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Sources and manufacturers
a. VitaLite, Duro-Test Corp., New Bergen, NJ.
b. Becton Dickinson Microbiology Systems, Cockeysville, MD.
c. GIBCO/BRL, Grand Island, NY.
d. Hemostat Laboratories, Dixon, CA.
e. American Type Culture Collection, Rockville, MD.
f. Phillips 300, Phillips Electronic Instruments, Mahwah, NJ.

References


Prevalences of some virulence genes among Escherichia coli isolates from swine presented to a diagnostic laboratory in Iowa

Harley W. Moon, Lorraine J. Hoffman, Nancy A. Cornick, Sheridan L. Booher, Brad T. Bosworth

Escherichia coli strains that carry genes encoding for specific virulence attributes cause diarrhea and edema disease in swine.1,2,8 Enterotoxigenic E. coli (ETEC) have genes for enterotoxins that stimulate secretion of electrolytes and water by the small intestine.6 To colonize the small intestine and cause diarrhea, ETEC must also produce fimbriae (pili).16 Escherichia coli strains that cause edema disease produce E. coli Shiga toxin (Verotoxin) and are designated as STEC.7 Shiga toxin is absorbed from the intestine into blood and causes systemic vascular damage resulting in edema disease. STEC must also produce fimbriae to colonize the small intestine and cause disease.2 Some E. coli strains are designated as attaching/effacing E. coli (AEEC) because of their ability to attach intimately to the surface of intestinal epithelial cells and efface microvilli.10 The attaching/effacing attribute is encoded by a series of chromosomal genes located in a pathogenicity island called the locus of enterocyte effacement. ETEC, STEC, and AEEC are considered to be different pathotypes of E. coli. However, some of the virulence genes that characterize them can be located on mobile genetic elements (plasmids, transposons, bacteriophages), and combinations of pathotypes occur. For example, some AEEC such as the human pathogen E. coli O157:H7 also have genes for Shiga toxin production,11,14 and some strains associated with edema disease of swine have genes for both Shiga toxin and enterotoxin production.2

The objectives of the work reported here were to determine 1) the prevalences of ETEC, STEC, and AEEC among swine E. coli isolates obtained at the Iowa State University Veterinary Diagnostic Laboratory, 2) the comparative prevalences of genes for different enterotoxin and pilus types among such isolates, and 3) whether there are differences in the prevalences of toxin and fimbrial gene types isolated from pigs in different age groups.

Escherichia coli isolates recovered from 539 swine fecal or tissue samples submitted to the Iowa State University Veterinary Diagnostic Laboratory from August 1996 through December 1997 were analyzed. More than 95% of the specimens were obtained from swine herds in the midwestern...
region of the United States. Each isolate came from a separate pig. The E. coli isolates were analyzed in a multiplex polymerase chain reaction (PCR) assay.³ The PCR detects genes for heat labile E. coli enterotoxin (LT), heat stable E. coli enterotoxins of the STa and STb types, Shiga toxin 2, and fimbriae of the F4 (K88), F5 (K99), F6 (987P), F18, and F41 types.

Isolates with 1 or more of the genes of interest were obtained from specimens representing 249 different swine herds. Specimens from 14 of these herds were examined on 2 or more occasions. These multiple submissions yielded isolates with the same virulence genes on each submission from 4 herds. Isolates with more than 1 combination of virulence genes were recovered from each of the other 10 multiple submission herds. In addition to the E. coli reported here, 138 of the specimens from the 249 herds cited above were also infected with other bacterial, viral, or protozoan pathogens (a total of 15 different agents).

The results of the PCR analysis are summarized in Table 1. Slightly more than half of the isolates did not have any of the toxin genes represented in the PCR and were therefore classified as nontoxigenic E. coli (NTEC). The prevalence of ETEC (42% of all isolates) was about 10 times greater than that of STEC (4% of all isolates). This finding is consistent with the prevailing notion that diarrhea caused by ETEC is a common swine disease problem and that edema disease occurs less frequently. The occurrence of 13 isolates with genes for Shiga toxin and F18 fimbriae suggests that edema disease pathogens are prevalent in the swine population in spite of the current comparatively low prevalence of edema disease among US swine. Signs or lesions characteristic of edema disease were detected in 7 of the 13 cases that provided these Stx/F18⁺ isolates. Presumably the 9 STEC and 15 ETEC isolates that did not have genes for fimbriae would not have been able to colonize the small intestine and therefore were nonpathogenic. Alternatively, they may have carried genes for new or as yet unrecognized fimbriae (or known fimbriae with novel gene sequences) that were not amplified by the primers used in this study but did mediate colonization of pig intestine.

