Widespread Rotavirus H in Domesticated Pigs, United States

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We investigated the presence in US pigs of rotavirus H (RVH), identified in pigs in Japan and Brazil. From 204 samples collected during 2006–2009, we identified RVH in 15% of fecal samples from 10 US states, suggesting that RVH has circulated in the United States since 2002, but probably longer.

Rotaviruses (RVs) belong to the *Reoviridae* family and are a major cause of severe diarrhea in humans and animals worldwide (*1*). According to the International Committee on Taxonomy of Viruses, the *Rotavirus* genus is divided into 5 antigenically distinct groups or species (RVA, RVB, RVC, RVD, RVE), 2 tentative species (RVF, RVG), and an unassigned species (ADRV-N), recently confirmed to be distinct from the other RV species, and now referred to as RVH (*2*,*3*).

Three human RVH strains from Asia (ADRV-N, J19, B219) (*4*–*8*) and a porcine RVH strain (SKA-1) (*9*) were identified during 1997–2002. In 2012, three Brazil porcine RVH strains BR63, BR60, and BR59 (GenBank accession nos. KF021621, KF021620, and KF021619) were identified, bringing to only 7 the total number of known RVH strains. To investigate the presence of RVH in US swine, we screened 204 porcine samples collected during 2006–2009.

The Study

We identified RVH in a porcine intestinal sample (RVH/Pig-wt/USA/AR7.10-1/2012/GXP[X]) submitted from a farm in Arkansas in 2012. Subsequently, we rescreened 204 available RVA-, RVB-, and/or RVC-positive porcine samples collected during 2006–2009 from 16 US states for RVH. The samples were from 5 different age groups of pigs: 1–3 days (21 samples), 4–7 days (23), 8–20 days (19), 21–55 days (110), and >55 days (9); 22 samples were from pigs of unknown age. Sample selection, histologic examination, extraction of genomic material, reverse transcription PCR (RT-PCR) amplification, sequencing of viral protein (VP) 6 gene, and statistical and sequence analysis are described in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/20/7/14-0034-Techapp1.pdf).

We identified RVH in 30 (15%) of the 204 samples, including sample AR7.10-1 (online Technical Appendix Table). RVH strains were identified in samples from 10 US states (Figure 1, panel A). The first US sample was identified on November 7, 2006. Of samples from age groups in which we detected positive results, most (20/111, 18%) were from 21–55-day-old pigs; RVH was not detected in 1–3-day-old piglets. We also detected RVH-positive samples in 4–20-day-old (5/42, 12%) and >55-day-old (5/9, 56%) pigs. The number of positive and negative samples differed significantly between age groups ($p = 0.036$, Fisher exact test). The odds of 21–55 day-old pigs being RVH positive was not significant (odds ratio [OR] 1.63, $p = 0.36$); however, in the >55-day group, the odds of being RVH positive was significant (OR 5.92, $p = 0.031$), compared with odds for the 4–20-day group. The trend for increased RVH positivity by age group was not significant ($p = 0.94$, Wald χ^2 test).

Although we identified only 5 samples with RVH in pigs co-infected with RVA and RVB, co-infections with RVH and RVA, RVB, both RVA and RVC, or both RVB and RVC (1 sample each) also were identified but did not differ significantly ($p>0.05$, Fisher exact test) (Figure 1, panel B). We did not identify RVH co-infected with only RVC. Most RVH samples (21 [70%]) were identified from pigs co-infected with RVA, RVB, and RVC, which was significantly higher from any other RVH co-infections with RVA, RVB, RVC, RVAB, RVAC, or RVBC ($p<0.001$, Fisher exact test). Of these 21 RVA, RVB, RVC, and RVH co-infected samples, 15 were from 21–55-day-old pigs (Figure 1, panel B).

The US porcine RVH VP6 sequences (GenBank accession nos. KF757260–KF757289) exhibited 91%–100% nt identity with each other and shared 89%–92% nt identity with Japan porcine strain SKA-1 and 85%–87% nt identity with Brazil porcine strains BR63, BR60, and BR59 (Table 1). The US porcine and human RVH VP6 sequences shared 70%–73% nt identity. The US porcine RVH VP6 sequences were 97%–100% aa identical with each other and 97%–98% and 96%–98% aa identical with the Japan and the Brazil porcine strains, respectively. The US porcine and human RVH VP6 sequences were 75.3%–76.8% aa identical (Table 1). The nucleotide and amino acid pairwise

