An Outbreak of Gastroenteritis Associated with a Novel GII.8 Sapovirus Variant-Transmitted by Vomit in Shenzhen, China, 2019

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Abstract

Human Sapovirus (SaVs) has been reported as one of the causative agents of acute gastroenteritis worldwide. We investigated an outbreak of SaV that affected 428 students of a primary school during spring activities from 24th February to 11th March 2019 in Shenzhen city, China. The single factor analysis showed that the students which saw others vomiting in classroom or playground, saw vomitus less than 1.5 meters are susceptible ($P < 0.05$). Seven of eleven fecal samples from patients were positive for GII.8 SaV genotype. In this study, we obtained the genome sequence of a sapovirus GII.8 strain SZ08 comprehensively analyzed the genetic diversity. The phylogenetic analysis showed that the GII.8 strain SZ08 formed an independent branch and became a novel variant of GII.8 genotype.

1 Introduction

There has been increasing concern about Sapoviruses (SaVs), a member of the family Caliciviridae, as a causative agent of gastroenteritis in humans in both sporadic cases and outbreaks worldwide, especially among infants and young children[1–5]. SaVs are recognized as the second most commonly etilolgical virus behind Norovirus in children with acute diarrhea after the successful deployment of the Rotavirus vaccine[6]. SaVs-associated outbreaks have occured in semi-closed institutions such as kindergardens, schools, hospitals and nursing homes for the elderly, and happen through the fecal-oral route, the primary route of transmission. SaVs transmit via exposure to SaVs-positive aerosols from feces, vomitus or the consumption of SaVs-contaminated food and water[7, 8]. SaVs-associated Acute gastroenteritis (AGE) has increased and has been accepted as a major public health problem worldwide, especially in developing countries[6, 9, 10].

The genome of SaVs consists of a single-stranded positive-sense RNA genome of approximately 7.1–7.5 kb in size and has a 3'-end poly (A) tail[7]. The SaVs genome contains either 2 or 3 open reading frames (ORFs). ORF1 encodes nonstructural proteins and a major capsid protein (VP1). ORF2 encodes a minor structural protein (VP2). ORF3 predicted for several SaVs strains encodes proteins with unknown function[2, 7, 9]. Based on complete capsid gene (VP1) sequences, multiple genetic clusters of human SaVs are divided into 4 genogroups (GI,GII,GIV,GV) with 20 genotypes (GI.1 to GI.7, GII.1 to GII.8, GIV.1 and GV.1 to GV.4)[11–13].

Recently, a novel genotype of SaVs, GII.8 strians were reported in some countries and one GII.8 SaV strain (GZ2014-l231) was detected in 2014 in Guangzhou city, Guangdong province of China[13]. In this study, an acute gastroenteritis outbreak caused by a novel GII.8 SaV variant occurred in primary school in the other city, Shenzhen city, Guangdong province of China in 2019. The genome of the GII.8 strain was analyzed and the epidemiology of this outbreak was described. Since rarely detected Norovirus genotypes, GII.17[P17] and GII.2[P16] caused sharp increasing of outbreaks in the season 2014/15 and 2016/17 in China. Therefore, the emerging of novel GII.8 SaV variant made a warning for SaV outbreaks control and prevention.

2 Materials And Methods

2.1 Ethics statement

This work was conducted in agreement with the research Ethics committee and the Institutional Review Board at the Baoan district Center for Disease Control and Prevention for human subject projection. We informed each potential subject of the details of study and written informed consent was obtained from the parents or legal guardians of all subjects.

2.2 Epidemiological investigation

Cases were defined as at least three bouts of unformed (loose and watery) stool and/or vomiting in a 24-hour period during the outbreaks. A standardized questionnaire was prepared to collect demographic data (gender and age), and illness onset data (symptoms and duration of symptom).

2.3 Nucleic acid extraction

A 0.1-g fecal specimens was diluted with 1.0 ml phosphate-buffered saline (PH 7.2) to a 10% suspension. The supernatants were collected and the viral genomes were extracted by using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and the QIAamp DNA Mini Kit (Qiagen), according to the manufacturer's instructions.

