International Journal of Food Microbiology 290 (2019) 105–115

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Risk factors associated with *Salmonella* spp. prevalence along smallholder pig value chains in Vietnam

Sinh Dang-Xuan[a,b], Hung Nguyen-Viet[a,c], Phuc Pham-Duc[a], Fred Unger[c], Ngan Tran-Thi[a], Delia Grace[d], Kohei Makita[b,*]

[a] Center for Public Health and Ecosystem Research, Hanoi University of Public Health, 1A Duc Thang Road, Duc Thang Ward, North Tu Liem District, Hanoi 100000, Viet Nam
[b] Veterinary Epidemiology Unit, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[c] International Livestock Research Institute, 298 Kim Ma Street, Hanoi 10000, Viet Nam
[d] International Livestock Research Institute, 30709 Naivasha Street, Nairobi 00100, Kenya

ARTICLE INFO

Keywords:
Pig farm
Pig slaughterhouse
Pig carcass
Pork shop
Mixed-effects model

ABSTRACT

The objective of this study was to investigate the prevalence of, and risk factors for, *Salmonella* contamination along the smallholder pig value chain in northern Vietnam. Repeat cross-sectional (for farm and pork shops) and longitudinal (for slaughterhouses) studies were 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 at the farm, slaughterhouse, and pork shop levels were performed using generalized mixed-effects models with the significant levels 10%.

Salmonella prevalence was 36.1%, 38.9%, and 44.7% 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 a pig pen next to a household ($p = 0.06$) and free access to the farm by visitors ($p = 0.06$). Our slaughterhouse model found a single risk factor for carcass contamination: slaughter area close to lairage without hygienic measures ($p = 0.03$). For pork shops, presence of flies or insects on pork at shop ($p = 0.02$) and use of a cloth at pork shop ($p = 0.02$) were risk factors. The *Salmonella* prevalence on pig carcass and cut pork was significantly lower in winter compared to that in other seasons. Our study results highlighted the need of improving farm hygiene at farm level, and pork hygiene practices to avoid cross-contamination at the slaughterhouse and market levels, to reduce the risk of salmonellosis through pork consumption in northern Vietnam.

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 (Majowicz et al., 2010), whereas reported cases of human salmonellosis in the United States in 2009 and in the European Union in 2015 were approximately 40,000 (CDC, 2009) and 94,000 (EFSA and ECDC, 2016), respectively. Pork has been implicated as one of the most important sources of *Salmonella* (together with egg and poultry) in several countries (Davidson et al., 2011; EFSA, 2008; Havelaar et al., 2008; Pires et al., 2014). The estimated annual cost of human *Salmonella* infections in 2008 from all sources was about € 608 million in the European Union (FCC, 2010) and about $3.4 billion in the US in 2013 (USDA, 2013). This economic burden of *Salmonella* infection is significant in both low and middle income countries (LMIC), and high income countries, implying the need for enhanced monitoring and reporting systems, improved food safety, and greater consumer awareness (Schwartz, 1999). However, intervention programs to control *Salmonella* in pork production are costly, requiring investment in biosecurity facilities and training on hygiene practices in farms, slaughterhouses, processing plants, and retail outlets. Therefore, understanding risk factors is expected to facilitate the targeting of effective intervention points and reducing associated costs.

Salmonella prevalence and related risk factors in the pig value chain have been well characterized in the United States, Australia, and Canada, as well as in European Union countries. *Salmonella* contamination of

* Corresponding author.
E-mail addresses: dxs@huph.edu.vn (S. Dang-Xuan), H.Nguyen@cgiar.org (H. Nguyen-Viet), pdp@huph.edu.vn (P. Pham-Duc), F.Unger@cgiar.org (F. Unger), ttn@huph.edu.vn (N. Tran-Thi), D.GRACE@cgiar.org (D. Grace), kmakita@rakuno.ac.jp (K. Makita).

https://doi.org/10.1016/j.ijfoodmicro.2018.09.030
Received 3 February 2018; Received in revised form 6 August 2018; Accepted 29 September 2018
Available online 06 October 2018

finished carcasses can be linked to farm level *Salmonella* infection in pigs destined for slaughter (Berends et al., 1996). At the slaughterhouse, cross-contamination has been shown to significantly affect the occurrence of *Salmonella* on pig carcasses (Duggan et al., 2010). At the distribution level, *Salmonella* contamination has been found to be related to the type of retail outlet (Hansen et al., 2010). Contamination of pork has important health and financial implications: in the Netherlands and Germany, it has been estimated that 15–20% of *Salmonella* infections in humans are caused by consumption of contaminated pork or processed pork (Berends et al., 1996; Steinbach and Kroell, 1999).

Pork consumption in Vietnam is relatively high compared to that in other countries with similar GDP (29.1 kg pork per capita yearly) (OECD, 2016); most (80%) of this pork is produced by small-scale producers and sold by small-scale retailers (Lapar and Tiongco, 2011). Recent studies in the Mekong Delta revealed poor hygiene at smallholder pig farms: 8.2% of drinking water for pigs sourced from local rivers or ponds was contaminated with *Salmonella* (Tran et al., 2004). Pig abattoirs in Hanoi that processed 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 (Le Bas et al., 2006). In Hue province in central Vietnam, similar results were found after sampling various surfaces in slaughterhouses, such as cooking boards (28.6%), weighing bowls (38.1%), and floors (47.4%) (Takeshi et al., 2009). At the retail level, most pork in Vietnam is sold in informal "wet" markets. These open-air markets, which can take the forms of central markets, village markets, or roadside vendors, can consist of as many as 20 pork stalls or as few as 1–2 (Dang-Xuan et al., 2017). Studies in northern Vietnam found prevalences of *Salmonella* in pork wet markets of 39.6% (Thai et al., 2012) and 25% (Yokozawa et al., 2016), and a study in southern Vietnam detected a prevalence of 69.9% (Phan et al., 2005). The *Salmonella* serotypes reported in Vietnam were *S. typhimurium*, *S.* Anatum and *S.* Weltevreden in pig faeces at farm (Tran et al., 2004; Vo et al., 2006); *S. typhimurium*, *S. derby* on pig carcasses at slaughterhouse (Dang-Xuan, 2013); and *S. derby*, *S.* Weltevreden, *S.* London (Dang-Xuan, 2013; Phan et al., 2005), *S.* Anatum and *S. infantis* (Thai et al., 2012) in pork at market. In particular, *S. typhimurium* was one of the most isolated *Salmonella* serotype (21/56, 37.5%) from diarrheal and febrile patients in Vietnam (Vo et al., 2006). These studies illustrate that *Salmonella* prevalence varies widely in different settings along the Vietnamese pig value chain.

This study was conducted as a part of a project, entitled "Reducing disease risks and improving food safety in smallholder pig value chains in Vietnam (PigRisk)", that sought to assess the impacts of pork-borne diseases on human health and to identify effective and feasible risk management options. The specific aim of this study was to investigate the prevalence of, and risk factors for, *Salmonella* contamination along the smallholder pig value chains in northern Vietnam.

