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Phenotypic and Genotypic Characterization of *Salmonella* spp. Isolated from Pigs and their Farm Environment in Korea

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This study's objective was to determine the prevalence of *Salmonella* spp. in pigs and their farm environments in Korea, and to investigate the relationship between the strains based on their phenotypic and genotypic characteristics. A total of 36 *Salmonella* spp. were isolated in this study: 18 isolates from 492 pigs (3.7%) and 18 isolates from 418 (4.3%) farmhouse environmental samples from 16 different pig farms. Of the *Salmonella* strains isolated from the numerous environmental samples, the highest prevalence was observed in slurry or manure, followed by partitions, farmer's hands, floors, water/ nipples, ventilation sources, and feed, respectively. All the *Salmonella* isolates originating from different farms were genetically distinct. In three farms, however, identical phage types and pulse-field gel electrophoresis patterns were observed among *Salmonella* isolates from pig feces and environmental samples. This study suggests that environments contaminated with *Salmonella* could pose an infection risk to pigs on pig farms.

Keywords: *Salmonella* spp., pig, environment, Korea

Salmonella is one of the major foodborne pathogens in humans and is also an important causative agent of enteric disease in animals [5]. A variety of biotic or abiotic reservoirs of *Salmonella* have been reported within the environment of pig production systems [4]. *Salmonella* infections in pig farms can persist in the herd environment for several months, or even for years [2, 19]. Identical pulse-field gel electrophoresis (PFGE) patterns among fecal and environmental isolates have been observed [5, 19]. Although it has been difficult to determine whether the source of *Salmonella* infections in pigs is other infected pigs, or the farm environment, a contaminated environment may constitute an important source of infection. In Korea, *Salmonella* is the second most common cause of food poisoning in humans [11], with the *S.* Typhimurium serotype being one of the most common causation agents for human salmonellosis [6]. *S.* Typhimurium has also been observed to be the most prevalent serotype in Korean pig farms [12]. Thus, pigs may be one of the most important reservoirs of human salmonellosis in Korea. To successfully reduce the carriage of pathogens in animals, control measures should focus not only on the target animals, but also on their environment. Information on the prevalence of *Salmonella* in the environment of pigs, the major animal reservoir of the pathogens, would be particularly useful in the control of outbreaks, and to reduce the spread of the pathogens. However, no such study has been conducted on the prevalence of *Salmonella* in the pig farmhouse environment in Korea. Hence, the aim of this study was to investigate the prevalence of *Salmonella* in pigs and their environment in Korea, and to examine the phenotypic and genetic relationships of the *Salmonella* isolates through the use of phage typing and PFGE.

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Materials and Methods

A total of 910 samples (492 fecal samples and 418 farmhouse environmental samples) were collected from 16 different pig farms in Korea between 2006 and 2007. At least one farm, and typically two, was randomly selected from each province within the country. Prior information on the *Salmonella* infection status of the pigs in the farms was not available. Fecal samples were randomly taken from 5–10 apparently healthy pigs in each farm. One or two samples of feed, water, slurry, and manure were also collected from each farm. Swab samples were obtained from the pen floors, ventilation systems, nipples, iron partitions, and farmers' hands, in each herd. Four gauze pads (10 cm×10 cm) wetted with 1% sterile skim milk were used to swab the samples, and the samples were then placed in a sterile plastic bag.

Isolation of *Salmonella* spp. was performed using conventional methods [9]. All of the samples were pre-enriched with buffered peptone water (Becton Dickinson, CA, USA) and incubated at 37°C for 24 h. The samples were then transferred to a Modified Semi-solid Rappaport Vassiliadis medium (MSRV; Becton Dickinson, CA, USA) and incubated at 42°C for 24–48 h. Suspect samples were plated onto Rambach agar (Merck, Darmstadt, Germany) and the plates were incubated at 37°C for 24 h. Colonies exhibiting a red color were further identified biochemically. Identification of isolates for serotypes was performed by slide and tube agglutination using a commercial antiserum (Difco Laboratories, Detroit, MI, USA), in accordance with the latest versions of the Kauffmann–White scheme [16].

