Occurrence and Characteristics of Enterotoxigenic
Escherichia coli Isolated from Calves with Diarrhea

L. L. MYERS* AND P. A. M. GUINÉE

Veterinary Research Laboratory, Montana State University, Bozeman, Montana 59715* and Laboratory of Bacteriology, National Institute of Public Health, Bilthoven, The Netherlands

Received for publication 12 December 1975

Of 1,004 isolates of Escherichia coli obtained during the spring of 1975 in seven different states from calves with diarrhea, 124 isolates were enterotoxigenic based upon ability to cause distention of the calf ligated intestinal segment. Isolates of enterotoxigenic E. coli (ETEC) were obtained from calves in six of the seven states. ETEC were detected in calves in 118 of 355 herds in Montana during the 1974 and 1975 spring beef calving seasons. The occurrence and serotypes of ETEC isolated from calves in states outside Montana were similar to ETEC isolated from calves in Montana. One hundred and fourteen of the 124 isolates of ETEC were placed in one of six different groups upon agglutination in OK antiserum. Serotyping of 35 of the 124 isolates of ETEC indicated the following serotype for isolates in each group: group 1, O9:K35; group 2, O101:K30; group 3, O8:K85; group 4, O20:K7; group 5, O8:K25; and group 6, O101:K28. Determination of the presence of K99 antigen indicated that 28 of 35 isolates of ETEC had K99 antigen, whereas the antigen was not detected in any of the 10 isolates of non-ETEC studied.

Enteric colibacillosis (diarrheal disease caused by enteropathogenic Escherichia coli) in calves is generally thought to be common in occurrence throughout many countries of the world. There is, however, little definitive information concerning the importance of enteric colibacillosis in calves, primarily due to the difficulty in accurately differentiating enteric colibacillosis from diarrheal syndrome in calves caused by agents other than E. coli.

It was reported earlier that enterotoxigenic E. coli (ETEC) isolated in Montana from calves with diarrhea could be placed in six different groups based upon agglutination with OK antiserum (5). Because of a lack of serotyping information these groups were numbered 1 through 6 for convenience. In this study the occurrence and certain characteristics (including serotyping information) are reported for E. coli isolated in several states during the spring of 1975 from calves with diarrhea.

MATERIALS AND METHODS

Obtaining cultures of E. coli. Isolates of E. coli were obtained from fecal specimens or intestinal contents of calves with diarrhea in 200 different herds in Montana. In addition, 204 E. coli cultures on agar slants were supplied by personnel in six states in addition to Montana. E. coli were streaked on Tergitol-7 agar plates

1 Contribution of the Montana Agricultural Experiment Station, submitted as Journal Series no. 645.

(Difco Laboratories, Detroit, Mich.) with 100 mg of triphenyltetrazolium chloride added per liter. After overnight incubation at 37 C, an isolated colony representative of each colonial type or color present was restreaked on a Tergitol-7 agar plate and incubated overnight. Pure cultures were stored in tryptose agar slabs or slants at room temperature in the dark. Confirmation that the isolates were E. coli was done using standard biochemical tests (2).

Colonial characteristics. Colonies were studied prior to initial storage and described as smooth, smooth and mucoid, intermediate, or rough. Colonies were described as mucoid if a strand of mucous-like material was evident when a wire inoculating loop was withdrawn from a colony after overnight incubation at 37 C on a Tergitol-7 agar plate.

Determination of enterotoxigenicity. The calf ligated intestinal segment test (6) was used routinely to differentiate ETEC from non-ETEC (NETEC). All E. coli isolates were inoculated into one ligated segment of the small intestine of at least one calf. Isolates that caused significant fluid accumulation (6) in the ligated intestinal segment of one calf were checked in one or more additional calves. An isolate was considered enterotoxigenic if it caused significant fluid accumulation in the ligated intestinal segment of two calves.

K99 antigen. The presence of K99 antigen (7) was evaluated by one of us (P. A. M. G.), without prior information regarding enterotoxigenicity, in 45 E. coli isolates, 35 of which were isolates of ETEC. The strains were cultured on a slightly enriched synthetic medium to prevent masking of the K99 antigen by abundant K-polysaccharide formation. The K99 antiserum was prepared with strain WS10,
kindly provided by W. J. Sojka, Weybridge, England. The serum was absorbed with an ultrasonicate of strain WS10 grown at 18 C. Details of the medium and antiserum will be published elsewhere (Guinée, manuscript in preparation).

Agglutination of *E. coli* using OK antisera. Rabbit OK antisera were prepared, using standard procedures (3), against 50 isolates of ETEC obtained earlier in Montana from calves with diarrhea (5). It was shown that OK antiserum prepared against six different isolates of ETEC agglutinated all of the isolates of ETEC. For this study these six different OK antiserum were reacted in the direct bacterial agglutination test as previously described (5) in the various states.

Serotyping of *E. coli*. The O antigen was determined using a mechanized microtechnique described earlier (4). The technique was further developed for typing of the K antigen. K antigen suspensions were prepared by suspending the growth of a nutrient agar slant in 9 ml of formalized (0.5% formalin) NaCl (0.5% solution containing gentian violet (0.009%, wt/vol). Each isolate was tested against the complete set of standard OK antiserum. By means of the multiple dropper, 0.037 ml of each antiserum was delivered into a cup in a plastic tray. The tray was mechanically shaken on a rotary shaker (150 shakes/min) for 10 min and then read on an illuminated screen. Positive or suspect positive reactions have to be confirmed by means of a slide agglutination test, since the mechanical procedure gives no information about the type of agglutination (O, K, or H agglutination). The antiserum used in the multiple dropper were diluted 50% less than those used in the slide agglutination.

