SUPPLEMENTAL MATERERIAL:

Application of a Microbial and Pathogen Source Tracking 'Toolbox' to Identify Infrastructure Problems in Stormwater Drainage Networks: A Case Study

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Supplemental Information:

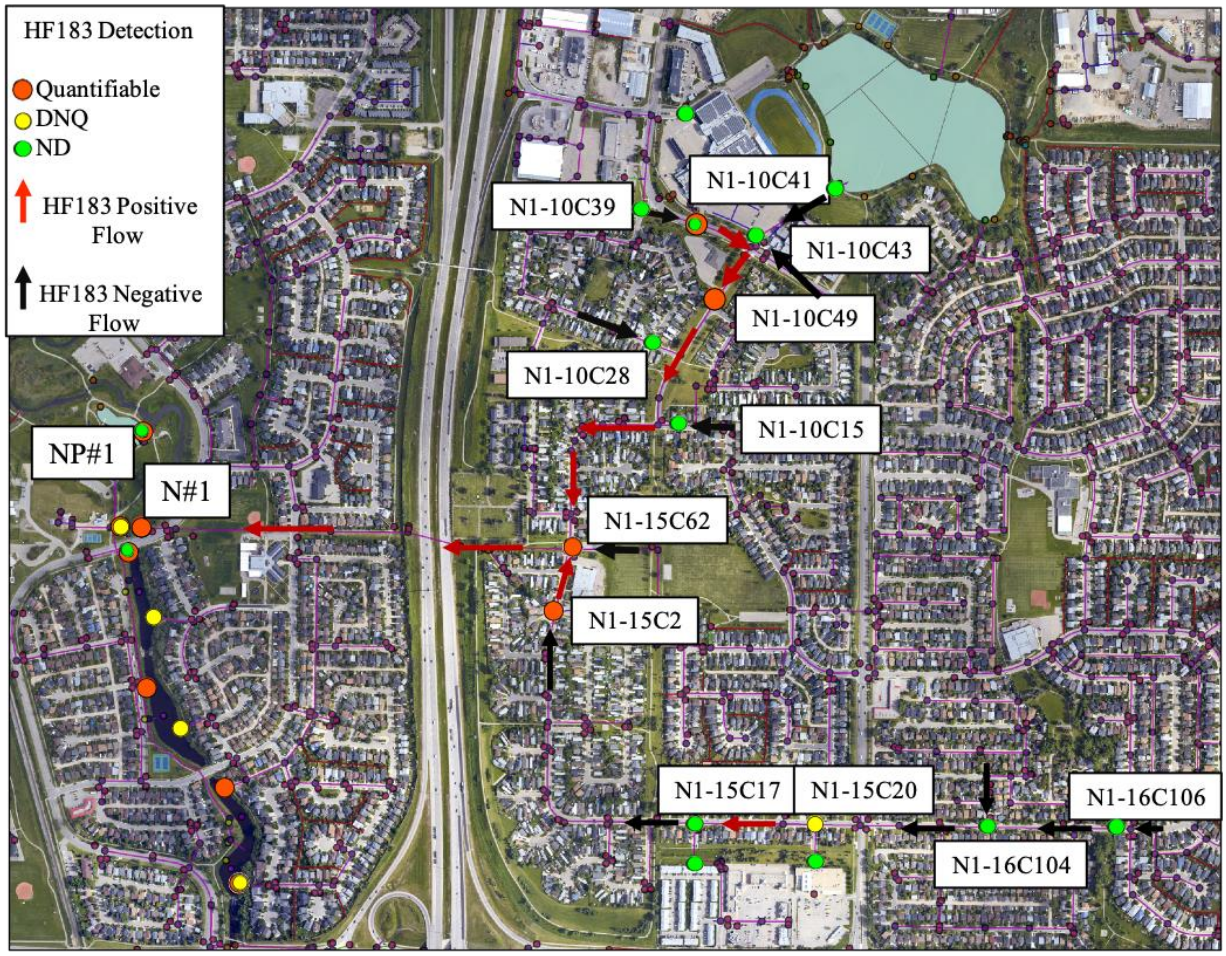

Fig. S1 Map of Nose Creek stormwater drainage network and the manholes tested for human sewage marker HF183 upstream of HF183 positive outfall site N#1 from the final two weeks of the investigation. Note the manholes upstream of N#1 where HF183 was positive and quantifiable (red dots), detectable but not quantifiable (yellow dots), or not detected (green dots). Note also the flow of stormwater through the system, represented by presumed HF183 positive flow (red arrows) based on upstream site HF183 detection and concentrations, as well as flow with no demonstrable HF183 detection (black arrows). Note that all maps presented were created and co-owned with the City of Airdrie and have been used with permission.

Table S1 Evidence of human sewage contamination and FIB results from investigative samples taken upstream of the N#1 site in Nose Creek during the first half of the investigation (weeks 1-3)

Sampling Date	Site	HF183	HumM2	*Enterococcus*	*E. coli*
		log_{10} copies/100 mL	log_{10} copies/100 mL	log_{10} CCE/100 mL	log_{10} MPN/100 mL
26/7/21	N1-15C2-N	4.06	DNQ	4.25	0.99
	N1-15C62-N	5.25	4.32	2.65	2.51
	N1-15C62-E	ND	ND	1.93	1.27
9/8/21	N1-10C39-N	ND	ND	2.33	0.80
	N1-10C43-E	ND	ND	ND	ND
	N1-10C43-W	ND	ND	1.71	0.30
	N1-15C54-N	ND	ND	3.79	0.61
	N1-15C17-E	ND	DNQ	4.57	1.78
24/8/21	N1-10C39-W	ND	ND	3.09	2.44
	N1-GenEdge-S	ND	ND	2.82	0.72
	N1-GenEdge-N	ND	ND	3.45	1.96
	N1-NoFrills-N	ND	ND	2.86	1.51

