1	Supporting Information
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3	Respiratory human adenovirus outbreak captured in wastewater surveillance
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HAdV-4 in Influent and Primary Solids

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Figure S1. Comparison of HAdV-4 in Influent and Primary Solids. Influent samples shown in blue
circles and primary solids samples shown in red circles. Open circles indicate the targets were
below the limit of detection (LOD) and are plotted at the value below the LOD for that assay. The
y-axis values are reported as gene copies per gram dry weight or gene copies per milliliter and are

48 log₁₀-scaled for convenience.



Figure S2. ddPCR Inhibition Control. 183 primary solids samples were diluted 1:40 and measured for adenovirus target HAdV-40/41. Diluted samples were compared to undiluted samples and the ratio of the estimated gene copies from the dilution divided by the actual gene copies in the undiluted sample is plotted. Ratios >1 represent inhibition from the sample disrupting the assay while ratios <1 represent little to no inhibition from the samples. The geometric mean for all values is plotted as a horizontal black bar at 1.108.

57 Influent Sample Collection and Processing

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59 24-hour time-weighted composite influent samples were collected daily at a treatment plant in 60 Southeast Michigan in 50 mL conical tubes and stored at 4°C and transported 2x weekly to the 61 University of Michigan campus. Samples were pasteurized at 56°C for 30 minutes. NaCl and PEG 62 were added to reach final concentrations of 0.2 M NaCl and 8% PEG and mixed by inversion until 63 all PEG was dissolved. Following PEG addition, samples were refrigerated at 4°C for 4+ hours or 64 overnight. Following refrigeration, the precipitated samples were centrifuged at 4,700xg for 45 minutes at 4°C. A majority of the supernatant was removed and discarded, while the remaining 65 pellet was resuspended with 1-5 mL supernatant kept in the bottom of each sample. The final 66 67 sample volume was recorded and gene targets are reported as gene copies per mL. Concentrated influent samples were extracted similarly to settled solids samples as described in nucleic acid 68 69 extraction methods and gene targets were amplified with ddPCR and RT-ddPCR.

- *Table S1*. Sequence of gBlock standards, gene location, and GenBank accession numbers for
- 71 adenovirus targets

Assay	gBlock sequence	Gene location	GenBank accession no.
HAdV-4	5' - TCC TTT TGC CAT GGA AAT CAA CAT CCA AGC CAA CCT GTG GAG GAA CTT CCT CTA TGC CAA TGT TGC CCT CTA TTT GCC TGA TAA ATA CAA ATA CAC ACC GGC CAA CAT CAC CCT GCC CAC CAA CAC C - 3'	19,567-19,693	AY594253
HAdV-14	5' - GAT GTG CGT GTT ATT GAA AAT CAT GGT GTG GAA GAT GAA CTT CCC AAC TAC TGT TTT CCA CTG GAC GGC ATC GGT CCG CGA ACA GAT AGT TAC AAG GAG ATT CAG TTA AAT GGA GAC CAA GCT TGG AAA GAT GTA AAT CCA AAT GG - 3'	19,428-19,573	AY803294
HAdV-21	5' - GTA TGG TGG TAG AGC TCT AAA GCC AGA AAC TAA AAT GAA ACC CTG CTA TGG GTC TTT TGC TAA ACC CAC TAA CGT CAA AGG CGG ACA GGC AAA ACA AAA AAC TAC TGA ACA ACC GCA AAA CCA GCA GGT TGA ATA TGA TAT TGA CAT G - 3'	19,107-19,254	AY601633
HAdV-40	5' - CAA GTT CAG AAA CCC CAC CGT GGC TCC CAC CCA CGA TGT AAC CAC AGA CAG GTC GCA GCG ACT GAC GCT GCG CTT CGT GCC CGT CGA CCG CGA GGA AAC CGC CTA CTC TTA CAA AGT GCG CTT TAC GCT GGC CGT GGG CGA CAA CCG G - 3'	17,771-17,918	NC_001454
HAdV-41	5' - CAA GTT CAG AAA TCC CAC TGT GGC TCC GAC CCA CGA TGT AAC CAC AGA CAG GTC ACA GCG ACT GAC GCT GCG ATT CGT GCC AGT CGA CCG CGA GGA CAC CGC TTA TTC TTA CAA AGT GCG CTT TAC GCT GGC CGT GGG CGA CAA CCG G - 3'	17,771-17,918	NC_001454
HAdV-pan	5' - AGA TGG CCA CCC CAT CGA TGA TGC CCC AAT GGG CAT ACA TGC ACA TCG CCG GAC AGG ATG CTT CGG AGT ACC TCA GTC CGG GTC TGG TGC AGT TCG CCC GTG CAA CAG ACA CCT ACT TCA GTA TGG GGA ACA AAT TTA GAA ACC CCA CAG TGG CGC CCA CCC ACG ATG TGA CCA - 3'	18,416-18,589	NC_011203



