BIOTYPE, SEROTYPE AND PATHOGENICITY OF ATTACHING EFFACING ENTEROPATHOGENIC ESCHERICHIA COLI STRAINS ISOLATED FROM DIARRHEIC COMMERCIAL RABBITS

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Introduction

Attaching effacing enteropathogenic *Escherichia coli* (AEEC) are now considered to be an important cause of diarrhea in suckling and weanling rabbits (Camguilhem et al., 1986, Okerman, 1987). 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 has to be slaughtered and replaced (Peeters et al., 1986). So, early differentiation of the pathogenicity of the strains is important for prognosis and treatment.

At this moment definite diagnosis of AEEC is rather cumbersome and based on histopathology. Histology is expensive and not available in every veterinary laboratory. Moreover, histopathology does not always give sufficient information on the pathogenicity of the strain involved.

Okerman & Devriese (1985) compared the fermentation patterns of 45 enteropathogenic *E.coli* (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. Biotyping of strains of *E.coli* is highly discriminatory and reliable and defines types of *E.coli* that are extremely stable both in vivo and in vitro (Crichton & Old, 1985). So, 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

In total 568 strains of *E.coli* were included in this study: 191 strains have been 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 caecal contents. All the rabbits from which these strains have been 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 (015:H-) was kindly provided by J. Cantey, University of South Carolina, Charleston, strains V2700 and N6651 (both 0103:K-:H2) by L. Renault, C.R.C.B., Athis-Mons, France and strains 5/1 and 5/2 (both 0103) from E. Facchin, Istituto Zooprofilattico Sperimentale delle Venezia, Verona, Italy. Finally 154 strains were isolated by faecal swabs from healthy weaned rabbits in 8 rabbitries without demonstrable problems of colibacillosis.

Serotyping

O:K:H serotypes of 62 selected strains showing attaching effacing properties after experimental infection were examined by standard methods (Ørskov & Ørskov, 1984).

Biotyping

Biotyping was performed according to the scheme of Okerman & Devriese (1985). After primary isolation each strain was passaged twice on blood agar (tryptose blood agar base Difco with 5 % (vol/vol) sheep blood). Then two sterile tubes with nutrient broth were inoculated with one colony respectively and incubated for 4 h aerobically 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 % (w/v) 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 % (w/v). The completed media were dispensed in 5 ml volumes to sterile capped 10 ml tubes. Results were read after 48 hours incubation at 37°C. Motility and ornithine decarboxylase were tested on MIO-medium (Gibco) 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 lyophilized after primary isolation. The inoculum was prepared from second-passage organisms grown on blood agar by inoculating colonies into nutrient broth and incubating for 6 h aerobically at 37°C.

A total of 336 coccidia-free New Zealand white rabbits were used. After weaning at 4 weeks, the rabbits were housed individually in heat sterilized, wire-floored metal cages 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 antimicrobic additive. Fecal examination confirmed the absence of coccidia.

For each strain two rabbits were infected orally with 2 ml of inoculum containing approximately 2 x 10⁶ CFU. In each series of experiments 4 rabbits remained uninfected and served as negative control. Rabbits were checked for feed intake and weight gain 7 and 10 days post infection (p.i.) and for diarrhea on a daily basis. Seven and 10 days p.i. one rabbit was killed and necropsied. Segments of terminal small intestine, cecum and proximal colon were processed for histology. Coliform bacteria attached to the intestinal mucosa were traced in haematoxylin and eosin-stained sections at magnifications of 500 x and 1000 x with a Leitz Laborlux 12 microscope. 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 streaking plates of G2SN (Gassner agar Merck, 77 g; yeast extract Gibco, 3 g; sodium thiosulfate 5 H20, 5 g; ferric citrate, 0.5 g and distilled water 1000 ml, pH 7.2; after autoclaving 20 ml of 25 % novobiocin was added) with intestinal contents and incubation at 37°C for 18 h. The number of lactose-positive colonies on the plates was evaluated semi-quantitatively as follows: 0, no growth; 1, widely spaced colonies; 2, closely spaced colonies and 3, confluent growth of colonies. Coliform colonies were identified by the method of MacKenzie et al. (1948).

