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The effect of sewage source on HF183 risk-based threshold estimation for recreational water quality management

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Abstract

Host-associated fecal indicator measurements can be coupled with quantitative microbial risk assessment to develop risk-based thresholds for recreational use of potential sewage-contaminated waters. These assessments require information on the relative concentrations of indicators and pathogens in discharged sewage, typically based on data collected from wastewater treatment plant influent samples. However, most untreated sewage releases occur from within the collection system itself (i.e. compromised sewer laterals, compromised gravity and force mains, sanitary sewer overflows), where these relationships may differ. This study therefore analyzed the concentrations of a selected reference pathogen (norovirus) and fecal indicator (HF183) in sewage samples from upper and lower segments of gravity sewage collection systems, wastewater pumpstations, and the influent and effluent of treatment plants, to characterize variability in their relative concentrations. Norovirus detection rates were lower and more variable in upper collection system samples due to the smaller population represented; whereas, HF183 was routinely detected at all sites with higher concentrations in the collection system compared to treatment plant influent, resulting in variable comparative relationships across sample locations (types). Mean HF183:NoV ratios ranged from 1.0×10^5 for sewer lateral samples to 7 × 10° for force main samples. Results were used to develop risk-based thresholds for HF183 based on estimated recreational exposure to norovirus following a release from each potential sewage source, with higher thresholds for treatment facility influent compared to forced mains, or effluent. Consequently, this approach can allow for the rapid application of potential risk-based thresholds for recreational water quality applications based on different types of sewage discharge events.

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CRediT authorship contribution statement

Kyle Curtis: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization, Software. **Michael Jahne:** Writing – review & editing, Writing – original draft, Validation, Conceptualization, Software. **David Keeling:** Visualization, Software. **Raul Gonzalez:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization, Validation, Supervision.

Declaration of competing interest

The authors declare no conflicting interests that could in appropriately bias the submitted work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2024.100315.

Keywords

Quantitative microbial risk assessment; Norovirus; HF183; Sewage spill; Public health

1. Introduction

Sewage discharged into environmental waters used for recreation impose a public health burden due to enteric pathogens including viruses, bacteria, and protozoa. Microbial water quality has been traditionally evaluated using fecal indicator bacteria (FIB), such *E. coli* and enterococci, as proxies for the presence of human pathogens. Recognized limitations of FIB, including lack of host specificity and differing persistence in secondary environments, have driven interest in the use of alternative indicators. HF183 and crAssphage are examples of alternative indicators that are highly abundant in and more associated with human feces, making them a more reliable indicator of sewage contamination (Bernhard and Field, 2000; Stachler et al., 2017). Still, assessing the extent and duration of human health risk following sewage discharge remains challenging due to complex pathogen die off kinetics (Boehm et al., 2018; Dean and Mitchell, 2022), mixing patterns of sewage in environmental waters, and pathogen and indicator organism persistence/survival variability (Ferguson and Signoretto, 2011).

The quantitative microbial risk assessment (QMRA) framework offers a numerical approach for estimating public health risk by integrating empirical data into a comprehensive risk model. QMRA has been widely applied to investigate the likelihood of gastrointestinal illness after exposure to sewage-contaminated waters (Federigi et al., 2019). A strength of QMRA is that the model can be built to accommodate a range of sewage-borne pathogens and fecal indicators to generate a risk estimation. Work by Boehm et al. (2015) used literature sewage concentrations of *Salmonella* spp., *Campylobacter, E. coli* 0157:H7, *Cryptosporidium, Giardia,* and norovirus to estimate the probability of illness given exposure to water contaminated with various concentrations of sewage. From this they were able to estimate health risk using the concentration of two microbial source tracking genetic indicators, HF183 and HumM2, providing a practical means of assessing swimming risk without the need to conduct analyses of multiple fecal pathogens. QMRA is a useful tool for translating pathogen or indicator data into actionable estimates of health risk but, like all models, its efficacy hinges on the quality and accuracy of input data.

Most risk-based thresholds for recreational water quality management applications use pathogen and indicator distributions found in untreated influent entering a treatment facility. Influent concentrations are often used as model inputs as these data are more readily available than sewer collection system data. However, using influent distributions to estimate risk-based thresholds has two limitations; distributions of pathogens and indicators are heterogeneous across sewage collection systems, and treatment facility influent is rarely the proximate source of sewage released into the environment. The United States Environmental Protection Agency (EPA) estimates that there are at least 23,000 - 75,000 sanitary sewer overflow (SSO) events per year being primarily driven by severe weather, system blockages, breaks in sewer lines, and improper system operation and maintenance (US EPA, 2004). In

each of these cases, the sewage source discharged into the environment is from the sewer collection system, not from the influent of a treatment facility. Sewage discharges from the upper reaches of the collection system could occur due to a blockage or compromised lateral while downstream releases could be the result of sewer pipe failure or an extreme precipitation event causing SSO discharges. It is also likely that treated effluent discharges affect recreational waters. Estimation of health risk due to exposure to sewage contaminated waters could be improved by considering these different sewage sources and their associated distribution of pathogens and fecal indicators.

