Micro-organisms in gastroenteritis

M E ELLIS, B WATSON, B K MANDAL, E M DUNBAR, J CRASKE, A CURRY, J ROBERTS, AND J LOMAX

Regional Department of Infectious Diseases and Tropical Medicine, Monsall Hospital, and Regional Virus Laboratory, Public Health Laboratory, Withington, Manchester

SUMMARY We present bacteriological and virological findings together with salient clinical features from a prospective study of 447 children aged under 2 years admitted to hospital with infectious gastroenteritis. Putative pathogenic micro-organisms were identified in the stools of 75% of these children. Eight identifiably distinct groups of viruses, found on electron microscopy and tissue culture were present in 67% of patients—rotavirus was detected most frequently. Pathogenic bacteria (salmonellas, shigellas, Escherichia coli, and Campylobacter jejuni—but excluding Clostridium difficile) were found in 16% only. Altogether 4.9% of 390 patients had gastroenteritis associated with Cl difficile toxin.

The mean duration of diarrhoea was shortest in patients with identifiable virus, with rotavirus having a mean of 5.01 days, and was longest in patients with pathogenic bacteria in the stools (11.14 days). The finding of more than one type of virus did not seem to be associated with a significantly increased duration of diarrhoea. There are few clinical features which can be associated specifically with any particular micro-organism or groups of these. Multiple organism isolation was common, but the severity of the illness in those patients with at least two types of organism was not greater. Certain viruses, including the norwalk-like virus, known to be associated with outbreaks of gastroenteritis were found as frequently in a group of patients who did not have diarrhoea studied for comparison. Virus was still detectable in the stools of up to 40% of asymptomatic children on the day of discharge.

In 1967 a major study by Ironside et al1 from this unit showed that pathogenic organisms could be identified in only 16% of children aged under 2 years admitted with infectious gastroenteritis. Since that time there have been major advances in both virological (mainly electron microscopy and tissue culture techniques) and bacteriological laboratory techniques, with the identification of several new pathogens. It was appropriate, therefore, to carry out a modern study in the same unit.

Patients and methods

The Regional Infectious Diseases Unit at Monsall Hospital serves a population of 1¼ million people drawn from a mixed racial, industrial and business population mainly from the north of Greater Manchester.2

Children aged less than 2 years who were admitted with acute diarrhoea (frequent watery or unformed offensive stools) with or without vomiting, judged to be caused by primary infectious gastroenteritis were entered into this prospective study over the 12 month period December 1981 to November 1982. The referring general practitioner was briefly questioned about the child's illness, drugs prescribed, and reason for hospital admission. Mother was interviewed within 24 hours of admission and a detailed questionnaire relating to the management and course of the illness before hospital admission was completed. Clinical examination of the child was followed by appropriate treatment and their child's status was monitored until discharge, when he or she was asymptomatic. Assessment of dehydration was based on the Medical Research Council criteria previously described.1

A sample of faeces was obtained on admission to hospital and again on the day of discharge (when symptom free). Routine phlebotomy was performed for full blood count, serum electrolytes, and urea. Blood cultures, throat swab, and midstream urine sample were taken for bacteriology.
Stools were routinely cultured for enteropathogenic *Escherichia coli*, salmonellae, campylobacter, shigellas, and yersinia; they were examined microscopically for ova, cysts, and parasites. The technique used for isolation of *Clostridium difficile* and detection of toxin has been described elsewhere.3

**Preparation of faecal specimens for electron microscopy.** All faecal specimens were stored at 4°C. Twenty per cent faecal suspensions in faecal transport medium were centrifuged at 1500 g for 15 minutes at 8°C (clarification spin). Supernatant (2 ml) was transferred to a polycarbonate ultracentrifuge tube and spun at 65 000 g for one hour at 8°C. The supernatant from the ultracentrifuge spin was tipped off and the pellet resuspended in the small amount of fluid remaining. Viruses were adsorbed onto Formvar-carbon coated electron microscope specimen grids, stained with 3% PTA (pH 6 to 6-5), and examined at 63 000 magnifications in an AEI (Kratos) EM801 electron microscope.

**Tissue culture technique.** A thawed 20% faecal emulsion (0-1 ml) was inoculated into three cell lines: primary baboon kidney (BK), diploid fibroblasts (MRC 5), and continuous human epithelial cells (HEp 2). These were examined twice a week for evidence of cytopathic effect and were discarded after two weeks.

For comparison, all other children aged under 2 years and admitted over the same period with non-gastrointestinal illness had their faeces examined as above. The most common diagnosis in the comparison group was that of a respiratory illness— whooping cough being most frequent.