Results

Biochemistry

The biochemical characteristics of 414 strains of *E.coli* isolated from diarrheic rabbits and of 154 strains of healthy rabbits were determined (Table 1.). The sucrose test generally gave the same result as the 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 other fermentation patterns (biotypes 5 to 21) than described by Okerman & Devriese (1985).

Table 1. Biotypes of 568 strains of *Escherichia coli* isolated from intestinal contents of rabbits after extension of the system of Okerman & Devriese (1985) from 4 to 21 fermentation patterns

| Biotype | Ability of ferment | | | | | | Decarboxy- | |
|---------------------------------|--------------------|---------------|----------------|---------------|--------------|---------|---------------------|---------|
| | Cello- biose | Dulci- tol | Raffi- nose | Rham- nose | Sor- lose | Sucrose | lation of ornithine | Motile |
| 1 | - | - | + | + | - | + | 110/113 | 111/113 |
| 2 | - | + | + | + | - | + | 203/207 | 196/207 |
|] 3] | - | + | + | + | + | + | 156/163 | 65/163 |
| 2 3 4 5 6 7 8 | - | - | + | - | + | + | 0 | 0 |
| 5 | - | - | - | - | - | - | 0/1 | 1/1 |
| 6 | - | + | - | + | - | - | 2/12 | 10/12 |
| 7 | - | + | - | + | + | - | 11/11 | 9/11 |
| | - | + | + | - | - | + | 19/19 | 19/19 |
| 11 | + | + | + | + | - | + | 0/1 | 1/1 |
| 12 | + | + | + | + | + | + | 1/11 | 11/11 |
| 13 | - | - | + | + | + | + | 2/5 | 5/5 |
| 15 | - | + | + | + | + | - | 6/6 | 1/6 |
| 16 | - | - | - | + | | + | 0/4 | 1/4 |
| 17 | - | + | - | + | - | + | 4/6 | 0/6 |
| 18 | - | + | + | - | + | + | 1/1 | 1/1 |
| 19 | - | - | - | + | + | - | 1/2 | 1/2 |
| 20 | - | - | - | + | - | - | 0/5 | 3/5 |
| 21 | + | + | - | + | + |] - | 0/1 | 1/1 |

The percentage occurrence of these biotypes in 154 weaned healthy rabbits and in 191 diarrheic rabbits with confirmed histologic lesions of AEEC is listed in Table 2. In healthy rabbits 7 fermentation patterns were detected, 7 in weaned diarrheic rabbits and 3 in suckling diarrheic rabbits (less than 4 weeks of age). Biotype 1 was predominant in suckling diarrheic rabbits and biotype 3 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.

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 on the severity of histologic lesions and on the presence of AEEC in five different intestinal compartments. The results of the positive rabbits are listed in Table 3 and related to biotype and clinical signs.

Table 2. Percentage occurrence of biotypes of *E.coli* isolated from healthy rabbits and trom diarrhoeic rabbits with histologic lesions of attaching effacing *E.coli*

| Biotype | Motility | Healthy rabbits | Diarrhoeic rabbits with AEEC | | | |
|-------------------|----------|--------------------|------------------------------|--------------|--------|--|
| | | | < 4 weeks | 4 - 11 weeks | | |
| | | | of age | of age | of age | |
| 1 | + | 14,3 | 50 | 21.9 | | |
|] | | 0.0 | 3 | 0 | | |
| 2 | + | 62.3 | 20 | 25.8 | 17 | |
| | - | 0.0 | 10 | 2.6 | | |
| 3 | + | 10.4 | 3 | 3.9 | | |
| | - | 0.0 | 13 | 35.5 | 67 | |
| 6 | + | 1.3 | 0 | 0.6 | | |
| 7 | + | .3.9 | 0 | 0 | | |
| | - | 1.3 | 0 | 0 | | |
| 8 | + | 0.0 | 0 | 7.1 | 17 | |
| 12 | + | 5.9 | 0 | 0 | | |
| 15 | - | 0.0 | 0 | 1.3 | | |
| 17 | - | 0.6 | 0 | 1.3 | | |
| | | | | | | |
| Number of strains | | 154 | 30 | 155 | 6 | |

Suckling rabbits colonized by strains of biotype 1+ showed high mortality: in such rabbitries up to 20 % of the litters were affected and mortality within the litters reached almost 100 %. Usually the weanling rabbits of the affected rabbitries showed no clinical signs. Other biotypes were isolated sporadically from suckling rabbits, but always in association with enteric problems in weaned rabbits. In these cases mortality was low to moderate. Independently of the biotype, AEEC in suckling rabbits were often (13/30) found attached to the intestinal mucosa in a continuous layer from duodenum to colon.

