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Norovirus epidemiology in South African children <5 years hospitalised for diarrhoeal illness between 2009 and 2013

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SUMMARY

Public health interest in norovirus (NoV) has increased in recent years following improved diagnostics, global burden estimates and the development of NoV vaccine candidates. This study aimed to describe the detection rate, clinical characteristics and environmental features associated with NoV detection in hospitalized children <5 years with diarrhoea in South Africa (SA). Between 2009 and 2013, prospective diarrhoeal surveillance was conducted at four sites in SA. Stool specimens were collected and screened for NoVs and other enteric pathogens using molecular and serological assays. Epidemiological and clinical data were compared in patients with or without detection of NoV. The study detected NoV in 15% (452/3103) of hospitalized children <5 years with diarrhoea with the majority of disease in children <2 years (92%; 417/452). NoV-positive children were more likely to present with diarrhoea and vomiting (odds ratio (OR) 1·3; 95% confidence interval (CI) 1·1–1·7; $P = 0{\cdot}011$) with none-to-mild dehydration (adjusted OR 0·5; 95% CI 0·3–0·7) compared with NoV-negative children. Amongst children testing NoV positive, HIV-infected children were more likely to have prolonged hospitalization and increased mortality compared with HIV-uninfected children. Continued surveillance will be important to consider the epidemic trends and estimate the burden and risk of NoV infection in SA.

Key words: Caliciviruses, diarrhoea, Norwalk agent and related viruses, virology (human) and epidemiology.

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INTRODUCTION

Globally, norovirus (NoV) are a common cause of viral diarrhoea and vomiting in children <5 years [1]. Awareness of NoV has heightened with the improvement of diagnostics to detect the virus and the identification of NoV as an important cause of food-borne illness globally [2, 3]. A systematic review of NoV in sporadic gastroenteritis cases conducted in 2014 estimated that NoV was responsible for 18% (95% confidence interval (CI) 15–20) of gastroenteritis among children <5 years [1]. A previous review in 2008 estimated that NoV was responsible for 12% (95% CI 10–15) of severe diarrhoea in children <5 years and projected 218 000 deaths in children <5 years in developing countries annually [4].

While NoV infections are generally self-limiting, outcomes can be severe in individuals with comorbidities, extremes of age (<5 and >65 years) or following the emergence of new strains [5, 6]. A study conducted in the USA demonstrated that children <5 years have the highest rates of NoV-associated health care visits and adults ⩾65 years have the greatest risk of death due to NoV [7]. NoV disease in children is generally mild, however, severe outcomes do occur with hospitalization rates between 7·2 and 16 hospitalizations/10 000 population <5 and an estimated 9·9–12% of diarrhoeal deaths annually [7, 8].

Mans *et al*. recently conducted a review of NoV epidemiology in Africa [9]. While data on NoV prevalence in patients with diarrhoea in sub-Saharan Africa are available, most of these studies have duration of <2 years or <300 specimens available for evaluation. Despite these limitations, the review revealed overall NoV prevalence in individuals with diarrhoea of 13·5% (961/7141; 95% CI 12·7–14·3), with children <1 year mainly affected in African settings [9].

Research on NoV in South Africa (SA) dates back to 1993 [10] and originally focused on seroprevalence rates in all age groups, which ranged from >50% to >90% [11, 12]. An early virus detection study applied a combination of electron microscopy and recombinant enzyme immunoassays against Norwalk (NV)-like and Mexico (MX)-like NoVs and RT–PCR (reverse transcription–polymerase chain reaction) for the detection of caliciviruses in paediatric stool specimens collected between 1991 and 1995 [13]. NV-like and MX-like NoVs were detected in 0·2% (3/1296) and 2·2% (29/1296) specimens, respectively [13].

Between November 2009 and February 2012, the multisite birth cohort study investigating pathogen-specific burdens of community diarrhoea (MAL-ED) included a site in Venda, SA [14]. NoV incidence at this site was 9·52 (95% CI 7·06–12·83) NoV GII detected per 100 child-months and 5·09 NoV GI detected per 100 child-months [14]. Severity of NoV diarrhoea was comparable with other enteric pathogens detected, except RV, and undernutrition was identified as a risk factor for NoV disease [14]. NoV infections peaked in children 6–11 months of age [14].

In immunocompromised hosts, NoV infections are reported to be more severe with prolonged symptoms and viral shedding [6]. However, the majority of these studies have been conducted in transplant recipients with limited information in HIV-infected individuals [6]. Persistent diarrhoea, lasting 7 months, was described in an HIV-infected adult patient with poor compliance to antiretroviral therapy and a chronic NoV infection [15]. NoVs were detected more often in HIV-infected compared with HIV-uninfected children [16, 17].

