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EXPERIMENTAL ESCHERICHIA COLI ENTEROPATHY IN WEANLING RABBITS

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INTRODUCTION

Gastrointestinal disorders in rabbits generally appear multifactorial and due to a variety of conditions, including viruses, bacteria, protozoa, nutrition and husbandry (11). In a previous study Escherichia coli was shown to play a predominant role in the etiology of diarrhoea of commercial rabbits (18). In total 40.0 % of the rabbits showed coccobacilli attaching to the luminal intestinal border of ileum, caecum and colon. This was associated with anorexia, weight loss, diarrhoea and moderate to high mortality.

In order to obtain more accurate data on the pathogenicity of these strains, we decided to study their characteristics and their ability to attach to the intestinal border after experimental infection. Moreover the sequential pathomorphologic events after experimental infection with one of these strains and its influence on growth and feed intake of weanling rabbits have been followed.

MATERIAL AND METHODS

In total 35 strains of E. coli were included in this study :

a. 3 strains isolated from healthy rabbits from 3 different rabbitries, where no attaching colibacilli have been observed.
b. 6 strains isolated from suckling rabbits from 5 different rabbitries (strains E232, E243 and E297 were kindly provided by courtesy of dr L. Okerman, State University of Ghent, Belgium).
c. 26 strains isolated from weanling rabbits from 12 different rabbitries (strain RDEC-1 received by courtesy of dr J.R. Cantey, Medical University of South Carolina, U.S.A.).

They were tested on following characteristics :

a. production of heat-stable enterotoxins according to the method

of Gianella (7).
b. production of heat-labile enterotoxins according to the methods of Donta (5) and Speirs (24).
c. production of verotoxins according to the method of Konowalchuk (9).
d. ability to attach to intestinal microvilli of rabbits in vitro according to the method of Girardeau (8).
e. invasivity (Serenytest)(22)
f. O-antigen according to the method of Sojka (23) with antisera 0.1 to 0.157.

A total of 143 coccidia-free New Zealand White rabbits were used in four experiments. After weaning at 4 weeks, the rabbits were housed individually in heat sterilized wire-floored metal cages. They were kept at 18 °C ambient temperature and received a commercial pelleted ration with 16 % crude protein and 15 % crude fiber ad libitum. The feed did not contain any antimicrobial additive. Individual coprological examination confirmed the absence of coccidia.

In a first experiment 35 rabbits were used. Each of them received 2 ml of broth culture of one of the 35 strains of E. coli respectively. Doses of bacteria were given with 10 ml of 10 % $NaHCO_3$, an amount that elevates gastric pH to more than 7 for 1 to 2 hours (1). Rabbits were observed daily for feed intake and presence of diarrhoea. Seven days post-infection (p.i.) rabbits were killed and necropsied. Specimens of ileum, caecum and colon were taken for histology. In a second experiment the same procedure was followed, but this time no $NaHCO_3$ was administered at the time of inoculation. Experiment 3 was executed as experiment 2, but rabbits were observed during 3 weeks and no necropsy was performed.

In experiment 4 sixteen 4-week-old rabbits were divided into two groups, each of 8 animals. One week later each rabbit of group B received 2 ml of a suspension with 2×10^6 colony forming units (CFU) of E. coli U83/39 (0.15/130:NM), a strain which has been isolated during an outbreak of extensive diarrhoea in weaned rabbits. Group A served as uninfected control and was housed in an identical but separate room. During 4 weeks weight gain and feed consumption were determined individually. The rabbits were checked for diarrhoea on a daily basis. Diarrhoea was quantified as follows : 0 = no diarrhoea, 1 = increased water content of faecal pellets, 2 = pappy diarrhoea, 3 = liquid diarrhoea. At the same time rectal swab specimens were taken and streaked on G2SN, a selective medium for enterobacteriaceae (19). The numbers of lactose positive CFU on the plates were scored as follows : 0 = no growth, 1 = widely spaced colonies, 2 = closely spaced colonies, 3 = confluent growth of colonies.