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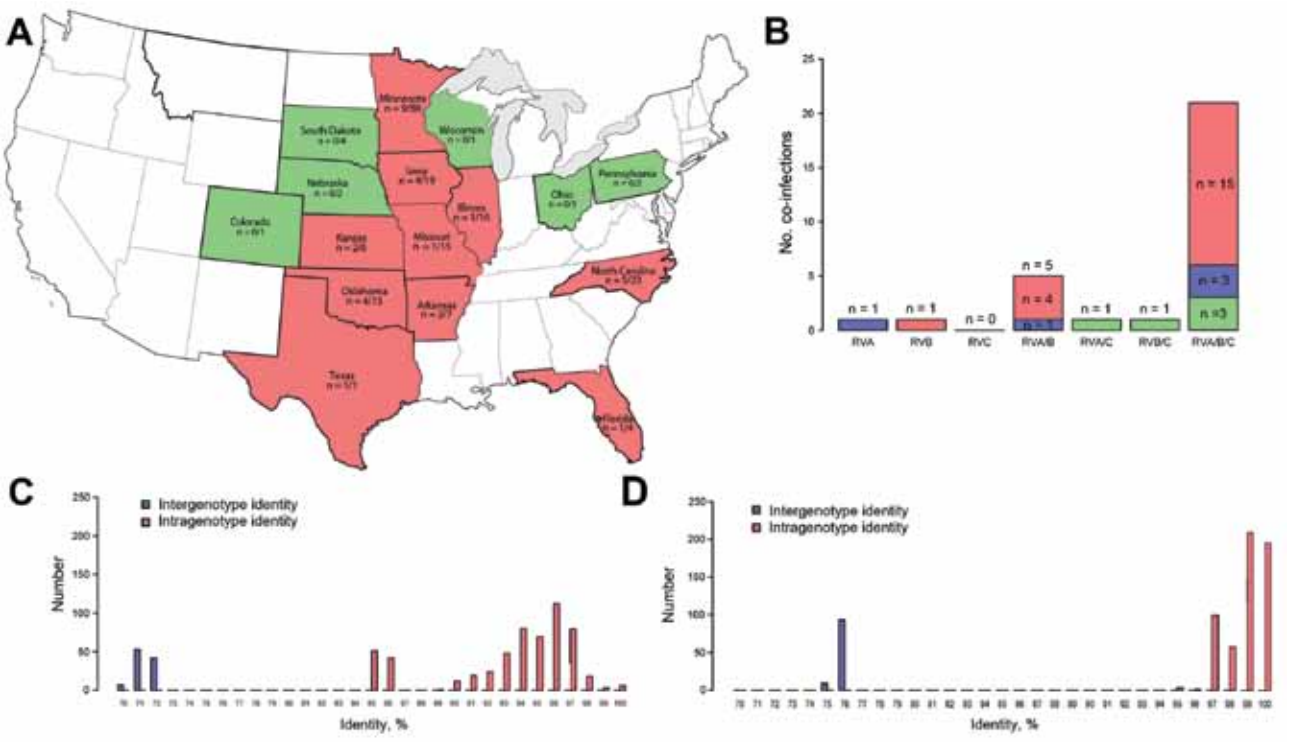

Figure 1. Epidemiologic and molecular distribution of porcine rotavirus H (RVH) strains, United States, 2006–2009. A) Geographic distribution of RVH-positive porcine samples/total number of samples tested. Pink indicates states containing positive samples; green indicates states negative samples; white indicates states from which samples were not submitted. B) Distribution of RVH-positive samples and age group in pigs co-infected with RVA, RVB, and/or RVC. Blue indicates samples from the 4–20-day age group; pink indicates samples from the 21–55-day age group; green indicates samples from the >55-day age group. C) RVH viral protein 6 nt pairwise identity. D) RVH amino acid pairwise identity.

identity charts (Figure 1, panels C and D) and phylogenetic trees (Figure 2, panel A) suggest the existence of at least 2 distinct RVH VP6 (I) clusters/genotypes containing human and porcine strains, respectively.

Compared with other RV species, the US RVH VP6 sequences shared the highest nucleotide and amino acid identities with RVG (51%–53% and 39%–41%, respectively) and RVB (47%–52% and 34%–39%, respectively) (Table 2). In the RV VP6 phylogenetic tree, The RVH, RVG, and RVB VP6 sequences clustered in 1 large branch, whereas the RVA, RVC, RVF, and RVD sequences clustered separately in another large branch (Figure 2, panel A). The RVH evolutionary rate (substitution/site/year) from BEAST (http://tree.bio.ed.ac.uk/) was estimated at 2.6×10^{-3} (95% CI 5.83×10^{-4} to 4.46×10^{-3}). On the basis of the estimate of the time from the most recent common ancestor for the VP6 gene segment, we believe that US RVH strains circulated in US swine for at least a decade and possibly much longer (the time from the most recent common ancestor 1963–2002, 95% highest posterior

Table 1. Nucleotide and amino acid percentage identities of RVH*

RVH type	US porcine RVH, %	Japan porcine RVH, %	Brazil porcine RVH, %	Human RVH, %
US porcine RVH				
Nucleotide	91–100	89.2–91.9	85.2–86.8	70.4–72.8
Amino acid	97–100	96.5–98.2	95.7–97.7	75.3–76.8
Japan porcine RVH				
Nucleotide	89.2–91.9	NA	85.5	71.7–72.3
Amino acid	96.5–98.2	NA	97	76.5-76.8
Brazil porcine RVH				
Nucleotide	85.2–86.8	85.5	100	71.1–71.2
Amino acid	95.7–97.7	97	100	75.8-76
Human RVH				
Nucleotide	70.4–72.8	71.7-72.3	71.1-71.2	94–100
Amino acid	75.3–76.8	76.5–76.8	75.8–76	98.7–100

