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Rotavirus and Other Viruses in the Stool of Premature Babies

L. Van Renterghem, P. Borre, and J. Tilleman

Laboratory of Microbiology and Virology, State University of Ghent, Akademisch Ziekenhuis, Belgium

In a 12-month study, 363 stools of 199 premature babies nursed in a separate ward of a paediatric clinic were examined by electron microscopy and on cell culture to detect virus. Twenty-four (6.6%) were positive for rotavirus, in one winter epidemy. From four stools Echo 22 was isolated, and in six cases virus-like particles were detected by electron microscopy. These virus infections are not a major problem in newborns, requiring special care, as they are mostly symptomless or mild.

Key words: **rotavirus, Echo 22, premature baby**

INTRODUCTION

Many reports have described the presence of viruses in the faeces of children [9, 16, 17, 22]. Rotavirus is generally accepted as the commonest cause of acute infantile gastroenteritis [1, 5, 6, 10, 12, 20]. Other viruses – eg, adenovirus and enteroviruses – also have been associated with outbreaks of gastroenteritis [7]. The role of many other viruses is still being explored.

The presence of virus in the faeces is not always associated with disease. Especially in neonates excretion of virus has been observed, though the infection is often asymptomatic [3, 4, 15, 19, 21, 23].

In this study, presence of virus was investigated during a period of one year in premature babies nursed in a separate ward of a paediatric clinic. The purpose of this study was to establish which viruses are excreted in faeces and how they are spread in a ward where special care is taken to prevent infection. Because of the greater susceptibility to infection in this group of patients, attention was paid to the clinical manifestation of the infection.

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Address reprint requests to L. Van Renterghem, Laboratory of Microbiology and Virology, State University of Ghent, Akademisch Ziekenhuis, Blok A, De Pintelaan 135, 9000 Ghent, Belgium.

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MATERIALS AND METHODS

Patients

A prospective study of all premature babies was carried out in one ward of the paediatric clinic from January 1978 to December 1978.

In the ward individual babies are isolated, and most of them are in incubators. Visitors can view the babies only through glass from a corridor, movement of the nursing staff in the ward is restricted, and attention is paid to gowning and hand-washing.

Stool specimens for virus isolation and electron microscopy were collected every fortnight from all infants. As the hospitalization time varied from a few days up to seven months, more than one stool specimen was obtained from some patients. In this way 363 stool specimens from 199 patients were examined.

Preparation of the Stool Suspension

A suspension is prepared by mixing the stool with an approximate five-fold volume of Hanks balanced salt solution containing 100 IU penicillin and 100 μg streptomycin/ml. The stool is thoroughly homogenized for five minutes. Thereafter the suspension is clarified in a centrifuge for 30 minutes at 12,800g to remove debris and bacteria. The supernatant is the material to be examined. One part is used for electron microscopy, the other part is used for virus isolation.

Electron microscopy

Four milliliters of supernatant is layered onto 1 ml of a 25% (w/v) sucrose solution and centrifuged at 190,000g for 150 minutes in a Beckman L-2 ultracentrifuge with a 50.1 swinging rotor. The supernatant is carefully discarded. The pellet or sediment is resuspended in one drop of distilled water, and placed on a 300-mesh Formvar carbon-coated grid; the excess fluid is removed with the edge of a filter-paper disk. The grid is negatively stained with phosphotungstic acid (2%, pH 7.4) and examined at a magnification of 40,000 in an electron microscope (Siemens, Elmiskop 1A) [1, 2, 8, 23, 24].

Virus Isolation on Cell Culture

The second part of the stool supernatant is inoculated into cell culture tubes following a conventional technique for isolation of enterovirus and adenovirus [11, 13, 14]; 0.2 ml is inoculated into secundary monkey kidney cells, HeLa cells, and human lung fibroblasts. After one hour adsorption, the inoculum is withdrawn and 2 ml of maintenance medium (containing 2% FBS) is added. The cultures are incubated at 37°C on roller drums and daily microscopic observation for viral cytopathic effect is performed.

Secondary monkey kidney cells and human lung fibroblasts are kept for three weeks. If there is no evidence of a cytopathic effect on HeLa cells by the end of the second week, a blind passage is carried out and the new culture is further examined for one more week.

RESULTS

Patients and Specimens

Three-hundred and sixty-three stools of 199 babies were examined. Eighty-eight (44.2%) were hospitalised from two weeks up to seven months, so that for these babies two to 12 stool specimens could be examined, but virus was never detected in more than one sample from the same baby however.

