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Nosocomial infantile gastroenteritis associated with minirovirus and calicivirus

A prospective study was carried out to determine the epidemiology and etiology of acute gastroenteritis on the general infant ward of The Montreal Children's Hospital in the late fall of 1976. Diarrhea occurred in 41 of 165 infants (25%), with probable nosocomial acquisition in 26 patients. Two infants each had two episodes of diarrhea, and one had three. A putative pathogen was found in 31 of 45 case episodes (69%). Virus-like particles were present in 28 of 45 patients, and in 24 of 74 asymptomatic room contacts. Particles belonging to six morphologic classes were identified: adenovirus, rotavirus, minirovirus, calicivirus, picorna-parvovirus, and coronavirus. More than one agent was identified in 12 infants with diarrhea and in five asymptomatic room contacts. No wardwide etiologic pattern was evident, but minirovirus or calicivirus or both were associated with diarrhea in 20 patients, accompanied by vomiting in 15 of these infants. Moreover, spread of individual agents was almost entirely limited to minirovirus and calicivirus, with diarrhea in six of ten, and four of seven, virus positive room contacts, respectively. These viruses were also identified in stools from 12 infants without diarrhea, seven of whom had repeated vomiting. Data support the etiologic role of minirovirus and calicivirus in diarrhea or vomiting or both in hospitalized infants.

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NOSOCOMIAL GASTROENTERITIS remains a problem in many pediatric units. In the fall of 1976 a prospective study was undertaken to determine the epidemiology and etiology of diarrheal disease on the general infant ward of The Montreal Children's Hospital. Most studies have dealt with epidemics, which have often been attributed to viral agents.¹⁻⁴ Rotaviruses have been associated with nosocomial and community acquired diarrhea,³⁻⁶ but other candidate particles have also been identified.⁷⁻¹⁰ Most of these particles are not amenable to routine

laboratory culture; the standing of some of the small virus-like particles, both as viruses and pathogens, is unclear.¹⁰ Careful examination of some of these newer particles shows consistent morphologic characteristics, permitting a preliminary classification based on electron-microscopic appearance.^{8, 9} Thus far only two reports have made a morphologic distinction amongst the small viruses.^{9, 10}

We report an investigation of nosocomial infantile gastroenteritis carried out on the general infant ward of The Montreal Children's Hospital in the late fall of 1976. Our data support the validity of the morphologic classification of enteritis viruses proposed by others,⁸⁻¹⁰ and add information on the clinical features and epidemiology (transmissibility) of infantile gastroenteritis associated with minirovirus and calicivirus, and their potential to cause clinical enteritis in close contacts.

MATERIALS AND METHODS

All ward patients were studied during the period October 8 to November 30, 1976. Accommodation comprised six three-crib rooms and one four-crib room,

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situated either side of a walk-through corridor. Diarrhea was defined as an increase in the usual daily stool frequency by two or more, and excessive water loss in stool. When symptoms began more than 24 hours after admission to hospital, the infections were considered to be nosocomial.

Stool specimens were obtained from resident infants on the first or second day of the study, and from newly admitted patients as soon as available after admission to the study ward. When possible, specimens were obtained from infants with diarrhea at daily intervals for the first three days of their illness, and at variable intervals for up to four days after diarrhea had ceased. Specimens were also obtained from asymptomatic room contacts of infants with diarrhea, usually on the first and fourth days following the onset of symptoms in the index case. Specimens were not obtained from medical or nursing staff.

Virus identification. Stool was suspended in phosphate-buffered saline (at 10% w/v) and stored at -20°C . Specimens were subsequently thawed, clarified by low-speed centrifugation (3,000 g) for 10 minutes, and 2 ml aliquots concentrated by ultracentrifugation (100,000 g) for 1 hour. The pellet was resuspended in a drop of 1% ammonium acetate solution, and examined using a Philips 201 or 300 electronmicroscope.¹¹ Virus-like particles were sought in at least 5 grid squares, and classified as described by Flewett⁸ and by Middleton et al.⁹

Specimens containing adenovirus particles were inoculated into tissue cultures of HEp-2 and human embryonic lung cells. Cytopathogenic effect was confirmed by a second passage, and adenovirus isolates were typed by neutralization tests. Specimens from room contacts of adenovirus positive infants with diarrhea were cultured in the same way.

