

Prevalence of *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Salmonella* in swine herds

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The prevalence of *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Salmonella* spp. were investigated by multiplex PCR using fecal samples of pigs with diarrhea or a history of diarrhea. The overall herd prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were 46.5%, 37.2% and 51.1%, respectively. Also, the prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. among all sampled pigs were 19.9%, 10.8% and 17.7%, respectively. Seventeen of 43 herds were positive with 2 enteric organisms, and 2 herds were positive with *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. simultaneously. It was notable that 11 of 12 herds with more than 2,000 pigs were affected with *Salmonella* spp., and that only 2 of 12 the herds were affected with *B. hyodysenteriae*. This study suggested that herds positive for *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were distributed throughout Korea, although the relationship among other pathogens such as viral or parasitic ones and/or with metabolic disorders was not determined.

Key words: *Brachyspira hyodysenteriae*, *Lawsonia intracellularis*, prevalence, *Salmonella*, swine

Introduction

Porcine proliferative enteropathy (PPE), swine dysentery (SD) and porcine salmonellosis (PS) are bacterial enteric diseases with severe diarrhea, and similar in clinical aspects. These diseases have occurred at a growing and finishing stage in swine production cycle, and the causative bacteria are transmitted by fecal-oral route. Therefore, diagnosis of PPE, SD and PS in laboratory requires special handling of intestinal specimens in time-consuming procedures [7,17].

Caused by *Lawsonia intracellularis*, an obligate intracellular bacterium, PPE has been characterized by adenomatous proliferation of immature intestinal epithelial cells in the distal small intestines [15]. Farm prevalence studies in several countries in Europe, Asia and Northern America indicated that 24 to 47% of pig farms had a serious incidence with PPE in the past years. Informed estimates of economic losses due to PPE, using specific production data and disease diagnostic information, have ranged from an annual loss of 4 millions GB pounds to the British industry, and US \$ 98 million to the USA industry [16].

Duijkeren *et al.* [6] studied serotypes of *Salmonella* strains isolated from humans and animals in the Netherlands. They reported that the most prevalent serotypes in humans were serovars Typhimurium and Enteritidis; in cattle, serovars Typhimurium and Dublin; in pigs, serovar Typhimurium; and in chickens, serovars Enteritidis, Infantis, and Typhimurium. Schwartz [19] pointed out considerable confusion about the etiology and epidemiology of clinical salmonellosis in swine. Also, prevalence of *Salmonella* infection showed different rates depending on which isolation/sampling methods were examined [10].

SD caused by *Brachyspira hyodysenteriae*, a anaerobic, beta-hemolytic spirochete, is a severe mucohemorrhagic diarrheal disease that primarily affects pigs during the growing and finishing period. Clinical signs of SD seem to occur in a cyclic manner at 3 to 4 week intervals in large groups of pigs. This recurring symptom often occurs only after removal of therapeutic drugs. Therefore, SD has been acknowledged as an important cause of sub-optimal performance and mortality in swine production. For example, 35% of pig herds with a history of diarrhea have the disease in Brazil [1]. Although lots of studies on epidemiology of those diseases have been reported overseas, there was relatively little published information on the prevalence of 3 etiologic agents in Korea. The purpose of this study was to determine the prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. from swine farms with diarrhea or a history of diarrhea by the multiplex PCR previously developed [18].

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Materials and Methods

Pig herds

A total of 43 farrow to finish herds with between 500 and 5,000 pigs per herd in Gyeongsang-do were selected on the basis of a history of diarrhea in a growing and finishing herd or presence of diarrhea at the time of the study. Gyeongsang-do is located on the southern areas in Korea, and about 25% of total swine heads of Korea were raised in the province.

Fecal samples

A total of 462 fecal samples from 43 pig herds were collected between March 1999 and December 2000. Fecal specimens were randomly sampled from 8-16 growing pigs of each herd. All specimens were taken from freshly defecated feces using sterile cotton swab and were submitted to the laboratory.

DNA extraction of fecal specimens

Total DNA was extracted from each fecal sample, and processed as previously described [18]. Briefly, fecal specimen (0.2 g) was suspended in lysis buffer (5 M guanidine thiocyanate, 22 mM EDTA, 0.05 M tris-Cl, pH 6.4, and 0.65% triton X-100), vortex-mixed and centrifuged at $14,000 \times g$ for 20 sec after standing for 1 h at room temperature. The supernatant was placed in a tube containing 50 μ l of 20% diatomaceous earth suspension in 0.17 M HCl. The specimen was held at room temperature for 10 min, vortex-mixed and centrifuged at $14,000 \times g$ for 20 sec. The lysis buffer was drawn off with a pipette, dried at 56°C for 15 min and dissolved in TE buffer (100 mM tris-Cl, pH 7.0, 1 mM EDTA). After centrifugation at $12,000 \times g$ for 2 min, the supernatant containing DNA was stored at -20°C until subjected to multiplex PCR.

