Novel G10P[14] Rotavirus Strain, Northern Territory, Australia

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We identified a genotype G10P[14] rotavirus strain in 5 children and 1 adult with acute gastroenteritis from the Northern Territory, Australia. Full genome sequence analysis identified an artiodactyl-like (bovine, ovine, and camelid) G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genome constellation. This finding suggests artiodactyl-to-human transmission and strengthens the need to continue rotavirus strain surveillance.

Group A rotavirus infection is the major cause of acute gastroenteritis in children worldwide. The rotavirus genome consists of 11 segments of double-stranded RNA encoding 6 structural viral proteins (VP1–4, VP6, VP7) and 6 nonstructural proteins (NSP 1–5/6) (*1*). Genotypes are assigned on the basis of 2 outer capsid proteins into G (VP7) and P (VP4) genotypes; these proteins also elicit type-specific and cross-reactive neutralizing antibody responses (*1*). Strains that include genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] cause most rotavirus disease in humans (*1*). Since 2008, rotaviruses have been classified by using the open reading frame of each gene. The nomenclature Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx represents the genotypes of the gene segments encoding VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 (*2*). To date, 27 G, 35 P, 16 I, 9 R, 9 C, 8 M, 16 A, 9 N, 12 T, 14 E, and 11 H genotypes have been described (*2*).

Two live oral vaccines are available globally: Rotarix (GlaxoSmithKline Biologicals, Melbourne, Victoria, Australia) and RotaTeq (Merck, Whitehouse Station, NJ, USA). Rotarix is a monovalent vaccine that contains a single human G1P[8] strain (*3*). RotaTeq is a pentavalent vaccine comprised of 5 human–bovine reassortant virus strains (*3*). Both vaccines were introduced into the Australian National Childhood Immunization Program in July 2007. The strategy of a rotavirus vaccination program is to target the most frequently circulating rotavirus strain(s) and provide homotypic and heterotypic protection.

G10P[14] rotavirus strains are rarely reported as the source of infection in humans. Of 7 previously reported G10P[4] rotavirus infections, 1 each was in the United Kingdom and Thailand and 5 were in Slovenia (*4*). During 2011, the Australian Rotavirus Surveillance Program identified 6 G10P[14] strains in the Northern Territory (NT). We report the characterization of G10P[14] strains detected in Australia.

The Study

Six rotavirus-positive specimens collected from NT were genetically untypeable by reverse transcription PCR (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/8/12-1653-Techapp1.pdf). Sequence analysis of the VP7 and VP4 genes of these strains demonstrated highest nucleotide identity with G10 and P[14] rotaviruses, respectively. The G10P[14] strains were from specimens collected from 5 children and 1 adult (84 years of age) during August and September 2011 (Table 1). Of the 6 G10P[14] case-patients, 5 were from Tennant Creek, NT, ≈1,000 km south of Darwin in northern Australia; the residence of the other case-patient is unknown. All strains were detected in indigenous Australians. Specimens V585, V582, and WDP280 were collected from case-patients who had received 2 doses of Rotarix, and specimen SA179 was collected from a case-patient who had received 1 dose. No vaccination data were available for the case-patient from whom specimen SA175 was collected.

Sanger sequencing was used to generate the complete genome of specimen V585 (online Technical Appendix). For the other 5 G10P[14] strains, the complete open reading frames of VP7, VP4, NSP4, and NSP5 and partial reading frames of VP1, VP2, VP3, VP6, NSP1, and NSP2 were sequenced (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/8/12-1653-T2.htm). These 5 strains demonstrated >99.5% sequence identity to V585, confirming that V585 was representative of all 6 strains. The genotype of each segment of V585 was determined by using RotaC version 2.0 (http://rotac.regatools.be), a web-based genotyping tool for group A rotaviruses; a G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 constellation was identified. Maximum-likelihood phylogenetic analyses were performed by using full-length open reading frame nucleotide sequences of V585 and other group A rotavirus strains (online Technical Appendix). The nucleotide sequences of the 11 gene segments of V585 and the VP7 and VP4 genes of the other 5 G10P[14] strains were deposited in GenBank (accession nos. JX567748–JX567768).

