Virulence Properties of Enterotoxigenic Escherichia coli O8:KX105 Strains Isolated from Diarrheic Piglets

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Twenty-two enterotoxigenic Escherichia coli (ETEC) O8:KX105 strains isolated from 1- to 7-week-old diarrheic piglets were examined for virulence properties. Thirteen strains caused acute watery diarrhea in orally infected, colostrum-deprived newborn piglets, whereas the remaining nine did not. The enteropathogenic strains colonized the small intestine, albeit with lower intensity than classical porcine ETEC. They produced the heat-stable STa and heat-labile LT enterotoxins, whereas the nonenteropathogenic strains produced the STb enterotoxin alone. None of the E. coli O8:KX105 strains exhibited mannose-resistant hemagglutination with erythrocytes from 12 species. Ten of the enteropathogenic and two of the nonenteropathogenic strains were positive for mannose-sensitive hemagglutination. These strains produced rodlike fimbriae 3 to 5 nm in diameter, whereas no fimbriae were detected on the other strains. None of the 22 strains produced the fimbrial antigens F4, F5, F41, F2, F3, FY(Att25), and F165. Of the 13 enteropathogenic strains, 10 expressed the F6 antigen in the intestines of infected piglets but not in cultures. The other three enteropathogenic strains apparently lacked all of the known fimbrial antigens from porcine ETEC.

Enterotoxigenic Escherichia coli strains (ETEC) are recognized as an important cause of diarrhea in piglets (21). Porcine ETEC produce one or more of the thermostable STa and STb enterotoxins and the thermodetable LT enterotoxin which cause a net fluid secretion by the intestine, resulting in diarrhea. STa enterotoxin is methanol soluble and causes intestinal secretion in infant mice and in piglets, whereas STb enterotoxin is methanol insoluble and is active in piglets but not in infant mice (13, 24). LT enterotoxin causes intestinal secretion in piglets and induces cytotoxic changes in Y1; CHO and Vero cell cultures (6, 29).

ETEC colonize the small intestine by means of surface components that promote adherence to the intestinal mucosa (5). Four distinct types of fimbriae, designated F4 (K88), F5 (K99), F6 (987P), and F41, have been recognized as important colonizing factors in porcine ETEC (see reference 25 for a discussion of antigen nomenclature) (12, 20, 22, 23). In addition, certain capsular antigens and F1 (type 1) fimbriae may be involved (10, 11, 32). Fimbrial antigens can be differentiated by their pattern of mannose-sensitive hemagglutination (MSHA) or mannose-resistant hemagglutination (MRHA) and by various immunological methods (5, 25).

In addition to producing one or more of the fimbrial antigens F4, F5, F6, and F41, most ETEC associated with porcine neonatal diarrhea belong to a limited number of O and K serogroups (classical ETEC) (2, 5, 19, 31). ETEC apparently lacking these fimbrial antigens and belonging to other serogroups (nonclassical ETEC) are occasionally isolated from diarrheic piglets, but evidence of their pathogenicity has generally not been established (7, 8, 19). One group of such nonclassical ETEC, E. coli O8:KX105 (previously K°104242), first described in The Netherlands, is being isolated more and more frequently from diarrheic piglets in Quebec (7, 8). The objective of the present study was therefore to investigate the pathogenicity and virulence properties of E. coli O8:KX105 strains isolated in Quebec.

MATERIALS AND METHODS

Bacterial strains. Twenty-two strains belonging to the E. coli O8:KX105 serogroup were examined. One strain obtained from P. A. M. Guénette was originally isolated in The Netherlands, whereas the remainder originated in Quebec. The latter were isolated from the intestinal contents of diarrheic piglets aged from 1 to 7 weeks, and each isolate originated from a different farm. They were collected from 1979 to 1986 and were stored at −70°C in tryptic soy broth (TSB) (Difco Laboratories, Detroit, Mich.) or freeze-dried until investigated. Isolates were serotyped by the serum agglutination test (SAT) (4). Strains used for the production of antisera or toxin assays are listed in Table 1.

