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Comprehensive Review of Human Sapoviruses

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SUMMARY

Sapoviruses cause acute gastroenteritis in humans and animals. They belong to the genus *Sapovirus* within the family *Caliciviridae*. They infect and cause disease in humans of all ages, in both sporadic cases and outbreaks. The clinical symptoms of sapovirus gastroenteritis are indistinguishable from those caused by noroviruses, so laboratory diagnosis is essential to identify the pathogen. Sapoviruses are highly diverse genetically and antigenically. Currently, reverse transcription-PCR (RT-PCR) assays are widely used for sapovirus detection from clinical specimens due to their high sensitivity and broad reactivity as well as the lack of sensitive assays for antigen detection or cell culture systems for the detection of infectious viruses. Sapoviruses were first discovered in 1976 by electron microscopy in diarrheic samples of humans. To date, sapoviruses have also been detected from several animals: pigs, mink, dogs, sea lions, and bats. In this review, we focus on genomic and antigenic features, molecular typing/classification, detection methods, and clinical and epidemiological profiles of human sapoviruses.

INTRODUCTION

Sapoviruses cause acute gastroenteritis in humans and animals. They belong to the genus *Sapovirus* within the family *Caliciviridae*. Sapovirus infections are a public health problem because they cause acute gastroenteritis in people of all ages in both outbreaks and sporadic cases worldwide. Outbreaks often occur in semiclosed settings. Outbreaks caused by foodborne transmission of sapovirus have also been reported. In this comprehensive review, we focus mainly on human sapoviruses.

HISTORY

Sapovirus particles are small (about 30 to 38 nm in diameter) and icosahedral and have cup-shaped depressions on the surface, which is a typical calicivirus morphology (Fig. 1) (1). Sapovirus particles were first detected in human diarrheic stool samples in 1976 in the United Kingdom using electron microscopy (EM) (2), and the virus was soon recognized as a new gastroenteritis pathogen (3–11). However, the prototype strain of the *Sapovirus* genus

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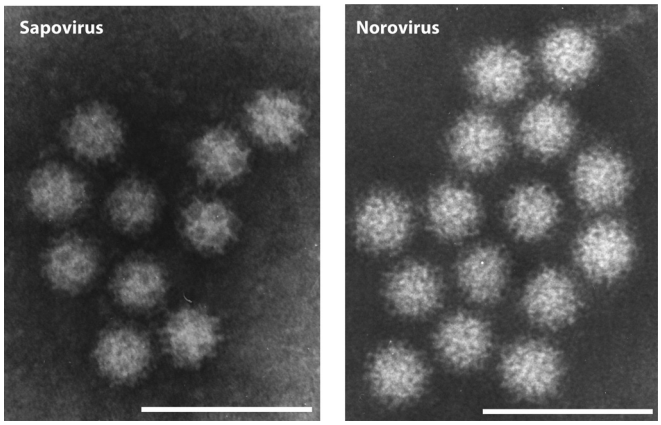

FIG 1 Transmission electron micrographs of sapovirus and norovirus particles from clinical samples. Scale bars indicate 100 nm. (Courtesy of Yasutaka Yamashita, Ehime Prefectural Institute of Public Health and Environmental Science, Japan.)

was from another outbreak in Sapporo, Japan, in 1982 (strain Hu/SaV/Sapporo/1982/JPN), because it has been studied extensively for sapovirus virological and genetic characteristics (10, 12–14).

TAXONOMY

Sapoviruses were previously called "typical human caliciviruses" or "Sapporo-like viruses." In 2002, The International Committee on the Taxonomy of Viruses assigned these viruses to the species *Sapporo virus*, genus *Sapovirus*, in the family *Caliciviridae* (15). Currently, the family *Caliciviridae* consists of five established genera, *Sapovirus*, *Norovirus*, *Lagovirus*, *Vesivirus*, and *Nebovirus* (http://www.ictvonline.org/virusTaxonomy.asp), whereas five new genera (*Bavovirus*, *Nacovirus*, *Recovirus*, *Valovirus*, and *Secalivirus*) have been proposed (16–20).

CELL CULTURE AND ANIMAL INFECTION TRIALS

Attempts to grow human sapoviruses in cell cultures (2, 4, 5, 9, 21–24), have been reported, and two studies describe the propagation of sapoviruses in green monkey kidney cells (23) or primary human embryo kidney cells in the presence of trypsin and actinomycin D (Table 1) (24); however, no confirmed reproduction of these data is available. Currently, only a few porcine sapovirus strains have been grown successfully in primary porcine kidney cells or a porcine kidney cell line (i.e., LLC-PK1) in the presence of porcine intestinal contents or bile acids (Table 1) (25–28). Bile acids likely support porcine sapovirus replication via escape from endosomes during the virus entry step (29). Also, a cellular cyclic AMP (cAMP) signaling pathway induced by intestinal contents or bile acids likely causes downregulation of innate immunity (25, 30). An infection trial of human sapoviruses in mice did not succeed (5). Currently, only the specific porcine sapovirus (Cowden strain) has been studied for its pathogenesis in gnotobiotic pigs (31–33).

PHYSICAL CHARACTERISTICS AND STABILITY

Sapovirus is a nonenveloped virus, and the virus has a buoyant density of 1.36 to 1.41 g/cm^3 (5, 22, 34, 35). The stability of porcine sapovirus under physicochemical treatment is as follows: (i) stable at pH 3.0 to 8.0 at room temperature for 1 h, (ii) sensitive to ethanol treatment (60% and 70%) at room temperature for 30 s, (iii) inactivated by 200 mg/liter (or ppm) sodium hypochlorite at room temperature for 30 min, and (iv) inactivated by heating at 56°C for 2 h (36).

GENOMIC ORGANIZATION

The sapovirus genome has a positive-sense, single-stranded RNA genome, which is approximately 7.1 to 7.7 kb in size and has a 3′-end poly(A) tail. The sapovirus genome contains two open reading frames (ORFs) (Fig. 2). ORF1 encodes a large polyprotein containing the nonstructural proteins followed by the major cap-

TABLE 1 Cell culture trials for human and animal sapoviruses

Origin	Virus growth	Tested cells	Supplement in the culture medium	Reference(s)
Human	No[a]	Human and rhesus monkey amnion, fetal cat cells	None	2
	Yes[a,b]	Green monkey kidney cells	None	23
	No[a]	Human fetal intestinal organ culture, human embryonic kidney cells, monkey kidney cells, human embryonic lung cells, HEp-2 cell line	None	21
	No[a]	Primary rhesus monkey kidney and human embryonic kidney cells	None	4
	No[a]	Primary monkey kidney, primary baboon kidney, primary human embryo intestine, human embryo kidney cells, MRC5, Hep, Vero, and feline lung cells	None	5
	No[a]	HeLa, green monkey kidney cells, and HEL-R66	None	22
	No[a]	Monkey kidney and MRC5 cells	None	9
	Yes[a,b]	primary human embryo kidney cells	Trypsin and actinomycin D	24
Porcine	Yes[c]	Primary porcine kidney cells	Porcine intestinal contents	26
	Yes[c]	LLC-PK1	Porcine intestinal contents	35, 27
	Yes[d]	LLC-PK1	Bile acids	25

[a] Confirmed by electron microscopy.
[b] This result could not be reproduced.
[c] Confirmed by immunofluorescent staining of the virus-infected cells and immune electron microscopy.
[d] Confirmed by immunofluorescent staining of the virus-infected cells and ELISA.

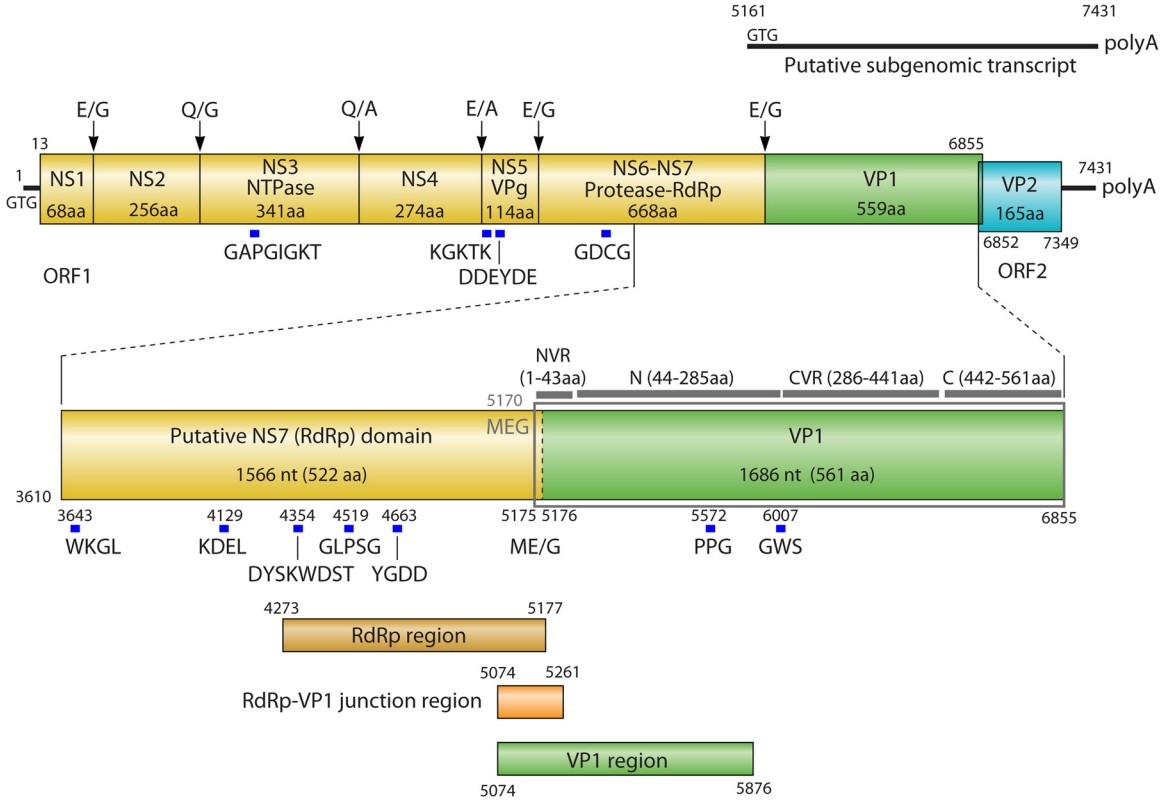

FIG 2 Diagram of sapovirus genomic organization and the RT-PCR target regions for human sapoviruses based on the GI.1 Manchester strain (GenBank accession no. X86560). A schematic diagram of the sapovirus genomic organization, including the putative subgenomic transcript, two common open reading frames (ORF1 and ORF2), the predicted viral nonstructural proteins (NS1, NS2, NS3 [NTPase], NS4, NS5 [VPg], and NS6-NS7 [protease–RNA-dependent RNA polymerase {RdRp}]), and structural proteins VP1 and VP2 is shown. The putative cleavage sites in the ORF polyprotein and their predicted sizes are indicated, according to previous reports (37, 41, 42, 49–51, 55, 272). Typical amino acid motifs for NS3 (GAPGIGKT), NS5 (KGKTK and DDEYDE), NS6 (GDCG), NS7 (WKGL, KDEL, DYSKWDST, GLPSG, and YGDD) and VP1 (PPG and GWS) are also shown. An overview of the RT-PCR target regions (RdRp region, RdRp-VP1 junction region, and VP1 region) is shown, and the detailed primer information is summarized in Tables 2 to 4. The putative first amino acids of VP1 from subgenomic transcript "MEG" and the cleavage site of this motif (ME/G; the slash indicates the cleavage site) from the ORF1 polyprotein are also shown with their nucleotide positions. The putative NS7 (RdRp) region and the cleavage site between RdRp and VP1 are based on previous reports (50, 52, 55, 58). The proposed subdomains in the sapovirus VP1 from the subgenomic transcript (N-terminal variable region [NVR] [1 to 43]), N-terminal region [N] [44 to 285]), central variable region [CVR] [286 to 441]), and C-terminal region [C] [442 to 561]) (47) are also indicated.

sid protein, VP1 (Fig. 2). ORF2 is predicted to encode the minor structural protein VP2 (Fig. 2) (28, 37). A similar genomic organization (i.e., two ORFs, with the first ORF encoding the nonstructural proteins and VP1) is found in other calicivirus genera, such as *Lagovirus*, *Nebovirus*, and the newly proposed genera *Valovirus*, *Bavovirus*, and *Nacovirus* (17, 19, 20, 38, 39). The genomic organization of *Norovirus*, *Vesivirus*, and *Recovirus* differs from that of *Sapovirus*: ORF1 encodes nonstructural proteins, and ORF2 and ORF3 encode structural proteins VP1 and VP2, respectively (18, 37, 40). A third ORF (ORF3) has been predicted in several human (12, 41–47) and bat (48) sapovirus strains; however, its function is unknown.

The ORF1-encoded polyprotein is expressed and processed into at least six nonstructural (NS) proteins (NS1, NS2, NS3, NS4, NS5, and NS6-NS7) and a structural protein (VP1) by virus-encoded protease (Fig. 2) (49–54). *In vitro* studies failed to show cleavage of the NS6-NS7 protein by the viral protease (28, 49, 50, 52, 55, 56), although both the NS6 and NS7 proteins can carry out their respective functions (proteolytic and polymerase) when expressed individually *in vitro* (56–58). The NS6-NS7 protein was also detected in porcine sapovirus-infected cells (28). Similar to the case for sapoviruses, vesivirus also produces the NS6-NS7 protein (fused protease-polymerase) (52, 53, 59–61), whereas noroviruses and lagoviruses produce an individual protease and polymerase, NS6 and NS7, respectively (51, 53, 62–67). The biological functions of the other sapovirus NS proteins have not been experimentally determined; however, NS3 and NS5 have a typical calicivirus NTPase motif (GAPGIGKT) and VPg motifs (KGKTK and DDEYDE), respectively (Fig. 2) (37, 49, 68, 69). VPg is linked to the 5′ end of the viral RNA and is critical for calicivirus genome replication, transcription, and translation (37, 70).

VP1, an approximately 60-kDa protein, is a major component of the complete virus (34, 35). Two mechanisms can be considered in the production of sapovirus VP1. One is that VP1 is cleaved from the ORF1-encoded polyprotein, and the other is that VP1 is translated from a subgenomic RNA (from the 3′-coterminal RNA corresponding to VP1 to the genome end region) (Fig. 2) (71, 72). A subgenomic RNA was confirmed for the sapovirus Cowden strain during replication (25). The VP2 protein has not yet been identified in sapovirus virions; however, the expression of this protein was detected in the *in vitro* translation products of a porcine sapovirus full-length genomic cDNA construct and from

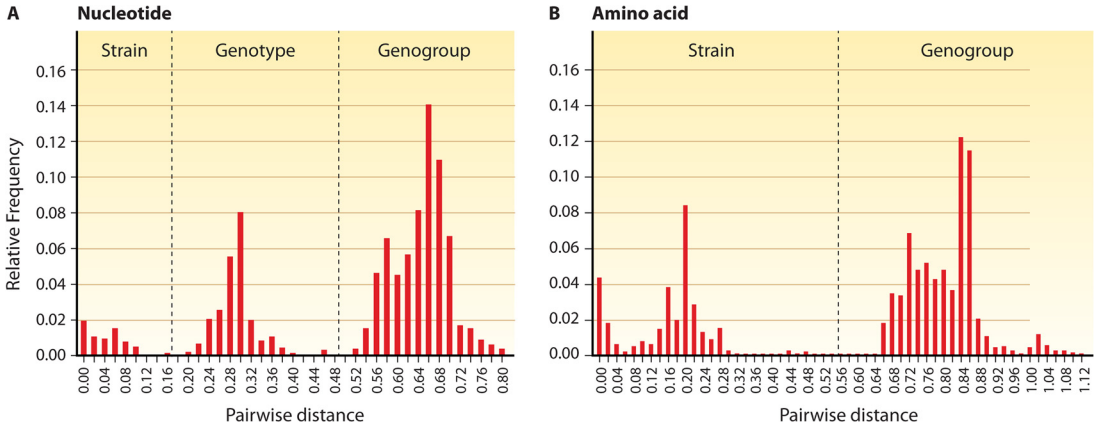

FIG 3 Pairwise distance distribution histograms of 59 representative sapovirus complete VP1 sequences. The vertical dashed lines indicate the cutoff limits for genogrouping and/or genotyping. (A) Limits based on nucleotide sequences, with three peaks (0 to 0.159, 0.198 to 0.471, and 0.522 to 0.807) corresponding to the strain, genotype, and genogroup range, respectively. The mean values ± 3 SD for the pairwise distance peaks were 0 to 0.151, 0.170 to 0.416, and 0.489 to 0.801, and the cutoff values for the genotype and genogroup clusters were designated ≤0.169 and ≤0.488, respectively. (B) Limits based on amino acid sequences, with two major peaks (0 to 0.480 and 0.652 to 1.115) corresponding to the strain and genogroup range, respectively.

porcine sapovirus-infected cells (28). VP2 is predicted to be a strong basic protein and is identified as an interior component of the norovirus particles (73).

The expression of VP1 in insect or mammalian cells resulted in spontaneously assembled virus-like particles (VLPs) (12, 71, 72, 74–81). The sapovirus VLPs are morphologically and antigenically indistinguishable from those of the native sapovirus virions found in clinical specimens (12, 74). Digitized electron cryomicrographs of the human sapovirus VLPs revealed that the icosahedral capsid is formed from 180 molecules of VP1, the same as in norovirus (76). Sapovirus VP1 could be separated into several domains: the N-terminal variable region (NVR), N-terminal region (N), central variable region (CVR), and C-terminal region (C) (Fig. 2) (47). The conserved amino acid motif "GWS" was found in the predicted N and CVR junction (Fig. 2). The "G" in this motif is strictly conserved among caliciviruses (76). Norovirus VP1 has also been separated into several domains, the N-terminal domain, shell domain, and protruding (P) domain, which is further divided into P1 and P2 subdomains (76, 82, 83). The sapovirus VP1 CVR region likely corresponds to the highly variable P2 domain of norovirus VP1 (47, 76).

GENOMIC SEQUENCE AND ANTIGENICITY

The first complete genome of a sapovirus was determined for the Manchester strain detected in the United Kingdom in 1993 (Hu/Manchester/93/UK; GenBank accession no. X86560) (41, 42), which is closely related genetically to the prototype Sapporo strain (14). Thus far, 26 (21 from humans and five from animals [porcine and bat]) complete sapovirus genomes are available in GenBank (as of 1 September 2013). The VP1-encoding region is the most diverse region in the genome (84–86), and sapoviruses are divided into multiple genogroups based on complete VP1 sequences. Five genogroups (GI to GV) are recognized (46, 87), and nine additional genogroups (GVI to GXIV) were recently proposed (88). To date, human sapoviruses have been classified into four genogroups (GI, GII, GIV, and GV).

Distinct antigenicity among sapovirus strains has been demonstrated by using clinical specimens (9, 43, 89–91), recombinant VP1 proteins (77, 92), or virus-like particles (VLPs) (74, 77, 80, 81, 93). Antigenicity differs among GI, GII, GIV, and GV strains (93, 94) and is also distinct among different genotypes within GI and GII (80, 81, 94). These experimental results also support that VP1 determines sapovirus antigenicity. The antigenic differences between human and animal sapoviruses have not yet been determined.

