Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Journal of Infection (1979) **1**, 339–351

Gastroenteritis in Auckland: an aetiological and clinical study

Paul N. Goldwater*

Department of Medicine, Auckland Hospital, Auckland, New Zealand

Summary

Faecal specimens from 60 patients (under six years old), most of whom were Maoris and Pacific Islanders admitted to Auckland Hospital with gastroenteritis during the months of June and July 1977, were examined for the presence of faecal viruses, bacterial pathogens and parasites. Faecal specimens from 18 non-diarrhoeal control patients were also examined, of which three contained rotavirus. Forty-three (72 per cent) gastroenteritis patients had rotavirus detectable in stools by electron microscopy or immune electron microscopy. Of the remainder, 17 patients were regarded as having non-rotavirus diarrhoea. Enterotoxigenic *Esch. coli.* was isolated from seven patients of whom six yielded stable toxin producers (ST+), four labile toxin producers (LT+) and two dual toxigenic strains (ST+/LT+). All ST+ isolates appeared to be of low enterotoxigenicity as indicated by low gut weight/carcass weight ratios in the infant mouse assay.

Rotavirus was the commonest aetiological agent (72 per cent), bacterial pathogens (alone) accounted for only five per cent and no enteric pathogens were found in 15 per cent of cases. Non-agglutinable rotavirus, presumably a different serotype, was seen in both gastroenteritis and control patients. Rotavirus 'satellite' particles previously undescribed were demonstrated in a number of stool samples.

Introduction

There have been no studies of the relative incidence of agents of gastroenteritis in New Zealand. Prior to this survey, in the majority of cases admitted to Auckland Hospital, no pathogen has been found using traditional bacteriological and virological techniques. This study was designed to give some indicaton of the relative importance of aetiological agents and clinical features in cases of gastroenteritis requiring hospital admission.

Rotavirus has been shown to be a major cause of gastroenteritis in other parts of the world (Bishop, Davidson, Holmes and Ruck, 1974; Flewett, Bryden, Davies, Woode, Bridger and Derrick, 1974; Middleton, Szymanski, Abbott, Bortolussi and Hamilton, 1974; Kapikian, Kim, Wyatt, Rodriguez, Ross, Cline, Parrott and Chanock, 1974; Echeverria, Blacklow and Smith, 1975; Ryder, Wachsmuth, Buxton, Evans, Du Pont, Mason and Barrett, 1976; *Lancet* 1975; WHO 1975/76; *British Medical Journal,* 1977)—but with the advance of knowledge in this area and the finding of the virus in large

*Current address: Senior Registrar in Virology, Department of Virology, St. Thomas' Hospital, London SE1 7EH.

0163–4453/79/040339+15 $01·00/0 ©1979 The British Society for the Study of Infection

numbers of non-diarrhoeal neonates (Totterdell, Chrystie and Banatvala, 1976; Murphy, Albrey and Crewe, 1977), uncertainty of its relationship with its host is evolving. Like rotavirus, exterotoxigenic *Esch. coli* has been implicated as a cause of gastroenteritis in young children in various parts of the world (Gorbach and Khurana, 1972; Nalin, McLaughlin, Rahaman, Yunus and Curlin, 1975; Sack, Hirschhorn, Brownlee, Cash, Woodward and Sack, 1975; Ryder, Wachsmuth, Buxton, Evans, Du Pont, Mason and Barrett, 1976; Evans, Olarte, Du Pont, Evans, Galindo, Portnoy and Conklin, 1977; Echeverria, Ho, Blacklow, Quinnan, Portnoy, Olson, Conklin, Du Pont and Cross, 1977). The significance of rotavirus and enterotoxigenic *Esch. coli* as causal agents of gastroenteritis was studied.

In June and July, 1977, patients admitted to Auckland Hospital with gastroenteritis were studied to determine the relative isolation rates of (1) rotavirus, (2) other viruses identifiable by electronmicroscopy of stools, (3) enterotoxigenic *Esch. coli* producing heat stable (ST) and /or heat labile (LT) enterotoxins, (4) salmonella species, (5) shigella species and (6) parasites as aetiological agents. In addition, the presence of red blood cells, leucocytes and mucus strands in stools was recorded.

Patients and methods

Patients

Sixty patients (30 males and 30 females) admitted to the infectious disease unit of Auckland Hospital with gastroenteritis were studied. Gastroenteritis was defined as acute development of unusually frequent and loose stools with or without vomiting. The first stool passed after admission was collected in a sterile plastic container and processed on the day of collection or the day following overnight storage at 4°C. Eighteen control patients (11 males and seven females) with non-diarrhoeal diseases were studied. Clinical features were obtained on review of the patients' notes.

Electronmicroscopy of stool specimens

Viral pellets of stool specimens from patients with diarrhoea and from control patients were prepared for electronmicroscopy on a Philips EM 300 by the method of Totterdell, Chrystie and Banatvala (1976). The pellet was resuspended in a few drops of distilled water and stored at −20° until examined. Specimens were negatively stained with three per cent potassium phosphotungstate (pH6). At least five suitable grid squares were scanned at approximately 41,000 magnification. Electronmicrographs were prepared for measurements of virus size and identification of morphologically distinct viruses.