*RVH, rotavirus H; NA, not applicable

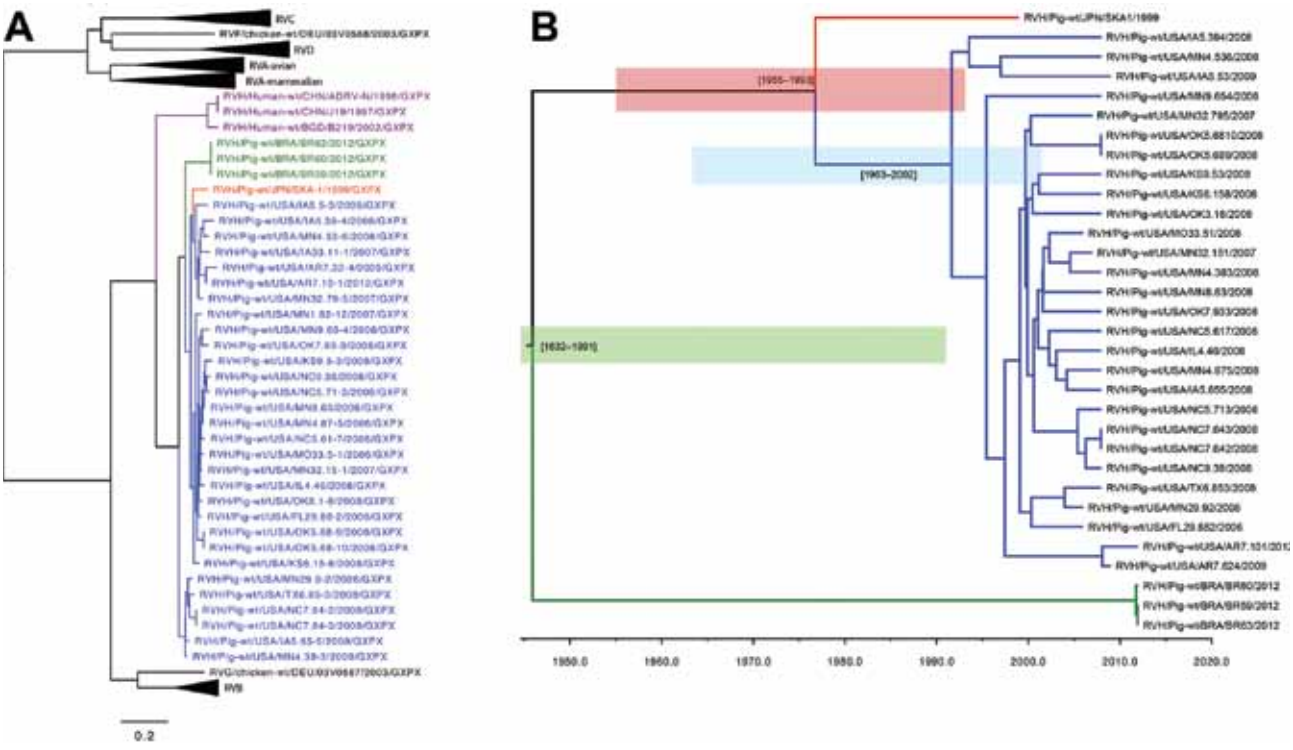

Figure 2. A) Nucleotide neighbor-joining phylogenetic tree of rotavirus (RV) A–D and F–H viral protein (VP) 6 sequences. Blue strains are from the United States; green strains are from Brazil; and the red strain is from Japan. Purple strains are from humans. Scale bar indicates percentage of dissimilarity between sequences. B) Time-scaled phylogeny of swine RVH VP6 sequences using a Bayesian Markov chain Monte Carlo approach. Blue shaded region indicates the time from the most recent common ancestor range (tMRCA) of the US strain; red shaded region indicates the US and Japan RVH tMCRA range; green shaded region indicates the tMRCA range for all swine RVH VP6 sequences.

density [HPD]) (Figure 2, panel B). The US and Japan RVH VP6 sequences diverged during 1955–1993, 95% HPD, and the estimated divergence of the Brazil RVH VP6 sequences from the US and Japan RVH VP6 sequences was 1832–1991, 95% HPD.

Conclusions

Our data indicate that RVH is widespread in US swine herds. Although the samples analyzed already were known to be positive for RV species A, B, and/or C, our identification of RVH in 15% of samples is remarkable. In the United States, piglets are weaned at 21 days of age and then mixed with other piglets from different production sites, which may explain the higher rate of RV co-infections in 21–55-day-old pigs (*10*,*11*). These findings suggest that RVH is underdiagnosed in US swine herds and requires further surveillance.

