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Original Article

Distribution, quantitative load and characterization of *Salmonella* associated with swine farms in upper-northern Thailand

Pakpoom Tadee[1], Kittipong Kumpapong[1], Danai Sinthuya[1], Panuwat Yamsakul[1], Nipa Chokesajjawatee[2], Supachai Nuanualsuwan[3], Suchawan Pornsukarom[4], Bayleyegn Z. Molla[4], Wondwossen A. Gebreyes[4], Prapas Patchanee[1,*]

[1]*Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*
[2]*National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani 12120, Thailand*
[3]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
[4]*Department of Veterinary Preventive Medicine, Faculty of Veterinary Medicine, Ohio State University, Columbus, OH 43210, USA*

This study was conducted to analyze the prevalence and quantitative loads of *Salmonella* spp. on pig farms in Chiang Mai, Lamphun, Thailand to assess loading levels before slaughtering. The serotype diversity, antimicrobial-resistance pattern and pulse-field type of *Salmonella* spp. were also characterized to assess the dynamic propagation of the pathogen. The *Salmonella*-positive prevalence was 246/805 (30.56%), and the quantitative loads varied from 1.48～4.04 Log_{10}MPN/g, with a mean ± standard deviation of 2.11 ± 0.57. AMP/S/TE (ampicillin/streptomycin/tetracycline) was the highest frequency antimicrobial resistance pattern found in this study. In addition, *Salmonella* Rissen was the primary serotype in this region. PFGE results indicated the occurrence of infection by cross contamination among pig farms. Our study showed that pork is easily contaminated with this pathogen. Farm control programs must be based on strict biosecurity and hygienic measures, which could further reduce the contamination pressure at slaughterhouses or retail shops.

Keywords: characterization, pig, prevalence, quantitative load, *Salmonella*

Introduction

Salmonella spp. comprises one of the most important bacterial-zoonotic pathogens, causing acute food-borne diseases in humans [28], and is recognized as a major public health problem [10]. Salmonellosis is the group of clinical conditions caused by *Salmonella* spp., and an estimated 80.3 million cases of foodborne Salmonellosis occur worldwide annually [23]. 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 [14]. Although contaminated eggs and raw or undercooked poultry are the primary sources of Salmonellosis in humans, pork causes an estimated 15～20% of all cases [16]. While contamination can occur during any process along the food production line [1,21], infected pigs on the farm are the origin of the contaminated pork that leads to human infections [12].

Several studies have assessed *Salmonella* prevalence on farms. García-Feliz reported a *Salmonella* prevalence of 43.1% in finishing pig herds in Spain [9]. In contrast, Visscher reported a *Salmonella* prevalence of 5.58% in fattening pigs in Lower Saxony, Germany [28]. However, *Salmonella* spp. data is insufficient for quantitative measurement and development of strategies to reduce the risk of this pathogen.

Pig farmers routinely use antibiotics for both treatment and prophylactic purposes. Excessive and incorrect uses of antibiotics are probably a primary cause of increasing bacterial resistance [23,27]. In addition, further study of

*Corresponding author: Tel: +66-53-948002; Fax: +66-53-948065; E-mail: patprapas@gmail.com

the antibiotic resistance profiles of isolates is needed to develop antibiotic resistance profiles of pigs for porcine and human cases.

Pathogen characterization is essential for investigation of foodborne bacteria epidemiology. Serotyping is one of the most common techniques used for *Salmonella* characterization. However, this technique has a lower discriminatory power than molecular techniques such as pulsed field gel electrophoresis (PFGE). PFGE has long been accepted as a molecular characterization method for a wide range of bacterial species, including *Salmonella*. This technique clearly and precisely distinguishes bacterial genotypic diversity and is more appropriate for epidemiological investigations of foodborne pathogens, such as *Salmonella* spp. [8].

The objectives of this study were to determine the prevalence and quantitative loads of *Salmonella* spp. at the farm level in Chiang Mai - Lamphun to assess loading levels before going to the slaughterhouse and define the diversity of *Salmonella* spp. by phenotypic and serotypic characterizations. In addition, the pulse-field types were assessed to determine dynamic propagation, including origin, evaluation and common source of infection or transmission patterns of *Salmonella* spp. in the study area.

