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RESEARCH ARTICLE

Assessment of pathogens in flood waters in coastal rural regions: Case study after Hurricane Michael and Florence

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Abstract

The severity of hurricanes, and thus the associated impacts, is changing over time. One of the understudied threats from damage caused by hurricanes is the potential for cross-contamination of water bodies with pathogens in coastal agricultural regions. Using microbiological data collected after hurricanes Florence and Michael, this study shows a dichotomy in the presence of pathogens in coastal North Carolina and Florida. *Salmonella typhimurium* was abundant in water samples collected in the regions dominated by swine farms. A drastic decrease in *Enterococcus spp*. in Carolinas is indicative of pathogen removal with flooding waters. Except for the abundance presence of *Salmonella typhimurium*, no significant changes in pathogens were observed after Hurricane Michael in the Florida panhandle. We argue that a comprehensive assessment of pathogens must be included in decision-making activities in the immediate aftermath of hurricanes to build resilience against risks of pathogenic exposure in rural agricultural and human populations in vulnerable locations.

Introduction

Hurricanes cause significant economic damage [1], traditionally measured in terms of loss of civil infrastructure, primarily in urban locations in the United States. The vulnerability of highly dense metropolitan coastal communities to hurricanes under both current and changing climates is well-documented [2, 3]. However, an understanding of the susceptibility of rural, agricultural-dominant human communities to the short- and long-term impacts of hurricanes is still lacking [4]. Rural regions tend to receive limited financial resources and government assistance, which may decrease the resilience of these communities to recover from the effect of extreme natural events [4]. In livestock-dominant agricultural communities,

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hurricane-induced flooding poses the risk of contaminating natural water sources with run-off from lagoons and barnyards containing animal fecal material. In turn, this could increase the exposure of humans to pathogens. A handful of studies have highlighted the role of pathogens [5] in agricultural regions after hurricanes, despite the ubiquitous presence in rural communities. In 2018, there was concern that water from Hurricane Michael may overtop wastewater treatment plants and sanitary sewer systems, which may lead to contamination of drinking water supply lines [6, 7].

In 2018, the Atlantic Hurricane season was dominated by hurricanes Florence and Michael. Hurricane Florence made landfall near Wrightsville Beach, NC, as a Category 1 storm on September 14, 2019 [8]. Despite the storm's relatively low wind speeds, Hurricane Florence wreaked havoc with towering storm surges and historical rainfall [8, 9]https://weather.com/storms/hurricane/news/2018-09-15-florence-north-carolina-tropical-rain-record. Less than four weeks later, Hurricane Michael made landfall near Mexico Beach, FL, as a Category 4 hurricane [10]. Both hurricanes were destructive, but the means by which each storm caused the damage was unique. Hurricane Michael produced only a fraction of the rainfall that deluged North Carolina [11] compared with hurricane Florence in the Florida panhandle.

The lack of adequate information on the presence and prevalence of pathogens of clinical importance after hurricanes is the key motivation for the current study. Therefore, the objective here was to provide a survey assessment for the genes from key bacterial pathogens after hurricane-induced flooding in the rural coastal locations of North Carolina and Florida. Our anticipated goal is to initiate the dialogue through the characterization of the vulnerability of humans in terms of exposure to clinically significant pathogens after hurricanes.

Materials and methods

Selection of sampling locations

The first step toward accurately identifying sampling locations was to characterize the flooded regions after hurricanes. Inundation resulting from extreme rainfall can be modeled using traditional hydrological and hydrodynamic models. However, here we used a relatively new method for mapping inundation based on the landform's geomorphometric principles [12–14]. The basic premise is to let topography dictate how water will fill a particular landscape. It allows a fast but static computation and accurate identification of locations likely to be inundated after heavy rainfall. Digital elevation model (DEM) data at 30 m resolution was used to spatially capture the locations and estimate the inundation depths due to any known amount of rainfall. Details on inundation mapping are provided in previously published work [15]. Sampling locations for North Carolina and Florida were selected by mapping the flood extents of each hurricane, identifying agriculture or wastewater infrastructure exposed to flooding, and then selecting accessible water bodies downstream of the point of interest. Twenty-six locations were sampled in North Carolina on October 7, 2018 (3 weeks post-hurricane), ranging from the coast to about 100 miles inland. In the Florida panhandle, 11 locations were sampled on October 27, 2018 (2 weeks post-hurricane), with the locations tailored to investigate the impacts of storm surge on pathogen transport in coastal communities and flooded water treatment facilities, covering a geographical extent of Pensacola to Port St. Joe, Florida.

The locations of swine farms were obtained from the North Carolina Department of Environmental Quality (NCDEQ) Animal Feeding Operations Program [16]. The 2283 permitted farm locations were overlaid onto the flood map, which was then queried by flood depth. All swine farms with flood depths of five feet or greater were selected as potential sampling sites because there was high certainty about flooding at the location, resulting in the selection of 40 swine farms. This number is similar to the number arrived at by the post-hurricane report

given by the NCDEQ, in which 28 swine facilities reported lagoon discharging. An additional eight reported inundation (surface water surrounding and flowing into the lagoon), and eight more reported that lagoons were at full capacity and likely to overtop [17]. Similarly, unflooded farms were identified, and 23 locations were selected from the list of unflooded farms by visual inspection to meet two criteria. First, the unflooded location must be relatively close to flooded farms of interest, making travel and collection of samples less burdensome. Second, the flood maps must show the location as free of flooding to a high certainty (i.e., not located on or near the boundary of the flood extent). While in the field, water samples were taken at publicly accessible locations downstream and near the selected farms. The sampling locations are shown in Figs 1 and 2.

