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Biotype, Serotype, and Pathogenicity of Attaching and Effacing Enteropathogenic *Escherichia coli* Strains Isolated from Diarrheic Commercial Rabbits

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A total of 568 strains of *Escherichia coli* isolated from healthy and diarrheic rabbits were separated into 11 different biotypes according to the fermentation patterns of four carbohydrates. Strains belonging to biotypes 1 to 3, 6, and 8 induced lesions characteristic for attaching and effacing *E. coli* (AEEC). They attached to the intestinal epithelium of the terminal small intestine and the large intestine of 5-week-old rabbits after experimental infection and caused effacement of the microvillous brush border. However, pathogenicity for weaned rabbits, as judged by diarrhea score, anorexia, and reduced weight gain, varied according to the biotypes of the strains. Strains belonging to biotypes 1 and 6 produced only discrete clinical signs, strains belonging to biotypes 2 and 3^+ (motile) induced diarrhea and growth depression, whereas strains belonging to biotypes 3^- (immotile) and 8 caused severe clinical signs and high mortality. This confirms evidence from the field. Biotypes 3^- and 8, accounting for 35.5 and 7.1% of AEEC strains in weaned diarrheic rabbits, respectively, were not detected in weaned healthy rabbits, while biotype 2 was the predominant strain in weaned healthy rabbits (62.3%). Finally, serotyping showed a close relationship between biotype and serotype of the AEEC examined. Most strains of biotypes 1^+ and 2^+ tested were O109:K^-:H2 and O132:K^-:H2, respectively, whereas all strains tested of biotype 3– were O15:K^-:H^- and those of biotype 8 were O103:K^-:H2. These data indicate that specific clones of AEEC might be involved in juvenile rabbit enteritis. It was concluded that determination of biotypes allows the screening of highly pathogenic AEEC in weaned rabbits (biotypes 3^- and 8).

In 1979 Cantey and Blake (3) described a highly pathogenic strain of *Escherichia coli* in weaned rabbits. This strain, RDEC-1, was able to induce watery diarrhea and high mortality after experimental infection. Similar observations have been made with other strains in England (18), Belgium and The Netherlands (16), and France (2). None of these strains produced heat-labile or heat-stable enterotoxins, nor were they enteroinvasive. So they are considered to be enteropathogenic *E. coli* (EPEC), according to the definition of Levine et al. (5). Histology and electron microscopy showed these strains to be tightly adherent to the brush border of intestinal epithelial cells after experimental infection and to cause effacement of microvilli, followed by epithelial desquamation, villous atrophy, and malabsorption. Moon et al. (7) called such strains attaching and effacing *E. coli*. Attaching and effacing enteropathogenic *E. coli* (AEEC) are now considered to be an important cause of diarrhea in suckling and weanling rabbits (2, 9). Mortality varies from very low to very high, according to the strains involved. Strains of low pathogenicity mostly cause problems in rabbitries with poor hygiene and can easily be controlled by antibiotic treatment and hygienic measures. In case of highly pathogenic strains, on the contrary, most antibiotics fail to overcome the disease and often the whole rabbit stock must be killed and replaced (14). Therefore, early differentiation of the pathogenicity of the strains is important for prognosis and treatment.

At present the definite diagnosis of colibacillosis in rabbits is based on the demonstration of the typical lesions of AEEC by histology. Nevertheless, infection experiments confirmed the existence of AEEC strains with different tropisms for age group and intestinal compartment. AEEC isolated from suckling rabbits attach to the full length of the epithelial lining of the small and large intestines, which is followed by yellow diarrhea and high mortality (11, 13), whereas strains isolated from weanling rabbits have been shown to attach only to the epithelium of the distal small intestine and the large intestine (2, 3, 10, 15). The rate of mortality depends on the strains inoculated. Cross infection experiments showed that suckling rabbit strains may also attach to the intestinal mucosa of weaned rabbits and vice versa (10, 13, 17). In this case mortality is quite low. This means that histological demonstration of AEEC only confirms that EPEC are involved in a particular case of enteritis, but describes nothing of the pathogenicity of the strains involved.

Strains of different serotypes have been shown to be involved in rabbit enteritis by experimental infection experiments. Serotype O109:K-:H2 is mainly associated with yellow diarrhea in suckling rabbits, whereas other serotypes (O15:H^-, O20:H7, O103:H2, O128:H2, O132:H2, O153) are associated with diarrhea in weanling rabbits (2, 3, 17, 18, 19). Strains belonging to serotypes O15:H^- and O103:H2 are highly pathogenic, and low numbers of the strains may cause mortality of 50% or greater. Strains belonging to the other serotypes show moderate pathogenicity and require higher levels of infection to cause clinical signs.

