Microbial Source Tracking in Sparkill Creek: Increasing evidence supporting contributions of human sewage contamination beyond background levels of fecal indicators in Sparkill Creek.

Study Conducted by: Lawrence Vail, James Elling and Gregory O'Mullan

Summary

This project continues a preliminary Molecular Source Tracking (MST) study completed in 2020 that found evidence of human fecal contamination in multiple areas of the Sparkill watershed particularly after wet weather. Tests include a cultivation based fecal indicator bacteria (FIB) determination commonly-used to screen for contamination and a genetic based MST test that is specific for bacterial contamination related to human sources. The main objectives for the 2021 project are: 1) *to examine the contaminant level in **stormwater runoff** from road surfaces into the creek and,* 2) *to look more closely at an area in the **upper watershed*** where human fecal contamination was found in 2020***.*** Despite the elevated levels of fecal indicators, stormwater samples collected at street level during wet weather did not have detectable levels of human specific fecal bacteria. Five wet weather samples were collected between the Marsico Court site, where previous high human signal was detected, and the upstream reservoir outlet that previously had no human signal. The area mostly had high FIB values and increasing MST readings for samples collected further downstream. However, on one sample date human contamination was detected even at the most upstream reservoir site - complicating the interpretation of these results. Other exploratory work was done on the Blauvelt Arm and some of its stormwater sources. Taken as a whole the data strongly supports the presence of human fecal waste as a component of the FIB signal near Marsico Ct. Examination of possible septic contamination or malfunctioning of sewer lines near the pump station are possible next steps along with additional MST testing.

Introduction

Over the last decade, community scientist monitoring has demonstrated widespread fecal contamination in Sparkill Creek using EPA approved cultivation-based enumeration of the Fecal Indicator Bacteria (FIB), enterococci. These cultivation methods do not differentiate human from animal sources of contamination, making the optimization of management/mitigation efforts to reduce contamination difficult. In 2020, a preliminary Molecular Source Tracking (MST) study was conducted to validate the use of human specific MST methods in this system and to determine if there was any evidence from MST approaches for human contributions within the broader fecal contamination signal established using traditional cultivation based monitoring in the creek. The 2020 data provided general validation for two MST assays with both detecting high levels of human signal in positive control samples (human sewage samples from Orangetown) and no detection in negative control (sterile water) samples. The 2020 data also provided evidence of relatively widespread human contamination (detection, at least once, at 5 of 6 sites tested) within the creek and increasing range (greater number of sites with human detection) and increasing concentration of human fecal detection in wet weather compared to dry weather conditions. Although fecal indicators were found at all sites, human detection did not occur at some sites (e.g. Tackamack South Park) even during wet weather. It must be noted that these data do not suggest that other sources or fecal contamination are absent or that human signals are primarily responsible for the broader fecal indicator signal, only that human contamination appears to be present as a component of the larger fecal signal at some sites in the creek.

The 2021 Project:

To build upon this prior effort, we selected one (HF183, the method with higher detection levels) of the two MST assays used in 2020 to conduct a follow up study in 2021. In 2021, we had two main objectives: 1) *to examine the level of human signal in* ***stormwater runoff*** *from road surfaces into the creek as a possible non-point source contribution of fecal contamination* and; 2) *to confirm a sub-set of areas where human sewage signal was detected in the* ***upper watershed*** *with an intent to constrain possible inputs that could be targets for management follow up*. The concept was that the upper watershed may have source patterns that were easier to differentiate due to less development and less opportunity for transport from upstream sources and from a confluence of tributaries with mixed sources. Data from 2021 demonstrate that while stormwater runoff from road and parking lot surfaces do contain high levels of traditional fecal indicators, there were not detectable levels of human fecal contamination in the tested stormwater. Stormwater runoff does significantly contribute to the broader, non-human, background signal of fecal indicators found in prior monitoring data but these MST data suggest that the human fecal signal in the creek is likely occurring primarily from subsurface sources or specific point (pipe) sources rather than being widespread in stormwater runoff. The 2021 data also provide evidence for a common human fecal signal in the upper watershed near Marsico Court following rain events and identifies an area that would benefit from additional management attention to search for subsurface inputs of fecal contamination to the creek, including the possibility of septic influence in a region where municipal sewer lines are now available. These data did not clearly identify a point source but do provide an area of interest worthy of management follow up.

