



# Investigation on the preponderance of high-pathogenicity island and *Escherichia coli* infection associated with porcine diarrhea

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## Abstract

One hundred and sixty four rectal swab samples were collected from 110 live diarrheic piglets and 54 healthy piglets during an investigation on the prevalence of HPI-harboring *Escherichia coli* (HPI<sup>+</sup> *E. coli*) infection associated with porcine diarrhea. The data of polymerase chain reaction (PCR) revealed the presence of HPI<sup>+</sup> *E. coli* in 58.18% of swabs taken from diarrheic piglets, while 44.44% of samples taken from healthy pigs were so positive. Further PCR examination of 600 bacterial isolates from diarrheic samples revealed that 25 isolates were HPI<sup>+</sup> *E. coli* while 4 (16%) were F4<sup>+</sup>, 2 (8%) were F4<sup>+</sup> and F6<sup>+</sup>, 2 (8%) were F4<sup>+</sup> and F6<sup>+</sup>, and one (4%) was F6<sup>+</sup>, LTa<sup>+</sup> and STb<sup>+</sup>. Among the 480 bacterial isolates from non-diarrheic samples, 20 isolates were HPI<sup>+</sup> *E. coli*, and only one (5%) was LTa<sup>+</sup> and STb<sup>+</sup>, while absence of isolates belonging to the F4<sup>+</sup> and F6<sup>+</sup> groups was noted. O138 was the vast prevalent serotype among the HPI<sup>+</sup> *E. coli* isolates. It is suggested that HPI<sup>+</sup> *E. coli* maybe an opportunistic pathogen in swine.

**Keywords:** *Escherichia coli*, HPI, prevalence, piglet.

## INTRODUCTION

Pathogenicity islands (PAIs) are a distinct class of genomic islands (GEIs), which are acquired by horizontal gene transfer. PAIs harbor virulence genes and some antibiotic resistance genes (Oelschlaeger et al., 2004). The high-pathogenicity island (HPI) was previously described in *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* (Bearden et al., 1998; Buchrieser et al., 1998a).

In addition, HPI have been horizontally transferred to other bacterial species, such as *Shigella*, *Citrobacter*, *Klebsiella* and *Escherichia* (Bach et al., 2000; Karch et al., 1999; Schubert et al., 1998). It was also reported that some HPI<sup>+</sup> *E. coli* isolates from patients or animals with diarrhea were closely correlated with clinical symptoms (Xu et al., 2000; Cheng et al., 2006).

But the role of HPI<sup>+</sup> *E. coli* isolates play in the piglet diarrhea need to be deeply investigated and researched in detail. In this study, we detected HPI<sup>+</sup> *E. coli* infection in diarrheic and healthy piglets, and investigate the prevalence of the other virulence factors in the HPI<sup>+</sup> *E. coli* isolates.

## MATERIALS AND METHODS

### Collection of samples

From March - June, 2009, a total of 164 rectal swab samples were collected from piglets with a mean age of 6.5±3.4 days, including 110 live diarrheic piglets from 28 swine farms and 54 healthy piglets from 18 swine farms in the Jiangsu Province, China. This brings each test sample to a final volume of 500 µl with LB broth (10 g tryptone, 10 g NaCl, 5 g yeast extract, H<sub>2</sub>O to 1 L, pH 7.4) and store at -70°C.

### Bacterial pre-culturing and extraction of DNA templates

100 µl dilution of each rectal swab sample was transferred into separate tubes containing 2 ml of LB broth and the liquid cultures were grown with vigorous agitation at 37°C for 6 h. 0.5 ml of the liquid cultures was transferred to labeled micro centrifuge tubes, and centrifuged at 10,000 rpm for 5 min. The supernatants were discarded and each pellet was resuspended in 200 µl of distilled water. After boiling for 10 min, the suspensions were chilled on ice for 5 min, centrifuged at 10,000 rpm at 4°C for an additional 5 min, and the supernatants used directly or stored at 4°C before PCR detection.

