O-serogroups, virulence genes of pathogenic *Escherichia coli* and Pulsed-field gel electrophoresis (PFGE) patterns of O149 isolates from diarrhoeic piglets in Korea

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ABSTRACT: A total of 116 *Escherichia (E.) coli* isolates isolated from neonatal diarrhoeic piglets were serogrouped and tested for the presence of virulence genes for fimbrial and non-fimbrial adhesins, intimin, and enterotoxins. Pulsed-field gel electrophoresis (PFGE) pulsotypes were also analyzed within O149 enterotoxigenic *E. coli* (ETEC) isolates. In total, Sixty eight (58.6%) isolates were serotyped. Among them, forty three (63.2%) belonged to 12 serogroups in the descending order: O149, O8, O157, O101, O60, O9, O117, O127, O138, O167, O27 and O97. The predominant pathotype was ETEC (68, 58.6%) which is closely associated with F4 (37, 31.9%) and LT:STb:EAST1 (23, 19.8%) out of the isolates harbouring at least one gene for toxin and/or fimbria. Among non-fimbrial adhesins, porcine attaching and effacing-associated factor (paa) was closely associated with F4-positive isolates (64.7%) rather than F18-positive isolates (5.9%). Adhesion involved in diffuse adherence (AIDA) was only detected in 3 isolates. No *eae*-positive isolates were detected. The PFGE pattern of 15 O149 isolates was grouped into 12 pulsotypes at 88% similarity level. The results show a wide variety of distinct restriction patterns though all belonged to the same serogroup O149. It is believed that a broad array of O serogroup and virulence genes are associated with neonatal diarrhoea in Korea.

Keywords: *Escherichia coli*; piglet; O-serogroup; pathotype; virulence gene

List of abbreviations

AIDA = adhesion involved in diffuse adherence, **EAEC** = enteroaggregative *E. coli*, **EAST1** = enteroaggrative *E. coli* heat-stable enterotoxin 1, **ETEC** = eEnterotoxigenic *E. coli*, **ND** = neonatal diarrhoea, **paa** = porcine attaching and effacing-associated factor, **PFGE** = pulseed-field gel electrophoresis, **STEC** = shiga toxin-producing *E. coli*

Escherichia (E.) coli is one of the enteric commensals of warm blood animals and constitutes the normal flora which plays an important role in preventing enteric infections (Gyles 1994). However, some strains cause diarrhoea and oedema disease in which symptoms are exacerbated under condition of environmental stress such as feed change and weaning in pigs. They have been divided into several pathotypes according to their combinations of virulence factors which include fimbriae, toxins and intimin.

Among pathotypes, enterotoxigenic *E. coli* (ETEC) is a well-known agent of neonatal diarrhoea (ND) and it produces two groups of enterotoxins: heat-labile (LT) and heat-stable toxins (ST) which are subdivided into STa and STb. In addition, they can

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produce one or more fimbriae: F4, F5, F6, F18 and F41 (Gyles 1994, 2010; Fairbrother and Gyles 2006). Fimbriae are surface proteins that play a pivotal role in adhesion to intestinal mucus and epithelial cells (Nagy and Fekete 2005). ETEC strains which express F4 or F18 are commonly associated with diarrhoea in many countries (Francis 2002). F4-positive strains frequently produce LT and STb enterotoxins with or without STa. F18-positive ETEC produce STa and STb toxins, sometimes with STX2 toxin. The most commonly reported serotypes of ETEC associated with neonatal diarrhoea include O8, O9, O20, O64, O101, O138, O141, O147, O149 and O157 (Francis 2002; Fairbrother and Gyles 2006; Gyles 2010). Shiga toxin-producing *E. coli* (STEC), also called verotoxin-producing *E. coli* (VTEC), are usually associated with post-weaning diarrhoea (PWD) and oedema disease (ED). They produce the Stx2e variant which causes systemic vascular damage resulting in oedema disease and frequently express F18 and adhesion involved in diffuse adherence (AIDA).