Antiserum. Anti-O8:KX105 serum was produced in rabbits with strain K442 (Table 1) by standard procedures (4). Antisera against the fimbrial antigens F4, F5, F6, F41, F165, FY(Att25), F2 (colonization factor antigen 1) and F3 (colonization factor antigen II) were produced in rabbits with strains K-12(F4), K-12(F5), 603A, B41M, 4787d, 25KH9, H10407, and PB176, respectively (Table 1). To promote the expression of fimbrial antigens, we grew strains K-12(F4) and 603A at 37°C overnight on blood agar base (Difco) plus 5% calf blood and strains K12(F5), B41M, 4787d, 25KH9, H10407, and PB176 at 37°C overnight on Minca agar plus 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) (Minca-Is agar) (4, 5, 26). Immunization was carried out as recommended for the preparation of OK antisera (4). Antisera were exhaustively absorbed as follows: anti-K-12(F4) and -K-12(F5) sera were absorbed with the parent K-12 strain grown at 37°C in tryptic soy agar (Difco) plus 0.1% glucose; anti-603A, -B41M, -4787d, -25KH9, -H10407, and -PB176 sera were absorbed with the homologous strains grown at 15°C on tryptic soy agar plus 0.1% glucose (4). In addition, rabbit antisera raised against purified F6 fimbriae was used. Immunizations with F6 fimbriae were carried out as described previously (4).

Purification of F6 fimbriae. F6 fimbriae were purified from strain 603A by a modification of the method described by...
Korhonen et al. (15). Bacteria were grown in TSB in a microfermentor (New Brunswick Scientific Co., Inc., Edison, N.J.), collected by centrifugation, and suspended in 50 mM Tris hydrochloride (Sigma Chemical Co., St. Louis, Mo.). The suspension was sheared in an Omnimixer (Ivan Sorvall Inc., Norwalk, Conn.) at half speed for 5 min and centrifuged. The supernatant was treated with ammonium sulfate (Sigma) at 25% saturation overnight. The precipitate was recovered by centrifugation, suspended in Tris buffer, and exhaustively dialyzed. After dialysis, sodium deoxycholate (Fisher Scientific Co., Pittsburgh, Pa.) was added to the suspension to a final concentration of 0.5% (wt/vol), and the suspension was dialyzed against Tris buffer-0.3% sodium deoxycholate (wt/vol) for 72 h. The sodium deoxycholate-insoluble material was removed by centrifugation, and the supernatant was applied to a Superoxide-12 gel filtration column (FPLC system; Pharmacia, Uppsala, Sweden) equilibrated in 50 mM phosphate-0.15 M NaCl. The fractions eluting in the voided volume were tested for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and for the presence of fimbriae by electron microscopy. Samples were denaturated with HCl (pH 2) before being boiled in Tris buffer-2% sodium dodecyl sulfate-5% ß-mercaptoethanol, and the gels were stained with Coomassie brilliant blue R250 (Pharmacia). Only one peptide band with a molecular weight of approximately 22,000 was observed.

Serological tests. The serological detection of fimbrial antigens F2, F3, F4, F5, F6, F41, F165, and Fy(Att25) was performed in cultures by using the SAT and the indirect fluorescent-antibody test (IFAT) as described previously (4). Strains were grown under culture conditions promoting the expression of the respective antigens as described above, with the exception of the F6 antigen. For this antigen, strains were grown in TSB incubated statically at 37°C for several days, with three subcultures. The SAT and IFAT were performed on cells recovered after a final overnight culturing at 37°C in TSB and on blood agar base plus 5% calf blood (19).