In April 2009, a prospective sentinel surveillance system was established to monitor diarrhoeal disease in hospitalized children <5 years of age in SA. We aimed to describe the epidemiology of NoV between 2009 and 2013.

METHODS

Study participants and sites

Hospitalized children <5 years with acute diarrhoea were enrolled in the sentinel diarrhoea surveillance study. Acute diarrhoea was defined according to the World Health Organization (WHO) definition of '*three looser than normal stools within a 24 h period*'. The sentinel sites, located in three provinces, included: Chris Hani Baragwanath Academic Hospital (CHBAH; 2009–2013), Mapulaneng Hospital (MPH; 2009–2013), Matikwane Hospital (MKH; 2009–2013) and Edendale Hospital (EDH; 2010–2013).

Study enrolment and data collection

Written informed consent was obtained from the parents of patients prior to enrolment. Systematic sampling was used to enrol patients on a daily basis from Monday to Friday between 8 am and 5 pm. Patients who refused to participate in the study were noted, but no additional information was collected. Surveillance officers collected demographic, socio-economic and risk factor data from

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the parents by interview and additional clinical data from medical records on standardized questionnaires. Data on HIV status of the mother and child were obtained during the interview or review of the medical records. If the status of the child was unknown and the parent gave consent, then a dried blood spot was collected for anonymised HIV testing.

Laboratory screening

A stool specimen was collected for enteric pathogen detection. Nucleic acids were extracted from 10% stool suspensions using the QIAamp® Viral RNA Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions and eluted in 60 µl RNase-free water. Briefly, 10 µl extracted RNA was reverse transcribed with random primers using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany) according to the manufacturers' instructions. Samples were screened for NoV GI and GII in monoplex reactions using 5 µl cDNA, LightCycler® 480 Probe Master Kit (Roche) and primers and probes from published methods [18–20]. Screening of other enteric viruses, bacteria and parasites has been described elsewhere [21, 22].

A fully screened specimen was defined as a specimen where all viral, bacterial and parasite testing had been performed ($n = 1354$).

Dried blood spots were screened using the AmpliPrep® (Roche) for automated nucleic acid extraction and COBAS® TaqMan® (Roche) for automated real-time detection of HIV-1.

Data analysis

NoV detection rate was calculated using data from years with 12 months of surveillance (2010–2013). Demographic data, clinical characteristics and environmental features associated with NoV detection were compared in patients with or without NoV, genogroup I (GI) or II (GII) using Stata12 (StataCorp LP, College Station TX). In addition, the characteristics of HIV-infected and HIV-uninfected children were compared in NoV-positive cases. NoV-negative cases were defined as cases where neither NoV GI nor GII strains were detected. Mixed infections were defined as detection of NoV GI or GII strains with any of the following pathogens: RV, human adenovirus (any group), astrovirus, sapovirus (SaV), bocavirus, bacteria (*Campylobacter* spp., diffusely adherent *Escherichia coli*, enteroaggregative *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli, Salmonella* spp. and *Shigella* spp.) and parasites (*Ascaris lumbricoides, Cryptosporidium* spp., *Entamoeba coli, Giardia lamblia* and *Isospora belli*).

The χ^2 tests were used to determine statistical significance ($P \leqslant 0{\cdot}05$) of categorical data, while t tests with unequal variance and Welch's approximation were used for means. A Wilcoxon rank-sum test was used to assess differences between medians. Univariate analysis and stepwise multivariable logistic regression analysis was performed to identify environmental features associated with NoV detection in patients with diarrhoea and a separate analysis restricted to NoV-positive cases compared the characteristics of HIV-infected and HIV-uninfected children. Variables that did not yield statistically significant P-values on univariate analysis were not included in the multivariable models or reported in the tables.

RESULTS

Between 20 April 2009 and 31 December 2013, 3103 children <5 years provided stool samples and were included in the surveillance study. All samples were screened for NoV GI and GII, SaV, human adenovirus, astrovirus and bocavirus. In addition, specimens were screened for RV (99·7%; 3093/3103), bacteria (88·6%; 2748/3103) and parasites (48·5%; 1508/3103).

Overall, 2·6% (79/3019) of children admitted to the sentinel hospitals for the treatment of diarrhoea died over the study period. While 10% (8/79) of these children had NoV detected, only two children had NoV GII strains as the sole pathogen detected. Of the eight children that died, four were HIV infected.

