Diverse sapovirus genotypes identified in children hospitalised with gastroenteritis in selected regions of South Africa

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Abstract:

Background: Sapoviruses (SaVs) are recognised as causative agents of gastroenteritis worldwide. However, data on the genetic diversity of this virus in Africa is lacking, particularly in the form of current long-term studies.

Objective: To determine the genetic diversity of SaVs in children hospitalised with gastroenteritis in South Africa (SA).

Study design: From April 2009 to December 2013, SaVs were characterised from stool specimens from children hospitalised with gastroenteritis in four provinces of SA.

Results: Fourteen different SaV genotypes were identified from the 221 strains that were characterised. Genogroup (G) IV predominated overall and was detected in 24% (53/221) of specimens. The other identified genotypes included six belonging to GI (GI.1, GI.2, GI.3, GI.5, GI.6, and GI.7) and seven belonging to GII (GII.1, GII.2, GII.3, GII.4, GII.5, GII.6 and GII.7).

Conclusion: This study has provided the first comprehensive data on the genetic diversity of SaVs in a clinical setting in SA, contributing to the global knowledge of this virus.

Keywords: Genotyping, molecular diversity, paediatric, sapovirus, South Africa

Abbreviations: Adenovirus, AdV; Doctor George Mukhari hospital, DGM; Genotype, G; Gauteng, GP; KwaZulu Natal, KZN; Mpumalanga, MP; Norovirus, NoV; Polymerase Chain Reaction, PCR; Rotavirus, RV; Rotavirus Sentinel Surveillance Programme, RSSP; South Africa, SA; Sapovirus, SaV; Western Cape, WC.

1. Background

Sapovirus (SaV), a member of the *Caliciviridae* family, is a causative agent of gastroenteritis [1]. The virus is predominantly reported in outbreaks and sporadic cases of gastroenteritis in children [2-6], but has more recently been reported in adult populations [7-10]. Sapoviruses are small, non-enveloped, single-stranded RNA viruses. The genome is 7.3-7.5 kb in length and is arranged in two or three open reading frames [11]. The SaV genus is divided into at least five genogroups (GI-GV) based on the capsid gene sequence, of which GI, GII, GIV and GV include strains that infect humans [12]. Genogroups I and II are each further divided into seven genotypes [13].

There have been few long-term studies on the genetic diversity of SaVs in children with sporadic acute gastroenteritis. Currently the largest study, spanning nine years (2002-2011), has originated from Japan where SaVs were analysed in outpatients with gastroenteritis and GIV predominated until 2007 after which it was replaced by GII.3 [4]. In a two-year (2005-2007) study in Denmark, SaVs were characterised from children with gastroenteritis referred by general practitioners and GI.1 predominated [2]. These studies did not focus primarily on hospitalised children with severe gastroenteritis and even fewer studies on SaVs have been conducted in Africa. A recent study (2009-2010) from Burkina Faso reported nine SaV genotypes in children with gastroenteritis, with infections generally associated with milder symptoms [14]. In Tunisia, SaVs were reported in paediatric outpatients with gastroenteritis and only GI.1 was identified [15]. Sapoviruses have also been reported in patients hospitalised with gastroenteritis in Tanzania [16] and in Malawi [17]. Genotypic characterisation was not conducted in the Tanzanian study [16] and in Malawi, only GII strains were identified [17]. Five different SaV genotypes were identified in HIV-seropositive children in Kenya [18]. In South Africa (SA), SaVs were first reported in 1997 in 0.4% of patients with sporadic gastroenteritis [19]. In 2008, SaVs were reported in 4% (10/245) of specimens from children hospitalised with gastroenteritis in the Pretoria region of Gauteng (GP), SA, but the strains were not further characterised [20].

There are no recent comprehensive studies on SaVs in southern Africa. Many studies on SaVs span less than two years and are restricted to one geographical region of a country. This may influence the diversity of genotypes identified.

2. Objectives

To determine the genetic diversity of SaVs in children hospitalised with gastroenteritis over a five-year period, from several provinces within SA.

