COVID-19 Part 2
Another Special Edition of Abstracts from the Northwest Biosolids Library
Discussion by Sally Brown, Ph.D., University of Washington
April 15, 2020

I promise that the next library will be something light and entertaining. Biosolids-based soil blends perhaps or how biosolids can save the world. Right now, my obsession -- one likely shared by many of you -- is still on this virus. Every day I check for the daily case counts in King County and Washington State (gradually decreasing I am happy to report). We are all still stuck at home and all still concerned about our own health and safety. And, as residual professionals, still wondering how waste and wastewater plays into this. The first library I did on the virus was about corona viruses in general. Now there is some information out there on this virus specifically. So here is what we know.

First article -- this is primarily a respiratory virus. But not totally a respiratory virus. Diarrhea is one of the lesser reported symptoms. The article -- a short summary of findings -- points out that gastrointestinal symptoms are present to varying degrees. More importantly, they note that rectal swabs or samples of fecal material can test positive for the virus. They refer to a number of cases where fecal samples continued to test positive even after nasal swabs were negative. In other words, fecal oral route of transmission is viable with COVID- 19. Yet another reason to wash your hands.

The second article details progression in 9 patients in Germany who had relatively mild cases of the virus. Sampling began as symptoms presented. The authors took repeated swabs and samples from the throat, spit and as fecal samples. The virus was present in high number in the throat in initial sampling. They did not find live virus in the stools. They conclude that respiratory spread is the big concern here but note that they had a limited sample size and that only one of the patients that they sampled had diarrhea. So not a clear result but indications that fecal transmission is possible if not common. Also important here is that the virus can be detected in fecal material. That is the introduction to the next three articles. These articles focus on detection of the virus in wastewater.

We've seen articles before where the authors suggest that wastewater can be used as a window onto society. For example, testing wastewater for illegal drugs has been suggested as a way to determine how wide spread drug use is in a community. It can also be used as a way to measure the presence of disease in a community – termed wastewater-based epidemiology (WBE). These next three articles focus on whether wastewater can be used to detect how widespread the COVID-19 virus is in communities. No work yet on viability of the virus or how long viable copies of this particular virus persist in wastewater -- Ian Pepper is likely hard at work as I write. Even so, this is still of interest.

The third article comes from the Netherlands. The authors used RT- PCR (reverse transmission polymerase chain reaction) to look for fragments of the actual protein as well as for the envelope. They looked in wastewater from 7 cities as well as from the airport. That is one big

airport and that was back in the day when planes were still flying. They found no samples on February 6th, three weeks prior to the first positive case in the Netherlands (February 27th). By March 5th, they detected portions of the virus in 5 of the sampled sites. By the 15th of March, that had increased to 6 sites with other portions of the virus also detected. The authors suggest that monitoring wastewater is a potential means to monitor for the presence of the virus in communities.

The fourth article does just that in Boston. The authors refer to previous work where WBE has been used to track disease spread in different communities. They collected wastewater from the central plant in Boston, using influent samples collected before anyone had ever heard of COVID-19 as a control. They used RT-qPCR (real time) to test for fragments of the virus -- remember here this is a technique to study the presence of portions of the virus, not viability of those fragments. There were no hits from samples collected before the first known US case of the virus. However, all 10 samples collected from March 18-25th tested positive. They do a fascinating calculation to back track the detection in wastewater to estimate the number of people infected:

> "Nonetheless, we can estimate an abundance based on the lowest observed values across these samples of ~10 copies/mL. If we assume typical stool sizes of 200g, diluted into an average volume of $1.36*10^9$ L, and a population of $2.3*10^6$ individuals each producing one stool per day, and we further assume that there is no loss of viral RNA in sewer lines and that excreted viruses are fully suspended in sewage, then we expect the viral titer in feces to be about 3000 times higher than that in sampled raw sewage, or about 30,000 particles per mL."

In order to really get this right, they would need to understand how much virus shows up in the poop and if that number changes over the course of infection. They noted that one study found as many as 600,000 viral genomes in each mL of poop. By their estimates, about 5% of Boston was infected -- much higher than the 0.026% of cases in Massachusetts confirmed using tests. If instead they used a higher rate of viral genomes in the poop, they get an estimate of 0.1% positive -- much closer to the reported rate of infection. If we have a good idea of quantity of virus in fecal material, this could be a really powerful tool to test for outbreaks. Talk about source control.

The final paper in the library talks about the potential to use a simpler test to detect the virus in wastewater. That would really make this an excellent tool. They have a list of different diseases that have been detectable using rapid, easy systems in wastewater. Rotavirus A uses a special piece of paper and the naked eye to get a positive. Others like human papillomavirus use specially developed papers and smart phones. Conclusions here are that we wastewater types may be part of a solution to contain the virus. That sounds pretty sweet to my ears.

NEW ITEMS IN THE NBMA RESOURCE LIBRARY

Covid- 19 Part 2 April 2020

TITLE: CoVID-19: faecal–oral transmission?

Author: Hindson, J.

Source: Nature Reviews Gastroenterology & Hepatology. 2020 https://doi.org/10.1038/s41575-020-0295-7

Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes coronavirus disease 2019 (COVID-19), first emerged in China in December 2019 and has now spread worldwide, with a reported 351,731 confirmed cases and 15,374 deaths as of 23 March 2020 according to John Hopkins University. The infection is typically characterized by respiratory symptoms, which indicates droplet transmission. However, several case studies have reported gastrointestinal symptoms and/or evidence that some patients with SARS-CoV-2 infection have viral RNA or live infectious virus present in faeces, which suggests that another possible route might be faecal–oral transmission.

