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Astrovirus and norovirus infections and their association with diarrheal symptoms in immunocompromised children

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ABSTRACT

Human astroviruses are a common cause of viral gastroenteritis and are often underdiagnosed. While infections are typically mild or asymptomatic in immunocompetent hosts, astrovirus can cause prolonged and severe disease in immunocompromised individuals. Here, we conducted a case-control study to evaluate the clinical presentation of astrovirus infections in paediatric oncology patients. Our findings were compared to two groups: (1) norovirus infections in the same immunocompromised cohort, and (2) a cohort of immunocompetent children with and without diarrheal symptoms. Astrovirus was detected in 9.8% of immunocompromised patients, with prolonged shedding seen in multiple cases. Astrovirus infection was associated with an increased odds of diarrhoea, although this association weakened when adjusting for co-infections. In contrast, norovirus was not significantly associated with diarrhoea in immunocompromised patients, despite prolonged shedding in 24% of the cohort. High rates of co-infection with adenovirus and *Clostridium difficile* may have influenced symptom patterns. In the immunocompetent cohort, astrovirus was not associated with diarrhoea, while norovirus showed a strong association with symptoms. These findings indicate that astrovirus infections are more likely to cause diarrhoea in immunocompromised paediatric patients, while often asymptomatic in immunocompetent children. Norovirus showed the opposite trend, highlighting distinct roles of host immune status in shaping clinical outcomes. These findings highlight the importance of considering astrovirus as a potential contributor to gastrointestinal symptoms in immunocompromised children and call for broader diagnostic testing and further research on viral co-infections and persistence.

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KEYWORDS Human astrovirus; norovirus; diarrhoea; enteric viral infections; immunocompromised children; viral co-infection; gastroenteritis

Introduction

Astroviruses, non-enveloped positive-sense RNA viruses, are a leading cause of viral gastroenteritis worldwide. With 90% of children developing antibodies to at least one human astrovirus (HAstV) genotype by age 5, these infections are among the most common in early childhood [1-3]. Despite their prevalence, HAstV infections remain underdiagnosed due to their typically mild, self-resolving symptoms and inadequate diagnostic testing that fail to detect the complete range of known HAstV strains [4] although tests are improving [5].

Astroviruses are associated with a spectrum of symptoms, and their co-detection with other pathogens likely contributes to disease outcomes. Data indicates that HAstV infections can remain subclinical in a large proportion of cases [6,7]. In fact, similar rates of HAstV infection in symptomatic and asymptomatic individuals have been observed across the globe, including in Brazil, Kenya & the Gambia, and Nigeria [8-10]. Growing evidence indicates that astrovirus infections often occur as co-infections with other enteric pathogens, particularly with rotavirus, but has also been reported with norovirus, adenovirus, and sapovirus [11-18]. Co-infections occur in 41% to 77% of HAstV cases [11-13] and are associated with increased disease severity, including higher rates of emesis and more severe gastroenteritis [19-21] compared to HAstV infections alone.

Astrovirus infections present risks to immunocompromised individuals. Similar to norovirus, which causes prolonged illness [22,23] and extended viral shedding [24,25] in immunocompromised hosts, astrovirus can be shed for over 100 days [6] and

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progress to severe infections leading with systemic spread [6,26]. In immunocompromised hosts, astroviruses have been detected in multiple extraintestinal sites [27-29], including a concerning propensity for neuroinvasion of the central nervous system ([21,27] Reuter, 2018 #103, [30-34]). The wide spectrum of symptoms, from asymptomatic to severe, underpins the importance of understanding HAstV epidemiology in immunocompromised populations. Paediatric cancer patients already experience high diarrhoea rates (up to 44-50%) [35,36] due to various factors including other infections, medications, and post-transplant graft-versus-host disease [35,37,38]. In most cases, no definitive aetiology is established, and the consequences can include higher complication rates, poorer overall quality of life, and potential for delayed treatment plans [38,39].

