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INTESTINAL COLONIZATION WITH DIFFERENT RABBIT ENTEROPATHOGENIC ESCHERICHIA COLI BIOTYPES AND CROSS PROTECTION INDUCED BY DIFFERENT STRAINS

L. Okerman and L.A. Devriese.

from the Department of Poultry Pathology, Bacteriology and Infectious Diseases, Faculty of Veterinary Medicine, University of Ghent, Casinoplein 24, B-9000 Gent, Belgium.

Introduction.

During the past 5 years the occurrence of enteropathogenic Escherichia coli (EPEC) as causes of diarrhea in weaned and unweaned rabbits has been reported in different countries [2,4,10,14]. EPEC are one of the most important causes of losses among broiler rabbits in large-scale farms [13].

Cantey and Hosterman [5] studied the course of colonization in rabbits experimentally infected with RDEC-1, an O15:H- strain, by measuring semi-quantitatively the number of colonies grown on selective plates inoculated with rectal swabs. This is possible in rabbits, because healthy animals do not harbour large numbers of E. coli in their intestinal tract [8,17]. RDEC-1 was excreted in high numbers from the 2nd-5th day post infectionem (p.i.) and was only eliminated from the intestinal tract after approximately 14 days of heavy colonization. Rabbits that had recovered from such a colonization period, had developed a strong immunity against a second colonization with the same strain RDEC-1 [5].

In previous experiments, a biotyping method was developed to recognize rabbit EPEC strains from the apathogenic E. coli that occur also in this species [12]. It was found that the rabbit EPEC, that had been isolated in Belgium and the Netherlands till 1984, belonged to four biotype groups, with different virulence for newborn and for weaned rabbits, but all of them produced identical microscopical lesions.

The present report describes colonization experiments in weaned rabbits with EPEC strains belonging to our four biotype groups. In order to detect further similarities or differences between these groups, we wanted to investigate 1. if colonization in non-immune animals with other rabbit EPEC types followed the same course as with RDEC-1; 2. if rabbits that had recovered from an infection with one of the EPEC types were protected against subsequent infection with one or more other types.

Materials and methods.

Rabbits: the experimental animals were all EPEC-free from birth till use. They originated from a farm where during the last five years no mortalities had been recorded among the weanlings, or had been bred at our department and had been checked weakly from the 2nd week after birth by the rectal swab method (see below) for excretion

of E. coli belonging to one of the four pathogenic biotype groups. All the experimental animals were 6-8 weeks old and weaned when they received their first challenge. During the experiments they were kept in a protected environment to be sure that no direct or indirect contact was possible between groups or with rabbits from other sources. They were fed ad libitum. Control animals that had not received the first infection but which were of the same age and origin as the test animals were included when the second challenge infection was given.

Strains: four strains belonging to different biogroups, were used for experimental infections. Pathogenic properties of E232 and E300 (biotype 1, serotype O109:H2), E326 (biotype 2, not typeable), and E452 (biotype 4, serotype O26:O119:H11) have been described in previous papers. E622 (serotype O15:H-) belongs to our biotype 3 group, which includes also the well known RDEC-1 [3,12]

All E. coli were maintained in the lyophilized form and were reconstituted on Trypticase Soy Agar (TSA, Oxoid, Basingstoke) when needed.

Experimental infections: The reconstituted EPEC strains were swab-inoculated on TSA and grown for 16-18h at 37°C. Growth from one plate (Ø 90 mm) was harvested with 3ml of physiological saline. Each experimental animal was infected with 0,5ml of a suspension, which contained approximately 10^9 colony forming units of the respective EPEC strain.

The suspensions were administered perorally; a syringue was brought deeply into the mouth cavity, and was emptied at the moment that the animal started swallowing.

Measurement of intestinal colonization: The degree of intestinal colonization was assessed by the rectal swab method, as described by Cantey and Hosterman [5]. Sterile cotton swabs were introduced gently into the anal opening, and turned one or two times. They were then streaked on a Mac Conkey plate (Oxoid), or, when appropiate, on a selective medium for biotype 3 strains that was developed by J. Peeters (personal communication). This selective medium was composed of Simmons Citrate medium (Oxoid) to which sorbose was added to a concentration of 0,2% (w/v). Sorbose fermenting biotype 3 and 4 strains grew as yellow colonies on this medium and changed its colour from green to yellow when growth was abundant. A similar medium, with adonitol instead of sorbose, has been described for detection of K99+ E. coli [16].