Materials and Methods

Sample collection

The present study was performed on six farms (A ~ F) in Chiang Mai - Lamphun (Thailand). A total of 606 fecal samples were randomly collected from the rectum of pigs by the individual finger palpation method. Fecal samples from pigs aged 4, 8, 12, 18 and 24 weeks, sows and boars were collected for further microbiological assay for detection and quantification purposes. In addition, 199 environmental samples were collected, including swabs from the floor of the animal house, feeder, nipple-drinker, and worker's hands and boots (100 cm^2/samples). Samples were also collected from the drinking water, feed and pests (such as flies). All samples were shipped in an icebox to the Central Laboratory, Chiang Mai University, for *Salmonella* isolation within 24 h of collection.

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 [13].

For the qualitative assay, solid samples of fresh feces, feed and flies were obtained. Next, nine times the 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). 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, UK). 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) and brilliant-green phenol red lactose sucrose agar (BPLS; Merck) 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), urease and motile indole lysine decarboxylase (MIL; Merck).

Environmental samples such as drinking-water were also added into nine times the quantity of BPW as pre-enrichment media and incubated at 37°C for 24 h. Next, aliquots of 0.1 mL and 1 mL were transferred to 9.9 mL of Rappaport-Vassiliadis broth (RV; Merck) and 9 mL of tetrathionate broth (TT; Merck), respectively. After incubation at 42°C for 24 h for RV and 37°C for 24 h for TT, material taken from each broth was streaked onto selective solid media (XLD and BPLS agar), and a biochemical test for presumptive colonies was conducted.

Environmental swab samples were subjected to the same procedure used for drinking-water samples, except that these samples were prepared with 100 mL of BPW, rather than nine times the weight, in the pre-enrichment process.

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 [17].

Serotyping and antimicrobial susceptibility testing

A total of 200 *Salmonella*-positive isolates were randomly serotyped as appropriate by the WHO National *Salmonella* and *Shigella* Center Laboratory (NSSC), Nonthaburi, Thailand. In addition, each serotype was submitted to antimicrobial susceptibility testing. Susceptibility to a panel of ten antimicrobial agents was investigated and interpreted by disk diffusion [4]. If isolates showed intermediate resistance [4], they were grouped with the susceptible isolates to avoid overestimation of resistance. The antibiotics were abbreviated as follows: ampicillin (AMP); amoxicillin-clavulanic acid (AUG); chloramphenicol (C); ciprofloxacin (CIP); cefotaxime (CTX); nalidixic acid (NA); norfloxacin (NOR); streptomycin (S); tetracycline

(TE); sulfamethoxazole-trimethoprim (SXT) (Oxoid).

PFGE genotyping

DNA fingerprinting of the first major serotype isolated in this study was conducted using PFGE at the Infectious Diseases Molecular Epidemiology Laboratory (IDMEL) of Ohio State University. Twenty-five isolates were selected at random and subjected to PFGE according to the CDC's standardized PulseNet protocol for *Salmonella* [19]. The PulseNet "Universal" standard strain *Salmonella enterica* serovar Braenderup H9812 was used as a reference marker, and *XbaI* was used as a digestion enzyme. Gel images were transferred to Bionumerics software ver. 3.5 for cluster analysis. Cluster analysis was performed using the unweighted pair group method, with optimization with 1.0% band position tolerances and 2.5% optimization values. Similarity coefficients were obtained within Bionumerics by calculating Dice coefficients. PFGE banding patterns with a similarity index $>75\%$ were grouped within the same genotypic cluster.

Statistical analyses

The data were collected and analyzed for descriptive statistical analysis of *Salmonella* in both prevalence and numbers by Microsoft Excel and PHstat2. A Chi-square test and ANOVA were used to compare the proportion of the presence of *Salmonella* and the mean of the MPN numbers, respectively, in each group of samples by StataSE9.0 (StataCorp, USA).

Results

The overall prevalence of *Salmonella* spp. in pig farms in Chiang Mai - Lamphun was 30.56% (246/805). For the fecal samples, 34.98% (212/606) of the positives samples were included. These were classified into seven groups included in this study. According to the Chi-square test, the greatest prevalence was in finishing pigs aged 12 weeks (57.73%, 95% CI: 47.90～67.56). The prevalence of *Salmonella*-positive samples in the environment was 17.08% (34/199), which was lower than that of the fecal samples. No positive results were observed in the feed samples, and the prevalence of *Salmonella* in the environmental samples was not significantly different among the six farms ($p > 0.05$).

