Epidemiology of human astroviruses among children younger than 5 years: Prospective hospital-based sentinel surveillance in South Africa, 2009-2014

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ABSTRACT

Background: The epidemiology of human astroviruses (HAstVs) in hospitalised patients <5 years of age from selected sites in South Africa (SA) was investigated. Diarrheagenic stool specimens collected from April 2009 to May 2014 were screened retrospectively for selected viruses, bacteria and parasites.

Method: Patient data were analysed to identify epidemiologic factors most frequently detected with HAstV infections. The following case-comparisons were investigated; HAstV-positive and HAstV-negative children, human immunodeficiency virus (HIV)-infected and HIV-uninfected (HAstV-positive) children and HIV-exposed and unexposed (HAstV-positive HIV-uninfected) children.

Results: Astrovirus was identified in 7.0% (234/3 340) of cases and most frequently in ages 7 to 12 months (9.2%, 90/975) compared with 5.8%-6.6% in other 6-month age groups. No seasonal trends were observed. More HAstVs were detected in children from homes that used outdoor water sources (7.6%) compared to indoor sources (5.7%, aOR1.5, 95% CI 1.1-2.1, p=0.009). Astroviruses were detected in 8.4% (67/799) of HIV-uninfected patients that were exposed to HIV compared with 5.9% (74/1 257) of HIV-unexposed patients (p=0.032).

Conclusion: Astroviruses were most prevalent in children aged 7 to 12 months and were detected throughout the study period. The study was limited as only hospitalised patients were investigated and no comparisons were made to diarrhoea-free control groups. Future HAstV surveillance should include community-based studies and children presenting at outpatient facilities.

KEY WORDS:
Human astrovirus (HAstV), South Africa (SA), epidemiology, human immunodeficiency virus (HIV); **childhood diarrhoea**

1. Introduction

Human astroviruses (HAstVs) were first identified as a viral cause of human diarrhoea in 1975[1] and have since been recognised as the second leading agent of acute viral gastroenteritis among out-patients <5 years in Georgia, United States of America (USA).[2] Diarrhoeal infections associated with HAstVs have mostly been reported in children <5 years, elderly persons[3-4] and among immunocompromised persons of all ages.[5-7] Recent reviews of the global epidemiology of HAstVs have shown a shift in strains circulating, with the potential for HAstVs to cause disseminated and complicated infections.[3,7-8] The evolving epidemiology of HAstVs and its role in human disease needs clarity and continued surveillance.

The transmission of HAstVs is mainly from person-to-person through the faecal-oral route[4] and outbreaks due to contaminated food and water sources have been reported.[3,9] Clinically, HAstV-associated diarrhoea in children presents similarly to rotavirus (RV) and norovirus (NoV) infections but with lesser severity.[3,10] Astroviruses have been detected in asymptomatic children[11] and death due to protracted diarrhoea[12] and disseminated disease has been reported.[13]

Classic HAstVs are distributed worldwide and are associated with 2.9% to 5.0% of acute diarrhoea in children.[3,7] In the MAL-ED birth cohort study that investigated community acquired diarrhoea in children from South America, Africa and Asia, the adjusted attributable fraction (AF) for HAstVs in age group 0 to 11 months was 2.7% and in ages 12 to 24 months, 4.2%. In both age groups, *Campylobacter* spp., norovirus GII (NoV GII) and RV preceded HAstVs as the foremost causes of diarrhoea.[14] A multi-centre study in USA of children <5 years of age, detected HAstVs in 4.9% (38/782) of diarrhoeal cases compared with 3.0% (15/499) in healthy controls ($p<0.001$).[15]

Many countries on the African continent have identified HAstVs in children with diarrhoea. In Mali, 0.8% to 3.2% of HAstV cases were detected in children aged 59 months and younger.[16] Varied prevalences have been reported from Kenya (6.3%, 30/476),[17] Ghana (3.3%, 12/367),[18] Egypt (6.3%, 23/364),[19] Nigeria (40.4%, 65/161),[20] Burkina Faso (2.1%, 1/48),[21] Kenya and The Gambia (9.9%, 94/949)[11] and Tunisia (4%, 2/50).[22]

Data on HAstV-associated diarrhoea in human immunodeficiency virus (HIV)-infected individuals are limited.[6,23-24] An investigation of enteric opportunistic infections in HIV–infected adults showed that HAstVs were detected more frequently in persons with diarrhoea

(12.0%) than in persons without diarrhoea (2%; p=0.003).[5] In HIV-infected children, HAstVs infections were associated with diarrhoea and an increase in the severity of clinical symptoms.[6,23] Astrovirus infections in all persons with compromised immune systems are important due to the potential for extra-intestinal pathogenesis.[24-25]

The first report of HAstVs in Johannesburg, South Africa (SA) was in 1979. The virus was identified by electron microscopy (EM) in stool specimens of a 6 month old infant with gastroenteritis.[26] In 1997, HAstVs were detected co-infecting with RV and adenovirus (HAdV) in a multi-pathogen outbreak of gastroenteritis at a child care centre in Pretoria.[27] The prevalence of HAstVs has been investigated in selected areas of SA; namely Tshwane[28-30] and Johannesburg[31] but to date no multi-regional investigations have been carried out.