The susceptibilities of the *E. coli* isolates were tested against 14 different antimicrobials using the standard Kirby–Bauer disk diffusion method [3]: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cephalothin (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), cefepime (30 μg), streptomycin (10 μg), gentamicin (10 μg), amikacin (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), and tetracycline (30 μg). Cartridges of antimicrobial-containing disks were obtained from Becton Dickinson (BBL Sensi-Disk). The diameter of inhibition zones surrounding the antimicrobial disks was interpreted in accordance with the guidelines of the Clinical Laboratory Standards Institute [7]. The quality control strain was *Escherichia coli* ATCC 25922.

Phage types were determined by the extended phage typing scheme of Anderson *et al*. [1] through the use of 30 *S.* Typhimurium phages obtained from the Public Health Laboratory Service (PHLS, London, UK). All *S.* Typhimurium isolates displaying unusual phage types, of which lysed patterns did not match with the standardized ones, were assigned as untypable.

The PulseNet method of the Center for Disease Control and Prevention [17] was used for PFGE typing of the *Salmonella* spp. isolates. *Xba*I and *Avr*II were used for genomic DNA digestion. DNA band patterns were analyzed and compared using BioNumerics (Applied Maths, Belgium). Isolates with band patterns that were 100% identical were considered to be of identical PFGE types.

Results and Discussion

A total of 36 *Salmonella* spp. were isolated in this study: 18 isolates from 492 pigs (3.7%) and 18 isolates from 418 (4.3%) farmhouse environmental samples of 16 different pig farms. *Salmonella* spp. were isolated from 6 of the 16 farms (37.5%) examined (Table 1). Most of the isolates were identified as *S.* Typhimurium, although two isolates were identified as *S.* Ardwick and *S.* Rissen. These two non-*S.* Typhimurium strains originated from samples of manure and slurry, respectively. No *Salmonella* spp. was isolated from groups of neonate pigs and sow, whereas 16 (16.1%) and 2 (1.4%) *Salmonella* were isolated from 99 weaned pigs and 138 growing-finishing pigs, respectively (Table 1). Although the sample size of this study was small, our results were similar to the findings of other studies conducted in Korea [20] and in other countries [14, 18], where the highest prevalence of *Salmonella* was observed among weaning group pigs. These results suggest that strategies to prevent and control *Salmonella* on pig farms should focus on weaned pigs.

Previous studies reported that *Salmonella* spp. were distributed in not only animals but also in various environments such as farmhouses and vectors [4]. In this study, we also found that the environment of some pig farms was contaminated with *Salmonella* spp. *Salmonella* strains were isolated from various environmental samples, and the highest prevalence was observed in slurry and manure (22.7%), followed by partitions (7.6%), farmer's hands (4.0%), floors (2.5%), water/nipples (2.3%), ventilation sources (1.7%), and feed (1.5%).

In three of six *Salmonella*-positive farms, very closely related isolates were distributed amongst pigs and their farmhouse environments. These findings are similar to the results of a study by Sandvang *et al.* [19], where identical

Table 1. Prevalence of *Salmonella* spp. in pigs and their farm environment of 16 different pig farms.

