A major decrease in viral acute gastroenteritis in hospitalized Finnish children as rotavirus returns as the most detected pathogen

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A R T I C L E   I N F O

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A B S T R A C T

Introduction: This study was performed to assess the prevalence and circulating genotypes of rotavirus, norovirus, and sapovirus in children. The results were compared to those of previous surveillance studies covering the years 2006–2008, 2009–2011, and 2012–2014 with similar methodology and setting, encompassing the start of universal vaccination with RotaTeq in 2009.

Methods: Stool samples were collected from children aged 0–16 years with acute gastroenteritis at Tampere University Hospital, Finland, from January 1, 2017 to December 31, 2018. The samples were analysed using reverse transcription PCR and positive amplicons were sequenced.

Results: A total of 178 stool samples were collected from 214 children. Rotavirus was detected in 56 (32%) stool samples, norovirus in 48 (27%), and sapovirus in 11 (6.3%). Rotavirus G9P[8] and G12P[8] were the most detected genotypes in vaccinated and unvaccinated children. GI.4 comprised 96% of the norovirus detections.

Conclusions: The prevalence of all-cause acute gastroenteritis in a hospital setting decreased by 51% compared to 2012–2014, and by 88% compared to 2006–2008. Rotavirus returned as the most common cause of viral acute gastroenteritis in children, but the prevalence remains at a low level. No considerable changes were seen in the genotyping results of norovirus and sapovirus.

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1. Introduction

Globally, diarrhoea is the second-most common cause of death among children from 1 month to 5 years of age (Liu et al., 2016). Rotavirus infection is a common cause of diarrhoea in infants and young children, and before the licensure of rotavirus vaccines it was estimated to cause 527 000 deaths annually in children under 5 years old (Parashar et al., 2009). To date, 95 countries have included a rotavirus vaccine in their national immunization programme (Soares-Weiser et al., 2019), but rotavirus still remains one of the leading causes of death in children of young age globally, causing 128 000 deaths in 2016 (GBD 2016 Diarrhoeal Disease Collaborators, 2018).

Following rotavirus mass immunization, two human caliciviruses – norovirus and sapovirus – have become the leading causes of viral acute gastroenteritis and related hospitalizations, with norovirus present in 21–34% and sapovirus in 4–23% of cases (Hemming-Harlo et al., 2016; Heusinkveld et al., 2016; Payne et al., 2013; Platts-Mills et al., 2018). Previous observations strongly suggest that rotavirus vaccination does not reduce the absolute numbers of norovirus and sapovirus gastroenteritis (Rucardo et al., 2014; Hemming et al., 2013). Noroviruses present a single globally dominant genotype, GI.4, that shows the chronological emergence of viral variants, but a similar phenomenon has not been reported in sapoviruses (Tohma et al., 2020). Regarding both norovirus and sapovirus, the human immune response is known to be very genotype-specific (Blazevic et al., 2015; Sanchez et al., 2018).

RotaTeq (Merck & Co., Kenilworth, NJ, USA) was introduced into the Finnish National Immunization Programme in 2009 and is given in a three-dose schedule at the ages of 2, 3, and 5 months, and the current coverage is 92–93% (The Finnish Institute for Health and Welfare, 2021). In comparison to the pre-vaccination years, rotavirus detections were reduced by 90%, and a major 94% reduction in wild-type rotavirus gastroenteritis cases was also seen among children too old to be vaccinated.
In Finnish children, norovirus has become the leading cause of severe acute gastroenteritis, accounting for 29–34% of cases seen in the hospital, followed by sapovirus at 5.5% (Hemming-Harlo et al., 2016; Pitkänen et al., 2019).

Several changes in the epidemiology of circulating rotavirus genotypes have been reported since the introduction of large-scale rotavirus vaccination. The previously predominant rotavirus genotypes appear to gradually subside and the previously less detected or undetected strains, e.g. G9P[8] and G12P[8], emerge. Yet, the vaccines have been shown to retain their high efficacy against rotavirus acute gastroenteritis (Leshem et al., 2014; Markkula et al., 2020a).

