2014 Graduation Thesis

A study of *Salmonella* prevalence in pork value chain from pig slaughterhouses to markets in Hung Yen, Vietnam

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Contents

Ⅰ. Introduction

Salmonella spp. which exists in livestock intestine is one of important hazardous pathogens causing food poisoning. Clinical characteristics of salmonellosis in human are as same as general food poisoning symptoms such as fever, stomachache, diarrhea and vomiting and they are presented 12 to 72 hours after infection. Salmonellosis in human is usually mild, but it can be fatal to young children, the elderly, and the immunocompromised people. Moreover, in contrast to *Shigella*, which competes with phagocytes for survival, *Salmonella* has the capacity to survive in cells. In the intracellular space, they move to distant foci and are protected from the lethal effects of therapeutic antimicrobial substances, when administered. Although the actual number of infection has not been revealed because many mild cases are not reported, it was estimated that there are approximately 93.8 million cases of salmonellosis and 155,000 persons die each year with acute salmonellosis around the world [15].

The genus *Salmonella* belongs to the family Enterobacteriaceae and consists of two species, *Salmonella enterica* and *S. bongori*. Furthermore, *S. enterica* is divided into six subspieces (subsp. *enterica*, subsp. *salamae*, subsp. *arizonae*, subsp. *diarizonae*, subsp. *houtenae*, subsp. *indica*). Serovars are determined by combination of O-antigen and H-antigen and more than 2,500 serovars are known and most of them are capable of causing infection in humans [1, 9]. *Salmonellae* are facultative anaerobic, gram-negative, oxidase-negative, rod-shaped bacteria having peritrichous flagellation and mobility. *Salmonella* can grow between 5°C and 46°C, but the generation time is longer than 10 hours below 10°C; pH for growth is between 4.0 and 9.0; A_w-minimum for growth is 0.93 and salt at concentrations of 5% or more can inhibit growth but cannot inactive the organisms. Although *Salmonella* is tolerant to drying, it is not much resistant to heat like other enterobacteriaceae [23].

Most human *Salmonella* outbreaks occur due to consumption of contaminated animal origin food such as meat and eggs. In Vietnam, pork is the most consumed meat which accounts for over 70% of total meat consumption [4, 19], and contamination of pork with *Salmonella* spp. in pork value chain is a serious public health problem. A report shows the prevalence of *Salmonella* spp. exceeded 50 % in samples of pig origin such as caecal contents and carcass swabs in pig slaughterhouses of Hanoi [3] and other report the prevalence was around 40 % of carcasses in pig slaughterhouses in Hung Yen Province [16]. From these reasons, elucidating of the dynamics of *Salmonella* contamination on pork

value chain is urgently required in Vietnam. Furthermore, understanding hygiene in the operations at slaughterhouses and in markets would be the keys for improvements for their practice, because the majority of contamination may occur during slaughtering, processing and sales. The aims of this study are to trace the value chain forward from pig slaughterhouses to the markets in order to elucidate the dynamics of *Salmonella* contamination on pork value chain, to determine the prevalence of *Salmonella* on pork in pig slaughterhouses and the prevalence and Most Probable Number (MPN) in markets and to understand current practices in slaughterhouses and markets.

Ⅱ. Materials and Methods

2.1. Study sites

The study site was Hung Yen Province which is located in the northern part of Vietnam. Hung Yen is closed to Hanoi (33km from the central of Hanoi) and it is one of the important pork supply base. This study was conducted in Van Giang District, Hung Yen Province in February 2014. Two slaughterhouses were selected randomly from the list of slaughterhouses which slaughter more than ten pigs/ day and adapt to floor slaughtering style. Pig carcasses were traced from these slaughterhouses to the markets which located Hanoi or Van Giang District.

2.2. Sample size

The sample size for the study was determined by using following formula [10].

$$n=[1.96^2*P_{exp}(1-P_{exp})/d^2]$$ Where: n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

According to the previous study, 35% was taken as expected prevalence and 10% absolute precision was used [16]. So the appropriate sample size was 88 carcass samples. These samples were collected from two slaughterhouses through seven visits altogether. To trace carcasses forwards to the markets, we randomly selected three swabbed carcasses per a visit to collect pork samples again at the markets and 21 pork samples were collected in total.

2.3. Sampling methods

Two teams which were composed of people having veterinary background such as veterinarians and veterinary students conducted sampling at each slaughterhouse and 50 and 38 carcass samples were collected through four-times visiting Slaughterhouse A and three times visiting Slaughterhouse B respectively. For carcass samples, a carcass-swabbing method using cotton surgical gauzes and a 100cm^2 steel frame was applied and four sites on the medial of carcass were swabbed, so one carcass swab sample covered 400 cm^2 of surface. Collected pork in markets included many parts such as shoulder, belly and loin and was around 200-300 grams. The collected samples were kept in a cool box and then microbiological tests were conducted within 24 hours. For carcass samples at slaughterhouses, the presence of *Salmonella* was analyzed, while for pork at markets, MPN was examined besides the presence.

2.4. Microbiological test

2.4.1. *Salmonella* qualitative determination

For qualifying *Salmonella*, three steps were conducted (enrichment, isolation and confirmation) following ISO 6579:2002 [6].

Enrichment of *Salmonella*

For non-selective enrichment, buffered peptone water (BPW) was used. Twenty five grams of pork was added with 225 ml BPW while carcass swabs were added by 100 ml BPW. Then, the BPW containing sample was homogenized and was incubated at 37 °C for 24 hours. For selective enrichment, modified semi-solid Rappaport Vassiliadis (MSRV) medium base and Muller-Kauffman Tetrathionate-Novobiocin broth (MKTT) was used. Three drops incubated medium were dispensed to MSRV medium base and were incubated at 42 °C for 24 hours and 1ml of incubated medium was added to 9 ml of MKTT and be incubated at 37 °C for 24 hours.

