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Short Communication

Epidemiological Characteristics of Sapovirus and Human Astrovirus Detected among Children in Nara Prefecture, Japan, during the 2009/2010–2014/2015 Seasons

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SUMMARY: The current study elucidated the epidemiological characteristics of sapovirus (SaV) and human astrovirus (HAstV) associated with gastroenteritis among children in regional populations of Nara Prefecture, Japan, during the 2009/2010–2014/2015 seasons. The SaV detection rate was 7.5% (71/948) according to reverse transcription-polymerase chain reaction. A high SaV detection rate of 13.5% was observed among children 4 years of age. The highest SaV detection rate was observed in June (19.2%), followed by July (11.1%). The detected SaV included 7 genotypes: GI.1, GI.2, GII.3, GII.1, GI.3, GII.2, and GV, in order of decreasing prevalence. In comparison, the HAstV detection rate was 4.2% (40/948). The HAstV detection rate among children 4 years of age was 12.2%. The HAstV detection rate was highest in July (13.9%), followed by May (10.5%) and August (6.7%). The detected HAstVs included genotypes 1, 4, 6, and 8. The most prevalent genotype was 1, followed by 4 and 8. This report provides an epidemiological overview of SaV and HAstV infection in Nara Prefecture, Japan.

Sapovirus (SaV), belonging to the family *Caliciviridae*, is recognized as an important causative agent of acute gastroenteritis. SaVs are classified into 5 genogroups (GI, GII, GIII, GIV, and GV). Genogroups GI, GII, GIV, and GV have been reported in human infections, whereas genogroup GIII has been identified in swine infections. A previous study in Japan reported that the GI and GII strains accounted for 95% of human infections (1). These strains were further classified into 7 genotypes each (2). Human astrovirus (HAstV), belonging to the family *Astroviridae*, causes diarrhea in young children. HAstVs are classified into 8 serotypes, with serotype 1 being the most prevalent worldwide (3). In a previous report of diarrhea-causing viruses detected in Japan, norovirus (NoV) was the most common, followed by group A rotavirus (RVA), sapovirus, and astrovirus (4). In Japan, methods for rapid diagnosis of SaV and HAstV are not popular in medical institutions. Information regarding these viruses, including their epidemiological characteristics, is limited when compared with that regarding NoV and RVA (5,6). The aim of the current study was to reveal the epidemiological characteristics of SaV and HAstV detected among children in Nara Prefecture, Japan.

During gastroenteritis surveillance in Nara Prefecture over 6 epidemic seasons, from September 2009 to August 2015, we tested 948 fecal samples from gastroenteritis patients aged below 15 years for SaV and HAstV. This study was conducted as a part of national surveillance in compliance with the Infectious Diseases Control Law. The annual observation period of gastroenteritis in the current study began in September and ended in August of the following year. Viral RNA was extracted as previously described (7). Reverse transcription-polymerase chain reaction (RT-PCR) was performed to screen for SaV and HAstV using SV-F11 and SV-R1 primers for SaV (8) and AC1′ and AC230 primers for HAstV (9). The RT-PCR was performed using the PrimeScript One Step RT-PCR Kit Ver.2 (Takara Bio Inc., Shiga, Japan). Electrophoresis, amplified DNA purification, and direct sequencing were performed as previously described (7). Direct sequencing was performed by RT-PCR using the primers for SaV strains. For these strains, phylogenetic analysis of the partial nucleotide sequences of the capsid (428 bp) region was performed using MEGA software version 6 (10). GI.4 was used as an outgroup. The genetic distances were calculated according to Kimura 2-Parameter + Gamma model, and the tree was plotted using the maximum likelihood method. One thousand bootstrap re-samplings were performed for each tree. SaV strains were genotyped based on the partial capsid region, with the reference strain corresponding to 17 genotypes (GI.1–7, GII.1–7, GIII, GIV, and GV), as previously described (2). Sequence data were submitted to the DNA Data Bank of Japan database with accession numbers LC071854–LC071924. For HAstV genotyping, RT-PCR was performed with serotype-specific primers (11).

The monthly occurrence and genotype distribution of SaV from 2009/2010 to 2014/2015 is presented in Table 1. Of the 71 detected strains, 34 (47.9%) and 35 (49.3%) were isolated from samples from male and female patients, respectively. The origins of 2 samples were unknown. There was no significant difference in the patient gender ratio (male vs. female: 0.971 vs.

