Supporting Information: Particle association and size fraction of molecular viral fecal pollution indicators in wastewater

Justin Greaves[a,b], Devin North[b], Kyle Bibby[b]*

[b]School of Environmental Sustainability, Loyola University Chicago, 6364 N. Sheridan Rd, Chicago, IL 60660

[a]Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, IN 46556, USA

*Corresponding author KBibby@ND.edu; 574-631-1130

Keywords

Microbial source tracking, Transport, Water quality, PMMoV, crAssphage

Methods:

Sampling methods

Wastewater samples from this study were taken from an anonymous conventional activated sludge wastewater treatment plant that served a population of approximately 50,000 people. Primary influent samples were taken from one of the four clarifiers present at the plant (Greaves et al., 2020).

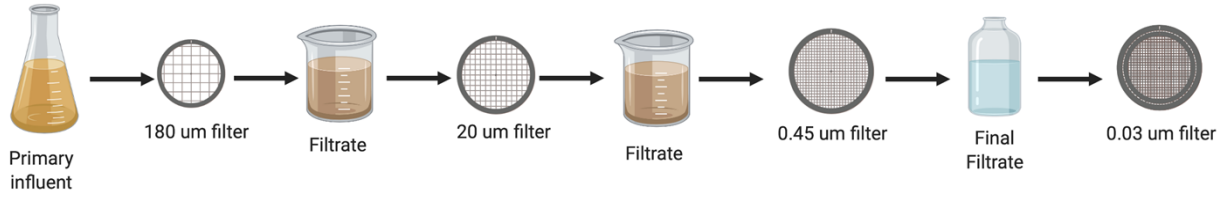

Figure S1: Schematic of procedure used throughout experiment.

Molecular methods

Thermocycler conditions for each assay are described in **Table S1**. Values were generated by allowing program to automatically set threshold. Detection limit for molecular assays was assumed to be 1.70 $\log_{10}$ GC/100mL (Wu et al., 2020).

Results:

Size distribution results

PMMoV had its highest mean concentration on the 0.45 μm filter (40.0%). The second highest, third highest and lowest concentration of PMMoV was on the 20 (36.7%), 180 (14.2%), 0.03 (11.2%) μm filters, respectively. NoV GII had its highest mean concentration on the 0.45 μm filter (58.8%). The second highest, third highest and lowest concentration of NoV GII was on the 20 (20.9%), 180 (11.4%), 0.03 (8.9%) μm

filters, respectively. AdV had its highest mean concentration on the 0.45 μm filter (46.6%). The second highest, third highest and lowest concentration of AdV was on the 20 (39.4%), 180 (10.0%), 0.03 (3.9%) μm filters, respectively. HPyV had its highest mean concentration on the 0.45 μm filter (55.5%). The second highest, third highest and lowest concentration of HPyV was on the 20 (23.6%), 0.03 (11.9%), 180 (9.0%) μm filters, respectively. CrAssphage had its highest mean concentration on the 0.45 μm filter (55.2%). The second highest, third highest and lowest concentration of crAssphage was on the 20 (24.6%), 180 (17.7%), 0.03 (2.4%) μm filters, respectively. HF183 had its highest mean concentration on the 0.45 μm filter (80.5%). The second highest, third highest and lowest concentration of HF183 was on the 180 (15.9%), 20 (3.5%), 0.03 (0.04%) μm filters, respectively.

Settling velocity method calculations

To put our data in context, settling velocities for each particles size (180, 20, 0.45 and 0.03 μm) were calculated using stokes equation below:

$$v_t = \frac{g d^2 (\rho_p - \rho_m)}{18u} \quad (1)$$

Where g is the acceleration of gravity (9.81 m/s^2), d is the diameter of the particle, ρ_p is the density of the particle (assumed to be 1,038 kg/m^3), ρ_m is the density of the matrix (assumed to be 1,000 kg/m^3) and u is the matrix viscosity (assumed to be 0.001 kg/ms). The settling velocity of the smallest sized particles (assumed d=180 μm) captured on the 180 μm filter is 4.03 cm/min and hence represents particles that will settle over a residence time in wastewater treatment plants or in surface water. Settling velocity for

particles captured on the 0.45 (assumed d=0.45 μm) and 20 μm (assumed d=20 μm) filters is 3.5×10^{-5} and 0.05 cm/min, respectively, and represent particles that may settle depending on the residence time and mixture within the system. The settling velocity of particles trapped on the 0.03 μm (assumed d=0.03 μm) filter is 1.1×10^{-7} cm/min and represent non-settling particles in all waters.