Isolation of *Salmonella*

XLT4 agar and Rambach agar were used for isolation of *Salmonella.* The enriched bacteria in MSRV medium base were inoculated to XLT4 agar and Rambach agar and were incubated at 37 °C for 24 hours. After incubation, each media was examined to identify the presence of typical and

atypical colonies of *Salmonella*. Their positions were marked on the bottom of the media. Typical *Salmonella* colonies (H_2S-positive) appeared black or black-centered with a yellow periphery after 18 -24 hours of incubation. Upon continued incubation, the colonies became entirely black or pink to red with black centers. Colonies of H_2S-negative *Salmonella* strains appeared pink-yellow. On the other hand, typical *Salmonella* spp. colonies were opaque and pink. The few lactose and/or sucrose fermenting Organisms that grow were readily differentiated due to formation of green colonies.

Confirmation of *Salmonella*

After *Salmonella* isolation, the typical or suspect colonies were streaked onto pre-dried nutrient agar (NA) plates and were incubated plates at 37 °C for 24 hours. Pure cultures were used for biochemical confirmation.

Biochemical confirmation

The pure colonies from NA agar were picked up and were inoculated into three media: Triple Sugar Iron agar (TSI), Motility Indole Lysine agar (MIL) and Urea broth. All biochemical tests were incubated at 37 °C for 24 hours. After invocation, Salmonella presents following reaction (Table 1).

Table 1. Biochemical results for *Salmonella*. (ISO 6579: 2002(E) 4rd ed.).

Test	**Positive or Negative reaction**	**Percentage of *Salmonella* inoculations showing the reaction**
TSI glucose (Acid information)	+	100
TSI glucose (Gas information)	+	91.9
TSI lactose	-	99.2
TSI sucrose	-	99.5
TSI hydrogen sulfide	+	91.6
Urea splitting	-	99

Lysine decarboxylation	+	94.6
β 4Galactosidase reaction	-	98.4
Voges-Proskauer reaction	-	100
Indole reaction	-	98.9

Serological confirmation

Strains were divided into O multigroup from OMA to OMG using *Salmonella* antisera which is intended for serological confirmation by means of slide agglutination (Table 2).

Table 2. The details of O multigroup

O multi group	Groups
OMA	Group A, B, D, E, L (1, 2, 12 + 4, 5, 12 + 9, 12 + 9, 46 + 3, 10 + 3, 15 + 1, 3, 19 + 21)
OMB	Group C, F, G, H (6, 7 + 6, 8 + 11 + 13, 22 + 13, 23 + 6, 14, 24 + 8, 20)
OMC	Group I, J, K, M, N, O, P (16 + 17 + 18 + 28 + 30 + 35 + 38)
OMD	Group Q, R, S, T, U, V, W (39 + 40 + 41 + 42 + 43 + 44 + 45)
OME	Group X, Y, Z, 51 to 53, 61 (47 + 48 + 50 + 51 + 52 + 53 + 61)
OMF	Group 0:54 to 0:59 (54 + 55 + 56 + 57 + 58 + 59)
OMG	Group 0:60 to 0:67 (60 + 62 + 63 + 65 + 66 + 67)

Antigen-antibody complexes were formed when a bacterial culture was mixed with a specific antiserum directed against bacterial surface components and agglutinations were invisible to the naked eyes.

2.4.2. *Salmonella* quantitative determination

Enumeration of *Salmonella*

Beside qualitative determination of *Salmonella,* this study also quantified the number of *Salmonella* from positive samples of pork at markets. The three tube-most probable number (MPN) technique was used for quantitative analysis. Three replicates of three ten-fold dilutions (1:10, 1:100

and 1:1000) were pre-enriched with BPW and be incubated at 37 °C for 24 hours. After incubation, other two steps were performed following the same procedure of ISO 6579:2002 (as described above). The number of *Salmonella* was confirmed based on the MPN table [13, 14, 20].

2.5. Checklist survey

For slaughterhouses, workers' practices during slaughtering were observed by using checklists (Annex1). In addition, some information such as pig origin and workers' practice after or before slaughtering was collected by interviews. For markets, sellers' practice and facilities were checked as well as workers in slaughterhouses.

2.6. Statistical analysis

2.6.1. *Salmonella* prevalence and MPN

Statistical analysis was conducted using statistical software R (ver. 3. 0.2). The prevalence was calculated by dividing the number of positive samples by the number of total samples. Chi-squared test or Fisher's exact test was used for a comparison of prevalence. *Kappa* value was estimated to understand the agreement between prevalence in slaughterhouses and in markets. A MPN table for 3 tube tests was referred to measure the MPN in each tube. Considering the error structure of MPNs of the samples, the methods for the mean calculation was selected.

2.6.2. Survey for slaughterhouses and markets

The aim of this survey was to understand the level of hygiene in slaughterhouses and markets in Northern Vietnam, and to elucidate the practice affecting *Salmonella* prevalence in the markets. The data was collected from nine pork sellers in Hung Yen and Hanoi. Although some markets were visited a few times in different days, the same market was counted as different markets because market's situation was thought to change from day to day. For this reason, the number of checklists from markets is identical to the number of collected pork samples. As the number of slaughterhouses worked on was only two, statistics to compare the results from these two checklists were not performed, but was done only for markets. In addition, some sellers didn't answer about D-1 and D-2 in checklists (see Annex) for markets so these items were not used. For markets' data, two by two tables were made for each item and prevalence was shown and then Chi-squared test or Fisher's

exact test was conducted for univariate analysis to estimate *p*-value. In addition, odds ratio was estimated to understand how each factor affects *Salmonella* presence. After estimating the factors which is likely to affect *Salmonella* presence, Pearson product-moment correlation coefficient and *p*-value were estimated to understand the correlation among these factors.