Compared to Hurricane Florence's effects in North Carolina, Hurricane Michael did not trigger inland flooding to the same depth or extent as observed in the Florida Panhandle. This, combined with the absence of a singular dominating industry at flood risk comparable to swine farming in North Carolina, led to an eclectic array of sampling locations in Florida. Furthermore, widespread road closures forced many sampling locations to be selected based on the accessibility. Water samples were taken downstream near five wastewater treatment plants

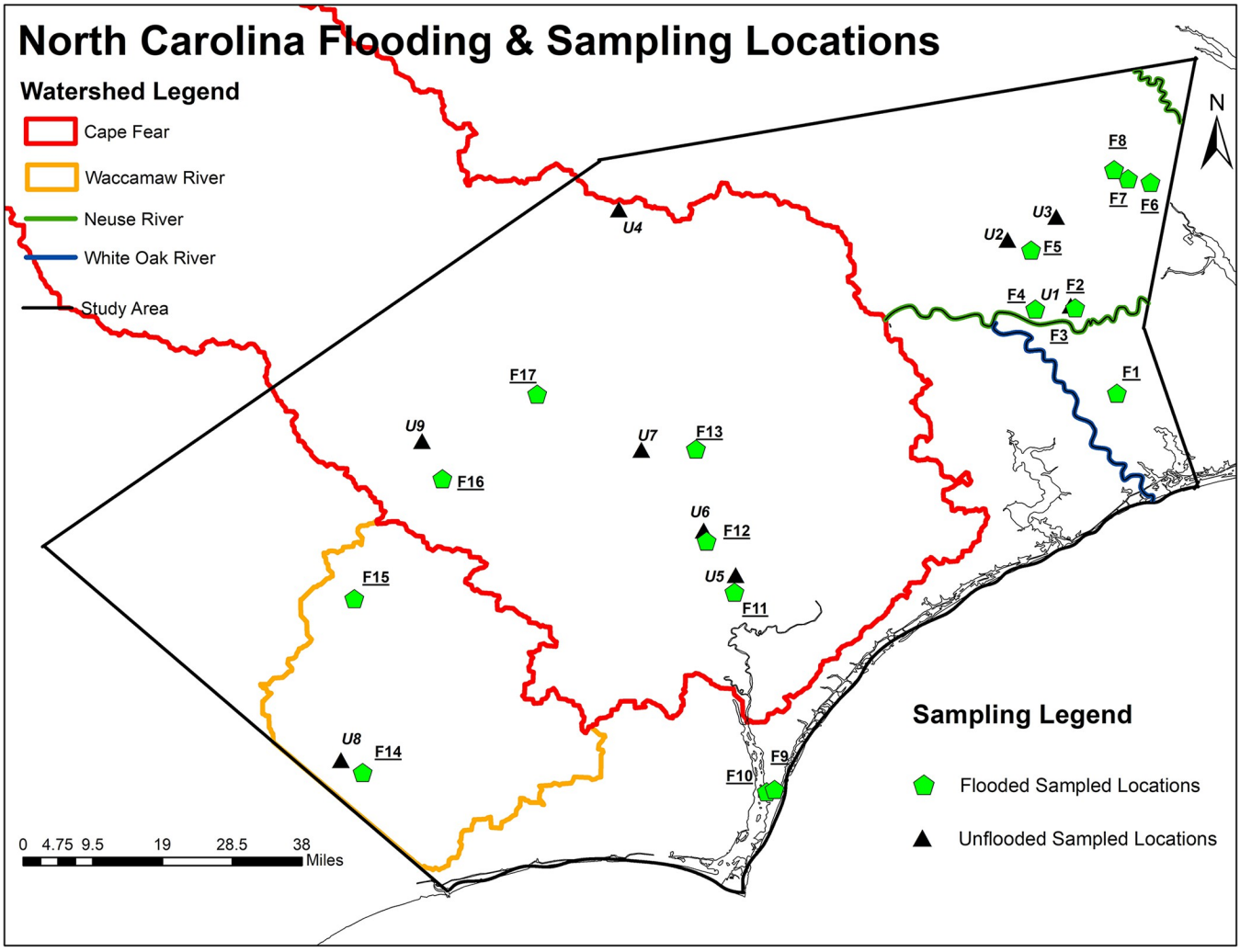

Fig 1. North Carolina flooding and sampling locations.

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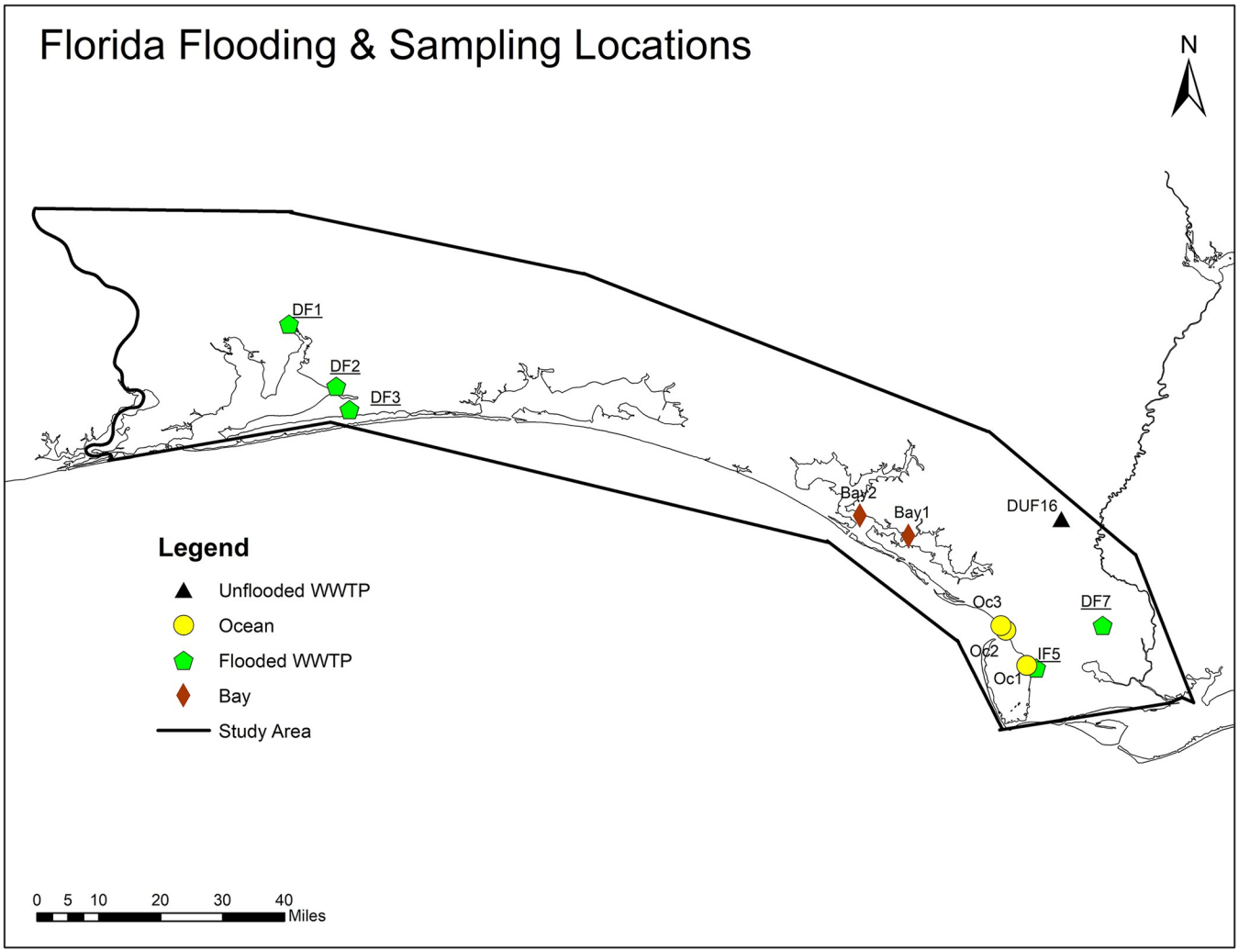

