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Astrovirus in White-Tailed Deer, United States, 2018

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We report the identification of astrovirus WI65268 in a white-tailed deer with respiratory disease in the United States in 2018. This virus is a recombinant of Kagoshima1-7 and Kagoshima2-3-2 (both bovine astroviruses from Japan) and was characterized as a potential new genotype. Further surveillance of deer might help identify related isolates.

Astrovirus is a positive-sense, single-stranded RNA virus first identified in feces of children with gastroenteritis in 1975. Since then, astrovirus has been found in a wide variety of mammals and birds (1). The family *Astroviridae* comprises 2 genera, *Mamastrovirus* and *Avastrovirus*, and classification is based on host origin. Astroviruses cause diarrhea and neurologic diseases in mammals and a spectrum of diseases, including diarrhea, hepatitis, and nephritis, in birds (2). Astrovirus is associated with respiratory disease in humans, cattle, and pigs (3–5) and has also been found in fecal samples from roe deer with gastrointestinal illness in Denmark (6). Whether astrovirus circulates in other species of deer remains unclear.

In September 2018, the Veterinary Diagnostic Laboratory at Iowa State University (Ames, Iowa, USA) received 5 sets of tissue samples collected from deer of the same farm for identification of the infectious cause of death of 5 male white-tailed deer 8–14

weeks of age. The pen-raised deer experienced pneumonia and sudden death. Postmortem examinations showed pleural fluid in the lungs, pneumonia, and purple-mottled lungs. Histopathologic observations revealed that 3 deer had necrotizing bronchopneumonia, and 2 had interstitial pneumonia.

Although different combinations of the bacterial pathogens *Bibersteinia trehalosi*, *Tureperella pyogenes*, *Fusobacterium necrophorum*, and *Pasteurella multocida* were found in all cases, an underlying viral cause could not be excluded. Therefore, we used next-generation sequencing, first with pooled lung samples and then with individual lung samples, using Nextera XT DNA Library Preparation Kit with the MiSeq platform and MiSeq Reagent Kit v2 (Illumina, <https://www.illumina.com>). A bioinformatic analysis indicated the presence of an astrovirus along with the bacteria. The complete genome sequence (6,246 nt) of this astrovirus (WI65268; GenBank accession no. MN087316) was found in the pooled lung tissue sample and 1 lung tissue sample, and partial genomes were found in the other 4 lung samples. A complete-genome comparison revealed that BoAstV/JPN/Ishikawa24-6/2013 (bovine isolate from Japan) had the highest identity (60.9%) to WI65268. Further nucleotide sequence analysis revealed that WI65268 had a similar genome organization as other astroviruses (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/26/2/19-0878-App1.pdf>).

Sequence comparisons of the amino acid sequences of the 3 open reading frames (ORFs) showed that WI65268 was closely related to 4 bovine astroviruses from Asia: B18 (ORF1a 71.9% sequence identity), Kagoshima1-7 and B76-2 (ORF1b 87.8% sequence identity), and Hokkaido11-55 (ORF2 46.8% sequence identity, distance value 0.479) (Appendix Table). In contrast, WI65268 showed low amino acid sequence identities to US bovine strain BSRI-1 for all 3 ORFs (ORF1a 37.0%, ORF1b 68.3%, ORF2 38.8%) (Appendix Table). The 2 available astrovirus sequences from roe deer (GenBank accession nos. HM447045 and HM447046) from Europe comprised only partial genomic sequences. WI65268 had low identities (34.0% HM447045 and 34.4% HM447046) and pairwise distances (0.787 HM447045 and 0.813 HM447046) to these isolates. On the basis of the International Committee on Taxonomy of Viruses p-distance criteria (new genotypes are assigned at a value of ≥ 0.378) (7), WI65268 represents a novel astrovirus genotype.

Phylogenetic analysis of the complete genome showed that WI65268 is distantly related to other bovine, dromedary, takin, and yak strains (Appendix Figure 2). In phylogenetic analyses of ORF1a and ORF1b protein sequences, WI65268 clustered with

bovine, yak, and takin astrovirus isolates from Asia (Appendix Figure 3). However, in an analysis of ORF2 (capsid) protein, WI65268 clustered with 2 bovine isolates from Japan and was distantly related to the cluster formed by the bovine, yak, and takin isolates from Asia (Figure), strongly indicating that WI65268

is a recombinant. We used Recombination Detection Program 5 (<http://web.cbio.uct.ac.za/~darren/rdp.html>) to confirm that WI65268 was a recombinant and characterize the recombination event (Kagoshima1-7 at 1-5,031 and 5,651-7,967 and Kagoshima2-3-2 at 5,032-5,650; Appendix Figure 4). Reverse transcription