**RESULTS**

A total of 1,004 isolates of *E. coli* were obtained in seven different states during the spring of 1975 from calves with diarrhea, with 124 of these isolates being enterotoxigenic based upon ability to distend the ligated intestinal segment of the young calf (Table 1). One hundred and fourteen of the 124 isolates of ETEC were agglutinated by one of the six different OK antiserum. Seven of the 10 *E. coli* isolates that could not be grouped had the intermediate colony type and were agglutinated by more than one of the six OK antiserum. Most of the *E. coli* isolates in groups 1, 2, 4, and 5 were enterotoxigenic, whereas there was little correlation between enterotoxigenicity and group number for *E. coli* isolates in groups 3 and 6 (Table 1).

The group number and colony type for Montana isolates of ETEC were as follows: 1, smooth; 2, smooth and mucoid; 3, intermediate; 4, intermediate; and 5, intermediate. All but one isolate of ETEC in group 6 had the smooth colony type. Seventeen of the 23 *E. coli* isolates with the smooth, mucoid colony type were enterotoxigenic. The six NETEC isolates with the smooth, mucoid colony type could not be classified in any of the six antigentic groups.

**Serotype.** Serotyping results given in Table 2 for 35 isolates of ETEC indicate that there are six different serotypes of ETEC. These six serotypes correspond with group numbers 1 through 6 from the stand point of K antigen content. Less heterogeneity exists between isolates in different groups concerning the content of O antigen, since all isolates of ETEC have one of only four different O antigens. Serotyping of a total of 10 isolates of NETEC in groups 1 through 6 indicated that seven of the 10 isolates of NETEC have the same K antigen as isolates of ETEC in the respective groups; however, only 2 of the 10 isolates of NETEC have both the O and K antigens characteristic of isolates of ETEC in the respective groups.

K99 antigen determination. K99 antigen was detected in 28 of the 35 isolates of ETEC studied (Table 2). There were seven isolates of ETEC (representing four of the six different serotypes of ETEC) in which K99 antigen was not detected. K99 antigen was not detected in any of the 10 isolates of NETEC studied.

**DISCUSSION**

Results of a study of *E. coli* isolated in Montana during the 1974 (5) and 1975 calving seasons from calves with diarrhea indicate that ETEC were detected in 118 of 355 (33%) of the herds. The isolates of ETEC represent six dif-

---

**Table 1. Number of isolates of ETEC and NETEC with antigen numbers 1 through 6**

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of isolates</th>
<th>No. of isolates of ETEC</th>
<th>Antigen no.*</th>
<th>Unclassified ETEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Montana</td>
<td>800</td>
<td>8,3</td>
<td>17,6</td>
<td>33,41</td>
</tr>
<tr>
<td>South Dakota</td>
<td>119</td>
<td>8,0</td>
<td>4,0</td>
<td>1,17</td>
</tr>
<tr>
<td>Colorado</td>
<td>49</td>
<td>2,0</td>
<td>0,0</td>
<td>5,3</td>
</tr>
<tr>
<td>Kentucky</td>
<td>18</td>
<td>0,0</td>
<td>0,0</td>
<td>0,1</td>
</tr>
<tr>
<td>Idaho</td>
<td>13</td>
<td>2,0</td>
<td>0,0</td>
<td>0,1</td>
</tr>
<tr>
<td>Oregon</td>
<td>4</td>
<td>0,0</td>
<td>0,0</td>
<td>0,1</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1</td>
<td>0,0</td>
<td>0,0</td>
<td>1,0</td>
</tr>
</tbody>
</table>

* The first number in each column represents the number of isolates of ETEC and the second number represents isolates of NETEC.
The occurrence and antigenic group of isolates of ETEC from six states other than Montana were similar to that found in Montana, indicating that enteric colibacillosis may be important in other states. Too few cultures of E. coli were studied from states other than Montana to accurately evaluate the occurrence and types of ETEC on an individual state basis. Earlier reports (1, 7) suggest that ETEC with serotypes O9:K35, O101:K30, and O8:K85 (groups 1, 2, and 3) are associated with colibacillosis in calves throughout the world. This association with colibacillosis has not previously been shown for strains of ETEC in groups 4, 5, and 6 (O20:K?, O8:K25, O101:K28).

There is a good correlation between group number and enterotoxigenicity for E. coli isolates in groups 1, 2, and 5, with 56 of the 67 (84%) isolates in these three groups being enterotoxigenic. In contrast, only 56 of 164 (34%) of the E. coli isolates in groups 3 and 6 were enterotoxigenic. We have made only three isolations of E. coli in group 4 during the past 2 years (one isolation in each of three different states, Montana in 1974 and Wisconsin and Kentucky in 1975). All three isolates in this group had the intermediate colony type and were enterotoxigenic.

Serotyping results indicated that one isolate of NETEC in group 2, three isolates of NETEC in group 3, and four isolates in group 6 had a different serotype than the isolates of ETEC in the respective groups. The NETEC usually had the same K antigen but a different O antigen than ETEC in the same group.

There appears to be a positive correlation between presence of the K99 antigen and enterotoxigenicity, since none of the 10 isolates of NETEC had the K99 antigen whereas 80% (28 of 35) of the isolates of ETEC were positive for the K99 antigen.

ACKNOWLEDGMENTS

This research was performed as a contribution to Western Regional Project W-112. The technical assistance of Patricia Shadoan is appreciated. Appreciation is expressed to personnel of the Montana Department of Livestock, Animal Health Division, Diagnostic Laboratory, for supplying cultures of E. coli isolates from calves with diarrhea. The cooperation of individuals in supplying E. coli cultures from the several states in addition to Montana is also acknowledged.

LITERATURE CITED