NoV detection

Between 2009 and 2013, NoV was detected in 452/3103 (14·6%) of specimens. The detection rate when only years with 12 months of data were included was 16% (396/2468; 2010–2013). NoV GII strains were detected in 13·4% (330/2468; 95% CI 12·1–14·8) of specimens and NoV GI in 3·4% (84/2468; 95% CI 2·4–3·6) of specimens between 2010 and 2013. An additional 47 GII and 10 GI cases were detected between April and December 2009, resulting in 377 NoV GII and 94 NoV GI cases available for analysis (Supplementary Tables 1 and 2). In fully screened NoV-positive specimens, NoV GII strains were detected as the sole pathogen in 35·4% (61/172)

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and GI in 23·4% (11/47) of cases. Mixed GII and GI NoV detected in 19 (0·6%; 19/3103) diarrhoea cases.

The median cycle threshold (C_t) values in these specimens were compared in single and mixed pathogen NoV cases. The median C_t values of single (C_t = 27·1; IQR 18·6–31·1) pathogen GII cases were significantly lower than mixed (C_t = 30·6; IQR 22·0–33·4) pathogen NoV GII cases (P = 0·005). Similarly, the median C_t values of single pathogen GI cases were significantly lower than mixed pathogen NoV GI cases (n = 47; single: C_t = 27·2 (IQR 21·0–29·0); mixed: C_t = 32·0 (IQR 28·0–33·9); P = 0·008).

NoV epidemiology

NoV detection was higher in children 0–6 months and 7–12 months compared with children older than 24 months (0–6 months adjusted odds ratio (aOR) 2·2; 95% CI 1·4–3·6; P = 0·001; 7–12 months aOR 2·2 95% CI 1·3–3·5; P = 0·002; Table 1). A similar age trend was noted in NoV GII cases (Supplementary Table 1). Unlike NoV GII, the odds of NoV GI detection were higher in the second year of life (19–24 months; aOR 2·3; 95% CI 1·2–4·5; P = 0·011; Supplementary Table 2) compared with the 0–6-month age group (Supplementary Table 2).

NoV strains were frequently detected in 2010 and 2011 compared with 2013 (2010; aOR 2·2 (95% CI 1·5–3·1); P<0·001 and 2011; aOR 2·1 (95%CI 1·4–3·0); P < 0·001; Table 1). This result was echoed in the individual analyses of GII and GI strains although the result was not statistically significant in multivariable analysis of GI (Supplementary Tables 1 and 2). The NoV detection rate by sentinel site was similar ranging from 10·0% in MPH to 16·1% in EDH over the study period (Table 1).

NoV GII were frequently detected from September to December each year (Fig. 1). The percentage-positive NoV GII during this period was 20·5% (163/797) compared with the rest of the year (9·3%; 214/2306; P < 0·001). The monthly detection rate of NoV GI strains in SA was less pronounced than NoV GII although the average detection in late summer (January–April) was 4·3% (43/1009) compared with 2·4% for the rest of the year (May–December; 51/2094; P = 0·01; Fig. 1).

Clinical characteristics of NoV infections

Vomiting was more frequently identified in NoV-positive compared with NoV-negative children (OR 1·3; 95% CI 1·1–1·7; P = 0·011; Table 1). NoV GII-positive patients were more likely to report five or more vomiting episodes per day (OR 1·9; 95% CI 1·2–2·8; P = 0·003) and vomiting duration of up to 2 days (aOR 1·8; 95% CI 1·2–2·7; P = 0·005) compared with NoV-negative patients (Supplementary Table 1). However, NoV-positive cases were less likely to be dehydrated when compared with NoV-negative cases (aOR 0·7–0·5; 95% CI 0·3–0·9; P = 0·009; Table 1). Children with NoV detected were admitted to hospital for a median of 2 days (IQR 1–4) compared with 3 days (IQR 1–6) in children without NoV detected (P < 0·001). Low weight at birth (aOR 0·6; 95% CI 0·4–0·9; P = 0·017) compared with normal weight at birth was significantly less common in NoV GII-positive compared with NoV GII-negative patients (Supplementary Table 1).

Environmental features associated with NoV detection

Informal housing compared with brick houses (aOR 0·7; 95% CI 0·5–1·0; P = 0·021; Table 1) was significantly less common in NoV-positive than NoV-negative cases. Water from outdoor taps and boreholes (aOR 0·7; 95% CI 0·5–1·0; P = 0·045) compared with indoor taps and mixed infections with RV (aOR 0·6; 95% CI 0·4–0·8; P = 0·002) and human adenovirus (aOR 0·3; 95% CI 0·2–0·6; P < 0·001) were all significantly less common in NoV GII-positive than NoV GII-negative patients (Supplementary Table 1). No statistically significant environmental or behavioural features were associated with NoV GI detection (Supplementary Table 2).

Compared with NoV-negative cases, mixed pathogen infections were frequently associated with NoV detection (NoV GII + one other pathogen (OR 1·8; 95% CI 1·3–2·6; P = 0·001) and NoV GII + ⩾2 other pathogens (OR 5·2; 95% CI 3·5–7·7; P < 0·001) compared with NoV only; Table 1). These results were also significant when NoVs were stratified according to genogroup (Supplementary Tables 1 and 2).