MOLECULAR CHARACTERIZATION

Genogroups and Genotypes

The partial RNA-dependent RNA polymerase (RdRp) or partial VP1 region (Fig. 2) or both of these regions can be used to partially characterize detected sapoviruses, as well as to investigate the similarity of the detected sapovirus for epidemiological surveys. In contrast, the RdRp-VP1 junction region (Fig. 2) is too short for such sequence analysis.

For genetic classification of sapoviruses, VP1 sequences are widely used, because this region is more diverse than the RdRp region (45, 46) and the VP1 sequence correlates with virus phenotype (i.e., antigenicity) (43, 74, 77, 80, 81, 92–95). The International Calicivirus Conference Committee proposed that at least the entire VP1 region sequence is necessary to designate new genogroups or genotypes. We recently established a human sapovirus classification scheme based on the complete VP1 nucleotide sequences (87). In this review, we include newly available sapovirus strains and updated sapovirus genotype numbering along with our previous analytical methods and criteria (87). The frequency histogram with pairwise distance values of 59 representative complete capsid nucleotide sequences of GI, GII, GIII, GIV, and GV sapoviruses resulted in three clearly distinct and nonoverlapping peaks (0 to 0.159, 0.198 to 0.471, and 0.522 to 0.807) (Fig. 3A). These three peaks can be considered to represent the strain, genotype, and genogroup, respectively, as previously described (87). The mean values ± 3 standard deviations (SD) for the pairwise distance peaks were 0 to 0.151, 0.170 to 0.416, and 0.489 to 0.801, respectively (Fig. 3A), and the cutoff values for the genotype and genogroup clusters were designated ≤0.169 and ≤0.488, respectively. Based on the criteria, human sapovirus GI and GII were each subdivided into seven genotypes (GI.1 to GI.7 and GII.1 to

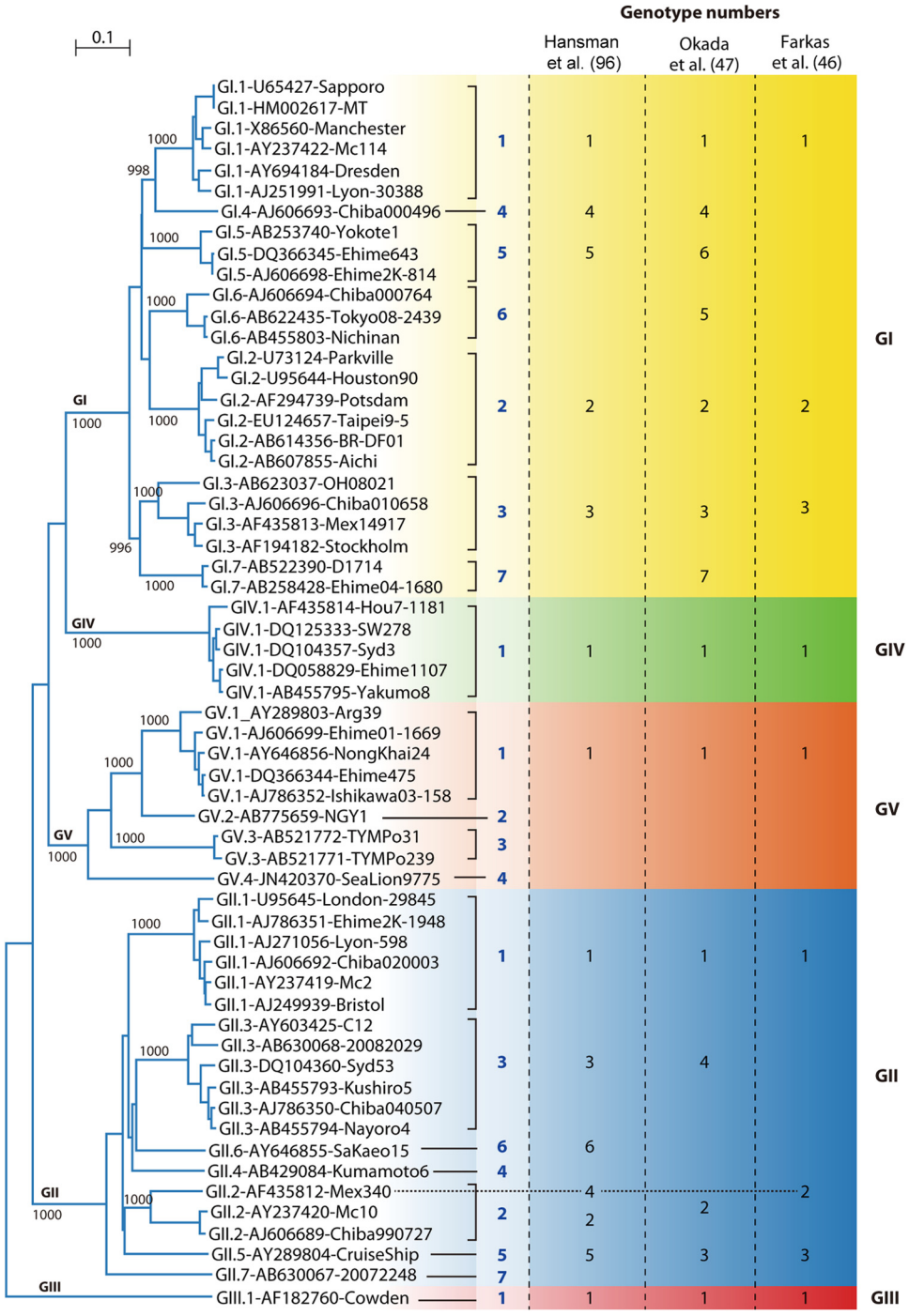

FIG 4 Genogroup and genotypes of GI, GII, GIII, GIV, and GV sapovirus strains based on complete VP1 nucleotide sequences. The phylogenetic tree is based on the complete VP1 nucleotide sequences (approximately 1,690 nt) of a total of 59 sapovirus strains (representing 58 sapovirus strains corresponding to all genotypes within GI, GII, GIV, and GV and one porcine strain representing GIII). The phylogenetic tree was constructed by the neighbor-joining method with 1,000 bootstrap replications using NJPlot software (http://pbil.univ-lyon1.fr/software/njplot.html) (273). The numbers on each branch indicate the bootstrap values of ≥950. The scale represents the nucleotide substitutions per site. Each sapovirus strain is indicated as genogroup/genotype-GenBank accession number-strain name (i.e., GI.1-U65427-Sapporo). Genotyping numbers are updated based on a recent classification scheme (87). Genotyping numbers from three other reports (46, 47, 96) are summarized for comparison.

GII.7). GIV was placed into a single genotype (GIV.1), and GV was subdivided into two genotypes (GV.1 and GV.2) (Fig. 4). GV also includes sapoviruses detected from pigs (GV.3) and sea lions (GV.4). As summarized in Fig. 4, genotype numbering based on the entire VP1 sequences is inconsistent among research groups for GI.5, GI.6, and GII.2 to GII.5 (46, 47, 87, 96). The phylogenetic tree pattern based on the 59 complete VP1 amino acid sequences is similar to that based on the nucleotide sequences (data not

shown); however, the pairwise distance histogram showed only two major peaks (0 to 0.480 and 0.652 to 1.115), and we cannot define the genotype range statistically (Fig. 3B). This differs from the case for noroviruses, because genotypes could be defined statistically by both VP1 nucleotide and amino acid sequences (97).

Evolution and Emergence of Predominant Sapovirus Strains

Genogroup and genotype analysis is important to characterize the currently circulating sapoviruses in the population. Emergence of genetically similar sapoviruses in multiple countries in Europe (98) and dynamic changes of genogroups and genotypes in different years among gastroenteritis patients in the same geographical area in Japan have been reported (99–101). Interestingly, GIV.1 strains were detected predominantly in Japan, Canada, the United States, and Europe around 2007 (98, 101–104). The dynamic change of the detected sapovirus genogroups in 2007 was also identified by national surveillance through regional diagnostic labs network in Japan (http://www.nih.go.jp/niid/en/iasr-table/2784-iasrtve.html; see "IASR Tables Virus" "By Season" "Gastrointestinal Pathogens" "PDF"). This is a distinct trend compared to noroviruses, in which a specific genogroup and genotype (i.e., genogroup II and genotype 4 [GII.4]) have been predominant in the past decade in Japan (http://www.nih.go.jp/niid/en/iasr-table/2784-iasrtve.html) and in multiple other countries (105–108). In the case of norovirus GII.4, time-ordered genetic and antigenic change of VP1 was identified (109, 110). Recent studies from Europe reported similar time-ordered genetic change in the VP1 region of the sapovirus GI.2 strains (98), as reported for norovirus GII.4 strains (111–115).

Due to the inconsistent genotype numbering systems used by different research groups for GI.5, GI.6, and GII.2 to GII.5 (46, 47, 87, 96) (Fig. 4), it is important to indicate which numbering system was used for genotyping, and a harmonized genotype numbering system will facilitate comparison and exchange of information from sapovirus surveillance at national and international levels.

Recombinant Strains

Sapoviruses with inconsistent grouping between the nonstructural protein-encoding region (including the RdRp region) and the VP1 encoding region have been designated "recombinant" or "chimeric" strains. Both intra- and intergenogroup recombinant strains have been reported (Fig. 5). All reported intergenogroup recombinant strains were GIV (based on VP1 sequence), whereas they were clustered together with GII strains in the RdRp region (46, 85, 102, 116, 117). Intragenogroup recombinant strains within GI (118–120), GII (84, 121, 122), and GIII (123) have been identified.

Recently, a norovirus classification scheme has been reported (97). The authors used nucleotide sequences of nearly complete RdRp (1,300 nucleotides [nt]) and both amino acid and nucleotide sequences of VP1 for classification (97). Although sapovirus RdRp (NS7) is fused with protease (NS6) (Fig. 2), we defined the putative complete RdRp-encoding region (1,566 nt) for sapoviruses (Fig. 2) based on our previous *in vitro* studies (52, 58). Among GI, GII, GIV, and GV sapoviruses, the nucleotide sequences spanning the putative complete RdRp- and VP1-encoding regions (Fig. 2) of 26 strains (10 GI, 5 GII, 5 GIV, and 6 GV based on VP1) were available in GenBank as of 1 September 2013. All of these strains and a representative GIII Cowden strain of pig origin were used for phylogenetic analysis based on the RdRp and VP1 regions. We found conserved amino acid motif "WKGL" (Fig. 2) at amino acid positions 12 to 15 in the putative compete RdRp (NS7) region among these sapovirus strains. As shown in Fig. 5, several strains clustered differently on the phylogenetic trees based on RdRp- and VP1-encoding regions. For example, based on the RdRp region, GII and GIV strains are not well separated, as discussed previously (85). The GII.2 Mc10 and GII.3 C12 strains also cluster together in the RdRp region and were previously reported as intragenogroup recombinant strains (84). The GV.4 strain clustered together with other GV strains (GV.1, GV.2, and GV.3) in the VP1 region, but it is separated from other GV strains in the RdRp region. However, the RdRp sequence-based classification is less reliable due to the fewer available sequences compared to the complete VP1 sequences. Further accumulation of sufficient sequence data spanning the complete or sufficient length of the RdRp- to the VP1-encoding regions for all the genogroups and genotypes are critical to provide a better understanding of "recombinant" or "chimeric" strains and to establish a reliable classification scheme for the sapovirus RdRp region in the future, because the putative complete RdRp sequence data for GI.3, GI.4, GI.6, GI.7, GII.4, GII.5, and GII.7 sapoviruses are not yet available (Fig. 5). In addition, it is also critical to amplify a single PCR fragment covering the partial RdRp- and VP1-encoding region for recombination analysis to avoid the possibility of coinfection of different genogroups and/or genotypes of sapovirus strains, as discussed previously (124).

LABORATORY DIAGNOSIS

Virus Particle Detection

Sapoviruses are morphologically distinguishable from other gastroenteritis pathogens (e.g., norovirus, rotavirus, astrovirus, or adenovirus) by their typical "Star of David" surface morphology under the electron microscope (1, 33, 90, 125) (Fig. 1). However, this has low sensitivity compared to nucleic acid detection methods (116, 126–128, 130, 131).

Antigen Detection Methods

Enzyme-linked immunosorbent assays (ELISAs) have been developed for the detection of human sapovirus antigens (91, 93, 132, 190) and have been used for the detection of sapoviruses from clinical samples (43, 91, 132–135, 190). However, these assays are not widely used for diagnosis due to the difficulty in detection of antigenically diverse sapovirus strains, low sensitivity compared to nucleic acid detection methods (43, 91, 93), and current lack of commercial availability. The development of a broadly reactive ELISA or immunochromatography system for the detection of sapovirus antigens depends on the combination of a panel of genogroup/genotype-specific antisera and/or using broadly reactive monoclonal antibodies. These approaches may be feasible, because a common epitope(s) likely exists among GI, GII, GIV, and GV sapovirus strains (94). Broadly reactive norovirus-specific monoclonal antibodies that recognize VP1s of different genogroups of noroviruses were also reported (136–140).

Nucleic Acid Detection Methods

Reverse transcription-PCR (RT-PCR), especially real-time RT-PCR, has become a major and routine method for sapovirus de-

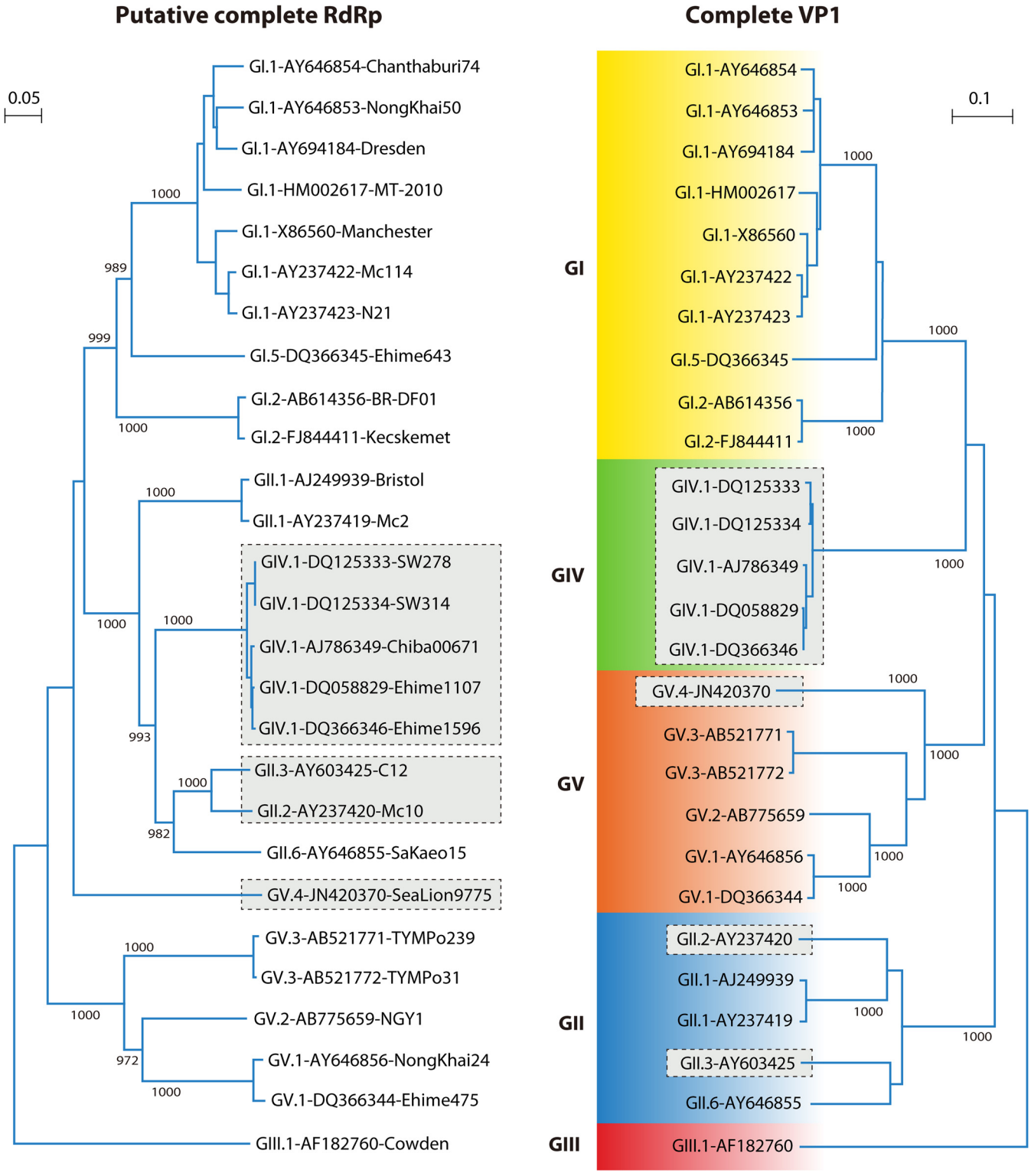

FIG 5 Comparative phylogenetic analysis of sapoviruses based on complete RdRp and VP1 nucleotide sequences. Phylogenetic trees of 27 sapovirus strains (10 GI, 5 GII, 5 GIV, and 6 GV strains and 1 representative GIII strain) whose sequences covering the putative complete RdRp to the end of VP1 had been available at GenBank (www.ncbi.nlm.nih.gov/) by 1 September 2013 are shown. The trees on the left and right are based on the nucleotide sequences of the putative complete RdRp region (approximately 1,570 nt) and the entire VP1 region (approximately 1,690 nt), respectively. The phylogenetic trees were constructed by the neighbor-joining method with 1,000 bootstrap replications using NJPlot software. The numbers on each branch indicate the bootstrap values of ≥950. The scale represents the nucleotide substitutions per site. The sapovirus strains showing inconsistent clustering patterns on the two trees are indicated within dashed boxes.