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 present in 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. Most of the weaned rabbit strains attached only to the mucosa of ileum, caecum and colon and in severe cases also to the lower half of small intestine. Only biotype 8 also caused lesions in the upper half of small intestine.

Table 3. Clinical signs and lesions associated with 191 strains of *E.coli* isolated from diarrhoeic rabbits with histologic lesions of attaching effacing *E.coli*

| | | | I | | Histo- logic lesions | AEEC in duo- denum |
|---------------|---|---|--|---|--|-----------------------------|
| | strains | rabbitries | in suckling and weanling rabbits | | | |
| 0109 | 15 | 6 | 2/6 | High | +++ | 6/15 |
| 08 | 1 | 1 | 1/1 | ? | +++ | |
| ND | 6 | 5 | 5/5 | Low | ++ | 4/6 |
| ND | 3 | 1 | 1/1 | Moderate | ++ | 0/3 |
| 02 | | 1 | 1/1 | Low | + | 1/1 |
| ND | 4 | 3 | 3/3 | Moderate | +++ | 1/4 |
| 0100 000 0150 | 24 | | 0/15 | | | 0.01 |
| | _ | | | | | 0/34 |
| | | | | | | 0/41 |
| | | 3 | | | | 0/4 |
| | | l . | | - | | 0/6 0/59 |
| | | , | | A CT A HIGH | | 0/39 |
| | - | | | Very high | | 7/12 |
| | | | | ory mgn | | 0/2 |
| ND | 2 | i | 0/1 | ? | ++ | 0/2 |
| | 08 ND ND 02 ND 0109, 020, 0153 0132, 0128 ND 02, 0128 015 ND 0103 015 | 08 1 ND 6 ND 3 02 1 ND 4 0109, 020, 0153 34 0132, 0128 41 ND 4 02, 0128 6 015 59 ND 1 0103 12 015 2 | 08 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | 08 1 1 1 1/1 ND 6 5 5/5 ND 3 1 1/1 02 1 1 1 1/1 ND 4 3 3/3 0109, 020, 0153 34 17 2/17 0132, 0128 41 13 5/13 ND 4 3 1/3 02, 0128 6 6 1/6 015 59 21 3/21 ND 1 1 0/1 0103 12 2 0/2 015 2 1 0/1 | 08 1 1 1/1 ? ND 6 5 5/5 Low ND 3 1 1/1 Moderate 02 1 1 1/1 Low ND 4 3 3/3 Moderate 0109, 020, 0153 34 17 2/17 Moderate 0132, 0128 41 13 5/13 Moderate ND 4 3 1/3 Moderate 02, 0128 6 6 1/6 Low 015 59 21 3/21 Very high ND 1 1 0/1 ? 0103 12 2 0/2 Very high 015 2 1 0/1 ? | 08 |

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. Only two of the 31 healthy rabbit strains were able to induce lesions of AEEC against 72 of 122 diarrheic rabbit strains. Almost all strains belonging to biotype 3- and 8+ attached to the intestinal mucosa. A much smaller proportion of the other biotypes did so (Table 4).

None of the 6 attaching suckling rabbit strains 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. Infection of weaned rabbits with attaching weaned rabbit strains 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, biotypes 2+ and 3+ induced diarrhea, anorexia and growth depression, but no mortality within 10 days p.i., whereas biotypes 3- and 8+ generally caused liquid diarrrhea, severe growth depression and 12 % mortality (5/42) within 10 days p.i. for biotype 3- and 44 % (8/18) for biotype 8+. In contrast with the other biotypes, confluent growth of *E.coli* in the upper and mid small intestine was not very frequent in rabbits infected

with biotypes 1+. Finally, there was a good correlation between biotype and serotype: most strains tested of biotypes 1+ and 2+ belonged to serotype 0109:K-:H2 and 0132:K-:H2 resp., whereas all strains tested of biotype 3- were 015:K-:H-, and those of biotype 8, 0103:K-:H2.