2. Material and methods

2.1. Study sites and target population

This 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 (ILRI, 2013). Three communes were randomly selected from each of these selected districts, yielding a total of 18 communes: nine out of 161 communes in Hung Yen, and nine out of 469 communes 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 (Fig. 1). The scope of the research was the smallholder pig value chain (i.e., pig farm, slaughterhouse, and market), and slaughtering process, illustrated in Fig. 2. Therefore, farms, slaughterhouses, and markets in this study were selected to represent both small- to medium-scale farms (i.e., ≤10 pigs and 11–100 pigs, respectively), small- to medium-scale slaughterhouses (i.e., 1–10 pigs/day and 11–50 pigs/day, respectively) and wet markets (Dang-Xuan et al., 2017).

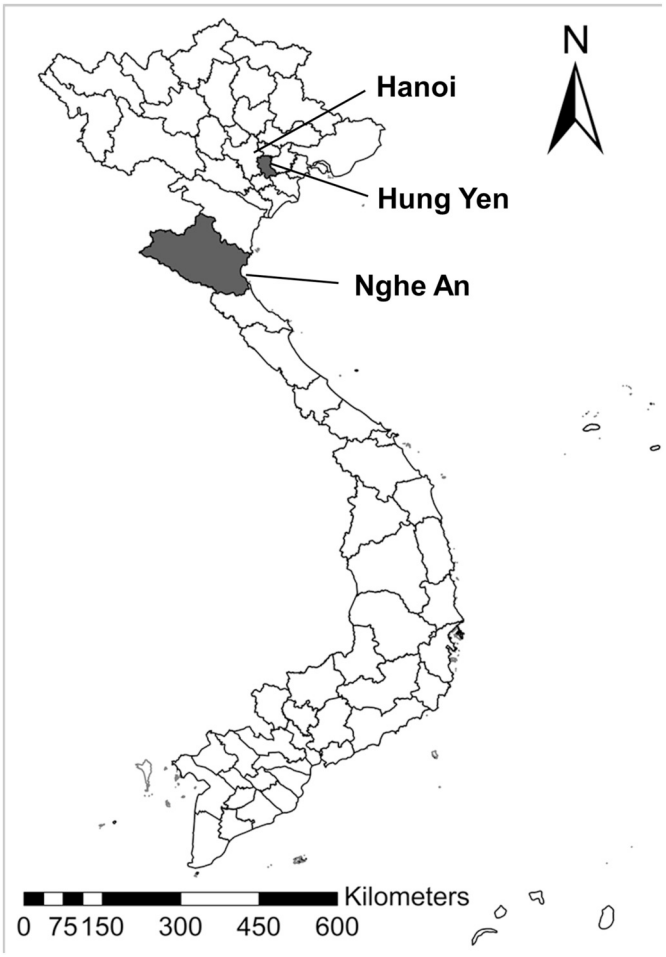

Fig. 1. A map showing the locations of study sites: Hung Yen and Nghe An provinces, and the capital city of Vietnam, Hanoi.

2.2. Study design

2.2.1. Study design and sample sizes

The study design included a repeat cross-sectional study for the farm and pork shops, and a longitudinal study for slaughterhouses. Sample sizes were based on a comparison of two proportions with a precision of minimum detectable difference of 10% in prevalence 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% (Dohoo et al., 2009).

For the sample size at the farm level, the expected *Salmonella* prevalence on the pig pen floor was set at 25%, a value that fell approximately in the middle of reported range of prevalences (8.2% (Tran et al., 2004) and 49.4% (Vo et al., 2006)), and the difference in prevalence between exposed and non-exposed groups to detect was set at 15%. The expected *Salmonella* prevalences on slaughtered pig carcasses and retailed cut pork were determined to be 34.9% (Dang-Xuan, 2013) and 32.8% (Takeshi et al., 2009), respectively, as previously described, and the difference in prevalences between exposed and non-exposed groups to detect was set at 10% both for carcass and cut pork. Using epitools package in R (Aragon et al., 2017), the minimum required sample sizes were calculated as 60 farms, 146 pig carcasses, and 143 pork shops. The actual number of samples included 72 farms, 149 carcasses from 13 abattoirs, and 217 pork samples from 145 shops.

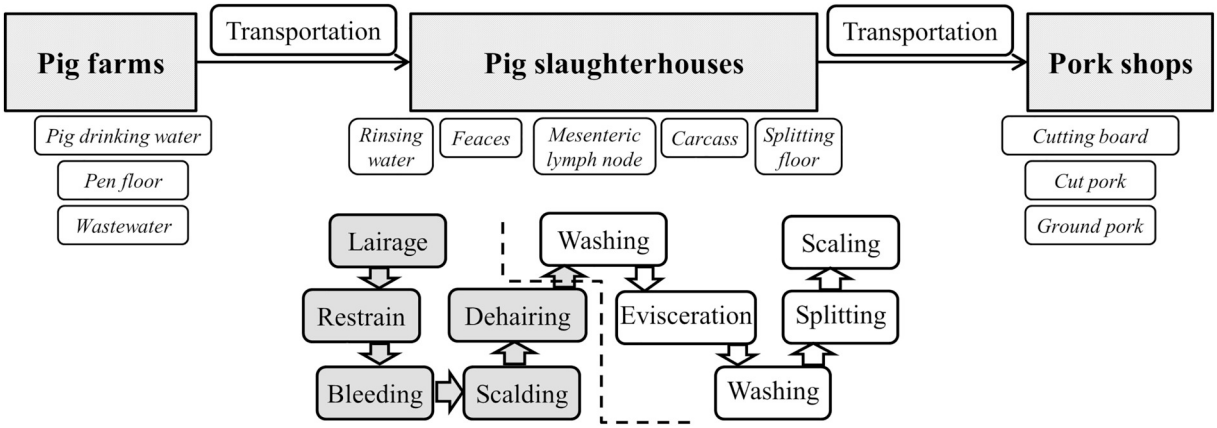

Fig. 2. Smallholder pig value chain and slaughtering process. Wider allows show the process in slaughtering. The dashed line shows ideal demarcation of clean and dirty zones in a slaughterhouse, although slaughtering was commonly practiced without separation of these zones.

2.2.2. 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. 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., equally split between exposed and no-exposed). With these assumptions, the farm and slaughterhouse (carcass swab) 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 the relevant calculations are available on request.

2.2.3. Sampling framework

Sampling was performed in four periods over the course of a year (2014 April to June 2014 July to September 2014 October to November, and 2014 December to 2015 February). Samples were collected from farms and wet markets in all 18 communes, and from slaughterhouses in 13 communes. No samples were collected in March of either year. During each sampling visit, the researchers visited the slaughterhouses from 2:00 a.m. to 6:00 a.m., the market from 6:30 a.m. to 9:00 a.m., and the farms between 9:00 a.m. and 11:00 a.m., to avoid the variabilities of *Salmonella* detection and concentration, as *Salmonella* can increase over time.

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

Up to two slaughterhouses were visited per district during a given sampling period (except for the first visit, when the maximum three were visited), permitting visits to a total of 13 slaughterhouses, of which seven were in Hung Yen and six in Nghe An. Samples were collected as follows: from mesenteric lymph nodes (MLNs), faeces, and carcass of a given pig; the slaughterhouse splitting floor; and from carcass rinse water. These slaughterhouses were visited repeatedly (four times) over the course of the study period.