Immune electronmicroscopy

All faecal viral pellets were examined after reacting with specific anti-rotavirus guinea pig serum kindly supplied by Dr M. D. Holdaway, Dunedin Hospital. Using Kayline 96 U-welled microtitre plates, 25 μl anti-rotavirus

serum (complement fixation titre 1:512) was diluted in phosphate buffered salines pH 7·01 to 1:64 and incubated with 25 μl faecal pellet at 37°C for three hours. After negatively staining and coding, grids were stored in LKB 4828B specimen grid boxes at room temperature until examined 'blind'.

Stool light microscopy

A wet preparation of stool stained with one per cent Loeffler's methylene blue (Harris and Coleman, 1963; Harris, Du Pont and Hornick, 1972) was examined for the presence of red blood cells, leucocytes, mucus strands and parasites.

Bacteriology

Stool speciments were examined for the presence of *Salmonella* sp. and *Shigella* sp. using MacConkey and XLD agar plates and selenite broth. These species were identified by standard methods (Edwards and Ewing, 1972).

Enterotoxigenic isolates

Ten lactose-fermenting colonies with the typical appearance of *Esch. coli* were discriminately selected from the last two streaks on MacConkey plates and were stored on nutrient (Columbia) agar slopes and inoculated into 3 ml Syncase medium (glucose substituted for sucrose) to make a pool suspensions of the 10 colonies for enterotoxin screening. Pure and predominant non-lactose fermenting growths were treated in a similar manner. Each pool was incubated stationary at 37°C for 48 hours, a 1 ml aliquot of broth suspension was removed and stored at −20°C for LT assay screening. The remainder of the suspension was centrifuged at 6000 rev/min for 30 minutes and the supernatant withdrawn for ST assay screening. All specimens were coded and tested 'blind'.

Suckling mouse assay

The assay for ST was carried out as described by Dean, Ching, Williams and Harden (1972). Pools of 10 isolates from each patient were tested for ST production. Each individual isolate from ST positive pools was then tested. Four mice were used per assay. A mean ratio of $> 0{\cdot}0700$ was regarded as weakly positive, $> 0{\cdot}0800$ was regarded as positive. Positive and negative control cultures were included in each assay. ST + /LT + strains B7a (serotype 0148 K? H28), H10407 (serotype 078 H11) and ST −/LT − U5/41 (serotype 01K1H7) were kindly supplied by Dr B. Rowe, Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT and ST +/LT + strain 408-3 and ST −/LT − strain 408-4 were kindly supplied by Dr R. B. Sack, Baltimore City Hospital, 4940 Eastern Avenue, Baltimore, MD 21224.

Y1-mouse adrenal cell assay

A miniculture assay was carried out using whole bacterial pool culture as

described by Sack and Sack (1975). Individual strains of LT+ pools were tested later.

All enterotoxigenic isolates were identified by standard biochemical tests.

Results

Clinical features

The male:female ratio in diarrhoeic patients with stools positive for rotavirus was 0·75 and for non-rotavirus gastroenteritis was 2·4. For the purpose of comparing clinical features, gastroenteritis patients were placed into two diagnostic categories according to the presence or absence of rotavirus in their stools: (1) rotavirus gastroenteritis group and (2) non-rotavirus gastroenteritis group. Further subdivisions into age groups provided comparisions.

Table I shows the main clinical features associated with rotavirus diarrhoea

Table I *Percentage positive clinical and other features by age*

Age group	<6M		6M – <2Y		2Y – 6Y	
Clinical group	R	NR	R	NR	R	NR
Clinical features						
Respiratory						
Pharyngitis	31	14	35	71	70	0
Coryza	69	29	40	43	20	100
Cough	38	57	35	86	20	33
Tachypnoea	23	14	10	43	10	0
Otitis media	15	0	20	0	10	0
Any of above respiratory symptoms or signs	77	71	80	100	80	100
Other						
Vomiting	69	86	80	100	100	67
Abdominal pain	8	14	0	0	0	67
Shock	15	0	15	0	10	0
Convulsion	0	0	5	14	10	33
Fever	100	71	95	100	100	100
Antibiotics						
Pre-admission	31	43	20	57	20	0
Post-admission	46	57	20	57	10	33
Dehydration						
Clinical	54	43	70	71	90	33
⩾ five percent by weight	23	14	15	29	50	0
Requiring IV therapy	85	71	65	100	100	33
Ethnic groups						
Caucasians	15	57	45	14	80	67
Maoris	46	29	30	14	10	0
Pacific Islanders	44	14	25	57	10	33
Asians	8	0	0	14	0	0
Maoris and Pacific Islanders	78	43	55	71	20	33
No. in group	13	7	20	7	10	3

R = Rotavirus gastroenteritis.
NR = Non-rotavirus gastroenteritis.

