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FULL PAPER

Quantification of contamination levels and particular risk of *Salmonella* spp. in pigs in slaughterhouses in Chiang Mai and Lamphun provinces, Thailand

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Abstract

***Salmonella* spp. is one of the important foodborne pathogens, and the slaughtering process is recognized as a potential point of contamination and the spread of the pathogens. The three objectives of this study are first, to quantify the contamination levels of *Salmonella* spp. in pig skins and carcasses, second, to evaluate the outcomes from different pig supply sources and different practices at three critical steps (scalding, splitting, and washing) for *Salmonella* spp. contamination, and third, to assess risk of *Salmonella* spp. contamination in pork products after slaughtering level. The study was performed in three representative slaughterhouses in Chiang Mai and Lamphun provinces, Thailand. Investigation conducted from May 2013 through October 2013 found the overall prevalence and contamination levels mean to be 11.85% and 0.34 MPN/cm^2, respectively. There was no statistically significant in *Salmonella* spp. prevalence and contamination levels detected with different patterns at the slaughterhouses which were supplied pigs from either co-operative or integrated farms. Factors found to reduce *Salmonella* spp. loads on carcasses included good practices, e.g., regular changing of water in the scalding tank after each batch and the use of chlorine in the washing step. Risk of *Salmonella* spp. contamination of pork products at the final stage of slaughtering was nearly 10%. Good practices and proper hygiene measures should be applied to minimize the risk of *Salmonella* spp. exposure in the slaughtering line, which can reduce the contamination pressure downstream at retail shops as well as for end consumers.**

Key Words: Prevalence, Contamination levels, *Salmonella* spp., Risk, Pig, Slaughterhouse

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Introduction

Salmonella spp. is an important bacterial zoonotic pathogen that causes acute food-borne diseases in humans[5,9,24] and is recognized as a major public health problem[7,17]. Approximately that worldwide of 80 million foodborne salmonellosis cases occurred annually[19]. Contaminated eggs and raw or undercooked meat are involved in most human cases [16] and it has been estimated that pork causes 15–20% of all human cases of salmonellosis[13]. That estimate is supported by several studies which have reported that pork products are a major source of infection[1,4,10].

Surveillance data in northern Thailand, found a high prevalence of *Salmonella* spp. in pigs at the farm level. In two studies, 55%[15] and 63%[4] of samples from pigs were found to be positive. Those high levels of infection suggest that the *Salmonella* spp. menace at the pre-harvest level is not easy to eradicate. A high prevalence at the pre-harvest level could be expected to cause a high prevalence at the harvest level[18]. The idea that "*Salmonella* spp. positive carcasses result from infected pigs" has been mentioned in several studies[1,3,8,23].

In the slaughtering process, inadequate routine practices such as improper evisceration techniques, poor conditions in the lairage area, etc., have also been found to play an important role in the colonization and spread of *Salmonella* spp. to pork[12,19,22]. Steps that can reduce the numbers of *Salmonella* spp. such as maintaining a high temperature in the scalding tank and a low temperature in the chilling step after processing, have been widely reported[3,6,9,12]. The objectives of this study are to quantify contamination levels of *Salmonella* spp. on pig skins and carcasses during the slaughtering process, and to evaluate the outcomes from different in pig supply sources and with different practices in three critical processing steps (scalding, splitting and washing). Additionally, this study attempted to define the risk of *Salmonella* spp. contamination in pork products after slaughtering level in three representative slaughterhouses in Chiang Mai and Lamphun provinces, Thailand, as a part of an epidemic investigation conducted between May 2013 and October 2013 and to identify appropriate preventive measures to help control salmonellosis in this region.

Materials and Methods

Sample collection: This study was conducted in three slaughterhouses in Chiang Mai-Lamphun provinces, in northern Thailand (Table 1). The steps in the slaughtering process were similar at all three slaughterhouses (Fig. 1). However, there were significant differences in the supply chain of pigs, i.e., slaughterhouse A received pigs from a co-operative pig farm, while slaughterhouse B and C received pigs from integrated pig farms. From each pig tested, pig skin swab-samples were obtained from five sites, two from each shoulder and two from each hip plus one from the backside, from an area of 100 cm^2 per site. The samples were collected from live pigs and also from carcasses at each of the following steps: transportation, lairage, scalding, dehairing and evisceration (Fig. 2a). No samples were taken during stunning, bleeding or head removal. In addition, six carcass swab-samples (three from the skin and three from the internal parts of