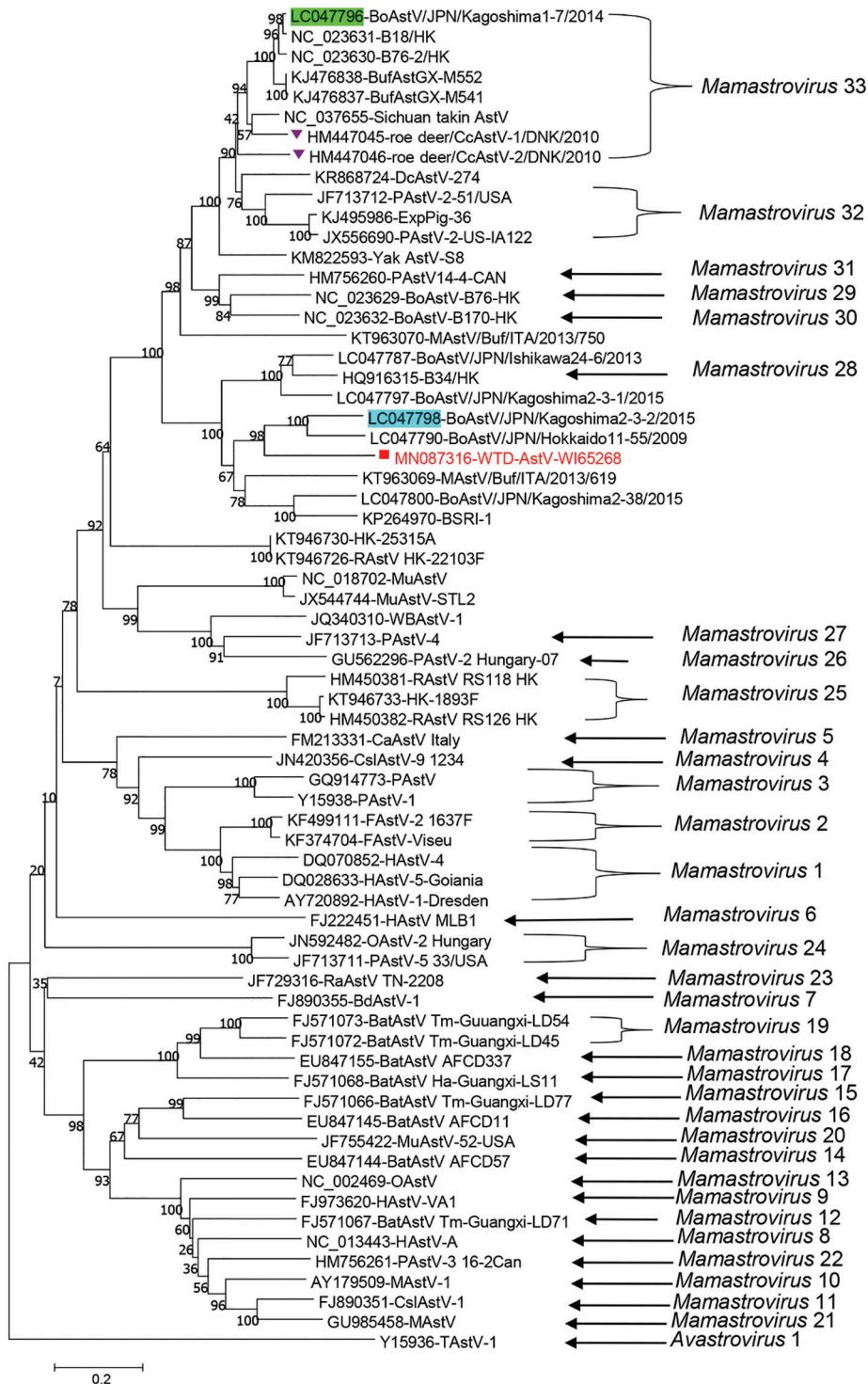


Figure. Phylogenetic analysis of amino acid sequence of open reading frame 2 of WTD-AstV WI65268 from deer in the United States, 2018 (red square), and potential parent viruses, including Kagoshima1-7 (green highlight), Kagoshima2-3-2 (blue highlight), and CcAstVs (purple triangles). Genus type is provided for viruses where that information was known. GenBank accession numbers are indicated, and bootstrap values are provided at nodes. Scale bar indicates amino acid changes per site. AstV, astrovirus; BdAstV, bottlenose dolphin astrovirus; BoAstV, bovine astrovirus; BufAst, water buffalo astrovirus; CaAstV, canine astrovirus; CcAstV, *Capreolus capreolus* astrovirus; CslAstV, California sea lion astrovirus; DcAstV, dromedary camel astrovirus; FAstV, feline astrovirus; HAstV, human astrovirus; MAstV, mink astrovirus; MuAstV, murine astrovirus; OAstV, ovine astrovirus; PAstV, porcine astrovirus; RAstV, rat astrovirus; RaAstV, rabbit astrovirus; TAstV, turkey astrovirus; WBAstV, wild boar astrovirus; WTD, white-tailed deer.

PCR and sequencing results confirmed that sequences at the 2 junctional sites were the same as those found by next-generation sequencing.

Pathogens causing respiratory disease in domesticated animals, such as cattle and pigs, are relatively well studied. However, pathogens causing these diseases in wildlife animals, such as deer, are not well characterized. In this study, the new astrovirus we found or the bacterial pathogens could have contributed to the respiratory disease observed. Whether astrovirus plays a major or just synergistic role in respiratory disease in deer should be explored further.

Astrovirus was previously identified in roe deer with gastrointestinal illness in Europe and found to be closely related to bovine astrovirus isolates from Hong Kong, China, of the same genus (*Mamastrovirus* 33) (7,8). WI65268 was also closely related to bovine isolates from Japan but distantly related to roe deer and Hong Kong bovine astrovirus isolates. An additional analysis of genetic distances of related isolates on the basis of ORF2 tentatively classified WI65268 as a novel species (Appendix Table).

Determining the evolution of WI65268 any further is difficult without further epidemiologic data. Bovine or bovid astroviruses might be able to cross species barriers and replicate in deer, as suggested in a previous study (9), in which a bovine astrovirus isolate clustered with a porcine astrovirus type 5 instead of other bovine astroviruses. Further surveillance of white-tailed deer for astrovirus is needed for field monitoring.