Another group (C) of twenty rabbits was inoculated at the same time as group B with 2×10^6 CFU of strain U83/39. Rabbits were killed 0, 1, 2, 3, 4, 5, 6, 7, 8, 11, 14, 28 and 35 days p.i. Blood cultures were made with heart blood. The intestines were removed from the animal immediately after killing and segments of duodenum, midjejunum, ileum, Peyer's patch, caecum (anterior, medium and posterior part), appendix, colon ascendens and descendens were fixed in 10 % formaline in phosphate buffered saline for paraffin sections. They were stained with hematoxylin and eosin. Pieces of ileum, Peyer's patch, midcaecum and colon ascendens were also fixed in cold cacodylate buffered glutaraldehyde 2.5 % at pH 7.6 within 20 minutes after killing the animals

and processed for scanning electron microscopy (17). Presence of E. coli was evaluated semiquantitatively in duodenum, jejunum, ileum and caecum as described above.

RESULTS

Characterization of the different strains of E. coli isolated from diarrhoeic commercial rabbits revealed following results : strains from suckling rabbits all belonged to serogroup 0.109 and attached to intestinal microvilli in vitro, whereas the 25 strains from weanling rabbits did not. Strains from weanling rabbits belonged to at least 7 different serogroups : 0.15 (9 strains), 0.103 (1 strain), 0.109 (3 strains), 0.130 (3 strains), 0.132 (5 strains), 0.142 (1 strain) and 0.145 (1 strain). Eight strains did not belong to serogroups 0.1 to 0.157. Two strains isolated from healthy rabbits belonged to serogroups 0.7 and 0.9 respectively, whereas the third one could not be identified. None of the strains examined was invasive or produced thermostable or thermolabile enterotoxins.

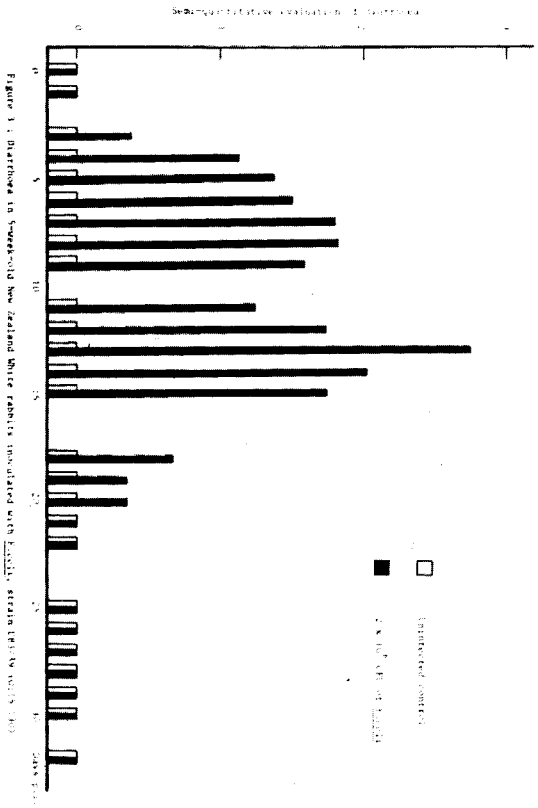
Experimental infection with the 35 strains of E. coli revealed following results :