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DOI: http://dx.doi.org/10.3201/eid1908.121653

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Table 1. Cohort and vaccination status for novel G10P[14] rotavirus strain, Northern Territory, Australia, 2011*

Case-patient specimen ID	Case-patient age	Date specimen collected	Location (postal code) of specimen collection†	Rotavirus vaccine (no. doses)‡
V582	8 mo	Aug 13	0860	Rotarix (2)
WDP280	10 mo	Aug 19	0872	Rotarix (2)
V585	2 y	Aug 19	0860	Rotarix (2)
SA175	3 mo	Sep 2	Unknown	Unknown
SA179	4 mo	Sep 6	0872	Rotarix (1)
D355	84 y	Sep 11	0860	Not applicable

*ID, identification.
†Postal code zone 0860 encompasses Tennant Creek, Northern Territory; samples WDP280 and SA179 were collected in post code zone 0872, in communities near code zone 0860.
‡Rotarix, GlaxoSmithKline Biologicals, Melbourne, Victoria, Australia.

Phylogenetic analysis of the VP7 gene identified 10 lineages (Figure 1, panel A). The 6 G10P[14] strains from NT (lineage IX) were distinct from human G10P[14] rotaviruses RVA/human-tc-GBR/A64/1987/G10P14 (lineage II) and RVA/human-tc/THA/Mc35/1987–1989/G10P[14] (lineage V), and they were most closely related to bovine strains identified predominantly in Ireland, China, and Australia (lineage IV). V585 had the highest level of nucleotide