Enteropathogenic *E. coli* (EPEC) have also been associated with diarrhoea in pigs. They harbour the *eae* gene encoding intimin, which plays a role in intimate binding to enterocytes and in the effacement of microvilli resulting in an impaired absorption system. In addition, porcine attaching and effacing-associated factor (paa) has also been implicated in porcine diarrhoea (An et al. 1999; Batisson et al. 2003)

Additionally, enteroaggregative *E. coli* (EAEC) has been reported as a causative agent of human diarrhoea in developing countries. EAEC produce enteroaggrative *E. coli* heat-stable enterotoxin 1 (EAST1) which has also been found in ETEC isolates from pigs suffering from diarrhoea (Yamamoto and Nakazawa 1997; Veilleux and Dubreuil 2006)

Pulsed-field gel electrophoresis (PFGE) has been performed to analyse the degree of genetic relatedness or variability among various serotypes as well as among isolates of the same serogroups originating from diarrhoeic piglets (Osek 2000; Blanco et al. 2006; Vu-Khac et al. 2007). Enteric colibacillosis is the most significant disease of newborn and post-weaning piglets in pig industry. Although some reports have focused on virulence genes of pathogenic *E. coli* in Korea, there are limited studies on the prevalence of the O-serogroup and virulence factors in recent years. The objectives of this study were to determine the prevalence of the O-serogroup, virulence genes among *E. coli* isolated from diarrhoeic piglets and PFGE patterns of O149 ETEC which is the predominant serogroup of diarrhoeic piglets in Korea. It is hoped that this study will provide valuable insight into pathogenic porcine *E. coli* and help to establish preventative measures in Korea.

MATERIAL AND METHODS

***E. coli* isolates**. Between 2007 and 2010, 116 *E. coli* strains were isolated from diarrhoeic piglets at ages of between five and 21 days. The farms consisted of 100 different pig herds (50 to 100 sows per each herd). The farms were divided into three areas: northern (24 farms encompassing the Gangwon, Gyeonggi and Incheon provinces), middle (26 farms, Chungbuk and Chungnam provinces) and southern part (50 farms, Chonbuk, Chonnam, Gyeongbuk and Gyeongnam provinces). The aseptically collected intestinal contents and faeces were inoculated on MacConkey (BBL, USA) and blood agar (Asan, Korea). After overnight incubation at 37 °C, only pure and nearly pure cultured colonies were selected and transferred to blood agar. Suspected colonies were identified as *E. coli* using VITEK II systems (bioMéreiux, France). Haemolysis was also determined in blood agar. The tested isolates were stored in 50% glycerol at –70 °C until further characterisation.

Reference strains and O-serogrouping. Reference *E. coli* strains provided by Dr. J.M. Fairbrother (EcL, Canada) were used for positive controls for PCR: 7805 (F4:LT:STa:STb:EAST1:Paa), 6611 (STX1:STX2:eae:EAST1:Paa), 1033 (F18:AIDA), 2316 (F6:STa:STb:EAST1:Paa), 13316 (F5:F41:STa:Paa) and 3463 was used as a negative control. O-group was determined using the slide agglutination technique in an *E. coli* reference laboratory (EcL, Canada) and tube agglutination using rabbit antisera purchased from SSI (Serum Staten Institute, Denmark).

Determination of virulence genes. The genes for the toxins (LT, STa, STb, Stx1, Stx2 and EAST1) and adhesins (F4, F5, F6, F18, F41, as well as eae, paa and AIDA) were amplified using PCR. The primers used in this study are listed in Table 1. PCR was carried out using previously described protocols with some modifications (Woodward et al. 1992; Beaudry et al. 1996; Zhang et al. 2007). All colonies were suspended in 200 µl of water and boiled for 10 min. After centrifugation at 8000 × g,

Table 1. Primers used in this study

Primer	Oligonucleotide sequence	Product size (bp)	Reference
K88 (F4)	TGAATGACCTGACCAATGGTGGAACC GCGTTTACTCTTTGAATCTGTCCGAG	484	Zhang et al. (2007)
K99(F5)	GCGACTACCAATGCTTCTGCGAATAC GAACCAGACCAGTCAATACGAGCA	230	
987P (F6)	GCCAGTCTATGCCAAGTGGATACTTC GTTTGTATCAGGATTCCCTGTGGTGG	391	
F18	TGGCACTGTAGGAGATACCATTCAGC GGTTTGACCACCTTTCAGTTGAGCAG	230	
F41	TTAGCAGCGAAGATGAGTGATGGG GTACTACCTGCAGAAACACCAGATCC	515	
LT	ACGGCGTTACTATCCTGTCTATGTGC TTGGTCTCGGTCAGATATGTGATTC	275	
STa	GTCAGTCAACTGAATCACTTGACTCT CATGGAGCACAGGCAGGATTACAACA	152	
STb	GCTACAAATGCCTATGCATCTACACA CATGCTCCAGCAGTACCATCTCTAAC	125	
Stx1	TTAGACTTCTCGACTGCAAAG TGTTGTACGAAATCCCCTCTG	531	Woodward et al.(1992)
Stx2	TTATATCTGCGCCGGGTCTG AGACGAAGATGGTCAAAACG	327	
eae	CATTATGGAACGGCAGAGGT ATCTTCTGCGTACTGCGTTCA	791	Beaudry et al. (1996)
AIDA	AGTGGCGGGGCTCAGAACATCT CTCAGTGGCATTAGCGCCAGCA	273	modified for this study
paa	TGGCTGGACCAGGAAAGGCACT AAGTGCGGGTGCGTTGAGGATG	584	
EAST1	CTGGCCGAAAATGAAGGGGCGA TAACTGGATGCGGGCCTTCGGA	153	