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Table 1. SaVs isolated from gastroenteritis among children during 2009/2010 to 2014/2015 seasons in the Nara Prefecture

Season	Samples in each season	Total numbers of SaVs in each season	Total numbers of SaVs genotype in each season	Month Total samples in each month											
				Sep 14	Oct 42	Nov 88	Dec 96	Jan 82	Feb 56	Mar 153	Apr 204	May 95	Jun 52	Jul 36	Aug 30
2009/2010	153	11 (7.2%)	GI.1 (7)				GI.1 (2)					GI.1 (2)	GI.1 (3)		
			GI.3 (2)								GI.3 (1)	GI.3 (1)			
			GII.1 (2)							GII.1 (1)		GII.1 (1)			
2010/2011	217	13 (6.0%)	GI.1 (5)								GI.1 (1)		GI.1 (2)	GI.1 (2)	
			GI.2 (4)				GI.2 (1)		GI.2 (1)		GI.2 (1)	GI.2 (1)			
			GII.3 (3)				GII.3 (1)	GII.3 (1)					GII.3 (1)		
			GV (1)							GV (1)					
2011/2012	150	13 (8.7%)	GI.1 (8)			GI.1 (1)	GI.1 (1)	GI.1 (2)					GI.1 (3)	GI.1 (1)	
			GI.2 (2)									GI.2 (2)			
			GII.2 (2)										GII.2 (1)	GII.2 (1)	
			GII.3 (1)								GII.3 (1)				
2012/2013	143	3 (2.1%)	GI.2 (3)								GI.2 (2)	GI.2 (1)			
2013/2014	81	8 (9.9%)	GI.1 (6)			GI.1 (4)	GI.1 (1)	GI.1 (1)							
			GII.1 (2)			GII.1 (1)						GII.1 (1)			
2014/2015	204	23 (11.3%)	GI.1 (19)			GI.1 (1)	GI.1 (1)	GI.1 (2)	GI.1 (3)	GI.1 (9)	GI.1 (3)				
			GII.1 (1)						GII.1 (1)						
			GII.3 (3)		GII.3 (1)		GII.3 (1)		GII.3 (1)						
Total number	948	71 (7.5%)		0 (0%)	1 (2.4%)	7 (8.0%)	8 (8.3%)	6 (7.3%)	6 (10.7%)	11 (7.2%)	9 (4.4%)	9 (9.5%)	10 (19.2%)	4 (11.1%)	0 (0%)

Table 2. HAstVs isolated from gastroenteritis among children during 2009/2010 to 2014/2015 seasons in the Nara Prefecture

Season	Samples in each season	Total numbers of HAstVs in each season	Total numbers of HAstVs genotype in each season	Month Total samples in each month											
				Sep 14	Oct 42	Nov 88	Dec 96	Jan 82	Feb 56	Mar 153	Apr 204	May 95	Jun 52	Jul 36	Aug 30
2009/2010	153	6 (3.9%)	G4 (3)									G4 (1)	G4 (1)	G4 (1)	
			G6 (2)								G6 (2)				
			NT (1)												NT (1)
2010/2011	217	3 (1.4%)	G1 (2)			G1 (1)				G1 (1)					
			G4 (1)							G4 (1)					
2011/2012	150	11 (7.3%)	G1 (5)							G1 (1)				G1 (3)	G1 (1)
			G4 (2)									G4 (2)			
			NT (4)								NT (1)	NT (1)	NT (1)	NT (1)	
2012/2013	143	8 (5.6%)	G1 (4)							G1 (1)		G1 (2)	G1 (1)		
			G4 (1)								G4 (1)				
			NT (3)								NT (2)	NT (1)			
2013/2014	81	3 (3.7%)	G1 (3)							G1 (2)		G1 (1)			
2014/2015	204	9 (4.4%)	G1 (3)							G1 (2)	G1 (1)				
			G8 (5)								G8 (3)	G8 (2)			
			NT (1)								NT (1)				
Total number	948	40 (4.2%)		0 (0%)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)	0 (0%)	8 (5.2%)	11 (5.4%)	10 (10.5%)	3 (5.8%)	5 (13.9%)	2 (6.7%)

Table 3. Age distribution of SaVs and HAstVs during 2009/2010 to 2014/2015 seasons in the Nara Prefecture

age (*n*)	0 (130)	1 (295)	2 (154)	3 (117)	4 (74)	5 (56)	6 (37)	7–15 (83)	Total (948)
SaV (%)	8 (6.2)	24 (8.1)	13 (8.4)	11 (9.4)	10 (13.5)	2 (3.6)	1 (2.7)	2 (2.4)	71 (7.5)
HAstV (%)	1 (0.8)	1 (0.3)	8 (5.2)	8 (6.8)	9 (12.2)	5 (8.9)	4 (10.8)	4 (4.8)	40 (4.2)

1.00).

