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Serotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from piglets with diarrhoea in Slovakia

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Abstract

Two hundred and fifty *Escherichia coli* isolates from diarrhoeic and healthy piglets were serotyped and tested for the presence of virulence genes for fimbriae, intimin, heat-labile (LT) and heat-stable (STa and STb) enterotoxins, Stx toxins, and enteroaggregative heat-stable 1 (EAST1) enterotoxin by polymerase chain reaction (PCR). Although 220 isolates from diarrhoeic piglets belonged to 43 O serogroups and 77 O:H serotypes, 60% were of one of the 10 serogroups O2, O8, O15, O54, O84, O101, O141, O147, O149 and O157, and 60% belonged to only 10 serotypes (O8:H-, O54:H-, O84:H7, O101:H-, O141:H-, O141:H4, O147:H-, O149:H10, O163:H-, and ONT:H-).

PCR showed that 79% of 220 isolates carried genes for at least one of the virulence factors tested. The gene encoding for EAST1 was the most prevalent (65%) followed by those encoding for STb (49%), LT (42%), STa (13%), and Stx2e (4%). Eighty-three (38%) of the 220 *E. coli* isolates carried the gene for F4 (K88), whereas genes for F18, F5 (K99), F41, F6 (P987), F17, and intimin (*eae*) were detected in 9%, 3%, 3%, 3%, 1%, and 3%, respectively. Seropathotype O149:H10:F4:LT/STb/EAST1 (70 isolates) was the most common, representing 32% of isolates. Pulsed-field gel electrophoresis (PFGE) analysis with XbaI of 15 O149:H10 representative isolates from diarrhoeic piglets distinguished 14 types. The 15 isolates exhibited a wide variability of distinct restriction patterns though all belonged to the same serotype (O149:H10), and all but one showed identical virulence determinants (F4, LT, STb, and EAST1). Among 30 isolates from healthy piglets only two virulence genes were detected: EAST1 (26%) and *eae* (17%). In total, 12 isolates were positives for the *eae* gene: five isolates had intimin β1, four possessed intimin θ and three showed intimin type ξB. This is believed to be the first study describing the presence of intimin type ξB in *E. coli* of porcine origin.

Keywords: *E. coli*; Enterotoxins; Enteropathogenic *E. coli*; *Escherichia coli*; ETEC; PFGE; *eae* gene; Intimin; Porcine diarrhoea; STEC; VTEC

1. Introduction

Escherichia coli is a common porcine enteric pathogen, causing diarrhoea in new-born and weaned pigs, and oedema disease in pigs after weaning (Bertschinger, 1999; Fairbrother, 1999; Mainil and Daube, 2005). Enterotoxigenic *E. coli* (ETEC) and Shiga toxin-producing *E. coli* (STEC) are the main categories of diarrhoeagenic *E. coli* that cause enteric infections in pigs.

ETEC is defined as a pathogen containing *E. coli* isolates that elaborate at least one member of two defined groups of enterotoxins, namely heat-labile (LT) and heat-stable enterotoxins (STs) (Smith and Gyles, 1970). STs are classified as STa (also called STI) and STb (also called STII) (Nair and Takeda, 1998). Enterotoxins are

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extracellular proteins or peptides, which are able to exert their action on the intestinal epithelium (Blanco et al., 1991; Gyles, 1994; Nagy and Fekete, 1999). Most ETEC isolated from diarrhoeic pigs can produce one or more of the following fimbriae: F4 (K88), F5 (K99), F6 (987P), F17, F18 and F41 (Wilson and Francis, 1986; Blanco et al., 1991; Ojeniyi et al., 1994; Garabal et al., 1997; Nagy and Fekete, 1999). Fimbriae are surface proteins that are responsible for adhesion of pathogenic isolates to intestinal epithelial cells (Nagy and Fekete, 1999).

ETEC associated with neonatal diarrhoea belong to a limited number of serogroups with O8, O9, O20, O64, O101, O138, O141, O147, O149, and O157 being the most commonly found in several countries (Wilson and Francis, 1986; Ojeniyi et al., 1994; Garabal et al., 1996; Blanco et al., 1997; Nagy and Fekete, 1999; Francis, 2002). A second pathotype is Shiga toxin-producing *E. coli* (STEC), also called verotoxin-producing *E. coli* (VTEC). STEC isolates have been associated mainly with post-weaning diarrhoea and oedema disease (González and Blanco, 1985; Linggood and Thompson, 1987; Garabal et al., 1995; Mainil and Daube, 2005). Porcine STEC produce the STxe2e (VT2e) variant, which is absorbed from intestine and enters the bloodstream where it causes systemic vascular damage resulting in oedema disease (Bertschinger, 1999; Mainil and Daube, 2005).