Enteric infections can present with or without symptoms, and there is clinical concern that these infections can lead to severe and/or prolonged diarrhoea within high-risk groups. Based on this, the present study examined the symptomatology of HAstV infections in paediatric oncology patients using a case–control study design. We additionally placed the results in the context of two comparison studies: (1) a case–control study of norovirus infections in the same immunocompromised population, serving as a well-characterized enteric viral comparison group, and (2) a retrospective cohort study of HAstV and norovirus infections identified in non-hospitalized immunocompetent children with and without diarrhoea symptoms. This approach allows us to distinguish the unique clinical presentation of astrovirus in the context of immunosuppression. Overall, this study highlights the potential role of astrovirus in contributing to diarrheal symptoms in paediatric oncology patients, with implications for diagnosis and management in high-risk paediatric populations.

Materials and methods

Patient cohort and samples

Stool samples were collected from immunocompromised paediatric patients at St Jude Children's Research Hospital (Memphis, TN, USA) under three IRB-approved protocols with consent from parents (and when age-appropriate, assent from children) (Supplementary Figure 1). All remnant samples were de-identified and designated as non-human subject research by the St Jude IRB. Samples from the following studies were used for these studies: ALLGUT (2012–2016) [40], which provided 513 stool samples from acute lymphoblastic leukaemia patients collected at predetermined timepoints throughout treatment regardless of symptoms; EPIGUT (2015–2018) which contributed 1,349 samples from patients (1) undergoing haematopoietic stem cell transplants collected at predetermined timepoints in the year after transplant regardless of symptoms [41] and (2) at the onset of diarrheal symptoms; and FAECIS (2012–2018), which supplied 2,782 leftover diagnostic samples from immunocompromised paediatric patients that were ordered for clinical evaluation, which included but was not limited to diarrheal symptoms or suspected gastrointestinal graft-versus-host disease. For comparison, de-identified leftover stool samples from healthy children and those presenting to emergency room with diarrhoea collected from previous IRB approved research studies (2011–2016) at Seattle Children's Hospital and designated as non-human subject research by the St. Jude's IRB were included as an immunocompetent control group. An independent reviewer examined patient medical records to document infection onset, demographics, diagnoses, co-infections, and gastrointestinal symptoms (including fever, vomiting, and diarrhoea).

Molecular detection of astrovirus and norovirus

Stool samples obtained from patients were diluted and homogenised in 1x Dulbecco's phosphate-buffered saline (PBS) to a final concentration of 10-20% (w/v). Viral RNA was extracted from stool samples using MagMAX AI/ND Viral RNA Isolation Kit (Applied Biosystems), according to the manufacturer's protocol, in 96-well plates loaded onto a KingFisher Flex Magnetic Particle Processor. Norovirus screening was included as a comparative control virus because, like astrovirus, its clinical presentation is well-characterized in immunocompetent hosts but less understood in immunocompromised populations. This comparison helps contextualize astrovirus findings and determine whether observed patterns are specific to astrovirus or common to enteric viruses in immunocompromised patients. Infection was defined as having at least 1 or more time points positive for astrovirus or norovirus regardless of any symptoms. A positive infection was based on quantitative real-time PCR assay with primers specific to conserved regions of the canonical (HAstV1-8) and noncanonical (MLB1 and VA2) astrovirus genotypes (4) or GI- and GII-norovirus genotypes [42]. Two positive controls and two negative controls were present on each 96-well plate. Samples that presented a cycle threshold (Ct) value of 38 or less to either virus were considered positive and further analyzed to determine precise viral genotype.

Sequencing and genotyping

Samples that were identified as positive to astrovirus infection were subsequently amplified by hemi-nested end-point PCR using primers specific to the open reading frame (ORF) 1 [43] and ORF2 [44]. Norovirus-positive samples were further amplified using a

set of primers specific to ORF2 (corresponding to VP1 capsid region D) that amplify viruses from both norovirus GI and GII genogroups [45]. Amplicons were run on a 2% agarose gel and appropriate bands (422 bp for astrovirus, 177 bp for norovirus GI, 253 bp for norovirus GII) were excised and purified using QIAquick Gel Extraction Kit and submitted for Sanger sequencing. To further resolve the length of a single infection, sequencing information was used to compare across samples. If the same virus was detected, then sequential samples were not counted as new infections.