Okerman and Devriese (10) compared the fermentation patterns of 45 EPEC strains from 26 rabbitries with those of 42 healthy rabbit strains from mainly 2 rabbitries. They concluded that biotyping can be used to recognize rabbit EPEC. In other species it has been shown that biotyping of *E. coli* strains is highly discriminatory and reliable and

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defines types of *E. coli* that are extremely stable both in vivo and in vitro (4). Therefore, we decided to relate the biotype of EPEC, isolated from diarrheic rabbits from 61 commercial rabbitries during a field survey, with their pathogenicity in the field and after experimental infection.

MATERIALS AND METHODS

Strains. A total of 568 strains of *E. coli* were included in this study; 191 strains were isolated from 191 diarrheic rabbits during a field survey in Belgium and The Netherlands. The strains were picked out at random from primary plates inoculated with cecal contents. All the rabbits from which these strains were isolated showed histologic lesions of AEEC and belonged to different age groups; 6 strains were isolated from reproduction stock in 3 rabbitries, 155 strains originated from weaned rabbits from 50 rabbitries, and 36 strains were isolated from suckling rabbits from 14 rabbitries. Another 218 strains were isolated from 449 diarrheic rabbits without demonstrable histologic lesions of AEEC, but 152 of these strains were isolated in rabbitries with simultaneous problems of AEEC. Five enteropathogenic strains were received from other laboratories. Strain RDEC-1 (O15:H^-) was kindly provided by J. Cantey (University of South Carolina, Charleston), strains V2700 and N6651 (both O103:K^-:H2) were provided by L. Renault (Laboratoires Vétérinaires Sanders, Athis-Mons, France), and strains 5/1 and 5/2 (both O103) were from E. Facchin (Istituto Zooprofilattico Sperimentale delle Venezie, Verona, Italy). Finally, 154 strains were isolated with fecal swabs from 386 healthy weaned rabbits in 8 rabbitries without demonstrable problems of colibacillosis.

Serotyping. The O:K:H serotypes of 62 selected strains showing attaching and effacing properties after experimental infection were examined by standard methods (12).

Biotyping. Biotyping was performed by the scheme of Okerman and Devriese (10). After primary isolation each strain was passaged twice on blood agar (tryptose blood agar base [Difco laboratories, Detroit, Mich.] with 5% [vol/vol] sheep blood). Then, two sterile tubes with nutrient broth were inoculated with one colony each and incubated aerobically for 4 h at 37°C. A standard volume (about 0.10 ml) of the resulting bacterial suspension was used to inoculate the media. All tests were made in duplicate, and only concordant results were recorded.

Fermentation of carbohydrates was tested on phenol red broth base (Difco). The following carbohydrates were prepared as 10% (wt/vol) solutions in deionized water: D-cellobiose, dulcitol, D-raffinose, L-rhamnose, sorbose, and sucrose. They were sterilized by filtration and added to sterile basal medium at a final concentration of 0.5% (wt/vol). The completed media were dispensed in 5-ml volumes into sterile, capped, 10-ml tubes. Results were read after 48 h of incubation at 37°C. Motility and ornithine decarboxylase were tested on MIO medium (GIBCO Laboratories, Paisley, Scotland) and read after 24 h of incubation at 37°C. In case of questionable motility, strains were subjected to transmission electron microscopy to confirm the presence or absence of flagellae.

Experimental infection studies. Experimental infections were carried out with strains that were lyophilized after primary isolation. The inoculum was prepared from second-passage organisms grown on blood agar by inoculating colonies into nutrient broth and incubating them aerobically for 6 h at 37°C.

A total of 336 coccidia-free New Zealand White rabbits were used. After the rabbits were weaned at 4 weeks, they were housed individually in heat-sterilized, wire-floored, metal cages for 1 week before experimental infection. They were kept at an ambient temperature of 18 to 20°C and received a commercial pellet ration with 16% crude protein and 15% crude fiber ad libitum. The feed did not contain any antimicrobial additive. Fecal examination confirmed the absence of coccidia.

