Partial Analysis of the Capsid Protein (VP1) of Human Sapovirus Isolated from Children with Diarrhoea in Rural Communities of South Africa

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Background. Viral diarrhoea is a concern in acute gastroenteritis cases among children younger than 5 years of age. Sapovirus has been noted as an emerging causative agent of acute gastroenteritis worldwide. Objective/Study Design. The aim of this study was to characterize human sapoviruses targeting the VP1 (NVR and N-terminal) region. Twenty-five samples were randomly selected from 40 sapovirus-positive samples previously detected and analyzed for the VP1 region using the One-Step RT-PCR assay. The PCR products were subjected to Sanger sequencing analysis. Results. The polyprotein segment (NVR and N-terminal) was successfully amplified from 10/25 samples. Sapovirus GI.1 was the most predominant strain (6/10; 60%), followed by SV-GII.1 (2/10; 20%) and 10% of each GI.3 and GII.3. Conclusion. Through the partial analysis of the VP1 region, this study provides more data to add on the human sapovirus genetic characterization of circulating strains in South Africa, with the proposition of further analysis of sapovirus VP1 fragments for the viral structure and function.

1. Introduction

Human sapovirus (SV) as one of the leading causative agents of diarrhoea in young children is becoming notable worldwide [1, 2]. Information on the distribution of SV genotypes can give insights into the patterns of probable transmission, immunity amongst exposed people, relevant diagnosis of the circulating strains, and development of vaccine to regulate or eradicate virulent strains [3]. Human SV has four well-known genogroups, namely, genogroups I, II, IV, and V. Although SV-GI is the most detected genogroup followed by SV-GII, these genogroups are commonly associated with acute gastroenteritis across age groups and often detected in infants [2, 4–8]. There are few studies on the detection of human SV in South Africa which have been reported in different settings, that is, on rural outpatients [9], in a longitudinal study [10], on urban hospitalized children [11, 12], and on all age groups [13].

Human SV, a single-stranded positive-sense RNA virus classified in the Caliciviridae family, has a genome estimated to be 7.7 kb in size. Amongst the three open reading frames (ORF-1, 2, and 3) documented, ORF-1 amongst other proteins contains the major capsid protein (VP1). The VP1 region is used for the classification of sapoviruses [14], and it is the most common targeted area for detection of this virus. Moreover, VP1 contains a segment which correlates with the genetic diversity and antigenicity of SV [4, 15, 16].

A variety of primers have been designed from previously accessible nucleotide sequences and tested for the detection of SVs [1, 15]. The capsid protein is described to have four regions, namely, N-terminal variable region (NVR) with 1–43 amino acid aa sequence containing 6.1% conserved residues, followed by the well-conserved section of the N-
followed by initial PCR activation at 95 °C for 15 min; then 39 cycles of three-step cycling (denaturation for 30 sec at 93°C, annealing for 30 sec at 53.5°C, and extension for 60 sec at 72°C); and final extension at 72°C for 10 min.

The location of part of the genome targeted for PCR amplification is important to understand the diversity and virulence of pathogens which are detected. Figure 1 displays the targeted locations by selected primers aligned against the VP1 segment, for amplification of human sapoviruses. In previous studies, the most reported target region of the SV genome has been the RdRp/VP1 junction [15].

2.4. Sequencing. PCR products of the amplified fragments were directly purified with a master mix of ExoSAP (Nucleics, Australia). Using the same primers (Table 1), Sanger sequencing was performed on the ABI 3500XL Genetic Analyzer POP7TM (Thermo-Scientific). The obtained nucleotide of the successful sequenced amplicons were compared against those of the reference strains available in the NCBI GenBank, using BLAST available at https://www.ncbi.nlm.nih.gov/blast [18]. The reference strains with sequences of ≥709 nucleotides (SV-GI) and ≥644 (SV-GII) nucleotides were randomly selected among BLAST hits with ≥85% similarities on the query sequences of SV strains detected from this study. For confirmation of SV genotypes, the human calicivirus typing tool available at https://norovirus.ng.philab.cdc.gov [19] was used. Phylogenetic analysis was performed to check for close relatedness of human SV strains using MEGA 11 [20]. The confirmed nucleotide sequences were submitted to GenBank under the accession numbers OK180480–OK180489.