Fig 2. Florida flooding and sampling locations. DF = Domestic, Flooded WWTP. DUF = Domestic, Unflooded WWTP.

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(WWTP) [18], three in the ocean between Port St. Joe and Mexico Beach and two in the bays surrounding Panama City, FL, as seen in Fig 2.

Lab testing procedure

We sampled water in N.C. and F.L. in October 2018, post-Hurricane Florence and Michael, respectively. As with our previous fieldwork [19, 20], we used sterilized plastic bags to collect and store four to eight liters of water from each source. Bags were kept in a cooler with ice packs and transferred within 24 hours to a 4˚C cold room in our lab. The volume of sampled water was tracked when samples were filtered. Water samples were flocculated with 25 mM $MgCl_2$ for 30 minutes before settling. Subsequently, they vacuum-filtered sequentially through a glass fiber filter with a 1.6 μm pore size (Fisher Scientific, Hampton, NH) to collect and concentrate bacteria. Every time the filter was clogged, it would be changed to a new filter to continue filtering until all the water sample in the bag was filtered. The number of filters used for each sample was concluded in S1 Table. A quarter of 1.6 μm filter of all filters used for a given water sample were subjected to DNA extraction by MPI FastDNA Kit for Soil Extraction (M. P. Biomedicals, Santa Clara, CA). The rest of the filters were kept for other ongoing analyses.

Quantitative polymerase chain reaction (qPCR) [21] assays were adapted from previous studies to detect *Enterococcus spp*., two genes of *E. Coli*, Enteropathogenic *E. Coli*, Shiga-toxin producing *E. Coli*, *Shigella spp*, *Shigella flexneri*, two genes of *Campylobacter jejuni*, *Campylobacter lari*, two genes of *Salmonella typhimurium*, *Clostridium perfringens*, *Listeria monocytogenes*, two genes of *Vibrio cholerae*, *Mycobacterium* spp., *Pseudomonas* spp., *Legionella* and *Giardia lamblia* [15, 22–24]. Forward and reverse primers for all assays were obtained as Custom DNA Oligos (Integrated DNA Technologies, Coralville, IA). Probes were obtained from the Universal Probe Library (UPL) (Roche, Basel, Switzerland) and were labeled with 6-FAM at the 5' end and a dark quencher dye at the 3' end and contained a short sequence (8–9 nucleotides) of locked nucleic acids [25]. Standards were obtained as gBlock Gene Fragments (Integrated DNA Technologies). Standard curves were generated by qPCR using serial dilutions (2 x 10^0 to 2 x 10^6 copies/μl) of a standard pool containing 24 DNA standards to validate the assays prior to use in MFQPCR. PCR inhibition was evaluated for the STA and MFQPCR analysis by including *Pseudogulbenkiania* NH8B as an internal amplification control in all environmental sample extracts and nuclease-free water. Prior to enumeration by mfqPCR, all DNA samples and standard pool dilutions underwent standard target amplification (STA) PCR to increase template DNA yields. Standard pool dilutions (2 x 10^0 to 2 x 10^6 copies/μl) amplified in the 14-cycle STA were used to generate standard curves for MFQPCR. 20X assays (18 μM of each primer and 5 μM probe) were pooled using 1 μl per assay and 179 μl of DNA Suspension Buffer (Teknova, Hollister, CA) to make a 0.2X TaqMan primer-probe mix. The reaction (5 μl) contained 2.5 μl 2X TaqMan PreAmp Master Mix (Thermo Fisher), 0.5 μl 0.2X TaqMan primer-probe mix, and 1.25 μl of template DNA. The PCR plate was processed with the following thermal cycle on an M.J. Research Tetrad thermal cycler (M.J. Research, Waltham, MA): 95˚C for 10 min and 14 cycles of 95˚C for 15 sec and 60˚C for 4 min. The STA products were diluted 25-fold with100 μl of T.E. buffer and were used for mfqPCR. The sample premix (5 μl) contained 2.5 μl 2X TaqMan Master Mix, 0.25 μl 20X Gene Expression Sample Loading Reagent (Fluidigm, South San Francisco, CA), and 2.25 μl 25-fold diluted STA product. The assay mix (5 μl) contained 2.5 μl 2X Assay Loading Reagent (Fluidigm) and 2.25 μl 20X TaqMan primer-probe mix. Aliquots (5 μl) of each sample and duplicates of each assay were loaded onto a 48.48 chip (Fluidigm). mfqPCR was performed in a Biomark HD Real-Time PCR (Fluidigm) using the following thermal conditions: 70˚C for 30 min, 25˚C for 10 min, 95˚C for 1 min, followed by 35 cycles of 96˚C for 5 sec and 60˚C for 20 sec. All the genes and primers used for pathogens tested in this study are listed in S2 Table.

