

A monitoring report for *Salmonella* spp. in an HACCP standard pig slaughterhouse

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Abstract

To monitor the prevalence of *Salmonella* spp. in a Hazard Analysis and Critical Control Point (HACCP) standard pork slaughterhouse, a comprehensive inspection of an HACCP slaughterhouse was carried out. One hundred and eighty pairs of fecal and pork samples (n=360) and 65 pairs of important hygiene samples from the before and after working area (n=130) were collected, isolated and serotype identified, in an HACCP standard slaughterhouse in northeast Thailand. These isolates were then antimicrobial susceptibility tested against ampicillin, ciprofloxacin, chloramphenicol, nalidixic acid, streptomycin, tetracycline, sulfamethoxazole/trimethoprim, and colistin. As a result, there were 61.11% *Salmonella* spp. isolated from total fecal samples (110/180), 12.78% of pork (23/180) and 6.15% from hygiene test (8/130), and the most common antimicrobial resistance was against ampicillin, streptomycin, and tetracycline. The PFGE result shows the *Salmonella* isolates of pork were contaminated from their own farm, and these dominant isolates can be sorted into 3 major sources with high similarity. Furthermore, nine fecal and ten pork *Salmonella* spp. isolates shared the same serotype and PFGE pattern in the paired samples of the dual positive fecal and carcass isolates from the total 180 pairs. The relativity rate of *Salmonella* spp. isolates from feces to pork was 5.00% (9 pairs of 180 pairs). In conclusion, there is a low rate of *Salmonella* spp. from farm *via* direct slaughter contamination, and the most reported cases could be from cross-contamination during the next process of transporting and storage. More work should be carried out focusing on improving meat production, transportation, and storage.

Keywords: *Salmonella* spp., Hazard Analysis and Critical Control Point, pig slaughterhouse

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Introduction

Salmonella spp. is the most common and threatening pathogenic bacteria to human beings, which can lead to salmonellosis. The non-typhoidal *Salmonella* enterica results in the highest burden of all the foodborne diseases, causing 4.07 million disability-adjusted life years (Kirk et al., 2015). The data from the World Health Organization show that there are approximately 11,800 non-typhoidal *Salmonella* enterica cases per 100,000 persons in the world. Human outbreaks are caused by consuming incompetently cooked meat mostly, while pork is one of the most popular meats (EFSA et al., 2017). And 95% of all cases of salmonellosis are caused by contaminated animal products (Foley et al., 2008).

The Hazard Analysis and Critical Control Point (HACCP) program was introduced to produce safe meat products for consumers. HACCP is a management system to provide safe meat products by controlling the biological, chemical, and physical hazards of meat, though monitoring and organizing the whole procedure. This management system was accepted after the USDA's Food Safety and Inspection Service (FSIS) announced the rule in 1996 for chicken products. After that, there were 190,000 cases less over 1996-2000 (Williams & Ebel, 2012), and little affliction afterwards (Ollinger et al., 2017).

HACCP is not a perfect system to stop all of the spread of *Salmonella* spp. after slaughtering, even though it is the best one for now. Systematic reviews (Wilhelm et al., 2011) from 14 studies of evaluating the effectiveness from beef, pork and poultry HACCP abattoirs were carried out, and most of the studies reported positive *Salmonella* spp. outcome, which indicates multiple factors that would contribute to contaminated meat production in the HACCP procedure. To assess the efficiency of the HACCP system in the quality of pork production, and obtain more detailed information about the production process, a comprehensive inspection of a pork HACCP slaughterhouse was performed. The fecal and carcass samples of the slaughter process were monitored in pairs, while hygiene samples before and after work were taken as well.

Materials and Methods

Sample collection: One hundred and eighty fecal and 180 pork samples were collected in pairs (n=360) from an HACCP standard slaughterhouse in northeast Thailand, and 30 pairs each time from 6 farms. Additionally, 65 pairs from 5 important hygiene zones from before and after working areas (n=130) were tested. These 5 zones included 9 points in the personal hygiene semi-clean zone, 11 points in the personal hygiene clean zone, 15 points in the equipment clean zone, 16 points in the machine clean zone and 14 in the equipment unclean zone. The fecal samples were collected by sterile rectal swab, the carcass samples were collected by sterile swab covering a 600 cm² area of each carcass, and for the hygiene-test samples were collected by wiping over a 25 cm² area of the target. The procedures have been approved by the Institutional Animal Care and Use Committee of Khon Kaen University, ID: IACUC-KKU-13/62.

***Salmonella* isolation:** All samples were isolated and identified for *Salmonella* spp., following the ISO standard 6579-1:2002 (ISO, 2002). The positive *Salmonella* spp. colonies were further sent to the WHO National Salmonella and Shigella Center for serotype identification.

