A NATURALLY OCCURRING CASE OF EPIZOOTIC ENTEROPATHY IN A SPECIFIC-PATHOGEN-FREE RABBIT COLONY

Een geval van natuurlijk optredende epizoötische enteropathie in een specifieke-pathogenen-vrije konijnenkolonie

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SUMMARY

In 1996 and 1997 a new epizootic enteric rabbit disease appeared in Europe. Since then the disease has also been reported in other parts of the world. Its main symptoms are caused by paresis of the intestinal tract. The causative agent is still unknown. The disease has been reported in meat rabbits and does, as well as in pet rabbits. The present report concerns a naturally occurring case of this disease in a specific-pathogen-free (SPF) rabbit colony. In the intestinal contents of one animal sacrificed for thorough examination, rotavirus and an apathogenic type of *Escherichia coli* were detected. From the ileal wall *Providencia rettgeri* was isolated, which is unlikely, however, to be of pathogenic significance. Histological and electron-microscopical analyses did not reveal any pathognomonic lesions.

SAMENVATTING

In 1996 en 1997 verscheen in Europa een nieuwe epizoötische spijsverteringsziekte bij de konijnen. Sindsdien is de ziekte ook gemeld in andere delen van de wereld. De voornaamste symptomen worden veroorzaakt door parese van het spijsverteringskanaal. De oorzaak ervan is nog steeds niet bekend. De ziekte werd gerapporteerd bij vleeskonijnen en voedsters, evenals bij gezelschapskonijnen. In dit artikel beschrijven we een natuurlijk optredend geval van deze ziekte in een kolonie van specific-pathogen-free (SPF) konijnen. In de intestinale inhoud van één dier, dat werd geëuthanaseerd voor een grondig onderzoek, werden rotavirus en een niet-pathogeen type van *Escherichia coli* aangetroffen. Uit de ileumwand werd *Providencia rettgeri* geïsoleerd, maar de kans dat deze bacterie van betekenis is, lijkt klein. Histologische en elektronenmicroscopische analysen leverden geen detectie van pathognomonische lesies op.

INTRODUCTION

A new epizootic enteric rabbit disease appeared in Portugal, Spain and France in late 1996, and by late 1997 had spread to most of Europe. Since then, the disease has also been reported in Canada, Mexico, Tunisia, India and other countries. Due to the absence of a detectable causative agent, the disease has been called "mucoid enteropathy", "enterocolitis" (Lebas and Coudert, 1997) or "epizootic rabbit enterocolitis" (Coudert *et al.*, 2000; Duperray *et al.*, 2000; Macchioni *et al.*, 2000). However, during a recent meeting of rabbit pathologists it was agreed to use the term "enteropathy" rather than "enterocolitis", since experimental reproduction of the disease in specific-pathogen-free (SPF) rabbits does not cause inflammatory lesions of the digestive system (Anonymous, 2000).

Rabbit epizootic enteropathy (REE) is characterized by an acute mortality (reaching 30 to 80 %), depression, inappetentia, abdominal distention, limited diarrhoea and sometimes the discharge of mucus. Periods of increased mortality are often interspaced with periods of limited losses. This suggests that REE induces a certain level of immunity, which has been confirmed experimentally (Licois *et al.*, 1999). At necropsy, the lesions are compatible with intestinal paresis. There is usually an overfilling of the gastrointestinal tract with large quantities of fluids and gas,

which is sometimes associated with mucus plugs in the small intestine or in the colon. The caecal content can be entirely or partially desiccated (Boucher and Nouaille, 1997; Coudert *et al.*, 1997). Occasionally the lungs show an interstitial pneumonia, but these lesions have also been seen in uninfected control animals (Licois *et al.*, 1999), so they should be interpreted with care. Histologically, lesions are found mainly in the small intestine, and seem to point in the direction of a viral etiology. They consist of villus atrophy and hyperplasia of the goblet cells. This hyperplasia is found throughout the entire length of the intestinal mucosa. Mucosal gland cells show caryorhexis, and down in the crypts large cells with granular and acidophilic cytoplasm can be found (Dedet, 1998). As for neuronal degeneration in the intestinal autonomous nervous system, information is contradictory. Boucher and Nouaille (1997) report the finding of lesions in the ganglion coeliacum and mesentericum, a report which is corroborated by the finding of gastrointestinal paresis and sometimes bladder paralysis. On the other hand there are also reports of researchers who have not found any proof of neuronal degeneration (Dedet, 1998; Licois *et al.*, 1999).

