure 12).

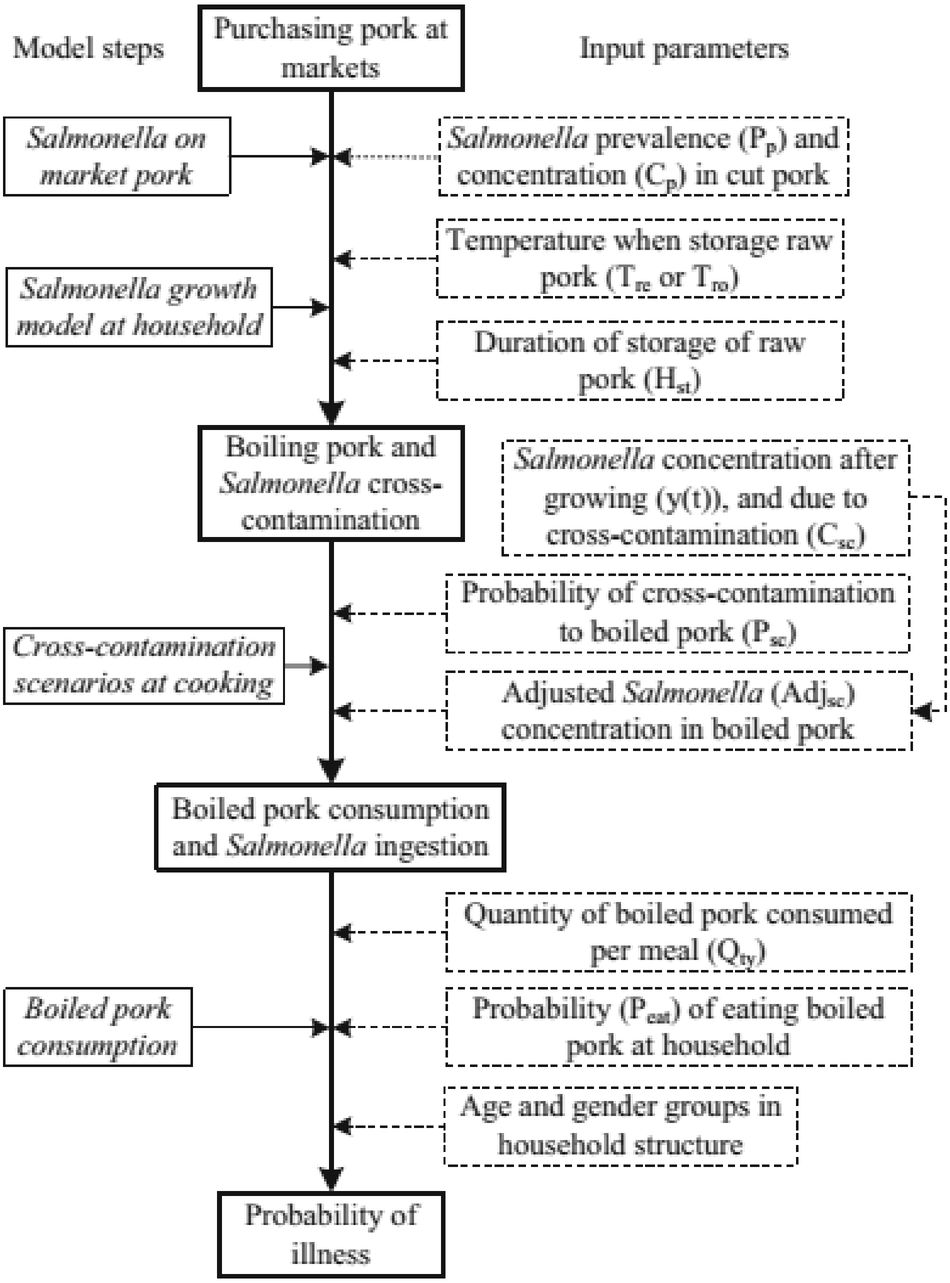

Figure 12. Steps and input parameters of the developed salmonellosis risk assessment model from retail pork to consumption in urban Hung Yen, Vietnam (thin solid arrow model steps, dotted arrow input parameters, thick solid arrow model flow)

Salmonella growth was modelled using below formula [137].

$$y(t) = y_0 + \mu_{max} F(t) - \ln\left(1 + \frac{e^{\mu_{max} F(t)} - 1}{e^{(y_{max} - y_0)}}\right) \qquad (1)$$

$$F(t) = t + \frac{1}{\mu_{max}} \ln\left(e^{-\mu_{max} t} + e^{-h_0} - e^{(-\mu_{max} t - h_0)}\right) \qquad (2)$$

where, y_0 is initial cell concentration in $\log_e$CFU/g; $y(t)$ is cell concentration in $\log_e$CFU/g at time t, y_{max} is maximum cell concentration in $\log_e$CFU/g, μ_{max} is maximum specific growth rate in $\log_e$CFU/g. The maximum CFU/g $10^{9.1}$ to calculate y_{max} was taken from Thayer et al. (1987) [124]. To model y_0, *Salmonella* concentration on sold pork was quantified and adjusted by the reduction rate in the experiments. To describe the effect of temperature on the maximum specific growth rates of the organism, the modified Ratkowsky equation was used as in Formula 3 [107].

$$\mu_{max} = a(T - T_{min})^2 (1 - \exp(b(T - T_{max}))) \qquad (3)$$

where, T_{min} and T_{max} represent theoretical minimum and maximum temperatures beyond which organism's growth is impossible and T represent the range of actual temperature, while a (0.00245) and b (0.2038) are regression coefficients that were obtained from the author group of Velugoti et al. (2011) by personal communication. According to this, they used these coefficients for their modelling, but did not present them in their paper. For *Salmonella*, T_{min} is 6.97 and T_{max} is 47.44 [137].

