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1 # Molecular detection and characterization of sapovirus in hospitalized
2 children with acute gastroenteritis in the Philippines

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ABSTRACT

Background: Human sapovirus (SaV) is a causative agent of acute gastroenteritis. Recently, SaV detection has been increasing worldwide due to the emerging SaV genotype I.2. However, SaV infection has not been reported in the Philippines.
Objectives: To evaluate the prevalence and genetic diversity of SaV in hospitalized children aged less than 5 years with acute gastroenteritis.
Study design: Stool samples were collected from children with acute gastroenteritis at three hospitals in the Philippines from June 2012 to August 2013. SaV was detected by reverse transcription real-time PCR, and the polymerase and capsid gene sequences were analyzed. Full genome sequencing and recombination analysis were performed on possible recombinant viruses.
Results: SaV was detected in 7.0% of the tested stool samples (29/417). In 10 SaV-positive cases, other viruses were also detected, including rotavirus (n = 6), norovirus (n = 2), and human astrovirus (n = 2). Four known SaV genotypes (GI.1 [7] (Oka et al., 2015), GI.2 [2] (Sakai et al., 2001), GII.1 [12] (Gallimore et al., 2006), and GV [2] (Sakai et al., 2001)) and one novel recombinant (n = 3) were identified by polymerase and capsid gene sequence analysis. Full genome sequencing revealed that the 5′ nontranslated region (NTR) and nonstructural protein region of the novel recombinant were closely related to the GII.1 Bristol/98/UK variant, whereas, the structural protein region and 3′ NTR were closely related to the GII.4 Kumamoto6/Mar2003/JPN variant.
Discussion and conclusions: SaV was regularly detected in hospitalized children due to acute gastroenteritis during the study period. A novel recombinant, SaV GII.1/GII.4, was identified in three cases at two different study sites.

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26 ## 1. Background

27 Acute gastroenteritis remains an important cause of childhood
28 morbidity and mortality in developing countries. Sapovirus (SaV),
29 first reported in the late 1970s, is a causative agent of acute gas-
30 troenteritis [1]. Although SaV has been reported to cause milder
31 illness than rotavirus and norovirus [2], severe SaV cases and SaV-
32 associated outbreaks in different age groups have been reported,
33 which suggests its importance [3,4], However, the epidemiol-
34 ogy of SaV remains poorly understood, particularly in developing
35 countries due to the need for expensive molecular methods for
36 diagnosis.
37 SaV belongs to the Caliciviridae family and it infects both
38 humans and animals [5]. Human SaV can be divided into four
39 genogroups (GI, GII, GIV, and GV) and further subdivided into 17
40 genotypes with 20.1% or more nucleotide variation in the cap-
41 sid gene [6]. The genomes of SaV GI, GIV, and GV contain three
42 open reading frames (ORFs), whereas, that of SaV GII has two ORFs.

Abbreviations: SaV, human sapovirus; ORF, open reading frame; G, genogroup; PCR, polymerase chain reaction; nt, nucleotide; NTR, nontranslated region.

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Table 1
Primers used in this study to detect and characterize sapovirus.

Use	Primer/probe	Nucleotide sequence (5′–3′)	Position	Source
Real-time PCR	Sav1F	TTGGCCCTCGCCACCTAC	5077–5094[a]	[19]
	Sav5F	TTTGAACAAGCTGTGGCATGCTAC	5112–5135[a]	
	Sav124F	GAYCASGCTCTCGCYACCTAC	5074–5094[a]	
	Sav1245R	CCCTCCATYTCAAACACTA	5159–5177[a]	
	Sav124 probe	VIC-CCRCCTATRAACCA-MGB	5101–5117[a]	
	Sav5 probe	VIC-TGCCACCAATGTACCA-MGB	5142–5157[b]	
Capsid genotyping	SV-F13	GAYYWGGCYCTCGCYACCTAC	5074–5094[a]	[21]
	SV-F14	GAACAAGCTGTGGCATGCTAC	5074–5094[a]	
	SV-R13	GGTGANAYNCCATTKTCCAT	5876–5861[a]	
	SV-R14	GGTGAGMMYCCATTCTCCAT	5876–5861[a]	
	SV-F22	SMWAWTAGTGTTTGARATG	5154–5172[a]	
	SV-R2	GWGGGRTCAACMCCWGGTGG	5591–5572[a]	
Polymerase genotyping	SaV4579a	CCATCTGGGATGCCATTYAC	4525–4544[a]	Current study
	SaV4579b	CCNTCDGGNATGCCNTTYAC	4525–4544[a]	
	SaV1245R	CCCTCCATYTCAAACACTA	5159–5177[a]	[19]
Amplification/sequencing of whole genome	TX30SXN	GACTAGTTCTAGATCGCGAGCGGCCGCCCT$_{29}$	Oligo dT	Current study
	1F	GTGATTGGTTRGTATGGCTTC	1–21[c]	
	600R	TTTACCACYTGTCGCCACAT	581–600[c]	
	401F	TACTCCACCGATTCGCTC	401–418[c]	
	2922R	CGCCACTCATCRTACTC	2906–2922[c]	
	2709F	ATCACCCGCAACATGAC	2693–2709[c]	
	2099R	TAGCWGCCATGTAKGATTTC	2080–2099[c]	
	3491F	GCATCATYAATGGTTACCC	3489–3507[c]	
	4292R	ACCTGGCAACTRTCCATGTT	4292–4311[c]	
	5744F	CARCCVYTNATCAACCCSTT	5750–5769[c]	
	6650F	ATGGCAGTGTTCAATGTGGA	6650–6669[c]	

