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A NATURALLY OCCURRING CASE OF MUCOID ENTEROPATHY IN A SPECIFIC PATHOGEN FREE (SPF) RABBIT

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ABSTRACT

A specific pathogen free doe, showing signs of mucoid enteropathy, was necropsied. Lesions compatible with mucoid enteropathy were found, i.e. gastrointestinal paralysis, presence of mucus in the small intestine, and desiccated contents of the caecum. Bacteriological analysis yielded none of the classical pathogens, only a non-pathogenic type of *Escherichia coli*. The presence of rotavirus was detected through ELISA. Histology did not produce any pathognomonic results. Electron microscopy (EM) revealed the presence of viral particles 65 - 75 nm in diameter in enterocytes in the small intestine, caecum and colon, which are very likely rotavirus particles. Additionally, EM revealed coliform bacteria in vacuoles in the enterocytes, as well as in macrophages, but only in the ileum. In the liver numerous vacuoles were seen, some of which contained paracrystalline structures. In the brain tissue a severe degeneration was noted. Further tests to isolate and identify the intracellular bacteria and the viral particles are to be performed.

INTRODUCTION

At the end of 1996 a new and severe enteric disease appeared in Portugal, Spain and France. Since 1997 rabbitries in Belgium and several other countries were reached by its epizootic spread throughout the European mainland. There have been reports of the disease in the United Kingdom and Canada as well. Due to absence of a detectable causative agent, the disease has been called "mucoid enteropathy" (EP) or "epizootic enterocolitis". It is characterized by a sudden onset of mortality which can reach 30 to 80 %, depression, decreased feed intake, abdominal distention, limited diarrhoea and sometimes the discharge of mucus (BOUCHER and NOUAILLE, 1997 ; COUDERT *et al.*, 1997). Often periods of increased mortality are interspaced with periods of limited losses. This indicates that EP induces a certain level of immunity, which has recently been confirmed under controlled conditions (LICOIS *et al*., 1999). At necropsy, enteritis is not often seen. Usually there is an overfilling of the stomach, with large quantities of fluids and gas in the stomach and the intestines, in some cases associated with mucus plugs in the small intestine or in the colon. The caecal content can be entirely or partially desiccated ; in the latter case the other part consists of fluids (BOUCHER and NOUAILLE, 1997 ; COUDERT *et al.*, 1997). Occasionally the lungs show an interstitial pneumonia, but these lesions should be interpreted with care, for they have also been seen in uninfected control animals (LICOIS *et al.*, 1999).
The disease has been reported in meat rabbits and does, as well as in pet rabbits. Reproduction of the disease was fairly easy when conventional rabbits were used, but only a low percentage of specific pathogen free (SPF) rabbits showed symptoms after experimental infection (LICOIS, 1998). After immunodepression of SPF rabbits for a period of three weeks with corticosteroids (LICOIS *et al.*, 1998), a useful disease reproduction protocol could be established. To our knowledge, the case presented here is the first report of a naturally occurring case of mucoid enteropathy in an SPF colony.

MATERIAL AND METHODS

The animal concerned was an SPF doe, aged 7 months. It arrived at our laboratory alive, and was euthanized for necrospy.

Bacteriological examination was performed on the kidneys, the stomach, the small intestines and the caecum. Caecal contents were analyzed for enteropathogenic *Escherichia coli* (EPEC), *Clostridium spiroforme* and coccidiosis as described by PEETERS *et al.*(1986). Typing of *E. coli* was performed according to the method of PEETERS *et al.* (1988). Control on the presence of rota and coronavirus was performed using a commercial ELISA kit (Pathasure Enteritis ELISA kit, Vetoquinol Diagnostics). For *Cryptosporidium* detection we used the method described by GARCIA and CURRENT (1989). *Saccharomycopsis guttulatus* detection was done using Gram staining.

Histological examination was performed on liver, kidney, brains, duodenum, jejunum and ileum, using haematoxylin-eosin (HE) staining techniques. Additionally a luxol fast blue staining was used to examine the neuronal tissues of the intestines.