Most of the ETEC had genes for more than 1 type of enterotoxin, and several STEC also had enterotoxin genes. STb and LT were the most prevalent enterotoxin types. More than 80% of the ETEC had genes for F4 fimbriae, and about 10% of them had genes for F18. In contrast, F18 was the only fimbrial type detected among STEC. Presumably the Shiga toxin genes detected were of the variant stx2b, associated with edema disease.¹¹¹⁴ However, the presence of this gene was not confirmed because the PCR is not specific for the stx2b variant. It also detects stx2 genes of E. coli O157:H⁷ strains from humans and cattle (unpublished data). More than 10% of the NTEC had genes for F18, and a few of them had genes for F4. The PCR test of the F18⁺ NTEC isolates was replicated to check the possibility that such isolates may have had toxin genes that were not detected in the initial PCR test. There was reason to be concerned about the sensitivity of the PCR for the Shiga toxin gene. The stx2 gene produces the largest amplicon in the PCR assay and occasionally gives negative PCR results with the stx2e⁻ control strain (data not shown). No Shiga toxin or enterotoxin genes were detected when the PCR test of the F18⁺NTEC isolates was replicated.

Information was available on the age and/or weaning status of the pigs that provided 231 of the 286 isolates that were PCR positive for at least 1 of the virulence genes. Isolates from pigs known to be at least 3 weeks old and/or weaned were assigned to 1 age group. Those from pigs known to be <3 weeks old and/or not weaned were assigned to another. The distribution of isolates among the 2 groups is summarized in Table 2. STb was the most prevalent and STa the least prevalent enterotoxin type in both age groups. F4 was the most prevalent fimbriae type in both age groups (64% and 82% of isolates). Shiga toxin and F18 fimbriae (11% and 29% of isolates, respectively) were more frequently identified in the older and/or weaned age group than in the younger/not weaned group (1% and 4% of isolates, respectively). These data suggest that, in contrast to F4 and the other fimbrial types, F18⁺ isolates have a predilection to colonize pigs several weeks old more frequently than those in the early neonatal period. These findings are consistent with the experimental data indicating that the receptivity of pig intestinal epithelial cells to adhesion by F18⁺ ETEC increased with age.¹²

The prevalences of AEEC, ETEC, and STEC were compared among an additional group of E. coli isolates obtained from 570 swine samples submitted to the Iowa State Uni-

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Table 1. Virulence genes detected by polymerase chain reaction assay of 539 Escherichia coli isolates from swine samples submitted to the Iowa State University Veterinary Diagnostic Laboratory, August 1996–December 1997.

<table>
<thead>
<tr>
<th>E. coli isolates</th>
<th>Enterotoxins</th>
<th>Shiga toxin</th>
<th>Fimbriae†</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patho-type²</td>
<td>No.</td>
<td>LT</td>
<td>STa</td>
<td>STb</td>
</tr>
<tr>
<td>ETEC‡</td>
<td>227</td>
<td>161</td>
<td>77</td>
<td>220</td>
</tr>
<tr>
<td>STEC§</td>
<td>22</td>
<td>0</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>NTEC</td>
<td>290</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>539</td>
<td>161</td>
<td>87</td>
<td>231</td>
</tr>
</tbody>
</table>

* ETEC = enterotoxigenic E. coli; STEC = Shiga toxin-producing E. coli; NTEC = nontoxigenic E. coli.
† Several isolates had genes for 2 types of fimbriae.
‡ Most of these isolates had genes for 2 or 3 enterotoxins.
§ Isolates with genes for both Shiga toxin and enterotoxin were listed as STEC.
versity Veterinary Diagnostic Laboratory from January through May of 1998. These isolates were first tested in the PCR assay. Isolates that tested negative for all genes in that initial PCR analysis were tested in an alternative multiplex PCR assay that detects the eae gene required for attaching/effacing activity and genes encoding Stx1 and 2, F5, and F41. Because the initial PCR did occasionally miss genes of interest (data not shown), 54 isolates that tested either positive for toxin genes and negative for fimbrial genes or positive for fimbrial genes and negative for toxin genes were also tested in the alternative PCR.

The data from analysis of the 1998 isolates in the initial and alternative PCR assays are summarized in Table 3. Two isolates that tested negative for all genes in the initial PCR were positive for Stx1 when tested in the alternative PCR. One isolate classified as an F18* NTEC on the basis of the initial PCR was positive for Stx2 when tested in the alternative PCR. None of the other isolates, classified as NTEC on the basis of the initial PCR, were positive for toxin or fimbrial genes when tested in the alternative PCR. The comparative prevalences of ETEC and STEC were similar to those among isolates collected during 1996–1997 (Table 1). The comparative prevalences of different enterotoxin and fimbrial types were also similar to those of the 1996–1997 isolates, except that the proportion of ETEC that did not have genes for any of the fimbrial types was somewhat higher (15/227 ETEC in 1996–1997 vs. 42/153 ETEC in 1998).

