Preliminary observations on enteritis associated with a coronavirus–like agent in rabbits

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Summary

An outbreak of fatal enteritis in 3–8-week old rabbits in a barrier-maintained breeding colony is described. The laboratory findings and treatments attempted suggest that a viral agent was the primary pathogen.

A closed breeding colony of laboratory rabbits (Chinchilla-Bastarde and Russian) with 1200 does was housed in 2 separate barrier rooms for 8 years. During this period productivity improved to give an average litter size of 7·2 and a pre-weaning mortality rate of 12%. Severe enteric disease occurred in 1 room in the spring of 1979 and several months later in the 2nd room. Routine laboratory investigations failed to demonstrate any known pathogen but electron microscopy revealed a coronavirus-like particle in the intestinal content of 94% of the samples examined.

Clinical picture

The colony was housed in mesh cages, fed on a pelleted diet *ad libitum* (Deuka Zuchtpellets: Deutsche Kraftfutter GmbH, Werk Worms, FRG) and maintained at 18 ± 1°C and 45–60% RH.

Animals were sick for up to 24 h prior to death. They were reluctant to move, had a staring coat and frequently a wet perineum. The abdomen appeared slightly swollen. Between 40–60% were affected and virtually all clinically sick animals died. Deaths occurred between 3–8 weeks of age. The distribution of mortality by age was as shown in Table 1.

Post-mortem findings

Carcases were cachectic and dehydrated. The perianal region was wet and soiled with faeces. The thoracic cavity and contents were normal. The stomach and small intestinal contents were normal in volume and consistency. The caecum was slightly distended and contained watery fluid varying in colour from off-white to normal faecal khaki.

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Table 1. Distribution of mortality by age

Age (weeks)	*% mortality*
3	3·5
4	10·4
5	27·5
6	34·5
7	13·8
8	8·6
9	1·7

There was only rarely a vascular disturbance in the serosa or mucosal epithelium of the intestine, although petechiae were sometimes seen in the thymus. Liver, kidney, spleen and lymph nodes were macroscopically normal. The pH values of intestinal lumen contents were as follows: stomach 1·0–2·0; duodenum 7·5; ileum 7·0; caecum 7·0–8·0.

Laboratory investigations

Endoparasites

Small numbers of coccidial oocysts were regularly found in faeces examined by centrifugation in a saturated salt solution. Smears from scrapings of the caecal wall in dead rabbits did not reveal significant numbers of oocysts.

Bacteria

Aerobic and anaerobic culture of intestinal contents yielded the range and density of flora associated with normal rabbits. Small numbers of bacteria were also isolated from the liver, kidneys and lungs. The following organisms were identified in the colony but not in each individual animal: *Escherichia coli* (haemolytic and non-haemolytic), *Clostridium perfringens* Type A, α-haemolytic streptococci, *Bordetella bronchiseptica, Proteus* spp., *Bacillus* spp., *Neisseria* spp. Giemsa-stained smears of intestinal contents did not reveal typical *Bacillus piliformis* bacterial clusters associated with Tyzzer's disease. *Clostridium perfringens* was isolated from 16 out of 26 caecal samples.

Caecal contents were centrifuged at 3000 *g* for 30 min and 0·2 ml of the supernatant inoculated intravenously into 6 week-old mice. No clinical

signs nor deaths occurred in the 5 days following inoculation. Iota toxin was detected in bacteria-free filtrates of caecal content by intradermal inoculation of guineapigs in 3 of the 26 samples. In each case the level of toxin was extremely low, as demonstrated by weak but typical reactions after 24 h.

Viruses

Faecal samples were treated by a combination of fractional centrifugation and agar concentration (Arens & Krauss, 1980). Negatively-stained preparations were screened for virus particles which were identified by size and morphology.

Coronavirus-like particles, 60–220 nm in diameter, with characteristic surface projections were found in 15 of 16 caecal samples (94%) and reovirus-like particles, 50–70 nm in diameter, in 3 of the 16 (19%).

Supernatants of faecal material were inoculated onto various cell culture systems including primary rabbit kidney and whole rabbit embryo. No cytopathic effects were observed on primary or secondary passage.

Histology

In the small and large intestines a diffuse infiltration of mixed inflammatory cells (neutrophil polymorphs and mononuclear cells) and oedema of the mucosa were observed. Patchy superficial epithelial necrosis was also present. No lesions were detected in the liver, kidney and spleen. In the thymus focal areas of pooled blood were seen without any features of an inflammatory response.

Treatment

Diet and husbandry

Hay was normally used for nesting and to encourage solid feeding in the young prior to weaning. It was made available to post-weaning animals in the susceptible period. 2 alternative commercial diets were given to rabbits in different groups of cages for 4 weeks. The weaning age was extended from 4–6 weeks of age for a period of 2 months.

The mortality rate was unaffected by any of these treatments.

Coccidiostats

Although there was no evidence of clinical coccidiosis the drinking water was medicated with sulphaquinoxyline (0·15% Sulka N: TAD Pharmazeutisches Werk GmbH, Cuxhaven, FRG) for 2 weeks. At the end of the treatment faecal samples were negative for oocysts but the mortality rate had not changed.

