

INFECTIOUS AGENTS ASSOCIATED WITH DIARRHOEA IN COMMERCIAL RABBITS: A FIELD STUDY

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Résumé

AGENTS INFECTIEUX ASSOCIÉS A LA DIARRHÉE CHEZ DES LAPINS DE CHAIR: ÉTUDE SUR LE TERRAIN. — Pendant une période de 9 mois la présence de parasites, bactéries et virus a été recherchée dans des lapereaux provenant de 21 élevages de type industriel et qui présentaient de la diarrhée. Ces micro-organismes ont été mis en relation avec les signes cliniques et les lésions macroscopiques et microscopiques. Des agents infectieux ont été trouvés chez 71,5 % des lapereaux. Des colibacilles s'attachant aux parois intestinales (ATEC) ont été détectés chez 40 % des lapereaux. Cette infection était associée à une mortalité modérée à sévère, de l'œdème cæcal, du gonflement sévère des ganglions mésentériques et des quantités parfois importantes de colibacilles s'attachant aux entérocytes de l'iléon, du cæcum et du côlon. Des rotavirus ont été trouvés dans 35,4 % des cas. La maladie se présentait normalement sous forme bénigne en infection pure et affectait surtout l'intestin grêle. Des coronavirus associés avec des ATEC ont été détectés dans un élevage seulement, alors que des coccidies étaient présentes dans 18,5 % des animaux examinés. Des infections multiples ont été constatées dans 18,5 % des cas.

Disease associated with diarrhoea, mainly after weaning, is a major cause of economic losses in commercial rabbit production. The highest mortality due to enteritis and diarrhoea occurs at five to eight weeks of age, but may also be present in younger rabbits. Whitney (1970) refers to this condition as the «enteritis complex» of rabbits and Löliger (1980) classifies it as acute dysentery. Most authors agree that the etiology of the disease is complex and includes viruses, bacteria and coccidia (Ostler, 1961; Whitney, 1976; Prescott, 1978; Löliger, 1980; Renault, 1980). Also dietary factors as the crude fiber content and the digestive HCl requirement of the feed and environmental factors play a major etiological part in rabbit diarrhoea (Colin and Renault, 1980; Prohaszka and Baron, 1981).

Until now no global studies have been performed on the importance of the different categories of infectious agents in the enteritis complex. The purpose of this paper is to deal with the results of an investigation on the occurrence of parasites, bacteria and viruses in diarrhoeic rabbits kept in intensified husbandry conditions and to relate the presence of these agents with clinical signs, gross pathology and histopathological lesions.

Materials and Methods

Over a period of nine months live diarrhoeic rabbits were collected in 21 commercial rabbitries with between 60 and 400 does. In total 130 rabbits (14 suckling rabbits included) arrived alive at the laboratory and were kept for this study. In the rabbitries New-

Zealand or Dendermonde White rabbits were housed in wire cages, most of which had droppings pits underneath and were fed *ad libitum* with a commercial pelleted ration containing 66 ppm robenidine as anticoccidial drug. Rabbits were weaned at between 4 and 5 weeks of age. During each visit one rabbit with acute signs of diarrhoea was taken to the laboratory and necropsied. None of the animals necropsied had been treated with antimicrobial drugs.

Within 20 min after killing the animals, specimens of duodenum, mid-jejunum, ileum, caecum, colon, heart, liver and kidney were fixed in 10% (v/v) formaline in phosphate buffered saline and processed routinely for paraffin sections. They were cut at 5 µm and stained with haematoxylin and eosin. Some sections were stained with Warthin-Starry silver stain or with Gram's stain. Presence of facultative aerobic bacteria in duodenum, jejunum, ileum and caecum and of anaerobes in caecum were evaluated according to standard procedures. Samples of caecal content were taken for parasitology and virology. Native preparations were made for semi-quantitative evaluation of numbers of *Saccharomyces guttulata*. Smears were made for Gram's stain and other smears were stained with carbol-fuchsin for demonstration of cryptosporidia (Heine, 1982).

Caecal content was examined using the salt-flotation-concentration technique for demonstration of coccidia and helminths. Numbers of parasites were counted by the McMaster egg counting technique (MAFF, 1977). Samples with coccidia were made up in 2% (w/v) potassium bichromate and allowed to sporulate in a water bath at 27 °C for subsequent differentiation of species as described before (Peeters *et al.*, 1981). Samples of caecal content were clarified by low speed centrifugation (8 000 × g). The supernatants were treated for negative staining with 2% (w/v) uranyl acetate in bidistilled water and examined for the presence of viral particles by transmission electron microscopy.

Results

All the rabbits necropsied showed various degrees of diarrhoea. The frequency of the isolated infectious agents is listed in table 1 and the age distribution of the animals in which the most important pathogens were present is shown in table 2.