The aim of this study was to examine the current epidemiological position of rotavirus and human calciviruses in children treated as outpatients or admitted to hospital with acute gastroenteritis. Further, the clinical appearance and the genetic development of the circulating pathogens were investigated.

2. Materials and methods

2.1. Clinical methods

A prospective study following the regulations of the Declaration of Helsinki was approved by the Ethics Committee of Pirkkamaa Hospital District, Finland, and was conducted at Tampere University Hospital, Finland, from January 1, 2017 to December 31, 2018. All children under 16 years of age who visited the emergency room (ER) or were admitted to a paediatric ward with symptoms of acute gastroenteritis were eligible for the study. A case of acute gastroenteritis was defined as the occurrence of ≥3 diarrheal stools or ≥2 episodes of vomiting, or one episode of both diarrhea and vomiting in the last 24 hours, and the symptoms had lasted a maximum of 7 days before admission. Subsequent hospital visits from a single participant were considered to be separate if there were seven or more symptomless days between the visits. The Vesikari score (Ruuksa and Vesikari, 1990) was used to analyse the severity of the gastroenteritis cases. Children with chronic illnesses that present symptoms of acute gastroenteritis were excluded. Informed written consent was obtained from the legal guardian of each participant and a stool sample was collected from every patient eligible for the study. If a stool sample could not be collected during the hospital visit, a home-sample kit was provided to send a stool specimen within the following week. The vaccination status of the children was confirmed from the records of the child welfare clinics.

This study is the fourth follow-up study on acute gastroenteritis in children conducted in the same setting using a similar methodology. The first studies were performed in the seasons 2006–2008 and 2009–2011, before and after the introduction of national rotavirus vaccination in Finland (Hemming et al., 2013; Räsänen et al., 2011). The results of the present study were compared to those of the previous prospective surveys covering the years 2006–2008 (Räsänen et al., 2011) and 2012–2014 (Hemming-Harlo et al., 2016).

2.2. Laboratory methods

After collection, the stool samples were stored at −70°C until examination with a previously used methodology and settings (Hemming et al., 2013; Pitkänen et al., 2019). Briefly, reverse transcription PCR was used as the primary detection method with modified rotavirus primer mixtures targeting the rotavirus viral protein (VP) 7 and VP4 genome segments and primer mixtures targeting the conserved RNA-dependent RNA polymerase region of the calcivirus genome. The VP6 genome segments of the rotavirus-positive samples were analysed to determine the presence of vaccine-derived virus (Hemming and Vesikari, 2012). The positive amplicons were sequenced using the same primers. The amplicons were purified using the Qiaqen QiAgel Extraction Kit (Qiagen, Hilden, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, MA, USA) on an ABI 3500XL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Sequences were analysed with Sequencher 4.10.1 (Gene Codes Corp. Inc., Ann Arbor, MI, USA) and compared to reference strains from the GenBank nucleotide database (National Center for Biotechnology Information, Bethesda, MD, USA) and NoroNet, using the Basic Local Alignment Search Tool and the Norovirus Automated Genotyping Tool (Kroneman et al., 2011), respectively. The patients were stratified by age group as determined by the World Health Organization (2008).

2.3. Statistical methods

IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. All tests were two-tailed and P-values below 0.05 were considered as statistically significant. The Pearson Chi-square test, Kruskal–Wallis test, and Fisher's exact test were used to calculate statistical differences in clinical data, as applicable.

3. Results

A total of 214 patients were recruited, from whom 178 stool samples were collected: 104 (58%) were collected in 2017 and 74 (42%) in 2018. Clinical data were available for 175 (98%) cases, forming the final study population. The median age of the children was 2 years 2 months (range 1 month to 14 years 5 months); 52% were female and 48% were male. Ninety-three children (53%) were admitted to a ward and 82 children (47%) were treated in the ER. Two patients were also treated in the intensive care unit, one positive for norovirus and the other for rotavirus. Overall, 132 children (75%) had received at least one dose of RotaTeq.