3. Study design

3.1. Specimen collection and processing

From April 2009 to December 2013, 296 out of 477 SaV-positive specimens were referred from the Centre for Enteric Diseases, National Institute for Communicable Diseases, for further genotypic characterisation. As part of the ongoing Rotavirus Sentinel Surveillance Programme (RSSP), all specimens were screened for SaV and other enteric viruses [21]. Specimens were collected from children younger than six years old who were hospitalised with gastroenteritis in four provinces of SA (GP; KwaZulu Natal, KZN; Mpumalanga, MP and the Western Cape, WC). In addition, SaV-positive specimens were also received from a hospital on the GP-North West border (DGM) for characterisation of the strains present. The SaV-positive study population had a median age of 10 months and gender ratio of 1:0.66 male:female. All specimens were stored at 4°C until processing. Stool suspensions were prepared as approximate 10% weight/volume in sterile water.

3.2. Nucleic acid extraction and reverse transcription

Total nucleic acid was extracted from 200 µl stool suspension using the MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) on the automated MagNA Pure platform (Roche Diagnostics) or from 160 µl stool suspension using the Qiagen Viral RNA Mini Kit (Qiagen, Hilden, Germany) on the automated QIAcube platform (Qiagen). Reverse transcription was performed as previously described [24].

3.3. Amplification of SaV partial capsid gene

Approximately 300 bp of a partial 5′-region of the SaV capsid gene was amplified for characterisation. The region was amplified by nested polymerase chain reaction (PCR) using published primers [22,23] as previously described [24], with minor adjustments. Briefly, in the first round of PCR 5 µl cDNA was added to a 50 µl reaction with 0.4 µM of each primer (SV-F13, SV-F14, SV-DS3 and SV-DS4) and 1.25 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA), with cycling parameters as previously described [24]. The second round of PCR was performed in a 50 µl reaction containing 2 µl of product from the first round of PCR, 0.4 µM of each primer (SaV1245Rfwd, SV-DS5 and SV-DS6) and 1.25 U AmpliTaq Gold DNA polymerase. Amplicons were visualised under UV light following electrophoresis through a 1.5% agarose gel and staining with ethidium bromide. If no bands of the correct size (430 bp) were present, PCR was repeated with a different primer set (SaV124F, SaV1F, SaV5F, SV-R13 and SV-R14 for the first PCR and SaV1245Rfwd and SV-R2 for the second PCR) and the same cycling conditions to yield a 340 bp amplicon.

3.4. Sequencing and phylogenetic analysis

Sapovirus amplicons were directly sequenced in both directions using the ABI PRISM BigDye® Terminator v. 3.1 Cycle sequencing kit (Applied Biosystems) on an ABI 3130 automated analyser (Applied Biosystems). M13(-21) and M13-Rev primer sequences were added to the 5′-end of the genotyping primers to facilitate sequencing. Nucleotide sequences were edited and analysed using Sequencher™ 4.9 (Gene Codes Corporation, Ann Arbor, MI) and BioEdit Sequence Alignment Editor (V.7.0.9.0) [25]. BLAST-n was used to determine the most closely related nucleotide sequences in GenBank [26]. Sequences were aligned with reference SaV strains, selected according to Oka et al. [13], using MAFFT Version 6 (https://align.bmr.kyushuu.ac.jp/mafft/online/server/). Phylogenetic analysis was performed in MEGA6 [27] using the neighbour-joining method with 1000 bootstrap replicates. Genotypes were assigned based on clustering with reference strains in the phylogenetic tree. Nucleotide sequences were submitted to GenBank: KC962463-510 and KP196379-566.

3.5 Statistical analysis

Descriptive statistics were analysed using STATA12 software. Chi-square test was used to determine statistical significance (p values ≤ 0.05).

4. Results

From April 2009 to December 2013, 221 SaV strains were characterised from 296 SaV-positive specimens. The median age of the children from whom SaVs strains were genotyped was 11 months (Interquartile range: 7.2-16.5 months) and the male:female ratio was 1:0.65. Genotypes were assigned based on clustering with reference strains in the phylogenetic tree (Fig.1A) and 14 different SaV genotypes were identified from the 221 characterised strains (Table 1).