The odds of detecting NoV GI strains increased 2·0 times when NoV GII strains were present (95% CI 1·2–3·3) and 2·0 times when SaV was present (95% CI 1·1–3·7; Supplementary Table 2) compared with single NoV GI cases. The association between NoV GI and SaV was maintained when adjusted for month of collection (aOR 1·9 (95% CI 1·0–3·5); P = 0·041). Similarly, the NoV GI/NoV GII association also maintained statistical significance when adjusted

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Table 1. *Univariate and multivariable analysis of demographic data, clinical characteristics and environmental features associated with NoV detection (n = 452)*

Parameter	NoV detection *n/N* (%)	Univariate analysis		Multivariable analysis	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Demographic characteristics					
Age (in months)					
>24	29/302 (9·6)	Ref		Ref	
19–24	38/232 (16·4)	1·8 (1·1–3·1)	0·020	1·8 (1·0–3·3)	0·051
13–18	56/459 (12·2)	1·3 (0·8–2·1)	0·267	1·7 (1·0–2·9)	0·063
7–12	149/915 (16·3)	1·8 (1·2–2·8)	0·005	2·2 (1·3–3·5)	0·002
0–6	179/1191 (15·0)	1·7 (1·1–2·5)	0·016	2·2 (1·4–3·6)	0·001
Year					
2013	56/537 (10·4)	Ref		Ref	
2012	56/459 (12·2)	1·2 (0·8–1·8)	0·378	1·2 (0·8–1·9)	0·343
2011	101/553 (18·3)	1·9 (1·4–2·7)	<0·001	2·1 (1·4–3·0)	<0·001
2010	183/919 (19·9)	2·1 (1·5–2·9)	<0·001	2·2 (1·5–3·1)	<0·001
Sentinel site					
CHBH	274/1764 (15·5)	Ref			
EDH	50/310 (16·1)	1·0 (0·8–1·5)	0·790		
MPH	36/359 (10·0)	0·6 (0·4–0·9)	0·008		
MKH	92/670 (13·7)	0·9 (0·7–1·1)	0·267		
Clinical characteristics					
Diarrhoea duration (in days)					
⩾6	34/203 (16·8)	Ref			
5	31/260 (11·9)	0·6 (0·4–1·1)	0·140		
1–4	375/2571 (14·6)	0·8 (0·6–1·2)	0·403		
Vomiting					
No	119/981 (12·1)	Ref		Not included in model	
Yes	324/2079 (15·6)	1·3 (1·1–1·7)	0·011		
Vomiting duration (in days)					
0	119/981 (12·1)	Ref		Ref	
1	67/437 (15·3)	1·3 (0·9–1·8)	0·100	1·3 (0·9–1·8)	0·228
2	111/655 (17·0)	1·4 (1·1–2·0)	0·006	1·4 (1·0–1·9)	0·041
⩾3	143/971 (14·7)	1·3 (1·0–1·6)	0·093	1·1 (0·8–1·5)	0·540
Maximum number of vomits per 24 h					
0	119/981 (12·1)	Ref		Not included in model	
1	39/337 (11·5)	0·9 (0·6–1·4)	0·786		
2–4	221/1410 (15·7)	1·3 (1·1–1·7)	0·015		
⩾5	45/233 (19·3)	1·7 (1·2–2·5)	0·004		
Admission temperature (°C)					
⩽37·0	151/942(16·0)	Ref			
37·1–38·4	90/588 (15·3)	0·9 (0·7–1·3)	0·705		
38·5–38·9	18/82 (22·0)	1·5 (0·8–2·6)	0·168		
⩾39·0	19/92 (20·7)	1·4 (0·8–2·3)	0·255		
Dehydration (as assessed by a clinician)					
None	84/414 (20·1)	Ref		Ref	
1–5% (mild)	244/1658 (14·7)	0·7 (0·5–0·9)	0·006	0·7 (0·5–0·9)	0·009
⩾6% (moderate/severe)	79/649 (12·2)	0·5 (0·4–0·8)	<0·001	0·5 (0·3–0·7)	0·001
Child's HIV status					
Negative	374/2467 (15·2)	Ref			
Positive	44/357 (12·3)	0·8 (0·6–1·1)	0·159		
Child's HIV exposure					
Uninfected, unexposed	260/1693 (15·4)	Ref			
Uninfected, exposed	128/924 (13·9)	0·9 (0·7–1·1)	0·301		
Infected	44/357 (12·3)	0·8 (0·6–1·1)	0·144		
Number of days admitted to hospital					
⩾3	190/1559 (12·2)	Ref			

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Table 1 (*cont.*)