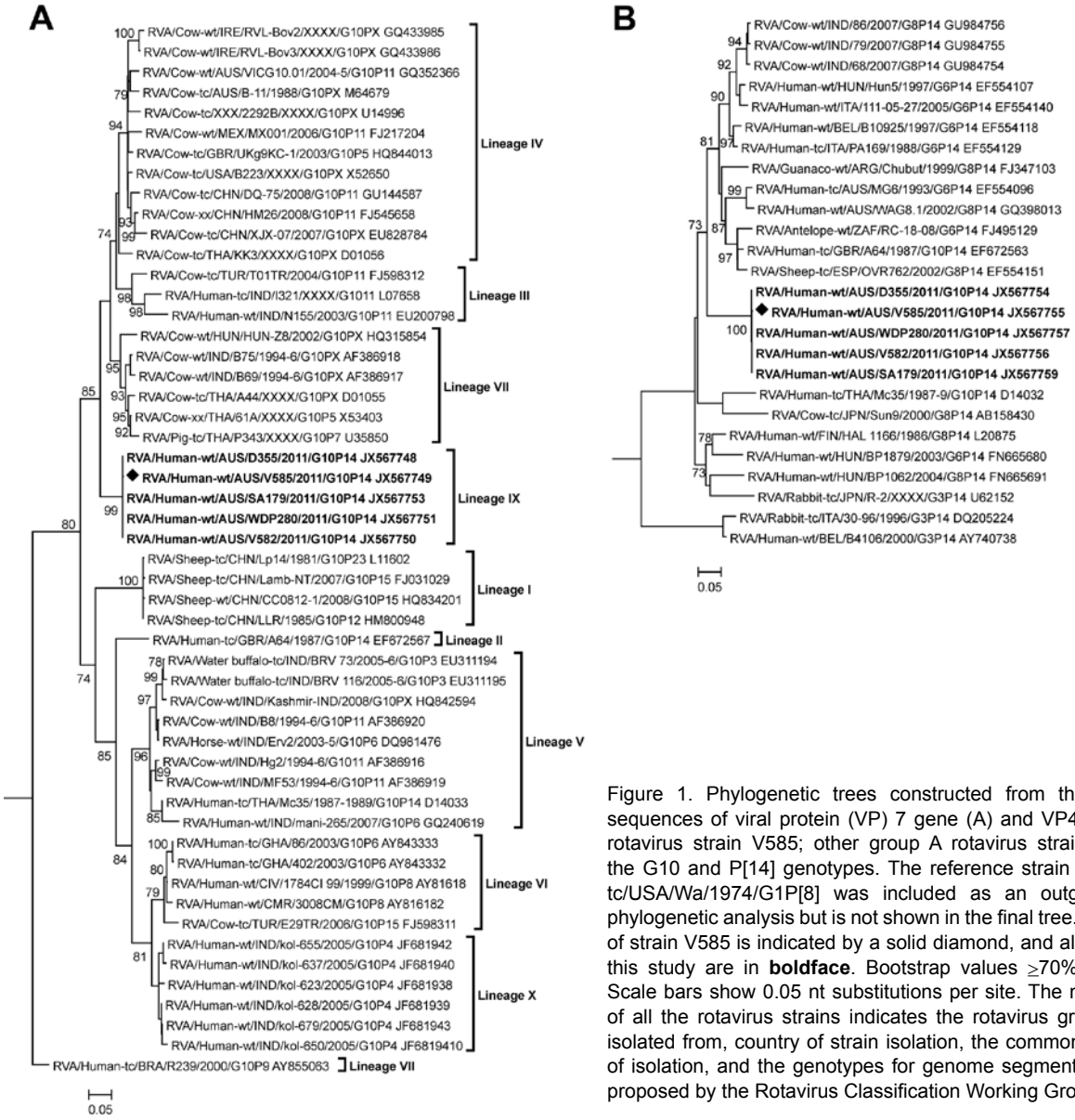

Figure 1. Phylogenetic trees constructed from the nucleotide sequences of viral protein (VP) 7 gene (A) and VP4 gene (B) of rotavirus strain V585; other group A rotavirus strains represent the G10 and P[14] genotypes. The reference strain RVA/human-tc/USA/Wa/1974/G1P[8] was included as an outgroup in the phylogenetic analysis but is not shown in the final tree. The position of strain V585 is indicated by a solid diamond, and all strains from this study are in **boldface**. Bootstrap values ≥70% are shown. Scale bars show 0.05 nt substitutions per site. The nomenclature of all the rotavirus strains indicates the rotavirus group, species isolated from, country of strain isolation, the common name, year of isolation, and the genotypes for genome segment 9 and 4, as proposed by the Rotavirus Classification Working Group (*2*).

Table 3. Nucleotide identity of 11 genome segments of the G10P[14] rotavirus strain V585, Northern Territory, Australia*

Gene encoding	Genotype of V585	Cutoff value†	Identity of V585 against indicated strains	
			Genotype reference strain	GenBank strains‡
VP1	R2	83	85.6 (DS-1)	96.9 (GirRV)
VP2	C2	84	84.9 (DS-1)	89.5 (B12)
VP3	M2	81	85.1 (DS-1)	88.9 (MG6)
VP4	P[14]	80	87.4 (A64)	88.9 (B10925)
VP6	I2	85	86.6 (DS-1)	93.8 (RotaTeq BrB-9/SC2-9/W17-9)
VP7	G10	80	86.8 (A64)	92.5 (RVL-Bov3)
NSP1	A11	79	79.9 (Hun5)	80.2 (BP1879)
NSP2	N2	85	87.4 (DS-1)	94.3 (B12)
NSP3	T6	85	92.4 (WC3)	94.0 (GirRV/A64)
NSP4	E2	85	88.7 (DS-1)	90.8 (Azuk-1)
NSP5	H3	91	92.9 (AU-1)	94.1 (RUBV81/Egy3399)

*VP, viral structural protein; NSP, nonstructural protein.
†Numeric values are given as percentage nucleotide identity. Percentage nucleotide cutoff values, genotype designation, and genotype reference strains proposed in (*2*).
‡Strains that shared the highest nucleotide identity with the Australian G10P[14] strain V585.

identity (92.5%) to the bovine strain RVA/cow-wt/IRE/RVL-Bov3/XXXX/G10P[X] (Table 3). Nucleotide identity was lower to Australian bovine G10 strains RVA/cow-wt/AUS/VICG10.01/2004-5/G10P[11] (91.1%) and RVA/cow-tc/AUS/B-11/1099/G10P[X] (90.6%).