Ambience temperature was measured as mean of 25°C with a standard deviation of 4.25°C (based on temperature data in the study region in 2015) [51]. Temperature in the refrigerator was assumed to be 4ºC. Data on *Salmonella* multiplication by time (hour) was adapted from fitted data in the Baranyi model; h_0 mean was 2.14 and standard deviation was 0.71 [7]. The temperature was also included to determine the growth model which followed the modified Ratkowsky equation 3 [107].

Salmonella prevalence and number was obtained from the conducted biological sampling and analysis as mentioned above. *Salmonella* number was analysed as MPN/g which was assumed equal to CFU/g for fitting into the growth model. At household level, pork handling and consumption information was used from the consumer survey (see above). To model pork consumption patterns according to gender and age groups, an actual data were sampled using non-parametric bootstrapping. In the risk model sheet, 100 individuals by four gender and age groups (children, adult males and females, and elders) were modeled, and the means were used for the simulation of mean salmonellosis incidence probability (P_{ill}) using the Beta-Poisson dose response model (Formula 4). Parameters, statistic, distribution and data sources used in the risk modeling are shown in Table 15.

$$P_{ill} = 1 - (1 + dose/\beta)^{-\alpha} \quad (4)$$

where, P_{ill} is probability of illness (salmonellosis), $dose$ is a number of *Salmonella* (CFU) ingested per meal, $\alpha = 0.0085$ and $\beta = 3.14$ as described by Teunis et al. (2010) [122].

5.2.7. Data management and analysis

Collected data were managed, processed and analysed using MS Excel 2010 and RStudio version 3.2.2 (R Core Team). Descriptive statistical analysis was used to describe *Salmonella* prevalence. The risk model was developed and Monte Carlo simulation was performed using @Risk (Palisade, Corporation, US) for 10,000 iterations. Sensitivity analysis was conducted selecting all the uncertainty parameters and run for 1000 iterations at seven quantile values.

Table 15. Parameters, statistics, distribution and data sources using in the risk model in urban Hung Yen, Vietnam

Parameters	Statistics/Distribution	Source
Market		
Salmonella prevalence of cut pork at central market (P_{pc})	Pork from small SH: Beta(13, 13) Pork from medium SH: Beta(10; 13)	Survey
Salmonella concentration in cut pork at central market (Cpc) *(LogCFU/g)*	LogNormal(0.24, 0.32)	Survey
Salmonella prevalence of cut pork at commune market (P_{pm})	Pork from small SH: Beta(16, 9) Pork from medium SH: Beta(1; 1)	Survey
Salmonella concentration in cut pork at commune market (Cpm) *(LogCFU/g)*	LogNormal(0.92, 0.32)	Survey
Status of *Salmonella* contamination in cut pork (Spo) from market types	Central market: Binomial(1, P_{pc}) Commune market: Binomial(1, P_{pm})	Survey
Growth model at household		
Temperature when store raw pork in refrigerator at household (Tre) *(°C)*	Fixed at 4°C	Opinion
Temperature when store raw pork at ambience condition at household (Tro) *(°C)*	Normal(24.4, 4.9)	
Duration of storage raw pork at household before cooking (Hst) *(Hour)*	Actual data: mean = 2.1, min=0, max =5	Survey
Salmonella grow rate in food matrices (h0) *(LogCFU/g)*	Normal(2.14, 0.71)	Baranyi 1994 [7]
Cooking and consumption at household		
Probability of *Salmonella* cross-contamination after boiling pork in cooking scenarios (P_{sc})	Scenario 1: P_{sc1} =Beta(8, 3) Scenario 2: P_{sc2} =Beta(1, 10) Scenario 3: P_{sc3} =Beta(3, 8) Scenario 4: P_{sc4} =Beta(7, 4)	Survey
Status of *Salmonella* cross-contamination after boiling pork in cooking scenarios (C_{sc})	Scenario 1: C_{sc1}=Binomial(1, P_{sc1}) Scenario 2: C_{sc2}=Binomial(1, P_{sc2}) Scenario 3: C_{sc3}=Binomial(1, P_{sc3}) Scenario 4: C_{sc4}=Binomial(1, P_{sc4})	Survey
Probability of eating boiled pork per meal by Hung Yen urban consumer (P_{eat}) ($0< P_{eat} \leq 1$)	Non-parametric bootstrapping from household data (using DUniform)	Survey
Status of eating boiled pork in the meal by Hung Yen urban consumer (S_{eat})	Binomial(1, P_{eat})	Survey
Quantity of boiled pork consumed per meal by Hung Yen urban consumer (Qty) *(gram/meal)*	Non-parametric bootstrapping from household data (using DUniform)	Survey
Illness probability from dose response model (I_{pro})	Beta Poisson(α,β) equation, α =0.00853 and β= 3.14	Teunius 2010 [122]

SH: Slaughterhouse, CFU: Colony Forming Unit

5.3. Results

5.3.1. Smallholder pig value chains to urban Hung Yen

In this study, the portions of fattening pigs raised in the small, medium and large scale farms were 5.5, 29.2 and 65.3%, respectively. About two thirds of finishing pigs were sent to slaughterhouses inside Hung Yen province while the remainder was sent outside. There were no large scale (over 50 pigs/day) slaughterhouses or supermarkets observed in the study site. Medium and large farms provided live pigs for both small and medium scale slaughterhouses. Moreover, most pigs from small scale farms were sent to the small-scale slaughterhouses. The proportion of pigs numbers from medium and large scale farms slaughtered in small slaughterhouse were 76.2% and 40.4%, and for medium slaughterhouses 23.8% and 59.6%, respectively. At the market, 53.9, 25.6 and 20.5% of pork from small slaughterhouse were sold at central, commune markets and roadside vendor, respectively, whereas, almost all pork (93.9%) from medium slaughterhouse was sold at central market and a only small portion (6.1%) was sold at commune market. Among interviewed households, three forth of them usually buy pork at central markets, and the remain usually buy pork at commune markets (Figure 13).