2.4 Bacteria isolation

The stool samples were analyzed for major bacterial pathogens (Escherichia coli, Salmonella, Shigella, Campylobacter and Yersinia enterocolitica) with MacConkey agar (MAC, Oxoid Ltd, Basingstoke, UK), Salmonella Shigella agar (Oxoid Ltd), Campylobacter-selective agar (Oxoid Ltd) and Yersinia Selective agar (Schiemann's CIN agar, Oxoid Ltd), respectively.

2.5 Virus detection

TaqMan real-time reverse-transcription polymerase chain reaction (RT-PCR) have been employed to test all viral nucleic acid for Norovirus and SaVs with primers and probes as described previously[14, 15]. By using enzyme immunoassay Kits and RT-PCR, Rotavirus A was identified; and Astrovirus was identified using RT-PCR; PCR was performed to detect Adenovirus[16–18]. Conventional RT-PCR was performed using primer sets including P289 and P290 (in polymerase region) and SLV5317 and SLV5749 (in capsid region) to further detect all positive samples for SaVs as described previously[19]. The PCR cycling conditions were as follows: 42℃ for 30 minutes, 95℃ for15 minutes followed by 40 cycles of 95℃ for 1 minute, 50℃ for 1 minute, and 72℃ for 1 minute, and a final 10-minute elongation at 72℃. The temperature was held at 4℃ until use.

2.6 Nearly complete genome amplification

SaV-positive samples were amplified for the complete genome. Fourteen primer sets (Table 1) were designed based on the whole genome MG674584 and MF462287. The primer set of SLV5317 and VN_3T_{20}[19] were performed to amplify the complete capsid and ORF2 sequences. A one-step RT-PCR to amplify the complete genome was performed with a OneStep RT-PCR Kit (Qiagen) according to the manufacturer's instructions.

Table 1
Primers used in this study for sapovirus detection and amplification of whole genome

	primer	Nucleotide sequence (5'-3')	position	source
Amplification of complete genome	orf-1	GAGTTGAACACGCAGTCC	42-59[a]	**This study**
		ATGATGGCACCAGTAAGG	1009-1026[a]	
	orf-2	CGCAGTGTCAGTGGTGTC	880-897[a]	
		AAGGTATGGTCGAACGAA	1880-1897[a]	
	orf-3	CGTCGTGTTCTAACTGTTGA	1763-1782[a]	
		GAATCTTGTGATAACCTCCATA	2740-2761[a]	
	orf-4	CCAATTAGTAGCTGAAACCCTT	2719-2740[a]	
		CCCTTCCACGCAAACACG	3634-3651[a]	
	orf-5	CAGAGGGCACTTATGAGAC	3432-3450[a]	
		CAGAGGCAGTTATGGGAG	4434-4451[a]	
	orf-6	GGGTCGTGTATTGTTTGG	4335-4352[a]	
		CCGAGTTTGGCATTTCTA	5280-5297[a]	
	orf-7	TAGTGTTTGAAATGGAGGGC	5157-5176[a]	
		TTGGGAAGTGACTGCTGA	6164-6181[a]	
	orf-8	AGGGCATCATCTTTCCAC	6114-6131[a]	
		TTTGCGAGACAGTCCATTA	7029-7047[a]	

[a]Position in the complete sapovirus GII genogroup sequence (accession no: MG674584).

[b]Position in the complete sapovirus GII genogroup sequence (accession no: MF462287).

	primer	Nucleotide sequence (5'-3')	position	source
	orf-9	GGAATCAACTGCGGAAAT GAATAAGACAGCGATGGTC	6577-6594[a] 7429-7447[a]	
	AP1	CTCGCCACCTATGAAGCA AATCGGCAGTGATGTCCT	5081-5098[a] 7004-7021[a]	
	Orf1-1	CTTCTAAGCCATTCTACTCAA CGTGCCATGAACTGTTTG	5-25[b] 1179-1196[b]	
	Orf1-2	GGCAGCATCACATCAGTC TGTTTGCAGACATGAACG	1093-1110[b] 2078-2095[b]	
	Orf1-1,2	CTTCTAAGCCATTCTACTCAA GCTCTGTTTGCAGACATG	5-25[b]	
	Orf1-4	ACCGTGGGTGGTATGACT GGCGACAACCGTTGAAAT		

[a]Position in the complete sapovirus GII genogroup sequence (accession no: MG674584).