the supernatant was used as a template for PCR reactions. The total volume of 20 µl was composed of 2 × EmeraldAmp Master Mix (Takara, Japan), 2µM of each primer and 3 µl of the DNA template. The amplified products were visualised using electrophoresis in 2% agarose gels which were stained with ethidium bromide.

Pulsed-field gel electrophoresis (PFGE). PFGE was performed in a CHEF MAPPER system (Bio-Rad) at 14 °C in 0.5X TBE using the Enternet-proposed standard protocol for PFGE (http://www.foodborne-net.de/content/e25/e70/e580/index_ger.html). Cleavage of the agarose-embedded DNA was carried out using 1.2 IU/µl XbaI (TAKARA, Japan) according to the instructions of the manufacturer. Run and pulse time were 2.21 to 54.19 s for 19 h with linear lamping. PFGE was used to establish clonal relatedness and diversity among a representative group of O149 isolates. To compare the PFGE pulsotypes, TIFF files were analysed with BioNemerics software (Applied Maths, Belgium). Cluster analysis of the Dice similarity indices based on the unweighted pair group method using arithmetic average (UPGMA) was undertaken to generate a dendrogram describing the relationship among O149 ETEC pulsotypes

RESULTS

Serogroups and haemolytic activity

Of the 116 *E. coli* isolates, O-serogroup was confirmed for 68 (58.6%) but not for 48 (41.4%). Sixty eight isolates belonged to 29 different O-serogroups (Table 2 and 3). Among them, forty three isolates (63.2%) belonged to 12 serogroups in the descending order: O149, O8, O157, O101, O60, O9, O117, O127, O138, O167, O27 and O97. The other serogroups were O1, O2, O45, O54, O63, O76, O78, O86, O100,

Table 2. O-serogroups, haemolysis and fimbrial adhesins of 116 *E. coli* isolates from piglets with neonatal diarrhoea

O-serogroup	Number of isolates (%)	Number of haemolysis	Number of isolates expressing the fimbrial gene							
			F4	F5	F6	F18	F41	F4:F41	F5:F41	None
O149	15 (12.9)	14	14	0	0	0	1	0	0	0
O8	4 (3.4)	2	0	0	0	1	0	0	0	3
O157	3 (2.6)	1	2	0	0	0	0	0	0	1
O101	3 (2.6)	0	0	0	0	0	0	0	0	3
O60	3 (2.6)	0	0	0	0	0	0	0	0	3
O9	3 (2.6)	0	0	0	1	0	0	0	1	1
O117	2 (1.7)	0	0	2	0	0	0	0	0	0
O127	2 (1.7)	1	0	0	0	2	0	0	0	0
O138	2 (1.7)	2	2	0	0	0	0	0	0	0
O167	2 (1.7)	0	0	0	0	0	0	0	0	2
O27	2 (1.7)	0	0	0	0	0	0	0	0	2
O97	2 (1.7)	0	0	0	1	0	0	0	0	1
OS*	25 (21.6)	11	5	0	0	1	0	0	1	18
NT**	48 (41.5)	14	13	5	0	1	0	1	1	27
Total	116 (100)	45	36	7	2	5	1	1	3	61

*other serogroup (number of isolates): O1, O2, O45, O54, O63, O76, O78, O86, O100, O116, O119, O120, O142, O147, O148, O159, nonspecific reaction (3)
**non typable

O116, O119, O120, O142, O147, O148 and O159. The predominant serogroup was O149 from which 15 isolates were associated with F4 (14 isolates, 93.3%) and LT:STb:EAST1 (seven isolates, 46.7%).