Parameter	NoV detection *n*/*N* (%)	Univariate analysis		Multivariable analysis	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
⩽2	248/1465 (16·9)	1·5 (1·2–1·8)	<0·001	1·3 (1·0–1·7)	0·023
Child's birth weight					
⩾2·5 kg	263/1548 (17·0)	Ref			
<2·5 kg	36/303 (11·9)	0·7 (0·5–1·0)	0·028		
Environmental features associated with NoV detection					
Housing material					
Brick	355/2316 (15·3)	Ref			
Informal	85/736 (11·6)	0·7 (0·6–0·9)	0·011	0·7 (0·5–1·0)	0·021
Water source					
In-door tap	225/1414 (15·9)	Ref			
Other	217/1637 (13·3)	0·8 (0·7–1·0)	0·038		
Sanitation type					
Flush toilet	253/1591 (15·9)	Ref			
Other	190/1462 (13·0)	0·8 (0·6–1·0)	0·023		
NoV mixed pathogen infections					
NoV only	72/592 (12·2)	Ref		Not included in model	
NoV + 1	75/370 (20·3)	1·8 (1·3–2·6)	0·001		
NoV + 2 or more	68/163 (41·7)	5·2 (3·5–7·7)	<0·001		
NoV + rotavirus	100/895 (11·2)	0·7 (0·5–0·8)	0·001		
NoV + adenovirus	71/579 (9·3)	0·8 (0·6–1·0)	0·082		
NoV + astrovirus	45/214 (21·0)	1·6 (1·1–2·3)	0·006		
NoV + parasites	18/190 (9·5)	0·6 (0·4–1·0)	0·039		

CHBH, Chris Hani Baragwanath Hospital; EDH, Edendale Hospital; MPH, Mapulaneng Hospital; MKH, Matikwane Hospital; HIV, human immunodeficiency virus.
Only variables with *P*-values <0·2 in the univariate analysis were reported in the table and included in the multivariable model.

for month of collection (aOR 1·9 (95% CI 1·1–3·3); $P = 0·017$).

NoV infection in HIV-infected and -uninfected children

The HIV status was available for 91% (2824/3103) of the children enrolled and for 96% (2974/3103) of mothers. The HIV status was missing in 7% (26/377) of the NoV GII cases and 9% (8/94) of the NoV GI cases. Mixed GII and GI infections were noted in 19 cases with 418 cases available for analysis.

The detection of single (11% *vs.* 10%) pathogen NoV GII cases was similar in HIV-infected and HIV-uninfected children ($P = 1·0$). The detection of mixed pathogen NoV GII cases was lower in HIV-infected (9%; 26/278) compared with HIV-uninfected children (14%; 267/1954; $P = 0·046$). There were no differences in the median NoV GII C_t values in HIV-infected ($C_t = 27·9$; IQR 21·4–32·0) compared with HIV-uninfected children ($C_t = 26·1$; IQR 19·7–32·0; $P = 0·45$).

The detection of single pathogen NoV GI infections was higher in HIV-infected (9%; 6/67) compared with HIV-uninfected (1%; 4/410; $P < 0·001$) children while the detection of mixed pathogen NoV GI infections was lower in HIV-infected (2%; 5/257) compared with HIV-uninfected children (4%; 71/1758; $P = 0·115$). Univariate analysis indicated that the odds of detecting single pathogen NoV GI in HIV-infected children were 10·0 times greater (95% CI 2·7–36·4) compared with HIV-uninfected children ($P < 0·001$). In addition, the median NoV GI Ct values in HIV-infected children were significantly lower ($C_t = 28·0$; IQR 24·4–31·1) compared with HIV-uninfected children ($C_t = 32·0$; IQR 26·9–34·2; $P = 0·05$) on univariate analysis.

Amongst children with NoV detected, HIV-infected children displayed a higher case fatality rate (9·3% *vs.* 1·0%; aOR 6·9; 95% CI 1·5–31·9; $P = 0·013$) and longer hospitalizations (⩾3 days 74·4% *vs.* 39·9%; aOR 4·3; 95% CI 2·1–8·9; $P < 0·001$; Table 2) than HIV-uninfected children on multivariable analysis. In addition, HIV-infected children with NoV had