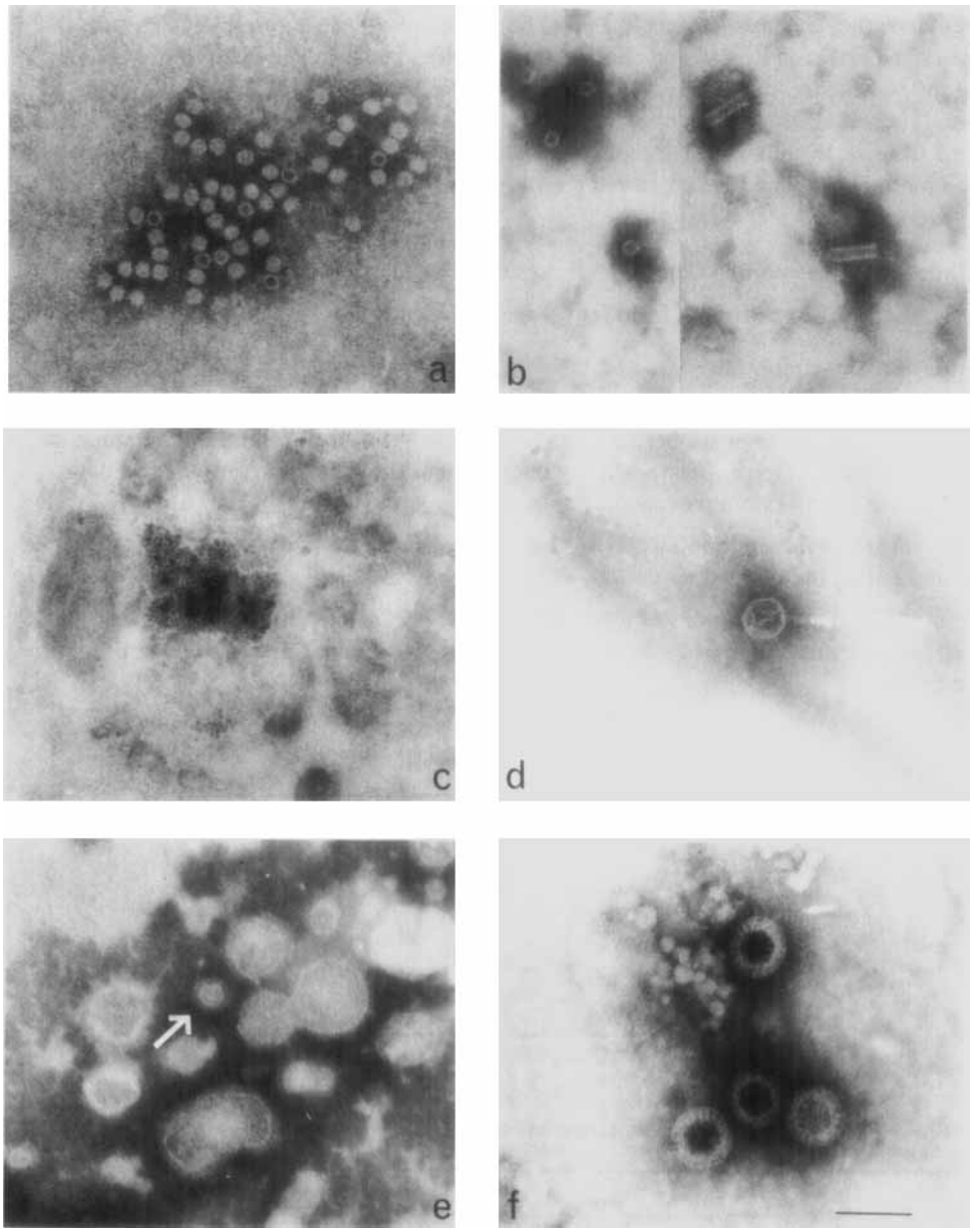

Fig. 1. Virus and virus-like particles in faeces of premature babies. a, b, c, and e: Unidentified particles; d: bacteriophage; e: rotavirus. (Natrium-phosphatungstate staining; bar represents 100 nm.)

Viruses Observed by Electronmicroscopy

Twenty-four (6.6%) stools were found to contain rotavirus, two stools contained small round virus particles, and four contained virus-like structures (Fig. 1).