Ⅲ. Results

3.1. Microbiological test results

3.1.1. *Salmonella* prevalence in slaughterhouses and markets

Daily *Salmonella* prevalence in slaughterhouses is shown in Table 3. The prevalence was in the range of 0% to 72.7% and it was very different depending on the day even in the same slaughterhouse.

Table 3. The daily *Salmonella* prevalence in each slaughterhouse

	Slaughterhouse A			Slaughterhouse B		
	No. Salmonella positive carcass samples	No. all carcass samples	Prevalence (%)	No. Salmonella positive carcass samples	No. all carcass samples	Prevalence (%)
Visit1	4	13	31.8	8	11	72.7
Visit2	5	13	38.5	4	20	20
Visit3	1	11	9.1	0	12	0
Visit4	0	8	0			

Figure 1 shows *Salmonella* prevalence on carcasses and pork in each slaughterhouse. Overall, the prevalence in slaughterhouse B was higher than the one in slaughterhouse A. However, there was no significant difference in the prevalence of carcass samples between two slaughterhouses (22.2% (10/45), and 27.9% (12/43), x^2=0.18, df=1, p=0.71 (chi-squared test)). In addition, there were not also significant differences between the prevalence of pork derived from each slaughterhouse and between the prevalence of all pork products (Pork: 23.1% (3/13), 37.5% (3/8), p=0.63 (Fisher's exact test); Total: 22.4% (13/58), and 29.4% (15/51), x^2=0.38, df=1, p=0.54 (chi-squared test)). The prevalence on all carcass samples and on all pork samples is shown in Figure 2. The prevalence of

pork in markets was slightly higher than the one of pig carcasses in slaughterhouse but there was no significant difference between them (25.0% (22/88), and 28.6% (6/21), x^2=0.0034, df=1, p=0.95 (chi-squared test)).

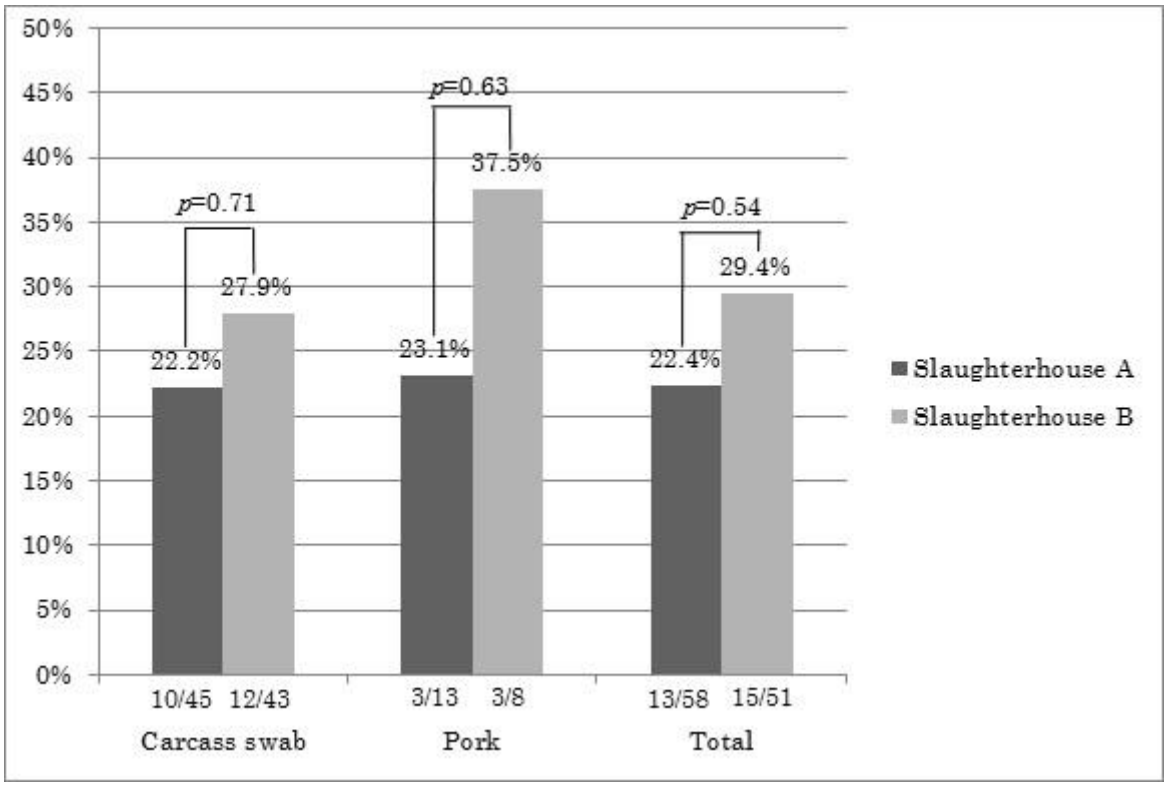

Figure 1. *Salmonella* prevalence on pig carcasses and pork in each slaughterhouse