We were unable to re-isolate *Salmonella* spp. from 87 samples (74 fecal samples and 13 environmental samples) during the quantification assays. Consequently, some sample types could not be quantified, including the drinking-water, worker's hands and fly samples. In the remaining 159 positive samples, the number of *Salmonella* ranged from 1.48～4.04 Log_{10}MPN/g, with a mean ± standard deviation (SD) of 2.11 ± 0.57 (data not shown). In addition, there was no significant difference between

Table 1. Distribution and quantification of *Salmonella*-positive samples isolated from pig farms in Chiang Mai, Lamphun, Thailand

Type of samples		Prevalence % (n)	95% Confidence intervals	Average Log_{10}MPN/g	95% Confidence intervals
Feces	Sow	30.00 (24/80)^{b}	19.95～40.04	2.07^{x}	1.77～2.38
	Boar	40.32 (25/62)^{b}	28.11～52.53	2.36^{x}	1.95～2.78
	3 weeks	33.75 (27/80)^{b}	23.38～44.11	1.97^{x}	1.80～2.16
	8 weeks	14.73 (14/95)^{a}	7.60～21.86	1.68^{x}	1.54～1.83
	12 weeks	57.73 (56/97)^{c}	47.90～67.56	2.08^{x}	1.91～2.26
	18 weeks	28.13 (27/96)^{b}	19.13～37.12	2.02^{x}	1.81～2.24
	24 weeks	40.62 (39/96)^{b}	30.80～50.45	1.98^{x}	1.76～2.21
	Sub-total	34.98 (212/606)	31.19～38.78	2.07	1.98～2.16
Environment	Feed	0 (0/30)^{d}	0	0	0
	Drinking-water	6.67 (2/30)^{de}	−2.25～15.59	−	−
	Nipple-drinker	16.67 (5/30)^{def}	3.33～30.00	2.28^{y}	1.44～3.13
	Feeder	13.33 (4/30)^{def}	1.17～25.50	1.95^{y}	1.51～2.39
	Floor	26.67 (8/30)^{ef}	10.84～42.49	2.58^{y}	1.88～3.30
	Worker's hands	10.53 (2/19)^{def}	−3.27～24.33	−	−
	Worker's boots	42.11 (8/19)^{f}	19.90～64.30	2.55^{y}	1.86～3.25
	Flies	45.45 (5/11)^{f}	16.03～74.88	−	−
	Sub-total	17.08 (34/199)	11.85～22.31	2.38	2.10～2.67
	Grand-total	30.56 (246/805)	27.38～33.74	2.11	2.02～2.20

Superscripts (a,b,c) and (d,e,f) in each column indicate significant differences ($p < 0.05$) of prevalence among sample types determined using the Chi-square test. The superscripts (X) and (Y) indicate that ANOVA was used to determine the numbers of *Salmonella* in each sample type.

Table 2. Serodiversity of *Salmonella* (*S.*)-positive samples (n = 200) isolated from pig farms in Chiang Mai, Lamphun, Thailand

Serotype	Distribution of *Salmonella*-positive samples					
	Fecal samples		Environmental samples		All samples	
	Number	%	Number	%	Number	%
S. I. 4,12 : i : -	1	0.60	0	0	1	0.5
S. I. 4,5,12 : i : -	24	14.29	3	9.38	27	13.5
S. I. ser. 3,10:-:1,7	2	1.20	0	0	2	1.0
S. IV. ser 43:z4z23:-	0	0	1	3.12	1	0.5
S. Agona	1	0.60	0	0	1	0.5
S. Amsterdam	2	1.20	0	0	2	1.0
S. Anatum	17	10.11	0	0	17	8.5
S. Augustenborg	1	0.60	1	3.12	2	1.0
S. Derby	3	1.78	1	3.12	4	2.0
S. Enteritidis	3	1.78	1	3.12	4	2.0
S. Give	7	4.16	0	0	7	3.5
S. Krefeld	1	0.60	0	0	1	0.5
S. Lexington	10	5.95	3	9.38	13	6.5
S. Mbandaka	0	0	1	3.12	1	0.5
S. Panama	9	5.35	0	0	9	4.5
S. Rissen	44	26.19	12	37.50	56	28
S. Senftenberg	2	1.20	2	6.25	4	2.0
S. Stanley	1	0.60	3	9.38	4	2.0
S. Typhimurium	31	18.44	0	0	31	15.5
S. Weltevreden	9	5.35	4	12.50	13	6.5
Total	168	100	32	100	200	100

sample groups (Table 1).