For each strain two rabbits were infected orally with 2 ml of inoculum containing approximately 2×10^6 CFU. In each series of experiments, four rabbits remained uninfected and served as negative controls. Rabbits were checked for feed intake and weight gain 7 and 10 days postinfection and for diarrhea on a daily basis. At 7 and 10 days postinfection one rabbit was killed and necropsied. Segments of the terminal small intestine, cecum, and proximal colon were processed for histology. Coliform bacteria that attached to the intestinal mucosa were traced in hematoxylin- and eosin-stained sections at magnifications of ×500 and ×1,000 with a microscope (Laborlux 12; Leitz/Cpto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.). A bacterium was considered attached to an epithelial cell if it was immediately adjacent to the surface of the cell and if there was no mucus or other material between the bacterium and the cell surface.

The presence of *E. coli* in the duodenum, jejunum, ileum, and cecum was evaluated after plates of G2SN (77 g of Gassner agar [E. Merck AG, Darmstadt, Federal Republic of Germany], 3 g of yeast extract [GIBCO], 5 g of sodium thiosulfate · $5H_2O$, 0.5 g of ferric citrate, and 1,000 ml of distilled water [pH 7.2]; after autoclaving, 20 ml of 0.025% novobiocin was added) were streaked with intestinal contents and incubated at 37°C for 18 h. The number of lactose-positive colonies on the plates was evaluated semiquantitatively as follows: 0, no growth; 1, widely spaced colonies; 2, closely spaced colonies; 3, confluent growth of colonies. Coliform colonies were identified by the method of MacKenzie et al. (6). Previous experiments showed that a score of 1 was correlated with 10^2 to 10^4 CFU/g of intestinal content, a score of 2 with 10^5 to 10^7 of CFU/g, and a score of 3 with more than 10^7 CFU/g.

RESULTS

Biochemistry. The biochemical characteristics of 414 strains of *E. coli* isolated from diarrheic rabbits and of 154 strains from healthy rabbits were determined (Table 1). The strains generally gave the same result in the sucrose test as they did in raffinose test, whereas only 2% of the strains fermented cellobiose. Most strains belonged to biotypes 1 to 3, whereas none of the strains showed the fermentation pattern of biotype 4. The remaining 81 strains showed 14 fermentation patterns (biotypes 5 to 21) other than those described by Okerman and Devriese (10).

The occurrence of these biotypes in 154 weaned healthy rabbits and in 191 diarrheic rabbits with confirmed histologic lesions of AEEC is given in Table 2. In healthy rabbits seven fermentation patterns were detected; seven were detected in weaned diarrheic rabbits, and three were detected in suckling diarrheic rabbits (less than 4 weeks of age). Biotype 1 was predominant in suckling diarrheic rabbits and biotype 3 was predominant in weaned diarrheic rabbits. Immotile strains of *E. coli* belonging to biotype 3 (3^-) and motile strains of biotype 8 (8^+) were frequently detected in weaned diarrheic rabbits, whereas such strains were absent in healthy rabbits. Biotype 2 was the predominant biotype in healthy rabbits. Only three strains (two of biotype 1^+ and one of biotype 3^+) isolated from sick rabbits with lesions of AEEC were not able to decarboxylate ornithine.

TABLE 1. Biotypes of 568 strains of *E. coli* isolated from intestinal contents of rabbits[a]

Biotype	Ability to ferment:						No. positive/total no. for:		
	Cellobiose	Dulcitol	Raffinose	Rhamnose	Sorbose	Sucrose	Decarboxylation of ornithine	Motility	Simplified biotype[b]
1	−	−	+	+	−	+	110/113	111/113	1
2	−	+	+	+	−	+	203/207	196/207	2
3	−	+	+	+	+	+	156/163	65/163	3
4	−	−	+	−	+	+	0	0	4
5	−	−	−	−	−	−	0/1	1/1	5
6	−	+	−	+	−	−	2/12	10/12	6
7	−	+	−	+	+	−	11/11	9/11	7
8	−	+	+	−	−	+	19/19	19/19	8
13	−	−	+	+	+	+	2/5	5/5	13
18	−	+	+	−	+	+	1/1	1/1	18
19	−	−	−	+	+	−	1/2	1/2	19
20	−	−	−	+	−	−	0/5	3/5	20
11	+	+	+	+	−	+	0/1	1/1	2
12	+	+	+	+	+	+	1/11	11/11	3
15	−	+	+	+	+	−	6/6	1/6	3
16	−	−	−	+	−	+	0/4	1/4	20
17	−	+	−	+	−	+	4/6	0/6	6
21	+	+	−	+	+	−	0/1	1/1	7

[a] The system of Okerman and Devriese (10) was extended from 4 to 21 fermentation patterns.
[b] Biotype only was based on the fermentation of dulcitol, raffinose, rhamnose, and sorbose.

