

Supporting Information: AEM-00563-15
Wastewater analysis indicates that genetically diverse astroviruses, including strains belonging to novel clades MLB and VA, are circulating within Japanese populations.

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Running title: Detection of astroviruses in wastewater

SUPPORTING FIGURES

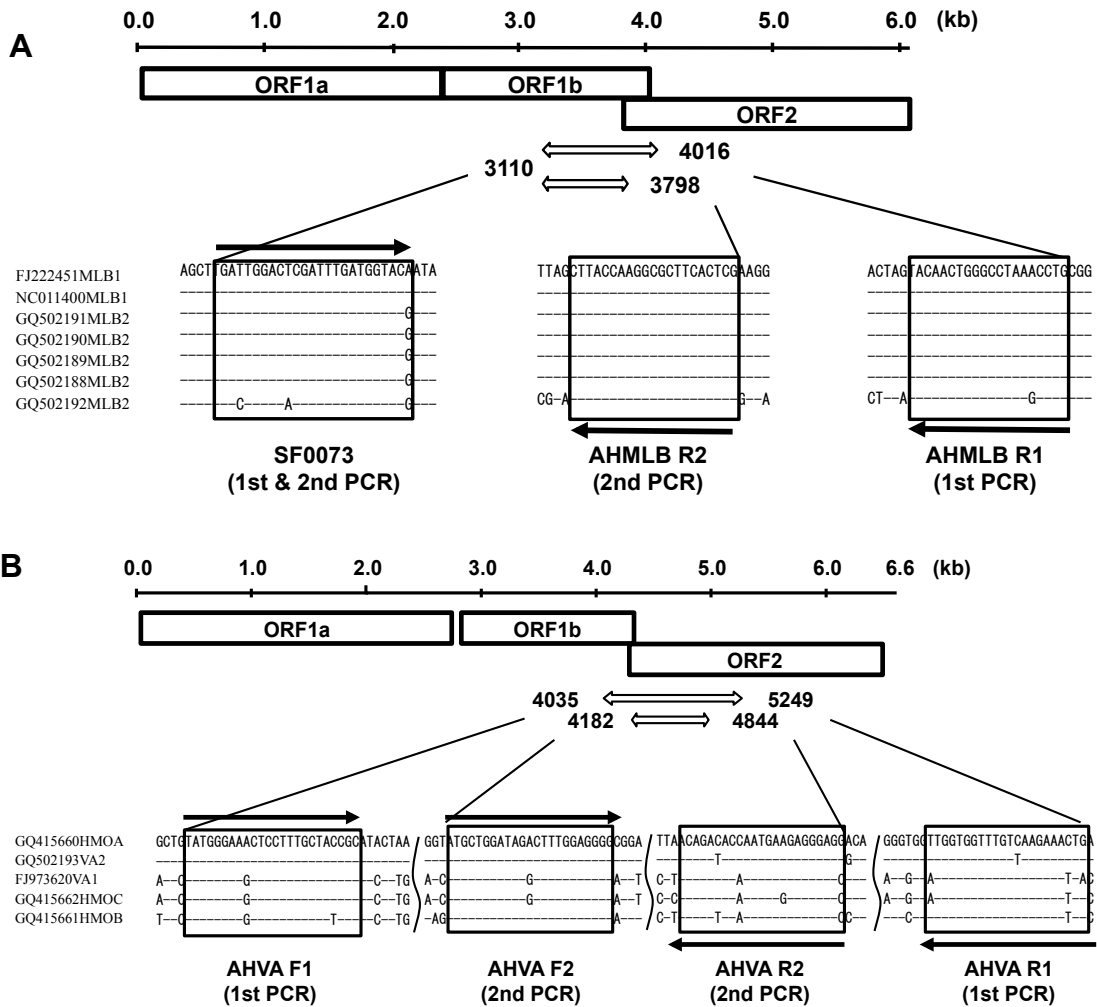


Fig. S1 Alignment of nucleotide sequences of partial ORF1b–ORF2 junction region of MLB- and VA(HMO)-AstV genotypes (Fig. S1 A and B, respectively). Primer binding sites (SF0073, AHMLB R1, and AHMLB R2 for MLB-AstVs and AHVA F1, AHVA F2, AHVA R1, and AHVA R2 for VA- AstVs) are indicated with arrows. Numbers indicate nucleotide positions corresponding to MLB-AstV-1 and VA-AstV-1 (GenBank accession number: FJ222451 and FJ973620, respectively).