Table 1. Slaughterhouses included this study

Slaughterhouses	Capacity (animals/d.)	Locations	Suppliers
A	40–60	Meung Dist, Lamphun	Co-operative pig farms
B	120–150	Sankampang Dist, Chiang Mai	Integrated pig farm
C	250–280	Sansai Dist, Chiang Mai	Integrated pig farm

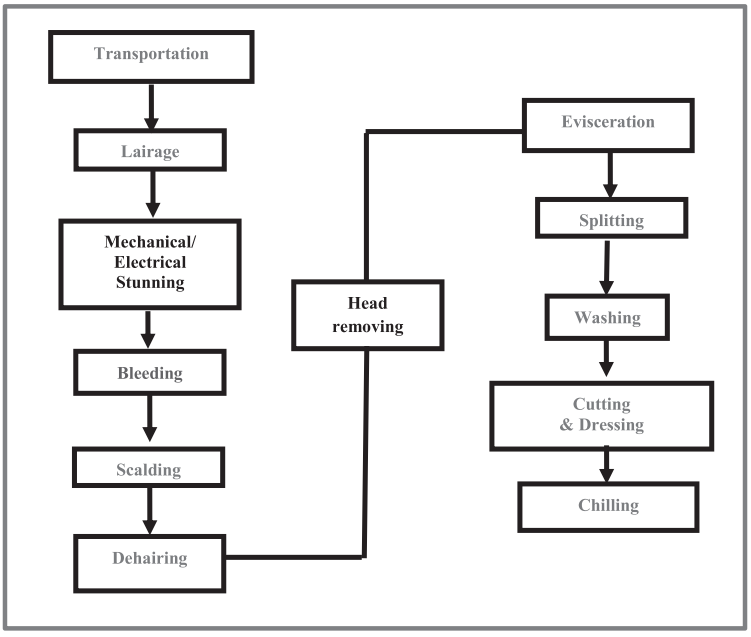

Fig. 1. The slaughtering process used in slaughterhouses in Chiang Mai and Lamphun.

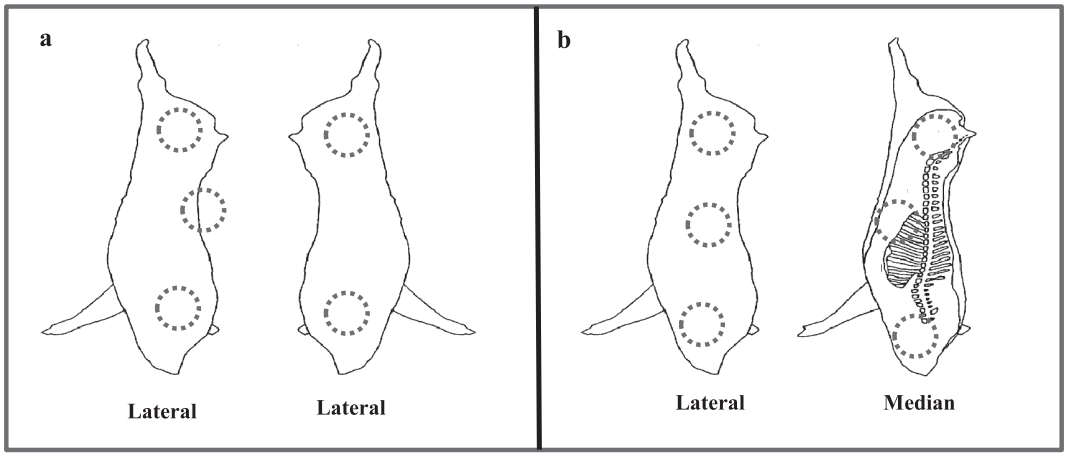

Fig. 2. Sites of sample collection. Fig. 2a; from transportation process to evisceration. Fig. 2b; from splitting process to the end of slaughtering process.

the carcass) were collected during the splitting, washing, cutting and dressing and chilling steps (Fig. 2b). At least five pigs from each slaughterhouse were included in the study; a total of 230 samples was collected. All samples were transported in an icebox to the Bacteriology Laboratory, Chiang Mai University, and cultured for isolation and identification of *Salmonella* spp. within 24 hr of sample collection.