Statistical analysis

Descriptive statistics were used to report medians with ranges, as well as means with standard deviations for continuous variables. To compare the difference of medians among groups, the non-parametric Wilcoxon rank sum test was utilized. Frequency tables were generated to summarize categorical data. The Chi-square test was performed to evaluate the relationship between two categorical variables, except when the expected count in table cells was less than 5, in which case Fisher's exact test was implemented. Logistic regressions were used to predict the probability of categorical outcome measures based on the values of risk factors of interest. Statistical significance was determined at the 5% level for two-sided *P*-values. All analyses were conducted in SAS version 9.4 (SAS Institute, Inc).

Results

Immunocompromised cohort description

Three cohorts of patients in treatment at St. Jude Children's Research Hospital between January 2012 and April 2018 were retrospectively tested for HAstV and norovirus infections. The population of our study included 1,140 immunocompromised patients (494 females, 646 males). A total of 4,644 faecal samples were collected over the 6-year period with a patient age range of 0.14–29.04 years old at the time of sample collection (mean 9.15 years, median 8.02 years).

We identified an overall positivity rate of 3.51% (163/4,644 samples) for HAstV and 4.01% (186/4,644 samples) for norovirus. For HAstV infections, nearly 10% of our study's population (9.74%, 111/1,140 patients) was identified as having at least 1 HAstV infection during their treatment. Of these 111, 8 patients developed 2 sequential HAstV infections and 1 patient was identified as experiencing 3 sequential HAstV infections. In total, this accounted for 121 distinct HAstV infections among 111 patients. Long-term shedding, defined as detection beyond 14 days after the initial positive sample, was identified in 3 patients, with a shedding duration of 36, 47, and 137 days. Of the 121 HAstV infections identified within our population, we were able to identify genotypes for 78 infections (Figure 1(a)). Classical astrovirus HAstV serotype 1 (HAstV1) was the most prevalent with 47 infections among 45 patients, with 2 patients experiencing 2 separate infections with HAstV1. In the additional 31 astrovirus infections, HAstV serotypes 2-5, MLB 1 and 2, as well as VA2 infections were detected.

We next found that 10.9% of our study population (124/1,140) screened positive for at least one norovirus infection. This included 24 patients infected with norovirus GI, 95 with norovirus GII, and 5 who tested positive for both. Among the 5 patients infected with both genogroups, 3 were simultaneously positive for both genogroups, while 2 patients experienced an initial infection with norovirus GI, followed by a later infection with norovirus GII. In the norovirus GII subgroup, 2 patients experienced 2 separate infections during their treatment. Long-term shedding of norovirus GII, defined as detection beyond 14 days after the initial positive sample, was observed in 23 patients (range 14-418; median shed duration 63 days). Sequencing analysis of norovirus GII identified genotypes for 32 samples, with norovirus II genotype 4 being the most prevalent within the cohort (n = 14, Figure 1(b)).

Case–control study population

To examine whether HAstV and norovirus infections were associated with diarrheal symptoms, we utilized a case–control study. The analysis was performed on 124 HAstV-positive samples from 111 immunocompromised patients, compared with 124 HAstV-negative samples that were specifically selected to match samples from our patient population. Only one sample per infection was included in the analysis. Multiple infections from the same patient were included if they met criteria for distinct infections, defined as either having an astrovirus-negative sample between two positive samples or at least 14 days between two positive samples. Each patient was matched based on age group (0–5, 5–10, 10–15, >20), diagnosis, and reason for faecal collection (i.e. clinical, diarrheal episode, or interval). Astrovirus cases and control patient characteristics are summarized in Table 1. Overall, we noted effective matching between cases and controls, with no significant differences in age group, diagnosis, or reason for faecal collection. We additionally modelled age as a continuous variable and found that there was no significant difference between the cases and controls (Wilcoxon rank sum test, $p = 0.937$). However, patients co-infected with other gastrointestinal pathogens and co-infected with non – gastrointestinal pathogens overall were more frequently detected in cases compared to controls (Chi-square test, $p = 0.0017$ for gastrointestinal; $p = 0.0002$ for co-infection).