Antimicrobial susceptibility test: The *Salmonella* spp. isolates were examined for antimicrobial susceptibility against ampicillin (AMP) 10 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (C) 30 µg, nalidixic acid (NA) 30 µg, streptomycin (S) 10 µg, sulfamethoxazole/trimethoprim (SXT) 25 µg, tetracycline (TE) 30 µg and colistin (CT) 10 µg, following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014).

Genetic relatedness test: The predominant isolates, the positive paired sample units and some related isolates were tested by pulsed field gel electrophoresis (PFGE), following the CDC standard (Control & Prevention, 2013). The 'positive paired sample units' means the paired fecal and pork samples which share the same serotype. The DNA fragments were separated by a CHEF-DRIII Pulsed-Field Electrophoresis System. The gel was stained with ethidium bromide and documented by ChemiDoc™ XRS+ (Bio-Rad). The dendrograms were produced using the band cluster method by BioNumerics version 7.6.3

Data analysis: The fecal and pork positive isolates were compared, in which relativity results were analyzed by Calculate and Draw Custom Venn Diagrams online (Ghent University, 2018).

Results

There were 61.11% *Salmonella* spp. isolated from total fecal samples (110/180), 12.78% from pork (23/180) and 6.15% from hygiene tests (8/130) in one slaughterhouse. The predominant serotypes identified in feces were Enterica type I 1,4,[5],12:- (50.00%), Rissen (13.64%), Kedougou (12.73%), Enterica 4,12:- (9.09%) and Bovismorbificans (8.18%); in pork they were Enterica type I 1,4,[5],12:- (60.87%) and Enterica 4,12:- (34.78%); while in the hygiene test there was Enterica type I 1,4,[5],12:- (75.00%) (Table 1).

These *Salmonella* isolates were then tested against 8 antimicrobial agents. The positive isolates from feces were resistant to AMP (76.36%), C (11.82%), S (60.00%), SXT (17.27%) and TE (85.45%), the positive *Salmonella* spp. from pork were resistant to AMP (95.65%), S (95.65%) and TE (95.65%), while those from the hygiene test were resistant to AMP (37.50%), S (25.00%), SXT (12.50%) and TE (87.50%) (Table 2). In summary, the most common resistance was similar to the prevalence in the north of Thailand, AMP (53%), S (44%) and TE (53%) (Patchanee et al., 2016).

Twenty one *Salmonella* Typhimurium and Typhimurium variant isolates were selected and analyzed by PFGE (monophasic variants of *S. Typhimurium* grouped by the European Food Safety Authority (Authority et al., 2017). The PFGE result shows the *Salmonella* isolates of pork were

contaminated from their own farm. Moreover, these Typhimurium and Typhimurium variants can be

sorted into three clusters and keep a high level of similarity (Figure 2).

Table 1 Serotypes of *Salmonella* spp. isolated from an HACCP pig slaughterhouse

Source	No. of isolates (%)	Serotypes (Number of isolates)
Feces	110 (61.11)	Enterica type I <u>1</u> ,4,[5],12:i:- (55), Rissen (15), Kedougou (14), Enterica 4,12:-:1,2 (10), Bovismorbificans (9), Derby (4), Krefeld (1), Typhimurium (1), Enterica 4,5,12:-:1,2 (1)
Carcass	23 (12.78)	Enterica type I <u>1</u> ,4,[5],12:i:- (14), Enterica 4,12:-:1,2 (8), Bovismorbificans (1)
Hygiene	8 (6.15)	Enterica type I <u>1</u> ,4,[5],12:i:- (6), Enterica 3,10:-:1,2 (1), Rissen (1)

Table 2 Antimicrobial resistance of *Salmonella* spp. isolated from an HACCP pig slaughterhouse by serotypes

Source	Serotype (n)	Antimicrobial resistance agents*							
		AMP	C	CIP	NA	S	SXT	TE	CT
Feces (110)	total	84 (76.36)	13 (11.82)	0	0	66 (60.00)	19 (17.27)	94 (85.45)	0
	I <u>1</u> ,4,[5],12:i:- (55)	51 (60.71)	0	0	0	50 (75.76)	2 (10.53)	52 (55.32)	0
	Rissen (15)	15 (17.86)	0	0	0	4 (6.06)	12 (63.16)	15 (15.96)	0
	Kedougou (14)	1 (1.19)	13 (100)	0	0	0	0	14 (14.89)	0
	4,12:-:1,2 (10)	10 (11.90)	0	0	0	9 (13.64)	1 (5.26)	10 (10.64)	0
	Bovismorbificans (9)	0	0	0	0	0	0	0	0
	Derby (4)	4 (4.76)	0	0	0	0	4 (21.05)	0	0
	Krefeld (1)	1 (1.19)	0	0	0	1 (1.52)	0	1 (1.06)	0
	Typhimurium (1)	1 (1.19)	0	0	0	1 (1.52)	0	1 (1.06)	0
	4,5,12:-:1,2 (1)	1 (1.19)	0	0	0	1 (1.52)	0	1 (1.06)	0
Pork (23)	total	22 (95.65)	0	0	0	22 (95.65)	0	22 (95.65)	0
	4,12:i:- (14)	14 (63.64)	0	0	0	14 (63.64)	0	14 (63.64)	0
	4,12:-:1,2 (8)	8 (36.36)	0	0	0	8 (36.36)	0	8 (36.36)	0
	Bovismorbificans (1)	0	0	0	0	0	0	0	0
Hygiene (8)	total	3 (37.50)	0	0	0	2 (25.00)	1 (12.50)	7 (87.5)	0
	I <u>1</u> ,4,[5],12:i:- (6)	2 (66.67)	0	0	0	2 (100)	0	6 (85.71)	0
	3,10:-:1,2 (1)	0	0	0	0	0	1 (100)	0	0
	Rissen (1)	1 (33.33)	0	0	0	0	0	1 (14.29)	0