[b]Position in the complete sapovirus GII genogroup sequence (accession no: MF462287).

2.7 Genotype and sequence analysis

These nucleotide sequences were first verified using Geneious 10.1.3 (www.geneious.com). Phylogenetic analysis of these sequences was performed in the MEGA6.0 software package (http://www.megasoftware.net/). Sequence Alignment was performed using Clustal W. Phylogenetic trees with bootstrap analysis from 1,000 replicates were generated by using the neighbor-joining method.

2.8 Statistical analysis method

The statistical package for the Social Sciences softwares (SPSS version 18.0) was required for statistical analysis of the collected data. The chisquare test showed significant differences between the variables and $P < 0.05$ was regarded as statistically significant.

3 Results

3.1 Epidemiological investigation

This outbreak of acute gastroenteritis consisting of 482 cases occurred in a primary school which had 1850 students and 95 teachers from 34 classes of 6 grades in Shenzhen city, China during 24th February and 11th March 2019. There were three Buildings in which students of grade 3 and 4 were in Building A and students of 5–6 were in Building B. The students from grade 4–6 were organized to participate in military training activities during February 26–27 while the students from grade 1–3 were organized to participate in spring outing on February 27 after the school opened on February 25. The age of 482 cases ranged from 9 to 14 years. The first case from class 4 grade five (C4G5) emerged on February 24, 2019 with the occurrence of vomiting at the classroom and the bus on the way to the activity, respectively. The students of grade 5 got sick first and then grade 4, 6 and 3. There was no case from grade 1 and 2. The case peak occurred on March 1 on which local district CDC was invited to investigate this outbreak and the second case peak occurred on March 4 on which classes of G4-6 were suspended and were resumed on March 8. Classes of G3 were suspended on March 8 and resumed on March 11 (Fig. 1). The characteristic symptoms within the cohort were diarrhea (67%, 321/482), vomiting (45%, 216/482), dizziness (30%, 144/482), stomach pain (22%, 106/482) and nausea (18%, 86/482). The results showed that the attack rate of each grade was 36% in Grade 5, 23% in Grade 6, 21% in grade 4 and 6% in grade 3, respectively ($P < 0.05$). The attack rate of each floor of Building A was 3% for the 4th floor, 20% for the 5th floor ($x^2 = 45.09$, $P < 0.01$), and the attack rate of each floor of Building B was 41% for the 3th floor, 38% for the 4th floor, and 20% for the 2th floor ($x^2 = 16.44$, $P < 0.05$). The difference of incidence among different floors was statistically significant.

During the activity, 218 students vomited. Vomiting occurred in plastic bags (13%), floor (10%) and window (1%) during taking the bus and vomiting occurred in the bathroom (3%) and dormitory (2%) during activity. Vomiting occurred in Classrooms (9%), playgrounds (3%), hallway (3%) after returning to school (Table 2).

Table 2
Distribution of vomiting places of students

Place of vomiting		Vomiting number	%
Bus	Plastic bag	28	13
	ground	10	5
	Outside the window	2	1
Military training	bathroom	6	3
	Dorm	4	2
	Playground	4	2
	Canteen	1	0
School	toilet	61	28
	Classroom	20	9
	Playground	7	3
	doorway	6	3
	Other	13	6

In the group with common exposure to vomit, the single factor analysis showed that the students which saw others vomiting in classroom or playground, saw vomitus less than 1.5 meters are susceptible ($P < 0.05$) (Table 3).