5.3.2. *Salmonella* prevalence in the smallholder pig value chains

Salmonella prevalence on pig pen floors and carcasses at slaughterhouse were 33.3% and 41.7%, respectively (Table 16). Overall *Salmonella* prevalence on cut pork at market was 44.4%. There was no significant difference on *Salmonella* prevalence among the three retail types (χ^2 = 0.77, df = 2, p = 0.68). The *Salmonella* prevalence tended to be higher at the end of the pork value chain (feces: 38.9%, carcass: 41.7%, and cut pork: 44.4%; $\chi^2 = 0.55$, df = 2, p = 0. 76).

5.3.3. Exposure assessment

Data from focus group discussion (FGD) and non-parametric bootstrapping showed that each person consumed an average of 74 (minimum 20 to maximum 200) g boiled pork/meal. Amount of boiled pork consumed varied by age and gender group: 37 g/meal (children), 100 g/meal (adult male), 87 g/meal (adult female) and 73 g/meal (elder). The frequency of eating boiled pork was 117 (minimum of 50 to maximum of 205) times/year.

5.3.4. Risk characterization

The overall mean estimated annual incidence rate of salmonellosis due to eating boiled pork for urban consumer in Hung Yen was estimated at 17.7% (90% CI: 0.89 – 45.96, Table 17). The estimated annual incidence rate was lowest in children, followed by adult female, however, adult male and elder groups showed similar results (Table 17).

Table 16. *Salmonella* prevalence on pen floor at farm, feces and carcass at slaughterhouse and cut pork at market in Hung Yen, Vietnam

Sample type	***Salmonella* prevalence (No.of positive/n, %)**			
	Small	**Medium**	**Large**	**Overall**
Pig pen floor swab at farm	1/2 (50.0)	6/22 (27.3)	5/12 (41.7)	12/36 (33.3)
Fecal sample at slaughterhouse*	13/39 (33.3)	15/33 (45.5)	-	28/72 (38.9)
Pig carcass swab at slaughterhouse	14/39 (35.9)	16/33 (48.5)	-	30/72 (41.7)
Cut pork at wet market**	6/17 (35.3)	10/23 (43.5)	32/68 (47.1)	48/108 (44.4)

(*) Fecal sample was collected from rectum after evisceration, (**) At wet market, small scale was defined as roadside vendor (1-2 stalls), medium scale as commune market (3-20 stalls) and large scale as central market (over 20 stalls)

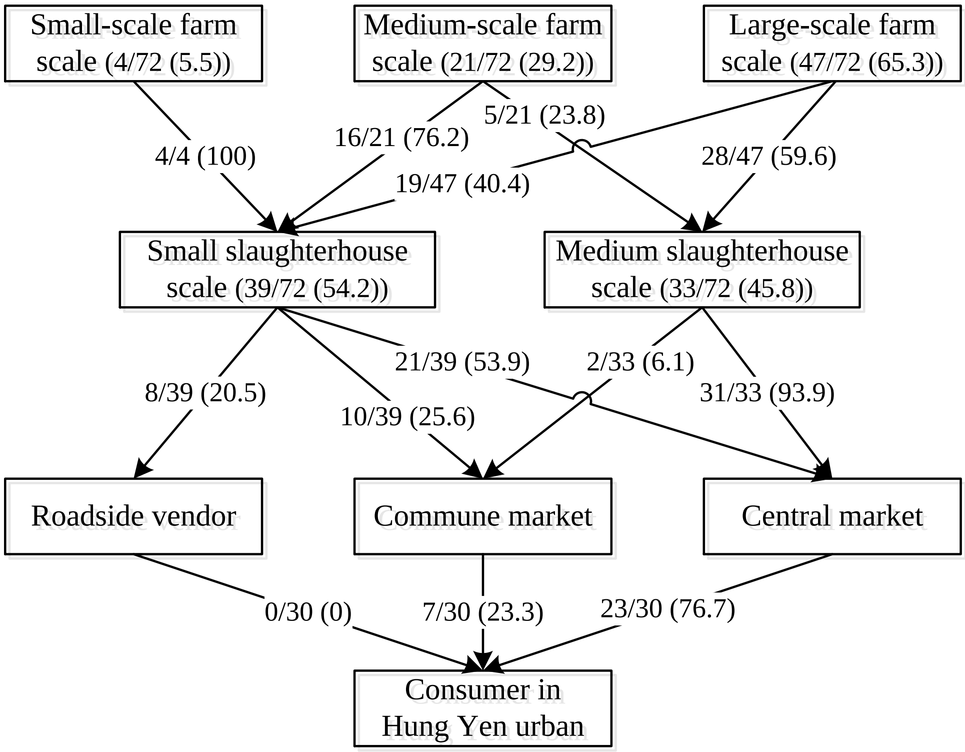

Figure 13. Smallholder pig value chains flow provides pork to urban Hung Yen, Vietnam (Numbers in blankets are in percentage)

Table 17. Annual incidence rate of human salmonellosis due to boiled pork consumption by age and gender groups in urban Hung Yen, Vietnam

Age and gender groups	**Estimated annual salmonellosis incidence rate (Mean (90% CI)) (%)**
Children (under 5 years old)	11.18 (0 - 45.05)
Adult female (6-60 years old)	16.41 (0.01 - 53.86)
Adult male (6-60 years old)	19.29 (0.04 - 59.06)
Elder (over 60 years old)	20.41 (0.09 - 60.76)
Overall	**17.7 (0.89 - 45.96)**

CI: Confidence interval

5.3.5. Sensitivity analysis

Sensitivity analysis results revealed the factors with the greatest influence on estimated salmonellosis incidence were cross-contamination rate in scenario 1 (using the same, both knife and cutting board, for raw and cooked pork), followed by the prevalence of *Salmonella* on pork in central market, where 76.7% of urban consumers purchase their pork and the *Salmonella* prevalence on pork at commune market. Two less important factors were cross-contamination rate in scenario 2 and scenario 3 (Table 18). By changing these seven uncertainty parameters into fixed mean values, the confidence interval of annual incidence rate (18.0%, 90% CI: 1.16% - 45.51%) became only slightly narrower, which means the larger confidence interval was due to the variability such as amount of pork and reduction of *Salmonella* concentration at cross-contamination on a boiled pork.