Table 2 shows the serodiversity of 200 randomly selected *Salmonella* strains. Up to 20 serotypes were found in this study. The highest frequency isolate was *Salmonella* Rissen (28%), followed by *Salmonella* Typhimurium (15.5%) and *Salmonella* I. 4,5,12 : i : - (13.5%). *Salmonella* I. 4,12 : i : -, *Salmonella* IV. ser 43:z4z23, *Salmonella* Agona, *Salmonella* Krefeld and *Salmonella* Mbandaka were present at the lowest frequencies (0.5% each). In addition, 9 of 20 serotypes were found in both the fecal and environmental samples. However, 9 and 2 serotypes were unique to the fecal and environmental samples, respectively.

Fig. 1 shows the percentage of antimicrobial resistant *Salmonella* strains (n = 200). Most isolates were resistant to ampicillin (AMP) (81.5%), followed by tetracycline (TE) (74.5%), streptomycin (S) (70.5%) and sulfa-trimethoprim (SXT) (35.5%). However, none of the isolates showed resistance to norfloxacin (NOR), ciprofloxacin (CIP) or amoxicillin-clavulanic acid (AUG).

The distribution of antimicrobial resistance patterns is summarized in Table 3. This study found 25 different resistance patterns among the 200 isolates. One hundred

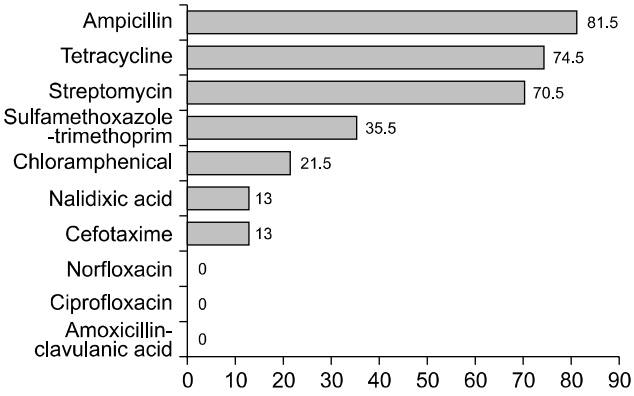

Fig. 1. The percentage antimicrobial resistance of *Salmonella* strains (n = 200) from pig farms in Chiang Mai, Lamphun, Thailand.

sixty-nine isolates showed resistance to at least one antimicrobial drug, while 31 isolates were susceptible to all drugs tested. Most samples in this study showed patterns common to both fecal and environmental samples (145 isolates in eight resistance-patterns), followed by samples showing resistance patterns only observed in fecal samples

Table 3. Distribution of antimicrobial resistance patterns from *Salmonella*-positive isolates (n = 200) in pig farms in Chiang Mai, Lamphun, Thailand

	Fecal samples (number)	Environmental samples	All samples (number)
A. Common pattern in isolates from fecal and environmental samples			
AMP C CTX S TE	10	3	13
AMP S TE SXT	28	5	33
AMP S TE	47	3	50
AMP TE SXT	6	3	9
AMP TE	1	3	4
AMP S	1	2	3
NA	1	1	2
Pansusceptible to all test	21	10	31
Subtotal A	115	30	145
B. Pattern only observed in fecal sample isolates			
AMP C NA S TE SXT	5	0	5
AMP C S TE SXT	9	0	9
AMP C NA TE SXT	3	0	3
AMP CTX NA S TE	1	0	1
AMP NA S TE SXT	1	0	1
C NA S TE SXT	1	0	1
AMP CTX S TE	8	0	8
AMP NA S TE	8	0	8
AMP C S SXT	8	0	8
AMP C CTX TE	1	0	1
AMP CTX TE	1	0	1
AMP C SXT	1	0	1
AMP CTX	1	0	1
AMP NA	3	0	3
NA TE	2	0	2
Subtotal B	53	0	53
C. Pattern only observed in environmental sample isolates			
AMP C CTX S TE SXT	0	1	1
AMP C S	0	1	1
Subtotal C	0	2	2
Grand total	168	32	200

AMP: ampicillin, C: chloramphenicol, CTX: cefotaxime, S: streptomycin, TE: tetracycline, SXT: sulfamethoxazole-trimethoprim, NA: nalidixic acid.

