

# Structured surveillance of infantile gastroenteritis in East Anglia, UK: incidence of infection with common viral gastroenteric pathogens

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## SUMMARY

The aim of this study was to investigate the burden of disease associated with gastroenteric viruses (rotavirus, norovirus, sapovirus, astrovirus and enteric adenovirus) using structured surveillance of children aged <6 years in the community. Faecal samples were collected between 2000 and 2003 from 685 children with symptoms of gastroenteritis. The children comprised three groups; 223 in the structured surveillance cohort, 203 in a community cohort and 259 in a cohort of hospitalized children. All samples were tested for the presence of viral pathogens using molecular methods. Questionnaires were sent to the parents/carers of the children recruited to the structured surveillance cohort in order to collect data that would allow an estimation of the severity of illness by means of the Vesikari score, and of the cost associated with gastrointestinal disease in this age group. A viral aetiological agent was detected in 53·5% of samples tested. Rotavirus was the most common pathogen found in all three cohorts followed by norovirus and enteric adenoviruses. Multiple viruses were found in 8% of the samples, and commonly involved rotavirus and any other virus. G1P[8] was the most commonly detected rotavirus strain and there was no significant difference in the distribution of rotavirus genotypes among the three cohorts. Analysis of the questionnaires indicated that rotavirus infections were likely to be more severe than any other virus infection, and children from whom a viral pathogen was identified were more likely to require rehydration therapy.

## INTRODUCTION

Infectious intestinal disease is common in childhood with each child probably experiencing four or more episodes of gastroenteritis before the age of 5 years.

Rotaviruses, adenoviruses, astroviruses, noroviruses and sapoviruses are the major viral aetiological agents associated with childhood gastroenteritis.

Rotaviruses are members of the Reoviridae family and are classified into groups (A–E) on the basis of antigenic differences of the viral middle layer [1]. Group A rotaviruses are the most common cause of human gastroenteritis, but groups B and C rotaviruses also infect humans. Group A rotaviruses are further classified into subgroups (SG) on the basis

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of immunological reactivities of the middle layer protein VP6, and into G- and P-types according to the diversity of the outer layer proteins VP7 (*glycoprotein*) and VP4 (*protease-sensitive protein*), respectively [1]. To date, four different SGs (I, II, I+II and non-I/non-II), 15 G-types (G1–G15) and 20 P-types (P[1]–P[20]) have been identified among group A rotaviruses [1].

Noroviruses (formerly known as Norwalk-like viruses or small round structured viruses) and sapoviruses (formerly known as Sapporo-like viruses) are members of the Caliciviridae family. Noroviruses are classified into three genogroups, and at least 16 genotypes [2]. Similarly sapoviruses have been classified into three genogroups and several genotypes [3, 4] although there is no firm agreement on the classification of these viruses as yet.

Astroviruses are members of the Astroviridae family and are classified into eight serotypes [5]. Human adenoviruses are members of the Adenoviridae family which are classified into six different subgroups or species (A–F) and within these into 51 distinct serotypes according to immunological, biochemical and biological differences [5]. Among these, adenoviruses of subgroup F, serotypes 40 and 41, have been associated with gastroenteritis and are termed enteric or fastidious adenoviruses [6].

Electron microscopy (EM) has traditionally been used for the detection of enteric viruses in the faeces of infected individuals, however, this method is relatively insensitive, as detection requires  $\sim 10^6$  virus particles/g faeces. This is a problem particularly for the detection of caliciviruses. Serological methods have been developed for the detection of some of these viruses. Enzyme immunoabsorbent assays (EIA) and passive particle agglutination tests (PPAT), some of which are available commercially, provide sensitivity comparable to EM for the detection of rotaviruses, noroviruses, astroviruses and adenoviruses. More recently, molecular methods, reverse transcription–polymerase chain reaction (RT–PCR) or PCR have been developed for the detection of enteric viruses [7–10]. These methods provide improved sensitivity for the detection of all enteric viruses, but have had their greatest impact on the detection of human caliciviruses.