Table 3
Case control study on risk factors of acute gastroenteritis outbreak caused by Sapovirus

No.	case		Non case			95%CI		X^2	P value
	Expose	%	Expose	%	OR value	lower limit	Upper limit		
See others vomiting	278	58	365	46	1.58	1.26	1.99	15.60	0.00
See others vomiting in the classroom	120	25	138	17	1.56	1.19	2.06	10.14	0.00
See others vomiting in the playground	36	7	35	4	1.74	1.08	2.81	5.22	0.02
Less than 1.5 m from vomit	156	32	166	21	1.42	1.04	1.95	4.81	0.03
Handling vomitus	45	9	36	5	2.17	1.37	3.41	11.57	0.00
See others Vomiting on bus	86	18	116	15	1.25	0.92	1.70	2.11	0.15
See others Vomiting during activity	14	3	30	4	0.76	0.40	1.44	0.72	0.40
See others Vomiting in bathroom	56	12	71	9	1.33	0.92	1.93	2.28	0.13
Self protection in handling	12	27	7	19	1.93	0.69	5.40	1.62	0.20
Family members in the same school	116	24	170	22	1.15	0.88	1.51	1.06	0.30

3.2 Virology investigation

A total of these virologies, including E coil, Salmonella, Shigella, Campylobacter, Yersinia enterocolitica, Norovirus, Rotavirus, Adenovirus and Astrovirus were detected to be negative. Sequence analysis of several positive RT-PCR samples revealed identical sequences, which were identified as SaVs. SaVs were detected in 7 of 11 stool specimens collected from sporadic cases.

3.3 Analysis of nearly complete genome sequence

The nearly complete genomic sequence of strain SZ08 was obtained and its length was 7338 nt excluding the sequences of 5'UTRs and the 3'-end poly (A) tail in this study. The sequenced genomes was predicted to contain two major open reading frames (ORF) from 1 to 6837 (ORF1, encoding the nonstructural proteins and major capsid protein VP1) and from 6838 to 7337 (ORF2, encoding the minor structural protein). Its 3'-UTR had 52 nt. The reference strain MF462288/ GII.8/Peru330/PNV010961/2008 shared the highest query cover and nucleotide identities (92.4%) with strain SZ08 based on complete genome. The nucleotide identities between them were 93.13% in the nonstructural proteins and major capsid protein VP1, 93.30% in the minor structural protein and 94.61% in the partial VP1. According to the amino acid identities of the protein alignment, a great degree of similarity (96.95% at the nearly complete genome, 98.46% in the nonstructural proteins and VP1 and 97.54% in the VP2) were showed between them. We observe 48 aa mutations in the nonstructural proteins and VP1 and 25 aa mutations in the minor structural protein.

3.4 Analysis of complete capsid protein

According to the phlylogenetic analysis, Strain SZ08 clustered into GII.8 branch and was independent with other clusters (A, B, C and D) named previously and was named after cluster E (Fig. 2). The nucleotide identity of Strain SZ08 was ranged from 92.94–94.02% with cluster A-D and the amino acid identity was ranged from 96.76–97.48%. However, cluster A-D shared the high nucleotide identity (96.87–99.84%) and amino acid identity (98.31–99.88%). Strain SZ08 introduced 11 specific amino acid mutations (S249A, A275S, S299A, A301T, G302T, V335N, L465I, S497N, I503V, A528V and V552I) compared with cluster A-D. However, five original amino acids were reused (S43T, S183A, E341D, T403S, I520V) from the earliest strain Peru330/PNV010961/2008 (cluster A).Cluster B-D had low amino acid mutations (Fig. 3).

4 Discussion

The epidemiology of cases of AGE of viral etiology is a relevant public health issue[20]. Of note, reports on SaV-associated AGE across all age groups have recently increased worldwide[6, 11, 13], which indicated that the cases caused by SaVs may become extensive and global. And Shenzhen is also one of the high-incidence areas of SaV-associated outbreaks recently in mainland China[19, 21]. Human SaVs cause AGE in all age groups in both sporadic cases and outbreaks worldwide as well as predominantly occurs in children[22, 23], which correspond to this study. On the other hand, the outbreak described by the study occured in primary school and expose to vomit was recognized as the cause, which are similar to the previous studies[24, 25].