Salmonella spp. isolation and identification (Quantitative assays): The isolation and identification of *Salmonella* spp. was performed following the ISO 6579:2002 Amendment 1:2007, Annex D technique[11] (Detection of *Salmonella* spp. in animal feces and in environmental samples from the primary production stage) to determine the prevalence of *Salmonella* spp.-positive samples in this study. All positive samples were quantified using the most probable number (MPN) technique. Additionally, 100 ml of

buffered peptone water (BPW; Merck, Germany) was added to swab samples as pre-enrichment media in which the first dilution was prepared. Two portions, of 0.1 ml and 0.01 ml of the first dilution were taken aseptically and added individually to tubes with BPW and homogenized using stomacher machine for 2 min. After incubation at 37°C for 24 hr, an aliquot of 0.1 ml from a homogenized mixture was transferred to Modified Semi-solid Rappaport-Vassiliadis (MSRV; Oxiod, United Kingdom). After the incubation at 42°C for 24 hr, the turbid, gray matter taken from broth was streaked on Xylose Lysine Deoxycholate agar (XLD; Oxiod, United Kingdom) and Brilliant green Phenol red Lactose Saccharose agar (BPLS; Merck, Germany) incubated at 37°C for 24 hr; Presumptive *Salmonella* spp. colonies, black in color, were then subjected to biochemical and serum agglutination tests. Finally, *Salmonella* spp.-positive results were used to quantify the estimated *Salmonella* spp. quantification using MPN table[21].

Statistical Analysis: Descriptive data were analyzed using PHStat®. Differences in *Salmonella* spp. prevalence among three slaughterhouses and the odds ratio in both of the differences in pig supply sources from the outcomes in the first step of slaughtering process (the transportation step) and differences in slaughtering practices were analyzed using Fisher's exact test by R-Studio®. In addition, R-Studio® was used to analyze of the differences in the mean of *Salmonella* spp. contamination levels among three slaughterhouses and the difference in those outcomes from different pig supply sources and the slaughtering practices, using the Kruskal-Wallis test.

Modeling Method: The software program @Risk 5.5® was used to simulate the binomial distribution model of *Salmonella* spp. contamination in pork products after the slaughtering level on overall representative slaughterhouses. Distribution of *Salmonella* spp. prevalence in chilling step (which recognized as the final stage at slaughtering step) was modeled and used as an output to simulate, with 10,000 iterations of Monte Carlo sampling[25] as the formula:

$$p = \beta\,(x + 1,\, n - x + 1)$$

Where p is the probability of exposure, β represents the "riskbeta" command in @Risk 5.5®, x denotes number of positive samples and n are total sample-numbers.

Results

The overall prevalence of *Salmonella* spp. on pig skin and carcasses from slaughterhouses in Chiang Mai and Lamphun between May 2013 and October 2013 was 11.85%. Values for each slaughterhouse, A, B and C, were 8.51%, 23.91% and 3.40%, respectively. The overall mean of *Salmonella* spp. contamination level found in this study was 0.34 MPN/cm^2; the values for each of the slaughterhouses, A, B and C, were 0.08, 0.80 and 0.14 MPN/cm^2, respectively (Table 2).

The highest prevalence of *Salmonella* spp. contamination was found at the cutting and dressing step followed by the scalding step, both at slaughterhouse B (Fig. 3). The highest contamination level was found at the scalding step followed by the evisceration step, also from slaughterhouse B (Fig. 4). At slaughterhouse C, the values obtained were quite low, except for the splitting step (Fig. 3 and Fig. 4). There was no statistically significant difference between slaughterhouses supplied with pigs by co-operatives and those supplied by integrated farms (Table 3). Table 4 demonstrated the statistical differences in the outcomes compared with different processing practices in representative slaughterhouses. The relationship between *Salmonella* spp. contamination and the quality of management of the scalding tank was found to be statistically significant both with on Fisher's exact test and with the Kruskal-Wallis test (OR = 11.29, average 0.77 MPN/cm^2 differences

Table 2. *Salmonella* spp. prevalence (%) and means of contamination levels (MPN/cm^2) in slaughterhouses in Chiang Mai and Lamphun