Haemolytic activity was demonstrated in 45 (38.7%) out of the 116 isolates tested (Table 2). Among them, F4-positive isolates (34 of 37 isolates, 91.8%) were more likely to be haemolytic than F5- and F6-positive isolates (0 of 9 isolates, 0%). Twelve isolates that carried both fimbriae and toxin genes were not haemolytic. Two isolates that did not carry virulence genes were haemolytic.

Adhesion and toxin genes

Among 116 isolates, 71 isolates (61.2%) harboured at least one gene for toxin and/or fimbria (Table 2 and 3). Among fimbrial genes, the predominant gene was F4 (37, 31.9%) followed by F5 (10, 8.6%), F18 (5, 4.3%) and F41 (5, 4.3%). Of toxin genes, the predominant gene was EAST1 (48, 41.4%), followed by LT (36, 31.0%), STb (36, 31.0%), STa (28, 24.1%) and Stx2 (3, 2.6%). Although EAST1 was commonly detected, 10 isolates (8.6%) harboured EAST1 as the only virulence gene. One isolate harboured the gene for Stx1 and was typed as O167 (Table 3). Of non-fimbrial genes, *paa* was detected in 34 isolates (29.3%) and AIDA was only isolated from three isolates (2.6%) from diarrheic piglets (Table 5). No *eae* genes were identified.

Frequency of virotypes (combination of toxin and adhesion)

The frequency of virotypes is shown in Table 4. Of 116 isolates, the predominant virotype was F4:LT:STb:EAST1 (20, 17.2%) followed by F5:STa (7, 6.0%) and F4:LT:STa:STb:EAST1 (3, 2.6%) and LT:STb:EAST1(3, 2.6%). The F18:STa and F5:F41:STa virotypes were only detected in two isolates, respectively. The virotype of STb:EAST1 with or without AIDA was detected in three isolates (2.6%)

Association between fimbrial and non-fimbrial adhesion genes

All the non-fimbrial adhesion genes were present together with the other virulence genes (Table 5).

Table 3. Relationship between toxin gene profile and O-serogroup of 116 *E. coli* isolates identified from piglets with neonatal diarrhoea

O-serogroup	Number of isolates (%)	Toxin gene profile																
		LT	STa	STX1	EAST1	LT:STa	LT:STb	STa:STb	STa:EAST1	STb:EAST1	STX2:EAST1	LT:STa:EAST1	LT:STb:EAST1	LT:STa:STX2	STa:STb:EAST1	LT:STa:STb:EAST1	LT:STa:STX2:EAST1	Number of toxin gene
O149	15 (12.9)	1	0	0	1	1	1	0	0	1	0	2	7	0	1	0	0	0
O8	4 (3.4)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O101	3 (2.6)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O157	3 (2.6)	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0
O60	3 (2.6)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O9	3 (2.6)	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
O117	2 (1.7)	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O127	2 (1.7)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
O138	2 (1.7)	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
O167	2 (1.7)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
O27	2 (1.7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O97	2 (1.7)	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OS*	25 (21.6)	1	1	0	7	1	0	0	0	0	1	0	3	1	0	1	0	8
NT**	48 (41.5)	0	5	0	2	0	1	3	2	1	0	0	7	0	1	2	0	24
Total	116 (100)	2	12	1	10	2	2	3	2	3	1	3	23	1	2	3	1	45

Relationship of toxin gene profile and O-serogroup of 116 *E. coli* isolates identified from neonatal diarrhoea
*other serogroup (number of isolates): O1, O2, O45, O54, O63, O76, O78, O86, O100, O116, O119, O120, O142, O147, O148, O159, nonspecific reaction (3)
**non typable

Although 34 isolates (29.3%) of 116 isolates tested had a *paa* gene, they accounted for 61.9% of 42 isolates harbouring F4 or F18 genes. The *paa* gene was more frequently detected in the F4-positive isolates (64.7%) than in F18-positive isolates (5.9%). AIDA was detected only in three isolates of the 116 isolates tested and two of three were in combination with F4. The *eae* gene was not detected in this study.

PFGE pattern of O149 ETEC

Fifteen O149 ETEC isolates were selected for analysis using PFGE. The XbaI-restriction pattern of the other 15 O149 isolates was clustered in 12 groups at 88% similarity level according to the Dice similarity index. There was a high degree of polymorphism among isolates of the same serogroup O149. The highest homogeneity was observed among seropathotype O149 F4:LT:STb:EAST1:Paa with two strains showing 100% similarity.