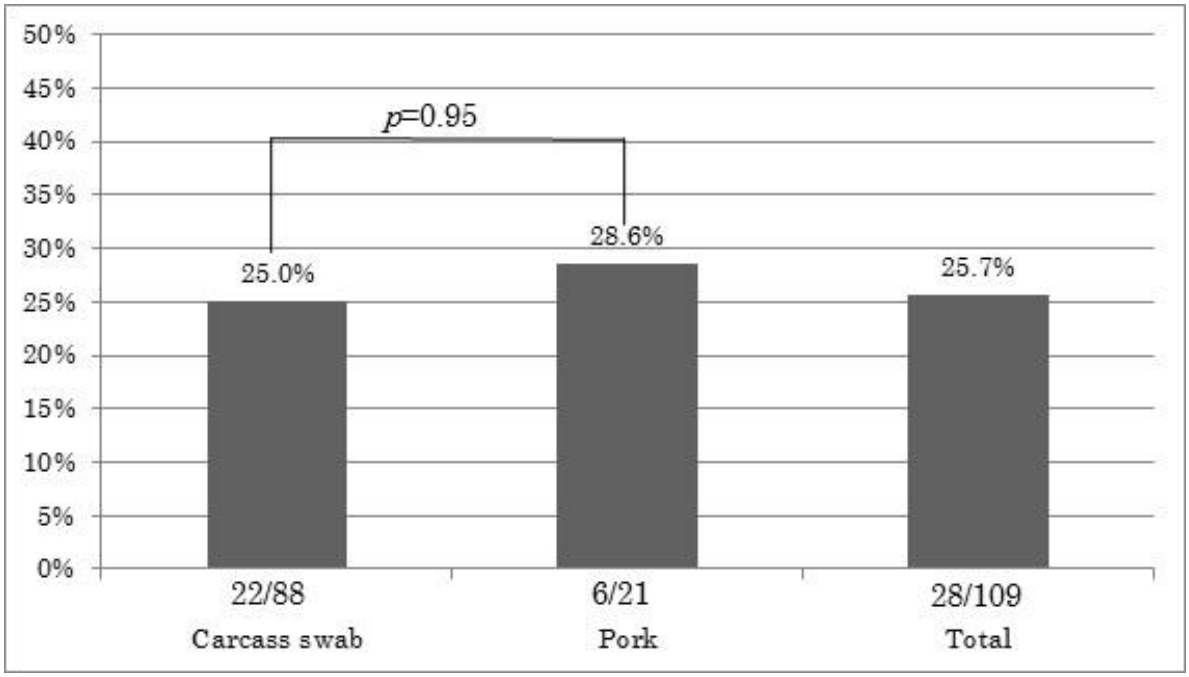

Figure 2. The comparison of *Salmonella* prevalence on pig carcasses and pork

3.1.2. MPN in markets

The results of *Salmonella* quantitative test are shown in Table 4. MPN of positive pork samples was in the range from less than 3.0 to 15 MPN/g (95%CI: <38 MPN/g).

Table 4. MPN on *Salmonella* positive pork samples

The number of pork samples	10^{-1}	10^{-2}	10^{-3}	MPN/g	95% Confidence interval	
					Low	High
1	2	0	0	9.2	1.4	38
1	2	1	0	15	3.7	42
4	0	0	0	<3.0	-	9.5

3.1.3. Serological test result

Figure 3 shows the result of serological test. In carcass samples, about 64% of strains belonged to OMA, while strains from pork belonged to OMA or OMB evenly. Furthermore, there was one carcass sample which was contaminated by more than two *Salmonella* which belonged to distinct O multi group.

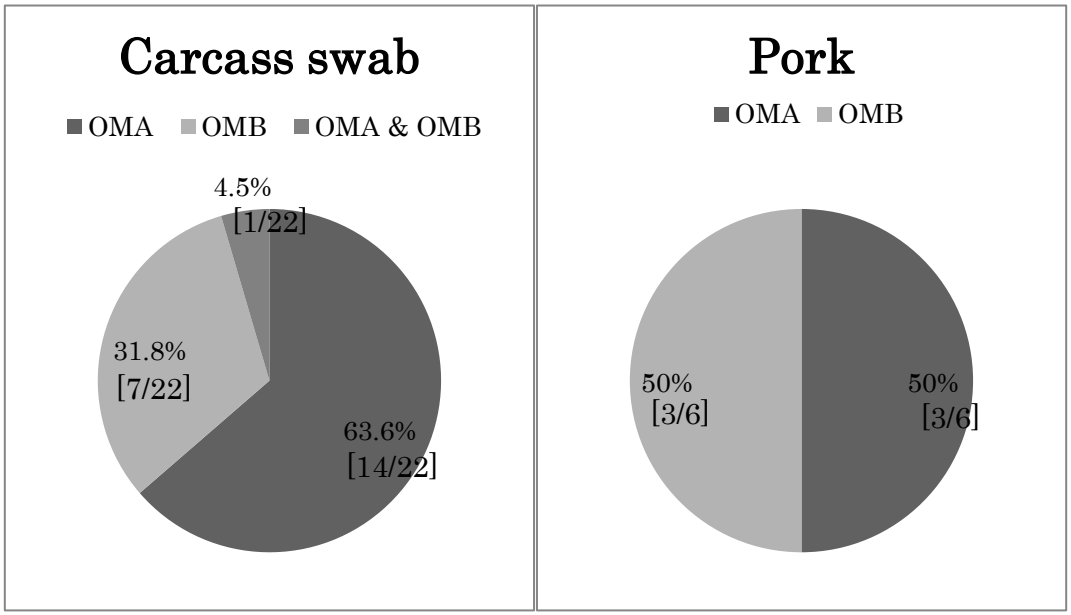

Figure 3. The proportion of O multi group for pig carcasses and pork

3.1.4. The transition of status of *Salmonella* contamination

Transition of status of contamination with *Salmonella* in pork is shown in Figure 4. Out of six positive carcass samples in slaughterhouses, only four pork samples were positive and other two samples became negative in markets. On the other hand, out of fifteen carcass samples in slaughterhouses, two samples changed into positive.