2. Objective

We investigated the epidemiology of HAstVs in paediatric patients aged <5 years that were hospitalised with diarrhoea, from four sentinel sites in SA. The objective of this investigation was to describe the clinical features and environmental factors identified most frequently in HAstV-positive children as compared with HAstV-negative children.

3. Study design

3.1 Study sites and population

Stool specimens collected between April 2009 and May 2014 as part of the Rotavirus Sentinel Surveillance Program (RSSP),[32] were tested retrospectively for the presence of HAstVs. Four hospital sites were selected; namely Chris Hani Baragwanath Academic Hospital (CHBAH) in Gauteng Province, Mapulaneng Hospital (MPH) and Matikwane Hospital (MKH) in Mpumalanga Province and Edendale Hospital (EDH) in Kwa-Zulu Natal Province.

Surveillance officers systematically screened all hospital admissions for children meeting the surveillance case definition. Acute diarrhoea was defined as “having three or more looser stools than normal in the past 24 hours with a duration of less than seven days” as per definition by the World Health Organization (WHO). The demographic and clinical information for each patient was obtained from parent or guardian interviews and the patient medical records.

3.2 Samples and specimens

Stool specimens and inoculated Cary & Blair bacterial transport media (Diagnostic Media Products [DMP], Sandringham, SA) were collected within 48 hours of admission and transported to the Centre for Enteric Diseases (CED), National Institute for Communicable Diseases (NICD), Johannesburg, SA for testing. When the patients' HIV status was unknown and if consent given, dried blood spots were collected for HIV screening. The HIV test screened for HIV-1 Total Nucleic Acid by real-time PCR using the Cobas® TaqMan® instrument (Roche) and the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test Kit v2.0 (Roche). As per the RSSP protocol, all specimens were anonymised.

3.3 Nucleic acid extraction and pathogen screening

Nucleic acid was extracted from 160 µl of 10% clarified stool suspensions prepared in nuclease-free water using the QIAamp Viral RNA Mini QIAcube Kit (Qiagen Inc., Valencia, CA) on the QIAcube instrument (Qiagen) as per manufacturer's instructions. Complementary deoxyribonucleic acid (cDNA) was made using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany) incorporating random primers (Roche). Real-time PCR was performed for HAstV detection using 5 µl cDNA in the LightCycler ® ProbeMaster kit (Roche) and primers and probe mix as previously described.[33] The nucleic acid was screened for HAdV, norovirus GI (NoV GI), NoV GII, sapovirus (SaV) and RV as previously described.[34]

The inoculated Cary & Blair media was sub-cultured on bacterial growth media and colonies grown were tested for *Escherichia coli* (*E coli)*, *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. on the VITEK-2 compact system (BioMérieux, Marcy L'Étoile, France). Bacteria identified by the Vitek (BioMérieux) were further characterised by PCRs.[34] When available a stool specimen aliquot was tested for *Ascaris lumbricoides*, *Entamoeba coli*, *Giardia lamblia*, *Cryptosporidium* spp. and *Isospora belli*.[34]

3.4 Data analysis

The clinical and demographic characteristics and surrounding environmental factors were compared between HAstV-positive and HAstV-negative cases. Within the HAstV-positive cases, HIV-infected patients were compared with HIV-uninfected patients. Among HIV-uninfected patients that were HAstV-positive, the differences between HIV-exposed and unexposed cases were investigated. When a patients' HIV status or HIV-exposure was unknown, the record was excluded from further analyses.

Patients who were HIV-uninfected, and born to a HIV-infected mother were defined as HIV-exposed HIV-uninfected (HEU). The mother's HIV status result was determined by an enzyme-linked immunosorbent assay (ELISA) or from information recorded during the interview. Patients that were unexposed were HIV-uninfected and their mothers were HIV-uninfected.

Chi-square and Wilcoxon rank-sum tests compared means and medians respectively. Bivariate and stepwise multivariable logistic regression analyses identified characteristics most commonly detected with HAstV infections. Variables were compared between patients comprising the selected groups by bivariate pairwise analysis and resulting p-values ≤0.2 were included in the multivariate regression model using the forward selection method. Odds ratios (OR) were calculated for differences within groups and p-values <0.05 were considered statistically significant. The selected patient information and laboratory test results were analysed using STATA v11 (StataCorp LP, College Station TX, USA). Specimens with missing information were not included in the analysis for that variable in the bivariate analysis. As a result of missing data, certain variables were not included in the multivariate analysis, even though the p-value was ≤ 0.2 in the bivariate analysis.

Stool specimens were defined as fully screened if the full panel of viruses, bacteria and parasite testing was completed. Partially screened referred to specimens that were only tested for viruses and did not have results for either bacteria or parasite testing. Only fully screened specimens were included for the analysis of HAstVs presenting as single or co-infections with other pathogens. For all other clinical, demographic and environmental investigations, fully and partially screened specimens were included.