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

| Biotype | Diarrheic rabbits | Healthy rabbits | |
|----------------|---------------------|-----------------|--|
| 1+ | 17/25 | 0/2 | |
| 1- 2+ 2- | 1/1 22/38 0/2 | 2/10 | |
| 3+ 3- | 5/15 15/17 | 0/11 | |
| 6+ 6- | 1/4 0/1 | | |
| 7+ 7- | 0/1 | 0/2 0/1 | |
| 8+ 11- | 9/9 0/1 | | |
| 12+ 13+ | 0/3 | 0/3 0/1 | |
| 15- 16- | 2/2 0/1 | 0/1 | |
| 17- 18+ | 0/1 0/1 | | |
| Total positive | 72/122 | 2/31 | |

Discussion

Since the discovery of an attaching effacing enteropathogenic strain of *E.coli* RDEC-1 in weaned diarrheic rabbits by Cantey and Blake (1977) in the United States, similar observations have been done in England (Prescott, 1978), Belgium and The Netherlands (Peeters et al., 1984b) and France (Camguilhem et al., 1986). 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 small and large intestine 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 healthy rabbit strains did not produce such effects.

AEEC have also been detected in suckling rabbits (Okerman et al., 1982). All suckling rabbit strains belonged to the same serotype 0109:K-:H2 (Peeters et al., 1984c), whereas different serotypes have been detected in weaned diarrheic rabbits showing lesions of AEEC: 015:H- in the United States (Cantey & Blake, 1977), 0153 in England (Prescott, 1978), 015:H-, 020:H2, 0109:H2, 0128:H2 and 0132:H2 in Belgium (Peeters et al., 1984c) and 0103:K-:H2 in France (Camguilhem et al., 1986). Serotypes 0103:H19, 0128:H2 and 0132:H2 occurred frequently in Hungarian diarrheic weaned rabbits, produced diarrhea after experimental infection, but their attaching effacing properties have not been examined (Varga & Pesti, 1982). The same serotypes have been detected in diarrheic rabbits during this study. Epidemics of colibacillosis associated with serotype 0103 or serotype 015 have been reported from France and from Belgium and The Netherlands respectively, indicating that some special serotypes may spread among rabbitries.

Infection experiments in suckling and weanling rabbits with both suckling and weaned enteropathogenic *E.coli* strains indicated that a tropism might exist for different age groups: suckling rabbit strains do attach to the intestinal microvillous border of both suckling and weanling rabbits, although to a far lesser extent in the latter, but cause clinical signs and mortality in suckling rabbits only (Peeters et al., 1984a, c). The reverse has been shown for weaned rabbit strains. So, histologic demonstration of AEEC in the gut of diarrheic rabbits alone is not sufficient to conclude that they are responsible for the observed losses. Therefore the severity of lesions, the clinical evolution and possibly serotyping as well also have to be taken in account. This makes diagnosis rather cumbersome, slow and expensive. On the other hand, evidence from the field indicates that in weanling and suckling rabbits strains of different pathogenicity do occur (Table 3). Medication of outbreaks associated with these strains requires a different approach according to the strains involved. So early differentiation of strains is necessary for prognosis and treatment.

Crichton & Old (1985) showed that biotyping of strains of *E.coli* is highly discriminatory and reliable to define types of *E.coli* that are extremely stable both in vivo and in vitro. Moreover, this method is also available in small laboratories. Okerman & Devriese (1982) showed that biotyping can be used to recognize rabbit EPEC. They distinguish four different biotypes: biotype 1, mainly affecting suckling rabbits and biotypes 2 to 4, occurring in diarrheic weaned rabbits. Biotype 3, to which belongs RDEC-1 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* we isolated from healthy and diarrheic rabbits revealed 14 new biotypes. Biotype 4 has not been detected. A total of 86 % of the strains belonged to biotypes 1 to 3. In healthy rabbits 7 different biotypes were found and 62 % of these 154 strains belonged to biotype 2. In diarrheic rabbits showing lesions

of AEEC 7 biotypes were established: 54 % of 30 suckling rabbit strains belonged to biotype 1, confirming earlier results. To this biotype belonged also 22 % of 155 weaned rabbit strains. For a great deal of them attachment could be reproduced (Table 4), but clinical signs were only discrete as we described before.