Samples	No. of positive farms/No. of tested farms (%)[a]	No. of positive samples/No. of tested samples (%)
Pigs	4/16 (25.0)^{L,M,O,Q}	18/492 (3.7)
Piglet	0/16 (0)	0/124 (0)
Weaned pig	3/16 (18.8)^{M,O,Q}	16/99 (16.1)
Growing-finishing pig	1/16 (5.9)^{L}	2/138 (1.4)
Sow	0/16 (0)	0/131 (0)
Environmental	5/16 (31.3)^{E,L,O,P,Q}	18/418 (4.3)
Floor	2/16 (12.5)^{L,Q}	2/80 (2.5)
Ventilation system	1/16 (6.3)^{Q}	1/60 (1.7)
Iron partition	1/16 (6.3)^{E}	6/79 (7.6)
Water/nipple	1/16 (6.3)^{Q}	2/87 (2.3)
Feed	1/16 (6.3)^{Q}	1/65 (1.5)
Farmer hand	1/16 (6.3)^{Q}	1/25 (4.0)
Slurry/manure	3/16 (18.8)^{O,P,Q}	5/22 (22.7)

[a]Farms.

Table 2. Antimicrobial resistance patterns, phage types, and PFGE patterns of *Salmonella* spp. isolated from pigs and their farm environment.

Isolates	Farms	Origin (age group)[a]	Serotypes	Antimicrobial phenotypes[b]	Phage type[c]	PFGE[d]	
						(*Xba*I)	(*Avr*II)
06-E-P-1	E	Iron partition (W)	*S.* Typhimurium	SM-NA-CM-TE	UT	PX7	PA8
06-E-P-2	E	Iron partition (W)	*S.* Typhimurium	SM-NA-CM-TE	PT193	PX7	PA8
06-E-P-3	E	Iron partition (W)	*S.* Typhimurium	SM-NA-CM-TE	PT194	PX7	PA8
06-E-P-4	E	Iron partition (W)	*S.* Typhimurium	SM-NA-CM-TE	PT194	PX7	PA8
06-E-P-5	E	Iron partition (W)	*S.* Typhimurium	SM-NA-CM-TE	PT194	PX7	PA8
06-E-P-6	E	Iron partition (W)	*S.* Typhimurium	SM-NA-CM-TE	PT193	PX7	PA8
07-L-96-1	L	Feces (G-F)	*S.* Typhimurium	SM-NA-TE	PT194	PX1	PA1
07-L-101-1	L	Feces (G-F)	*S.* Typhimurium	SM-GM-NA-TE	PT302	PX2	PA2
07-L-243-1	L	Floor (G-F)	*S.* Typhimurium	SM-NA-TE	PT194	PX1	PA1
07-L-165-1	M	Feces (W)	*S.* Typhimurium	AM-S-NA-TE	PT302	PX6	PA6
07-O-5-1	O	Feces (W)	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-O-5-2	O	Feces (W)	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-O-5-3	O	Feces (W)	*S.* Typhimurium	SM-TE	PT195	PX1	PA3
07-O-5-4	O	Feces (W)	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-O-5-5	O	Feces (W)	*S.* Typhimurium	SM-TE	PT195	PX3	PA3
07-O-5-6	O	Feces (W)	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-O-5-7	O	Feces (W)	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-O-5-8	O	Feces (W)	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-O-125-1	O	Slurry	*S.* Typhimurium	SM-TE	UT	PX3	PA7
07-P-147	P	Manure	*S.* Ardwick	TE	NT	NT	NT
07-P-148	P	Slurry	*S.* Rissen	SM-NA-TE	NT	NT	NT
07-Q-76-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	UT	PX4	PA5
07-Q-77-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-78-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	PT193	PX4	PA4
07-Q-79-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-80-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-81-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	PT194	PX5	PA4
07-Q-82-1	Q	Feces (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-83-1	Q	Feed (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-84-1	Q	Water (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-151-1	Q	Nipple (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-152-1	Q	Floor (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	U302	PX4	PA4
07-Q-153-1	Q	Ventilation system (W)	*S.* Typhimurium	AM-SM-GM-NA-CM	PT193	PX4	PA4
07-Q-162-1	Q	Farmer hand	*S.* Typhimurium	AM-CF-SM-GM-NA-CM	PT194	PX4	PA4
07-Q-170-1	Q	Manure	*S.* Typhimurium	AM-SM-GM-NA-CM	PT193	PX4	PA4
07-Q-171-1	Q	Slurry	*S.* Typhimurium	AM-SM-GM-NA-CM	PT193	PX4	PA4

[a]W, weaned pig; G-F, growing-finishing pig.
[b]SM, streptomycin; NA, nalidixic acid; CM, chloramphenicol; TE, tetracycline; AM, ampicillin; CF, cephalothin; GM, gentamicin.
[c]UT, untypable; NT, not tested.
[d]PX and PA refer to patterns of *Xba*I and *Avr*II, respectively.