A modified Vesikari scoring system[35] was used to calculate the clinical severity of diarrhoeal infections using the clinical features recorded. Diarrhoeal cases were not followed until the resolution of symptoms and thus no value for the duration of symptoms was available. Using the modified Vesikari method, scores of less than 10 points were graded mild to moderate diarrhoea while scores of 11 or more marked severe diarrhoea. All available specimens from hospitalised patients were included in the analyses irrespective of the Vesikari score assigned.

Table 1: Bivariate and multivariable analysis of epidemiologic characteristics associated with astrovirus detection

Parameter	Astrovirus prevalence _n/N (%)	Bivariate analysis		Multivariate analysis	
		Odds Ratio (OR; 95% Confidence Interval(95%CI))	p-value	Adjusted OR (aOR; 95%CI)	p-value
Demographic characteristics					
Site					
CHBAH	142/1 909 (7.4%)	Ref.		Ref.	
MPH	21/385 (5.5%)	0.7 (0.5-1.2)	0.169	0.7 (0.4-1.1)	0.163
MTK	46/714 (6.4%)	0.9 (0.6-1.2)	0.379	0.9 (0.6-1.2)	0.364
EDH	25/332 (7.5%)	1.0 (0.7-1.6)	0.953	1.0 (0.7-1.6)	0.876
Collection year					
2009 (April to December)	27/635 (4.3%)	Ref.		Ref.	
2010	98/921 (10.6%)	2.7 (1.7-4.2)	<0.001	2.7 (1.7-4.3)	<0.001
2011	29/552 (5.3%)	1.2 (0.7-2.1)	0.418	1.3 (0.7-2.2)	0.364
2012	21/460 (4.6%)	1.1 (0.6-1.9)	0.803	1.1 (0.6-2.0)	0.755
2013	29/535 (5.4%)	1.3 (0.8-2.2)	0.352	1.3 (0.8-2.3)	0.313
2014 (January to May)	30/237 (12.7%)	3.3 (1.9-5.6)	<0.001	2.8 (1.5-5.1)	0.001
Age in months					
0 to 6	75/1 270 (5.9%)	Ref.		Ref.	
7 to 12	90/975 (9.2%)	1.6 (1.2-2.2)	0.003	1.7 (1.2-2.4)	0.001
13 to 18	33/501 (6.6%)	1.1 (0.7-1.7)	0.590	1.1 (0.7-1.7)	0.686
19 to 24	15/260 (5.8%)	1.0 (0.6-1.7)	0.932	0.9 (0.5-1.7)	0.728
≥25	21/333 (6.3%)	1.1 (0.7-1.8)	0.784	1.1 (0.7-1.9)	0.626
Clinical characteristics					
Fed after four months					
Breast	32/629 (5.1%)	Ref.		Ref.	
Breast + other	140/1 850 (7.6%)	1.5 (1.0-2.3)	0.036	1.4 (1.0-2.0)	0.080
Dehydration					
None	23/421 (5.5%)	Ref.		Ref.	
1 - 5% (Mild)	131/1 772 (7.4%)	1.4 (0.9-2.2)	0.165	1.5 (0.8-2.6)	0.182
≥6% (Moderate/severe)	45/702 (6.4%)	1.2 (0.7-2.0)	0.520	1.3 (0.7-2.3)	0.461
Maximum vomits per day					
1 per day	23/434 (5.3%)	Ref.		Ref.	
2 to 4 per day	109/1 506 (7.2%)	1.4 (0.9-2.2)	0.159	0.3 (0.8-2.1)	0.303
≥5 per day	19/260 (7.3%)	1.4 (0.8-2.6)	0.285	1.3 (0.7-2.4)	0.478
Severity scores based on Vesikari scale					
≤10	103/1 617 (6.4%)	Ref.		Ref.	
≥11	131/1 722 (7.6%)	1.2 (0.9-1.6)	0.162	1.2 (0.9-1.5)	0.269
Environmental features					
Water source					
Indoor	87/1 517 (5.7%)	Ref.		Ref.	
Outdoor	134/1 753 (7.6%)	1.4 (1.0-1.8)	0.031	1.4 (1.0-1.8)	0.033

CHBAH - Chris Hani Baragwanath Academic Hospital, MPH - Mapulaneng Hospital, MKH - Matikwane Hospital, EDH - Edendale Hospital
Only variables with p-values <0.2 in the bivariate analysis were included in the multivariable model
Total number of observations: 3 270

4. Results

4.1 HAstV-positive compared with HAstV-negative patients

From 3 498 specimens collected during the study period, 95.5% (3 340/3 498) were available for testing. Of the 3 340 stools collected, 7.0% (234/3 340) tested positive for HAstVs. The median age of all HAstV-positive patients was 9 months (IQR 5-14), similar to the age of HAstV-negative patients (9 months, IQR 4-15; p=0.757). The highest HAstV detection rate was in the age group 7 to 12 months (90/975; 9.2%) as compared to other ages (aOR 1.7, CI 95% 1.2–2.4; p=0.001, Table 1).

