

## A search for faecal viruses in new-born and other infants

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(Received 12 January 1978)

### SUMMARY

Faecal specimens were collected at weekly intervals over the winter months from 141 new-born infants without diarrhoea. Contrary to the findings in other studies, no viruses were detected by electron microscopy or culture in any of these specimens. Over the same period faecal specimens were collected from 84 infants up to four years of age admitted to hospital. Rotaviruses or adenoviruses were found in 48 % of infants with gastroenteritis. Enteroviruses and other small round virus-like particles were found in infants both with and without gastroenteritis. No viruses or pathogenic bacteria could be found in 34 % of specimens from infants with gastroenteritis.

### INTRODUCTION

Rotaviruses have been well established as important aetiological agents of non-bacterial gastroenteritis in infants. We were interested in the reports from London and Glasgow that rotaviruses were found in the faeces of normal symptomless newborn babies (Chrystie *et al.* 1975, Madeley & Cosgrove, 1975).

We therefore decided to collect and examine faecal specimens from new-born infants at weekly intervals over the winter period when rotavirus infection is most likely to occur (Davidson *et al.* 1975; Bryden *et al.* 1975). Over this period we were also receiving specimens from other infants up to four years of age admitted to hospital. Many of these infants had gastroenteritis but some were admitted to hospital for other reasons.

The results of electron microscopy and virus isolation on specimens from these children are reported in this paper.

### MATERIALS AND METHODS

#### *Specimens*

Faecal samples were collected weekly from seven- to ten-day-old babies in both the normal nursery and special care baby unit at the Princess Alexandra Hospital,

Harlow, from September 1975 until February, 1976. Specimens were collected from 62 babies in the normal nursery and 47 babies in the special care unit.

From January to April 1976 specimens of faeces were also collected from 32 six-day-old babies in the special care unit of the Royal Sussex County Hospital, Brighton. None of these babies, either at Brighton or Harlow, had diarrhoea.

From July 1975 until April 1976 faecal specimens were obtained from 84 infants up to four years of age admitted to the Royal Alexandra Children's Hospital, Brighton. Fifty-nine of these children were admitted with diagnoses of gastroenteritis or diarrhoea. Twenty-five had other conditions and did not have diarrhoea.

### *Bacteriology*

All specimens from neonates at Harlow were cultured for enteropathogenic *Escherichia coli* (EPEC) and for salmonellas and shigellas. None of the faeces from the special care babies at Brighton were examined bacteriologically although specimens obtained from 15 of the babies were cultured for EPEC at other times during their stay in the unit.

The same samples of faeces as were sent for virus examination, from the 84 infants in the Royal Alexandra Children's Hospital, were cultured for EPEC and for salmonellas and shigellas. Most of the infants with gastroenteritis had, in addition, admission rectal swabs examined for EPEC and further specimens of faeces cultured throughout their hospital stay.

### *Preparation of specimens for virus study*

Ten per cent faecal extracts were made in medium 199 and clarified by centrifuging at 3000 rev/min for 30 min. Supernatants were stored at  $-40^{\circ}\text{C}$ .

### *Virus isolation*

Faecal extracts were inoculated on monolayer cultures of primary rhesus monkey kidney, Hela cells and a human embryo lung fibroblast line. Selected specimens were also inoculated on primary or secondary human embryo kidney cells. Inoculated cultures were incubated at  $37^{\circ}\text{C}$  and observed for 28 days for the development of cytopathic effects.

Specimens from the newborn babies in the Harlow unit and from 53 of the 84 infants (63 %) admitted to hospital in Brighton, were also inoculated into one day old mice by the intracerebral route. The mice were observed for 14 days for signs of paralysis.

### *Electron microscopy*

2 ml samples of faecal extracts were further clarified by centrifuging at 7000 rev/min for 20 min. The supernatants were then centrifuged at 20000 rev/min for 90 min. Pellets were resuspended in a few drops of distilled water, stained with 3 % phosphotungstic acid pH 6.5 and examined on formvar carbon coated grids in an AEI 801 electron microscope.

## RESULTS

*New born infants without diarrhoea*

No viruses were detected either by electron microscopy or by culture and no enteropathogenic strains of *Escherichia coli* or other recognized intestinal pathogens were isolated.

*Other infants admitted to hospital*

Viruses detected by electron microscopy are shown in Table 1 and viruses isolated from specimens are shown in Table 2.

