Abstract

Background: Diarrhoea in children is a common disease; understanding the incidence of causative viruses can aid infection control and vaccine development.

Objectives: Describe the incidence and characteristics of gastroenteric viruses including norovirus genotypes in a paediatric hospital cohort.

Study Design: Norovirus, adenovirus, sapovirus, astrovirus, rotavirus qPCR and norovirus genotyping results for all stool specimens (n = 4,786; 1,393 patients) at a UK paediatric tertiary referral hospital June 2014– July 2015.

Results and Discussion: 24% (329/1393) of patients were positive for a GI virus; the majority were positive for norovirus (44%, 144/329) or adenovirus (44%, 146/329). The overall incidence of rotavirus (2%) is reduced compared to pre-vaccination studies; however the incidence of other GI viruses has not increased. Norovirus infections had a significantly higher virus burden compared to other GI viruses (P ≤0.03); sapovirus infections had the lowest viral burden.

The number of norovirus cases per month did not follow the typical winter seasonal trend of nationally reported outbreaks. The number of cases per month correlates with the number of hospital admissions (R = 0.703, P = 0.011); the number of admissions accounts for 50% of the variability in number of cases per month. The breadth of genotypes seen (48% non-GII.4), suggests a community source for many norovirus infections and has implications for vaccine development.

All GI viruses caused chronic infections, with the majority (50–100%) in immunocompromised patients. Incidence or duration of infection in chronic norovirus infections did not differ between genotypes, suggesting host-mediated susceptibility.

Keywords: Viral gastroenteritis, paediatric, polymerase chain reaction, PCR, norovirus, genotypes

Background

In European children under five years, there are an estimated four episodes of diarrhoea per child per year [1]; norovirus is the leading causative agent in children [2]. Norovirus is highly transmissible thus is associated with outbreaks of diarrhoea and vomiting in enclosed settings such as cruise ships, schools and in particular healthcare institutions. The morbidity associated with infections in immunocompetent individuals is limited, however the financial burden to healthcare settings is considerable; in the UK acute gastroenteritis is estimated to cost £115 million per year, 63% of which is attributable to norovirus [3]. Outbreaks in healthcare settings typically follow a characteristic winter peak, although this is not mirrored in community outbreaks [4].

Norovirus is a single stranded RNA virus belonging to the calicivirus family. The genome is 7.5 kb, comprised of three open reading frames; ORF1, ORF2 and ORF3 coding for a non-structural polyprotein, major and minor capsid proteins, respectively. There are five genogroups (GI –GV) of which GI, GII and to a lesser extend GIV infect humans. Each genogroup is further classified into genotypes; GI.1–9 and GII.1–21 based on capsid and/or polymerase gene sequences [5]. Outbreaks worldwide have been dominated by GII.4 since the mid-1990s [6].

Objectives

We describe the incidence of gastroenteric viruses in a paediatric UK hospital, with extended analysis of norovirus genotypes, seasonality and PCR Ct values. Understanding the molecular epidemiology of viral gastroenteritis in children will contribute to improving infection control practices and vaccine development.

Study Design

Sampled population

Great Ormond Street Hospital is a large tertiary referral paediatric hospital in the UK. The hospital does not have an accident and emergency department therefore acute gastroenteritis is not the primary reason for admission. As part of the infection control screening policy at GOSH, all children are tested for gastrointestinal viruses on admission for inpatient stay, regardless of whether they are symptomatic or asymptomatic. Patients with a positive stool virus are followed up weekly until they become negative, however, patients with underlying immunodeficiency are tested weekly and thereafter at outpatient appointments irrespectively of whether they have previously been positive for a stool virus. Any child who develops gastrointestinal symptoms during their inpatient stay or in outpatients is also tested. Between 1/7/2014 and 30/6/2015 a total of 4,786 stool samples from 1,393 patients (8% outpatients) at Great Ormond Street Hospital for Children, UK, were tested during routine diagnostic analyses for the presence of gastroenteric viruses (Table 1). 1–46 samples were tested per patient (median 1) (Supplementary Figure 1).

PCR results and accompanying clinical data for this study were exported retrospectively from the laboratory information system. Detection of more than one virus during the study period was treated as an independent episode in the analysis.

