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Sent: Thursday, October 20, 2016 12:17 AM
To: Strockbine, Nancy (CDC/OID/NCEZID) <nas6@cdc.gov>
Cc: Karwowski, Mateusz P. (CDC/OID/NCEZID) <ydh4@cdc.gov>; Shawn Patrick McElmurry <s.mcelmurry@wayne.edu>; Terese Olson <tmolson@umich.edu>; Paul Evan Kilgore <paul.kilgore@wayne.edu>; Matt Seeger <matthew.seeger@wayne.edu>; Garcia, George <gagarcia@med.umich.edu>; masten@egr.msu.edu
Subject: Request for assistance with critiquing our analysis of samples from Flint

Dear Dr. Strockbine:

I found your contact information on the web and believe you may have heard about me from Dr. Matt Karwowski, who is currently assisting with the Shigellosis outbreak assessment in Flint, Michigan. I am writing to ask for your assistance, through critiquing our analytical approach to selected samples (72) that were collected across point-of-use (PoU) filters deployed in Flint Michigan in July 2016, which coincides with the spike in Shigellosis in the city and surrounding county. To date, we understand that no correlation between drinking water and Shigellosis has been made. We also understand that a source for the outbreak has also not been identified. At the same time, the baseline levels of Shigellosis seem to be somewhat elevated relative to normal baselines. Finally, there are multiple and unusual risk factors associated with management and status of the infrastructure in Flint's distribution system that make this assessment worth exploring.

Dr. Terri Olson and I have a National Science Foundation-supported grant to look at how point-of-use filters deployed in Flint affect the microbial water quality of drinking water. Dr. Shawn McElmurry at Wayne State University and Susan Masten at Michigan State University have a complementary grant to evaluate the chemistry across these same filters. Our three institutions have partnered to take field samples together and share data. This work follows on work we have been doing at U-M on a separate NSF grant that affirms work shown by many others that PoU filters enhance microbial abundance in waters that pass through the filter. Our work, conducted initially on water from Ann Arbor, also shows that PoU filters change the microbial community and actually enhance the abundance of Mycobacteria in filtered effluents (we are still assessing whether the opportunistic pathogens species are enhanced).

Our RAPID grant for Flint is focused on identifying best management practices for using the filters, and the frequency that cartridges should be replaced (assuming it could be more frequent than manufacturer recommendations) based on both microbiological and chemical data. We considered the changes in microbial composition across the filters as part of our assessment. It was in the process of doing this analysis that we obtained results that indicated the possible presence of Shigella in the water.

Independent of the shigellosis outbreak in Flint, we had detected possible Shigella or E. coli through a high level biomolecular screen (16S Illumina sequencing) in some samples from our July sampling events. The levels were extremely low and below what we would normally consider significant. In fact, in discussing the results with a bioinformatics expert, his assessment was that the samples could represent contamination at the core lab that ran our samples (in case samples were run before ours that included human fecal waste and some residual DNA interfered with our run). Nevertheless, the presence surprised us since we never saw that taxa in all our Ann Arbor-based samples. We also have very significant Enterobacteriaceae hits (up to 21% relative abundance in one sample) that we have not yet evaluated by PCR as we have focused our time on the Shigella/E. coli hits (and we never detected Enterobacteriaceae in Ann Arbor water either). Given the shigellosis outbreak in Flint and the high abundance of contamination risk factors from an infrastructure perspective, we felt obligated to further assess these samples using more targeted analyses. We engaged Dr. George Garcia on our campus, who has studied virulence factors in Shigella. Together, we decided to pursue a method that targets ipaH (see attached paper) using PCR (George's lab) and qPCR (Terri's and my lab), followed by running gels and sequencing bands by Sanger sequencing. We have run samples multiple times, anticipating

negative results so that we could emphatically say that Shigella was not detected when we reported back to residents. However, we continue to see unexpected results with ipaH positives, albeit at levels below typical infectious doses (estimated at around 2 cells per 2L water sample by the qPCR method outlined in the attached paper and applied to a subset of samples). Before we discuss with the county and state public health officials, we want to open up a discussion and critique of our approach with your group, since you have substantive expertise with Shigella/EIEC detection. We believe we have been diligent and careful with all our analyses. At the same time, my expertise is not Shigella and we are not used to working with agents that have such low infectious doses. We hope that you and others in your lab will listen to what we've done, look at our results and assess whether further analysis is warranted (and, if so, what method), or indicate why our method may not be valid. We are committed to getting the most accurate and truthful information to the citizens of Flint and, frankly, could use your help to this end. Furthermore, we have limited DNA and do not want to compromise the availability of those samples. I note that we have not analyzed all the biofilms that grow on the PoU fabric filters, which have much higher biomass amounts and are environments largely devoid of disinfectant residual since the residual is destroyed on the activated carbon block surface within the PoU device.

We would be happy to set up a webinar with you, appropriate members of your lab, and our team, and give you a presentation of our results. I believe we could do this as early as this Friday morning. We prefer to keep this a highly technical discussion, after which we will share our results with the city, public health officers in Michigan involved in this case, and community organizers to frame how to discuss results with the homeowners whose homes we sampled.

We thank you, in advance, for your assistance.

Nancy

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