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# Detection and genetic characterization of *Lawsonia intracellularis* from swine in Korea

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## Abstract

A total of 191 samples collected from the commercial swine farms located in Chungnam province were investigated by PCR to estimate the prevalence of *Lawsonia* (*L.*) *intracellularis* infection. In the group of the pigs with proliferative enteritis, 14 (93.3%) of 15 intestinal samples and 12 (80.0%) of 15 feces were positive in PCR. In contrast, a relatively low positive rate (18.0%, 29 of 161 samples) was determined in the group of normal healthy pigs. The group of pigs over 120 days showed the highest positive rates (26.8%, 15 of 56 samples). In the comparison of the sequences of 210bp for species specific fragments and 301bp for outer membrane protein, the isolates (L1. L2) showed almost 100% identity with the reference *L. intracellularis* (L08049, USA). For the sequences of partial 16s rDNA, the homologies among the 5 isolates (L1-L5) were 97.4% to 99.3%, and those of 5 sequences (L1-L5) versus 5 overseas reference strains of *L. intracellularis* ranged from 98.6% to 99.8%. In the comparison of the nucleotide sequences among 5 isolates and other species in Desulfovibrionales showed 82.4 to 99.5% identities. The 5 isolates shared relatively low identities (76.9% to 84.4%) with the species of alpha-proteobacteria. In phylogenetic analysis based on the 16s rDNA sequences, all of the 5 isolates (L1-L5) were located in the same branch with the strains of *L. intracellularis* that were previously isolated from the pigs in USA and China. Seven strains of *Desulfovibrio* sp. were clustered in the neighboring branches, whereas alpha and gamma Proteobacteria showed distant relationship with *L. intracellularis* strains. The present findings suggest that *L. intracellularis* infection is endemic in the swine farms in the regions, and that the domestic isolates maintained very limited genetic variation.

**Key words** : *L. intracellularis* infection, PCR, 16s rDNA gene

## INTRODUCTION

Porcine proliferative enteritis (PPE) caused by *Lawsonia* (*L.*) *intracellularis* is a commercially important disease in the swine industry worldwide, since it can affect large numbers of pigs, particularly those reared intensively (Knittel et al, 1997; Moreno et al, 2002; Jones et al, 1993). The disease is characterized by thickened intestinal mucosa with gross and histopathological evidence of hyperplastic mucosal epithelium (Lawson and Gebhart, 2000). The disease is of economic importance due to death loss, increased medication costs, poor weight gain, and decreased feed conversion (Roland and Lawson, 1992). Estimates of the reduction in the weight gain and feed conversion efficiency were generally 20 to 30% (Murakata et al, 2008; Lee et al, 2001; Connor 1991). Various treatment programs to control the clinical signs of PPE were hampered by the lack of data on the causative agent like antimicrobial susceptibility etc. (Roland and Lawson, 1992; Ward and Winkelman, 1990).

*L. intracellularis* is characterized by a slender, curved,

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microaero-philic, obligate intracellular, gram-negative rod with 1.25~1.75μm length and the width is about 0.25~0.43μm, without kinetism (Widdel and Pfennig, 1984). This bacteria associated with PPE have been referred to as "Campylobacter-like organisms" in 1989. Recently, these novel bacteria have been given the taxonomic name *L. intracellularis* in 1995. This bacterium is classified into Family Desulfovibrionaceae in Order Desulfovibrionales, being confirmed aetiological agent of proliferative enteropathy (PE) in various animal species (Lawson and Gebhart, 2000; Klein et al, 1999). The pig and hamster are the most frequently affected animals (McOrist et al, 1995). *L. intracellularis* can be grown in cells cultures including rat intestinal ephithilum cell (IEC-18), and some other cell lines (GPC-16, HEP-2, McCoy), but not in cell-free media.

Ordinarily, PPE is diagnosed by observation of gross lesions and confirmed by typical histopathological changes in which the intracellular curved rods is demonstrated by the special stainings such as the modified acid-fast stain, silver stains, and immunohistochemistry (Wattanaphansak et al, 2008). However, the diagnosis of PPE by gross lesion and detection of the bacteria by micro-observation and the culture methods are generally impracticable for a large number of samples (Wattanaphansak et al, 2009).