Although the association of these viruses with gastroenteritis is well established, the burden of disease associated with the different viruses is poorly described. Current data on the burden of disease are in the main derived from the routine voluntary reporting

by clinical microbiology laboratories, and only a few structured surveillance studies have been undertaken in order to better define the burden of disease associated with the common viral pathogens [11–16]. Moreover, the differing sensitivities of detection methods used to diagnose enteric viral infections can produce a false picture of the incidence of infection with the different viruses. Many studies have used sensitive molecular methods for the detection of caliciviruses but EM or EIA to detect rotaviruses, adenoviruses and astroviruses. The use of techniques of lower sensitivity is highlighted by the ‘diagnostic gap’ which can result in more than 60% of gastrointestinal infection from which the aetiology is not determined. The use of molecular detection methods such as PCR can reduce the diagnostic gap to between 30% and 40% [10].

The current study was undertaken in order to define the burden of viral gastroenteritis in children aged <6 years in the community, using sensitive molecular methods for the detection of rotaviruses, noroviruses, adenoviruses, astroviruses and sapoviruses. Data on disease severity and the cost associated with gastrointestinal disease obtained through detailed questionnaires completed by the parents/carers was collected and analysed in order to assess the severity of illness associated with the different viral agents of gastroenteritis, and to estimate the societal costs associated with an episode of infantile gastroenteritis.

## MATERIALS AND METHODS

### Patients and samples

Faecal samples collected in the winter months (December–April) during three consecutive winter seasons (2000–2003) from 685 patients aged <6 years with symptoms of gastroenteritis were tested for the presence of enteric viral pathogens. An episode of gastroenteritis was defined as acute onset of diarrhoea and vomiting or more than one episode of diarrhoea or vomiting within a 24-h period.

A total of 223 samples were from children recruited to a general practice-based structured surveillance study, of infantile gastroenteritis in the East Anglia region. The inclusion criteria were children aged <6 years who presented to a general practitioner (GP) with symptoms of gastroenteritis, diarrhoea and vomiting or diarrhoea. In addition, 203 and 259 samples were analysed from community and hospital-based cohorts, respectively. The samples from the

community cohort were samples referred to Addenbrooke's Hospital, Cambridge by general practices in East Anglia not recruited to the structured surveillance, for the investigation of gastroenteritis. The hospital samples were from children with gastroenteritis admitted as in-patients or attending the A&E department at Addenbrooke's Hospital. For the hospital cohort, there was insufficient data to allow distinction between nosocomial cases and admissions due to gastroenteritis.

### Data collection

Questionnaires were sent to the parents/carers of the children recruited to the structured surveillance study in order to collect data that would allow an estimation of the severity of illness and of the costs associated with gastrointestinal disease in this age group.

Parents were asked to complete a series of questions relating to the child's illness, medical care received, personal history, and socio-economic details (see Appendix). All information collected in the questionnaires was processed by computer in accordance with the Data Protection Act, 1990. Ethical approval for the study was obtained from the Regional Multi-centre Research Ethics Committee.

The only data available for the non-structured surveillance cohorts was clinical diagnosis, age and sex.

### Calculation of severity scores

Parents/carers were asked to record parameters, which allowed us to assign a score to disease severity. Information on the quantity of liquid feeds prior to infection and during illness was collected, and this was used to determine the likelihood of dehydration. A significant reduction in the intake of fluids during illness in concert with diarrhoea and vomiting was regarded as an indicator of possible dehydration. Severity scores were calculated using a modification of the method of Ruuska & Vesikari [17]. Accurate temperature measurements were not available, therefore children with or without fever, reported by carers, were given scores of 1 and 0 respectively. This resulted in a maximum score of 18 points rather than the 20 originally described (Table 1). An episode of gastroenteritis is considered mild when the severity score is  $\leq 5$ , moderate when severity scores range between 6 and 10, and severe when the severity scores are  $> 10$ .