The VP4 genes of the G10P[14] strains from NT formed a cluster distinct from other characterized P[14] sequences identified globally from humans and animals (Figure 1, panel B). V585 had the highest level of nucleotide identity (88.9%) to the human strain RVA/human-wt/BEL/B10925/1997/G6P[14] (Table 3). Nucleotide identity was lower to other Australian P[14] sequences, RVA/human-tc/AUS/MG6/1993/G6P[14] (87.5%) and RVA/human-wt/AUS/WAG8.1/2002/G8P[14] (87.2%).

Phylogenetic analysis of VP1, VP2, NSP2, and NSP3 demonstrated that V585 clustered with genes of rotaviruses identified in the mammalian order Artiodactyla (bovine, ovine, and camelid) and human strains derived from zoonotic infections (Figure 2, Appendix, panels A, B, F, G, wwwnc.cdc.gov/EID/article/19/8/12-1653-F2.htm). Similarly, VP3, which clustered with the RVA/human-tc/AUS/MG6/1993/G6P[14], was thought to be the result of zoonotic transmission (*5*) (Figure 2, Appendix, panel C). NSP1, NSP4, and NSP5 clustered with sequences from artiodactyl hosts, however branching was not supported by significant bootstrap values (Figure 2, Appendix, panels E, H, I). The NSP1 and NSP5 genes were divergent from sequences that define their respective genogroups (Table 3). Overall, the 11 genome segments of the G10P[14] strains from NT had relatively low nucleotide identity (80.2%–96.9%) to other strains in each of the respective genogroups, demonstrating that this G10P[14] strain identified in Australia was divergent from other strains identified globally (Table 3).

Conclusions

The V585 strain possessed a G10-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genome constellation. With the exception of the VP7 gene, the constellation is consistent with G6P[14] and G8P[14] strains identified globally: G6/G8-P[14]-I2-(R2/R5)-C2-M2-(A3/A11)-N2-T6-(E2/E12)-H3 (*6*). Human P[14] strains are related to rotavirus strains isolated from even-toed ungulates belonging to the mammalian order Artiodactyla (*6*). Consistent with this observation, each individual genome segment of V585 was most closely related to artiodactyl-derived strains or human zoonotic rotavirus strains characterized to be derived from artiodactyl hosts. In Australia, G10P[11] strains have been isolated from calves, and G8P[14] strains and G6P[14] strains have been isolated from children (*7,8*). However, the V585 strain demonstrated modest nucleotide identity with these 3 strains identified in Australia. These data suggest that V585 is novel and probably derived from a strain circulating in an artiodactyl host and transmitted to humans. A large feral animal population, including goats, rabbits, and camels, exists in the region where these specimens were collected, thereby supporting the possibility of an interspecies transmission event (*9*).

Vaccination with the monovalent G1P[8] Rotarix is available in NT, where 2-dose vaccine coverage is 74% for indigenous Australian infants (*10*). Rotarix vaccination status was available for 4 of the 5 children in this study: 3 were fully vaccinated, and 1 had received the primary dose. The heterotypic G10P[14] strain identified in these vaccinated children suggests a lack of protective immunity, although it cannot be excluded that vaccination provided protection against severe disease from other genotypes. Vaccine effectiveness against gastroenteritis leading to hospitalization has been variable in NT; vaccine was estimated to be 77.7% effective during a 2007 G9P[8] outbreak (*11*) and 19% effective against a fully heterotypic G2P[4] strain in 2009 (*12*). Rotarix has been effective for decreasing rotavirus infection notification rates in Darwin, NT (*10*), and New South Wales (*13*). However, in 1 location in central NT, reported rotavirus infection rates have remained similar in the vaccine era to

those in the prevaccine era (*10*), suggesting low vaccine uptake, low vaccine take, or waning immunity. The living conditions of the indigenous Australian population in central NT are typically crowded, with inadequate facilities for sanitation and food preparation (*14*). The number of diarrheal disease cases is high: admissions coded for enteric infections in NT indigenous Australian infants occur at a rate 10-fold higher than among nonindigenous Australian infants (*15*). The concurrent medical conditions present in the NT indigenous Australian population, combined with diversity of circulating rotavirus types, may have contributed to a lack of immunity. Detection of these unusual G10P[14] strains emphasizes the need for continued rotavirus surveillance to help guide current and future vaccination strategies.