The highest HAstV detection prevalence was seen at EDH, 7.5% (25/332) and CHBAH, 7.4% (142/1 909) with lower detection prevalence at MTK, 6.4% (46/714) and MPH, 5.5% (21/385). These prevalences were not significantly different (Table 1). For complete 12-month periods investigated (2010 to 2013), the highest prevalence of HAstVs was 10.6% (98/921) in 2010 and a range of 4.6% to 5.4% for 2011 to 2013. The monthly pattern of HAstV detection from April 2009 to April 2014 did not demonstrate any distinct trends in seasonality (Figure 1). Compared with 2009, HAstV detection was higher in 2010 (aOR 2.7, CI 95% 1.7-4.3; p<0.001) and in 2014 (aOR 2.8, CI 95% 1.5-5.1; p=0.001, Table 1).

Where possible the genotypes of strains comprising the monthly prevalences greater than 15% (Figure 1) were investigated further by HAstV characterisation assays to determine if the strains were related to each other by genotype. Not all HAstVs comprising the peaks could be re-amplified in the characterisation assays. The genotyping process was complex and resulted in the identification of a large number of putative recombinant strains. There was no indication of the predominance of a single putative recombinant strain during the study period.

There were no similarities observed among the genotypes characterised from March, April and August 2010, December 2011 or February 2014. Of the strains that could be characterised, the following genotypes were identified (ORF1a/ORF2 for putative recombinant strains) March 2010: T8/B55, T8/T4; April 2010: HAstV-4; August 2010: T8/B55, KS106209/T3; December 2011: KS106210/T3, KS106207/T4, T8/T1; February 2014: HAstV-1, HAstV-2, T1/T8, T1/T3, KS106207/T2, T2/T3. Further in depth characterisation of strains is in progress to clarify putative recombinant strains with discordant ORF1a/ORF2 genotypes.

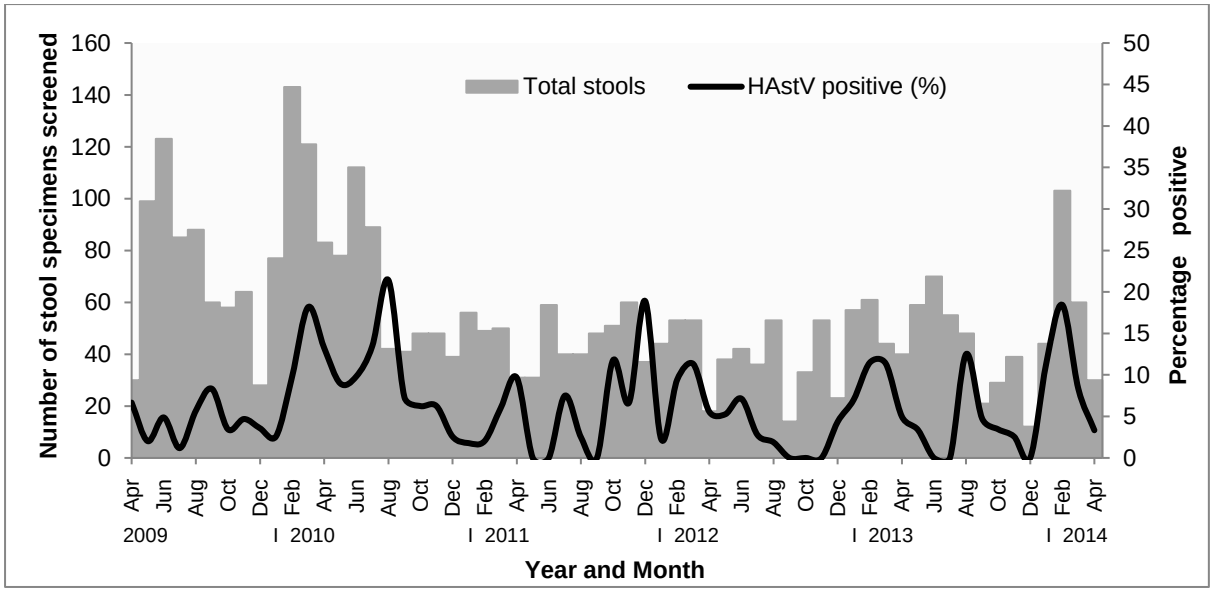

Figure 1: Monthly distribution of total stools collected, percentage positive HAstV screened by month between 2009 and 2014

- Secondary axis scale drawn to 50%
- Percentage positive HAstV = number of HAstV positive cases per month/total number of specimens screened*100

No significant differences in clinical characteristics were observed between HAstV-positive and HAstV-negative children (Table 1). However, HAstVs were detected more frequently in patients whose households reported the use of outdoor water sources (7.6%, 134/1 753) as compared to having taps inside the house (5.7%, 87/1 517). The source of water was significantly associated with HAstV infections in the final regression model (aOR 1.4, CI 95% 1.0-1.8; p=0.033, Table 1).

Astroviruses were present in 5.7% (5/87) of the children who died during the study. The age of the deceased HAstV-positive patients' ranged from 6 to 29 months with a median of 7 months (IQR 11-20). All five fatalities were from Mpumalanga province. Astrovirus was the only pathogen identified in a fully screened specimen while co-infections with RV, NoV GII and SaV were present in the remaining four partially screened specimens. The duration of hospitalisation in the deceased HAstV-positive patients ranged from one to 20 days with a median of four days (IQR 2-10) while in HAstV-positive patients that survived the median was three days (IQR 1-7).