(53 samples in 15 resistance-patterns). Only two patterns were observed in only one isolate from each environmental sample. The highest frequency antimicrobial resistance pattern among the tested isolates in this study was AMP/S/TE (ampicillin/streptomycin/tetracycline).

Salmonella Rissen, the most common type observed in this study, was randomly selected (n = 25) from fecal samples for pulse-field gel electrophoresis characterization. The PFGE *Xba*I macrorestriction banding patterns consisted of 10～12 DNA fragment bands. PFGE generated two major genotypic clusters (X-Y) with a dice coefficient index cut-off point of 75% (Fig. 2). The similarity among cluster X was about 75～100%, and represented the predominant group in this study, which comprised 18 isolates (from farms A, B and D in 8, 2, and 8 isolates, respectively). The *Salmonella* Rissen isolated from farm A and B showed identical DNA fingerprint profiles clustered in only one genotypic group (cluster X). Cluster Y contained two indistinguishable isolates from farm C. Interestingly, among samples from farm D, all isolates (8/8) from cluster X and all isolates from cluster Y (5/5) belonged to different pig type samples, suggesting a different source of infection among starters and sows in farm D. Finally, the antimicrobial resistance patterns of isolates from the same farm were partially different, except for six isolates from farm A and two isolates from farm C, which showed a

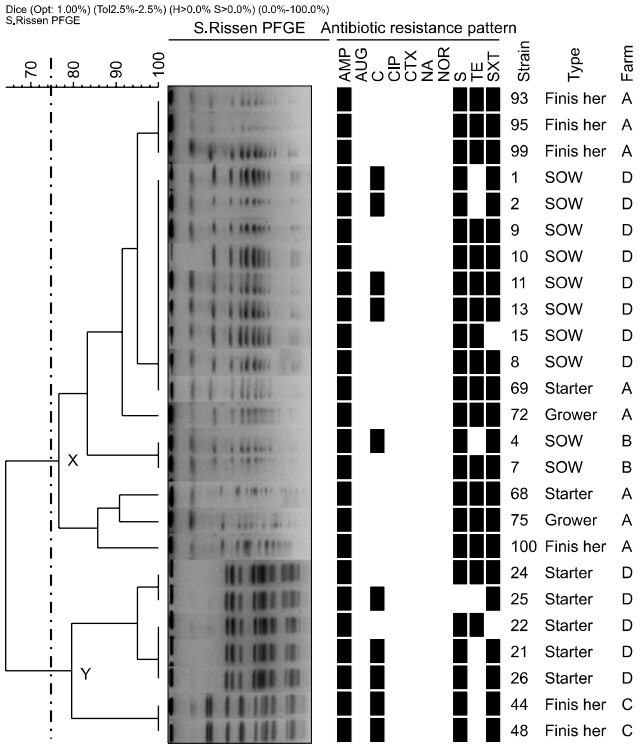

Fig. 2. Dendrogram representing PFGE-*Xba*I identified in the 1st majority serotype with antimicrobial resistance patterns of *Salmonella* Rissen (n = 25) from farms A ~ D in Chiang Mai, Lamphun, Thailand, with similarity determined by the Dice co-efficient and UPGMA clustering. The antibiotic resistance patterns include 10 antibiotics: ampicillin (AMP); amoxicillin-clavulanic acid (AUG); chloramphenicol (C); ciprofloxacin (CIP); cefotaxime (CTX); nalidixic acid (NA); norfloxacin (NOR); streptomycin (S); tetracycline (TE); sulfamethoxazole-trimethoprim (SXT).

similar pattern.