For markets, nine pork shops were selected per district in each sampling period, except for one district in the third period, in which ten shops were visited. 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. Given the limited number of pork shops in selected communes, forty-one shops were visited more than once during the year of study; specifically, 14, 23 and four shops were visited two, three, and four times, respectively. In addition, to permit tracking of contamination from finished carcasses at a slaughterhouse to the contamination status of cut pork at a market, a sub-set of 63 carcasses was tracked to permit parallel sampling of these carcasses and the resulting cut pork.

2.2.4. Sampling methods for bacteriological samples

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 premoistened gauze (5 cm × 5 cm, four layers) with 20 ml Buffered Peptone Water, BPW (Merck-Germany), forceps, and a 5 cm × 5 cm steel frame. Separately, samples consisting of 1 l of pig drinking water and 0.1 l of wastewater (from a pig pen wastewater tank or biogas effluent tank) were collected aseptically into sterile bottles.

We aimed to sample three pigs per slaughterhouse in each sampling period over the course of the year. However, sometimes fewer pigs were sampled due to the limited availability of animals, and occasionally up to five pigs were sampled per slaughterhouse to catch up with the schedule. Practically, in each slaughterhouse, pigs were selected for sampling either from all pigs when five or fewer pigs slaughtered, or every second or third pig until the number of pig sampled reaches five when more than five pigs were slaughtered. Immediately after evisceration, a rectal faecal sample (approximately 50 g) was collected using sterile forceps and a wooden stick, and a MLN sample (approximately 30 g) using sterile forceps and scalpel. Immediately after the final washing step, each split carcass was systematically swabbed at four positions (100 cm^2 each) along the medial carcass surface (i.e., lower part of neck, mid-back, abdomen, and hind limb), using the non-destructive technique described above, yielding a total sample surface area of 400 cm^2 (ISO-17604, 2003). A 25-cm^2 area of the slaughterhouse floor (from an area in the middle of slaughtering operation; where carcass splitting was performed) was swabbed, and 1 l of carcass rinsing water was collected, using the sampling procedures used on farms (described above).

At each market, approximately 400 g 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 by the researchers. A 25-cm^2 section of the cutting board surface in the pork shops was swabbed using the same procedure as that described for the pen floor (above).

Each surface sample was placed in a sterile bag 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 h. The laboratory tests were performed at the National Institute of Veterinary Research, Hanoi, Vietnam.

2.3. Sample preparation and microbiological analysis

All surface samples were diluted in the original sterile plastic bags by addition of BPW to yield a total volume of 100 ml. An aliquot (10 ml) of farm wastewater was pipetted into a sterile plastic bag containing 90 ml BPW. An aliquot (100 ml) of the pig drinking water or carcass rinse water was filtered through a membrane (0.45 µm pore size; Millipore, USA), and each membrane was placed in a sterile plastic bag containing 100 ml BPW. A 10-g portion of rectal faeces or MLN was added to 90 ml BPW in a sterile plastic bag. A 25-g portion of cut or ground pork was combined with 225 ml BPW in a sterile plastic bag, and homogenised using stomacher (400 Circulator, Seward, UK).

Salmonella isolation followed the ISO procedure (ISO-6579, 2002), which has been abrogated in 2017 with the revision of the enrichment and isolation media. The BPW homogenate was incubated for 16–20 h 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 1 ml aliquot, and a Modified Semisolid Rappaport-Vassiliadis (MSRV; Merck, Germany) agar plate was inoculated with three pipette drops (approximately 50 µl). Both media were incubated for 16–20 h at 37 °C. This selection step was repeated, by using one loop (approximately 10 µl) of 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, and Urease phenotypes) and another one to two colonies to inoculate Nutrient Agar (NA; Merck, Germany) to grow *Salmonella* for serological confirmation, using Antiserum *Salmonella* Polyvalent-O (Bio-Rad, France). *Salmonella* enumeration was performed only for the pork (cut and ground) samples using a traditional 3-tube MPN (Most Probable Number) method (Pavic et al., 2010), and the calculation table was used to determine the MPN (de Man, 1983).

2.4. Data collection

Observation checklists were used to collect information on management, facilities, equipment, and hygienic practices at farms, slaughterhouses and markets. Information on live pig or pork management during transportation from a farm to a slaughterhouse, and from a slaughterhouse to a market were obtained from questionnaires (Supplement Table 1). 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 pretested in Hung Yen province. Checklist data came from direct observation on farms, in slaughterhouses and at market operations by experienced researchers, whereas the questionnaire was administered face-to-face with slaughterhouse owners during each sampling visit.

2.5. Data management and statistical analysis

Checklist results, questionnaire data, and laboratory results were recorded and processed in Microsoft Excel 2010. Data from checklists and questionnaires were screened to eliminate variables that were considered redundant or low variation (Supplement Table 1). Descriptive statistics were performed, and statistical computing was interpreted with *p*-value of 0.1. Statistical software RStudio version 1.1.456 (RStudio Team, 2018) using R version 3.5.1 (R Foundation, 2018), 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-bred pigs), and Wilcoxon Rank Sum tests were performed to compare the average weight of live pigs and the number of pigs slaughtered between the 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 the generalized linear mixed-effects models (GLMMs) in lme4 package (Bates et al., 2017) in R. For the farm-level model, the outcome variable was *Salmonella* contamination status (positive or negative) on the pen floor, and commune was set as random effect. For the slaughterhouse-level model, *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 model, *Salmonella* contamination status on cut pork samples was used as the outcome variable and commune was set as a random effect. 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, Fig. 3). 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 (Dohoo et al., 2009) also was calculated.

2.6. Ethical considerations

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. Results

3.1. Smallholder pig value chains

The pig farms in Hung Yen were characterized by more intensified farming than those in Nghe An; the farm size in Hung Yen is larger than Nghe An, and exotic-bred pigs dominated in Hung Yen (33/36 farms, 91.7%), while cross-bred pigs dominated in Nghe An (20/36 farms, 5.6%, Table 1). Out of 26 variables on farm checklist, we subjectively excluded six redundant and five low variability variables. Thus, 13 variables were included for the univariable analysis, and other two variables were used for descriptive purposes.

Of the 13 slaughterhouses involved in the study, six had the capacity to slaughter 11–50 pigs/day, and seven slaughtered ≤ 10 pigs/day. In total, slaughterhouses were visited 49 times, and 149 pigs were sampled. The results summarized in Table 1 are presented based on the number of visits. Out of 60 variables (17 from questionnaire, and 43 from checklist) from slaughterhouses, we subjectively excluded 18 redundant and 19 low variability variables. Thus, 23 variables were included for general description and the univariable analysis.