Results and Discusson:

<u>*FIB in stormwater runoff-*</u>

Four samples, three in June and one in October, of street-water were collected during rainfall from runoff moving across impervious surfaces in the watershed before the water had the chance to enter a storm drain or runoff directly into the nearby creek. These samples represent recent rainfall that interacts with possible contaminants that have accumulated on the impervious surface or were carried from adjacent vegetated areas by the rainwater. This type of stormwater contributes to the extra volume in the creek following rainfall and is one, widely distributed, pathway for contaminants, including fecal contamination, to enter the creek. While it might seem unlikely that this type of stormwater input could the carry significant fecal waste, prior studies in similar environments have demonstrated that the levels of fecal indicators can be quite high in the stormwater from suburban, urban and agricultural areas (Montero and O'Mullan, 2018; Sidhu et al 2012; Parker et al 2010). This pattern of elevated FIB was also found in the subset of stormwater samples examined in this study with enterococci values ranging from 474 to >24,196/100ml. This confirms that stormwater can carry significant levels of FIB into the creek and is one mechanism accounting for the elevated indicator levels found in the creek after rainfall.

Despite the elevated levels of fecal indicators, these samples did not have detectable levels of human specific fecal bacteria (Table 1). These FIB may come from animal sources (e.g. bird droppings) or from background levels of the indicator that have become naturalized on street surfaces or adjacent vegetated areas and are mobilized in stormwater following rain. These data are important to understanding patterns of FIB in the creek and confirm that not all sources of FIB to the creek are an indication of human fecal waste contamination. Although prior data (MST data from 2020) indicate that levels of human specific fecal waste increase in the creek following rainfall, there are also sources of FIB (including stormwater) that do not appear to be linked to human waste and contribute the overall elevated levels of FIB in the creek found following rain. These data, together with the lack of human detection at many sites during dry weather (in MST data from the 2020 study) despite detectable FIB in these same samples, indicate that the FIB signal from prior monitoring data include widespread FIB from a non-human origin. When combined with the elevated human signal detected following rainfall at some sites (MST data from 2020), these data suggest that FIB levels in the watershed come from a complex mixture of human and non-human sources. Therefore, caution must be used in interpreting the traditional FIB data to understand that not all FIB signal is an indication of human fecal input. In fact, half of the stormwater samples had FIB levels at the maximum detection level (at the assay dilution level most commonly used in prior monitoring), suggesting that even the highest detections of FIB are not an indication, by itself, of a human contribution to the monitoring data signal. Although animal sources, common in stormwater, are thought to have a lower health risk than waters containing human fecal contamination (Soller et al 2010; Soller et al 2015), even animal sources may require management action. These data also reinforce the importance of including MST information when sources are of FIB are not easy to determine.

Table 1: Stormwater runoff fecal bacteria sampling in the Sparkill Creek watershed.