All SaV strains were successfully genotyped. Overall, 7 SaV genotypes were observed. During each season, 1–4 genotypes were observed. The 71 strains were classified as GI.1 (n = 45, 63.4%), GI.2 (n = 9, 12.7%), GII.3 (n = 7, 9.9%), GII.1 (n = 5, 7.0%), GI.3 (n = 2, 2.8%), GII.2 (n = 2, 2.8%), and GV (n = 1, 1.4%). GI.1 was the predominant genotype during all seasons except for the 2012/2013 season, in which GII.3 was the predominant genotype.

The monthly and genotype distribution of HAstV during 2009/2010–2014/2015 is presented in Table 2. Of the 40 detected strains, 21 (52.5%) and 18 (45.0%) were isolated from samples obtained from male and female patients, respectively. The origin of 1 sample was unknown. Therefore, the male: female ratio was approximately 1.167:1. Overall, 4 HAstV genotypes were observed. During each season, 1 to 2 genotypes were observed. Of the 40 strains, 31 were classified as HAstV-1 (n = 17, 54.8%), HAstV-4 (n = 7, 22.6%), HAstV-8 (n = 5, 16.1%), and HAstV-6 (n = 2, 6.5%). HAstV-1 was the predominant genotype. Of the 40 patients with HAstV infections, 3 were also positive for SaV.

The age distribution of SaV- and HAstV-positive patients during 2009/2010–2014/2015 is presented in Table 3.

Fecal samples from children with gastroenteritis were examined; 7.5% were SaV-positive, while 4.2% were HAstV-positive. These rates were similar to those reported previously in Japan (11,12). The detection rate of SaV among the 6 seasons was the lowest during the 2012/2013 season (2.1%) and highest during the 2014/2015 season (11.3%). In contrast, the detection rate of HAstV among the 6 seasons was lowest during the 2010/2011 season (1.4%) and highest during the 2011/2012 season (7.3%). These rates were low when compared with the previously reported detection rates of NoV or RVA in Nara Prefecture (7,13). However, a total of 111 strains were detected, and these viruses were present during all the 6 seasons studied. From the monthly distribution of the samples collected in the present study, 54.9% (39/71) of SaV was detected between March and June, whereas 72.5% (29/40) of HAstV was detected from March to May. These trends differed from that of NoV and were similar to that of RVA as previously reported. In particular, almost all HAstV patients demonstrated an apparent seasonal distribution, occurring during spring and summer. In the current study, HAstV was not detected during the winter months. Further epidemiological data are required to elucidate the seasonal distribution of HAstV in Japan. The age distribution of HAstV-positive patients was slightly higher than that of SaV-positive patients. In a previous report on detection rates in Japan from 1995 to 1998 (12), the highest detection rate of HAstV was observed in children 0–1 years of age. In the current study, only 2 HAstV strains were detected within this age range, although the reason for this discrepancy remains unknown. Further and continuing epidemiological studies are necessary to examine this discrepancy. In this study, we were unable to assess the characteristics of clinical symptoms caused by these viruses. Generally, the clinical symptoms of NoV and SaV are indistinguishable. However, the clinical symptoms of HAstV are typically milder than those of NoV (14). The low detection rates of SaV and HAstV compared with those of NoV and RVA has made detection of these viruses difficult in various epidemiological studies. For the efficient detection of these viruses, it is important to accumulate epidemiological data such as their monthly distribution with specimen collections.

Despite the limited number of positive samples, our results show the diversity of SaV strains in a local area of Japan. From the results of phylogenetic analysis of SaV, GI.1 was shown to be the predominant genotype in Nara Prefecture during the 6 epidemic seasons. This trend is supported by previous reports from different regions of Japan (15). Phylogenetic analysis of all 45 SaV GI.1 strains is shown in Fig. 1. The GI.1 strains detected during the 2014/2015 season were clustered with another clade belonging to the GI.1 strains detected during the other 4 seasons. However, we could not confirm obvious amino acid changes in SaV strains detected during the 2014/2015 season. In Japan, GIV SaVs emerged in Kumamoto Prefecture in 2007 (5), and an outbreak of GV SaVs occurred among primary school children in Yokohama City during 2010 (16). Therefore, it is necessary to focus attention not only on the common genotype GI.1 but also on rare genogroups such as GIV and GV. Further epidemiological study is required to assess the local area shifts of each genogroup. Based on the genotyping analysis of 40 HAstV strains, our data show HAstV-1 to be the most common genotype in Nara Prefecture, as previously reported in other areas of Japan (12,17). However, the predominant genotype changed from genotype 4 to 1 and from 1 to 8 during the 6 seasons included in the current study. Around 2008, new astrovirus (AstV) species such as MLB1, VA1, and VA2 were detected in diarrhea specimens from children (18–20). There are few reports in Japan regarding these newly discovered AstV (21). Therefore, it is necessary to conduct epidemiological research on not only HAstV but also these newly discovered AstV.