About the Author

Dr. Wang is a clinical assistant professor in the College of Veterinary Medicine at the University of Illinois, Urbana, Illinois, USA. His research interests focus on diagnosis of viral infectious diseases and novel pathogen discovery.

References

1. Bosch A, Pintó RM, Guix S. Human astroviruses. *Clin Microbiol Rev.* 2014;27:1048-74. <https://doi.org/10.1128/CMR.00013-14>
2. Donato C, Vijaykrishna D. The broad host range and genetic diversity of mammalian and avian astroviruses. *Viruses.* 2017;9:102. <https://doi.org/10.3390/v9050102>
3. Ng TF, Kondov NO, Deng X, Van Eenennaam A, Neiberghs HL, Delwart E. A metagenomics and case-control study to identify viruses associated with bovine respiratory disease. *J Virol.* 2015;89:5340-9. <https://doi.org/10.1128/JVI.00064-15>
4. Padmanabhan A, Hause BM. Detection and characterization of a novel genotype of porcine astrovirus 4 from nasal swabs from pigs with acute respiratory disease. *Arch Virol.* 2016;161:2575-9. <https://doi.org/10.1007/s00705-016-2937-1>
5. Cordey S, Brito F, Vu DL, Turin L, Kilowoko M, Kyungu E, et al. Astrovirus VA1 identified by next-generation sequencing in a nasopharyngeal specimen of a febrile Tanzanian child with acute respiratory disease of unknown etiology. *Emerg Microbes Infect.* 2016;5:e99. <https://doi.org/10.1038/emi.2016.98>
6. Smits SL, van Leeuwen M, Kuiken T, Hammer AS, Simon JH, Osterhaus AD. Identification and characterization of deer astroviruses. *J Gen Virol.* 2010;91:2719-22. <https://doi.org/10.1099/vir.0.024067-0>
7. To KKW, Chan WM, Li KSM, Lam CSF, Chen Z, Tse H, et al. High prevalence of four novel astrovirus genotype species identified from rodents in China. *J Gen Virol.* 2017;98:1004-15. <https://doi.org/10.1099/jgv.0.000766>
8. Tse H, Chan WM, Tsoi HW, Fan RY, Lau CC, Lau SK, et al. Rediscovery and genomic characterization of bovine astroviruses. *J Gen Virol.* 2011;92:1888-98. <https://doi.org/10.1099/vir.0.030817-0>
9. Nagai M, Omatsu T, Aoki H, Otomaru K, Uto T, Koizumi M, et al. Full genome analysis of bovine astrovirus from fecal samples of cattle in Japan: identification of possible interspecies transmission of bovine astrovirus. *Arch Virol.* 2015;160:2491-501. <https://doi.org/10.1007/s00705-015-2543-7>

