Biotypes of Enteropathogenic *Escherichia coli* Strains from Rabbits

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Forty-five enteropathogenic (enteropathogenic *Escherichia coli*-like) strains isolated in commercial rabbit farms were subdivided into four biotypes with the help of six carbohydrate fermentation tests, ornithine decarboxylase tests, and motility tests. *E. coli* strains which caused neonatal diarrhea in baby rabbits belonged to a single biotype, biotype 1, and displayed the same serotype antigens. Biotype 2 strains provoked diarrhea of variable severity in weaned rabbits and were also pathogenic for baby rabbits under laboratory conditions. Biotype 1 and biotype 2 strains resembled each other in many respects. Biotype 3 and biotype 4 (represented by a single strain) were highly pathogenic for weaned rabbits, but they provoked no symptoms or lesions in baby rabbits. Biotype 3 formed a homogeneous group of nonmotile strains which possessed the O15 antigen. Forty-two strains from nondiarrheic rabbits constituted a very heterogeneous collection of biotypes, most of which could easily be differentiated from the pathogenic types. Biotyping can be used to recognize rabbit pathogenic strains.

*Escherichia coli* strains, enteropathogenic for rabbits, with an unknown mechanism resembling that of the human enteropathogenic *E. coli* strains (EPEC), have been described by several authors (1, 5, 7). Disease provoked by these strains seems to be widespread in industrial rabbit farms in Belgium (7). Renault et al. (10) reported findings which suggest that other *E. coli* strains isolated in France are of the enterotoxigenic *E. coli* type.

The diagnosis of the EPEC-like disease requires histological examination of the intestines of a diarrheic rabbit immediately after its death. Indeed, easily demonstrable bacteriological properties which could distinguish the enteropathogenic from nonpathogenic strains are not known: the EPEC strains do not produce heat-stable or heat-labile toxins nor are they invasive. Moreover, the strains belong to several O groups (7, 9, 10, 12). Serotypes of rabbit *E. coli* strains have been reported by several other investigators as well (12), but these authors have not tried to assess the pathogenic importance and virulence of their strains.

Human EPEC strains isolated during a period of 9 years and in 22 countries from different continents were biotyped by Stenderup and Ørskov (11). They proved that these strains all belonged to a limited number of clones. A similar relationship was sought for the rabbit EPEC-like strains. It was of interest to establish the relationship of different biotypes with different properties, i.e., pathogenicity for newborn or weaned rabbits and differences in virulence.

Therefore, enteropathogenic strains isolated from scouring rabbits on different farms or on the same farms on different occasions were biotyped together with strains from rabbits without intestinal symptoms. Biotypes of strains from diarrheic rabbits were correlated with pathogenic properties described in the literature and with results of some additional virulence tests described in this paper.

**MATERIALS AND METHODS**

**Strains.** *E. coli* RDEC-1 was received from J. Cantey, University of South Carolina, Charleston. Twenty enteropathogenic strains were isolated in our laboratory from 15 different farms situated in Belgium and the Netherlands. Effects of two representative strains of this group on neonatal and weaned rabbits have been described (4, 5). Twenty-five strains from 10 farms were kindly provided by J. Peeters, National Institute of Veterinary Research, Brussels, Belgium. The pathogenic properties and serotypes of this second group have already been described (7). The presence of coccobacilli attaching to the brush border of the intestinal wall had been proved for 42 of the original 45 rabbits from which these strains were isolated. Enteropathogenicity of the three other strains was demonstrated by experimental infection (see below). Six strains were isolated in four farms from newborn or unweaned (less than 2 weeks old) rabbits. The remaining 39 strains originated from the cecal contents of recently weaned rabbits (usually 5 to 8 weeks of age) in 21 rabbit farms.

Another 42 strains were isolated from rectal swabs from normal rabbits. The majority of these rabbits were healthy and originated from our SPF unit or a small rabbit farm free of diarrheal epidemics for the last 6 years and with an extremely low mortality rate after weaning.

**Experimental infections.** Suspensions in 0.9% NaCl prepared from growth on Tryptone Soya Agar (Oxoid, Basingstoke, England) containing approximately 10⁹ CFU per ml were given orally to weaned rabbits (0.5 ml) or 4- to 7-day-old baby rabbits (0.1 ml). The rabbits used for experimental infections were bred at our SPF unit or were Belgian Silver rabbits (a local breed related to Argenté de Champagne) originating from the small rabbit farm mentioned above. Those rabbits were free from *Pasteurella multocida* but not *Bordetella bronchiseptica* and *Eimeria* spp. Mortalities were recorded over 2 weeks. The rabbits which died or were moribund were necropsied to check for the presence of typical macroscopic and microscopic lesions for *E. coli* diarrhea as described previously (4).