No parasites, viruses or attaching *Escherichia coli* (ATEC) on the intestinal mucosal surface were established in 28.5% (38/130) of the rabbits examined. In some of them proliferation of *Saccharomyces guttulata* (12/37) was evident. *Clostridium perfringens* was shown in 3 of 37 animals, but no specific gross or microscopic pathology could be assigned to those organisms. Stomach contents were mostly normal (28/37). In some of the animals the stomach was found to be empty. No gross lesions were present in the small intestine, unless in one animal which showed ileo-ileal intussusception. Caecal contents were watery, liquid or impacted in 13, 10 and 7 out

of 37 animals respectively. Mesenteric lymph nodes were normal (19/37) or moderately (12/37) to severely swollen (6/37). Light microscopy showed no lesions in 16 animals, an acute intestinal inflammatory response in 9 animals and basophilic debris (increasing from duodenum to colon) in the enterocytes of 12 out of 37 animals.

Coccidiosis

Coccidia have been found in 18.5% (24/130) of the animals sampled. Only three species have been detected: *Eimeria magna* (12/24), *E. media* (12/24) and *E. perforans* (19/24). Infection and also lesions were usually slight. In five out of the infected animals more than 10⁵ oocysts per gram of faeces have been counted. Other parasites such as cryptosporidia or helminths were absent. Coccidia were associated with ATEC or with rotaviruses in 15 out of 24 animals. In this case the intestinal lesions were dominated by those typical for rota or ATEC-infection as will be reported later. In 12 out of 24 animals with coccidia, *Cl. perfringens* and/or increase of the number of *S. guttulata* was present. In 4 out of 24 animals there was also simultaneous proliferation of non attaching colibacilli.

Escherichia coli

Intestinal proliferation of *E. coli* as shown by semiquantitative evaluation on selective media was present in 72.3% (94/130) of the animals examined. In 55% of these 94 rabbits, histology showed gram-negative organisms attached to the intestinal epithelium. In suckling rabbits the attached bacteria formed a continuous layer of huge numbers of coccobacilli along the surface of the epithelium from duodenum to colon. In weaning rabbits colonization was diffuse in the caecum and rather patchy in the ileum and colon. Different grades of colonization were present according to the strain and the animal. Duodenum and jejunum mostly remained free from colonization in weaned rabbits. Infiltration of the lamina propria by polymorphonuclear leucocytes was often great, especially beneath areas of heavy bacterial attachment. Some affected epithelial cells showed a decreased amount of cytoplasm and were desquamating. Severe oedema of the caecal lamina propria and submucosa was often present, together with congestion of blood vessels and extravasation of erythrocytes. In the ileum there was moderate to strong villous atrophy. Diffuse infiltration of the liver by polymorphonuclear leucocytes was found in five out of 52 animals affected.

Animals affected by ATEC (attaching *E. coli*) showed anorexia, apathia and liquid diarrhoea with sometimes traces of blood or strands of mucus. In some animals only splashing sounds were heard. Suckling rabbits mostly died within

48 h after showing yellowish diarrhoea. Mortality in weanling rabbits was mostly moderate (5 to 12.2%) but reached up to 50% in some rabbitries. Rabbits died mostly 24 to 72 h after the onset of diarrhoea. Infection by ATEC was associated with rotaviruses or with coccidia in 17 out of 52 animals and with *Cl. perfringens* and/or proliferation of *S. guttulata* in 22 of the rabbits. However, in 16 of the animals infected by ATEC, no such agents were found.

Gross lesions were identical in rabbits infected

by ATEC alone, or by ATEC associated with parasites or viruses. In suckling rabbits the stomach contained normal amount of curdled milk, whereas the caecum was filled with a watery yellowish liquid. No lesions were found in the small intestine, except for congestion in one case. In weanling rabbits the stomach contained a watery food bolus and the small intestine was dilated with sometimes a slight congestion of the ileal segment. Moderate to severe oedema was evident in the caecum and the mesenteric lymph

Table 1. — Percentage occurrence of infectious agents in diarrhoeic rabbits

Agent	Rabbitry	Rabbits
Coronavirus	5	0.8
Rotavirus	81	35.4
<i>Escherichia coli</i>	100	72.3 ^a
ATEC ^b	71	40.0
<i>Clostridium spp.</i>	86	33.8
<i>Clostridium perfringens</i>	71	16.2
<i>Saccharamycopsis guttulata</i>	100	32.3 ^c
<i>Cryptosporidium sp.</i>	0	0.0
<i>Eimeria spp.</i>	43	18.5
Helminths	0	0.0
Total number	21	130

a: confluent growth of *E. coli* from at least the caecum on selective media.

b: Attaching *E. coli* to the luminal intestinal border.