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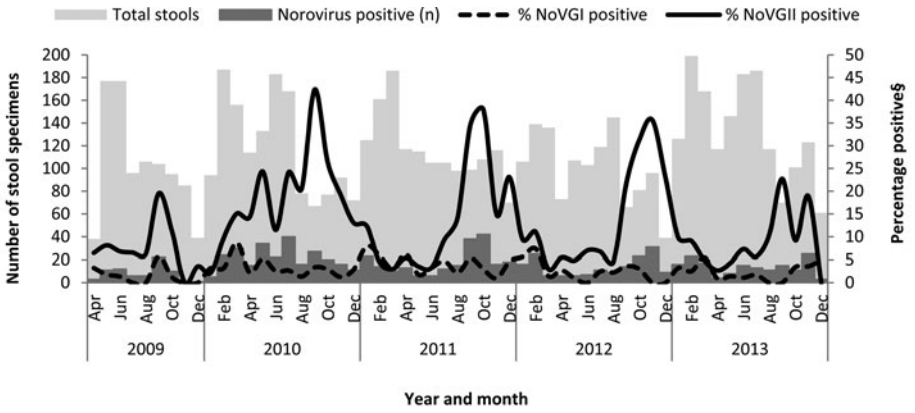

Fig. 1. Total stools tested, number of NoV-positive specimens and the percentage positive for NoV GI and GII by month between 2009 and 2013. §Percentage-positive NoV GII or GI = number of NoV GII- or GI-positive cases per month/total number of specimens screened per month × 100; secondary axis scale drawn to 50%.

2·4 times greater odds than HIV-uninfected children of experiencing diarrhoea duration of 3 days or more compared with 2 days or less (95% CI 1·2–4·9; $P = 0{\cdot}018$; Table 2).

DISCUSSION

The study provides epidemiological features of NoV infection in hospitalized children <5 years in a setting with high HIV prevalence. The study demonstrated the importance of NoV in hospitalized children aged <2 years and identified diarrhoea and vomiting with limited dehydration as prominent clinical characteristics in NoV cases. Amongst NoV-infected children, HIV-infection was associated with prolonged hospitalization and increased mortality. It is the first study of its kind in SA that investigates NoV detection in HIV-infected children and NoV diarrhoea post rotavirus vaccine introduction. The study also spans more than 12 months, includes more than one hospital and geographic area and assesses data on clinical characteristics and the environmental features associated with NoV detection in children <5 years.

An earlier study in SA in 2008 detected NoV in 14% of hospitalized children <2 years [23]. Previous studies in Africa have also identified NoV in an average of 13·5% of diarrhoea cases [9]. Similarly, a worldwide systematic review of studies conducted between 1990 and 2008 estimated that NoV was responsible for 12% of severe diarrhoea cases in children <5 years [3]. These results are comparable with the current study findings with NoV detection (GI and GII strains) at 15% in hospitalized South African children.

NoV has often been described as 'winter vomiting disease' due to the winter seasonality and occurrence of vomiting symptoms [6]. Globally, NoV infections seem to be more common during the winter months [24, 25]. However, reports from Spain in 2001 and the UK in 2002 show that summer peaks are occasionally present [26, 27]. In SA, NoVs were detected throughout the year with detection increasing during the warmer spring and summer months in SA (between September and November). The results obtained in this study are similar to those obtained by Mans *et al.* at two hospitals in Gauteng Province in 2008 [23].

During 2010, NoV GII strains were detected at levels above 20% for 5 months (May and July–October), with an uncharacteristic autumn/winter predominance. Whether or not this increase was related to increased tourist activity and the introduction of new GII and GI strains during the Soccer World Cup in SA in June and July of 2010 or the decrease in rotavirus due to the introduction of the rotavirus vaccine in 2009 is unknown. Since the New Orleans 2009 GII.4 variant was first detected in SA in 2008 and was regularly detected from April 2009 to December 2012, the increase is unlikely to be related to changes in the dominant GII.4 variant [28].

NoV GII strains identified in a 2008 study in Pretoria indicated that eight GII (GII.1, GII.4, GII.6, GII.7, GII.10, GII.13, GII.14, GII.16) strains

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Table 2. *Univariate and multivariable analysis of demographic data, clinical characteristics and environmental features associated with NoV detection in HIV-infected and HIV-uninfected children (n = 418)*