**Statistical analysis.** Fisher’s exact test and Student’s *t* test were used as appropriate.

**Results**

The reasons for admission to hospital given by the referring doctor included: (1) dehydration (11%); (2) failure of symptoms to settle on home management (52%); (3) adverse social factors (20%); (4) for isolation (12%); and (5) poor general condition (7%). No specific reason was given in 11% of the children.

There were 447 hospital admissions (including 21 children with two admissions); the boy:girl ratio was 1:36; and most children were aged under 1 year (Table 1; Fig. 1). There were 162 children in the comparison group, most of whom had a respiratory infection including whooping cough (117). The most common diagnoses in the remainder were measles, chickenpox, meningitis, or miscellaneous dermatological disorders.

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<tr>
<th>Micro-organisms in gastroenteritis 849</th>
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<th>Table 1 Prevalence of viruses and bacteria</th>
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<tr>
<td>Gastroenteritis group</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>447</td>
</tr>
<tr>
<td>One or more agents* (no (%))</td>
</tr>
<tr>
<td>Two or more agents (no (%))</td>
</tr>
<tr>
<td>Viruses only (no (%))</td>
</tr>
<tr>
<td>Pathogenic bacteria only (no (%))</td>
</tr>
<tr>
<td>Viruses and bacteria (no (%))</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> toxin only (no (%))</td>
</tr>
<tr>
<td>No agent* (no (%))</td>
</tr>
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<td>*Agent=potential viral or bacterial pathogen.</td>
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<th>Fig. 1 Age distribution of micro-organisms found in gastroenteritis patients.</th>
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See text for definition of groups.
pathogenic micro-organisms had at least two concurrently, and in seven per cent there were at least three (Table 1, Figs. 2 and 3).

Several morphologically distinctive viruses were identified, namely: rotavirus, adenovirus, norwalk-like virus, calicivirus, coronavirus, astrovirus, culturable enterovirus (including echovirus, coxsackievirus, and untypable (through unavailability of antisera)), and a heterogeneous group including parvovirus, picornovirus, and some non-culturable enteroviruses—the ‘small round structureless virus particles’.3-5

Rotavirus was the most commonly identified organism (153 of 447 patients: 34%); adenovirus and enterovirus were found in 17.2% and 12.5% respectively. Astroviruses, norwalk-like viruses, caliciviruses, coronaviruses, and small round structureless virus particles collectively were found in 25%. E.coli and Campylobacter jejuni were isolated from 6.9% and 5.1% of patients respectively: apart from Cl difficile these were the two most common pathogenic bacteria (Table 2).

Rotavirus, adenovirus, enterovirus, salmonellas, and shigellas were the five organisms which were usually found alone. The remainder were found more often in combination with others, in particular the small round structureless virus particles, astrovirus, and C jejuni (Fig. 2).

The adenoviruses found in association with gastroenteritis were usually identified by electron microscopy but either failed to grow or proved untypable on tissue culture (55 of the 85 patients). Table 3
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gives details of the adenoviruses found. Thirty were typable (mainly type 2). This contrasts with findings in the comparison group where 14 of the 18 were typable, and these patients had a significantly higher prevalence of respiratory symptoms (72%) compared with the gastroenteritis patients (16%).

In the comparison group, all organisms were found significantly less often apart from the norwalk-like virus, small round structureless virus particles, coronavirus, astrovirus, and enteroviruses (Table 2). In none of these patients were pathogenic bacteria, apart from Cl difficile, isolated. Cl difficile toxin was present in three comparison group patients.

Patients with gastroenteritis were divided into six groups, according to the micro-organisms isolated, thus permitting a comparison of the main clinical and biochemical features. Group 1 comprised patients in whom rotavirus was present alone; group 2, those in whom only any one virus—not rotavirus—was isolated, expressed as cumulative singles; group 3, patients in whom two or more viruses were isolated; group 4, those in whom one or more bacteria were present, alone or in combination; group 5, patients with one or more bacteria plus any one or more viruses in combination; and group 6 comprised patients in whom no pathogenic bacteria or viruses were present. Cl difficile was isolated from all six groups. These findings are summarised in Table 4. Patients excreting any virus tended to present during the winter months; those excreting bacteria presented during the warmer season (Fig. 3).

Diarrhoea persisted longer than vomiting in all groups. The mean duration was shortest in those with rotavirus alone (5-01 days) and longest with bacterial isolates (11-14 days). Viruses other than rotavirus tended to produce a significantly longer duration of diarrhoea (7-05 days) but the simultaneous finding of at least two different types of viruses was not associated with longer duration of diarrhoea (7-23 days). Furthermore, the presence of virus and bacteria did not seem to alter the duration of diarrhoeal symptoms (10-81 days) when compared with patients in whom bacteria alone were found (11-14 days). The non-specific gastroenteritis group seemed to have less diarrhoea than the bacteria groups and approximated to that for viruses, with a mean duration of 6-60 days.