Apart from one adenovirus isolated from a symptomless child, rotaviruses and adenoviruses were only found in specimens from infants with gastroenteritis. Although adenoviruses were isolated from a total of five specimens they could be isolated from only one of the four specimens in which they were seen by electron

Table 1. *Viruses seen by electron microscopy in faecal specimens from infants with and without diarrhoea*

	Infants with diarrhoea	Infants without diarrhoea	Total
Rotavirus	18 (31 %)	0	18 (21 %)
Rotavirus + SRP*	3 (5 %)	0	3 (4 %)
SRP	7 (12 %)	3 (12 %)	10† (12 %)
Adenovirus	4 (7 %)	0	4‡ (5 %)
Negative	27 (46 %)	22 (88 %)	49 (58 %)
Total	59	25	84

\* SRP, small round virus-like particles. † Echovirus isolated from 3 specimens.

‡ Adenovirus isolated from 1 specimen.

Table 2. *Viruses isolated from infants with and without diarrhoea*

	Infants with diarrhoea	Infants without diarrhoea	Total
Enterovirus	9 (15 %)	5 (20 %)	14 (17 %)
Adenovirus	4 (7 %)	1 (4 %)	5 (6 %)
Negative	46 (78 %)	19 (76 %)	65 (77 %)
Total	59	25	84

microscopy, but the adenovirus isolated need not necessarily have been the same as that visualized. The adenoviruses isolated were identified as three type 1, one type 2 and one type 26.

Small round virus-like particles were seen in ten specimens but enteroviruses were isolated from only three of these. Enteroviruses were however isolated from other specimens in which small round particles had not been seen. The enteroviruses and other small round particles were found in both infants with gastroenteritis and in infants without symptoms of gastroenteritis. The enteroviruses were identified as one echo 15, two echo 19, two echo 22, three echo 30, one coxsackie B2, three coxsackie B4 and one polio type 3.

Enteropathogenic *Escherichia coli* were isolated from seven children, five of whom had diarrhoea. Viruses were also detected in six of these children as shown in Table 3.

Most of the rotaviruses were excreted by children over six months of age (Table 4); the small round particles visualized by electron microscopy but not cultured were mainly found in children under one year.

No virus could be found by electron microscopy or by culture, and no bacterial pathogens could be isolated from specimens obtained from 20 infants (34 %) with gastroenteritis.

Table 3. *Viruses detected in faecal specimens from infants excreting enteropathogenic E. coli*

<i>E. coli</i> group O	Electron microscopy	Virus culture	Diarrhoea
18ac	Adenovirus	Echo 15	Yes
26	SRP	—	No
55	Adenovirus	Adeno 1	Yes
111	SRP	Echo 30	Yes
126	—	Echo 30	Yes
126	Rotavirus + SRP	—	Yes
142	—	—	No

Table 4. *Faecal viruses in children with diarrhoea – age distribution*

	0–6 months	7–12 months	1–4 years
Total number of children in group	19	8	32
Rotavirus	2 (11 %)	3 (38 %)	13 (41 %)
Rotavirus + SRP	—	—	3 (9 %)
SRP	3 (16 %)	3 (38 %)	1 (3 %)
Enterovirus	2 (11 %)	2 (25 %)	5 (16 %)
Adenovirus (EM)	1 (5 %)	—	3 (9 %)
Adenovirus (culture)	2 (11 %)	1 (13 %)	—

#### DISCUSSION

It has been reported that normal symptomless newborn babies excrete rotaviruses in their faeces (Chrystie *et al.* 1975; Madeley & Cosgrove, 1975). The specimens studied in those surveys came from babies in two city hospitals, one in London and one in Glasgow, where other babies with gastroenteritis associated with rotavirus were present. It appears that if rotavirus infection is present in a unit some babies will excrete rotavirus without symptoms, but our results suggest that newborn babies do not normally excrete rotavirus if the virus is not already present in the unit.

Babies in special care units are in a protected environment with restricted access for other people, so there is less chance of these particular babies acquiring virus infections compared with babies in a normal nursery. The nursery in our study was in a maternity unit servicing a new town, which is a rather different environmental background from that in the other studies. An outbreak of gastroenteritis

had occurred in this unit the previous year, and small virus-like particles had been observed in the faeces of affected babies (Appleton & Higgins, 1975), and so perhaps there was a greater awareness of how easily such infections could be introduced into the unit.

In contrast to our findings with the neonates, rotaviruses, adenoviruses, enteroviruses and other small round virus-like particles were found in specimens from other infants and children admitted to hospital. The rotaviruses and all but one adenovirus came only from those with gastroenteritis, whereas enteroviruses were found randomly in this group. Enteroviruses and adenoviruses isolated in tissue cultures were rarely seen by electron microscopy in the original specimens. Other small round viruses and adenoviruses were seen in other specimens however, but viruses were not isolated from these specimens. The failure to find any viruses or enteropathogenic bacteria in 34 % of specimens from infants with gastroenteritis is similar to the findings of Dr Madeley and his colleagues (1977) who also were unable to find any potentially pathogenic organisms in a third of infants with diarrhoea admitted to hospital in Glasgow.

We wish to acknowledge the help of the nursing staff in collecting specimens.

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