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Short Communication

Prevalence of the *paa* gene (porcine attaching and effacing associated) in porcine enteropathogenic *Escherichia coli* (PEPEC) associated with postweaning diarrhea in south Brazil

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Abstract

Paa (porcine attaching and effacing associated) may be an important virulence factor *E. coli* of piglets with diarrhea. This study showed for the first time in Brazil the prevalence of the *paa* gene (22%) in *E. coli* strains isolated from piglets and these isolates also harboured genes for other adhesins and toxins LT II, STa and STb.

Key words: *Escherichia coli*, pig, PEPEC, *paa* gene, postweaning diarrhea.

Neonatal diarrhea and postweaning diarrhea (PWD) in pigs are diseases of considerable economic importance and are characterized by watery diarrhea, dehydration, loss of body weight and sometimes death of infected pigs (Nagy and Fekete, 1999). Enterotoxigenic *E. coli* (ETEC) is an important cause of PWD, and its pathogenicity involves the adherence of the pathogen to the small intestine by means of specific adhesion factors (fimbriae) and production of one or several exotoxins responsible for disease development. ETEC produce heat-stable (Sta or Stb) and/ or heat-labile (LT) enterotoxin that cause fluid and electrolyte secretion (Nagy and Fekete, 1999)

However, non-enterotoxigenic porcine *E. coli* strains have been associated with PWD and neonatal diarrhea in swine by adhesion to intestinal epithelial cells in a characteristic attaching and effacing (A/E) pattern. This porcine enteropathogenic *E. coli* (PEPEC) produces an outer membrane protein (intimin), which is involved in the intimate attachment of the bacteria to enterocytes and induced typical A/E lesion in a pig ileal explant model (Zhu *et al.*, 1994, 1995). The A/E lesion contributes to the initial phases of PEPEC pathogenicity (Batisson *et al.*, 2003).

The gene that induces this lesion was designated *paa* (porcine A/E-associated gene) (Nagy and Fekete, 1999; An *et al.*, 1999), and its sequence revealed an open reading frame of 753 bp encoding a 27.6-kDa protein, that shows similarity with Paa of enterohemorrhagic *E. coli* O157:H7 strains (Batisson *et al.*, 2003). The A/E activity of PEPEC is highly correlated with the presence of the LEE (locus of enterocyte effacement) detected by DNA probes derived from the LEE of human enteropathogenic *E. coli* (EPEC) strain E2348/69 (An *et al.*, 2000).

In Brazil there is a lack of information about the prevalence of the paa gene of porcine *E. coli*. The objective of the present study was to evaluate the presence of *paa* gene, and its correlation with the presence of enterotoxin STa, STb and LT encoding genes of *E. coli* strains isolated from piglets with diarrhea in Northern region of Paraná State, Brazil, described in a previous study (Vidotto *et al.*, 2009).

Three hundred *Escherichia coli* strains isolated from 100 piglets with diarrhoea from different farms in Paraná State (Vidotto *et al.*, 2009) were tested for the presence of the *paa* gene by polymerase chain reaction assay (PCR). The *E. coli* HB101strain (Boyer and Roulland-Dssoix, 1969) was included as negative control.

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The base sequences for specific oligonucleotide primers used in this study were constructed based on the regions of conserved sequences between the *paa* gene of ETEC and PEPEC 045 (GeneBanK U82533.4), PAA PEPEC O45-F: 5'- TCTTCTGCTGCTTATGCTGATA TC-3' and PAA PEPEC O45-R: 5'- TTACCAGCCATA TTTTTTGAATGC-3', annealing at nucleotides 37 to 60 and 718 to 738 of the *paa* gene, respectively. Bacterial DNA to be amplified was released from whole organisms by boiling, and PCR was carried out in a total volume of 25 μL containing 5 μL template DNA, each of the primers at 20 pmol, 200μM dNTPs, PCR buffer and 1.5 U Taq DNA polymerase (Invitrogen). PCR amplifications consisted of 30 cycles of 94 °C for 1 min, annealing temperature specific for each primer for 1 min and 72 °C for 2 min. The amplified DNA was visualized in 1.5% agarose gels stained with ethidium bromide. The 100-pb ladder (Promega, Madison, WI) was used as standard.