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Actinomycetoma Caused by *Actinomyces mexicanus*, a Neglected Entity in the Caribbean

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Appendix

Targeted Amplification

PCR products of 5' end 597 bp and 3' end 576 bp were amplified using primers 5' end forward primer TAGTTGAGATTGAGCGTAATAAATA and reverse primer CTCAAGCCATATGTAAAGCTTAAGCA and 3' end forward primer GTCGCACAGTTTATGTGTGTTG and reverse primer CCCTTCACCTATGCAATCAAATCACAA, respectively, and sequenced on MiSeq. The sequence analysis showed that both ends of sequences are exactly same as the original complete sequence obtained by metagenomic sequencing. The junction sites between open reading frame (ORF) 1a and ORF1b as well as between ORF1b and ORF2 were amplified using primer sets DeerAstV-ORF1ab forward and reverse primers CAATATTTAGACCGGGACTATGATGC and CAAAAGCGGGATGGCTCGGCA, respectively, and DeerAstV-ORF12 forward and reverse primers GTGGCTTTACAGTTGGGAACAAC and TATTTGACGCTGAGACGGAGCAA, respectively. The reverse transcription PCR and sequencing results revealed that the sequences at the 2 junctional sites (ORF1a-1b, ORF1-2) were same as those from next-generation sequencing, which confirmed that there was only one strain in the sample instead of 2 distinct strains.

Detailed Genomic Information of WTD-AstV-WI65268

WTD-AstV-WI65268 has three complete overlapping ORFs: ORF1a at position 25–2478 nt encoding 817aa, ORF1b at position 2433–3941 nt encoding 502aa, and ORF2 at position 3886–6168 encoding 760aa. WTD-AstV-WI65268 had the conserved 'slipper heptamer' AAAAAAC sequence near the 3' end of the ORF1a for inducing ribosomal frameshift during polyprotein ns1ab translation. The highly conserved promoter sequence for subgenomic RNA

synthesis among mammalian AstVs UUUGGAGNGGNGGACCNAAN11AUGNC was present at the start of ORF2 in the WTD-AstV-WI65268.

Recombination in Astrovirus (AstV)

In addition to genomic mutation, recombination has been reported in different viral families. Recombination in ORF2 of bovine AstV was previously reported (1). WTD AstV was found to a recombinant between 2 Japan bovine strains with the recombination junction in ORF2. Recombination in ORF2 will allow generation of divergent viral progeny to enhance viral immune evasion function.

Reference

1. Tse H, Chan WM, Tsoi HW, Fan RY, Lau CC, Lau SK, et al. Rediscovery and genomic characterization of bovine astroviruses. *J Gen Virol.* 2011;92:1888–98. [PubMed](https://doi.org/10.1099/vir.0.030817-0)
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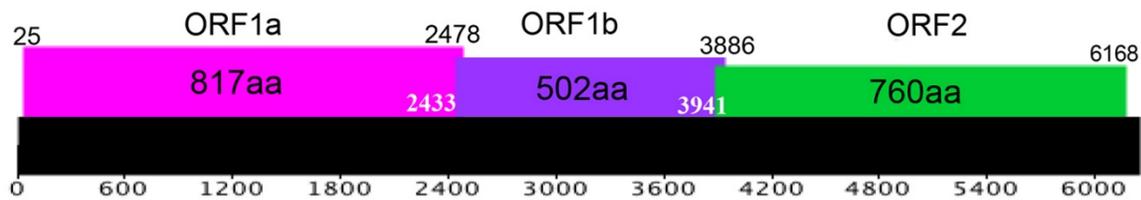
Appendix Table. Comparison of amino acid sequence identity of ORF2, ORF1a, ORF1b and Pairwise Distance of WTD-AstV-WI65268 to other strains in GenBank*