TABLE 2 Primer combinations targeting the RdRp region for human sapovirus detection

Primer or probe	Sequence (5′ to 3′)	Function[a]	Location in sapovirus genome	Strain	Accession no.	Reference
Sapp36	GTT GCT GTT GGC ATT AAC A	Forward	4273–4291	Manchester	X86560	141
Sapp35	GCA GTG GGT TTG AGA CCA AAG	Reverse	4740–4760	BR-DF01	AB614356	
4490	ACA CGT GGT GGT CTA CCA TCT GG	Forward	4510–4532	Manchester	X86560	143
4485	CAC ACT GTA CAT GCA RTC ATC ACC	Reverse	4666–4689	Manchester	X86560	
p290	GAT TAC TCC AAG TGG GAC TCC AC	Forward	4354–4376	Manchester	X86560	144
p289	TGA CAA TGT AAT CAT CAC CAT A	Reverse	4663–4684	Manchester	X86560	
SR80	TGG GAT TCT ACA CAA AAC CC	Forward	4366–4385	Manchester	X86560	147
JV33	GTG TAN ATG CAR TCA TCA CC	Reverse	4666–4685	Manchester	X86560	
SV-s1	CCC TGG CAA TAT TGG AGA GAT TTG	Forward	4403–4426	Manchester	X86560	148
SV-s2	AAG GCC TAT GAA CCA CAT GGG GC	Forward	4601–4623	Bristol	AJ249939	
SV-s3	GAC ATC AAG TTT GAT ACC AAG GA	Forward	4495–4517	BR-DF01	AB614356	
SV-r-c	GCA TTG TAG GTG GCG AGA GCC	Reverse	5079–5099	Manchester	X86560	
Sapp128	GAT TAC ACC AAA TGG GAT TCC AC	Forward	4354–4376	Manchester	X86560	149
Sapp129	GCA GTC ATC ACC ATA CGT GTG AA	Reverse	4655–4677	Manchester	X86560	
SLV-OFa	ACT SCA AAT GGG ATT CCA CWC AAM ACC	First, forward	4358–4384	Manchester	X86560	150
SLV-OFb	ACW CTA AAT GGG AYT CCA CAC AGA ATC C	First, forward	4358–4385	Manchester	X86560	
SLV-ORa	GTG RAA GAT KGA WGC RGT GGC AGG	First, reverse	4693–4716	Manchester	X86560	
SLV-ORb	TCA AAG ATG GAA GCG GTT GCC G	First, reverse	4694–4715	Manchester	X86560	
SLV-IF	TGG HCT MCC WTC WGG SAT GCC	Second, forward	4518–4538	Manchester	X86560	
SLV-IRa	CAC ACR CTG TAS ATG CAG TCA TCA CC	Second, reverse	4666–4691	Manchester	X86560	
SLV-IRb	CAC AAG GAG TAT ATG CAA TCA TCA CC	Second, reverse	4666–4691	Manchester	X86560	
p290h	GAT TAC TCC AGG TGG GAC TCC AC	Forward	4354–4376	Manchester	X86560	46
p290i	GAT TAC TCC AGG TGG GAC TCA AC	Forward	4354–4376	Manchester	X86560	
p290j	GAT TAC TCC AGG TGG GAT TCA AC	Forward	4354–4376	Manchester	X86560	
p290k	GAT TAC TCC AGG TGG GAT TCC AC	Forward	4354–4376	Manchester	X86560	
p289h	TGA CGA TTT CAT CAT CAC CAT A	Reverse	4663–4684	Manchester	X86560	
p289i	TGA CGA TTT CAT CAT CCC CGT A	Reverse	4663–4684	Manchester	X86560	
SR80	TGG GAT TCT ACA CAA AAC CC	First, forward	4366–4385	Manchester	X86560	152
JV33	GTG TAN ATG CAR TCA TCA CC	First, reverse	4666–4685	Manchester	X86560	
SR80 GI 1–3	CTR KCV GAT ATT GGA RAG ATT T	Second, forward	4404–4425	Manchester	X86560	
SR80 GI 2	AGT CTY TCC ATC TTA GAG AGA	Second, forward	4402–4422	BR-DF01	AB614356	
SR80 GII 1–2	GCT GCR TCY TTG KCA ATC CT	Second, forward	4400–4419	Bristol	AJ249939	
JV33	GTG TAN ATG CAR TCA TCA CC	Second, reverse	4666–4685	Manchester	X86560	
SV36	GTT TCT GTT GGC ATT AAC A	First, forward	4273–4291	Manchester	X86560	153
SRGA	CWG TAB ATG CAR TCA TCA CC	First, reverse	4666–4685	Manchester	X86560	
SVS1	CCC TGG CAA TAT TGG AGA GAT TTG	Second, forward	4403–4426	Manchester	X86560	
SRGE	TAC WGY AAA TGG GAT TCCAC	Second, forward	4357–4376	Manchester	X86560	
SRGA	CWG TAB ATG CAR TCA TCA CC	Second, reverse	4666–4685	Manchester	X86560	
SRGS2A	CAC ATT ACC AGT GTA AGG TGC CC	Second, reverse	4620–4642	Bristol	AJ249939	
Sapp36	GTT GCT GTT GGC ATT AAC A	Forward	4273–4291	Manchester	X86560	100
SV-s1	CCC TGG CAA TAT TGG AGA GAT TTG	Forward	4403–4426	Manchester	X86560	
SV-s2	AAG GCC TAT GAA CCA CAT GGG GC	Forward	4601–4623	Bristol	AJ249939	
SV-s3	GAC ATC AAG TTT GAT ACC AAG GA	Forward	4495–4517	BR-DF01	AB614356	
GV-GLPSGM	GGT CTC CCC TCG GGC ATG	Forward	4560–4577	NongKhai24	AY646856	
SaV1245R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	

[a] Primers used for nested RT-PCR are designated first and second.

tection from clinical specimens (i.e., feces), because of its specificity, sensitivity, and broad reactivity. Numerous primers have been designed for the detection of human sapoviruses (Tables 2 to 4). These primers are designed to amplify the partial RdRp (44, 46, 141–144, 146–153), RdRp-VP1 junction (86, 154–162), or partial VP1 (126, 152, 163–166) region (Fig. 2). Due to the high genetic diversity of sapoviruses, most of the assays include multiple or degenerate primers (Tables 2 to 4). The primers targeting the con-

TABLE 3 Primer and probe combinations targeting the RdRp-VP1 junction region for human sapovirus detection

Primer or probe	Sequence (5′ to 3′)[a]	Function	Location in sapovirus genome	Strain	Accession no.	Reference
CU-SV-F1	GAC CAG GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	154
CU-SV-F2	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	
CU-SV-R	CCC TCC ATY TCA AAC ACT AWT TTG	Reverse	5154–5177	Manchester	X86560	
CU-SV-Probe	TGG TTY ATA GGY GGT AC	Probe	5101–5117	Manchester	X86560	
SaV124F	GAY CAS GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	86
SaV1F	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	
SaV5F	TTT GAA CAA GCT GTG GCA TGC TAC	Forward	5112–5135	NongKhai24	AY646856	
SaV1245R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	
SaV124TP	CCR CCT ATR AAC CA	Probe	5101–5114	Manchester	X86560	
SaV5TP	TGC CAC CAA TGT ACC A	Probe	5142–5157	NongKhai24	AY646856	
sapo.fwdA	ACC AGG CTC TCG CCA CCT A	Forward	5075–5093	Manchester	X86560	155
sapo.fwdB	ATT TGG CCC TCG CCA CCT A	Forward	5075–5093	BR-DF01	AB614356	
sapo.rev	GCC CTC CAT YTC AAA CAC TAW TTT	Reverse	5155–5178	Manchester	X86560	
sapo.probeA	CTG TAC CAC CTA TGA ACC A	Probe	5101–5119	Manchester	X86560	
sapo.probeB	TTG TAC CAC CTA TGA ACC A	Probe	5101–5119	Manchester	X86560	
sapo.probeC	TGT ACC ACC TAT AAA CCA	Probe	5101–5118	Manchester	X86560	
sapo.probeD	TGC ACC ACC TAT GAA C	Probe	5107–5122	Ehime1107	DQ058829	
F1	CCA GGC TCT CGC CAC CTA C	Forward	5076–5094	Manchester	X86560	248
F2	CCA GGC TCT CGC TAC CTA C	Forward	5076–5094	Manchester	X86560	
F3	TTT GGC CCT CGC CAC CTA C	Forward	5076–5094	BR-DF01	AB614356	
R1	GCC CTC CAT CTC AAA CAC TAT TTT G	Reverse	5154–5178	Manchester	X86560	
R2	GCC CTC CAT TTC AAA CAC TAA TTT G	Reverse	5154–5178	Manchester	X86560	
probe	TGG TTY ATA GGY GGT RCA	Probe	5101–5118	Manchester	X86560	
SLVCF	GAY CWG GCY CTC GCC ACC T	Forward	5074–5092	Manchester	X86560	156
SLVCR	GCC CTC CAT YTC AAA CAC TA	Reverse	5159–5178	Manchester	X86560	
SVLCP	TGY ACC ACC TAT RAA CCA VG	Probe	5099–5118	Manchester	X86560	
SAPOs	CAG GCT CTC GCC ACC TAC	Forward	5077–5094	Manchester	X86560	157
SAPOas	CCC TCC ATY TCA AAC ACT AWT TT	Reverse	5155–5177	Manchester	X86560	
SAPO-XS-QS705	TGG TTC ATA GGT GGT RC	Probe	5101–5117	Manchester	X86560	
F1	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	158
F2	GAY CAS GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	
R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	
P	CCR CCT ATR AAC CA	Probe	5101–5114	Manchester	X86560	
F	GAY CAG GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	160
F	TTG GCC CTC GCC ACC TAC	Forward	5077–5094	BR-DF01	AB614356	
F	TTT GAA CAA GCT GTG GCA TGC TAC	Forward	5112–5135	NongKhai24	AY646856	
R	CCC TCC ATY TCA AAC ACT A	Reverse	5159–5177	Manchester	X86560	
P	CYT GGT TCA TAG GTG GTR CAG	Probe	5099–5119	Manchester	X86560	
P	CAG CTG GTA CAT TGG TGG CAC	Probe	5138–5158	NongKhai24	AY646856	
SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	Forward	5083–5105	Manchester	X86560	159
SMP-R	CMW WCC CCT CCA TYT CAA ACA C	Reverse	5161–5182	BR-DF01	AB614356	
F	GAY CAG GCT CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	161
F	TTT GAA CAA GCT GTG GCA TGC TAC	Forward	5112–5135	NongKhai24	AY646856	
R	CCC TCC ATY TC AAA CAC TA	Reverse	5159–5177	Manchester	X86560	
P	CYT GGT TCA TAG GTG GTR CAG	Probe	5099–5119	Manchester	X86560	
P	CAG CTG GTA CAT TGG TGG CAC	Probe	5138–5158	NongKhai24	AY646856	
GI-F	Tag-CTC GCC ACC TAC AAT GCY TGG TT	Forward	5083–5105	Manchester	X86560	162
GII, GIV, V-F	Tag-ACR GCC AAR GCT GAG GGG	Forward	5036–5053	Ehime1107	DQ058829	
GI-R	Tag-TGG GAT GTG GTC GGV CCA GT	Reverse	5242–5261	Manchester	X86560	
GII, GIV, V-R	Tag-CCC TCC ATT TCA AAC ACT AAT T	Reverse	5160–5181	Ehime1107	DQ058829	
GI probe	CCG AGC CTA GTG TTT GAG ATG GAG GGC AAT GGC TCG G	Probe	5152–5188	Manchester	X86560	
GII, GIV, V-Probe	CCC TGG GCC CCA GTG AAG AGA CCA CCA GGG	Probe	5133–5162	Ehime1107	DQ058829	

[a] Tag, GCAAGCCCTCACGTAGCGAA.

TABLE 4 Primer and probe combinations targeting the VP1 region for human sapovirus detection

Primer or probe	Sequence (5′ to 3′)	Function[a]	Location in sapovirus genome	Strain	Accession no.	Reference
SV-F11	GCY TGG TTY ATA GGT GGT AC	First, forward	5098–5117	Manchester	X86560	126
SV-R1	CWG GTG AMA CMC CAT TKT CCA T	First, reverse	5857–5878	Manchester	X86560	
SV-F21	ANT AGT GTT TGA RAT GGA GGG	Second, forward	5157–5177	Manchester	X86560	
SV-R2	GWG GGR TCA ACM CCW GGT GG	Second, reverse	5572–5591	Manchester	X86560	
SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	Forward	5083–5105	Manchester	X86560	163
SLV5749	CGG RCY TCA AAV STA CCB CCC CA	Reverse	5494–5516	Manchester	X86560	
SV-F13	GAY YWG GCY CTC GCY ACC TAC	First, forward	5074–5094	Manchester	X86560	165
SV-F14	GAA CAA GCT GTG GCA TGC TAC	First, forward	5074–5094	Manchester	X86560	
SV-R13	GGT GAN AYN CCA TTK TCC AT	First, reverse	5857–5876	Manchester	X86560	
SV-R14	GGT GAG MMY CCA TTC TCC AT	First, reverse	5857–5876	Manchester	X86560	
SV-F22	SMW AWT AGT GTT TGA RAT G	Second, forward	5154–5172	Manchester	X86560	
SV-R2	GWG GGR TCA ACM CCW GGT GG	Second, reverse	5572–5591	Manchester	X86560	
SV-F13	GAY YWG GCY CTC GCY ACC TAC	Forward	5074–5094	Manchester	X86560	165
SV-F14	GAA CAA GCT GTG GCA TGC TAC	Forward	5074–5094	Manchester	X86560	
SV-G1-R	CCC BGG TGG KAY GAC AGA AG	Reverse	5561–5580	Manchester	X86560	
SV-G2-R	CCA NCC AGC AAA CAT NGC RCT	Reverse	5483–5503	Mc10	AY237420	
SV-G4-R	GCG TAG CAG ATC CCA GAT AA	Reverse	5413–5432	Ehime1107	DQ058829	
SV-G5-R	TTG GAG GWT GTT GCT CCT GTG	Reverse	5384–5404	NongKhai24	AY646856	
No name	GCT GTT SCY ACT GGT GCA	Forward	5317–5334	Manchester	X86560	164
No name	GGC ATC CTG TCR TTC CAA GCA	Reverse	5391–5411	Manchester	X86560	
No name	CCA ATC SAA TGT CCC TGA GGC AAT ACG SAA	Probe	5337–5366	Manchester	X86560	
SLV5317	CTC GCC ACC TAC RAW GCB TGG TT	First, forward	5083–5105	Manchester	X86560	152
SLV5749	CGG RCY TCA AAV STA CCB CCC CA	First, reverse	5494–5516	Manchester	X86560	
SVpol3′-A	AAG GMR CSY MCA AAA ATA GTG	Second, forward	5144–5164	Manchester	X86560	
SVpol3′-B	GAA GRK RCW MCC AAA TTA GTG	Second, forward	5147–5167	Bristol	AJ249939	
SLV5749	CGG RCY TCA AAV STA CCB CCC CA	Second, reverse	5494–5516	Manchester	X86560	
SaV124F	GAY CAS GCT CTC GCY ACC TAC	First, forward	5074–5094	Manchester	X86560	175
SaV1F	TTG GCC CTC GCC ACC TAC	First, forward	5077–5094	BR-DF01	AB614356	
SaV5F	TTT GAA CAA GCT GTG GCA TGC TAC	First, forward	5112–5135	NongKhai24	AY646856	
SV-R13	GGT GAN AYN CCA TTK TCC AT	First, reverse	5857–5876	Manchester	X86560	
SV-R14	GGT GAG MMY CCA TTC TCC AT	First, reverse	5857–5876	Manchester	X86560	
1245Rfwd	TAG TGT TTG ARA TGG AGG G	Second, forward	5159–5177	Manchester	X86560	
SV-R2	GWG GGR TCA ACM CCW GGT GG	Second, reverse	5572–5591	Manchester	X86560	
SV-F13	GAY YWG GCY CTC GCY ACC TAC	First, forward	5074–5094	Manchester	X86560	180
SV-F14	GAA CAA GCT GTG GCA TGC TAC	First, forward	5074–5094	Manchester	X86560	
SVR-DS3	GGT GAV AVM CCA TTY TCC AT	First, reverse	5849–5868	Ehime1107	DQ058829	
SVR-DS4	GGH GAH ATN CCR TTB TSC AT	First, reverse	5849–5868	Ehime1107	DQ058829	
SaV 1245Rfwd	TAG TGT TTG ARA TGG AGG G	Second, forward	5159–5177	Manchester	X86560	
SVR-DS5	CCC CAC CCK GCC CAC AT	Second, reverse	5482–5498	Manchester	X86560	
SVR-DS6	CCC CAM CCM GCM MAC AT	Second, reverse	5482–5498	Manchester	X86560	
Forward	CAA TCC AAT CCA ATG TCC CT	Forward	5333–5352	Manchester	X86560	166
Reverse	ACY TCA AAV STA CCB CCC CA	Reverse	5494–5513	Manchester	X86560	
probe	ATT AAC CCG TAC ACT TCT CA	Probe	5452–5471	Manchester	X86560	

[a] Primers used for nested RT-PCR are designated first and second.

served motifs of the RdRp region (e.g., p290 and p289 [Table 2]) also amplify other human gastroenteritis viruses (norovirus, rotavirus, and astrovirus) (144, 167, 168). Numerous primers with distinct names are quite similar, especially for RdRp-VP1 junction-targeting primer sets (Table 3).

Multiplex RT-PCR or PCR assays, whose products were differentiated by agarose gel electrophoresis (101, 159, 163, 169), real-time RT-PCR or PCR (156, 158, 162, 170), and a microsphere-based fluorescent PCR product detection assay (e.g., Luminex technology) (160, 166), have been reported for the detection of human sapoviruses together with other gastroenteritis viruses. Although these assays aimed for simultaneous detection of multiple

viruses, it is unclear whether these assays can detect all genogroups of human sapoviruses.

Sapoviruses were also detected by specific primer-independent techniques (i.e., the metagenomic sequence approach) from untreated sewage (16), sewage sludge (171), and feces from California sea lions (172), dogs (173), and humans. These approaches are not widely used for diagnosis but may be applicable for routine clinical diagnosis in the future, when the cost of such assays and data analysis is comparable to that of traditional assays.

Selection of Detection Methods

The nucleic acid detection method is more sensitive than EM (116, 126–128, 130, 131) or ELISA (91). Different detection rates among different PCR assays using the same panel of specimens (clinical specimens, environmental water, and shellfish) were reported (99–101, 174–177). Assays targeting the RdRp-VP1 junction region have the highest detection rate and can be used as the first choice for sapovirus screening from clinical specimens (101, 174). The VP1-targeting RT-PCR is preferred because the products can be sequenced for reliable genotyping (99–101). Similar results were reported for environmental water samples (i.e., river water) (175). RdRp-VP1 junction-targeting real-time RT-PCR was also used for the detection of sapoviruses from shellfish (178, 179): however, the nested RT-PCR targeting the partial VP1 region is superior to the real-time RT-PCR and single-round RT-PCR because of the low level of viral RNA in shellfish compared to clinical specimens (177, 178). Currently, limited primer sets (47, 86, 100, 165, 175, 180) have demonstrated the ability to detect all genogroups of human sapoviruses.

Full-Genome Sequencing Approaches

Full genomic sequence analysis is still not practical for routine diagnosis. A long single-round or nested RT-PCR to amplify a 2- to 2.5-kb PCR fragment to determine the complete VP1 sequences of various sapovirus strains from clinical specimens is feasible by using forward primers targeting the RdRp and/or RdRp-VP1 junction region (Tables 2 to 4) and a reverse primer hybridized to the 3′-end poly(A) tail (Fig. 2) (46, 47, 87, 130, 174, 181, 182). In contrast, the amplification of the 5′-end 5- to 5.5-kb fragment corresponding to the beginning of the genome to the VP1 upstream region is variable because of the lack of universal primers. As a new technology, the specific primer-independent metagenomic sequencing approach (i.e., next-generation sequencing techniques) can be used to determine the nearly complete genome sequences (lacking the 5′ end or both the 5′ and 3′ ends) from fecal specimens (172, 173). 5′ rapid amplification of cDNA ends (RACE) techniques (14, 41, 42) are still necessary to determine 5′ ends to obtain the complete sapovirus genomic sequences.

CLINICAL AND EPIDEMIOLOGICAL OBSERVATIONS

Symptoms and Severity of Disease

Based on the epidemiological data from patients with sapovirus gastroenteritis, the incubation period ranges from less than 1 day to 4 days (5, 8, 44, 130, 135, 178, 183, 184). Major clinical symptoms include diarrhea and vomiting; however, additional constitutional symptoms (i.e., nausea, stomach/abdominal cramps, chills, headache, myalgia, or malaise) are also frequently reported.

TABLE 5 Reported clinical severity scores for sapovirus-, norovirus-, and rotavirus-associated gastroenteritis

Subject age	Clinical severity score (range)			Reference
	Sapovirus	Norovirus	Rotavirus	
<2 yr	6[b]	8[b]	10[b]	185
<2 yr	5.2 (3–10)[b]	7.9 (3–16)[b]	8.4 (1–16)[b]	186
Not specified[a]	6 (0–15)[c]	6 (0–12)[c]	8 (0–14)[c]	191

[a] Described as 0 to 65 and >65 years (191).
[b] Scored with a 0- to 20-point numerical system (192).
[c] Scored with a 0- to 21-point numerical system (191).