Quantification cycle (C_q) values and standard pool dilutions (log copies/μl) were used to generate standard curves for each assay. C_q values were determined by Real-Time PCR Analysis software (Fluidigm) and MFQPCR. Linear regression analysis was performed to fit the standard curves and calculate the goodness of fit (R^2). Assay efficiencies were calculated based on the slopes of the standard curves for each MFQPCR assay to validate adequate target amplification [26]. Standard curves were accepted as quantifiable if the efficiency achieved was greater than or equal to 90% and if the lower detection limit was less than or equal to 30 copies/μl. Consistent detection of NH8B throughout multiple assays indicates insignificant inhibition for qPCR amplification. The concentrations of detected bacterial genes were reported in the unit of gene copies per L of water sample.

Results

Water samples collected from creeks, ponds, and rivers immediately after hurricanes were tested for the presence of six common pathogenic genes [*Enterococcus spp*., *Legionella pneumophila*, Mycobacteria (atpE), Pseudomonas (gyrB), *Salmonella Typhimurium* (ttrC), *E. coli*

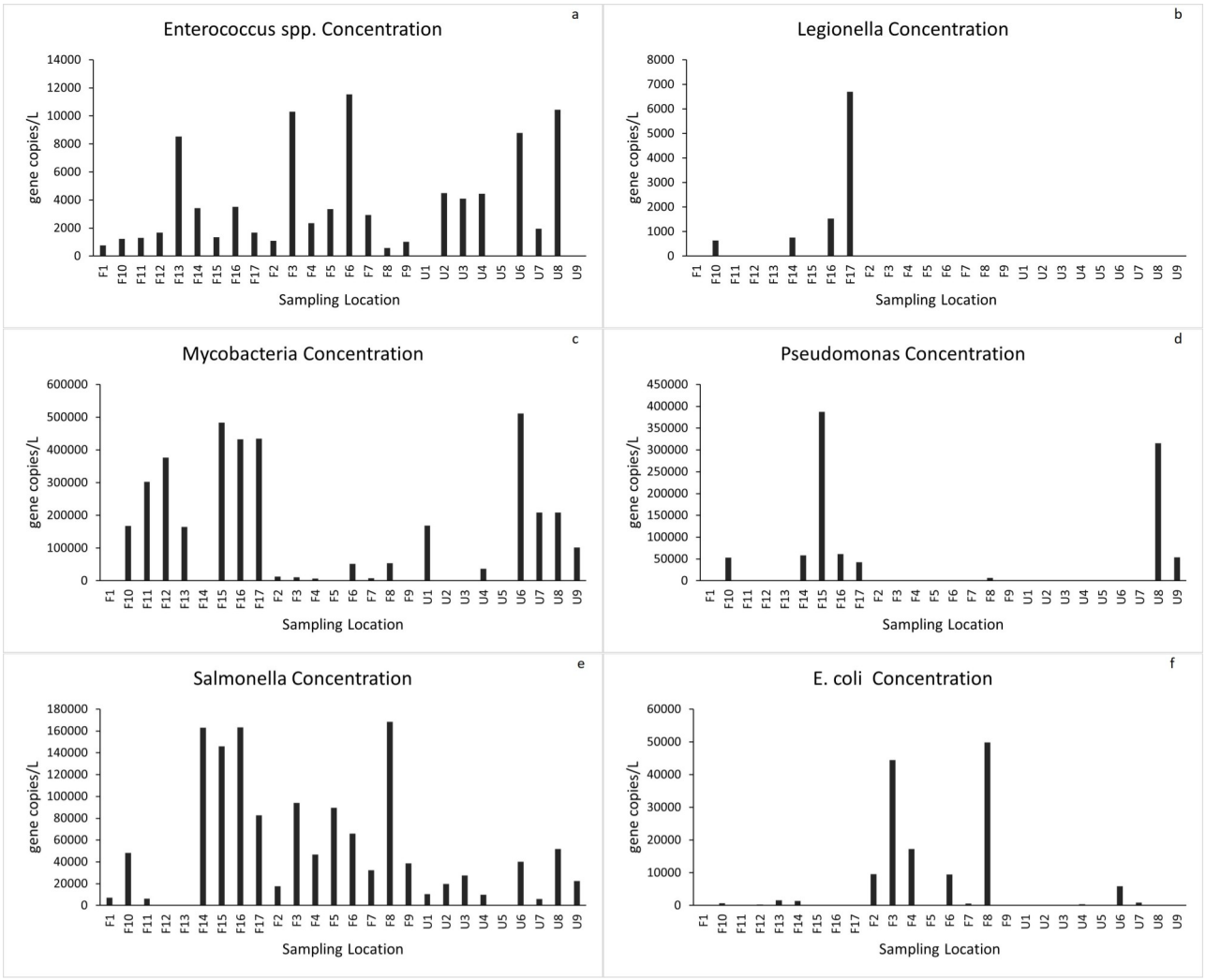

Fig 3. North Carolina max. pathogen concentrations. (a) *Enterococcus spp.* **(b)** *Legionella pneumophila*, **(c)** Mycobacteria (atpE), **(d)** Pseudomonas (gyrB), **(e)** *Salmonella typhimurium* (ttrC), **(f)** *E. coli* (eaeA, uidA, ftsZ) [F1, F2. . . F12 implies flooded location; U1 to U9 are unflooded sampling point).