Discussion

The overall prevalence of *Salmonella* spp. was 30.56%, comprised of 34.98% from fecal samples and 17.08% from environmental samples. The prevalence of *Salmonella* in the fecal samples was similar to the 43.1% prevalence reported in finishing pigs from a study of fattening units in Spain [9], with comparable isolation technique. However, our study revealed substantially lower prevalence than other studies in Northern Thailand, which recorded prevalence in pre-slaughter pigs of 55% [20] and 63% [6]. These differences might be due to the timing of sampling ("on farm" in our study and pre-slaughter in their study), with the stress during transportation and lairage potentially increasing the shedding of *Salmonella* from the intestinal lumen [15,26]. In contrast, a study of finishing pigs conducted in Germany revealed lower prevalence (5.65%) than our study [28]. Fecal swabs from the rectum may 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.

Upon comparison of sample types, prevalence from environmental samples was generally lower than fecal samples, except for flies and worker's boot samples. Flies are a major vehicle for foodborne pathogens, and boots of workers easily come into contact with animal feces; thus, their prevalence may be higher than that of other environmental samples.

In this study, *Salmonella* spp. could not be recovered from 87 samples using quantitative assays, although corresponding portions of the same samples were positive in the qualitative assays. The heterogeneous distribution and the overall low number of *Salmonella* in the samples may have been due to a failure of re-isolation procedures [16]. The MPN range of the remaining *Salmonella*-positive samples was quite low (1.48 ~ 4.04 Log_{10}MPN/g for the fecal samples and 1.56 ~ 3.38 Log_{10}MPN/g for the environmental samples). However, under the right conditions, even 1 CFU can grow to several million [25]. 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 [21]. Some such conditions may include longer waiting times in lairage, contributing to increased shedding of the pathogen from the intestinal lumen [15,23], inadequate processing of the carcasses in the slaughterhouse, such as evisceration, resulting in carcass contamination [3,15,26], and temperature abuse in retail shops, leading to increasing contamination levels [2,5,7,11,15].

This study demonstrated that two major serotypes, *Salmonella* Rissen and *Salmonella* Typhimurium, were the most common observed during pig production in Chiang Mai - Lamphun, Thailand. These serotypes have been reported as the dominant serotype in pigs in the same region [6,18], and *Salmonella* Rissen was also the most common *Salmonella* serotype found in healthy humans in Upper Northern Thailand [18]. The serodiversity of *Salmonella*-positive samples from feces and the environment were quite similar, suggesting that the environment is a potential source of *Salmonella* infection in pigs [28]. The post-infected animal could be highly susceptible to re-infection when exposed to the environment [6]. Interestingly, *Salmonella* Typhimurium was only found in fecal samples, which differs from several studies in which *Salmonella* Typhimurium was also present in the environment [6,21,28]. Organisms in these samples may have been destroyed by exposure to sunlight or disinfectants, or there may have been no common source of infection with this serotype between pigs and the environment. In this study, some serotypes were found only in environmental samples, suggesting that

other sources not sampled in this study played roles as important shedders, such as wild birds, lizards or invertebrates.

We also demonstrated the widespread occurrence of antimicrobial resistance. Specifically, resistance (21.5% to 81.5%) to ampicillin, tetracycline, streptomycin, sulfa-trimethoprim and chloramphenicol was observed, which is concordant with the results of previous studies of *Salmonella* epidemiology in pigs, pork and humans in Belgium and Thailand [22,27]. Antimicrobial drugs from the same groups as ampicillin, streptomycin, tetracycline and sulfa-trimethoprim have been widely used on pig farms in Thailand. Sub-dosing or extra-label usage could explain the high rates of resistance [23,24,27]. In contrast, chloramphenicol was banned from animal production more than 10 years ago; however, resistance to this antibiotic could be due to horizontal gene transmission [27]. The observed absence of resistance to norfloxacin, ciprofloxacin and amoxicillin-clavulanic acid may have been due to the limited use of these antimicrobial drugs in pig production in Thailand. The highest frequency resistance was reported in AMP/S/TE, and in both sample types (pigs and environment). This finding suggests that there is horizontal transmission of the antimicrobial resistance-gene between *Salmonella* strains from feces to the environment.