Duration of vomiting in all groups ranged from 2-21 to 3-65 days, viruses tending to have a longer duration of symptoms than bacteria—these differences, however, were not significant, apart from the group with bacteria and viruses in whom the duration (2-21 days) was significantly less than with rotavirus alone.

Moderate to severe dehydration occurred in 14% of patients but acidosis was not a frequent finding. Hypernatraemia was found in less than one per cent and in patients excreting virus alone.

Of other associated clinical findings, the prevalence of stool mucus was significantly increased in viruses other than rotaviruses and in the group with bacteria and virus found together. Macroscopic blood in the stool was highly significantly increased in patients with bacteria compared with rotavirus. Lower respiratory infection occurred in up to 21% of patients and its prevalence was significantly

<table>
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<th>Table 2</th>
<th>Micro-organisms found in patients</th>
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<tbody>
<tr>
<td>Organism</td>
<td>Gastroenteritis patients Group</td>
</tr>
<tr>
<td>Rotavirus*</td>
<td>153 (34)</td>
</tr>
<tr>
<td>Adenovirus*</td>
<td>77 (17-2)</td>
</tr>
<tr>
<td>Enterovirus†</td>
<td>56 (12-5)</td>
</tr>
<tr>
<td>Norwalk-like virus*</td>
<td>16 (3-6)</td>
</tr>
<tr>
<td>Calicivirus*</td>
<td>16 (3-6)</td>
</tr>
<tr>
<td>Coronavirus*</td>
<td>11 (2-5)</td>
</tr>
<tr>
<td>Astrovirus*</td>
<td>16 (3-6)</td>
</tr>
<tr>
<td>Small round structureless virus particles*</td>
<td>44 (9-8)</td>
</tr>
<tr>
<td>Salmonellas</td>
<td>19 (4-3)</td>
</tr>
<tr>
<td>Shigellas</td>
<td>9 (2)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>31 (6-9)</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>23 (5-1)</td>
</tr>
<tr>
<td>None</td>
<td>112 (25)</td>
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*Identified by electron microscopy; †identified by tissue culture; ‡See Table 3 for breakdown; §see text for definition.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Details of adenoviruses found and associated respiratory infection</th>
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<tbody>
<tr>
<td></td>
<td>Gastroenteritis patients Group</td>
</tr>
<tr>
<td>Identified by tissue culture and typable (group A)</td>
<td>30 (35)*</td>
</tr>
<tr>
<td>Identified by tissue culture and untypable (group B)</td>
<td>38 (45)†</td>
</tr>
<tr>
<td>Identified by electron microscopy alone (group C)</td>
<td>17 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>85 (100)</td>
</tr>
<tr>
<td>Associated respiratory infection</td>
<td>14 (16)‡</td>
</tr>
<tr>
<td>Serotypes: group A type 1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
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<td></td>
<td>4</td>
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<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
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</table>

*20 had adenovirus also been seen by electron microscopy, which may not have been the same virus as that identified by tissue culture.
†35 had adenovirus also been seen by electron microscopy, which may not have been the same virus as that identified by tissue culture.
‡Significant difference P<0-001.
increased in patients with adenovirus infections. A greater proportion of patients with viruses and with non-specific gastroenteritis had a higher blood urea concentration compared with those with bacteria. The proportion of patients with a peripheral white cell count over 13 × 10⁹/l was significantly greater in all groups compared with the rotavirus group.

The duration of hospital stay was determined primarily by whether symptoms had settled and not by continuing faecal excretion of the organism. Seventy-six per cent of patients with rotavirus and 75% patients with non-specific gastroenteritis had a short hospital stay (less than seven days); in those with at least two viral agents the stay was longer, and in those with bacteria more than 50% remained in hospital for over seven days.

There were no other significant differences in the clinical and biochemical features between the six groups (Table 5). In particular, the patient’s general condition was not more severe in those who had at least two micro-organisms present.