Note: The clear zone of disk diffusion test of Colistin in this study followed the standard of *Escherichia coli*, which also belongs to *Enterobacteriaceae* (CLSI, 2014).

Discussion

The positive rate of *Salmonella* spp. from feces was 61.11% and from carcass was 12.78%, which showed the HACCP system could block the spread of pathogens during slaughter. According to the results (Figure 1), the HACCP system can stop these pathogens most of the time, but the most cross-contamination is concentrated in one time process, revealing that a tiny mistake can reduce the pork quality. Additionally, these isolates of the predominant prevalent serotypes are similar to the report from Chiang Mai, Thailand (Patchanee *et al.*, 2016), that

major serotypes included Enterica type I 1,4,[5],12:i:-, Kedougou, and Rissen. These serotypes belong to the top 10 most common serotypes from patients in Thailand (Hendriksen *et al.*, 2009). This reveals a close correlation between retail pork and patients. Furthermore, these serotypes (Enterica type I 1,4,[5],12:i:-, Enterica 1,4,[5],12:-:1,2 and Enterica 1,4,[5],12:-:1,2) are variants of Enterica Typhimurium (Bugarel *et al.*, 2012). This indicates a high prevalence of Enterica Typhimurium variant isolates in northeast Thailand during the sample collection time.

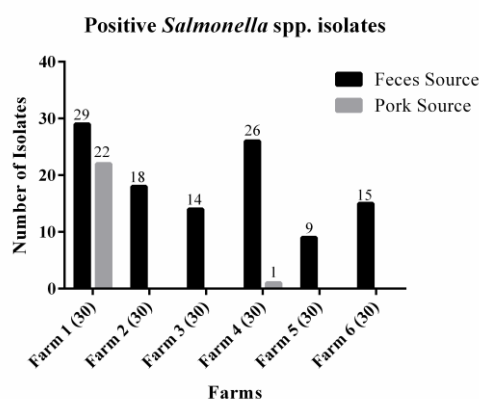
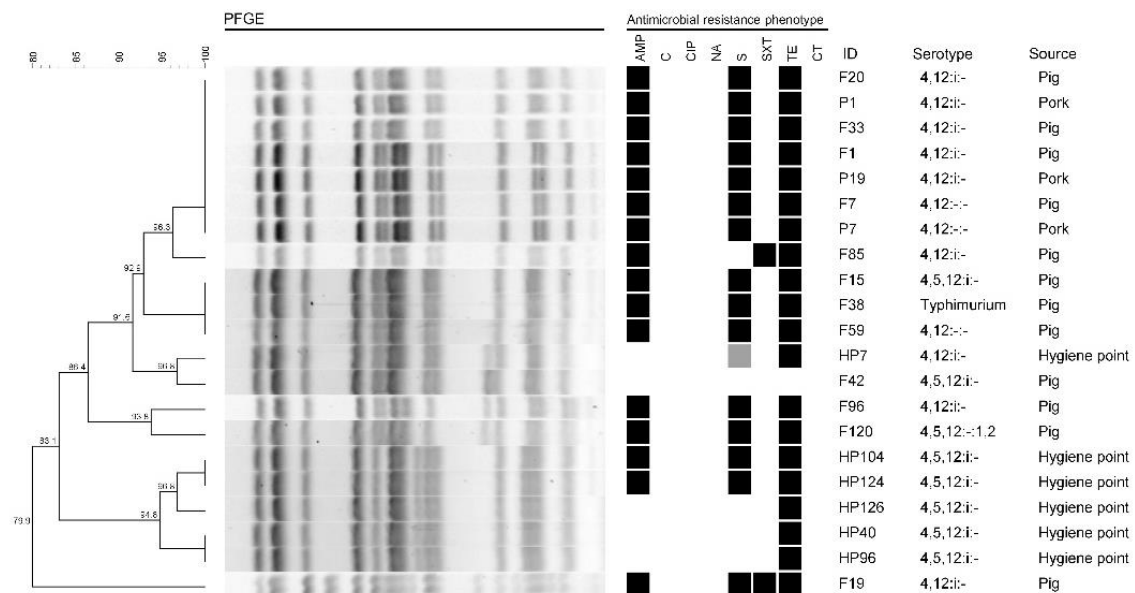


Figure 1 Positive *Salmonella* spp. isolates from feces and pork sources



Note: Monophasic variants of *S. Typhimurium* are marked by antigenic formula.