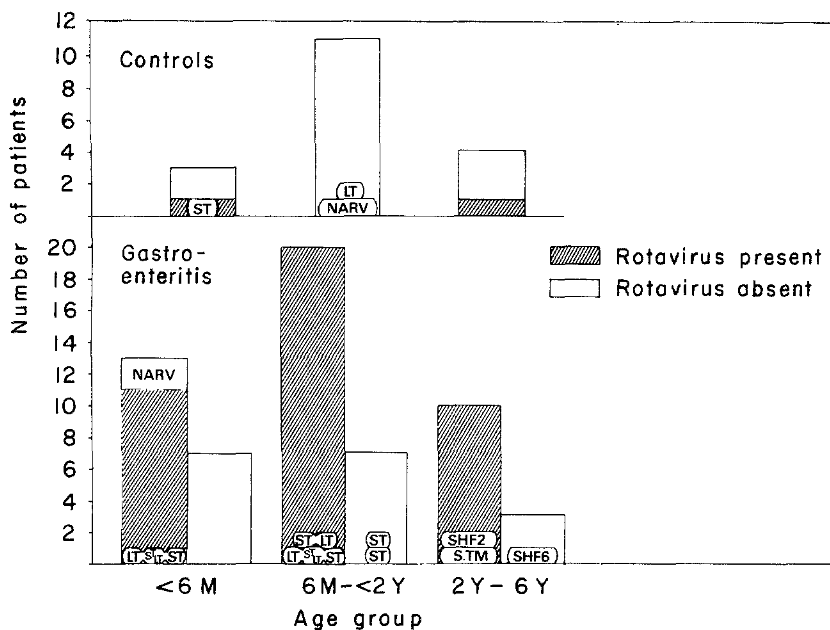

Fig. 1 Distribution of enteric pathogens according to age (Auckland June–July 1977). SHF6 = shigella flexneri 6; SHF2 = shigella flexneri 2; STM = salmonella enteritidis ser typhimurium; NARV = non-agglutinable rotavirus (on IEM); ST, LT, ST/LT = stable, labile and dually enterotoxigenic *E. coli* respectively. Merged ovals indicate toxigenic strains are from one patient.

and gastroenteritis due to other causes. A high incidence of upper respiratory symptoms and/or signs was seen in both gastroenteritis groups (71–100 per cent). Otitis media was exclusive to rotavirus gastroenteritis.

Diarrhoeic patients (in all age groups) with rotavirus in their stools had a propensity to present with marked dehydration and circulatory shock which was not encountered in other forms of diarrhoea.

On the whole, rotavirus gastroenteritis patients required intravenous fluids more often than the non-rotavirus group, but the numbers of patients in each group are small. Control patients had mainly respiratory infections and other non-diarrhoeal conditions. The relative distribution of enteric pathogens detected according to patient age is illustrated in Fig. 1.

Rotavirus was seen in non-diarrhoeal control stools in all age groups. Three of 18 (17 per cent) control stools contained rotavirus (one of which had non-agglutinable virus detectable on IEM). There was no evidence of gastroenteritis in these three patients, however, a three month old female infant had had transient loose bowel motions one week prior to admission to hospital with whooping cough syndrome.

Antibiotic treatment

The proportion of patients receiving antibiotics prior to and during admission to hospital is shown in Table I.

Table II *Complications*

Complication	Rotavirus gastroenteritis	Non-rotavirus gastroenteritis
2° disaccharidase deficiency	2	1
Malabsorption requiring hyperalimentation	—	1
Hypernatraemia	3	1
Hypoglycaemia	—	1
Pneumonia	2	2
Convulsion	3	2

Complications

Table II shows the complications arising in the two gastroenteritis groups of patients.

Racial groups

Table I shows the distribution of various ethnic groups. Maoris and Pacific Islanders predominated in the younger age groups affected by both rotavirus and non-rotavirus gastroenteritis. Controls showed a similar ethnic pattern.

Enterotoxigenic strains

Of the 78 pools tested, eight were weakly ST+ (ratios $> 0{\cdot}0700$ but $< 0{\cdot}0800$) and six were LT+. Assay of the individual ten strains of each pool identified enterotoxigenic strains. Table III summarises the enterotoxigenic

Table III *Enterotoxigenic* Esch. coli *isolates*

Age	Patient group	Pool number	ST+ pool	LT+ pool	Number of individual strains from pool: ST+	LT+	ST+/LT+
3M	Rotavirus gastroenteritis	26	+	+	3/10	4/10	1/10
16M	Non-rotavirus gastroenteritis	28	+	−	1/10	0/10	0/10
7M	Rotavirus gastroenteritis	51	+	+	4/10	3/10	0/10
4Y	Rotavirus gastroenteritis	54*	+	+	10/10	10/10	10/10
16M	Non-rotavirus gastroenteritis	65	+	−	2/10	0/10	0/10
7M	Control	78	−	+	0/10	5/10	0/10
23M	Rotavirus gastroenteritis	86	+	+	4/9	8/9	3/9
3M	Control	88	+	−	1/10	0/10	0/10

**Salmonella typhimurium.*

isolates. Dual enterotoxigenicity of a strain (ST + /LT +) was encountered only in two patients' stools. Mixtures of 'toxitypes' within one pool were encountered.

Interpertation of gut weight/remaining body weight ratios

Strain U5/41 (serotype 01K1H7) was used as the non-toxigenic control and

gave a mean ratio of 0·0612 (range 0·0487–0·0700, standard deviation ± 0·0062). Strains B7a (serotype 0148 K?H28), H10407 (serotype 078H11) and 408-3 (?serotype) were used as positive enterotoxigenic (ST + /LT +) strains and gave mean ratios of 0·1077, 0·0843 and 0·1203 respectively with ranges of 0·0769–0·1466, 0·0700–0·1066 and 0·0707–0·2877 respectively, with standard deviations of ± 0·022, ± 0·011 and ± 0·0516 respectively.