DISCUSSION

As in other countries, *E. coli* infection is one of the main enteric problems of pig industry in Korea. To diagnose enteric colibacillosis, the detection of O-serogroup and virulence genes including adhesins and toxins has been recommended worldwide. In the present study, the prevalence of O-serogroups, virulence genes and the PFGE pattern of O149 ETEC were investigated. Although a variety of O-serogroups have been associated with diarrhoea, only a limited number of serogroups

Table 4. Relationship between fimbriae and enterotoxins of 116 *E. coli* isolated from piglets with neonatal diarrhoea

Toxins	Fimbriae							
	F4	F5	F6	F18	F41	F4:F41	F5:F41	None
None	1	0	0	0	0	0	0	45
EAST1	0	0	0	0	1	0	0	9
LT	2	0	0	0	0	0	0	0
STa	1	7	2	2	0	0	2	0
STX1	0	0	0	0	0	0	0	1
LT:STa	1	0	0	0	0	0	0	0
LT:STb	2	0	0	0	0	0	0	0
STa:EAST1	0	0	0	1	0	0	1	0
STa:STb	2	0	0	0	0	0	0	0
STb:EAST1	1	0	0	0	0	0	0	1
STX2:EAST1	0	0	0	0	0	0	0	1
LT:STa:STb	1	0	0	0	0	0	0	0
LT:STa:STX2	0	0	0	1	0	0	0	0
LT:STa:EAST1	2	0	0	0	0	0	0	0
LT:STb:EAST1	20	0	0	0	0	0	0	3
STa:STb:EAST1	1	0	0	0	0	1	0	0
LT:STa:STb:EAST1	3	0	0	0	0	0	0	0
LT:STa:STX2:EAST1	0	0	0	1	0	0	0	0

have been reported in enteric infections of piglets (Gyles 1994, 2010; Frydendahl 2002; Chen et al. 2004). In this study, only 12 serogroups (O8, O9, O27, O60, O97, O98, O101, O117, O127, O138, O167, O149 and O157) accounted for 63.2% of the typable isolates. Of these 12 serogroups, O8, O9, O101, O149 and O157 have been commonly isolated in cases of neonatal diarrhoea in different countries (Francis 2002; Frydendahl 2002; Fairbrother et al. 2005; Vu-Khac et al. 2007; Gyles 2010). The frequencies of serogroups could vary from area to area and over time in specific regions (Harel et al. 1991; Chen et al. 2004; Fairbrother et al. 2005). This implies that regional differences or other selective advantages may result in *E. coli* strains with certain O-serogroups adapting to survival in the swine intestine and their environment (Chen et al. 2004). Kwon et al (Kwon et al. 1999) reported that the major serogroups were O101, O8, O20 and O45, all of which were isolated from diarrhoeic piglets from 1995 to 1997. In the current study, the predominant serogroup was O149 followed by O8, O157 and O101. It is apparent that the predominant serogroup has shifted from O101 to O149 over the last 10 years on Korean farms although the O101 serogroup has mainly been isolated from fields. The O149 serogroup has been determined to be the dominant serogroup in cases of neonatal and postweaning diarrhoea in many countries (Chen et al. 2004; Fairbrother et al. 2005); Frydendahl 2002; Vu-Khac et al. 2007; Gyles 2010)

Table 5. Relationship between fimbrial and non-fimbrial adhesins

	Non-fimbrial adhesions (%)		
	AIDA	paa	eae
F4 (n = 37)	2 (66.6)	22 (64.7)	0
F18 (n = 5)	0	2 (5.9)	0
Toxin genes	1 (33.3)	2 (5.9)	0
Number of fimbriae	0	8 (23.5)	0
Total	3 (100)	34 (100)	0

In this study, 31.9 % of all isolates were positive for the F4 gene, indicating that F4-positive *E. coli* are the most prevalent in Korea. This is in accordance with earlier reports from other countries such as the US (Zhang et al. 2007), Denmark (Frydendahl

2002), and Slovak Republic (Vu-Khac et al. 2007). Although F5 and F6 fimbria have frequently been detected in isolates from suckling piglets with diarrhoea (Garabal et al. 1997; Francis 2002), F5 and F6 were only detected in 10 isolates (8.6%) and two isolates (1.7%) in this study, respectively. It is interesting that a shift in the major fimbria has been reported in Korea over the last decade. F6 was the predominant adhesion factor in the late 1990s which was replaced by F5 in the mid-2000s (Kwon et al. 1999, 2002; Lee et al. 2009). Thereafter, F5 was in turn substituted by F4 in the late 2000s (Kim et al. 2010). Those reports support our data in that F4 appears to be the predominant adhesion factor of *E. coli* in recent years. Although the causes are unclear, it is possible that the use of indigenous and commercial vaccines could induce antigenic variation and the resistance of pigs with F5 and F6 from two to four weeks of age (Francis 2002).