Slaughterhouse	No. of samples	No. of positive samples	% prevalence (95% confidence interval)	Means of contamination levels (95% confidence interval)
A	235	20	8.51^a (4.94–12.07)	0.08^c (0.04–0.12)
B	230	55	23.91^b (18.40–29.42)	0.80^d (0.49–1.11)
C	235	8	3.40^a (1.08–5.72)	0.14^c (0.06–0.35)
Total	700	83	11.85 (9.46–14.25)	0.34 (0.21–0.46)

Superscripts (a) and (b) in each column indicate significant differences ($p < 0.05$) of prevalence among slaughterhouses, obtained using Fisher's exact test. Superscripts (c) and (d) in each column indicate that significant differences in *Salmonella* spp. contamination levels by Kruskal-Wallis test among slaughterhouses,

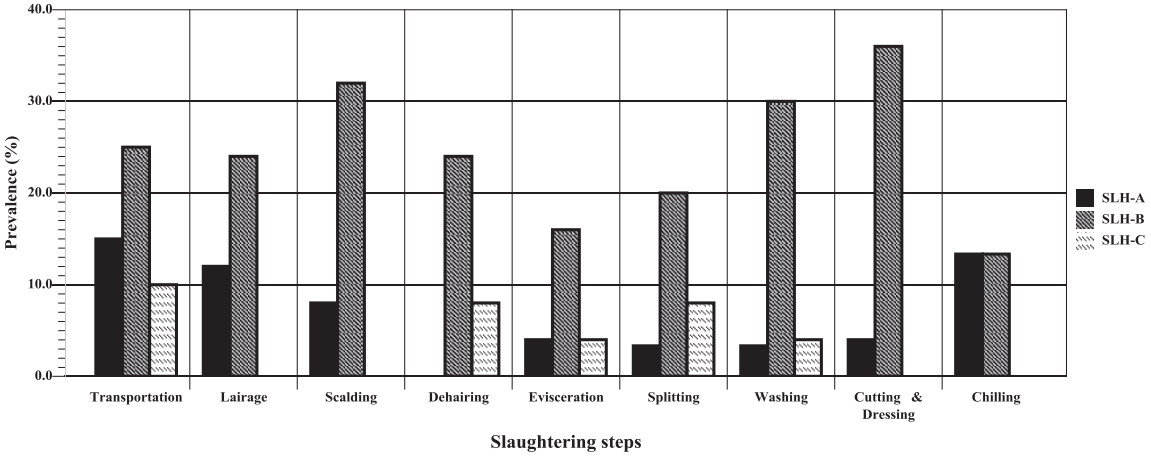

Fig. 3. Prevalence (%) of *Salmonella* spp. isolated from swabs of pig skins and carcasses taken at each step of the slaughtering process.

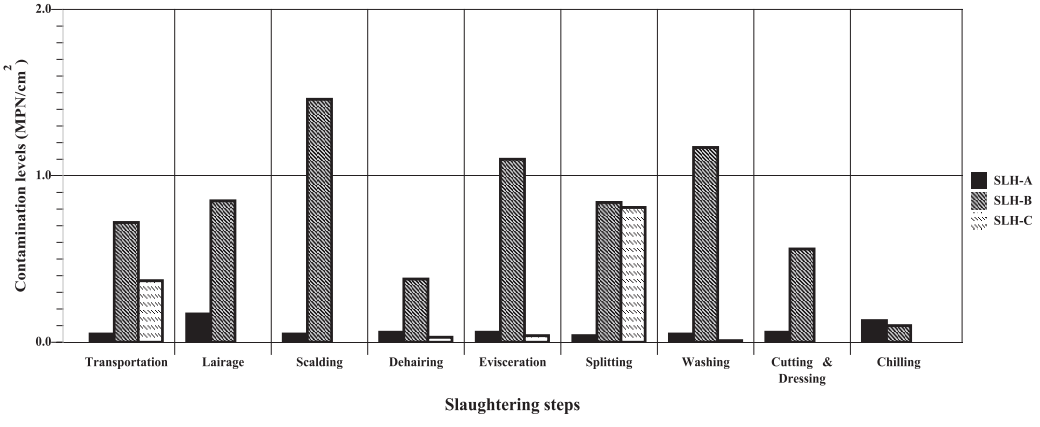

Fig. 4. Contamination levels (MPN/cm^2) of *Salmonella* spp. isolated from swabs of pig skins and carcasses taken at each step of the slaughtering process.