Detection of gastroenteric viruses by real-time PCR

All stool samples were tested by real-time PCR for the presence of norovirus, rotavirus, adenovirus, astrovirus and sapovirus (supplementary methods).

Norovirus genotyping

The first norovirus positive stool from each patient was genotyped by PCR amplification and capillary sequencing of the capsid shell domain (supplementary methods). Eleven norovirus positive samples had insufficient residual volume for genotyping and were excluded from analysis.

National norovirus genotyping data

The number of norovirus outbreaks reported nationally to Public Health England (PHE) and the proportion of each genotype for these incidences was provided by the Virus Reference Department (VRD), PHE, from their national surveillance data.

Rotavirus vaccine detection

Rotavirus positive specimens were genotyped by the PHE VRD, and GIP8 positives confirmed as vaccine or wildtype by sequencing the genes encoding VP4 and VP7.

Categorisation of patients

The clinical specialty of each patient was assigned based on the clinical specialty of the ward to which they were admitted at the time of specimen collection. Immunodeficiency patients consisted of specialties associated with profound immunodeficiency; bone marrow transplant, oncology, haematology and immunology specialties. Medical patients consisted of respiratory medicine, cardiac medicine, renal medicine, intensive care, neurology, dermatology, rheumatology, ear nose and throat and ophthalmology. It is likely that some patients in the medical category will have some degree of suppressed immunity.

Norovirus infections detected less than 48 hours after admission to hospital were considered positive on admission (POA); detection more than two days after admission was considered a hospital acquired infection (HAI). Since many of the patients at GOSH have complex medical histories, many of them have previously been admitted to local hospitals or had several outpatient visits prior to admission at GOSH, therefore earlier acquisition of infection in another healthcare facility cannot be excluded.

Infections that were detected for longer than one month were considered chronic infections; less than one month were acute.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v23 (supplementary methods).

Results

Prevalence of gastroenteric viruses

Twenty-four percent (329/1393) of all patients tested in the twelve month period were positive for a gastroenteric virus, among which norovirus and adenovirus predominated with 144 and 146 episodes each over the 12 month period, each constituting 44% of all viral gastrointestinal infections (Table 2).

Forty-four of the 329 infections (13%) were mixed infections with more than one virus detected (Table 2). The predominant mixed infections (23 /44, 52%) were norovirus and adenovirus, followed by norovirus and sapovirus (7/44, 16%) and adenovirus and sapovirus (6/44, 14%). Rotavirus was least frequently detected as part of a mixed infection (5/44, 11%). An equal number of mixed infections were from medical and immunocompromised patients; 16/44 (36%) each. Given that only 19% of patients tested were in the 'immunocompromised' category, this suggests mixed infections are likely to be more frequently associated with immune dysfunction.

The median age of patients with a rotavirus infection, 0.7 years, was significantly younger than other infections with a median age of 2–3 years ($P \leq 0.015$, Supplementary Figure 2).

Norovirus infections had a significantly higher virus burden, median Ct 23, compared to other infections ($P \leq 0.03$, Figure 1a). Sapovirus infections had the lowest viral burden; median Ct 35.

Rotavirus vaccine-derived infections

Four rotavirus positive patients had insufficient residual specimen for genotyping, thus 29 of 33 samples were genotyped. 28% (8/29) of rotavirus infections were identified as vaccine strain.

Prevalence of norovirus genotypes

Eighty-seven percent (117/133) of norovirus infections were genogroup II (GII), which had a significantly higher virus burden (median Ct 22) compared to genogroup I (GI) infections (median Ct 28)(P = 0.004, Figure 1b).

The majority of norovirus infections were GII.4 and GII.3; 52% and 26%, respectively (63/133 and 32/133), with the remaining 22% (38/133) identified as GI.1, GI.2, GI.3, GI.4, GII.1, GII.2, GII.6 or GII.17 (Figure 2). Eleven samples (8%) could not be amplified by PCR; these had a significantly lower viral burden compared to other samples (median Ct 35 and 22 for failed and successful typing, respectively, P ≤ 0.001).