Biotype 2 was frequently detected (28 %) in weaned diarrheic rabbits, but only 22 of 40 strains produced lesions of AEEC after experimental infection. This is not surprising as biotype 2 is the predominant healthy rabbit biotype. Moreover, 2 of 10 healthy rabbit strains showed attachment after experimental infection, while all healthy rabbit strains of other biotypes reacted negatively. This means that biotype 2 is of limited diagnostic significance. Possibly biotype 2 has to be separated in different subtypes. Anyhow biotype 2 is mostly involved with moderate problems of enteritis in the field, as was also evident after experimental infection.

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 have not been detected in healthy rabbits, while they account for 35.5 and 7.1 % respectively of weaned diarrheic rabbit strains. Moreover, they are associated with high mortality in the field (Table 3) and they cause severe growth depression, liquid diarrhea and considerable mortality after experimental infection. Medication is difficult. All fully serotyped strains of biotypes 3- and 8+ belong to serotypes 015:K-:H- and 0103:K-:H2 respectively, indicating that specific clones might be involved. Except for biotype 1+ (0109:K-:H2) in suckling rabbits, the other weaned rabbit biotypes were less homogeneous if serotype is concerned. Strains of biotype 2 mainly belonged to serotypes 0132:K-:H2 and 0128:K-:H2, as was also true for strains of biotype 2 isolated from healthy rabbits (P. Pohl, non published evidence). Other biotypes able to attach in weaned rabbits were biotypes 1+ (0109:K-:H2, 020:K-:H2 and 0153:K-:H7), 3+ (02, 0128), 6+ (r:H26) and 15-(015:K-:H-). Of these biotypes only biotype 15- has not been detected in healthy rabbits.

The biotyping system of Okerman & Devriese involves the fermentation of 6 different sugars, making the detection of 64 primary biotypes possible. Crichton & Old, only use the fermentation of 3 sugars, dulcitol, raffinose and sorbose and the decarboxylation of ornithine resulting in only 16 primary biotypes. In our hands ornithine negative reactions were only detected in 39 strains of 409 diarrheic rabbits and in only 3 of these 39 rabbits AEEC were detected (twice 1+ and once 3+). So this character seems of limited importance in rabbit AEEC. On the other hand the scheme of Crichton & Old does not include the fermentation of rhamnose. This makes the differentiation between the highly pathogenic biotype 8+ and the moderately pathogenic biotype 2+, which is the predominant biotype in healthy rabbits, impossible. So, this scheme seems less suitable to recognize AEEC in rabbits.

Analysis of the characteristics of our 568 rabbit strains makes clear that the number of primary biotypes of Okerman & Devriese can be reduced to 16 by omitting sucrose and cellobiose from the scheme without touching the essential. Most rabbit strains reacted analogeously for both

raffinose and sucrose. Only biotypes 15, 16 and 17 showed an opposite reaction. If we omit sucrose, these biotypes have to be classified as biotypes 3, 20 and 6 respectively. This is acceptable as most strains of biotype 15 are immotile and as the AEEC-strains of this biotype belong to serotype 015:K-:H- as all 3- strains do. Moreover biotype 15- has not been isolated from healthy rabbits either. Biotypes 16 and 17 don't seem to have any diagnostic value as is the case for biotypes 20 and 6. For the same reasons also cellobiose can be omitted: only 2 % of the 568 strains reacted positively. Omission of the sugar makes the differentiation of biotype 12 and 3 impossible, but this is not inconvenient as all biotype 12 strains are motile. So, the highly pathogenic immotile strain 3- can still be distinguished. The change of biotypes 11 and 21 to biotypes 2 and 7 can also be performed without problems.