Similar to the case for norovirus illness, fever is a rare clinical symptom. Diarrhea usually resolves within 1 week (4, 5, 7–9, 44, 104, 117, 127, 135, 183, 185–189); however, individuals showing symptoms for a longer time (i.e., from over a week to up to 20 days) were also reported (9, 21, 127, 186, 188, 267). In general, the severity of sapovirus gastroenteritis is milder than that for rotavirus and norovirus (Table 5) (185, 186, 191). Gastroenteritis symptoms are usually self-limiting, and patients usually recover within a couple of days; however, the symptoms, severity, and duration of disease are dependent on the individual, and sapovirus infection sometimes leads to hospitalization (22, 152, 167, 193–209). Mortality is rare, but it was reported from outbreaks that occurred in a long-term-care facility for the elderly (104). Human noroviruses are associated with more serious clinical complications in susceptible groups (i.e., premature neonates and immunocompromised patients) (210–212). No such information is available for human sapoviruses, and this requires investigation in the future.

Subclinical (asymptomatic) sapovirus infection was also detected (4, 6, 21, 134, 135, 213–216). Quantitative PCR analysis revealed that asymptomatic individuals also shed sapovirus in the feces at levels comparable to those shed by individuals with gastroenteritis (182, 183).

Shedding Levels and Patterns in Feces

Sapovirus shedding in feces may continue after symptoms disappear (1 to 4 weeks after onset of illness) (6, 22, 174, 191). Sapovirus shedding levels in clinical stool specimens range from 1.32×10^5 to 1.05×10^{11} genomic copies/gram of stool (80, 99, 101, 116, 117, 127–130, 174, 178, 181–183). Sapovirus RNA shedding levels in feces gradually decreased after onset of illness (174). During the prolonged excretion period (i.e., 25 days and 28 days after onset of illness) in some individuals in an outbreak, both synonymous and nonsynonymous nucleotide substitutions in the VP1-encoding region have been identified (174), and this is a possible mechanism for the generation of new variants of sapovirus *in vivo*. Similar to the case for noroviruses, sapoviruses were also detected from an immunocompromised patient who showed prolonged diarrhea (147 days) (217), although further studies with quantitative analysis are necessary.

Sporadic Cases

Sapoviruses are detected worldwide (i.e., in more than 35 countries), and more than 100 papers have described sapovirus detection from clinical specimens. Among them, 13 studies detected more than 30 sapovirus strains from patients with sporadic gastroenteritis (Table 6) (89, 99, 101, 102, 126, 131, 132, 165, 185, 191, 215, 216, 218–226). Although different methods (electron microscopy, ELISA, and PCR assays with different primer sets)

TABLE 6 Sapovirus positive rates in gastroenteritis patients from 13 studies that detected more than 30 strains during the study period

Country	Study period	Age (yr)	Sapovirus positive rate, % (no. positive/total)	Screening method	Genogroup[g]	Positive rate (%) for other viral pathogens	Reference(s)
UK	1979-1981	Unknown	6.6 (39/592)	EM	NA[h]	NA	89
Kenya	1991-1994	≤6	2.2 (32/1431)	ELISA	NA	NA	132
Finland	1993-1995	<2	9.3 (72/775)	Nested RT-PCR[a]	NA	Rotavirus, 23.0; norovirus, 20.2; astrovirus, 8.8; adenovirus, 6.3	185
UK	1993-1996	All	3.8 (92/2422)	RT-PCR[b]	NA	Norovirus, 36.0; rotavirus, 31.3	218
Netherlands	1998-1999	All	6.3 (43/687)	RT-PCR[b]	NA	Norovirus, 16.1; rotavirus, 7.3; adenovirus, 3.8; astrovirus, 2.0	216
Japan	1998-2005	All	9.3 (195/2100)	Nested RT-PCR[c]	GI, GII, GIV, GV	NA	126, 165
Japan	2002-2007	All	12.7 (81/639)	RT-PCR[d]	GI, GII, GIV, GV	Norovirus, 40.7; rotavirus, 7.7; adenovirus, 3.0; enterovirus, 2.0; astrovirus, 1.4	101
Japan	2002-2009	≤15	3.7 (146/3895)	RT-PCR[e]	GI, GII, GIV	NA	102, 220–224
Denmark	2005-2007	≤3	8.8 (97/1104)	qRT-PCR[f]	GI, GII, GV	NA	225
UK	2006-2007	<5	12.7 (74/583)	RT-PCR[b]	NA	Norovirus, 24.5; rotavirus, 19.0	219
USA	2008-2009	<5	5.4 (42/782)	qRT-PCR[f]	NA	Norovirus, 21.4; rotavirus, 18.0; adenovirus, 11.8; astrovirus, 4.9	215
UK	2008-2009	All	8.8 (77/874)	qRT-PCR[f]	NA	Norovirus, 12.4; rotavirus, 7.3; adenovirus, 3.4; astrovirus, 2.5	226
Canada	2008-2009	All	4.2 (107/2486)	qRT-PCR[f]	NA	Norovirus, 17.6; rotavirus, 6.8; astrovirus, 2.0; adenovirus, 1.4	131

[a] First, Sapp36/SLV-r-c; second, S1,S2,S3-Sapp-rc (148).
[b] SR80-JV33 (147).
[c] First, F11/R1 or F13,14,R13,14; second, F21/R2 or F22/R2 (126, 165).
[d] SaV1F, -1,2,4F, -5F, -1245R (86, 101).
[e] SLV5317-5749 (163).
[f] SaV1F, -1,2,4F, -5F, -1245R, and SaV124TP, SaV5TP (86).
[g] Genogroups were determined based on partial VP1 region sequences.
[h] NA, not available.

were used in these studies, the sapovirus positive rates ranged from 2.2% to 12.7%. Eight studies also detected other gastroenteritis pathogens, and sapoviruses ranked second to fourth as the major viral pathogens among patients with sporadic gastroenteritis (Table 6). Similar to the case for noroviruses (101, 131, 185, 198), sapoviruses were detected mainly in the cold season among patients with sporadic gastroenteritis (89, 99–101, 131, 198, 225–227), although different seasonal peaks among years have also been reported (132, 185). Sapovirus illnesses occur more frequently in younger children than in older children and adults (131, 191, 216).

Outbreaks

Although the reported outbreak numbers are less for sapoviruses than for noroviruses (145, 228–230), sapovirus gastroenteritis outbreaks occur throughout the year in all ages of people in various settings, such as child day care centers, kindergartens, schools, colleges, hospitals, nursing homes, restaurants, hotels, wedding halls, and ships (3–7, 9, 80, 98, 103, 104, 116, 117, 127, 129, 130, 145, 174, 181, 182, 186, 188, 189, 228, 231–237). Suspected foodborne sapovirus outbreaks have also been reported (44, 145, 178, 230, 232, 238). The largest foodborne sapovirus outbreak (n = 665) has been reported in Japan in 2010 (183). An epidemiological investigation pointed to contaminated box lunches which were prepared by food handlers who were shedding sapovirus.

Data from four studies suggest that sapovirus caused 1.3 to 8.0% of the gastroenteritis outbreaks (Table 7) (98, 145, 228, 230), and data from the other three studies reported that sapovirus was detected in 5.9 to 22.6% of outbreak samples that tested negative for norovirus or both norovirus and pathogenic bacteria (103, 104, 234) (Table 7).

Coinfections of sapoviruses and multiple enteric viruses (e.g., noroviruses, rotaviruses, astroviruses, adenoviruses, enteroviruses, kobuviruses, etc.) have also been reported among acute gastroenteritis outbreaks (10, 178, 230, 231, 235–237, 239). Coinfections with different sapovirus strains (i.e., different genogroups/genotypes) were also identified from oyster/clam-associated gastroenteritis outbreaks (178, 236).

Sapoviruses in Seafood, Environmental Water, and Animals

Sapoviruses genetically indistinguishable (i.e., similar or identical based on partial virus genome sequences) from those detected in

TABLE 7 Sapovirus positive rates in gastroenteritis outbreaks

Country	Study period	Sapovirus positive rate, % (no. positive/total)	Screening method	Genogroup[g]	Positive rate (%) for other viral pathogens	Reference
Sweden	1994-1998	1.3 (9/676)	EM	NA[h]	Norovirus, 89	145
USA	2000-2004	1.8 (4/226)	RT-PCR[c]	NA	Norovirus, 79.6	228
Netherlands	2007-2009	4.0 (19/478)	qRT-PCR[d]	GI, GIV	NA	98
Germany	2002-2003	5.9 (2/34)[a]	RT-PCR[e]	NA	NA	234
Canada	2004-2007	17.6 (43/244)[b]	qRT-PCR[f]	GI, GII, GIV, GV	NA	103
USA	2002-2009	22.6 (21/93)[b]	qRT-PCR[f]	GI, GII, GIV, GV	NA	104
Japan	2001-2012	8.0 (7/88)	qRT-PCR[f]	GI, GII, GIV	Norovirus, 96.6; kobuvirus, 21.6; astrovirus, 5.7; rotavirus, 1.1	230

[a] Norovirus- and pathogenic bacterium-negative outbreaks.
[b] Norovirus-negative outbreaks.
[c] p290/289 (144).
[d] SLV-CF/SLV-CR, SLV-CP (156).
[e] SR80/JV33 (147).
[f] SaV1F, -1,2,4F, -5F, -1245R, SaV124TP, SaV5TP (86).
[g] Genogroups were determined based on partial VP1 region sequences.
[h] NA, not available.

human clinical specimens have also been detected from shellfish (oysters and clams) (177, 178, 240, 241) and environmental water samples (river water and wastewater) (16, 171, 175, 176, 180, 241–246). These sapoviruses were likely viruses of human fecal origin that were discharged into environmental waters and accumulated in shellfish (i.e., oysters or clams). As evidence, sapoviruses were detected more frequently with higher viral RNA levels from environmental water samples (i.e., sewage and river water) in the cold season (175, 176, 180, 243, 247, 248), when the number of patients with sapovirus-associated sporadic gastroenteritis increased (89, 99–101, 131, 198, 225–227). In addition, similar sapovirus strains were detected from gastroenteritis patients, wastewater, and oysters, which were collected from geographically related areas in the same season (241). In contrast, sapoviruses genetically indistinguishable from those detected in human clinical specimens have not been discovered in other animals (i.e., swine, mink, bats, dogs, and sea lions) (48, 88, 123, 172, 173, 249–253). Based on complete VP1 sequences, GV.3 porcine sapoviruses are closest to human strains; however, they can be clearly separated into a different genotype (Fig. 4). These results suggest the existence of interspecies barriers among human and animal sapoviruses, although further epidemiological studies for other animals and experimental infection studies using human sapoviruses in various animals are necessary. Sapovirus contamination levels were $\sim 1.6 \times 10^4$ copies/g of digestive tissue in various types of shellfish (oyster, cockle, and smooth clam) (179), up to 1.3×10^5 copies/liter in wastewater treatment plant influent (248), and $\sim 1.3 \times 10^9$ copies/liter in untreated wastewater (247).

Transmission Route and Host Susceptibility

Transmission of sapovirus is through the fecal-oral route. Sapoviruses can be transmitted from person to person via contact with sapovirus-positive feces, vomitus, or sapovirus-contaminated materials/surfaces or via contaminated food and drinking water (44, 104, 129, 130, 145, 178, 182, 183, 231, 232, 236–239). These transmission routes are similar to those for norovirus (254), and sapovirus may also have a low infectious dose similar to that of norovirus (i.e., 1,015 to 2,800 genomic copies) (255, 256); however, similar volunteer studies are necessary to confirm this speculation for sapoviruses. No host genetic factors for susceptibility or resistance to human sapovirus infection and disease have been identified. Susceptibility to human sapoviruses is not associated with histo-blood group antigen (HBGA) phenotypes (214). *In vitro* data also support no binding of sapovirus to HBGAs (257, 258). This differs from the case for the prototype norovirus (Norwalk virus): certain HBGA phenotypes (e.g., nonsecretor) of an individual are clearly related to resistance to virus infection (259, 260). Other different genogroups/genotypes of norovirus strains can also bind to HBGAs (113, 261–263) but lack a clear relatedness between the HBGA phenotypes and resistance to infection (264–266). Sialic acids have recently been reported as binding factors for porcine sapovirus (258).

Immunity

The serological responses to sapovirus infection were demonstrated by immune electron microscopy, ELISA, or radioimmunoassay using paired sera (i.e., acute- and convalescent-phase sera) with purified virus from clinical specimens (3, 4, 7, 22, 90, 190, 267, 268). The seroprevalence studies of human sapoviruses using purified virus or recombinant capsid proteins demonstrated a gradually increasing seroprevalence rate with age, and it reached a high level (>90%) in school-age children, and remained high (80 to 100%) in sera or pooled immunoglobulin collected from adults (92, 132, 190, 269–271). These results suggest that sapovirus infection is common during early childhood.

Protective immunity/resistance mechanisms to sapovirus infection at the putative primary infection site (e.g., intestinal lumen) remain to be clarified, but the presence of preexisting serum antibodies to sapoviruses was associated with reduced frequencies of sapovirus infection and illness, at least for antibodies to antigenically homologous sapoviruses (267). A similar phenomenon was also observed in gastroenteritis outbreaks that occurred in mother and baby units (7). Adults who had serum antibodies to antigenically indistinguishable human sapoviruses did not show any clinical symptoms on reinfection (7). Symptomatic reinfections with a different genogroup/genotype of sapovirus were recently reported in a study from Japan (99).

CONCLUSIONS AND FUTURE DIRECTIONS

Recent epidemiological studies with improved diagnostic assays have highlighted the impact of sapovirus-associated gastroenteritis. Genetically highly diverse sapovirus strains were identified through epidemiological surveillance studies. Continuous surveillance with a broadly reactive detection system(s) and molecular characterization will permit the identification of changes in major strains as well as the emergence of new strains and an understanding of the evolution of sapoviruses among humans and animals. However, in contrast to the significant improvement in sapovirus detection methods, the basic understanding of infection/replication sites, pathological changes in infected persons, immunological responses and protective immunity to sapovirus infections in humans, infectious dose, and stability in the environment remain unknown. To date, no vaccines or antiviral drugs are available for the control and prevention of human sapovirus infections. The mechanisms of virus binding and entry into target cells and viral RNA replication and translation are undefined, partially due to the lack of a cell culture system. Extensive studies of human sapoviruses in clinical cases, the use of the cell culture-adapted porcine sapovirus strain as a model, and establishment of a human sapovirus cell culture system will improve our knowledge of sapoviruses and may lead to more targeted control measures for prevention of sapovirus gastroenteritis in the future.

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REFERENCES

1. **Madeley CR.** 1979. Comparison of the features of astroviruses and caliciviruses seen in samples of feces by electron microscopy. J Infect Dis **139:**519–523. http://dx.doi.org/10.1093/infdis/139.5.519.
2. **Madeley CR, Cosgrove BP.** 1976. Caliciviruses in man. Lancet **i:**199–200. (Letter.) http://dx.doi.org/10.1016/S0140-6736(76)91309-X.
3. **McSwiggan DA, Cubitt D, Moore W.** 1978. Calicivirus associated with winter vomiting disease. Lancet **i:**1215. http://dx.doi.org/10.1016/S0140-6736(78)91012-7.
4. **Chiba S, Sakuma Y, Kogasaka R, Akihara M, Horino K, Nakao T, Fukui S.** 1979. An outbreak of gastroenteritis associated with calicivirus in an infant home. J Med Virol **4:**249–254. http://dx.doi.org/10.1002/jmv.1890040402.
5. **Cubitt WD, McSwiggan DA, Moore W.** 1979. Winter vomiting disease caused by calicivirus. J Clin Pathol **32:**786–793. http://dx.doi.org/10.1136/jcp.32.8.786.
6. **Chiba S, Sakuma Y, Kogasaka R, Akihara M, Terashima H, Horino K, Nakao T.** 1980. Fecal shedding of virus in relation to the days of illness in infantile gastroenteritis due to calicivirus. J Infect Dis **142:**247–249. http://dx.doi.org/10.1093/infdis/142.2.247.
7. **Cubitt WD, McSwiggan DA, Arstall S.** 1980. An outbreak of calicivirus infection in a mother and baby unit. J Clin Pathol **33:**1095–1098. http://dx.doi.org/10.1136/jcp.33.11.1095.
8. **Humphrey TJ, Cruickshank JG, Cubitt WD.** 1984. An outbreak of calicivirus associated gastroenteritis in an elderly persons home. A possible zoonosis? J Hyg (Lond) **93:**293–299. http://dx.doi.org/10.1017/S0022172400064822.
9. **Cubitt WD, Pead PJ, Saeed AA.** 1981. A new serotype of calicivirus associated with an outbreak of gastroenteritis in a residential home for the elderly. J Clin Pathol **34:**924–926. http://dx.doi.org/10.1136/jcp.34.8.924.
10. **Nakata S, Honma S, Numata KK, Kogawa K, Ukae S, Morita Y, Adachi N, Chiba S.** 2000. Members of the family caliciviridae (Norwalk virus and Sapporo virus) are the most prevalent cause of gastroenteritis outbreaks among infants in Japan. J Infect Dis **181:**2029–2032. http://dx.doi.org/10.1086/315500.
11. **Chiba S, Nakata S, Numata-Kinoshita K, Honma S.** 2000. Sapporo virus: history and recent findings. J Infect Dis **181**(Suppl 2)**:**S303–S308. http://dx.doi.org/10.1086/315574.
12. **Numata K, Hardy ME, Nakata S, Chiba S, Estes MK.** 1997. Molecular characterization of morphologically typical human calicivirus Sapporo. Arch Virol **142:**1537–1552. http://dx.doi.org/10.1007/s007050050178.
13. **Matson DO, Zhong WM, Nakata S, Numata K, Jiang X, Pickering LK, Chiba S, Estes MK.** 1995. Molecular characterization of a human calicivirus with sequence relationships closer to animal caliciviruses than other known human caliciviruses. J Med Virol **45:**215–222. http://dx.doi.org/10.1002/jmv.1890450218.
14. **Nakanishi K, Tatsumi M, Kinoshita-Numata K, Tsugawa T, Nakata S, Tsutsumi H.** 2011. Full sequence analysis of the original Sapporo virus. Microbiol Immunol **55:**657–660. http://dx.doi.org/10.1111/j.1348-0421.2011.00358.x.
15. **Mayo MA.** 2002. A summary of taxonomic changes recently approved by ICTV. Arch Virol **147:**1655–1663. http://dx.doi.org/10.1007/s007050200039.
16. **Ng TF, Marine R, Wang C, Simmonds P, Kapusinszky B, Bodhidatta L, Oderinde BS, Wommack KE, Delwart E.** 2012. High variety of known and new RNA and DNA viruses of diverse origins in untreated sewage. J Virol **86:**12161–12175. http://dx.doi.org/10.1128/JVI.00869-12.
17. **Wolf S, Reetz J, Hoffmann K, Grundel A, Schwarz BA, Hanel I, Otto PH.** 2012. Discovery and genetic characterization of novel caliciviruses in German and Dutch poultry. Arch Virol **157:**1499–1507. http://dx.doi.org/10.1007/s00705-012-1326-7.
18. **Farkas T, Sestak K, Wei C, Jiang X.** 2008. Characterization of a rhesus monkey calicivirus representing a new genus of Caliciviridae. J Virol **82:**5408–5416. http://dx.doi.org/10.1128/JVI.00070-08.
19. **L'Homme Y, Sansregret R, Plante-Fortier E, Lamontagne AM, Ouardani M, Lacroix G, Simard C.** 2009. Genomic characterization of swine caliciviruses representing a new genus of Caliciviridae. Virus Genes **39:**66–75. http://dx.doi.org/10.1007/s11262-009-0360-3.
20. **Wolf S, Reetz J, Otto P.** 2011. Genetic characterization of a novel calicivirus from a chicken. Arch Virol **156:**1143–1150. http://dx.doi.org/10.1007/s00705-011-0964-5.
21. **Spratt HC, Marks MI, Gomersall M, Gill P, Pai CH.** 1978. Nosocomial infantile gastroenteritis associated with minirotavirus and calicivirus. J Pediatr **93:**922–926. http://dx.doi.org/10.1016/S0022-3476(78)81212-8.
22. **Suzuki H, Konno T, Kutsuzawa T, Imai A, Tazawa F, Ishida N, Katsushima N, Sakamoto M.** 1979. The occurrence of calicivirus in infants with acute gastroenteritis. J Med Virol **4:**321–326. http://dx.doi.org/10.1002/jmv.1890040410.
23. **Kjeldsberg E.** 1977. Small spherical viruses in faeces from gastroenteritis patients. Acta Pathol Microbiol Scand B **85B:**351–354.
24. **Cubitt WD, Barrett AD.** 1984. Propagation of human candidate calicivirus in cell culture. J Gen Virol **65:**1123–1126. http://dx.doi.org/10.1099/0022-1317-65-6-1123.
25. **Chang KO, Sosnovtsev SV, Belliot G, Kim Y, Saif LJ, Green KY.** 2004. Bile acids are essential for porcine enteric calicivirus replication in association with down-regulation of signal transducer and activator of transcription 1. Proc Natl Acad Sci U S A **101:**8733–8738. http://dx.doi.org/10.1073/pnas.0401126101.
26. **Flynn WT, Saif LJ.** 1988. Serial propagation of porcine enteric calicivirus-like virus in primary porcine kidney cell cultures. J Clin Microbiol **26:**206–212.
27. **Parwani AV, Flynn WT, Gadfield KL, Saif LJ.** 1991. Serial propagation of porcine enteric calicivirus in a continuous cell line. Effect of medium supplementation with intestinal contents or enzymes. Arch Virol **120:**115–122. http://dx.doi.org/10.1007/BF01310954.
28. **Chang KO, Sosnovtsev SS, Belliot G, Wang Q, Saif LJ, Green KY.** 2005. Reverse genetics system for porcine enteric calicivirus, a prototype sapovirus in the Caliciviridae. J Virol **79:**1409–1416. http://dx.doi.org/10.1128/JVI.79.3.1409-1416.2005.
29. **Shivanna V, Kim Y, Chang KO.** 2014. The crucial role of bile acids in the entry of porcine enteric calicivirus. Virology **456:**268–278. http://dx.doi.org/10.1016/j.virol.2014.04.002.
30. **Chang KO, Kim Y, Green KY, Saif LJ.** 2002. Cell-culture propagation of porcine enteric calicivirus mediated by intestinal contents is dependent on the cyclic AMP signaling pathway. Virology **304:**302–310. http://dx.doi.org/10.1006/viro.2002.1665.
31. **Flynn WT, Saif LJ, Moorhead PD.** 1988. Pathogenesis of porcine enteric