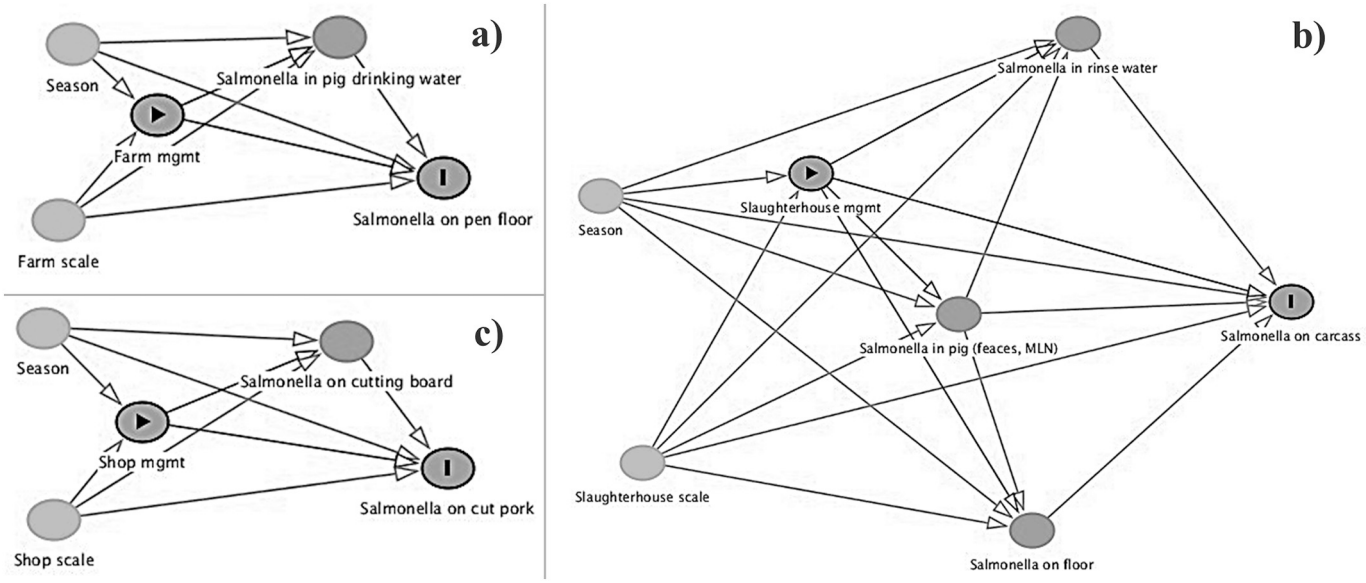

Fig. 3. Causal diagrams, for farm (a), slaughterhouse (b) and market (c), shows exposure variables of interest (dark circles with a triangle), intervening variables (dark grey circles), as well as potential confounders (light grey circles) related to the outcome variables (dark circles with a vertical bar, adapted from source: http://www.dagitty.net/dags.html). *Farm mgmt* includes variables related to farm management, facilities, and biosecurity; *Slaughterhouse mgmt* includes variables related to live pig management during transportation, slaughterhouse management, facilities and practices; *Shop mgmt* includes variables related to management, equipment, and hygiene practices at shop; MLN: mesenteric lymph node.

All of the pork shops surveyed in both provinces were 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) (Table 1). Out of 43 variables from the shop checklist, we excluded 16 redundant and 13 low variability variables. Thus, 14 variables at pork shops were included for general description and the univariable analysis.

3.2. Salmonella prevalence

Table 2 shows the *Salmonella* prevalence along the smallholder pig value chain. In general, prevalence was high at all stages (farm, slaughterhouse, and market). In pig farms, the prevalences on the pen floor (36.1%) and in wastewater (38.9%) were significantly higher than that in the drinking water (19.4%, $p = 0.04$; and $p = 0.02$, respectively). The results of *Salmonella* positivity in drinking water had high level of agreement with the results on the pen floor ($p = 0.02$), as well as in wastewater ($p = 0.02$, McNemar test).

In slaughterhouses, the prevalence on pig carcasses (38.9%) was not significantly different from that in rectal faeces (33.6%, $p = 0.40$). 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 also used for washing hands and equipment). The McNemar test showed no significant agreement between the *Salmonella* infection status in faeces and MLNs ($p = 0.77$).

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%, $p < 0.01$, and $p = 0.01$, respectively, Table 2). The *Salmonella* prevalence in cut pork and on cutting boards showed a high level of agreement by the McNemar test ($p < 0.01$).

3.3. Univariable analysis

Tables 3, 4, and 5 show the univariable GLMM analysis results for pig farms, slaughterhouses, and markets, respectively. At farms, there were two significant factors related with *Salmonella* prevalence on pig pen floors: medium-scale pig farms (odds ratio: OR = 7.1, $p = 0.03$), and free entry onto the farm by visitors (OR = 4.4, $p = 0.04$). At slaughterhouses, winter season (sampling from December to February) was associated with decreased risk of *Salmonella* positivity (OR = 0.1, $p = 0.01$). At markets, the significant factor associated with reduction

Table 1
General information and descriptive statistics comparing 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 carcasses sampled	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 visits in which the table used was observed to be higher than 60 cm	105	89	194 (89.4)**

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

Table 2
Salmonella prevalence by sample type in smallholder pig value chain in Vietnam.

Sample type	No. of positive/no. of samples	*Salmonella* prevalence (95% CI)*	No. of negative farm, slaughterhouse, shop/no. of visits**
Pig farm			
Pig drinking water	14/72	19.4 (12.0–30.0)[a]	30/72
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]	32/49
Splitting floor	11/49	22.5 (13.0–35.9)[a]	
Rectal faeces	50/149	33.6 (26.5–41.5)[b]	73/149***
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			
Ground pork	33/80	41.3 (31.1–52.2)[a]	
Cutting board	55/217	25.3 (20.0–31.5)[b]	108/217
Cut pork	97/217	44.7 (38.2–51.4)[a]	

CI: Confidence interval.
* Prevalences with different letters were significantly different ($p < 0.05$).
** Farms, slaughterhouses, and shops with no positive samples, versus positive ones had at least one positive sample.
*** Number of pigs.

of *Salmonella*-positive cut pork was winter season (OR = 0.2, $p < 0.01$), while presence of flies or insects on pork or table was a risk factor for *Salmonella*-positive cut pork (OR = 1.8, $p = 0.05$).

3.4. Multivariable analysis

At farms, there were two risk factors associated with the contamination of a pen floor with *Salmonella*: having pig pens located next

Table 3
Univariable GLMM results at the farm level.

Variables	*Salmonella* positive/tested	Prevalence (%)	Adjusted odds ratio	95% CI	*p*-Value
Sampling season					0.46
April–June	5/18	27.8	Ref	–	–
July–September	8/18	44.4	2.4	0.5–11.1	0.26
October–November	8/18	44.4	2.4	0.5–11.3	0.26
December–February	5/18	27.8	1.0	0.2–4.8	0.99
Farm management					
Scale of fattening farm					
Medium (11–100 pigs)	24/54	44.4	7.1	1.2–41.3	**0.03**
Small (≤10 pigs)	2/18	11.1			
Keeping sows at farm for breeding					
Yes	20/51	39.2	1.6	0.5–5.6	0.45
No	6/21	28.6			
Keeping pigs and other animals in the same area					
Yes	7/17	41.2	1.4	0.4–5.1	0.63
No	19/55	34.5			
Mix pig batches in the same pen					
Yes	2/3	66.7	4.8	0.2–94.8	0.30
No	24/69	34.8			
Using biogas system to treat waste from pens					
Yes	18/48	37.5	1.3	0.4–4.5	0.68
No	8/24	33.3			
Farm facility and biosecurity					
Pig pens located next to household					
Yes	24/58	41.4	4.7	0.8–29.2	**0.10**
No	2/14	14.3			
Pig drinking water stored in open tank					
Yes	15/35	42.9	1.9	0.6–6.3	0.26
No	11/37	29.7			
Visitors can freely enter farm					
Yes	15/31	48.4	4.4	1.1–18.5	**0.04**
No	11/41	26.8			
Having boot disinfection bath at farm					
Yes	8/23	34.8	0.8	0.2–2.7	0.67
No	18/49	36.7			
Worker wear uniform and boots					
Yes	8/23	34.8	0.8	0.2–2.7	0.67
No	18/49	36.7			
Farm facilities are clean					
Yes	16/45	35.6	1.0	0.3–3.4	0.97
No	10/27	37.0			
Presence of insects at farm					
Yes	20/53	37.7	1.5	0.4–5.7	0.52
No	6/19	31.6			

CI: Confidence interval, *p*-value in bold: selected variables (with $p \leq 0.2$) for multivariable analysis.