Recently, polymerase chain reaction (PCR) techniques have been successfully used to detect the DNA derived from the causative agent in specimen from the intestinal lesions and stools (Jones et al, 1993; Pusterla et al, 2008). The nested-PCR, one-step PCR, multiplex PCR and real-time PCR have been applied for detection of DNA encoding species-specific fragments, outer membrane protein, and 16s rDNA, of *L. intracellularis* (Pusterla et al, 2008; Elder et al, 1997). The one-step PCR for detection of 210bp DNA encoding species-specific fragments has been reported as a specific and sensitive method to detect *L. intracellularis* in specimens, and applied as a rapid diagnostic methods for *L. intracellularis* (Elder et al, 1997).

In the present study, the prevalence of *Lawsonia* infection in commercial swine farms was investigated by detection of 210bp species specific fragments. The sequences of 301bp for outer membrane protein and 591bp for a part of 16s rDNA of the isolates were compared with those of various strains of *L. intracellularis* and alpha and gamma Proteobacteria. Based on the sequences of a part of 16s rDNA and data available from Genbank, the phylogenetic analysis was performed for the bacteria.

## MATERIALS AND METHODS

### Sample collection

All samples used in this study were collected from the commercial swine farms located in Chungnam province during January to December 2008. The samples from intestinal mucosa and feces were collected from 15 pigs affected with PE. One hundred and sixty one fecal samples were randomly collected from normal healthy pigs. The specimens were kept in the sterile plastic vials with saline, and were diluted with 10% calcium- and magnesium-free phosphate-buffered saline. The mixture was centrifuged at 3,000rpm for 15min at room temperature, and then stored at −70°C until used.

**Table 1.** Oligonucleotide primer sequences for PCR of *L. intracellularis*

| Primer | Nucleotide sequence | Position | Expected size |
|---|---|---|---|
| La210 (F) | 5′-GCAGCACTTGCAAACAATAAACT-3′ | 1142939-1142961 | 210bp* |
| La210 (R) | 5′-TTCTCCTTTCTCATGTCCCATAA-3′ | 1143126-1143148 | |
| La301 (F) | 5′-CAGCCGTTATTCTTCTCA-3′ | 1122334-1122351 | 301bp** |
| La301 (R) | 5′-GCAAAGTTTACACCACGA-3′ | 1122617-1122634 | |
| La591 (F) | 5′-AGGGATGAAGGTCTTTGGA-3′ | 414-432 | 591bp*** |
| La591 (R) | 5′-AGATGTCAAGCCTGGGTAAG-3′ | 985-1004 | |

*The nucleotide for species specific fragments of *L. intracellularis* (GeneBank accession No. : L08049)
**The nucleotide for outer membrane protein of *L. intracellularis* (GeneBank accession No. : AM180252.1)
***The nucleotide location for 16s rDNA of *L. intracellularis* (GeneBank accession No. : L15739)

## DNA extraction and PCR primers

Total DNA was extracted by using DNeasy® Minikit (QIAGEN, Germany). The following three oligonucleotide primer pairs were used for amplification of DNA fragments of *L. intracellularis*: 210bp for species specific fragments: forward primer, 5′-GCAGCACTT-GCAAACAATAAACT-3′ and reverse primer, 5′-TTCTCCTTTCTCA TGTCCCATAA-3′ (Suh et al, 2000); 301bp for outer membrane protein; forward primer, 5′-CAGCCGTTATTCTTCTCA-3′ and reverse primer, 5′-GCAAAGTTTACACCACG A-3′ (Koyama et al, 2006); 591bp fragment of the 16s rDNA; forward primer, 5′-AGGGATGAAGGTCTTTGGA-3′ and reverse primer, 5′-AGATGTCAAGCCTGG GTAAG-3′ (Koyama et al, 2006) (Table 1).

## PCR for target fragments amplification

PCR mixture (50μl) contained 5μl of 10×PCR buffer, 3μl of 25mM $MgCl_2$, 4μl of 10mM deoxynucleotide triphosphate mixture, 20pmol of each primers, 5μl of DNA template, and 0.5 unit of Taq polymerase (Takara Co. Japan). PCR reaction was performed using an automatized thermal cycler (Gradient Thermal Block, U.S.A). The initial mixture was heated at 94℃ for 5min. this step was followed by 45 cycles, each step consisting of denaturatioin at 95℃ for 30 sec, annealing at 56℃ for 30 sec, and polymerization at 72℃ for 1min, followed by additional polymerization at 72℃ for 5min. Electrophoresis was performed on 5μl of the PCR product in a 1.8% metaphore agarose gel with Tris acetate electrophoresis buffer (TAE, 0.04M Tris, 0.001M EDTA, pH 7.8). The agarose gels were stained with ethidium bromide and the DNA band pattern was visualized by Image analyzer (Pharmacia Biotech, U.S.A.).