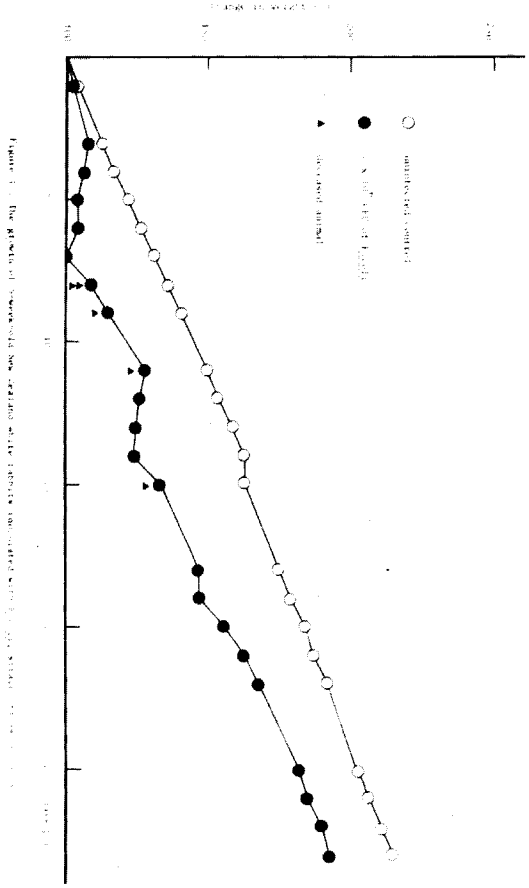
1. Rabbits infected with strains from suckling rabbits showed some degree of anorexia. Almost no lesions were found at autopsy (slight enlargement of mesenteric lymph nodes) and histology revealed only few patches of coccobacilli attached to the brush borders.

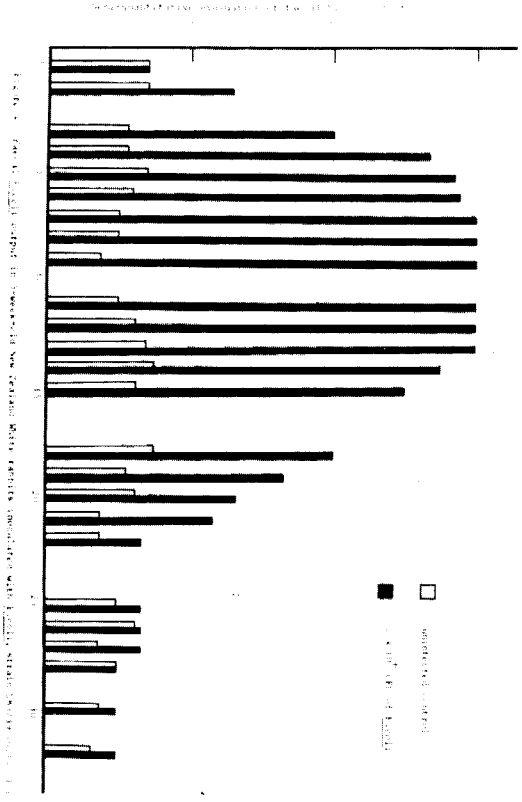
2. Rabbits infected with strains from healthy rabbits also showed some degree of anorexia, but no colibacilli were found attached.

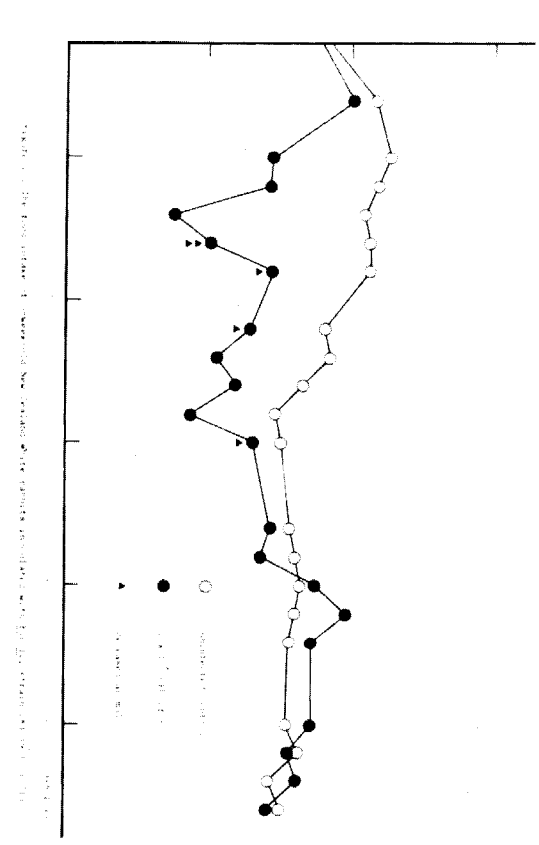
3. Rabbits infected with one of the 25 strains from weaned rabbits showed reduced feed intake and produced moderate to severe diarrhoea. At necropsy watery caecal contents and caecal oedema was a common finding. For all strains tested, histology showed different degrees of attachment of E. coli to enterocytes of ileum, caecum and colon as described before (18). Almost no difference was found between rabbits treated with 10 ml of 10 % $NaHCO_3$ at inoculation and the not treated ones. Haemorrhages in the caecal serosa were only observed in $NaHCO_3$ treated animals (5/25).