Table 4 Clinical and other features in 447 gastroenteritis patients classified into six major groups by micro-organisms, showing significant differences from group 1 in which rotavirus was found alone

<table>
<thead>
<tr>
<th>Group</th>
<th>1 Rotavirus alone</th>
<th>2 All viruses except rotavirus (cumulative singles)</th>
<th>3 Combination of viruses</th>
<th>4 All bacteria</th>
<th>5 Bacteria and viruses</th>
<th>6 Non-specific gastroenteritis</th>
</tr>
</thead>
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<tr>
<td></td>
<td>No. (%)</td>
<td>Duration of diarrhoea (days). Mean (SEM)</td>
<td>Duration of vomiting (days). Mean (SEM)</td>
<td>Mucus present in stools. No. (%)</td>
<td>Urea &gt; 6 mmol/l. No. (%)</td>
<td>White cell count</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.01 (0.53) 7.05 (1.03)²</td>
<td>7.23 (0.66)² 11.14 (1.35)²</td>
<td>17 (24) ns</td>
<td>18 (21) ns (adenovirus²)</td>
<td>18 (18)</td>
</tr>
</tbody>
</table>

*p = 0.005; *p = 0.025; *p = 0.05; *p = 0.0005 (Student’s t test).
*p = 0.05; *p = 0.025; *p = 0.01; *p = 0.001 (Fisher’s exact test).
ns = no significant difference from group 1.
See text for fuller definition of groups.

Ten children on admission and a further 20 within 48 hours of this (six per cent in total) required intravenous fluid replacement for one to two days in preference to oral rehydration treatment, to correct dehydration or to overcome persistent or severe symptoms. Most, including many with moderate to severe dehydration, were successfully rehydrated with a standard regimen of sodium chloride and glucose solution (Dioralyte, Armour Pharmaceutical) for 24 hours combined with temporary food and milk withdrawal, followed by a graduated dilutional re-introduction of either normal milk feeds or solid diet as appropriate.

Antibiotics were not routinely prescribed for gastroenteritis in this unit; only 16 patients received antimicrobial treatment as dictated by their clinical condition (salmonellas (five); C jejuni (two); shigellas (four); enteropathogenic E coli (one); C difficile colitis (six)).

There were no deaths during the study period, but complications were recorded in 85 patients. Five children had a convulsion before hospital admission; four of these had a fever in excess of 39.5°C on admission and this may have been the cause. None
of these five children was severely dehydrated or hypernatraemic. The associated micro-organisms were: rotavirus (three) and shigella (one). There were no previous disposing central nervous system factors in these patients. One other child sustained a brief convulsion after admission—he had gastroenteritis associated with rotavirus, was severely dehydrated on admission (sodium 164 mmol/l; urea 21 mmol/l), but made a full and complete recovery. Neither the type of intravenous fluid nor the rate of replacement was felt to have been contributory to this child’s convulsion. The more common complications were temporary secondary lactose intolerance resulting in a recrudescence of diarrhoea and necessitating the withdrawal of lactose-containing milk feeds.

Those patients who did not prove to have any pathogenic micro-organisms in their faeces did not seem to show any significant difference in the time that solid feeding had first been introduced or in the incidence of breast feeding.

Discussion

A decade ago the most commonly isolated micro-organism in infantile gastroenteritis was the enteropathogenic E coli, accounting for 11 to 16% of cases. In the vast majority of children, no pathogens were isolated (non-specific gastroenteritis). Our study indicates that the incidence of non-specific gastroenteritis is now much less common, but still accounts for a considerable core of patients. The reason for this changed pattern is the discovery of several new pathogens which cause human diarrhoeal disease.

Most of the micro-organisms identified were viruses, among which the rotavirus was the most common. Individual bacteria made a small overall contribution; C jejuni is now included among these.

The association of Cl difficile toxin with human diarrhoea has only recently been appreciated. In this study it was felt to have been the major factor in 19 patients, a frequency comparable with other established bacterial pathogens. The role of Cl difficile is detailed in another paper.

It is of some interest that in many instances the simultaneous presence of more than one agent occurred in the same patient; this was most notable in the patients excreting C jejuni, E coli, small round structureless virus particles, calicivirus, and coronavirus. This phenomenon of multiple organism isolation makes assessment of the contribution of each micro-organism to the illness difficult. There was, however, no difference in disease severity between those who excreted a single organism and those with at least two. The occasional reported finding, therefore, that those patients who have more than one micro-organism may have more severe disease does not seem to be a general phenomenon.

Numerous studies have established the role of rotavirus as an important human pathogen but the role of the newer viruses is uncertain. Viruses resembling the norwalk agent (norwalk-like viruses) have been previously identified with some outbreaks of gastroenteritis, usually in older children and adults. Their low and equal prevalence in patients and in the comparison group reinforces the view that it is not an important cause of sporadic diarrhoea among infants. Although coronavirus have also been incriminated previously, their role is very debatable and this is also supported by our findings. Nevertheless, they may constitute an important community reservoir from which outbreaks may arise, given favourable conditions. On the contrary, all the caliciviruses and astroviruses, though small in number, were found almost without exception in patients with gastroenteritis, strengthening the view that they are pathogenic. These latter two viruses are not usually associated with such a young age group, however, occurring more often in older children.