[a] Position in complete sapovirus GI genogroup sequence (Manchester, accession no. X86560).
[b] Position in complete genome of sapovirus GV genogroup (NongKhai-24/Thailand, accession no. AY646856).
[c] Position in complete genome of GII genogroup (Bristol/98/UK, accession no. AJ249939).

ORF1 encodes the nonstructural proteins and a major capsid protein, and ORF2 and ORF3 encode proteins whose functions are still unknown [7]. The capsid protein is thought to be cleaved from the ORF1 polyprotein by a protease and/or directly translated from subgenomic RNA [8,9]. Although several genotypes were identified in a longitudinal surveillance study in Japan during 2003–2009, SaV GI.1 was the predominant genotype, except in the winters of 2004–2005 and 2007–2008 [10]. SaV GI.1 was also the most frequently detected genotype in sporadic gastroenteritis cases [11,12]. Since 2007, several SaV GI.2 outbreaks have been reported in Europe, Asia, and North America [4,13], and GI.2 is regarded as an emerging genotype [4]. In the SaV genome, recombination typically occurs within ORF1 at the junction between the polymerase and capsid genes [14,15], which can generate antigenically distinct strains [16]. Some of these recombinants have caused outbreaks in various countries [13,17].

2. Objectives

To define the prevalence and genetic characteristics of SaV among hospitalized children with acute gastroenteritis in the Philippines from June 2012 to August 2013.

3. Study design

3.1. Study sites and sample collection

We conducted a prospective etiological study of hospitalized children aged less than 5 years due to acute gastroenteritis between June 2012 and August 2013 at three hospitals (Supplementary Fig. 1): Eastern Visayas Regional Medical Center (EVRMC), Tacloban City on Leyte Island; Ospital ng Palawan (ONP), Puerto Princesa City on Palawan Island; and Philippine Children's Medical Center (PCMC), Metro Manila. Informed consent was obtained from the parents or legal guardians of the study children before stool sampling. The age (date of birth), gender, and history of rotavirus vaccination of the participants were recorded. Stool specimens were collected within 72 h of illness onset and were transported at 4 °C to the Research Institute for Tropical Medicine (RITM) and then stored at −80 °C.

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3.2. Detection of gastroenteric viruses using reverse transcription PCR or real-time PCR

Viral nucleic acid was extracted from 20% clarified stool suspensions prepared in phosphate-buffered saline (PBS) using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). For denaturation of double-stranded RNA of rotavirus, 0.5 μL (0.3 mg/mL) of random primers (Life Technologies, Carlsbad, CA, USA), 0.5 μL of deoxynucleotide triphosphates (10 mM, Life Technologies), and 4.25 μL of the extracted viral RNA were heated at 98 °C for 5 min, followed by cooling on ice for 2 min. Then, 4.75 μL of the RT mixture, which contained 2 μL of 5× First-Strand buffer (Life Technologies), 0.5 μL of dithiothreitol (0.1 M, Life Technologies), 0.25 μL of RNaseOUT (40 U/μL, Life Technologies), 0.25 μL of SuperScript III reverse transcriptase (200 U/μL, Life Technologies), and 1.75 μL of RNase free water was added. The mixture was incubated at 25 °C for 5 min, and at 50 °C for 60 min, and then the enzyme was inactivated by incubation at 70 °C for 15 min.

Samples were screened for SaV and human astrovirus by duplex real-time PCR using described primers and TaqMan probes [18,19]. The duplex real-time PCR was performed in an ABI 7500 Fast Real-Time PCR system with TaqMan Fast Advanced master mix (Life Technologies). The detection limit was determined by standard curves generated with 10-fold serial dilutions (10^7–10) of SaV GII standard plasmid, and 100 copies/μL GII corresponded to a cycle threshold (Ct) value of 38. Therefore, a sample with a Ct value <38 was considered to be positive for SaV. Three positive and one negative controls were included to confirm the expected Ct values.

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