Hemagglutination tests. Hemagglutination tests were performed by the rocked-tile method as described previously (4). To demonstrate MSHA, we used guinea pig, horse, and chicken erythrocytes and grew bacteria serially in TSB incubated statically at 37°C for 48 to 72 h, with up to five subcultures. To demonstrate MRHA, we used guinea pig, mouse, rabbit, chicken, dog, cat, sheep, goat, calf, horse, pig, and human (group O) erythrocytes. Bacteria were grown on Minca-Is agar and in Minca broth plus 1% IsoVitalex (Minca-Is broth) incubated overnight at 37°C. MRHA tests were also performed with erythrocytes pretreated with 0.003% (wt/vol) tannic acid (J. T. Baker Chemical Co., Phillipsburg, N. J.) for 10 min at 37°C and for 30 min at room temperature.

TEM. The production of fimbriae in cultures was determined by using negative-stain transmission electron microscopy (TEM) as described previously (4). Bacterial cells were grown on Minca-Is agar and in TSB at 37°C overnight, harvested, and examined after negative staining with 2% phosphotungstic acid (pH 7.0) by using a Philips 201 TEM operated at 60 kV.

Detection of entero-toxins. Strains were tested for enterotoxicty by using the gut loop technique in 5- to 6-week-old piglets as described previously (17). The infant mouse assay was used for the detection of STa enterotoxin as previously described (27). The production of STb enterotoxin was determined by using the gut loop test in weaned piglets (13). For the latter test, strains were grown under agitation (200 rpm) in TSB at 37°C for 18 h. Bacterial cells were removed by centrifugation (10,000 g for 20 min), and culture supernatants were filtered (0.45-µm pores) and heated for 30 min at 65°C. Heated filtrates were stored at 4°C and tested within 24 h. Weaned piglets (5 to 6 weeks old) were deprived of feed but were allowed free access to water overnight before surgery. After halothane anesthesia, about 20 ligated intestinal segments (8 to 10 cm) were formed in each piglet beginning about 75 cm distal to the pylorus. Each bacterial strain was tested in various areas of the jejunum in at least three piglets by inoculating an 8-ml volume of culture filtrate into each loop. Filtrates from strains M432 and P16CG were used as negative and positive controls, respectively (Table 1). Piglets were euthanized 5 to 6 h after inoculation, and the

### TABLE 1. Relevant characteristics of *E. coli* strains used for antiserum production and toxin assays

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Fimbrial type</th>
<th>Toxin</th>
<th>Origin</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>K442</td>
<td>O:8;KX105&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>Pig</td>
<td>P. A. M. Guinée&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>K-12(F4)</td>
<td>O-;F4</td>
<td>F4</td>
<td>ND</td>
<td>Laboratory</td>
<td>C. L. Gyles&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>K-12(F5)</td>
<td>O-;F5</td>
<td>F5</td>
<td>ND</td>
<td>Laboratory</td>
<td>C. L. Gyles&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>B41M</td>
<td>O101;K-;F41</td>
<td>F41</td>
<td>STa&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Calf</td>
<td>J. A. Morris&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>603A</td>
<td>O9;K-;F1-;F6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>F6</td>
<td>ND</td>
<td>Pig</td>
<td>S. Larivièrè&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>4787d</td>
<td>O115;K&quot;V165&quot;;F165</td>
<td>F165</td>
<td>None</td>
<td>Pig</td>
<td>J. M. Fairbrother&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>25KH9</td>
<td>O101;K?;F4(Att25)</td>
<td>FY(Att25)</td>
<td>ND</td>
<td>Calf</td>
<td>P. Pohl&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>H10407</td>
<td>O78;H11;F2</td>
<td>F2</td>
<td>ND</td>
<td>Human</td>
<td>D. G. Evans&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PB176</td>
<td>O6;H15;F3</td>
<td>F3</td>
<td>ND</td>
<td>Human</td>
<td>D. G. Evans&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>K-12</td>
<td>O-</td>
<td>ND</td>
<td>ND</td>
<td>Laboratory</td>
<td>C. L. Gyles&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>P16CG</td>
<td>O9;K103</td>
<td>ND</td>
<td>STb&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Pig</td>
<td>C. L. Gyles&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>M432</td>
<td>O139;K82</td>
<td>ND</td>
<td>None</td>
<td>Pig</td>
<td>S. Larivièrè&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>TD427C2</td>
<td>O25;H&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>LT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Human</td>
<td>W. M. Johnson&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>H30</td>
<td>O26;H11</td>
<td>ND</td>
<td>VT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Human</td>
<td>W. M. Johnson&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>86-6136</td>
<td>O128;H&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>CLDT&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Human</td>
<td>W. M. Johnson&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Serotyping was confirmed by the International Escherichia and Klebsiella Centre, Copenhagen, Denmark.