Our phylogenetic analysis indicates that the RVH strains circulating in US swine is evolutionarily distinct from that found in humans, as well as from swine in Brazil and Japan. Although our low sample number and sequencing of a single gene (VP6) makes the genetic diversity of RVH in US swine herds difficult to fully assess, the lack of spatial structure in the tree indicates extensive gene flow of RVH between swine herds in different US regions. Inferring the circulation of RVH in US swine herds is difficult because of the small sample size, although our time-structured phylogenetic analysis indicates at least 1 decade of circulation. Although US swine are routinely transported to South America, the phylogeny indicates that the VP6 gene of US swine RVH viruses is more closely related to that of Japan strain SKA-1 than to those of the 3 Brazil strains included in this analysis.

In conclusion, we identified RVH in 30 samples from pigs co-infected with RVA, RVB, and/or RVC in the United States, which indicates that RVH has been circulating in US swine for at least 1 decade and perhaps for longer. The human and porcine RVH VP6 sequences clustered into separate branches in the phylogenetic tree, but the presence of RVH in swine clearly raises the possibility of interspecies transmission. Because the swine samples were co-infected with RVA, RVB, and/or RVC, the role of RVH in pathogenesis remains unknown but this circumstance illustrates the need for molecular epidemiologic studies.

Table 2. Nucleotide and amino acid percentage identities of RVs*

RV type	RVA	RVB	RVC	RVD	RVF	RVG	RVH
RVA							
Nucleotide	65.2–100	29.7–36.2	48.5–55.7	46.4–52.1	46.3–50.8	32.9–36.7	31.7–36.2
Amino acid	65–100	7.5–11.3	36.3–42.9	33.3–39.9	31.8–37.2	11.1–13.5	9.9–13.1
RVB							
Nucleotide	29.7–36.2	64.8–100	30.5–34.4	29.2–32.9	30.1–32.9	50.7–57.1	47.4–51.7
Amino acid	7.5–11.3	66.2–100	10.6–13.9	10.4–12.7	11.3–13.4	46.1–49.4	34.4–39.4
RVC							
Nucleotide	48.5–55.7	30.5–34.4	81.4–100	47.2–49.8	47.4–48.3	33.8–34.2	31.5–34.6
Amino acid	36.3–42.9	10.6–13.9	87.1–100	34.7–35.4	32.7–33.9	14.4–14.6	13.4–14.7
RVD							
Nucleotide	46.4–52.1	29.2–32.9	47.2–49.8	90.1–99.6	49.8–50.7	33–34	31.9–34.4
Amino acid	33.3–39.9	10.4–12.7	34.7–35.4	98.2–99.7	36.6–37.6	12–12.5	14.5–16.8
RVF							
Nucleotide	46.3–50.8	30.1–32.9	47.4–48.3	49.8–50.7	NA	32.3	31–32.2
Amino acid	31.8–37.2	11.3–13.4	32.7–33.9	36.6–37.6	NA	11.1	12.6–14
RVG							
Nucleotide	32.9–36.7	50.7–57.1	33.8–34.2	33–34	32.3	NA	50.7–52.2
Amino acid	11.1–13.5	46.1–49.4	14.4–14.6	12–12.5	11.1	NA	39.1–41.4
RVH							
Nucleotide	31.7–36.2	47.4–51.7	31.5–34.6	31.9–34.4	31–32.2	50.7–52.2	70.4–100
Amino acid	9.9–13.1	34.4–39.4	13.4–14.7	14.5–16.8	12.6–14	39.1–41.4	75.3–100

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Dr Marthaler is a scientist at the University of Minnesota Veterinary Diagnostic Laboratory. His primary research interests include rotavirus and other swine pathogens.

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Widespread Rotavirus H in Commercially Raised Pigs, United States

Technical Appendix

Sample Selection

The University of Minnesota Veterinary Diagnostic Laboratory routinely receives porcine intestinal or fecal samples from pig farms across the United States to determine the causative agents of gastrointestinal disease, which are screened by reverse transcription PCR (RT-PCR) for transmissible gastroenteritis coronavirus; rotavirus (RV) A, RVB, and RVC; and a variety of bacterial pathogens by bacterial cultures (*1*,*2*). In June 2012, a porcine intestinal sample (MN123) from a pig on a farm in Minnesota showed histologic RV-like lesions (blunt or nude villus tips) by light microscopy, but the sample was negative for RVA, RVB, and RVC by RT-PCR. Another porcine intestinal sample (AR7.10-1) from a pig on a farm in Arkansas was positive for RVA, RVB, and RVC. Both samples (MN123 and AR7.10-1) were screened for RVH with RVH viral protein (VP) 7–, VP6-, and nonstructural protein (NSP) 4-specific primers. The porcine intestinal sample MN123 was negative for RVH with all primer sets; however, the AR7.10-1 sample was positive for RVH with the RVH VP6-specific primers. The RVH VP6-specific primers amplified a band of ≈1,200 nt, and sequencing of the band revealed a 90% nt identity to the swine Japanese RVH SKA-1 strain, identifying an RVH strain for the first time in the United States. From a previous RV study, the extracted RNA from 204 RVA-, RVB- and/or RVC-positive samples collected during 2006–2009 was readily available and used to determine whether RVH also was present in those samples. For the screening of RVH, only the RVH VP6-specific primers were used because the RVH VP7- and NSP4-specific primers had failed to detect RVH in the sample AR7.10-1.