Table 18. Sensitivity analysis result of the influence factors on salmonellosis incidence in urban Hung Yen, Vietnam

Rank	**Influence factors on salmonellosis incidence**	**Values at 50th (1st - 99th) percentiles**	**Mean (90% CI) daily incidence of salmonellosis per 10,000 people**
1	Probability of cross-contamination in scenario 1	0.74 (0.39 - 0.95)	6.47 (4.69 - 7.79)
2	Prevalence of *Salmonella* on pork (from medium slaughterhouse) at central market	0.43 (0.21 - 0.67)	6.36 (5.67 - 7.17)
3	Prevalence of *Salmonella* on pork (from small slaughterhouse) at commune market	0.64 (0.41 - 0.84)	6.32 (5.5 - 7.11)
4	Prevalence of *Salmonella* on pork (from small slaughterhouse) at central market	0.5 (0.28 - 0.72)	6.36 (6 - 6.79)
5	Probability of cross-contamination in scenario 4	0.64 (0.3 - 0.91)	6.31 (5.98 - 6.7)
6	Probability of cross-contamination in scenario 2	0.07 (0 - 0.37)	6.35 (6.33 - 6.42)
7	Probability of cross-contamination in scenario 3	0.26 (0.05 - 0.61)	6.36 (6.34 - 6.38)

CI: Confidence interval

Scenario 1 presented a practice of after cutting raw pork, the knife, cutting board and hands used for it were washed once with soap and clean water. That same knife and cutting board were used to slice boiled pork without disinfection of hands.

Scenario 2 presented after cutting raw pork, the hands were washed once with soap and clean water. A new knife and a new cutting board were used for cutting boiled pork, but without hand disinfection.

Scenario 3 implied after cutting raw pork, the knife used for it was washed once with soap and clean water. Hands were washed and disinfected. Boiled pork was sliced using the same knife used for raw pork, but on a new cutting board.

Scenarios 4 indicated after cutting raw pork, the knife used was washed once with soap and clean water. Hands were washed and disinfected. Boiled pork was sliced using the same cutting board used for raw pork, but with a new knife.

5.4. Discussion

The domestic pork value chain is important for food and nutritional security in Vietnam. Our study confirmed the importance of small and medium farms and slaughterhouses in the pork value chain. Many agri-food systems in low and middle income countries (LMIC) are characterized by a large number of small operators, operating mainly in the informal sector [48]. This can make promotion of good practices and monitoring of food safety difficult, and shifting to large-scale, modern food production and retail is often seen as a way to mitigate food safety risks [150]. However, there is limited empirical evidence showing the effect of this approach on food safety risk [48].

Our study found significant levels of *Salmonella* along the pork value chain. Around 40% of carcass swabs were also positive. This is comparable to high-income countries, where a systematic review found an average of 55% of pork carcasses were *Salmonella* positive at the point of bleeding [100]. Our study also found a tendency of increase in prevalence along value chain, and that around 40% of retailed pork was *Salmonella* positive: this is higher than findings from high income countries (where typically 1-10%) of retailed pork is positive [92]. However, it is lower than found in some other LMIC [111, 155]. In our study, degree of intensification in sellers was not associated with pork hygiene sold at central markets in terms of *Salmonella* prevalence compared to roadside vendor or commune markets.

We estimated that the probability of acquiring salmonellosis from consumption of boiled pork was 17.7% in a given year. In high income countries, several studies have suggested that consumption of pork is one of the major sources of human salmonellosis [96, 132]. However, there is little good evidence on the incidence of salmonellosis in LMIC. The recent WHO report on the global assessment of foodborne disease, estimated that the annual incidence of foodborne salmonellosis in the Asian region including Vietnam was 1% (range 0.2-7%) [56]. This is much lower than our estimate of 17.7%, but the methods are not comparable and the WHO report is acknowledged to give conservative estimates. Another study in Vietnam estimated that the annual risk of infection by *Salmonella* from pork in an urban areas of Hanoi was 9.5 (0.4-30)% due to lack of separation knife, hands and cutting board at a consumption level of 86 g/person/day and of a frequency of 219 times of eating pork/person/year [127]. However the risk scenarios of this study were not comparable with those from our study.

Our 90% confidence interval of annual incidence was wide. The variability parameters in our QMRA model such as quantity of pork consumption and *Salmonella* concentration (MPN/g) contaminated on boiled pork in cooking experiments had a wide variety, and the structure of model simulating salmonellosis in the sets of 100 individuals reflects these variations.

In our study, much of the burden of salmonellosis was due to cross- contamination at the consumer level, when the same knife and cutting board was used for both raw and boiled pork. Other QMRA studies of pork have also identified this as a key process in amplifying risk [118]. A previous study in Hanoi, found that pork consumption was not associated with self-reported diarrhea, but consumption of vegetables was strongly associated with diarrhea [39]. This also supports the possible importance of pork as a source of bacteria which contaminate other foods which are eaten with minimal or no cooking.

Further, sensitivity analysis showed the importance of prevalence in marketed pork as the second most influential factor. This study did not identify the most critical stage for intervention; e.g. market, slaughterhouse or farm. The intervention targeted the value chains, e.g. farm, slaughterhouse, market or household will be incorporated in the intervention model which will be published elsewhere. However, the model developed in this study can be utilized in answering this question, and the risks will be studied under other settings in Vietnam further in future.

This study is the first published QMRA applied for food safety in Vietnam in international peer-reviewed literature. Risk-based approaches are now standard for food-safety issues in developed countries, as well as being the basis of rules governing international trade in food products. However, use of risk assessment, and especially quantitative risk assessment, has been limited in LMIC [47]. This study shows that QMRA can be applied to informal value chains and give credible information as well as insights into managing risk as concentration and prevalence in pork at central market were sensitive. In addition, although the model developed in this paper is specific for *Salmonella*, other microbiological pathogens in pork also present a risk of disease to consumers. The information from this study could provide valuable insight into risk factors and behaviors for other microbiological pathogens in pork. In Vietnam, food safety is one an important concern of the society and attracts great attention of all the stakeholders. Part of the problem is that hazards in food are often reported, however, there is little information on the magnitude of health risk caused by the hazards reported. Hence, the QMRA results here would offer a step forward by providing estimates of health impact.