Assuming that greater ratios are associated with a higher degree of toxigenicity, a comparison of the mean ratios for each control strain was carried out. The mean ratios of strains H10407 and B7a were significantly different ($P < 0{\cdot}001$). This suggests that these differences are true and that the idea of degrees of toxigenicity thus can be entertained (Klipstein, Engert and Short, 1977). The mean ratio of test strains regarded as being ST+ was 0·0736 (range 0·0701 to 0·0793, standard deviation ± 0·0025) being significantly greater than that of the control ST negative strain U5/41 ($P < 0{\cdot}001$). From Table III it is seen that enterotoxigenic isolates were found in both groups of gastroenteritis patients and also in non-diarrhoeal controls.

Table IV *Combinations of potential enteric pathogens isolated*

	Number of patients		
	Rotavirus gastroent.	Non-rotavirus gastroent.	Controls
Enterotoxigenic *E. coli*			
LT+	3	0	1
ST+	3	2	1
ST+/LT+	2	0	0
Shigella spp.	1	1	0
Salmonella enteritidis (see typhimurium)	1	0	0
Salmonella typhi	0	0	0
Rotavirus	41	0	2
Non-agglutinable rotavirus on IEM	2	0	1
Adenovirus	5	7	4
Reovirus	0	0	1
Unknown	0	9	—
Number in group	43	17	18

Table IV shows combinations of enteric pathogens isolated from gastroenteritis patients and control patients.

Electronmicroscopy (EM) and immune electronmicroscopy (IEM)

Mean rotavirus diameters for smooth and rough particles (Flewett, 1977), were 64·5 nm and 53·8 nm respectively (standard deviation ± 2·4 nm and ± 4·3 nm respectively). Rotavirus-like particles that failed to agglutinate on IEM were seen in two gastroenteritis patients' stools and in one control patient's stool. Solitary particles indicated non-agglutination.

Small round particles (which I shall call 'satellite particles') were seen in

close proximity to rotaviruses. These satellite particles were between 13 and 26 nm in diameter (mean 16·9 nm, standard deviation ± 3·8 nm) and appeared to have a surface substructure and were seen with increasing frequency with patient age. (Plates 1 and 2).

On four occasions direct EM of faecal viral pellets failed to show rotavirus that was later easily seen in large clumps on IEM. Table V summarises the per cent incidence of viruses seen by EM and IEM according to age and patient group.

Table V *Percent incidence of viruses seen by EM and IEM according to patient age and group*

Age group	<6/12			6/12 – <2Y			2Y – 6Y		
Patient group	R	NR	C	R	NR	C	R	NR	C
Rotavirus	100	0	33	100	0	0	100	0	25
Non-agglutinable rotavirus (on IEM)	15	0	0	0	0	9	0	0	0
Adenovirus	23	43	0	5	29	18	0	33	25
Corona-like virus	54	29	0	45	100	91	60	100	75
Reovirus	0	0	—	0	0	9	0	0	0
Rotavirus 'Satellite' particle	31	—	0	55	—	0	70	—	0
Capsomeres	31	57	33	65	43	36	60	33	75
Phage	62	57	33	45	43	55	20	33	25
Small round viruses	85	86	100	100	100	100	90	100	100
No. of patients in group	13	7	3	20	7	11	10	3	4

R = Rotavirus gastroenteritis; NR = Non-rotavirus gastroenteritis; C = Control.

Tubular capsid protein structures as described Flewett (1977) were common to all groups of stool whether or not rotavirus was present and failed to agglutinate on IEM. Adenovirus (54–78 nm) were seen in stools from all patient groups. Their link with diarrhoea could not be established. Coronavirus-like particles (mean size 93 × 161 nm) were commonly seen in all three patient groups. Reovirus (80 nm) was seen in one control patient's stool. It was distinguished from rotavirus by its size, capsid structure and failure to react with immune rotavirus serum on IEM. Small round viruses (SRV) 13–29 nm not identifiable as astroviruses or calciviruses were an almost invariable inhabitant of all stool types. Bacteriophages of various shapes and sizes were common inhabitants of stools regardless of clinical condition.

Stool light microscopy

Table VI shows the per cent incidence of stool red blood cells, leucocytes and mucus strands according to age and clinical group. Generally, all three features were common to rotavirus gastroenteritis where comparisons with non-rotavirus gastroenteritis and controls were possible. No parasites were recorded.