Both scanning (SEM) and transmission electron microscopy (TEM) were performed on stomach, duodenum, jejunum, ileum, caecum, and colon. For the examination of the brain and liver only TEM was used.

RESULTS

The doe showed severe depression, dehydration, pale mucosae, distention of the abdomen, and traces of mucus on the perianal region. She was the third animal in the SPF colony to show these symptoms, but the first to be examined in our laboratory. The two other animals had died in the laboratory of origin two to three days after onset of the disease. At necropsy, the animal was well-fed. No macroscopic lesions could be noted in the lungs. The stomach was filled with a dry content interspersed with hairs. The gastric mucous membrane showed erosions, especially in the fundus. Duodenum, jejunum, ileum and caecum were congested with subserosal and mucosal petechiae and some small haemorrhages. The small intestines contained mostly gas and some small mucus plugs, but no blood. The content of the caecum was partly desiccated, while the other part consisted of fluids and gas. The liver was very pale. The spleen was slightly congested and both kidneys showed multiple focal cortical degeneration. The other organs showed no macroscopic lesions.

Bacteriological analysis of stomach, duodenum, jejunum, ileum and caecum yielded a non-pathogenic type of *E. coli*, biotype 2 (non-mobile). All other bacteriological as well as parasitological analyses gave negative results. ELISA detected the presence of rotavirus. Coronavirus was not detected.

Histological examination of the liver showed fatty degeneration of the hepatocytes and capillary congestion, but no inflammation. In the kidneys tubulary degeneration without deposits of amyloid was found, with presence of capillary congestion and limited interstitial infiltrates of lymphocytes. In the brains there was capillary congestion as well, without any inflammatory infiltrate. In the duodenum, jejunum and ileum congestion and villus atrophy was detected, with a diffuse mucosal lymphoid infiltrate. Some crypts showed necrosis, and contained macrophages with enlarged eosinophilic cytoplasm. Hyperplasia of the goblet cells varied according to the intestinal segment ; it was virtually absent in the jejunum, but was more pronounced in the ileum and clearly present in the duodenum. In some places the submucosa presented a similar but more focal inflammatory reaction with occasional

implication of the Meissner's plexi, including an infiltration of lymphocytes in continuity of the inflammatory reaction in the surrounding submucosa. The tunica muscularis and additional Auerbach's plexi did not show any pathological lesions. These pathological changes are compatible with an enteropathy, but are not pathognomonic. Based on the inflammatory infiltrate, a viral component was suggested. The luxol fast blue staining yielded no clear signs of neuronal degeneration.

Electronmicroscopically the stomach showed no lesions, no bacteria and no viral particles. In the duodenum, a few areas with epithelial desquamation were found with SEM, some with coliform bacteria. The microvilli on the enterocytes were mostly intact. TEM revealed a few small areas containing viral particles with a diameter of 65 to 75 nm in dilated cisterns of the coarse endoplasmic reticulum of the enterocytes (figure 1). In the jejunum a more frequent presence of viral particles was detected in the epithelium. Some viral particles were present in a vacuole surrounded by a membrane. The epithelium of the ileum partly lacked its microvilli, and some enterocytes had burst. Coliform bacteria were found intracellularly in enterocytes (figure 2) and in macrophages. Viral particles were present in the enterocytes. In the caecum there was far less damage to the epithelium. Only two groups of viral particles were found in the epithelium. More viral particles were found in the enterocytes of the colon, but here too the epithelium was virtually intact. Neuronal degeneration was seen in the brain, but no viral particles were found. In the liver numerous vacuolized hepatocytes were found, some of them with paracrystalline structures (figure 3), but no viral particals could be detected.