PFGE profiling was used to identify similarities of *Salmonella* in the *Salmonella* Rissen isolates. The results also indicated the occurrence of cross contamination among pig farms. There were 12 isolates with identical PFGE patterns (similarity indexes of $\geq$95) from two different farms (A & D) classified in the same group (cluster X). This finding demonstrated that sources of Salmonellosis may spread over a wide area via the same supply chain (*e.g.*, gilt, feed or feed-ingredients) or a common source of infection among farms (*e.g.,* a transportation truck). In addition, all isolates from farm D were separated into two distinguishable genotypic groups among starters and sows, indicating no common source of infection between the two age groups. This may indicate that there is no common source of infection between the fattening and farrowing units, which are located approximately 10 km apart. However, in this study, infection in pigs resulting from environmental exposure could not be determined precisely. Thus, further investigation might be needed to assess the genotypic profiles of the positive environmental samples. When we compared the PFGE results and antimicrobial resistance patterns, the same antimicrobial resistance patterns with the same farm origin were observed, contrary to a previous study by Pulsrikarn *et al*. [22], who demonstrated that there was no association between PFGE and antimicrobial resistance patterns. This finding suggests that horizontal resistance gene transmission may be occurring among these farms.

Salmonella on farms is the first-origin of Salmonellosis in human cases, and is unlikely to be alleviated effectively in the short term. Farm control programs 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 at the farm level, which can reduce the contamination pressure downstream at slaughterhouses and retail shops.

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Conflict of interest

There is no conflict of interest.

References

1. **Berends BR, Van Knapen F, Mossel DAA, Burt SA, Snijders JMA.** Impact on human health of *Salmonella* spp. on pork in The Netherlands and the anticipated effects of some currently proposed control strategies. Int J Food Microbiol 1998, **44**, 219-229.
2. **Berends BR, Van Knapen F, Mossel DAA, Burt SA, Snijders JMA.** *Salmonella* spp. on pork at cutting plants and at the retail level and the influence of particular risk factors. Int J Food Microbiol 1998, **44**, 207-217.
3. **Berends BR, Van Knapen F, Snijders JM, Mossel DA.** Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. Int J Food Microbiol 1997, **36**, 199-206.
4. **CLSI.** Performance Standards for Antimicrobial Disc Susceptibility Test; Approved standard-Ninth Edition. CLSI document M2-A9. Clinical and Laboratory Standards Institute, Wayne, 2006.
5. **Delhalle L, Saegerman C, Farnir F, Korsak N, Maes D, Messens W, De Sadeleer L, De Zutter L, Daube G.** *Salmonella* surveillance and control at post-harvest in the Belgian pork meat chain. Food Microbiol 2009. **26**, 265-271.
6. **Dorn-in S, Fries R, Padungtod P, Kyule MN, Baumann MPO, Srikitjakarn L, Chantong W, Sanguangiat A, Zessin KH.** A cross-sectional study of *Salmonella* in pre-slaughter pigs in a production compartment of northern