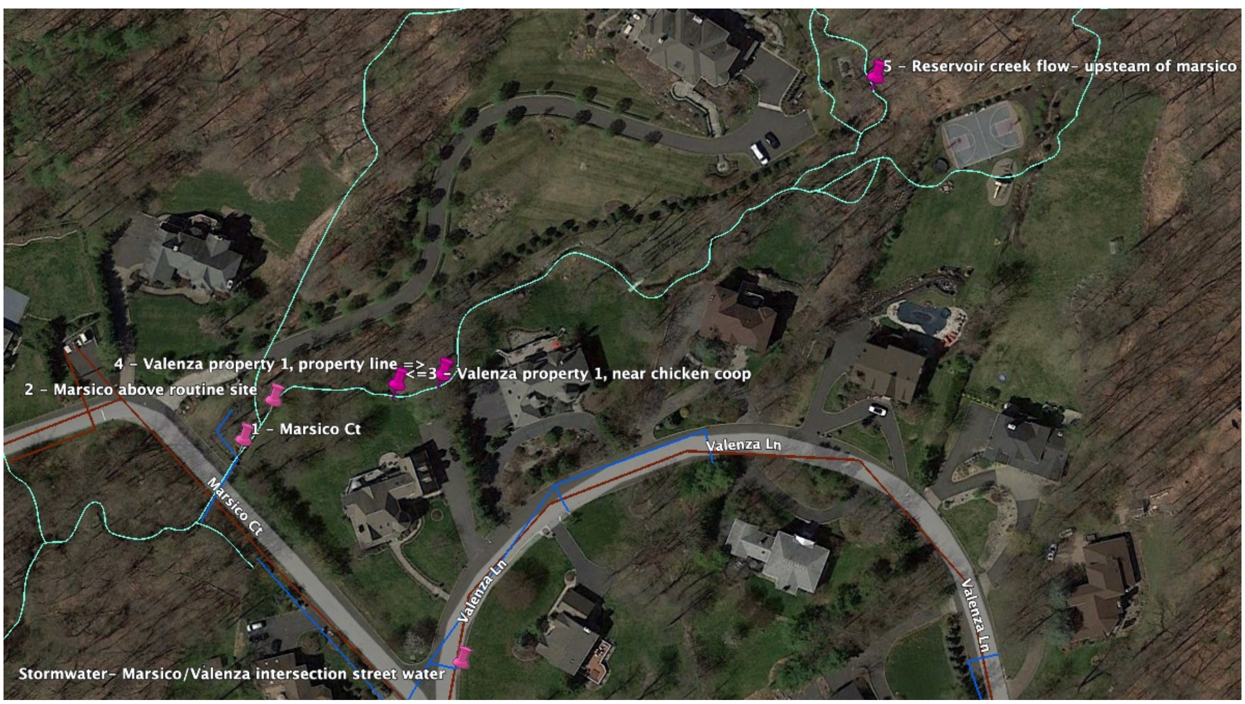
Sample site	**Sample type**	**Latitude/ Longitude**	**Date sampled**	**ENT / 100ml**	**HF183 gene copies/100ml**
Stop & Shop parking lot	Street water	41.040375°, -73.946616°	6/4/21	474	Not detected
Stop & Shop parking lot	Street water ?	41.040375°, -73.946616	10/26/21	1500	Not detected
South Greenbush Road	Street water	41.052192° -73.944697°	6/4/21	>24,196	Not detected
Marsico Ct/ Valenza Ln intersection	Street water	41.0658°, -73.9400°	6/4/21	>24,196	Not detected

<u>*MST confirmation of human-specific fecal waste in the upper watershed-*</u>
In 2020, no human fecal signal was detected at our site in Tackamack South Park under any of the conditions sampled (3 samples combining wet and dry conditions) despite the consistent, although often low, presence of traditional FIB. This suggests that locations with very limited human development at the site or above the site are likely to be free of human signal but not entirely free of FIB. In contrast, the 2020 data provided evidence, after rainfall, of human contamination in the Creek adjacent to Marsico Court, an area of the upper watershed where the creek pass through a few residential yards and where there is a nearby sewer pumping station. Over the period of 2012-2019 routine monitoring at this site had an enterococci geometric mean of 552 colony forming units/100ml, more than 5 times the federal guideline for safe recreational contact. Although most sites lower in the watershed had higher geometric means, these long term data indicate that the creek adjacent to Marsico Court is worthy of additional management attention. Therefore this site had both long term elevated FIB levels and recent preliminary evidence (2020) of human contamination after rainfall. This area became a focus in 2021 sampling to confirm detection of a human fecal signal and to attempt to constrain the area where it may be most concentrated or entering the creek. To be clear, this is not expected to be the most concentrated human signal in the watershed. This area was targeted for sampling because of its location in the upper watershed where fewer human sources are likely (and therefore they may be easier to identify and correct; a useful place to begin more concentrated MST sampling) and the creek just above this area passes through mostly undeveloped forested land where human signal would not be expected (similar to Tackamack South Park).

Eleven new samples, on three sampling dates in 2021, were analyzed in the upper watershed near Marsico Court (Table 2; Figure 1). All 2021 sampling was conducted following significant precipitation because that was the condition in which the highest detection occurred in 2020 sampling. The first sampling for 2021 occurred on 6/4/21 and included samples at the routine monitoring site adjacent to Marsico Court and upstream where a nearby reservoir feeds the creek and was expected to be above most likely sources of human contamination or at least just upstream of currently occupied residential neighborhoods. On this sampling date (6/4/21, light green in Table 2) there was a quantifiable human signal detected at the creek site adjacent to Marsico Court, but no human signal detected upstream where the reservoir feeds into the creek. This result matched expectations based on 2020 MST sampling. The enterococci levels on this date were typical for a wet weather day at the routine Marsico monitoring site and decreased upstream near the reservoir. Site 1 has a series of small input pipes, thought to be stormwater, entering the creek that might contribute to the human signal. There is a nearby sewer pump station and there are houses bordering the creek between sites 1 and 5. To follow up on this initial 2021 sampling, additional sites were added between sites 1 and 5 for the next sampling (8/19/21, light blue in table 2) in an attempt to determine if the human signal could be detected above the routine monitoring site, and in an attempt to constrain the spatial location of possible inputs. August 19 received heavy rainfall (4" or more overnight in Tappan) and the creek was unusually turbid on this day. The enterococci FIB signal on 8/19 was much higher than on the prior (6/4) event, reaching maximum FIB levels (>24,196/100ml) for the dilution used at all four locations. In contrast to the prior wet weather sampling events, human signal was not detected at the routine Marsico sampling site and was only detected (but not quantifiable) just upstream (site 3).

