Phenotype and Genotype of *Escherichia coli* Isolated from Pigs with Postweaning Diarrhea in Hungary

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A total of 205 *Escherichia coli* isolates from 88 diarrheal weanling (4- to 10-week-old) pigs from 59 farms were tested by slide agglutination for K88, K99, F41, and 987P antigens. K88 antigen was detected in 61% of the isolates representing 60% of the pigs and 56% of the farms. K88 antigen was associated with serogroup O149 and 91% of the K88⁺ isolates. K99, F41, and 987P were not detected. Of the K88⁻ isolates, 70 were additionally tested by colony hybridization with DNA probes for adherence factors K88, K99, 987P, and F41 and for enterotoxin genes STaP, STaH, STb, and LT and by Vero cell assay for verotoxins (VT). The 70 K88⁻ isolates could be divided into three categories: LT⁻, VT⁻, STaP⁺, and/or STb⁺ (34 isolates); LT⁻, STaP⁺, STb⁺, and/or VT⁺ (17 isolates); and nontoxicogenic (19 isolates). Only one of the K88⁻ isolates carried a known adherence factor (987P) detectable with DNA probes. Most of the STaP⁺ and STb⁺ isolates belonged to O groups O138, O139, O141, and O149. Only three of the VT⁺ strains were isolated from pigs with edema disease. We concluded that 73% of the K88⁻ isolates had the capability to produce enterotoxins or VT that could have contributed to weanling pig diarrhea.

Postweaning diarrhea (PWD) in pigs is common. Hemolytic enterotoxigenic *Escherichia coli* has been regarded as a primary etiologic agent of PWD for about 4 decades (19).

Virulence attributes of the *E. coli* associated with PWD are poorly defined and seem to be far more complex than those of neonatal colibacillosis. Some of these isolates produce K88 adhesin (16) and one or more of three enterotoxins, a heat-labile enterotoxin (LT) and two heat-stable enterotoxins (STa and STb). Some produce cytotoxins (VT) detectable by Vero cell assay (6, 8, 15), but the role of VT in diarrhea and its relationship to the VT produced by edema disease strains (4) are unknown. Some enterotoxigenic strains associated with the PWD do not have known adhesion factors (18). The prevalence in Sweden and Japan of PWD enterotoxigenic *E. coli* (ETEC) strains without detectable adhesins was estimated to be 29 to 38% (14, 17).

The objective of the present study was to estimate the prevalence and to identify possible virulence attributes of K88⁻ toxicogenic (enterotoxins or VT) *E. coli* strains in PWD of pigs in Hungary.

**MATERIALS AND METHODS**

*E. coli* strains. *E. coli* isolates from cases of postweaning deaths due to diarrhea or to edema disease were obtained from the small intestines of pigs within 24 h after death at diagnostic institutes in Hungary. Altogether, 205 isolates were obtained from 88 pigs from 59 herds.

**Phenotyping.** Phenotyping included determination of hemolysis and of the O groups. Plasmid antigens K88, K99, K41, and 987P (5, 7, 13) were determined by slide agglutination with absorbed antipili sera produced by the following *E. coli* strains: 263 (O8:K87, K88ab:H19) for antigen K88, B41 (O101:K99, F41) for K99, and F41 and 987 (O9:K103, 987P) for 987P.

The VT tests were performed by the method of Konwalchalk et al. (9). The results on coded samples were read by two persons. All VT tests were repeated with at least two different cell-free supernatants from each isolate and run in four wells each time. When cell rounding was observed, toxin neutralization was attempted with anti-LT rabbit serum produced against *E. coli* 263 (O8:K87, K88ab:H19) LT. Equal amounts of the culture supernatant (VT) and the anti-LT serum were mixed and incubated for 1 h at 37°C and then used for VT assay. Isolates were regarded as VT⁺ when anti-LT serum did not neutralize the cytotoxic activity. *E. coli* 263 (O8:K87, K88ab) was used as the VT⁺ control. *E. coli* 430 (supplied by J. Blanco and E. A. Gonzalez, University Santiago de Compostela, Lugo, Spain) was used as the VT⁻ control (1). *E. coli* 124 (O8:K50:NM) was used as a negative control in the enterotoxin and VT assays.

**Genotyping.** Colony blot hybridizations with gene probes for STaP (baby mouse-positive, porcine type, ST enterotoxin), STaH (baby mouse-positive, human type, ST enterotoxin), STb (baby mouse-negative, pig-positive, ST enterotoxin), and LT enterotoxin and for adherence factors K88, K99, and F41 were done as described earlier (10, 12). The 987P probe was a 360-base-pair *HpaII-BglII* fragment from a recombinant plasmid (pPM200) which expresses 987P (11). This fragment is contained entirely within the 987P subunit gene, and its nucleotide sequence is identical to that reported by deGraaf and Klaasen (3) for this part of the 987P subunit gene (R. A. Schneider and T. A. Casey, unpublished data).