## Sequencing and phylogenetic analysis

The PCR products for direct sequencing were separated on 1.5% (W/V) agarose gel and then extracted with Gel extraction kit (GENEALL, Korea). The nucleotide sequences of 210bp (1142939-1143148 nt), 301bp (1122 334-1122634 nt), and 591bp (414-1004 nt) were determined by COSMO CO (Korea). The sequences of the isolates detected in this study were compared with the corresponding regions of other known bacterial strains available in GenBank by the MegAlign computer program (DNASTAR Inc., Madison, Wis.). The percentages of nucleotide and amino acid sequence identities among

**Table 2.** Source of the nucleotide sequeneces used for sequence alignments and phylogenetic analysis

| Organisms (Strains) | Genbank accession no. (Country) |
|---|---|
| Desulfovibrionales | |
| *Lawsonia intracellularis* (1482/89) | L15739 (USA) |
| *Lawsonia intracellularis* (NCTC 12657) | U30147 (USA) |
| *Lawsonia intracellularis* | U65995 (USA) |
| *Lawsonia intracellularis* (GX) | EU348664 (China) |
| *Lawsonia intracellularis* | L08049 (USA) |
| *Lawsonia intracellularis* (PHE/MN1-00) | AM180252 (USA) |
| *Desulfovibrio intestinalis* | KMS2 (USA) |
| *Desulfovibrio* sp. | U07570 (USA) |
| *Desulfovibrio hydrothermalis* | AM13 (Japan) |
| *Desulfomicrobium norvegicum* | DSM 1741 (USA) |
| *Desulfobulbus mediterraneus* | 86FS1 (Germany) |
| *Desulfofaba hansenii* | NR_025142 (USA) |
| *Desulfovibrio bastinii* | SRL4225 (France) |
| α-Proteobacteria | |
| *Agrobacterium larrymoorei* | NR_026519 (USA) |
| *Bartonella bovis* | NR_025121 (USA) |
| *Brucella suis* | ATCC 23445 (Belgium) |
| γ-Proteobacteria | |
| *Yersinia pestis* | NCTC5923 (USA) |
| *Pseudomonas jessenii* | CIP 105274 (USA) |
| *Escherichia coli* | E24377A (USA) |
| *Coxiella brunetii* | NC_010115 (USA) |

different bacterial strains were determined with the MegAlign program. The accession numbers in GenBank data base and the sources of 16s rDNA sequences used for the phylogenetic analysis are given in Table 2.

## RESULTS

### Detection by PCR

A total of 191 samples were tested by one-step PCR to estimate the prevalence of *L. intracellaris* infections in the commercial swine farms located in Chungnam province (Table 3). The positive bands were visualized by electrophoresis at 210bp, 301bp and 591bp, as expected (Fig. 1). The pigs subjected to the test were classified into two groups; the pigs with PE and normal healthy pigs, Then, the groups were classified further into five subgroups according to their ages. In the group of pigs with PE, 14 (93.3%) of 15 intestinal samples and 12 (80.0%) of 15 feces were positive for *L. intracellaris* genes. In contrast, a relatively low positive rate (18.0%, 29 of 161 samples) was determined in the group of normal healthy pigs. However, in this group, pigs over 120 days were determined with the highest positive rate (26.8%, 15 of 56 samples). In addition, the 41~70 days old subgroup showed the lower distribution of positive cases.

### Genetic analysis

For genetic characterization of the isolates, the comparisons of nucleotide sequences for 210bp (species specific fragments) and 301bp (a part of outer memrane protein) were made between two isolates of *L. intracellularis* named as L1 and L2 and previously published sequences (GenBank accession no.: L08049, USA). The isolates, L1 and L2, showed 100% identity each other.