S. Typhimurium phage types and PFGE patterns were found in both fecal and environmental isolates. However, in the other three farms (E, M, and P farms), *Salmonella* strains were isolated only in either pigs or the farm environment. In farms M and P, pathogens were found in only pigs, whereas in farm E the pathogens were recovered from an iron partition in the weaning house. In the latter case, the lack of detection of the pathogen in pigs, despite the contamination of the partition, may be associated with the health conditions and susceptibility to *Salmonella* of the pigs, the infectivity of *Salmonella*, and the grade of contamination in the farm [10].

Salmonella strains isolated from pen floors, and from pigs staying on those pen floors, exhibited identical phenotypes and genotypes. This suggests, as previous experimental studies have shown [8], that depositing feces

contaminated with *Salmonella* on floors may result in the transmission of the organism to other pigs on the same floor.

In the present study, five *Salmonella* strains (three *S.* Typhimurium, one *S.* Ardwick, and one *S.* Rissen) were isolated from manure or slurry samples in three pig farms. They are reported to be the most prevalent serotypes in pigs in Korea [12]. More importantly, the *Salmonella* strains isolated from manure and slurry samples shared an identical phage-PFGE type (PT193-PX4-PA4) with strains isolated from a pig and ventilation source in farm Q. This finding suggests that *Salmonella* strains excreted by pigs might have survived in manure and slurry on the farm. Although we cannot draw any firm conclusion from the results owing to the small size of samples and farms in this study, the potential survival of *Salmonella* in manure and slurry poses a significant threat to public health because animal manure is often used as an organic fertilizer in agriculture in Korea. In particular, *S.* Typhimurium has been known to survive for considerable periods of time in pig slurries [13], and three of the five strains isolated from manure or slurry of three farms (O, P, and Q farms) in this study were *S.* Typhimurium. Although no information is available on the manure/slurry storage period in those three farms, pig fecal waste might play an important role in the survival or transmission of *Salmonella*. Further studies are needed to investigate the importance of waste disposal systems for the survivability and transmission of the pathogen.

Feed and water are known to be one of the reservoirs of *Salmonella* spp. in livestock environments, and the isolation of *S.* Newport and *S.* Typhimurium from animal feed has been reported in Canada [16] and Poland [21], respectively. Feed ingredients and dust can be a source of *Salmonella* contamination, and *Salmonella* can survive in feed of low water activity for a long period [21]. In this study, *S.* Typhimurium was isolated from a feed and a water sample in farm Q. These isolates exhibited an identical phage and PFGE type (U302-PX4-PA4) to pig isolates from the same farm, suggesting that the *Salmonella* present in the feed or water may have infected the pigs, or contaminated the environment on the farm. This is in agreement with previous observations in Poland, where *S.* Typhimurium DT 104 isolates with identical PFGE types were observed both in swine and feed [21]. Conversely, infected pigs, or a contaminated environment, may be the origin of the *Salmonella* in the feed or water on the farm.

This study shows that the environment of some pig farms is contaminated with *Salmonella* spp., and that some of the *Salmonella* strains isolated both from pigs and their environment are closely related to each other genetically. Our findings suggest that an environment contaminated with *Salmonella* could pose an infection risk for pigs on pig farms. In addition, *Salmonella*-contaminated slurry and manure can play a potential role in the transmission of this pathogen to the environment, to other animals, and to humans.

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