Quantitation of human enteric viruses as alternative indicators of fecal pollution to evaluate wastewater treatment processes

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Introduction

The North End Sewage Treatment Plant (NESTP) in Winnipeg, Manitoba provides 70% of the city's wastewater treatment (serving over 400,000 people) (City of Winnipeg, 2019). The wastewater treatment involves raw sewage undergoing primary treatment, in which solids are removed. It then gets processed to the stage of activated sludge, in which a heterotrophic cocktail of bacteria and protozoa degrade organic matter present in solid waste. After this treatment cycle, activated sludge is removed. A sludge cake is also produced, which is a by-product of activated sludge and raw sewage using anaerobic digestion. Once the treated water is disinfected, it is discharged (known as effluents) into bodies of water, such as Lake Winnipeg. Approximately 200 million liters of effluents are discharged per day. (City of Winnipeg, 2019). The main indicator of contamination used in wastewater treatment screening is *Escherichia coli*. However, this excludes other possible fecal markers present, such as human enteric viruses. Targeting these viruses in effluents could be a potential method used to monitor wastewater treatment. Samples of raw sewage, activated sludge, effluents, and sludge cake from the NESTP were collected (during the fall and winter season) to investigate the potential of quantitating human enteric viruses in wastewater samples as a complementary indicator of contamination to evaluate the processing stages of wastewater treatment. DNA enteric viruses (Adenovirus & CrAssphage); RNA enteric viruses [Pepper Mild Mottle Virus (PMMV), GII & GII Norovirus, Astrovirus, Rotavirus, and Sapovirus (Sav1, Sav124, Sav5)]; and gene *uidA* (which is found in *E. coli*) were targeted in the samples collected from the NESTP.

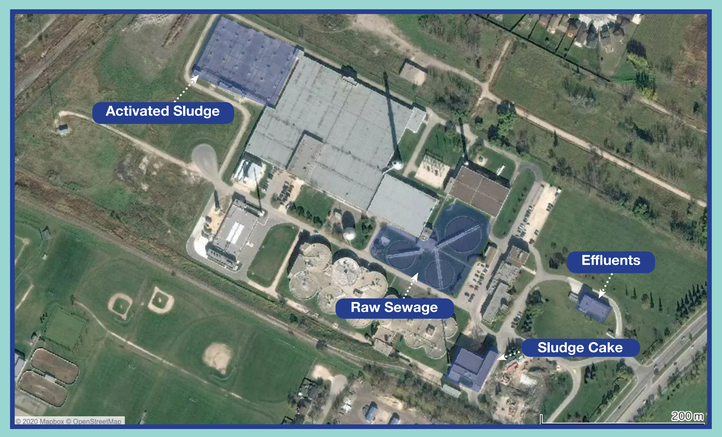

Figure 1. Satellite photo of the North End Sewage Treatment Plant.

Materials and Methods

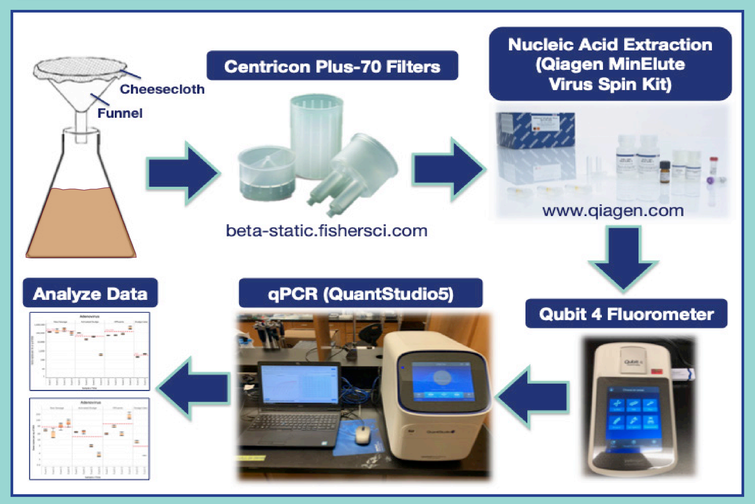

Figure 2. Diagram of the experimental design. Photos of the Qubit 4 Fluorometer and qPCR machine were taken at the Antimicrobial Resistance and the Environment Plus Laboratory (ARE+) at the University of Manitoba.