Growth on the rectal swab inoculated Mac Conkey plates was recorded as follows: score 0 - no growth; score 1 - less than 20 colonies; score 2 - widely spaced colonies; score 3 - closely spaced colonies; score 4 - confluent growth. At least one colony of each Mac Conkey plate inoculated with a rectal swab was biotyped, to confirm its identity.

Results.

1. Evolution of intestinal colonization with EPEC belonging to each of the four biotype groups.

As expected, the biotype 3 strain showed a similar colonization pattern as was found with the related RDEC-1 strain [5]. All infected rabbits were heavily colonized at 7 days and at 14 days p.i. but completely free at 3 weeks p.i. (Table 1). However, the use of the

sorbose containing selective medium permitted to detect single E. coli colonies, belonging to biotype 3, at 48 and at 56 days p.i. At least some of the animals harboured the organism for a prolonged period, without being colonized extensively and without showing any symptom. Colonization with the biotype 4 strain, which shows virulence characteristics similar to those of the biotype 3 strains, took a similar course (Table 2). Although the surviving rabbits had become negative on day 26, one of them excreted a biotype 4 strain in low numbers on day 40.

On the other hand, experimental infections with biotype 1 and 2 strains resulted in a somewhat different colonization pattern: the period of heavy colonization (scores 3 and 4) did not last longer than a few days in some of the animals (Tables 3, 4, 5 and 6). Some infected rabbits continued to excrete low quantities of the neonatal biotype 1 strains till the end of the observation period (36 days p.i.) but once the period of heaviest colonization had stopped they did not show diarrhea or any other symptom of EPEC disease (Tables 3 and 5).

2. Course of a second infection with a strain belonging to a different biogroup.

With only one exception, rabbits that had recovered from a first colonization were completely resistant against colonization when challenged in the 4th week after the first infection with a strain belonging to a different serobiogroup. Control rabbits of the same age and origin, that were infected simultaneously, were as susceptible to colonization with the infective strain as freshly weaned animals, receiving a first infection. The only animal that was not completely protected against the second challenge had first received a biotype 1 strain, but had shown a colonization score of 4 at only one occasion (day 15). The higly pathogenic biotype 4 strain, which was given at 28 days after the first inoculation, had colonized the animal at day 31, but excretion lowered quickly and was again zero at day 36, while both the control rabbits were still heavily colonized and had severe diarrhea at that time.

Our results indicated that rabbits that had recovered from a colonization with a biotype 1 strain (serotype O109:H2) were resistant against infection with EPEC belonging to biotype 2 (table 3), biotype 3 (table 4) and biotype 4 (table 5) groups; rabbits that had been colonized with a biotype 2 strain (serotype unknown) were protected against infection with a biotype 3 strain (table 6), and biotype 3 (serotype O15:H-) strains similarly protected rabbits against biotype 4 strains (serotype unknown) (table 1) and vice-versa (table 2).

Discussion.

It appears from the experiments described above that a previous colonization with an EPEC strain not only protects rabbits against colonization by the same strain as decribed by Cantey [3] but also against other strains belonging to different serotypes and biogroups. In some of our experiments only 2 or 3 rabbits survived the first challenge, but the results were always confirmed with each strain tested. The second inoculation resulted in intestinal colonization in only one animal, that had first been infected with a neonatal strain and 28 days later with a highly virulent biotype 4 strain; but unlike what was seen in non-immune animals, this colonization rapidly

diminished and disappeared. Cantey also mentioned that one animal of 15, that had been reinfected with RDEC-1, was colonized for one day [3]. The inocula, that were administered to the groups that had recovered from an infection with a different EPEC, never failed to colonize controls of the same age and origin that had not received a previous infection. Thus the absence of colonization in the first groups cannot be attributed to a loss of a virulence factor during preservation, nor to age dependent susceptibility.