<sup>b</sup> ND, Not determined.

<sup>c</sup> National Institute for Public Health, Bilthoven, The Netherlands.

<sup>d</sup> Central Veterinary Laboratory, United Kingdom.

<sup>e</sup> University of Montreal, Saint-Hyacinthe, Quebec, Canada.

<sup>f</sup> National Institute for Veterinary Research, Brussels, Belgium.

<sup>g</sup> University of Texas, Houston, Tex.

<sup>h</sup> Laboratory Centre for Disease Control, Tunney’s Pasture, Ottawa, Ontario, Canada.

<sup>i</sup> Institut Pasteur, Paris, France.
length and volume of the contents of each segment were recorded. A volume/length ratio greater than the mean value of the negative control strain plus two standard deviations was considered positive. Strains producing at least one positive loop were considered STb producers. LT enterotoxin was detected by using the culture assay with CHO, Y1, and Vero cells (6). Bacteria were grown as stationary cultures in Evans toxin medium at 37°C for 48 h (3). Sterile filtrates were prepared by passage of culture supernatants through 0.22-µm-pore cellulose acetate filters (Gelman Sciences, Inc., Ann Arbor, Mich.). Filtrates were stored at 4°C and tested for toxins within 2 days. Reference strain E. coli TD427C2 was used as a positive control (Table 1). Neutralization studies were performed by using the culture assay with burro antitoxin (CT) antitoxin obtained from J. B. Robbins, Bureau of Biologics, Bethesda, Md. (9). Toxin-antitoxin mixtures were preincubated at 37°C for 1 h prior to the assay for residual toxin activity. In addition, LT enterotoxin activity was confirmed by the gut loop test in 5- to 6-week-old piglets with heated and unheated whole-cell lysates as described previously (16).

Detection of cytotoxins. Assays were performed as previously described (11a, 14). Cultures were grown in Evans toxin medium with shaking at 37°C for 24 h, and sterile filtrates were prepared and stored as described above. Reference strains E. coli H30 and 86-6136 were used as positive controls for Verotoxin (VT) and cytotoxins (CLDT), respectively (Table 1). Vero and CHO monolayers were grown in 96-well plates at 200 µl per well. Aliquots (20 µl) of bacterial filtrates were added to duplicate wells without changing the medium. Plates were incubated at 37°C in a humidified 5% CO₂ atmosphere and examined daily for up to 4 days for cytotoxicity.

Experimental infection of piglets. Strains were tested for enteropathogenicity by experimental infection of colostrum-deprived newborn piglets (19). Piglets either were collected aseptically at birth from naturally farrowed litters or were hysterectomy derived. When 3 to 6 h old, they were given 1 ml of an overnight TSB culture of E. coli (8 to 9 log_{10} CFU/ml) in 20 ml of 0.1% peptone water intragastrically via a syringe-tubing catheter inserted at the time of birth. The catheter was returned to at least two piglets. Piglets were kept in plastic isolators under heat lamps at 30 to 35°C without feeding. At 16 to 24 h postinoculation, they were examined for diarrhea, euthanized, and necropsied immediately. Segments of mid-jejunum and terminal ileum were subjected to bacteriological examination, histopathology, immunofluorescence microscopy, and scanning electron microscopy (SEM).