Table 5. Biotyping results of 568 strains of E.coli from intestinal contents of rabbits after simplifying of the biotyping system of Okerman & Devriese (1983)

| Biotype | | Number of | | | | |
|--|--------------------------------------|--------------------------------------|---|--------------------------------------|--|--|
| | Dulcitol | Pulcitol Raffinose Rhamnose | | Sorbose | strains | |
| 1 2 3 4 5 6 7 8 13 18 19 20 | - + - + + - - - | + + + - - + + - | + + + - + + + + + + + + + + + + + + + + | - + + - - + - + | 113 208 180 0 1 18 12 19 5 1 2 | |

The proposed simplified biotyping scheme (Table 5) avoids unnecessary complication and still allows the primary screening for AEEC originally described by Okerman & Devriese and 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 serotype also have been detected in other species (Ørskov, unpublished evidence). So biotyping results still have to be compleded with other data, such as growth of E.coli in distal small intestine, clinical signs, mortality rate and histological evidence and if possible with serotyping.

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Summary

A total of 568 strains of *Escherichia coli* isolated from healthy and diarrheic rabbits have been separated into 11 different biotypes according to the fermentation pattern of four carbohydrates. Strains belonging to biotypes 1 to 3, 6 and 8 induced lesions characteristic for attaching effacing *E.coli* (AEEC). However, pathogenicity for weaned rabbits as judged by diarrhea score, anorexia and reduced weight gain varied according to the strains: biotypes 1 and 6 produced only discrete clinical signs, biotypes 2 and 3+ (motile) induced diarrhea and growth depression, whereas biotypes 3- (immotile) and 8 caused severe clinical signs and high mortality. Biotypes 3- and 8, accounting for 35.5 and 7.1% of AEEC in weaned diarrheic rabbits, were not detected in weaned healthy rabbits, while biotype 2 was the predominant weaned healthy rabbit strain (62.3%). Finally, serotyping showed a close relationship between biotype and serotype: most strains tested of biotype 1+ and 2+ were 0109:K-H2 and 0132:K-:H2 resp., whereas all strains tested of biotype 3- were 015:K-:H- and those of biotype 8, 0103:K-:H2. These data indicate that specific clones of AEEC might be involved in juvenile rabbit enteritis. It has been concluded that biotyping allows the screening of highly pathogenic AEEC in weaned rabbits (biotypes 3- and 8).

Résumé

Un total de 568 souches d'Escherichia coli a été isolé de lapereaux sains et de lapereaux atteints de diarrhée. Elles ont été reparties en 11 biotypes différents sur base des résultats de la fermentation de quatre carbohydrates différents. Les souches appartenant aux biotypes 1, 2, 3, 6 et 8 sont capables de provoquer les lésions caractéristiques des E. coli attachants et effacants (AEEC) aprés infection expérimentale. Prenant la réduction du gain de poids, le manque d'apétit et l'intensité de la diarrhée comme critères, ces souches peuvent être sousdivisées en différents groupes : les biotypes 1 et 6 induisent seulement des signes cliniques discrets, les biotypes 2 et 3+ (motile) provoquent de la diarrhée et un retard de croissance, alors que les biotypes 3- (immotile) et 8 causent des signes cliniques sévères et une mortalité importante. Les biotypes 3- et 8, représentant 35.5 et 7.1 % des AEEC isolés de lapereaux atteints d'entérite, n'ont pas été détectés chez des lapereaux sains, alors que le biotype 2 est le biotype dominant (62.3 %) des lapereaux sains. Finalement, le sérotypage a démontré une corrélation étroite entre biotype et sérotype : la plupart des souches testées du biotype 1+ et 2+ appartiennent aux sérotypes O109:K-:H2 et O132:K-:H2 respectivement, alors que toutes les souches du biotype 3- possèdent le sérotype O15:K-:H- et celles du biotype 8 le sérotype O103:K-:H2. Ces données saggèrent que des clones spécifiques d'AEEC sont associés avec la diarrhée juvéniles du lapereau. On peut donc conclure que le biotypage permet le tri des souches d'E. coli hautement pathogènes appartenant aux biotypes 3- et 8.

BIOTYPE, SEROTYPE AND PATHOGENICITY OF ATTACHING EFFACING ENTEROPATHOGENIC ESCHERICHIA COLI STRAINS ISOLATED FROM DIARRHEIC COMMERCIAL RABBITS

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BIOTYPE, SEROTYPE ET PATHOGENICITE DE SOUCHES ATTACHANTES, EFFAÇANTES ET ENTEROPATHOGENES DE ESCHERICHIA COLI ISOLEES DE LAPEREAUX PRESENTANT DE LA DIARRHEE EN ELEVAGE INTENSIF

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