One hundred and seven cases (61%) tested positive for one or more of the investigated pathogens. Rotavirus was the most common pathogen during the study period and was detected in 56 (32%) samples, followed by norovirus in 48 (27%) samples and sapovirus in 11 (6.3%). Rotavirus and sapovirus were most often detected in children over 60 months of age and norovirus in children aged from 24 to 59 months. The age distribution of the children positive for the investigated viruses is depicted in Figure 1. According to the clinical data, all but one patient with acute gastroenteritis had vomiting, whereas fever was seen mostly in rotavirus infections. The children with rotavirus infection were also significantly older on average. The clinical presentation of sapovirus infections was milder than those of norovirus and rotavirus infections, as depicted in Table 1.

Rotavirus and norovirus showed distinct seasonality with higher prevalence during the winter months, while the number of sapovirus was low overall (Figure 2).

3.1. Rotavirus

Rotavirus was detected in 34% (n = 34) of the samples in 2017 and 30% (n = 22) in 2018; females made up 50% of the cases. Thirty-three children (59%) were treated in a paediatric ward (Figure 1). Of all rotavirus infections, seven (13%) were coinfections: four with norovirus, two with sapovirus, and one with both norovirus and sapovirus. In 28 (50%) cases, the patient had been vaccinated with one or more doses of RotaTeq; two had re-
ceived just the first dose, one had received two doses, and 25 were fully vaccinated.

G9P[8] was the predominant genotype with 31 (55%) detections, followed by nine (16%) cases caused by G12P[8]. The other genotypes were detected more sporadically: five (9%) cases of G9P[4], four (7%) cases of G3P[8], three (5%) cases of G1P[8], two (4%) cases of G2P[4], and one (2%) case of G8P[8]. Two nosocomial infections caused by G3P[8] and G9P[4] were also detected. In one case, a mono-infection with vaccine-derived G1 was detected in a stool sample of a 2-month-old infant, who had received the first dose of RotaTeq 3 weeks prior to hospitalization due to acute gastroenteritis. There was no association between the vaccination status of the children and the specific rotavirus strains (P = 0.60). G9P[8] and G12P[8] were the most detected genotypes in both the vaccinated and unvaccinated children, and were also equally common in both groups, with 16 vs 15 cases and 4 vs 5 cases, respectively.

### 3.2. Norovirus

Norovirus was detected in 29% (n = 29) of samples during the first study year (2017) and 26% (n = 19) of samples during the second year (2018). A clear seasonality was seen (Figure 2). Norovirus was the leading cause of acute gastroenteritis in children aged from 6 months to 5 years, with most detections between 12 and 24 months of age (Figure 1). Twenty-seven (56%) patients were admitted to a paediatric ward and 48% of the patients were female. There were four mixed infections with rotavirus and one with rotavirus and sapovirus, and no nosocomial infections were detected. The median duration of the symptoms was the longest of the examined pathogens.

Genogroup GII (46 cases, 96%) comprised almost all of the norovirus detections. GII.4 was the most common capsid genotype with 36 (75%) cases, and genotype 2012 Sydney was present in all 33 GII.4 sequences that could be further assigned. Other capsid genotypes detected were GII.2 (three cases), GII.3 (six cases), GII.6,
GI.2, and GI.6 (one case each). The most detected P-genotypes were GII.16 (35%) and GII.21 (27%). The most common strains were GII.4 Sydney[P16] and GII.4 Sydney[P31] with 12 cases each, followed by GII.4 Sydney[P4 New Orleans] with six cases. More detailed results are shown in the Supplementary Material (Table S1).

3.3. Sapovirus

There were three sapovirus detections in the first year of the study and eight detections in the second year, and the cases appeared during the winter and spring months (Figure 2). Of those who were positive, 82% were female. Five patients (45%) were admitted to the ward, where no nosocomial infections were detected. The positive cases were not notably associated with any age group and there were no sapovirus detections in children aged between 3 years and 5 years.