The clinical response of the rabbits to experimental infection with 2×10^6 CFU of E. coli U83/39 is shown in Figures 1 to 3. All inoculated rabbits exhibited liquid diarrhoea which regularly contained blood









and mucus. Total excretion of faeces was reduced distinctly. The mean day of onset of diarrhoea was 6.25 ± 2.71 and the mean duration of diarrhoea was 6.13 ± 1.89 days. Al rabbits with diarrhoea had associated weight loss and five on eight infected rabbits died 8 to 15 days p.i. They always showed liquid diarrhoea immediately before death. No clinical signs were observed in control rabbits.

The mean scores of the rectal swab cultures are shown in Fig. 4. Rectal swabs taken before inoculation showed the presence of only few colonies of E. coli in 8 of 16 animals. The other samples were negative. Rectal swab cultures of infected animals increased regularly from day + 1 p.i., reached their maximum by 4 to 7 days p.i. and declined from 14 days p.i. to become equal to control rabbits 22 days p.i. The rectal swab culture score at the onset of diarrhoea was 3 and this was also observed on the day of death. Samples of blood for culture were taken from 17 infected rabbits from day 1 to 35 p.i. They were all sterile. Specimens from the small and large intestines of the same animals were cultured on G2SN. Growth became progressively heavier from duodenal to caecal cultures. From 8 to 14 days p.i. the four intestinal segments showed confluent growth of E. coli. Cultures became negative by day 28 p.i.

Rabbits killed 1 to 3 days p.i. showed no apparent lesions. From 4 to 14 days p.i. however slight to marked oedema was evident in caecum and mesenteric lymph nodes. Sometimes lymph nodes showed diffuse hemorrhage. Peyer's patches and the sacculus rotundus were also markedly swollen. The contents of the caecum was foul-smelling, watery and brown. In one rabbit there were paint brush hemorrhages in the caecal serosa and in another rabbit small necrotic spots were present in the appendix. The colon contained watery brown faeces and in some animals a mucoid jelly. The small intestine looked normal. In some animals the terminal ileum was slightly congested. The stomach mostly contained only a small watery food bolus. In total 8 rabbits died. Most of them showed the same features as described above, although lesions were more marked : 5 on 8 showed extensive paint-brush hemorrhages in the caecal serosa and petechiae on the caecal mucosa. Caecal content was mostly blood tinted. One animal which died 8 days p.i. showed an ileo-ileal intussusception.

Histologically, intestines from control rabbits were normal. The

morphology as seen by scanning electron microscopy (SEM) was as previously described (17). Twenty-four hours p.i. no morphologic changes were observed. Some isolated colibacilli were seen on the caecal epithelium. Observation of the specialized epithelium on the lymphoid follicles of the Peyer's patches was made difficult by the overlying villi. Light microscopy however showed some coccobacilli attached to this epithelium. The second day p.i. patches of E. coli were found on all the surfaces of the lymphoid follicles of the Peyer's patch and also some on the enterocytes of villi adjacent to the follicles. This was confirmed by SEM. Most of the colonized cells lacked their brush border, while the brush borders of other epithelial cells were in varying stages of degeneration. The epithelial surface surrounding the colonized zones appeared unaltered. There was slight infiltration of polymorphonuclear leucocytes (PMNL) beneath the affected epithelium. Adherent coccobacilli were not evident in ileum, caecum and colon until 3 days p.i. Coccobacilli were also attached to the lymphoid follicles of the sacculus rotundus and to the appendix, but not to the degree noted for Peyer's patches.