Astrovirus strain information	ORF2	ORF1a	ORF1b	Distance†
LC047790-BoAstV/JPN/Hokkaido11–55/2009	46.8	39.9	68.7	0.479
LC047798-BoAstV/JPN/Kagoshima2–3-2/2015	46.2	40.8	68.5	0.487
LC047787-BoAstV/JPN/Ishikawa24–6/2013	44.8	70.7	87.2	0.582
LC047797-BoAstV/JPN/Kagoshima2–3-1/2015	44.5	70.8	87.6	0.595
HQ916315-B34/HK	42.0	-	87.4	0.658
KT963069-MAstV/Buf/ITA/2013/619	40.2	-	-	0.644
LC047800-BoAstV/JPN/Kagoshima2–38/2015	38.8	39.6	68.3	0.658
KP264970-BSRI-1	38.8	37.0	68.3	0.626
NC_023629-BAstV-B76-HK	36.8	67.6	84.6	0.776
KJ476837-BufAstGX-M541	36.5	-	-	0.813
LC047796-BoAstV/JPN/Kagoshima1–7/2014	36.4	71.6	87.8	0.803
KJ476838-BufAstGX-M552	36.2	-	-	0.813
NC_023631-BAstV-B18 HK	36.0	71.9	87.2	0.803
NC_023630-B76–2/HK	35.8	70.7	87.8	0.808
NC_023632-BAstV-B170-HK	35.8	67.4	84.4	0.792
KM822593-YakAstV-S8	35.4	71.0	87.4	0.803
HM447046-deer/CcAstV-2/DNK/2010	34.4	-	-	0.813
HM447045-deer/CcAstV-1/DNK/2010	34.0	-	-	0.787
KR868724-DcAstV-274	33.4	64.5	82.6	0.776
JX556690-PAstV-2-U.S.-IA122	32.8	65.5	82.3	0.852
JF713712-PAstV-2–51/USA	32.3	66.8	81.8	0.835
KJ495986-ExpPig-36	31.3	66.7	80.5	0.835
HM756260-PAstV14–4-CAN	31.2	-	-	0.881
KT963070-MAstV/Buf/ITA/2013/750	30.4	-	-	0.922
KT946730-HK-25315A	24.5	30.4	58.6	0.978
KT946726-RAstV HK-22103F	24.5	8.6	58.4	0.978
JF713713-PAstV-4	23.9	29.4	58.0	0.922
NC_018702-MuAstV	23.2	27.2	57.1	0.991
JX544744-MuAstV-STL2	23.2	26.4	56.9	0.985
JQ340310-WBAstV-1	22.3	27.9	54.9	0.991
GU562296-PAstV-2 Hungary-07	21.3	-	-	0.991
KT946733-HK-1893F	21.2	25.5	57.5	1.052
HM450382-RAstV RS126 HK	21.0	-	57.5	1.052
AY720892-HAstV-1-Dresden	20.9	18.2	57.8	1.087