Bacteriological examination. Intestinal segments (10 cm) were opened, placed in 0.1% peptone water, and homogenized in an Omnimixer (Ivan Sorvall) at half speed for 2 min. Ten-fold serial dilutions were made in phosphate-buffered saline (pH 7.4), inoculated onto tryptic soy agar plus 0.1% glucose, and incubated overnight at 37°C. Colonies were counted and identified on the basis of their morphology. Their identities were confirmed by slide agglutination of at least five colonies per sample in OK antisera (19).

Histopathology. Intestinal segments were fixed in 10% Formalin in phosphate-buffered saline (pH 7.4) and processed for paraffin tissue sectioning by standard techniques. Sections were stained with hematoxylin, phloxine, and safranine (21).

Immunofluorescence microscopy. The association of bacteria with the intestinal mucosa and the production of fimbrial antigens F4, F5, F6, and F41 in vivo were evaluated by using the IFAT (19, 22). Intestinal segments were embedded in O.C.T. (Tissue Tek, Miles Laboratories, Inc., Elkhart, Ind.) and frozen at −20°C. Sections 6 µm thick were cut in a cryostat microtome, mounted on glass slides, fixed in methanol for 1 min, and air dried. Slides were incubated with appropriate dilutions of rabbit anti-O:8:KX105, F4, F5, F6, or F41 serum for 20 min in a moist chamber at 37°C, washed in phosphate-buffered saline and distilled water, and incubated with appropriately diluted fluorescein-labeled goat anti-rabbit immunoglobulin G (Miles) in a moist chamber at 37°C for 20 min. After being washed as described above, they were examined with a Labolux 12 fluorescence microscope (Leitz, Wetzlar, Federal Republic of Germany) with epi-illumination. The association of bacteria with the intestinal mucosa was evaluated by using an association index of 0 to 5 as previously described (23).

SEM. Intestinal segments were fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0) at 4°C overnight. Tissues were dehydrated through graded ethanol, critical point dried in CO₂, mounted on aluminum stubs, sputter coated with gold-palladium, and examined with a Hitachi S-530 SEM at an accelerating voltage of 20 kV.

RESULTS
Pathogenicity of O8:KX105 strains in newborn piglets. Acute watery diarrhea with dehydration developed 16 to 24 h postinoculation in piglets infected with 13 of the 22 E. coli O8:KX105 strains (Table 2). Colonization of the small intestine by the infecting strain was observed in all piglets with diarrhea, with mean counts of about 8 and 7 log_{10} CFU/10 cm in the ileum and jejunum, respectively (Table 2). However, microscopic examination of IFAT-stained intestinal sections revealed only occasional packets of bacteria associated with the tips of the intestinal villi or randomly distributed in the intestinal lumen (mean association index, 1.1) (Fig. 1 and Table 2). Similarly, only a few bacteria adherent to the intestinal mucosa were observed upon examination of histopathological sections or by SEM (Fig. 2). On the other hand, piglets infected with the other nine strains remained clinically healthy until 24 h postinfection. No intestinal colonization was observed in any of these piglets, for which intestinal bacterial counts were <6 log_{10} CFU/10 cm of jejunum and ileum.

Hemagglutination reactions. Of the 22 strains, 12 were MSHA positive with guinea pig, chicken, horse, and dog erythrocytes. These strains also reacted with an antiserum raised against F1 fimbriae purified from a porcine O115 strain (data not shown). Of the 12 F1-positive strains, 10 produced diarrhea in newborn infected piglets, whereas only 3 of the 10 F1-negative strains were enteropathogenic (Table 3). All of the strains were MRHA negative with native and tanned erythrocytes from the human and 11 animal species, both when grown on Minca-Is agar and in Minca-Is broth at 37°C.