c: presence of at least 5 blastospores per microscopic field at 540 ×

Table 2. — Percentage distribution of rotavirus, *E. coli* (ATEC) and coccidia in diarrhoeic rabbits

Age group (days)	Rotavirus	<i>E. coli</i>	Coccidia
0-14	4	4	...
15-21	...	6	...
22-28	4	10	...
29-35	24	25	38
36-42	50	33	29
43-49	11	10	12
50-56	...	4	8
57-63	2	6	8
≥ 63	4	4	4
Total number	46	52	24

nodes were markedly swollen. The caecal contents were foul-smelling, watery and brown. In some animals paint-brush hemorrhages were present on the caecal serosa. In some others miliary necroses were found in the heart and the liver (3/52). Warthin-Starry silver stain did not reveal *Bacillus piliformis*-like organisms at the border of these lesions.

Coronavirus

In one rabbitry coronaviruses were detected in caecal contents of a diarrhoeic rabbit. Simultaneously infection by ATEC was present. Lesions were as described above for ATEC infection.

Rotavirus

Rotaviruses were found in 81% of the rabbitries monitored and in 35.4% (46/130) of the rabbits sampled. Clinical signs included watery diarrhoea from weaning time on. In uncomplicated infections, diarrhoea stopped spontaneously 2 to 3 days after the onset of the symptoms and mortality was quite low. Animals showed anorexia for some days. Pure rota-infections were found in 18 out of 46 infected animals. Association with coccidia or ATEC occurred in 17 out of the animals and with *Cl. perfringens* and/or increase of the numbers of *S. guttulata* in 18 animals. In 10 animals there was also proliferation of non attaching *E. coli*.

Gross lesions were mostly limited to watery (11/25) or liquid (8/25) caecal contents in the rabbits which were only infected by rotaviruses without involvement of coccidia or ATEC. Caecal impaction was found in one animal. Mesenteric lymph nodes were slightly (9/25) to moderately swollen (10/25). Rotavirus-infection associated with ATEC showed lesions as described above for ATEC infected rabbits. Histology of the small intestine revealed moderate to severe villous atrophy. Lesions were more marked and more frequent in the posterior part of the small intestine than in the anterior part. Apical enterocytes on the tips of the villi were swollen, rounding and desquamating. Occasionally denuded tips were found. Usually the lamina propria was infiltrated by round cells with occasionally some neutrophils. Lesions in the caecum were discrete and limited to focal areas of desquamation of enterocytes (8/32). Presence of basophilic debris in epithelial cells was regularly found in 40% of the animals.

Discussion

Clinical signs and pathology of the diarrhoeic rabbits examined in this study correspond well with the general description of the enteritis complex given by Whitney (1976). In our situa-

tion, three different infectious agents seem to play a predominant role in the enteritis complex: coccidia, attaching *E. coli* and rotaviruses. Each of them seems to be associated with more or less typical gross and microscopic lesions, although two or three of them were often present at the same time. In 28.5% (37/130) of the animals examined however no such agents were found, although nine of these 37 animals showed acute intestinal inflammation. It is not excluded that this was linked with one or more infectious agents which were not established. Diarrhoea in the remaining 28 of them might be linked with dietary (Prohaszka and Baron, 1981; Renault, 1980) or environmental factors (Colin and Renault, 1980), which were not the subject of this study.

Cl. perfringens was found in 16.2% of diarrhoeic rabbits. In rabbits which showed only clostridia as potential pathogens, typical enterotoxaemia lesions such as epithelial desquamation, haemorrhage and oedema in the caecum (McDonnel and Duncan, 1975; Patton *et al.*, 1978) were completely absent. Lesions were similar in rabbits infected with coccidia, ATEC or rotaviruses alone and in rabbits infected with one of these agents in association with *Cl. perfringens*. So these clostridia were considered as normal habitants of the gut and no further attempts were made to quantitate them.

Proliferation of *S. guttulata* was present in 32.3% of the rabbits examined. As for *Cl. perfringens* no specific gross or microscopic pathology could be assigned to those organisms. *S. guttulata* is considered as a part of the normal intestinal flora of weaned rabbits, guinea pigs and chinchillas (Shifrine and Phaff, 1958). Experimental infection of rabbits with large numbers of blastospores of *S. guttulata* does not induce any clinical sign (Burgisser, 1961; Riche and Schöler, 1961). This has been confirmed by own experiments (unpublished). However, it is not excluded that abundant growth of *S. guttulata* in rabbits with intestinal disorders might aggravate enteric disease.