Figure 2 Genetic relatedness of the predominant and paired serotype *Salmonella* spp. Isolates

The serotype and antimicrobial resistance results from the hygiene test are presented in Tables 1 and 2, while the sources of hygiene test isolates are shown in Table 3, and some weak points of the HACCP system in this slaughterhouse are mentioned. There were 3 *Salmonella*-contaminated points in the hygienic clean part (belt conveyor No. 1, belt conveyor metal detector No. 3 and pork grinder production staff). Multiple pathogen subtypes could infect the procedure. The belt is not only the transportation equipment that transports the product in the working line, but it also provides convenience to spread pathogens. Since the pork grinder is a gathering part, it would crossly contaminate to other pork and staff. For a better hygienic product, this system can improve the belt conveyor part, the pork grinder part, and their peripheral devices. It is suggested that the part for grinding each carcass should be separated and disinfected, and could be separated by a tray during

transportation *via* the belt. For example, the grinder machine could be improved with a disinfection function, and the staff should disinfect their hands thoroughly before handling or dressing other carcasses.

Nine feces and 9 pork *Salmonella* spp. isolates were serotype and PFGE matched in the paired samples of the 110 fecal and 23 carcass isolates from the total 180 pair samples ($n=360$) (Figures 1 & 3). The relativity rate of *Salmonella* spp. isolates from feces to pork was 5.00% (9/180) (Figure 1 & 3). It reveals a low relativity rate from feces to pork directly in the process of the HACCP standard slaughtering. However, farm source (from feces) contributes 39.13% of total contaminations (9/23) and the remaining 60.87% (14/23) is from cross-contamination during the slaughter process. This indicates that the direct way from farm to pork is still a dominant source of contamination.

Table 3 Positive *Salmonella* spp. hygiene test of 5 zones isolated from a pig HACCP slaughterhouse

Region	time	Positive points	Serotype
Machine Clean Zone	After work	Belt conveyor NO.1	3,10:-:-
Machine Clean Zone	After work	Belt conveyor metal detector NO.3	I 1,4,[5],12:i:-
Personal Hygiene Clean Zone	After work	Pork grinder production staff	I 1,4,[5],12:i:-
Personal Hygiene Semi Clean Zone	Before work	Pork grinder production staff	I 1,4,[5],12:i:-
Equipment Unclean Zone	After work	Pork grinder production staff	I 1,4,[5],12:i:-
Equipment Unclean Zone	Before work	Pig stomach washing machine	Rissen
Equipment Unclean Zone	After work	Pig stomach washing machine	I 1,4,[5],12:i:-
Equipment Unclean Zone	After work	Roller after pig dehairing	I 1,4,[5],12:i:-

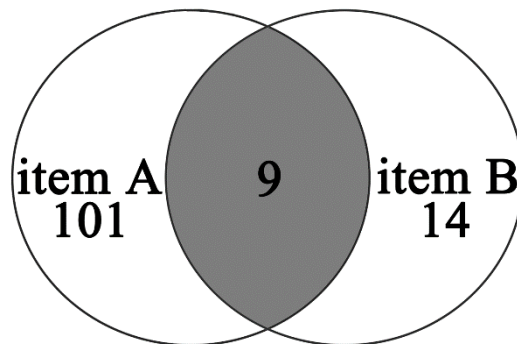
Conclusion

The findings from this study suggest that the HACCP system needs to be improved to stop pathogens spreading, especially at some weak points during the production procedure including the belt

conveyor, pork grinder and their peripheral devices. To our knowledge, this is the first study to estimate the direct and cross contamination rate revealing the probability of the contamination source during the production process. The rate of the *Salmonella* spp.

from farm to retail via direct contamination is low, and most cases are stopped by HACCP slaughter. However, the high prevalence report of retail infection

should be from the following cross-contamination. Further works should focus on improving the meat production, transport and storage.



Note: The 110 feces source isolates (item A) and 23 pork source isolates (item B) were isolated and serotypes identified from 180 pairs of fecal and pork samples. Nine pair isolates were serotype and PFGE matched.

Figure 3 Proportional relationship of *Salmonella* spp. isolates between feces and pork source shown by Venn diagram.

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