The total number of infections with norovirus GII vastly outnumbered infections with norovirus GI,

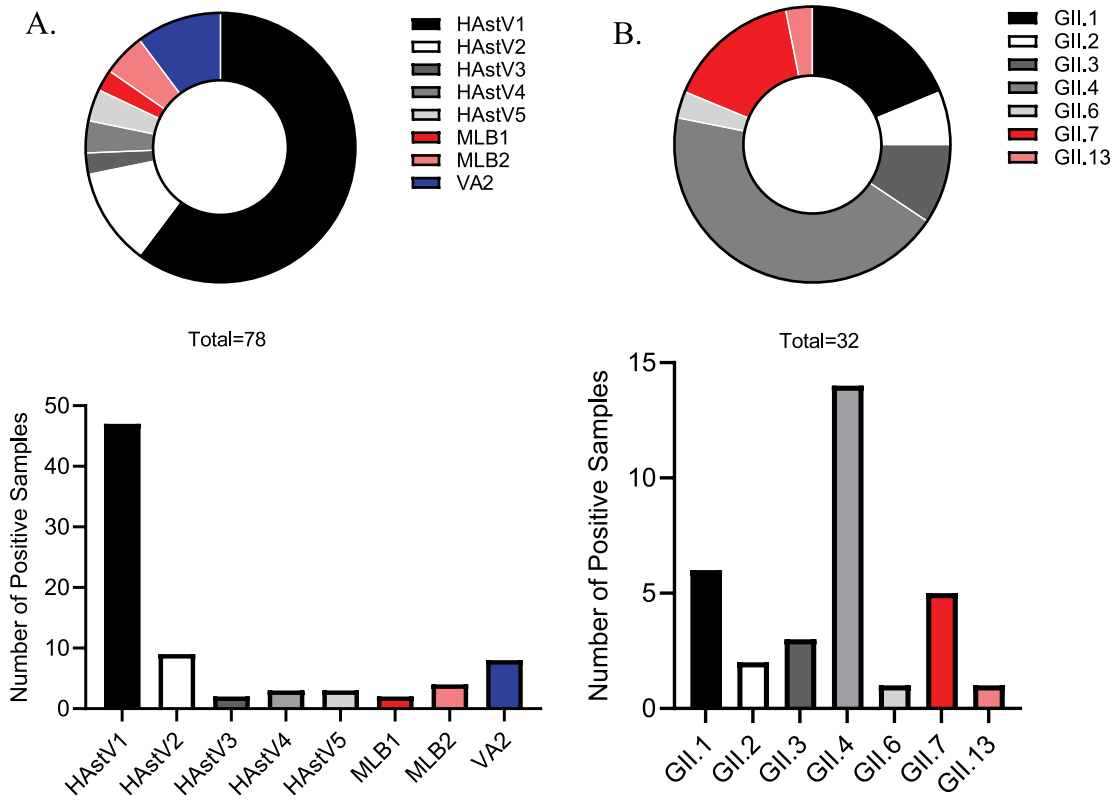

Figure 1. Detection of viral genotypes within patient samples. Patient stool samples were screened for (a) astrovirus and (b) norovirus using primers specific to ORF1 and ORF2 of the astrovirus genome and the ORF2 of the norovirus genome. Sanger sequencing was performed on positive samples to identify viral genotype.

reflecting previously reported trends in norovirus epidemiology [46,47]. Due to the larger sample size in norovirus GII, we compared the 106 norovirus-positive samples from 102 patients alongside 106 norovirus-negative samples using the same matching scheme applied in the astrovirus analysis. Norovirus cases and control patient characteristics are summarized in Table 2. Overall, we noted effective matching between cases and controls, with no significant differences in age group, diagnosis, reason for faecal collection, gastrointestinal infection, or co-infection. Unlike HAstV, there was no significant difference between the cases and controls in any of the parameters.