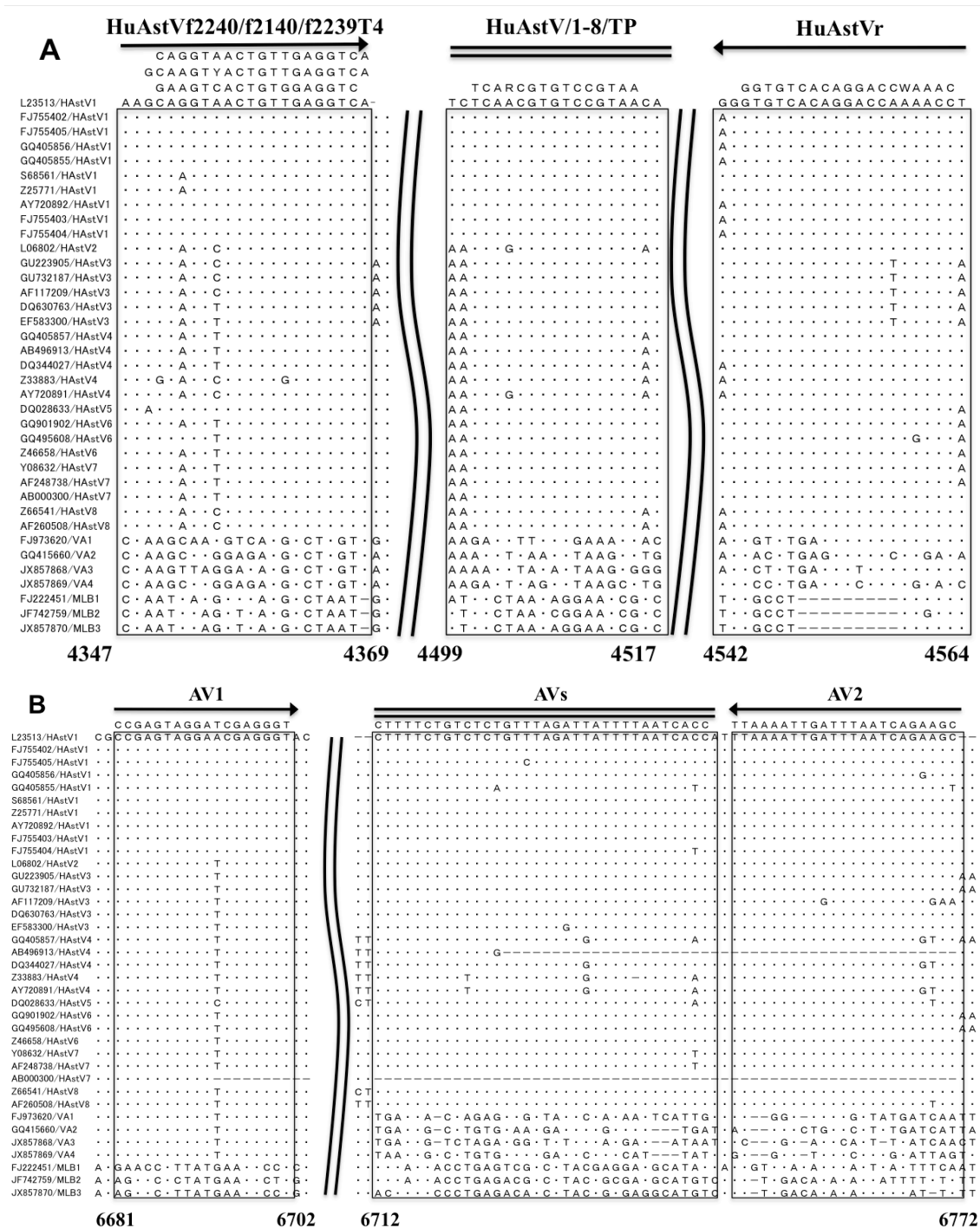


FIG. S2. Primers and TaqMan probe designed by Yokoi and Kitahashi (1) (A) and Le Cann et al. (2) (B) targeting the 5'- end and the 3'-end of ORF 2 region, respectively. Alignment of HAstV nucleotide sequences including complete ORF 2 region in GenBank database are selected and shown. Numbers shown at the bottom of the figures indicate nucleotide positions corresponding to HAstV-1 Oxford strain (GenBank accession number: L23513).

