11-1999

Prevalences of Some Virulence Genes among Escherichia Coli Isolates from Swine Presented to a Diagnostic Laboratory in Iowa

Harley W. Moon  
*Iowa State University*

Lorraine J. Hoffman  
*Iowa State University*, lhoffman@iastate.edu

Nancy A. Cornick  
*Iowa State University*, ncornick@iastate.edu

Sheridan L. Booker  
*Iowa State University*

Follow this and additional works at: [http://lib.dr.iastate.edu/vmpm_pubs](http://lib.dr.iastate.edu/vmpm_pubs)

Part of the Large or Food Animal and Equine Medicine Commons, Veterinary Infectious Diseases Commons, Veterinary Microbiology and Immunobiology Commons, and the Veterinary Preventive Medicine, Epidemiology, and Public Health Commons

The complete bibliographic information for this item can be found at [http://lib.dr.iastate.edu/vmpm_pubs/101](http://lib.dr.iastate.edu/vmpm_pubs/101). For information on how to cite this item, please visit [http://lib.dr.iastate.edu/howtocite.html](http://lib.dr.iastate.edu/howtocite.html).

This Article is brought to you for free and open access by the Veterinary Microbiology and Preventive Medicine at Iowa State University Digital Repository. It has been accepted for inclusion in Veterinary Microbiology and Preventive Medicine Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Lambert, Jason Scott, Dee Stevenson, Dr. Jim Dugan, Dr. Jonathan Sleeman, Melanie Gregory, Bernt Tryon, and Kreis Weigel for technical assistance in the preparation of this manuscript.

Sources and manufacturers


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. Enterotoxigenic E. coli (ETEC) have genes for enterotoxins that stimulate secretion of electrolytes and water by the small intestine. To colonize the small intestine and cause diarrhea, ETEC must also produce fimbriae (pili). Escherichia coli strains that cause edema disease produce E. coli Shiga toxin (Verotoxin) and are designated as STEC. 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. 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. 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, and some strains associated with edema disease of swine have genes for both Shiga toxin and enterotoxin production.

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. 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 do 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 stx2e, associated with edema disease. However, the presence of this gene was not confirmed because the PCR is not specific for the stx2e 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 repilicated 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.

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>Pathotype</th>
<th>Enterotoxins</th>
<th>Virulence genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT</td>
<td>STa</td>
</tr>
<tr>
<td>ETEC‡‡</td>
<td>227</td>
<td>161</td>
</tr>
<tr>
<td>STEC§</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>NTEC</td>
<td>220</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>539</td>
<td>161</td>
</tr>
</tbody>
</table>

‡‡ Most of these isolates had genes for 2 or 3 enterotoxins.
§ Isolates with genes for both Shiga toxin and enterotoxin were listed as STEC.
E. coli. Fifty-nine percent of the isolates were positive for STa when tested in the alternative PCR.

The comparative prevalences of different enterotoxins and Shiga toxins among isolates from pigs of 2 age groups submitted to the Iowa State University 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 Sta, Shiga toxins 1 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 Sta 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 enterotoxins 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).

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

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>Enterotoxins</th>
<th>Shiga toxin</th>
<th>Fimbriae†</th>
<th>Attaching/effacing activity (eae gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT</td>
<td>Sta</td>
<td>Stb</td>
<td>1</td>
</tr>
<tr>
<td>ETEC†‡</td>
<td>153</td>
<td>92</td>
<td>56</td>
<td>148</td>
</tr>
<tr>
<td>STEC§</td>
<td>18</td>
<td>0</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>NTEC</td>
<td>400</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>
</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 diarrhea and edema disease in some herds.1,15

Acknowledgements. This work was conducted with the technical assistance of Sophi M. Franck, Timothy J. Klinefelter, Matthew D. Mettenburg, and Dawn R. Wiarda. Serologic analysis of some isolates was conducted by Kimberly Seebart, E. coli Reference Center, Pennsylvania State University. The work was supported by the Frank K. Ramsey endowment and by NIH grant AI 41328.

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.

From the Departments of Pathology (Schultheiss), Microbiology (Jones), and Clinical Sciences (Kesel, Olson), Colorado State University, Fort Collins, CO 80523.

Received for publication October 23, 1998.