Specimens containing picorna-parvovirus particles were inoculated onto primary Rhesus monkey kidney cells, and isolates identified by cytopathogenic effect on second passage and electronmicroscopic appearance. Particles were classified as polioviruses or non-polio enteroviruses by neutralization tests.

Bacterial identification. All specimens were examined for *Salmonella* and *Shigella* by conventional methods, and for enteropathogenic serotypes of *Escherichia coli* by slide agglutination using a battery of 16 commercially prepared antisera (Difco). The predominant *E. coli* colony type from an early specimen culture of each infant with diarrhea was sent to The Laboratory Centre for Disease Control, Ottawa, for complete serotyping. Incubation at 4°C was used for enhanced recovery of *Yersinia enterocolitica*.¹²

Five lactose-fermenting colonies from the first stool

Table I. Bacterial pathogens and virus-like particles identified in stool from infants with diarrhea and asymptomatic room contacts

Stool pathogens	41 infants with diarrhea (45 episodes)	74 asymptomatic room contacts
<i>S. typhimurium</i>	1	0
<i>Y. enterocolitica</i>	1	0
Enteropathogenic <i>E. coli</i>	4	7
Enterotoxigenic <i>E. coli</i>	1	Not examined
Adenovirus	7	4
Rotavirus	7	4
Minirovirus	12	8
Calicivirus	8	4
Picorna-parvovirus	4	7
Coronavirus	1	1
Patients with pathogens	29	31

Multiple pathogens were present in 12 index patients and five asymptomatic room contacts.

culture of each patient with diarrhea were tested for enterotoxin production, using the infant mouse assay,¹³ and the Y1 adrenal cell assay methods.¹⁴ Control strains of *E. coli* were kindly provided by Dr. D.A. Sack. Assays were carried out in triplicate.

The predominant lactose-fermenting colony type from each infant with diarrhea was tested for invasiveness by the guinea pig eye inoculation (Serény) test.¹⁵ An inoculum of 1 drop of a very heavy suspension of the organism in Mueller-Hinton broth (at 5×10^8 cells per ml) was used. Control strains of *E. coli* were kindly provided by Dr. H.C. Dupont.

RESULTS

One hundred and sixty-five infants aged 9 days to 24 months (median 4 months) were studied; 41 had diarrhea, comprising 8 of 16 infants resident at outset, and 33 of 149 newly admitted infants. Two to eight new cases occurred per week; 26 cases were considered nosocomial. Two infants each had two periods of diarrhea, and one had three, for a total of 45 diarrhea episodes. Eighty-one room contacts of infants with diarrhea remained asymptomatic, and specimens from 74 were available for examination.

A putative stool pathogen was identified in 31 of 45 case episodes (69%), and in 31 of 74 (42%) asymptomatic room contacts (Table I). Bacterial pathogens were isolated from seven infants, four of whom coincidentally carried a virus. Concurrent infection with two agents occurred in 10 patients, and with three agents in two, without evidence of an agent:agent pattern.

Viruses. Six morphologic classes of virus-like particles were present in stools from 28 of 45 episodes of diarrhea

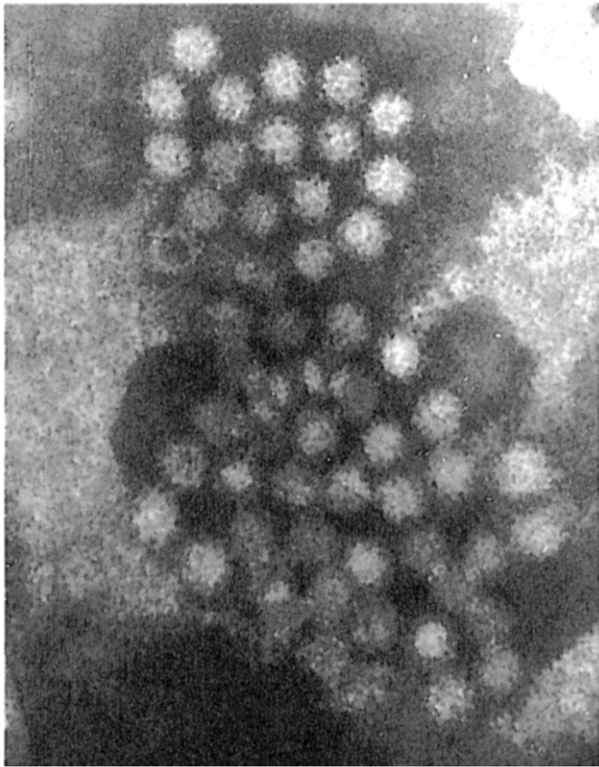


Fig. 1. Electronmicrograph of minirotavirus particles ($\times 200,000$).