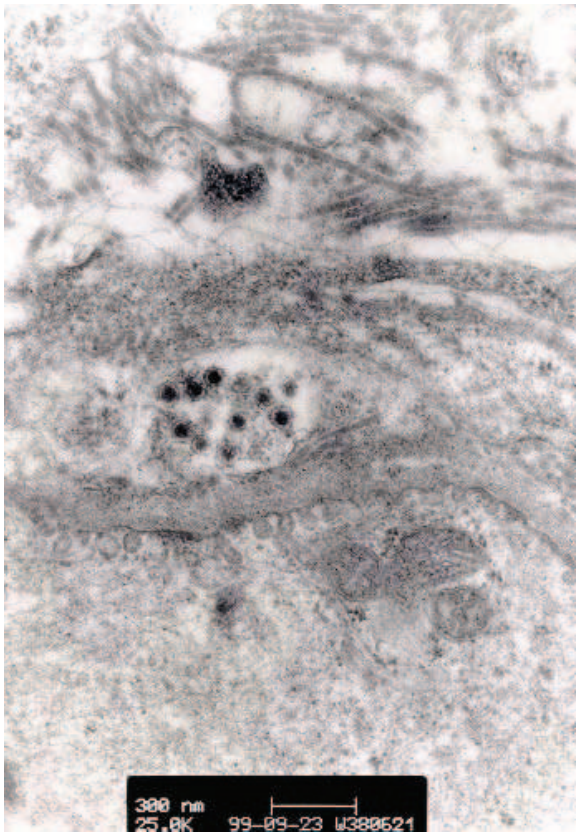

Figure 1. Transmission electron micrograph of the duodenum. Bar represents 300 nm. Viral particles approximately 65 - 75 nm in diameter were detected in dilated cisterns of the coarse endoplasmic reticulum of enterocytes.

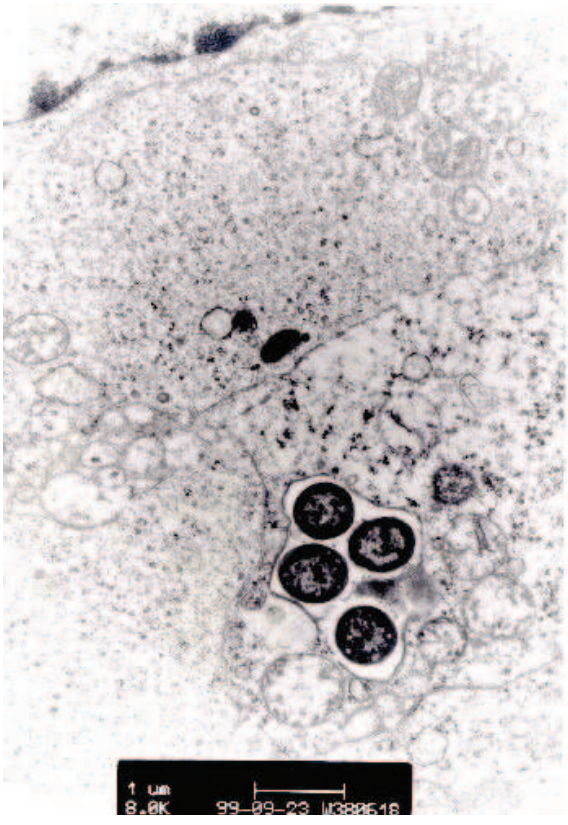

Figure 2. Transmission electron micrograph of the ileum. Bar represents 1 µm. Intracellular coliform bacteria were detected in enterocytes.