The goal of this study is to describe the variability of human norovirus (NoV) and the human-associated HF183 genetic marker across sewage collection and treatment. Distributions of NoV and HF183 are then used to simulate four of the most common causes of sewage discharge into the environment (SSO, unknown gravity pipe break, compromised sewer lateral, force main break) and compared with treatment plant influent and effluent. Risk estimations based on NoV are then correlated with HF183 concentrations in the corresponding sewage source and used to calculate risk-based thresholds for each sewage pathway scenario. HF183 measurements coupled with pathogen distributions specific to the type of sewage spill can be used to yield a more robust risk-based threshold estimation for recreational water quality applications. The result is a more accurate framework for risk estimation after a sewage spill.

2. Methods

Data generated for this study has been assimilated from multiple projects conducted between March 2016 and August 2018 in the Hampton Roads metropolitan area in Virginia. Analytical methods remained consistent between projects, allowing for pooling and generation of a more robust dataset.

2.1. Wastewater collection system and treatment facility samples

Grab samples were collected from upper and lower segments of gravity sewage collection systems and from wastewater pumpstations to characterize HF183 and NoV concentrations prior to reaching a wastewater treatment facility (n = 7 sites per collection system segment). Upper system, lower system, and pumpstation samples were collected in each of the seven cities comprising the Hampton Roads region of southeastern Virginia (Fig. 2) during three dry weather and three wet weather events (n = 21 per site for dry and 21 per site for wet weather samples). Wet weather was defined as a storm generating least 0.64 cm rainfall accumulation in the antecedent twenty-four hours. Mean rainfall accumulations for the three wet weather events were 9.17, 3.78, and 1.85 cm. The respective duration for each of these events was 12.5, 3.2, and 7.5 h. Samples were collected during morning hours (approximately 0800 – 1100) to increase the likelihood that upper system sites serving a smaller population would have sampleable flow. For this study, the upper segments of a sewershed were defined as those servicing approximately 25 (SD=12.5) homes or businesses, comprising a mean sewershed area of 0.45 km^2 (SD=0.36 km^2). Similarly, lower segments of a sewershed were downstream of upper system sites and serviced on average 218 (SD = 108) homes or businesses and comprised a mean sewershed area of 3 km^2 (SD

= 1.2km^2). A visual example of upper and lower collection systems is provided in Fig. 1. Pumpstations sites were further downstream in the sewer collection systems, receiving wastewater from a broader area and pumping towards wastewater treatment facilities.

Wastewater treatment plant influent and sodium hypochlorite-disinfected effluent data were collected from monthly grab sampling over a year (n = 12 for 3 of 4 facilities) as previously described in Worley-Morse et al. (2019). For an overview of treatment train and sewershed characteristics for each facility see Worley-Morse et al. (2019). In addition to the three facilities from the Hampton Roads region (facilities G, H, and I) six months of data (December – June) from a fourth facility was included in the dataset (n = 6). The fourth facility was collected concurrently with the second half of the Worley-Morse et al. (2019) study. Wastewater treatment plant samples were collected during dry conditions having no measurable rainfall in the antecedent 72 h.

2.2. Microbiological analyses

One liter grab samples were transported to the laboratory on ice within 6 h. Immediately upon arrival, samples were concentrated using mixed cellulose ester HA filters (HAWP04700; Millipore, Billerica, MA, USA) as described by Worley-Morse et al. (2019) and stored in a −80 °C freezer. Samples were analyzed within one month of collection.

Prior to extraction, 10^7 copies of hepatitis G Armored RNA (Asuragen, Austin, TX, USA) and 0.1 μg of salmon sperm DNA (Sigma-Aldrich, St. Louis, MO, USA) were added to the lysis buffer for all samples and controls to quantify matrix inhibition. Total nucleic acid was extracted using the NucliSENS easyMag (bioMerieux, Inc., Durham, NC, USA) following a modified protocol documented by Worley-Morse et al. (2019). NoV GI, NoV GII, and HF183 were quantified on a QX200 droplet digital PCR system (Bio-Rad, Hercules, CA, USA) according to protocols in Worley-Morse et al. (2019) and Gonzalez et al. (2020).

Each run included two no-temple controls (NTC), two negative extraction controls (NEC), a filter blank, and a positive control. Positive genomic RNA standards for NoV GI (VR-3234SD) and NoV GII (VR-3235SD) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HF183 Taqman OSTD1 genomic DNA reference standard (Layton et al., 2013) is from Integrated DNA Technology (IDT, Coralville, IA, USA).