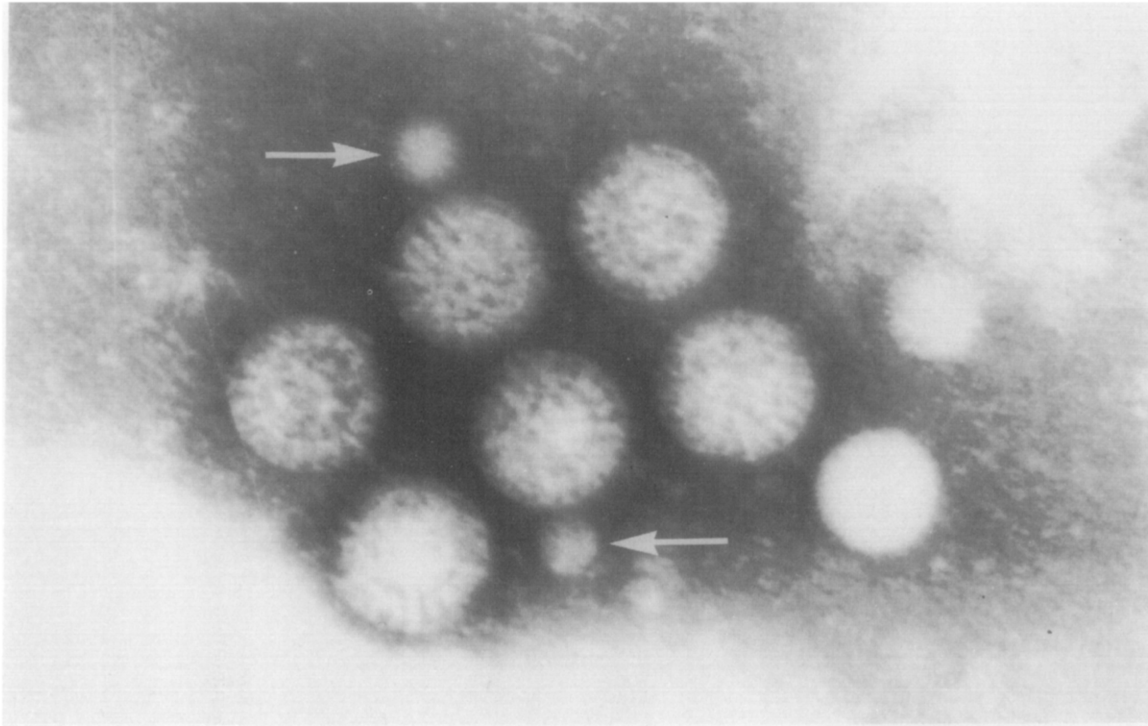

Plate 1 Electronmicrograph showing 'satellite particles' approximately 25 and 26 nm diameter associated with 'smooth' rotaviruses. Magnification approximately × 340,000.

[*facing page 346*]

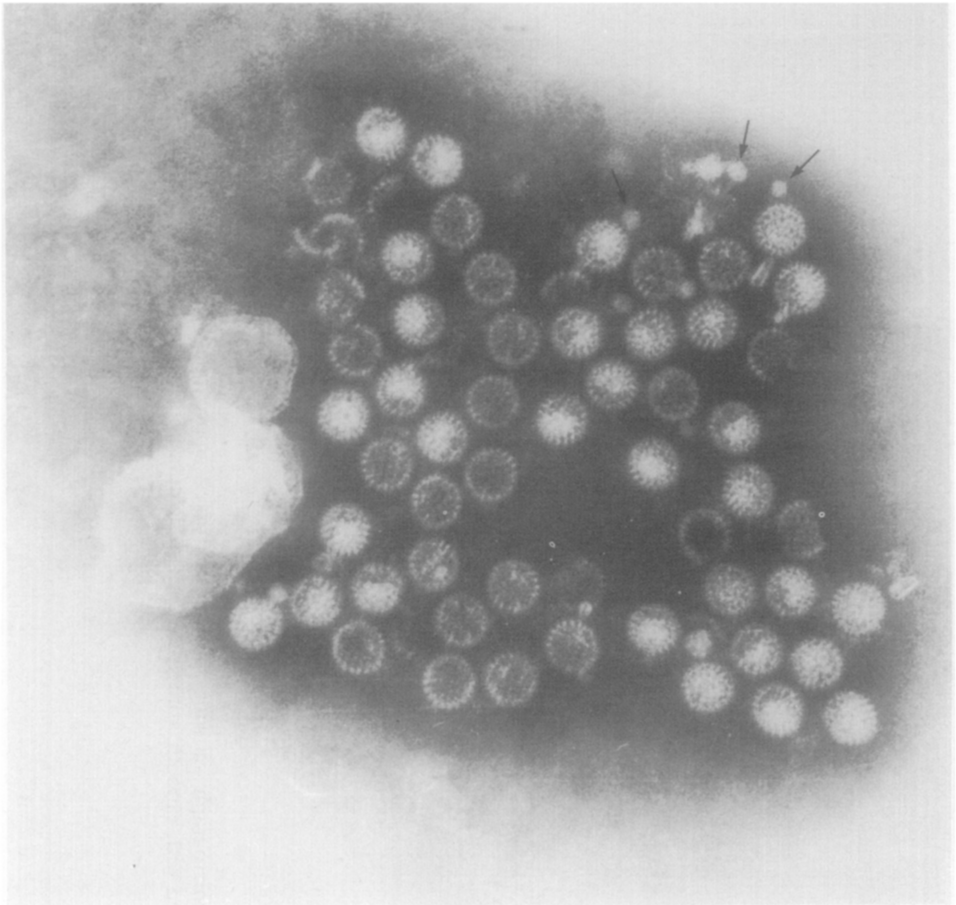

Plate 2 Immune electronmicrograph showing rotavirus satellite particles in association with 'rough' rotaviruses. Magnification approximately × 136,000.