Biotype and pathogenicity in the field. All diarrheic rabbits from which strains of *E. coli* were isolated arrived alive in the laboratory and were carefully examined for the severity of histologic lesions and for the presence of AEEC in five different intestinal compartments. The results for the positive rabbits are given in Table 3 and are related to biotype and clinical signs.

Suckling rabbits colonized by strains of biotype 1^+ had a high mortality. In such rabbitries up to 20% of the litters were affected and mortality within the litters reached almost 100%. Usually no clinical signs of infection were established in the weaned rabbits from the affected rabbitries. Besides biotype 1, strains also belonging to biotypes 2 and 3 were isolated sporadically from suckling rabbits, but in contrast to strains of biotype 1, their occurrence was always associated with enteric problems in weaned rabbits. Mortality associated with these strains was only low to moderate. In suckling rabbits AEEC were often found (13 of 30 rabbits) to be attached to the full length of the epithelial lining of the small and large intestines.

In weaned rabbits high mortality (up to 50%) was detected in rabbitries infected with immotile strains of biotype 3 and motile strains of biotype 8. Biotype 3^- was detected in Belgian and Dutch rabbitries, whereas biotype 8 was isolated from diarrheic rabbits from Belgium, France, and Italy. Usually only weaned rabbits were affected. The other biotypes were associated with low to moderate mortality (Table 3) and mostly occurred in less hygienic or continuously occupied rabbitries. In most cases the AEEC were only found attached to the mucosa of the ileum, cecum, and colon and in severe cases were also attached to the mid-small intestine. Only biotype 8 was found to be associated with lesions in the proximal small intestine.

Biotype, serotype, and experimental pathology. The attaching properties of 122 strains isolated from diarrheic rabbits showing lesions of AEEC and of 31 strains isolated from healthy rabbits were examined after experimental infection of 4- to 5-week old rabbits. Strains from only 2 of the 31 healthy rabbits induced lesions of AEEC, whereas 72 of the 122 strains from diarrheic rabbits induced lesions. Almost all strains belonging to biotypes 3^- and 8^+ were shown to attach to the intestinal mucosa. A much smaller proportion of strains of the other biotypes did so (Table 4). There was a good correlation between biotype and serotype. Most strains of biotypes 1^+ and 2^+ tested belonged to serotypes O109:K^-:H2 and O132:K^-:H2, respectively, whereas all strains of biotype 3^- tested were 015:K^-:H^-, and those of biotype 8 were O103:K^-:H2 (Table 5).

More details on the pathogenicity of the attaching strains of *E. coli* are listed in Table 5. None of the strains in suckling rabbits belonging to biotype 1 caused distinct clinical signs in weaned rabbits, although discrete to moderate intestinal attachment was evident. Only a strain of biotype 3^+ caused growth depression.

TABLE 2. Number of *E. coli* strains belonging to different biotypes and isolated from healthy rabbits and diarrheic rabbits with histologic lesions of AEEC

Biotype	Motility	No. of healthy rabbits	No. of diarrheic rabbits with AEEC at age (wk):		
			<4	4–11	>11
1	+	22	15	34	0
	−	0	1	0	0
2	+	96	6	40	1
	−	0	3	4	0
3	+	16	1	6	0
	−	0	4	55	4
6	+	2	0	1	0
7	+	6	0	0	0
	−	2	0	0	0
8	+	0	0	11	1
12	+	9	0	0	0
15	−	0	0	2	0
17	−	1	0	2	0

TABLE 3. Clinical signs and lesions associated with 191 strains of *E. coli* isolated from diarrheic rabbits with histologic lesions of AEEC

Age group, biotype, and motility	No. of:		No. with diarrhea/total no.[a]	Mortality	Histologic lesions	No. with AEEC in duodenum/total no.
	Strains	Rabbitries				
Suckling rabbits						
1+	15	6	2/6	High	+++	6/15
1−	1	1	1/1	?	+++	1/1
2+	6	5	5/5	Low	++	4/6
2−	3	1	1/1	Moderate	++	0/3
3+	1	1	1/1	Low	+	1/1
3−	4	3	3/3	Moderate	+++	1/4
Weaned rabbits						
1+	34	17	2/17	Moderate	++	0/34
2+	41	13	5/13	Moderate	++	0/41
2−	4	3	1/3	Moderate	++	0/4
3+	6	6	1/6	Low	+	0/6
3−	59	21	3/21	Very high	+++	0/59
6+	1	1	0/1	?	+	0/1
8+	12	2	0/2	Very high	+++	7/12
15−	2	1	0/1	?	++	0/2
17−	2	1	0/1	?	++	0/2

[a] Diarrhea in both suckling and weanling rabbits.