REE has been reported in meat rabbits and does, as well as in pet rabbits. Experimental reproduction of the disease was fairly easy in conventional rabbits, but SPF animals showed low morbidity and low mortality (Licois, 1998), unless they were immunosuppressed (Licois *et al.*, 1998). To our knowledge, the case presented here is the first report of a naturally occurring case of REE in an SPF colony.

MATERIALS AND METHODS

The subject was an SPF doe, aged 7 months, and the third animal in the colony showing signs of enteropathy. It arrived at our laboratory alive, and was euthanized for necropsy.

Bacteriological examination was performed on the stomach, the small intestines, the caecum and the kidneys. 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 done according to the method of Peeters *et al.* (1988). Possible presence of rota and coronavirus was checked using a commercial ELISA kit (Pathasure Enteritis ELISA kit, Vétoquinol Diagnostics, Aartselaar, Belgium). For *Cryptosporidium* detection, carbolfuchsine staining (Eckert *et al.*, 1995) was used. *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 nervous 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. Two other animals had died in the laboratory of origin two to three days after onset of the same symptoms. At necropsy, the animal was well fed. The stomach was filled with a dry content interspersed with hairs. The gastric mucosa 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. Part of the caecal content was desiccated, while the other part consisted of fluids and gas. The liver was pale. The spleen was slightly congested and both kidneys showed multiple foci of cortical degeneration. The other organs displayed no macroscopic lesions.

Bacteriological analysis of stomach, duodenum, jejunum, ileum and caecum yielded a non-pathogenic type of *E. coli*, i.e. biotype 2 (non-motile). All other bacteriological and parasitological analyses of the intestinal contents produced negative results. ELISA detected the presence of rotavirus. Coronavirus was not detected. The kidneys were bacteriologically sterile.

Histological examination of the liver showed fatty degeneration of the hepatocytes and capillary congestion, but no inflammation. In the kidneys, tubular degeneration was found, with capillary congestion and limited interstitial infiltrates of lymphocytes. In the brain 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 macro-

phages with enlarged eosinophilic cytoplasm. Hyperplasia of the goblet cells varied according to the intestinal segment: it was virtually absent in the jejunum, but 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.

Electron-microscopically, in the duodenum, a few areas with epithelial desquamation were found with SEM, some with rod-like 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. Their morphology and diameter were compatible with rotavirus. In the jejunum, more viral particles were detected within 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. Rod-like bacteria were found intracellularly in enterocytes and in macrophages. After homogenization of the ileal wall, *Providencia rettgeri* was isolated. 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. In the liver, numerous vacuolized hepatocytes were found, some of them with paracrystalline structures.

DISCUSSION

The SPF rabbit presented here had not been used in any experiment. It was diagnosed as suffering from REE based on the clinical symptoms of depression, abdominal distention, mucus discharge, and the lesions seen at necropsy, (the overfilling of the gastrointestinal tract with fluids and gas, some mucus, and a partially desiccated caecal content) as described by Boucher and Nouaille (1997) and Coudert *et al.*, (1997). The absence of the "classical" enteropathogenic agents also corroborates this diagnosis. Lebas (2001) reports that a reference inoculum is now available for experimental reproduction of REE, which is tested for the absence of parvovirus, coronavirus, calicivirus, clostridia, EPEC, and a number of other potential pathogens. Several attempts to immunohistochemically identify the viral particles in the enterocytes using a Goat anti-Rotavirus antibody (Chemicon International, Inc., Temecula, CA, USA), and to isolate them through centrifugation, have been unsuccessful. In view of their morphology and dimensions, they are most likely to be rotavirus particles. However, the presence of rotavirus alone cannot explain the extent of the symptoms. Lebas (2001) also reports the detection of "banal" rotavirus in two rabbits originating from an REE-affected rabbitry, which did not show any clinical symptoms of rotavirosis. The *P. rettgeri* isolated from this case is likely to be an accidental finding. The SPF colony whence the doe originated, has had periods of normal mortality, alternated with periods of increased mortality since the first cases were reported. Licois *et al.* (1999) report the development of a protective immune response in animals surviving a previous challenge. The paracrystalline structures found in the liver may have been caused by hyperlipoproteinemia, as reported in human cases of endogenous hyperlipoproteinaemia by Kemmer and Hanefeld (1977).