calicivirus-like virus in four-day-old gnotobiotic pigs. Am J Vet Res **49:** 819–825.
32. **Guo M, Hayes J, Cho KO, Parwani AV, Lucas LM, Saif LJ.** 2001. Comparative pathogenesis of tissue culture-adapted and wild-type Cowden porcine enteric calicivirus (PEC) in gnotobiotic pigs and induction of diarrhea by intravenous inoculation of wild-type PEC. J Virol **75:**9239–9251. http://dx.doi.org/10.1128/JVI.75.19.9239-9251.2001.
33. **Saif LJ, Bohl EH, Theil KW, Cross RF, House JA.** 1980. Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. J Clin Microbiol **12:**105–111.
34. **Terashima H, Chiba S, Sakuma Y, Kogasaka R, Nakata S, Minami R, Horino K, Nakao T.** 1983. The polypeptide of a human calicivirus. Arch Virol **78:**1–7. http://dx.doi.org/10.1007/BF01310853.
35. **Parwani AV, Saif LJ, Kang SY.** 1990. Biochemical characterization of porcine enteric calicivirus: analysis of structural and nonstructural viral proteins. Arch Virol **112:**41–53. http://dx.doi.org/10.1007/BF01348984.
36. **Wang Q, Zhang Z, Saif LJ.** 2012. Stability of and attachment to lettuce by a culturable porcine sapovirus surrogate for human caliciviruses. Appl Environ Microbiol **78:**3932–3940. http://dx.doi.org/10.1128/AEM.06600-11.
37. **Green KY.** 2007. Caliciviridae: the noroviruses: specific virus families, p 949–979. *In* Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (ed), Fields virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
38. **Smiley JR, Chang KO, Hayes J, Vinje J, Saif LJ.** 2002. Characterization of an enteropathogenic bovine calicivirus representing a potentially new calicivirus genus. J Virol **76:**10089–10098. http://dx.doi.org/10.1128/JVI.76.20.10089-10098.2002.
39. **Meyers G, Wirblich C, Thiel HJ.** 1991. Rabbit hemorrhagic disease virus—molecular cloning and nucleotide sequencing of a calicivirus genome. Virology **184:**664–676. http://dx.doi.org/10.1016/0042-6822(91)90436-F.
40. **Clarke IN, Lambden PR.** 2000. Organization and expression of calicivirus genes. J Infect Dis **181**(Suppl)2:S309–S316. http://dx.doi.org/10.1086/315575.
41. **Liu BL, Clarke IN, Caul EO, Lambden PR.** 1995. Human enteric caliciviruses have a unique genome structure and are distinct from the Norwalk-like viruses. Arch Virol **140:**1345–1356. http://dx.doi.org/10.1007/BF01322662.
42. **Liu B, Clarke IN, Caul EO, Lambden PR.** 1997. The genomic 5′ terminus of Manchester calicivirus. Virus Genes **15:**25–28. http://dx.doi.org/10.1023/A:1007946628253.
43. **Jiang X, Cubitt WD, Berke T, Zhong W, Dai X, Nakata S, Pickering LK, Matson DO.** 1997. Sapporo-like human caliciviruses are genetically and antigenically diverse. Arch Virol **142:**1813–1827. http://dx.doi.org/10.1007/s007050050199.
44. **Noel JS, Liu BL, Humphrey CD, Rodriguez EM, Lambden PR, Clarke IN, Dwyer DM, Ando T, Glass RI, Monroe SS.** 1997. Parkville virus: a novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. J Med Virol **52:**173–178. http://dx.doi.org/10.1002/(SICI)1096-9071(199706)52:2<173::AID-JMV10>3.0.CO;2-M.
45. **Schuffenecker I, Ando T, Thouvenot D, Lina B, Aymard M.** 2001. Genetic classification of "Sapporo-like viruses." Arch Virol **146:**2115–2132. http://dx.doi.org/10.1007/s007050170024.
46. **Farkas T, Zhong WM, Jing Y, Huang PW, Espinosa SM, Martinez N, Morrow AL, Ruiz-Palacios GM, Pickering LK, Jiang X.** 2004. Genetic diversity among sapoviruses. Arch Virol **149:**1309–1323. http://dx.doi.org/10.1007/s00705-004-0296-9.
47. **Okada M, Yamashita Y, Oseto M, Ogawa T, Kaiho I, Shinozaki K.** 2006. Genetic variability in the sapovirus capsid protein. Virus Genes **33:**157–161. http://dx.doi.org/10.1007/s11262-005-0051-7.
48. **Tse H, Chan WM, Li KS, Lau SK, Woo PC, Yuen KY.** 2012. Discovery and genomic characterization of a novel bat sapovirus with unusual genomic features and phylogenetic position. PLoS One **7:**e34987. http://dx.doi.org/10.1371/journal.pone.0034987.
49. **Oka T, Katayama K, Ogawa S, Hansman GS, Kageyama T, Ushijima H, Miyamura T, Takeda N.** 2005. Proteolytic processing of sapovirus ORF1 polyprotein. J Virol **79:**7283–7290. http://dx.doi.org/10.1128/JVI.79.12.7283-7290.2005.
50. **Oka T, Yamamoto M, Katayama K, Hansman GS, Ogawa S, Miyamura T, Takeda N.** 2006. Identification of the cleavage sites of sapovirus open reading frame 1 polyprotein. J Gen Virol **87:**3329–3338. http://dx.doi.org/10.1099/vir.0.81799-0.
51. **Oka T, Yokoyama M, Katayama K, Tsunemitsu H, Yamamoto M, Miyashita K, Ogawa S, Motomura K, Mori H, Nakamura H, Wakita T, Takeda N, Sato H.** 2009. Structural and biological constraints on diversity of regions immediately upstream of cleavage sites in calicivirus precursor proteins. Virology **394:**119–129. http://dx.doi.org/10.1016/j.virol.2009.08.018.
52. **Oka T, Yamamoto M, Yokoyama M, Ogawa S, Hansman GS, Katayama K, Miyashita K, Takagi H, Tohya Y, Sato H, Takeda N.** 2007. Highly conserved configuration of catalytic amino acid residues among calicivirus-encoded proteases. J Virol **81:**6798–6806. http://dx.doi.org/10.1128/JVI.02840-06.
53. **Oka T, Murakami K, Wakita T, Katayama K.** 2011. Comparative site-directed mutagenesis in the catalytic amino acid triad in calicivirus proteases. Microbiol Immunol **55:**108–114. http://dx.doi.org/10.1111/j.1348-0421.2010.00295.x.
54. **Yokoyama M, Oka T, Kojima H, Nagano T, Okabe T, Katayama K, Wakita T, Kanda T, Sato H.** 2012. Structural basis for specific recognition of substrates by sapovirus protease. Front Microbiol **3:**312. http://dx.doi.org/10.3389/fmicb.2012.00312.
55. **Oka T, Katayama K, Ogawa S, Hansman GS, Kageyama T, Miyamura T, Takeda N.** 2005. Cleavage activity of the sapovirus 3C-like protease in Escherichia coli. Arch Virol **150:**2539–2548. http://dx.doi.org/10.1007/s00705-005-0591-0.
56. **Robel I, Gebhardt J, Mesters JR, Gorbalenya A, Coutard B, Canard B, Hilgenfeld R, Rohayem J.** 2008. Functional characterization of the cleavage specificity of the sapovirus chymotrypsin-like protease. J Virol **82:**8085–8093. http://dx.doi.org/10.1128/JVI.00693-08.
57. **Fullerton SW, Blaschke M, Coutard B, Gebhardt J, Gorbalenya A, Canard B, Tucker PA, Rohayem J.** 2007. Structural and functional characterization of sapovirus RNA-dependent RNA polymerase. J Virol **81:**1858–1871. http://dx.doi.org/10.1128/JVI.01462-06.
58. **Bull RA, Hyde J, Mackenzie JM, Hansman GS, Oka T, Takeda N, White PA.** 2011. Comparison of the replication properties of murine and human calicivirus RNA-dependent RNA polymerases. Virus Genes **42:**16–27. http://dx.doi.org/10.1007/s11262-010-0535-y.
59. **Green KY, Mory A, Fogg MH, Weisberg A, Belliot G, Wagner M, Mitra T, Ehrenfeld E, Cameron CE, Sosnovtsev SV.** 2002. Isolation of enzymatically active replication complexes from feline calicivirus-infected cells. J Virol **76:**8582–8595. http://dx.doi.org/10.1128/JVI.76.17.8582-8595.2002.
60. **Sosnovtsev SV, Garfield M, Green KY.** 2002. Processing map and essential cleavage sites of the nonstructural polyprotein encoded by ORF1 of the feline calicivirus genome. J Virol **76:**7060–7072. http://dx.doi.org/10.1128/JVI.76.14.7060-7072.2002.
61. **Oehmig A, Buttner M, Weiland F, Werz W, Bergemann K, Pfaff E.** 2003. Identification of a calicivirus isolate of unknown origin. J Gen Virol **84:**2837–2845. http://dx.doi.org/10.1099/vir.0.19042-0.
62. **Konig M, Thiel HJ, Meyers G.** 1998. Detection of viral proteins after infection of cultured hepatocytes with rabbit hemorrhagic disease virus. J Virol **72:**4492–4497.
63. **Belliot G, Sosnovtsev SV, Mitra T, Hammer C, Garfield M, Green KY.** 2003. In vitro proteolytic processing of the MD145 norovirus ORF1 nonstructural polyprotein yields stable precursors and products similar to those detected in calicivirus-infected cells. J Virol **77:**10957–10974. http://dx.doi.org/10.1128/JVI.77.20.10957-10974.2003.
64. **Chang KO, Sosnovtsev SV, Belliot G, King AD, Green KY.** 2006. Stable expression of a Norwalk virus RNA replicon in a human hepatoma cell line. Virology **353:**463–473. http://dx.doi.org/10.1016/j.virol.2006.06.006.
65. **Sosnovtsev SV, Belliot G, Chang KO, Prikhodko VG, Thackray LB, Wobus CE, Karst SM, Virgin HW, Green KY.** 2006. Cleavage map and proteolytic processing of the murine norovirus nonstructural polyprotein in infected cells. J Virol **80:**7816–7831. http://dx.doi.org/10.1128/JVI.00532-06.
66. **Katayama K, Hansman GS, Oka T, Ogawa S, Takeda N.** 2006. Investigation of norovirus replication in a human cell line. Arch Virol **151:**1291–1308. http://dx.doi.org/10.1007/s00705-005-0720-9.
67. **Meyers G, Wirblich C, Thiel HJ, Thumfart JO.** 2000. Rabbit hemorrhagic disease virus: genome organization and polyprotein processing of a calicivirus studied after transient expression of cDNA constructs. Virology **276:**349–363. http://dx.doi.org/10.1006/viro.2000.0545.