Full screening for viruses, bacteria and parasites was completed in 37.4% (1 248/3 340) of specimens and HAstVs were detected in 6.9% (86/1 248) of these specimens. Of the 86 HAstVs identified, 17.4% (15/86) were detected as single infections, 39.5% (34/86) as co-infections with another pathogen and 43.0% (37/86) as co-infections with two or more

pathogens. Among fully screened HAstVs, co-infections with bacteria were most common (10.5%, 9/86). When co-infection with two or more pathogens was examined in fully screened HAstVs, HAstVs were detected more frequently with HAdVs (11.5%, 27/234, Table 2) as compared with HAstVs being detected in HAdV-negative specimens (5.8%, 59/1 014). Co-infections among fully screened HAstVs were not examined in the multivariate analysis together with other factors identified in HAstV-positive children, as fully screened specimens represented a subset of the total HAstVs detected. A multivariate analysis of co-infections with HAstVs (Table 2) showed that in fully screened specimens, HAdVs and HAstVs were most frequently detected together as compared with the other enteric pathogens screened (aOR 2.1, CI 95% 1.3-3.4; p=0.003, Table 2).

Table 2: Bivariate and multivariate analysis of co-infections with astroviruses

Co-infections in astrovirus-positive specimens	Astrovirus co-infections _n/N (%)	Bivariate analysis		Multivariate analysis	
		Odds Ratio (OR; 95% Confidence Interval(95%CI))	p-value	Adjusted OR (aOR; 95%CI)	p-value
Astrovirus single	15/487 (3.1%)	Ref.			
Astrovirus plus one	34/377 (9.0%)	3.1 (1.7-5.87)	<0.001	Not included in model	
Astrovirus plus two or more	37/170 (21.8%)	8.8 (4.7-16.4)	<0.001		
Astrovirus and Bacteria					
Astrovirus in Bacteria-negative	53/740 (7.2%)	Ref.		Ref.	
Astrovirus in Bacteria-positive	33/508 (6.5%)	0.9 (0.6-1.4)	0.648	0.9 (0.5-1.3)	0.486
Astrovirus and Parasites					
Astrovirus in Parasite-negative	72/1 056 (6.8%)	Ref.		Ref.	
Astrovirus in Parasite-positive	14/8 153 (9.2%)	1.4 (0.8-2.5)	0.296	1.2 (0.7-2.3)	0.490
Astrovirus and Adenovirus					
Astrovirus in Adenovirus-negative	59/1 014 (5.8%)	Ref.		Ref.	
Astrovirus in Adenovirus-positive	27/234 (11.5%)	2.1 (1.3-3.4)	0.002	2.1 (1.3-3.4)	0.003
Astrovirus and Rotavirus					
Astrovirus in Rotavirus-negative	64/845 (7.6%)	Ref.		Ref.	
Astrovirus in Rotavirus-positive	22/403 (5.5%)	0.7 (0.43-1.2)	0.170	1.1 (0.8-1.5)	0.757
Astrovirus and Norovirus GI					
Astrovirus in Norovirus GI-negative	83/1 209 (6.9%)	Ref.		Ref.	
Astrovirus in Norovirus GI-positive	3/39 (7.7%)	1.1 (0.3-3.7)	0.841	1.1 (0.3-3.8)	0.846
Astrovirus and Norovirus GII					
Astrovirus in Norovirus GII-negative	74/1 089 (6.8%)	Ref.		Ref.	
Astrovirus in Norovirus GII-positive	12/159 (7.6%)	1.1 (0.6-2.1)	0.727	1.2 (0.6-2.2)	0.614
Astrovirus and Sapovirus					
Astrovirus in Sapovirus-negative	81/1 145 (7.1%)	Ref.		Ref.	
Astrovirus in Sapovirus-positive	5/103 (4.9%)	0.7 (0.3-1.7)	0.397	0.6 (0.2-1.6)	0.315

The relative concentration of the HAstVs detected, as indicated by cycle threshold (Ct) value, was compared between single and mixed infections in fully screened specimens. No difference between the median Ct values was observed for single, Ct=31.86 (IQR 18.05-

34.52) or mixed infections Ct=31.01 (IQR 20.49-34.81). The Ct values did not vary with a distinct trend relative to the severity of infection based on Vesikari scores calculated.

4.2 HAstVs detected in HIV-infected and HIV-uninfected patients

Of the 3 340 patients screened, 10.3% (345/3 340) were HIV-infected, 64.3% (2 147/3 340) were HIV-uninfected and in 25.4% (848/3 340) of patients, the HIV status was unknown. Of the total HAstV-positive individuals (n=234), HIV-infected patients contributed 10.7% (25/234), 63.7% (149/234) were collected from HIV-uninfected children and the HIV status was unknown in 25.6% (60/234) of cases. Specimens collected from children with unknown HIV status, were excluded from further analysis (n=60). All other HAstV-positive patients (n=174) were included in the analysis, irrespective of full or partial screening of the specimens for enteric pathogens.