Table 4
Univariable GLMM results at the slaughterhouse level.

Variables	*Salmonella* positive/tested	Prevalence (%)	Adjusted odds ratio	95% CI	*p*-Value
Sampling season					**0.02**
April–June	19/37	51.4	Ref		
July–September	16/39	41.0	0.5	0.1–2.1	0.33
October–November	17/36	47.2	0.6	0.1–2.8	0.55
December–February	6/37	16.2	0.1	0.0–0.5	0.01
Source pig information					
Live pig farm scale					
Medium (11–100 pigs)	46/103	44.7	2.8	0.8–9.3	**0.10**
Small (≤10 pigs)	12/46	26.1			
Breed of pig					
Indigenous	16/50	32.0	0.6	0.2–2.0	0.41
Exotic	42/99	42.4			
Clean and disinfected transport vehicle					
Yes	40/97	41.2	1.3	0.4–3.9	0.70
No	18/52	34.6			
Time of pig arrival to slaughterhouse					
Afternoon (of a previous day)	14/34	41.2	1.2	0.3–4.4	0.77
Morning (of a previous day)	44/115	38.3			
Duration of transportation of live pigs					
> 1 h	4/9	44.4	1.6	0.2–15.0	0.70
< 1 h	54/140	38.6			
Slaughterhouse management					
Scale of slaughterhouse					
Small (≤10 pigs/day)	16/54	29.6	0.5	0.2–1.4	**0.18**
Medium (11–100 pigs/day)	42/95	44.2			
Slaughterhouse in the same house's compartment					
Yes	31/84	36.9	0.8	0.2–2.5	0.68
No	27/65	41.5			
Keep > 1 pig per m^2 in lairage					
Yes	40/106	41.9	1.6	0.4–5.6	0.49
No	18/43	37.8			
Slaughter area closes to lairage without hygienic measures					
Yes	53/123	43.1	5.0	0.9–27.6	**0.06**
No	5/26	19.2			
Presence of flies, blue flies, or rats in slaughter areas					
Yes	23/53	43.4	1.5	0.4–5.0	0.54
No	35/96	36.5			
Slaughterhouse facilities					
Slaughtering is processed on table or shelf					
Yes	3/5	60.0	4.1	0.2–67.1	0.33
No	55/144	38.2			
Tank water used for washing carcass and floor					
Yes	43/111	38.7	0.7	0.2–2.5	0.58
No	15/38	39.5			
Scalding vat water used in slaughtering					
Yes	23/46	50.0	2.2	0.7–7.6	**0.19**
No	35/103	34.0			
Slaughtering practices					
Live pig washed before slaughtering					
Yes	12/41	29.3	0.4	0.1–1.6	**0.199**
No	46/108	42.6			
Separate internal organs and carcass					
Yes	19/51	37.3	0.8	0.2–2.6	0.71
No	39/98	39.8			
Floor washed after slaughtering each pig					
Yes	38/109	34.9	0.4	0.1–1.5	**0.17**
No	20/40	50.0			
Tools, hands washed after each pig					
Yes	18/56	32.1	0.5	0.2–1.7	0.27
No	40/93	43.0			
Tools, hands washed in water tank intended for rinsing carcasses					
Yes	46/105	43.8	2.4	0.7–8.1	**0.17**
No	12/44	27.3			
Cloth used to wipe carcasses					
Yes	23/56	41.1	1.0	0.3–3.0	0.96
No	35/93	37.6			

CI: Confidence interval, *p*-value in bold: selected variables (with $p \leq 0.2$) for multivariable analysis.

to households (OR = 10.7, $p = 0.06$, Table 6), and visitors having free entry to the farm (OR = 6.5, $p = 0.06$). When farm scale was removed from the model, the estimates for having pig pens located next to households and visitors having free entry to the farm changed by 4.3% (estimate changed from 2.371 (OR 10.7) to 2.473) and 13.5% (estimate changed from 1.867 (OR 6.5) to 1.913), respectively, suggesting a medium level of confounding with the factor removed (data not shown).

Table 5
Univariable GLMM results at market.

Variables	*Salmonella* positive/tested	Prevalence (%)	Adjusted odds ratio	95% CI	*p*-Value
Sampling season					**< 0.01**
April–June	31/54	57.4	Ref	–	–
July–September	30/54	55.6	0.9	0.4–2.0	0.85
October–November	23/55	41.8	0.5	0.2–1.1	0.11
December–February	13/54	24.1	0.2	0.1–0.5	**< 0.01**
Shop management					
Market scale					0.38
Commune market	36/76	47.4	Ref	–	–
Central market	53/116	45.7	0.9	0.5–1.7	0.82
Roadside vendor	8/25	32.0	0.5	0.2–1.4	0.18
Shop located in the area for selling pork					
Yes	60/126	47.6	1.3	0.8–2.3	0.31
No	37/91	40.7			
Shop is next to sewerage or stagnant water					
Yes	12/19	63.2	2.3	0.9–6.0	**0.10**
No	85/198	42.9			
Presence of flies or insects on pork, table					
Yes	41/76	53.9	1.8	1.0–3.1	**0.05**
No	56/141	39.7			
Equipment and hygiene practices at shop					
Shop uses a pork grinder					
Yes	23/55	41.8	0.9	0.5–1.6	0.62
No	74/162	45.7			
Tap water used at shop					
Yes	43/106	40.6	0.7	0.4–1.2	0.23
No	54/111	48.6			
Table is higher than 60 cm					
Yes	85/194	52.2	0.7	0.3–1.7	0.45
No	12/23	43.8			
Type of table surface material					0.79
Carton	34/80	42.5	Ref	–	–
Granite or steel	29/66	43.9	1.1	0.5–2.0	0.86
Wood	34/71	47.9	1.2	0.7–2.4	0.51
Cutting board used for cutting pork					
Yes	75/166	45.2	1.1	0.6–2.0	0.80
No	22/51	43.1			
Storage of pork meat in close proximity of offal					
Yes	26/48	54.2	1.6	0.9–3.1	**0.14**
No	71/169	42.0			
Same cloth used for wiping both pork and hands					
Yes	88/186	47.3	2.2	1.0–5.0	**0.06**
No	9/31	29.0			

CI: Confidence interval, *p*-value in bold: selected variables (with $p \leq 0.2$) for multivariable analysis.