68. **Pfister T, Wimmer E.** 2001. Polypeptide p41 of a Norwalk-like virus is a nucleic acid-independent nucleoside triphosphatase. J Virol **75:**1611–1619. http://dx.doi.org/10.1128/JVI.75.4.1611-1619.2001.
69. **Marin MS, Casais R, Alonso JM, Parra F.** 2000. ATP binding and ATPase activities associated with recombinant rabbit hemorrhagic disease virus 2C-like polypeptide. J Virol **74:**10846–10851. http://dx.doi.org/10.1128/JVI.74.22.10846-10851.2000.
70. **Leen EN, Kwok KY, Birtley JR, Simpson PJ, Subba-Reddy CV, Chaudhry Y, Sosnovtsev SV, Green KY, Prater SN, Tong M, Young JC, Chung LM, Marchant J, Roberts LO, Kao CC, Matthews S, Goodfellow IG, Curry S.** 2013. Structures of the compact helical core domains of feline calicivirus and murine norovirus VPg proteins. J Virol **87:**5318–5330. http://dx.doi.org/10.1128/JVI.03151-12.
71. **Oka T, Yamamoto M, Miyashita K, Ogawa S, Katayama K, Wakita T, Takeda N.** 2009. Self-assembly of sapovirus recombinant virus-like particles from polyprotein in mammalian cells. Microbiol Immunol **53:**49–52. http://dx.doi.org/10.1111/j.1348-0421.2008.00086.x.
72. **Hansman GS, Oka T, Takeda N.** 2008. Sapovirus-like particles derived from polyprotein. Virus Res **137:**261–265. http://dx.doi.org/10.1016/j.virusres.2008.07.002.
73. **Vongpunsawad S, Venkataram Prasad BV, Estes MK.** 2013. Norwalk virus minor capsid protein VP2 associates within the VP1 shell domain. J Virol **87:**4818–4825. http://dx.doi.org/10.1128/JVI.03508-12.
74. **Jiang X, Zhong W, Kaplan M, Pickering LK, Matson DO.** 1999. Expression and characterization of Sapporo-like human calicivirus capsid proteins in baculovirus. J Virol Methods **78:**81–91. http://dx.doi.org/10.1016/S0166-0934(98)00169-4.
75. **Guo M, Qian Y, Chang KO, Saif LJ.** 2001. Expression and self-assembly in baculovirus of porcine enteric calicivirus capsids into virus-like particles and their use in an enzyme-linked immunosorbent assay for antibody detection in swine. J Clin Microbiol **39:**1487–1493. http://dx.doi.org/10.1128/JCM.39.4.1487-1493.2001.
76. **Chen R, Neill JD, Noel JS, Hutson AM, Glass RI, Estes MK, Prasad BV.** 2004. Inter- and intragenus structural variations in caliciviruses and their functional implications. J Virol **78:**6469–6479. http://dx.doi.org/10.1128/JVI.78.12.6469-6479.2004.
77. **Hansman GS, Natori K, Oka T, Ogawa S, Tanaka K, Nagata N, Ushijima H, Takeda N, Katayama K.** 2005. Cross-reactivity among sapovirus recombinant capsid proteins. Arch Virol **150:**21–36. http://dx.doi.org/10.1007/s00705-004-0406-8.
78. **Hansman GS, Oka T, Katayama K, Takeda N.** 2006. Enhancement of sapovirus recombinant capsid protein expression in insect cells. FEBS Lett **580:**4047–4050. http://dx.doi.org/10.1016/j.febslet.2006.06.040.
79. **Oka T, Hansman GS, Katayama K, Ogawa S, Nagata N, Miyamura T, Takeda N.** 2006. Expression of sapovirus virus-like particles in mammalian cells. Arch Virol **151:**399–404. http://dx.doi.org/10.1007/s00705-005-0613-y.
80. **Hansman GS, Saito H, Shibata C, Ishizuka S, Oseto M, Oka T, Takeda N.** 2007. Outbreak of gastroenteritis due to sapovirus. J Clin Microbiol **45:**1347–1349. http://dx.doi.org/10.1128/JCM.01854-06.
81. **Oka T, Miyashita K, Katayama K, Wakita T, Takeda N.** 2009. Distinct genotype and antigenicity among genogroup II sapoviruses. Microbiol Immunol **53:**417–420. http://dx.doi.org/10.1111/j.1348-0421.2009.00133.x.
82. **Prasad BV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK.** 1999. X-ray crystallographic structure of the Norwalk virus capsid. Science **286:**287–290. http://dx.doi.org/10.1126/science.286.5438.287.
83. **Hardy ME.** 2005. Norovirus protein structure and function. FEMS Microbiol Lett **253:**1–8. http://dx.doi.org/10.1016/j.femsle.2005.08.031.
84. **Katayama K, Miyoshi T, Uchino K, Oka T, Tanaka T, Takeda N, Hansman GS.** 2004. Novel recombinant sapovirus. Emerg Infect Dis **10:**1874–1876. http://dx.doi.org/10.3201/eid1010.040395.
85. **Hansman GS, Takeda N, Oka T, Oseto M, Hedlund KO, Katayama K.** 2005. Intergenogroup recombination in sapoviruses. Emerg Infect Dis **11:**1916–1920. http://dx.doi.org/10.3201/eid1112.050722.
86. **Oka T, Katayama K, Hansman GS, Kageyama T, Ogawa S, Wu FT, White PA, Takeda N.** 2006. Detection of human sapovirus by real-time reverse transcription-polymerase chain reaction. J Med Virol **78:**1347–1353. http://dx.doi.org/10.1002/jmv.20699.
87. **Oka T, Mori K, Iritani N, Harada S, Ueki Y, Iizuka S, Mise K, Murakami K, Wakita T, Katayama K.** 2012. Human sapovirus classification based on complete capsid nucleotide sequences. Arch Virol **157:**349–352. http://dx.doi.org/10.1007/s00705-011-1161-2.
88. **Scheuer KA, Oka T, Hoet AE, Gebreyes WA, Molla BZ, Saif LJ, Wang Q.** 2013. Prevalence of porcine noroviruses, molecular characterization of emerging porcine sapoviruses from finisher swine in the United States, and unified classification scheme for sapoviruses. J Clin Microbiol **51:**2344–2353. http://dx.doi.org/10.1128/JCM.00865-13.
89. **Cubitt WD, McSwiggan DA.** 1981. Calicivirus gastroenteritis in North West London. Lancet **ii:**975–977. http://dx.doi.org/10.1016/S0140-6736(81)91167-3.
90. **Cubitt WD, Blacklow NR, Herrmann JE, Nowak NA, Nakata S, Chiba S.** 1987. Antigenic relationships between human caliciviruses and Norwalk virus. J Infect Dis **156:**806–814. http://dx.doi.org/10.1093/infdis/156.5.806.
91. **Hansman GS, Guntapong R, Pongsuwanna Y, Natori K, Katayama K, Takeda N.** 2006. Development of an antigen ELISA to detect sapovirus in clinical stool specimens. Arch Virol **151:**551–561. http://dx.doi.org/10.1007/s00705-005-0630-x.
92. **Farkas T, Deng X, Ruiz-Palacios G, Morrow A, Jiang X.** 2006. Development of an enzyme immunoassay for detection of sapovirus-specific antibodies and its application in a study of seroprevalence in children. J Clin Microbiol **44:**3674–3679. http://dx.doi.org/10.1128/JCM.01087-06.
93. **Hansman GS, Oka T, Sakon N, Takeda N.** 2007. Antigenic diversity of human sapoviruses. Emerg Infect Dis **13:**1519–1525. http://dx.doi.org/10.3201/eid1310.070402.
94. **Kitamoto N, Oka T, Katayama K, Li TC, Takeda N, Kato Y, Miyoshi T, Tanaka T.** 2012. Novel monoclonal antibodies broadly reactive to human recombinant sapovirus-like particles. Microbiol Immunol **56:**760–770. http://dx.doi.org/10.1111/j.1348-0421.2012.00499.x.
95. **Hansman GS, Natori K, Ushijima H, Katayama K, Takeda N.** 2005. Characterization of polyclonal antibodies raised against sapovirus genogroup five virus-like particles. Arch Virol **150:**1433–1437. http://dx.doi.org/10.1007/s00705-005-0506-0.
96. **Hansman GS, Oka T, Katayama K, Takeda N.** 2007. Human sapoviruses: genetic diversity, recombination, and classification. Rev Med Virol **17:**133–141. http://dx.doi.org/10.1002/rmv.533. (Erratum, **17:**2364, http://dx.doi.org/10.1002/rmv.552.)
97. **Kroneman A, Vega E, Vennema H, Vinje J, White PA, Hansman G, Green K, Martella V, Katayama K, Koopmans M.** 2013. Proposal for a unified norovirus nomenclature and genotyping. Arch Virol **158:**2059–2068. http://dx.doi.org/10.1007/s00705-013-1708-5.
98. **Svraka S, Vennema H, van der Veer B, Hedlund KO, Thorhagen M, Siebenga J, Duizer E, Koopmans M.** 2010. Epidemiology and genotype analysis of emerging sapovirus-associated infections across Europe. J Clin Microbiol **48:**2191–2198. http://dx.doi.org/10.1128/JCM.02427-09.
99. **Harada S, Oka T, Tokuoka E, Kiyota N, Nishimura K, Shimada Y, Ueno T, Ikezawa S, Wakita T, Wang Q, Saif LJ, Katayama K.** 2012. A confirmation of sapovirus re-infection gastroenteritis cases with different genogroups and genetic shifts in the evolving sapovirus genotypes, 2002-2011. Arch Virol **157:**1999–2003. http://dx.doi.org/10.1007/s00705-012-1387-7. (Erratum, **158:**2641–2642, 2013, http://dx.doi.org/10.1007/s00705-013-1757-9.)
100. **Harada S, Tokuoka E, Kiyota N, Katayama K, Oka T.** 2013. Phylogenetic analysis of the nonstructural and structural protein encoding region sequences, indicating successive appearance of genomically diverse sapovirus strains from gastroenteritis patients. Jpn J Infect Dis **66:**454–457.
101. **Harada S, Okada M, Yahiro S, Nishimura K, Matsuo S, Miyasaka J, Nakashima R, Shimada Y, Ueno T, Ikezawa S, Shinozaki K, Katayama K, Wakita T, Takeda N, Oka T.** 2009. Surveillance of pathogens in outpatients with gastroenteritis and characterization of sapovirus strains between 2002 and 2007 in Kumamoto Prefecture, Japan. J Med Virol **81:**1117–1127. http://dx.doi.org/10.1002/jmv.21454. (Erratum, **85:**1685–1686, 2013, http://dx.doi.org/10.1002/jmv.23677.)
102. **Chanit W, Thongprachum A, Khamrin P, Okitsu S, Mizuguchi M, Ushijima H.** 2009. Intergenogroup recombinant sapovirus in Japan, 2007–2008. Emerg Infect Dis **15:**1084–1087. http://dx.doi.org/10.3201/eid1507.090153.
103. **Pang XL, Lee BE, Tyrrell GJ, Preiksaitis JK.** 2009. Epidemiology and genotype analysis of sapovirus associated with gastroenteritis outbreaks in Alberta, Canada: 2004-2007. J Infect Dis **199:**547–551. http://dx.doi.org/10.1086/596210.
104. **Lee LE, Cebelinski EA, Fuller C, Keene WE, Smith K, Vinje J, Besser JM.** 2012. Sapovirus outbreaks in long-term care facilities, Oregon and

Minnesota, USA, 2002-2009. Emerg Infect Dis **18:**873–876. http://dx.doi.org/10.3201/eid1805.111843.
105. **Siebenga JJ, Vennema H, Duizer E, Koopmans MP.** 2007. Gastroenteritis caused by norovirus GGII.4, The Netherlands, 1994-2005. Emerg Infect Dis **13:**144–146. http://dx.doi.org/10.3201/eid1301.060800.
106. **Glass RI, Parashar UD, Estes MK.** 2009. Norovirus gastroenteritis. N Engl J Med **361:**1776–1785. http://dx.doi.org/10.1056/NEJMra0804575.
107. **Siebenga JJ, Vennema H, Zheng DP, Vinje J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M.** 2009. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. J Infect Dis **200:**802–812. http://dx.doi.org/10.1086/605127.
108. **Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinje J.** 2014. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol **52:**147–155. http://dx.doi.org/10.1128/JCM.02680-13.
109. **Lindesmith LC, Beltramello M, Donaldson EF, Corti D, Swanstrom J, Debbink K, Lanzavecchia A, Baric RS.** 2012. Immunogenetic mechanisms driving norovirus GII. 4 antigenic variation. PLoS Pathog **8:**e1002705. http://dx.doi.org/10.1371/journal.ppat.1002705.
110. **Lindesmith LC, Donaldson EF, Baric RS.** 2011. Norovirus GII.4 strain antigenic variation. J Virol **85:**231–242. http://dx.doi.org/10.1128/JVI.01364-10.
111. **Siebenga JJ, Vennema H, Renckens B, de Bruin E, van der Veer B, Siezen RJ, Koopmans M.** 2007. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol **81:**9932–9941. http://dx.doi.org/10.1128/JVI.00674-07.
112. **Motomura K, Oka T, Yokoyama M, Nakamura H, Mori H, Ode H, Hansman GS, Katayama K, Kanda T, Tanaka T, Takeda N, Sato H.** 2008. Identification of monomorphic and divergent haplotypes in the 2006-2007 norovirus GII/4 epidemic population by genomewide tracing of evolutionary history. J Virol **82:**11247–11262. http://dx.doi.org/10.1128/JVI.00897-08.
113. **Bok K, Abente EJ, Realpe-Quintero M, Mitra T, Sosnovtsev SV, Kapikian AZ, Green KY.** 2009. Evolutionary dynamics of GII.4 noroviruses over a 34-year period. J Virol **83:**11890–11901. http://dx.doi.org/10.1128/JVI.00864-09.
114. **Motomura K, Yokoyama M, Ode H, Nakamura H, Mori H, Kanda T, Oka T, Katayama K, Noda M, Tanaka T, Takeda N, Sato H.** 2010. Divergent evolution of norovirus GII/4 by genome recombination from May 2006 to February 2009 in Japan. J Virol **84:**8085–8097. http://dx.doi.org/10.1128/JVI.02125-09.
115. **van Beek J, Ambert-Balay K, Botteldoorn N, Eden JS, Fonager J, Hewitt J, Iritani N, Kroneman A, Vennema H, Vinje J, White PA, Koopmans M.** 2013. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. Euro Surveill **18**(1):pii=20345. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20345.
116. **Hansman GS, Ishida S, Yoshizumi S, Miyoshi M, Ikeda T, Oka T, Takeda N.** 2007. Recombinant sapovirus gastroenteritis, Japan. Emerg Infect Dis **13:**786–788. http://dx.doi.org/10.3201/eid1305.070049.
117. **Mikula C, Springer B, Reichart S, Bierbacher K, Lichtenschopf A, Hoehne M.** 2010. Sapovirus in adults in rehabilitation center, upper Austria. Emerg Infect Dis **16:**1186–1187. http://dx.doi.org/10.3201/eid1607.091789.
118. **Phan TG, Okitsu S, Muller WE, Kohno H, Ushijima H.** 2006. Novel recombinant sapovirus, Japan. Emerg Infect Dis **12:**865–867. http://dx.doi.org/10.3201/eid1205.051608.
119. **Phan TG, Trinh QD, Yagyu F, Sugita K, Okitsu S, Muller WE, Ushijima H.** 2006. Outbreak of sapovirus infection among infants and children with acute gastroenteritis in Osaka City, Japan during 2004-2005. J Med Virol **78:**839–846. http://dx.doi.org/10.1002/jmv.20632.
120. **Dey SK, Mizuguchi M, Okitsu S, Ushijima H.** 2011. Novel recombinant sapovirus in Bangladesh. Clin Lab **57:**91–94.
121. **Hansman GS, Takeda N, Katayama K, Tu ET, McIver CJ, Rawlinson WD, White PA.** 2006. Genetic diversity of sapovirus in children, Australia. Emerg Infect Dis **12:**141–143. http://dx.doi.org/10.3201/eid1201.050846.
122. **Nguyen TA, Hoang L, Pham le D, Hoang KT, Okitsu S, Mizuguchi M, Ushijima H.** 2008. Norovirus and sapovirus infections among children with acute gastroenteritis in Ho Chi Minh City during 2005-2006. J Trop Pediatr **54:**102–113. http://dx.doi.org/10.1093/tropej/fmm096.
123. **Wang QH, Han MG, Funk JA, Bowman G, Janies DA, Saif LJ.** 2005. Genetic diversity and recombination of porcine sapoviruses. J Clin Microbiol **43:**5963–5972. http://dx.doi.org/10.1128/JCM.43.12.5963-5972.2005.
124. **Gallimore CI, Iturriza-Gomara M, Lewis D, Cubitt D, Cotterill H, Gray JJ.** 2006. Characterization of sapoviruses collected in the United Kingdom from 1989 to 2004. J Med Virol **78:**673–682. http://dx.doi.org/10.1002/jmv.20592.
125. **Roingeard P.** 2008. Viral detection by electron microscopy: past, present and future. Biol Cell **100:**491–501. http://dx.doi.org/10.1042/BC20070173.
126. **Okada M, Shinozaki K, Ogawa T, Kaiho I.** 2002. Molecular epidemiology and phylogenetic analysis of Sapporo-like viruses. Arch Virol **147:**1445–1451. http://dx.doi.org/10.1007/s00705-002-0821-7.
127. **Wu FT, Oka T, Takeda N, Katayama K, Hansman GS, Muo CH, Liang SY, Hung CH, Dah-Shyong Jiang D, Hsin Chang J, Yang JY, Wu HS, Yang CF.** 2008. Acute gastroenteritis caused by GI/2 sapovirus, Taiwan, 2007. Emerg Infect Dis **14:**1169–1171. http://dx.doi.org/10.3201/eid1407.071531.
128. **Ootsuka Y, Yamashita Y, Ichikawa T, Kondo R, Oseto M, Katayama K, Takeda N, Oka T.** 2009. Molecular characterization of sapoviruses detected in sporadic gastroenteritis cases in 2007 in Ehime Prefecture, Japan. Jpn J Infect Dis **62:**246–248.
129. **Miyoshi M, Yoshizumi S, Kanda N, Karino T, Nagano H, Kudo S, Okano M, Ishida S.** 2010. Different genotypic sapoviruses detected in two simultaneous outbreaks of gastroenteritis among schoolchildren in the same school district in Hokkaido, Japan. Jpn J Infect Dis **63:**75–78.
130. **Yamashita Y, Ootsuka Y, Kondo R, Oseto M, Doi M, Miyamoto T, Ueda T, Kondo H, Tanaka T, Wakita T, Katayama K, Takeda N, Oka T.** 2010. Molecular characterization of Sapovirus detected in a gastroenteritis outbreak at a wedding hall. J Med Virol **82:**720–726. http://dx.doi.org/10.1002/jmv.21646.
131. **Pang XL, Preiksaitis JK, Lee BE.** 2014. Enhanced enteric virus detection in sporadic gastroenteritis using a multi-target real-time PCR panel: a one-year study. J Med Virol 86:1594–1601. http://dx.doi.org/10.1002/jmv.23851.
132. **Nakata S, Honma S, Numata K, Kogawa K, Ukae S, Adachi N, Jiang X, Estes MK, Gatheru Z, Tukei PM, Chiba S.** 1998. Prevalence of human calicivirus infections in Kenya as determined by enzyme immunoassays for three genogroups of the virus. J Clin Microbiol **36:**3160–3163.
133. **Matson DO, Estes MK, Glass RI, Bartlett AV, Penaranda M, Calomeni E, Tanaka T, Nakata S, Chiba S.** 1989. Human calicivirus-associated diarrhea in children attending day care centers. J Infect Dis **159:**71–78. http://dx.doi.org/10.1093/infdis/159.1.71.
134. **Matson DO, Estes MK, Tanaka T, Bartlett AV, Pickering LK.** 1990. Asymptomatic human calicivirus infection in a day care center. Pediatr Infect Dis J **9:**190–196. http://dx.doi.org/10.1097/00006454-199003000-00009.
135. **Grohmann G, Glass RI, Gold J, James M, Edwards P, Borg T, Stine SE, Goldsmith C, Monroe SS.** 1991. Outbreak of human calicivirus gastroenteritis in a day-care center in Sydney, Australia. J Clin Microbiol **29:**544–550.
136. **Kitamoto N, Tanaka T, Natori K, Takeda N, Nakata S, Jiang X, Estes MK.** 2002. Cross-reactivity among several recombinant calicivirus virus-like particles (VLPs) with monoclonal antibodies obtained from mice immunized orally with one type of VLP. J Clin Microbiol **40:**2459–2465. http://dx.doi.org/10.1128/JCM.40.7.2459-2465.2002.
137. **Parker TD, Kitamoto N, Tanaka T, Hutson AM, Estes MK.** 2005. Identification of genogroup I and genogroup II broadly reactive epitopes on the norovirus capsid. J Virol **79:**7402–7409. http://dx.doi.org/10.1128/JVI.79.12.7402-7409.2005.
138. **Yoda T, Suzuki Y, Terano Y, Yamazaki K, Sakon N, Kuzuguchi T, Oda H, Tsukamoto T.** 2003. Precise characterization of norovirus (Norwalk-like virus)-specific monoclonal antibodies with broad reactivity. J Clin Microbiol **41:**2367–2371. http://dx.doi.org/10.1128/JCM.41.6.2367-2371.2003.
139. **Shiota T, Okame M, Takanashi S, Khamrin P, Takagi M, Satou K, Masuoka Y, Yagyu F, Shimizu Y, Kohno H, Mizuguchi M, Okitsu S, Ushijima H.** 2007. Characterization of a broadly reactive monoclonal antibody against norovirus genogroups I and II: recognition of a novel

