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Edema Disease Caused by a Clone of *Escherichia coli* O147

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Edema disease is a systemic disease of weaned pigs caused by host-adapted strains of *Escherichia coli*, most commonly belonging to serogroup O138, O139, or O141. In the late 1990s, *E. coli* O147 strains containing the virulence genes *f18, sta, stb,* and *stx2* were recovered from outbreaks of edema disease in the United States. Pulsed-field gel electrophoresis (PFGE) was used to determine that the majority of these strains (34/43) were closely related to one another. Subsequent analysis by multilocus restriction typing confirmed the PFGE results and indicated that the cluster of edema disease strains were only distantly related to other *E. coli* O147 strains. Serogrouping of edema disease isolates from the Iowa State University Veterinary Diagnostic laboratory recovered between 1996 and 2000 indicated that 42% belonged to serogroup O147. Our data suggest that these strains may be a common serotype of edema disease-causing *E. coli* in the United States.

Edema disease is a systemic disease of weaned piglets caused by host-adapted strains of *Escherichia coli* that produce a variant of Shiga toxin 2, Stx2e. This toxin is closely related to Stx2, produced by human isolates of *E. coli* capable of causing hemolytic uremic syndrome (10). Clinical signs of edema disease include swollen eyelids, neurological symptoms (such as ataxia and stumbling), recumbency, and/or sudden death (2). These *E. coli* strains colonize the ileum via host-specific F18 fimbrae. Many edema disease isolates also produce heat-stable toxins that cause diarrhea (7, 11). Historically, edema disease has been caused by *E. coli* strains belonging to serogroups O138, O139, and O141 (2, 7, 8). Evidence suggests that these *E. coli* strains can be transmitted from both the sow and the environment to the piglets. Once this organism is in the pig’s environment, it is difficult to eliminate and can be carried to other areas on the farm or off the farm to other swine herds.

Since the mid-1990s, there have been several reports of large outbreaks of severe edema disease in the Midwest and North Carolina. Many of the *E. coli* strains recovered from these farms had identical virotypes (*f18 sta stb stx2*) and belonged to serogroup O147. This is not a serogroup of *E. coli* that is commonly associated with edema disease in the United States, although it has been described in Europe (12). In the United States, serogroup O147 *E. coli* strains are usually isolated from piglets with postweaning diarrhea and are considered an enterotoxigenic pathotype of *E. coli* (ETEC). Such strains typically produce F18 or F4 fimbriae and heat-stable and/or heat-labile enterotoxins, but not Stx2e (6). Postweaning diarrhea affects pigs in the same age group as those affected by edema disease. The reported high severity of the clinical symptoms in the edema disease outbreaks, the unusual serogroup (O147), and the identical virotypes of these isolates from several surrounding states (Iowa, Minnesota, Nebraska, and Oklahoma) suggested that the strains may be clonal.

The purpose of this study was to determine if the serogroup O147 edema disease isolates (i) were closely related to one another and (ii) could have evolved from ETEC strains belonging to the same serogroup by acquiring the *stx2e* gene. In addition, we determined the prevalence of the O147 serogroup among edema disease-causing isolates in Iowa in recent years. (Preliminary reports of this work were presented at the 104th General Meeting of the American Society for Microbiology, 2004, and the Conference on Gastrointestinal Function, Chicago, IL, 2003.)

**MATERIALS AND METHODS**

**Bacterial strains.** Forty-three *E. coli* O147 strains isolated between 1996 and 2001 from edema disease cases in Iowa (10 strains), Minnesota (2 strains), Nebraska (1 strain), Oklahoma (1 strain), and North Carolina (29 strains) were included in this study. This set of strains (strain set 1) comprises the initial group of isolates that were suspected of belonging to the same clone. All of the isolates in this study were independent, with a single isolate from one pig on a farm experiencing edema disease included. Isolates from subsequent cases of edema disease from the same farm recovered a year or more after the first isolate was collected were also included, as were isolates from the same farm with different virotypes. Additional *E. coli* O147 strains that were not associated with current edema disease outbreaks were obtained from the National Animal Disease Center (6 strains) and from the *E. coli* Reference Center at Penn State University (11 strains). These additional strains were isolated prior to 1996 and included four F18* ETEC strains, eight F4* ETEC strains, one F18* Shiga toxin-producing *E. coli* (STEC) strain, and four strains lacking virulence genes.