Thirty patients had culturable and typable adenoviruses in their stools, mainly of serotype 2 (group A, Table 3). Thirty eight patients had non-typable or poorly growing adenovirus (group B)—they may not have grown because of their fastidious nature, insufficient faecal concentration, or unavailability of specific antisera. Fifty five of these 68 patients (groups A and B) had adenovirus identifiable by electron microscopy as well, but it is uncertain whether the adenovirus seen by electron microscopy was the same as the one cultured. In the remaining 17 patients (group C) adenovirus was not culturable but was detected by electron microscopy alone. In contrast, most adenoviruses found in comparison group patients grew and were typable—mainly serotype 7. The relevance of these findings is not entirely clear but it is likely that adenoviruses found in group C were responsible for gastroenteritis, those in group B are more dubious, and those in group A unlikely. Our findings support the work of others who argue that the adenoviruses associated with primary gastroenteritis are distinct from those associated with primary extragastrointestinal illness. There may well be other adenoviruses responsible for gastroenteritis which, owing to their fastidious nature, fail to grow under our tissue culture conditions, and these remain unrecognised.

A large proportion of children were discharged asymptomatic as convalescent excretors. This may
be of public concern and is at variance with the findings of others, who report a much lower percentage of children still excreting virus at this stage of convalescence.\textsuperscript{14}

Overall, the clinical features indicate that gastroenteritis in this age group is nowadays a relatively benign self-limiting illness associated with a short stay in hospital and few complications. Apart from one child, convulsions occurred before admission; in only one child did there seem to be the precipitating factor of hypernatraemia. This is in striking contrast to the situation described a decade ago from this unit, when hypernatraemic dehydration associated with cerebral disturbance and related to high solute milk feeds and concentrated glucose drinks was common and carried an appreciable mortality.\textsuperscript{1, 15}

This important aspect of management is discussed in detail elsewhere.\textsuperscript{16}

The presence of macroscopic blood in the stools, a normal plasma urea concentration, a peripheral white cell count greater than $13 \times 10^9/l$, longer duration of diarrhoeal symptoms, and a longer stay in hospital all tend to suggest a bacterial rather than a viral aetiology for the gastroenteritis. This is not absolute, however, and there were no specific or characteristic clinical, biochemical, or haematological features in any particular group to indicate unequivocally a particular agent. Thus, for example, the widely held view that adenovirus infections are suggested by the presence of lymphadenopathy and a maculopapular rash seems untenable from our findings (Table 5). Also, our results indicate that it is not possible to make an emphatic diagnosis of rotavirus diarrhoea or rotavirus syndrome on clinical findings alone,\textsuperscript{17} since upper and lower respiratory infection and otitis media were not found more commonly in those subsequently shown to have faecal rotavirus.

The group with gastroenteritis in whom no organisms were identified merits particular comment. This group was not associated with a higher incidence of extragastrointestinal features (otitis media, urinary infections etc) so that a 'parenteral' aetiology is unlikely, there was no increased prevalence of antibiotic usage in this group (16%) compared with the other groups (9 to 24%), and there was no increased delay in stool analysis excluding virological 'fall off'. Recent change in bowel flora precipitated by recent alterations in feeding schedules ('weanling diarrhoeae') or a change from breast to bottle feeding were no more common in this group—hence acute alterations in bowel flora were unlikely to be the cause. On the other hand, the seasonal and age distribution together with the broadly similar clinical features of these patients compared with those who had an identifiable viral agent suggests a viral aetiology. It may be that some of these patients were 'missed' cases of rotavirus, adenovirus, or other viral gastroenteritis since electron microscopy is relatively insensitive\textsuperscript{18} and existing tissue culture techniques may not identify some viruses, notably the enteric adenoviruses. Electron microscopy, however, is the only 'catch all' method currently available for the identification of viral associated gastroenteritis. The possibility is that some other agent, as yet undiscovered, was responsible.

We gratefully thank Dr K Whale, Consultant Microbiologist for her help with the bacteriological cultures; Dr M Addison, Consultant Pathologist for help with biochemistry measurements; Mr A Mokowski, Senior Research Officer for expert statistical analysis; and Sisters Coyle, Fosbrook, and Thompson and nursing staff at Monsall Hospital for their invaluable assistance.

References


Correspondence to Dr M E Ellis, Regional Department of Infectious Diseases and Tropical Medicine, Monsall Hospital, Newton Heath, Manchester M10 8WR.

Received 6 June 1984