The investigation showed the difficulty to prove the person-to-person transmission by contact or from aerosols generated by vomit[26–28]. In this study, we investigated the vomiting places and the single factor analysis showed that the participation in the treatment of vomitus and the distance of vomitus were the main factors causing gastroenteritis infection (logistic regression, $P < 0.05$). It has been proposed that the formation of aerosols that can remain in the air for some time and can then be breathed in and swallowed or through contamination of surface causes the outbreaks[29–31]. The aerosols transmission caused by vomiting could responsible for the majority of gastroenteritis outbreaks which are not caused by foodborne

or waterborne outbreaks as described previously[31–34]. Vomit was considered only when exposure was at < 1 m, however, possible exposures at greater distances have also been reported[26, 27]. Our study showed the distance under 1.5 m could cause the infection. Therefore, the study gave the evidence that the greater distance also could cause the cases. Since vomiting is a common symptom in calicivirus outbreaks, vomiting aerosols transmission may exist in most Norovirus outbreaks. Therefore, vomiting aerosols transmission should be valued in the investigation of gastroenteritis outbreaks.

Emerging virus strains often have a risk of causing a pandemic. SaV GII.8 was first identified in two hospitalized children's samples in Peru, in 2008[35]. In mainland China, GII.8 was first reported in Shenzhen, in 2011[21]. Additionally, in 2019, Xue al. acquired the first GII.8 SaV genome from mainland China[13]. Based on the phylogenetic analysis, the strain SZ08 in this study was classified as a member of GII.8 SaV. In addition, strain SZ08 isolated in 2019 was clustered as independent branch and was different from the strain GZ2014-L231 isolated in 2014 although strain of Shenzhen and strain GZ2014-L231 of Guangzhou were both from the same province, Guagndong. GII.8 strains were also detected in other countries not only in clinical but also in environmental samples[36, 37]. SaVs outbreak caused by GII.8 strains occurred in long term center in previous report[35] and the GII.8 SaV outbreak occurred in primary school. It suggested the GII.8 strain could cause the children and the elderly to infect. Despite the low detection rate, the wide spread distribution of the virus in different countries and wide age groups, make it as important concern to understand its genetic diversity and evolutionary characteristics.

Recombination and amino acid mutations are the two important way of viral evolution not only in SaVs but also in Noroviruses. According to the genomic analysis, there was no recombination occurred in stain SZ08.

Amino acid point mutations in proteins are the important way of viral evolution. In this study, by comparing the differences between the strain SZ08 as new cluster and GII.8 human SaV representative strains of other clusters, more amino acid mutations occurred in VP1 capsid region. GII.8 strain might obtain new antigenicity and receptor binding activity through the variation of amino acid sites in the capsid region, just as it occurs in norovirus, and gained greater susceptibility in the population. This might explain there was the large scale of cases in this SaV outbreak in this study.

In conclusion, vomiting could be one of the transmission mechanisms which may explain a large number of cases of SaV outbreaks. It is noteworthy that SaV-associated diarrhea is generally mild, while large-scale outbreaks by SaV may also occur. In addition, we obtained the genome of a novel GII.8 SaV variant from China and comprehensively analyzed the genomic characteristics. The results of this study could not only provide reference data for SaV researches in the future, but also deepen the understanding of evolution mechanisms of the new GII.8 variant. Constant surveillance is required to monitor the emergence of these strains and will permit the identification of changes in major strains and improve our knowledge of the evolution of SaVs among humans.

Declarations

Conflicts of interest

The authors declare that they have no conflict of interests.