### PCR detection of HPI-harboring *E. coli*

To detect HPI-harboring *E. coli* in the liquid cultures of rectal swab

**Table 1.** Summary of the O serotype and virulence factors of the 25 HPI<sup>+</sup> *E.coli* isolates from diarrheic piglets.

Virulence factor O serotype						Numbers of isolates
	LTa	STa	STb	F4	F6	
O138	-	-	-	-	-	12
O138	-	-	-	-	+	2
O138	-	-	-	+	+	1
O138	+	-	+	-	+	1
O65	-	-	-	-	-	3
O139	-	-	-	-	+	2
O9	-	-	-	+	-	2
O55	-	-	-	+	+	1
O141	-	-	-	-	-	1
Total	1	0	1	4	7	25

samples, PCR assays were performed in micro centrifuge tubes for the Applied Biosystems (2720 Thermal Cycler America). The primers (F: 5'-AAGGATTCGCTGTTACCGGAC-3', R: 5'-TCGTCGGGCAGCGTTTCTTCT-3') were used for PCR amplification to detect HPI<sup>+</sup> *E. coli* (Schubert et al., 1998) and synthesized by Sangon Biological Engineering Technology and Service Co. Ltd. (Shanghai, PR China). All the PCR reagents were purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. The PCR mixture contained 5 µl of 10× PCR buffer (Mg<sup>2+</sup> plus), 5 IU of *Taq* polymerase, 4 µl of dNTP mixture (each 2.5 mmol/l), 1 µl of HPI primers set (each 50 mmol/l), 2 µl of DNA template, and deionized water to a final volume of 50 µl. After denaturation at 94°C for 3 min, 30 cycles of the PCR was performed using the following program: denaturation at 94°C for 30 s, annealing at 62°C for 30 s and elongation at 72°C for 60 s (10 min for the final cycle). The expected size of PCR products should be 287 bp following separation in 1% agarose gel electrophoresis along with DL2000 DNA markers and visualization on a UV transilluminator after staining with ethidium bromide.

#### Virulence genes determination and O serotyping of HPI-harboring *E. coli*

Following rapid PCR detection, 30 HPI-positive diarrheic samples and 24 HPI-positive healthy samples were submitted to separative cultivation of bacteria by streaking on MacConkey agar plates. After incubation at 37°C overnight, twenty colonies were picked from each sample and cultured on LB agar plates at 37°C for 24 h. All the bacterial isolates were submitted to PCR detection (Cheng et al., 2005b, 2006) for the genes of HPI, enterotoxins (STa, STb and LTa) and fimbriae (F18, F4, F5, F6 and F41). In addition, all the HPI-harboring *E. coli* were serotyped by using uni-factor serum of O antigen of *E. coli*.

## RESULTS

Among the 110 rectal swab samples from live diarrheic piglets, 64 (58.18%) samples (from 22 farms) were infected with HPI-harboring *E. coli*, while 24 (44.44%) samples (from 9 farms) were HPI-positive in the 54 healthy samples.

Six hundred bacterial isolates were picked out from the diarrheic samples, and the data for PCR examination

determined that only 25 isolates (from 22 samples) were HPI<sup>+</sup> *E. coli*, thereinto, 4 (16%) were F4<sup>+</sup>, 2 (8%) were F4<sup>+</sup> and F6<sup>+</sup>, and 1 (4%) was F6<sup>+</sup>, LTa<sup>+</sup> and STb<sup>+</sup> (Table 1). In addition, the 25 HPI<sup>+</sup> *E. coli* isolates were O serotyped and O138 was the most prevalent serotype accounting for 64% (16/25), followed by O65 (12%), O139 (8%), O9 (8%), O55 (4%) and O141 (4%) (Table 1).