Table VI *Percent incidence of light microscopic features of stools according to age and clinical group*

Age group	<6M			6M – 2Y			2Y – 6Y		
Clinical group	R	NR	C	R	NR	C	R	NR	C
Stool red blood cells	31	0	0	25	0	0	30	33	0
Stool leucocytes	31	14	33	35	29	0	70	33	0
Stool mucus strands	38	0	0	25	29	18	80	33	0

R = Rotavirus gastroenteritis; NR = non-rotavirus gastroenteritis; C = control.

Discussion

This article points out the high incidence of upper respiratory symptoms and signs associated with rotavirus gastroenteritis and other causes of diarrhoea. This was alluded to (without reference) in a leading article (1977) but a respiratory mode of transmission of rotavirus was not suggested. From this study there is clinical evidence for this, but the hypothesis would also support a similar mode of transmission of non-rotavirus gastroenteritis and its largely unknown cause or causes. It should be noted that this study took place during winter when respiratory admissions are most common. However, respiratory symptoms and signs were also a common finding in Washington children with rotavirus gastroenteritis reported by Rodriguez, Kim, Arrobio, Brandt, Chanock Kapikian, Wyatt and Parrott (1977) and Lewis, Parry, Davies, Parry, Mott, Dourmashkin, Sanderson, Tyrrell and Valman (1979) showed a significant excess of rotavirus infected children with respiratory illness. A female predominance in the younger age groups affected by rotavirus was noted. The finding of rotavirus or non-agglutinable rotavirus in non-diarrhoeal stools in all age groups suggests asymptomatic infection. This is contrary to current evidence elsewhere except that newborn infants in neonatal units appear to be colonised by rotavirus with quite variable consequences (Chrystie Totterdell and Banatvala, 1975; Totterdell, Chrystie and Banatvala 1976; Murphy, Albrey and Crewe, 1977).

The predominance of Maoris and Pacific Islanders in the younger age groups with or without gastroenteritis may be a reflection of socio-economic, nutritional or other factors. Evidence of spread within families was seen with equal frequency in Maoris and Caucasians. Rotavirus more often spread to siblings than other agents of gastroenteritis.

Enterotoxigenic strains were isolated from six patients with diarrhoea and three control patients. No strain producing ST had mouse mean gut weight/remaining body weight ratios exceeding 0·0800 indicating a low level of ST production. From the work of Klipstein, Engert and Short, 1977, relative degrees of enterotoxigenicity seem to occur amongst toxigenic enterobacteriaceae. Whether or not the weakly ST+ strains found in this study produced the symptoms in those patients with gastroenteritis remains to be seen. To date there is no information concerning the amount of toxigenic activity

required to classify a strain as toxigenic except arbitrary infant mouse mean gut weight body ratios which vary from one published study to another (Dean, Ching, Williams and Harden, 1972; Morris, Merson, Sack, Wells, Martin, De Witt, Feeley, Sack, Bessudo, 1976; Donta, Wallace, Whipp and Olarte, 1977). The finding of enterotoxigenic isolates in control patients indicates that LT and ST plasmids are probably circulating freely in the community, and as pointed out by Pickering, Du Pont, Evans, Evans and Olarte (1977), asymptomatic subjects are probably important in transmission of infection. Combinations of enteric pathogens isolated from gastroenteritis patients and control patients were a common finding. The isolation of multiple enteric pathogens supports the findings of Evans, Olarte, Du Pont, Evans, Galindo, Portnoy and Conklin (1977); Schoub, Greef, Lecatsas, Prozesky, Hay, Prinsloo and Ballard (1977); Echeverria, Ho, Blacklow, Quinnan, Portnoy, Olson, Conklin, Du Pont and Cross (1977); and Madeley, Cosgrove, Bell and Fallon (1977). Rotavirus was responsible for a large proportion of non-bacterial gastroenteritis in most age groups accounting for 74 per cent of diarrhoeal illness in the six months to less than two year age group, and 77 per cent in the two to six year group. In the youngest age group (less than six months) its prevalence was 65 per cent. Rotavirus' high overall prevalence (72 per cent) may be explained by the winter season during which the study took place. From overseas reports (Middleton, Szymanski, Abbott, Bortolussi and Hamilton, 1974; Bryden, Davies, Hadley, Flewett, Morris and Oliver, 1975; Davidson, Bishop, Townley, Holmes and Riuck, 1975; Kapikian, Kim, Wyatt, Cline, Arrobio, Brandt, Rodriguez, Sack, Chanock and Parrott, 1976), rotavirus diarrhoea appears to occur at the cooler times of the year in temperate climates but neonates in Sydney showed no seasonal variation of rotavirus excretion (Murphy, Albrey and Crewe, 1977). It remains uncertain whether seasonal variation of rotavirus infection occurs in New Zealand. However, this high detection rate may be reflected by the use of IEM. The increased sensitivity of IEM over EM was shown by the detection of rotavirus in four patients which would otherwise have been missed by the latter method. Rotaviruses of two gastroenteritis patients and one control patient failed to agglutinate on IEM. The particles were identical in structure to agglutinable rotavirus and the severity of attributable disease appeared to be similar. Zissis and Lambert (1978) have shown that IEM is a valid means of serotyping rotavirus. At least two serotypes of rotavirus (one predominant) were responsible for a large proportion of paediatric gastroenteritis admissions in Auckland at the time of the study.