The prevalence of detecting HAstVs was similar in HIV-infected (7.3%, 25/345) and HIV-uninfected patients (6.9%, 149/2 147, p=0.836). Among the HAstV-positive patients, the median age in HIV-infected patients was 8 months (IQR 6-13) compared to a median of 10 months (IQR 5-15) in HIV-uninfected patients (p=0.735). Among the HAstV positive patients, a higher proportion of HAstVs was seen in HIV-infected patients in MTK, 27.3% (12/44) and MPH, 19.1% (4/21) as compared to CHBAH, 9.2% (8/87) and EDH, 4.6% (1/22) (p=0.006). For the complete 12-month periods investigated, between 2010 and 2013, the prevalence of HAstVs in HIV-infected children decreased from 23.4%, (15/64) in 2010 to 3.5% (1/29) in 2013. No HAstVs were identified in HIV-infected children during 2011.

Among HAstV-positive cases the median Vesikari score was 11 (IQR 8-12) in HIV-infected patients and 11 (IQR 8-13) in HIV-uninfected patients indicating similar severity (p=0.122). The median Ct for HAstV detection in HIV-infected patients was 33.2 (IQR 26-5-37.0) compared with Ct = 29.3 (IQR 20.0-33.3) in HIV-uninfected patients (p=0.735). No significant differences in clinical characteristics were observed between HAstV-positive patients that were HIV-infected or HIV-uninfected. Data collected from HAstV-positive cases revealed that fewer HIV-infected mothers (32.5%, 26/80) were practising breastfeeding as compared with HIV-uninfected mothers (67.5%, 54/80, p<0.001).

Two of the five HAstV-positive patients who died during the study were HIV-infected. In the first HIV-infected fatality, HAstV was the only pathogen detected (fully screened specimen) in the 20-month old patient who died ten days after admission to MPH. The patient was not vomiting before hospitalisation and diarrhoea was already continuous for four days with a maximum of eight stools per day. The patient's clinical symptoms scored 10 points on the

Vesikari scale. In the second fatality that was HIV-infected a RV and HAstV co-infection was identified in the partially screened specimen. The 6-month old patient presented at MPH and was in hospital for 20 days before death. Prior to hospitalisation, the patient vomited for one day with four episodes reported and diarrhoea had occurred for three days already, with a maximum of seven stools per day. The patient's clinical symptoms on admission scored 11 Vesikari points. The patient's home used a communal outdoor water supply and a pit latrine served for sanitation purposes.

4.3 HAstVs detected in HIV-exposed uninfected and unexposed patients

From the HIV-uninfected patients (n=2 147), HAstVs were identified in 8.4% (67/799) of cases exposed to HIV (HEU) and in 5.9% (74/1257) of HIV-unexposed patients (p=0.032). The HIV-exposure status in eight HAstV-positive patients was unknown. Overall, from 63.7% (149/234) HAstVs identified in HIV-uninfected cases, 45.0% (67/149) were collected from HEU patients, 49.6% (74/149) from unexposed patients (p=0.680) and in 5.4% (8/149) HIV-exposure was unknown. Only HAstV-positive patients were included in the investigation of HAstVs identified in HEU and unexposed children, with the exclusion of children with unknown exposure status. Both fully and partially screened specimens were analysed for all variables except for comparisons between single and co-infections.

Among the HAstV-positive cases, the median age in HEU patients was 8 months (IQR 5-12) compared with 10 months (IQR 7-18) in unexposed patients (p=0.611). The median Vesikari scores in HEU and unexposed patients was the same (11) which could infer that the severity of diarrhoeal illness was similar in both groups.

Among HAstV-positive cases, HEU patients (71.4%, 45/63) were more likely to have mixed feeding patterns in the first four months of life as compared with unexposed patients (28.6%, 18/63). The absence of exclusive breastfeeding remained associated with HEU patients in the multivariate model (aOR 8.0, CI 95% 3.4-19.0; p<0.001, Table 3).

Among the HAstV-positive cases HEU patients (58.4%, 45/77) were more likely to have non-flushing toilet facilities as compared with unexposed children (41.6%, 32/77, p=0.005). Non-flushing toilets remained significantly associated in HEU children (aOR 4.5, CI 95% 1.9-10.7; p=0.001, Table 3).

Table 3: Bivariate and multivariable analysis of epidemiologic characteristics associated with HAstV-positive HIV-uninfected HIV-exposed and HIV-unexposed children

