CHAPTER 2

CROSS SECTIONAL STUDY OF *Salmonella* PREVALENCE AND CONTAMINATION LEVELS FROM PIG PRODUCTION CHAIN IN UPPER-NORTHERN, THAILAND

2.1 Abstract

Salmonella spp. is an important bacterial-zoonotic pathogen that causes human gastroenteritis. Pork products are the main source of salmonellosis. Based on the ideas of "The *Salmonella* positive carcasses results from the *Salmonella* infected pigs" and "Slaughtering level is the major point to spreading the organism from *Salmonella* infected pigs to the pork via contaminated feces", the cross-sectional study was explored *Salmonella* occurrence in the swine production chain at pre-harvest and harvest level in Chiang Mai and Lamphun Provinces, Thailand. Investigations were conducted from June 2011 through June 2012 at six representative farms (805 samples) and May 2013 through October 2013 at three representative slaughterhouses (1,875 samples). *Salmonella* positive samples were detected in 30.56% of the farm samples and 18.83% of the slaughterhouse samples. The *Salmonella* detected in pig feces was significantly different ($p<0.01$) between the farm and slaughterhouse samples as demonstrated using Fisher's and Kruskal-Wallis tests. In addition**,** differences in contamination levels on swabs taken from the floor and workers' hands at the production level were also detected using the Kruskal-Wallis test. Study results suggest that pork products are easily contaminated with the organism. *Salmonella* control programs should be implemented across the entire pig production chain, including timely monitoring of large populations of farm animals and surveillance to insure good hygiene practices are followed at slaughterhouses.

2.2 Introduction

Salmonella spp. is one of the most important food-borne zoonotic pathogens causing acute gastroenteritis in humans [1], and is recognized as a major public health problem [2]. Approximately 80 million human cases of foodborne salmonellosis occur worldwide annually [3]. In Thailand, the Bureau of Epidemiology reported an estimated 100,000 human cases in 2012 [4]. Clinically, salmonellosis in humans may start with an acute onset of fever, nausea, headache, vomiting and profuse diarrhea within 8 ~ 48 h of ingesting the pathogen. The severity of the disease depends on the ingested dose and the host's immune status [7]. Although contaminated farm animal products are the primary sources of Salmonellosis in humans, pork causes an estimated 15 ~ 20% of all cases [28]. While contamination can occur during any process along the food production chain [5, 20], infected pigs on the farm is the origin of the contaminated pork that leads to human infections [21].

Several studies have assessed *Salmonella* prevalence on pig farms and at slaughterhouses. García-Feliz reported a *Salmonella* prevalence of 43.1% in finishing pig herds in Spain in 2007 [44]. Visscher reported a *Salmonella* prevalence of 5.58% in fattening pigs and 13.2% in slaughtered pigs in Lower Saxony, Germany in 2011 [1]. In addition, Padungtod reported a *Salmonella* prevalence of 28% in pig slaughterhouses from Chaing Mai, Thailand in 2006 [29]. However, *Salmonella* spp. prevalence data is insufficient for quantitative measurement and development of strategies to reduce the risk of this pathogen. The objectives of this study were to determine the prevalence and quantitative loads of *Salmonella* spp. on farms and in slaughterhouses in Chiang Mai and Lamphun Provinces, Thailand, and to compare contamination levels at these two points of the pork production chain.

2.3 Materials and Methods

2.3.1 Farm samples collections

A total of 805 samples from six farms in Chiang Mai and Lamphun Provinces, Thailand from June 2011 through June 2012 were collected. Fecal samples (*n*=606)

were randomly obtained from the rectum of pigs by the individual finger palpation method. Environmental samples (*n*=199) were collected in 100 cm^2 swabs from the floor of the animal house, feeder, nipple-drinker and workers' hands and boots, and from the drinking water, pig feeds and flies. Table 2.1 shows the sample types and frequency.

2.3.2 Slaughterhouses samples collections

The study collected 1,875 samples from three slaughterhouses in Chiang Mai and Lamphun Provinces, Thailand from May 2013 through October 2013. Five replication of sampling time was conducted in each slaughterhouse. Samples were collected from pig feces, mesenteric lymph nodes and scalding water as well as 100-cm^2 swabs from pig skin, pig carcasses, transportation trucks, knives, workers' hands, cutting blocks and lairage floors (Table 2.1).