All negative controls (NEC, NTC, filter blanks) yielded non-detectable results, and any run with detectable signals was re-run until a negative result was achieved. Positive controls were required to produce positive results from the initial run or were re-run to confirm positivity. All samples were run in duplicate, and reactions were considered positive if at least three droplets (out of more than 10,000) were identified as positive. If a well contained fewer than 10,000 droplets, the sample was re-run to confirm the original concentrations. The threshold was manually set at the lower third of the space between the negative and positive droplets.

Inhibition was determined by calculating the hepatitis G and salmon sperm DNA recovery in the samples compared to the NEC. For inhibited samples (less than 10% recovery), the

samples were diluted and re-run until they exceeded the 10% recovery threshold. Limit of Detection (LOD) for the NoV GI, NoV GII, and HF183 assays are 1.31, 3.23, and 1.66 copies per reaction, respectively. Any sample with results below the detection limit was reported using the LOD values in place of concentrations.

2.3. Data analysis

Lognormal distributions were fitted to NoV concentrations for each collection system and treatment plant location, for dry and wet weather conditions, using a Shapiro-Wilk test to assess model fit and plotted using the ggridges package (Wilke, 2022) in R Statistical Software Version 4.0.4 (R Core Team, 2021); distribution goodness of fit statistics are provided in Table S1. Sewage sources for which the Shapiro-Wilk test indicated that the data were not well-approximated by the lognormal distribution have been reported (Table 2, Fig. 3) but excluded from the risk-based threshold analysis. NoV GI and GII concentrations were summed, yielding a single NoV value. Hypothetical sewage sources were categorized to describe a range of spill scenarios (i.e., broken sewage force main or precipitation-driven sewer overflow) and resulting distributions were created. Kruskal-Wallis and Dunn's pairwise comparison tests have been run for all combinations of sewage sources to test for significant differences in HF183 or NoV concentration (Table S3). For data below the analytical limit of detection, the limit of detection value has been used for analyses and plotting.

2.4. Norovirus-based risk estimates

The probability of illness given NoV exposure and infection was estimated using fitted NoV concentration distributions for each sewage source scenario. NoV concentrations (copies mL^{-1}) for each sewage source were approximated using 10^5 random draws from the lognormal distributions. Exposure was modeled assuming a lognormal distribution (mean = −2.78 L, standard deviation = 0.93 L) following the most recent EPA recreational water criteria study (USEPA, 2019) and modeled via 10^5 iterations. The NoV dose (copies) was calculated as NoV concentration (copies mL^{-1}) times the ingested volume of water (L^{-day}). Hypergeometric dose-response models were used to estimate the probability of illness following the models of Teunis et al. (2020) as described by Schoen et al. (2023). Probabilities of illness given exposure to water contaminated by various levels of each sewage source were modeled based on simulated NoV dilutions into receiving waters (undiluted to 9x serial dilution). Model inputs are summarized in Table 1. All calculations were performed in R. Model code is available upon request.

2.5. Deriving an HF183 risk-based threshold

The median values for risk estimates resulting from the hypothetical dilutions of sewage-derived NoV were regressed against the median HF183 concentration for the same sewage source which was similarly scaled to range from undiluted to the equivalent of a 9x serial dilution. A linear regression was fitted to these data to assess the relationship between NoV-derived risk and HF183 concentration. Regression lines were fit using base R and assessed based on multiple R^2 values; see Supplementary Table 2 for regression fit statistics. For all cases the equation describing the association was solved to determine the median

HF183 concentration that corresponds to the EPA Recreational Water Quality benchmark (USEPA, 2012) of 32 illnesses per 1000 recreators.

3. Results/discussion

3.1. Norovirus and HF183 distribution across collection and treatment

Fig. 3 shows the distributions of HF183, NoV GI, and NoV GII across the collection system (upper system [US], lower [LS], pumpstation [PS]) and treatment (raw influent, final effluent) with fitted lognormal models presented in Table 2. Within the collection system, average dry weather HF183 concentrations were within the same order of magnitude across all sites (US mean = 1.04E+08, SD = 1.57E+08, LS mean = 1.03E+08, SD = 1.01E+08, PS mean = 1.07E+08, SD = 1.05E+08, all units copies $100mL^{-1}$). Upper system samples had a higher standard deviation and larger range (2.19E+05 – 6.50E+08 copies $100mL^{-1}$) compared with the lower system (5.75E+06 – 3.13E+08 copies $100mL^{-1}$) and pumpstation samples (1.86E+06 – 2.97E+08). This is likely due to the heterogenous nature of sewage and a reduced dilution effect at these small sub-sewershed catchments, resulting in greater variability. HF183 wet weather collection system data (US mean = 2.26E+07, SD = 2.20E+07, LS mean = 2.31E+07, SD = 1.44E+07, PS mean = 2.20E+07, SD = 1.98E+07, all units copies $100mL^{-1}$) were similar to dry weather distributions in their relative variability but with the central tendency shifted approximately an order of magnitude lower. Average NoV GI and GII concentrations increased (one-tailed Wilcoxon signed rank test, $W = 15$, $p < 0.001$) moving from the upper system to pumpstations. This was driven by an increased detection rate moving from upper to lower system samples (US GI and GII detection rates = 25%, 25%, LS GI and GII detection rates = 60%, 60%, PS GI and GII detection rates = 75%, 75%). Finding greater NoV detection as population served increases was expected as a larger population yields a greater chance of NoV infections and therefore occurrence of the target in each grab sample. For a given collection system site, NoV distributions did not appear to significantly change during wet weather. In general, NoV has a greater range than HF183 as the human-associated genetic marker is likely consistently shed by collection system users and thus is ubiquitous in wastewater while NoV is only shed by those experiencing infection, therefore concentrations are driven by the infection rate within sewershed population.