PCR

PCR amplification of total DNA extracted from fecal samples for the detection of 3 organisms was performed according to the previous publication [18]. Briefly, primers used in multiplex PCR for specific amplification of DNAs from *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were 5'-GCAGCACTTGCAAACAATAAACT-3', 5'-TCTCCTTTCTCATGTCCCATAA-3'; 5'-GCTGGAGATGATGCTTCTGG-3', 5'-GTCCAAGAGCTTGGCTGTTC-3'; 5'-TTGGTGTTTATGGGGTCGTT-3'; and 5'-GGGCATACATCCAGAGAAA-3', respectively. The 50 μ l of PCR

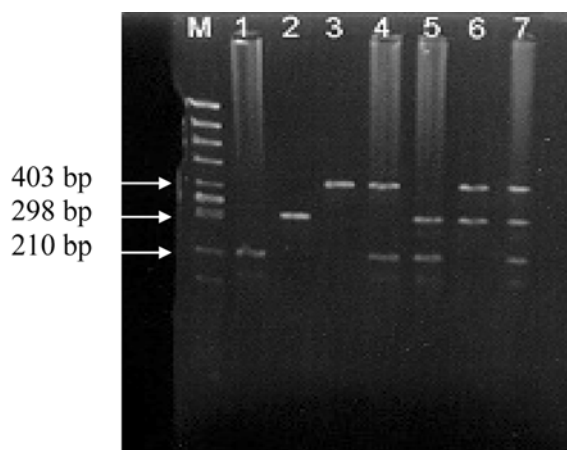


Fig. 1. Multiplex PCR for detection of individual or combinations of bacterial DNAs from porcine fecal samples. M; ϕ X174 digested by *Hae* III, Lane 1; *L. intracellularis*, Lane 2; *Salmonella* spp., Lane 3; *B. hyodysenteriae*, Lane 4; *L. intracellularis* and *B. hyodysenteriae*, Lane 5; *L. intracellularis* and *Salmonella* spp., Lane 6; *Salmonella* spp. and *B. hyodysenteriae*, Lane 7; *L. intracellularis*, *Salmonella* spp. and *B. hyodysenteriae*.

mixture contained 5 μ l of $10 \times$ PCR buffer, 3 μ l of 25 mM $MgCl_2$, 4 μ l of 10 mM deoxynucleotide triphosphate mixture, 1 μ l each of 20 pM sense and antisense primers, 1 μ l of DNA template and 0.5 unit of *Taq* DNA polymerase (Takara, Japan). PCR amplification was conducted on a DNA thermocycler (Robocycler; Stratagene, USA). The initial mixture was heated to 94°C for 5 min. This step was followed by 35 cycles, each consisting of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec and polymerization at 72°C for 1 min with final polymerization at 72°C for 5 min. The amplified DNAs were analyzed on 1% agarose gel and visualized using the Eagle Eye II (Stratagene, USA).

Results

Detection of bacterial DNA in the feces of pigs by multiplex PCR

Multiplex PCR assay yielded PCR products alone or in combination corresponding to the expected molecular weight of DNAs from *L. intracellularis*, *Salmonella* spp. and *B. hyodysenteriae*; 210-bp, 298-bp and 403-bp bands, respectively (Fig. 1)

Table 1. Effect of herd size on the frequency of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp.

Herd size	No. of herds	<i>L. Intracellularis</i>	<i>B. hyodysenteriae</i>	<i>Salmonella</i> spp.	Negative
<1,000	19	8	9	7	4
1,001-2,000	12	7	5	4	2
>2,000	12	5	2	11	.

Table 2. Concomitant infection of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. in swine farms

Organisms	No. of positive herds (%)	No. of positive pigs (%)
<i>L. intracellularis</i>	8 (18.6)	70 (15.2)
<i>B. hyodysenteriae</i>	3 (7.0)	24 (5.2)
<i>Salmonella</i> spp.	7 (16.3)	52 (11.3)
<i>L. intracellularis</i> & <i>Salmonella</i> spp.	6 (13.9)	10 (2.2)
<i>L. intracellularis</i> & <i>B. hyodysenteriae</i>	4 (9.3)	6 (1.3)
<i>Salmonella</i> spp. & <i>B. hyodysenteriae</i>	7 (16.3)	14 (3.0)
<i>L. intracellularis</i> & <i>B. hyodysenteriae</i> & <i>Salmonella</i> spp.	2 (4.7)	6 (1.3)
Negative	6 (13.9)	280 (60.6)
Total	43 (100.0)	462 (100.0)

Prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. in pig herds

The overall herd prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were 46.5%, 37.2% and 51.1% of 43 herds, respectively. Also, the average prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were 19.9%, 10.8% and 17.7% of 462 feces, respectively. DNAs of 3 enteric bacteria tested were not detected in 6 of the 43 herds.