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Figures

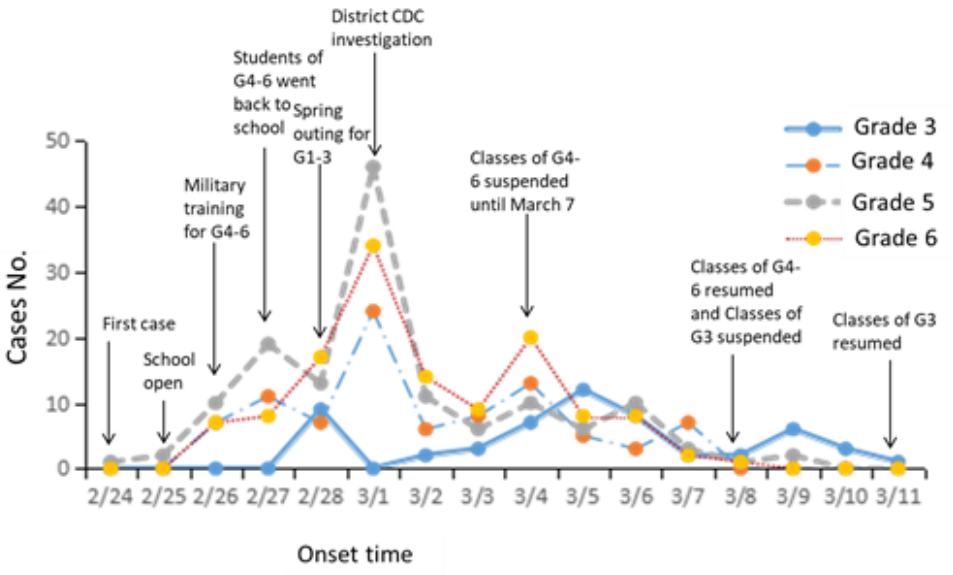

Figure 1

Epidemic curve of acute gastroenteritis cases in the outbreak occurred in Shenzhen, China, 2019

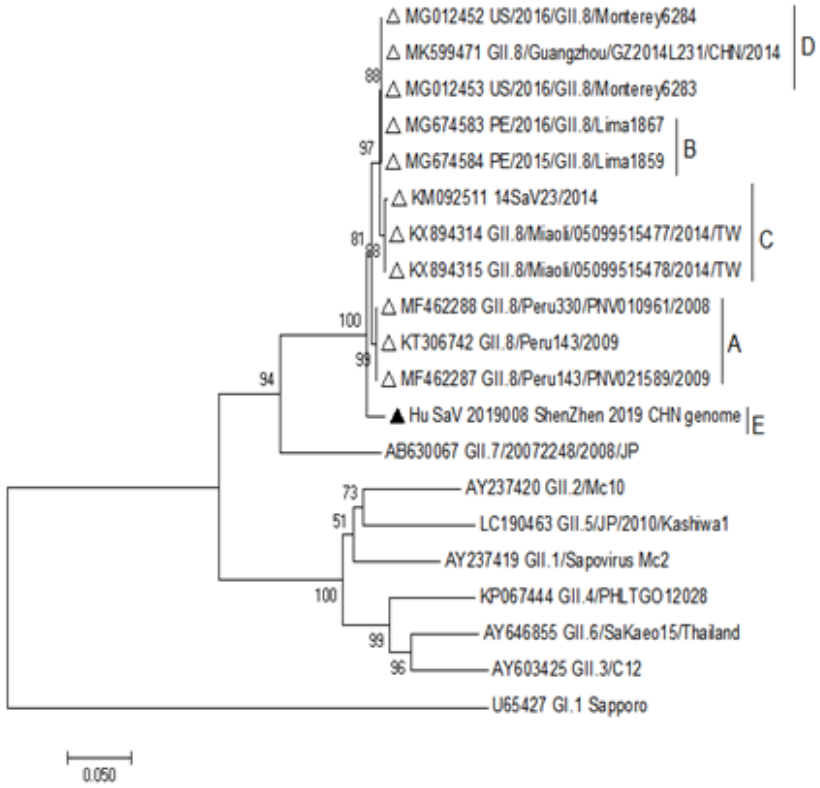

Figure 2

Phylogenetic analysis of SaVs based on nucleotide of compete VP1.

Cluster	name	43	107	183	249	275	296	299	301	302	335	341	403	445	465	497	503	520	528	552
A	MF462288_GII.8/Peru330/PNV010961/2008	S	Y	A	S	A	F	S	A	G	V	D	S	M	L	S	I	V	A	V
C	KX894314_GII.8/Miaoli/05099515477/2014/TW	T	F	S	S	A	Y	S	S	G	I	E	T	M	L	S	I	I	A	V
D	MK599471_GII.8/Guangzhou/GZ2014L231/CHN/2014	S	F	S	S	A	Y	S	S	G	V	E	T	M	L	S	I	I	A	V
B	MG674583_PE/2016/GII.8/Lima1867	S	F	S	S	A	Y	S	S	G	V	E	T	M	L	S	I	I	A	V
E	SaV_2019008_ShenZhen_2019_CHN	S	F	A	A	S	Y	A	T	T	N	D	S	M	I	N	V	V	V	I

Figure 3

Amino acid variation in complete capsid protein gene compared to reference strains of SaV GII.8