**Biotyping.** Fermentation of carbohydrates was tested on phenol red broth base (Difco Laboratories, Detroit, Mich.) in repli dishes (Sterilin; Teddington, Middlesex, England). Results were read on 3 successive days. Positive reactions seen after 1 or 2 days which became negative upon further incubation were still considered positive.

Initially the following 15 carbohydrates were tested:
TABLE 1. Characteristics of different pathogenic E. coli biotypes from rabbits

<table>
<thead>
<tr>
<th>Biotype</th>
<th>Ability to ferment:</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>Dulcitol</td>
</tr>
<tr>
<td>1 (neonatal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (RDEC-1 type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Positive after 2 or 3 days.

† Only 16 of the 18 biotype 2 strains were positive.

Adonitol, L-arabinose, D-cellulose, dulcitol, n-inositol, lactose, maltose, D-raffinose, L-rhamnose, salicin, D-sorbitol, sorbose, sucrose, D-trehalose, and D-xylose. Cellulose, dulcitol, raffinose, rhamnose, sorbose, and sucrose were retained in the biotyping scheme (Table 1). Salicin was eliminated because the results could not always be reproduced, and the others were eliminated because the results were always negative or always positive.

Motility and ornithine decarboxylase were tested on MIO medium (GIBCO Laboratories, Paisley, Scotland) and read after 24 h of incubation at 37°C. Lysine decarboxylase was tested on lysine decarboxylase broth (Difco). As the tested strains were all positive, this last property was not included in the biotyping procedure.

Representative strains of different groups were tested with the 50 carbohydrates of the API-50 CH system (API, La Balme les Grottes, France) to detect further differences or similarities.

RESULTS

Biotypes. All E. coli strains which had been found to be pathogenic for rabbits by histological examination or by means of experimental infection belonged to four biotypes, as shown in Table 1. Their origins and serotypes are tabulated in Table 2.

Six strains isolated from cases of E. coli diarrhea in newborn rabbits from four farms and three strains from diarrhea in weaned rabbits from three farms belonged to the neonatal biotype 1. One of the latter strains originated from one of the farms at which the neonatal E. coli diarrhea had been diagnosed, but its flagellar antigen differed from those of the neonatal strains. The fermentation patterns of the strains from newborn and weaned rabbits were identical when tested with the API-50 CH system.

The reaction pattern of biotype 2 strains differed from that of biotype 1 only in its slow fermentation of dulcitol. Eighteen strains belonged to this group. They were isolated from weaned rabbits in nine different farms. E. coli E 326, used in the pathogenicity experiments described by Okerman et al. (4), belonged to this biotype.

Biotype 3 contained the RDEC-1 strain described by Cantey and Blake (1) and 16 strains from weaned rabbits in 13 Belgian and Dutch farms. This group was also called RDEC-1 biotype.

Only three strains from weaned rabbits originating from one farm were found to belong to biotype 4. Eleven different biotyping patterns were demonstrated in the 42 strains isolated from rabbits without intestinal troubles in four rabbit farms (Table 3). All strains were motile.

Four strains belonged to the biotype 1 seen in the neonatal rabbit diarrhea. They differed, however, from the neonatal pathogenic strains in their production of acid from D-arabinose (after 48 h) and β-gentiobiose (after 48 h).

Virulence tests. The biotype 2 strains E 326 (not typeable), 266/3380 (0132:H+), and U 82/172 (O109:H2) were highly pathogenic for baby rabbits. All infected animals, comprising three litters of three different does, died or were moribund when killed for histological examination. The bacterial cells adhered to the villous border of cecum, colon, and to a lesser extent small intestine and caused a watery diarrhea. No symptoms or mortality were seen after experimental infection of baby rabbits with the biotype 3 strain 266/3305 (O15:H−) and the biotype 4 strain E 452 (not typeable).

Differences in virulence for weaned rabbits found in simultaneously conducted experiments with rabbits of the same origin and age are shown in Table 4. In experiment 1, the biotype 3 strains E 503 and E 398 (O15:H−) gave more symptoms than the biotype 2 strain 266/3380. Similar results were obtained in experiment 2 with two other biotype 2 strains E 326 and 266/2336 (not serotyped). In experiment 3, the biotype 2 strain E 326 did not cause any symptoms, whereas the biotype 3 strain killed one of five infected animals, and the biotype 4 strain killed or severely affected all inoculated rabbits. The results of experiment 4 confirmed the lack of effect of neonatal strains (biotype 1) on weaned rabbits, as described earlier (5). They also demonstrated the
difference in pathogenicity of biotypes 1 and 2, which differ only slightly otherwise from each other.

**DISCUSSION**

Pathogenic *E. coli* from rabbits apparently are not a homogeneous group. We concluded from our experiments that three different types of *E. coli* rabbit enteropathy are important in Belgium and the Netherlands.