Acknowledgments

We acknowledge the assistance of Heather Cook, Centre for Disease Control, Department of Health, NT, for collection and supply of patient information.

C.D.K. is a codeveloper of an investigational rotavirus vaccine.

C.D.K. is director of the Australian Rotavirus Surveillance Program, which is supported by research grants from vaccine manufacturers CSL and GlaxoSmithKline Biologicals. This work was supported by grants from the National Health and Medical Research Council of Australia (1031473); the Australian Commonwealth Department of Health and Aging; GlaxoSmithKline Biologicals; and CSL (Melbourne). C.D.K. is supported by a Career Development Award from the National Health and Medical Research Council of Australia (607347). This study was supported by the Victorian Government's Operational Infrastructure Support Program.

Dr Cowley is a molecular virologist at the Murdoch Childrens Research Institute. His research interests include the molecular characterization and evolution of rotavirus strains in the community of Australia.

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Article DOI: http://dx.doi.org/10.3201/eid1908.121653

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Technical Appendix

Methods and Materials

Faecal Specimens

Faecal specimens collected from individuals with acute diarrhea were frozen, stored at –70°C and forwarded to the Australian Rotavirus Surveillance Program in Melbourne, Australia. Patient information including age, date of sample collection, sex, immunisation status with regard to the Rotarix rotavirus vaccine and Indigenous status was obtained. The fecal specimens were screened for the presence of other enteric pathogens including ova, cysts, parasites, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Vibrio* and shiga-like toxin producing *E. coli* by the pathology services at the site of collection.

Nucleic Acid Extraction

Faecal suspensions (20% wt/vol) were prepared in virus dilution buffer (0.01 mol/L Tris-HCl (pH 7.5), 0.1 mol/L $CaCl_2$, 0.15 mol/L $NaCl_2$). The homogenate was clarified by centrifugation at 16,000 *x* g for 3 min and the supernatant was stored at -20ºC. RNA was extracted from 140 µl fecal suspension using the QIAamp® Viral RNA Mini Kit (QIAGEN, Inc., Hilden, Germany) in accordance with the manufacturer's spin protocol. RNA was eluted in 40 µl RNase-free distilled water and stored at -20 ºC.

G and P Genotyping RT-PCR

All samples were subjected to a two-step hemi-nested multiplex RT-PCR to determine G and P genotype using the One-Step RT-PCR Kit (QIAGEN, Inc., Hilden, Germany), following an established protocol in a PCR thermocycler (96-Well GeneAmp® PCR system 9700, Applied Biosystems). The amplification of the VP7 gene yielded a 881 bp fragment using the oligonucleotide primer set VP7F/VP7R and G genotyping was performed using a pool of primers specific for G1, G2, G3, G4, G8 and G9 (*1*-*3*). A 663 bp fragment of the VP4 gene was generated using the oligonucleotide primer set VP4F/VP4R and P genotyping was performed using a pool of primers specific for P[4], P[6], P[8], P[9], P[10] and P[11] (*4*,*5*).

Amplification of VP4 and VP7 Genes

For each of the six G10P[14] samples the full length VP4 and VP7 genes were reverse transcribed and amplified by PCR using the SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity kit (Invitrogen, USA). Briefly, 5 µl of RNA was denatured at 97°C for 3 min and quenched on ice. Samples were reverse transcribed at 45°C for 30 min, followed by Taq Polymerase activation at 94°C for 2 min, 40 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec and extension at 68°C for 5 min were followed by a final extension of 5 min at 68°C. The oligonucleotide primers sets Beg9/End9 (*2*) were used to amplify the VP7 gene and the oligonucleotide primer set VP4 P14 1F/VP4 P14 3R (*6*) were used to amplify the VP4 gene.

