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Detection and Transmission of 30 nm Virus Particles (Astroviruses) in Faeces of Lambs With Diarrhoea

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With 2 Figures

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Summary

An outbreak of diarrhoea in lambs was investigated, and electron microscopic examination revealed small round virus-like particles in the faeces from eight of seventeen lambs. A bacteria-free filtrate of faeces from one lamb was given orally to a gnotobiotic lamb, which subsequently excreted virus in faeces. Intestinal contents were collected from this lamb and a filtrate given orally to two further gnotobiotic lambs, which subsequently developed diarrhoea and excreted virus in faeces. The mean diameter of the virus particles was 29.7 nm, and 12 per cent of them showed their surface structure arranged in the form of a six-pointed or five-pointed star. They were similar to those particles previously observed in human infant faeces which were referred to as "astroviruses".

The passage through gnotobiotic lambs with development of diarrhoea showed that these particles were animal viruses which were probably pathogenic for lambs.

Introduction

Direct electron microscopic (EM) examination of faeces from man and animals has resulted in the acceptance of rotaviruses as a cause of neonatal diarrhoea (1). In addition to rotaviruses, small round virus-like particles have been observed by EM examination of stools from cases of infectious non-bacterial gastroenteritis in man. In most instances, these have been 22—28 nm in diameter with no distinguishing morphological features. A serological relationship between some of these particles has been shown by immune EM techniques (2, 10), and by cross-challenge experiments in volunteers (12). Some have been suggested as parvovirus-like (3, 10), others as probable enteroviruses (3, 7).

There are other reports of the observation in human stools of small round virus-like particles with a more characteristic structure; some with a similarity to

caliciviruses (6), and others, 28 nm in diameter, with a five- or six-pointed stellate configuration superimposed on the circular shape, for which the name astrovirus was suggested (4, 5). There are at the moment no reports of astroviruses in faeces of species other than man. We record their detection in faeces from diarrhoeic lambs, and their successful transmission to gnotobiotic lambs with the production of diarrhoea.

Materials and Methods

Outbreak of Diarrhoea in Lambs

Diarrhoea occurred in a group of 4—6 week old Suffolk lambs on a commercial farm. *Escherichia coli* of unknown enteropathogenicity was isolated from the diarrhoeic faeces, and helminth egg counts and coccidial oocyst counts were below clinically significant levels. A viral involvement in the aetiology of this diarrhoea was considered, and investigations undertaken.

Electron Microscopy

Faeces from both naturally- and experimentally-infected lambs were mixed with distilled water to give an approximate 20 per cent suspension. The large debris was allowed to settle before a drop of the supernatant fluid was transferred to a carbon collodion-coated grid and stained with either 1 per cent potassium phosphotungstic acid (pH 7.0) or 1 per cent ammonium molybdate (pH 5.3).

Infection of Experimental Lambs

Virus-containing faeces from one naturally-infected lamb were diluted to 20 per cent in distilled water, and filtered through a 0.22 μm membrane. 1 ml of this filtrate was given orally to a 2-day-old gnotobiotic lamb (No. 1), which was killed on the third day after infection and the intestinal contents collected. A bacteria-free 20 per cent filtrate of these intestinal contents was prepared in a similar manner and given orally in 3 ml amounts to 3-day-old gnotobiotic lambs (Nos. 2 and 3). Lamb 2 was killed on the 4th day after infection. Lamb 3 was bled for antiserum preparation 18 days after infection.

No bacteria were isolated from lamb 1, and a *Bacillus* sp was isolated from faeces of lambs 2 and 3. No pathogenic significance is attached to this organism.

Cell Culture

Filtrates of intestinal contents were inoculated onto foetal lamb kidney (FLK) cells which were grown and maintained as described (9). Cells from five successive virus passages were examined for cytopathic effect, and were also examined by immunofluorescence using antiserum from lamb 3. In addition, filtrates of intestinal contents were centrifuged in microtitre plate wells containing FLK cells (8). After incubation for 24 hours at 37° C, these cells were examined in an immunofluorescence test using antiserum from lamb 3 or gnotobiotic lamb antiserum to lamb rotavirus (9).

