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Prevalence of Virulence Factors Among Escherichia coli

Abstract
Polymerase chain reaction (PCR) assays were used to characterize 575 Escherichia coli isolates from swine submitted to the veterinary diagnostic laboratory from January through May 1998. About one-third of the isolates carried genes characteristic of enterotoxigenic (ETEC), or Shiga toxin-producing (STEC), or attaching and effacing (AEEC) E. coli pathotypes. The ETEC were the most common pathotype. The data indicate that vaccination or vaccine development based on F18 and K88 pilus antigens continues to be appropriate for the control of ETEC infections. The STEC and AEEC infections also contribute to intestinal diseases in swine but apparently at lower prevalences than ETEC in this population. Nearly 25% of the ETEC lacked genes for any of the pilus types included in the assay. It is not known if these are nonpathogenic ETEC or if they produce other (as yet undiscovered) pilus antigens. If the latter interpretation is correct, they could represent an emerging population of ETEC pathogens not reflected in current pilus-based vaccines and diagnostic tests. There were also a number of strains that carried genes for K88 or F18 pili but did not have genes for enterotoxins. It was speculated that such strains may be acting as naturally occurring live oral pilus vaccines in herds where they occur.

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Prevalence of Virulence Factors
Among *Escherichia coli*

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**ASL-R1601**

**Summary and Implications**

Polymerase chain reaction (PCR) assays were used to characterize 575 *Escherichia coli* isolates from swine submitted to the veterinary diagnostic laboratory from January through May 1998. About one-third of the isolates carried genes characteristic of enterotoxigenic (ETEC), or Shiga toxin-producing (STEC), or attaching and effacing (AEEC) *E. coli* pathotypes. The ETEC were the most common pathotype. The data indicate that vaccination or vaccine development based on F18 and K88 pilus antigens continues to be appropriate for the control of ETEC infections. The STEC and AEEC infections also contribute to intestinal diseases in swine but apparently at lower prevalences than ETEC in this population. Nearly 25% of the ETEC lacked genes for any of the pilus types included in the assay. It is not known if these are nonpathogenic ETEC or if they produce other (as yet undiscovered) pilus antigens. If the latter interpretation is correct, they could represent an emerging population of ETEC pathogens not reflected in current pilus-based vaccines and diagnostic tests. There were also a number of strains that carried genes for K88 or F18 pili but did not have genes for enterotoxins. It was speculated that such strains may be acting as naturally occurring live oral pilus vaccines in herds where they occur.

**Introduction**

*Escherichia coli* cause diarrhea and edema disease (gut edema) in swine. The *E. coli* that cause these diseases have specific characteristics (called virulence attributes) that enable them to cause disease. Therefore, the presence of genes encoding for virulence attributes can be used to distinguish these pathogens from the nonpathogenic or commensal *E. coli* normally carried in the intestine. One type of pathogen is enterotoxigenic *E. coli* (ETEC). The enterotoxins produced by ETEC stimulate the small intestine to secrete electrolytes and water resulting in diarrhea. A second pathotype is Shiga toxin-producing *E. coli* (STEC). Shiga toxins are absorbed from intestine into blood where they cause systemic vascular damage resulting in edema disease. Deaths from edema disease are thought to result from edema of the brain. To cause diarrhea or edema disease both ETEC and STEC must colonize the small intestine. They do so through a second set of virulence attributes called pili. Pili are filamentous bacterial appendages that enable the *E. coli* to adhere to and thus colonize the lining of the small intestine. Pili are the basis for many of the vaccines that are used to prevent ETEC-induced diarrhea of newborn pigs.

*E. coli* that do not produce either enterotoxin or Shiga toxin are called nontoxigenic (NTEC). Many NTEC are nonpathogenic. Some, called attaching/effacing *E. coli* (AEEC), however, have virulence attributes that enable them to attach to and damage (efface) the surfaces of intestinal epithelial cells, resulting in diarrhea.