A final wet weather sampling event was conducted on 10/26/21 (light aqua in table 2) in an effort to better constrain the potential human inputs to this area. Five sites were sampled during another major rain event (at least 5/8” prior to and continuing through sampling), in this case including substantial roadway and backyard flooding. FIB levels were again near maximum detection and in human signal was detectable at all five locations (quantifiable at sites 1 and 3). Unlike the prior sampling events, human contamination was detected even at the most upstream site - complicating the interpretation of these results. It is possible that the extreme precipitation and surface flooding may have altered flow paths creating detectable human signal even at the most upstream location, but it is also possible that some rarely detected human source enters the creek near the reservoir. In combination the 2020 and 2021 sampling demonstrate a strong case for human fecal inputs near Marsico Court despite the relatively low density of development. This signal is most common between sites 1 and 3. Additional management attention to consider the possible presence of septic connections or influence from the nearby pumping station seem appropriate in this area, as the number of possible sources would seem to be quite limited.

Figure 1: Aerial view of Marsico Court Sampling Area



From 2016 Google Earth image. GPS locations determined from Google Earth.

Table 2: Upper watershed MST 2021 sampling near Marsico Court.

Table is shaded to highlight the three 2021 wet weather sampling events (6/4.21 in light green; 8/20/21 in light blue; 10/26/21 in light aqua). The Marsico Area Site 1 being most downstream and 5 most upstream) correspond to the sites in Figure 1. MST data is color coded as: red = human signal detected and quantified; orange = detected but not quantifiable; and green = not detected.

Sample site	Marsico Area Site #	Sample type	Latitude/ longitude	Date sampled	ENT / 100ml	Human specific MST: HF183 gene copies/100ml
Marsico Ct, routine monitoring site	1	Creek	41.066412°, -73.940717°	6/4/21	1334	8.18×10^2
Creek flowing from Reservoir upstream of Marsico Ct	5	Creek	41.067359°, -73.938527°	6/4/21	146	Not detected
Marsico Ct, routine monitoring site	1	Creek	41.066412°, -73.940717°°	8/19/21	>24,196	Not detected
Behind Valenza Property 1, near Chicken Coop	3	Creek	41.066557°, -73.940185°	8/19/21	>24,196	Detected, Not Quantified
Behind Valenza, between property 1 and 2	4	Creek	41.066582°, -73.940029°	8/19/21	>24,196	Not detected
Creek flowing from Reservoir upstream of Marsico Ct	5	Creek	41.067359°, -73.938527°	8/19/21	>24,196	Not detected
Marsico Ct, routine monitoring site	1	Creek	41.066412°, -73.940717°°	10/26/21	24,196	7.78×10^2
Marsico Ct, just above routine monitoring site- above stormwater pipes	2	Creek	41.066515°, -73.940613°	10/26/21	15,531	Detected, Not Quantified
Behind Valenza Property 1, near Chicken Coop	3	Creek	41.066557°, -73.940185°	10/26/21	14136	9.11×10^2
Behind Valenza, between property 1 & 2	4	Creek	41.066582°, -73.940029°	10/26/21	24,196	Detected, Not Quantified
Creek flowing from Reservoir upstream of Marsico Ct	5	Creek	41.067359°, -73.938527°	10/26/21	19,863	Detected, Not Quantified

Results from other 2021 sampling sites-
In 2020, most sites sampled in the lower watershed following precipitation were found to have detectable human signal, including the Clausland Arm between South Greenbush Road and and NY Rte 303. In 2021, on 8/19 following heavy precipitation, both the Clausland and Blauvelt arms of the Sparkill Creek were tested just upstream of their confluence. FIB were near maximum detection in both samples and human fecal signal was detected, but not quantified, consistent with human sources upstream in each arm of the creek. An exploratory sample was collected upstream at one of the sources of the Blauvelt Arm from a pipe entering a drainage culvert near the Blauvelt Library. This pipe had an FIB signal at maximum detection but no human MST signal was detected in this sample. These limited data are consistent with 2020 data, showing widespread human signal in the creek lower in the watershed following wet weather.