To determine the prevalence of serogroup O147 among recent edema disease isolates, 55 strains of *E. coli* recovered from edema disease outbreaks between 1996 and 2000 were obtained from the Iowa State University (ISU) Veterinary Diagnostic Laboratory (strain set 2). To ensure that only isolates with the potential to cause edema disease were examined, only porcine isolates positive for the *stx2e* gene were included.

**Virulence genes and serogrouping.** A multiplex PCR to detect genes for F18 (F107), F4, F4 (K88), F5 (K99), and F6 (987P) fimbriae; heat-stable enterotoxins a and b (STa, STb), heat-labile enterotoxin, and Stx2 was performed on all of the isolates (4). The Stx2 primers also detected the edema disease variant, Stx2e. Strains were serogrouped using standard methods to determine if they

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belonged to serogroups commonly associated with edema disease (O138, O139, and O141) or to serogroup O147 (3). Typing for the H antigen was performed on representative strains by the E. coli Reference Center at Penn State University.

**PFGE.** All of the serogroup O147 isolates recovered from outbreaks of edema disease between 1996 and 2001 (strain set 1) were analyzed by pulsed-field gel electrophoresis (PFGE) using standard methods (14). Briefly, the strains were grown overnight in tryptic soy broth, washed, and embedded in agarose plugs. The plugs were treated with lysozyme (1 mg/ml) in a lysis buffer with 10 mM Tris (pH 7.5), 50 mM NaCl, 100 mM EDTA (pH 8.0), 0.2% sodium deoxycholate, and 0.5% (wt/vol) N-lauroylsarcosine for 2 h at 37°C and then with proteinase K (0.5 mg/ml) in a buffer with 0.5 mM EDTA (pH 8.0) and 1% (wt/vol) N-lauroylsarcosine overnight at 55°C. The plugs were washed with 1 mM phenylmethylsulfonyl fluoride and 10 mM Tris-1 mM EDTA buffer (pH 8.0) and then digested with 5 U of XbaI restriction enzyme overnight at 37°C. The DNA was separated using a 1% agarose gel on a CHEF-DR II system (Bio-Rad, Hercules, CA) and the DNA bands were visualized by staining the gel with ethidium bromide.

**DNA amplification.** All of the seven housekeeping genes were selected for the additional strains isolated prior to 1996) were analyzed using multilocus restriction typing (MLRT) (5). The seven housekeeping genes were selected for previous work by Reid et al. (13). Primers were designed using DNAMAN software (Lynnon Corp., Quebec, Canada) and were selected from the published sequence of the E. coli K12 sequence.

**RESULTS.**

Characterization of E. coli O147 edema disease isolates. The majority of the isolates in strain set 1 (41/43) had a virotype of f18 sta stb stx2 (Table 2). The two remaining isolates carried virulence gene combinations of f18 sbt stx2 and f18 stx2, respectively. PFGE analysis indicated that the majority of the isolates in strain set 1 (26/43) shared the same banding pattern (Fig. 1), and this was designated the major banding pattern (outbreak strain). Of the remaining edema disease isolates in strain set 1, 8/43 differed from the major banding pattern by one genetic event (a one- to two-band difference), suggesting that these isolates were closely related to the outbreak strain. Another six isolates differed by two genetic events (a three- to five-band difference) and were possibly related to the outbreak strain. The banding patterns of 3/43 isolates from strain set 1 differed by more than five bands from the outbreak strain, and these isolates were unlikely to be related.