### Virological investigations

Faecal samples were prepared as 10% suspensions in balanced salt solution (M199, Sigma, Poole, Dorset, UK) and stored at 4 °C. Nucleic acid was extracted using guanidinium isothiocyanate/silica and cDNA synthesized in the presence of random primers [18, 19]. The cDNA was used in PCRs for the detection of enteric adenoviruses, astroviruses, noroviruses, group A rotaviruses, and sapoviruses using oligonucleotide primers and methods described previously [10]. Also, a PCR for the detection of group C rotaviruses was optimized and used in this study. Oligonucleotide primers were designed to amplify a region of the gene encoding the VP6 [RV-CVP6F: 5'-ATW GAA GCY GTA TGT GAT G-3' (nt 296-314) and RV-CVP6R: 5'-ATT RTT TGG TGC ATT YTC AG-3' (nt 615-634)]. Rotavirus-positive samples were genotyped using method described previously [20].

### Statistical analysis

$\chi^2$  test and Student's *t* test were performed to determine the significance of differences observed between the different groups of patients and/or incidence of infection with the different viruses (STATS™ v.1.1, Decision Analyst '98, Arlington, TX, USA).

## RESULTS

A viral aetiological agent was detected in 367/685 (53·6%) samples tested (Table 1). A viral pathogen was detected in a significantly greater number of patients from the structured surveillance group than from either the community or the hospital cohort ( $P < 0·01$ ) and also in the community cohort than in the hospital cohort ( $P < 0·05$ ).

Rotavirus was the most common viral pathogen found in all three groups, followed by norovirus and enteric adenoviruses (Table 2). No significant differences were observed in the relative incidences of the different viruses between the three cohorts.

Multiple viral pathogens were found in 55/686 (8%) of the samples. Among these, two viral pathogens were found in 53 samples, and three viral pathogens in a further two samples (Table 2). Multiple infections involving rotavirus and any other virus were most common ( $n = 40$ ), followed by norovirus with any other virus ( $n = 28$ ) and adenovirus with other viruses ( $n = 24$ ) (Table 2).

Although samples were collected from children aged  $\leq 5$  years, they were predominantly from

Table 1. Criteria used for calculating disease severity scores; comparison with previously published criteria

Symptoms	20 points (Ruuska & Vesikari [17])	Symptoms	18 points (this study)
Duration of diarrhoea (days)		Duration of diarrhoea (days)	
1–4	1	1–4	1
5	2	5	2
≥6	3	≥6	3
Max no. diarrhoeal stools/24 h		Max no. nappies/24 h	
1–3	1	1–3	1
4–5	2	4–5	2
≥6	3	≥6	3
Duration of vomiting (days)		Duration of vomiting (days)	
1	1	1	1
2	2	2	2
≥3	3	≥3	3
Max no. vomiting episodes/24 h		Max no. vomiting episodes/24 h	
0	0	0	0
1	1	1	1
2–4	2	2–4	2
≥5	3	≥5	3
Fever		Fever	
≤37 °C	0	No	0
37·1–38·4 °C	1	Yes	1
38·5–38·9 °C	2		
≥39 °C	3		
Dehydration		Dehydration	
None	0	No change/increase in fluid intake	0
1–5 %	2	Decrease of 1 liquid feed	1
>6 %	3	Decrease of between 2–4 liquid feeds	2
		Decrease of ≥5 liquid feeds	3
Treatment		Treatment	
None	0	None	0
Rehydration	1	Rehydration	1
Hospitalization	2	Hospitalization	2

children aged ≤2 years. The numbers of children presenting with gastroenteritis to their GPs declined as age increased.

Rotavirus was the most common viral pathogen in all age groups, followed by norovirus in ≤1- and 2-year-olds, and enteric adenovirus in 3- and 4-year-olds (Table 3). No significant differences were seen in the incidence of mixed infections between age groups (Table 3). In children aged ≤1 year the number of samples in which no viral pathogen was identified was significantly higher in the hospital cohort compared to the same age group in the structured surveillance and community cohorts (hospital cohort, 75%; structured surveillance cohort, 37·4%; community cohort, 45·6%;  $P<0·01$ ) and also compared to any other age groups ( $P<0·01$ ) (excluding the 5-year-olds as the numbers of children in this group were insufficient for meaningful comparison). In the structured

surveillance cohort, the mean age of children for which a pathogen was identified was the same as that of children for whom no aetiological agent was identified, 1·9 years, and the median age was 2 and 1 years, respectively.