3. Results

3.1. RT-PCR Amplification. This study reports on 40% (10/25) successful amplification of partial VP1 using SV-F11 and SV-R1 primers, while other 15 samples failed to amplify. In addition, other pairs of primers used failed to amplify in all selected samples. The unsuccessful amplifications are suggested to be as a result of degraded or limited RNA genome copies. Sixty percent (6/10) of the results was identified as SV-positive [9], were subjected to further RT-PCR amplification targeting the fragments which form the capsid protein (VP1).

2.2. Sample Selection. Twenty-five (62.5%) randomly selected and extracted RNA samples, from 40 samples which were previously identified as SV-positive [9], were subjected to further RT-PCR amplification targeting the fragments which form the capsid protein (VP1).

2.3. Amplification of Partial VP1 by RT-PCR. The One-Step RT-PCR (QIAGEN, Germany) kit was used for amplification of the selected SV-positive samples. Selected primers (Table 1) were used on the attempt to target region of the VP1 sequence [17]. The reagent mixture for SV amplification was as follows: 25 µl of reaction volume containing 5 µl of 5X One-Step RT-PCR buffer, 1 µl of One-Step RT-PCR enzyme mix, 1 µl of dNTP mix (containing 10 mM of each dNTP), 1.5 µl of 0.6 µM of each primer, 10 µl of RNase-free water, and 5 µl of the RNA sample. Amplification was done using the following conditions set on a T100 Thermal cycler (Bio-Rad, USA): reverse transcription at 50°C for 30 min; followed by initial PCR activation at 95°C for 15 min; then 39 cycles of three-step cycling (denaturation for 30 sec at 94°C, annealing for 30 sec at 53.5°C, and extension for 60 sec at 72°C); and final extension at 72°C for 10 min.
gave a hint of 87–98% similarities of all featured sequences on a phylogenetic tree (Figures 2 and 3).

The phylogenetic tree (Figure 2) presents two distinctive clusters of genotypes, SV-GI.1 and SV-GI.3, showing relatedness of their strains. The detected SV-GI.1 genotypes (OK180480–OK180485) from this study presented an isolated cluster showing their close relatedness and a slight relatedness to a strain detected in Congo from a chimpanzee (KJ858686), with a common ancestor. The GI.1 strains detected in this study showed similarities of between 93 and 96.43% with KJ858686 strain on BLAST. Although other reference strains had similarities of between 90 and 98% (BLAST) with the detected strains from this study, they showed distinct clusters when rooted by a porcine SV strain (MF766258).

The phylogenetic tree (Figure 3) displays two distinctive clusters of genotypes, SV-GII.1 and SV-GII.3, showing their strains’ relatedness. The detected GII.1 strains (OK180486 and OK180488) from this study did not cluster with any of the reference strains although sharing a common ancestor and similarity hints of >85% on BLAST. Within a GII.3 cluster, relatedness of a strain (OK180487) detected in this study with a strain (MF944258) detected in China is shown by a distinct cluster and these strains had a similarity of 89.62% on BLAST. Reference strains used on the phylogenetic tree (Figure 3) gave similarity hints of between 85% and 92.55% (BLAST) to the detected strains from this study, although they present distinct clusters from strains reported in this study.

4. Discussion

The detection of circulating human SVs has been previously reported based on the analysis of small fragments (especially the RdRP/VP1 junction) on the ORF1 segment of the viral genome [15]. In this study, the successful amplification of partial VP1 provides valuable data on circulating SV strains in South Africa.
This study reports on 40% (10/25) partial sequencing of the VP1 (polyprotein) fragment of the human SV strain circulating in the rural areas of South Africa. It has been previously reported that single-stranded RNA is generally known to be very unstable, which may lead to difficulties of generating positive results [21]. This may explain the reason that some amplicons failed to generate successful sequence in this study.

The SV GI.1, reportedly associated with gastroenteritis [23], was noted as a dominant genotype (60%; 6/10) from this study. SV-GI.1 strains were detected from 66.7% (4/6) cases of patients admitted in hospitals and 33.3% (2/6) cases of patients in clinics (Table 2). However, other strains (SV-GI.1 (20%: 2/10), SV-GI.3 (10%: 1/10), and SV-GI.3 (10%: 1/10)) were detected from patients aided in clinics. SV-GI.1 seems to be the most widespread genotype, since it was also reported as the predominant strain in a study from Brazil [4], with other genotypes detected at a low rate similar to the findings of this study.