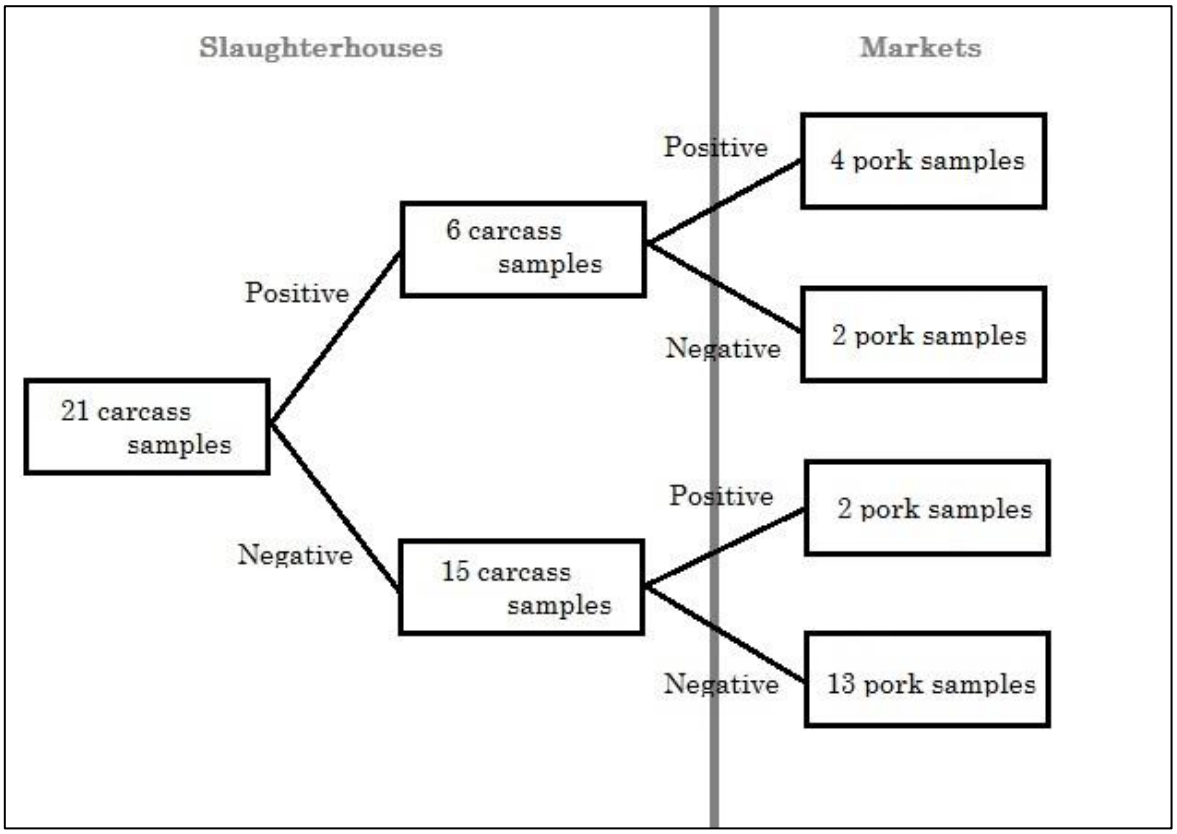

Figure 4. Transition of status of contamination with *Salmonella* on pork

Table 5 is two by two table which was converted from Figure 4 and *kappa* value was estimated from this table. *Kappa* value was 0.53 and it means there was substantial agreement between the prevalence in slaughterhouses and in markets.

Table 5. The result of transition of status of *Salmonella* contamination on pork

		At markets	
		Positive	Negative
At slaughterhouses	Positive	4	2
	Negative	2	13
		Kappa value	0.53

3.2. Descriptive summary of slaughterhouses

Slaughtering included following stages: restraining, bleeding, scalding, flaying, evisceration, washing, wiping and splitting. All stages were conducted on the floor because slaughterhouses which were adapted to floor slaughtering style were targeted in this survey. Information on the origin (farm level) of pigs was collected from the interviews with slaughterhouse owners. Both slaughterhouses used specific trucks which were cleaned and disinfected for transporting pigs. Pigs transported to both slaughterhouses were raised in the commercial farms with more than 50 pigs, located within 2 hours distance by these trucks. Table 6 shows the results of checklists for slaughterhouses.

Table 6. The practices in slaughterhouses and *Salmonella* prevalence on pig carcasses of each slaughterhouse

No	**Observations**	**Slaughterhouse A**	**Slaughterhouse B**
A	**Lair**		
1	Mixing of pigs from different origins in the same lair	No	Different from day to day
2	Presence of abnormal pigs (sick, too thin or dead)	No	No
3	Pigs entry lair in separate way to avoid contamination on slaughtering area	Yes	No
4	Wash live pigs just before slaughtering	No	Different from day to day
B	**Slaughterhouse and slaughtering**		
1	Workers wear an apron	No	No

2	Workers wear a uniform	No	No
3	Workers wear boots	Yes	Yes
4	Separate workers in each step of slaughtering	No	No
5	Washing floor after slaughtering each pig	No	No
6	Washing knife, hook and hand after slaughtering each pig	No	Yes (without soap)
7	Use cloth for wiping carcass and hands/equipment	Yes	Yes
8	People enter freely without cleaning and disinfection of their body, boots and clothing	Yes	Yes
9	People can walk freely on every slaughter areas	Yes	Yes
10	Carcasses are stored in chilled room.	No	No
11	Transport carcasses outside without cover or cooling measures	Yes	Yes
C	**Others**		
1	Presence of pest control measures	No	No
2	Presence of meat inspectors/vet authority officers	No	It wasn't answered

Slaughterhouse workers and sellers in markets worked to slaughter pigs. Sellers generally choose pigs to buy and then sellers did all stages of slaughtering by themselves with the help of other workers. They didn't wear protective gears such as apron, gloves and uniform but they only wore boots. People could enter every slaughter areas freely without cleaning or disinfection of their body, boots and clothing. They didn't wash floor and equipment with detergent after slaughtering each pig and kept slaughtering without intermission. Furthermore, facilities were primitive and there were no cool room or no pest control measures.

