SUPPLEMENTARY MATERIALS

Supplementary Table S1: Primers and probes used for the quantitative PCR (qPCR) assays

Assays	Target gene/region	Primer and probe sequences (5'-3')	Concentration (nM)	PCR Conditions	References
ENT 23S rRNA	23S rRNA	F:AGA AAT TCC AAA CGA ACT TTG R:CAG TGC TCT ACC TCC ATC ATT P:FAM-TGG TTC TCT CCG AAA TAG CTT TAG GGC TA-TAMRA	500 500 400	95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 2 min	1
E. coli H8	Sodium/Hydrogen exchanger precursor	F:ACA GTC AGC GAG ATT CTT C R:GAA CGT CAG CAC CAC CAA	400 400	95°C for 5 min, 40 cycles of 95°C for 10 s and 60°C for 30 s	2
HF183	16S rRNA	F:ATC ATG AGT TCA CAT GTC CG R:TAC CCC GCC TAC TAT CTA ATG	300 300	95°C for 10 min, 40 cycles of 95°C for 30 s, 53 °C for 1 min and 60°C for 1 min	3, 4
nifH	*nifH*	F:GAAAGCGGAGGTCCTGAA R:ACTGAAAAACCTCCGCAAAC P:FAM-CCG GAC GTG GTG TAA CAG TAG CTA-BHQ-1	800 800 240	95°C for 10 min, 50 cycles of 95°C for 10 sec and 57°C for 30 s	5
HAdVs (A-F)	Hexon	F:GCC ACG TGG GGG TTT CTA AAC TT R:GCC CCA GTG GTC TTA CAT GCA CAT C P:FAM-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-TAMRA	200 200 200	95°C for 10 s, 45 cycles of 95°C for 10 s, 55°C for 10 s and 65°C for 1 min	6
HPyVs	Homologous T antigen	F:AGT CTT TAG GGT CTT CTA CCT TT R:GGT GCC AAC CTATGGAACAG P:FAM-TCATCACTGGCAAACAT-MGBNFQ	240 240 160	95°C for 10 min, 45 cycles of 95°C for 15 s, 55°C for 20 s and 60°C for 1 min	7
PMMoVs	Replication-associated protein	F:GAG TGG TTT GAC CTT AAC GTT GA R:TTG TCG GTT GCA ATG CAA GT P:CCT ACC GAA GCA AAT G	200 200 80	50°C for 10 min, 95°C for 10 min, 45 cycles of 95°C for 30 s, 53°C for 1 min, 72°C for 1 min and 72°C for 10 min	8, 9
HAdVs 40/41	Fibre gene	F:AAC TTT CTC TCT TAA TAG ACG CC R:AGG GGG CTA GAA AAC AAA A P:CTG ACA CGG GCA CTC TTC GC	400 400 100	95°C for 10 min, 45 cycles of 95°C for 15 s, 55°C for 30 s and 72°C 30 s	10
HNoVs	ORF1-ORF2 junction region	F:CAA GAG TCA ATG TTT AGG TGG ATG AG R:TCG ACG CCA TCT TCA TTC ACA P:FAM-TGG GAG GGC GAT CGC AAT CT-BHQ	250 250 100	50°C for 10 min, 95°C for 15 min, 45 cycles of 94°C for 10 s, 55°C for 20 s	11
EVs	5' LTR	F:CCT CCG GCC CCT GAA TG R:ACC GGA TGG CCA ATC CAA P:FAM-CGG AAC CGA CTA CTT TGG GTG TCC GT-TAMRA	300 900 125	50°C for 10 min, 95°C for 5 min, 45 cycles of 95°C for 1 min, 60°C for 30 s	12

Supplementary Table S2: Quantitative PCR (qPCR) performance characteristics determined from the slope of the standard curves

qPCR assays	qPCR performance characteristics		
	Amplification efficiencies (E)	Correlation coefficient (r^2)	Slope
ENT 23S rRNA	94.5 to 98.1	0.999 to 1.000	-3.410 to -3.418
E. coli H8	93.5 to 98.1	0.979 to 0.983	-3.430 to -3.420
HF183	86.9 to 101	0.962 to 0.966	-3.506 to -3.472
nifH	90.0 to 92.8	0.994 to 0.998	-3.541 to -3.549
HAdVs	98.1 to 104	0.994 to 0.998	-3.293 to -3.303
HPyVs	96.9 to 107	0.921 to 0.937	-3.276 to -3.268
PMMoVs	81.2 to 101	0.945 to 0.976	-3.301 to -3.872
HAdVs 40/41	91.5 to 94.9	0.986 to 0.988	-3.493 to -3.591
HNoVs	106 to 119	0.983 to 0.986	-2.931 to -3.193
EVs	83.9 to 109	0.985 to 0.995	-3.465 to -3.869

E: Amplification efficiency = $10^{(1/-slope)}/2$.
r^2: denotes the correlation coefficient of determination representing the proportion of variability accounted for by the linear model
SD: Standard deviation

Supplementary Table S3: Environmental sampling site descriptions and potential sources of fecal pollution

Site Name	Description	Potential sources of fecal pollution
Bribie Island	White sandy public swimming beach	Storm water drain
Sandstone Point	White sandy beach adjacent to a small mangrove system	Wastewater pumping station
Godwin Beach	White sandy beach	Storm water drain
Deception Bay	White sandy beach	Storm water drain
Scarborough	Yellow sandy beach adjacent to a public park	Storm water drain
Shorncliffe	White sandy beach 200 m from a public access boat marina	Storm water drain
Breakwater Park	Yellow sandy beach adjacent to a public picnic area	Storm water drain
Pandanus Beach	Popular swimming beach adjacent to a children's water playground	Storm water drain
Jabiru Island	White sandy beach with a popular public swimming enclosure	Storm water drain
Paradise Point	White sandy beach with a popular public swimming enclosure	Storm water drain
Labrador	White sandy beach	Storm water drain
Southport	Yellow sandy beach adjacent to a popular public park	Storm water drain

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