**MLRT analysis of E. coli O147 strains.** A dendrogram constructed from the MLRT analysis of all of the E. coli O147 strains is shown in Fig. 2. The 60 isolates, including all of the isolates in strain set 1, as well as the additional O147 E. coli isolates Pathotype

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### TABLE 1. E. coli housekeeping genes analyzed by MLRT

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amplified base pair</th>
<th>Product</th>
<th>Primers</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspC</td>
<td>1076</td>
<td>Aspartate amino transferase</td>
<td>F'---AAA TTA ACC TCG GGA TTG GTG</td>
<td>HhaI</td>
</tr>
<tr>
<td>clpX</td>
<td>1213</td>
<td>ATP binding subunit of Clp protease</td>
<td>R'---AGC CAT GTT ATC TGG TGT CAT C</td>
<td>HhaI</td>
</tr>
<tr>
<td>fadD</td>
<td>1176</td>
<td>Acyl-coenzyme A synthetase</td>
<td>F'---ACG TAA TTC TGA CCC GTA TGG</td>
<td>HpaII</td>
</tr>
<tr>
<td>icdA</td>
<td>1166</td>
<td>Isocitrate dehydrogenase</td>
<td>R'---TGC GTA ACT CAT CAC GAA ACT C</td>
<td>HpaII</td>
</tr>
<tr>
<td>lysP</td>
<td>1413</td>
<td>Lysine-specific permease</td>
<td>F'---ACA AGG CAA GAA GAT CAC CC</td>
<td>HpaII</td>
</tr>
<tr>
<td>mdh</td>
<td>917</td>
<td>Malate dehydrogenase</td>
<td>R'---CAT CAG ACG CTC GAA GTC ATA G</td>
<td>HpaII</td>
</tr>
<tr>
<td>uidA</td>
<td>1455</td>
<td>β-Glucuronidase</td>
<td>F'---GGG TTA AAG GCG GCT CAC C</td>
<td>HpaII</td>
</tr>
</tbody>
</table>

---

### TABLE 2. Virulence genes detected in E. coli O147 strains analyzed by MLRT

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>Pathotype</th>
<th>f18</th>
<th>f4</th>
<th>lt</th>
<th>sta</th>
<th>sb</th>
<th>stx2</th>
<th>MLRT dendrogram clade</th>
</tr>
</thead>
<tbody>
<tr>
<td>41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>STEC/ETEC</td>
<td>+&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A, D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>STEC/ETEC</td>
<td>+&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>STEC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>STEC/ETEC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ETEx</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ETEx</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ETEx</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ETEx</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>C</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>B, D</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The letters in this column reflect which clades the strains belong to in the MLRT dendrogram (Fig. 1).

<sup>b</sup> Isolate belonging to strain set 1.

<sup>c</sup> Gene present.

<sup>d</sup> None, no virulence genes detected.
isolates, fell into four distinct clades. The majority of the isolates (42/60) clustered together in clade D, and 41/42 of these were isolates from strain set 1 (STEC) recovered from outbreaks of edema disease; 40 of these were related by PFGE analysis. Three representative strains were H typed, and all belonged to the H17 flagellin type. The strain that was unrelated based on PFGE analysis belonged to the H19 flagellin type. The lone isolate within clade D that was not associated with strain set 1 was a strain from the Penn State University collection. This strain did not have any of the virulence genes associated with the outbreak strains. Two of the recent edema disease isolates included in strain set 1 did not cluster within clade D but instead clustered tightly with the clade of F18 ETEC isolates (clade A). In addition, these isolates differed from the major PFGE banding pattern by >5 bands. Both of these strains were identified as H14 flagellin type. Clade A also contained an additional edema disease isolate that was not associated with the recent outbreaks but was from the NADC culture collection. Clade B contained the other three isolates that did not have virulence genes and that were isolated from birds, cattle, or soil. The F4* ETEC strains clustered tightly together in clade C. The recent edema disease strains in clade D were more closely related to E. coli K12 (93%) than they were to either of the other two clades containing the ETEC strains (88%).

Prevalence of serogroup O147 among edema disease isolates. Fifty-five isolates (strain set 2) submitted to the ISU Diagnostic Laboratory from 1996 to 2000 were serotyped to determine the prevalence of serogroup O147 compared to the other serogroups commonly associated with edema disease in the United States (Table 3). The most common serogroup recovered from this collection was O147 (42%), followed by O141 (18%), O138 (13%), and O139 (9%). Regardless of the serogroup, the most abundant virotype was f18 sta stb stx2 (24/55 strains).