At slaughterhouses, the sole risk factor for *Salmonella* contamination on finished carcasses was slaughter area closes to lairage without hygienic measures (OR = 5.6, $p = 0.03$, Table 6). At markets, there were two risk factors for *Salmonella* contamination on cut pork: presence of flies or insects on pork or table (OR = 2.3, $p = 0.02$), and the use of a cloth to wipe pork, hands, and equipment at the shop (OR = 2.8, $p = 0.02$). Winter season (December to February) was associated with decreased risk of *Salmonella* positivity both for carcass at slaughterhouse (OR = 0.1, $p < 0.01$) and cut pork at market (OR = 0.2, $p < 0.01$).

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

Salmonella concentrations on most cut pork samples (77.3%, 75/97) were < 3.0 MPN/g. In terms of the proportion of highly contaminated samples (that is, those with *Salmonella* concentrations exceeding 30 MPN/g), there was no significant difference between cut and ground pork samples (10/97, 10.3% versus 5/33, 15.2%, respectively; $p = 0.5$, Fisher's Exact test). The overall *Salmonella* concentrations also did not differ significantly between cut and ground pork samples (W = 1859, $p = 0.15$, Wilcoxon Rank-Sum test). Between the two provinces, the proportions of cut pork samples with *Salmonella* exceeding 30 MPN/g were not significantly different: Nghe An yielded 16.3% (8/49) and Hung Yen 4.2% (2/48, $p = 0.09$, Fisher's Exact test, Table 7).

Out of the 63 carcasses traced, 25 carcasses (39.7%) were contaminated with *Salmonella*, 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 that 42.4% of contaminated pork at shops was attributable to the contamination of carcasses at the slaughterhouse (data not shown in tables).

4. Discussion

This study elucidated the prevalence of *Salmonella*, hygiene practices, and risk factors for contamination with *Salmonella* along the informal smallholder pig value chain ending in wet markets in northern Vietnam. As described in the Introduction, this smallholder pig 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 greater evidence of agri-food system transformation than did Nghe An. However, the capacity of slaughterhouses and levels of contamination at markets were not different between the two provinces. At the slaughterhouses and markets, we observed lower *Salmonella* prevalences in colder seasons. This result is consistent with the report from a study that examined 12 European pig slaughterhouses (Hald et al., 2003). However, at the pig farms, there was no significant

Table 6
Multivariable GLMM results at farm, slaughterhouse and market.

Factors	Adjusted odds ratio	95% CI	*p*-Value
Pig farm (pen floor)			
Sampling season (April–June as a reference)			
July–September	3.2	0.5–21.3	0.23
October–November	3.2	0.5–20.8	0.23
December–February	1.9	0.3–13.1	0.51
Scale of farm (middle scale as a reference)			
Small scale	0.2	0.0–1.5	0.11
Pig pens located next to household	10.7	0.9–121.0	**0.06**
Visitors can freely enter farm	6.5	1.1–37.8	**0.06**
Pig slaughterhouse (pig carcass)			
Sampling season (April–June as a reference)			
July–September	0.6	0.2–2.2	0.44
October–November	0.6	0.2–2.3	0.48
December–February	0.1	0.0–0.4	< **0.01**
Scale of slaughterhouse (medium scale as a reference)			
Small scale	0.4	0.2–1.2	0.10
Slaughter area closes to lairage without hygienic measures	5.6	1.2–26.8	**0.03**
Market (cut pork)			
Sampling season (April–June as a reference)	0.5	0.2–1.3	0.14
July–September	0.9	0.4–2.0	0.77
October–November	0.7	0.3–1.7	0.42
December–February	0.2	0.1–0.4	< **0.01**
Scale of market (commune market as a reference)			
Central market	0.9	0.5–1.7	0.79
Street vendor	0.5	0.2–1.3	0.14
Presence of flies or insects on pork or table	2.3	1.1–4.7	**0.02**
Same cloth used for wiping pork, hands and equipment at shop	2.8	1.2–6.9	**0.02**

OR: Odds ratio, CI: Confidence interval, *p*-value in bold font: statistically significance at $p \leq 0.1$.

difference of *Salmonella* prevalence on pen floor among sampling seasons. This may suggest that *Salmonella* prevalence and concentration are influenced by the ambient temperature of the environment which determines the speed of *Salmonella* multiplication.

In the small-scale pig farms studied, bio-security measures were generally not adequate to keep farming environments hygienic, and contamination with *Salmonella* could occur easily both from outside and within farms. It has been reported that poor biosecurity measures are important risk factors for *Salmonella* prevalence at farms (Andres and Davies, 2015). Our study also indicated the importance of improving bio-security measures at small-scale pig farms, particularly regarding farm location and visitor access.

The *Salmonella* prevalence on finished carcasses in this study was comparable to the reports from Belgium (37%) (Botteldoorn et al., 2003), and Thailand (28%) (Padungtod and Kaneene, 2006), but much higher than the prevalences examined between 2001 and 2009 on chilled carcasses in the United States (2 to 4%) (FSIS, 2010). The multivariable risk factor analysis for the contamination of finished carcasses in slaughterhouses found that location of the slaughter area close to lairage without hygienic measures was a risk factor. This finding presumably indicates that cross-contamination of finished carcasses occurred directly with high-risk materials (e.g., faeces, wastewater) from lairage in unclean slaughtering areas, and/or indirectly by workers and equipment contacted with these materials. This contamination risk is common to swine processing worldwide because intestinal tracts carrying *Salmonella* can be accidentally lacerated during processing, resulting cross-contamination of carcasses (Baptista et al., 2010; Berends et al., 1997; Botteldoorn et al., 2004).

The prevalence on MLN (35.6%) in our study was higher than the reports from European and North American countries (10.9%, (Fosse et al., 2009)). *Salmonella*-positive MLNs are considered a proxy for subclinical levels of *Salmonella* infection in apparently healthy pigs (Garrido et al., 2014), as infected pigs can asymptomatically carry *Salmonella* in the tonsils, intestines and the gut-associated lymphoid tissue (Boyen et al., 2008; Rostagno and Callaway, 2012; Wood et al., 1989). 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 (Berends et al., 1996; Wales et al., 2011), which may explain our finding. Moreover, considering that the reactivation of *Salmonella* excretion may occur due to stress, the observed higher prevalence of *Salmonella* in pigs from larger farms might be due to elevated animal stress in intensive farming environments. Therefore, raising and transporting pigs in a low-stress environment may be one of the manageable options for decreasing swine salmonellosis (Boyen et al., 2008; Wales et al., 2011).

Several slaughtering practice issues were observed. First, the proportion of slaughterhouses washing live pigs was low. Second, splitting carcasses was conducted exclusively on the floors that typically already were contaminated with *Salmonella*, where no segregation between clean and dirty zones was practiced. Third, slaughterhouse workers typically washed tools and hands in the same water tank from which the water for rinsing carcasses was obtained. A previous report from Vietnam also indicated that these practices were common during processing at conventional slaughterhouses in this country (Dang-Xuan et al., 2016). Cleaning and disinfection procedures along the slaughter line are beneficial (Arguello et al., 2013; De Busser et al., 2013), and hygiene in slaughtering must be continuously improved in Vietnam, regardless of the results of the present risk factor analysis.