The objectives of this study were to determine (1) the prevalences of ETEC, STEC, and AEEC among *E. coli* isolates suspected to cause or contribute to disease among swine presented to the veterinary diagnostic laboratory; and (2) the comparative prevalences of different pilus and toxin types among such ETEC and STEC.

**Materials and Methods**

*E. coli* isolates. *E. coli* recovered from swine tissue and submitted to the Veterinary Diagnostic Laboratory at Iowa State University from January through May 1998 were assayed for genes encoding virulence attributes characteristic of ETEC, STEC, and AEEC.

**PCR assays.** All isolates were tested in a multiplex polymerase chain reaction (PCR) assay (1) to determine if they carried genes for: *E. coli* enterotoxins of the heat labile (LT) or heat stable (STa or STb) types, Shiga toxin type 2 (associated with edema disease), and pili of the K88, F18, K99, 987P and F41 types. Isolates that did not react positively for any of the genes of interest in the initial PCR assay were tested a second time by using a different multiplex PCR assay (3) that detects genes for attaching/effacing activity (eae), STa enterotoxin, Shiga toxins 1 and 2, and pili of the K99 and F41 types.

**Results and Discussion**

The results are summarized in Table 1. Thirty-two percent of the isolates was found to have genes for enterotoxin, Shiga toxin or attaching/effacing activity and
were classified as ETEC, STEC, or AEEC. Most of the ETEC had genes for more than one type of enterotoxin, with STb and LT being the most prevalent. The pilus K88 was the most prevalent type, followed by F18. Twenty-four percent of the ETEC did not have genes for any of the pili. Presumably these isolates would not have been able to colonize the small intestine and, therefore, were nonpathogenic. Alternatively, they may have carried genes for other (new or unrecognized) pilus types that mediate colonization of pig small intestine.

The ETEC were much more prevalent than either STEC or AEEC. This finding is consistent with the current notion that ETEC are the most common *E. coli* pathotype causing disease among U.S. swine. The occurrence of 11 STEC with genes for F18 pili indicates that pathogens capable of causing edema disease persist in the swine population in spite of the comparatively low incidence of edema disease among U.S. swine during the last 2 decades. That 6% of the isolates classified as NTEC on the basis of the initial PCR assay were subsequently found to be AEEC confirms reports that AEEC are associated with disease in swine (4,6). Studies have shown, however, that most AEEC do not produce enterotoxin (6).

Several of the NTEC had genes for K88 or F18 pili. We suspect that these isolates were not pathogenic. We speculate that these F18+ or K88+ NTEC may act as naturally occurring immunogens (pilus vaccines) protecting against diarrhea and edema disease in some herds (2,5).

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This work was conducted with the technical assistance of Sophi M. Franck, Timothy J. Klinefelter, Matthew D. Mettenburg, and Dawn R. Wiarda.

### References


### Table 1. Virulence genes detected by polymerase chain reaction assay of 575 *E. coli* isolates from swine.

<table>
<thead>
<tr>
<th>Pathotype</th>
<th>No. of Isolates</th>
<th>Enterotoxins*</th>
<th>Shiga toxin</th>
<th>Pili</th>
<th>Attaching/Effacing Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETEC</td>
<td>144</td>
<td>80 51 141</td>
<td>1 2</td>
<td>K88 14 1 2 1 34</td>
<td></td>
</tr>
<tr>
<td>STEC</td>
<td>18</td>
<td>0 7 8</td>
<td>18</td>
<td>0 11 0 0 0 7</td>
<td></td>
</tr>
<tr>
<td>NTEC</td>
<td>413*</td>
<td>0 0 0</td>
<td>0 6 20 0 0 1 386</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEEC</td>
<td>24*</td>
<td>2 0 2 0</td>
<td>0 0 0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Most ETEC have genes for two or three enterotoxins.
* Nine isolates had genes for enterotoxin and Shiga toxin but are listed only as STEC.
* 362 NTEC isolates were tested in a different PCR assay that detects eae, STa, Shiga toxins 1 and 2, K99, and F41.