Table S2 Human sewage MST and FIB results from investigative samples taken upstream of the N#1 site in Nose Creek during the second half of the investigation (weeks 4-5)

Sampling Date	Site[a]	HF183	HumM2	*Enterococcus*	*E. coli*
		log_{10} copies/100 mL	log_{10} copies/100 mL	log_{10} CCE/100 mL	log_{10} MPN/100 mL
7/9/21	N#1	3.64	ND	2.19	0.98
	N1-10C39-E	ND	ND	2.35	ND
	N1-10C28-E	ND	ND	4.18	3.24
	N1-10C49-S	4.75	DNQ	2.32	1.71
	N1-10C15-E	ND	ND	1.43	0[b]
8/9/21	N1-15C62-N	4.40	ND	2.23	1.30
	N1-15C62-E	ND	ND	1.90	0.80
	N1-15C2-E	ND	ND	1.93	0[b]
	N1-15C2-S	ND	ND	2.57	0.93
	N1-15C17-W	ND	ND	2.67	0.99
	N1-15C20-W	DNQ	ND	2.57	1.38
	N1-16C104-E	ND	ND	1.84	0.61
	N1-16C104-N	ND	ND	2.45	0.72
	N1-16C106-E	ND	ND	2.12	1.13
27/9/21	N1-10C49-S	5.36	4.31	4.36	>3.38
	N1-10C41-N	6.08	6.05	5.60	>3.38
	N1-10C41-W	ND	ND	3.20	ND

N1-10C43-N	ND	ND	1.72	0.88
N1-10C39-E	ND	ND	2.81	0[b]

[a] Letters at the end of each manhole site name (i.e., N, E, S, or W) represent the direction of the specific trunk sampled at said manhole

[b] Due to $\log_{10}$ transformation, values of 0 represent detection at 1.00 MPN/100 mL for *E. coli*, and are not equivalent to an absence of detection at <1.00 MPN/100 mL.

Table S3 Conditions for all qPCR assays using the ABI 7500, including target, primer, and probe names, sequences, and concentrations where applicable

Target Name	Target	Target Organism (locus)	Primer/Probe	Primer/Probe Target Sequnce (5'-3')	Primer/Probe Conc.	Reference
Fecal Indicator Bacteria (FIB)						
Entero1	*Enterococcus* spp.	*23S* rRNA	Entero1-F	GAGAAATTCCAAACGAACTTG	1 uM	1, 2
			Entero1-R	CAGTGCTCTACCTCCATCATT	1 uM	
			Entero1-P	6FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA	80 nM	
Microbial Source Tracking (MST)						
HF183	Humans	*Bacterioides* spp. (*16S* rRNA)	HF183-F	ATCATGAGTTCACATGTCCG	1 uM	3
			BFDrev	CGTAGGAGTTTGGACCGTGT	1 uM	
			BFD-FAM	FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA	80 nM	
HumM2	Humans	Unknown (alpha-1,2-mannosidase analog)	HumM2-F	CGTCAGGTTTGTTTCGGTATTG	200 nM	4
			HumM2-R	TCATCACGTAACTTATTTATATGCATTAGC	200 nM	
			HumM2-P	FAM-TATCGAAAATCTCACGGATTAACTCTTGTGTACGC-TAMRA	125 nM	
Pathogen Markers						
VD16S	*Campylobacter* spp.	*16S* rRNA	VD16S-F	CTGCTTAACACAAGTTGAGTAGG	300 nM	5
			VD16S-R	TTCCTTAGGTACCGTCAGAA	300 nM	
			VD16S-P	6FAM-CGCTCCGAAAAGTGTCATCCTCC-BHQ1	100 nM	
invA	*Salmonella* spp.	*invA*	invA-F	GCGTTCTGAACCTTTGGTAATAA	450 nM	6
			invA-R	CGTTCGGGCAATTCGTTA	450 nM	
			invA-P	6FAM-TGGCGGTGGGTTTTGTTGTCTTCT-MGBNFQ	125 nM	
hsp60	*A. butzleri*	*hsp60*	Abutz-F	CTCTTCATTAAAAGAGATGTTACCAATTTT	300 nM	7
			Abutz-R	CACCATCTACATCTTC**W**GCAATAATTACT	300 nM	
			Abutz-P	6FAM-CTTCCTGATTGATTTACTGATT-NFQMGB	100 nM	
Stx1	STEC	*Stx1*	Stx1-F	CATCGCGAGTTGCCAGAAT	450 nM	8
			Stx1-R	GCGTAATCCCACGGACTCTTC	450 nM	
			Stx1-P	6FAM-CTGCCGGACACATAGAAGGAAACTCATCA-TAMRA	125 nM	
Stx2	STEC	*Stx2*	Stx2-F	CCGGAATGCAAATCAGTC	450 nM	8
			Stx2-R	CAGTGACAAAACGCAGAACT	450 nM	
			Stx2-P	6FAM-ACTGAACTCCATTAACGCCAGATATGA-TAMRA	125 nM	
Sample Processing and Inhibition Controls						

Sketa	*Onchorhynchus keta*	rRNA ITS	Sketa-F	GGTTTCCGCAGCTGGG	1 µM	2, 9
			Sketa-R	CCGAGCCGTCCTGGTC	1 µM	
			Sketa-P	VIC-AGTCGCAGGCGGCCACCGT-TAMRA	80 nM	

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