Haemolysis has been considered as one of the determinants of virulence (Frydendahl 2002; Nagy and Fekete 2005). In this work, only 45 isolates (38.7%) were haemolytic. However, F4-positive isolates were more likely to be associated with haemolysin (94.4% of F4-positive isolates). On the other hand, All the F5- and F6-positive isolates were non-haemolytic (Francis 2002; Gyles 2010).

The predominant virotype identified was F4:LT:STb:EAST1 (26.7%) among the isolates containing at least one of the adhesion and toxin genes. F5-positive isolates were exclusively associated with STa. The virotype of STb:EAST1 with or without AIDA was also detected in three isolates (2.6%), which may be pathogenic and induce diarrhoea (Ngeleka et al. 2003). Although some pathotypes tend to produce multiple enteroroxins capable of inducing diarrhea, F4ac strains that express only LT or STb enterotoxin are virulent for gnotobiotic pigs (Zhang et al. 2006, 2007). The association between F4 and either LT:STb:EAST1 or LT:STa:STb:EAST1 has been reported previously (Fairbrother and Gyles 2006; Gyles 2010). LT- and STb-positive isolates were more prevalent than STa in ETEC from pigs older than one week (Dubreuil 2008). This is in agreement with the studies of Harel et al. (1991) and, Osek and Truszczynski (1992) who also found STb as the most prevalent toxin isolated from diarrhoeic piglets in Canada and Poland, respectively.

The Stx1 and Stx2 toxins were detected in one (0.8%) and three (2.6%) of 116 isolates, respectively. Although the prevalence was lower than in a previous report (Kim et al. 2010), it is still possible that piglets harbour reservoirs of STEC which are pathogenic for humans.

Paa-positive isolates were more likely to associate with F4 (64.7%) than with F18 (5.9%). The *paa* gene was first identified in porcine enteropathogenic *E. coli* and has been found in ETEC of O149:H10 (An et al. 1999). However, the role of porcine *paa* in ETEC pathogenesis is not fully known. In previous reports, it was commonly found in F4-positive strains (An et al. 1999; Leclerc et al. 2007).

AIDA has been detected in ETEC and STEC from ND and PWD in Korea (Ha et al. 2003, 2004). It is also known that F18 and AIDA are localised on the same plasmid (Mainil et al. 2002). In this work, we detected AIDA in three isolates, two of which were F4-positive. This is in accordance with the results of Ha et al. (2004)

No eae-positive isolates were detected in this study. Although the *eae* gene has been detected in *E. coli* from diarrhoeic ND piglets, they do not carry toxin genes (Vu-Khac et al. 2007; Ha et al. 2008). The *eae* gene can also be detected in non-diarrhoeic piglets which thus may be carriers of eae-positive *E. coli* (Vu-Khac et al. 2007).

EAST1 toxin belongs to the STa family of enterotoxins and has been recovered from porcine ETEC (Yamamoto and Nakazawa 1997; Choi et al. 2001). However, *E. coli* strains from pigs without diarrhoea also carried the EAST1 gene (Ngeleka et al. 2003; Zajacova et al. 2012). In this study, the EAST1 gene was most commonly present together with other toxin genes (LT, STa and STb). In only 10 (20.8%) out of 38 isolates was EAST1 present as the only toxin.

PFGE has been used to reveal the inter- and intra-serogroup genetic relatedness of porcine *E. coli* (Osek 2000; Blanco et al. 2006; Vu-Khac et al. 2007). The results obtained in this study revealed a high degree of polymorphism among 15 O149 serogroup isolates from diarrhoeic piglets with virulence genes (F4, LT, STa, STb, Paa and EAST1). These results are in accordance with the findings of Vu-Khac et al. (2007) in Slovak Republic. In contrast to our results, Osek (2000) showed a strong genetic homogeneity within 25 O149:K91 isolates in Poland. Therefore, there exist different clusters in the most prevalent serogroup isolated from piglets with diarrhoea in Korea even though they harbour the same virulence genes.