As we have only investigated strains that were isolated in Belgium and the Netherlands before 1984, we cannot extend our conclusions to the O103 strains that are probably the most common diarrhea-inducing E. coli in French rabbitries [2] and that still belong to another biogroup (unpublished observations).

Many animals were still excreting the strain with which they had been colonized at later occasions, but only in low quantities and not continuously. They did not suffer any ill effect at this time, but this phenomenon explains the spreading of rabbit EPEC clones from infected to healthy farms, through the purchase of new breeding stock.

The cross-protection induced by our four biogroups suggest that they all share a common virulence characteristic, probably a colonization factor. This is surprising because biotype 3 and 4 strains are not pathogenic for newborns, while biotype 1 strains cause the most serious effects at a very young age [12,15]. RDEC-1, a biotype 3 strain, produces fimbriae called AF/R1, which mediate the initial attachment to the Peyer's patches [1,7]. Isolated AF/R1 agglutinate microvillous borders from the intestinal epithelium of weaned rabbits, but not from rabbits younger than 3 weeks, thus confirming the lack of pathogenicity of these strains for suckling rabbits [7]. The possession of AF/R1 fimbriae has not been investigated in other rabbit EPEC strains.

Acquired resistance against intestinal colonization by RDEC-1 is mediated by secretory IgA [3]. In their experimental model with RDEC-1, Cantey et al. measured IgA against the O15 antigen in lung and intestinal secretions [4]. As rabbits that have been colonized with one of our EPEC belonging to different serotypes are protected as well, we can suppose that it is not the O15 antigen that induces the immune response against colonization but another, still unknown factor. Further investigations should be carried out to find the exact role of AF/R1 fimbriae in this respect.

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Table 1: Course of colonization after experimental infection with E622 (biotype 3) and 27 days later with E452 (biotype 4).

Days after first inoculation	Experimental infection with	Number of surviving rabbits	Number of rabbits showing the respective colonization scores with E622					with E452				
			0	1	2	3	4	0	1	2	3	4
0	E622	8	8									
5		8					8					
14		7					7					
21		7	7									
27	E452	7	7					7				
30*		7	7					7				
33*		7	7					7				
37		7	7					7				
48		7	6	1				7				
52		7	7					7				
56		7	6	1				7				

* Seven control rabbits that received only E452 on day 27 had colonization scores of 4 when tested on day 30 and 33. Three of them died.

Table 2: Course of colonization after experimental infection with E452 (biotype 4) and 26 days later with E622 (biotype 3).

Days after first inoculation	Experimental infection with	Number of surviving rabbits	Number of rabbits showing the respective colonization scores with E452					with E622				
			0	1	2	3	4	0	1	2	3	4
0	E452	7	7									
4		7					7					
7		6					6					
11		5					5					
14		5					5					
18		4	1		1	1	1					
21		3	1	1	1							
26	E622	3	3					3				
30*		3	3					1	2			
31*		3	3					3				
32		3	3					3				
34		3	3					3				
40		3	2	1				3				
46		3	3					3				

* Four control rabbits that recieved only E622 on day 27 had colonization scores of 4 when tested on day 30 and 31 and all of them died within 10 days with haemorrnagic diarrhea.

Table 3: Course of colonization after experimental infection with E232 (biotype 1) and 28 days later with E326 (biotype 2).

Days after first inoculation	Experimental infection with	Number of surviving rabbits	Number of rabbits showing the respective colonization scores with E232					with E326				
			0	1	2	3	4	0	1	2	3	4
0	E232	5	5									
2		5	5									
4		5	4				1					
6		5				1	4					
9		5				1	4					
12		4					4					
15		2					2					
19		2		1			1					
22		2				2						
26		2	2									
28	E326	2	2					2				
31‡		2		2				2				
32		2	2					2				
33		2	1	1				2				
34‡		2	1	1				2				
36‡		2	2					1	1			

‡ Two control rabbits, infected at day 28 with E326, became positive at day 31 and 34. At day 36, both had colonization scores of 4.

Table 4. Course of colonization of rabbits after experimental infection with E232 (biotype 1) and 27 days later with E622 (biotype 3).