From 3 days p.i. epithelial cells on the lymphoid follicles of the Peyer's patch were still covered by numerous coccobacilli. In the ileum, there was villus atrophy and apical enterocytes were swollen, desquamating and covered by numerous coccobacilli. Cells extensively colonized lacked a brush border. From the third day p.i. continuous layers of E. coli were found in the caecum. There was slight oedema of the caecal lamina propria with infiltration by PMNL, vacuolisation and ulceration of epithelial cells with extensive attachment of coccobacilli. The epithelium was flattened and disorganized. In later stages there was a decreased amount of cytoplasm in epithelial cells with adherant bacteria and luminal borders of the cells presented a ragged, irregular appearance. Coccobacilli were never found in the lamina propria. From 4 days p.i. also patches of E. coli were found on the tips of the colonic villi.

This situation was present in all rabbits slaughtered until 14 days p.i. Maximal lesions with extensive involvement of caecum and colon ascendens were found between 7 and 14 days p.i. with severe oedema of the caecal lamina propria and submucosa, congestion of blood vessels and extravasation of erythrocytes into the lamina propria and submuco-

sa. Haemorrhages were found in the serosa 8 days p.i. The attachment of bacteria was maximal when diarrhoea was present. Necrosis of lymphoid follicles of the appendix was found in one animal. In most animals duodenum, jejunum and colon descendens remained free from colonization. Only 7 days p.i. some small numbers of coccobacilli were found attached to the enterocytes of the colon descendens and 8 days p.i. the epithelial cells of the midjejunum were diffusely colonized with strong villar atrophy and severe infiltration by PMNL. Rabbits examined 28 and 35 days p.i. showed no lesions nor E. coli colonization any more.

DISCUSSION

Strains isolated from suckling rabbits attached to microvilli in vitro, whereas strains from weaned rabbits did not. In the field strains from suckling rabbits colonize the intestine from duodenum to colon, whereas strains from weaned rabbits colonize only ileum, caecum and colon and exceptionally the jejunum (18). Experimental infection with strains E232 and U82/123 isolated from suckling rabbits caused high mortality in one day old rabbits and only discrete lesions in weaned rabbits (15, 16, 18), whereas strain U 83/39 isolated from weaned rabbits caused high mortality in weanling rabbits and seemed not pathogenic in one-day-old rabbits (16). Mortality associated with colibacillosis occurs within 48 hours p.i. in suckling rabbits and 8 to 15 days p.i. in weanling rabbits. All these data indicate that possibly two different mechanisms of E. coli enteropathy might exist in rabbits. Only E. coli-enteropathy in weaned rabbits is further studied in detail in this communication.

Experimental infection with E. coli U83/39 was associated with colonization of the epithelium of ileum, caecum and colon and with the excretion of huge numbers of E. coli in the faeces. Until now four factors have been described, which increase faecal E. coli output in weanling rabbits : treatment with some antibiotics, such as ampicillin (6, 12, 13), coccidiosis (10, 17), high digestive HCl requirement of the diet, which indirectly causes increase of caecal pH (20, 21) and infection with RDEC-1. Both former factors were not involved. High digestive HCl requirement of the diet may be ruled out as in control rabbits the mean rectal E. coli score was always lower than 1.

In 1977 Cantey and Blake (1) isolated a piliated strain of E. coli O.15:NM from diarrhoeic rabbits. The strain produced diarrhoea in 48 of 62 rabbits 5.8 ± 3.1 days p.i. The mean duration of diarrhoea was 4.1 ± 2.8 days and mortality was at least 12 %. The bacterium was not invasive and did not synthetize either heat labile or heat stable enterotoxins. The strain produces a small amount of Shigella dysenteriae-like enterotoxin, but whether the toxin is important to the ability of RDEC-1 to produce diarrhoea is unknown (14). Infection by the strain caused an acute intestinal inflammatory response and large numbers of RDEC-1 were adhering to the mucosal surfaces of ileum, caecum and colon. The brush borders were in varying stages of degeneration (25). In vitro the intestinal brush border sucrase/isomaltase enzyme complex, which becomes first detectable at weaning, is capable to serve as host receptor for RDEC-pili (4). The strain first attaches to the tips of the Peyer's patch lymphoid follicles by 24 hours p.i., but not to ileal, caecal or colonic mucosa until 3 days p.i. Afterwards these sites were colonized too (3). Colonization as shown by faecal E. coli output reached a maximum 3 to 4 days p.i. and did not decrease until 15 days p.i. (2).