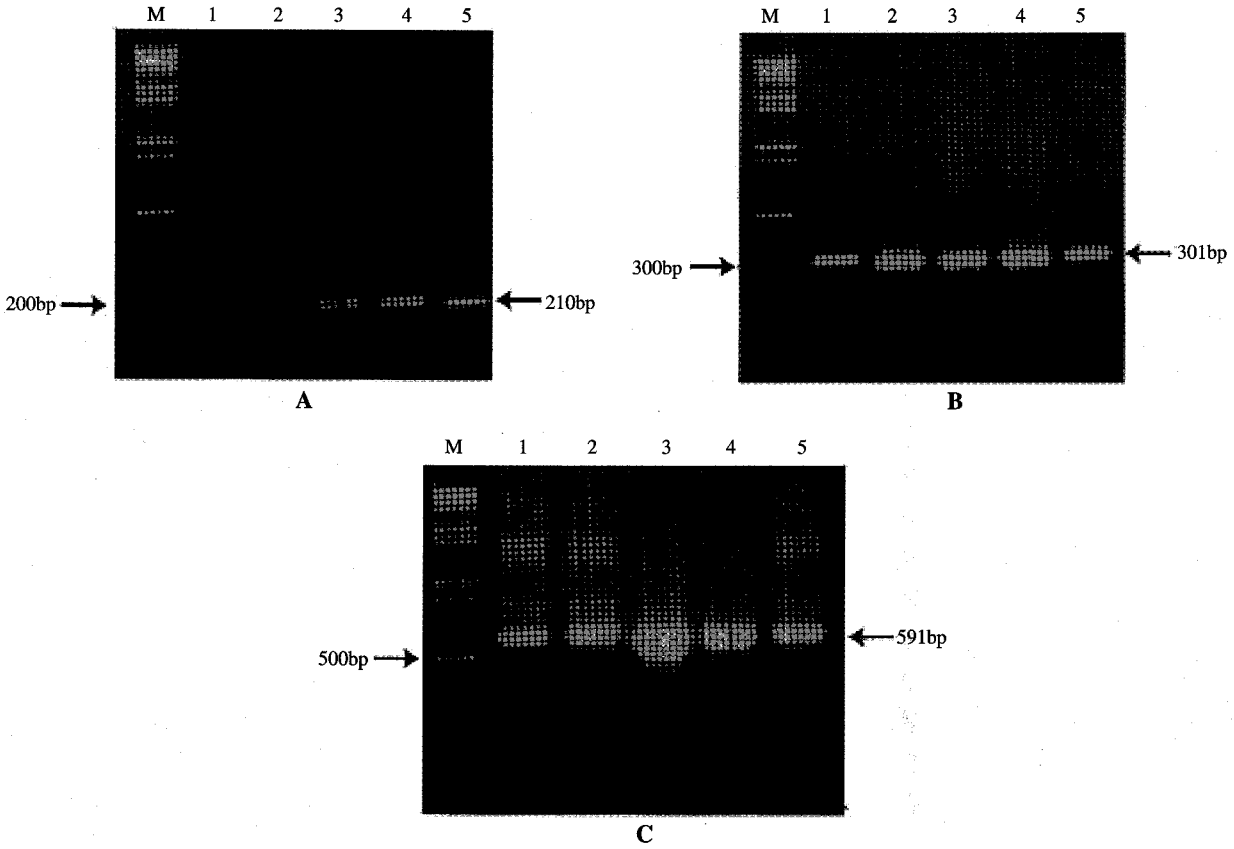


**Fig. 1.** PCR for *L. intracellularis* DNA in the samples from pigs. A: 210bp (species specific fragments), B: 301bp (outer membrane protein), C: 591bp (16s rDNA). Lane M: 1kb DNA ladder (SolGent Co, USA). Lanes 1, 2, 3, 4 and 5: L1, L2 L3, L4, and L5 isolates, respectively.

**Table 3.** Detection of *L. intracellularis* gene in the specimens collected from the commercial swine farms located in Chungnam province during January to December 2008

| Animals | Age groups (Days) | Specimens | No. of samples | No. of positive | % Positive |
|---|---|---|---|---|---|
| Pigs with PE | 90-150 | Intestinal mucosa | 15 | 14 | 93.3 |
| | | Feces | 15 | 12 | 80.0 |
| Normal healthy pigs | 25-40 | Feces | 19 | 3 | 15.8 |
| | 41-70 | Feces | 47 | 5 | 10.6 |
| | 71-120 | Feces | 39 | 6 | 15.4 |
| | >120 | Feces | 56 | 15 | 26.8 |
| Subtotal | | | 161 | 29 | 18.0 |
| Total | | | 191 | 55 | 28.8 |

