SARS-CoV-2 Wastewater Surveillance in a remote municipality in the Aleutian Islands of Alaska

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Introduction

The City of Unalaska is a municipality located in the Aleutian Island chain approximately 800 miles southwest of Anchorage, Alaska. The City owns and operates a chemically enhanced primary treatment sewage facility which screens and disinfects an average of 0.483 million gallons of domestic wastewater per day.
Dutch Harbor is the largest commercial fishing port in the Pacific and is number one in the US for volume of fish. There are approximately 4,768 full time residents on the island and during peak seasons of the year, an additional 5-6,000 people are added to this population. The community's health care services are provided by a local clinic operated by Iliuliuk Family & Health Services, Inc. (IFHS). The clinic obtained two types of "rapid" COVID-19 testing devices at the beginning of the Covid 19 Pandemic, the Abbott IDNow system and the Cepheid GeneXpert IV-2 Molecular system and to date have performed 8,890 local clinical tests. Wastewater-based epidemiology (WBE) began being explored as a new tool to track the spread of COVID-19 from the onset of the pandemic (Medema et al. 2020). Many studies report viral detection in sewage across the world. Detection of SARS-CoV-2 RNA in wastewater has been shown to be a valuable tool in early detection and informing public health decisions (Wu et al. 2020).
Weekly samples were collected at the Unalaska WWTP Influent and two lift stations from July 2000 until the present. Two methods were used to quantify SARS-CoV-2 in raw sewage. We developed a sensitive, consistent and reliable method to allow for pooled surveillance of wastewater which now serves as an early warning system.

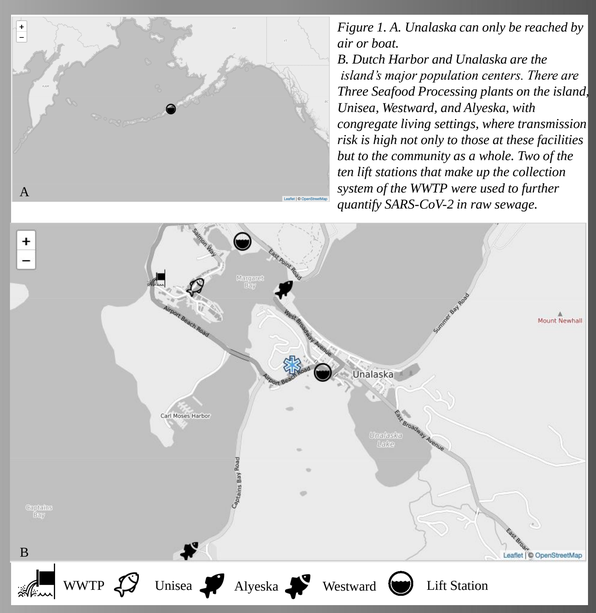

Figure 1. A. Unalaska can only be reached by air or boat.
B. Dutch Harbor and Unalaska are the island's major population centers. There are Three Seafood Processing plants on the island, Unisea, Westward, and Alyeska, with congregate living settings, where transmission risk is high not only to those at these facilities but to the community as a whole. Two of the ten lift stations that make up the collection system of the WWTP were used to further quantify SARS-CoV-2 in raw sewage.

Methods

Viral precipitation, RNA extraction, reverse transcription and quantitative PCR (qPCR)

24-hour composite samples were collected during high flow times at two lift stations and the WWTP Influent and stored at 4C.
Samples were mixed well and added to 50 mL centrifuge tubes. Tubes were centrifuged at 8300G for 30 minutes with no breaking force. Supernatant (40 mL) was carefully decanted and mixed with 4g of Polyethylene glycol 8000 and 0.9 g NaCL and centrifuged at 13,000 G for 2.5 hours to precipitate viral particles. In most samples the 6-tube capacity was repeated 4-5 times (total sample- 960- 1,200 mL). The entire volume of viral pellet was transferred into a single tube and allowed to settle overnight at 4C. Before extraction the settled pellet was spun at 8300G for 30 minutes.

Method I
The viral pellet was then resuspended in 1.5 mL Trizol reagent for RNA extraction. cDNA was synthesized by reverse transcription: 10 μL of RNA was mixed with random hexamers and then incubated at 70C for 5 min and 4C for 3 min. After that, the RNA-hexamer mixture was mixed with 5X ProtoScript II buffer (5 μL), 0.1M DTT (2.5 μL), ProtoScript II Reverse Transcriptase (200 U/μl. 1.25 μL), 10 mM dNTP (1.25 ul), RNase Inhibitor (40 U/μl, 0.5ul), and Nuclease-free water (2.5 μl) to a total volume of 25 μl. The mixture was then incubated at 42C for 1 hour and inactivated at 65C for 20 minutes.
The quantitative PCR (qPCR) was performed with TaqMan assay techniques. The Taqman Fast Advanced Master Mix (4444557, Thermofisher Scientific) was mixed with the primers and water and 2 ul of cDNA template from RT was added.