Results

Outbreak of Diarrhoea in Lambs

By electron microscopic examination, low numbers of small round virus-like particles with the morphology of astroviruses (5) were seen in faeces from eight of seventeen lambs examined.

Clinical Response to Experimental Infection

Lamb 1 remained clinically normal, but passed faeces that became loose though not diarrhoeic. Faeces from lambs 2 and 3 became loose in consistency on

the third day after infection, and a yellowish diarrhoea developed in both lambs on the fourth day, persisting for two days in lamb 3. No other clinical abnormality was noted in either lamb.

Virus Excretion

Small, round virus-like particles were observed in the faeces of lamb 1 on the third day after infection. On the second lamb passage, similar particles were observed in the faeces of lamb 2 from the second day after infection. These were present in large numbers in the contents of the large intestine at necropsy, but only in small numbers in the contents of the small intestine. Lamb 3 excreted similar virus-like particles from the 3rd to 9th days after infection.

Virus Morphology

The virus-like particles observed in faeces and intestinal contents were roughly circular in outline (Fig. 1). About 12 per cent of the particles showed their surface structure arranged in the form of a six-pointed or occasionally five-pointed star. Bridging structures were frequently seen between adjacent particles (Fig. 2). This morphology is similar to that described by MADELEY and COSGROVE (5) for human astroviruses, although there was no evidence of quasicrystalline arrays in our preparations. The similarity of the lamb virus to the astroviruses observed in human stools has been confirmed (C. R. Madeley personal communication). The

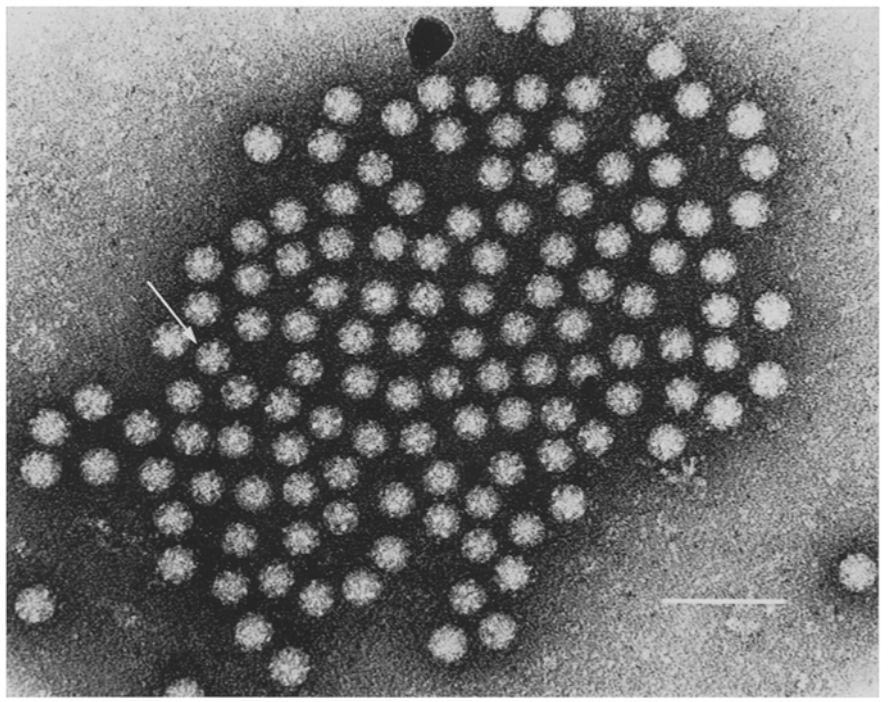

Fig. 1. Astrovirus particles in intestinal contents of lamb 1. The arrow indicates a group of particles showing star-like surface structure. Bar represents 100 nm. Stained ammonium molybdate

size of the virus particles was determined by comparing them with the lattice spacing of beef-catalase crystals photographed in the same field (11). The mean size of 25 particles was 29.7 ± 0.8 nm.