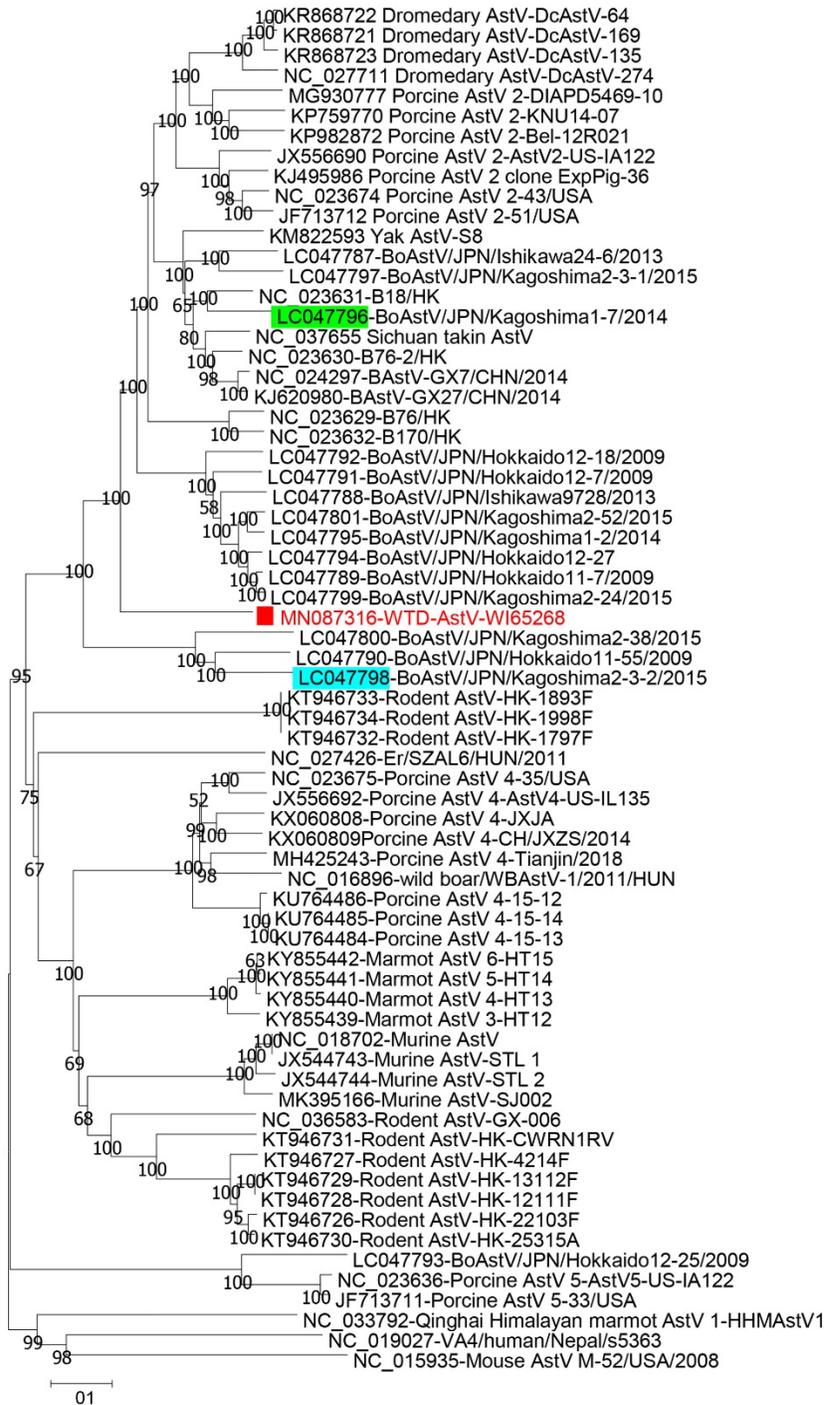
Astrovirus strain information	ORF2	ORF1a	ORF1b	Distance†
HM450381-RAstV RS118 HK	20.8	-	56.9	1.080
JF713711-PAstV-5 33/USA	20.6	18.3	46.4	1.184
DQ028633-HAstV-5-Goiania	20.3	18.8	56.0	1.094
JN592482-OAstV-2 Hungary	20.2	-	-	1.208
KF374704-FAstV-Viseu	20.1	19.4	56.1	1.072
KF499111-FAstV-2 1637F	20.0	18.9	55.2	1.087
DQ070852-HAstV-4	19.7	18.6	57.6	1.101
FM213331-CaAstV Italy	19.5	-	-	1.101
FJ890355-BdAstV-1	19.4	-	-	1.116
JN420356-CsIAstV-9 1234	19.2	19.5	53.5	1.130
Y15938-PAstV-1	18.6	-	-	1.200
GQ914773-PAstV	17.7	-	-	1.249
JF729316-RaAstV TN-2208	17.6	18.4	55.1	1.161
FJ222451-HAstV MLB1	16.9	20.7	51.1	1.301
FJ571068-BatAstV Ha-Guangxi-LS11	16.7	-	-	1.249
EU847144-BatAstV AFCD57	16.7	-	-	1.249
FJ571066-BatAstV Tm-Guangxi-LD77	16.4	-	-	1.292
FJ571072-BatAstV Tm-Guangxi-LD45	16.3	-	-	1.208
FJ973620-HAstV-VA1	15.9	20.0	48.2	1.310
FJ571073-BatAstV Tm-Guangxi-LD54	15.4	-	-	1.249
FJ890351-CsIAstV-1	15.3	-	-	1.328
NC_013443-HMOAstV-A	15.0	19.4	48.1	1.346
FJ571067-BatAstV Tm-Guangxi-LD71	14.9	-	-	1.365
EU847155-BatAstV AFCD337	14.9	-	-	1.283
AY179509-MAstV-1	14.9	18.6	50.8	1.337
NC_002469-OAstV	14.5	19.5	48.9	1.374
JF755422-MouseAstV-52-USA	14.5	15.5	47.6	1.384
HM756261-PoAstV-3_16-2Can	14.4	-	-	1.346
EU847145-BatAstV AFCD11	14.3	-	-	1.337
GU985458-SMS-AstV	13.9	-	-	1.346
Y15936-TAstV-1	12.8	11.3	36.4	1.540

*ORF, open reading frame.