There were several limitations in the study. First was the uncertainty of reduction in cooking that we don’t know how accurate the reduction at household is. We based this only on the experiences that stimulated the cross-contamination. The study did not sample at households for *Salmonella* cross-contamination since it was challenging to conduct *Salmonella* sampling, e.g. costly and impossible for ethical consideration. Secondly, since the speculative nature of modeling, particularly as the model hasn't been validated, attribution studies based on field data would be more robust although much more expensive and challenging when implementing. There is also a huge gap between government reports and hospital cases as well as the limitation of cross-contamination of cooking at household base on the experiment due to the limited sample size used. Thirdly, the amount and frequency of pork eating also varied by individual and time. Therefore, the actual *Salmonella* cross-contamination and concentration might be lower which might lead to over-estimate and larger confidence interval of incidence in our findings. Moreover, this model has been applied for *Salmonella* in general using the Beta-Poisson dose-response and not specified for any *Salmonella* strains. In addition, our model was not able to simulate the differing susceptibility in different consumer groups (e.g. children or elder) as well as to the specific *Salmonella* strains. However, we propose the magnitude of salmonellosis was not much of our interest rather than for the future intervention along the pork value chains.

This study shows high levels of *Salmonella* from farm to final product (pork at market) along the smallholder pig value chains. The risk of salmonellosis in humans due to boiled pork consumption appears to be high. Feasible mitigations to improve hygiene practices are required to reduce the risk for the consumer. Control at farm may benefit from good agricultural practices as well as technological innovations such as water acidification [150]. Similarly good practices and adequate infrastructure can improve hygiene at slaughter and retail. Given the important role of cross-contamination in the kitchen, public education should address household practices.

5.5. Summary of Chapter 5

In this chapter, we have developed and used a quantitative microbial risk assessment to quantify salmonellosis risk in humans through consumption of boiled pork in urban Hung Yen province, Vietnam. We collected 302 samples along the pork value chain in Hung Yen between April 2014 and February 2015. We developed a model in @Risk, based on microbiological, market, and household surveys on cooking, cross-contamination and consumption, and conducted sensitivity analysis. *Salmonella* prevalence of pen floor swabs, slaughterhouse carcasses and cut pork were 33.3%, 41.7% and 44.4%, respectively. The annual incidence rate of salmonellosis in humans was estimated to be 17.7% (90% CI: 0.89 - 45.96). Parameters with the greatest influence risk were household pork handling practice followed by prevalence in pork sold in the central market. Wide confidence interval in the incidence estimate was mainly due to the variability in the degree of reduction in bacteria concentration by cooking, and pork consumption pattern. The risk of salmonellosis in humans due to boiled pork consumption appears to be high. Control measures may include improving the safety of retailed pork and improving household hygiene.

Chapter 6. General Discussions

6.1. Highlights of this thesis

Food safety is an important public health concern worldwide, especially in emerging economies. In Vietnam, pork production (provided over 80% by smallholders [78], and pork safety are of great interest to consumers, in policy discussions [61, 147]. Moreover, pork has been implicated as one of the most important sources of *Salmonella* (together with egg and poultry) in several countries [29, 37, 55, 105]. Risk assessments have been used very successfully in developed countries to help address issues of food safety. In recent years, this approach has also been used to assess risks of infections with microbial pathogens in informal markets [85]. Development and implementation of risk assessment will help scientists and policy makers involved in food safety by providing insight into the risks present in the value chain and help identify potential areas for successful mitigation of the risk.

Throughout studies conducted in this PhD thesis, I assessed the extent of *Salmonella* contamination and salmonellosis risk along the smallholder pork value chain in order to identify the optimal risk reduction strategies. To quantify the salmonellosis risks, hazard identification, characterization, and exposure assessment of QMRA framework [18] involving field investigations of smallholder pig farm, slaughterhouse and market was essential to obtain the contamination level, risk factors as well as consumption practices. I have used both participatory and field epidemiological approaches to explore knowledge, perceptions, and practices regarding disease in animals, food safety, and health risks among smallholder pork value chain actors and consumers, to identify *Salmonella* prevalence and potential risk factors along the pork value chain (Chapter 2 and 3). Second, I designed a laboratory experiment to determine cross-contamination of *Salmonella* in pork at household level (Chapter 4). Third, I applied QMRA methodology to quantify salmonellosis risks at the household level through pork consumption, using developed risk models (Chapter 5).

The outputs in Chapter 3 and Chapter 4 were used to develop the baseline QMRA models in Chapter 5. Thereafter, the optimal risk reduction strategies can be attained in consideration of affordable and practical intervention approaches. In this chapter, the main findings of my studies are highlighted and discussed among methods used and proposed opportunities on further future investigations with the links of the food safety risk assessment, management and communication.

6.1.1. Food safety perceptions and practices among smallholder pork value chain actors in Vietnam (Chapter 2)

Given recognition of the dominant role of smallholder pork production system in Vietnam in providing nutrition to the public, understanding the perception of food safety and risk-mitigating behaviors among the value chain actors and consumers are important for food safety risk assessment and management. This cross-sectional study by direct observation and questionnaire survey, combined with a participatory approach (key informant interviews, and focus group discussions) has characterized the structure, operation and role of smallholder pork value chain in Hung Yen province, Vietnam. More importantly, our study captured valuable insights into practices and perceptions of pork safety and quality among smallholder pork value chain actors. Subtle but important differences in perceptions of pig diseases and food safety among smallholder pork value chain actors were noted. There were considerable misperceptions surrounding zoonotic and foodborne diseases among farmers, slaughterhouse workers, and pork sellers. Slaughterhouse workers and pork sellers knew about some food safety risks associated with their pork handling practices, but they might not be aware of the degree of the risks.