Parameter	HIV-infected n/N (%)	HIV-uninfected n/N (%)	Univariate analysis		Multivariable analysis	
			OR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
Gender						
Male	20/44 (45·5)	218/374 (58·3)	Ref			
Female	24/44 (54·5)	156/374 (41·7)	1·7 (0·9–3·1)	0·107		
Sentinel site						
CHBH	16/44 (36·4)	232/374 (62·0)	Ref			
EDH	5/44 (11·4)	42/374 (11·2)	1·7 (0·6–5·0)	0·311		
MKH	19/44 (43·2)	69/374 (18·5)	4·0 (1·9–8·2)	<0·001		
MPH	4/44 (9·1)	31/374 (8·3)	1·9 (0·6–6·0)	0·289		
Year						
2013	3/44 (6·8)	51/374 (13·6)	Ref			
2012	6/44 (13·6)	46/374 (13·6)	2·2 (0·5–9·4)	0·279		
2011	7/44 (15·9)	92/374 (24·6)	1·3 (0·3–5·2)	0·718		
2010	25/44 (56·8)	140/374 (37·4)	3·0 (0·9–10·5)	0·079		
2009	3/44 (6·8)	45/374 (12·0)	1·1 (0·2–5·9)	0·882		
Diarrhoea duration (in days)						
⩽2	12/44 (27·3)	170/369 (46·1)	Ref		Ref	
⩾3	32/44 (72·7)	199/369 (53·9)	2·3 (1·1–4·6)	0·020	2·4 (1·2–4·9)	0·018
Vomiting duration (in days)						
⩽2	25/44 (56·8)	261/372 (70·2)	Ref			
⩾3	19/44 (43·2)	111/372 (29·8)	1·8 (0·9–3·4)	0·074		
Dehydration (as assessed by a clinician)						
None–mild (1–5%)	27/40 (67·5)	280/344 (81·4)	Ref			
⩾6% (moderate/severe)	13/40 (32·5)	64/344 (18·6)	2·1 (1·0–4·3)	0·041		
Bloody stools						
No	36/44 (81·8)	333/370 (90·0)	Ref			
Yes	8/44 (18·2)	37/370 (10·0)	2·0 (0·9–4·6)	0·105		
Number of days admitted to hospital						
⩽2	11/43 (25·6)	221/368 (60·1)	Ref		Ref	
⩾3	32/43 (74·4)	147/368 (39·9)	4·4 (2·1–9·0)	<0·001	4·3 (2·1–8·9)	<0·001
Number of inhabitants living in house						
⩽6	31/35 (88·6)	268/283 (94·7)	Ref			
⩾7	4/35 (11·4)	15/283 (5·3)	2·3 (0·7–7·4)	0·160		
Sanitation type						
Flush toilet	14/44 (31·8)	222/372 (59·7)	Ref			
Other	30/44 (68·2)	150/372 (40·3)	3·2 (1·6–6·2)	0·001		
Outcome						
Discharged	39/43 (90·7)	365/369 (98·9)	Ref		Ref	
Died	4/43 (9·3)	4/369 (1·0)	9·4 (2·3–38·9)	0·002	6·9 (1·5–31·9)	0·013
NoV GII mixed pathogen infections						
NoV GII +rotavirus	5/44 (11·4)	89/372 (23·9)	0·4 (0·2–1·1)	0·067		

CHBH, Chris Hani Baragwanath Hospital; EDH, Edendale Hospital; MKH, Matikwane Hospital; MPH, Mapulaneng Hospital; HIV, human immunodeficiency virus.
Only variables with *P*-values <0·2 in the univariate analysis were reported in the table and included in the multivariable model.

were circulating [23]. While genotyping data from 2010 revealed that GII.2, GII.3, GII.4, GII.12 and GII.17 strains were circulating [29], only limited typing was performed on the 2010 GII strains (39/216; 18%). Therefore, with the current data available, the introduction of new virus genotypes or variants as the cause of the increased detection of NoV GII and GI infections in 2010 could not be established. Continued surveillance and genotyping of NoV strains is recommended to identify the introduction of new

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variants or types in the South African paediatric population.

In agreement with recent NoV data [6, 30], vomiting was a significant symptom associated with NoV detection in South African children hospitalized for diarrhoea. However, children with NoV were not dehydrated and admitted for a median of 2 days. These results suggest that children with NoV detected are probably brought to the hospital for the treatment of numerous vomiting episodes rather than severe dehydration or diarrhoea. In NoV challenge studies, vomiting was more prevalent than diarrhoea and nearly half of the participants who experienced vomiting did not report diarrhoea symptoms [30]. Based on the surveillance case definition, NoV cases presenting as only vomiting or treated in the outpatients department will have been missed. Furthermore, a large proportion of children with NoV may not have required medical treatment resulting in an under estimation of the true prevalence of NoV diarrhoea in children <5 years in SA.

NoV GI strains have been detected at similar levels to GIIs in river water in SA [28] but were not seen at comparable levels in hospitalized patients (12% *vs.* 3%). These results suggest that either NoV GI strains survive longer in the environment or patients shed NoV GI strains at higher titres [31] or NoV GI strains cause less severe or asymptomatic infections. Furthermore, these cases may be treated at home or at a clinic level, which means that they would not be captured by the study surveillance system.

The exception to the perceived mild nature of GI infections may be in HIV-infected children. The current study indicated increased detection of single GI infections in HIV-infected children compared with HIV-uninfected children (9% *vs.* 1%) in children admitted for the treatment of diarrhoea. In addition, the median NoV GI C_t values in HIV-infected children were also significantly lower compared with HIV-uninfected children ($P = 0{\cdot}05$). These results combined could be used as an indicator of NoV GI disease severity in HIV-infected children. Similar differences in disease severity in RV and NoV GII infections in HIV-infected and HIV-uninfected patients have not been reported. However, this may be due to our inability to distinguish subtle differences in clinical severity between the two populations rather than the absence i.e. diarrhoea infections associated with RV and NoV GII strains are severe regardless of immune status.