The genetic distribution of SaVs was not consistent for the different provinces in SA and the number of detected genotypes varied per province (Fig. 2). Thirteen genotypes were identified in GP, 12 in WC, 11 in KZN and eight in MP. Seven (GI.1, GI.2, GI.3, GII.1, GII.2, GII.4 and GIV) of the 14 genotypes were detected in all four provinces. KwaZulu Natal had the most varied genetic distribution when compared to the other provinces. Genotype II.1 predominated (25%), followed by GII.4 (19%) and GIV (18%). This differed from the other provinces where GIV was one of the two most frequently identified genotypes. The two predominant genotypes in KZN, GII.1 and GII.4, were significantly more frequently reported in KZN than WC (11% for GII.1; 6% for GII.4), the province with the second most frequent occurrence of these particular genotypes ($p = 0.0368$ for GII.1; $p = 0.0156$ for GII.4). These significant differences emphasise the different genotypic profile for SaV in KZN when compared to the other provinces.

Other enteric viruses were present in 43% (95/221) of SaV-positive specimens, the majority (76%) of which were co-infected with one other virus. Co-infections included adenovirus (AdV), astrovirus, bocavirus, norovirus (NoV) GI, NoV GII or rotavirus (RV) [21]. The most frequent co-infection was AdV (43%), followed by NoV GII and RV in 21% of specimens each. There was no significant correlation between the SaV genotype identified in a specimen and the presence of multiple viruses in that specimen ($p = 0.3778$).

Table 1. Sapovirus genotypes identified in children hospitalised with gastroenteritis from 2009 to 2013 in South Africa.

	No. of strains (%)					
Genotype	**2009**	**2010**	**2011**	**2012**	**2013**	**Total**
GIV	3 (27)	5 (25)	4 (15)	13 (28)	28 (24)	53 (24)
GI.2	1 (9)	4 (20)	10 (37)	2 (4)	23 (20)	40 (18)
GI.1	3 (27)	1 (5)	2 (7)	7 (15)	17 (15)	30 (14)
GII.1	1 (9)	2 (10)	1 (4)	9 (20)	14 (12)	27 (12)
GII.4	1 (9)			1 (2)	17 (15)	19 (9)
GII.3			3 (11)	8 (17)	3 (3)	14 (6)
GII.5		3 (15)	2 (7)	2 (4)	1 (1)	8 (4)
GI.5			1 (4)	2 (4)	3 (3)	6 (3)
GI.3	1 (9)		1 (4)	1 (2)	3 (3)	6 (3)
GII.2		1 (5)	2 (7)	1 (2)	2 (2)	6 (3)
GI.6		3 (15)	1 (4)			4 (2)
GI.7	1 (9)	1 (5)			2 (2)	4 (2)
GII.6					2 (2)	2 (1)
GII.7					2 (2)	2 (1)
Total	11	20	27	46	117	221