Document#: WWT.VIR.5.11

TITLE: Virological assessment of hospitalized patients with COVID-2019

Author: Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier et al.

Source: Nature 2020 https://doi.org/10.1038/s41586-020- 2196-x

Abstract: Coronavirus disease 2019 (COVID-19) is an acute respiratory tract infection that emerged in late 2019[1,2]. Initial outbreaks in China involved 13.8% cases with severe, and 6.1% with critical courses3. This severe presentation corresponds to the usage of a virus receptor that is expressed predominantly in the lung[2,4]. By causing an early onset of severe symptoms, this same receptor tropism is thought to have determined pathogenicity, but also aided the control, of severe acute respiratory syndrome (SARS) in 20035. However, there are reports of COVID-19 cases with mild upper respiratory tract symptoms, suggesting the potential for pre- or oligosymptomatic transmission[6–8]. There is an urgent need for information on body site-specific virus replication, immunity, and infectivity. Here we provide a detailed virological analysis of nine cases, providing proof of active virus replication in upper respiratory tract tissues. Pharyngeal virus shedding was very high during the first week of symptoms (peak at 7.11 × 108 RNA copies per throat swab, day 4). Infectious virus was readily isolated from throat- and lung-derived samples, but not from stool samples, in spite of high virus RNA concentration. Blood and urine never yielded virus. Active replication in the throat was confirmed by viral replicative RNA intermediates in throat samples. Sequence-distinct virus populations were consistently detected in throat and lung samples from the same patient, proving independent replication. Shedding of viral RNA from sputum outlasted the end of symptoms. Seroconversion occurred after 7 days in 50% of patients (14 days in all), but was not followed by a rapid decline in viral load. COVID-19 can present as a mild upper respiratory tract illness. Active virus replication in the upper respiratory tract puts the prospects of COVID-19 containment in perspective.

Document#: WWT.VIR.5.12

TITLE: Presence of SARS-Coronavirus-2 in sewage

Author: Medema, G., L. Heijnen, G. Elsinga, R. Italiaander

Source: medRxiv preprint. 2020 https://doi.org/10.1101/2020.03.29.20045880

Abstract In the current COVID-19 pandemic, a significant proportion of cases shed SARS-Coronavirus- 2 (SARS-CoV-2) with their faeces. To determine if SARS-CoV-2 is present in sewage during the emergence of COVID-19 in the Netherlands, sewage samples of 7 cities and the airport were tested using RT-PCR against three fragments of the nucleocapsid protein gene (N1-3) and one fragment of the envelope protein gene (E). No SARS-CoV-2 was detected in samples of February 6, three weeks before the first case was reported in the Netherlands on February 27. On March 5, the N1 fragment was detected in sewage of five sites. On March 15/16, the N1 fragment was detected in sewage of six sites, and the N3 and E fragment were detected at 5 and 4 sites respectively. This is the first report of detection of SARS-CoV-2 in sewage. The detection of the virus in sewage, even when the COVID-19 prevalence is low, indicates that sewage surveillance could be a sensitive tool to monitor the circulation of the virus in the population.

Document#: WWT.VIR.5.13

TITLE: SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases

Author: Wu, F.Q., A. Xiao, J.B. Zhang, X.Q. Gu et al.

Source: medRxiv 2020 https://doi.org/10.1101/2020.04.05.20051540.

Abstract: Wastewater surveillance may represent a complementary approach to measure the presence and even prevalence of infectious diseases when the capacity for clinical testing is limited. Moreover, aggregate, population-wide data can help inform modeling efforts. We tested wastewater collected at a major urban treatment facility in Massachusetts and found the presence of SARS-CoV-2 at high titers in the period from March 18 - 25 using RT-qPCR. We then confirmed the identity of the PCR product by direct DNA sequencing. Viral titers observed were significantly higher than expected based on clinically confirmed cases in Massachusetts as of March 25. The reason for the discrepancy is not yet clear, and until further experiments are complete, these data do not necessarily

indicate that clinical estimates are incorrect. Our approach is scalable and may be useful in modeling the SARS-CoV-2 pandemic and future outbreaks.

Document#: WWT.VIR.5.14

TITLE: Can a paper-based device trace COVID-19 sources with wastewater-based epidemiology?

Author: Mao, K., Zhang, H. and A. Yang.

Source: Environ. Sci. Tech. 2020 54:3733-3735

Abstract: A recent outbreak of novel coronavirus pneumonia (COVID-19) caused by SARS-CoV-2 infection has spread rapidly around the globe, with cases now confirmed in 130 countries worldwide. Although public health authorities are racing to contain the spread of COVID-19 around the world, the situation is still grim. About 158111 confirmed cases and 5946 cumulative deaths (81 059 confirmed cases and 3204 cumulative deaths from China) have been reported around the globe as of March 15, 2020. Some clinical cases have found that some carriers of the virus may be asymptomatic, with no fever, and no, or only slight symptoms of infection. Without the ability to screen these asymptomatic patients quickly and effectively, these unsuspecting carriers have the potential to increase the risk of disease transmission if no early effective quarantine measures are implemented. Therefore, to trace unknown COVID-19 sources, fast and accurate screening of potential virus carriers and diagnosis of asymptomatic patients is a crucial step for intervention and prevention at the early stage.

Document#: WWT.VIR.5.15