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(eaeA, uidA, ftsZ)] from 26 different sampling locations in North Carolina and Florida (location in Fig 3). *Enterococcus spp*. (Fig 3A), Mycobacteria (Fig 3C) and *Salmonella typhimurium* (Fig 3E) were detected in almost all the flooded locations in high concentrations relative to the unflooded ones in North Carolina. Legionella (Fig 3B), Pseudomonas (Fig 3D), and *E. coli* (Fig 3F) were present in a few of the flooded locations in higher concentrations than the unflooded sampling points. Unlike North Carolina, there was no clear distinction in the detection of pathogens in the waters of Florida after Hurricane Florence. *Enterococcus spp* (Fig 4A), *Salmonella typhimurium* (Fig 4E), and *E. coli* (Fig 4F) were the most prevalent pathogens among the eleven sampling locations (see map in Fig 2). Presence-wise, all flooded and unflooded locations had *Salmonella typhimurium* in water system.

Flooded and unflooded sites did not have significant differences in *Enterococcus spp*. concentrations in Carolinas. However, our findings in FL showed that the abundance of *Enterococcus spp*. was less than those in the Carolinas. This may be an indication of the flushing role of flood waters resulting in low *Enterococcus spp*. concentration. This was consistent with the

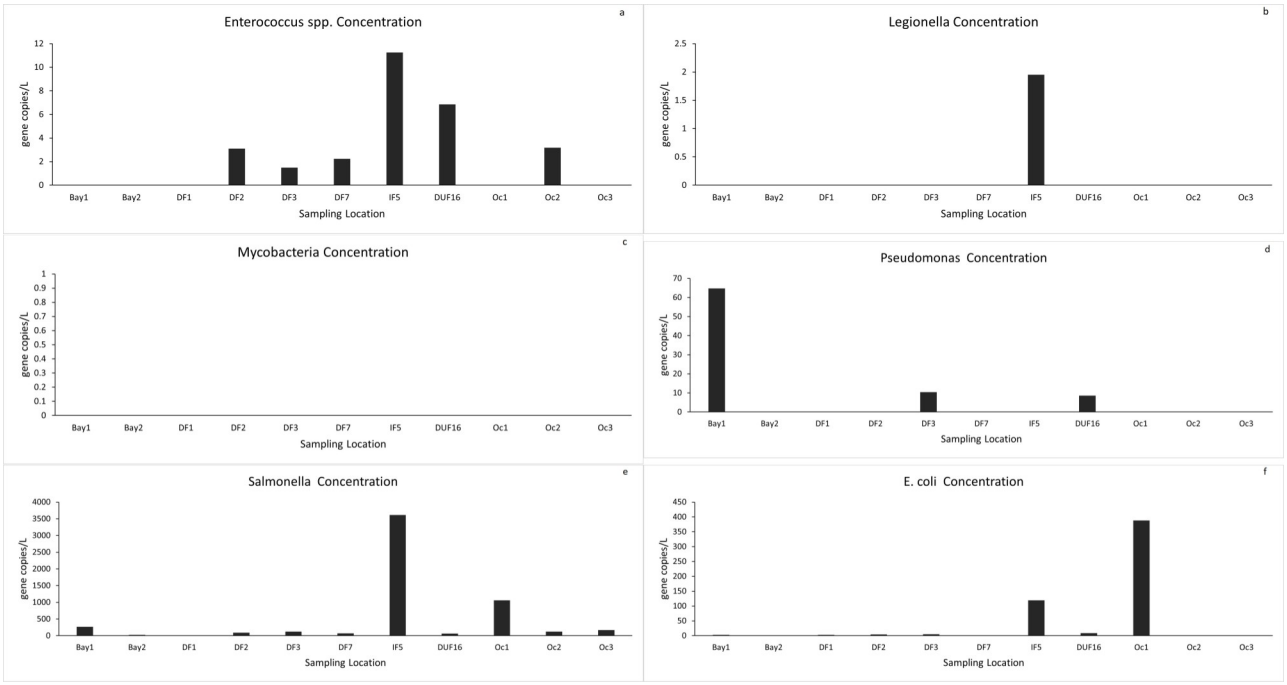

Fig 4. Maximum concentrations of pathogens in Florida. (a) *Enterococcus spp*. **(b)** *Legionella pneumophila*, **(c)** Mycobacteria (atpE), **(d)** Pseudomonas (gyrB), **(e)** *Salmonella typhimurium* (ttrC), **(f)** *E. coli* (eaeA, uidA, ftsZ) [DF = Domestic, Flooded WWTP. DUF = Domestic, Unflooded WWTP. IF = Industrial Flooded; WWTP = waster water treatment plant].

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previous study where it was observed that the fecal indicator bacteria level decreased a few weeks after the flood event when the dewatering process was done [5, 27]. In NC samples, *E. coli* concentration were observed higher in the flooded sites (F2, F3, F4, F6, and F8) than in unflooded locations. The flooded sites were located in the Neuse River watershed. The two unflooded sites with *E. coli* detected were U6 and U7 in the Cape Fear watershed.

In order to explore the impacts of floods on pathogens, a probability of exceedance analysis was performed on the samples collected from North Carolina. There appears to be no difference in the presence of Mycobacteria with inundation (Fig 5A), as the probability of non-exceedance for flooded and unflooded samples was very similar. However, there was a marked difference in the presence and detection of *Salmonella typhimurium*(Fig 5B) in the rural agricultural regions. Flooding appears to have strengthened the abundance of *Salmonella typhimurium* when the probability of non-exceedance was greater than 50%. On further examination, the odd's ratio analysis suggested that the presence of *Salmonella typhimurium* in surface water bodies increased by 2.3 times during flooding. The increased likelihood of *Salmonella typhimurium* during flooding may be attributable to cross-contamination of litter and associated swine activities, including run-off water from livestock farms. However, additional experiments are required to ascertain this observation. One of the interesting findings is with the *Enterococcus spp*. (Fig 5C) where the abundance of pathogens decreased during flooding, especially when the probability of exceedance increased to 50%. It is plausible that flooding water washed off the pathogen from its natural environment to coastal waters. This, therefore, decreased its concentration in the terrestrial surface water bodies. The odds ratio analysis suggested a three-fold decrease in this pathogen during floods following hurricanes.