*PCR conducted by a 210bp for species specific fragment

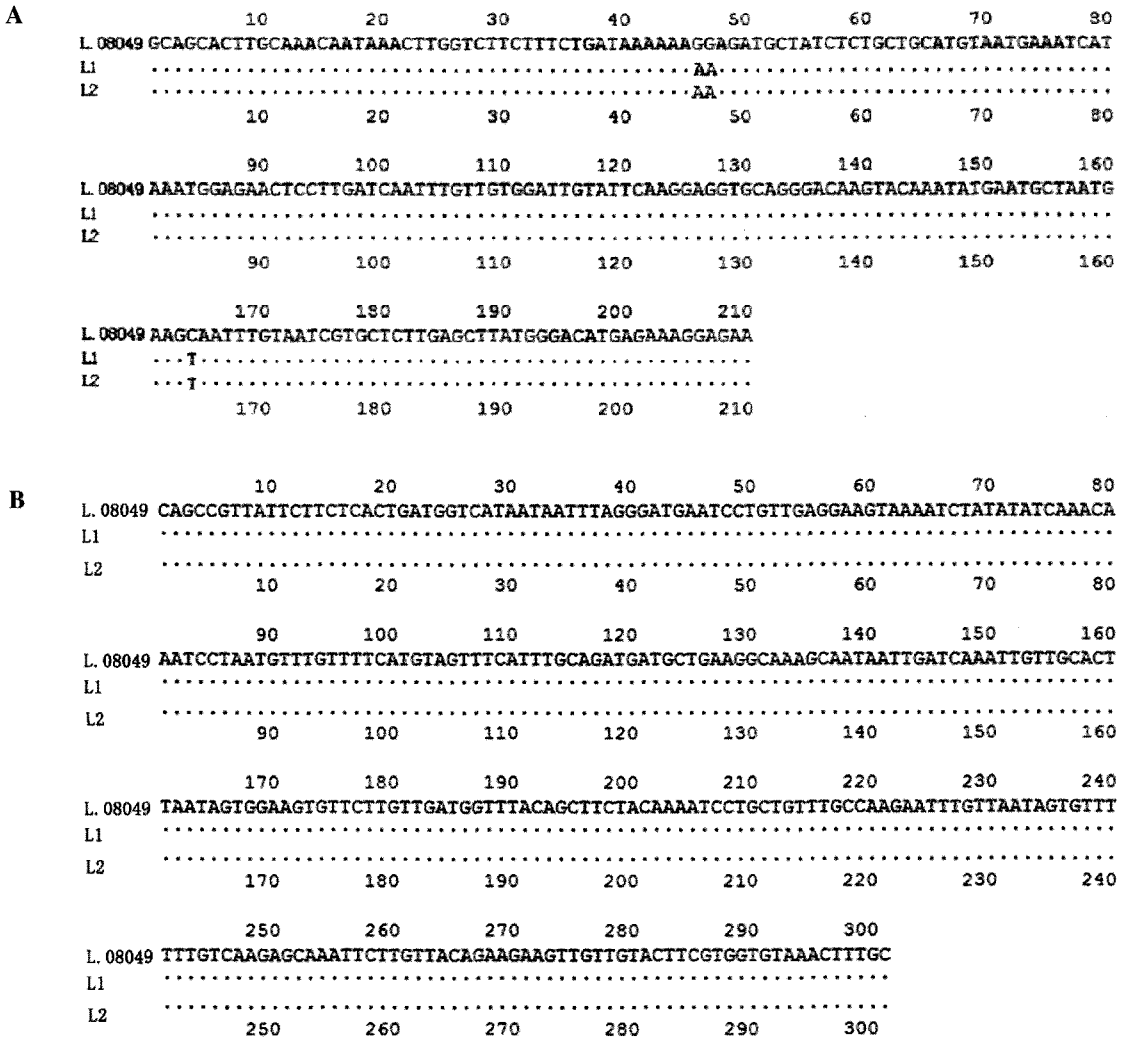


**Fig. 2.** Nucleotide sequence alignments of 210bp for species specific fragments (A) and 301bp for outer membrane protein (B). *L. intracellularis* DNA sequences of the isolates (L1 and L2) were compared with the consensus sequences of a pig isolate of *L. intracellularis* (L08049, USA). Dots indicate identical bases.

The comparison between the isolates and the reference strain showed the complete identity in 301bp nucleotides, whereas three different sequences were found in 210bp for species specific fragments (Fig. 2).

