January 2021 Update
NC Policy Collaboratory, UNC Hurricane Florence Response Team

Jill Stewart[1] and Rachel Noble[2]

[1]Department of Environmental Sciences & Engineering, Gillings School of Global Public Health
[2]Institute of Marine Sciences, University of North Carolina at Chapel Hill

Executive Summary: Flooding and storm surges associated with extreme events are associated with an increased risk of infection for the public. In the State of North Carolina, this risk can be compounded by compromised stormwater and sewage conveyance systems, as well as compromises in poultry and hog fecal waste lagoons. The major risk factors for outbreaks associated with flooding are 1) contamination of drinking-water systems, including both municipal and private drinking water wells[1], 2) exposure to floodwater and consumption of foods contaminated by floodwaters, and 3) legacy contamination of food, shellfish and recreational waters. There is an increased risk of infection of water-borne diseases contracted through direct contact with polluted waters, such as wound infections.

In order to advance our ability to mitigate the risks associated with floodwaters, it is vital to prioritize such risks through the use of quantitative analysis tools. Fortunately, a few laboratories across the State of North Carolina, including the laboratories of Drs. Stewart and Noble, have devoted efforts to developing the capacity for direct pathogen measurements in wastewater, stormwater, hog lagoon wastewaters, recreational waters, drinking water, and shellfish. ***This project was originally designed to conduct storm-associated sampling to begin to characterize the sources of fecal material and pathogens present in floodwaters.*** The project demonstrated the capacity for quantification of pathogens associated with flooding versus background levels of microbial contaminants in water systems, and began the process of understanding the distribution and duration of impacts associated with flooding events. A summary of our overall research findings are:

- Precipitation-related effects on concentrations of *E. coli* and other fecal indicator bacteria are not limited to large hurricane events but can occur across a range of precipitation events, particularly when groundwater levels are high and antecedent rainfall creates saturated soils.
- There is merit in conducing direct measures of bacterial and viral pathogens as well as antimicrobial resistance to supplement fecal indicator bacteria analysis to identify locations with pathogen hazards and to better characterize risks to human health.
- In order to develop mitigation strategies for microbial contaminants from flooding, it will be vital to quantify sources of fecal contamination. At this juncture, both human and hog waste are implicated in floodwaters from different sampling events.
- Flooding can be associated with increased levels of pathogens and antimicrobial resistance in surface waters, with effects lasting for days to weeks.

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[1] see collaborative work of Sozzi, Stewart, Noble and others as part of NSF RAPID
Rapid: Impact of Hurricane Florence on Drinking Water Safety in Eastern and Central North Carolina: Rapid Assessment and Recommendations for Recovery and Resilience, May 2021

- Risks from microbial hazards including pathogens and antimicrobial resistance are higher in locations proximal to human and animal wastewaters. This is a concern in North Carolina in areas with food animal production that use waste lagoon systems and in municipalities with aging and decaying wastewater infrastructure.
- Septic, package treatment plant, and low resource sewage infrastructure deserve further review for their potential impacts on human health from the perspectives of drinking water and shellfish consumption.
- Of particular interest to the research being conducted as part of this program is that both laboratories have now invested in molecular analysis equipment that permits real-time, fully quantitative analysis for a range of viral pathogens important to public health concerns, including SARS-CoV-2 and related variants. The application of this technology moving forward will permit NC to lead an advanced set of studies on the impacts of flooding and extreme events.

Research Methods: We conducted sample collection during and in response to Hurricane Florence. Samples included for this work were collected from selected locations in the Lumber, Cape Fear, and Neuse river basins (Figure 1). These basins include almost 4 million NC residents, over 2,000 confined swine operations, over 1,600 confined poultry operations, and almost 25,000 miles of NC rivers and streams, with an estimated 1.4 million residents who likely depend on on-site septic systems for wastewater management. These locations were highlighted for sample collection due to the precipitation patterns from Hurricane Matthew, the complex contributions of wastes from multiple (human and hog) sources, and concerns about contamination of private drinking water wells. Over 250 samples were collected and analyzed in response to extreme events for microbial contaminants. The project team is building a database for selection of pathogen response variables that will allow the development of a new monitoring system, as well as future predictive models. Our research currently includes regulatory targets such as total coliforms, fecal coliforms, and *E. coli*, as well as other targets of public health concern including antibiotic resistant bacteria, HF183 human fecal marker, *Vibrio* sp., somatic and male-specific coliphages, *Campylobacter* sp., *Salmonella* sp. and respiratory and gastrointestinal viral pathogens.