A comparison between hurricane Florence and Michael is provided in Table 1. Hurricane Florence was characterized by record-breaking rainfall and inland flooding, whereas the

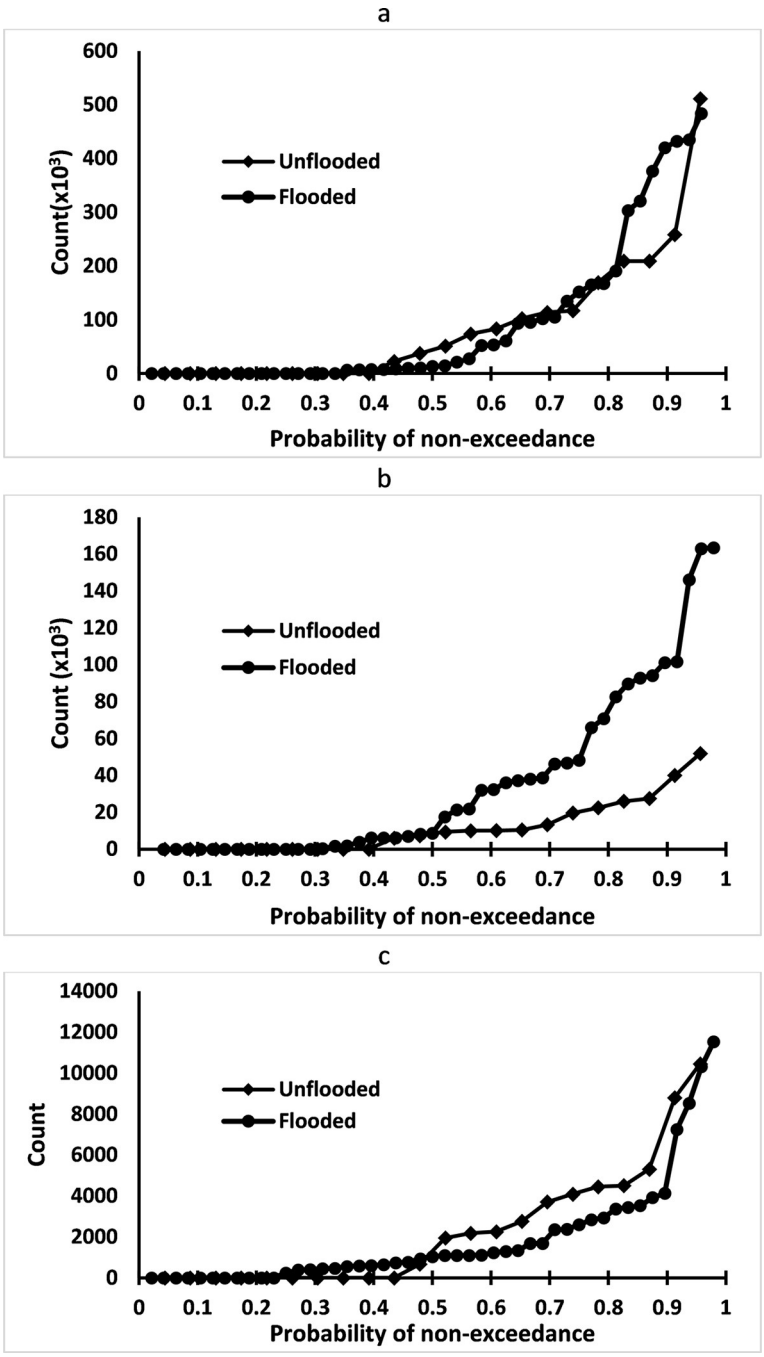

Fig 5. Probability of exceedance analysis for the samples collected from North Carolina. (a) Mycobacteria, (b) *Salmonella typhimurium* and (c) *Enterococcus spp*.

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damage caused by Hurricane Michael was attributed primarily to coastal (storm surge) flooding and wind damage. North Carolina has a massive agriculture industry dominated by swine farming, while Florida lacks a singular industry with obvious potential for introducing pathogens into surface waters. These two dissimilarities are evident in the remarkable differences in pathogen concentrations between the two study areas.

Table 1. Hurricanes Florence and Michael summary.

2018 Atlantic Hurricane	*Florence*[1]	*Michael*[3]
Landfall Date	September 14	October 10
Landfall Site	Wrightsville Beach, NC	Mexico Beach, FL
Category at Landfall	1	4
Rainfall	20–30 inches, 35 inches + local	< 7 inches
Storm Surge	9–13 feet	9–14 feet
Max. Wind	106 mph, less severe	155 mph, severe
Inland Flooding	Severe, 28 flood records broken[2]	Limitted/minor
Study Area	Coastal and Sub-coastal NC	Coast of Florida panhandle
Dominant Local Industry	Swine farming[4]	N/A

1, *Historic Hurricane Florence*, *September 12–15*, *2018*. September 2018. National Oceanic and Atmosphere Administration. web. February 28 2019. <https://www.weather.gov/mhx/Florence2018>.

2, *USGS*: *Florence set at least 28 flood records in Carolinas*. November 13 2018. web. February 28 2019. <https://www.usgs.gov/news/usgs-florence-set-least-28-flood-records-carolinas>.

3, *Catastrophic Hurricane Michael Strikes Florida Panhandle October 10*, *2018*. October 2018. web. March 3 2019. <https://www.weather.gov/tae/20181010_Michael>.

4, National Agriculture Statistics Service, Agriculture Statistics Board, United States Department of Agriculture. "Quarterly Hogs and Pigs." Quarterly Report. December 20, 2018. web. March 4 2019. <https://downloads.usda.library.cornell.edu/usda-esmis/files/rj430453j/bc386p647/rf55zc904/hgpg1218.pdf>.