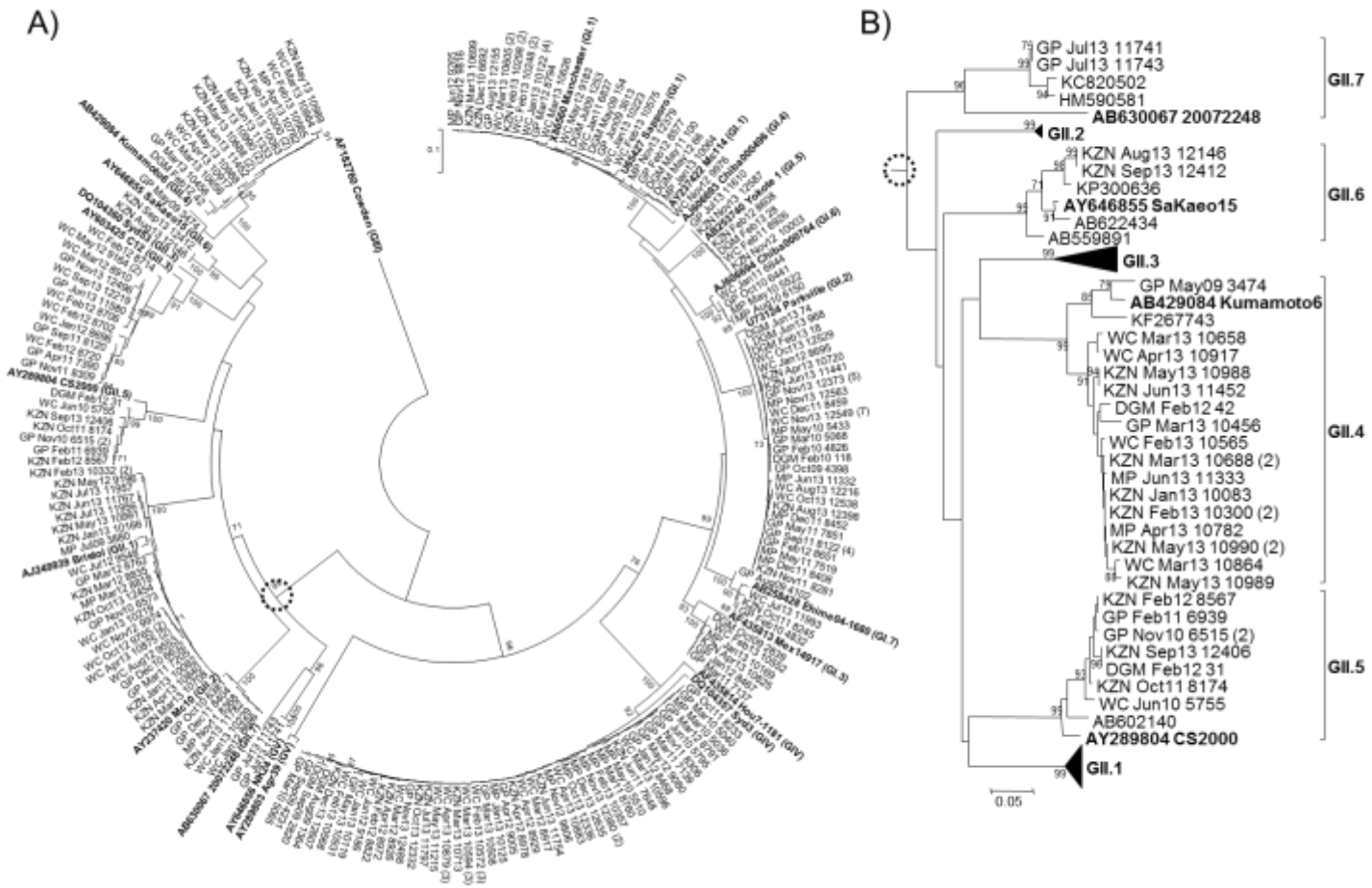

Figure 1A) Phylogenetic analysis of the partial capsid gene of sapoviruses (SaVs) detected in South Africa and **B)** an enlargement of the SaV genogroup II section of the tree. Neighbour-joining phylogenetic analysis of the partial capsid region (approximately 300 bp) of 221 SaV strains. The SaV reference strains are indicated by GenBank accession numbers and are shown in bold. The tree was condensed by removing strains from the study that clustered together and originated from the same province and in the same month of the same year. The number of strains was then indicated in brackets next to the representative strain. Statistical significance was evaluated with a 1000 bootstrap replicates and bootstrap support of >70% is indicated. The scale bar represents nucleotide substitutions per site.