Histology, Extraction of Genomic Material, and RT-PCR Amplification

Samples of small intestine were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained by using Harris' hematoxylin and eosin, as described previously (*1*,*2*). Porcine intestinal or fecal samples were homogenized with Hyclone donor equine serum (Thermo Fisher Scientific, Waltham, MA, USA) at a 2:3 ratio for 15 min, then underwent 3,000 × g centrifugation for 1 h, as described previously (*1*,*2*). The total nucleic acid was extracted by using the Ambion MagMax 1836 extraction kit following the manufacturer's instructions (Life Technologies, Carlsbad, CA, USA).

For the molecular detection of RVH strains, VP7, VP6, and NSP4-specific primers were designed (forward RVH_VP7_1-20: 5′-GGCAATTTGAAGCCATGTTG-3′ and reverse RVH_VP7_804-781: 5′-CATAACGGATTTCTCAACGTTATG-3′, forward RVH_VP6_1-23: 5′-GGCAATTTCTTGCTACAAGTGAC-3′ and reverse RVH_VP6_1203-1181: 5′-GGGTATATTTTATTTGCTATACTACTACGG-3′, and forward RVH_NSP4_1-24: 5′-GGCATTTTGTTCATCACAAATCACG-3′ and reverse RVH_NSP4_721-698: 5′-CTACCAAGCTATGTTTCCATCCAT-3′) based on sequence alignment with ClustalW of the VP7, VP6, and NSP4 sequences of the single porcine (SKA-1) and the 3 human (ADRV-N, J19, B219) RVH strains (*3*–*8*). The RT-PCR reaction used the QIAGEN OneStep RT-PCR kit (QIAGEN. Valencia, CA, USA) in accordance with the manufacturer's recommended instructions and following the previously described thermal cycling conditions (*1*,*2*). RT-PCR products were visualized, purified, and Sanger sequenced by using previously described methods (*1*,*2*). The nucleotide peaks from the traces files were visually evaluated and had a minimum of 2 times consensus coverage in Lasergene Seqman 10.0 program (DNASTAR, Madison, WI, USA).

In Geneious Pro (http://www.geneious.com)Tre, the novel, and GenBank RVH (SKA-1, AB576626; B219, DQ168033; ADRV-N, AY632080; J19, DQ113902; BR63, KF021621; BR60, KF021620; BR59, KF021619) RV VP6 nucleotide sequences were aligned with ClustalW (*8*). The nucleotide and amino acid phylogenetic trees were constructed with the neighbor-joining method (*9*). A time-scaled phylogeny was inferred for the swine RVH VP6 sequences by using a Bayesian Markov Chain Monte Carlo (MCMC) approach, available in the BEAST package (BEASTv1.8.0) (*10*–*16*). A general-time reversible model of nucleotide substitution was implemented, with a γ distributed among-site rate variation. Using a relaxed molecular clock and

a Bayesian skyline population prior, the MCMC chain was run for 300 million generations, with subsampling every 30,000 iterations. The initial 10% of the chain was discarded as burn-in, and a maximum clade credibility tree was summarized by using TreeAnnotator (v.1.8.0) (*10–16*). Times to the most recent common ancestor were identified on key nodes using FigTree (v1.4.0) (http://beast.bio.ed.ac.uk/software/figtree/).

Statistical Analysis

The Fisher exact test was used to determine whether the number of positive and negative samples significantly differed among age groups. By using the age groups that contained RVH-positive samples (4–20-day, 21–55-day, and >55-day age groups), a logistic regression model was used to estimate the odds of RVH infection by age group, and the trend of RVH infection by age was tested by using Wald χ^2.

Sequence Analysis

The open reading frame alignment (1,191 nt) of the 3 human (ADRV-N, J19, B219), 4 porcine (SKA-1, BR63, BR60, BR59), and the 30 novel porcine US RVH VP6 sequences revealed 682 (37%) identical sites. When compared with the human RVH sequences, the US porcine RVH VP6 sequences had 2 nt deletions at 1223 and 1224 in the 3′ untranslated region. The RVH VP6 396 amino acid alignment of the US (n = 30), Chinese (n = 2), Bangladeshi (n = 1), Japanese (n = 1), and Brazilian (n = 3) strains revealed 291 (74%) identical sites. Although most of the identified polymorphisms involved 2 different amino acids, polymorphisms involving 3 amino acids were identified at positions 44, 48, 150, 201, 220, 258, 353, and 378 (data not shown). Sequencing of the 30 novel RVH strains revealed 6 samples (IA5.5-3, MN4.53-6, MN4.87-5, MN32.15-1, AR7.32-4, and AR7.10-1) that contained mixed RVH infections or viral population, as indicated by ambiguous nucleotides within each the sequences (data not shown). Each ambiguous nucleotide translated only 1 aa.