The ORF1 of SV is proposed to encode a polyprotein that is processed by the viral protease, resulting in manifestation of proteins needed for the viral genome’s replication [14, 24]. In this study, the analysis of long amino acid sequences of NVR and N terminals was performed. It has been proposed that analysis of viral polyproteins provides understanding in mechanisms and clues leading to the drug design against viral diseases [24]. Moreover, the NVR is reportedly common to all SV genotypes [15, 16], and N-terminal has been identified as an area which can undergo significant conformational variation [14]. The analysis of the capsid protein region (VP1) is very crucial, since genotyping of SV is based on the capsid, which strongly correlates with the viral antigenic properties [16].

Sapovirus GI is known to be associated with severe diarrhoeal disease. Furthermore, GI.1 strains have previously been detected in acute gastroenteritis cases in studies...
conducted in Africa [23, 25, 26]. The phylogenetic analysis on this study showed a variability of strains detected. A discrete cluster of the detected strains (OK180480–OK180485) within genotype GI.1 suggests slight mutations on strains circulating in the Vhembe rural communities, South Africa (Figure 2). In addition, a slight relation of the cluster of GI.1 strains from this study with a strain (KJ858686) detected from a nonhuman host proposed a possibility of zoonotic transmission. Although closely relatedness of human genogroups to the nonhuman genogroups have been predictable [27], more analysis on SV strains detected from human and other mammalians should be performed to confirm zoonotic transmission.

Sapovirus-GII is mostly associated with nonsevere diarrhea cases in Africa [23, 25, 28]. Among other strains of genogroup-II, the most commonly detected is GII.1 as also reported in South Africa by Murray et al. [25]. The identified SV-GII.1 in this study clustered away from each other and reference strains, which could suggest possible mutations based on the occurred number of substitutions per site measured by the branch lengths (Figure 3).

A more successful detection rate of SVs has been commonly achieved by targeting the RdRp/VP1 junction [29, 30]. However, the analyzed segment in this study is reliable for accurate strain identification, since it is most variable and contains the maximum conserved residues on the VP1 sequence [15, 29]. From BLAST search list, there were no available data on SV strains previously reported in South Africa that could be used for phylogenetic relatedness. Moreover, analysis of a larger fragment on SV should be considered, as it creates a better possibility of genetic characterization by sequence analysis and comparison of strains around the globe. To our knowledge, this is the first study in South Africa to report on analysis of the large fragment (≥644 nucleotide long) of human sapovirus VP1.

Figure 3: Phylogenetic analysis of the partial polyprotein of human SV-GII detected in Vhembe district, South Africa, and reference strains selected from the GenBank database. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. A bar scale representing a genetic distance scale. The phylogenetic tree was deduced by the maximum likelihood method and the Kimura 2-parameter model using MEGA 11 [20, 21], based on a 644-nucleotide sequence fragment. The porcine SV (MF766258) was selected as an outgroup strain.
5. Conclusion

Our study reports on partial analysis of VP1 protein (polypeptide), suggested to be responsible for viral protein folding, dimer formation, and viral particle assembly. Predominance of SV-G1.1 was determined in this study. Studies on the antigenicity and VP1 variations of SV are needed for accessing the role of SV as an emerging virulent agent associated with diarrhoeal diseases in young children. Analysis of SV’s capsid protein should be carried out for epidemiological reference, to identify the diversity of virulent proteins produced and possibly to give insight into vaccine proposition.

Data Availability

All data supporting this results are included within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] M. Diez-Valcarce, A. K. Kambhampati, L. E. Calderwood, A. J. Hall, S. A. Mirza, and J. Vinje’, “Global distribution of sporadic sapovirus infections: a systematic review and meta-analysis,” *PloS One*, vol. 16, no. 8, Article ID e0255436, 2021.

[2] M. H. Razizadeh, A. Khiami, and M. Zarei, “Global molecular prevalence and genotype distribution of Sapovirus in children with gastrointestinal complications: a systematic review and meta-analysis,” *Reviews in Medical Virology*, vol. 32, Article ID e2302, 2022.