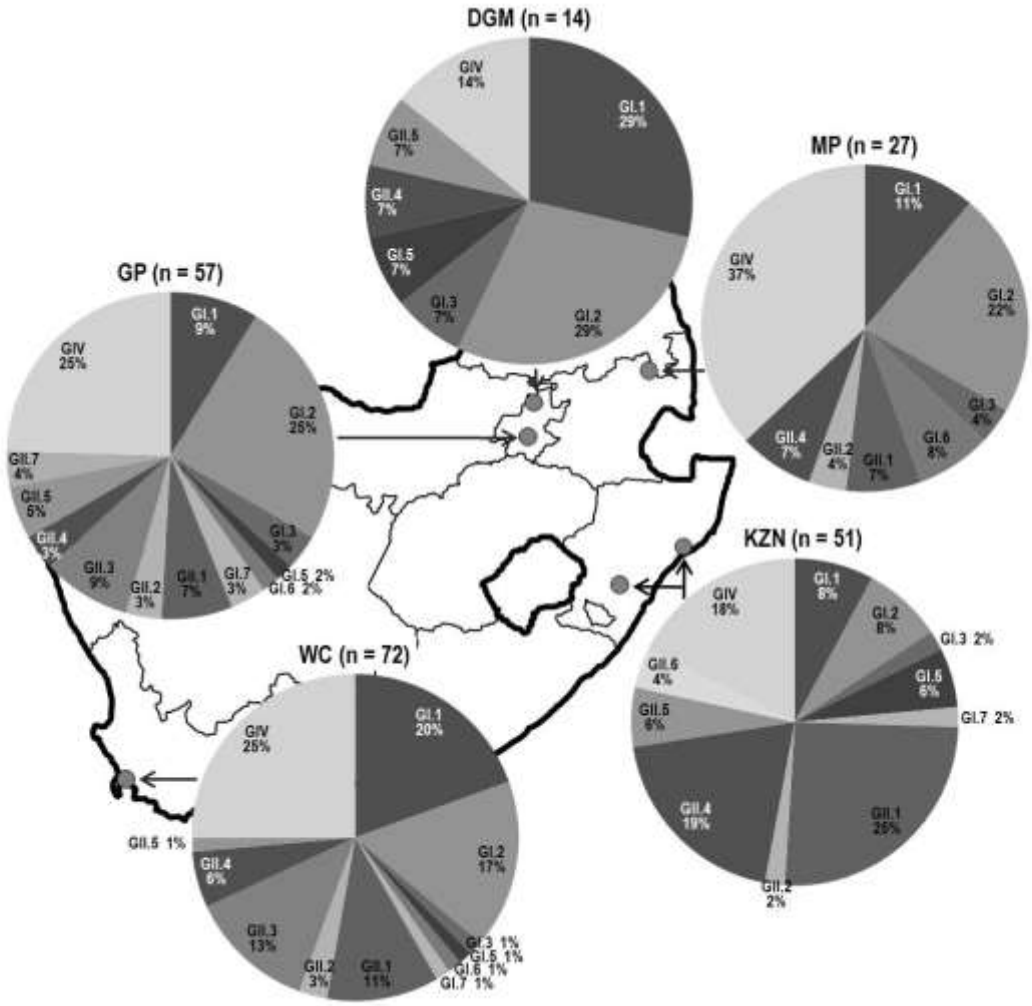

Figure 2 A Map indicating the provincial distribution of SaV genotypes within South Africa. Each grey circle represents a specimen collection site within a province (GP, Gauteng; MP, Mpumalanga; KZN, KwaZulu Natal; WC, Western Cape). Specimens from DGM (a site on the GP-North West province border) were not routinely available. However, those that were available and were SaV-positive were genotyped. The number of genotyped strains (n) is indicated next to the province/collection site name.

Table 2
Sapovirus genotypes detected in South Africa and their closest matches from other countries.