Nucleotide Sequence Accession Numbers

The 30 RVH VP6 sequences were deposited into GenBank under accession nos. KF757260–KF757289.

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RVA/Humantc/IDN/57M/1980/G4P[10], JQ863313; human/Bethesda/DC1563/1974/G3P[8], FJ947180; RVA/Humantc/USA/P/1974/G3P1A[8], EF583040; RVA/Human/JPN/Hosokawa/1983/G4P1A[8], DQ870492; human/Bethesda/DC4613/1980/G4P[8], HM773914; human/Bethesda/DC1285/1980/G4P[8], FJ947169; RVA/Humantc/GBR/ST3/1975/G4P2A[6]EF583048; RVA/Humantc/USA/WI61/1983/G9P1A[8], EF583052; RV3, FJ998275; HR3RV3, U04741; RVA/Humantc/AUS/McN13/1980/G3P2A[6], JX416221; Hu/RUS/Nov04H297/2004/G1P[8], JQ818163; Hu/RUS/Nov03H269/2003/G1P[8], JQ818162; Hu/RUS/Nov03H223/2003/G1P[8], JQ818158; Hu/RUS/Nov11N2817/G4P[8], JQ230101; CMH110/07, JQ043299; CMH060/07, JQ043297; Omsk08350, GQ477110; Nov10N415, HQ611016; Nov10N375, HQ611014; CU957KK/11, JN706552; CU956KK/11, JN706551; CU875BK/10, JN706550; Hu/RUS/O917/2010/G1P[8], JX867347; human/Victoria/CK00019/2005/G1P[8], JF490265; human/Victoria/CK00020/2005/G1P[8], JF490277; human/Vanderbilt/VU050657/2005/G1P[8], JF490764; human/Victoria/CK00015/2005/G1P[8], JF490222; human/Victoria/CK00016/2005/G1P[8], JF490233; RVA/Human-wt/USA/2008747323/2008/G1P[8], JN258392; RVA/Human-wt/ITA/AV28/2010/G9P8, JX195078; RVA/Human-wt/BEL/BE00045/2009/G1P[8], HQ392404; RVA/Human-wt/BEL/BE00031/2008/G1P[8], HQ392262; RVA/Human-wt/ITA/AV21/2010/G9P8, JX195067; RVA/Human-wt/USA2007719907/2007/G1P[8], HM773848; RVA/Human-wt/USA2007719945/2007/G1P[8], HM773837; human/Vanderbilt/VU060732/2006/G1P[8], JF490937; human/Vanderbilt/VU050640/2005/G1P[8], JF490704; human/Vanderbilt/VU050613/2005/G1P[8], JF490584; human/Vanderbilt/VU050619/2005/G1P[8], JF490629; human/Vanderbilt/VU050620/2005/G1P[8], JF490640; human/Vanderbilt/VU050633/2005/G1P[8], JF490693; human/Vanderbilt/VU050623/2005/G1P[8], JF490650; human/Vanderbilt/VU05062/2005/G1P[8], JF490563; human/Vanderbilt/VU050667/2005/G1P[8], JF490794; US6588, FJ152109; CAU160, EU679384; CAU200, EU679387; RVA/Human-wt/AUS/CK00086/2009/G1P[8], JX027852; Hu/CI81/2011/KOR, JN887819; CAU163, EU679385; CAU136, EU679383; RVA/Human-wt/BEL/BE00004/2005/G1P[8], HQ392007; CAU214, EU556222; CAU195, EU556221; RVA/Human-wt/BEL/B4633/2003/G12P[8], DQ146642; RVA/Human-wt/USA2008747307/2008/G9P[8], HM773639; RVA/Human-wt/BEL/BE00044/2009/G1P[8], HQ392389; RVA/Human-wt/ZMB/MRCDPRU3491/2009/G12P[6], KF636150; RVA/Human-wt/Bel/BE00090/2003/G1P[8], JN258880; RVA/Human-wt/Bel/BE00112/2009/G1P[8], JN258948; human/Bethesda/DC1730/1979/G3P[8], FJ947323; human/Bethesda/DC1359/1980/G4P[8], HM773870; RVA/Human-wt/USA/DC4315/1988/G1P[8], KC579654; RVA/Human-wt/USA/DC3975/1988/G1P[8], KC580258; RVA/Human-wt/USA/DC4089/1988/G1P[8], KC580361; RVA/Human-wt/USA/DC4092/1988/G1P[8], KC579479; RVA/Human-wt/USA/DC4094/1988/G1P[8], KC579524; RVA/Human-wt/USA/DC4065/1988/G1P[8], KC579579; RVA/Human-wt/USA/DC4081/1988/G1P[8], KC579612; RVA/Human-wt/USA/DC3723/1989/G1P[8], KC579706; RVA/Human-wt/USA/DC4087/1988/G1P[8], KC579750; RVA/Human-wt/USA/DC4005/1988/G1P[8], KC579774; RVA/Human-wt/USA/DC3687/1989/G1P[8], KC579818; RVA/Human-wt/USA/DC4085/1988/G1P[8], KC579859; RVA/Human-wt/USA/DC3827/1989/G1P[8], KC579998; RVA/Human-wt/USA/DC4053/1988/G1P[8],