(62%), and in 24 of 74 (32%) asymptomatic room contacts (Table I). These included adenovirus, rotavirus, minirotavirus (Fig. 1), calicivirus (Fig. 2), picorna-parvovirus, and coronavirus. Minirotavirus is the name we have chosen for a 32 nm particle, which resembles particles identified in Toronto (minireovirus)⁹ and in Glasgow.¹⁰ It is distinguished from other small round viruses by its slightly larger size and its irregular margin, at times resembling a palisade of very small capsomeres. Caliciviruses tend to be smaller than minirotaviruses, and are distinguished by their scalloped or coarsely indented surface appearance. Both viruses have variations in surface detail, depending on their lie on the grid and their state of preservation. Picorna-parvoviruses are small dense particles, with an entire margin, and no detectable surface structure.

Ten of 26 nosocomial cases showed virus particle concordance with their presumed index source (Table II). Eight of 24 virus positive asymptomatic room contacts showed similar concordance. With the exception of one asymptomatic acquisition of rotavirus, viral concordance between infants with diarrhea and their room contacts was limited to minirotavirus and calicivirus. Concordance between asymptomatic infants excreting virus and their

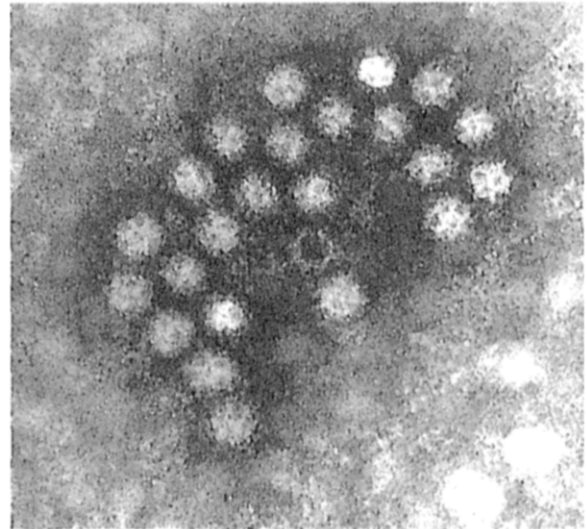


Fig. 2. Electronmicrograph of calicivirus particles ($\times 200,000$).

respective room contacts was also limited to minirotavirus, which was found in three of 15 contacts, none of whom developed diarrhea. Correlation of symptoms with virus acquisition was variable: 10 of 17 infants with either minirotavirus or calicivirus developed diarrhea (Table II).

Twelve infants with diarrhea had no identifiable pathogen in the stool, of whom nine exposed 26 room contacts. Twenty-five contacts remained asymptomatic and had negative stool examinations. One had diarrhea associated with picorna-parvovirus.

Bacteria. Two serotypes of enteropathogenic *E. coli* were identified in specimens from four infants with diarrhea, three of whom coincidentally carried a virus. These and two other enteropathogenic serotypes were also identified as the only pathogen in seven of 74 asymptomatic contacts. There was no evidence of spread of these enteropathogenic serotypes in the infants studied, and complete serotyping of 55 isolates of *E. coli* from 35 infants with diarrhea also failed to show an epidemiologic pattern in this symptomatic population. None of the commonly recognized enterotoxigenic serotypes was identified.¹⁶ Also none of these same 55 isolates of *E. coli* was invasive, evidenced by negative Serény tests.

Stools from one infant with diarrhea were consistently positive for a strain of *E. coli* (O?:K?:H4) producing heat stable and labile enterotoxins. The same specimens contained adenovirus (tissue culture negative). Isolates of *E. coli* obtained from parents and two siblings were enterotoxin negative, and none was found to carry *E. coli* (O?:K?:H4).

Salmonella typhimurium and *Y. enterocolitica* were the only pathogens identified in one index patient each.

Table II. Communicability of viral agents from infants with diarrhea to room contacts

Virus	Index patients		Room contacts			
	No. of infants	Duration of viral shedding (days)	No. of infants	Duration of contact (days)	No. with same pathogen	
					Symptomatic	Asymptomatic
Adenovirus	7	2-5	17	1-5	0	0
Rotavirus	7	3-10	16	1-7	0	1
Minirovirus	11	3-16	25	1-7	6	4
Calicivirus	8	4-13	30	1-6	4	3
Picorna-parvovirus	4	2-6	9	1-5	0	0

Neither case was nosocomial and transmission to room contacts did not occur.