Symptoms associated with astrovirus and norovirus infections

We next examined whether diarrhoea, fever, or vomiting were associated with HAstV infection in immunocompromised patients. We found that diarrhoea was the most frequently observed symptom reported in 46.22% (n = 55) of cases, while 53.78% (n = 64) of HAstV-infected patients did not experience diarrhoea. Fever was present in 28.81% (n = 34) of cases, and vomiting occurred in 22.41% (n = 26). Chi-square analysis showed that neither fever nor vomiting was significantly associated with HAstV infection but showed significant association between diarrhoea with HAstV infection (Table 3). Additionally, we performed a logistic regression analysis and found that the odds of HAstV cases had a 1.74-fold increase in diarrheal symptoms as compared to controls (95% CI: 1.04–2.93, $p = 0.037$). However, when adjusted for the presence of gastrointestinal infections, there was an attenuated association (OR = 1.56, 95% CI: 0.91-2.65, $p = 0.104$). In addition, when adjusted for the presence of all co-infections, we observed a further attenuated association (OR = 1.51, 95% CI: 0.88-2.59, $p = 0.140$). Indeed, we found that among astrovirus-positive samples that did not have gastrointestinal co-infections, 34 (38.63%) had diarrhoea versus 54 (61.36%) that did not have diarrhoea. This was significantly different among the samples with co-detected gastrointestinal infections (Chi-square analysis, $p = 0.0052$), which had twice as many samples (n = 21, 67.74%) that were associated with diarrhoea as compared to the 10 samples (32.25%) that did not have diarrhoea. There was no association between detectable gastrointestinal infections and symptom status in the astrovirus-negative analysis (Supplementary Table 1). When comparing Ct value as a proxy for viral load, there was no significant difference between patients with and without diarrheal symptoms (Median: 27.36 vs. 29.71, Wilcoxon rank sum, $p = 0.2153$).

For norovirus infections, we focused only on diarrheal symptoms, which were reported in 44.55% (n = 45) of norovirus GII-positive cases (Table 4). Regardless of whether we examined all cases or the subset without GI

Table 1. Astrovirus case-control characteristics.

Variable	HAstV-positive samples (n = 124)	HAstV-negative controls (n = 124)	Total (N = 248)	*p*-value
Age				0.9990*
0–5	52 (41.94%)	54 (43.55%)	106	
5–10	28 (22.58%)	27 (21.77%)	55	
10–15	28 (22.58%)	28 (22.58%)	56	
15–20	14 (11.29%)	13 (10.48%)	27	
>20	2 (1.61%)	2 (1.61%)	4	
Sex				0.4422*
F	51 (41.13%)	57 (45.97%)	108	
M	73 (58.87%)	67 (54.03%)	140	
Diagnosis				0.8867**
Brain tumour	5 (4.03%)	7 (5.65%)	12	
Hematologic disorder	4 (3.23%)	5 (4.03%)	9	
Hematologic malignancy	94 (75.81%)	89 (71.77%)	183	
Inherited immunodeficiency	2 (1.61%)	4 (3.23%)	6	
Solid tumour	19 (15.32%)	19 (15.32%)	38	
Collection type				0.8070*
Clinical	68 (54.84%)	63 (50.81%)	131	
Diarrheal episode	6 (4.84%)	6 (4.84%)	12	
Interval	50 (40.32%)	55 (44.35%)	105	
Gastrointestinal infections				0.0691**
Adenovirus	10 (31.25%)	7 (53.85%)	17 (37.78%)	
C.diff	13 (40.63%)	3 (23.08%)	16 (35.56%)	
C.diff, Adenovirus	4 (12.5%)	0 (0%)	4 (8.89%)	
C.diff, Adenovirus, Norovirus	1 (3.13%)	0 (0%)	1 (2.22%)	
C.diff, Norovirus	1 (3.13%)	0 (0%)	1 (2.22%)	
Norovirus	3 (9.38%)	0 (0%)	3 (6.67%)	
Rotavirus	0 (0%)	1 (7.69%)	1 (2.22%)	
Sapovirus	0 (0%)	2 (15.38%)	2 (4.44%)	
Gastrointestinal co-infection				0.0017*
Yes	32 (25.81%)	13 (10.48%)	45 (18.15%)	
No	92 (74.19%)	111 (89.52%)	203 (81.85%)	
Co-infection[a]				0.0002*
Yes	46 (37.1%)	20 (16.13%)	66 (26.61%)	
No	78 (62.9%)	104 (83.87%)	182 (73.39%)	

*p-value is for Chi-Square test. *P*-value less than 0.05 provides evidence of a statistically significant association between two categorical variables.

