Concurrent Infection of Pigs with Enterotoxigenic *Escherichia coli* of Different Serogroups†

DAVID H. FRANCIS1†* AND RICHARD A. WILSON2

Department of Veterinary Science, South Dakota State University, Brookings, South Dakota 57007,7 and Department of Veterinary Science, The Pennsylvania State University, University Park, Pennsylvania 168022

Received 24 January 1985/Accepted 17 June 1985

Naturally occurring dual infections with *Escherichia coli* of different serogroups occurred in 12 pigs 2 to 14 days of age. In each case, one isolate was hemolytic and produced K88 pili and the other was nonhemolytic and produced either K99 or 987P pili.

Mixed infections involving more than one pathogenic agent in enteritis of pigs occur frequently and have been mentioned in several reports (1, 8, 9, 15). Naturally occurring coinfection of pigs by more than one strain of the same enteric pathogen, however, has not been previously reported. The purpose of this communication is to report the occurrence of dual enterotoxigenic *Escherichia coli* infections in young pigs.

Specimens from diarrheic piglets submitted to the South Dakota State University Veterinary Diagnostic Laboratory from various farms in the upper Midwest were examined for microbial pathogens by standard procedures as reported previously (4, 5, 10, 13). Impression smears prepared from piglet ileum (and frequently jejunum) were Gram stained and field, identical smears were fixed in cold acetone and stained by indirect immunofluorescence for K88, K99, and 987P antigens (5). (Although the designations for K88, K99, and 987P antigens have been recently changed to F-4, F-5, and F-6, respectively [12], the more familiar nomenclature will be used in this article when referring to these pili.) Intestines were also cultured for *E. coli* with sheep blood agar (heart infusion base) and tergitol-7 agar. Isolates were subcultured onto E agar (6) for promotion of the expression of K99 antigen and blood agar for promotion of the expression of K88 and 987P antigens. Cultured bacteria were tested for pilus antigens by seroagglutination as previously described (6). Isolates from dually infected pigs were serotyped by standard methods (8) and tested for ability to produce enterotoxins. The Y-1 adrenal cell assay (3) was used to test for heat-labile toxin. The suckling mouse assay was used to test for heat-stable toxin (ST I) (2). A gene probe was used to identify isolates harboring genes for ST II (personal communication, C. W. Maddox and R. A. Wilson, Pennsylvania State University, University Park). In addition, heat-treated culture supernatant of some of the strains was tested for ST II in piglet intestinal loops (11).

### Table 1. Dual *E. coli* infections and characteristics of isolated organisms

<table>
<thead>
<tr>
<th>Strain</th>
<th>Piglet age (days)</th>
<th>Immunofluorescence results</th>
<th>Characteristic of isolatesa</th>
<th>Organism 1 Serotype</th>
<th>Organism 1 Enterotoxin</th>
<th>Organism 2 Serotype</th>
<th>Organism 2 Enterotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>81-9228</td>
<td>4</td>
<td>Negative</td>
<td>O149:K88</td>
<td>LT, ST II</td>
<td>O9:987P</td>
<td>ST I, ST II</td>
<td></td>
</tr>
<tr>
<td>81-18084</td>
<td>8–10</td>
<td>4+, K88</td>
<td>O149:K88</td>
<td>LT, ST II</td>
<td>O20:987P</td>
<td>ST I</td>
<td></td>
</tr>
<tr>
<td>82-830</td>
<td>4</td>
<td>4+, K88; 4+, 987P</td>
<td>O149:K88</td>
<td>LT, ST II</td>
<td>O7:987P</td>
<td>ST I, ST II</td>
<td></td>
</tr>
<tr>
<td>82-8425B</td>
<td>10–14</td>
<td>3+, K88; 2+, K99</td>
<td>O149:K88</td>
<td>LT, ST II</td>
<td>O8:K99</td>
<td>ST I, ST II</td>
<td></td>
</tr>
<tr>
<td>82-10616</td>
<td>7</td>
<td>3+, K88; 4+, 987P</td>
<td>O149:K88</td>
<td>LT, ST II</td>
<td>O7:K99, F41</td>
<td>ST I, ST II</td>
<td></td>
</tr>
<tr>
<td>82-13156A</td>
<td>3</td>
<td>4+, K88; 4+, 987P</td>
<td>O149:K88:NM</td>
<td>LT, ST II</td>
<td>O7:987P:NM</td>
<td>ST I</td>
<td></td>
</tr>
<tr>
<td>82-14423</td>
<td>2</td>
<td>3+, K88; 2+, K99</td>
<td>O149:K88:H43</td>
<td>LT, ST II</td>
<td>O101:K99:NM</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>82-16163</td>
<td>2</td>
<td>4+, K88; 4+, K99</td>
<td>O?:K88:NM</td>
<td>LT, ST II</td>
<td>O101:K99:NM</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>82-1633B</td>
<td>3</td>
<td>3+, K88; 3+, 987P</td>
<td>O149:K88:NM</td>
<td>LT, ST II</td>
<td>O7:987P:NM</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>84-4754</td>
<td>NGd</td>
<td>3+, K88; 2+, K99</td>
<td>None</td>
<td>LT, ST II</td>
<td>None</td>
<td>ST I</td>
<td></td>
</tr>
<tr>
<td>84-5989</td>
<td>10</td>
<td>3+, K88; 3+, K99</td>
<td>None</td>
<td>LT, ST II</td>
<td>O6:K99:NM</td>
<td>ST I</td>
<td></td>
</tr>
<tr>
<td>84-12592-2</td>
<td>6</td>
<td>4+, K88; 4+, 987P</td>
<td>O?:K99:NM</td>
<td>LT</td>
<td>O7:987P:NM</td>
<td>ST I</td>
<td></td>
</tr>
</tbody>
</table>

