Proceedings 3rd World Rabbit Congress, 4-8 April 1984, Rome – Italy, Vol. 2, 265-273

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

J.E. Peeters, P. Pohl, G. Charlier, R. Geeroms & B. Glorieux

National Institute of Veterinary Research
Groeselenberg 99 - 1180 Brussels, Belgium

INTRODUCTION

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 (18) refers to this condition as the 'enteritis complex' of rabbits. Most authors agree that the etiology of the disease is complex and includes viruses, bacteria and coccidia (8, 10, 14, 16, 18). Also dietary factors as the crude fiber content (17) and the digestive HCl requirement (15) and environmental factors (1) play a major etiological part in rabbit diarrhoea.

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 communication 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.

MATERIAL AND METHODS

During a period of nine months living diarrhoeic rabbits were collected in 21 commercial rabbitries with between 60 and 400 does. In total 130 rabbits arrived alive at the laboratory and were hold back 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 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 antibiotics.

Within 20 minutes after killing the animals, specimens of duodenum, midjejunum, ileum, caecum, colon, heart, liver and kidney were fixed in 10 % formaline in phosphate buffered saline and processed routinely for paraffin sections. They were cut at 5 µm and stained with haematoxylin and eosin. 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 semiquantitative evaluation of numbers of Sacharomycopsis guttulatus. Smears were made for Gram's stain, whereas other smears were stained with carbol-fuchsine for demonstration of cryptosporidia (5). 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. Samples with coccidia were made up in 2 % potassium bichromate and allowed to sporulate at 27 °C for subsequent differentiation of species as described before (11). Samples of caecal content were clarified by low speed centrifugation. The supernatants were treated for negative staining with 2 % 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. No parasites, viruses or ATEC (attaching Escherichia coli to the intestinal mucosa) have been established in 28.5 % (37/130) of the rabbits examined. In some of them proliferation of S. guttulatus (12/37) was evident. Clostridium perfringens has been shown in 3 of 37 animals, but no specific gross or microscopic pathology could be assigned to those organisms. Stomachal contents was mostly normal. No gross lesions were present in the small intestine, unless in one animal which showed intussusception. Caecal contents was watery, liquid or impacted in 35.1, 27.0 and 18.9 % of the 37 animals respectively. Mesenteric lymph nodes were normal (51.4 %) or moderately (32.4 %) to severely swollen (16.2 %). Light micrcscopy showed no lesions in 42.3 %, an acute intestinal inflammatory response in 24.3 % and basophilic debris (increasing from duodenum to colon) in the enterocytes of 32.4 % of the 37 animals.

Coccidia have been found in 18.5 % (24/130) of the animals sampled. Only 3 species have been detected : Eimeria magna (12/24), E. media

Table 1 : Percentage occurrence of infectious agents in diarrhoeic rabbits

AGENT	RABBITRY	RABBITS
Coronavirus	4.8	0.8
Rotavirus	81.0	35.4
Escherichia coli	100.0	72.3 (1)
ATEC (2)	71.4	40.0
Clostridium spp.	85.7	33.8
Clostridium perfringens	71.4	16.2
Sacharomycopsis guttulatus	100.0	32.3 (3)
Cryptosporidium sp.	0.0	0.0
Eimeria spp.	42.9	18.5
Helminths	0.0	0.0
Total number	21	130

(1) confluent growth of E. coli from at least the caecum on selective media
(2) attaching E. coli to the luminal intestinal border
(3) presence of at least 5 blastospores per microscopic field at 540X

(12/24) and E. perforans (19/24). Infection and also lesions were usually slight. Other parasites as cryptosporidia or helminths were not observed. 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-infections as will be reported further. In 50 % of the 24 animals with coccidia, Cl. perfringens and/or an increase of the numbers of S. guttulatus was present. In 4 out of the 24 animals there was also simultaneous proliferation of non attaching colibacilli.

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 55.3 % of these 94 rabbits, histology showed these organisms to be 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 weanling rabbits colonization was dif-

fuse in the caecum and rather patchy in 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 erythro cytes. In the ileum there was moderate to strong villous atrophy. Diffuse infiltration of the liver by polymorphonuclear leucocytes was found in 9.6 % (5/52) of the animals affected.

Rabbits affected by such attaching E. coli (ATEC) showed anorexia, apathia and liquid diarrhoea with sometimes traces of blood or strands of mucus. Suckling rabbits mostly died within 48 hours after showing yellowish diarrhoea. Mortality in weanling rabbits was mostly moderate (5.0 to 12.2 %), but reached up to 50 % in some rabbitries. Rabbits died mostly 24 to 72 hours after the onset of diarrhoea. Infection by ATEC was associated with rotaviruses or with coccidia in 32.7 % of the 52 animals and with Cl. perfringens and/or proliferation of S. guttulatus in 42.3 % of the rabbits. However, in 30.8 % 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 a 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 coecum and the mesenteric lymph nodes were markedly swollen. The caecal contents was 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 heart and liver (3/52).

In one rabbitry a coronavirus was detected in caecal contents of a diarrhoeic rabbit. Simultaneously infection by ATEC was present.

Lesions were as described above for ATEC infection.

Rotaviruses were found in 81.0 % of the rabbitries followed 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 rotavirusinfections were found in 39.1 % of the 46 infected animals. Association with coccidia or ATEC occurred in 37.0 % of the animals and association with Cl. perfringens and/or increase of the numbers of S. guttulatus in 39.1 %. In 10 animals (21.7 %) there was also proliferation of non attaching E. coli.