### Rotavirus genotyping

G1P[8] was the most commonly detected rotavirus strain in the structured surveillance group and the community and hospital cohorts and there were no significant differences among rotavirus genotypes detected in each patient group.

Temporal distribution indicated that although G1P[8] was the most frequently found rotavirus each year, significantly higher numbers of G1P[8] strains were found during the 2002/2003 season than in the previous two seasons. This was accompanied

Table 2. Virus distribution in cases of gastroenteritis (including multiple infections)

	Structured surveillance		Community cohort		Hospital cohort		Total	
	n	% positive	n	% positive	n	% positive	n	% positive
<b>Viral aetiology</b>								
Rotavirus A	106	47·5	60	29·6	59	22·8	225	32·8
Norovirus	31	13·9	18	8·9	36	13·9	85	12·4
Adenovirus	20	9·0	26	12·8	20	7·7	66	9·6
Astrovirus	11	4·9	4	2·0	7	2·7	22	3·2
Sapovirus	2	0·9	8	3·9	5	1·9	15	2·2
Rotavirus C	1	0·4	2	1·0	2	0·8	5	0·7
<b>Single virus infection</b>								
Rotavirus A	90	40·4	50	24·6	46	17·8	186	27·2
Norovirus	22	9·9	13	6·4	25	9·7	60	8·8
Adenovirus	12	5·4	18	8·9	12	4·6	42	6·1
Astrovirus	8	3·6	1	0·5	5	1·9	14	2·0
Sapovirus	1	0·4	3	1·5	1	0·4	5	0·7
Rotavirus C	0	0·0	2	1·0	2	0·8	4	0·6
<b>Infection with multiple viruses</b>								
Rotavirus A + Adenovirus	6	2·7	5	2·5	5	1·9	16	2·3
Rotavirus A + Norovirus	8	3·6	2	1·0	4	1·5	14	2·0
Norovirus + Sapovirus	1	0·4	3	1·5	2	0·8	6	0·9
Norovirus + Adenovirus	0	0·0	3	1·5	2	0·8	5	0·7
Rotavirus A + Astrovirus	1	0·4	2	1·0	1	0·4	4	0·6
Rotavirus A + Sapovirus	1	0·4	1	0·5	1	0·4	3	0·4
Adenovirus + Astrovirus	2	0·9	0	0·0	0	0·0	2	0·3
Rotavirus C + Sapovirus + Astrovirus	1	0·4	0	0·0	0	0·0	1	0·1
Norovirus + Astrovirus	0	0·0	0	0·0	1	0·4	1	0·1
Astrovirus + Sapovirus	0	0·0	1	0·5	0	0·4	1	0·1
Rotavirus A + Norovirus + Adenovirus	0	0·0	0	0·0	1	0·4	1	0·1
Rotavirus A + Norovirus + Sapovirus	0	0·0	0	0·0	1	0·4	1	0·1
No virus detected	70	31·4	99	48·8	150	57·9	319	46·6
Total	223		203		259		685	

by a decrease in the number of infections with G9P[8] strains detected in 2002/2003 with respect to the two previous seasons (Table 4). A G12P[6] rotavirus strain was detected for the first time in the United Kingdom. This strain failed to amplify in the G-type-specific multiplex second-round PCR, and was characterized through sequencing of the first-round amplicon.

Severity scores were available for 49 patients from whom a rotavirus was detected and genotyped. The majority ( $n=37$ ) were G1P[8] and severity scores ranged from 6 (mild) to 16 (severe).

#### Structured surveillance: analysis of data obtained through completed questionnaires

A total of 142 (64%) completed questionnaires were returned of which 136 provided sufficient information on disease severity to be evaluable.

#### Days between onset of symptoms and sample collection

There was no statistically significant difference between the means of days after onset when samples were collected from children with or without an identified viral aetiology. The mean number of days between onset of symptoms and sample collection was 5·4 days (median 5) for samples from which a viral pathogen was identified, and 6·1 days (median 5) for samples from which no viral pathogen was detected.

#### Severity of illness

In total there were 136 respondents providing information on the duration and number of episodes of diarrhoea and vomiting, the presence or absence of fever and the use of oral rehydration therapy which allowed the calculation of severity scores (Table 5).