Twenty-two of the isolates tested in the alternative PCR assay had the eae gene and were designated as AEEC (Table 3). Thus, the prevalence of AEEC (4%) was lower than that of ETEC (27%) and similar to that of STEC (3%). Pathogenic E. coli that have attaching/effacing activity as their only virulence attribute are also called enteropathogenic E. coli (EPEC). The AEEC identified in this study did not have genes for Shiga toxin or enterotoxin. The AEEC identified in previous studies of swine were also nontoxigenic. Enteric disease in pigs has been reproduced by experimental infection with porcine EPEC. In aggregate, the evidence indicates that EPEC cause enteric disease in swine. However, EPEC appeared to be less prevalent than ETEC among the swine submissions to the laboratory during the reporting period covered here.

In conclusion, the data confirm that STEC and AEEC occur in diseased pigs but apparently at lower prevalences than ETEC. The results are consistent with experimental data indicating that F18* E. coli are more suited to colonizing older and/or weaned pigs than those in the immediate neonatal period, whereas F4* strains readily colonize both age groups. The results suggest that F4 and, to a lesser extent, F18 continue to be the major fimbrial antigen types among problem ETEC and STEC infections referred to the diagnostic laboratory. The significance of the ETEC and STEC that lacked genes for fimbriae as represented in the PCR assays is unknown. They may be nonpathogenic or they may

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### Table 2. Distribution of virulence genes* among Escherichia coli isolates from pigs of 2 age groups submitted to the Iowa State University Veterinary Diagnostic Laboratory, August 1996–December 1997.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. isolates†</th>
<th>Enterotoxins</th>
<th>Shiga toxin</th>
<th>Fimbriae</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥3 wk old or weaned</td>
<td>160</td>
<td>58</td>
<td>26</td>
<td>78</td>
<td>11</td>
</tr>
<tr>
<td>&lt;3 wk old or not weaned</td>
<td>71</td>
<td>65</td>
<td>35</td>
<td>94</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enterotoxins</th>
<th>Stx1</th>
<th>Stx2</th>
<th>F4</th>
<th>F18</th>
<th>F5</th>
<th>F6</th>
<th>F41</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Detected by multiplex polymerase chain reaction (PCR) assay for genes encoding E. coli enterotoxins LT, STa, STb; Shiga toxin 2; and fimbriae K88, F18, K99, 987P, F41.
† Those containing virulence genes (PCR positive).

### Table 3. Virulence genes detected by polymerase chain reaction assays of 570 Escherichia coli isolates from swine samples submitted to the Iowa State University Veterinary Diagnostic Laboratory, January–May 1998.

<table>
<thead>
<tr>
<th>Pathotype*</th>
<th>E. coli isolates</th>
<th>Enterotoxins</th>
<th>Shiga toxin</th>
<th>Fimbriae†</th>
<th>Attaching/effacing activity (eae gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETEC‡</td>
<td>153</td>
<td>92</td>
<td>56</td>
<td>148</td>
<td>... 0</td>
</tr>
<tr>
<td>STEC§</td>
<td>18</td>
<td>0</td>
<td>7</td>
<td>8</td>
<td>... 18</td>
</tr>
<tr>
<td>NTEC</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AEEC</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* ETEC = enterotoxigenic E. coli; STEC = Shiga toxin-producing E. coli; NTEC = nontoxigenic E. coli; AEEC = attaching/effacing E. coli.
† Several isolates had genes for 2 types of fimbriae.
‡ Most ETEC had genes for 2 or 3 enterotoxins.
§ Isolates with genes for both enterotoxin and Shiga toxin were listed as STEC.
be pathogens that have novel fimbrial antigens. NTEC with genes for F18 or F4 may be nonpathogens and may be acting as naturally occurring immunogens protecting against diarrhoea and edema disease in some herds.4,15

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References


Normal bacterial flora in canine and feline uteri

Patricia C. Schultheiss, Robert L. Jones, M. Lynne Kesel, Patricia N. Olson

Many cases of inflammatory disease in the reproductive tract of dogs and cats are considered to be caused by infectious agents. Knowledge of identity and antibiotic susceptibility patterns of infecting bacteria is useful in diagnosing and treating reproductive disease in animals but results of cultures must be interpreted in light of the normal flora present in the tract. The stage of the estrous cycle and associated patency of the cervix may also influence whether bacteria are found in a normal uterus.

A variety of organisms are normal inhabitants of the vagina of cats and dogs.1,3,8,10 The normal flora of the canine uterus has not been thoroughly characterized,1,8,10 and even less is known about the uterine flora of cats.1 The purpose of this study was to identify uterine bacterial flora of clinically normal dogs and cats at various stages of the estrous cycle using aerobic, anaerobic, and mycoplasma isolation techniques and to correlate culture results with the stage of the estrous cycle and any gross or histologic lesions.

Samples were obtained from 69 dogs and 37 cats 4 months to 6 years of age on which elective ovariohysterectomies (OHE) were performed in a routine sterile manner at the Colorado State University Veterinary Teaching Hospital. All animals were clinically normal, and there were no gross

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