Pig slaughtering and pork handling on transportation and sales were often performed without using adequate protective equipment. Sellers preferred to use wood tables over other materials to maintain perceived ‘‘freshness’’ of pork, despite the high risk of wood tables for the maintenance of microbial contamination of pork. Both slaughterhouse workers and pork sellers often used the same cloths for cleaning equipment and drying meat, presenting risks for cross-contamination. Misperceptions of slaughterhouse workers and pork sellers surrounding the risks of zoonoses for pork safety were observed. These findings suggested that training programs incorporating perception, animal and human health risks, and technical aspects for pork value chain actors are needed. However, training alone is unlikely to change behavior unless there are some additional motivations or incentive in place.

6.1.2. Risk factors associated with *Salmonella* spp. prevalence along smallholder pork value chains (Chapter 3)

In this study, I investigated the prevalence of, and risk factors for, *Salmonella* contamination in the smallholder pig farms, slaughterhouses and markets in Vietnam. The results showed that *Salmonella* prevalence was high along the chain, 36.1% (26/72), 38.9% (58/149), and 44.7% (97/217) on pig pen floors, pig carcasses in slaughterhouses, and cut pork in pork shops, respectively. In colder seasons, *Salmonella* prevalence was lower in slaughterhouses and markets, but not at farms. Our study also indicated the importance of improvement of bio-security measures in the small scale pig farms, particularly on the farm location and limiting the access. In slaughterhouses, the risk factor related to contamination of finished carcasses was slaughter area closed to lairage without hygienic measures, which implies frequent cross-contamination of finished carcasses with the source (e.g., faeces and wastewater) either directly or dispersed by workers, equipment to slaughterhouse surface. The risk factors at the pork markets were associated with not practicing control options such as fly control, and discouragement of using a common cloth to wipe pork, hands, and equipment.

This study provided useful information in planning applicable and effective intervention programs at each step of the value chain. However, planning interventions requires several more considerations, as smallholder pork value chains are characterized by complex relationships - many-to-many interactions among actors. First, designing effective and feasible form of intervention may be needed through single research project or collaborative trans-disciplinary research programs. Second, risk managers should decide which interventions at which points in the value chain should be targeted to achieve the best outcome. Third, they also need to carefully discuss a balance between intervention costs (development, implementation, and—most importantly—monitoring and evaluation of compliance) and the subsequent risk reduction in terms of both the number of illnesses or deaths avoided, and the public health costs saved. Moreover, economic impacts on the livelihood of smallholder actors due to the cost of compliance should be considered [48], as they may be vulnerable to the change even for a better public health.

6.1.3. A simulation of *Salmonella* cross-contamination on boiled but originally contaminated pork through cooking at home kitchen (Chapter 4)

The third study was conducted to assess the magnitude of cross-contamination of *Salmonella* from raw to boiled pork in households, by household questionnaire survey and laboratory experiment. Simulation experiments indicated that hands, wash-water, knives and cutting boards exposed to raw contaminated pork were the main sources of spread to boiled pork, even after once *Salmonella* were eliminated from pork surface. The level of *Salmonella* cross-contamination at the household kitchen was depending on the re-use of kitchen utensils between raw and cooked pork.

In this experiment, cross-contamination mainly occurred through use of same cutting board (scenarios 1 and 4). The step of washing equipment such as cutting boards and knives, and hands generally helps to reduce bacterial contamination [8, 17]. However in our study, washing of these equipment and hands, even using dish detergent, was not enough to remove *Salmonella* (Scenario 1 and 4).

The information both on the probability and magnitude of cross-contamination were used for quantitative risk assessment of salmonellosis through household pork consumption (presented in Chapter 5). The levels of cross-contamination in different scenarios will allow us to estimate the potential effects of possible risk-mitigating strategies. These findings therefore can help advocating the importance of improving food handling practices at household, as well as support risk communication and food safety education for consumers [19, 93], to minimize adverse health risk consequences. The findings also can help countering the common misperception that if pork is boiled well before eating, it does not present a risk.

6.1.4. Quantitative risk assessment for human salmonellosis in the smallholder pig value chains in urban of Vietnam (Chapter 5)

Based on the findings on the levels of *Salmonella* contamination along the smallholder value chain (Chapter 3) and experiment of *Salmonella* cross-contamination between raw and cooked pork at household (Chapter 4), I have developed a quantitative microbial risk assessment model to quantify salmonellosis risk in humans through consumption of boiled pork (as described in Chapter 5). The annual incidence rate of salmonellosis in humans was estimated to be 17.7% (90% CI: 0.89 - 45.96). In high income countries, several studies have suggested that consumption of pork is one of the major sources of human salmonellosis [96, 132]. However, there is little good evidence on the incidence of salmonellosis in LMIC. The recent WHO report on the global assessment of foodborne disease, estimated that the annual incidence of foodborne salmonellosis in the Asian region including Vietnam was 1% (range 0.2-7%) [56]. This is much lower than our estimate of 17.7%, but the methods are not comparable, and the WHO report is acknowledged to give conservative estimates. Parameters with the greatest influence on the risk were household pork handling practice followed by *Salmonella* prevalence in pork sold in the central market. The study has shown that, much of the burden of salmonellosis were due to cross-contamination at the household handling level, when the same knife and cutting board was used for both raw and boiled pork, and the importance of prevalence of *Salmonella* in marketed pork (as the second most influential factor).

This study is the first published QMRA applied for food safety in Vietnam in international peer-review. Risk-based approaches are now standard for food-safety issues in developed countries, as well as being the basis of rules governing international trade in food products. The study shows that QMRA can be applied to informal value chains and give credible information for managing risks. For the planning of interventions to reduce the risk for consumers, QMRA model can be used further to pre-evaluate the efficacy of the control options. The discussions in selection should take into account the feasibility. Control at farm may benefit from good agricultural practices as well as technological innovations [150]. Similarly good practices and adequate infrastructure can improve hygiene at slaughter and retail. Given the important role of cross-contamination in the kitchen, public education should address household practices.