The sequences of partial 16s rDNA sequences of *L. intracellularis* isolates named as L1, L2, L3, L4, and L5 and other sequences deposited in the GenBank were compared in pairs. As shown in Table 4, the homologies among the 5 isolates (L1~L5) were 97.4~99.3%, and 5 isolates versus 5 oversea strains of *L. intracellularis* showed identities from 97.4% to 99.8%. In the comparison of the nucleotide sequences among 5 isolates and other species in the same order of Desulfovibrionales showed 82.4 to 99.5% identities. The 5 isolates shared relatively low identities (76.9% to 84.4%) with other two species, *Agrobacterium larrymoorei* and *Bartonella bovis* in the class of α-proteobacteria.

## Phylogenetic analysis

Phylogenetic analysis based on the partial 16s rDNA sequences was conducted for the 5 isolates, the selected strains of *L. intracellularis* and other species in alpha and gamma Proteobacteria (Fig. 3). A phylogenetic tree was inferred from the results of a maximum likelihood analysis of more extensive sequence data (591 nts per sequence). All of the 5 isolates were located in the same branch with the selected known representative strains of *L. intracellularis* that were previously isolated from the pig in USA and China. Seven strains of *Desulfovibrio* sp. were clustered in the neighboring branches, whereas *Agrobacterium larrymoorei* and *Bartonella bovis* in alpha Proteobacteria and other gamma Proteobacteria showed distant relationship with *L. intracellularis* and *Desulfovibrio* sp.

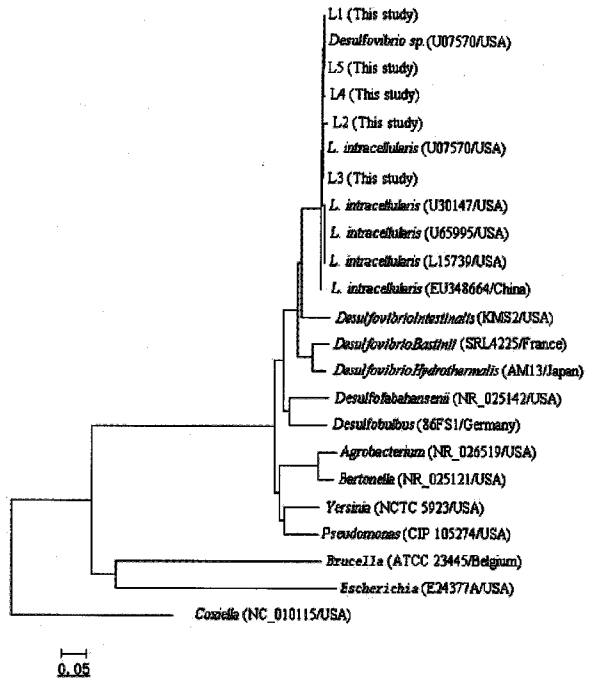


**Fig. 3.** A phylogenetic tree analysis based on the 16s rDNA sequence from 5 isolates of *L. intracellularis* detected in this study and the members of the Desulfovibrionales and some Proteobacteria. The tree was inferred from the results of a maximum-likelihood analysis of 16s rDNA homo-logous sequence positions for each organism. GenBank accession numbers and countries are shown in parentheses. The scale bar indicates a genetic distance of 0.05% nucleotide substitution per position.

## DISCUSSION

Porcine proliferative enteritis (PPE) caused by *L. intracellularis* infection is considered an important disease for morbidity in swine in many pig-producing countries. Farm prevalence studies in several countries including Europe, Asia, and North America indicated that 24 to 47% of pig farms showed a serious incidence with ileitis in the past several years (Lee et al, 2001; MØller et al, 1998; Chang et al, 1997). A study in Korea has reported that 53% of finisher pigs in the disease-harboring farms were infected with *L. intracellularis* and PPE was one of major economic impacts on pig production in the regions (Lee et al, 2001).

Detection of *L. intracellularis* in pigs with PPE is extremely important for the diagnosis of the disease. However, the disease has been poorly detected, because the diagnosis depends on observation of gross lesions and histopathological changes in which the intracellular curved rods is demonstrated by specific stainings, and cultivation of the organism require special cell culture technique.