Dry weather raw influent HF183 (mean = 1.31E+07, SD = 9.50E+06) and NoV concentrations (mean = 4.16E+04, SD = 6.77E+04 copies $100mL^{-1}$) were lower in concentration and less variable than the upstream collection systems. While the majority of the raw influent data fell within the range of collection system concentrations the raw influent central tendency of the data is shifted left. This is likely due to dilution and the increased age of sewage (US mean 3.3hr, maximum 36hr) once it has reached the headworks of a treatment facility (Nielsen et al., 1992; Kapo et al., 2017). Final effluent concentrations were significantly lower than influent concentrations (NoV GI $\chi^2 = -0.50$, $p < 0.001$; NoV GII $\chi^2 = -0.64$, $p < 0.001$; HF183 $\chi^2 = -5.73$, $p < 0.001$), with HF183 showing a larger difference than NoV. This is likely due to the differing susceptibility of the targeted bacteria and viruses to treatment (Worley-Morse et al., 2019). Influent HF183 concentrations were more variable than final effluent concentrations potentially due to the homogenizing effect

of wastewater treatment during long retention times. Raw influent NoV concentrations for this study were generally higher than literature values (Pouillot et al., 2015; Eftim et al., 2017) likely due to differences in NoV quantification methodology such as the use of more appropriate processing volumes (1 L compared to 50 mL), improved nucleic acid extraction methods, and utilization of digital PCR instead of qPCR (Jahne et al., 2020).

3.2. HF183 risk-based thresholds

The HF183 and NoV distributions used for each sewage source are summarized in Table 2 and visualized in Supplemental Fig. 1. For each sewage source the relationship between NoV-derived risk and HF183 concentration were well-approximated by a linear relationship with R^2 values ranging 0.73–0.97 (Table S2). Given the varying distributions of NoV and HF183, the resulting HF183 risk-based thresholds range two orders of magnitude (Table 4). Upper collection system sites had highly variable NoV concentrations which included many non-detect observations. This was expected given that NoV concentrations are driven by infections rates and the population served by upper system sites is relatively small. As a result, NoV data for these sites were poorly modeled by the lognormal distribution, which was used to approximate NoV data for other sewage sources. Further, generating appropriately protective risk-based thresholds for upper system sites would require significant sampling to understand the influence of rare but impactful outbreak events on sewage pathogen concentrations. Given these limitations, risk-based thresholds have not been estimated for sewer lateral, gravity main, and sanitary sewer overflow datasets.

As sewage ages, the relative concentration of NoV increases and becomes less variable compared to HF183 concentrations (Kapo et al., 2017). Data presented here support this finding with less variable NoV concentrations in pumpstations resulting in a lower HF183:NoV ratio (Table 3). A lower indicator:pathogen relationship indicates that greater concentrations of HF183 likely mean greater concentrations of NoV, requiring a stringent risk-based threshold (Table 4, Fig. 4). For a force main impacted site, the resulting HF183 risk-based threshold is 68 copies 100 mL^{-1} as NoV concentrations for this sewage source are high and the HF183:NoV ratio is low; thus, a more stringent HF183 threshold is required to achieve the risk threshold of 32/1000. The HF183 threshold for a force main spill scenario is 2 orders of magnitude more stringent than the threshold derived using treatment facility influent data (2630 copies 100 $mL^{-1)}$, representing an important discrepancy in public health protection hinging on which sewage source data are used. The authors hypothesize that the location of force mains within the sewage collection system may be driving this result. Force mains and pumpstations receive flow from a large enough population that there are sufficient NoV infections to create concentrations higher than upstream sites (gravity pipes and sewer laterals) which may not always include shedding users in their smaller service areas. However, concentrations observed in treatment plant influent are slightly decreased due to degradation and dilution associated with the remaining travel time to get from pumpstations/force mains to the treatment plant. This finding emphasizes the importance of using the appropriate sewage source data to model public health risk due to a sewage release.