Parameter	Proportion HIV-exposed among HIV-uninfected_n/N (%)	Bivariate analysis		Multivariate analysis	
		Odds Ratio (OR; 95% Confidence Interval(95%CI))	p-value	Adjusted OR (aOR; 95%CI)	p-value
Demographic characteristics					
Collection year					
2009 (April to December)	2/6 (33.3)	Ref.		Ref.	
2010	19/46 (41.3)	1.4 (0.2-8.5)	0.709	0.5 (0.1-2.0)	0.343
2011	11/26 (42.3)	1.5 (0.2-9.5)	0.688	0.5 (0.1-2.4)	0.402
2012	11/16 (68.8)	4.4 (0.6-32.5)	0.146	2.7 (0.6-13.6)	0.217
2013	16/28 (57.1)	2.7 (0.4-17.0)	0.300	0.9 (0.2-3.6)	0.835
2014 (January to May)	8/19 (42.1)	1.5 (0.2-10.0)	0.703	omitted	-
Age in months					
0 to 6	25/43 (58.1%)	Ref.		Ref.	
7 to 12	28/55 (51.1%)	0.75 (0.3-1.7)	0.476	1.4 (1.0-3.8)	0.463
13 to 18	9/22 (41.0%)	0.50 (0.2-1.4)	0.191	1.0 (0.3-3.3)	0.923
19 to 24	1/6 (16.7%)	0.14 (0.02-1.3)	0.089	0.2 (0.02-3.1)	0.273
≥25	4/15 (26.7%)	0.26 (0.07-1.0)	0.043	0.6 (0.1-3.0)	0.563
Gender					
Female	22/58 (37.9%)	Ref.		Ref.	
Male	45/83 (54.2%)	1.9 (1.0-3.8)	0.058	1.7 (0.7-4.0)	0.215
Clinical characteristics					
Fever duration					
<3 days	21/53 (39.6%)	Ref.		Ref.	
≥3 days	12/20 (60%)	2.29 (0.8-6.5)	0.123	1.3 (0.4-4.6)	0.665
IV fluids received					
None	22/51 (43.1%)	Ref.		Ref.	
Yes	37/66 (56.1%)	1.7 (0.8-3.5)	0.167	0.9 (0.3-2.2)	0.754
Other features					
Feeding (first four months)					
Breast	18/63 (28.6%)	Ref.		Ref.	
Breast + other	45/63 (71.4%)	6.2 (2.9-13.5)	<0.001	8.0 (3.4-19.0)	<0.001
Feeding (after four months)					
Breast	4/21 (19.1%)	Ref.			
Other	50/92 (54.4%)	5.1 (1.6-16.2)	0.006	1.7 (0.4-6.8)	0.450
Rotavirus vaccination received					
None	12/36 (33.3%)	Ref.			
Full/Partial	55/103 (53.4%)	2.3 (1.0-5.1)	0.041	1.7 (0.6-5.2)	0.343
Environmental features					
Water source					
Indoor	20/53 (37.7%)	Ref.			
Outdoor/Other	47/88 (53.4%)	1.9 (1.0-3.8)	0.073	1.2 (0.5-3.0)	0.668
Sanitation					
Flush	22/64 (34.4%)	Ref.			
Other	45/77 (58.4%)	2.7 (1.4-5.3)	0.005	4.5 (1.9-10.7)	0.001
Housing material					
Brick	43/95 (45.3%)	Ref.			
Tin/Wood/Iron	16/34 (47.1%)	1.1 (0.5-2.4)	0.857	0.7 (0.3-1.9)	0.463
Mud/Traditional	8/12 (66.7%)	2.4 (0.7-8.6)	0.172	1.0 (0.2-4.4)	0.990

CHBAH - Chris Hani Baragwanath Academic Hospital, MPH - Mapulaneng Hospital, MKH - Matikwane Hospital, EDH - Edendale Hospital, HIV - Human immunodeficiency virus
Only variables with p-values <0.2 in the bivariate analysis were included in the multivariable model.
Total number of observations: 126

From the HAstV-positives that were detected in HIV-uninfected children (n=149), 47 isolates from patients with known HIV exposure were fully screened. The most common co-infection observed in this sub-group of HAstV-positive specimens was with parasites, 50.0% (5/10) followed by bacteria, 47.6% (10/21).

5. Discussion

The study presents new epidemiologic information on HAstVs from selected sites in SA among children <5 years of age. These data present baseline information that may be explored further when HAstV disease prevention measures are being investigated. A review of the limitations and merits of this study will guide the design of future HAstV investigations. Astroviruses need close monitoring, especially with the mounting evidence of the extra-intestinal activity of these viruses, often with fatal outcomes.[36]

The results show that the prevalence of HAstVs was similar in HIV-infected children as compared with HIV-uninfected children with diarrhoea. The success of the prevention of mother to child transmission (PMTCT) program has most likely contributed to the decreased number of HIV-infected children (Communicable Diseases Communication., 2015; SA DOH, 2014). The risk of HAstV disease among HEU patients was not calculated as incidence estimates by HIV status of all patients was not performed.

Among HEU cases, the largest proportion of HAstVs was identified from patients aged less than six months old (Table 3). This could be associated with the feeding patterns observed in HEU children, that is a milk formula or mixed breastfeeding diet in the first four months of life. These results are similar to an investigation in Iran that reported viral diarrhoeal infections were more frequent in patients that were not exclusively breastfed.[37] Patients exposed to HIV may be fed milk formulae on account of the HIV-infected mothers' fear of infecting her baby through breastfeeding.[38] The child may have decreased levels of maternal antibodies and be more susceptible to all infections, including HAstVs. While these speculations were not substantiated by the study design, these observations may support the promotion of breastfeeding by HIV-infected mothers.[38-40]

A cohort study in SA compared all infections in HEU and unexposed children and reported that while both groups were equally susceptible in the first year of life,[41] HEU children were at a higher risk for more severe infection events that would require hospitalisation.[41] In the present investigation of hospitalised children, more HAstV-positive cases were HEU than

unexposed children. Whether the HAstVs detected were associated with causality of hospitalisation could not be determined or validated as incidence rates were not calculated.