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RVA/Humantc/USA/Se584/1998/G6P[9], EF583044; RVA/Horse-tc/JPN/OH-4/1982/G6P[5], KC815662; OH4, D82975; Nov10N539, HQ611024; Nov10N413, HQ611017; Nov10N397, HQ611011; RVA/Humantc/ITA/PA169/1988/G6P[14], EF554130; bovine-tc/USA/NCDV/1971/G6P[1], JF693031; ROBMCP, K02254;
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AU32xUKreassortant(UKg9AU32)BRV10G9, GQ496291;
1290xUKreassortant(UKg91290)BRV1290G8, GQ496274;
ST3xUKreassortant(UKg9ST3)BRV4G4, GQ496257; DS1xUKreassortant(UKg9DS1)BRV5G2, GQ496223; AU32xUKreassortant(UKg9AU32), GQ225794; 1290xUKreassortant(UKg91290), GQ225793; ST3xUKreassortant(UKg9ST3), GQ225792; DS1xUKreassortant(UKg9DS1), GQ225790; UKtissueculturead apted(UKtc, X53667; KJ91, HM988974; KJ192, HM988973;
RVA/Vaccine/USA/RotaTeqSC29/1992/G2P7[5], GU565067;
RVA/Vaccine/USA/RotaTeqWI799/1992/G1P7[5], GU565056;
RVA/Vaccine/USA/RotaTeqWI788/1992/G3P7[5], GU565078; WC3, AF411322; RVA/Human-wt/AUS/CK20039/2008/G1P[8],, KC443602;
RVA/Vaccine/USA/RotaTeqWI794/1992/G6P1A[8],, GU565045; RVA/Human-wt/ITA/1110527/2005/G6P[14], EF554141; Sun9, AB374146;
RVA/Camelwt/SDN/MRCDPRU447/2002/G8P[11], KC257095; NCDV, AF317127; HQ09, JN790188; RVA/Human-wt/ITA/PAI58/1996/G3P[9], GU296429; B223, AF317128; BRV033, AF317126; RVA/Human-wt/ZAF/2371WC/2008/G9P[8], JN014003; Ecu534, EU805774; Ch03V0158G3, EU486966; Ch03V0358F3, EU486967; Ch06V0661, EU486969; 02V0002G3, FJ169858; Ch04V0027G6, EU486968; 02V0002G3, DQ096805; AvRV2, JQ085406; Ch-1, X98870; RK3, D38099; 993/83, L13765; pheasant-tc/GER/10V0112H5/2010/G23P[37], JX204815; Ty1, X98871; Ty1, D82980; Tu03V0001E10, EU486964;
turkeytc/GER/03V0002E10/2003/G22P[35], JX204826; Tu03V0002E10, EU486965; TY-3, D82981; genomiccloneTY3, X98872; Ch2, EU486970; AvianCH2, EF687020; ARO, D16329; RotavirusC, M88768; Toyama, AB738416; Y122, AB740143; Y121, AB740140; Y084, AB533512; Y083, AB533511; Y082, AB533510; Y081, AB533509; Y091, AB533513; Icheon, GU199224; V508, AY795898; RVC/Pigwt/USA/RV0143/2011, KC164677;
RVC/Pigwt/USA/RV0104/2011, KC164674; WD534tc, AF162434; 06-144-2, FJ494691;

chicken-wt/DEU/06V0064/2006, JN034680; chicken-wt/BGD/BS7/2010, JN034683; chicken-wt/BGD/MJ5/2010, JN034685; chicken-wt/BGD/HS58/2010, JN034684; chicken-wt/GBR/06V0274/2006, JN034681; chicken-wt/DEU/06V0047/2006, JN034679; chicken/03V0568/DEU/2003, NC021635; chicken/03V0567/DEU/2003, HQ403604; RUBV282, GQ358715; DB176, GQ358713; RUBV226, GQ358714; Nemuro, AB106542; NIV-005626, JQ904201; Bang544, FJ851392; 10913, JQ904209; NIV-005623, JQ904200; NIV-0948756, JQ904207; NIV-04623, JQ904202; IDH-084, GU377228; 9222, JQ904208; 11037, JQ904210; NIV-957971; 1995; JQ904199; NIV-04624, JQ904203; NIV-0632252; 2006; JQ904204; NIV-1048101, JQ904211; NIV-005625, JN009779; NIV-094456, JN009777; NIV-04622, JN009778; Bang373, AY238389; Bang117, GU391305; WH-1, AY539858; NIV-076222, JQ904205; CAL-1, AB037931; MMR-B1, FJ811827; IC-008, GU377217; NIV-0924341, JQ904206; ADRV, M55982; IDIR, M84456; 9222, JQ904221.