a Number of organisms per X400 microscopic field estimated: 2+, 10 to 100; 3+, 101 to 400; 4+, >400.

b ST II results listed are for the gene probe test. O?, Serogroup undeterminable; NM, Nonmotile; LT, heat-labile enterotoxin.

c K88 immunofluorescence was observed in a specimen from the ileum, whereas the 987P immunofluorescence was observed in a specimen from the jejunum.

d NG, Not given.

* Corresponding author.
† Journal article no. 2068, Agriculture Experiment Station, South Dakota State University.
‡ Present address: Department of Animal, Dairy, and Veterinary Science, UMC 56, Utah State University, Logan, UT 84322.
During the course of the study (1981 through 1984), 12 cases of dual *E. coli* infections were identified (Table 1). Most cases of dual infections were initially identified by immunofluorescence and later confirmed by culturing two strains of *E. coli* from the intestine. All dual infections included one strain that produced K88 pili and another strain that produced either K99 or 987P pili. Strains were easily separable from mixed cultures because the K88-positive isolates were always hemolytic on blood agar, whereas none of the K99- or 987P-positive isolates were hemolytic. When identifiable, serogroups were those expected for strains producing the respective adhesive pili (7). In all except two cases, each of the pairs of infecting *E. coli* strains were present in the small intestine in large numbers, suggesting a significant contribution to the diarrhea. Interestingly, in one case, the jejunal was heavily colonized with K88-bearing *E. coli* organisms, whereas the ileum was heavily colonized with 987P-bearing organisms. In all other cases, both the hemolytic and the nonhemolytic *E. coli* strains had colonized the ileum.

One or more of the tests for enterotoxins were positive for all except three strains. That the three enterotoxin-negative strains contributed to the diarrheal disease was suggested by their presence in large numbers in the ilea of the pigs from which they were isolated. Enterotoxin-encoding plasmids may have been lost after isolation or may have never been present. In an earlier study, Smith and Linggood (14) observed that some of the piglets experimentally inoculated with a K88-positive, enterotoxin-negative *E. coli* strain developed mild diarrhea.

Six strains, all of which were ST II probe-positive, were tested by piglet loop for biologically active ST II. Strains 81-18084 (O149:K88), 81-18084 (O20:987P), and 82-8425B (O149:K88) were ST II positive. Strains 81-9328 (O149:K88), 82-830 (O149:K88), and 82-10616 (O149:K88) were ST II negative. Discrepancies between the two ST II tests may have been the result of inactive ST II genes or ST II non-reactivity in the piglet loop. Piglets vary in their response to ST II (R. A. Wilson, unpublished data; personal communication, Shannon Whipp, National Animal Disease Center, Ames, Iowa).

A major goal associated with the diagnosis of colibacillosis in swine is isolation of the organism for antimicrobial susceptibility testing or for the production of an autogenous vaccine to protect the offspring of sows in subsequent farrowings. This study suggests that in some cases, more than one strain of *E. coli* may be contributing to the diarrhea. Therefore, diagnostic tests chosen for use by a diagnostic laboratory should be capable of differentiating among the various types of enterotoxigenic *E. coli* which may infect piglets.

We thank Carol W. Maddox for performing gene probes and Rita Miller for assistance in manuscript preparation.

**LITERATURE CITED**