Figure 1: Sampling locations for post-Florence sampling events
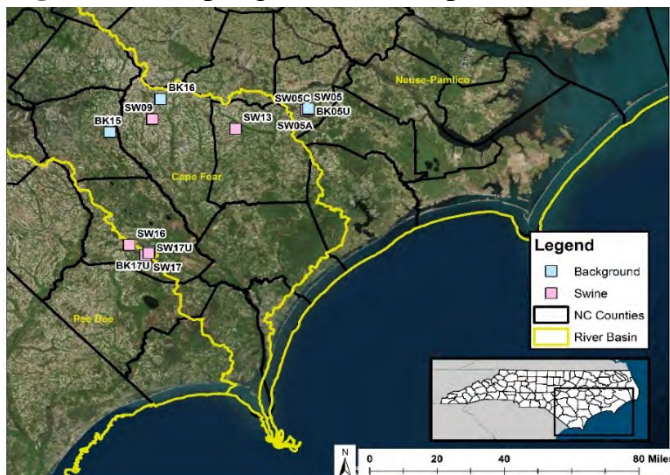
All samples were analyzed for fecal indicator bacteria *E. coli* using standard membrane filter techniques, or standard IDEXX enzymatic substrate techniques or both. Male-specific (F+) coliphage and somatic coliphage were quantified using vetted, published single-agar overlay approaches. Salmonella sp. concentrations were determined using vetted, published culture-based approaches. Salmonella sp., HF183, and Pig-2-Bac quantification for species and pathogenicity islands were completed following molecular approaches published in Steele et al. 2018 and XXXXX et al.

E. coli (CFU/100mL)
E. coli IDEXX (MPN/100mL)
Total Coliforms IDEXX (MPN/100mL)
F+ coliphage (PFU/100mL)
Somatic coliphage (PFU/100 mL)
Salmonella (MPN/100mL)
Salmonella ttr (copies/100mL)
Salmonella invA (copies/100mL)
Campylobacter (copies/100mL)
HF183 (copies/100mL)
Pig-2-bac (copies /100mL)

Table 1: Mean and 95% confidence interval (CI) for outcomes measured and observed precipitation for four sampling dates post-Hurricane Florence. BD= below detectable limit; NA=not applicable; TBD=to be determined, outcomes not yet assessed in laboratory

Measurement	9/24/2018		11/14/2018		4/24/2019		9/18/2019	
	Mean	CI	Mean	CI	Mean	CI	Mean	CI
Prior Precipitation: 48 h (inches)	0	0	1.5	1.0 - 2.1	0	0	0.004	0 – 0.014
Prior Precipitation: 7 days (inches)	0.04	0.02 - 0.06	2.3	1.9 - 2.7	1.3	1.0 - 1.6	0.2	0.06 - 0.2
E. coli (CFU/100mL)	650	0 - 1482	816	233 – 1,399	112	59 - 164	NA	NA
E. coli IDEXX (MPN/100mL)	779	0 - 2010	734	274 – 1,194	105	58 - 152	469	27 - 911
Total Coliforms IDEXX (MPN/100mL)	>24,196[1]	NA	15,753	9,885 – 21,621	11,423	6,852 – 15,994	19,896	15,606 – 24,186
F+ coliphage (PFU/100mL)	17	0 - 43	13	1 - 25	3.4	2 - 5	0.17	0 – 0.5
Somatic coliphage (PFU/100 mL)	145	0 - 408	169	20 - 319	26	0 – 68	58	0 – 135
Salmonella (MPN/100mL)	5.8	2.9 - 8.7	1.8	1.8 - 1.8	3.2	1.8 - 4.5	28	0 – 63
Salmonella ttr (copies/100mL)	BD	BD	BD	BD	BD	BD	BD	BD
Salmonella invA (copies/100mL)	BD	BD	BD	BD	BD	BD	BD	BD
Campylobacter (copies/100mL)	2,063	0 – 4,225	788	249 – 1,327	TBD	TBD	TBD	TBD
HF183 (copies/100mL)	1,130	0 – 3,287	301	174 - 428	TBD	TBD	TBD	TBD
pig-2-bac (copies /100mL)	17,652	0 – 54,555	21	5 – 37	TBD	TBD	TBD	TBD