	Closest GenBank matches			
Genotype	% Identity[a]	Year	Country	Accession number
GI.1	98–100%	2005	Denmark	GQ340699
			Nicaragua	JX029963
		2006	Russia	FJ214052
		2007	Thailand	KC608715
		2008	United Kingdom	FJ986146
		2010	Brazil	KF924389
			Burkina Faso	KP300617
GI.2	99–100%	2006	Russia	FJ214045
		2008	Iran	GU376748
		2010	Brazil	KF924388
		2012	China	KF974441
			Japan	AB894245
GI.3	99%	2009	Japan	AB622459
			Japan	AB689815
GI.5	98–99%	2001	Thailand	EU363881
		2003	Russia	AY538715
		2005	Japan	AB429094
GI.6	99%	2003	Russia	AY538717
			Vietnam	DQ372736
		2008	Japan	AB622435
GI.7	97–99%	1990	Pakistan	AB181133
		2001	Thailand	EU363885
		2004	Japan	AB258428
GII.1	96–99%	2005	Nicaragua	JX162645
		2006	Denmark	GQ340722
		2008	United Kingdom	FJ986149
			Vietnam	JF262614
		2010	China	KF495122
GII.2	98–99%	2000	Thailand	EU363877
		2001	Australia	DQ104362
		2003	United States	HM800904
		2006	Denmark	GQ340710
		2008	Japan	AB622433
GII.3	95–99%	2010	Japan	AB622456
			Nicaragua	KF361382-5
GII.4	90–94%	2000	Kenya	KF267743
		2003	Japan	AB429084
GII.5	93–95%	2005	Japan	AB602140[b]
GII.6	91–97%	2004	Thailand	AY646855
		2008	Japan	AB622434
			Spain	AB559891[b]
		2009	Burkina Faso	KP300636
GII.7	97–98%	2010	United States	HM590581
		2011	China	KC820500
GIV	96–99%	2003	Venezuela	HM214146
		2004	Australia	DQ104357
		2007	United States	HM800912
		2008	United Kingdom	FJ986151
		2010	Burkina Faso	KP300626
		2012	China	KF649156

[a] Denotes the range of percentages at which different South African strains identified in this study, within a specified genotype, are identical to the listed GenBank matches.
[b] Environmental samples, all other SaVs were detected in stool specimens.

The predominant genotype identified in this study was GIV, comprising 24% of the genotyped strains and was followed by GI.2 at 18% (Table 1). Many of the SaV strains from SA were closely related to SaVs identified elsewhere in the world, with nucleotide identities ranging from 90% to 100% over the typed region (Table 2). Within GI, SA strains were 97-100% identical over the genotyped region to the closest matches from GenBank. Genogroup IV strains from SA were also highly identical (96-99%) to SaVs from other countries on several continents. More diversity was seen in GII where nucleotide identities ranged from 90-99% between the SA SaVs and the closest GenBank matches. Within GII (GII.4, GII.5, GII.6 and GII.7), SA strains grouped separately from the closest SaVs from other countries (Fig. 1B). Sapoviruses from SA were not associated with the most closely related GenBank strains in terms of country of origin or time period of occurrence.

5. Discussion

This is the first report to provide comprehensive data on the genetic diversity of SaVs circulating in a paediatric population hospitalised with gastroenteritis in southern Africa. The study was conducted over a five-year period and includes specimens from several provinces within SA. A total of 221 of the 477 SaV-positive stool specimens were successfully genotyped. The median age and gender ratios of the SaV-positive population and the sub-population from which SaVs were characterised were highly similar. This suggests that the population from which SaVs were successfully genotyped is an adequate representation of the overall SaV-positive population. A notable diversity of SaVs was observed in the study, including 14 different genotypes. Currently 16 SaV genotypes are known to infect humans and GI.4 and GV were the only two that were not identified. Other studies have reported lower SaV genetic diversity [2,4,28-31], including a nine-year (2002-2011) study from Japan in which 11 genotypes were identified from 139 specimens [2] and a two-year (2005-2007) study from Denmark during which seven genotypes were characterised from 91 specimens [4]. The five-year time period of the study and the four different provinces in which specimens were collected are both likely to have influenced the genetic diversity seen in this study. This emphasises the need to conduct long term studies, preferably including more than one study site within a country. It is also possible that a higher genetic diversity of SaVs is circulating in children with gastroenteritis on the African continent. A recent study from Burkina Faso

reported nine different genotypes characterised over an 11-month period [14] and a previous study from Kenya reported five genotypes from six specimens [18].

A wide genetic distribution of SaVs was observed in each of the four provinces. The number of genotypes ranged from eight in MP to 13 in GP. The number of detected genotypes is likely influenced by the number of strains characterised from a particular province, which varied greatly from 27 strains (MP) to 72 strains (WC). The genotypic distribution of SaVs in KZN differed significantly from other provinces. In KZN specimens were collected from a combination of rural and urban sampling sites whereas in the other provinces, the collection sites were either urban (GP, WC) or rural (MP).