The results reported here provide not only epidemiological data regarding the prevalence of known serogroups and virulence factors but could also be

used to design control measures for enteric colibacillosis in Korean piggeries.

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REFERENCES

An H, Fairbrother JM, Desautels C, Harel J (1999): Distribution of a novel locus called paa (porcine attaching and effacing associated) among enteric Escherichia coli. Advances in Experimental Medicine and Biology 473, 179–184.

Batisson I, Guimond MP, Girard F, An H, Zhu C, Oswald E, Fairbrother JM, Jacques M, Harel J (2003): Characterization of the novel factor paa involved in the early steps of the adhesion mechanism of attaching and effacing Escherichia coli. Infection and Immunity, 71, 4516–4525.

Beaudry M, Zhu C, Fairbrother JM, Harel J (1996): Genotypic and phenotypic characterization of Escherichia coli isolates from dogs manifesting attaching and effacing lesions. Journal of Clinical Microbiology 34, 144–148.

Blanco M, Lazo L, Blanco JE, Dahbi G, Mora A, Lopez C, Gonzalez EA, Blanco J (2006): Serotypes, virulence genes, and pfge patterns of enteropathogenic Escherichia coli isolated from cuban pigs with diarrhea. International Microbiology 9, 53–60.

Chen X, Gao S, Jiao X, Liu XF (2004): Prevalence of serogroups and virulence factors of Escherichia coli strains isolated from pigs with postweaning diarrhoea in eastern China. Veterinary Microbiology 103, 13–20.

Choi C, Cho W, Chung H, Jung T, Kim J, Chae C (2001): Prevalence of the enteroaggregative Escherichia coli heat-stable enterotoxin 1 (EAST1) gene in isolates in weaned pigs with diarrhea and/or edema disease. Veterinary Microbiology 81, 65–71.

Dubreuil JD (2008): Escherichia coli stb toxin and colibacillosis: Knowing is half the battle. FEMS Microbiology Letters 278, 137–145.

Fairbrother JM, Gyles CL (2006): E. coli infections. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ (eds.): Diseases of Swine. 9th ed. Ames, Iowa State. 639–674.

Fairbrother JM, Nadeau E, Gyles CL (2005): Escherichia coli in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. Animal Health Research Reviews 6, 17–39.

Francis DH (2002): Enterotoxigenic E. coli infection in pigs and its diagnosis. Journal of Swine Health Production 10, 171–175.

Frydendahl K (2002): Prevalence of serogroups and virulence genes in Escherichia coli associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Veterinary Microbiology 85, 169–182.

Garabal JI, Vazquez F, Blanco J, Blanco M, Gonzalez EA (1997): Colonization antigens of enterotoxigenic Escherichia coli strains isolated from piglets in spain. Veterinary Microbiology 54, 321–328.

Gyles CL (1994): Escherichia coli in Domestic Animals and Humans. CAB International Wallingford, Oxon, UK. 666 pp.

Gyles CL (2010): Escherichia coli. In: Gyes CL, Prescott JF, Songer JG, Thoen CO (eds.): Pathogenesis of Bacterial Infections in Animals. 4th ed. 267–298.

Ha SK, Choi C, Chae C (2003): Prevalence of a gene encoding adhesin involved in diffuse adherence among Escherichia coli isolates in pigs with postweaning diarrhea or edema disease. Journal of Veterinary Diagnostic Investigation 15, 378–381.

Ha SK, Choi C, Jung K, Kim J, Han DU, Ha Y, Lee SD, Kim SH, Chae C (2004): Genotypic prevalence of the adhesin involved in diffuse adherence in Escherichia coli isolates in pre-weaned pigs with diarrhoea in Korea. Journal of Veterinary Medicine B 51, 166–168.

Ha Y, Ha SK, Lee YH, Kim D, Chae S, Kim CH, Ahn KK, Kim O, Chae C (2008): Prevalence of tir gene subtypes in Escherichia coli isolates from pigs. Veterinary Record 163, 424–425.

Harel J, Lapointe H, Fallara A, Lortie LA, Bigras-Poulin M, Lariviere S, Fairbrother JM (1991): Detection of genes for fimbrial antigens and enterotoxins associated with Escherichia coli serogroups isolated from pigs with diarrhea. Journal of Clinical Microbiology 29, 745–752.