At the markets, *Salmonella* prevalence on cut pork in our study was comparable to the prevalences reported other studies conducted in Vietnam: 32.8% (Takeshi et al., 2009), 39.6%, (Thai et al., 2012), 28.6% (Yokozawa et al., 2016), and 44.4% (Dang-Xuan et al., 2017). The risk factor analysis for markets suggested specific control options as follows: implementation of fly and insect vector control; and discouraging the use of a cloth to wipe pork, hands and equipment. Affordable and practical methods to allow effective cleaning and disinfection of shop equipment (e.g., table surfaces, cutting boards, knives,

Table 7
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

and weight scales) are needed. A recent study in Uganda showed that erecting wooden frames covered with deltamethrin-impregnated nets in the windows of pork shops reduced the number of flies in the shops (Heilmann et al., 2017). In Vietnam, informal pork shops conduct business in an open environment, and public health authorities should bear in mind the potential utility and effectiveness of these simple control options. Another recommendation is to focus on the effect of temperature on *Salmonella* prevalence, as shown in the present study. Limiting pork sales to cooler morning hours may reduce both prevalence and bacterial 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; this issue 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 that 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 (Hald et al., 2003; Lo Fo Wong et al., 2002). Samples were taken from a limited number of available sample sites at each value chain location due to funding limitations. Another limitation is that there can be potential biases in the selection of farms, slaughterhouses, and markets, as no statistical randomizing was performed. Specifically at abattoirs, potential clustering of samples from same farm could have occurred when sampling pigs. The GLMMs for slaughterhouse dealt with the random effect on the clustering within a slaughterhouse. However, it did not consider clustering of source farms, as information on the origin of pigs was not always available in our study.

This study provided useful information for planning applicable and effective intervention programs at each step of the value chain. However, planning interventions requires several additional considerations, given that smallholder pig value chains have complex relationships (characterized by many-to-many interactions among actors). First, the form of intervention may need to be considered, for example, in the form of a single project or a collaborative trans-disciplinary program. Second, risk managers and policy makers should decide which points in the value chain can be 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 illnesses or deaths avoided, and the public health cost saved. Finally, economic impacts on the livelihood of smallholder actors due to the cost of compliance should be considered (Grace, 2015), as they may be vulnerable to the changes, even when such changes are implemented in the interest of better public health. In the long term perspective, involvement of the industry/private sector into the interventions would be essential in order to make these more sustainable and effective.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.09.030.

Acknowledgements

This study was a part of the PigRISK project funded by the Australian Centre for International Agricultural Research (ACIAR) (Grant number: LPS/2010/047) and the Consultative Group on International Agricultural Research (CGIAR), Research Program Agriculture for Nutrition and Health (A4NH). SDX was supported by the Rakuno Gakuen University Guest Researcher Grant and JSPS RONPAKU Program (ID No. R11717) during the analysis and writing phases. The authors thank Professor Emeritus Ian Dohoo, University of Prince Edward Island, Canada, for his suggestions on data analysis and comments on the manuscript. We thank Ms. Julie Hood, who was supported by the Government of Canada through Global Affairs Canada and the Veterinarians Without Borders Volunteer Cooperation Program, for her review of the manuscript. The authors thank Mr. Nguyen Tien Thanh, and Mr. Nguyen Hai Nam for their great help in sample collection, and Mr. Max Barot for his primary data analysis contribution.

References

Andres, V.M., Davies, R.H., 2015. Biosecurity measures to control *Salmonella* and other infectious agents in pig farms: a review. Compr. Rev. Food Sci. Food Saf. 14, 317–335.

Aragon, J.T., Fay, P.M., Wollschlaeger, D., Omidpanah, A., 2017. epitools: Epidemiology Tools. Available at: https://CRAN.R-project.org/package=epitools.

Arguello, H., Alvarez-Ordonez, A., Carvajal, A., Rubio, P., Prieto, M., 2013. Role of slaughtering in *Salmonella* spreading and control in pork production. J. Food Prot. 76, 899–911.

Baptista, F.M., Dahl, J., Nielsen, L.R., 2010. Factors influencing *Salmonella* carcass prevalence in Danish pig abattoirs. Prev. Vet. Med. 95, 231–238.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2017. lme4: Linear Mixed-Effects Models Using 'Eigen' and S4. Available at: https://CRAN.R-project.org/package=lme4.

Berends, B.R., Urlings, H.A., Snijders, J.M., Van Knapen, F., 1996. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. Int. J. Food Microbiol. 30, 37–53.

Berends, B.R., Van Knapen, F., Snijders, J.M., Mossel, D.A., 1997. Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. Int. J. Food Microbiol. 36, 199–206.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003. *Salmonella* on pig carcasses: positive pigs and cross contamination in the slaughterhouse. J. Appl. Microbiol. 95, 891–903.

Botteldoorn, N., Herman, L., Rijpens, N., Heyndrickx, M., 2004. Phenotypic and molecular typing of *Salmonella* strains reveals different contamination sources in two commercial pig slaughterhouses. Appl. Environ. Microbiol. 70, 5305–5314.

Boyen, F., Haesebrouck, F., Maes, D., Van Immerseel, F., Ducatelle, R., Pasmans, F., 2008. Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control. Vet. Microbiol. 130, 1–19.

CDC, 2009. National *Salmonella* Surveillance Annual Summary 2009. https://www.cdc.gov/ncezid/dfwed/PDFs/SalmonellaAnnualSummaryTables2009.pdf.

Dang-Xuan, S., 2013. Quantifying *Salmonella* spp. in Pig Slaughterhouses and Pork Markets Associated With Human Health in Hung Yen, Vietnam. Chiang Mai University and Freie Universität Berlin, pp. 118.

Dang-Xuan, S., Nguyen-Viet, H., Meeyam, T., Fries, R., Nguyen-Thanh, H., Pham-Duc, P., Lam, S., Grace, D., 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.

Dang-Xuan, S., Nguyen-Viet, H., Unger, F., Pham-Duc, P., Grace, D., Tran-Thi, N., Barot, M., Pham-Thi, N., Makita, K., 2017. Quantitative risk assessment of human salmonellosis in the smallholder pig value chains in urban of Vietnam. Int. J. Public Health 62, 93–102.

Davidson, V.J., Ravel, A., Nguyen, T.N., Fazil, A., Ruzante, J.M., 2011. Food-specific attribution of selected gastrointestinal illnesses: estimates from a Canadian expert elicitation survey. Foodborne Pathog. Dis. 8, 983–995.

De Busser, E.V., De Zutter, L., Dewulf, J., Houf, K., Maes, D., 2013. *Salmonella* control in live pigs and at slaughter. Vet. J. 196, 20–27.

de Man, J.C., 1983. MPN tables, corrected. Eur. J. Appl. Microbiol. Biotechnol. 17, 301–305.

Dohoo, I.R., Martin, S.W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd ed. VER, Incorporated, PEI, CA.

Duggan, S.J., Mannion, C., Prendergast, D.M., Leonard, N., Fanning, S., Gonzales-Barron, U., Egan, J., Butler, F., Duffy, G., 2010. Tracking the *Salmonella* status of pigs and pork from lairage through the slaughter process in the Republic of Ireland. J. Food Prot. 73, 2148–2160.