[3] S. Becker-Dreps, F. González, and F. Bucardo, “Sapovirus: an emerging cause of childhood diarrhoea,” *Current Opinion in Infectious Diseases*, vol. 33, no. 5, pp. 388–397, 2020.

[4] A. Cilli, A. Luchs, S. G. Morillo et al., “Surveillance and molecular characterization of human sapovirus in patients with acute gastroenteritis in Brazil, 2010 to 2017,” *Journal of Clinical Virology*, vol. 140, Article ID 104844, 2021.

[5] M. Diez-Valcarce, C. J. Castro, R. L. Marine et al., “Genetic diversity of human sapovirus across the Americas,” *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, vol. 104, pp. 65–72, 2018.

[6] M. Magwalivha, J. P. Kabue, A. N. Traore, and N. Potgieter, “Prevalence of human sapovirus in low and middle income countries,” *Advances in Virology*, vol. 2018, Article ID 5986549, 12 pages, 2018.

[7] M. F. Varela, E. Rivadulla, A. Lema, and J. L. Romalde, “Human sapovirus among outpatients with acute gastroenteritis in Spain: a one-year study,” *Viruses*, vol. 11, no. 2, p. 144, 2019.

[8] Y. Yu, X. H. Guo, H. Q. Yan et al., “Systematic review on the characteristics of acute gastroenteritis outbreaks caused by sapovirus,” *Zhonghua Liuxingbingxue Zazhi*, vol. 40, no. 1, pp. 93–98, 2019.

[9] M. Magwalivha, J. P. K. Ngandu, A. N. Traore, and N. Potgieter, “Prevalence and genetic characterisation of human sapovirus from children with diarrhoea in the rural areas of Vhembe district, South Africa, 2017–2020,” *Viruses*, vol. 13, no. 3, p. 393, 2021.

[10] J. A. Platts-Mills, J. Liu, E. T. Rogawski et al., “The MAL-ED network investigators, Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study,” *Lancet Global Health*, no. 18, pp. 30349–30348, 2018.

[11] J. Mans, J. C. de Villiers, N. M. du Plessis, T. Avenant, and M. B. Taylor, “Emerging norovirus GII.4 2008 variant detected in hospitalised paediatric patients in South Africa,” *Journal of Clinical Virology*, vol. 49, no. 4, pp. 258–264, 2010.

[12] N. Page, M. J. Groome, T. Murray et al., “Sapovirus prevalence in children less than five years of age hospitalised for diarrhoeal disease in South Africa, 2009-2013,” *Journal of Clinical Virology*, vol. 78, pp. 82–88, 2016.

[13] J. Mans, W. B. van Zyl, M. B. Taylor et al., “Applicability of Bio-wipes for the collection of human faecal specimens for detection and characterisation of enteric viruses,” *Tropical Medicine and International Health*, vol. 19, no. 3, pp. 293–300, 2014.

[14] S. W. B. Fullerton, M. Blaschke, B. Coutard et al., “Structural and functional characterization of sapovirus RNA-dependent RNA polymerase,” *Journal of Virology*, vol. 81, no. 4, pp. 1858–1871, 2007.

[15] T. Oka, Q. Wang, K. Katayama, and L. J. Saif, “Comprehensive review of human sapoviruses,” *Clinical Microbiology Reviews*, vol. 28, no. 1, pp. 32–53, 2015.

[16] M. Okada, Y. Yamashita, M. Oseto, and K. Shinozaki, “The detection of human sapoviruses with universal and genotype-specific primers,” *Archives of Virology*, vol. 151, no. 12, pp. 2503–2509, 2006.

[17] H. L. Choi, C.-I. Suh, S.-W. Park, J.-Y. Jin, H.-G. Cho, and S.-Y. Paik, “Whole-genome sequencing analysis of sapovirus detected in South Korea,” *PLoS One*, vol. 10, no. 7, Article ID e0132328, 2015.

[18] Human calicivirus typing tool, https://norovirus.ng.philab.cdc.gov.

[19] National Center for Biotechnology Information (NCBI), “Nucleotide BLAST,” https://www.ncbi.nlm.nih.gov/blast.