All farm and slaughterhouse samples were shipped in an icebox to the Central Laboratory, Chiang Mai University, for *Salmonella* isolation within 24 h of collection.

2.3.3 *Salmonella* isolation (qualitative and quantitative assays)

Isolation and identification of *Salmonella* spp. was conducted following the ISO 6579:2002 Amendment 1:2007, Annex D technique (Detection of *Salmonella* spp. in animal feces and environmental samples from the primary production stage) to determine the prevalence and numbers of positive samples [45].

For the qualitative assay, samples of fresh feces, feed, flies, scalding water and mesenteric lymph nodes were obtained. Nine times amount of buffered peptone water (BPW; Merck, Germany) was added as pre-enrichment media (25 g of solid sample was added to 225 mL of BPW). Additionally, 100 ml of buffered peptone water was added to swab samples as pre-enrichment media in which the first dilution was prepared. The mixture was then homogenized using a stomacher machine for 2 min. Following incubation at 37°C for 24 h, an aliquot of 0.1 mL was transferred to a Modified Semi-solid Rappaport-Vassiliadis (MSRV; Oxiod, United Kingdom). The samples were then incubated at 42°C for 24 h, after which the material from this agar

was streaked onto xylose lysine deoxycholate agar (XLD; Oxiod, United Kingdom) and brilliant-green phenol red lactose sucrose agar (BPLS; Merck, Germany) and incubated at 37°C for 24 h. The presumptive *Salmonella* colonies were further processed for biochemical tests, including measurement of triple sugar iron (TSI; Oxiod, United Kingdom), urease and motile indolelysine decarboxylase (MIL; Merck, Germany).

In the quantitative assays, the number of *Salmonella* was determined using the most probable number (MPN) technique. From each positive sample, which was kept refrigerated, three replicates in three portions (3×0.1 mL, 3×0.01 mL and 3×0.001 mL) were taken aseptically and added individually to tubes with BPW. All processes of *Salmonella* identification were performed as qualitative tests, and all suspected colonies from selective media were continually confirmed as *Salmonella* by biochemical tests. *Salmonella*-positive results were used to estimate *Salmonella* quantification with the MPN calculator [46].

2.3.4 Statistical analysis

The data were collected and analyzed for descriptive statistical analysis of *Salmonella* in both prevalence and numbers by Microsoft Excel and PHstat2. Fisher's test and Kruskal-Wallis test were used to compare the proportion of the presence of *Salmonella* and the mean of the MPN numbers, respectively, in representative samples from pig, environment and person, by R-Studio®.

2.4 Results

The overall prevalence of *Salmonella* spp. in the sampled pig farms in Chiang Mai and Lamphun Provinces was 30.56% (246/805). Nine groups of sample types were included in this level. The highest prevalence of *Salmonella* spp. contamination was found in the fly samples (45.45%; 5/11), followed by workers' boot samples (42.11%; 8/19) and pig fecal samples (34.98%; 212/606) (Figure 2.1). In addition, quantification method was submitted in *Salmonella*-positive samples, contamination levels were varied up to sample types. Considering in unit of each samples types,

fecal samples and pen floor swabs were detected in the highest numbers of *Salmonella* contamination when considered as the difference unit in the output (1.07 logMPN/g and 1.58 logMPN/cm^2, respectively) (Figure 2.2)

The overall prevalence of *Salmonella* spp. in the sampled pig slaughterhouses in Chiang Mai and Lamphun Provinces was 18.83% (353/1,875). Nine groups of samples types were also comprised in this level. The finding of positive to *Salmonella* in pig mesenteric lymph node samples was most prevalent (61.33%; 46/75), followed by pig feces samples (56.00%; 42/75) and Lairage floor swab samples (39.33%; 59/150) (Figure 2.3). The quantification assays are shown in Figure 2.4. Pig feces samples and cutting block swab samples were detected in the highest numbers of *Salmonella* contamination (1.46 logMPN/g and 0.47 logMPN/cm^2, respectively) (Figure 2.4).