EFSA, 2008. Opinion of the Panel on Biological Hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: source attribution for human salmonellosis from meat. EFSA J. 625, 1–32.

EFSA, ECDC, 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA J. 14 (12), 4634 (231 pp).

FCC, 2010. FCC Consortium: Analysis of the Costs and Benefits of Setting a Target for the Reduction of *Salmonella* in Slaughter Pigs. pp. 1–198.

Fosse, J., Seegers, H., Magras, C., 2009. Prevalence and risk factors for bacterial foodborne zoonotic hazards in slaughter pigs: a review. Zoonoses Public Health 56, 429–454.

FSIS, 2010. Serotypes Profile of *Salmonella* Isolates From Meat and Poultry Products. Food Safety and Inspection Service, US Department of Agriculture, Washington, DC.

Garrido, V., Sanchez, S., San Roman, B., Zabalza-Barangua, A., Diaz-Tendero, Y., de Frutos, C., Mainar-Jaime, R.C., Grillo, M.J., 2014. Simultaneous infections by different *Salmonella* strains in mesenteric lymph nodes of finishing pigs. BMC Vet. Res. 10, 59.

Grace, D., 2015. Food safety in low and middle income countries. Int. J. Environ. Res. Public Health 12, 10490–10507.

Hald, T., Wingstrand, A., Swanenburg, M., von Altrock, A., Thorberg, B.M., 2003. The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses. Epidemiol. Infect. 131, 1187–1203.

Hansen, T.B., Christensen, B.B., Aabo, S., 2010. *Salmonella* in pork cuttings in supermarkets and butchers' shops in Denmark in 2002 and 2006. Zoonoses Public Health 57 (Suppl. 1), 23–29.
Havelaar, A.H., Galindo, A.V., Kurowicka, D., Cooke, R.M., 2008. Attribution of foodborne pathogens using structured expert elicitation. Foodborne Pathog. Dis. 5, 649–659.
Heilmann, M., Roesel, K., Grace, D., Bauer, B., Clausen, P.H., 2017. The impact of insecticide-treated material to reduce flies among pork outlets in Kampala, Uganda. Parasitol. Res. 116, 1617–1626.
ILRI, 2013. PigRISK Empirical Surveys in Hung Yen and Nghe An. Available at: https://pigrisk.wikispaces.com.
ISO-17604, 2003. International Standard Organization: Microbiology of Food and Animal Feeding Stuffs - Carcass Sampling for Microbiological Analysis, Geneva, Switzerland.
ISO-6579, 2002. International Standard Organization: Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection of *Salmonella* spp., Geneva, Switzerland.
Lapar, M., Tiongco, M., 2011. Private standards in pork value chains: role, impact and potential for local innovation to improve food safety and enhance smallholder competitiveness. Farm Pol. J. 8, 39–53.
Le Bas, C., Tran, T.H., Nguyen, T.T., Dang, D.T., Ngo, C.T., 2006. Prevalence and epidemiology of *Salmonella* spp. in small pig abattoirs of Hanoi, Vietnam. Ann. N. Y. Acad. Sci. 1081, 269–272.
Lo Fo Wong, D.M.A., Hald, T., van der Wolf, P.J., Swanenburg, M., 2002. Epidemiology and control measures for *Salmonella* in pigs and pork. Livest. Prod. Sci. 76, 215–222.
Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M., 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50, 882–889.
OECD, 2016. Meat Consumption (Indicator). https://doi.org/10.1787/fa290fd0-en. (Accessed on 01 March 2016).
Padungtod, P., Kaneene, J.B., 2006. *Salmonella* in food animals and humans in northern Thailand. Int. J. Food Microbiol. 108, 346–354.
Pavic, A., Groves, P.J., Bailey, G., Cox, J.M., 2010. A validated miniaturized MPN method, based on ISO 6579:2002, for the enumeration of *Salmonella* from poultry matrices. J. Appl. Microbiol. 109, 25–34.
Phan, T.T., Khai, L.T., Ogasawara, N., Tam, N.T., Okatani, A.T., Akiba, M., Hayashidani, H., 2005. Contamination of *Salmonella* in retail meats and shrimps in the Mekong Delta, Vietnam. J. Food Prot. 68, 1077–1080.
Pires, S.M., Vieira, A.R., Hald, T., Cole, D., 2014. Source attribution of human salmonellosis: an overview of methods and estimates. Foodborne Pathog. Dis. 11, 667–676.
R Foundation, 2018. The R Project for Statistical Computing. https://www.r-project.org/.
Rostagno, M.H., Callaway, T.R., 2012. Pre-harvest risk factors for *Salmonella enterica* in pork production. Food Res. Int. 45, 634–640.
RStudio Team, 2018. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL. http://www.rstudio.com/.
Schwartz, K.J., 1999. Salmonellosis. In: Straw, B.E., D'Allaire, S., Mengeling, W.L., Taylor, D.J. (Eds.), Diseases of Swine, 8th ed. Iowa State University Press, Ames, Iowa.
Steinbach, G., Kroell, U., 1999. *Salmonella* infections in swine herds-epidemiology and importance for human diseases. Dtsch. Tierarztl. Wochenschr. 106, 282–288.
Takeshi, K., Itoh, S., Hosono, H., Kono, H., Tin, V.T., Vinh, N.Q., Thuy, N.T.B., Kawamoto, K., Makino, S., 2009. Detection of *Salmonella* spp. isolates from specimens due to pork production chains in Hue City, Vietnam. J. Vet. Med. Sci. 71, 485–487.
Thai, T.H., Hirai, T., Lan, N.T., Yamaguchi, R., 2012. Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. Int. J. Food Microbiol. 156, 147–151.
Tran, T.P., Ly, T.L., Nguyen, T.T., Akiba, M., Ogasawara, N., Shinoda, D., Okatani, T.A., Hayashidani, H., 2004. Prevalence of *Salmonella* spp. in pigs, chickens and ducks in the Mekong Delta, Vietnam. J. Vet. Med. Sci. 66, 1011–1014.
USDA, 2013. Recent estimates of the cost of foodborne illness are in general agreement. Available at. https://www.ers.usda.gov/amber-waves/2013/november/recent-estimates-of-the-cost-of-foodborne-illness-are-in-general-agreement/.
Vo, A.T., van Duijkeren, E., Fluit, A.C., Heck, M.E., Verbruggen, A., Maas, H.M., Gaastra, W., 2006. Distribution of *Salmonella enterica* Serovars from humans, livestock and meat in Vietnam and the dominance of *Salmonella typhimurium* phage type 90. Vet. Microbiol. 113, 153–158.
Wales, A.D., Cook, A.J., Davies, R.H., 2011. Producing *Salmonella*-free pigs: a review focusing on interventions at weaning. Vet. Rec. 168, 267–276.
Wood, R.L., Pospischil, A., Rose, R., 1989. Distribution of persistent *Salmonella typhimurium* infection in internal organs of swine. Am. J. Vet. Res. 50, 1015–1021.
Yokozawa, T., Sinh, D.-X., Hung, N.-V., Lapar, L., Makita, K., 2016. Transition of *Salmonella* prevalence in pork value chain from pig slaughterhouses to markets in Hung Yen, Vietnam. J. Vet. Epidemiol. 20, 51–58.