Infection of weaned rabbits with strains from weaned rabbits, on the contrary, was almost always followed by clinical signs. The degree of clinical signs was related to the biotype. Rabbits infected with biotypes 1^+ and 6^+ exhibited only discrete clinical signs; those infected with biotpyes 2^+ and 3^+ induced diarrhea, anorexia, and growth depression, but no mortality within 10 days postinfection, whereas biotypes 3^- and 8^+ generally caused liquid diarrhea 3 to 7 days after inoculation, severe growth depression, and 12% mortality (5 of 42 rabbits) within 10 days postinfection for biotype 3^- and 44% (8 of 18 rabbits) for biotype 8^+. At necropsy watery cecal contents and cecal edema were common findings. Mesenteric lymph nodes were moderately to severely swollen, and so were Peyers patches. Histology showed ulceration of epithelial cells, with different degrees of attachment of *E. coli* to epithelial cells of the ileum, cecum, and ascending colon and with infiltration of polymorphonuclear leukocytes beneath the affected epithelium.

DISCUSSION

Since the discovery by Cantey and Blake (3) of AEEC strain RDEC-1 in weaned diarrheic rabbits in the United States, similar observations have been made in England (18), Belgium and The Netherlands (16), and France (2). These strains do not produce thermolabile or thermostable enterotoxins and are not invasive, as judged by the Sereny test. They are able to attach to the epithelial brush borders of the small and large intestines after experimental infection and to cause effacement of the microvillous border to colonized cells. This is followed by desquamation of enterocytes, villous atrophy, and diarrhea. Experimental infection with strains from healthy rabbits did not produce such effects.

AEEC have also been detected in suckling rabbits (11). All strains from suckling rabbits belonged to the same serotype, O109:K^-:H2 (17), whereas strains with different serotypes have been detected in weaned diarrheic rabbits showing lesions of AEEC:O15:H^- in the United States (3); O153 in England (18); O15:H^-, O20:H2, O109:H2, O128:H2, and O132:H2 in Belgium (17); and O103:K^-:H2 in France (2). The pathogenicity and the attaching properties of these strains have been confirmed by infection experiments. Serotypes O103:H19, O128:H2, and O132:H2 occurred frequently in Hungarian diarrheic weaned rabbits and produced diarrhea after experimental infection, but their attaching and effacing properties were not examined (19). The same serotypes were detected in diarrheic commercial rabbits during this study, and results of our infection experiments confirm their pathogenicities.

Epidemics of colibacillosis associated with serotype O15 have been reported in Belgium and The Netherlands, whereas in recent studies (1, 8) it has been shown that serotype O103 is largely spread among French rabbitries. According to the results of this study, serotype O103 is also being detected now in the neighboring countries of France: Belgium and Italy. This indicates that certain serotypes may spread. No data are available on the occurrence of AEEC in other countries.

TABLE 4. Attaching properties of *E. coli* strains belonging to different biotypes and isolated from diarrheic and healthy rabbits after experimental infection of 5-week-old rabbits

Biotype	No. of attaching strains/total no. of strains in:	
	Diarrheic rabbits	Healthy rabbits
1^+	17/25	0/2
1^-	1/1	
2^+	22/38	2/10
2^-	0/2	
3^+	5/15	0/11
3^-	15/17	
6^+	1/4	
6^-	0/1	
7^+	0/1	0/2
7^-		0/1
8^+	9/9	
11^-	0/1	
12^+	0/3	0/3
13^+		0/1
15^-	2/2	
16^-	0/1	0/1
17^-	0/1	
18^+	0/1	
Total positive	72/122	2/31

TABLE 5. Characteristics of strains of *E. coli* isolated from commercial diarrheic rabbits with lesions of AEEC and causing the same lesions in weaned rabbits after experimental infection

