Prevalence of *tir* gene subtypes in *Escherichia coli* isolates from pigs

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ATTACHING and effacing *Escherichia coli* (AEEC) are characterised by their capacity to infect intestinal epithelial cells, resulting in attaching and effacing (AE) lesions. AE histopathology is characterised by intimate adherence of the bacteria to enterocytes and disruption of the underlying cytoskeleton (Moon and others 1983). A chromosomal gene, *eae*, encodes the protein intimin, which contributes to this intimate adherence (Jerse and others 1990, Jerse and Kaper 1991, Zhu and others 1994). The *tir* gene encodes the intimin receptor (Tir), which is translocated into the cytoplasm of the host where it is phosphorylated and then incorporated into the host cell membrane (Kenny and others 1997).

The *eae* gene shows high conservation in the N-terminal region, which encodes the region of the intimin protein that binds to Tir, but there is variability in the last C-terminal 280 amino acids between *eae* gene subtypes (Frankel and others 1994). On the basis of deduced amino acid sequences and PCR analysis, at least 16 subtypes of *eae* have been described (Agin and Wolf 1997, Adu-Bobie and others 1998, China and others 1999, Oswald and others 2000, Zhang and others 2002, Garrido and others 2006). Among these subtypes, the *eae*_β gene was found to be the most common subtype among isolates of AEEC from pigs with diarrhoea, followed by *eae*_γ, *eae*_ε, and *eae*_ζ (Ha and others 2004). The *tir* gene has highly conserved central regions that encode the region that binds to intimin, and variable regions outside of these central intimin-binding regions (Liu and others 2002, Nielsen and Andersen 2003). On the basis of deduced amino acid sequences and PCR analysis, at least four subtypes of *tir* have been identified: α, β, γ, and θ (Goffaux and others 2000, Nielsen and Andersen 2003). To the best of the authors' knowledge, there have been no reports of subtypes of *tir* in isolates of AEEC from pigs. This short communication describes a study in which PCR was used to determine the subtypes of *tir* present in AEEC isolated from pigs with diarrhoea in the Republic of Korea.

A total of 59 AEEC isolates were analysed. Thirty-eight of the isolates were identified as AEEC out of 707 *E. coli* isolates from diarrhoeic preweaned pigs, and 21 were identified as AEEC out of 480 isolates from diarrhoeic postweaned pigs. The *eae* gene subtypes of the isolates were identified in a previous study by Ha and others (2004). All the farms were farrow-to-finish operations and nine farms were using an all-in/all-out rearing system. Fifty AEEC were isolated from Large White x Duroc crossbred pigs and nine were isolated from pigs of unknown breed. Among the 38 isolates from preweaned pigs, 12 were from Kyunggi province, eight were from Chungcheung province, seven were from Cholla province and 11 were from Kyungsang province. Among the isolates from postweaned pigs, six were from Kyunggi province, five were from Chungcheung province, four were from Cholla province and six were from Kyungsang province.

Pigs were submitted alive or dead for diagnosis of the cause of diarrhoea; those submitted alive were sedated by an intravenous injection of sodium pentobarbital, stunned electrically and then bled out as previously described (Anon 2001). Immediately after euthanasia or, for pigs submitted dead, immediately upon receipt, specimens of ileal content were collected for bacteriological culture on 5 per cent sheep blood agar and Tergitol-7 agar (Difco Laboratories). The

TABLE 1: PCR primers used in assays to amplify specific fragments from various subtypes of the *tir* gene of *Escherichia coli* in isolates from pigs with diarrhoea

Target gene	Primer sequence 5'-3'	Amplicon (bp)	Reference
<i>tir</i> _α	CAGCCTGGCACTTACCTTCACA CGCTAACCTCCAAACCAT	342	China and others (1999)
<i>tir</i> _β	CAGCCTGGCACTTACCTTCACA GATTTTCCCTCGCCACTA	560	China and others (1999)
<i>tir</i> _γ	CAGCCTGGCACTTACCTTCACA GTCGGCAGTTTCAGTTTCAC	781	China and others (1999)
<i>tir</i> _θ	CTAGCGCAGTGGTTAATCCATATG ATTCCCTGAGAAATTTGAATGACG	137	Nielsen and Andersen (2003)
bp Base pairs			

colony characteristics of isolates were noted, including haemolysis on blood agar and texture (smooth, rough, mucoid) on Tergitol-7 agar, for routine diagnostic procedures. Infections were considered to be potentially dual or multiple when colonies with different haemolytic patterns or morphology were observed on one plate.