Table 3. *Virus distribution by age*

	Age (years)									
	≤1		2		3		4		5	
	n	%	n	%	n	%	n	%	n	%
Adenovirus	7	2·7	5	4·6	7	10·3	4	14·8	0	0·0
Astrovirus	5	1·9	5	4·6	1	1·5	2	7·4	0	0·0
Norovirus	13	5·0	9	8·3	6	8·8	2	7·4	0	0·0
Rotavirus	83	32·0	46	42·2	19	27·9	5	18·5	1	7·7
Mixed infection	17	6·6	10	9·2	4	5·9	1	3·7	0	0·0
Any virus	125	48·3	75	68·8	37	54·4	14	51·9	1	7·7
No virus	134	51·7	34	31·2	31	45·6	13	48·1	12	92·3
Total	259	100·0	109	100·0	68	100·0	27	100·0	14	100·0

Table 4. *Distribution of G- and P-type combinations of 225 rotavirus-positive samples collected from 2000 to 2003 (including structured surveillance, community and hospital cohort samples)*

	2000/01		2001/02		2002/03		Total	
	n	%	n	%	n	%	n	%
G1P[8]	37	74·0	57	71·3	89	93·7	183	81·3
G1P[4]	1	2·0	0	0·0	0	0·0	1	0·4
G2P[4]	4	8·0	1	1·3	1	1·1	6	2·7
G2P[8]	0	0·0	2	2·5	0	0·0	2	0·9
G3P[8]	0	0·0	0	0·0	1	1·1	1	0·4
G4P[8]	1	2·0	2	2·5	1	1·1	4	1·8
G9P[8]	6	12·0	13	16·3	3	3·2	22	9·8
G2P[8]	0	0·0	2	2·5	0	0·0	2	0·9
G12P[6]	0	0·0	1	1·3	0	0·0	1	0·4
GNKP[4]	0	0·0	1	1·3	0	0·0	1	0·4
G1PNK	1	2·0	0	0·0	0	0·0	1	0·4
G1+G9P[8]	0	0·0	1	1·3	0	0·0	1	0·4
Total	50		80		95		225	

Infections with enteric adenoviruses, and astroviruses were more often associated with mild to moderate disease, and severe disease was more frequently associated with noroviruses and rotaviruses; however, infections of moderate or severe disease were associated with all viruses with the exception of astrovirus (Table 5). The median severity scores for children aged from <1 to 2 years were 10, compared with scores of 9, 8, and 7·5 for children aged 3, 4 and 5 years, respectively. The median severity score for patients from whom no virus was detected was 7 (range 3–15). There was no statistically significant increase or decrease in severity associated with astrovirus, adenovirus or norovirus infections ( $P>0·05$ ). Rotavirus infections were statistically more likely to be more severe than any other virus infection or in

symptomatic patients from whom no aetiological agent was found ( $P=0·0014$ ) (Table 5). The analysis of the symptoms reported showed associations between rotavirus and norovirus infections and the duration and number of episodes of vomiting and contributed to the higher severity scores associated with rotavirus infection. Children from whom a viral pathogen was identified were more likely to require rehydration therapy than those with gastroenteritis of unknown aetiology ( $P<0·01$ ) (Table 5).

## DISCUSSION

Previous studies of the burden of infectious intestinal disease associated with gastroenteritis viruses indicated that, in children aged <5 years, rotaviruses or

Table 5. Severity score determined for 136 patients with gastroenteritis

	Severity score			% requiring rehydration
	Median	Mean (S.D.)	Range	
Adenovirus	6.5	8.1 (3.3)	5–13	28.6
Astrovirus	7	7.3 (2.4)	4–10	33.3
Norovirus	11	10.1 (2.7)	5–15	53.3
Rotavirus	11	10.6 (2.9)	4–16	41.5
Mixed infection	10	10.5 (2.8)	5–14	54.5
Any virus	10	10 (3.0)	4–16	44.9
No virus	7	8.5 (3.2)	3–15	25.0

Table 6. Comparison of structured surveillance studies of infantile gastroenteritis