Several theories have been formulated on the possible cause of REE. A virus may be playing a key role in the disease syndrome (Legall *et al.*, 1998). So far, however, despite observations of virus-like particles in purified material from the digestive tract of REE cases, all attempts to isolate these particles in sufficient quantity to allow further analysis have failed (Licois *et al.*, 1999). The frequent presence of bacterial pathogens such as EPEC and *Clostridium spiroforme* (Anonymous, 1999a; Rossi *et al.*, 1999) should be taken into account, even if only as a complicating factor. The disease can be controlled to a certain extent through the use of antibiotics, in particular those with a predominantly gram-positive spectrum such as tilmicosin, tiamulin (Barral, 1999) and bacitracin (Duperray *et al.*, 2000; Richard *et al.*, 2000). However, the aminoside apramycin, primarily active against gram-negative bacteria, has also been successfully used (Badiola *et al.*, 2000). REE reappears very rapidly after termination of the antibiotic treatment (Coudert *et al.*, 2000; Licois *et al.*, 1999), which seems to contradict the hypothesis that only bacteria cause REE.

The theory that there could be a connection between REE and the dysautonomia syndrome is not entirely sustained by the available findings: as mentioned before, information about neuronal degeneration in the autonomous nervous system is contradictory. It is technically very difficult to collect autonomous gang-

lia without traumatizing them, a fact which does not facilitate their examination. In this case no neuronal degeneration was found either in the Meissner's or in the Auerbach's plexi. Nevertheless, the possible role of the autonomous nervous system in the disease syndrome requires further investigation.

A connection with mucoid enteropathy, a disease that was described as early as 1929 (van Kruiningen and Williams, 1972), seems more likely. The symptoms of mucoid enteropathy are similar to those of REE, be it without its epizootic character. But then the rabbit sector has thoroughly changed over the past decades, with increasing scales of transport and enterprises in general. Moreover, the cause of mucoid enteropathy was never found.

Several possible new routes for research on REE have been outlined (Anonymous, 2000). These include the investigation of the pathophysiological changes that occur during the disease, especially the role of bacterial toxins and their effect on neurons. Recently Rossi *et al.* (2001) reported that the paralysis of the intestines seen in REE cases is related to the impossibility of stimulating the smooth muscle cells with acetylcholine. Toxins such as the ones produced by *Clostridium spiroforme* and *C. perfringens* could cause such an effect.

Another possible route is the study of flagellata, whose importance is usually underestimated because they do not survive long after the death of the host. Bacteriophages could provide a link between the virus theory and the role played by bacteria. Since bacteriophages are ubiquitous, a comparison between those present in REE cases and those in clinically normal rabbits should be performed.

REFERENCES

Anonymous (1999a). Le rôle des clostridies est primordial dans la mortalité par entérocolite. *L'Eleveur de Lapins 11,* 28-29.

Anonymous (2000). COST 848 , Multi-facetted research in rabbits: a model for developing healthy and safe production with respect to animal welfare. *Report of the kick-off meeting. Website: http://www.etsia.upv.es/cost848/.*

Badiola J. I., Faus C., Pérez de Rozas A. M., Gorostiaga O., Rosell J. M. (2000). Mucoid enteropathy: treatment with apramycin of naturally infected rabbits. In: Universidad Polytécnica de Valencia, ed. *Proceedings of the 7th World Rabbit Congress. Valencia, 4-7July 2000, Vol. B,* 195-198.