**p-value is for Fisher's Exact test when some cells have expected counts less than 5. *P*-value less than 0.05 provides evidence of a statistically significant association between two categorical variables.

[a]Encompasses all gastrointestinal and non-gastrointestinal co-infections that included Human polyomavirus (BK virus), Human Herpesvirus, Epstein-Barr Virus, Human Metapneumovirus, Vancomycin-resistant enterococci (VRE), Human Rhinovirus, Cytomegalovirus.

co-infections, we found no significant association between diarrhoea and norovirus (Table 4, Supplementary Table 2). We performed logistic regression analysis and similarly found no significant association between norovirus GII infection and diarrhoea either in the presence (Table 4) or absence (Supplementary Table 2) of coinfections (OR = 0.87, 95% CI: 0.51-1.54, $p = 0.666$). Additionally, we did not observe a significant difference of Ct value between patients with and without diarrheal symptoms (Median: 23.44 vs. 26.99, Wilcoxon rank sum, $p = 0.1523$). Overall, these combined results indicate that HAstV and norovirus infections often present without clear clinical symptoms in immunocompromised paediatric patients, highlighting the necessity for careful diagnostic.

Retrospective analysis of diarrhoea associated with astrovirus and norovirus infection in children from the general population

To compare our findings within the context of the general population of non-hospitalized children, we examined a dataset collected from patients with diarrheal symptoms (n = 85) and patients without diarrheal symptoms (n = 77) who visited an emergency department (ED) in Seattle, Washington from 2011 to 2015 (Table 5). The median age of children with diarrhoea was significantly higher than children without diarrhoea (8.67 versus 3.5, Wilcoxon rank sum, $p < 0.0001$). Using logistic regression analysis, the odds of having a HAstV infection were not significantly different in children with diarrhoea as compared to children without diarrhoea (OR = 0.74, 95% CI: 0.22-2.53, $p = 0.629$). However, when using Ct value as a proxy for viral load, we observed a lower average Ct (22.92) in children with diarrhoea in comparison to children without diarrhoea (32.67), but this did not reach statistical significance (Wilcoxon rank sum, $p = 0.055$). In contrast, the odds of having a norovirus infection were 10.28 higher in children with diarrhoea in comparison to children without diarrhoea (95% CI: 2.96-35.69, $p = 0.0002$). The average Ct for norovirus detected in children with diarrhoea (23.76) was higher than in children without diarrhoea (28.79), but this did not reach statistical significance (Wilcoxon rank sum, $p = 0.236$). Overall, these data indicate there is a strong association between diarrhoea and norovirus, but not HAstV infection in non-hospitalized children.

Table 2. Norovirus case-control patient characteristics.

Variable	Norovirus-positive samples (n = 106)	Norovirus-negative controls (n = 106)	Total	*p*-value
Age				0.8216*
0–5	53 (49.07%)	55 (50.93%)	108	
5–10	21 (52.5%)	19 (47.5%)	40	
10–15	13 (56.52%)	10 (43.48%)	23	
15–20	16 (50%)	16 (50%)	32	
>20	3 (33.33%)	6 (66.67%)	9	
Sex				0.5816*
F	51 (52.04%)	47 (47.96%)	98	
M	55 (48.25%)	59 (51.75%)	114	
Diagnosis[#]				0.8784**
Brain Tumour	5 (41.67%)	7 (58.33%)	12	
Hematologic Disorder	2 (50%)	2 (50%)	4	
Hematologic Malignancy	76 (51.01%)	73 (48.99%)	149	
Infectious Disease	1 (25%)	3 (75%)	4	
Inherited Immunodeficiency	1 (100%)	0 (0%)	1	
Solid Tumour	20 (48.78%)	21 (51.22%)	41	
Collection type				0.4386*
Clinical	75 (48.39%)	80 (48.09%)	155	
Diarrheal episode	0 (0%)	0 (0%)	0	
Interval	31 (54.39%)	26 (45.61%)	57	
Gastrointestinal Infections				0.1857**
Adenovirus	2 (20%)	4 (33.33%)	17 (37.78%)	
Astrovirus	0 (0%)	1 (8.33%)	16 (35.56%)	
C.diff	3 (30%)	5 (41.67%)	4 (8.89%)	
C.diff, Adenovirus	1 (10%)	0 (0%)	1 (2.22%)	
C.diff, Astrovirus	1 (10%)	0 (0%)	1 (2.22%)	
Norovirus GI	3 (30%)	0 (0%)	3 (6.67%)	
Rotavirus	0 (0%)	1 (8.33%)	1 (2.22%)	
Sapovirus	0 (0%)	1 (8.33%)	2 (4.44%)	
Gastrointestinal co-infection				0.6524*
Yes	10 (9.43%)	12 (11.32%)	22 (10.38%)	
No	96 (90.57%)	94 (88.68%)	190 (89.62%)	
Co-infection[a]				0.0882*
Yes	12 (11.32%)	21 (19.81%)	33 (15.57%)	
No	94 (88.68%)	85 (80.19%)	179 (84.43%)	