Age group (rabbitry)	Strain	Biotype	Serotype	Score of attachment[a]	Score of diarrhea[b]	Anorexia[c]	Growth depression[d]	No. died/total no.	Confluent growth of *E. coli* in:	
									Jejunum	Ileum
Suckling rabbits										
2	82/123	1^+	O109:K^-:H2	+	−	−	−	0/3	−	+
2	82/158		O109:K^-:H2	+	+	−	−	0/3	−	+
2	82/160		O109:K^-:H2	+	−	+	−	0/2	−	+
3	82/215/1		O109:K^-:H2	+	−	−	−	0/1	−	−
8	82/190		Rough:H2	++	+	+	−	0/3	+	+
3	82/215/2	1^-	O8	+	−	−	−	0/1	−	+
20	84/177	3^+	O2:K1:H6	+	+	+	++	0/2	+	+
Weaned rabbits										
3	82/172	1^+	O109:K^-:H2	++	++	++	ND[e]	0/2	+	+
3	85/33		O109:K^-:H2	+	+	−	−	0/2	ND	ND
3	85/237/1		O109:K^-:H2	+	−	−	−	0/1	−	−
13	84/221		Rough$^-$:H7	+	−	+	+	0/2	−	−
17	82/95/2		ND	++	+	+	++	0/3	−	+
17	82/207		O109:Hagg	++	+	+	ND	0/2	−	−
18	010		ND	+	−	+	+	0/2	−	−
18	82/196		Rough:K^-:H7	+	−	−	−	0/2	−	+
18	82/260		O20:K^-:H7	+	+	++	−	0/3	−	+
24	83/146		O153:K^-:H7	+	+	+	+	0/2	−	−
1	AP1	2^+	ND	++	+	−	+	0/2	−	+
1	AP7		ND	++	++	+	+	0/2	−	+
1	AP24		ND	++	+++	++	+++	0/2	−	+
7	85/260		O?:K^-:H11	++	+	+	+	0/2	+	+
9	84/250		O132:K^-:H2	+	−	+	++	0/3	−	−
9	H14		O132	+	+	+	++	0/2	+	+
9	H41		O132	+	−	+	++	0/2	−	−
10	L60		O132	++	+	−	+	0/2	−	−
12	83/45		O128:K^-:H2	++	++	++	ND	0/3	+	+
15	82/90		O132:K^-:H2	++	++	++	+++	0/5	+	+
15	82/131		O132	+	+	−	ND	0/2	−	−
15	82/138		Rough:H2	+	+	−	ND	0/3	+	+
15	82/146		O132:K^-:Hagg	+++	++	++	ND	0/3	−	+
15	82/150		O132:K^-:Hagg	++	+	+	ND	0/3	−	−
15	82/168		O132:K^-:H2	++	++	++	ND	0/3	+	+
15	82/183		O128:K^-:H2	++	++	++	+++	0/6	+	+
15	82/211		O132:K^-:H2	++	++	++	ND	0/2	+	+
15	83/4		O132:K^-:H2	++	++	++	ND	0/2	+	+
15	83/8		Rough:H2	++	+	++	ND	0/3	+	+
15	84/244/1		ND	−	−	ND	+	0/2	ND	ND
15	84/244/2		O132:K^-:H2	+	−	ND	−	0/2	ND	ND
18	036	2^+	ND	+	−	−	−	0/2	−	−
19	84/156/3		O132:K^-:H2	++	+	+	++	0/2	−	+
26	83/220		O128abc:K^-:H2	++	+	−	+	0/2	−	+
32	RDEC-1	3^-	O15:K^-:H^-	++	++	+	+++	0/6	−	+
4	83/63/5		Rough:H^-	++	+++	+	+++	0/2	−	+
6	83/136		O15:K^-:H^-	++	++	+	+	0/2	+	+
6	84/15		O15:K^-:H^-	++	++	+	+	0/2	−	+
7	84/278/6		O15:K^-:H^-	+++	+++	+	+++	0/2	+	+
7	85/227		O15:K^-:H^-	++	++	+	++	0/2	+	+
7	85/11		ND	+++	+++	++	+++	1/2	+	+
7	D28		ND	+++	+++	++	+++	0/2	+	+
8	84/17		Rough:H^-	++	+	−	+	0/2	−	+
10	L63		ND	++	−	+	−	0/2	+	+
11	84/287/4		O15:K^-:H^-	+++	++	+	++	0/2	+	+
18	85/389		O15:K^-:H^-	++	−	+	+	0/2	−	−
21	83/68/2		O15:K^-:H^-	+++	+++	+++	+++	1/4	−	+
22	84/305		Rough:H^-	+++	+++	++	+++	1/2	+	+
25	83/39		O15:K^-:H^-	+++	+++	+++	+++	1/3	+	+
25	83/40		O15:K^-:H^-	+++	+++	+++	+++	1/2	+	+
27	82/85/2		O15:K^-:H^-	++	+++	++	ND	0/3	+	+
3	83/90	3^+	O2:K1:H6	+	−	−	−	0/2	−	+
7	DI		O2	+++	+	+	+	0/2	−	−
10	L53		Rough	++	++	+	++	1/2	−	+
15	82/72		O128	+	+	−	+	0/4	−	+
18	82/152		O2	+	+	+	−	0/4	+	+