Fig. 4 demonstrates the differences in probability of illness for each sewage source in terms of required dilution, as sites with higher risk of illness due to NoV require more dilution to reach the EPA acceptable illness rate for recreational waters. Previous work by Ahmed et. al (2018) and Schoen et al. (2020) supports the use of risk-based thresholds, finding that public health risk varies as the volume and age of sewage discharged into recreational waters changes. Their work also found that the age of sewage influenced the efficacy of genetic markers as indicators of elevated health risk. This trend differs for final effluent however, where both NoV and HF183 concentrations are lower relative to untreated sewage sources. Despite lower overall concentrations in final effluent, finding a lower ratio of HF183:NoV results in a lower, i.e. more protective, risk-based threshold for treatment plant effluent (301 copies $100mL^{-1}$) than influent (2630 copies 100 mL^{-1}). Conversely, final effluent risk-based thresholds are less stringent than those for force main (68 copies $100mL^{-1}$) impacted sites. These findings are driven by the indicator:pathogen ratios for each sewage source. Rather than relying on pathogen concentrations, understanding these ratios and their effect on estimated risk allows for the assessment of impact to a site, regardless of dilution. Since it is unlikely that raw influent is making its way into recreational waters, the use of appropriate HF183 thresholds specific to the contamination source of interest is an important consideration when assessing potential impacts. Sewage releases originate from a variety of sources (e.g., unknown compromised laterals and sewer gravity mains, combined or sanitary sewer overflows, force main breaks) however in practice upstream sites have populations that are too small and pathogen concentrations that are too variable for use in this framework. Using force main pathogen and indicator concentrations and ratios, rather than treatment plant influent, provides an appropriately conservative estimate of risk-based thresholds for determining extent of impact. An extension of this approach could incorporate an assessment of fecal age for samples taken across collection systems (Boehm et al., 2018; Boehm and Soller, 2020; Schoen et al., 2020).

4. Conclusions

HF183 has been supported by the EPA as a validated method (USEPA Method 1696.1) for assessing microbial water quality (USEPA, 2019). Establishing a risk-based threshold for HF183 should consider collection system or treated effluent samples rather than raw influent alone, as these are more likely to impact receiving recreational waters. Further characterization of pathogens and indicator genetic markers across collections systems is needed to better understand the distribution of these targets across differing populations and geographic regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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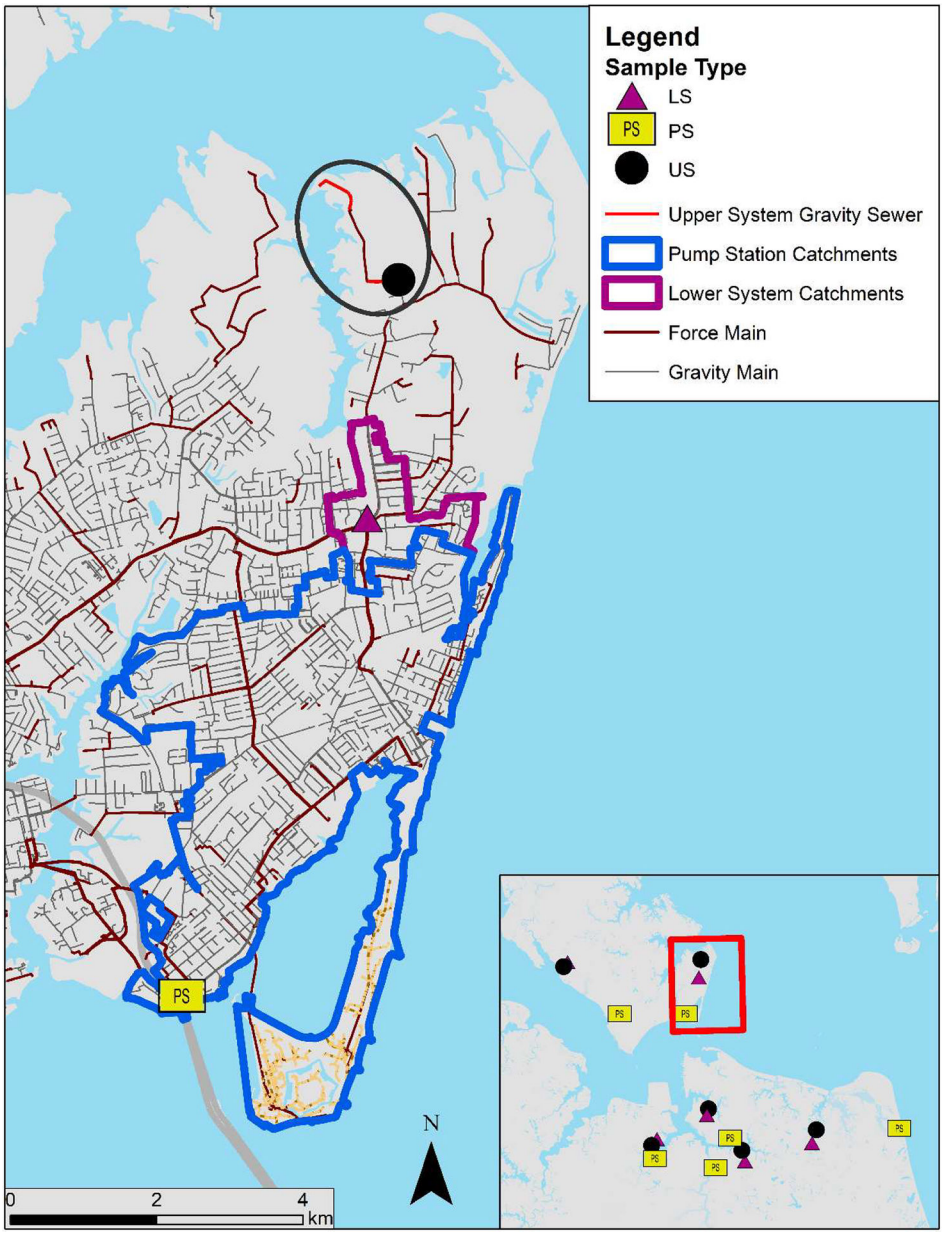

