Prevalence of (high-pathogenicity island) HPI-harboring Escherichia coli in diarrheic and healthy piglets

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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 + E. coli) infection associated with porcine diarrhea. The data of polymerase chain reaction (PCR) revealed the presence of HPI + 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 + E. coli while 4 (16%) were F4*, 2 (8%) were F4* and F6*, 2 (8%) were F4* and F6*, and one (4%) was F6*. LTa* and STb*. Among the 480 bacterial isolates from non-diarrheic samples, 20 isolates were HPI + E. coli, and only one (5%) was LTa* and STb*, while absence of isolates belonging to the F4* and F6* groups was noted. O138 was the vast prevalent serotype among the HPI + E. coli isolates. It is suggested that HPI + E. coli maybe an opportunistic pathogen in swine.

Key words: 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; Buchriesser 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* 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 + E. coli isolates play in the piglet diarrhea need to be deeply investigated and researched in detail. In this study, we detected HPI + E. coli infection in diarrheic and healthy piglets, and investigate the prevalence of the other virulence factors in the HPI + 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, H₂O 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
samples, PCR assays were performed in micro centrifuge tubes for the Applied Biosystems (2720 Thermal Cycler America). The primers (F: 5′-AAGGATTCCGTGTTACCGGAC-3′, R: 5′-TCGTGGGCAGGTTTTCTTCT-3′) were used for PCR amplification to detect HPI + 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 (Mg2+ 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-harbouring 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 Mackonkey 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-harbouring 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-harbouring 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 + E. coli, the entraino, 4 (16%) were F4+, 2 (8%) were F4+ and F6+, and 1 (4%) was F6+, LTA+ and STb+ (Table 1). In addition, the 25 HPI + E. coli isolates were O serotyped and O138 was the most prevalent serotype accounting for 46% (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-diarrheic samples, just 20 isolates (from 18 samples) were HPI + E. coli, thereinto, only one (5%) was LTA+ and STb+, while no single F4+ and F6+ isolate was detected (Table 2). Furthermore, the 20 HPI + 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 pesticin receptor (FyuA) and the irp (iron repressible protein, such as HMWP1 and HMWP2) loci encoding the siderophoreyersiniabactin (Carniel et al., 1992; Guilvout et al., 1993; Lucier et al., 1996). The HPI element is associated with asparagin-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 + E. coli in 58.18% of swabs taken from diarrheic piglets, while 44.44% of samples taken from healthy pigs were
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 + E. coli, while 50.00% healthy farms were confirmed to be HPI-positive. Furthermore, some HPI + 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+ and STb+, 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 + 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 + 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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is present in certain non-O157 Shiga toxin-producing *Escherichia coli* clonal lineages. Infection Immunity, 67: 5994-6001.