Norovirus seasonality

The proportion of norovirus genotypes each month in our paediatric population is not the same as those seen in nationally reported outbreaks, primarily attributable to the increased proportion of GII.3 in our population (Figure 3). A peak in incidence of GI.3 in nationally reported outbreaks from August to November 2014 is followed by a similar peak in our paediatric population from September to December 2014. Conversely, a peak in GII.6 episodes in our population from March to June 2015 is not seen in nationally reported outbreaks (Figure 3).

The overall number of norovirus cases per month in our population does not follow the typical winter peak seen in national outbreaks (Figure 3). Instead it was noted that in our population the number of cases of norovirus per month follows a similar trend to the number of hospital admissions, including outpatient visits and transfer between wards (Figure 4). There is a significant positive correlation between the number of admissions and number of norovirus cases per month (R = 0.703, P = 0.011). This suggests that the number of hospital admissions accounts for 50% of the variability in number of norovirus cases (R^2 = 0.494). Based on the Poisson regression coefficient ($y = -1.447 + 0.001x$) it is estimated that one case of norovirus occurs for every 100 admissions (95% CI 0.000–0.002, P = 0.002).

Seasonality of other viruses

Similarly to norovirus, adenovirus showed a summer peak in new infections. Conversely rotavirus infections had a spring peak, sapovirus peaked in winter and spring and astrovirus showed no distinct seasonal trends (Supplementary Figure 3).

Hospital and community acquired norovirus infections

Infections acquired before admission (POA) include a greater range of genotypes, with hospital acquired infections (HAI) showing a higher proportion of GII.4 infections (40% and 68%, respectively; Supplementary Figure 4); however this difference is not significant (P = 0.062).

Norovirus in clinical specialties

The incidence of norovirus infection is higher in immunocompromised compared to surgical or medical patients; 19% (51/270) of immunocompromised patients tested were found to be norovirus positive compared to 5% (10/202) and 7% (57/803) of surgical and medical patients.

There was no significant difference in the norovirus PCR Ct values between immunocompromised and non-immunocompromised patients (median Ct 23 and 24, respectively; P=0.226).

Chronic infections

Norovirus had the highest rate of chronic infections (38/144, 25%); adenovirus, rotavirus and sapovirus had similar rates whilst astrovirus had the fewest (1/18, 6%) (Table 2, Supplementary Figure 5a). With the exception of sapovirus, in which chronic infections occurred equally in immunocompromised and medical clinical specialties, the majority (67–100%) of chronic infections were in patients from immunocompromised clinical specialties (Supplementary Figure 5b).

There was no difference in proportion of chronic patients between the different norovirus genotypes (Supplementary Figure 6, P = 0.801). The median duration of infection in chronically infected patients was 5 months (range 1–21 months).

Discussion

We present the incidence of viral gastrointestinal infections and the prevalence of norovirus genotypes in a large cohort of 1,393 paediatric patients in a tertiary referral hospital over a 12 month period, which is dominated by norovirus and adenovirus infections. This is similar to previous reports of UK hospitalised children in which norovirus and adenovirus were detected in 15–16% and 14–15% of cases, respectively[7].

Following the introduction of the rotavirus vaccine to the UK childhood vaccination programme in July 2013 the incidence of rotavirus infections has reduced by 67%[8], which is reflected in the low incidence of 2% reported in this study; an earlier study of hospitalised UK children reported a 31% rotavirus positive rate[7]. Whilst the rate of rotavirus positive patients is reduced compared to the pre-vaccination UK study [7] the rate of detection of norovirus, adenovirus, sapovirus and astrovirus is similar; consequently the overall positivity rate for gastrointestinal viruses is lower than previously reported; 23% in this study compared to 53% reported previously[7]. This suggests that other gastrointestinal viruses have not increased in prevalence to replace rotavirus infections.