between groups). Similarity, contamination levels in facilities using tap water versus those using chlorinated water in the washing step was found to be statistically significant using the Kruskal-Wallis test (average 1.15 MPN/cm^2 differences between groups). Both the scalding and the washing steps are considered to be risk factors for *Salmonella* spp. contamination. On the other hand, levels of *Salmonella* spp. contamination with different types of splitting equipment,

Table 3. Odds ratio and means of contamination levels (MPN/cm^2) of the different type of pig supplier to slaughterhouses in Chiang Mai and Lamphun

Source of pig supply	[a]SLH	[b]No. of samples	[b]No. of Positive samples	Prevalence comparison		Contamination levels comparison	
				Odds ratio	P-value	Means	P-value
Integrated pig farm	B, C	40	7	1.2	1	0.54	0.64
Co-operative pig farm	A	20	3	[C]Ref		0.05	

Remarks: [a]SLH (slaughterhouses)
[b]*Salmonella* spp. prevalence in the transportation step was used as the outcome of the analysis
[c]Reference category

Table 4. Odds ratios and means of contamination levels (MPN/cm^2) of different practices in the major steps in the slaughtering process in slaughterhouses in Chiang Mai and Lamphun

Steps	Factors	[a]SLH	No. of samples	No. of Positive samples	Prevalence comparison		Contamination levels comparison	
					Odds ratio	P-value	Means	P-value
Scalding	Bad management in scalding tank	B	25	8	11.29	<0.01	0.85	0.01
	Good management in scalding tank	A, C	50	2	[b]Ref		0.08	
Splitting	Electric splitter	B, C	60	8	4.46	0.26	0.82	0.13
	Knife	A	30	1	[b]Ref		0.04	
Washing	Tap water	A, B	60	10	5.80	0.13	1.18	< 0.01
	Chlorined water	C	30	1	[b]Ref		0.03	

Remarks: [a]SLH (slaughterhouses)
[b]Reference category

however, was not statistically significant between an electric-splitter and a knife (p-value > 0.05).

The prevalence in chilling step was used as the outcomes for determining the risk of *Salmonella* spp. contamination model, in pork products after slaughtering process to next production level. With the iteration from β (9,83), the value varying from 4.62% to 16.59% with the mean of 9.78%, and the distribution was demonstrated in Fig. 5.

Discussion

The overall prevalence of *Salmonella* spp. on skin and carcass-swab samples from slaughterhouses in Chiang Mai and Lamphun was found to be 11.85%, lower than the 37.33% prevalence reported in a previous report by Padungtod *et al*. (2006)[14] for slaughtered pig carcasses in the same region. The difference might have arisen from the fact that the values for the slaughterhouses in this study (A, B and C) were pooled, and slightly lower levels of *Salmonella* spp. contamination was found in slaughterhouse C. In contrast, research carried out elsewhere in the world has found larger differences than in the present study in Thailand. For example, the prevalence of *Salmonella* observed on carcass swabs in the Netherlands was 1.4%[22]. Bung dropper was used in order to suck up feces from the rectum avoiding the *Salmonella* spp. contamination, and the splitted-carcasses were singeing at 1000°C for cleaning skin in deep layers. These indicated that better hygiene management can definitely have an influence on decreasing pathogen levels in slaughterhouses.

The MPN range of the samples taken in this study was relatively low (0–24 mpn/cm^2). Under