Presence of fimbriae in vitro. Examination of the strains by negative-stain TEM revealed roddlike fimbriae (3 to 5 nm in

<p>| TABLE 2. Relationship of intestinal colonization and association index to clinical status in piglets experimentally infected with E. coli O8:KX105 strains |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>No. of strains</th>
<th>No. of piglets</th>
<th>Colonization of:</th>
<th>Association index for ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>13</td>
<td>30</td>
<td>7.0 ± 1.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>No diarrhea</td>
<td>9</td>
<td>18</td>
<td>&lt;6</td>
<td>0</td>
</tr>
</tbody>
</table>

*Log_{10} mean CFU of E. coli per 10 cm of intestine ± standard deviation.  
Mean association index ± standard deviation.
associated and stained by moderately viable strains. In the intestinal lumen, bacteria were observed on the tips of the intestinal villi or randomly distributed in the intestinal lumen.

diameter) on bacteria from the 12 MSHA-positive F1-positive strains. These fimbriae were particularly abundant after several passages in TSB at 37°C but sparse after growth on Minca-Is agar at 37°C. No fimbriae were observed on bacteria from the other 10 strains under the culture conditions used. All of the 22 E. coli O8:KX105 strains were negative for fimbrial antigens F4, F5, F6, F41, F165, F2, F3, and FY(Att25) when cultures were tested by the SAT and IFAT.

**Presence of fimbriae in vivo.** Bacteria present in intestinal sections from infected piglets were negative for F4, F5, and F41 upon examination by the IFAT. However, coccobacilli reacting with F6 antiserum were observed in ileal sections from piglets infected with 10 of the 13 enteropathogenic strains (Fig. 3). When examined by the SAT and IFAT immediately after reisolation from experimentally infected piglets, all of these strains were F6 negative. All of the in vivo F6-positive strains were F1 positive in vitro (Table 3).

**Toxin reactions.** All of the 22 strains were found to be enterotoxigenic in pig intestinal loops when tested as live cultures. None of these strains produced detectable STa enterotoxin, VT, or CLDT. All were considered to produce STb enterotoxin, as their heated culture filtrates caused fluid secretion in pig intestinal loops. Poor repeatability of the gut loop test for STb was observed. Secretory responses varied considerably between different piglets, and some animals did not respond at all. Thirteen of the strains produced LT enterotoxin that was neutralizable with CT antitoxin. Neutralization of the cytopathic effect caused by LT enterotoxin

**TABLE 3. Relationship of enterotoxin, F1, and F6 production to the ability of E. coli O8:KX105 strains to cause diarrhea in piglets**

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>STb+ LT-</th>
<th>STb- LT+</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+ F6-</td>
<td>F1- F6+</td>
<td>F1- F6-</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No diarrhea</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

* +, Positive; −, negative. F1 production was determined in vitro by MSHA and IFAT. F6 production was determined in vivo by IFAT.

FIG. 1. Ileum section from a piglet experimentally infected with a moderately colonizing enteropathogenic E. coli O8:KX105 strain and stained by IFAT with OK antiserum. Packets of bacteria were associated with the tips of the intestinal villi or randomly distributed in the intestinal lumen.

FIG. 2. Scanning electronmicrograph of the ileal mucosa from a piglet experimentally infected with a moderately colonizing enteropathogenic E. coli O8:KX105 strain. Only a few bacteria are associated with the tips of the intestinal villi. Bar, 10 μm.

FIG. 3. Ileal smear from a piglet experimentally infected with a moderately colonizing enteropathogenic E. coli O8:KX105 strain and stained by IFAT with F6 antiserum. Fluorescent coccobacilli can be seen.
with CT antitoxin indicated that it belongs to the LT-1 class of LT enterotoxins. All 13 LT-positive, STb-positive strains were enteropathogenic, whereas none of the 10 LT-negative, STb-positive strains caused diarrhea in newborn piglets (Table 3).