6.2. Future research opportunities

Through the researches, several research opportunities on reducing the risk of salmonellosis in Vietnam through pork consumption were identified. First, the studies on food safety perceptions and practices suggested future research needs for risk perceptions and risky practices of pork value chain actors in the other communities in Vietnam as well as the other countries. Such qualitative approach should be useful for understanding willingness to change behaviors for potential intervention programs. Moreover, such perception study is applicable to other food categories, e.g., chicken, beef or vegetable.

Second, the risk factor study underlined the needs for evidence-based planning of effective and affordable intervention for salmonellosis through improvement of hygiene and management along the informal pork value chain, i.e., farm, slaughterhouse, and market. There are also needs of future evaluation on the attribution of risky practices at each stage of informal pork value chain.

Third, the present study on cross-contamination has clarified the research needs for intervention include studies on behavior-change theories, and trial intervention in the target population using validated instruments. For instance, food safety intervention programs focus on reducing the risk of cross-contamination on cutting boards in home kitchens may prove the effect of the intervention.

Fourth, this QMRA study did not model the most critical stage for planning intervention; risk reduction simulations at farms, slaughterhouses, or markets. Moreover, the QMRA model presents only urban Vietnam. The baseline QMRA model developed in this study provided necessary frameworks for *a-priori* intervention evaluation at the farm, slaughterhouse, market, and household level. These intervention models and the result of risk factors assessment can be used to evaluate the effectiveness on reducing human salmonellosis risk when certain intervention measures are taken placed, including social, cultural and economic aspects. With such information, intervention planning will become feasible and realistic to reduce the burden of salmonellosis in Vietnam and other parts of the world. The model developed in this study can be applied in answering the question on the risks under other food safety settings in Vietnam in the future. More importantly, risk assessment for food safety needs to be engaged in the linkages and interactions with risk management and risk communication, so that mutual understanding among policymakers, scientists, communication experts, value chain actors, and public is facilitated, which will result in minimizing health risks in smallholder pork value chain.

Other research outputs of the project that accommodated our study in this thesis, an ACIAR funded, coordinated by ILRI, abbreviated as PigRISK, include health economics and smallholder pork value chain economic assessment, and they are also useful for the future food safety implementations. This study also contribute to the risk management aspect under the national strategies for food safety, which currently addressed by the National food safety program. The finding in food safety health economics reported that the costs per treatment episode and per hospitalization day for foodborne diarrhea cases were US$ 106.9 and US$ 33.6, respectively [59]. The findings from system dynamics (SD) models of value chain assessment will be published elsewhere. The integration of the outputs from the QMRA including *a-priori* risk reduction, SD models, and the cost of illness (foodborne diarrhea) will be quite informative to discuss about cost-benefit and cost effectiveness. The considerations on ranking or prioritization of interventions will be carefully taken place, as quantitative methods may neglect social and cultural aspects.

The next project named as SafePork (2017-2022), which is designed based on the PigRISK project, aims to develop and evaluate market-based approaches to reduce the burden of foodborne diseases in informal, emerging formal, and niche markets targeting small and medium scale producers. The baseline and intervention QMRA models integrated with SD model will be used in the SafePORK project as a tool for a pre- and post-trials and/or pilot assessment [68]. The models can be also applied for the other CGIAR Research Programs (CRPs) looking for interventions for a better health outcomes and beneficial agri-food systems, such as CRPs on livestock, agriculture for nutrition and health, climate change, or policies, institutions and markets [21]. The pork value chain in Vietnam has been identified as systems where the "incentive-based, light touch" approaches for food safety [21] to meet the Sustainable Development Goals (SDGs) of the world [130].

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RESEARCH ACHIEVEMENTS

Publications related to this thesis

Dang-Xuan, S., Nguyen-Viet, H., Meeyam, T., Fries, R., Nguyen-Thanh, H., Pham-Duc, P., Lam, S., Grace, D. and Unger, F. 2016. Food safety perceptions and practices among smallholder pork value chain actors in Hung Yen province, Vietnam. *J. Food Prot.*, **79**:1490-1497.

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ABSTRACT

Food safety is an important public health concern worldwide, especially in emerging economies, including Vietnam, where pork production plays an important role in both livelihood and diet. Throughout studies conducted in this PhD thesis, the overall goals of this research was to assess the extent of *Salmonella* contamination along the smallholder pork value chain in Vietnam and determine the risk of salmonellosis in humans to aid in the production of the risk reduction strategies. We have used both participatory and field epidemiological approaches to explore food safety perceptions and practices among a variety of different occupational streams involved in the smallholder value chain and pork consumers, and to identify *Salmonella* prevalence and potential risk factors along the chain. A consumer survey and laboratory experiments were also conducted to determine pork handling practices and cross-contamination risks of *Salmonella* in pork in Vietnamese households. We have developed a quantitative microbial risk assessment (QMRA) model to quantify salmonellosis risks in the household through pork consumption.

The first study findings relating to food safety practices and perceptions held by pork value chain associated employees and consumers indicated that most slaughterhouse workers acquired knowledge and experience of food safety through ‘‘learning by doing’’ rather than from training. The workers and sellers often use the same cloths to dry the meat and clean equipment without thinking of contamination risks. They were found to possess some accurate perceptions about swine and foodborne diseases but had misperceptions of zoonoses risks. Consumers perceived that pork freshness was a strong indicator of food safety and perceived that sellers may have health issues that they are trying to conceal by wearing

protective equipment (e.g., gloves, masks). Veterinary and public health staff emphasized the gap between regulations and food safety practices.

The investigation results on *Salmonella* contamination and risk factors demonstrated *Salmonella* prevalence to be 36.1% (26/72), 38.9% (58/149), and 44.7% (97/217) on pig pen floors, pig carcasses in slaughterhouses and cut pork in pork shops, respectively. The risk factors for *Salmonella* prevalence on pig pen floors included having a pig pen next to the household (p = 0.055) and free access to the farm by visitors (p = 0.061). Slaughter areas close to lairage without hygienic measures was a risk factor for carcass contamination at the slaughterhouse (p = 0.031). For pork shops, presence of flies or insects on the pork at shop (p = 0.021) and use of cloths at pork shop (p = 0.023) were risk factors. The *Salmonella* prevalence on pig carcasses and cut pork was significantly lower in winter compared to other seasons.