Fisher's test and Kruskal-Wallis test were used to compare the proportion of the presence of *Salmonella* and the mean of the MPN numbers from the representative samples types at farms and slaughterhouses in the pork production chain. Pig feces, floor swabs and workers' hand swabs were selected for analysis. The association between *Salmonella* spp. contamination in pig feces (from both qualitative and quantitative tests) and the difference of production level was found to be statistically significant both with Fisher's exact test and with the Kruskal-Wallis test (OR=0.42, average 0.39 MPN/g differences between groups). Similarly, contamination levels in floor swabs and workers' hands were found to be statistically significant only using the Kruskal-Wallis test (average 1.13 and 0.54 MPN/cm^2 differences between groups of production level, respectively). For the comparison in prevalence from these samples, No significantly differences detected by Fisher's test demonstrated ($p>0.05$).

2.5 Discussion

The prevalence of *Salmonella* spp. contamination from pig fecal samples on the sampled farms was 30.56 %. This result is similar to the study of García-Feliz et al. who reported a 43.1% prevalence of *Salmonella* in the fecal samples from fattening

units in Spain [44], using a comparable isolation technique. In contrast, a study of finishing pigs conducted in Germany revealed a lower prevalence (5.65%) than our study [1]. Fecal swabs from the rectum might not be sufficient to compare with the amount of feces (up to 25 g) collected in our study, and good management practices in Germany may reduce pathogen levels on farms. Prevalence from the environmental samples in our study was generally low, except for the samples taken from flies and workers' boots. Flies are a major vehicle for transmitting foodborne pathogens, and boots of workers are easily exposed to animal feces [47]; thus, the prevalence of Salmonella from these sources may be higher than that of other environmental samples.

The overall prevalence of *Salmonella* in the sampled slaughterhouses in this study was 18.83%. This was lower than the 37.33% prevalence reported previously by Padungtod et al. [29] for slaughtered pig samples in the same region. The difference might derive from the different sample types and different sampling times (over 10 years apart). The revealing of *Salmonella* was high in pig feces and pig mesenteric lymph nodes. This finding indicated the positive test in each sample type might be considered as the predictor of positive results in another sample type. Feces and mesenteric lymph nodes have been recognized as the major reservoir of *Salmonella* origin in slaughtering levels [24, 26]. Additionally, contaminated equipment and improper routine practices can play a role in *Salmonella* cross contamination at this level [27].

The MPN range of *Salmonella*-positive samples on the sampled farms was relatively high in pig feces, pen floors and workers' boots. This finding indicated that the environment is a potential source of *Salmonella* infection in pigs [1]. The post-infected animal could be highly susceptible to re-infection when exposed to the environment [22]. Moreover, other sources not sampled in this study might also play roles as important shedders, such as wild birds, lizards or invertebrates [47]. Furthermore, the MPN range of *Salmonella*-positive samples in the sampled slaughterhouses was high in pig feces and mesenteric lymph nodes. *Salmonella* was detected at low levels in the other remaining sample types. It is not unusual, pig feces

and lymph nodes are renowned as the major areas of *Salmonella* multiplication [30, 31, 38]. However, under the right conditions, even 1 CFU can grow to several million [48]. Therefore, relatively low levels of *Salmonella* at any point in the production process can have a large impact, if they have the opportunity to proliferate to hazardous numbers under improper conditions [20].

Salmonella prevalence and contamination levels detected from pig feces on the farms was significantly lower than at the slaughterhouses. Longer waiting times in transportation and lairage, contributing to increased shedding of the pathogen from the intestinal lumen in slaughtered pigs [30], and high pig-density areas such as the lairage area might increase the probability of exposure of *Salmonella*-free pigs with *Salmonella*-infected pigs from direct contact, via feces or inhalation [3]. On the other hand, average contamination levels mean detected from floor swabs and workers' hands on the farms were significant higher than at the slaughterhouses. It is not uncommon, because of the better sanitary conditions such as resident cleaning or worker's hygienic cares were observed in slaughterhouses.