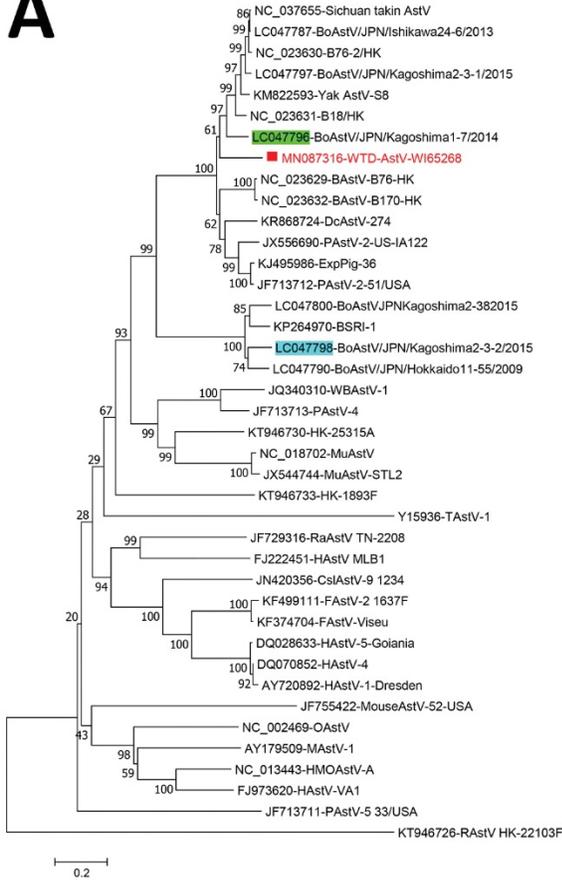
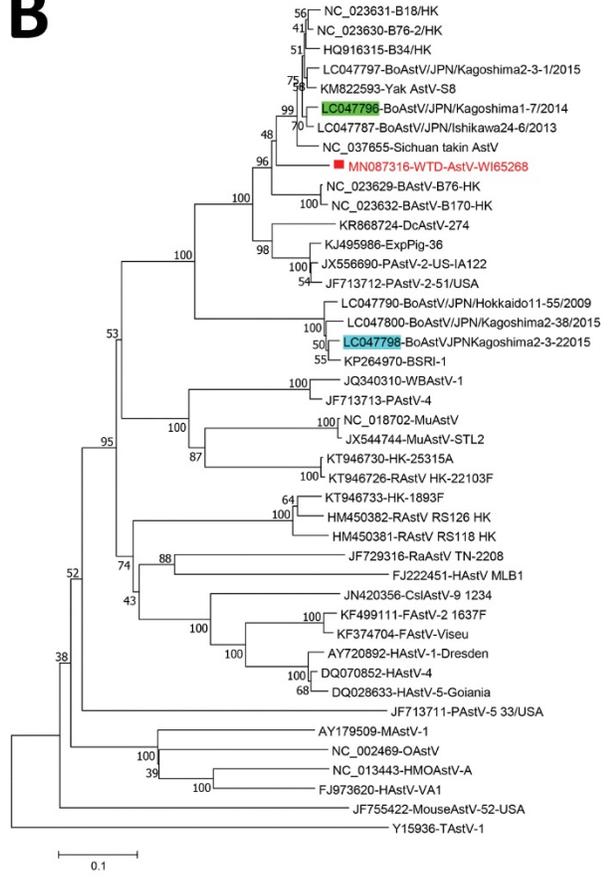
†Pairwise distance was calculated using the MEGA version 7.0.26