conformational epitope. J Virol **81:**12298–12306. http://dx.doi.org/10.1128/JVI.00891-07.
140. **Parra GI, Azure J, Fischer R, Bok K, Sandoval-Jaime C, Sosnovtsev SV, Sander P, Green KY.** 2013. Identification of a broadly cross-reactive epitope in the inner shell of the norovirus capsid. PLoS One **8:**e67592. http://dx.doi.org/10.1371/journal.pone.0067592.
141. **Berke T, Golding B, Jiang X, Cubitt DW, Wolfaardt M, Smith AW, Matson DO.** 1997. Phylogenetic analysis of the caliciviruses. J Med Virol **52:**419–424. http://dx.doi.org/10.1002/(SICI)1096-9071(199708)52:4<419::AID-JMV13>3.0.CO;2-B.
142. **Wolfaardt M, Taylor MB, Booysen HF, Engelbrecht L, Grabow WO, Jiang X.** 1997. Incidence of human calicivirus and rotavirus infection in patients with gastroenteritis in South Africa. J Med Virol **51:**290–296. http://dx.doi.org/10.1002/(SICI)1096-9071(199704)51:4<290::AID-JMV6>3.0.CO;2-0.
143. **Wright PJ, Gunesekere IC, Doultree JC, Marshall JA.** 1998. Small round-structured (Norwalk-like) viruses and classical human caliciviruses in southeastern Australia, 1980-1996. J Med Virol **55:**312–320. http://dx.doi.org/10.1002/(SICI)1096-9071(199808)55:4<312::AID-JMV10>3.0.CO;2-G.
144. **Jiang X, Huang PW, Zhong WM, Farkas T, Cubitt DW, Matson DO.** 1999. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. J Virol Methods **83:**145–154. http://dx.doi.org/10.1016/S0166-0934(99)00114-7.
145. **Hedlund KO, Rubilar-Abreu E, Svensson L.** 2000. Epidemiology of calicivirus infections in Sweden, 1994-1998. J Infect Dis **181**(Suppl 2): S275–S280. http://dx.doi.org/10.1086/315585.
146. **Honma S, Nakata S, Kinoshita-Numata K, Kogawa K, Chiba S.** 2000. Evaluation of nine sets of PCR primers in the RNA dependent RNA polymerase region for detection and differentiation of members of the family Caliciviridae, Norwalk virus and Sapporo virus. Microbiol Immunol **44:**411–419. http://dx.doi.org/10.1111/j.1348-0421.2000.tb02515.x.
147. **Vinje J, Deijl H, van der Heide R, Lewis D, Hedlund KO, Svensson L, Koopmans MP.** 2000. Molecular detection and epidemiology of Sapporo-like viruses. J Clin Microbiol **38:**530–536.
148. **Honma S, Nakata S, Sakai Y, Tatsumi M, Numata-Kinoshita K, Chiba S.** 2001. Sensitive detection and differentiation of Sapporo virus, a member of the family Caliciviridae, by standard and booster nested polymerase chain reaction. J Med Virol **65:**413–417. http://dx.doi.org/10.1002/jmv.2050.
149. **Martinez N, Espul C, Cuello H, Zhong W, Jiang X, Matson DO, Berke T.** 2002. Sequence diversity of human caliciviruses recovered from children with diarrhea in Mendoza, Argentina, 1995-1998. J Med Virol **67:**289–298. http://dx.doi.org/10.1002/jmv.2220.
150. **Ratcliff RM, Doherty JC, Higgins GD.** 2002. Sensitive detection of RNA viruses associated with gastroenteritis by a hanging-drop single-tube nested reverse transcription-PCR method. J Clin Microbiol **40:**4091–4099. http://dx.doi.org/10.1128/JCM.40.11.4091-4099.2002.
151. **Bon F, Giraudon H, Sancey C, Barranger C, Joannes M, Pothier P, Kohli E.** 2004. Development and evaluation of a new commercial test allowing the simultaneous detection of noroviruses and sapoviruses by reverse transcription-PCR and microplate hybridization. J Clin Microbiol **42:**2218–2220. http://dx.doi.org/10.1128/JCM.42.5.2218-2220.2004.
152. **Monica B, Ramani S, Banerjee I, Primrose B, Iturriza-Gomara M, Gallimore CI, Brown DW, Fathima M, Moses PD, Gray JJ, Kang G.** 2007. Human caliciviruses in symptomatic and asymptomatic infections in children in Vellore, South India. J Med Virol **79:**544–551. http://dx.doi.org/10.1002/jmv.20862.
153. **Rachakonda G, Choudekar A, Parveen S, Bhatnagar S, Patwari A, Broor S.** 2008. Genetic diversity of noroviruses and sapoviruses in children with acute sporadic gastroenteritis in New Delhi, India. J Clin Virol **43:**42–48. http://dx.doi.org/10.1016/j.jcv.2008.05.006.
154. **Chan MC, Sung JJ, Lam RK, Chan PK, Lai RW, Leung WK.** 2006. Sapovirus detection by quantitative real-time RT-PCR in clinical stool specimens. J Virol Methods **134:**146–153. http://dx.doi.org/10.1016/j.jviromet.2005.12.013.
155. **Logan C, O'Leary JJ, O'Sullivan N.** 2007. Real-time reverse transcription PCR detection of norovirus, sapovirus and astrovirus as causative agents of acute viral gastroenteritis. J Virol Methods **146:**36–44. http://dx.doi.org/10.1016/j.jviromet.2007.05.031.
156. **Svraka S, van der Veer B, Duizer E, Dekkers J, Koopmans M, Vennema H.** 2009. Novel approach for detection of enteric viruses to enable syndrome surveillance of acute viral gastroenteritis. J Clin Microbiol **47:**1674–1679. http://dx.doi.org/10.1128/JCM.00307-09.
157. **van Maarseveen NM, Wessels E, de Brouwer CS, Vossen AC, Claas EC.** 2010. Diagnosis of viral gastroenteritis by simultaneous detection of adenovirus group F, astrovirus, rotavirus group A, norovirus genogroups I and II, and sapovirus in two internally controlled multiplex real-time PCR assays. J Clin Virol **49:**205–210. http://dx.doi.org/10.1016/j.jcv.2010.07.019.
158. **Gustavsson L, Westin J, Andersson LM, Lindh M.** 2011. Rectal swabs can be used for diagnosis of viral gastroenteritis with a multiple real-time PCR assay. J Clin Virol **51:**279–282. http://dx.doi.org/10.1016/j.jcv.2011.05.025.
159. **Khamrin P, Okame M, Thongprachum A, Nantachit N, Nishimura S, Okitsu S, Maneekarn N, Ushijima H.** 2011. A single-tube multiplex PCR for rapid detection in feces of 10 viruses causing diarrhea. J Virol Methods **173:**390–393. http://dx.doi.org/10.1016/j.jviromet.2011.02.012.
160. **Liu J, Kibiki G, Maro V, Maro A, Kumburu H, Swai N, Taniuchi M, Gratz J, Toney D, Kang G, Houpt E.** 2011. Multiplex reverse transcription PCR Luminex assay for detection and quantitation of viral agents of gastroenteritis. J Clin Virol **50:**308–313. http://dx.doi.org/10.1016/j.jcv.2010.12.009.
161. **Liu J, Gratz J, Amour C, Kibiki G, Becker S, Janaki L, Verweij JJ, Taniuchi M, Sobuz SU, Haque R, Haverstick DM, Houpt ER.** 2013. A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. J Clin Microbiol **51:**472–480. http://dx.doi.org/10.1128/JCM.02658-12.
162. **Jiang Y, Fang L, Shi X, Zhang H, Li Y, Lin Y, Qiu Y, Chen Q, Li H, Zhou L, Hu Q.** 2014. Simultaneous detection of five enteric viruses associated with gastroenteritis by use of a PCR assay: a single real-time multiplex reaction and its clinical application. J Clin Microbiol **52:**1266–1268. http://dx.doi.org/10.1128/JCM.00245-14.
163. **Yan H, Yagyu F, Okitsu S, Nishio O, Ushijima H.** 2003. Detection of norovirus (GI, GII), sapovirus and astrovirus in fecal samples using reverse transcription single-round multiplex PCR. J Virol Methods **114:**37–44. http://dx.doi.org/10.1016/j.jviromet.2003.08.009.
164. **Gunson RN, Collins TC, Carman WF.** 2006. The real-time detection of sapovirus. J Clin Virol **35:**321–322. http://dx.doi.org/10.1016/j.jcv.2005.11.001.
165. **Okada M, Yamashita Y, Oseto M, Shinozaki K.** 2006. The detection of human sapoviruses with universal and genogroup-specific primers. Arch Virol **151:**2503–2509. http://dx.doi.org/10.1007/s00705-006-0820-1.
166. **Liu Y, Xu ZQ, Zhang Q, Jin M, Yu JM, Li JS, Liu N, Cui SX, Kong XY, Wang H, Li HY, Cheng WX, Duan ZJ.** 2012. Simultaneous detection of seven enteric viruses associated with acute gastroenteritis by a multiplexed Luminex-based assay. J Clin Microbiol **50:**2384–2389. http://dx.doi.org/10.1128/JCM.06790-11.
167. **Zintz C, Bok K, Parada E, Barnes-Eley M, Berke T, Staat MA, Azimi P, Jiang X, Matson DO.** 2005. Prevalence and genetic characterization of caliciviruses among children hospitalized for acute gastroenteritis in the United States. Infect Genet Evol **5:**281–290. http://dx.doi.org/10.1016/j.meegid.2004.06.010.
168. **Ludert JE, Alcala AC, Liprandi F.** 2004. Primer pair p289-p290, designed to detect both noroviruses and sapoviruses by reverse transcription-PCR, also detects rotaviruses by cross-reactivity. J Clin Microbiol **42:**835–836. http://dx.doi.org/10.1128/JCM.42.2.835-836.2004.
169. **Shigemoto N, Fukuda S, Tanizawa Y, Kuwayama M, Ohara S, Seno M.** 2011. Detection of norovirus, sapovirus, and human astrovirus in fecal specimens using a multiplex reverse transcription-PCR with fluorescent dye-labeled primers. Microbiol Immunol **55:**369–372. http://dx.doi.org/10.1111/j.1348-0421.2011.00325.x.
170. **Niwa S, Tsukagoshi H, Ishioka T, Sasaki Y, Yoshizumi M, Morita Y, Kimura H, Kozawa K.** 2014. Triplex real-time PCR assay for detection and quantification of norovirus (GI and GII) and sapovirus. Microbiol Immunol **58:**68–71. http://dx.doi.org/10.1111/1348-0421.12107.
171. **Bibby K, Peccia J.** 2013. Identification of viral pathogen diversity in sewage sludge by metagenome analysis. Environ Sci Technol **47:**1945–1951. http://dx.doi.org/10.1021/es305181x.
172. **Li L, Shan T, Wang C, Cote C, Kolman J, Onions D, Gulland FM, Delwart E.** 2011. The fecal viral flora of California sea lions. J Virol **85:**9909–9917. http://dx.doi.org/10.1128/JVI.05026-11.
173. **Li L, Pesavento PA, Shan T, Leutenegger CM, Wang C, Delwart E.** 2011. Viruses in diarrhoeic dogs include novel kobuviruses and sapo-

viruses. J Gen Virol **92:**2534–2541. http://dx.doi.org/10.1099/vir.0.034611-0.
174. **Iwakiri A, Ganmyo H, Yamamoto S, Otao K, Mikasa M, Kizoe S, Katayama K, Wakita T, Takeda N, Oka T.** 2009. Quantitative analysis of fecal sapovirus shedding: identification of nucleotide substitutions in the capsid protein during prolonged excretion. Arch Virol **154:**689–693. http://dx.doi.org/10.1007/s00705-009-0358-0.
175. **Kitajima M, Oka T, Haramoto E, Katayama H, Takeda N, Katayama K, Ohgaki S.** 2010. Detection and genetic analysis of human sapoviruses in river water in Japan. Appl Environ Microbiol **76:**2461–2467. http://dx.doi.org/10.1128/AEM.02739-09.
176. **Kitajima M, Haramoto E, Phanuwan C, Katayama H.** 2011. Genotype distribution of human sapoviruses in wastewater in Japan. Appl Environ Microbiol **77:**4226–4229. http://dx.doi.org/10.1128/AEM.00088-11.
177. **Iizuka S, Takai-Todaka R, Ohshiro H, Kitajima M, Wang Q, Saif LJ, Wakita T, Noda M, Katayama K, Oka T.** 2013. Detection of multiple human sapoviruses from imported frozen individual clams. Food Environ Virol **5:**119–125. http://dx.doi.org/10.1007/s12560-013-9109-1.
178. **Iizuka S, Oka T, Tabara K, Omura T, Katayama K, Takeda N, Noda M.** 2010. Detection of sapoviruses and noroviruses in an outbreak of gastroenteritis linked genetically to shellfish. J Med Virol **82:**1247–1254. http://dx.doi.org/10.1002/jmv.21791.
179. **Benabbes L, Ollivier J, Schaeffer J, Parnaudeau S, Rhaissi H, Nourlil J, Le Guyader FS.** 2013. Norovirus and other human enteric viruses in moroccan shellfish. Food Environ Virol **5:**35–40. http://dx.doi.org/10.1007/s12560-012-9095-8.
180. **Sano D, Perez-Sautu U, Guix S, Pinto RM, Miura T, Okabe S, Bosch A.** 2011. Quantification and genotyping of human sapoviruses in the Llobregat river catchment, Spain. Appl Environ Microbiol **77:**1111–1114. http://dx.doi.org/10.1128/AEM.01721-10.
181. **Ishida S, Yoshizumi S, Miyoshi M, Ikeda T, Okui T, Katayama K, Takeda N, Oka T.** 2008. Characterization of sapoviruses detected in Hokkaido, Japan. Jpn J Infect Dis **61:**504–506.
182. **Yoshida T, Kasuo S, Azegami Y, Uchiyama Y, Satsumabayashi K, Shiraishi T, Katayama K, Wakita T, Takeda N, Oka T.** 2009. Characterization of sapoviruses detected in gastroenteritis outbreaks and identification of asymptomatic adults with high viral load. J Clin Virol **45:**67–71. http://dx.doi.org/10.1016/j.jcv.2009.03.003.
183. **Kobayashi S, Fujiwara N, Yasui Y, Yamashita T, Hiramatsu R, Minagawa H.** 2012. A foodborne outbreak of sapovirus linked to catered box lunches in Japan. Arch Virol **157:**1995–1997. http://dx.doi.org/10.1007/s00705-012-1394-8.
184. **Lee RM, Lessler J, Lee RA, Rudolph KE, Reich NG, Perl TM, Cummings DA.** 2013. Incubation periods of viral gastroenteritis: a systematic review. BMC Infect Dis **13:**446. http://dx.doi.org/10.1186/1471-2334-13-446.
185. **Pang XL, Honma S, Nakata S, Vesikari T.** 2000. Human caliciviruses in acute gastroenteritis of young children in the community. J Infect Dis **181**(Suppl 2)**:**S288–S294. http://dx.doi.org/10.1086/315590.
186. **Sakai Y, Nakata S, Honma S, Tatsumi M, Numata-Kinoshita K, Chiba S.** 2001. Clinical severity of Norwalk virus and Sapporo virus gastroenteritis in children in Hokkaido, Japan. Pediatr Infect Dis J **20:**849–853. http://dx.doi.org/10.1097/00006454-200109000-00005.
187. **Akihara S, Phan TG, Nguyen TA, Hansman G, Okitsu S, Ushijima H.** 2005. Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. Arch Virol **150:**2061–2075. http://dx.doi.org/10.1007/s00705-005-0540-y.
188. **Johansson PJ, Bergentoft K, Larsson PA, Magnusson G, Widell A, Thorhagen M, Hedlund KO.** 2005. A nosocomial sapovirus-associated outbreak of gastroenteritis in adults. Scand J Infect Dis **37:**200–204. http://dx.doi.org/10.1080/00365540410020974.
189. **Yan H, Abe T, Phan TG, Nguyen TA, Iso T, Ikezawa Y, Ishii K, Okitsu S, Ushijima H.** 2005. Outbreak of acute gastroenteritis associated with group A rotavirus and genogroup I sapovirus among adults in a mental health care facility in Japan. J Med Virol **75:**475–481. http://dx.doi.org/10.1002/jmv.20292.
190. **Nakata S, Estes MK, Chiba S.** 1988. Detection of human calicivirus antigen and antibody by enzyme-linked immunosorbent assays. J Clin Microbiol **26:**2001–2005.
191. **Rockx B, De Wit M, Vennema H, Vinje J, De Bruin E, Van Duynhoven Y, Koopmans M.** 2002. Natural history of human calicivirus infection: a prospective cohort study. Clin Infect Dis **35:**246–253. http://dx.doi.org/10.1086/341408.
192. **Ruuska T, Vesikari T.** 1990. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis **22:**259–267. http://dx.doi.org/10.3109/00365549009027046.
193. **Kirkwood CD, Bishop RF.** 2001. Molecular detection of human calicivirus in young children hospitalized with acute gastroenteritis in Melbourne, Australia, during 1999. J Clin Microbiol **39:**2722–2724. http://dx.doi.org/10.1128/JCM.39.7.2722-2724.2001.
194. **Dove W, Cunliffe NA, Gondwe JS, Broadhead RL, Molyneux ME, Nakagomi O, Hart CA.** 2005. Detection and characterization of human caliciviruses in hospitalized children with acute gastroenteritis in Blantyre, Malawi. J Med Virol **77:**522–527. http://dx.doi.org/10.1002/jmv.20488.
195. **Guntapong R, Hansman GS, Oka T, Ogawa S, Kageyama T, Pongsuwanna Y, Katayama K.** 2004. Norovirus and sapovirus infections in Thailand. Jpn J Infect Dis **57:**276–278.
196. **Hansman GS, Katayama K, Maneekarn N, Peerakome S, Khamrin P, Tonusin S, Okitsu S, Nishio O, Takeda N, Ushijima H.** 2004. Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. J Clin Microbiol **42:**1305–1307. http://dx.doi.org/10.1128/JCM.42.3.1305-1307.2004.
197. **Kirkwood CD, Clark R, Bogdanovic-Sakran N, Bishop RF.** 2005. A 5-year study of the prevalence and genetic diversity of human caliciviruses associated with sporadic cases of acute gastroenteritis in young children admitted to hospital in Melbourne, Australia (1998-2002). J Med Virol **77:**96–101. http://dx.doi.org/10.1002/jmv.20419.
198. **Phan TG, Okame M, Nguyen TA, Maneekarn N, Nishio O, Okitsu S, Ushijima H.** 2004. Human astrovirus, norovirus (GI, GII), and sapovirus infections in Pakistani children with diarrhea. J Med Virol **73:**256–261. http://dx.doi.org/10.1002/jmv.20084.
199. **Khamrin P, Maneekarn N, Peerakome S, Tonusin S, Malasao R, Mizuguchi M, Okitsu S, Ushijima H.** 2007. Genetic diversity of noroviruses and sapoviruses in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand. J Med Virol **79:**1921–1926. http://dx.doi.org/10.1002/jmv.21004.
200. **Nguyen TA, Yagyu F, Okame M, Phan TG, Trinh QD, Yan H, Hoang KT, Cao AT, Le Hoang P, Okitsu S, Ushijima H.** 2007. Diversity of viruses associated with acute gastroenteritis in children hospitalized with diarrhea in Ho Chi Minh City, Vietnam. J Med Virol **79:**582–590. http://dx.doi.org/10.1002/jmv.20857.
201. **Phan TG, Okame M, Nguyen TA, Nishio O, Okitsu S, Ushijima H.** 2005. Genetic diversity of sapovirus in fecal specimens from infants and children with acute gastroenteritis in Pakistan. Arch Virol **150:**371–377. http://dx.doi.org/10.1007/s00705-004-0401-0.
202. **Gutierrez-Escolano AL, Velazquez FR, Escobar-Herrera J, Lopez Saucedo C, Torres J, Estrada-Garcia T.** 2010. Human caliciviruses detected in Mexican children admitted to hospital during 1998-2000, with severe acute gastroenteritis not due to other enteropathogens. J Med Virol **82:**632–637. http://dx.doi.org/10.1002/jmv.21743.
203. **Jin Y, Cheng WX, Yang XM, Jin M, Zhang Q, Xu ZQ, Yu JM, Zhu L, Yang SH, Liu N, Cui SX, Fang ZY, Duan ZJ.** 2009. Viral agents associated with acute gastroenteritis in children hospitalized with diarrhea in Lanzhou, China. J Clin Virol **44:**238–241. http://dx.doi.org/10.1016/j.jcv.2008.12.010.
204. **Li CS, Chan PK, Tang JW.** 2009. Prevalence of diarrhea viruses in hospitalized children in Hong Kong in 2008. J Med Virol **81:**1903–1911. http://dx.doi.org/10.1002/jmv.21611.
205. **Lorrot M, Bon F, El Hajje MJ, Aho S, Wolfer M, Giraudon H, Kaplon J, Marc E, Raymond J, Lebon P, Pothier P, Gendrel D.** 2011. Epidemiology and clinical features of gastroenteritis in hospitalised children: prospective survey during a 2-year period in a Parisian hospital, France. Eur J Clin Microbiol Infect Dis **30:**361–368. http://dx.doi.org/10.1007/s10096-010-1094-9.
206. **Medici MC, Tummolo F, Albonetti V, Abelli LA, Chezzi C, Calderaro A.** 2012. Molecular detection and epidemiology of astrovirus, bocavirus, and sapovirus in Italian children admitted to hospital with acute gastroenteritis, 2008-2009. J Med Virol **84:**643–650. http://dx.doi.org/10.1002/jmv.23231.
207. **Rimoldi SG, Stefani F, Pagani C, Chenal LL, Zanchetta N, Di Bartolo I, Lombardi A, Ruggeri FM, Di Lillo D, Zuccotti GV, Gismondo MR.** 2011. Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents. Arch Virol **156:**1583–1589. http://dx.doi.org/10.1007/s00705-011-1037-5.
208. **Trang NV, Luan le T, Kim-Anh le T, Hau VT, Nhung le TH, Phasuk**

P, Setrabutr O, Shirley H, Vinje J, Anh DD, Mason CJ. 2012. Detection and molecular characterization of noroviruses and sapoviruses in children admitted to hospital with acute gastroenteritis in Vietnam. J Med Virol **84:**290–297. http://dx.doi.org/10.1002/jmv.23185.