2.6 Conclusions

Salmonella on farms is the first origin of salmonellosis, while slaughterhouses are the major point for spreading *Salmonella*. It is unlikely to be alleviated effectively in the short term. Farm control programs and slaughtering routine practices must be based on strict biosecurity and hygiene measures to minimize the risk of *Salmonella* exposure to many potential infection sources. Moreover, these findings highlight the need for continuous monitoring, along with greater focus on problem solving on farms and at slaughterhouses, which can reduce the contamination pressure downstream at cutting plants or retail shops.

2.7 Acknowledgements

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Table 2.1 Sample types and their frequency in the study

Sample types	Farms	Slaughterhouses
Pig feces	606	75
Pig Mesenteric Lymphnodes	0	75
Pig skins/carcasses	0	705
Feeds	30	0
Drinking-water	30	0
Nipple-drinkers	30	0
Feeders	30	0
Floors	30	150
Scalding waters	0	30
Worker's hands	19	270
Worker's boots	19	0
Flies	11	0
Knives	0	450
Cutting blocks	0	60
Transporation trucks	0	60
TOTAL	805	1,875

Table 2.2 Odds ratio and means of contamination levels from the different production level in representative samples in the study

Samples	Production level[a]	No. of samples	Positive samples	Fisher's test		Kruskal-Wallis test	
				OR	P-value	Mean[b]	P-value
Feces	F	606	212	0.42	<0.01	1.07	<0.01
	SLH	75	42			1.46	
Floors	F	30	8	0.56	0.21	1.59	<0.01
	SLH	150	59			0.46	
Worker's hands	F	19	2	0.66	0.74	0.56	<0.01
	SLH	270	41			0.02	

[a]Production level: F (Farms); SLH (Slaughterhouses)

[b]Unit of mean: log MPN/g (from feces)

log MPN/cm^2 (from floor swabs and worker's hands swabs)

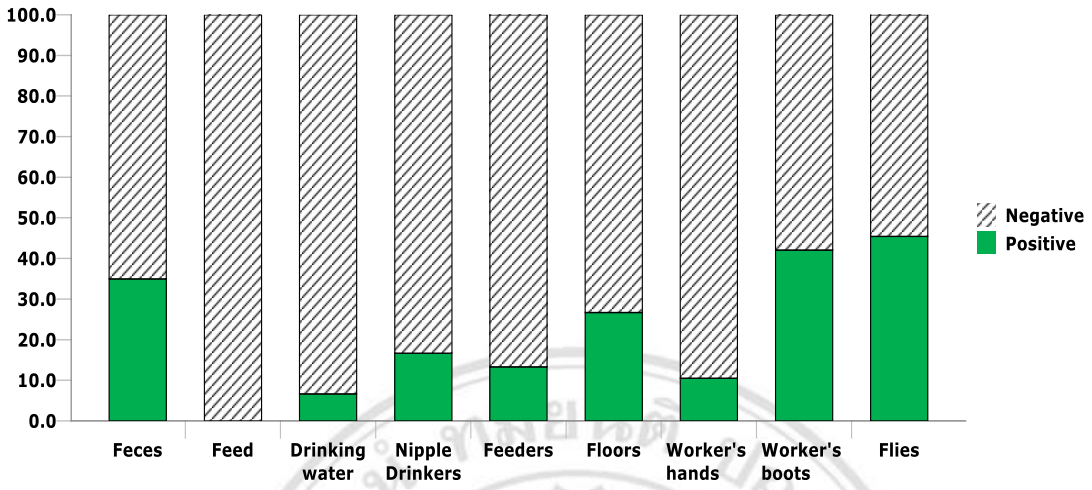

Figure 2.1 Prevalence (%) of *Salmonella* spp. from each sample types of farm level