Fig. 1.

Map of example upper system (US), lower system (US), and pumpstation (PS) service areas.

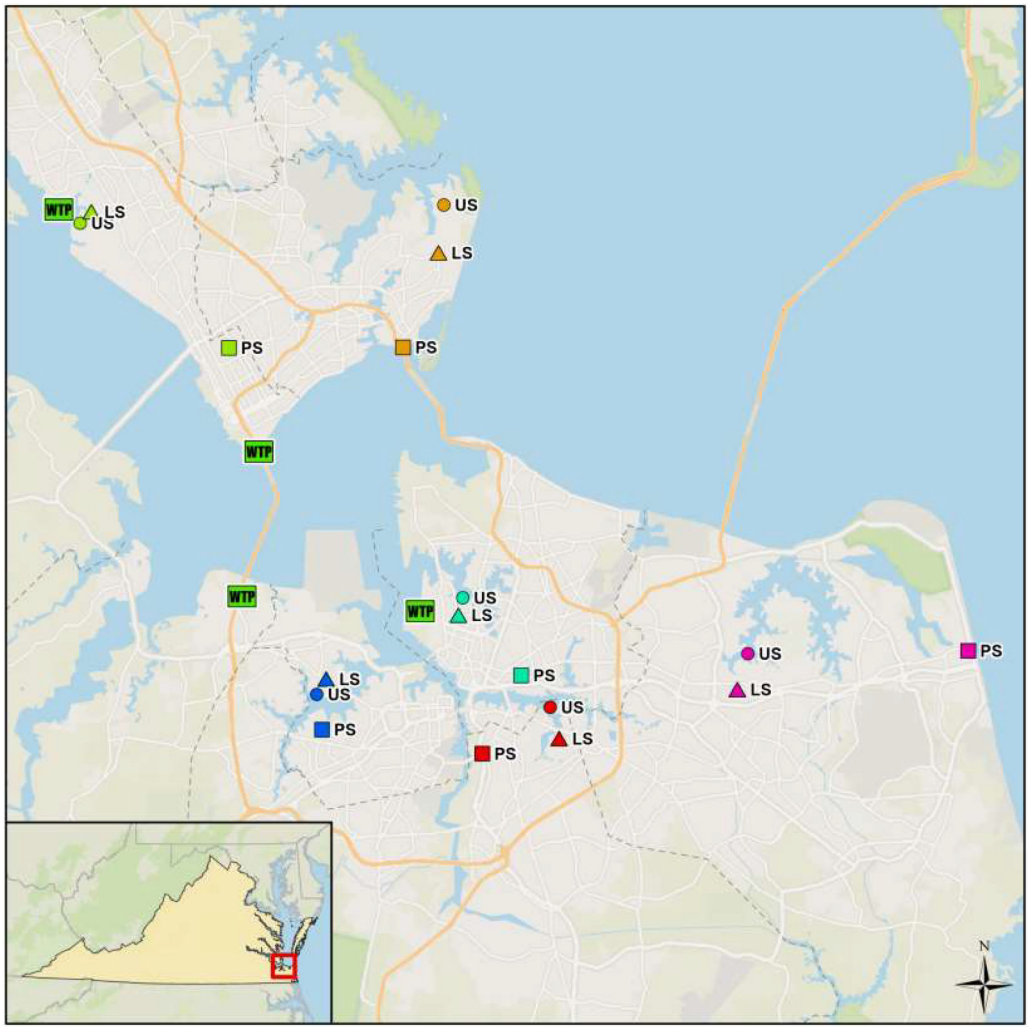

Fig. 2.

Map of all sampling sites in Hampton Roads metropolitan area, southeastern Virginia. Abbreviations: US, upper system; LS, lower system; PS, pump station, WTP, wastewater treatment plant. Colors denote distinct treatment facility sewersheds.