In addition to ETEC and STEC, strains inducing attaching and effacing lesions similar to those produced by enteropathogenic *E. coli* (EPEC) in humans, have also been associated with diarrhoea in pigs (Zhu et al., 1994). These attaching and effacing *E. coli* (AEEC) possess the *eae* gene encoding an outer membrane protein termed intimin, which is involved in attachment of the bacteria to enterocytes (Krause et al., 2005; Malik et al., 2006).

A new category of diarrhoeagenic *E. coli*, named enteroaggregative *E. coli* (EAEC), is being increasingly recognised as an agent of diarrhoea in young children in developing countries (Nataro and Kaper, 1998; Piva et al., 2003). EAEC possesses the ability to adhere to HEp-2 cells and other surfaces in an aggregative pattern, sometimes described as a stacked-brick configuration. EAEC isolates produce a low-molecular-weight, partially heat-stable, plasmid encoded enterotoxin named enteroaggregative heat-stable enterotoxin 1 (EAST1) (Nataro and Kaper, 1998). The gene encoding for EAST1 toxin is apparently not restricted to human EAEC and has also been detected in *E. coli* of different pathogenic groups (ETEC, EPEC, STEC) strains from humans and animals (Yamamoto and Nakazawa, 1997; Choi et al., 2001; Frydendahl, 2002; Ngeleka et al., 2003; Noamani et al., 2003; Osek, 2003).

Pulsed-field gel electrophoresis (PFGE) has been used effectively to analyse the degree of genetic relatedness or variability among *E. coli* isolates of different serotypes as well as among isolates of the same serogroups originating from weaned piglets with diarrhoea and oedema disease (Aarestrup et al., 1997; Osek, 2000). In Slovakia, as in other countries, enteric colibacillosis is the most common disease of newborn piglets. However, little is known about the prevalence of serogroups, serotypes and virulence factors. The aim of this work was to investigate the prevalence of serogroups, serotypes, and virulence genes among *E. coli* isolated from piglets. This kind of investigation has not been carried out previously in Slovakia and the results obtained in our study offer valuable epidemiological information of porcine enteropathogenic *E. coli*.

2. Materials and methods

2.1. E. coli isolates

Two hundred and twenty *E. coli* strains isolated from piglets (<14 days old) with diarrhoea and 30 isolates recovered from healthy piglets of the same age were examined. Each of the 250 *E. coli* strains was isolated from a different piglet and in intestinal content of dead piglets as well as from rectal swabs submitted to the Departments of Bacteriology (State Veterinary Institutes in Bratislava, Nitra, Zvolen, Slovakia) and to the Department of Food Hygiene and Technology (Institute of Microbiology and Immunology, University of Veterinary Medicine, Slovakia) during the years 2001 and 2003.

The 220 *E. coli* isolates from diarrhoeic piglets came mainly from 31 different pig farms (range 5-100 sows per farm). Of these, 15 farms were located in the East part of Slovakia, nine in the West and seven in the Central part of the country. Of the 30 *E. coli* strains isolated from healthy piglets, 16 isolates come from four farms located in the East and the remaining 14 were isolated in three farms in Central parts of Slovakia. The faecal strains were plated onto MacConkey agar (Oxoid) and the *E. coli* isolates identified by standard biochemical procedures. After isolation, the strains were stored in Luria-Bertani broth containing 20% glycerol at −70 °C for further characterization studies.

2.2. Reference strains

The *E. coli* strains used as controls were: 298 (F4), 329 (F5), 318 (F6), 320 (F41), 216 (F18 and Stx2e), 281 (LT), 256 (STa and STb), G491(F4/K88ac), P201 (F4/K88ad), 5138 (F18ab), 8813 (F18ac), 253KH85 (F17), 226KH85 (F17), 960205 (EAST1), 022206 (EAST1), EDL933 (Stx1, Stx2 and *eae*-γ1) and B49 (Stx1 and *eae*-ξB). *E. coli* C600 was used as a negative control. A number of the control strains were kindly supplied by Dr. J. Osek (National Veterinary Research Institute, Pulawy, Poland), Dr. P. Alexa (Veterinary Research Institute, Brno, Czech Republic), Dr. P.F. Lintermans (Veterinary and Agrochemical Research Center, Bruxelles, Belgium) and Dr. C. Chae (Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, Republic of Korea).