**RESULTS**

Pigs that died because of PWD were 4 to 10 weeks of age. One pig showed signs of edema disease. VT-producing *E. coli* O139:K⁺ was isolated from this pig. All of the other cases were classified as typical weanling diarrhea. Twenty-nine farms were represented by one pig each, and 15 of these farms were represented by one isolate each. An overview of the prevalence of K88⁺ *E. coli* and those isolates that did not have any known adhesin (4P⁻) is presented in Table 1. Only K88⁻ isolates were derived from 23 herds. There were no detectable adhesins in isolates from 26 herds. In the remain-
ing 10 herds, 13 pigs were infected only with K88+; 8 pigs were infected with both K88+ and 4P−, and 8 pigs were infected with 4P− E. coli only. Altogether, nearly two-thirds of the isolates produced K88. The presence of K88 was always associated with hemolysin. Hemolysis was observed with 90% of the 205 isolates.

Combined results of phenotyping and genotyping of 70 4P− isolates are shown in Table 2. These 70 isolates were divided into three categories: STaP and/or STb producer (34 strains); STaP, STb, and/or VT producer (17 strains); and nontoxic (19 strains). One isolate was found to possess the 987P gene.

Data from 14 farms (where at least two pigs and four isolates were tested) indicated that K88+ E. coli dominated on 11 farms and 4P− toxin-producing E. coli was dominant on 3 farms.

As expected, a vast majority (94%) of hemolytic E. coli isolates proved to be enterotoxigenic or cytotoxigenic. However, 4 of 20 nonhemolytic isolates (20%) produced either STaP only (one isolate) or both STaP and STb (three isolates). ETEC or VT+ strains were isolated from 78 of the 88 pigs investigated (88.6%).

**DISCUSSION**

These studies indicate that K88+ E. coli was common (61% of isolates) in cases of fatal PWD. These strains typically produce LT and STb. Furthermore, the vast majority of the K88+ strains that were serogrouped were O149:K+, similar to the findings of Soderlin et al. (17).

**TABLE 2. Toxin characteristics and serogroups of 4P− E. coli isolates**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no. of isolates</th>
<th>STaP, STb, STa, STb, VT, Non-toxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>O8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>O138</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>O139</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>O141</td>
<td>7</td>
<td>4</td>
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<tr>
<td>O147</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>O149</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>O157</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>OX−</td>
<td>17</td>
<td>10</td>
</tr>
</tbody>
</table>

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LITERATURE CITED


Twenty-five percent of the isolates produced toxin (STaP, STb, VT) without any known adhesins, and 9.3% were probably nonpathogenic (no detectable virulence factors).

Our data contrast with those of Svendsen et al. (18), who found that E. coli O149 without K88 was dominant in most pigs with PWD in Denmark. Nakazawa et al. (14) found that only 3% of the PWD strains from pigs in Japan were K88+ and that 23% were K99− (associated with O149). In their studies, it was calculated that ETEC could contribute to diarrhea in about 65% of the piglets with PWD. It should be noted that Nakazawa et al. did not test for STb and that, furthermore, they tested E. coli strains from rectal swabs of pigs with PWD rather than from ileal isolates. In contrast, in our studies using DNA hybridization, 88.6% of the pigs that died because of PWD were infected with ETEC. Interestingly, most of the K88− strains were LT− and possessed genes for both STaP and STb. Most of them belonged to serogroups O141, O147, and O157, indicating that they were not K88− LT− mutants of the predominant K88+ E. coli strains. Most isolates produced multiple toxin types. Only one isolate was STaP only, and three isolates were STb+ only.

A cell-free VT was detected in cultures from 14 (7%) PWD isolates representing five pigs from five farms. Furthermore, three isolates from one pig produced VT cytotoxin and hybridized with the STaP and STb probes. This prevalence of VT-producing E. coli in weanling pigs is similar to that described by Gannon et al. (6).

In this collection, the majority of E. coli strains belonged to the classic porcine hemolytic, K88+ LT− STb+ (O149) type (16, 17). However, 25% of the isolates did not possess known adhesins but did have characteristic (STaP and STb+, or VT+) toxic attributes. It remains to be determined whether these enterotoxin- or VT-producing K88− strains produce disease in weanling pigs, whether they do it by hitherto unknown colonization factors, and how the VT detected here is related to toxin produced by the edema disease strains (2, 4).