A household survey revealed that most people (71%) used the same knife and cutting board for both raw and cooked pork. Simulation experiments indicated that hands, wash-water, knives and cutting boards exposed to raw contaminated pork were the main source of *Salmonella* spread to cooked pork. 78% of cooked pork samples were contaminated with *Salmonella* when the same hands, knife and cutting board were used for both raw and cooked pork. Using the same cutting board resulted in 67% of cooked pork samples becoming contaminated with *Salmonella*. The results on quantifying salmonellosis using a QMRA model found the annual incidence rate of salmonellosis in humans to be estimated as 17.7% (90% CI: 0.89 - 45.96). Parameters with the greatest influence risk were household pork handling practices followed by prevalence of *Salmonella* in pork sold in the central market.

Our results highlighted the need for prioritization of education and training among pork value chain associated employees on food safety risks and proper handling. Risk factors for *Salmonella* contamination at farms, slaughterhouses and markets need to be addressed by planning effective and affordable control options for human salmonellosis by improving pork hygiene along the informal pork value chain. Control measures may include improving the safety of retail pork and improving household hygiene. Moreover, findings also provided information relating to the level of understanding about cross-contamination in households. From these insights, future education programs may be based on communication with households about strategies for improved food safety. Our work constitutes original evidence in food safety with the aim of understanding pork safety and its heath impact. From this research, intervention strategies to improve food safety with links to food safety risk assessments, management and communication may be put into place.

ABSTRACT IN JAPANESE（和文要旨）

食品衛生は世界中の国々にとって重要課題であるが、特に豚肉生産が生計・栄養・食糧確保の面で重要な位置を占める新興国ベトナムにとっては公衆衛生上大変重要である。本博士論文はベトナムの小規模養豚場で生産された豚肉バリューチェイン上のサルモネラ汚染程度の理解と、人の感染リスクの最適な低減措置を探るためのリスク評価を総合目標とした。目的遂行のため、本研究ではまずベトナムの典型的豚肉バリューチェインが存在するフン・イェン省とンゲ・アン省にて、小規模養豚場を基点とする農場から食卓までの各ポイントに携わる人々の食品衛生に対する意識と行動、サルモネラ汚染率とそのリスク因子を知るために、参加型および実地疫学的アプローチの両方を用いた。さらに家庭における豚肉の取り扱いと交差汚染の程度を理解するため、消費者調査と実験室での実験を実施した。そして最後に家庭での豚肉喫食によるサルモネラ症リスクの定量的微生物学的リスク評価 (QMRA) を実施した。

豚肉バリューチェインに携わる関係者と消費者の食品衛生に関する意識と行動については、と畜場で働くと夫は研修を受けずに実地経験から食品衛生の知識を得ていた。と夫と露天販売の精肉屋は肉の交差汚染の意識はなく、一般的に肉と道具を共通の布で拭いていた。これらの人々は豚病と食中毒について、ある程度正確な知識を有するものの、とりわけ人獣共通感染症については不正確に理解していた。消費者は食品衛生の指標として豚肉の鮮度を重要視しており、精肉販売者がグローブやマスクをしていると却って何か病気を持っているのではないかと疑っていた。

獣医師と公衆衛生担当者は、食品衛生に関する法律と実際の履行には乖離があることを強調していた。

サルモネラ汚染程度の調査では、サルモネラ汚染率は、豚房の床、と畜場でのと体、精肉店の豚肉ブロックにおいてそれぞれ 36.1% (26/72)、38.9% (58/149)、44.7% (97/217)であった。サルモネラ汚染のリスク因子は、豚房の床の汚染については母屋と豚房が隣接していること($p = 0.055$)と、訪問者の養豚場出入り制限がないこと($p = 0.061$)であった。と畜場においては、と畜エリアと豚の待機エリアが特段の衛生的配慮もなく近いことが枝肉汚染のリスク因子であった ($p = 0.031$)。豚肉販売店については、販売店におけるハエあるいは昆虫がいること ($p = 0.021$) と、豚肉を布で拭いていること ($p = 0.023$) がリスク因子であった。豚と体と豚肉ブロックにおけるサルモネラ汚染率は、気温が低い季節に低かった。

消費者調査では、71%の人々が生肉と茹でた豚肉の両方に同じ包丁とまな板を使っていた。再現実験の結果、生肉に触れた手、豚肉を洗った水、包丁とまな板が茹でた肉への交差汚染源であることが示唆された。生肉と茹でた肉の両方に同じ手、包丁、まな板を用いた場合の 78%で茹で豚肉へのサルモネラ交差汚染が起きた。また生肉と茹で豚肉に同じまな板を用いた場合、67%でサルモネラ交差汚染が起きた。ベトナムで始めて実施された QMRA モデルを用いたサルモネラ症定量化の結果、ベトナム人における年間サルモネラ症発生率は、17.7% (90% CI: 0.89 - 45.96) であった。最も感度の高い因子は家庭での豚肉の取り扱いで、次いで中央市場で販売されている豚肉における汚染率であった。

本論文での研究を通して、豚肉バリューチェインの関係者への食品衛生リスクと適切な豚肉の取り扱いに関する教育・研修機会を提供することが優先事項であると考えられた。農場、と畜場、市場レベルでのサルモネラ汚染リスク因子の把握は、非正規豚肉バリューチェインの衛生向上の効果的かつ経済的に可能な対策立案に有用である。対策には、小売豚肉と家庭での衛生向上も含まれる。また家庭での交差汚染による健康影響が明らかになったことから、家庭での食品衛生管理の教育、普及啓蒙の重要性が認識された。本研究は、ベトナム発の食品衛生に関する新知見であり、今後同国におけるリスク評価、マネジメント、コミュニケーションに基づく戦略的食品衛生の継続的向上の礎になる。