3.2. Association between hygiene practice and prevalence

3.2.1. Univariate analysis results from check lists at markets

Table 7 shows two by two tables for each item including prevalence and *p*-value which was estimated by Chi-squared test or Fisher's exact test.

Table 7. The result of univariate analysis

Factors	No. Positive pork	No. Negative pork	Prevalence (%)	*p*-value
A-1. Only pork are sold				
Yes	6	15	28.6	1
No	0	0	NA	
A-2. Contact with vegetable and other meat source while selling				
Yes	0	0	NA	1
No	6	15	28.6	
A-3. Pork is closed or next to internal organs				
Yes	0	0	NA	1
No	6	15	28.6	
A-4. Pork is put in cool cabinet				
Yes	0	0	NA	1
No	6	15	28.6	
B-1. Table is higher than 60cm				
Yes	5	12	29.4	1
No	1	3	25.0	
B-2.1. Table surface (pork contained) is wood				
Yes	5	8	38.5	0.34
No	1	7	12.5	
B-2.2. Table surface (pork contained) is granite/enameled tile				
Yes	4	7	36.4	0.64
No	2	8	20.0	
B-2.3. Table surface (pork contained) is steel				
Yes	2	1	66.7	0.18
No	4	14	22.2	
B-2.4. Table surface (pork contained) is cloth/paper				

Yes	0	3	0	0.53
No	6	12	33.3	
B-3. Usually use insect control equipment while selling				
Yes	0	0	NA	1
No	6	15	28.6	
B-4. Use wipe cloth during selling time				
Yes	6	10	37.5	0.26
No	0	5	0	
B-5. Use more than 1 knife				
Yes	6	13	31.6	1
No	0	2	0	
B-6. Use meat grinder				
Yes	1	5	16.7	0.62
No	5	10	33.3	
B-7. Use cutting board				
Yes	2	8	20.0	0.64
No	4	7	36.4	
B-8. Use water while selling				
Yes	1	2	33.3	1
No	5	13	27.8	
B-9.1. Wipe hands on apron				
Yes	5	9	35.7	0.61
No	1	6	14.3	
B-9.2. Wipe hands on cloth using for wipe pork/equipment				
Yes	6	9	40.0	0.12
No	0	6	0	
B-10. Seller wears gloves				
Yes	0	3	0	0.53
No	6	12	33.3	

B-11. Seller wears apron				
Yes	5	14	26.3	0.5
No	1	1	50.0	
C-1. Contain pork in basket to transport to the shop				
Yes	0	6	0	0.12
No	6	9	40.0	
C-2. Carcass was transported to the shop with covering				
Yes	0	3	0	0.53
No	6	12	33.3	
C-3. Pork/carcass was shipped to the shop by motorbike				
Yes	5	11	31.3	1
No	1	4	20.0	
Market is located in rural area				
Yes	5	7	41.7	0.18
No	1	8	11.1	

Odds ratio was estimated and shown in Table 8 but when some cell in two by two table included zero, odds ratio wasn't estimated.

Table 8. Odds ratio of each check list item

Factors	Odds ratio	95% Confidence Interval	
		Lower	Upper
B-1. Table is higher than 60cm	1.3	0.10	15
B-2.1. Table surface (pork contained) is wood	4.4	0.41	47
B-2.2. Table surface (pork contained) is granite/ enameled tile	2.3	0.32	17
B-2.3. Table surface (pork contained) is steel	7	0.50	99
B-6. Use meat grinder	0.4	0.036	4.4
B-7. Use cutting board	0.44	0.061	3.2

B-8. Use water while selling	1.3	0.095	18
B-9.1. Wipe hands on apron	3.3	0.31	36
B-11. Seller wears apron	0.36	0.019	6.9
C-3. Pork/carcass was shipped to the shop by motorbike	1.8	0.16	21
Market is located in rural area	5.7	0.53	61

From above results, the factors which were likely to have relationship with *Salmonella* presence were as follows:

- Table surface (pork contained) is steel (risk factor)
- Wipe hand on cloth using for wipe pork/equipment (risk factor)
- Contain pork in basket to transport to the shop(protective factor)
- Market is located in rural area(protective factor)

Moreover, Pearson product-moment correlations among above factors were estimated and the result is shown in Table 9. There was positive correlation between "Wipe hands on cloth using for wipe pork/equipment" and "Market is located in rural area", while there was negative correlation between "Contain pork in basket to transport to the shop" and "Market is located in rural area".