Table S1: Primers, probes, and cycling conditions for all molecular assays used in this study

Marker	Primers/Probes	Cycling condition	Source
HF183	Forward - ATCATGAGTTCACATGTCCG Reverse - CTTCCTCTCAGAACCCCTATCC Probe FAM-CTAATGGAACGCATCCC-BHQ-1	10 mins at 95 C, 40 cycles of 95C for 30s and 60C for 60 s, 10 mins at 98C	Green et al., 2014
AdV	Forward - GCC ACG GTG GGG TTT CTA AAC TT Reverse - GCC CCA GTG GTC TTA CAT GCA CAT C Probe - FAM-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-BHQ-1	10 mins at 95 C, 40 cycles of 95C for 30s and 58C for 60 s, 10 mins at 98C	Heim et al., 2003
HPyV	Forward - AGT CTT TAG GGT CTT CTA CCT TT Reverse - GGT GCC AAC CTATGGAACAG Probe - FAM-TCATCACTGGCAAACAT-BHQ-1	10 mins at 95 C, 40 cycles of 95C for 30s and 58C for 60 s, 10 mins at 98C	McQuaig et al., 2009
crAssphage	Forward - CAG AAG TAC AAA CTC CTA AAA AAC GTA GAG Reverse - GAT GAC CAA TAA ACA AGC CAT TAG C Probe - FAM- AAT AAC GAT TTA CGT GAT GTA AC-BHQ-1	10 mins at 95 C, 40 cycles of 95C for 30s and 60C for 60 s, 10 mins at 98C	Stachler et al. 2018
PMMoV	PMMV-FP1-rev - GAGTGGTTTGACCTTAACGTTTGA PMMV-RP1 - TTGTCGGTTGCAATGCAAGT PMMV-Probe1 - FAM-CCTACCGAAGCAAATG-MGB-NFQ	60 mins at 49 C, 10 mins at 95 C, 40 cycles of 95C for 30s and 60C for 60 s, 10 mins at 98C	Haramoto et al 2013
NoV GII	Forward - ATG TTC AGR TGG ATG AGR TTC TCW GA Reverse - TCG ACG CCA TCT TCA TTC ACA Probe - FAM - AGC ACG TGG GAG G GC GAT CG - BHQ1	60 mins at 49 C, 10 mins at 95 C, 40 cycles of 95C for 30s and 60C for 60 s, 10 mins at 98C	Loisy et al., 2005 Kageyama et al., 2003

Table S2: ddPCR summary Statistics of performance metrics

	Poisson Mean estimate				Number of Accepted droplets				Fluorescence amplitude difference between positive and negative droplets			
Target	min	median	mean	max	min	median	mean	max	min	median	mean	max
crAssphage	0.000	0.282	0.288	0.986	4854	13441	12937	18257	430	2108	1752	2458
HF183	0.000	0.060	0.203	0.993	4854	13507	12936	16250	819	13507	12936	16250
AdV	0.000	0.006	0.025	0.180	10618	12038	12169	14548	253	3023	2625	3547
HPyV	0.000	0.002	0.006	0.049	10618	12079	12283	15563	592	2316	2215	3544
norovirus	0.000	0.002	0.005	0.048	10426	13896	13858	17006	1338	4233	3730	6041
PMMoV	0.000	0.051	0.205	0.761	10760	13112	13432	16465	3089	6304	6509	7501

Physical characteristics

Sample conductivity and pH averaged 1.25 mS/cm and 7.71, respectively, for all samples throughout experiment. Air temperature ranged from 29°C in September to 3.9°C in December. Water temperature ranged from 12.1°C in the winter to 23.6°C in the summer. Average total suspended solids concentration was 106 (±10.4) mg/L.