Figure 2. Chai Open PCR QPCR Detection System

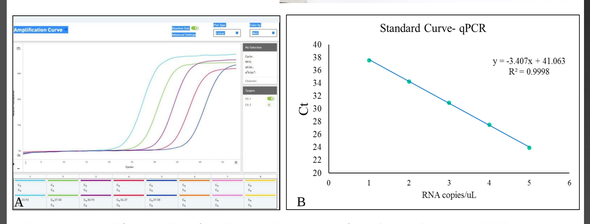

Figure 3. A. Amplification Plot of synthetic SARS-CoV-2 performed on a Chai Open QPCR machine. B. Standard Curve obtained with synthetic SARS-CoV-2 ((Integrated DNA Technologies (IDT)).

The qPCR reaction was carried out for 48 cycles using Chai Open PCR QPCR Detection System (Figure 2) based on the following program: polymerase activation (95C for 2 min), PCR (48 cycles, denature at 95C for 1 s), and anneal/extend at (55C for 30 s).

Method II
The viral pellet was then added to the PowerBead Bead Tube and RNA extracted with the Qiagen RNeasy PowerMicrobiome Kit (Cat. #26000-50). The eluted RNA (10uL-20uL) was immediately used for one-step RT-PCR with Taqman Fast Virus 1-Step Master Mix (Thermofisher, Cat. #4444436) based on the following protocol: 50 C 10 minutes for reverse transcription, 95C 20 s for RT inactivation and initial denaturation, and 48 cycles of denature (95C 1 s) and anneal/extend (55C 30 s).

The primers (N1 and N2) and DNA standards of SARS-CoV-2 nucleocapsid gene were used to quantify the titers of SARS-CoV-2 (Integrated DNA Technologies (IDT) 2019-nCoV CDC EUA qPCR Probe Assay primer/probe mix and 2019-nCoV_N Positive Control plasmid). Two replicates were performed. Positive and negative controls were included in each run as well as internal controls.

Standard Curve

MilliQ water was spiked with standard solution of synthetic SARS-CoV-2 ((Integrated DNA Technologies (IDT)) to perform a standard curve. The trend line has a slope of -3.407 and a R value of 0.999 (Figure 3). The linear trend between Ct values and RNA copy numbers allowed for quantitative analysis of virus RNA concentration and to track SARS-CoV-2 in the wastewater over time.

QA/QC

The Promega Quantus fluorometer was used to measure the concentration of RNA. A Hach spectrophotometer was used to measure purity of RNA OD260/OD280: 2.0; OD260/OD230 > 1.8
The method for recovery and concentration of SARS-CoV-2 from wastewater was validated and standardized using an internal process control and an internal amplification control. Pepper Mild Mottle Virus (PPMoV), the most abundant RNA virus in human feces, was used as an internal standardization control (Promega Wastewater SARS-CoV-2 N1 qPCR kit). Additionally, an extraction control containing synthetic SARS-CoV-2 from the Seracare AccuPlex SARS-CoV-2 Verification Panel (0505-0168) was used.

Results

The City remained insulated from the pandemic until December of 2020 at which time it experienced two outbreaks, the first at Unisea in January 2021 and the second and most significant at Alyeska in February 2021. Community spread remained low during the year and cases dropped off after vaccinations became widely available. Viral detection began at as low as ten reported active clinical cases. Viral load proceeded clinical cases by several days and closely followed clinical cases throughout the pandemic (Figure 4). WWTP Influent picked up both outbreaks. The Alyeska outbreak viral load could further be detected at Lift Station 4 on the Unalaska side of the island where Alyeska is located and Lift Station 2 directly behind the quarantine facilities (Figure 5). This Indicated that the ten lift stations in the WWTP collection system could be used to further pinpoint viral origin.

Figure 4. Active clinical cases vs. SARS-Co-2 Viral load in the WWTP Influent.

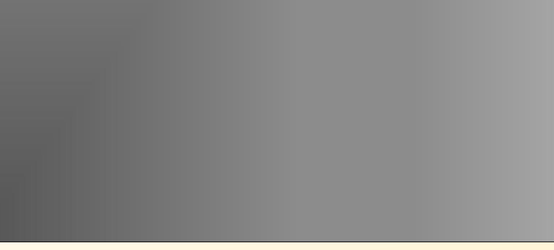

Figure 5. SARS-CoV-2 Viral load at the WWTP, Lift Station 2 and Lift Station 4.

Conclusion

The best PCR signals for viral pellets were obtained with Method II which proved to be more sensitive, consistent, and reliable. This method has been used exclusively from November 2020 until the present.

The recovery of internal standards was highly variable with low recoveries as seen in other studies. These variations are likely caused by the complexity of wastewater as an environmental matrix (Betancourt et al. 2021).

Wastewater testing has been successful in identifying new cases of COVID-19 in congregate living settings, and it is shown to be an important tool for preventing outbreaks in college dormitories, barracks, nursing homes and seafood processing plants (Betancourt et al. 2021).

Our study showed that we could identify new cases with high sensitivity, and in the future could serve useful in early warning detection of SARS-CoV-2 variants, understanding the efficiency of vaccination on transmission and surveillance for other infectious diseases.

Acknowledgements

Thank you to Erin Reinders, Dan Winters, Steve Tompkins, Mark Descoteaux, Brian Brown, Miguel Cristobal, James Esnardo, and Kim Gumera and to the staff at the IFHS clinic. This project was funded by the City of Unalaska and CARES ACT funding.