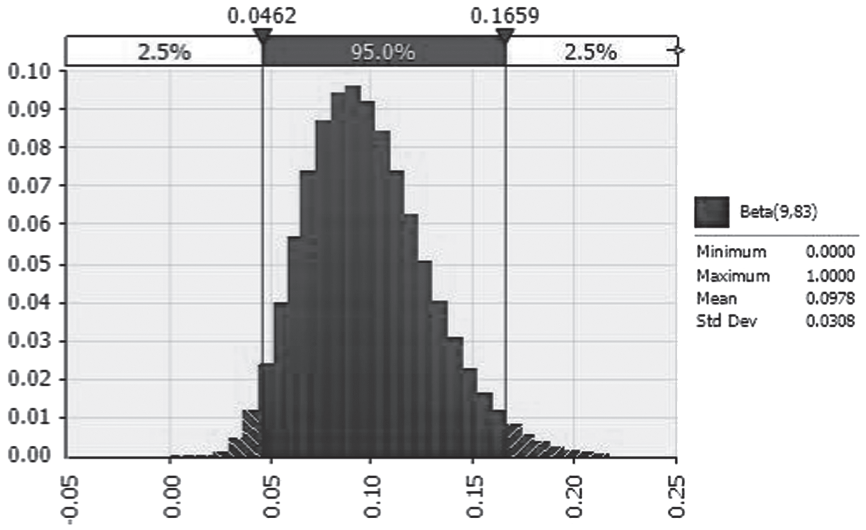

Fig. 5. Risk simulation of *Salmonella* spp. in pork products at the final stage of slaughtering level. X-axis; *Salmonella*'s prevalence. Y-axis; frequency of *Salmonella* samples.

appropriate conditions, however, even 1 CFU of *Salmonella* spp. can grow to several million[20]. Thus, even a relatively small number of *Salmonella* spp. at any production levels is highly significant if improper conditions during the process exist which provide an opportunity for proliferation to hazardous numbers[22].

Comparison of prevalence and contamination levels in samples from the different slaughterhouses, found that the levels were statistically significantly higher in slaughterhouse B ($p < 0.05$). High levels of contamination normally are found at the beginning of the transportation and lairage steps[23]. Stress due to longer waiting periods before processing could be a factor resulting in the pigs shedding increased numbers of pathogens in the intestinal lumen[19]. The scalding step in slaughterhouse B was found to have a high *Salmonella* spp. prevalence and high contamination levels. This finding was different from that of several studies which mention scalding as being considered a step that reduces the probability of contamination and the number of carcasses contaminated[1,12,23]. The reason for the higher levels in slaughterhouse B was probably that the water in the scalding tank in slaughterhouse B was reused during two or three batches of processing, allowing water contaminated with large amounts of organic matter (soil, pig feces or pig hairs) commonly found in the tank to contact many carcasses. The organic matter in the water could even have helped protect *Salmonella* spp. from being destroyed by the hot water[22]. The levels of *Salmonella* spp. contamination at the evisceration and splitting steps were higher than at the dehairing step. Improper practices and manipulation of contaminated material might have resulted in cross contamination[1,12,24]. In addition, the splitter might have played a role in spreading contamination from pig bellies to other sites on the carcasses[22]. Auto-cleaning of the electric splitter might not be sufficient to eliminate *Salmonella* spp. contamination, resulting in the lack of statistically significant difference observed with different types of splitting equipment (electric-splitter and knife). Interestingly, for slaughterhouse C, the values obtained were quite low, except for the splitting step. There were two positives out of 30 samples at that step, about 0.3 and 24 MPN/cm^2; these two *Salmonella* spp.-positive samples increased the overall mean contamination levels for this step. In the washing step, slaughterhouses A and B were found to be using tap water for processing. Tap water could not be expected to

bring about a decrease in the number of organisms; on the contrary, it could enhance the dissemination of organisms to other sites on the carcasses. Chlorinated water was used in slaughterhouse C, the numbers of *Salmonella* spp. were observed to decrease after this step[2]; *Salmonella* spp. contamination was found to be quite similar at all slaughterhouses in the cutting and dressing steps. Risk or Probability of *Salmonella* spp. contamination in pork products, after slaughtering level was considered low. Such value specified that in 95% of the sampling on pork products from the end process of slaughtering, probably at least 4.62% and in the maximum of 16.59% of products may be positive for *Salmonella* spp. This finding indicated the chilling step has been recognized as a suitable method for arresting colonization by *Salmonella* spp.[6], which compatible with the low levels of contamination observed in this study. However, acquiring output data can be used as the input data for assessing *Salmonella* spp. prevalence in the next production level.

Salmonella spp. in pigs during the slaughtering process is an important cause of salmonellosis in humans; however, this menace is unlikely to be alleviated effectively in the short term. This study found that pork is easily contaminated with this pathogen at every step of the slaughtering process. Good practices and appropriate hygiene measures at every step of slaughtering process should be promoted. These findings in this study highlight the need for continuous monitoring of slaughterhouses, along with an increased focus on problem solving to reduce downstream the contamination at retail shops as well as consumers.

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