In only 18.5% of the rabbits low numbers of coccidia were detected. In an earlier study we found coccidiosis in 83.8% of 191 commercial rabbits (Peeters *et al.*, 1981). Also the numbers of species present per animal were reduced: 1 to 2 species per sample against 2 to 4 previously. The very pathogenic species *E. flavescens* and *E. intestinalis* were also absent in this study, whereas they occurred in 26.2 and 21.5% of the rabbits respectively in 1981. As since 1982 82.5 ppm sulphaquinoxaline/pyrimethamine has been replaced by 66 ppm robenidine as anticoccidial drug in the pelleted feed, it seems likely that robenidine is responsible for this change. This

confirms non published evidence in a commercial rabbitry with 400 does, where 66 ppm robenidine has been used since more than 3 years. Other studies performed in two different rabbitries showed also that robenidine reduced mortality from enteric diseases with 30.6 and 52.0% respectively (Peeters *et al.*, 1980). These findings confirm that coccidiosis is an important factor in the enteritis complex of commercial rabbits and indicate that coccidiosis has to be kept under permanent control.

Rotaviruses seemed to be very spread out they were present in 81% of the rabbitries and 35.4% of the rabbits sampled. This is in agreement with serological evidence, as we regularly detect high titers of rotavirus antibodies in sera of commercial rabbits. This has also been shown in the USA, where 98% of 91 samples were positive (Petric *et al.*, 1978) and in Hungary, where 74.1% of 112 samples from 5 large scale rabbitries were positive (Kudron *et al.*, 1982). This evidence seems to indicate that rotaviruses are endemic in commercial rabbit populations. Our findings suggest that rotaviruses are only mildly pathogenic. This hypothesis is supported by the results of preliminary experimental infection studies in weaned rabbits with lapine rotavirus (Petric *et al.*, 1978). Histological lesions associated with rotavirus-infection in this study correspond well with those described in other species (Flewett and Woode, 1978). Generally rotaviruses destroy the cells which synthesise disaccharidases. Lack of these enzymes causes lactose or other disaccharides to remain in the lumen of the bowel, only monosaccharides being absorbed by the healthy intestine, and so causes an osmotic drain, attracting body fluid into the intestinal lumen. This can aggravate diarrhoea caused by other pathogens such as coccidia and ATEC. Viral infection may also cause an increased sensitivity of enterocytes to bacterial adhesion (Costerton *et al.*, 1978).

Coronavirus-infection has been detected in only one animal. The significance of this agent is difficult to establish as ATEC were simultaneously present. Data in the literature about its pathogenicity in rabbits are scarce. They were found in rabbits in Canada (Lapierre *et al.*, 1980), in

Germany (Eaton, 1982) and in The Netherlands (Osterhaus *et al.*, 1982). Eaton (1982) found a 50% mortality rate in weaned rabbits naturally infected with a coronavirus in the absence of any other established pathogen. Osterhaus *et al.* (1982) however found only mild symptoms after experimental infection with a filtered faecal suspension. So more data are needed to evaluate the pathogenicity of coronaviruses for rabbits.

The most important losses and the most severe gross and macroscopic lesions in this study were found in rabbits infected with ATEC. As infection by these coccobacilli was associated with the same lesions in animals infected either with ATEC alone or in rabbits infected with a mixture of ATEC and other pathogenic agents, we consider them as responsible for the pathology observed.

Until now four factors have been described in rabbits which increase the numbers of *E. coli* along the intestinal tract: treatment with some antibiotics, such as ampicillin (Escoula *et al.*, 1981; Milhaid *et al.*, 1976; Morisse, 1978), coccidiosis (Licois and Guillot, 1980; Peeters *et al.*, 1984), high digestive HCl-requirement of the diet, which indirectly causes increase of caecal pH (Prohaszka, 1980; Prohaszka and Baron, 1981) and infection with some strains of *E. coli* as RDEC-1 (Cantey and Blake, 1977). Antibiotics were not involved here, as the animals have not been treated before sampling. The histopathologic changes associated with ATEC resemble strongly RDEC-1 infection, which causes similar lesions in ileum, caecum and colon. However, coccidiosis and diet as primary etiological factors can only be ruled out by experimental infection studies and characterization of these strains of attaching *E. coli*. This is the subject of a separate study (Peeters *et al.*, 1984).

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Summary

Over a period of 9 months the occurrence of parasites, bacteria and viruses has been examined in diarrhoeic rabbits from 21 commercial rabbitries and related with clinical signs and gross and microscopic lesions. Infectious disease agents have been found in 71.5% of the animals. *Escherichia coli* (ATEC) were found to be attached to the luminal intestinal border of 40% of the rabbits examined. This was associated with moderate to high mortality, caecal oedema, severe swelling of mesenteric lymph nodes and high numbers of colibacilli attached to the epithelium of ileum, caecum and colon. Rotaviruses were detected in 35.4% of the animals. Disease associated with pure rota infection was usually mild and affected predominantly small intestine. A coronavirus associated with ATEC has been established in one rabbitry and coccidia were present in 18.5% of the animals. Multiple agents were found in 18.5% of the animals.

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