**DISCUSSION**

Our studies showed that certain *E. coli* 08:KX105 strains moderately colonized the small intestine and produced diarrhea in newborn piglets. Although this colonization was of a lower intensity than that observed with classical ETEC producing the F4, F5, F6, or F41 antigen, which usually have ileal counts of >9 log_{10} CFU/10 cm (19, 20, 23), it was nevertheless of a significantly higher intensity than that observed with the nonenteropathogenic 08:KX105 strains. However, little evidence for bacterial adherence to the intestinal villi was found in piglets with diarrhea when intestinal sections were examined histologically or by immunofluorescence microscopy and SEM. These results suggest that the enteropathogenic *E. coli* O8:KX105 strains either lack the ability to strongly adhere to the intestinal mucosa, possibly binding only to mucus, or are unable to multiply and survive in the intestinal environment (1, 33). Thus, we have demonstrated that certain ETEC may cause acute diarrhea in piglets even in the absence of extensive colonization of the small intestine (19, 30).

Although the enteropathogenic *E. coli* O8:KX105 strains colonized the ileum of infected piglets, when examined under the culture conditions used they produced none of the fimbrial colonization factors commonly associated with porcine ETEC. However, the positive F6 reaction observed in IFAT-stained intestinal sections suggested that the majority of the enteropathogenic strains produced F6, but only in the environment of the intestine. This idea is not surprising, as previous studies have shown that many F6-positive strains produce the F6 fimbrial antigen in the pig intestine but rapidly shift to an F6-negative phase after being cultured (19, 23; J. M. Fairbrother and S. Larivièr, submitted for publication). A more surprising finding was that the F6-positive O8:KX105 strains did not colonize the small intestine to the same extent as the F6-positive classical strains (10, 19, 23). Furthermore, strains are usually isolated from piglets less than 3 week old and produce STa enterotoxin (19). On the other hand, the F6-positive O8:KX105 strains in this study originated from weaned as well as suckling piglets and produced STb and LT enterotoxins but not STa enterotoxin. It is possible that F6 produced by such strains is modified, reducing its ability to adhere to intestinal villi in vivo.

An association among the production of F1 fimbriae, colonization, and enteropathogenicity in the *E. coli* O8:KX105 strains was noted. Although it has been reported that F1 fimbriae promote intestinal colonization by certain porcine ETEC, indisputable evidence of their role during intestinal infection is still lacking, and their role in the pathogenicity of the O8:KX105 strains should be investigated further (11, 32). Finally, we were unable to demonstrate any of the known fimbrial colonization factors of porcine ETEC in 3 of the 13 colonizing enteropathogenic strains.

There was also a high positive association between the production of LT enterotoxin and enteropathogenicity in the *E. coli* O8:KX105 strains. This correlation is noteworthy, as the production of LT enterotoxin is rarely observed in strains lacking the F4 antigen or producing the F6 antigen (8, 19, 24, 31). However, F4-negative, LT-positive O8 strains have been reported on several occasions (8, 17, 18). Our results suggest that LT enterotoxin may be an important virulence factor for the *E. coli* O8:KX105 strains. On the other hand, it is possible that the production of this toxin is genetically linked to that of another important virulence factor, such as a colonization factor. Indeed, only the LT-positive O8:KX105 strains were able to colonize the small intestines of infected piglets. Moreover, genes coding for such enterotoxins and colonizing factors as LT enterotoxin and colonization factor antigen II are genetically linked in certain human ETEC (28).

Thus, our study has shown that certain *E. coli* O8:KX105 strains produce LT and STb enterotoxins, colonize the small intestine to a moderate extent, do not strongly adhere to the intestinal epithelium, but nevertheless induce diarrhea in neonatal piglets. The majority of these strains produce the colonization factor F6, but only in the intestines of infected piglets. Three F6-negative enteropathogenic strains, however, apparently lack all of the known fimbrial colonization factors of porcine ETEC.

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**LITERATURE CITED**