*p-value is for Chi-Square test. P-value less than 0.05 provides evidence of a statistically significant association between two categorical variables.

** p-value is for Fisher's Exact test when some cells have expected counts less than 5. P-value less than 0.05 provides evidence of a statistically significant association between two categorical variables.

[#]Diagnosis data was not obtained from one patient

[a]Encompasses all gastrointestinal and non-gastrointestinal infections that included Human polyomavirus (BK virus), Human Herpesvirus, Epstein-Barr Virus, Human Metapneumovirus, Vancomycin-resistant enterococci (VRE), Human Rhinovirus, Cytomegalovirus.

Discussion

There are few published studies on symptom prevalence in hospitalized children with enteric viral infections, resulting in limited understanding of clinical presentation and the impact of co-infections on disease severity [48-51]. The findings presented here addresses knowledge gaps regarding enteric viral infections in immunocompromised paediatric patients. Nearly 10% of the immunocompromised children experienced at least one HAstV infection during treatment, with long-term viral shedding identified in three patients. Similarly, norovirus infections were detected in 10.9% of patients, with 23 patients exhibiting prolonged shedding, consistent with previous reports in immunocompromised hosts [49,50]. These findings highlight the high frequency of enteric viral infections in young immunocompromised hosts, potentially contributing to more severe and prolonged disease [6,52].

Case–control analysis revealed that patients with HAstV infections were 1.74-fold more likely to present with diarrhoea compared to matched controls.

Table 3. Symptoms associated with astrovirus infection.

Variable[#]	HAstV-positive samples (n = 124)	HAstV-negative controls (n = 124)	Total	*p*-value*
Fever				
Yes	34 (28.81%)	27 (21.77%)	61	0.2074
No	84 (71.19%)	97 (78.23%)	181	
Vomit				
Yes	26 (22.41%)	32 (25.81%)	58	0.5395
No	90 (77.59%)	92 (74.19%)	182	
Diarrhoea				
Yes	55 (46.22%)	41 (33.06%)	96	0.036
No	64 (53.78%)	83 (66.94%)	147	

*p-value is for Chi-Square test. P-value less than 0.05 provides evidence of a statistically significant association between two categorical variables.
[#]Symptom data was not available for all patients.

Table 4. Symptoms associated with norovirus infection.

Variable[#]	Norovirus-positive samples (n = 106)	Norovirus-negative controls (n = 106)	Total	*p*-value*
Diarrhoea				
Yes	45 (44.55%)	49 (47.57%)	94	0.6654
No	56 (55.45%)	54 (52.43%)	110	

*p-value is for Chi-Square test. P-value less than 0.05 provides evidence of a statistically significant association between two categorical variables.
[#]Symptom data was not available for all patients.

Table 5. Enteric infection associated with diarrhoea in children visiting an ER.

Variable	Diarrhoea (n = 85)	No diarrhoea (n = 77)	Total	*p*-value*
Astrovirus				
Yes	5 (45.45%)	6 (54.55%)	11	0.629
No	80 (52.98%)	71 (47.02%)	151	
Norovirus				
Yes	25 (89.29%)	3 (10.71%)	28	**<0.0001**
No	60 (44.78%)	74 (55.22%)	134	

*p-value is for Wilcoxon rank sum. P-value less than 0.05 provides evidence of a statistically significant association.