209. **Park S, Oh S, Cho S, Lee J, Ryu S, Song M, Jung H, Park G, Choi S, Chae Y, Kim H.** 2012. Genetic characterization of sapovirus detected in hospitalized children with acute gastroenteritis in Korea. Clin Lab **58:** 1219–1224. http://dx.doi.org/10.7754/Clin.Lab.2012.120118.
210. **Bok K, Green KY.** 2012. Norovirus gastroenteritis in immunocompromised patients. N Engl J Med **367:**2126–2132. http://dx.doi.org/10.1056/NEJMra1207742.
211. **Frange P, Touzot F, Debre M, Heritier S, Leruez-Ville M, Cros G, Rouzioux C, Blanche S, Fischer A, Avettand-Fenoel V.** 2012. Prevalence and clinical impact of norovirus fecal shedding in children with inherited immune deficiencies. J Infect Dis **206:**1269–1274. http://dx.doi.org/10.1093/infdis/jis498.
212. **Naing Z, Rayner B, Killikulangara A, Vunnam K, Leach S, McIver CJ, Scott GM, Craig ME, Lui K, Rawlinson WD.** 2013. Prevalence of viruses in stool of premature neonates at a neonatal intensive care unit. J Paediatr Child Health **49:**E221–E226. http://dx.doi.org/10.1111/jpc.12113.
213. **Akihara S, Phan TG, Nguyen TA, Yagyu F, Okitsu S, Muller WE, Ushijima H.** 2005. Identification of sapovirus infection among Japanese infants in a day care center. J Med Virol **77:**595–601. http://dx.doi.org/10.1002/jmv.20497.
214. **Bucardo F, Carlsson B, Nordgren J, Larson G, Blandon P, Vilchez S, Svensson L.** 2012. Susceptibility of children to sapovirus infections, Nicaragua, 2005-2006. Emerg Infect Dis **18:**1875–1878. http://dx.doi.org/10.3201/eid1811.111581.
215. **Chhabra P, Payne DC, Szilagyi PG, Edwards KM, Staat MA, Shirley SH, Wikswo M, Nix WA, Lu X, Parashar UD, Vinje J.** 2013. Etiology of viral gastroenteritis in children <5 years of age in the United States, 2008-2009. J Infect Dis **208:**790–800. http://dx.doi.org/10.1093/infdis/jit254.
216. **de Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J, van Leusden F, Bartelds AI, van Duynhoven YT.** 2001. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol **154:**666–674. http://dx.doi.org/10.1093/aje/154.7.666.
217. **Roos-Weil D, Ambert-Balay K, Lanternier F, Mamzer-Bruneel MF, Nochy D, Pothier P, Avettand-Fenoel V, Anglicheau D, Snanoudj R, Bererhi L, Thervet E, Lecuit M, Legendre C, Lortholary O, Zuber J.** 2011. Impact of norovirus/sapovirus-related diarrhea in renal transplant recipients hospitalized for diarrhea. Transplantation **92:**61–69. http://dx.doi.org/10.1097/TP.0b013e31821c9392.
218. **Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J.** 2007. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993-1996). Eur J Clin Microbiol Infect Dis **26:**311–323. http://dx.doi.org/10.1007/s10096-007-0290-8.
219. **Iturriza-Gomara M, Elliot AJ, Dockery C, Fleming DM, Gray JJ.** 2009. Structured surveillance of infectious intestinal disease in pre-school children in the community: 'The Nappy Study.' Epidemiol Infect **137:**922–931. http://dx.doi.org/10.1017/S0950268808001556.
220. **Phan TG, Nguyen TA, Nishimura S, Nishimura T, Yamamoto A, Okitsu S, Ushijima H.** 2005. Etiologic agents of acute gastroenteritis among Japanese infants and children: virus diversity and genetic analysis of sapovirus. Arch Virol **150:**1415–1424. http://dx.doi.org/10.1007/s00705-005-0514-0.
221. **Phan TG, Khamrin P, Quang TD, Dey SK, Takanashi S, Okitsu S, Maneekarn N, Ushijima H.** 2007. Emergence of intragenotype recombinant sapovirus in Japan. Infect Genet Evol **7:**542–546. http://dx.doi.org/10.1016/j.meegid.2007.02.004.
222. **Phan TG, Trinh QD, Yagyu F, Okitsu S, Ushijima H.** 2007. Emergence of rare sapovirus genotype among infants and children with acute gastroenteritis in Japan. Eur J Clin Microbiol Infect Dis **26:**21–27. http://dx.doi.org/10.1007/s10096-006-0235-7.
223. **Chan-it W, Thongprachum A, Okitsu S, Mizuguchi M, Ushijima H.** 2010. Epidemiology and molecular characterization of sapovirus and astrovirus in Japan, 2008-2009. Jpn J Infect Dis **63:**302–303.
224. **Dey SK, Phan GT, Nishimura S, Mizuguchi M, Okitsu S, Ushijima H.** 2010. Molecular and epidemiological trend of sapovirus, and astrovirus infection in Japan. J Trop Pediatr **56:**205–207. http://dx.doi.org/10.1093/tropej/fmp082.
225. **Johnsen CK, Midgley S, Bottiger B.** 2009. Genetic diversity of sapovirus infections in Danish children 2005-2007. J Clin Virol **46:**265–269. http://dx.doi.org/10.1016/j.jcv.2009.07.008.
226. **Tam CC, O'Brien SJ, Tompkins DS, Bolton FJ, Berry L, Dodds J, Choudhury D, Halstead F, Iturriza-Gomara M, Mather K, Rait G, Ridge A, Rodrigues LC, Wain J, Wood B, Gray JJ.** 2012. Changes in causes of acute gastroenteritis in the United Kingdom over 15 years: microbiologic findings from 2 prospective, population-based studies of infectious intestinal disease. Clin Infect Dis **54:**1275–1286. http://dx.doi.org/10.1093/cid/cis028.
227. **Dey SK, Phathammavong O, Nguyen TD, Thongprachum A, Chan-It W, Okitsu S, Mizuguchi M, Ushijima H.** 2012. Seasonal pattern and genotype distribution of sapovirus infection in Japan, 2003-2009. Epidemiol Infect **140:**74–77. http://dx.doi.org/10.1017/S0950268811000240.
228. **Blanton LH, Adams SM, Beard RS, Wei G, Bulens SN, Widdowson MA, Glass RI, Monroe SS.** 2006. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000-2004. J Infect Dis **193:**413–421. http://dx.doi.org/10.1086/499315.
229. **Hall AJ, Wikswo ME, Manikonda K, Roberts VA, Yoder JS, Gould LH.** 2013. Acute gastroenteritis surveillance through the National Outbreak Reporting System, United States. Emerg Infect Dis **19:**1305–1309. http://dx.doi.org/10.3201/eid1908.130482.
230. **Iritani N, Kaida A, Abe N, Kubo H, Sekiguchi JI, Yamamoto SP, Goto K, Tanaka T, Noda M.** 2014. Detection and genetic characterization of human enteric viruses in oyster-associated gastroenteritis outbreaks between 2001 and 2012 in Osaka City, Japan. J Med Virol **86:**2019–2025. http://dx.doi.org/10.1002/jmv.23883.
231. **Bon F, Ambert-Balay K, Giraudon H, Kaplon J, Le Guyader S, Pommepuy M, Gallay A, Vaillant V, de Valk H, Chikhi-Brachet R, Flahaut A, Pothier P, Kohli E.** 2005. Molecular epidemiology of caliciviruses detected in sporadic and outbreak cases of gastroenteritis in France from December 1998 to February 2004. J Clin Microbiol **43:**4659–4664. http://dx.doi.org/10.1128/JCM.43.9.4659-4664.2005.
232. **Gallimore CI, Pipkin C, Shrimpton H, Green AD, Pickford Y, McCartney C, Sutherland G, Brown DW, Gray JJ.** 2005. Detection of multiple enteric virus strains within a foodborne outbreak of gastroenteritis: an indication of the source of contamination. Epidemiol Infect **133:**41–47. http://dx.doi.org/10.1017/S0950268804003218.
233. **Gomes KA, Stupka JA, Gomez J, Parra GI.** 2007. Molecular characterization of calicivirus strains detected in outbreaks of gastroenteritis in Argentina. J Med Virol **79:**1703–1709. http://dx.doi.org/10.1002/jmv.20989.
234. **Ike AC, Hartelt K, Oehme RM, Brockmann SO.** 2008. Detection and characterization of sapoviruses in outbreaks of gastroenteritis in southwest Germany. J Clin Virol **43:**37–41. http://dx.doi.org/10.1016/j.jcv.2008.04.003.
235. **Lyman WH, Walsh JF, Kotch JB, Weber DJ, Gunn E, Vinje J.** 2009. Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers. J Pediatr **154:**253–257. http://dx.doi.org/10.1016/j.jpeds.2008.07.057.
236. **Nakagawa-Okamoto R, Arita-Nishida T, Toda S, Kato H, Iwata H, Akiyama M, Nishio O, Kimura H, Noda M, Takeda N, Oka T.** 2009. Detection of multiple sapovirus genotypes and genogroups in oyster-associated outbreaks. Jpn J Infect Dis **62:**63–66.
237. **Le Guyader FS, Krol J, Ambert-Balay K, Ruvoen-Clouet N, Desaubliaux B, Parnaudeau S, Le Saux JC, Ponge A, Pothier P, Atmar RL, Le Pendu J.** 2010. Comprehensive analysis of a norovirus-associated gastroenteritis outbreak, from the environment to the consumer. J Clin Microbiol **48:**915–920. http://dx.doi.org/10.1128/JCM.01664-09.
238. **Usuku S, Kumazaki M, Kitamura K, Tochikubo O, Noguchi Y.** 2008. An outbreak of food-borne gastroenteritis due to sapovirus among junior high school students. Jpn J Infect Dis **61:**438–441.
239. **Rasanen S, Lappalainen S, Kaikkonen S, Hamalainen M, Salminen M, Vesikari T.** 2010. Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. Epidemiol Infect **138:**1227–1234. http://dx.doi.org/10.1017/S0950268809991671.
240. **Hansman GS, Oka T, Okamoto R, Nishida T, Toda S, Noda M, Sano D, Ueki Y, Imai T, Omura T, Nishio O, Kimura H, Takeda N.** 2007. Human sapovirus in clams, Japan. Emerg Infect Dis **13:**620–622. http://dx.doi.org/10.3201/eid1304.061390.
241. **Ueki Y, Shoji M, Okimura Y, Miyota Y, Masago Y, Oka T, Katayama K, Takeda N, Noda M, Miura T, Sano D, Omura T.** 2010. Detection of

SApovirus in oysters. Microbiol Immunol **54:**483–486. http://dx.doi.org/10.1111/j.1348-0421.2010.00239.x.
242. **Hansman GS, Sano D, Ueki Y, Imai T, Oka T, Katayama K, Takeda N, Omura T.** 2007. Sapovirus in water, Japan. Emerg Infect Dis **13:**133–135. http://dx.doi.org/10.3201/eid1301.061047.
243. **Iwai M, Hasegawa S, Obara M, Nakamura K, Horimoto E, Takizawa T, Kurata T, Sogen S, Shiraki K.** 2009. Continuous presence of noroviruses and sapoviruses in raw sewage reflects infections among inhabitants of Toyama, Japan (2006 to 2008). Appl Environ Microbiol **75:**1264–1270. http://dx.doi.org/10.1128/AEM.01166-08.
244. **Di Bartolo I, Ponterio E, Battistone A, Bonomo P, Cicala A, Mercurio P, Triassi M, Pennino F, Fiore L, Ruggeri FM.** 2013. Identification and genotyping of human sapoviruses collected from sewage water in Naples and Palermo, Italy, in 2011. Food Environ Virol 5:236–240. http://dx.doi.org/10.1007/s12560-013-9124-2.
245. **Murray TY, Mans J, Taylor MB.** 2013. First detection of human sapoviruses in river water in South Africa. Water Sci Technol **67:**2776–2783. http://dx.doi.org/10.2166/wst.2013.203.
246. **Murray TY, Mans J, Taylor MB.** 2013. Human calicivirus diversity in wastewater in South Africa. J Appl Microbiol **114:**1843–1853. http://dx.doi.org/10.1111/jam.12167.
247. **Murray TY, Mans J, van Zyl WB, Taylor MB.** 2013. Application of a competitive internal amplification control for the detection of sapoviruses in wastewater. Food Environ Virol **5:**61–68. http://dx.doi.org/10.1007/s12560-012-9101-1.
248. **Haramoto E, Katayama H, Phanuwan C, Ohgaki S.** 2008. Quantitative detection of sapoviruses in wastewater and river water in Japan. Lett Appl Microbiol **46:**408–413. http://dx.doi.org/10.1111/j.1472-765X.2008.02330.x.
249. **Wang QH, Costantini V, Saif LJ.** 2007. Porcine enteric caliciviruses: genetic and antigenic relatedness to human caliciviruses, diagnosis and epidemiology. Vaccine **25:**5453–5466. http://dx.doi.org/10.1016/j.vaccine.2006.12.032.
250. **Martella V, Lorusso E, Banyai K, Decaro N, Corrente M, Elia G, Cavalli A, Radogna A, Costantini V, Saif LJ, Lavazza A, Di Trani L, Buonavoglia C.** 2008. Identification of a porcine calicivirus related genetically to human sapoviruses. J Clin Microbiol **46:**1907–1913. http://dx.doi.org/10.1128/JCM.00341-08.
251. **Nakamura K, Saga Y, Iwai M, Obara M, Horimoto E, Hasegawa S, Kurata T, Okumura H, Nagoshi M, Takizawa T.** 2010. Frequent detection of noroviruses and sapoviruses in swine and high genetic diversity of porcine sapovirus in Japan during fiscal year 2008. J Clin Microbiol **48:**1215–1222. http://dx.doi.org/10.1128/JCM.02130-09.
252. **Sisay Z, Wang Q, Oka T, Saif L.** 2013. Prevalence and molecular characterization of porcine enteric caliciviruses and first detection of porcine kobuviruses in US swine. Arch Virol **158:**1583–1588. http://dx.doi.org/10.1007/s00705-013-1619-5.
253. **Guo M, Evermann JF, Saif LJ.** 2001. Detection and molecular characterization of cultivable caliciviruses from clinically normal mink and enteric caliciviruses associated with diarrhea in mink. Arch Virol **146:**479–493. http://dx.doi.org/10.1007/s007050170157.
254. **CDC.** 2011. Updated norovirus outbreak management and disease prevention guidelines. MMWR Recommend Rep **60:**1–18.
255. **Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, Ramani S, Hill H, Ferreira J, Graham DY.** 2014. Determination of the 50% human infectious dose for norwalk virus. J Infect Dis **209:**1016–1022. http://dx.doi.org/10.1093/infdis/jit620.
256. **Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J, Calderon RL.** 2008. Norwalk virus: how infectious is it? J Med Virol **80:**1468–1476. http://dx.doi.org/10.1002/jmv.21237.
257. **Shirato-Horikoshi H, Ogawa S, Wakita T, Takeda N, Hansman GS.** 2007. Binding activity of norovirus and sapovirus to histo-blood group antigens. Arch Virol **152:**457–461. http://dx.doi.org/10.1007/s00705-006-0883-z.
258. **Kim DS, Hosmillo M, Alfajaro MM, Kim JY, Park JG, Son KY, Ryu EH, Sorgeloos F, Kwon HJ, Park SJ, Lee WS, Cho D, Kwon J, Choi JS, Kang MI, Goodfellow I, Cho KO.** 2014. Both alpha2,3- and alpha2,6-linked sialic acids on O-linked glycoproteins act as functional receptors for porcine sapovirus. PLoS Pathog **10:**e1004172. http://dx.doi.org/10.1371/journal.ppat.1004172.
259. **Hutson AM, Atmar RL, Graham DY, Estes MK.** 2002. Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis **185:**1335–1337. http://dx.doi.org/10.1086/339883.
260. **Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R.** 2003. Human susceptibility and resistance to Norwalk virus infection. Nat Med **9:**548–553. http://dx.doi.org/10.1038/nm860.
261. **Shirato H, Ogawa S, Ito H, Sato T, Kameyama A, Narimatsu H, Xiaofan Z, Miyamura T, Wakita T, Ishii K, Takeda N.** 2008. Noroviruses distinguish between type 1 and type 2 histo-blood group antigens for binding. J Virol **82:**10756–10767. http://dx.doi.org/10.1128/JVI.00802-08.
262. **Donaldson EF, Lindesmith LC, Lobue AD, Baric RS.** 2010. Viral shape-shifting: norovirus evasion of the human immune system. Nat Rev Microbiol **8:**231–241. http://dx.doi.org/10.1038/nrmicro2296.
263. **Huang P, Farkas T, Zhong W, Tan M, Thornton S, Morrow AL, Jiang X.** 2005. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. J Virol **79:**6714–6722. http://dx.doi.org/10.1128/JVI.79.11.6714-6722.2005.
264. **Takanashi S, Wang Q, Chen N, Shen Q, Jung K, Zhang Z, Yokoyama M, Lindesmith LC, Baric RS, Saif LJ.** 2011. Characterization of emerging GII.g/GII.12 noroviruses from a gastroenteritis outbreak in the United States in 2010. J Clin Microbiol **49:**3234–3244. http://dx.doi.org/10.1128/JCM.00305-11.
265. **Larsson MM, Rydell GE, Grahn A, Rodriguez-Diaz J, Akerlind B, Hutson AM, Estes MK, Larson G, Svensson L.** 2006. Antibody prevalence and titer to norovirus (genogroup II) correlate with secretor (FUT2) but not with ABO phenotype or Lewis (FUT3) genotype. J Infect Dis **194:**1422–1427. http://dx.doi.org/10.1086/508430.
266. **Lindesmith L, Moe C, Lependu J, Frelinger JA, Treanor J, Baric RS.** 2005. Cellular and humoral immunity following Snow Mountain virus challenge. J Virol **79:**2900–2909. http://dx.doi.org/10.1128/JVI.79.5.2900-2909.2005.
267. **Nakata S, Chiba S, Terashima H, Yokoyama T, Nakao T.** 1985. Humoral immunity in infants with gastroenteritis caused by human calicivirus. J Infect Dis **152:**274–279. http://dx.doi.org/10.1093/infdis/152.2.274.
268. **Kjeldsberg E.** 1986. Demonstration of calicivirus in human faeces by immunosorbent and immunogold-labelling electron microscopy methods. J Virol Methods **14:**321–333. http://dx.doi.org/10.1016/0166-0934(86)90034-0.
269. **Cubitt WD, McSwiggan DA.** 1987. Seroepidemiological survey of the prevalence of antibodies to a strain of human calicivirus. J Med Virol **21:**361–368. http://dx.doi.org/10.1002/jmv.1890210408.
270. **Sakuma Y, Chiba S, Kogasaka R, Terashima H, Nakamura S, Horino K, Nakao T.** 1981. Prevalence of antibody to human calicivirus in general population of northern Japan. J Med Virol **7:**221–225. http://dx.doi.org/10.1002/jmv.1890070306.
271. **Nakata S, Chiba S, Terashima H, Nakao T.** 1985. Prevalence of antibody to human calicivirus in Japan and Southeast Asia determined by radioimmunoassay. J Clin Microbiol **22:**519–521.
272. **Simmonds P, Karakasiliotis I, Bailey D, Chaudhry Y, Evans DJ, Goodfellow IG.** 2008. Bioinformatic and functional analysis of RNA secondary structure elements among different genera of human and animal caliciviruses. Nucleic Acids Res **36:**2530–2546. http://dx.doi.org/10.1093/nar/gkn096.
273. **Perriere G, Gouy M.** 1996. WWW-query: an on-line retrieval system for biological sequence banks. Biochimie **78:**364–369. http://dx.doi.org/10.1016/0300-9084(96)84768-7.

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