A study by Groome and Madhi [32] estimated that 26% of children admitted with acute gastroenteritis to CHBAH, Johannesburg between March 1998 and October 2000 were HIV infected, based on the prevalence rate in women attending antenatal clinics in the area. While the study did not detect more frequent RV infections in HIV-infected children, the absolute burden of disease in these children was twofold higher than in HIV-uninfected children [32]. In addition, HIV-infected children were more likely to be hospitalized for a longer period and had a higher case fatality rate [32]. A similar trend was noted in the current study where NoV strains were detected at similar levels in HIV-infected and HIV-uninfected children. However, NoVs were associated with a higher case fatality rate in HIV-infected children compared with HIV-uninfected children.

Population denominators were unavailable for the surveillance sites and; therefore, the incidence and increased risk of NoV associated with HIV infections could not be calculated. However, the mean HIV prevalence among children 0–4 years with NoV detected was 9·7% (43/443) between 2009 and 2013 while the HIV prevalence among children 0–4 years in the general population was 3·3% in 2008 [33], a threefold increase. These results suggest that HIV infection may be associated with hospitalization of NoV cases in children and further study may be warranted.

Analysis of the environmental features associated with NoV detection found that NoVs were less common in diarrhoeal patients living in informal housing (aOR 0·7; $P = 0{\cdot}021$) compared with brick housing or using external water sources (aOR 0·7; $P = 0{\cdot}045$) compared with indoor water. The role of continuous environmental exposure to NoV strains in these settings is unclear. Future study including control groups from the same community without diarrhoea would be required to interrogate these findings further.

The study has several limitations that should be considered when evaluating the findings. Missing data were dealt with by pairwise deletion and information selection bias may be present affecting the estimates and associations observed. The comparison groups used for the analysis were not strict control groups with the absence of diarrhoea and may have resulted in an underestimation of the clinical and environmental features associated with NoV infections. While patients who refused to participate in the study were noted, no additional information was gathered from these patients and, therefore, there may be non-participation biases unaccounted for in the analysis. The study enrolled children who were

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admitted to hospital overnight and was limited to moderate-to-severe diarrhoea. Therefore, any findings are restricted to this category of diarrhoeal infections and should be extrapolated to less severe outcomes with care. Not all the participants included in the study had specimens screened for all enteric pathogens. Limited clinical specimen volumes resulted in reduced screening for parasites. This may have resulted in an underestimation of mixed infections and affected the analysis of single pathogen infections.

Asymptomatic infections and prolonged shedding of enteric pathogens complicates epidemiological evaluations when trying to establish whether a pathogen is associated with an illness. Asymptomatic NoV infections have been described in paediatric patients at frequencies between 11·6% and 49·2% in a recent review [6]. An Australian study recorded NoV GII shedding in seven young children for 2–100 days [34]. In addition, NoV shedding in immunocompromised patients has been recorded for up to 898 days [35]. The current study did not have any data on asymptomatic NoV infections or duration of NoV shedding and the frequency of mixed pathogen NoV cases ranged from 77% to 66%.

However, efforts have been made to translate the faecal NoV viral load measurements or C_t values as a proxy measure into disease-attribution cut-offs [36]. An English study calculated an optimal C_t cut-off for children <5 of 30 [36]. As no South African C_t-disease-attribution analysis of the NoV real-time detection assay has been performed, the current study included all specimens positive for NoV irrespective of C_t value. An interesting finding was the statistically significant difference in the median C_t values between sole pathogen and mixed pathogen cases (27·2 *vs.* 32·0; $P = 0{\cdot}006$). Additional research establishing the C_t-disease-attribution cut-off and investigating NoV shedding in SA should be considered.

The study determined the detection rate, clinical characteristics and environmental features associated with NoV detection in hospitalized children <5 years in SA. Furthermore, the study identified NoV GI strains as a potentially serious pathogen in vulnerable HIV-infected patients and demonstrated an association between NoV detection and mortality in this group. Future monitoring of NoV detection rates and variants circulating in the South African population may aid in enumerating diarrhoea burden due to the introduction of new NoV strains.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0950268817000668.

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DECLARATION OF INTERESTS

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ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Ethical approval for the study was obtained from the Human Research Ethics Committee (Medical), University of Witwatersrand (M091018), the Biomedical Research Ethics Committee, University of Kwa-Zulu Natal (BF074/09) and the Faculty of Health Sciences Research Ethics Committee, University of Pretoria (278/2015).

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