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Technical Appendix Table. Distribution of RHV-positive samples

Strain name	Collection date	State	Age, d	RVA result	RVB result	RVC result	GenBank accession no.
RVH/Pig-wt/USA/NC7.64–2/2008/GXP[X]	3/27/2008	North Carolina	7	+	+	+	KF757279
RVH/Pig-wt/USA/MN29.9–2/2006/GXP[X]	11/7/2006	Minnesota	14	+	–	–	KF757260
RVH/Pig-wt/USA/NC7.64–3/2008/GXP[X]	3/27/2008	North Carolina	15	+	+	+	KF757280
RVH/Pig-wt/USA/IA5.39–4/2008/GXP[X]	3/3/2008	Iowa	20	+	+	–	KF757271
RVH/Pig-wt/USA/NC5.61–7/2008/GXP[X]	3/5/2008	North Carolina	20	+	+	+	KF757272
RVH/Pig-wt/USA/IL4.46/2008/GXP[X]	2/20/2008	Illinois	21	+	+	+	KF757268
RVH/Pig-wt/USA/MN32.15/2007/GXP[X]	11/28/2007	Minnesota	28	+	+	–	KF757264
RVH/Pig-wt/USA/MN8.63/2008/GXP[X]	4/7/2008	Minnesota	28	+	+	+	KF757283
RVH/Pig-wt/USA/KS9.5–3/2008/GXP[X]	4/15/2008	Kansas	35	+	+	–	KF757285
RVH/Pig-wt/USA/OK5.68–9/2008/GXP[X]	3/6/2008	Oklahoma	35	+	+	+	KF757274
RVH/Pig-wt/USA/AR7.10–1/2012/GXP[X]	4/6/2012	Arkansas	42	+	+	+	KF757289
RVH/Pig-wt/USA/OK5.68–10/2008/GXP[X]	3/6/2008	Oklahoma	42	+	+	+	KF757275
RVH/Pig-wt/USA/OK7.93–3/2008/GXP[X]	4/1/2008	Oklahoma	42	+	+	+	KF757281
RVH/Pig-wt/USA/TX6.85–3/2008/GXP[X]	3/19/2008	Texas	42	+	+	+	KF757278
RVH/Pig-wt/USA/MO33.5–1/2006/GXP[X]	12/13/2006	Missouri	49	–	+	–	KF757262
RVH/Pig-wt/USA/MN32.79–5/2007/GXP[X]	12/5/2007	Minnesota	21–28	+	+	+	KF757265
RVH/Pig-wt/USA/IA5.5–3/2009/GXP[X]	3/5/2009	Iowa	21–39	+	+	+	KF757287
RVH/Pig-wt/USA/KS6.15–8/2008/GXP[X]	3/12/2008	Kansas	21–42	+	+	+	KF757277
RVH/Pig-wt/USA/NC5.71–3/2008/GXP[X]	3/6/2008	North Carolina	21–42	+	+	–	KF757276
RVH/Pig-wt/USA/MN4.87–5/2008/GXP[X]	2/26/2008	Minnesota	21–55	+	+	+	KF757270
RVH/Pig-wt/USA/NC9.38/2008/GXP[X]	4/14/2008	North Carolina	21–55	+	+	+	KF757284
RVH/Pig-wt/USA/IA5.65–5/2008/GXP[X]	3/5/2008	Iowa	22–42	+	+	+	KF757273
RVH/Pig-wt/USA/MN4.38–3/2008/GXP[X]	2/19/2008	Minnesota	35–49	+	+	+	KF757267
RVH/Pig-wt/USA/MN4.53–6/2008/GXP[X]	2/21/2008	Minnesota	35–55	+	+	+	KF757269
RVH/Pig-wt/USA/FL29.88–2/2006/GXP[X]	11/7/2006	Florida	42–55	+	+	–	KF757261
RVH/Pig-wt/USA/IA33.11/2007/GXP[X]	12/10/2007	Iowa	60	+	+	+	KF757266
RVH/Pig-wt/USA/MN9.65–4/2008/GXP[X]	4/16/2008	Minnesota	63	+	+	+	KF757286
RVH/Pig-wt/USA/AR7.32–4/2009/GXP[X]	3/26/2009	Arkansas	70	+	+	+	KF757288
RVH/Pig-wt/USA/OK8.1–8/2008/GXP[X]	4/3/2008	Oklahoma	84	–	+	+	KF757282
RVH/Pig-wt/USA/MN1.82–12/2007/GXP[X]	1/22/2007	Minnesota	56–112	+	–	+	KF757263

*RHV, rotavirus H; +, positive; –, negative.