Structured surveillance group	Tomkins et al. 2000–2003	Pang et al. 2000 [12]	Waters et al. 2000 [13]	de Witt et al. 2001 [14]	de Witt et al. 2001 [15]	Román et al. 2003 [16]
Rotavirus group A	47.5%	17.4%	29.1%	32.0%	9.7%	16.7%
Rotavirus group C	0.4%	n.t.	n.t.	n.t.	n.t.	n.t.
Adenovirus	9.0%	3.7%	5.1%	4.0%	5.4%	9.5%
Astrovirus	4.9%	5.0%	8.8%	1.0%	1.9%	1.2%
Calicivirus	14.8%	15.0%	29.5%	2.5%	26.1%	14.9%
Norovirus	13.9%	n.d.	20.2%	2.0%	18.3%	10.1%
Sapovirus	0.8%	n.d.	9.3%	0.5%	7.3%	4.8%
No virus detected	31.4%	59.0%	40.0%	58.0%	44.6%	69.15%

n.d., Not differentiated; n.t., not tested.

noroviruses are the major causes of infectious gastroenteritis in this age group [11–16]. Incidences of rotavirus and norovirus infection among different studies ranged from 9.7% to 32.0% and from 2% to 20.2%, respectively. Adenoviruses, astroviruses and sapoviruses were each responsible for <10% of gastrointestinal infections in all studies (Table 6).

Detection methods of low or differing sensitivities were used in all of these studies and may have influenced the incidence rates determined for each viral pathogen. In the current study, sensitive molecular methods were used to detect rotaviruses (groups A–C), adenoviruses types 40 and 41, noroviruses, sapoviruses and astroviruses. It has previously been shown that the sensitivity of detection can be increased by 22–700% depending on the viral pathogen sought [10].

Analysis of the results of all samples (structured surveillance cohort and community and hospital cohorts) showed that a virus or viruses were identified in 53.5% of samples. As in other reported studies, rotaviruses and noroviruses were associated with the

majority of infections. Interestingly, analysis of the structured surveillance cohort alone showed that a virus was identified in 68.6% of samples. This difference may be associated with a firmer case definition used in the structured surveillance study including, acute onset of diarrhoea and vomiting or more than one episode of diarrhoea or vomiting in a 24-h period whereas controls were selected on a clinical diagnosis of ‘gastroenteritis’, ‘diarrhoea and vomiting’ or ‘diarrhoea’. Therefore, the community and hospital cohorts may have included children with chronic symptoms or non-infectious diarrhoeas or the lack of detection may reflect a time-lag between the onset of illness and sample collection.

No differences were observed in the distribution of rotavirus genotypes between the structured surveillance and the community or hospital cohorts and in all groups G1P[8] were the predominant strains. A rotavirus G12P[6] was detected for the first time in the United Kingdom from one of the patients in the community cohort. G12 strains in combination with either P[6] or P[9] have recently been described in

sporadic cases of infantile diarrhoea in several parts of the world [21–24]. G9P[8] strains which emerged in the United Kingdom in 1995 were found throughout the study, but their incidence decreased during the last year of the study. Previously we observed that these viruses first emerged in the United Kingdom predominantly in urban areas and that the incidence of infection with G9 strains increased in the first two years in the geographical areas where they were detected to become the second most common strain, followed by a significant decrease in their incidence during the third year [25, 26]. At the time G9 rotaviruses were first found in the United Kingdom in 1995/1996 to 1998/1999 they were not detected in the eastern region of the United Kingdom, which is mostly a rural area. The results may suggest that G9P[8] strains emerged in East Anglia later, and the incidence of infection with these viruses peaked in 2001/2002, decreasing subsequently.

The use of numerical scores for disease severity allows comparisons among studies and of different aetiological agents within studies and allows the whole range of symptoms associated with viral gastroenteritis to be taken into account. Median severity scores decreased with age and this probably reflects the impact of immunity, acquired previously, which modified the illness making it less severe rather than preventing infection and disease. Infections with rotaviruses and noroviruses were associated with higher severity scores than other viral agents and were similar to those described by Pang *et al.* [12]. In a limited study by Roman *et al.*, in which only tests for rotavirus, adenovirus and astrovirus were included, rotavirus infections again had the highest severity score [16].