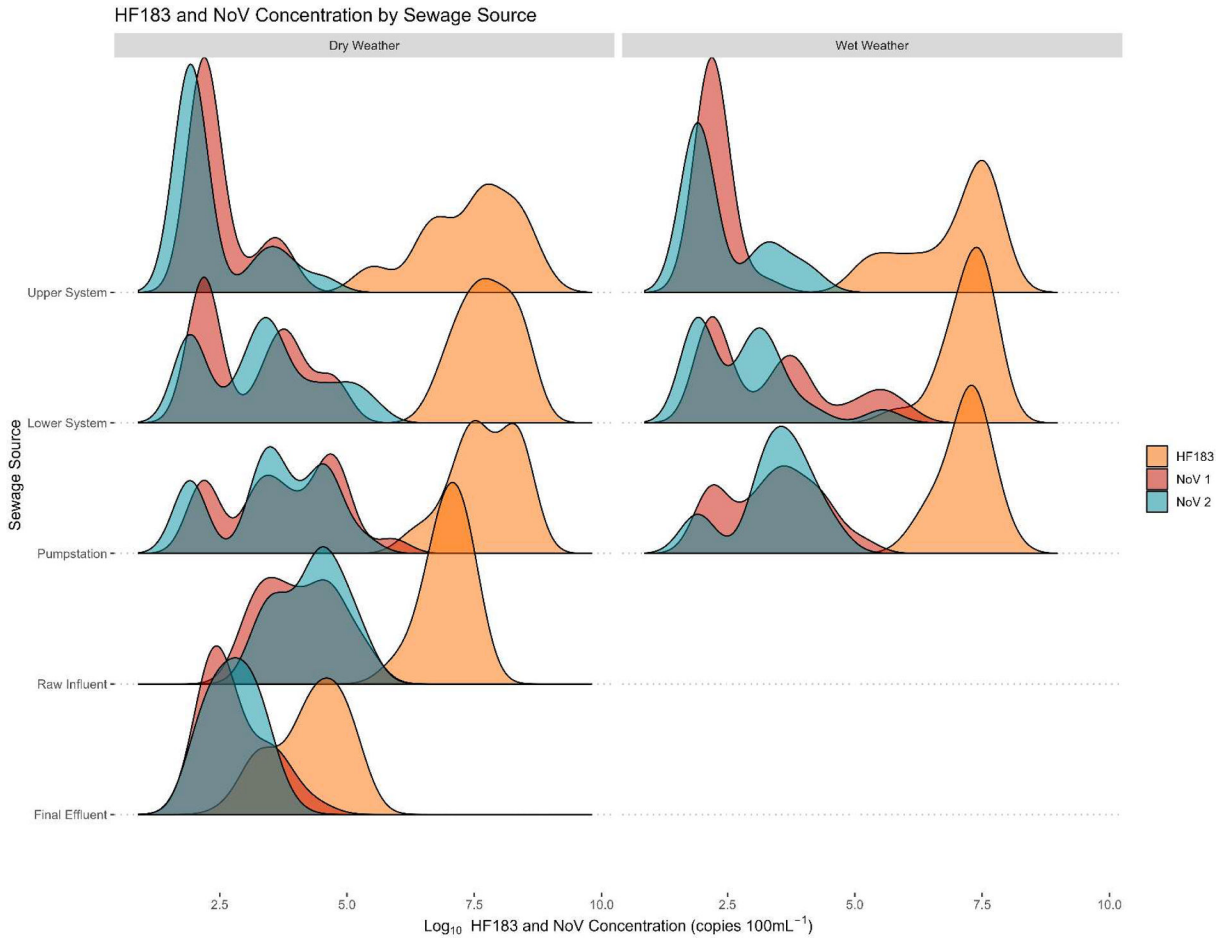

Fig. 3.

NoV and HF183 concentrations by collection system location. Collection system samples are broken out by wet and dry weather conditions.