DISCUSSION

Most studies of nosocomial gastroenteritis have concerned epidemics of diarrhea in closed patient populations, often attributed to single agents. This study differs in the open nature of the population. The ward continued to accept new patients throughout, while attempting to discharge, transfer, or cohort those with diarrhea. No infant with a history of recent loose stools was accepted into the ward; about half of the new patients had acute respiratory disease. Respiratory isolation priorities led to frequent room changes and may, in part, explain the high incidence of nosocomial diarrhea and acquisition of viruses by one third of asymptomatic infants studied. It does not, however, explain the variety of virus particles identified. It seems likely that once the large and varied reservoir of pathogens had been established, the continued admission of new patients and their rapid turnover permitted survival of individual agents at a low level of endemicity.

Infants were not followed after discharge from hospital, and our data are probably a low estimate of the prevalence of nosocomial diarrhea and communicability of viruses in the study population.

Almost half of the instances of diarrhea were associated with either minirovirus or calicivirus in stool. Their claim as true viruses and potential pathogens is supported by the temporal association of fecal carriage and presence of diarrhea, and evidence of communicability from infant to infant. In one sequence, lateral transmission of calicivirus could be traced through a chain of seven infants. In another exceptional instance, an infant had intractable diarrhea for several weeks, the course of which was punctuated by two periods of vomiting associated with fecal shedding of minirovirus and calicivirus, respectively. Two other infants had separate attacks of diarrhea and vomiting associated with minirovirus and calicivirus.

Seven of 12 infants without diarrhea but with miniro-

Table III. Vomiting in infants with minirovirus or calicivirus in stool

Virus	Infants with diarrhea		Infants without diarrhea	
	Total	No. with vomiting	Total	No. with vomiting
Minirovirus	12	9	8	5
Calicivirus	8	6	4	2

tavirus or calicivirus (Table I) had repeated vomiting coincident with the presence of virus in the stool, and two others had loose stools without an increase in daily frequency. Vomiting was as frequent an association as diarrhea in patients found to carry these viruses (Table III). Vomiting is also a prominent feature of rotavirus gastroenteritis,¹⁷ but low-grade fever was present in only three of 20 infants with diarrhea associated with minirovirus and calicivirus. We have been unsuccessful in attempts to cultivate minirovirus and calicivirus using human fetal intestinal organ culture, human embryonic kidney cells, monkey kidney cells, human embryonic lung, and HEp-2 cell lines.

Adenovirus was a relatively common finding in stool examined by electronmicroscopy, but poor correlation with symptoms and its occurrence with other agents obscure its significance. Communicability was not demonstrated by either electronmicroscopy or tissue culture methods. Culture using HEp-2 cells was successful in six of eight specimens from infants with diarrhea, and has been sustained with five isolates identified as adenovirus types 2 (two isolates), and 7 (three isolates).

Rotavirus played a smaller role than expected, and transmission could only be presumed in one asymptomatic room contact. This may reflect the bias of small numbers, for there is no doubt that rotavirus can assert its presence in an open infant ward.⁴ Seasonal variations seem to affect identification rates of enteritis-associated viruses in a similar manner.⁹

Picorna-parvoviruses particles appeared to be a random finding in stool and were not shown to be communicable. Enteroviruses were identified in five of eight specimens examined by tissue culture, two of which were identified as polioviruses.

The yield of bacterial pathogens in this study was meagre, and the data are largely negative. Classical enteropathogenic serotypes of *E. coli* occurred sporadically, and three of four affected infants with diarrhea carried a virus in the same specimen. Sequential specimens from one infant with diarrhea contained an enterotoxin-producing strain of *E. coli*, but the same specimen contained an adenovirus. Screening of five lactose-fermenting organisms from more than 400 infants with diarrhea seen at this hospital (1975-1976) has yielded only one other enterotoxin producing strain of *E. coli* (06:H16), in an infant who acquired diarrhea in Pakistan (unpublished data).

We found that multiple putative pathogens were operating concurrently and independently in the study population. Approximately half of the cases of diarrhea were associated with either minirovirus or calicivirus, whose standing as etiologic agents is strengthened by the data presented. Community-based epidemiologic studies, serology, and virus culture will be necessary to substantiate the pathogenic role of these viruses.

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