The description of the disease caused by RDEC-1 fits in very well with our results with E. coli U83/39 and it might be assumed that U83/39 acts according to a same mechanism. As in a previous epidemiological survey 40.0 % of weaned diarrhoeic rabbits showed lesions similar to RDEC-1 and U83/39 infection and as 13 out of 17 commercial rabbitries examined were infected by such strains (18), it might be concluded that this mechanism of E. coli infection is more important than assumed before and warrants further research.

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SUMMARY

Thirty-two different strains of E. coli isolated from diarrhoeic rabbits have been studied. None of the strains produced thermostable nor thermolabile enterotoxins, nor was invasive. Strains isolated from suckling rabbits attached to microvilli in vitro, whereas strains from weanling rabbits did not. The former strains all belonged to serogroup 0.109, while the latter belonged to at least 8 different serogroups. It was suggested that two different mechanisms of E. coli enteropathy might exist in rabbits.

After experimental infection of 5-week-old rabbits, the 26 strains isolated from weaned diarrhoeic rabbits attached to the intestinal epithelium. One strain has been studied further. E. coli strain 0.15/130 produces diarrhoea 6.25 ± 2.71 days after infection (p.i.) during 6.13 ± 1.89 days. Mortality was high. Sequential examination of the intestines by light and scanning electron microscopy showed the strain to attach first to the tips of the Peyer's patch lymphoid follicle epithelium by 24 hours p.i., but not to other sites until 3 days p.i. From 3 to 14 days p.i. the strain causes an acute intestinal inflammatory response with extensive colonization of ileum, caecum and colon. Most colonized cells lack microvilli, become rounded and desquamate. Severe oedema of the caecal lamina propria and submucosa was often present. Colonization of the intestines as shown by rectal swab cultures reaches a maximum 4 to 7 days p.i. and does not decrease until 14 days p.i.

RESUME

Trente-deux souches différentes d'E. coli provenant de lapins présentant de la diarrhée ont été étudiées. Aucune de ces souches n'a produit d'entérotoxines thermostables ni thermolabiles. Celles-ci ne sont pas invasives. Les souches isolées de lapereaux au nid s'attachent aux villosités intestinales de lapin in vitro, ce qui n'est pas le cas pour les souches isolées de lapereaux sevrés. Les souches de lapereaux non sevrés appartiennent au serogroupe 0.109 et ceux des lapereaux sevrés à au moins 8 sérogroupes différents. L'existence possible

de deux mécanismes d'entéropathogénicité colibacillaire a été suggérée.

Après infection expérimentale de lapereaux de 5 semaines, les 26 souches provenant de lapereaux sevrés se sont attachées aux cellules épithéliales de l'intestin. Une souche a été étudiée en détail. Cette souche (0.15/130) provoque de la diarrhée 6.25 ± 2.71 jours après l'infection (p.i.) pendant 6.13 ± 1.89 jours. La mortalité était élevée. La microscopie ordinaire et la microscopie électronique à balayage ont montré que la souche s'attache d'abord aux cellules épithéliales couvrant les follicules lymphoides des plaques de Peyer 24 heures p.i. Les autres segments n'ont été colonisés qu'à partir de 3 jours p.i. De 3 à 14 jours p.i., la souche a entraîné une réponse inflammatoire aigue de l'intestin avec colonisation importante de l'iléon, du caecum et du colon. La plupart des cellules colonisées étaient dépourvues de microvillosités, présentant une forme arrondie et désquamation. L'oedème caecal était fréquent. La colonisation de l'intestin comme l'ont montré les cultures de swabs rectales, a atteint un maximum 4 à 7 jours p.i. pour ne diminuer qu'à partir du 14ième jour.

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