Gross lesions were mostly limited to watery (44 %) or liquid (32 %) caecal contents in the 25 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 (36 %) to moderately (40 %) swollen. Rotavirusinfection 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. Occasionaly denuded tips were found. Usually the lamina propria was infiltrated by round cells with occasionally some neutrophils. Lesions in the caecum were only discrete and limited to focal areas of desquamation of enterocytes (32 %). Presence of basophilic debris in enterocytes was regularly found (40 %).

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 (18). In our situation, 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 2 or 3 of them were often present at the same time. In 28.5 % (37/130) of the animals examined how-

ever no such agents were found, although 24.3 % 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 75.7 % of them might be linked with dietary or environmental factors, which were not the subject of this study.

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 (11). 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 sulphaquinoxaline/pyrimethamine has been replaced by 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 2 different rabbitries showed also that robenidine reduced mortality from enteric disease with 30.6 and 52.0 % respectively (12). 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 : they were present in 81.0 % of the rabbitries and in 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 U.S.A. (13) and in Hungary (6) where 98 and 74.1 % of the samples respectively were positive for rotavirus antibodies. This evidence seems to indicate that rotaviruses are endemic in commercial rabbit populations. Our findings in the field suggest that rotaviruses are only mildly pathogenic for rabbits. This hypothesis is supported by the results of preliminary experimental infection studies in weaned rabbits with lapine rotavirus (13). Histological lesions associated with pure rotavirusinfection in this study correspond well with those described in other species (4). Generally rotaviruses destroy the cells which synthesise disacharidases. Lack of these enzymes causes lactose or other disaccharides to remain in the lumen of the bowel and so causes an osmo-

tic drain, attracting body fluid into the intestinal lumen. This can aggravate diarrhoea caused by other pathogens such as coccidia and ATEC. Viral infections may also cause an increased sensitivity of enterocytes to bacterial adhesion (2).

Coronavirusinfection 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 (7), in Germany (3) and in The Netherlands (9). Eaton (3) found a 50 % mortality rate in weaned rabbits naturally infected with a coronavirus in the absence of any other established pathogen. Osterhaus (9) 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 these coccobacilli were 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. Their characteristics and pathogenicity will be the subject of a separate communication.

REFERENCES

1. COLIN, M. & L. RENAULT, Le Point Vétérinaire, 1980, 10, 51-58.
2. COSTERTON, J.W., R.T. IRVIN & K.J. CHENG, Ann. Rev. Microbiol., 1981, 35, 299-324.
3. EATON, P., Zeitschr. Versuchstierk., 1982, 24, 31-32.
4. FLEWETT, T.H. & G.N. WOODE, Arch. Virol., 1978, 57, 1-23.
5. HEINE, J., Zbl. Vet. Med. B, 1981, 29, 324-327.
6. KUDRON, E., I. HORVATH, A. ANTAL, L. SZABO & J. MEHESFALVI, Mag. All. Lapja, 1982, 37, 248-254.
7. LAPIERRE, J., G. MARSOLAIS, P. PILON, J.P. DESCOTEAUX, Can. J. Microbiol., 1980, 26, 1204-1208.
8. LOLIGER, H.C., Proc. II World Rabbit Congress, Barcelona 1980, Vol II, 265-273.
9. OSTERHAUS, A.D.M.E., J.S. TEPPEMA & G. VAN STEENIS, Lab. Anim. Sci., 1982, 32, 663-665.
10. OSTLER, D.C., Vet. Rec., 1961, 73, 1237-1255.
11. PEETERS, J.E., R. GEEROMS, R. FROYMAN & P. HALEN, Res. Vet. Sci., 1981, 30, 328-334.
12. PEETERS, J.E., R. GEEROMS, P. LAMPO & P. HALEN, Proc. II World Rabbit Congress, Barcelona 1980, Vol II, 315-324.

13. PETRIC, M., P.J. MIDDLETON, C. GRANT, J.S. TAM & C.M. HEWITT, Can. J. Comp. Med., 1978, 42, 143-147.
14. PRESCOTT, J.F., Vet. Bull., 1978, 48, 475-480.
15. PROHASZKA, L. & F. BARON, Zbl. Vet. Med. B, 1981, 28, 102-110.
16. RENAULT, L. In 'Physiologie et pathologie digestive du lapin', ADEPRINA, pp 147-184, Paris 1980.
17. SINCOVICS, G., Z. SZEREMY & I. MEDGYES, Proc. II World Rabbit Congress, Barcelona 1980, Vol II, 358-371.
18. WHITNEY, J.C., Lab. Anim., 1976, 10, 209-221.

SUMMARY

During 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 40.0 % of the rabbits examined. This was associated with moderate to high mortality, caecal oedema, severe swelling of mesenteric lymph nodes and huge numbers of colibacilli attached to the epithelium of ileum, caecum and colon. Rotaviruses were detected in 35.4 % of the animals. Sisease associated with pure infection was usually mild and affected predominanly 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.

RESUME

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 40.0 % des lapereaux. Cette infection était associée à une mortalité modérée à sévère, de l'oedème caecal, du gonflement sévère des ganglions mésentériques et des quantités parfois importantes de colibacilles s'attachant aux entérocytes de l'iléon, du caecum et du colon. 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. Un coronavirus associé avec des ATEC a été détecté dans un élevage seulement, alors que des coccidies étaient présentes chez 18.5 % des animaux examinés. Des infections multiples ont été constatées dans 18.5 % des cas.