Table 9. Correlations among factors which were likely to have relationship with Salmonella presence

Factors	Pearson product-moment correlation coefficient	*p*-value
Table surface (pork contained) is steel	0.26	0.26
Wipe hands on cloth using for wipe pork/equipment		
Table surface (pork contained) is steel	-0.26	0.26
Contain pork in basket to transport to the shop		
Table surface (pork contained) is steel	0.35	0.12
Market is located in rural area		

Wipe hands on cloth using for wipe pork/equipment	-0.3	0.19
Contain pork in basket to transport to the shop		
Wipe hands on cloth using for wipe pork/equipment	0.73	<0.01*
Market is located in rural area		
Contain pork in basket to transport to the shop	-0.52	0.02*
Market is located in rural area		

3.2.2. Recommendation for markets

The markets' hygiene management in Vietnam seemed to be inadequate and it may relate to high *Salmonella* prevalence. From this reason, the improvement of hygiene management of markets was thought to be an urgent task. This would help decreasing *Salmonella* prevalence in markets.

Specific recommendations were summarized in Table 10 and moreover, the items which were not included in checklists of my study are shown in a red letter.

Table 10-1. Recommendations about infrastructure of markets

Infrastructure	
Recommendations	**Reasons**
■ The meat handling areas should be waterproof, without crevices and easy to clean.	
■ Wood should be avoided.	Wood may develop cracks which may become breeding place for microorganisms and pest.
■ Adequate water (safety, quality and pressure) should be available.	It is for cleaning and food preparation. At least, potable water should be available for drinking.
■ An adequate ventilation system is needed.	It is to prevent excessive water condensation which may encourage the growth of *Salmonella*.

■ Pest control system should be adapted.	Flies and rodents are frequently contaminated with *Salmonella*.
■ Toilets with hand-washing facilities should be furnished.	They are important for both food safety and well-being of market participants.
■ There should be hand-washing facilities in the working area for sellers.	It is for sellers to avoid contaminating from their fingers.
■ Drainage should be appropriately designed.	
■ Solid and liquid waste should be removed on a regular basis and stored in covered container.	
■ Thermometers or temperature recorders should be checked at least daily.	The temperature should be kept sufficiently low to inhibit the growth of *Salmonella*.

Table 10-2. Recommendations about operations in markets

Operations	
Recommendations	**Notes**
■ Clean food area should be separated from contaminated food areas as much as possible.	These practices are important to avoid cross contamination.
■ Ready-to-eat food should be separated from food which is likely to be contaminated.	
■ Separate equipment and utensils such as knives and cutting boards for handling raw and cooked food should be used.	
■ Cooling of all raw meats through	It aims to minimize the growth of

refrigeration, storage on ice should be promoted.	*Salmonella*.
■ Solid and liquid waste should be handled and stored in sanitized manner.	It is to keep food handling areas clean.
■ Sellers should wear protective clothing such as aprons and gloves.	Clothing should be washable or disposable at the end of selling.
■ Sellers should wash their hands frequently.	Washing of hands is important because it has been found that *Salmonella* survive on fingertips.
■ Cleaning and disinfection should be carried out at frequent intervals.	If it is thought desirable to have higher temperature, cleaning and disinfection should be carried out more frequently.
■ Equipment such as knives and meat grinder should be designed and it can be easily cleaned.	
■ Medical examination of personnel should be conducted.	If meat handler has been ill with an enteric disease, it is recommended that he should not be allowed to handle meat.
■ Education for food handlers is needed.	This is important for sellers to have awareness of good food hygiene.

Ⅳ. Discussion

Compared to other studies in Northern Vietnam, the *Salmonella* prevalence in this study was relatively lower both at slaughterhouse level and market level: the prevalence on pig carcass at slaughterhouse was reported in the range of 15.5% to 95.7% [3, 8, 16], while the prevalence on pork in markets was in the range of 32.8% to 42.9% [8, 16]. However, the prevalence in Vietnam remains high. In Hung Yen, *Salmonella* prevalence was kept in the same high level at between

slaughterhouse and market. In addition, estimated *kappa* value was in the range of 0.41 to 0.60 and it can be concluded that the prevalence at slaughterhouse level substantially affects the prevalence at market level although status of *Salmonella* contamination had changed on some pork. For this reason, it was found that contamination mainly occurred before the end of slaughtering and it may because the level of hygiene at slaughtering and meat processing were not adequate. A report shows "dirty polishing equipment" and "faulty techniques and sloppy hygiene during evisceration" were risk factors in slaughterhouses. In this study, the slaughterhouses processing pig carcasses on the floor were studied and it can be thought that *Salmonella* contamination easily occurs in such a condition. In considering effective improvement of hygiene, a comparison of microbiological study with the other type of slaughterhouses using hanging style, maybe needed, to examine its efficacy in hygiene, as contamination of pork with *Salmonella* can easily occur on the floor of slaughterhouses.

In this survey for markets, it was suggested that table surface with stainless steel could be risk factor even though WHO mentioned that wood surface relates to *Salmonella* high prevalence [23]. However, it is understandable because it was proved in some reports that *Salmonella* has the ability to adhere to steel [5, 7]. For this reason, the material of table surface is not very related but practices which keep clean might be sufficient to minimize *Salmonella* prevalence. In addition, the result for estimating correlation among factors gave suggestion that the markets in urban area were not likely to wipe their hands on their clothing and they took care of their pig carcasses when transporting. That's why it can be concluded that the markets located in urban area tended to have better hygiene awareness than the ones located in rural area and it may be the reason of lower prevalence in the urban markets. There were only markets which had similar operation in this study, for example, all shops didn't handle ready-to-eat and they sold only pork. If various markets were investigated, the more accurate results would be revealed.