Unexpectedly, the overall incidence of norovirus does not follow the characteristic seasonal trend seen in national outbreaks. Instead the number of infections per month strongly correlates with the number of hospital admissions, accounting for 50% of the variability in norovirus incidence. Our results suggest that the incidence of norovirus in a tertiary children's hospital is driven by traffic through the hospital, rather seasonal outbreaks. The breadth of genotypes seen in this study, more commonly seen in community cohorts compared to hospitals, backs this hypothesis; patients presenting to primary healthcare facilities, such as GP practices, reportedly have a lower proportion of GII.4 infections; 54% compared to 91% of hospital infections are GII.4 [9]. The true distribution of norovirus genotypes in the community is not known since all genotyping studies to date are based on patients presenting to healthcare facilities thus introducing a presentation bias. Genotyping of

184 norovirus infections in unbiased community cohorts is needed in order to determine whether
185 infections caused by a breadth of genotypes are a true reflection of the community.

186 In our cohort a quarter (26%) of norovirus infections were caused by GII.3, which has previously
187 been described in varying proportions in UK paediatric cohorts, from 0–20%[10]. This is different to
188 adult cohorts, in which outbreaks are largely dominated by GII.4[9]. Our data supports the notion
189 that GII.3 is more frequently associated with children; however the reason for this is unknown. We
190 speculate the reason could be immunity to GII.3 in the adult population following childhood
191 infection, differences in receptor binding between children and adults or lower transmissibility
192 compared to GII.4 resulting in fewer associated outbreaks and reporting bias.

193 All patients in this study have been categorised by clinical specialty; this was based on the ward to
194 which they were admitted at the time of specimen collection. It is clear that immunocompromised
195 patients are over-represented among patients with norovirus infection; 19% of
196 immunocompromised patients were found to be norovirus positive, compared to just 5% and 7% of
197 surgical and medical patients, respectively. Previous studies in smaller cohorts of 47 and 116
198 immunocompromised patients have reported incidence of norovirus as 23% and 22%, respectively
199 [11, 12] which suggests the categorisation of patients into clinical specialties in this study is reliable
200 and that our larger cohort of 270 immunocompromised patients corroborates earlier findings.

201 Norovirus, adenovirus, rotavirus and sapovirus show a similar rate of chronic infections (15–26% PCR
202 positive >1 month), with the highest rate in norovirus and the lowest (6%) in astrovirus; the vast
203 majority of chronic infections were in immunocompromised patients. Chronic norovirus infections in
204 immunocompromised patients is a recognised cause of morbidity, in whom a bi-phasic illness
205 develops [13] with an initial acute phase followed by a second chronic phase with viral shedding and
206 diarrhoea lasting weeks to years. The consequence of chronic norovirus infection can be
207 dehydration, malnutrition, dysfunction of intestinal barrier [14], dramatic weight loss [15], a
208 requirement for nutritional support [16] and, in extreme cases, death [15, 17]. Immunocompromised

patients in this study do not show a higher norovirus viral burden or difference in genotypes; suggesting the higher chronicity in immunocompromised patients is host, not virus, mediated.

The linear relationship between viral load and PCR Ct value makes Ct values a good semi-quantitative indicator of viral burden, with a difference of 3 Ct values equating to a log difference in viral load[18]. However, despite efforts to standardise stool volume in RNA extraction, differences in stool consistency make the input variable which may falsely indicate a higher or lower viral burden when comparing samples. Consequently small differences in viral burden, such as a two-fold difference estimated by a difference in Ct value of 1, are unlikely to be reliable when comparing stool samples. However major differences in viral burden, such as a log, are likely to be reliable since the input volume, whilst variable, is not expected to vary by such extremes.

We report the incidence of gastroenteric viruses in a large observational cohort of paediatric patients in a UK hospital following the implementation of routine rotavirus vaccination, showing rotavirus incidence of just 2%. All viruses are shown to establish chronicity, primarily in immunocompromised patients. We observe that new infections are not driven by seasonal trends, which may be specific to our population but has been reported in community cohorts[4]. The high proportion of non-GII.4 infections may have implications for vaccine development.

Conflict of Interest Declarations

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Competing Interests

The authors declare no competing interests

Ethical Approval

This study was approved by the NRES Committee London - Brent (REC reference 14/LO/1331).

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Figure 3(b) was reproduced, with permission, from the Public Health England (PHE) norovirus surveillance report. Rotavirus vaccine/wildtype typing was undertaken by the PHE Virus Reference Department.

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