(i) **Neonatal diarrhea.** Strains isolated from this type of diarrhea produce severe symptoms and death in rabbits less than 2 weeks of age and no disease and minimal histological lesions in weaned rabbits (2, 5–7).

(ii) **E. coli enteropathy caused by biotype 2 strains.** *E. coli* enteropathy caused by biotype 2 strains was characterized by variable but usually low mortality in weaned rabbits (4). Varga and Pesti (12), who tested the pathogenic effects of a serotype 132:H2 strain, found that the addition of NaHCO3 was necessary for the expression of virulence of *E. coli* in experimental infections. Strains of the same serotype were shown to belong to the low-pathogenic biotype 2 in our study (Table 2). The biotype 2 strains were serologically diversified, although all strains in which the flagellar antigens could be examined possessed flagellar antigen H2. This antigen was also present in the neonatal biotype 1 strain. Biotypes 1 and 2 seem to be related also in the virulence traits; although the biotype 2 strains were not found in neonatal diarrhea, they were as pathogenic to baby rabbits as biotype 1 strains isolated from neonatal diarrhea. Strain U 82/172, which belongs to biotype 2, was originally described as nonpathogenic for newborn rabbits (6), but it was found to be highly pathogenic for 5-day-old rabbits in our experiments.

(iii) **E. coli enteropathy characterized by high mortality in weaned rabbits caused by strains of biotype 3 and 4.** Strains belonging to these biotypes caused death or severe stunting in 15 of 20 weaned rabbits (Table 4), whereas in six experiments with the biotype 2 strain E 326 described by Okerman et al. (4), only 11 of 31 died or had severe diarrhea with growth retardation. Similar results were obtained in the present experiments. Considering the total number of rabbits infected with biotype 2 strains, described earlier and here, the difference in virulence between biotype 2 and biotype 3 strains is statistically significant (*P* < 0.05). The RDEC-1 strain of Cantey and Blake (1) and the highly pathogenic strain U 83/29 described by Peeters et al. (6) belong to biotype 3. These strains are not pathogenic for rabbits less than 2 weeks of age. Biotype 3 strains isolated from different farms and different countries were remarkably similar serologically (Table 2). The O antigen 15 and the lack of flagellar antigens and motility in the strains are useful diagnostic traits.

**TABLE 4. Comparison of pathogenic effect of strains belonging to four different biotypes for weaned rabbits (four experiments)**

<table>
<thead>
<tr>
<th>Expt and strain</th>
<th>Biotype</th>
<th>Effect on baby rabbits</th>
<th>No. of rabbits showing diarrhea/ no. of rabbits*</th>
<th>No. of dead/ no. of rabbits*</th>
<th>No. of surviving stunted rabbits/ no. of rabbits*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>E 326</td>
<td>2 + 5/5</td>
<td>0/5</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>266/3380</td>
<td>E 303</td>
<td>3 NT* 5/5</td>
<td>3/5</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>E 398</td>
<td>3</td>
<td>5/5</td>
<td>4/5</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>E 326</td>
<td>2 + 2/5</td>
<td>2/5</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>266/2336</td>
<td>E 326</td>
<td>2 NT* 2/5</td>
<td>1/5</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td>E 326</td>
<td>2 + 0/5</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>266/3305</td>
<td>E 452</td>
<td>4 + 5/5</td>
<td>4/5</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Experiment 4</td>
<td>E 326</td>
<td>2 + 4/5</td>
<td>2/5</td>
<td>2/3</td>
<td></td>
</tr>
<tr>
<td>E 300</td>
<td>1</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

* No pathogenic effects were found in the 4 control rabbits in experiment 1 or the 5 controls in experiments 2, 3, or 4.

* Not tested.
mortality rates to acceptable levels. Although strains of this biotype are pathogenic for newborn rabbits, diarrhea in baby rabbits was not reported by the owners. All rabbit farms infected with biotype 3 strains experienced high mortality throughout the broiler units. Most of the affected farms had obtained breeding material from the same farm. The farm from which the similarly highly virulent biotype 4 strain was recovered had an economically unacceptable high mortality despite continuous administration of antibiotics.

Biotyping of a strain is not sufficient to prove its enteropathogenicity. Attachment of colonizing E. coli by histological examination of the intestines of a diseased untreated rabbit is still required. However, biotyping can show which type of E. coli diarrhea affects the rabbit farm, and this information is useful for prognosis and treatment. In addition, repeated isolations of the same biotype from different diarrheic rabbits originating from the same farm is an indicator of pathogenicity of the strain involved.

ACKNOWLEDGMENTS

We thank W. Coussement, L. van den Broeck, J. Peeters, P. Pohl, and P. Lintermans for help in various ways.

LITERATURE CITED


