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

Tanya Y. Murray[a,*], Sandrama Nadan[a,b], Nicola A. Page[b], Maureen B. Taylor[a]

[a] Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Arcadia, 0007 Pretoria, South Africa
[b] Centre for Enteric Diseases, National Institute for Communicable Diseases, Private Bag X4, Sandringham, 2131 Johannesburg, 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 are 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.3, GI.5, GI.6, and GI.7) and seven belonging to GII (GII.1–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.

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 population [7–10]. Sapovirus is a small, non-enveloped, single-stranded RNA virus. 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 SaVs 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].

Abbreviations: AdV, adenovirus; DGM, Doctor George Mukhari Hospital; G, genotype; GP, Gauteng; KZN, KwaZulu Natal; MP, Mpumalanga; NoV, norovirus; PCR, polymerase chain reaction; RV, rotavirus; RSSP, Rotavirus Sentinel Surveillance Programme; SA, South Africa; SaV, sapovirus; WC, Western Cape.

* Corresponding author. Fax: +27 12 325 5550.
E-mail addresses: tanyaymurray@gmail.com (T.Y. Murray), sandran@nicd.ac.za (S. Nadan), nicolap@nicd.ac.za (N.A. Page), maureen.taylor@up.ac.za (M.B. Taylor).

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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.

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

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.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