Summary
Polymerase chain reaction for five fimbriae (K88, K99, 987P, F41, F18), two heat-stable enterotoxins (STa, STb), one heat-labile enterotoxin (LT), and Shiga-like toxin type II (SLT) was performed on Escherichia coli isolates to determine their genotype among isolates from weaned pigs with diarrhea and/or edema disease in North Carolina. A total of 175 isolates were tested. All but two isolates possessed genes for at least one of the enterotoxins or fimbrial adhesins. Nearly all (94.86%) of the isolates that carried enterotoxin genes also carried genes for one of the fimbrial adhesins. The two predominant genotypes were K88 LT STb and F18 STa STb SLT, respectively.

Keywords: swine, Escherichia coli, fimbrial adhesins, genotypes

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Postweaning diarrhea and edema disease due to enterotoxigenic (ETEC) and verocytotoxigenic (VTEC) strains of Escherichia coli are important causes of morbidity and mortality in weaned pigs. Pathogenesis of ETEC is mediated by enterotoxins (heat-labile toxin [LT] and heat-stable toxins [STa and STb]), which act locally in the gut, stimulating hypersecretion of water and electrolytes by enterocytes. ETEC manifests clinically as diarrhea and dehydration. Edema disease, on the other hand, is an enterotoxemia, in which strains of E. coli elaborate an exotoxin (verocytotoxin or Shiga-like toxin type II), which is absorbed from the gut and produces systemic lesions associated with vascular injury.

Nonpathogenic strains of E. coli are commonly found in the intestinal tracts of animals. It is important to identify the virulence factors in order to establish the etiology of diarrhea. Serologic tests have traditionally been used to identify virulence factors associated with specific clinical syndromes. The application of molecular methods, mainly the polymerase chain reaction (PCR) assay, has increased both the rapidity of characterization and the amount of information that can be gleaned from an individual isolate.

The objective of the present work was to determine the frequency of occurrence of selected virulence factors in strains of E. coli isolated from pigs with postweaning diarrhea and/or edema disease in North Carolina.

Materials and methods
Escherichia coli strains
We analyzed Escherichia coli isolates that were recovered from 175 swine clinical specimens, either from feces or ileum, and submitted to the Rollins Animal Disease Diagnostic Laboratory between March 1998 and March 1999. Each isolate came from an individual animal 21–42 days of age with postweaning diarrhea and/or edema disease.

Genotyping
Analysis of the isolates was performed by a recently developed multiplex PCR assay which detects genes for the enterotoxins LT, STa, STb, Shiga-like toxin type II and fimbrial adhesins K88 ac,ad (F4), K99 (F5), F41, 987P (F6), and F18 ab,ac — common virulence factors associated with swine enteric disease. Amplified products were electrophoresed in 2% agarose gel, stained with ethidium bromide, and examined under ultraviolet illumination. DNA fragment lengths were verified by a digested £-DNA standard run simultaneously. Control DNA from reference strains was included in each reaction.

Results
Genotypes are listed for all 175 isolates in Table 1. All but two isolates possessed genes for at least one of the enterotoxins or fimbrial adhesins. Isolates that carried fimbrial adhesin genes also carried a gene for at least one of the enterotoxins.

Nearly all (94.86%) of the isolates that carried enterotoxin genes also carried genes for one of the fimbrial adhesins. The two predominant genotypes observed were K88 LT STb and F18 STa STb SLT, respectively.

Discussion
The most prevalent fimbrial adhesin identified in this study was F18. K88 has previously been considered to be the predominant fimbrial adhesin associated with postweaning disease in the pig. More recent surveys, however, have increasingly incriminated F18 fimbriae in postweaning disease. F18 fimbriae cannot always be reliably identified in vitro; therefore, prior estimates of prevalence that were based upon immunologic methods may have been lower than those based upon genetic analysis.

These data indicate a positive relationship...
between fimbrial adhesion and enterotoxin genes. K88 positive *E. coli* usually produced both LT and STb. This is in contrast to a report by Wilson and Francis where K88-positive *E. coli* produced LT more frequently in animals of postweaning age than LT and STb concurrently, but is in agreement with a more recent report.

Historically, in the United States, a majority of edema disease isolates have been F18 SLT or F18 SLT STb (Bosworth, unpublished data, 1997). In our survey, F18 positive fimbriae were most often found in association with SLT STa STb. The increase in this specific genetic combination could be due to clonal expansion.

Nine isolates were negative for fimbrial genes. These could be strains that either have lost the capability to produce fimbriae due to loss of a plasmid during culture, or may suggest a novel fimbrial type not currently characterized. Another reason could be minor (even silent) changes in sequence, which cause primers to fail to bind. Results of this survey demonstrate the high frequency of strains containing F18 fimbriae in North Carolina swine. These strains typically are resistant in vitro to most of the currently approved swine antimicrobials (Post, unpublished data, 1998). Since there is no currently available commercial vaccine that contains this fimbrial type, most control methods have involved dietary management, sanitation, and the oral use of live, nontoxicogen strains to impart localized immunity in at-risk animals. Nontoxicogen strains, which may be naturally harbored in the intestines of some pigs, have been theorized to be natural immunogens that may protect against disease in herds.

### Implications
- There is a higher frequency of F18 strains than K88 strains in North Carolina hogs during the postweaning period.
- The majority of these strains carry SLT STa STb and could potentially cause both edema disease and diarrhea.
- The lack of commercially available vaccines make immunologic prophylaxis difficult.
- Additional research is needed to formulate adequate prevention and control measures.

### References—referred

### Table 1: Virulence factors detected in 175 *Escherichia coli* isolates from symptomatic postweaning-aged pigs

<table>
<thead>
<tr>
<th>Fimbriae</th>
<th>No. of strains</th>
<th>LT</th>
<th>SLT</th>
<th>STa</th>
<th>STb</th>
<th>LT</th>
<th>SLT</th>
<th>STa</th>
<th>STb</th>
<th>LT</th>
<th>SLT</th>
<th>STa</th>
<th>STb</th>
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<tr>
<td>F18</td>
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<td>1</td>
<td>5</td>
<td>8</td>
<td>5</td>
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<td>2</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>56</td>
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<td></td>
</tr>
<tr>
<td>K88</td>
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<td>72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>None</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>56</td>
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<tr>
<td>Total</td>
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<td>6</td>
<td>82</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>9</td>
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<td>3</td>
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### References—nonreferred