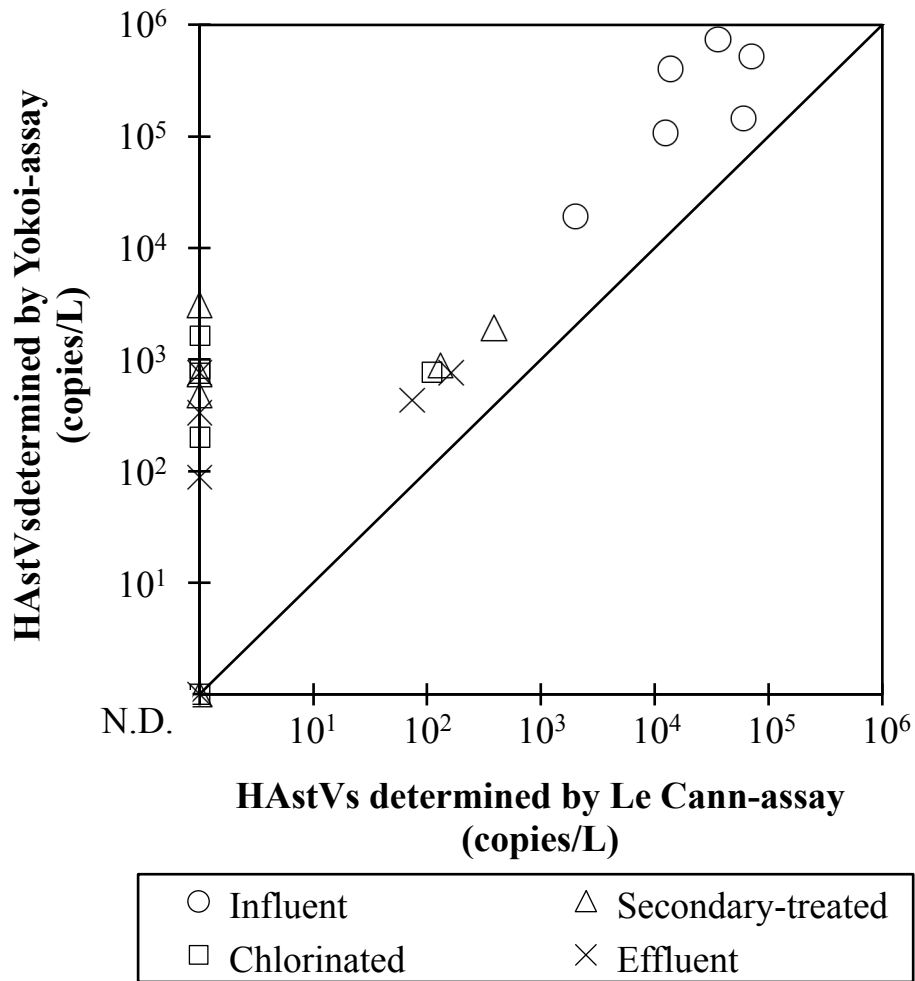


Fig. S3 Comparison of HAstVs concentrations in wastewater samples determined by RT-qPCR assays targeting the 5'-end of ORF2 (Yokoi-assay: Yokoi and Kitahashi, 2009 (1)) to the 3'-end of ORF2 (Le Cann-assay: Le Cann et al., 2002 (2)). "N.D." on x and y axes indicates "not detected".

SUPPORTING TABLE

TABLE S1 Description of the WWTP investigated.

Service population (person)	781,000
Treatment capacity (m ³ /day)	450,000
Hydraulic retention time (h)	9.3
Biochemical oxygen demand in the influent (mg/L)	220
Biochemical oxygen demand in the effluent (mg/L)	1

TABLE S2 Recovery efficiency of spiked MNV by RT-qPCR (%).

Month (2007-2008)	Influent	Secondary-treated	Chlorinated	Effluent
October	102	62	65	100
December	130	52	54	137
November	127	59	46	85
January	110	60	30	49
February	119	49	64	57
March	99	87	52	71

REFERENCES

1. **Yokoi, H., and T. Kitahashi.** 2009. Astrovirus RNA Detection Using Real-Time Reverse Transcription-Polymerase Chain Reaction. *J. Jpn. Assoc. Inf. Dis.* **83(2)**:120-126. [in Japanese]
2. **Le Cann, P., S. Ranarijaona, S. Monpoeho, F. Le Guyader, and V. Ferré.** 2004. Quantification of human astroviruses in sewage using real-time RT-PCR. *Res Microbiol.* **155(1)**:11-15.