Frequency of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. by herd size

The frequency of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. in 19 herds with less than 1,000 pigs were 42.1%, 47.4% and 36.8%, respectively, and in the 12 herds with 1,001-2,000 pigs, 58.3%, 41.7% and 33.3%, respectively. Also, the frequency of these bacteria in 12 herds with more than 2,000 pigs were 41.7%, 16.7% and 91.7%, respectively. It was notable that 11 of 12 herds with more than 2,000 pigs were positive with *Salmonella* spp., and that only 2 of 12 the herds were affected with *B. hyodysenteriae* (Table 1).

Concomitant detection of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. on farms

In 43 herds, 17 herds were positive for 2 bacteria mixed differently among *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp., and 2 herds were positive for these three bacteria. Interestingly, there were no diarrheic pigs observed at the time of sample collection in the 2 farms, which had history of severe diarrhea outbreaks before (Table 2).

Discussion

PPE was endemic in many countries. Among infectious enteropathies generally associated with diarrhea in weaner

and grower/finisher pigs, the most frequently reported disease was PPE, followed by swine dysentery and salmonellosis [21]. In Taiwan, a survey for *L. intracellularis* by PCR reported an overall herd prevalence of 30% and an average prevalence of 5.5% in all sampled pigs [2]. Chiriboga *et al.* [3] reported 20% herd prevalence and 7.2% of animals being infected, although 11% did not present any symptoms of PPE in Brazil. In Korea, PPE was first reported by Hwang *et al.* [9] in 1995, and a total of 19 cases were detected during 5-year period from 1991 to 1995. Also, Kim *et al.* [13] reported a 20% and 3.3% of *L. intracellularis* prevalence in herds and animals, respectively. The prevalence of *L. intracellularis* in the present herds and pigs, respective of 46.5% and 19.9%, were a somewhat higher than those reported by previous surveys. Although the present results probably do not accurately represent the current prevalence on all farms, the detection of *L. intracellularis* from herds with a history of diarrhea but no current outbreaks of diarrhea in this study indicated that subclinical or chronic infection with *L. intracellularis* was widespread. These subclinical infections can lead to the clinical outbreaks if certain predisposing factors intervene. The animals' weight and feed conversion rates were not recorded from any of the herds, so further studies should include these variables to detect the impact of infection with *L. intracellularis*, including subclinically infected herds.

In regard to the prevalence of *Salmonella*, *Salmonella* spp. were isolated from 6.0% of the lymph node of slaughter pigs in Korea [20]. Choi *et al.* [5] also reported that the isolation rates of *Salmonella* spp. from slaughter pigs were 8.1%. In 1999, *Salmonella* spp. was isolated from 20.7-23.1% of mesenteric lymph nodes and 12.3% of rectal contents of slaughter pigs [12]. It was recognized that prevalence of *Salmonella* spp. increased yearly in Korea. However, most of the previous reports containing estimates of the prevalence

of *Salmonella* spp. are in fact have compiled epidemiological data from slaughterhouse or carcasses. Choi *et al.* [5] reported farm prevalence of *Salmonella* spp. 1.1% to 4.5% between 1984 and 1985. In this study, fecal prevalence of *Salmonella* spp., 17.7% in pigs and 51.1% in herds, was higher compared with these previous reports. This might be explained by the fact that the selection of herds was not a random representation of all herds but herds with diarrhea or a history of diarrhea. It could be speculated that *Salmonella* spp. was most frequent in grower and finisher pigs in Gyeongsang-do, although the swine herds in this investigation comprise the small number of herds.

SD has been widely distributed throughout the world [1]. In Korea, since first outbreak of SD was reported at Kimhae in 1975, about 17-30% of pig herds were infected with *B. hyodysenteriae* in 1986 [4]. Jung *et al.* [11] reported that herd prevalence of 30.7% and pig prevalence of 10.6% in 1994. Similar results were shown in this study with herd prevalence of 37.2% and pig prevalence of 10.8%. This implies that prevalence of *B. hyodysenteriae* has somewhat increased in swine herds in Korea. To our knowledge, this is the first report about the prevalence of three major bacterial pathogens in pigs during growing and finishing stages by multiplex PCR in Korea.

It has been reported that outbreaks of PPE and SD occur more often in the large production unit [8,14] that is, the association between the herd size and disease outbreak. However, the results in this study showed the opposite results. Eleven of 12 herds with more than 2,000 pigs were positive with *Salmonella* spp., whereas only 5 (41.7%) and 2 (16.7%) herds showed positive with *L. intracellularis* and *B. hyodysenteriae*, respectively. Stressors to pigs, such as large numbers of susceptible pigs, the build-up of contamination in densely stocked facilities and poor sanitation, were thought to be responsible for the outbreaks of PS. Interestingly, 37.5% and 36.4% of the herds positive with *B. hyodysenteriae* and *Salmonella* spp., respectively, were co-affected with *L. intracellularis* in the present study. In particular, 2 herds were positive with *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. simultaneously. These farms were not suffered from typical clinical signs of each disease at the time of sample collection, but had the history of severe diarrhea with dead loss of pigs before. The present study could not detect a relation between any of these pathogens, but the high numbers of herds with multiple infections indicated that interactions between pathogens might occur.

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