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TABLE 5—*Continued*

Age group (rabbitry)	Strain	Biotype	Serotype	Score of attachment[a]	Score of diarrhea[b]	Anorexia[c]	Growth depression[d]	No. died/total no.	Confluent growth of *E. coli* in:	
									Jejunum	Ileum
27	83/64	6^+	Rough:H26	+	−	−	−	0/2	−	+
5	85/150	8^+	O103:K^-:H2	ND	+++	++	ND	2/2	ND	ND
28	84/40/1		Rough:H2	++	+++	++	+++	1/2	+	+
28	84/110/1		O103:K^-:H2	+++	+++	++	+++	1/2	+	+
28	84/128/2		Rough:H2	++	+++	++	+++	1/2	−	+
28	85/91/2		O103:K^-:H2	+++	+++	++	+++	0/2	+	+
29	V2700		O103:K^-:H2	+++	+++	++	+++	0/2	+	+
30	N6651		O103:K^-:H2	+++	+++	+++	+++	1/2	+	+
31	5/1		O103	+++	+++	++	+++	1/2	+	+
31	5/2		O103	++	++	+++	+++	1/2	+	+
14	83/11	15^-	O15:K^-:H^-	+++	++	++	ND	0/2	+	+
14	83/12		O15:K^-:H^-	++	++	++	ND	0/2	+	+

[a] Attachment of *E. coli* to the epithelium of ileum, cecum, or colon: +, few patches of AEEC; ++, numerous patches or continuous layers of AEEC; +++, massive colonization.

[b] −, No diarrhea; +, increased water content of fecal pellets; ++, pulpy diarrhea; +++, liquid diarrhea.

[c] −, No anorexia; +, feed intake equals 51 to 75% of that of the noninfected rabbits; ++, feed intake equals 26 to 50% of that of the noninfected rabbits; +++, feed intake equals 25% or less of that of the noninfected rabbits.

[d] −, No growth depression; +, weight gain equals 51 to 75% of that of the noninfected rabbits; ++, weight gain equals 26 to 50% of that of the noninfected rabbits; +++, weight gain equals 25% or less of that of the noninfected rabbits.

[e] ND, Not determined.

Infection experiments in suckling and weanling rabbits with EPEC strains from both suckling and weaned rabbits indicated a tropism for different age groups. Strains from suckling rabbits attached to the intestinal microvillous border of both suckling and weanling rabbits, although to a far lesser extent in the latter, but caused clinical signs and mortality in suckling rabbits only (13, 17). The reverse has been shown for strains from weaned rabbits (17). Therefore, histologic demonstration of the presence of AEEC in the gut of diarrheic rabbits alone is not sufficient to conclude that they are responsible for the observed deaths. Therefore, the severity of lesions, the clinical evolution, and preferably, serotyping as well must be taken in account. This makes diagnosis rather cumbersome, slow, and expensive. Moreover, evidence from the field indicates that in weanling and suckling rabbits, strains with different pathogenicities do occur (Table 3). Medication of rabbits in outbreaks associated with these strains requires a different approach, depending on the strains involved. Therefore, early differentiation of strains is necessary for prognosis and treatment, and determination of the biotypes may contribute to this differentiation.

Crichton and Old (4) showed that biotyping of *E. coli* strains is highly discriminatory and reliable for the definition of types of *E. coli* that are extremely stable both in vivo and in vitro. Moreover, small laboratories are capable of using this method. Okerman and Devriese (10) showed that biotyping can be used to recognize rabbit EPEC. They distinguished four different biotypes: biotype 1, which mainly affected suckling rabbits, and biotypes 2 to 4, which occurred in diarrheic weaned rabbits. Biotype 3, to which RDEC-1 belongs, and biotype 4 appeared highly pathogenic, whereas biotype 2 was only moderately pathogenic.

In this study, the analysis of the fermentation patterns of 563 strains of *E. coli* isolated from healthy and diarrheic rabbits allowed the detection of 14 new biotypes. Biotype 4 was not detected. A total of 86% of the strains belonged to biotypes 1 to 3. In healthy rabbits seven different biotypes were established, and 96 of these 154 strains belonged to biotype 2. In diarrheic weaned rabbits showing lesions of AEEC seven biotypes were shown, with a predominance of biotype 3, whereas 16 of 30 strains from suckling rabbits belonged to biotype 1. Although 34 of 155 strains from weaned rabbits also belonged to biotype 1, attachment could be reproduced for only 17 of 25 of these strains (Table 4) and clinical signs were only discrete. This confirms earlier results (17). Strains belonging to biotypes 3^- and 8^+ have not been detected in healthy rabbits.