Kim YJ, Kim JH, Hur J, Lee JH (2010): Isolation of Escherichia coli from piglets in South Korea with diarrhea and characteristics of the virulence genes. Canadian Journal of Veterinary Research 74, 59–64.

Kwon D, Kim O, Chae C (1999): Prevalence of genotypes for fimbriae and enterotoxins and of o serogroups in Escherichia coli isolated from diarrheic piglets in Korea. Journal of Veterinary Diagnostic Investigation 11, 146–151.

Kwon D, Choi C, Jung T, Chung HK, Kim JP, Bae SS, Cho WS, Kim J, Chae C (2002): Genotypic prevalence of the fimbrial adhesins (F4, F5, F6, F41 and F18) and toxins (Lt, Sta, Stb and Stx2e) in Escherichia coli iso-

lated from postweaning pigs with diarrhoea or oedema disease in korea. Veterinary Record 150, 35–37.

Leclerc S, Boerlin P, Gyles C, Dubreuil JD, Mourez M, Fairbrother JM, Harel J (2007): Paa, originally identified in attaching and effacing Escherichia coli, is also associated with enterotoxigenic E. coli. Research in Microbiology 158, 97–104.

Lee SI, Rayamahji N, Lee WJ, Cha SB, Shin MK, Roh YM, Yoo HS (2009): Genotypes, antibiogram, and pulsed-field gel electrophoresis profiles of Escherichia coli strains from piglets in korea. Journal of Veterinary Diagostic Investigation 21, 510–516.

Mainil JG, Jacquemin E, Pohl P, Kaeckenbeeck A, Benz I (2002): DNA sequences coding for the F18 fimbriae and AIDA adhesin are localised on the same plasmid in Escherichia coli isolates from piglets. Veterinary Microbiology 86, 303–311.

Nagy B, Fekete PZ (2005): Enterotoxigenic Escherichia coli in veterinary medicine. International Journal of Medical Microbiology 295, 443–454.

Ngeleka M, Pritchard J, Appleyard G, Middleton DM, Fairbrother JM (2003): Isolation and association of Escherichia coli AIDA-I/Stb, rather than EAST1 pathotype, with diarrhea in piglets and antibiotic sensitivity of isolates. Journal of Veterinary Diagnostic Investigation 15, 242–252.

Osek J (2000): Clonal analysis of Escherichia coli strains isolated from pigs with post-weaning diarrhea by pulsed-field gel electrophoresis. FEMS Microbiology Letters 186, 327–331.

Osek J, Truszczynski M (1992): Occurrence of fimbriae and enterotoxins in Escherichia coli strains isolated from piglets in Poland. Comparative Immunology Microbiology and Infectious Diseases 15, 285–292.

Veilleux S, Dubreuil JD (2006): Presence of Escherichia coli carrying the EAST1 toxin gene in farm animals. Veterinary Research 37, 3–13.

Vu-Khac H, Holoda E, Pilipcinec E, Blanco M, Blanco JE, Dahbi G, Mora A, Lopez C, Gonzalez EA, Blanco J (2007): Serotypes, virulence genes, intimin types and pfge profiles of Escherichia coli isolated from piglets with diarrhoea in Slovakia. Veterinary Journal 174, 176–187.

Woodward MJ, Carroll PJ, Wray C (1992): Detection of entero- and verocyto-toxin genes in Escherichia coli from diarrhoeal disease in animals using the polymerase chain reaction. Veterinary Microbiology 31, 251–261.

Yamamoto T, Nakazawa M (1997): Detection and sequences of the enteroaggregative Escherichia coli heat-stable enterotoxin 1 gene in enterotoxigenic E. coli strains isolated from piglets and calves with diarrhea. Journal of Clinical Microbiology 35, 223–227.

Zajacova ZS, Konstantinova L, Alexa P (2012): Detection of virulence factors of Escherichia coli focused on prevalence of EAST1 toxin in stool of diarrheic and non-diarrheic piglets and presence of adhesion involving virulence factors in astA positive strains. Veterinary Microbiology 154, 369–375.

Zhang W, Berberov EM, Freeling J, He D, Moxley RA, Francis DH (2006): Significance of heat-stable and heat-labile enterotoxins in porcine colibacillosis in an additive model for pathogenicity studies. Infection and Immunity 74, 3107–3114.

Zhang W, Zhao M, Ruesch L, Omot A, Francis D (2007): Prevalence of virulence genes in Escherichia coli strains recently isolated from young pigs with diarrhea in the US. Veterinary Microbiology 123, 145–152.

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