Table 3: Other 2021 MST samples collected in the Sparkill Creek watershed.

Sample site	Sample type	Latitude/ Longitude	Date sampled	ENT / 100ml	HF183 gene copies/100ml
Clausland Arm	Creek	41.05433, -73.94515	8/19/21	>24,196	Detected, Not Quantified
Blauvelt Arm	Creek	41.05440, -73.945136	8/19/21	19,863	Detected, Not Quantified
Blauvelt Library Culvert pipe	Pipe input to culvert	41.059118° -73.956451°	8/19/21	>24,196	Not detected

Summary Conclusions and Management Relevance:
These data suggest that stormwater input, likely without human fecal contributions in most locations, contributes to the widespread FIB signal in the creek. This high background level of FIB makes it difficult to use FIB data alone to identify possible human fecal sources. However, the MST sampling does suggest that human fecal waste contributes to the FIB signal at many locations in the watershed. Locations in the upper watershed, such as the creek near Marsico Court and Valenza Lane, where human signal is generally lacking at upstream sites and where there are fewer possible sources of human input provide a more constrained location to begin management efforts. Our data strongly support the presence of human fecal waste as a component of the FIB signal near Marsico Ct. It is expected that this human signal occurs as a component of a broader non-human FIB signal and therefore even if management actions remove the human waste inputs, it is not expected that traditional FIB monitoring data will drop to acceptable geometric mean levels. However, examination of possible septic contamination or malfunctioning of sewer lines near the pump station are possible next steps now that a human contribution has been clearly indicated.

In the lower watershed there appear to be widespread human inputs for example in both the Clausland and Blauvelt Arms before their confluence. Although MST data will be useful to test individual inputs, it is anticipated sampling in the creek will be more difficult to interpret due to a high likelihood of multiple upstream human inputs. As the concentration of human-specific fecal waste is expected to vary considerably across sampling events due to variable dilution, and human signal will be present at most sites lower in the watershed, MST sampling would likely require a high level of sampling from single events to allow interpretation of MST concentration data as a source identification tool. MST sampling of individual pipe inputs would still have management value and regional investigations of sewer leakage may be useful next steps given the widespread human signal in the watershed.

Sediment is known to contain high levels of FIB throughout the region in fecal impacted waterways (O'Mullan et al 2019) and in small volume systems like Sparkill Creek there is extensive water and sediment interaction that has the potential to influence FIB dynamics in the creek water. As another future step, MST approaches could be used to examine whether human fecal signal is retained in the sediment, which could then act as a reservoir (potentially resuspended during the higher flow following rainfall). Other components of the system, such as groundwater could also be tested to better understand the routes of contaminant delivery to Sparkill Creek.

Brief Description of Sampling and Analytical Methods:
Sampling and analytical methods are briefly described below. More complete methods are provided in the 2020 report. The Sparkill Creek watershed is located in southeastern Rockland County, NY and a small portion of Bergen County, NJ. The creek flows through a twelve square mile watershed of parkland, suburban and low density industrial/commercial landscapes before entering the Hudson River via a tidal wetland at Piermont NY. The creek is listed on the New York State Priority Waterbody List of stressed streams (NYS-DEC, 2013; USEPA 2020). Riverkeeper and SCWA have monitored enterococci concentrations, utilizing EPA approved IDEXX Enterolert cultivation-based methods, at twelve to sixteen sites since 2011 (Vail, 2015; Riverkeeper 2019). This MST study collected samples using gloved hand or sampling pole, into autoclave sterilized 250 or 1000 ml polyproplene bottles, triple rinsed with creek water before final sample collection, and immediately placed into an opaque ice filled cooler until processing. FIB negative control samples were included for each sampling date and consisted of an autoclaved sterile water sample that was transferred into a sample bottle in the field and handled in parallel to creek water samples. MST positive and negative control samples were included with the 2020 samples. FIB enumeration and filtration for MST occurred within six hours of collection for all samples. Enterococci were enumerated using the IDEXX Enterolert variant of EPA method 1600 (US-EPA, 2009), including a 1/10 dilution in sterile water of each creek sample and a negative (sterile water only) control with each sampling date, as previously described in Young et al (2013). The MST samples (60-200ml) were vacuum filtered onto sterile 0.45 um polycarbonate membranes, using sterile technique to handle samples, filtration funnels and membranes, and immediately following filtration membranes were transferred into 2ml sterile cryotubes and frozen before overnight shipping for DNA extraction and qPCR analysis.