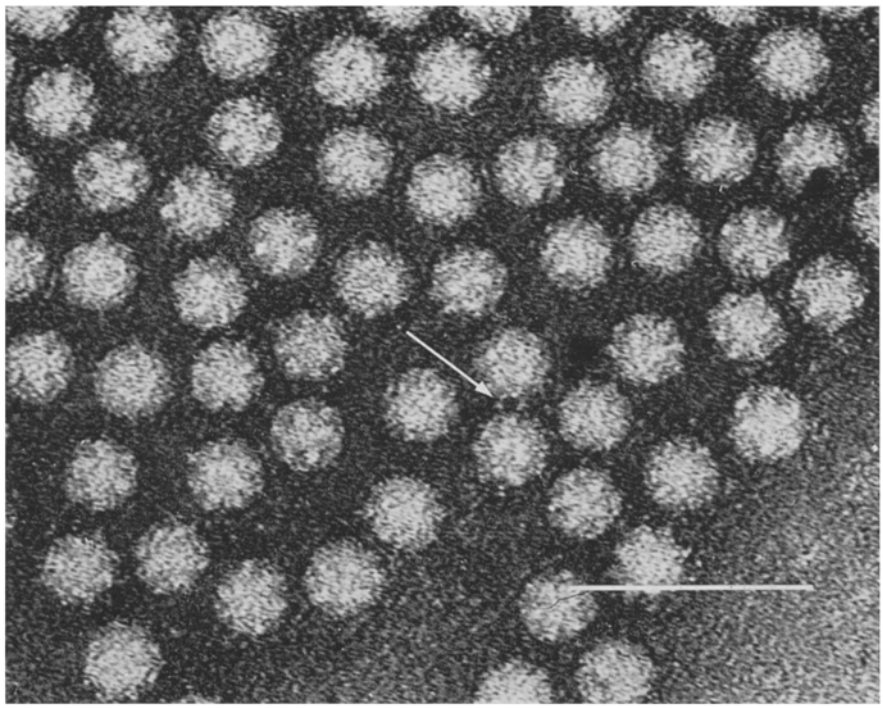

Fig. 2. Astrovirus particles in intestinal contents of lamb 1. The arrow indicates structures bridging particles. Bar represents 100 nm. Stained ammonium molybdate

Cell Culture

No evidence of virus multiplication in FLK cells was obtained by examination for cytopathic effect or specific immunofluorescence in tube cultures or microtitre plate wells. There was no immunofluorescence in cells inoculated with astrovirus filtrate and tested with rotavirus antiserum, or between FLK cells containing lamb rotavirus which were tested with antiserum from lamb 3.

Discussion

The virus studied in these experiments appears to conform to the type of small round viruses described as astroviruses (5). The only criteria at present for this classification are stellate morphology, a size of approximately 30 nm, and occurrence in faeces. All of these are fulfilled in the case of this lamb virus. Whereas Madeley and Cosgrove (4) could not be certain that these viruses were not bacteriophages, their transmission through gnotobiotic lambs in this case shows that they are animal viruses. Their structure appears unique, but their relationships and classification have to await further study.

The experimental lambs excreted astrovirus in faeces for several days, and in the case of lamb 2 had a large volume of intestinal contents containing astrovirus at necropsy. This indicates that the virus detected resulted from replication and was not merely residue from the small volume of inoculum. No other virus was observed in lengthy electron microscopic examination of faeces samples from the experimental lambs; no cytopathic virus was isolated in FLK cells; and the presence of rotavirus was specifically excluded by crossimmunofluorescence. Thus it is probable that the clinical disease observed in the experimental study was caused by the astrovirus, and that this virus can be considered an enteric pathogen of lambs. The natural occurrence of astrovirus and its role in aetiology of diarrhoea are unknown, although the temporal association of the astrovirus with diarrhoea in eight of the seventeen lambs examined in the original outbreak suggests an aetiological link in this case.

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