[1] All samples above detectable limit

Table 2: Mean and 95% confidence interval for measured outcomes among sprayfield-impacted and background sites among post-Florence sampling events

| Measurement	n Sample Dates	n Samples	Sprayfield-impacted Sites		Background Sites		p-value
			Mean	95% Confidence Interval	Mean	95% Confidence Interval	
E. coli (CFU/100mL)	3	36	401	119 – 682	776	33 – 1144	0.36
E. coli IDEXX (MPN/100mL)	4	48	658	208 – 1108	249	81 – 826	0.17
Total Coliforms IDEXX (MPN/100mL)	4	48	18,880	16,219 – 21,542	15,690	11,394 – 23,177	0.20
F+ coliphage (PFU/100mL)	4	48	9.1	0.3 – 18	7.8	0 – 17.5	0.39
Somatic coliphage (PFU/100 mL)	4	48	135	33 – 237	30	0 – 165	0.0095*
Salmonella (MPN/100mL)	4	48	7.7	1 – 14	13.5	0 – 28.5	0.48
Campylobacter (copies/100mL)	2	24	1,826	365 – 3,288	623	106 – 2,343	0.12
Salmonella ttr (copies/100mL)	4	48	BD[1]		BD		
Salmonella invA (copies/100mL)	4	48	BD		BD		
HF183 (copies/100mL, ddPCR)	2	24	226	139 – 313	1,693	0 – 3,088	0.54
pig-2-bac (copies /100mL, qPCR)	2	24	13,251	0 – 37,925	8	5 – 13,253	0.007*

[1]BD= below detectable limit; *significant difference between swine and background sites p<0.05 for Mann-Whitney non-parametric test for difference in mean rank

Table 3: Number, percent, and relative risk of *E. coli* isolates with observed resistance to tested antibiotics

	Background sites # isolates	Sites close to lagoons # isolates	Relative risk (95% CI)
Number of *E. coli* isolated	356	556	
Resistant to at least one class of antibiotics	20 (6%)	81 (15%)	2.59 (1.62 – 4.15)
Resistant to at least two classes of antibiotics	0 (0%)	11 (2%)	n/a
Resistant to at least three classes of antibiotics (multi-drug resistance)	1 (0.3%)	14* (2.5%)	8.96 (1.18 – 67.9)

*Eleven isolates were also positive for beta-lactamase production that confers resistance to a wide variety of penicillin and cephalosporin drugs. These bacteria are classified as extended-spectrum beta-lactamase (ESBL) *E. coli* and are listed as a serious threat by the US CDC.[41] We do not know the pathogenicity of these particular isolates.