One stool of one baby contained rotavirus together with an unidentified virus-like structure (Fig. 1e).

Some specimens contained coronavirus-like structures, but according to Professor Pensaert, they are mycoplasma-like particles of unknown identity, resembling those described earlier in human and animal fecal samples [18, 22]. The small round viruses found in two stools did not cause cytopathic effect on cell culture and did not cause paralytic lesions in suckling mice.

Figure 1b shows a small tubular structure, seen in great numbers in the stool of a dysmature baby, and Figures 1c and e show virus-like structures from babies with gastroenteritis.

Viruses Observed on Cell Culture

Enterovirus cultivated from four stools was identified as Echo type 22.

Rotavirus Infection

Age distribution. Table I shows the distribution, according to age, of rotavirus infection. Difference in age between the rotavirus-infected group and the total group is not significant (Wilcoxon test). The youngest patient shedding rotavirus was five days old on the day the stool specimen was obtained.

Seasonal distribution. Rotavirus had a seasonal variation (Table II). Virus was detected during the first winter period, with the highest level from January to March.

Clinical manifestations of infection. From the 24 babies infected with rotavirus, 19 were symptomless, two had mild diarrhea, and three had severe gastroenteritis, one with vomiting.

TABLE I. Detection of Rotavirus in Premature Babies, According to Age

Age in days	Total examined	Rotavirus positive	% of rotavirus positive
≤ 15	126	13	10.3
15–60	172	10	5.8
> 60	65	1	1.5
	363	24	

TABLE II. Monthly Occurrence of Rotavirus and Echo 22 in a Ward of Premature Babies, January 1978–December 1978

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Total examined	53	19	39	55	36	23	14	19	18	42	21	24
Rotavirus positive	11	2	6	3	1	0	0	1	0	0	0	0
% positive	20.7	10.5	15.4	5.5	2.8	0	0	5.3	0	0	0	0
Echo 22 isolated			1							1	1	1

DISCUSSION

In this survey of premature babies, rotavirus was demonstrated during the first winter period. This indicates that spread of the virus occurs in the ward, although special care is taken to prevent outbreaks of infection.

There was not a second outbreak during the same year, although rotavirus was isolated from patients in other wards of the same hospital during November and December. Sporadic cases of virus excretion might have been missed, but it is clear that the infection did not become endemic again.

The occurrence of the virus on the nursery for a long period on one hand and the self-limiting character of the rotavirus infection with no carrier-state on the other hand [5, 10] favour transmission of infection from baby to baby within the ward and argue against a continuous introduction of virus from an external source.

The clinical manifestation of rotavirus infection has been mostly asymptomatic or mild; only three infants, out of 24 infected, have had severe gastroenteritis. Rotavirus infection is self-limiting, as the virus has been detected only once in the same baby. These results agree with the findings of others in normal newborns nursed in maternity units [3, 4, 15, 19, 21, 23].

Therefore, we conclude that prematurity and dysmaturity do not influence the severity of clinical manifestations of rotavirus infection, although such babies are more susceptible to infection since they receive less passive immunity against infectious agents from their mothers.

As all of the babies, are bottle-fed, it seems that breast-feeding is not a major factor in preventing infection and decreasing the severity of the symptoms in the baby.

In this survey, rotavirus infection was the only one that became endemic. Echovirus type 22 was isolated on cell culture from four stools. The virus was detected during winter months, although enterovirus infection occurs mainly in summer.

During the year of the study, Echovirus of type 5, 6, 13, 17, and 22 was isolated from other patients of the same paediatric clinic. Only Echo 22 could be found on the premature nursery. The infections were asymptomatic. The isolation of the strain from neonates has already been reported, also without evidence of pathogenicity [3].

The sporadic findings of the same type of virus suggests the possibility that there is a carrier on the medical or nursing staff. Nevertheless, a mass infection or an endemic infection with transmission of the virus from one baby to another did not occur.

Adenovirus, another possible cause of gastroenteritis, was not detected, either in cell culture, or by electron microscopy.

In a few cases virus-like particles have been detected by electron microscopy, but without evidence that these structures are viral pathogens.

Finally bacteriophages, as shown in Figure 1d, occurred in one patient, but their presence in stool has no pathogenic significance [16].

We can conclude that gastroenteritis due to a virus infection, is not a major problem in newborns, even in premature and dysmature babies.

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