Finally, the current study analyzed SaV and HAstV among children in Nara Prefecture from the 2009/2010 season to the 2014/2015 season in a local area of Japan. Few institutions in Japan are conducting detailed epidemiological analysis of these viruses. Because many current surveys of SaV and HAstV focus on children, adult surveys are also important in order to understand the epidemiological characteristics of these viruses. We believe that a continual grasp of the epide-

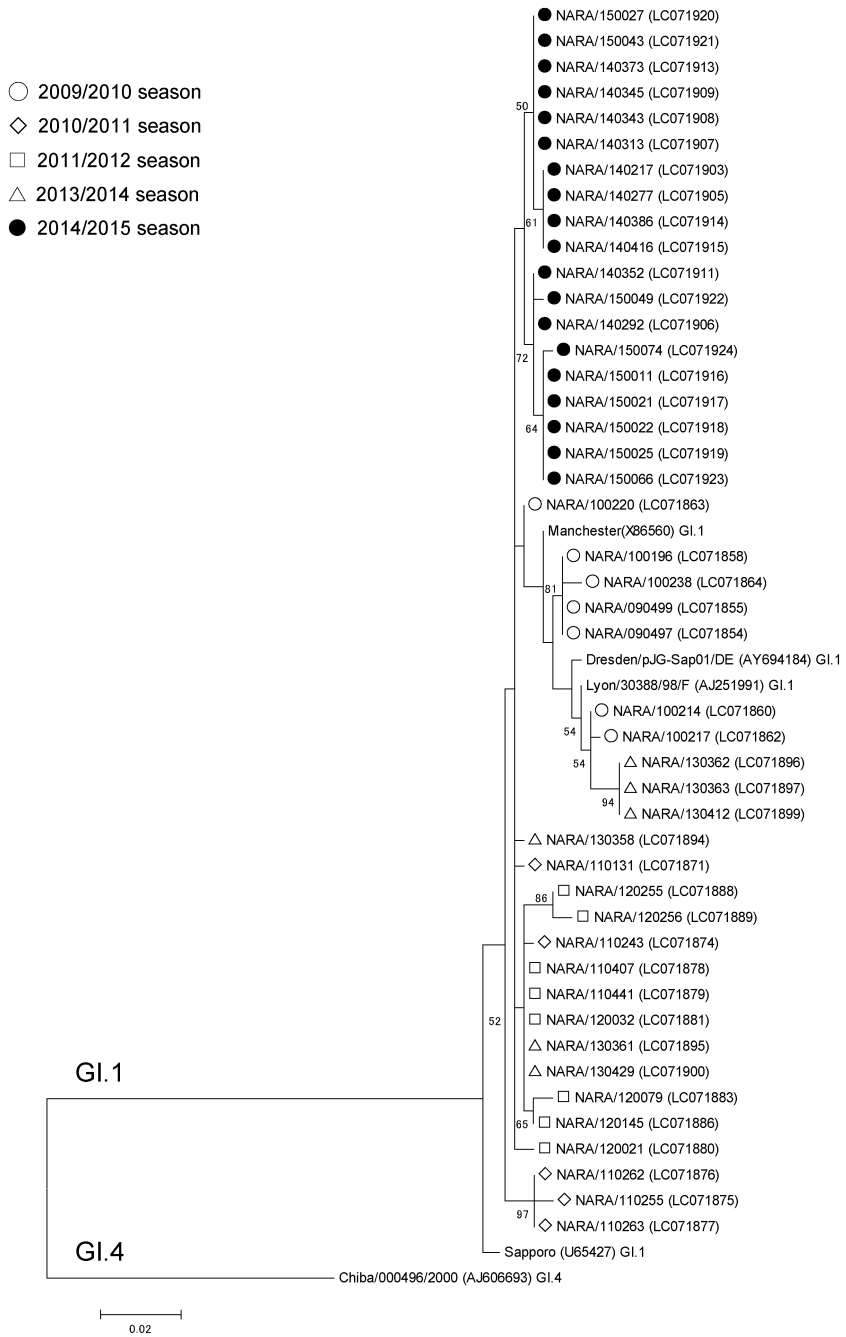

Fig. 1. Phylogenetic tree constructed using the partial nucleotide sequences capsid (428 bp) region of 45 SaV GI.1 strains. The tree was constructed by maximum likelihood method with labeling of the branches at least 50% bootstrap support. GI.4 strain (Chiba/00496/2000) was used as an out group. Scale bar indicate nucleotide/substitution/site/year.

miological considerations of SaV and HAstV will help inform prevention measures for food poisoning outbreaks caused by these viruses.

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Conflict of interest None to declare.

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