Figure 1: 48-hour prior precipitation on *E. coli* concentration at sites sprayfield-impacted (SW) and background (BK) sites: SW17, SW17U, BK17U (top) and sites SW05, SW05A, SW05C, BK05U (bottom) at sampling events and after Hurricane Florence
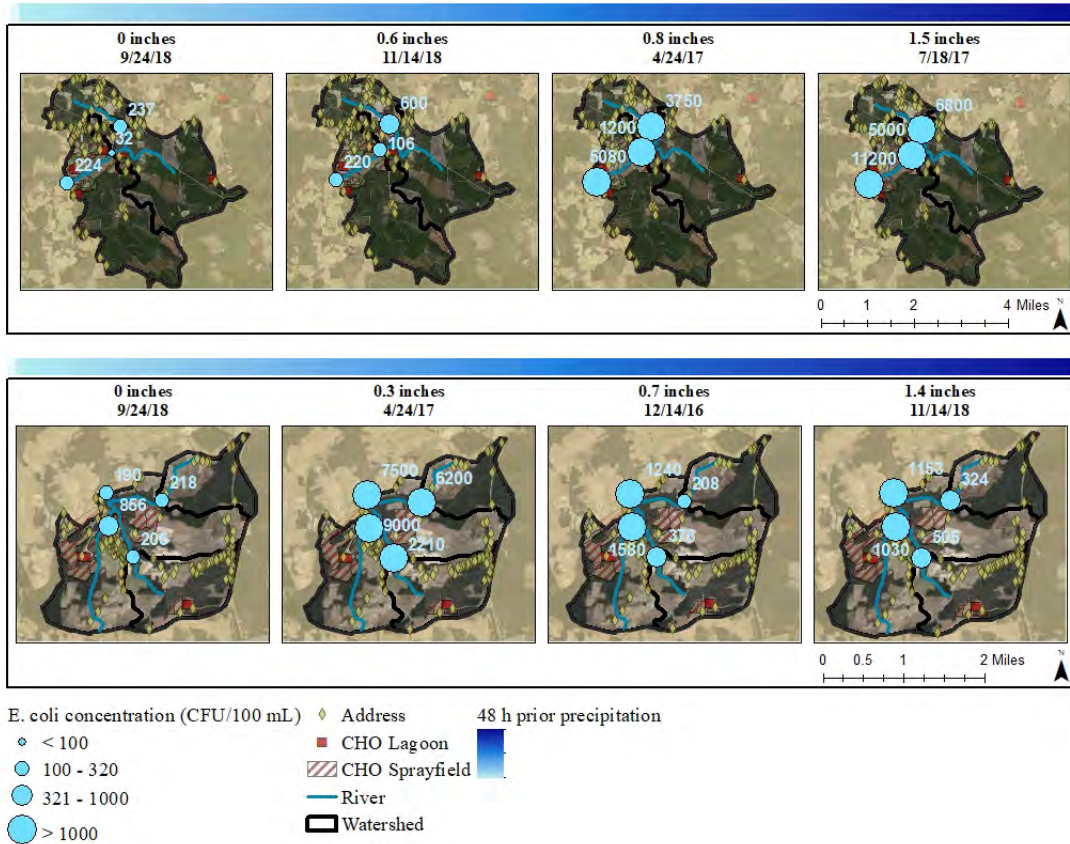
Findings:

- Although this is a pilot project with relatively few sampling events characterized, there is a trend toward higher concentrations of molecular markers and microbial contaminants in floodwaters immediately following Hurricane Florence, than during a non-storm impacted period.
- Our findings indicate that both human fecal and hog fecal contamination was prevalent in the floodwaters following Hurricane Florence. This is cause for serious concern, because these sources of fecal contamination would be expected to pose a serious risk to the public.

Recommendations:

(1) Surveillance and response
- **Now that we have developed advanced techniques for high throughput characterization and quantification of microbial contaminants in light of the COVID-19 pandemic, we recommend a stratified random sampling approach to characterize floodwaters and non-flood impacted waters with priority devoted to assessing the relative impact of sewage and hog fecal material on relevant systems.**
- **Improved system characterization of pathogens will benefit from a simultaneous assessment of the reports of GI pathogen, wound infection and floodwater related exposures and risks by analysis of the NC DETECT emergency room data.**

(2) Accurately quantifying pathogens and genes is a first step towards understanding whether water sources pose risks to human health. Especially during extreme events, it is important to efficiently determine health risks.
- *Vibrio***:** This is a pathogen with outcomes in the NC Surveillance System (e.g. NC DETECT). We are working on improving methods for quantifying the known virulence sub-strains of *Vibrio* that can cause infection which may be more useful than past approaches to quantify *Vibrio* at the species level.
- **Antimicrobial resistance genes:** We are hoping to use more advanced molecular diagnostic approaches for quantification of combinations of antimicrobial resistance genes that may be more indicative of risk to humans.

(3) Stakeholder engagement: It is certain that engagement with relevant stakeholders, including municipal wastewater utilities, hog and livestock farming operations, and county health departments will permit a further assessment of the risks posed by floodwaters. Thankfully, we have already accomplished much of this stakeholder relationship building with WWTP utilities through the COVID-19 pandemic, so working with them to address flooding and flood/stormwater resilience strategies will be a valuable next phase of effort.