Adenoviruses, small round viruses and coronavirus-like particles appeared (on EM) to a similar extent in gastroenteritis and control patients' stools. Without immune serum, identification of parvoviruses and their distinction from other small round viruses by IEM was not possible. Astroviruses and caliciviruses (Madeley and Cosgrove, 1975 and 1976) were not seen.

'Satellite particles' were a common accompaniment of rotavirus. The

proximity of these small isometric particles to rotavirus may only be an accidental encounter in the overcrowded tube journey to the outside world. But that this was seen with a frequency of 54 per cent suggests that some design was involved in their meeting. It is possible that these particles represent small aggregates of coat proteins. They have not been described previously. The author has not seen these particles in rotavirus positive specimens from patients at St. Thomas' Hospital.

The capsid protein described by Flewett (1977) as an association with rotaviruses of various animal species was seen in all types of stool whether or not rotavirus was detectable. This material failed to agglutinate on IEM suggesting that it is not of rotavirus origin.

In 15 per cent of gastroenteritis cases no pathogen could be found. It is possible that these infections were due to rotavirus excreted in numbers not great enough for detection even by IEM of faecal viral pellets. The features of most cases of rotavirus gastroenteritis and non-rotavirus gastroenteritis were so similar that a separate aetiology for each group could not be distinguished on clinical evidence. That only one stool specimen from each patient was examined may explain negative results, but the overall yield of potential pathogens is higher or similar to other published studies already cited where multiple specimens had been collected. The role of enteropathogenic serotypes of *Esch. coli* (EPEC) and their relationship with enterotoxin production may cast some light on the area of aetiologically unexplained gastroenteritis. Data on EPEC serotypes is being prepared for publication.

(My thanks to Dr R. B. Ellis-Pegler and Dr T. E. Miller for their helpful criticism. Additional thanks to Dr W. E. Lang, Dr C. Howden and Dr Keitha Farmer and other clinicians for giving me access to their patients and clinical notes. My gratitude to the nursing staff who collected the specimens and without whose help this work would not have been possible. Dr Sharon Hannan's help with ultracentrifugation is gratefully appreciated. My thanks to Merck, Sharp and Dohme who supported this work by means of a fellowship.)

References

Bishop, R. F., Davidson, G. P., Holmes, I. H. and Ruck, B. J. (1974). Detection of a new virus by electron microscopy of faecal extracts from children with acute gastroenteritis. *Lancet*, **i**, 149.

Bryden, A. S., Davies, H. A., Hadley, R. E., Flewett, T. H., Morris, C. A. and Oliver, P. (1975). Rotavirus enteritis in the west Midlands during 1974. *Lancet*, **ii**, 241.

Chrystie, I. L., Totterdell, B., Baker, M. J., Scopes, J. W. and Banatvala, J. E. (1975). Rotavirus infection in a maternity unit. *Lancet*, **ii**, 79.

Davidson, G. P., Bishop, R. F., Townley, R. R. W., Holmes, I. H. and Ruck, B. J. (1975). Importance of a new virus in sporadic enteritis in children. *Lancet*, **i**, 242.

Dean, A. G., Ching, Y. C., Williams, R. G. and Harden, L. B. (1972). Test for *Escherichia coli* enterotoxin using infant mice: Application in a study of diarrhoea in children in Honolulu. *The Journal of Infectious Diseases*, **125**, 407.

Donta, S. T., Wallace, R. B., Whipp, S. C. and Olarte, J. (1977). Enterotoxigenic *Escherichia coli* and diarrheal disease in Mexican children. *The Journal of Infectious Diseases*, **135**, 482.