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Discussion and conclusion

Using microbiological data collected after hurricanes Florence and Michael, results from this study show a dichotomy in the presence of pathogens in coastal North Carolina and Florida. *Salmonella typhimurium* was abundant in water samples collected in the regions dominated by swine farms. A drastic decrease in *Enterococcus spp*. in the Carolinas is indicative of pathogen removal with flooding waters. Except for the abundance presence of *Salmonella typhimurium*, no significant changes in pathogens were observed after Hurricane Michael in the Florida panhandle. Our results strengthen findings from previous studies where increase in human cases of infectious diseases were reported in human populations after hurricanes and include viral gastroenteritis and legionellosis in New York [28, 29]; nontuberculous mycobacteria after hurricanes in Louisiana, Florida and Oklahoma [30]; cholera in Haiti [31] and *E. coli*, Giardia, *Cryptosporidium* in New Orleans [5]. The results presented in this study, while only from two locations, are indicative of the comprehensive need for development of pathogenic libraries along the entire US coastal regions. Identification of pathogenic libraries and ecological active niches is likely to be helpful in development of protocols for mitigation and intervention strategies for infectious diseases. A recent example includes outbreak of *Vibrio vulnificus* in Florida after Hurricane Ian [32] is a stark reminder of absence of qualification of clinically important and climate processes modulated infectious pathogens in the environment.

Traditionally, studies of pathogens in floodwaters are generally reported in regions with poor water and sanitation infrastructure with known knowledge of the emergence of microbes after heavy rainfall. In the continental United States, speculative assessment of pathogens is conducted after floods from the standpoint of risk of diseases in the urban human population centers. Perhaps this is one of the few studies that has made an attempt to shed insights on the pathogenic dangers of hurricane-induced flooding in the rural agricultural region of the U.S. The agricultural livestock of the U.S. are under constant threat of changes in climatic patterns, and thus effective policies should be made to safeguard these commodities. A significant

portion of U.S. extensive livestock agriculture is located within a few hundred miles of the eastern coast (e.g., swine farms in N.C.). An increased occurrence of extreme events is likely to devastate rural economies. Therefore, the significant implications of this study include an ambitious plan to develop a database for threat assessment of pathogens in the immediate aftermath of hurricanes. Impacts of the two hurricanes along two prominent U.S. coastal regions have significant variability in the behavior of different pathogens. Hence, the predictive intelligence systems must be developed and should include information on microbes that may be prevalent in the water system after extreme events. A well-planned infrastructure plan should be in place to safeguard agricultural commodities so that pathogen spillover should be contained or anticipated in advance.

Supporting information

S1 Table. The number of filters used for each sample.
(DOCX)

S2 Table. List of genes and primers used for pathogens tested in the study.
(DOCX)

Author Contributions

Conceptualization: Moiz Usmani, Joanna Shisler, Avinash Unnikrishnan, Thanh H. Nguyen, Antarpreet Jutla.

Data curation: Moiz Usmani.

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Investigation: Nathan Bonham, Yusuf Jamal, Yuqing Mao.

Methodology: Moiz Usmani, Sital Uprety, Joanna Shisler, Avinash Unnikrishnan, Thanh H. Nguyen, Antarpreet Jutla.

Software: Moiz Usmani, Sital Uprety.

Supervision: Daisuke Sano, Joanna Shisler, Avinash Unnikrishnan, Thanh H. Nguyen, Antarpreet Jutla.

Validation: Moiz Usmani, Sital Uprety.

Writing – original draft: Moiz Usmani.

Writing – review & editing: Daisuke Sano, Joanna Shisler, Avinash Unnikrishnan, Thanh H. Nguyen, Antarpreet Jutla.

References

1. Pielke RA, Gratz J, Landsea CW, Collins D, Saunders MA, Musulin R. Normalized Hurricane Damage in the United States: 1900–2005. Nat Hazards Rev. 2008 Feb; 9(1):29–42.
2. Haines A, Kovats RS, Campbell-Lendrum D, Corvalan C. Climate change and human health: Impacts, vulnerability and public health. Public Health. 2006 Jul; 120(7):585–96. https://doi.org/10.1016/j.puhe.2006.01.002 PMID: 16542689
3. McGranahan G, Balk D, Anderson B. The rising tide: assessing the risks of climate change and human settlements in low elevation coastal zones. Environ Urban. 2007 Apr; 19(1):17–37.