Days after first inoculation	Experimental infection with	Number of surviving rabbits	Number of rabbits showing the respective colonization scores with E232					with E622				
			0	1	2	3	4	0	1	2	3	4
0	E232	5	5									
3		5					5					
6		5					5					
9		5			2	2	1					
11		5	1			1	3					
13		4	1	1			2					
15		4	1	1			2					
17		4	1		1		2					
19		3		1	1		1					
21		3	2			1						
24		3	2				1					
27	E622	2	2					2				
30‡		2	2					2				
32‡		2	2					2				
34		2	2					2				

‡ Two control rabbits that received only E622 on day 27 had colonization scores of 4 when tested on day 30 and day 32; one of them died.

Table 5. Course of colonization of rabbits after experimental infection with E300 (biotype 1), and 28 days later with E452 (biotype 4).

Days after first inoculation	Experimental infection with	Number of surviving rabbits	Number of rabbits showing the respective colonization scores with E300					with E452				
			0	1	2	3	4	0	1	2	3	4
0	E300	5	5									
2		5	3			1	1					
4		5	3				2					
6		4	2			1	1					
9		4			1	3						
12		4				1	3					
15		4				1	3					
19		4	1	1	1		1					
22		4	3		1							
26		4	4									
28	E452	4	4					4				
31‡		4	2	2				3				1
32‡		4	2	1	1			3			1	
33		4	2	2				3		1		
34		4	3	1				3	1			
36 ‡		4	4					4				

‡ Two control rabbits, that received only strain E452 at day 28, became postive resp. at day 31 and 32. AT day 36, both had colonization scores of 4 and had severe diarrhea.

Table 6. Course of colonization after experimental infection with E326 (biotype 2) and 26 days later with E622 (biotype 3).

Days after first inoculation	Experimental infection with	Number of surviving rabbits	Number of rabbits showing the respective colonization scores with E326					with E622				
			0	1	2	3	4	0	1	2	3	4
0	E326	7	7									
4		7	1				6					
8		7					7					
12		6	1				5					
15		5	1			2	2					
18		5	1	1	1		2					
21		5	5									
26	E622	5	5					5				
30‡		5	5					5				
31‡		5	5					4	1			
32		5	5					5				
34		5	5					5				
36		5	5					5				
40		5	5					5				
42		5	5					5				
48		5	5					5				

‡ Four control rabbits, that were infected at day 26 with E622, were heavily colonized at day 30 and 31 and all of them died within 10 days with haemorrhagic diarrhea.

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L. Okerman and L.A. Devriese, Department of Poultry Pathology, Bacteriology and Infectious Diseases, Faculty of Veterinary Medicine, University of Ghent, Casinoplein 24, B-9000 Gent, Belgium.

SUMMARY

Experimental infections with 4 different rabbit enteropathogenic *Escherichia coli* biotypes were carried out in EPEC free rabbits, and intestinal colonization was assessed by measuring semi-quantitatively the rectal colonization. When the animals were infected for the first time shortly after weaning, the excretion followed an approximately identical course with all four EPEC: at 3-9 days post infectionem all rabbits were excreting the EPEC infection strain in high quantities, and continued to do so for 1 - 2 weeks. Rabbits that had survived such a colonization with one EPEC, were protected against colonization with strains belonging to other EPEC groups.

RESUME.

Des lapins indemnes d' *Escherichia coli* entéropathogènes (EPEC) étaient infectés expérimentalement avec 4 biotypes différents de EPEC pathogénes pour cet espèce animale. La colonization intestinale était estimée en mesurant le nombre de colonies recouvert d'ecouvillons rectaux. Des animaux qui étaient inoculés dans la premiére semaine aprés le sevrage, montraient une excrétion qui était presque identique pour les 4 biotypes: entre 3 et 9 jours après l'infection expérimentale ils commencaient a excréter un grand nombre de colibacilles appartenant au biotype qui était administré, et cet excrétion continuait pour à peu près 2 semaines. Des lapins qui avaient survécu cette période de colonization étaient protégés complètement contre une nouvelle colonization avec une souche EPEC appartenant à d'autres biotypes.