Among the 480 bacteria isolates that were picked out from the non-diarrhoeic samples, just 20 isolates (from 18 samples) were HPI<sup>+</sup> *E. coli*, thereinto, only one (5%) was LTa<sup>+</sup> and STb<sup>+</sup>, while no single F4<sup>+</sup> and F6<sup>+</sup> isolate was detected (Table 2). Furthermore, the 20 HPI<sup>+</sup> *E. coli* isolates were O serotyped and O138 also was the most prevalent serotype accounting for 60% (12/20), followed by O65 (25%), O21 (10%), O9 (5%), and O74 (5%) (Table 2).

## DISCUSSIONS

HPI was first discovered in pathogenic *Yersinia* strains and has recently been found to be widespread in other enterobacteria (Buchrieser et al., 1998b; Hacker et al., 2000; Carniel et al., 1992; Fetherston et al., 1994; Perry et al., 1990; Schubert et al., 1998). HPI carries the gene *fyuA*, which is specific for the pestacin receptor (FyuA) and the *irp* (iron repressible protein, such as HMWP1 and HMWP2) loci encoding the siderophore yersiniabactin (Carniel et al., 1992; Guilvout et al., 1993; Lucier et al., 1996). The HPI element is associated with asparagine-specific tRNA loci and carries an integrase gene *int*, often associated with a phage genome (Buchrieser et al., 1998; Rakin et al., 2001). But whether HPI could contribute to the virulence of *E. coli* isolates responsible for piglet diarrhea remains deeply a research question.

In this research, the data revealed the presence of HPI<sup>+</sup> *E. coli* in 58.18% of swabs taken from diarrheic piglets, while 44.44% of samples taken from healthy pigs were

**Table 2.** Summary of the O serotype and virulence factors of the 20 HPI<sup>+</sup> *E.coli* isolates from non-diarrheic piglets.

Virulence factor O serotype	LTa	STa	STb	F4	F6	Numbers of isolates
O138	-	-	-	-	-	10
O138	+	-	+	-	-	1
O65	-	-	-	-	-	5
O21	-	-	-	-	-	2
O9	-	-	-	-	-	1
O74	-	-	-	-	-	1
Total	1	0	1	0	0	20

highly positive. Therefore, we are suspicious of whether HPI could contribute to the virulence of *E. coli* isolates that causes piglet diarrhea. But after the analysis on the other hand, it was found that 78.57% diarrheic farms were infected with HPI<sup>+</sup> *E. coli*, while 50.00% healthy farms were confirmed to be HPI-positive. Furthermore, some HPI<sup>+</sup> *E. coli* isolates from diarrheic samples more frequently contained other virulence-associated genes, such as toxin(s) and fimbria(e), while only one isolate from healthy samples was LTa<sup>+</sup> and STb<sup>+</sup>, although the prevalent O serotype all was O138 in diarrheic and healthy isolates. According to the previous investigation on the effects of different virulent factors of *Escherichia coli* on pathogenesis (Cheng et al., 2005a), it suggested that the HPI<sup>+</sup> *E. coli* most likely to be opportunistic pathogen. This attractive hypothesis has to be verified by comparing the virulence of the parental strain and that of the isogenic mutants in a suitable infection mode.

It is well known that most *E. coli* are the normal inhabitants of intestinal tracts of animals (Levine, 1987; Martins et al., 2000), and it is very difficult to obtain the pathogenic *E. coli* in the rectal swab samples from live diarrheic piglets by bacteria isolation and identification. As a matter of fact, the clinical diagnosis of *E. coli* infection in piglets, especially in the rectal swab samples from live diarrheic animals, usually just need confirming whether the pathogenic *E. coli* was/were existed in the sample(s), and not always need bacterial isolation and identification anymore, while it is so difficult to pick out the pathogenic isolate(s). This is perfectly supported by the data of this research, such as 58.18% diarrheic rectal swab samples could be confirmed to be HPI-positive by the rapid detection method, and only 22 HPI<sup>+</sup> *E. coli* were obtained in 600 bacteria isolates by bacteria isolation and identification; while most isolates were the important members of the normal microbiologic flora of piglets. The experience of this research could provide a constructive idea and use for the diagnosis of other pathogen infection.

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