Amplification of Nine Remaining Rotavirus Genome Segments

A representative sample of the six G10P[14] strains, V585, was selected for whole genome sequence analysis. RT-PCR was performed under the same conditions outlined above. The oligonucleotide primers used in the amplification of the nine remaining gene segments are listed in Table.

Nucleotide Sequencing

PCR amplicons were excised and purified via gel extraction and spin column purification using the QIAquick Gel extraction Kit (QIAGEN, Inc., Hilden, Germany) according to the manufacturer's protocol. Purified DNA together with oligonucleotide primers (detailed in Supplementary Table), were sent to the Australian Genome Research Facility, Melbourne and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA) in an Applied Biosystems 3730xl DNA Analyser (Applied Biosystems, Foster City, CA, USA). Primer walking was employed to cover the complete sequence of each gene.

Phylogenetic Analysis

The electropherograms generated were visually analysed and contiguous DNA sequence files were generated utilising the Sequencher® Software program version 5.0.1 (Gene Codes Corp Inc., Ann Arbor, MI, USA). Nucleotide similarity searches were performed using the BLAST server on the GenBank database at the National Center for Biotechnology Information, USA (www.ncbi.nlm.nih.gov). The deduced nucleotide and amino acid sequences of each gene were compared with sequences available in GenBank that possessed the entire open reading frame (ORF). Multiple nucleotide and amino acid alignments were constructed using ClustalW algorithms in the MEGA5.10 program (*7*). The model of nucleotide substitution

was chosen by following Bayesian information criterion ranking of each alignment as implemented in MEGA5.10. The models selected for each genome segment were Tamura 3-parameter (VP1, VP3, VP7, NSP1, NSP5), Tamura-Nei (VP2, VP4, VP6, NSP4), Hasegawa-Kishino-Yano (NSP2) and General Time Reversible (NSP3). Phylogenetic trees were generated using the Maximum likelihood method and the trees were statistically supported by bootstrap values >70% using 1000 pseudoreplicate runs.

Assignment of Genotypes

In accordance with the recommendations of the Rotavirus Classification Working Group (RCWG), the genotypes of each of the 11 genome segments were determined using the online RotaC v2.0 rotavirus genotyping tool (http://rotac.regatools.be).

Accession Numbers

The nucleotide sequences of the 11 gene segments of V585 and the VP7 and VP4 genes of the other five G10P[14] strains described in this study were deposited in GenBank under the accession numbers JX567748–JX567768.

Technical Appendix Table. Primers used for full genome characterization of G10P[14]