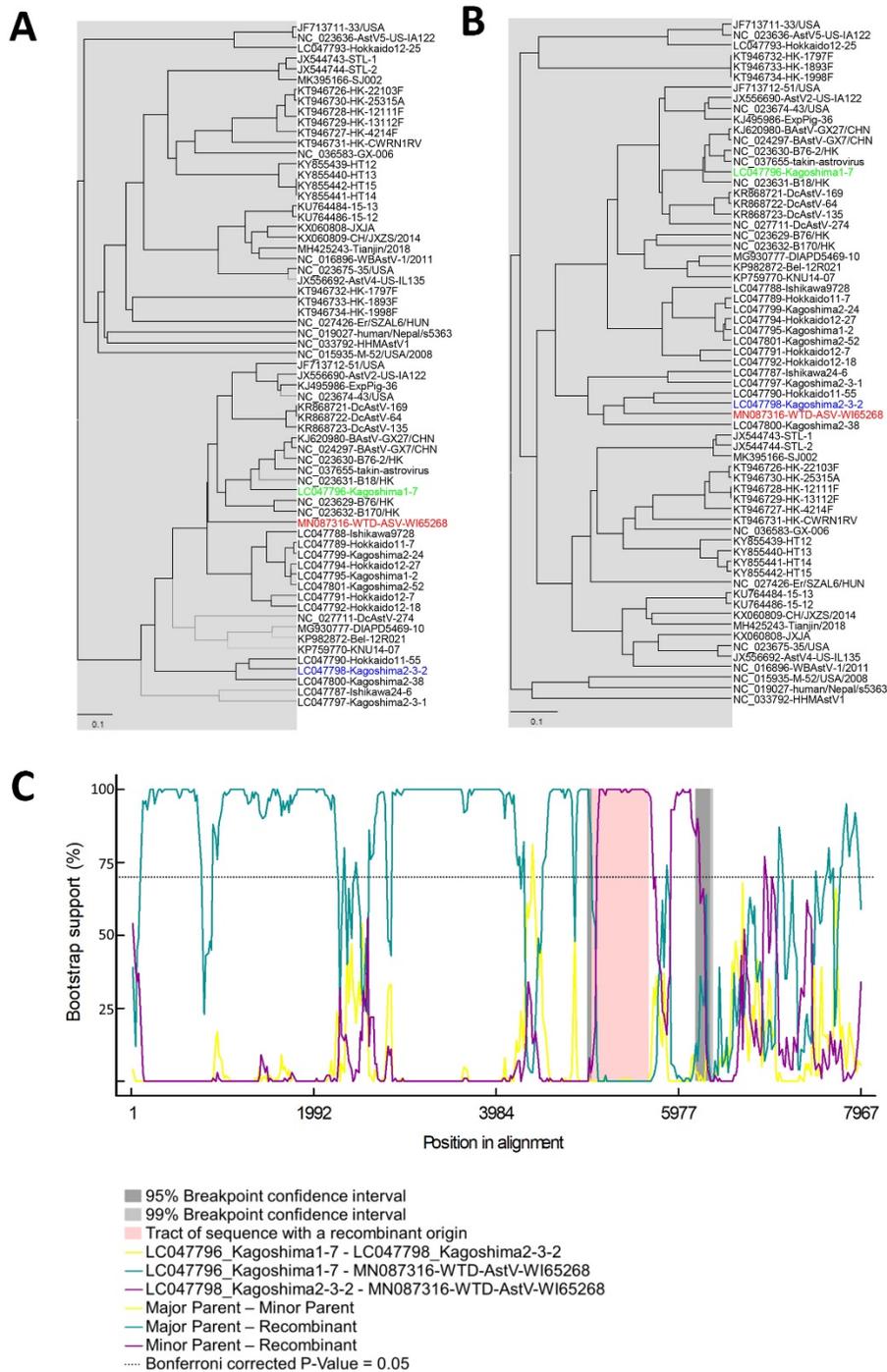
Appendix Figure 1. Schematic diagram of WTD-AstV-WI65268 genome. ORF, open reading frame.



Appendix Figure 2. Phylogenetic tree analysis of complete genome sequences of astrovirus including WTD-AstV-WI65268 (indicated with a red square) and its potential parent virus strains Kagoshima1–7 and Kagoshima2–3-2 were marked with green and turquoise colors. The sequences acquired from GenBank were labeled with their accession numbers. Bootstrap values are indicated at nodes. Scale bar indicates 0.1 nucleotide changes per site.

A**B**

Appendix Figure 3. Phylogenetic analyses of amino acid sequences of open reading frame 1a (A) and 1b (B) of astrovirus WI65268, United States, 2018 (red square), and potential parent viruses, including Kagoshima1-7 (green highlight) and Kagoshima2-3-2 (turquoise highlight). GenBank accession numbers of sequences are provided. Scale bar indicates 0.2 and 0.1 amino acid changes per residue site in panel A and B, respectively.



Appendix Figure 4. Recombination analysis of the nucleotide sequence of WTD-AstV-WI65268 using RDP v.5 software. A and B) Two trees of recombinant WTD-AstV-WI65268. Red, green, and blue color shade are used to label the recombinant, major, and minor parent strains on each tree, respectively. C) Bootscan plot. Turquoise blue lines are Major Parent-Recombinant; purple lines are Minor Parent-Recombinant, yellow lines are Major Parent-Minor Parent.