DNA extraction and MST qPCR were performed at Source Molecular Corp (Miami Lakes, FL) in 2020 and at LuminUltra (which acquired Source Molecular) in 2021, an ISO 17025 accredited testing laboratory, using assays based on EPA Method 1696 (HF183; EPA, 2019). For each sample, DNA was extracted from filters using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), eluted in 100µl of sterile water. MST qPCR assays were run on duplicate reactions using 2µl of extract as template. An Applied Biosystems StepOnePlus real time thermocycler (Applied Biosystems, Foster City, CA) was used for qPCR assays with a final reaction volume of 20µl. For each batch of qPCR results assay controls including negative (no template), positive (positive control plasmid added), and a dilution series of calibration curve samples (to determine limits of detection and amplification efficiency) were included. For the purposes of this 2021 MST data set, samples with none, or only one, of the replicates positive (positive meaning fluorescence signal above background in the qPCR assay) are reported as "*No Detection" (ND)*; samples with both replicates positive but outside the range of quantification (generally meaning a quantitative cycle (Cq) above 34) are reported as "*Detected, Not Quantified" (DNQ)*; while samples with both non-diluted and replicates positive within the range of quantification (generally a Cq below 34) are reported as "*Detected and Quantified" (DQ)* and the number of gene copies per 100ml of creek water is reported based on extrapolation from the calibration curve. Samples in the "detected, not quantified" categories are considered to be low level detection near the minimum detection level of the assay.

Acknowledgements:

The authors would like to thank the Rockland County Soil and Water Conservation District for providing funds to conduct this study.

References:

Montero, A. and G. O'Mullan. 2018. Street Runoff as a Source of Fecal Indicator Bacteria to Urban Embayments. Section VIII: 1-38 pp. In S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2017. Hudson River Foundation.

O'Mullan, G.D., Dueker, M.E. and Juhl, A.R. 2017 Challenges to managing microbial fecal pollution in coastal environments: Extra-enteric ecology and microbial exchange among water, sediment, and air. *Current Pollution Reports*, 3(1), 1-16.

O'Mullan, G.D., A. Juhl, R. Reichert, E. Schneider, and N. Martinez. (2019) Patterns of sediment-associated fecal indicator bacteria in an urban estuary: benthic-pelagic coupling and implications for shoreline water quality. Science of the Total Environment 656:1168-1177.

Parker, J.K., D. Mcintyre, and R.T. Noble. 2010. Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA. Water Research 44:4186-4184.

Riverkeeper, 2019. Sparkill Creek Community Water Quality Monitoring Results 2012-2019. Available at: https://www.riverkeeper.org/wp-content/uploads/2020/05/2019-SPARKILL-Entero-Report-RIVERKEEPER.pdf

Sidhu, J.P.S., L. Hodgers, W. Ahmed, M.N. Chong, and S. Toze. 2012. Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia. Water Research 46: 6652–6660.

Soller, J.A., M.E. Schoen, T. Bartrand, J.E. Ravenscroft, and N. Ashbolt. 2010. Estimating human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research 44: 4674-4691.

Soller, J.A., T. Bartand, J. Ravenscroft, M. Molina, G. Whelan, M. Schoen, and N. Ashbolt. 2015. Estimated human health risks from recreational exposures to stormwater runoff containing animal faecal material. Environmental Modelling and Software 72:21-32.

U.S. Environmental Protection Agency 2019A. Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 Taqman Quantitative Polymerase Chain Reaction (qPCR) Assay. Environmental Protection Agency Office of Water 821-R-19-002.

U.S. Environmental Protection Agency 2020. National Summary of Impaired Waters and TMDL Information, available online at:
https://iaspub.epa.gov/waters10/attains_nation_cy.control?p_report_type=T#causes_303d

Vail, L. 2015. Sparkill Creek- Citizen Science Pathogen Indicator Project
https://www.hudsonriver.org/wp-content/uploads/2017/12/SCWA_CS_FinalReport.pdf