DISCUSSION

Based on PFGE and MLRT analyses, our data clearly indicate that 93% of the O147 edema disease isolates are closely

<table>
<thead>
<tr>
<th>Virotype</th>
<th>No. of strains positive in serogroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O138</td>
</tr>
<tr>
<td>f18 sta stb stx2</td>
<td>5</td>
</tr>
<tr>
<td>f18 stb stx2</td>
<td>1</td>
</tr>
<tr>
<td>sta stb stx2</td>
<td>0</td>
</tr>
<tr>
<td>sta stx2</td>
<td>0</td>
</tr>
<tr>
<td>stb stx2</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>7</td>
</tr>
</tbody>
</table>

* NT, nontypeable strains. These isolates either autoagglutinated with O138, O139, O141, or O147 sera or did not agglutinate with any of the sera.

FIG. 3. Dendrogram based on MLRT banding patterns. The numbers in parentheses indicate the number of strains in each clade. The scale at the top indicates the percent relatedness between the strains.
related. To our knowledge, this is the first documentation of *Escherichia coli* O147 strains being associated with edema disease in the United States. Although the interpretation of PFGE banding patterns is somewhat arbitrary and subjective, Tenover et al. proposed that isolates with ≤3 band differences (i.e., one or two random genetic events) can be considered closely related (15). Using this criterion, 34 of the recent edema disease isolates would be considered closely related and 6 additional strains would be considered possibly related. These guidelines were designed to be used when isolates are collected over a short period of time to minimize the number of random genetic events that can occur with numerous cycles of replication. Since our strains were collected over a 5-year period, the conservation of the PFGE banding patterns and virulence genes is further evidence that these strains are likely to be closely related.

MLRT analysis of *E. coli* housekeeping genes supported our PFGE data. MLRT is useful to determine more distant relationships between strains than PFGE because conserved housekeeping genes are analyzed (5). Since serogroup O147 is often associated with porcine ETEC strains, we wanted to determine if the strains recovered from edema disease outbreaks could have evolved from the F18* streptococcus ETEC strains by acquiring the stx2* gene. The majority of the O147 edema disease strains (41/43) clustered together in the dendrogram (Fig. 2, clade D). These included one isolate that was predicted by PFGE analysis to be unrelated to the majority of the edema disease strains. The remaining strains tended to cluster together based on pathotypes represented by F18* ETEC, F4* ETEC, and nonpathogens. Although the majority of the edema disease strains were not related to the F18* ETEC strains, two isolates did cluster within this clade (clade A). These same two strains were predicted by PFGE to be unrelated to the outbreak strain and belong to a different H type than the majority of edema disease strains. The edema disease outbreak strains clustered closer to *E. coli* strain K12 than to any of the other O147 clades. This suggests that this clone arose from strains more similar to *E. coli* K12 than to any of the other porcine pathogen strains analyzed in this study.

Other serogroups of *E. coli* that typically cause edema disease have been shown to have genetically diverse backgrounds despite identical lipopolysaccharide antigens and conserved virulence genes (9). Among this group of strains (Hungarian and U.S.), serogroup O138 appears to be less genetically diverse than serogroups O139 and O141. As in our study, there was some overlap in the genetic backgrounds of *E. coli* strains that cause postweaning diarrhea with those that cause edema disease. Serogroup O157 *E. coli* strains have also been shown to cluster genetically by pathotypes, and porcine F4* ETEC strains are not related to the O157:H7/H* clones associated with human food-borne illness or to the strains recovered from human urinary tract infections (16).

The appearance of the O147 clone of edema disease-causing *E. coli* in various geographic locations suggests that it may be spreading between farms. We do not know which herds contributed to the spread of these strains to naïve herds. However, under current swine management practices in the United States, pigs are frequently farrowed in one location and moved to other locations to be finished or put into breeding programs. Such movement could easily lead to the spread of a pathogenic edema disease clone, as was described previously in Denmark (1). Our serogrouping results for edema disease isolates submitted to the ISU Diagnostic Laboratory suggest that this O147 clone is widespread among swine production facilities in Iowa and the Midwest.

**ACKNOWLEDGMENTS**

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