Astroviruses are usually detected as milder infections[3] that do not require hospitalisation. Hospitalised patients with severe diarrhoeal infections were thus not the ideal population for investigating the clinical aspects of HAstV infections. The study limitation also highlights the possible incorrect use of the Vesikari scoring system for grading HAstV infections, which are milder in comparison with RV diarrhoeal infections.[3]

From 2009, a decrease in the total number of diarrhoea cases presenting at the surveillance sites was observed. This has been attributed to the introduction of the RV vaccine into the SA immunization schedule in 2009 and the concomitant reduction in the number of RV-associated diarrhoeal infections detected thereafter.[42] The effect of the RV vaccine on HAstV gastroenteritis was not investigated in this study. However, studies have reported that the introduction of the rhesus tetravalent RV vaccine had no remarkable effect on HAstV gastroenteritis prevalence.[43]

Astroviruses were detected at a median age of 9 months among patients. A cohort study in Egypt investigating community acquired diarrhoea in children less than 2 years, detected HAstVs with the highest annual incidence rates in patients aged 6 to 11 months.[44] This study also suggests that a HAstV vaccine must confer immunity very early in life. With further epidemiology support, a HAstV vaccine may be designed to confer protection at age 9 months, as highlighted in the data from this study, to prevent diarrhoeal disease and possibly extra-intestinal infections later on in life.

There was an isolated increase in the number of HAstVs detected in 2010, but this did not represent a seasonal trend for the study period. The genotypes of HAstVs reflected in the peak prevalence collected in 2010 and 2014 were not associated with each other. This showed that HAstVs circulated throughout the year independent of genotype. The complete surveillance years of 2011 to 2013 showed relatively similar detection rates, implying a stable and steady level of HAstV presence in SA. Overall, the monthly prevalence recorded for HAstVs did not demonstrate any distinct trends that indicated seasonality. Similar findings of HAstVs circulating throughout the year have been reported.[3,8,14,45-46]

A disease severity comparison between HAstV single and co-infections revealed that in this study population the diarrhoeal event was independent of the HAstV detection. In specimens that were HAstV-positive only, it is possible that other pathogens not screened

for were contributing to the diarrhoea. As only qualitative analysis was done with Ct values used as proxy for virus concentration, disease correlations in mixed infections could not be made as recommended by Vu et al.[7]

The absence of an indoor water supply was frequently identified with HAstV-positive patients. The main external sources of water used were boreholes, communal taps, rivers and water tanks. The quality of the water from these alternate sources was not tested and the transmission of HAstVs through contaminated water sources is documented.[47] The disruption of good hygiene practices may also occur with the lack of water for disinfection, washing and other domestic purposes in the home.

Astrovirus-positive HEU patients were more likely to have non-flushing sanitation facilities, as compared with unexposed cases. Based on the assumption that HAstV-positive HEU patients were more susceptible to infections with the absence of exclusive breastfeeding and the reported increased risk for severe hospitalised infections in these patients, it can be reasoned that HEU patients would also be more prone to person-to-person spread of HAstVs. The increased frequency of HAstVs among HEU cases with non-flushing toilets may allude to breaks in disinfection procedures after using non-flushing sanitation facilities and the subsequent spread to HEU children. The potential carriage of HAstVs by HIV-infected caregivers and subsequent transmission to HUE children is highlighted. Educating the community about infection control is the recommended solution.

In this study the HAstV-positive cases were not compared with a diarrhoea-free control group. The burden of diarrhoeal disease attributable to HAstVs must consider asymptomatic carriage in the community.[7,11,14] This limitation in the study may have resulted in an underestimation of the total prevalence of HAstVs as only a hospitalised population was included. Further, the absence of a control group restricts calculating the attribution to causality related to HAstVs. The prevalence calculated in the study is thus relevant for hospitalised cases only and does not reflect the prevalence of HAstV disease in the general population of the sites investigated. The clinical features associated with HAstV-diarrhoea were not distinct within the sample population. Astroviruses are associated with milder illness[3] and thus more suitable subjects for HAstV investigations would be from the community and children attending out-patient facilities. Many specimens in the study were partially screened and the regression model included variables that did not have information for all observations.

In conclusion, although the study highlights the group of children hospitalised for diarrhoea that were most frequently HAstV-positive, community studies and investigations at out-patient facilities are recommended to establish the burden of HAstV-diarrhoeal disease in SA.

Ethics section

Human Research Ethics Committee (Medical), University of Witwatersrand (M091018)
Faculty of Health Sciences Research Ethics Committee, University of Pretoria (174/2015)

Author's contribution

S.N. completed lab work, data analysis and writing
N.A.P. designed study, edited manuscript
M.B.T, M.J.G, C.C, S.A.M reviewed and edited manuscript

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Competing interests

The authors declare that they have no competing interests.

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