In 2017, all three detections of sapovirus presented genogroup GI. In 2018, GII was the leading genogroup, further divided into genotypes GII.1 and GII.3. The three mixed infections were associated with sapovirus genogroup I.

4. Discussion

In this fourth consecutive 2-year hospital-based epidemiological study, the prevalence of rotavirus, norovirus, and sapovirus in acute gastroenteritis in children was examined using a similar study setup to the three previous surveillance studies covering the years 2006–2008 (Räsänen et al., 2011), 2009–2011 (Hemming et al., 2013), and 2012–2014 (Hemming-Harlo et al., 2016), expanding the follow-up of viral acute gastroenteritis in children to 12 years in total and covering the introduction of national rotavirus vaccination. The all-cause acute gastroenteritis prevalence decreased by 51% compared to the post-vaccination seasons of 2012–2014, and by 88% compared to pre-vaccination seasons of 2006–2008. In contrast to the previous surveillance studies, a slight resurgence in the number of wild-type rotavirus was observed, and rotavirus returned as the most detected cause of viral acute gastroenteritis in children under 16 years of age. The prevalence of norovirus in all stool samples was 27% and similar to 29% in 2012–2014. Six percent of stool samples were positive for sapovirus, similar to the seasons 2012–2013 and 2013–2014, with 3% and 5%, respectively (Hemming-Harlo et al., 2016).

The overall proportion of rotavirus-positive samples increased from 12% in 2012–2014 to 32% in the present study, with an increase of 40% in the absolute number of detected cases. This is directly because of the increase in detected wild-type rotavirus cases, from 4.2% to 21% in vaccinated children and from 27% to 63% in unvaccinated children with gastroenteritis between seasons 2012–2014 and 2017–2018 (Hemming-Harlo et al., 2016). The vaccine coverage has remained at 92–93% over the past 6 years (The Finnish Institute for Health and Welfare, 2021), thus not explaining the detected increase. With the high vaccine coverage, wild-type rotavirus continues to circulate at a low level. An evident shift in the circulating rotavirus genotypes was seen, as the three most detected genotypes, G9P[8] (55% vs 8%), G12P[8] (16% vs 5%), and G9P[4] (9% vs not detected), notably increased in proportion when compared to 2012–2014, whereas the predominant genotype from the previous study, G4P[8] (28%), was not detected in this study. In Finland, all rotavirus-positive samples nationally are collected for further genotyping. Data from the database with similar detection methods to ours found that the G12P[8] strain was predominant during 2017–2018, with G9P[8] and a novel G9P[4] becoming increasingly common. Similar to the present findings, G12P[8] and G9P[8] were the predominant strains in the unvaccinated children (Markkula et al., 2020a). It has been documented that G9 and G12 capsid proteins show increased numbers of antigenic parts that differ from those of all the vaccine components (Ogden et al., 2018), which could explain the detected resurgence.

Viral shedding is very common after the administration of the oral vaccine (Markkula et al., 2015). However, vaccine-type rotavirus gastroenteritis cases remain rarely seen in the hospital, as shown in our results and supported by a national study, where the detection rate of RotaTeq vaccine-derived viruses was very low, accounting for <1% (6/827) of cases (Markkula et al. 2020a). In the only case detected in the present study population, it is likely that the symptoms were caused by another pathogen(s) because of the relatively long 3-week period between the oral vaccination and the hospitalization. The overall role of vaccine strains in symptomatic rotavirus gastroenteritis has remained unclear (Markkula et al. 2020b). In the previous surveillance study by (Hemming-Harlo et al., 2016), a significant difference was found in the rotavirus genotype distribution between the vaccinated and unvaccinated children, but similar results were not seen in this study. A further reduction in the vaccine genotypes in the unvaccinated
nated population due to herd immunity is a possible explanation, and also possibly the emergence of novel rotavirus strains in both groups.

It was noted that the children with rotavirus infections were older than the children with norovirus or sapovirus infections. Such a shift in the occurrence of rotavirus gastroenteritis towards the older age groups after vaccine introduction has been reported previously (Markkula et al., 2017; Aliabadi et al., 2019) and might be due to the continuous high vaccine coverage starting at the age of 6–8 weeks.