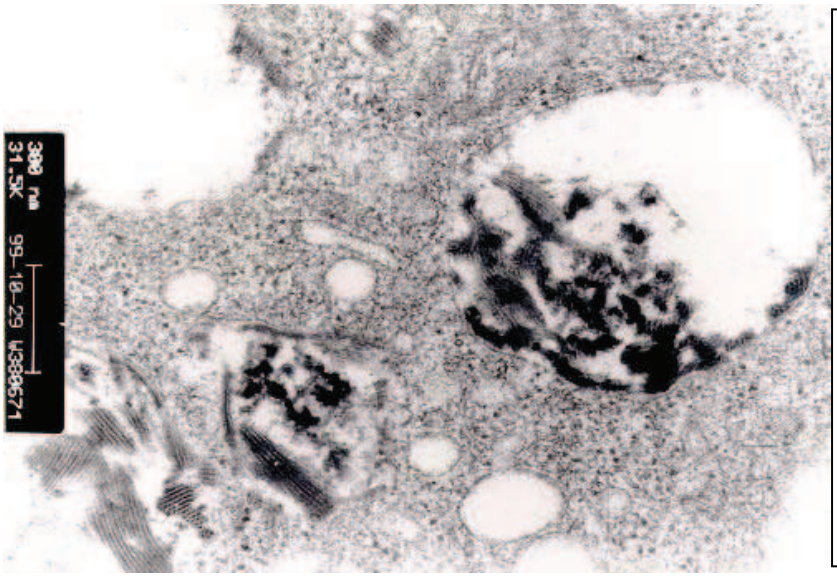

Figure 3 . Transmission electron micrograph of hepatocytes. Bar represents 300 nm. The hepatocytes contain numerous vacuoles, some of them with paracrystalline structures.

DISCUSSION

Several theories have been formulated on the possible cause of EP. We know that it spreads epizootically, and it induces immunity (LICOIS *et al.*, 1999). The disease can be controlled to a certain extent by the use of antibiotics, in particular those with a predominantly gram positive spectrum like tiamulin (BARRAL, 1999) and bacitracin (DUPERRAY, 1999). But EP also reappears very rapidly after termination of the treatment (LICOIS *et al.*, 1999). A virus may be playing an important role in the disease syndrome (LEGALL *et al.*, 1998), but the frequent presence of classical bacterial pathogens like EPEC and *Clostridium spiroforme* (ANONYMOUS, 1999 ; ROSSI *et al.*, 1999) should also be taken into account, even if it were only as a complicating factor. As for neuronal degeneration in the intestinal autonomous nervous system, information is contradictory. BOUCHER and NOUAILLE (1997) report he finding of lesions in the ganglion coeliacum and mesentericum, which is corroborated by findings by Dr. M. van der Hage at the University of Utrecht in the Netherlands (personal communication), and the finding of gastrointestinal paralysis and sometimes bladder paralysis. On the other hand there are also reports of researchers who haven't found any proof of neuronal degeneration, like Prof. M. Wyers of the Veterinary School of Nantes, France (DEDET, 1998 ; LICOIS *et al.*, 1999). Obviously it is technically very difficult to collect ganglia without traumatizing them, which does not facilitate their examination.

The case presented here is an SPF rabbit which had not been used in any experiment. Being SPF, it can be assumed that no "contaminating" pathogens were present, and that any pathogen found could have played a major role in the disease development. At first sight no pathogens were detected, except for rotavirus. However, its presence alone cannot explain the extent of the disease symptoms. Since the size of the viral particles found in the intestinal wall corresponds with that of rotavirus, and the rotavirus was detected by ELISA, it is likely that that they are rotavirus particles. The paracrystalline structures found in the liver could indicate that this organ is implicated in the disease pattern as well. There is a report of extra-mucosal spread of rotavirus and development of hepatitis in immunosuppressed mice (UHNOO *et al.* 1990). However, in this case no indications of an inflammatory reaction in the liver was found.

No pathogenic bacteria were isolated from the intestinal content ; only a non-pathogenic type of *E. coli* was found. However, coliform bacteria were detected intracellularly in the ileal

epithelium. Even EPEC invade cells only occasionally; therefore, it is not likely that the intracellular bacteria should be *E. coli*. Further steps will be taken to isolate the bacteria as well as the viral particles, and to identify them. In the intestines the Meissner's plexi are partially implicated in the inflammatory response, but no neuronal degeneration is found here or in the Auerbach's plexi. Nevertheless, the possible role of the autonomous nervous system in the disease syndrome needs further investigation.

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