Echeverria, P., Blacklow, N. R. and Smith, D. H. (1975). Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhoea in Boston children. *Lancet,* **ii**, 113.
Echeverria, P., Ho, M. T., Blacklow, N. R., Quinnan, G., Portnoy, B., Olson, J. G., Conklin, R., Du Pont, H. L. and Cross, J. H. (1977). Relative importance of viruses and bacteria in the etiology of pediatric diarrhoea in Taiwan. *The Journal of Infectious Diseases,* **136**, 383.
Editorial (1975). Rotaviruses of man and animals. *Lancet,* **i**, 257.
Editorial (1977). Rotavirus gastroenteritis. *British Medical Journal,* **ii**, 784.
Edwards, P. R. and Ewing, W. H. *Identification of enterobacteriaceae,* 3rd Ed. Burgess Publishing Co. (1972).
Evans, D. G., Olarte, J., Du Pont, H. L., Evans, D. J., Galindo, E., Portnoy, B. L. and Conklin, R. H. (1977). Enteropathogens associated with pediatric diarrhoea in Mexico City. *Journal of Pediatrics,* **91**, 65.
Flewett, T. H. Acute non-bacterial infectious gastroenteritis. An essay in comparative virology. In *Recent Advances in Clinical Virology.* (Waterson, A. P., Ed). Churchill Livingstone (1977), p. 151.
Flewett, T. H., Bryden, A. S., Davies, H., Woode, G. N., Bridger, J. C. and Derrick, J. M. (1974). Relation between viruses from acute gastroenteritis of children and newborn calves. *Lancet,* **ii**, 61.
Gorbach, S. L. and Khurana, C. M. (1972). Toxigenic *Escherichia coli.* A cause of infantile diarrhoea in Chicago. *The New England Journal of Medicine,* **287**, 791.
Harris, A. H. and Coleman, M. B. (Eds). *Diagnostic Procedures and Reagents* (4th Ed.) New York, American Public Health Association Inc. (1963), p. 43.
Harris, J. C., Du Pont, H. L. and Hornick, R. B. (1972). Fecal leucocytes in diarrheal illness. *Annals of Internal Medicine,* **76**, 697.
Kapikian, A. Z., Kim, H. W., Wyatt, R. G., Cline, W. L., Arrobio, J. O., Brandt, C. D., Rodriguez, W. J., Sack, D. A., Chanock, R. M. and Parrott, R. H. (1976). Human reovirus-like agent as the major pathogen associated with 'winter' gastroenteritis in hospitalized infants and young children. *New England Journal of Medicine,* **294**, 965.
Kapikian, A. Z., Kim, H. W., Wyatt, R. G., Rodriguez, W. J., Ross, S., Cline, W. L., Parrott, R. H. and Chanock, R. M. (1974). Reovirus-like agent in stools: Association with infantile diarrhoea and development of serologic tests. *Science,* **185**, 1049.
Klipstein, F. A., Engert, R. F. and Short, H. B. (1977). Relative enterotoxigenicity of coliform bacteria. *Journal of Infectious Diseases,* **136**, 205.
Lewis, H. M., Parry; J. V., Davies, H. A., Parry, R. P., Mott, A., Dourmashkin, R. R., Sanderson, P. J., Tyrrell, D. A. J. and Valman, H. B. (1979). A year's experience of the rotavirus syndrome and its association with respiratory illness. *Archives of Disease in Childhood,* **54**, 339.
Madeley, C. R. and Cosgrove, B. P. (1975). 28 nm particles in faeces in infantile gastroenteritis. *Lancet,* **ii**, 451.
Madeley, C. R. and Cosgrove, B. P. (1976). Caliciviruses in man. *Lancet,* **i**, 199.
Madeley, C. R., Cosgrove, B. P., Bell, E. J. and Fallon, R. J. (1977). Stool viruses in babies in glasgow. 1. Hospital admissions with diarrhoea. *Journal of Hygiene,* Cambridge, **78**, 261.
Middleton, P. J., Szymanski, M. T., Abbott, G. D., Bortolussi, R. and Hamilton, J. R. (1974). Orbivirus acute gastroenteritis of infancy. *Lancet,* **i**, 1241.
Morris, G. K., Merson, M. H., Sack, D. A., Wells, J. G., Martin, W. T., De Witt, W. E., Feeley, J. C., Sack, R. B. and Bessudo, D. M. (1976). Laboratory investigation of diarrhoea in travellers to Mexico: evaluation of methods of detecting enterotoxigenic *Escherichia coli. Journal of Clinical Microbiology,* **3**, 486.
Murphy, A. M., Albrey, M. B. and Crewe, E. B. (1977). Rotavirus infections of neonates. *Lancet,* **ii**, 1149.
Nalin, D. R., McLaughlin, J. C., Rahaman, M., Yunus, M. and Curlin, G. (1975). Enterotoxigenic *Escherichia coli* and idiopathic diarrhoea in Bangladesh. *Lancet,* **ii**, 1116.
Pickering, L. K., Du Pont, H. L., Evans, D. G., Evans, D. J. and Olarte, J. (1977). Isolation of

enteric pathogens from asymptomatic students from the United States and Latin America. *The Journal of Infectious Diseases,* **135**, 1003.

Rodriguez, W. J., Kim, H. W., Arrobio, J. O., Brandt, C. D., Chanock, R. M., Kapikian, A. Z., Wyatt, R. G. and Parrott, R. H. (1977). Clinical features of acture gastroenteritis associated with human reovirus-like agent in infants and young children. *Journal of Pediatrics,* **91**, 188.

Ryder, R. W., Wachsmuth, I. K., Buxton, A. E., Evans, D. G., Du Pont, H. L. Mason, E., Barrett, F. F. (1976). Infantile diarrhoea produced by heat-stable enterotoxigenic *Escherichia coli. New England Journal of Medicine,* **295,** 849.

Sack, R. B., Hirshhorn, N., Brownlee, I., Cash, R. A., Woodward, W. E., and Sack, D. A. (1975). Enterotoxigenic *Escherichia coli*-associated diarrheal disease in Apache children. *New England Journal of Medicine,* **292**, 1041.

Sack, D. A. and Sack, R. B. (1975). Test for enterotoxigenic *Escherichia coli* using Y1 adrenal cells in miniculture. *Infection and Immunity,* **11**, 334.

Schoub, B. D., Greef, A. S., Lecatsas, G., Prozesky, O. W., Hay, I. T., Prinsloo, J. G. and Ballard, R. C. (1977). A microbiological investigation of acute summer gastroenteritis in black South African infants. *Journal of Hygiene,* Cambridge, **78**, 377.

Totterdell, B. M., Chrystie, I. L. and Banatvala, J. E. (1976). Rotavirus infections in a maternity unit. *Archives of Disease of Childhood,* **51**, 924.

WHO (1975–76). The new program of the world health organization in medical virology. *Intervirology,* **6**, 137.

Zissis, G. and Lambert, J. P. (1978). Different serotypes of human rotaviruses. *Lancet,* **i**, 38.