In 2012–2014 (Hemming-Harlo et al., 2016), norovirus was the major causative agent of viral acute gastroenteritis in children, and similar results have been reported from other countries with high rotavirus vaccine coverage (Halasa et al., 2021; Quee et al., 2020; Halasa et al., 2021). Compared to the results from the years 2012–2014, we observed a major decline from 100 norovirus–positive cases to 46 cases. However, due to the simultaneous decrease in the number of overall cases, the proportion of norovirus infections remained at the same level. Norovirus remained as the most detected of the examined pathogens in children aged from 6 months to 5 years, and genotype GII.4 still accounted for the vast majority of cases. As no significant change was detected in the circulating norovirus genotypes, it is plausible that acquired immunity suppresses the otherwise widespread epidemics (Rouhani et al., 2016). Notably, the proportion of norovirus–positive cases admitted to a hospital ward increased by 15 percentage points from 41% in 2012–2014. In this study, the median age of norovirus gastroenteritis patients was 20 months compared to 27 months in 2012–2014, thus possibly explaining the detected shift (Hemming-Harlo et al., 2016). In 2012–2014 and 2017–2018, the complete Vesikari score was available for 27/41 (66%) and 19/24 (79%) hospitalized cases, with a mean severity score of 13.0 (range 6–19) and 12.9 (range 7–16), respectively, therefore not explaining the difference.

Sapovirus remained sporadically detected with no obvious seasonality. The clinical data showed that by the time of admission, the sapovirus infections appeared to be as severe as the rotavirus and norovirus infections, presenting less diarrhoeal symptoms but requiring invasive rehydration therapy just as often, according to the evaluating clinicians. Nevertheless, the median duration of symptoms was evidently the shortest. The white blood cell count appeared to be higher in these patients; however, this was caused by two highly severe cases within a small number of total infections. Multiple different sapovirus genotypes were detected, but as described previously (Pitkänen et al., 2019), only genogroups GI and GII were present. The primers used in this study are sensitive for the detection of sapovirus but are known to target a conserved region of the partial RNA-dependent RNA polymerase sequence in the sapovirus genome and therefore set a limitation for further conclusions of the circulating genotypes (Hansman et al., 2005).

The small overall number of positive cases could add weight to the occasional outbreaks in institutional settings concerning all examined viruses. The study hospital is the paediatric referral centre for approximately 550 000 people, and the results from a national database study (Markkula et al., 2020a) indicate that the present results could well reflect the situation at the national level. As seen in the low number of cases treated only in the ER, due to the strong role of primary health care centres in emergency services, it is likely that most of the mild to moderate cases of acute gastroenteritis are not seen in the hospital setting, especially cases in older children. Outside health centre office hours, all patients are directed to visit the ER, but the overall impacts of this were not studied. Stool samples were collected mainly by the time of admission and based on symptoms regardless of the primary reason for hospitalization. Data associated with disease of different severities were collected, and the main reason for not completing the clinical data was due to loss to follow-up. PCR was used for initial virus testing, which is more sensitive than enzyme immunoassays used in many setups.

In conclusion, there was a notable decrease in the total number of children hospitalized for acute gastroenteritis. An increase of 40% in the absolute number of rotavirus gastroenteritis cases was detected, and rotavirus returned as the most detected of the pathogens investigated. An increase in rotavirus gastroenteritis cases in older children was not observed, indicating that the vaccine-induced protection remains at a good level for years after vaccination.

Declarations

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval: The study followed the regulations of the Declaration of Helsinki and was approved by the Ethics Committee of Päikšnamma Hospital District, Finland.

Conflict of interest: Oskari Pitkänen and Jukka Markkula have no conflicts of interest to declare. Maria Hemming-Harlo has received speaker’s fees or research support from MSD Finland and is a deputy member of the Finnish National Advisory Committee on Vaccines.

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Supplementary materials


REFERENCES