4. Kapucu N, Hawkins CV, Rivera FI. Disaster Preparedness and Resilience for Rural Communities: Disaster Preparedness and Resilience. Risk Hazards Crisis Public Policy. 2013 Dec; 4(4):215–33.
5. Sinigalliano CD, Gidley ML, Shibata T, Whitman D, Dixon TH, Laws E, et al. Impacts of Hurricanes Katrina and Rita on the microbial landscape of the New Orleans area. Proc Natl Acad Sci. 2007 May 22; 104(21):9029–34. https://doi.org/10.1073/pnas.0610552104 PMID: 17488814
6. Farber M. Hurricane Michael's health risks: 3 things to watch out for [Internet]. Fox News. 2018 [cited 2019 Mar 26]. Available from: https://www.foxnews.com/health/hurricane-michael-and-health-risks-3-things-to-watch-out-for
7. Hurricane Michael—Safety and Health Advisory [Internet]. U.S. Department of the Interior. 2018 [cited 2019 Mar 26]. Available from: https://www.doi.gov/emergency/hurricane-michael-safety-and-health-advisory
8. US Department of Commerce N. Historical Hurricane Florence, September 12–15, 2018 [Internet]. National Weather Service. [cited 2019 Mar 26]. Available from: https://www.weather.gov/mhx/Florence2018
9. Erdman J. Florence Sets Preliminary North Carolina and South Carolina Tropical Cyclone Rain Records; Third, Fourth States to Do So in 12 Months [Internet]. The Weather Channel. 2018 [cited 2019 Mar 26]. Available from: https://weather.com/storms/hurricane/news/2018-09-15-florence-north-carolina-tropical-rain-record
10. US Department of Commerce N. Catastrophic Hurricane Michael [Internet]. National Weather Service. [cited 2019 Mar 26]. Available from: https://www.weather.gov/tae/20181010_Michael
11. Hurricane Michael Recap: Historic Category 4 Florida Panhandle Landfall; Swath of Wind Damage and Flooding Into the Carolinas, Mid-Atlantic [Internet]. The Weather Channel. 2018 [cited 2019 Mar 26]. Available from: https://weather.com/storms/hurricane/news/2018-10-11-hurricane-michael-recap-gulf-coast-southeast
12. Bolch T, Peters J, Yegorov A, Pradhan B, Buchroithner M, Blagoveshchensky V. Identification of potentially dangerous glacial lakes in the northern Tien Shan. Nat Hazards. 2011 Dec; 59(3):1691–714.
13. Clubb FJ, Mudd SM, Milodowski DT, Valters DA, Slater LJ, Hurst MD, et al. Geomorphometric delineation of floodplains and terraces from objectively defined topographic thresholds. Earth Surf Dyn. 2017 Jul 10; 5(3):369–85.
14. Sofia G, Fontana GD, Tarolli P. High-resolution topography and anthropogenic feature extraction: testing geomorphometric parameters in floodplains: LIDAR DTMS AND ANTHROPOGENIC FEATURE EXTRACTION. Hydrol Process. 2014 Feb 15; 28(4):2046–61.
15. Mao Y, Zeineldin M, Usmani M, Uprety S, Shisler JL, Jutla A, et al. Distribution and Antibiotic Resistance Profiles of *Salmonella enterica* in Rural Areas of North Carolina After Hurricane Florence in 2018. GeoHealth [Internet]. 2021 Feb [cited 2022 Aug 12]; 5(2). Available from: https://onlinelibrary.wiley.com/doi/10.1029/2020GH000294
16. NC DEQ: Animal Facility Map [Internet]. North Carolina Environmental Quality. 2019 [cited 2019 Mar 26]. Available from: https://deq.nc.gov/cafo-map
17. NC DEQ: DEQ Dashboard [Internet]. North Carolina Environmental Quality. 2018 [cited 2019 Mar 26]. Available from: https://deq.nc.gov/news/deq-dashboard#animal-operations—swine-lagoon-facilities
18. Wastewater Facility Information | Florida Department of Environmental Protection [Internet]. [cited 2019 Mar 26]. Available from: https://floridadep.gov/water/domestic-wastewater/content/wastewater-facility-information
19. Sadik NJ, Uprety S, Nalweyiso A, Kiggundu N, Banadda NE, Shisler JL, et al. Quantification of multiple waterborne pathogens in drinking water, drainage channels, and surface water in Kampala, Uganda, during seasonal variation. GeoHealth. 2017 Aug; 1(6):258–69. https://doi.org/10.1002/2017GH000081 PMID: 32158991
20. Uprety S, Hong PY, Sadik N, Dangol B, Adhikari R, Jutla A, et al. The Effect of the 2015 Earthquake on the Bacterial Community Compositions in Water in Nepal. Front Microbiol. 2017 Dec 6; 8:2380. https://doi.org/10.3389/fmicb.2017.02380 PMID: 29270153
21. Diviacco S, Norio P, Zentilin L, Menzo S, Clementi M, Biamonti G, et al. A novel procedure for quantitative polymerase chain reaction by coamplification of competitive templates. Gene. 1992 Dec; 122 (2):313–20. https://doi.org/10.1016/0378-1119(92)90220-j PMID: 1487146
22. González-Escalona N, Hammack TS, Russell M, Jacobson AP, De Jesús AJ, Brown EW, et al. Detection of Live *Salmonella* sp. Cells in Produce by a TaqMan-Based Quantitative Reverse Transcriptase Real-Time PCR Targeting *invA* mRNA. Appl Environ Microbiol. 2009 Jun; 75(11):3714–20.
23. Ishii S, Segawa T, Okabe S. Simultaneous Quantification of Multiple Food- and Waterborne Pathogens by Use of Microfluidic Quantitative PCR. Appl Environ Microbiol. 2013 May; 79(9):2891–8. https://doi.org/10.1128/AEM.00205-13 PMID: 23435884

24. Liu J, Gratz J, Amour C, Kibiki G, Becker S, Janaki L, et al. A Laboratory-Developed TaqMan Array Card for Simultaneous Detection of 19 Enteropathogens. J Clin Microbiol. 2013 Feb; 51(2):472–80. https://doi.org/10.1128/JCM.02658-12 PMID: 23175269

25. Mouritzen P, Noerholm M, Nielsen PS, Jacobsen N, Lomholt C, Pfundheller HM, et al. ProbeLibrary: A new method for faster design and execution of quantitative real-time PCR. Nat Methods. 2005 Apr; 2 (4):313–6.

26. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin Chem. 2009 Apr 1; 55(4):611–22. https://doi.org/10.1373/clinchem.2008.112797 PMID: 19246619

27. LaMontagne MG, Zhang Y, Guillen GJ, Gentry TJ, Allen MS. Hurricane Harvey Impacts on Water Quality and Microbial Communities in Houston, TX Waterbodies. Front Microbiol. 2022 Jun 14; 13:875234. https://doi.org/10.3389/fmicb.2022.875234 PMID: 35774461

28. Gaither JB, Page R, Prather C, Paavola F, Garrett AL. Impact of a Hurricane Shelter Viral Gastroenteritis Outbreak on a Responding Medical Team. Prehospital Disaster Med. 2015 Aug; 30(4):355–8. https://doi.org/10.1017/S1049023X15004872 PMID: 26132579

29. Greene SK, Wilson EL, Konty KJ, Fine AD. Assessment of Reportable Disease Incidence After Hurricane Sandy, New York City, 2012. Disaster Med Public Health Prep. 2013 Oct; 7(5):513–21. https://doi.org/10.1017/dmp.2013.98 PMID: 24274131

30. Honda JR, Bernhard JN, Chan ED. Natural Disasters and Nontuberculous Mycobacteria. Chest. 2015 Feb; 147(2):304–8.

31. Jutla A, Whitcombe E, Hasan N, Haley B, Akanda A, Huq A, et al. Environmental Factors Influencing Epidemic Cholera. Am J Trop Med Hyg. 2013 Sep 4; 89(3):597–607. https://doi.org/10.4269/ajtmh.12-0721 PMID: 23897993

32. DOH. DOH-LEE REMINDS PUBLIC OF POTENTIAL RISKS ASSOCIATED WITH VIBRIO VULNIFICUS [Internet]. Lee county; 2022 Oct [cited 2023 Jan 23]. Available from: https://lee.floridahealth.gov/newsroom/2022/10/100320228.html