Antibiotics

No specific bacterial cause was found, but broad spectrum antibiotic treatment was initiated in an attempt to reduce the mortality rate. The diet was premedicated with oxytetracycline (200 mg/kg) and furazolidone (200 mg/kg) for 3 weeks. Later dimetridazole (460 mg/kg) was included in the diet for 6 weeks. (All 3 antibiotics were supplied by Optivet GmbH & Co. KG, Hamm, FRG.)

The mortality rate was unchanged by either treatment.

Vaccination

Oral vaccine trials were undertaken using a modified live virus vaccine aginst corona- and reoviruses prepared in cells of bovine origin (Scourvax II: Norden, Lincoln, Nebraska, USA) for the immunization of calves. 2 trials were conducted.

In the 1st trial 30 does and 3 bucks were transferred from the main breeding area to a separate barrier maintained room. The offspring were vaccinated orally with 2 ml of reconstituted vaccine at 14, 17 and 21 days of age. Mortality, measured by survival to 10 weeks of age, is shown in Table 2a.

The 2nd trial was conducted in an affected breeding area where rabbits were inoculated in a modified pattern recorded in Table 2b.

Following the manufacturers' instructions, all animals in each area were inoculated at the same time. Control values were estimated from mortality prior to treatment.

Discussion

For many years intestinal disorders in the laboratory rabbit have been a serious problem. A

Table 2a. Mortality of rabbits after vaccination

Age at vaccination (days)	*No. of rabbits*	*% mortality*
14	71	35
17	74	12
21	68	16

Table 2b. Mortality of rabbits after vaccination

Age at vaccination (days)	*No. of rabbits*	*% mortality*
15	30	33
16	90	31
17	30	67
19	7	86

number of specific conditions have been identified, but many outbreaks of disease do not correspond with any established diagnosis. Viruses have been incriminated in the enteropathies of many domesticated animals and the paucity of reports for enteric viruses of rabbits is surprising.

3 enteroviruses have been identified in rabbits with enteric disease. Rotavirus infection was reported by Bryden, Thouless & Flewett (1976), adenovirus by Bodon, Prohaszka, Adam & Nasz (1979) and coronavirus by Lapierre, Marsolais, Pilon & Descoteaux (1980). Although the observations of Lapierre *et al.*, are similar to those reported here, in neither case was virus replication demonstrated by cytopathic effect in tissue culture. Bodon *et al.* were able to identify adenovirus by immunofluorescence and this method might find a useful application in the identification and localization of coronavirus. The possibility of synergism between different viruses has not been investigated in the rabbit. In this report, although 94% of samples contained coronavirus-like and only 19% reovirus-like particles, reovirus cannot be excluded as a concomitant pathogen.

The clinical picture in this colony was of an acute afebrile disease causing a marked disturbance of water balance affecting young animals in the susceptible post-weaning period. There was a limited inflammatory reaction in the intestinal epithelium, but no recognized enteric pathogen was detected. Coccidia had been present in the colony for several years, but neither caecal scrapings nor treatment with coccidiostats suggested that coccidia played any significant part in the disease process.

Clostridium perfringens was isolated from the caecum but failure to demonstrate any enterotoxins (except for trace quantities of iota toxin) precludes clostridial enterotoxaemia as a possible cause of the disease. Normal growth of enterobacteria on culture of intestinal content and survival of mice following intravenous inoculation of caecal supernatant do not suggest the presence of any other intestinal toxins. These observations, together with the high percentage of virus-like particles found in faeces, are very suggestive of a viral agent being the cause of the disease.

The inconsistent and inconclusive results of the vaccination trial have several possible explanations. First, the viral vaccine was of bovine origin; the cross reactivity tests of Thouless *et al.* (1977) show that, despite a common antigen, there are probably species differences in infectivity of rotaviruses. This may also be true for coronaviruses (Pederson, Ward & Mengeling, 1978). Secondly, the presence and possible influence of maternally acquired antibody was not known. In calves the variation in passive immunity and virus challenge produces inconsistent results (de Leeuw, Ellens, Talman & Zimmer, 1980). Thirdly, the infection pressure was probably very high, so that any vaccination schedule would have a reduced chance of success. It is of interest to note that 10 of the vaccinated animals, when returned to an 'infected' room 6 weeks after vaccination, developed a typical enteritis within 1 week and died; however, no virus particles could be demonstrated in their caecal content although particles were present in unvaccinated controls.

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Vorläufige Beobachtungen bei einer mit einem Coronavirus ähnlichen Agens assoziierten Enteritis bei Kaninchen

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Zusammenfassung

Es wird der Ausbruch einer tödlichen Enteritis bei 3–8 Wochen alten Kaninchen in einer Barriere-Zuchtkolonie beschrieben. Die Laborbefunde und Behandlungsversuche lassen vermuten, daß als ursächliches Pathogen ein Virus in Frage kommt. (G)