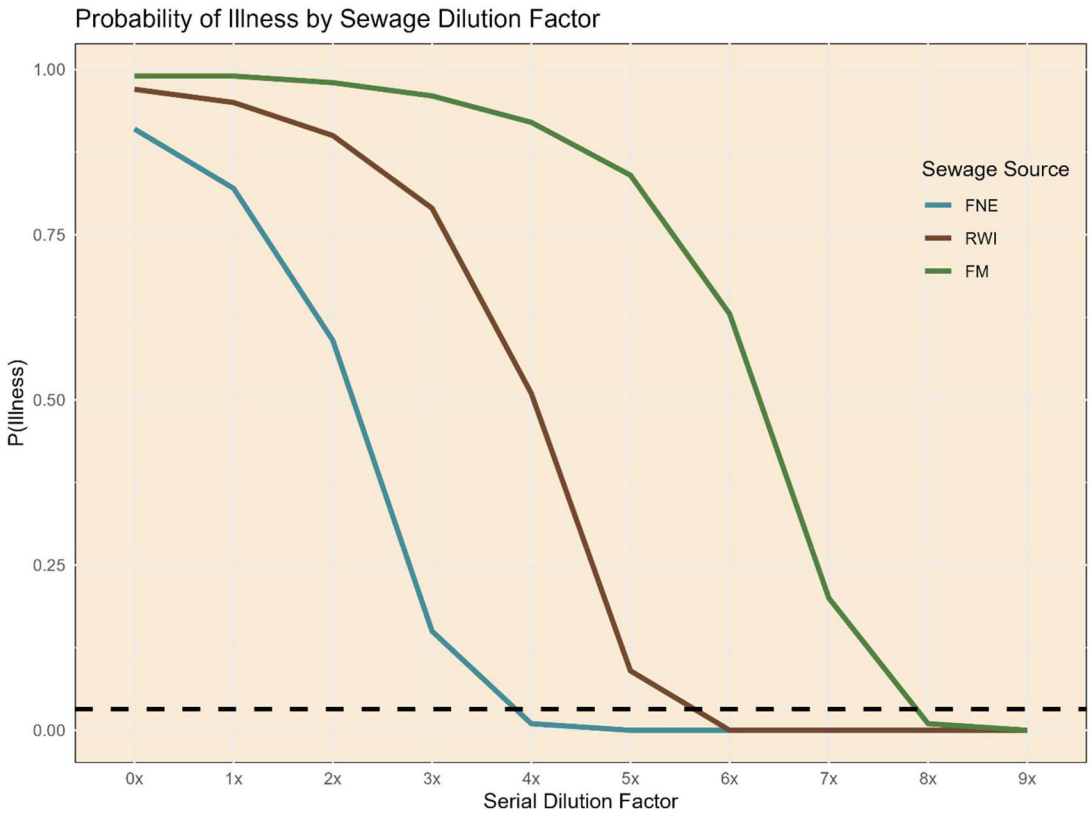

Fig. 4.

Probability of illness across 9x serial dilutions of sewage from each source type. The dashed line denotes the EPA Recreational Water Quality acceptable illness rate of 32/1000 recreators.

Table 1

QMRA model inputs.

Model Parameter	Description	References
NoV Concentration	Lognormal (mean and SD vary by sewage source)	Data generated in-house
Ingested Water Volume (L/Day)	Lognormal (−0.278, 0.929)	Dufour et al. (2006), and US EPA (2011) Exposure Factors Handbook
NoV Dose (copies)	NoV Concentration (copies/mL) * Ingested Water Vol (mL)	Calculated
P(Infection)	Hypergeometric Dose-Response Model[a]	Teunis et al. (2020), Schoen et al. (2023)
P(Illness)	Hypergeometric Dose-Response Model[a]	Teunis et al. (2020), Schoen et al. (2023)

[a]Refer to Schoen et al. (2023) for the selected model and its transformed parameters.

Table 2

Fitted HF183 and norovirus distributions for each sewage source type and which locations were used to generate the distributions.

Sewage Source	Description	Log10 HF183 Mean, Standard Deviation (copies / 100 m L)	Log10 NoV Mean, Standard Deviation (copies / 100 mL)
Raw Influent	Untreated sewage from the head of the treatment facility	7.1, 7.0	5.2, 5.3
Final Effluent	Treatment facility effluent post-chlorination	4.7, 4.7	3.5, 3.8
Force Main	Pumpstation influent/effluent	7.8, 7.9	7.7, 8.1
Gravity Sewer Pipe	Lower collection system samples	7.8, 8.0	4.6, 5.1
Sanitary Sewer Overflow	Wet weather collection system samples	7.4, 7.3	4.7, 5.2
Sewer Lateral	Upper collection system samples	7.8, 8.1	3.4, 3.8
All Data Pooled	All data sources Pooled	7.6, 7.9	6.9, 7.7

Table 3

HF183:NoV ratios across sewage sources. Values indicate the mean and standard deviation HF183:NoV in samples collected from each source.

Sewage Source	Mean HF:NoV Ratio	SD
Lateral	1.26×10^5	2.46×10^5
Gravity	8.24×10^4	1.91×10^5
SSO	4.18×10^4	7.44×10^4
FM	$7.42 \times 10°$	1.67×10^1
RWI	2.22×10^2	3.13×10^2
Final	5.88×10^1	1.09×10^2

Table 4

HF183 risk-based thresholds for force main (FM), treatment plant effluent (FNE), treatment plant influent (RWI), sanitary sewer overflows (SSO), gravity pipe sewage (Gravity), sewer lateral sewage (Lateral) and all sources combined.

Log10 HF183 (copies 100mL^{-1})	Risk-Based Threshold
FM	1.83
FNE	2.48
RWI	3.42