For improvement of food handling, WHO suggests five following keys: to keep clean, to avoid contamination, to destroy hazards when possible, to minimize growth of microorganisms in food and to use safe water and raw materials [21]. Moreover, WHO recommends the implementation of the HACCP approach because HACCP approach can be very sufficient for food safety [22]. HACCP will enable the systematic identification of potential hazards and their control measure. HACCP also provides guidance in selection of enforcement and education priorities, rather than general sanitation and superficial improvements. However, a change in attitude by decision-makers will be

needed to implement the HACCP system and it seems to be difficult to adapt HACCP approach when taking account current Vietnam situation. Improvement of food handlers' awareness for food safety is thought to be more important and more sufficient rather than changing system under the current circumstances.

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Ⅵ. Abstract

【Introduction】

Salmonella which exists in livestock intestine is one of important hazardous pathogens causing food poisoning, and most salmonellosis cases in humans occur due to consumption of contaminated animal origin foods such as meat and eggs. In Vietnam, pork is the most consumed meat and contamination of pork with *Salmonella* spp. is serious public health problem. This study aimed to trace the value chain forward from pig slaughterhouses to the markets in order to elucidate the dynamics of *Salmonella* contamination on pork value chain in Hung Yen, Vietnam.

【Materials and Methods】

This survey was conducted in February 2014 in two slaughterhouses which were randomly selected from the list of slaughterhouses in Hung Yen province and swab samples were collected from 88 carcasses after split there. Out of them, 21 carcasses were traced to the markets and pork samples were collected. Microbial tests were performed for the presence of *Salmonella* from carcass samples at slaughterhouses. MPN was determined in addition to the presence of *Salmonella* for pork in markets.

【Results】

For comparison of two slaughterhouses, there was no significant difference in the prevalence of pig carcass samples between them (22.2% (10/45), and 27.9% (12/43), x^2=0.18, df=1, p=0.71). In addition, there was also no significant difference between the prevalence on carcasses and pork (25.0% (22/88), and 28.6% (6/21), x^2=0.0034, df=1, p=0.95). There was transition of status of *Salmonella* contamination on pork but *kappa* value was 0.53 which means there was substantial agreement between the prevalence in slaughterhouses and in markets. MPN of positive pork samples was in the range of <3.0 to 15 MPN/g.

【Discussion】

In Hung Yen, *Salmonella* prevalence on pork was high and it was suggested that the level of hygiene at meat processing was not adequate. The prevalence was kept in the same level at between slaughterhouse and market, and it was found that contamination mainly occurred before the end of slaughtering. In this study, only the slaughterhouses processing pig carcasses on the floor were studied. In considering effective improvement of hygiene, a comparison of microbiological study with the other type of slaughterhouses using hanging style, maybe needed, to examine its efficacy in hygiene, as contamination of pork with *Salmonella* can easily occur on the floor of slaughterhouses.

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Annex 1

Part A. Pig origin information

1. What kind of vehicle to transport pig to this slaughterhouse?

   ☐ Special truck ☐ Motorbike ☐Other.....

2. Was vehicle cleaned and disinfection before transporting pig?

   ☐ No ☐ Yes

3. How long did it take to transport pigs from farm to slaughterhouse?

   ☐ Less than 1h ☐ 1-2h ☐ 3-4h ☐ More than 5h

4. Pig production system is:

   ☐ Free raising ☐ In traditional stable/indoor ☐Commercial style

5. Scale of pig production is:

   ☐ Backyard (<5 pigs) ☐ Small household (6-10 pigs) ☐ Farm (11-50 pigs) ☐ Intensive (>50 pigs)

6. When transported to the slaughterhouse, do you apply for quarantine or transportation documents from related authorities?

   ☐ No ☐ Yes

Part B. Pig slaughterhouse checklists

No	Observations	Yes	No	Note
A	**Lairage**			
1	Mixing of pigs from different origins in the same lairage			
2	Presence of abnormal pigs (sick, too thin or dead)			
3	Pigs entry lairage in separate way			
4	Wash live pig just before slaughtering			
B	**Slaughterhouse and slaughtering**			
1	Workers wear apron			
2	Workers wear uniform			

3	Workers wear boots			
4	Separate workers in each stage of slaughtering			
5	Washing floor after slaughtering each pig			
6	Washing knife, hook and hand after slaughtering each pig			
7	Use cloth for wiping carcass and hand/equipment			
8	People enter freely without cleaning and disinfection of their body, boots and clothing			
9	People can walk freely on every slaughter areas			
10	Carcasses are stored in cool room.			
11	Transport carcasses outside without cover or cooling measures			
C	**Slaughtering control and waste management**			
1	Presence of pest control measures			
2	Presence of meat inspectors/vet authority officers			

Part C. Market checklists

No	Observations	Yes	No	Note
A	**Selling condition**			
1	Only pork are sold			
2	Contact with vegetable and other meat source while selling			
3	Pork is closed or next to internal organs			
4	Pork is put in cool cabinet			
B	**Equipment**			
1	Table is higher than 60 cm			
2	Table surface pork contained is wood			
	Table surface pork contained is granit/enameled tile			

	Table surface pork contained is inox/steel			
	Table surface pork contained is cloth/paper			
3	Usually use insect control equipment while selling			
4	Use wipe cloth during selling time			
6	Use more than 1 knife			
7	Use meat grinder			
8	Use cutting board			
9	Use water while selling			If yes, water source is
10	Wipe hands on apron			
	Wipe hands on cloth using for wipe pork/equipment			
11	Seller wears gloves			
12	Seller wears apron			
C	**Transportation**			
1	Contain pork in basket to transport to the shop			
2	Carcass was transported to the shop with covering			
3	Pork/carcass was shipped to the shop by motorbike			If no, pork was shipped by
D	**Others**			
1	Wash table at the end of selling time by water and detergent			
	Clean table at the end of selling time by wiping cloth			
2	Shops' offal is put in bin/place for treatment			
	Shops' offal is put freely area around			