Figure S3. Comparison of RNA and DNA Supermix. Eleven samples are listed on the x-axis and were analyzed for three adenovirus targets: HAdV-3 (top), HAdV-4 (middle), HAdV-40/41 (bottom). Gene copies per gram dry weight are shown as bars in blue using one-step RT-ddPCR mastermix for RNA targets and in tan using ddPCR mastermix for DNA targets. Error bars represent the standard error of three independent sample replicates extracted and analyzed on the same day. Thermal cycler conditions varied since ddPCR mastermix for DNA targets did not need an initial reverse transcription (RT) step. All other thermal cycler conditions remained unchanged.



Figure S4. Comparison of HAdV-3 and HAdV-40/41 gene copies before and after two freeze/thaw
cycles. Sample IDs are listed on the x-axis and were analyzed for two adenovirus targets: HAdV3 (top) and HAdV-40/41 (bottom). Gene copies per gram dry weight are shown as bars in blue
quantified before freeze/thaw and in purple quantified after two freeze/thaw cycles. Error bars
represent the standard error of three independent sample replicates extracted and analyzed on the
same day.



Figure S5. Correlation between clinical cases and non-normalized HAdV-4, HAdV-4 normalized by PMMoV, and HAdV-4 normalized by crAssphage. The clinical cases as described in the outbreak in Figure 1 were correlated with HAdV-4 in gene copies per gram dry weight (top), HAdV-4 normalized with PMMoV gene copies (bottom, left), and HAdV-4 normalized with crAssphage gene copies (bottom, right). The Spearman's correlation coefficient was calculated and is noted on the plot. All correlation coefficients were significant.

Table S2. Kendall's tau correlation values

	HAdV-4	HAdV-4/PMMoV	HAdV-4/crAssphage
Clinical cases in	$\tau = 0.301,$	$\tau = 0.425,$	$\tau = 0.309,$
outbreak	p = 0.0077	p = 0.0001	p = 0.0061

	3-day middle- centered average HAdV-3 + 4	3-day middle- centered average HAdV-3 + 4/ PMMoV	3-day middle- centered average HAdV-3 + 4/ crAssphage
3-day middle- centered average RPAN incidence rate	au = 0.414, p < 0.0001	au = 0.422, p < 0.0001	au = 0.408, p < 0.0001

	3-day middle-	3-day middle-	3-day middle-
	centered average	centered average	centered average
	HAdV-40/41	HAdV-40/41/	HAdV-40/41/
		PMMoV	crAssphage
3-day middle-	$\tau = 0.185,$	$\tau = 0.195,$	$\tau=0.206,$
centered average	p = 0.0005	p = 0.0002	p = 0.0001
GIPAN incidence rate			



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108 Figure S6. Comparison between wastewater and RPAN/GIPAN data. In panels A and B, blue 109 squares represent human adenovirus targets in wastewater normalized by PMMoV and blue lines 110 represent a 3-day middle-centered average with the area shaded under the curve. Wastewater data 111 is plotted on the left y-axis. Red bar graphs represent clinical data shown as RPAN (respiratory 112 panel) incidence rate and GIPAN (gastrointestinal panel) incidence rate for adenovirus positives 113 based on a student population of 52,000. Clinical data is plotted on the right y-axis. All dates refer 114 to specimen collection dates for wastewater and clinical specimens. In panels C and D, a 115 corresponding correlation is shown for the data in panels A and B. The 3-day middle-centered 116 average normalized human adenovirus targets in gene copies per gram dry weight were correlated 117 with the 3-day middle-centered average incidence rate for the RPAN and GIPAN analyses. The 118 Spearman's correlation coefficient was calculated and is noted on the correlation plot in panels C 119 and D. Both correlation coefficients were significant.