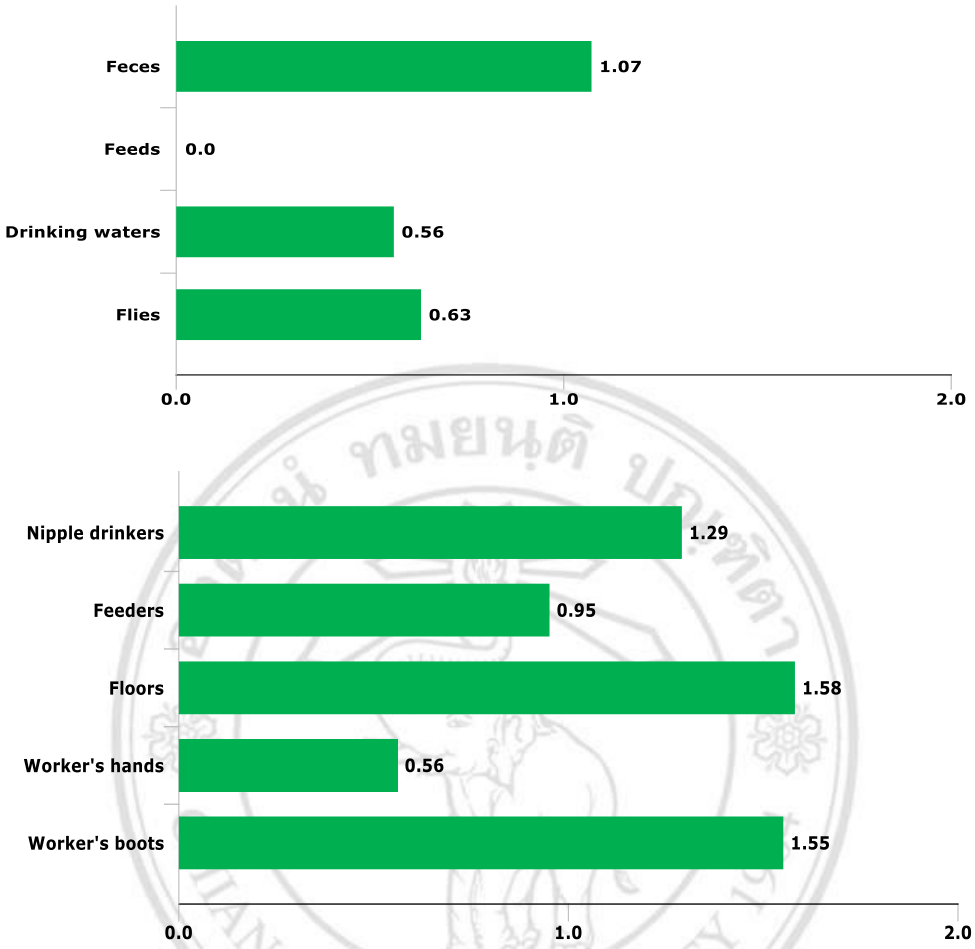

Figure 2.2 Contamination levels means (expected numbers means) of *Salmonella* spp. from each sample types of farm level

Unit of mean: log MPN/g (from feces, feeds, drinking waters and flies)

log MPN/cm^2 (from swab of the remaining samples)