Thailand. Prev Vet Med 2009, **88**, 15-23.
7. **Escartín EF, Lozano JS, Rodríguez O, Gonzáles NM, Torres JA.** Incidence and level of *Salmonella* serovars in raw pork obtained from mexican butcher shops. Food Microbiol 1995, **12**, 435-439.
8. **Foxman B.** Molecular Tools and Infectious Disease Epidemiology. 1st ed. pp. 23-40, Academic Press, Burlington, 2012.
9. **García-Feliz C, Collazos JA, Carvajal A, Vidal AB, Aladueña A, Ramiro R, De La Fuente M, Echeita MA, Rubio P.** *Salmonella enterica* infections in Spanish swine fattening units. Zoonoses Public Health 2007, **54**, 294-300.
10. **Giovannini A, Prencipe V, Conte A, Marino L, Petrini A, Pomilio F, Rizzi V, Migliorati G.** Quantitative risk assessment of *Salmonella* spp. infection for the consumer of pork products in an Italian region. Food Control 2004, **15**, 139-144.
11. **Gonzales-Barron UA, Redmond G, Butler F.** A risk characterization model of *Salmonella* Typhimurium in Irish fresh pork sausages. Food Res Int 2012, **45**, 1184-1193.
12. **Hauser E, Hebner F, Tietze E, Helmuth R, Junker E, Prager R, Schroeter A, Rabsch W, Fruth A, Malorny B.** Diversity of *Salmonella enterica* serovar Derby isolated from pig, pork and humans in Germany. Int J Food Microbiol 2011, **151**, 141-149.
13. **International Standard Organization.** Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp. 4th ed. ISO 6579:2002(E), ISO, Geneva, 2002.
14. **Jawetz E, Melnick JL, Adelberg EA.** Review of Medical Microbiology. 16th ed. pp. 244-248, Lange Medical Publication, Los Altos, 1984.
15. **Lo Fo Wong DMA, Hald T, van der Wolf PJ, Swanenburg M.** Epidemiology and control measures for *Salmonella* in pigs and pork. Livest Prod Sci 2002, **76**, 215-222.
16. **Mürmann L, dos Santos MC, Cardoso M.** Prevalence, genetic characterization and antimicrobial resistance of *Salmonella* isolated from fresh pork sausages in Porto Alegre, Brazil. Food Control 2009, **20**, 191-195.
17. **Oblinger JL, Koburger JA.** Understanding and teaching the most probable number technique. J Milk Food Technol 1975, **38**, 540-545.
18. **Padungtod P, Kaneene JB.** *Salmonella* in food animals and humans in northern Thailand. Int J Food Microbiol 2006, **108**, 346-354.
19. **Patchanee P, Molla B, White N, Line DE, Gebreyes WA.** Tracking *Salmonella* contamination in various watersheds and phenotypic and genotypic diversity. Foodborne Pathog Dis 2010, **7**, 1113-1120.
20. **Patchanee P, Zessin KH, Staak C, Srikijkarn L, Taravijitkul P, Tesaprateep T.** Pre-slaughther infection of *Salmonella* spp. and consideration of using the DANISH MIX-ELISA for monitoring *Salmonella* in pigs. Chiang Mai Vet J 2003, **1**, 33-38.
21. **Prendergast DM, Duggan SJ, Gonzales-Barron U, Fanning S, Butler F, Cormican M, Duffy G.** Prevalence, numbers and characteristics of *Salmonella* spp. on Irish retail pork. Int J Food Microbiol 2009, **131**, 233-239.
22. **Pulsrikarn C, Chaichana P, Pornruangwong S, Morita Y, Yamamoto S, Boonmar S.** Serotype, antimicrobial susceptibility, and genotype of Salmonella isolates from swine and pork in Sa Kaew province, Thailand. Wetchasan Sattawaphaet 2012, **42**, 21-27.
23. **Rostagno MH, Callaway TR.** Pre-harvest risk factors for *Salmonella* enterica in pork production. Food Res Int 2012, **45**, 634-640.
24. **Schwaiger K, Huther S, Hölzel C, Kämpf P, Bauer J.** Prevalence of antibiotic-resistant enterobacteriaceae isolated from chicken and pork meat purchased at the slaughterhouse and at retail in Bavaria, Germany. Int J Food Microbiol 2012, **154**, 206-211.
25. **Stephens PJ, Joynson JA, Davies KW, Holbrook R, Lappin-Scott HM, Humphrey TJ.** The use of an automated growth analyser to measure recovery times of single heat-injured Salmonella cells. J Appl Microbiol 1997, **83**, 445-455.
26. **Swanenburg M, van der Wolf PJ, Urlings HAP, Snijders JMA, van Knapen F.** *Salmonella* in slaughter pigs: the effect of logistic slaughter procedures of pigs on the prevalence of *Salmonella* in pork. Int J Food Microbiol 2001, **70**, 231-242.
27. **Van Boxstael S, Dierick K, Van Huffel X, Uyttendaele M, Berkvens D, Herman L, Bertrand S, Wildemauwe C, Catry B, Butaye P, Imberechts H.** Comparison of antimicrobial resistance patterns and phage types of *Salmonella* Typhimurium isolated from pigs, pork and humans in Belgium between 2001 and 2006. Food Res Int 2012, **45**, 913-918.
28. **Visscher CF, Klein G, Verspohl J, Beyerbach M, Stratmann-Selke J, Kamphues J.** Serodiversity and serological as well as cultural distribution of *Salmonella* on farms and in abattoirs in Lower Saxony, Germany. Int J Food Microbiol 2011, **146**, 44-51.