Barral E. (1999). Entérocolite: l'utilisation de la tiamuline se précise. *L'Eleveur de Lapins 5,* 42-43.

Boucher S., Nouaille L. (1997). Le syndrome entéropathie mucoïde. *L'Eleveur de Lapins 9-10,* 25-29.

Coudert P., Lebas F., Licois D. (1997). Une nouvelle pathologie ravage les élevages: la profession se mobilise. *Cuniculture 24,* 225-229.

Coudert P., Rideaud P., Raboteau D. (2000). Epizootic rabbit enterocolitis: spontaneous evolution and attempt to control the disease. In: Universidad Polytécnica de Valencia (ed.) *Proceedings of the 7th World Rabbit Congress. Valencia, 4-7July 2000, Vol. B,* 219-224.

Dedet V. (1998). Trois questions à Monique Wyers à propos de l'histopathologie de l'entérocolite du lapin. *Semaine Vétérinaire 895,* 2-3.

Duperray J., Eckenfelder B., Puybasset A., Richard A., Rouault M. (2000). Interest of zinc bacitracin in the treatment and the prevention of the epizootic rabbit enterocolitis syndrome in growing rabbits. In: Universidad Polytécnica de Valencia, ed. *Proceedings of the 7th World Rabbit Congress. Valencia, 4-7July 2000, Vol. B,* p. 233-240.

Eckert J., Braun R., Shirley M. W., Coudert P. (1995). Guidelines on techniques in coccidiosis research. *COST 89/820, Biotechnology,* 214-215.

Kemmer C., Hanefeld M. (1977). Ultrastructural results in liver biopsies of patients with hyperlipoproteinaemia. *Zentralblatt für Allgemeine Pathologie und Pathologische Anatomie 121,* p. 243-253.

Lebas F., Coudert P. (1997). Entérocolite: les données récentes. *Cuniculture 24,* 269-272.

Lebas F. (2001). Entérocolite: situation en juin 2001. *Cuniculture 28,* 183-188.

Legall G., Morisse J.-P., Picault J.-P., Allee C., Le Bichannic P., Colin P. (1998). Une maladie contagieuse, probablement virale. *L'Eleveur de Lapins 6-7,* 28-30.

Licois D. (1998). Séance d'actualité: l'Entérocolite Epizootique. Bilan des travaux réalisés à l'INRA, sur l'Entérocolite Epizootique, dans l'hypothèse d'une étiologie virale. In: ITAVI, Paris (ed.) *7è Journées de la Recherche Cunicole en France. Lyon, 13-14 mai 1998.* 20-26.

Licois D., Vautherot J. F., Coudert P., Dambrine G. (1998). Modèle de reproduction expérimentale de l'entérocolite épizootique chez des lapins EOPS. *World Rabbit Science 6,* 349-353.

Licois D., Lebas F., Coudert P., Le Gall G. (1999). Note d'information sur les travaux de recherche conduits sur l'Entérocolite Epizootique du Lapin. *Website: http://www.rabbit-science.com/info1.htm. 10,* 1-7.

Macchioni P., Mariani G., Finzi A. (2000). Epidemiology of epizootic rabbit enterocolitis in rabbits raised without chemical treatments. In: Universidad Polytécnica de Valencia (ed.) *Proceedings of the 7th World Rabbit Congress. Valencia, 4-7 July 2000, Vol. B,* 307-310.

Peeters J. E., Geeroms R., Carman R., Wilkins T. (1986). Significance of *Clostridium spiroforme* in the enteritis complex of commercial rabbits. *Veterinary Microbiology 12,* 25-31.

Peeters J. E., Geeroms R., Ørskov F. (1988). Biotype, serotype, and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrhoeic commercial rabbits. *Infection and Immunity 56,* 1442-1448.