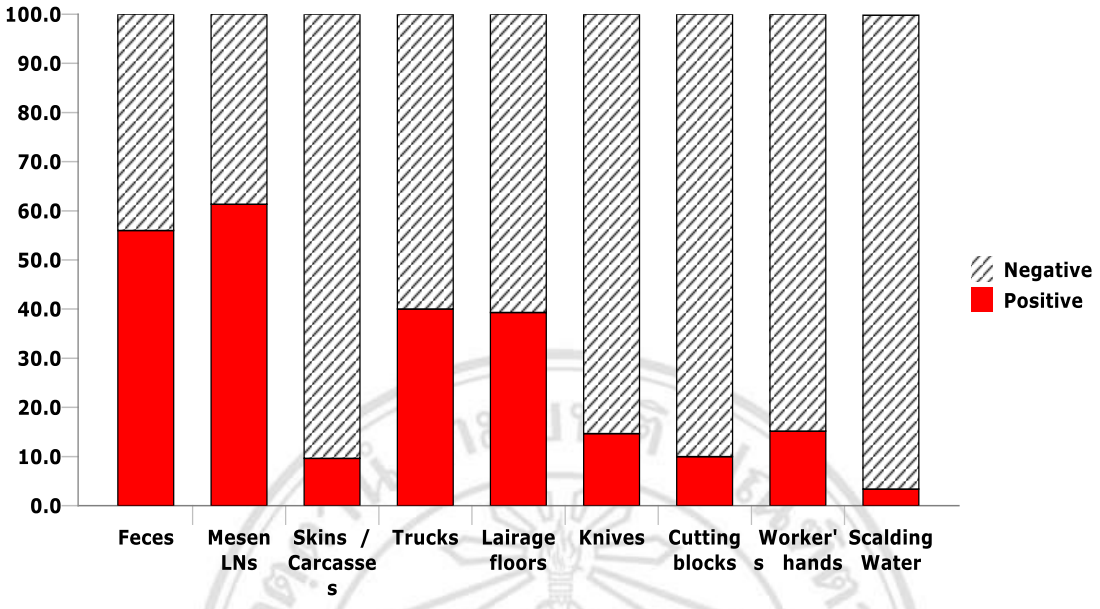

Figure 2.3 Prevalence (%) of *Salmonella* spp. from each sample types of slaughtering level

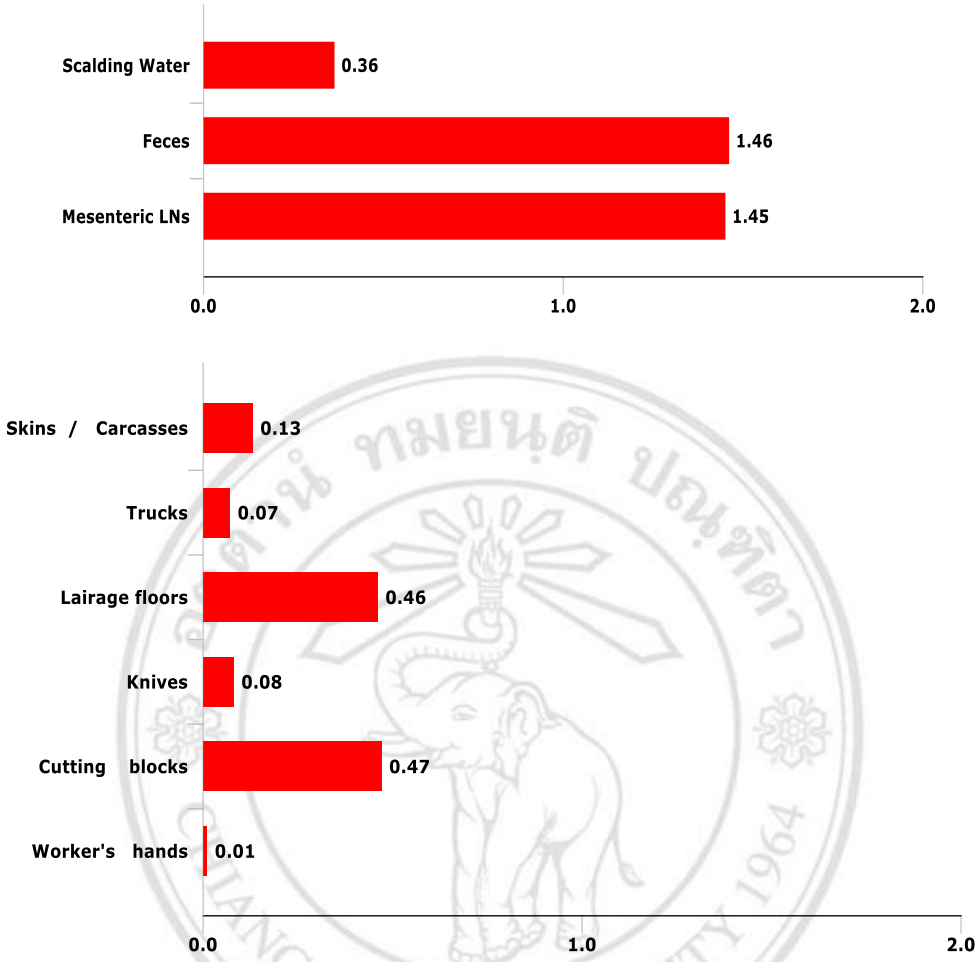

Figure 2.4 Contamination levels means (expected numbers means) of *Salmonella* spp. from each sample types of slaughtering level

Unit of mean: log MPN/g (from scalding water, feces and mesenteric lympnodes)

log MPN/cm^2 (from swab of the remaining samples)