Severity scores in this study (between 6·5 and 11) and the study of Pang *et al.* (between 5 and 10) [12], indicated mild or moderately severe disease associated with cases of gastroenteritis in the community. The duration of diarrhoea ranged from a median of 5 days for norovirus and astrovirus to 6 days for rotavirus, whereas, in the study of Pang *et al.*, the duration of astrovirus-associated diarrhoea had a median of 1 day, rotavirus 4 days and adenovirus 5 days [12].

There were no significant differences between this study and the study of Pang *et al.* [12] when the number of diarrhoea episodes, the duration of vomiting, or the number of vomiting episodes were compared. In both studies, vomiting was significantly associated with rotavirus infection and both rotavirus

and norovirus infections produced the highest numbers of vomiting episodes in a 24-h period.

Interestingly, children infected with adenovirus were less likely to require rehydration than children infected with any other virus, this may be directly associated with the finding that adenovirus infections were more prevalent in older children. Rehydration therapy is likely to be required more often and be more complicated in children of low weight, which includes the very young and the malnourished.

In comparison to previous studies in which virus detection included EM or EIA and PCR for selected virus targets, the current study used molecular methods only. There was no significant increase in the detection of caliciviruses among studies as molecular methods were used in all. The detection of rotaviruses was enhanced through the use of molecular methods with a significantly higher incidence of rotavirus infection identified in the current study. Also, the ‘diagnostic gap’ was reduced to 31·4% through the use of molecular methods in conjunction with structured surveillance. Interestingly, the median time from illness to the collection of a faecal sample was 5 days and might suggest that duration of illness as well as severity of symptoms are significant causes to seek medical attention. This late sample collection is likely to impact on the ability to detect viral pathogens, and if common, it would suggest that the more sensitive molecular detection methods should be used for diagnosis.

The ability to close further the ‘diagnostic gap’ may involve the improved detection of other enteric pathogens including bacteria and parasites. All the samples in the community and hospital cohorts, and in the first year of the current study, and all structured surveillance samples were submitted for routine enteric bacterial investigation, but no significant non-viral pathogens were found to cause disease in this age group. However, in subsequent years, two children recruited to the structured surveillance reported having been diagnosed with *Campylobacter* infection. Similar studies have found bacteria such as *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia* spp. and verotoxigenic *Escherichia coli* to be the aetiological agent associated with gastroenteritis in a total of 11·9% of cases in this age group [15]. Similarly, parasites such as *Cryptosporidium*, *Giardia* and *Entamoeba* have been associated with 7·7% of infectious enteric disease in young children [15].

Viral pathogens such as toroviruses, kobuviruses and parvoviruses, not sought routinely, may be

associated with a proportion of the undiagnosed cases. Waters *et al.*, detected toroviruses by EM in 3% of cases of community-acquired diarrhoea in children [13]. In the current study, all samples from the structured surveillance cohort were negative for torovirus by RT-PCR (data not shown).

With the exception of adenoviruses, the common gastroenteric viral pathogens have RNA genomes, which during replication have high mutation rates. This results in virus families of wide antigenic and genomic diversity, and it may be difficult to construct a single assay capable of detecting all members of the virus family. Antibody-escape mutants and mutations that result in detection failures in molecular assays have been described for rotaviruses [27–29].

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## DECLARATION OF INTEREST

None.

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## APPENDIX. Questionnaire sent to parents/carers