Recently, PCR and Southern blot hybridization methods have been reported for detection of *L. intracellularis*-specific DNA, and these tests are more sensitive than the conventional methods (Jones et al, 1993; Elder et al, 1997). The application of nested-PCR and one-step PCR techniques for the detection of *L. intracellularis* results in rapid detection in a large number of fecal and intestinal mucosal specimens (Wattanaphansak et al,

**Table 4.** Levels of 16rDNA similarity for *L. intracellularis* and other Proteobacteria

| No. | Organism (Strain/Isolates) Name | % 16s rDNA similarity[a] | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 1 | *L. intracellularis* (L1) | * | | | | | | | | | | | | | | | | | | |
| 2 | *L. intracellularis* (L2) | 97.4 | * | | | | | | | | | | | | | | | | | |
| 3 | *L. intracellularis* (L3) | 98.3 | 98.5 | * | | | | | | | | | | | | | | | | |
| 4 | *L. intracellularis* (L4) | 97.9 | 98.5 | 99.0 | * | | | | | | | | | | | | | | | |
| 5 | *L. intracellularis* (L5) | 99.0 | 98.1 | 99.3 | 99.0 | * | | | | | | | | | | | | | | |
| 6 | *L. intracellularis* (AM180252)[b] | 99.1 | 98.8 | 99.1 | 98.8 | 99.8 | * | | | | | | | | | | | | | |
| 7 | *L. intracellularis* (U30147)[b] | 99.1 | 98.8 | 99.1 | 98.8 | 99.8 | 100 | * | | | | | | | | | | | | |
| 8 | *L. intracellularis* (U07570.1)[b] | 99.1 | 98.8 | 99.1 | 98.8 | 99.8 | 100 | 100 | * | | | | | | | | | | | |
| 9 | *L. intracellularis* (U65995)[b] | 99.1 | 98.8 | 99.1 | 98.8 | 99.8 | 100 | 100 | 100 | * | | | | | | | | | | |
| 10 | *L. intracellularis* (EU348664)[b] | 98.9 | 98.6 | 99.0 | 98.6 | 99.6 | 99.0 | 99.8 | 99.8 | 99.8 | * | | | | | | | | | |
| 11 | *Desulfovibrio intestinalis* | 89.6 | 89.0 | 89.7 | 89.4 | 90.4 | 93.5 | 93.5 | 93.5 | 93.5 | 93.3 | * | | | | | | | | |
| 12 | *Desulfovibrio* sp. | 99.0 | 98.0 | 99.0 | 98.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.5 | 99.8 | 88.8 | * | | | | | | | |
| 13 | *Desulfovibrio hydrothermalis* | 89.5 | 88.5 | 89.5 | 89.1 | 90.1 | 89.5 | 89.5 | 89.5 | 89.5 | 89.8 | 88.5 | 86.9 | * | | | | | | |
| 14 | *Desulfomicrobium* | 89.0 | 88.2 | 89.2 | 88.7 | 89.7 | 88.8 | 88.8 | 88.8 | 88.8 | 89.1 | 89.7 | 87.2 | 88.8 | * | | | | | |
| 15 | *Desulfobulbus mediterraneus* | 84.7 | 84.0 | 85.0 | 84.6 | 85.5 | 83.5 | 83.5 | 83.5 | 83.5 | 83.9 | 84.5 | 84.1 | 84.5 | 85.1 | * | | | | |
| 16 | *Desulfofaba hansenii* | 83.0 | 82.4 | 83.2 | 82.9 | 83.9 | 88.7 | 88.7 | 88.7 | 88.7 | 88.5 | 83.7 | 82.4 | 84.3 | 84.6 | 86.7 | * | | | |
| 17 | *Desulfovibrio bastinii* | 89.0 | 88.0 | 89.0 | 89.0 | 89.6 | 89.0 | 89.0 | 89.0 | 89.0 | 89.3 | 89.0 | 87.0 | 93.9 | 89.6 | 85.2 | 84.4 | * | | |
| 18 | *Agrobacterium larrymoorei* | 84.4 | 76.9 | 77.8 | 80.5 | 81.4 | 87.1 | 87.1 | 87.1 | 87.1 | 86.9 | 76.0 | 75.0 | 74.8 | 76.4 | 75.6 | 75.3 | 74.5 | * | |
| 19 | *Bartonella bovis* | 81.2 | 80.0 | 81.2 | 80.9 | 81.9 | 88.7 | 88.7 | 88.7 | 88.7 | 88.5 | 74.8 | 75.0 | 75.2 | 76.0 | 76.5 | 74.8 | 74.8 | 93.5 | * |

[a]The values represent the percent identities of the nucleotide sequences for 16s rDNA, [b]Genbank accession No. L1 ~ L5 are the isolates detected in this study

2009; Pusterla et al, 2008).