Of 100 piglets with diarrhea, 22% presented *E. coli* that carried *paa* genes. The presence of the *paa* gene (Table 1) was correlated with the presence of genes that encode fimbrial adhesins F4, F5, F6, F18, F41 and the toxins LT II, STa and STb found in these studied strains (Vidotto *et al.*, 2009). Some strains that carried the *paa* gene also harboured genes for others adhesins and toxins (Table 1). There was no significant association of *paa* gene with the other virulence genes when analyzed by chi-square test ($p > 0.05$).

The presence of ETEC virulence factors, fimbriae and enterotoxins production, are common features of isolates associated with diarrhea during the pre-weaning and weaning periods (Nagy and Fekete, 1999, 2005), but some *E. coli* strains isolated from piglets with diarrhea were non-enterotoxigenic, and presented intimin (*paa* gene) responsible for A/E lesions (Zhu *et al.*, 1995). The serogroup O45 is particularly important among PEPEC strains, and based on their virulence gene content, O45 PEPEC strains displayed significant differences from typical EPEC and could be regarded as atypical EPEC, that are defined as LEE-positive *E. coli* lacking *stx*1 and *stx*2 genes (Bruant *et al.*, 2009).

In the present study, the *paa* gene was detected in 22% of *E. coli* isolated from diarrhoeic piglets, from the Northern Region of Paraná State, and this gene was associated with other virulence factors from ETEC. We found the *paa* gene in isolates that carried additional virulence encoding genes such as *faeG* (F4), *fanC* (F5), *fasA* (F6), *fed*A (F18), *f41*, *estA* (*STa*), *estB* (*STb*) and *elt* (LT). The pathotype $F4^+$ $F18^+$ $F41^+$ Paa^+ STa^+ STb^+ and LT^+ were found among the strains. These data suggest that the various virulence genes together can be link to resistance genes on plasmids. The *paa* gene was found on high molecular weight plasmids that codify tetracycline resistance (Leclerc *et al.*, 2007).

Similarly Leclerc *et al.* (2007) found the *paa* gene with virulence genes associated with pig ETEC, *estA* that encodes STa, *estB* that encodes STb, *elt* that encodes LT, astA that encodes EAST1 and faeG that is a part of the F4

Table 1 - Distribution of the *paa* gene and enterotoxins among *E. coli* strains isolated from diarrheic piglets.

Adhesins	Nº of strains	STb	LT	STa LT	STb LT	STa STb LT	None toxin
Paa	2						2
Paa	3		3				
Paa	2				2		
Paa	1	1					
Paa + F6	1			1			
Paa + F41	1					1	
Paa + F4+F5	1		1				
Paa + F5+F18	1	1					
Paa + F4+ F6+ F18	2			1		1	
Paa + F4+F18+F41	1					1	
Paa + F5+F18+F41	1			1			
Paa + F4+F5+F6+F41	1						1
Paa + F4+F6+F18+F41	2	1			1		
Paa + F4+F5+F18+F41	1					1	
Paa + F4+F5+F6+F41	2					2	
Total	22	3	4	3	3	6	3

Paa- porcine A/E lesion-associated adhesin, F4- K88, F5- K99, F6-987P.
LT-thermolabile enterotoxin , ST-termostable enterotoxin.
No statistically significant association detected.

operon. The occurrence of enterotoxins was also associated with specific fimbriae in *E. coli* from pigs and several strains produced more than one fimbrial antigen (Nagy and Fekete, 2005; Toledo *et al.*, 2012; Vidotto *et al.*, 2009).

The prevalence of the *paa* gene (22%) found in this study was different than that found by others, Zhang *et al.* (2007) found a prevalence of 60% in *E. coli* strains isolated from young pigs with diarrhea in the US, and Boerlin *et al.* (2005) detected *paa* in 92% of porcine ETEC isolated in Canada.

The *paa* gene sequence is similar to that of the EPEC *eae* gene that codifies intimin (Zhu *et al.*, 1994), and the *eae* gene also have been found in 25.7% of isolates in Brazil (Martins *et al.*, 2000), 28.33% in China (Cheng *et al.*, 2006); and 27% of isolates in Mexico (Toledo *et al.*, 2012).

In conclusion, this study showed for the first time in Brazil the prevalence of the *paa* gene in *E. coli* strains isolated from piglets with diarrhea, and confirms the combination of various virulence genes in ETEC and porcine EPEC, suggesting that the *paa* gene could play a role in virulence.

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