Community Survey of Rotavirus Gastroenteritis in Children Under 5 Years of Age	
Where possible, <u>circle</u> your response.	
Child's name:	Child's date of birth: ....../.......
Address:.....	
..... Sex of child: <u>Male</u> / <u>Female</u>	
GP name:..... GP practice:.....	
Has your child seen any other nurses or doctors? <u>Yes</u> / <u>No</u>	
If Yes, please give details:.....	
<b>Illness details</b>	
Date when illness started:....../.... Time it started: <u>morning</u> / <u>afternoon</u>	
Did your child have unusually loose or watery stools (diarrhoea)?: <u>Yes</u> / <u>No</u>	
Please record the maximum number of nappies/stools per day:.....	
Has your child been vomiting?: <u>Yes</u> / <u>No</u>	Please record the maximum number of vomiting episodes per day:.....
How many days did your child have symptoms? Diarrhoea:..... Vomiting:.....	
Was the child irritable (unusual crying)?: <u>Yes</u> / <u>No</u>	Was there a change in sleep pattern?: <u>Yes</u> / <u>No</u>
Fever: <u>Yes</u> / <u>No</u>	Highest temperature if taken:..... °C or ..... °F / Unknown
<b>Medical care</b>	
Estimate the <u>number</u> of liquid feeds/day:	1. before illness <input type="checkbox"/> milk <input type="checkbox"/> water/ juice 2. during illness <input type="checkbox"/> <input type="checkbox"/>
Was your child prescribed any medicines by your GP?: <u>Yes</u> / <u>No</u>	
If yes, what: a:..... b:..... c:.....	
Did you buy any medicines from the chemist or supermarket for the child?: <u>Yes</u> / <u>No</u>	
If yes, what: a:..... b:..... c:.....	
For how long did you treat the child?:.....days	
How much have you spent on medicines for your child's illness?: £.....	
GP visits. (Include dates of all GP visits as a result of this illness)	
1 <sup>st</sup> visit: Date:....../....	<u>Home</u> / <u>Surgery</u>
Reason: <u>Vomiting</u> / <u>Diarrhoea</u> / <u>No improvement</u> / <u>Worried</u> / <u>Other</u> (please say what).....	
2 <sup>nd</sup> visit: Date:....../....	<u>Home</u> / <u>Surgery</u>
Reason: <u>Vomiting</u> / <u>Diarrhoea</u> / <u>No improvement</u> / <u>Worried</u> / <u>Other</u> (please say what).....	
3 <sup>rd</sup> visit: Date:....../....	<u>Home</u> / <u>Surgery</u>
Reason: <u>Vomiting</u> / <u>Diarrhoea</u> / <u>No improvement</u> / <u>Worried</u> / <u>Other</u> (please say what).....	
Number of further GP visits as a result of this illness :      Number	
Home <input type="checkbox"/> Surgery <input type="checkbox"/>	

Did your child attend hospital during the illness?: Yes / No  
 If yes, give date(s): ....../.... Department: Accident & Emergency / Out Patients' / Admissions  
 ....../.... Department: Accident & Emergency / Out Patients' / Admissions

Name of hospital:..... Number of days in hospital: .....

Reason for attendance/ admission:.....

### Personal History

Was this child breast-fed: Yes / No      If yes, for how long: .....months  
 Are there any other children under 16 years of age in the household: Yes / No      Number:.....  
 Age child 1: .....months/years      Diarrhoea in the last two weeks: Yes / No      Date:....../....  
 Age child 2: .....months/years      Diarrhoea in the last two weeks: Yes / No      Date:....../....  
 Age child 3: .....months/years      Diarrhoea in the last two weeks: Yes / No      Date:....../....  
 Age child 4: .....months/years      Diarrhoea in the last two weeks: Yes / No      Date:....../....  
 Did anyone else in the house have diarrhoea?: Yes / No Who:..... When:.....

Does your child usually attend : Creche / Nursery / Childminder / School / Other / None  
 If yes to any, then how many days per week?: .....

Does anyone else look after your child on a regular basis?: Yes / No

If yes, then who?: ..... How many days per week?: .....

### Answer the remaining questions after the child has recovered

Did anyone in the family have to take time off work?: Yes / No  
 Mother: Yes / No / Not working      If yes, how many days?: .....

Father: Yes / No / Not working      If yes, how many days?: .....

Other: Yes / No / Not working      If yes, how many days?: .....

Time away from outside childcare: Yes / No / Does not apply      If yes, how many days?: .....

Have you changed any other plans or activities as a result of your child's illness?: Yes / No

If Yes, please specify: .....

### Please feel free to make any other comments:

.....  
.....  
.....

Completed by: ..... Telephone number: ..... Date: ....../....  
 Parent / Guardian / Carer

Thank you for completing this questionnaire.  
 Please return the form in the envelope provided (postage paid).

Information collected in this questionnaire is for research purposes only and data will be processed by computer in accordance with the Data Protection Act, 1990