Richard A., Remois G., Lafargue-Hauret P. (2000). Effect of zinc bacitracin on epizootic rabbit enterocolitis. In: Universidad Polytécnica de Valencia (ed.) *Proceedings of the 7th World Rabbit Congress. Valencia, 4-7July 2000, Vol. B*, 345-349.

Rossi G., Vandekerchove D., Köhler B., Hafez H. M. (1999). The appearance of enterocolitis with high mortality in middle Germany, Abstracts of 11th Symposium of Celle on Housing and Diseases of Rabbits, Furbearing and Pet Animals. *World Rabbit Science 7,* 123.

Rossi G., Grilli G., Galazzi D., Lavazza A., Köhler B. (2001). Untersuchungsergebnisse bei Enterocolitis, *12. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle 9. und 10. Mai 2001*, Ed. Deutsche Veterinär Medizinische Gesellschaft, Giessen.

van Kruiningen H. J., Williams C. B. (1972). Mucoid enteritis of rabbits. *Veterinary Pathology 9,* 53-77.

Uit het verleden

EEN SCHELLEKEN BOEBOE

"Een schelleken boeboe
Een schepken ratatoe
Menschen schept koerage
Gij zult ervan opblazen
Tutenstoverij
En rijst met mossels bij
Menschen schept koerage
Den oorlog gaat voorbij."

Zo zongen de Gentenaars naar het einde van de eerste wereldoorlog toe.

Een schelleken boeboe? Hondenvlees, inderdaad.

In het Gentse Woordenboek geeft de samensteller en tijdgenoot Lodewijk Lievevrouw – Coopman tekst en uitleg: "*Een schelleke boeboe: hondenvlees en worst, zoals men tijdens den oorlog 1914 – 1918 verkocht*" en hij verwees naar de *Saucietjesboulevard*, alias *Boulevard Boeboe* of de Elisabethlaan bij het Sint-Pietersstation.

Of het nu daar was dat er hondenvlees verkocht werd of elders, er is geen twijfel mogelijk: in de laatste twee oorlogsjaren werd er effectief hondenvlees gegeten. In maart 1917 had de politie verschillende clandestiene hondenslachterijen ontdekt. Men wou die praktijk niet verbieden, maar er moest controle en waarborg komen dat het om "gezond" hondenvlees ging.

De zaak werd met Duitse Gründlichkeit aangepakt. In het gemeentelijk slachthuis werd een hondenslachterij ingericht. Per geslachte hond diende 50 centimes slachtrecht betaald te worden. Alleen gezonde dieren kwamen in aanmerking en het vlees kreeg een bijzonder keurteken. De hondenhuiden mochten tegen betaling afgeleverd worden aan de *Häutesammelstelle* of aan erkende opkopers. In de landelijke gemeenten was het slachten van honden verboden. Het Gentse gemeentebestuur tenslotte, kreeg de opdracht winkels aan te wijzen waar het vlees kon verkocht worden. Zij dienden het opschrift *Hondenslachterij* of *Hondenbeenhouwerij* te dragen en ze mochten geen ander vlees verkopen. Alle preparaten met hondenvlees moesten effectief het opschrift *Hondenvleesch* dragen.

Dat was nog niet alles. De hondenbeenhouwerijen dienden een klantenboek bij te houden met de namen van kopers en verkochte hoeveelheden. Per gezin mocht er maximaal één kg per week verkocht worden. De maximumprijs werd op 2 fr/kg vastgesteld. Aan hotels, restaurants en Duitse militairen mocht er geen *boeboe* verkocht worden.

Het uitheemse en onbekende voedsel dat via Amerikaanse hulp toen binnen kwam (ratatoe: ratatouille, soldatenkost; rijst met mossels; tutenstoverij...) wekte heel wat weerstand en afkeer op. Hoe zou het dan geweest zijn met hetgeen rechtstreeks afkomstig was van 's mensen's beste vriend? Maar honger is de beste saus...zo wist men vroeger.

Bron: Despretz, A., Ghendtsche Tydinghen, jg. 21, 1992, p. 166 – 168.