However, this association weakened when adjusted for co-infections, suggesting that complex factors beyond astrovirus infections alone may contribute to symptom severity. It is notable that co-infections with *Clostridium difficile* (*C. diff*) were especially prominent in our patient cohort (Table 1), which on its own could be a significant driver of symptoms. Alternatively, it is possible that we are observing a synergistic effect that warrants additional study. Consistent with this idea, previous reports indicate that co-infections, particularly with rotavirus or norovirus, can exacerbate gastrointestinal symptoms [53,54].

Surprisingly, norovirus infections were not significantly associated with diarrhoea in our immunocompromised case–control analysis, contrasting with established findings in immunocompetent populations and our healthy cohort data [50,55]. The high prevalence of co-infections with adenovirus and *C. diff* in our immunocompromised cohort may have masked typical norovirus symptoms. Alternatively, the prolonged viral shedding observed in 24% of norovirus GII-infected patients may represent low-grade, asymptomatic infection, rather than active disease.

Our comparison with immunocompetent children provides important contrast for these findings: HAstV infections were not significantly associated with diarrhoea in the general paediatric population, while norovirus infections strongly correlated with diarrheal symptoms. This suggests that the host immune status may determine disease severity with astrovirus infections as they are often asymptomatic in immunocompetent children and associated with diarrhoea in immunocompromised children [6,56]. The inclusion of norovirus as a comparison reveals distinct differences in how these enteric viruses present in immunocompromised versus immunocompetent hosts. While norovirus showed strong association with diarrhoea in immunocompetent children but not in our immunocompromised cohort, HAstV showed the opposite pattern – more strongly associated with symptoms in immunocompromised patients. This reversed pattern suggests that the host immune response plays a crucial but potentially different role in the clinical manifestation of these two viral infections.

Several limitations and caveats must be acknowledged in interpreting our findings. First, small sample size for certain HAstV genotypes limited our ability to draw conclusions about strain-specific disease associations. Second, our study design likely underestimated asymptomatic infections, since samples were predominantly collected for clinical purposes rather than surveillance, potentially missing mild cases and affecting prevalence estimates. Third, we could not fully account for the temporal relationship between infection detection and symptom onset, complicating determining directionality of causal associations. Fourth, while we documented co-infections, the complex interactions between very diverse pathogens (which may contain some that are additive in terms of symptomology or only infect patients of the same exposure history or immunodeficiencies) and their collective impact on disease presentation were difficult to fully characterize within this dataset.

Future research should incorporate larger cohorts with prospective sampling at predefined intervals to provide a more accurate assessment of asymptomatic infections and shedding dynamics. Longitudinal studies tracking viral load, immune markers and detailed symptom profiles would enhance our understanding of infection progression and prevention in this vulnerable population. Additionally, metagenomic approaches examining the entire virome could provide deeper insights into how pathogen interactions influence disease manifestation in immunocompromised hosts.

Our findings emphasize the complexity of enteric viral infections in paediatric oncology patients. Astrovirus infections, while often asymptomatic in immunocompetent individuals, increase diarrhoea risk in immunocompromised hosts, particularly with co-infections. Norovirus, despite its known role in gastroenteritis, showed an unexpectedly weak association with diarrhoea in this cohort, warranting further investigation into the clinical impact of prolonged viral shedding in this population. This study highlights the need for comprehensive research into viral co-infections, host immune status, and viral persistence to inform better clinical management and treatment strategies for these vulnerable patients.

Author contribution

Conceptualization, V.C., S.S.C.; methodology, A.E.D., Y.S., V.C., E.M.; formal analysis, Y.S., L.T., V.C.; investigation, A.E.D., P.F., R.D., R.H.; resources, J.A.E., E.J.K, R.D., H.H., R.H., G.M.; data curation, A.E.D., Y.S., R.D.; writing – original draft preparation, A.E.D., Y.S., E.M., V.C.; writing – review and editing, all authors; visualization, A.E.D., Y.S.; supervision, E.M., L.T., V.C., S.S.C.; project administration, P.F., J.A.E., E.J.K., R.D., H.H., R.H., G.M., S.S.C.; funding acquisition, V.C., S.S.C.

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