[20] K. Tamura, G. Stecher, and S. Kumar, “Mega 11: molecular evolutionary genetics analysis version 11,” *Molecular Biology and Evolution*, vol. 38, no. 7, pp. 3022–3027, 2021.

[21] M. Kimura, “A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences,” *Journal of Molecular Evolution*, vol. 38, no. 2, pp. 111–120, 1980.

[22] P. Kralik and M. A. Ricchi, “Basic guide to real time PCR in the molecular reference, to identify the diversity of virulent proteins produced and possibly to give insight into vaccine proposition.”

[23] A. J. Hall, S. A. Mirza, and J. Vinje, “Global distribution of sporadic sapovirus infections: a systematic review and meta-analysis,” *PLoS One*, vol. 16, no. 8, Article ID e0255436, 2021.

[24] T. Oka, Q. Wang, K. Katayama, and L. J. Saif, “Comprehensive review of human sapoviruses,” *Clinical Microbiology Reviews*, vol. 28, no. 1, pp. 32–53, 2015.

[25] M. Okada, Y. Yamashita, M. Oseto, and K. Shinozaki, “The detection of human sapoviruses with universal and group-specific primers,” *Archives of Virology*, vol. 151, no. 12, pp. 2503–2509, 2006.

[26] H. L. Choi, C.-I. Suh, S.-W. Park, J.-Y. Jin, H.-G. Cho, and S.-Y. Paik, “Whole-genome sequencing analysis of sapovirus detected in South Korea,” *PLoS One*, vol. 10, no. 7, Article ID e0132328, 2015.

[27] Human calicivirus typing tool, https://norovirus.ng.philab.cdc.gov.

[28] National Center for Biotechnology Information (NCBI), “Nucleotide BLAST,” https://www.ncbi.nlm.nih.gov/blast.

[29] K. Tamura, G. Stecher, and S. Kumar, “Mega 11: molecular evolutionary genetics analysis version 11,” *Molecular Biology and Evolution*, vol. 38, no. 7, pp. 3022–3027, 2021.

[30] M. Kimura, “A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences,” *Journal of Molecular Evolution*, vol. 38, no. 2, pp. 111–120, 1980.

[31] P. Kralik and M. A. Ricchi, “Basic guide to real time PCR in the molecular reference, to identify the diversity of virulent proteins produced and possibly to give insight into vaccine proposition.”
[24] T. Crépin, C. Swale, A. Monod, F. Garzoni, M. Chaillot, and I. Berger, "Polyproteins in structural biology," Current Opinion in Structural Biology, vol. 32, pp. 139–146, 2015.

[25] T. Y. Murray, S. Nadan, N. A. Page, and M. B. Taylor, "Diverse sapovirus genotypes identified in children hospitalised with gastroenteritis in selected regions of South Africa," Journal of Clinical Virology, vol. 76, pp. 24–29, 2016.

[26] K. Sdiri-Loulizi, M. Hassine, H. Gharbi-Khelifi et al., "Molecular detection of genogroup I sapovirus in Tunisian children suffering from acute gastroenteritis," Virus Genes, vol. 43, no. 1, pp. 6–12, 2011.

[27] K. T. Lauritsen, M. S. Hansen, C. K. Johnsen, G. Jungersen, and B. Böttiger, "Repeated examination of natural sapovirus infections in pig litters raised under experimental conditions," Acta Veterinaria Scandinavica, vol. 57, no. 1, pp. 60–65, 2015.

[28] N. Ouédraogo, J. Kaplon, I. J. O. Bonkoungou et al., "Prevalence and genetic diversity of enteric viruses in children with diarrhoea in Ouagadougou, Burkina Faso," PLoS One, vol. 11, no. 4, Article ID e0153652, 2016.

[29] T. Oka, S. P. Yamamoto, N. Iritani et al., "Polymerase chain reaction primer sets for the detection of genetically diverse human sapoviruses," Archives of Virology, vol. 165, no. 10, pp. 2335–2340, 2020.

[30] R. Rahman, S. Rahman, M. M. H. Afrad et al., "Epidemiology and genetic characterization of human sapovirus among hospitalized acute diarrhoea patients in Bangladesh, 2012-2015," Journal of Medical Virology, vol. 93, no. 11, pp. 6220–6228, 2021.