Sapovirus single infections were present in 57% of the specimens from which SaVs were genotyped. Adenovirus was the most frequent co-infection and the possibility of a respiratory AdV co-infection cannot be excluded. Mixed infections, involving SaVs and other enteric viruses such as RV, AdV and NoV, at frequencies ranging from 17-26% have been reported previously [17, 30, 33].

The predominant genotype detected in children hospitalised with gastroenteritis in SA over the five-year period, GIV, was identified in each year and in all four provinces. The GIV strains from SA shared high nucleotide identity (96-99%) with SaVs identified in patients with gastroenteritis in several countries over the past ten years, including Burkina Faso [14], China [10], the United Kingdom (UK), the United States (US) [9] and Venezuela [33]. This confirms a worldwide distribution of the genotype as well as indicating its presence in southern Africa. It also suggests stability of the genotype as older strains from 2003 shared high nucleotide identities over the partial capsid gene region to strains as recent as 2012 and 2013. Genogroup IV has been frequently reported in children as well as adults with gastroenteritis [4,10,32,33] and particularly in SaV-associated outbreaks [7,8,34,35]. The second most prevalent and widely-distributed genotype, GI.2, is also frequently reported in patients with gastroenteritis worldwide [6,8,10,30,31]. Strains from SA shared even higher nucleotide identity (99-100%) with SaVs from other countries, particularly recent strains reported in Asia (2012) and earlier strains from Brazil, Iran and Russia (2006-2010).

Genotype II.1 was the most frequently detected GII genotype in SA. The SA strains shared high nucleotide identity (96-99%) with strains from Europe, Asia and South America (2005-2010) [2, 31, 36]. Genotype II.2 strains from SA were also closely-related (98-99% nucleotide identity) to SaVs from various countries from 2000 to 2008 [2, 9, 29], whereas the GII.3 strains matched closely (95-99% nucleotide identity) to SaVs recently detected in Japan (2010) and Nicaragua (2010) [31] only. The GII.4 SA strains shared 90-94% nucleotide identity with the two closest SaVs, older strains originating from Kenya (2000) [18] and Japan (2003) [5]. In GII.5, the most closely-related SaV (93-95% nucleotide identity) was identified from wastewater in Japan (2005) [22] and in GII.6 GenBank matches with < 90% nucleotide identity over the genotyped region included SaVs detected in stool from Burkina Faso [14], Thailand [37] and Japan and river water in Spain [23], from 2004 to 2009. In GII.7, closely-related SaVs from other countries included recent strains from the US (2010) and China (2011) [38]. Genotypes II.6 and II.7 were rarely found in SA, both emerging in 2013 only and each in a single province.

In SA, recent studies on the presence of SaVs in the environment have indicated a diversity of circulating genotypes which correlates to what was found in the clinical setting of this study. Twelve of the 14 SaV genotypes identified in this study have been identified in various environmental samples in SA [24,39]. The strains circulating in the environment are highly identical to those detected in this clinical setting, with nucleotide identities ranging from 95-100%. Interestingly, SaV GIV has not been reported in surface water [39] or effluent wastewater [24] in SA and was only recently identified in one water sample collected from an irrigation pivot on a fresh produce farm in SA [40]. The differing prevalence of SaV GIV in children with gastroenteritis and in the environment highlights the need to use passive environmental surveillance in combination with clinical studies to gain an accurate understanding of the genetic diversity of the virus in SA.

This study has provided new and comprehensive data on the genetic diversity of SaVs present in a population of children with gastroenteritis. Importantly, these results reflect the diversity of SaVs in severe gastroenteritis as the children were all hospitalised with the illness. The results provide valuable

insight on the high diversity of SaV genotypes circulating in this population and the close genetic relatedness of GIV and GI SaV genotypes to strains circulating in many different countries on several continents worldwide. The study has also shown a more varied genetic relationship between GII SaVs from SA and those reported worldwide. The study has provided recent data on SaV genotypes in Africa, where data is lacking.

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6.2. Competing interests

None declared

6.3. Ethical Approval

Ethical approval for the RSSP was obtained from research ethics committees of the University of the Witwatersrand (M091018), University of KZN (BF074/09), University of Cape Town (068/2010) and University of Limpopo (MREC/P/10/2009).

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