For each culture, all isolates with different phenotypes were selected for PCR analysis. PCR for *tir* gene subtypes was carried out as previously described by China and others (1999) and Nielsen and Andersen (2003) with the primers shown in Table 1. PCR for the Shiga toxin gene was also carried out as previously described by Kong and others (1999). The PCRs were performed in triplicate. Control DNA from reference strains was included in each reaction.

The primer pair for each *tir* subtype yielded PCR products of the expected size for the control strains and field isolates. The results of the analysis of *tir* gene subtypes are shown in Table 2. None of the AEEC isolates studied carried the gene for Shiga toxin.

The results of this study show the presence of various combinations of *eae* and *tir* subtype genes. The combination of *eae*_γ and *tir*_γ is the most common in *E. coli* isolated from human beings, while the pathotype *eae*_β and *tir*_β seems to be most frequently associated with diarrhoea in calves (China and others 1999). In these pigs, the *eae*_β gene was associated with the *tir*_θ gene in 14 of 59 isolates. Homologous combinations were also present: *eae*_β with *tir*_β in two isolates and *eae*_γ with *tir*_γ in three isolates. Four subtypes of *tir* were identified by PCR analysis of the AEEC isolates from diarrhoeic Korean pigs. The prevalence of *tir* subtypes in *E. coli* from the preweaned pigs was similar to that from the postweaned pigs. Fourteen of the isolates could not be typed because they did not react with the PCR primers used in this study. These results suggest that some *eae*-positive *E. coli* may possess another unique subtype or may have different sequences in their subtypes that prevent their recognition by PCR.

All the AEEC strains used in this study were isolated from the ileum, because all the samples were submitted for the

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TABLE 2: Prevalence of *tir* gene subtypes from attaching and effacing *Escherichia coli* isolates from pigs with diarrhoea

Pigs	<i>eae</i> gene subtype	Number of samples	<i>tir</i> gene subtype				
			α	β	γ	θ	NT
Preweaned	β	19	4	2	1	8	4
	γ	12	1	2	2	3	4
	ε	4	1	1	1	0	1
	NT	3	0	0	1	1	1
Postweaned	β	10	3	0	0	6	1
	γ	5	2	2	1	0	0
	ε	2	0	0	0	0	2
	ζ	1	0	0	0	1	0
	NT	3	0	0	0	2	1
NT Non-typable							

diagnosis of diarrhoea and ileal samples are usually collected for routine diagnostic procedures at the authors' diagnostic centre. Different intimin types have been shown to be an important factor for the initial tissue tropism of the bacteria in the host (Fitzhenry and others 2002). *E. coli* O157 produces γ -intimin and is considered to have a tropism toward the distal gastrointestinal tract, while *E. coli* O26 produces β -intimin and is considered to have a tropism toward the proximal gastrointestinal tract (Caprioli and others 2005). Therefore, β -intimin has a tropism for the ileum, and this may explain the high prevalence of isolates bearing the gene for β -intimin (*eae* _{β}) in the present study.

AEEC strains are classified as either enteropathogenic or enterohaemorrhagic *E. coli* depending on their ability to produce Shiga toxin (Nataro and Kaper 1998). Since all the *eae*-positive *E. coli* examined in this study were negative for the Shiga toxin gene, all the strains studied could be enteropathogenic. A total of 10 AEEC strains were serotyped in this study; four were serogroup O127, three were serogroup O26, two were serogroup O111 and one could not be serogrouped. Intestinal tropism is species-independent, at least between human and porcine intestinal tissues (Fitzhenry and others 2003). Therefore, pigs may be an important reservoir of AEEC strains that are responsible for *E. coli*-associated diarrhoea in human beings.

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