Biotype 2 constituted 28% of strains from weaned diarrheic rabbits, but only 22 of 40 strains produced lesions of AEEC after experimental infection. This was not surprising, as biotype 2 is the predominant biotype in healthy rabbits. Moreover, 2 of 10 strains from healthy rabbits showed attachment after experimental infection, while all strains of other biotypes from healthy rabbits reacted negatively. This means that biotype 2 is of limited diagnostic significance. It is possible that biotype 2 must be separated into different subtypes. Biotype 2 is mostly involved with moderate problems of enteritis in the field, as was also shown in infection experiments. Most problems associated with biotype 2 occur in constantly occupied rabbitries or in rabbitries with poor hygiene, indicating that clinical disease becomes apparent when the level of infection becomes high. In such cases disease can easily be treated with antibiotics and hygienic measures.

Immotile strains of biotype 3 (3^-) and motile strains of biotype 8 (8^+), on the contrary, were highly correlated with severe lesions of AEEC. These strains were not detected in healthy rabbits, while they accounted for 35.5 and 7.1% of the strains from weaned diarrheic rabbits, respectively. Moreover, they were associated with a high mortality in the field (Table 3); and they caused severe growth depression, liquid diarrhea, and considerable mortality after experimental infection. It was difficult to treat these strains with medication. All fully serotyped strains of biotypes 3^- and 8^+ belonged to serotypes O15:K^- : H^- and O103:K^-:H2, respectively, indicating that specific clones might be involved. Except for biotype 1^+ (O109:K^-:H2) in suckling rabbits, the biotypes of strains from other weaned rabbits were less homogeneous with regard to serotype (Table 5).

The biotyping system of Okerman and Devriese (10) involves the fermentation of six different sugars, making the

detection of 64 primary biotypes possible. Crichton and Old (4) only used the fermentation of three sugars, dulcitol, raffinose, and sorbose, and the decarboxylation of ornithine, resulting in only 16 primary biotypes. As ornithine-negative reactions were only detected in 39 of 409 strains of *E. coli* from diarrheic rabbits and as only 3 of these 39 strains were, in fact, AEEC (two strains were 1^+ and one was 3^+), ornithine seems of limited importance in screening for AEEC in rabbits. On the other hand, the scheme of Crichton and Old (4) does not include the fermentation of rhamnose. This makes the differentiation between highly pathogenic strains of biotype 8^+ and moderately pathogenic strains of biotype 2^+, which are also predominant in healthy rabbits, impossible. So, this scheme seems less suitable for the screening of AEEC in rabbits.

Analysis of the characteristics of our 568 rabbit strains makes clear that the number of primary biotypes of Okerman and Devriese (10) can be reduced to 16 by omitting sucrose and cellobiose from the scheme, without affecting the essential sugars. Most rabbit strains reacted analogously to both raffinose and sucrose. Only biotypes 15, 16, and 17 showed an opposite reaction. If we were to omit sucrose, these biotypes would be classified as biotypes 3, 20, and 6, respectively (Table 1). This is acceptable, as most strains of biotype 15 are immotile and as the AEEC strains of this biotype belong to serotype $O15:K^-:H^-$, as do all biotype 3^- strains. Moreover, biotype 15^- has not been isolated from healthy rabbits either. Biotypes 16 and 17 do not seem to have any diagnostic value, as is the case for biotypes 20 and 6. Cellobiose can also be omitted for the same reasons. Only 2% of the 568 strains reacted positively. Omission of cellobiose makes the differentiation of biotypes 12 and 3 impossible, but this is not inconvenient, as all biotype 12 strains were motile. So, the highly pathogenic immotile strain 3^- could still be distinguished. The change of biotypes 11 and 21 to biotypes 2 and 7, respectively, can also be performed without problems.

The proposed simplified biotyping scheme (Table 1) avoids unnecessary complications and still allows for the primary screening of AEEC originally described by Okerman and Devriese (10) and of those established during this study. The results discussed above make clear that biotyping allows such screening for very pathogenic AEEC (biotypes 3^- and 8^+ in weaned rabbits, biotype 1^+ in suckling rabbits), although biotyping results should be interpreted with caution. Indeed, strains of *E. coli* belonging to these biotypes, but with different serotypes, also have been detected in other species (F. Ørskov, unpublished data). Therefore, biotyping results still must be obtained with other data, such as proliferation of *E. coli* in the distal small intestine, clinical signs, mortality rate, histological evidence, and if possible, serotyping.

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