Gene	Primer	Sequence	Binding Position	Reference
VP1	VP1 RV3 Fwd start	GGCTATTAAAGCTATACAATG	1–21	(*8*)
	VP1 P2 R	GGCATCCAACATTTTCTGCT	1045–1025	(*9*)
	VP1 G1 F*	CGAGCNATAGTTCCNGACCA	847–867	(*9*)
	VP1 G5 F*	GCAGCKAATTCAATAGCRAA	1810–1830	(*9*)
	VP1 G6 R*	ATTCGCGCRTAYGTTTCTCT	2003–1984	(*9*)
	CD-VP1-2544 F	TGCACCAATATCACTTGAC	2544–2562	This study
	CD-VP1-2805 R	GCTGATTTTGAACCGTC	2805–2788	This study
	VP1 RV3 Rev end	GGTCACATCTAAGCGCTCTA	3302–3274	(*8*)
VP2	VP2 RV3 Fwd start	GGCTATTRAAGGYTCAATGG	1–20	(*8*)
	VP2 P2 R	AACAAAATGYAGCCAATTC	1441–1423	(*9*)
	VP2 G1 F	TCATTAATTTCAGGYATGTGG	1223–1244	(*9*)
	CD-VP2-2100R	GCACGTTCTATYTGATCC	2100–2083	This study
	DC-VP2-585R	TCCAATACCATCTGTTACGC	382–402	This study
	DC-VP2-585F	ATAACTTTGAGTCGCTGTGG	892–911	This study
	DC-VP2-585F	AGAATTAATGCGGACAGGCG	2165–2184	This study
	VP2 RV3 Rev end	GGTCATATCTCCACARTGG	2687–2669	(*8*)
VP3	CD-G10P14-VP3-23F	GTGTGTTTTACCTCTGATGG	23–43	This study
	VP3 RV3 Rev end	GGYCACATCATGACTAGTG	2591–2572	(*8*)
	CD-G10P14-VP3-917F	ATCAGCACCATCGTACTGGA	917–937	This study
	CD-G10P14-VP3-509R	CATCATCATCCGTAGCCG	509–491	This study
	CD-G10P14-VP3-1776R	CGTATTTGATGCGACCGT	1776–1758	This study
	CD-G10P14-VP3-2470F	CAGTGAGAATAGATACCACG	2470–2490	This study
VP4	VP4 P14 1F	GGCTATAAAATGGCTTCTTT	1–20	(*6*)
	VP4 P14 2R	AAAGATGGTTCACTAACAGC	1320–1300	(*6*)
	CD VP4 P14 1191F	CCAGTAATGAGTGGTGGC	1191–1208	This study
	VP4 P14 3R	GGTCACATCYTWARCAGACAG	2361–2341	(*6*)
	DC-VP4-585R	GAGCATACCCAGTCTGTGC	116–134	This study
	DC-VP4-585F	TACACTGCCGACAGATTTCG	988–1007	This study
	VP4 P14 1R	CTTCATTCATTCATTTGTGC	771–751	(*6*)
	VP4 P14 3F	ATGTCAGATGCGGCTTCTTC	1711–1730	(*6*)
VP6	VP6 F	GGCTTTWAAACGAAGAAGTCTT	1–19	(*8*)
	VP6 R	GGTCACATCCTCTCACT	1356–1340	(*8*)
	CD-G10P14-VP6-700F	CCAGATGCAGAAAGATTTAG	701–721	This study

Gene	Primer	Sequence	Binding Position	Reference
	CD-G10P14-VP6-521R	CAGTGTAAATGACGCTGAA	521–502	This study
VP7	Beg9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1–23	(*2*)
	End9	GGTCACATCATACAATTCTAATCTAAG	1062–1035	(*2*)
	DC-VP7 585 R	TGATACATCCATCGATCCAG	183–202	This study
	DC-VP7 585 F	ATCTGATCCAACGACTGCTC	820–839	This study
NSP1	NSP1_5'UTR	TGTAAAACGACGGCCAGTTATGAAAAGTCTT GTGGAAGC		JCVI
	NSP1_3'UTR	CAGGAAACAGCTATGACCCATTTTATGCTGC CTAGGCG		JCVI
	CD-G10P14-NSP1-1180F	ATGAAGAACGGTGGACTGAG	1180–1120	This study
	CD-G10P14-NSP1-695F	GGAATTGGTAAGAAACGAC	695–714	This study
	CD-G10P14-NSP1-531R	CCTGCTCCATAAAATCATAG	531–511	This study
NSP2	NSP2F	GGCTTTTAAAGCGTCTCAG	1–19	(*8*)
	NSP2R	GGTCACATAAGCGCTTTC	1059–1042	(*8*)
	DC-NSP2-585R	TAAATTGTGGCGGTGGTGC	152–170	This study
	DC-NSP2-585F	ATGAAGCGGGAGAGTAATCC	874–894	This study
NSP3	NSP3F	GGCTTTTAATGCTTTTCAGTG	1–21	(*8*)
	NSP3R	ACA TAA CGC CCC TAT AGC	1070–1054	(*8*)
	CD-G10P14-NSP3-923F	AGAGGACTAACGAAGCAATG	923–943	This study
NSP4	10.1	GGCTTTTAAAAGTTCTGTCC	1–20	Not previously published
	10.2	GGTCACACTAAGACCATTCC	750–730	Not previously published
NSP5	NSP5F	GGCTTTTAAAGCGCTACAG	1 – 19	(*8*)
	NSP5R	GGTCACAAAACGGGAGT	667–647	(*8*)

*Primer with degenerate sites inserted.

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