The PCR which amplifies 210bp bands, species specific fragments was employed in the present study for detection of *L. intracellularis* gene in the specimens collected from pigs. This method was reported to be so sensitive as to detect 100pg of *L. intracellularis* genomic DNA without nonspecific amplification, and suggested that the test can be used as a rapid diagnostic method for *L. intracellularis* infection (Suh et al, 2000). The sensitivity of the PCR was also proved in the present study, showing that the intestinal mucosa from the pigs with PE at necropsy revealed the high positive rate of 93.3% (14/15).

In the group of pigs with PE, 14 (93.3%) of 15 intestinal samples and 12 (80.0%) of 15 feces were positive to *L. intracellaris* genes. A relatively low positive rate (18.0%, 29 of 161 samples) was revealed in the group of normal healthy pigs. This value is some what higher than the previous study. The infection rate of *L. intracellularis* in the Korean pigs and herds in 1998 were 3.3% and 20% by PCR, respectively (Lee et al, 2001). In the comparison with other countries such as USA (12% in pigs, 29% in herds), Japan (14.9% in pigs), Brazil (7.2% in pigs) and Taiwan (5.5% in pigs, 30% in herds), the current data showed the slightly high prevalence of *L. intracellularis* infection. In addition, the positive rates of individual pig were analyzed to determine whether age was related with susceptibility to infection. As shown in Table 3, the infection rate of three age groups (25 ~ 40 days, 41 ~ 70 days, and 71 ~ 120 days) were 10.6% to 15.8% showing no significant differences among age groups. However, the finishing pigs (> 120 days) showed the particularly high prevalence of infection. This result is likely related with the fact that the symptoms frequently occur in pigs between 6 and 20 weeks and between 4 and 12 months of age.

Lee et al (2001) have reported that the prevalence of PE in Korea were 44% to 69% in individual pigs, and 100% in pig farms as investigated by indirect immunofluorescence antibody technique.

Since this results based on the serological method, direct comparison with the present data is somewhat

difficult. The epidemiological data for PE could be depending on regional variations, different swine breeding systems, use of antibiotics, size of samples, sampled population (sick or healthy animals), and diagnostic techniques. However, based on the present study, it is evident that PE is markedly prevalent in the herds in Korea and its infection widely distributed in individual pigs.

For genetic characterization and phylogenetic analysis of the isolates, L1~L5, the sequences of 210bp for species specific fragments, 301bp for outer membrane protein and 591bp for a part of 16s rDNA were compared with various strains of *Lawsonia* spp., *Desulfovibrio* spp, *Agrobacterium larrymoorei, Bartonella bovis* and the species in gamma Proteobacteria. In the comparison of sequence alignments of 210bp and 301bp, the isolates (L1. L2) showed almost 100% identity with the reference *L. intracellularis* (L08049, USA), even though 3 point mutations were observed in 210bp nucleotides.

Recently, comparisons of 16s ribosomal RNA sequence fragments of isolates from the pig, hamster, deer and ostrich have been conducted to demonstrate genetical and pathological relationships (Dale et al, 1998). In this study, the high genetic similarity was observed by comparison of a part of 16s rDNA from the isolates (L1 ~L5), and *L. intracellularis* from overseas, suggesting that the domestic *L. intracellularis* isolates have same genetic origin with *L. intracellularis* strains reported in USA and China strains. In phylogenetic analysis based on a part of 16s rDNA, it was evident that the isolates shared particularly low relationship with alpha and gamma Proteobacteria. Dale et al (1998) have suggested that, in analysis of the GroEL sequence relationship, *L. intracellularis* is not significantly related to other Proteobacteria, and *L. intracellularis* is a member of a novel group of enteric pathogens from 16S sequence analyses.

McOrist et al (1993) also have suggested that *L. intracellularis* is most closely related to *Desulfovibrio* spp, sharing over 91% sequence similarity. Even though the parts of nucleotides for comparison of genetic relationship are not exactly same among the different studies, the present data are generally correspondent to the previous data. Tomanová et al (2002) and McOrist et al (1995) have reported that a comparison of sequ-ences of the *L. intracellularis*-type strain from a dome-stic pig and from the wild pigs showed a high homo-geneity (99~100%). The similar results were also demon-strated in our data that the homologies among the 5 iso-lates were 97.4% to 99.3% (Table 4), suggesting that 16s rDNA of *L. intracellularis* might be very conserved.

The present findings suggest that *L. intracellularis* infection is endemic in the swine farms in the regions, and that the domestic isolates maintained very limited genetic variation.

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