Materials and Methods

Each wastewater treatment sample (raw sewage, activated sludge, effluents, and sludge cake) was first filtered via a funnel and cheesecloth in order to remove any solid waste or debris. A volume of 140 mL of each sample (including Milli-Q water as a negative control) was then concentrated down using Centricon Plus-70 filters using an ultrafiltration method. Once the volume was collected from each sample, a Qiagen virus spin kit was used to extract the nucleic acid (RNA or DNA) from each wastewater treatment sample. A Qubit 4 Fluorometer was used to measure the quantity of RNA or DNA extracted. Once viral RNA or DNA was assessed in the samples, an ABI QuantStudio 5 PCR system (Applied Biotechnologies) was used to quantitate enteric viruses in each wastewater treatment sample. The number of gene copies was expressed in terms of biomass (per ng of DNA or RNA) and volume (per milliliter of sample). However the number of gene copies for sludge cake, in terms of volume, was expressed as per gram of DNA or RNA since it is a solid material.

Results

The mean of the number of gene copies of each sample across all events are indicated by the red-dotted lines in Figures 3, 4 and 5.

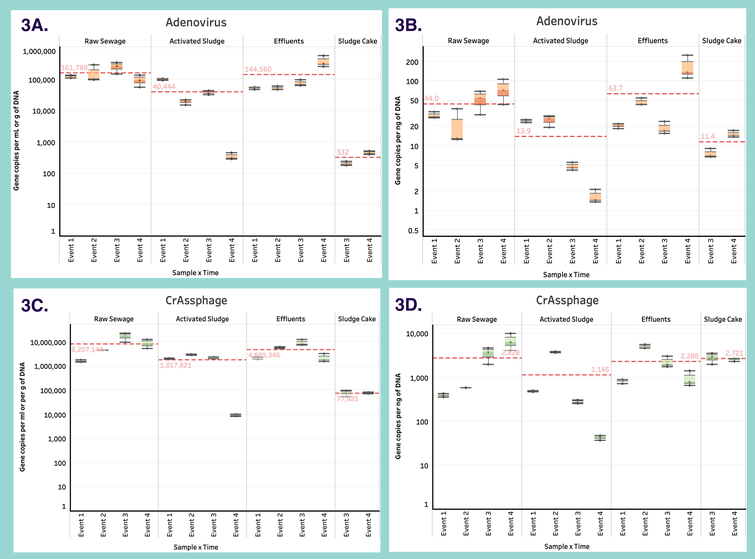

Figure 3. Box plot graphs of the number of genes copies of DNA enteric viruses across each wastewater stage throughout Events 1-4. The units for the sludge cake in Figures 3A and 3C are gene copies per gram of DNA.

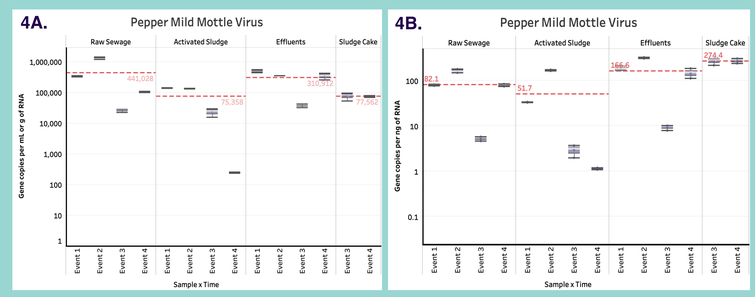

Figure 4. Box plot graphs of the number of genes copies of RNA enteric virus, PMMV, across each wastewater stage throughout Events 1-4. The units for the sludge cake in Figure 4A are gene copies per gram of RNA.

Acknowledgements

Special thanks to Dr. Qiuyan Yuan and graduate students Palwinder Singh and Kadir Yanaç in Civil Engineering at the University of Manitoba. Research start-up funds grant number 322388 were assigned to Dr. Miguel Uyaguari-Diaz.

Results

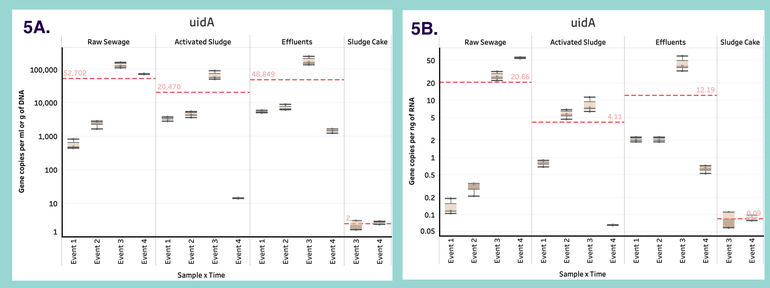

Figure 5. Box plot graphs of the number of genes copies of *uidA* (representative of *E. coli*) across each wastewater stage throughout Events 1-4. The units for the sludge cake in Figure 5A are gene copies per gram of DNA.

Due to the higher abundance and more stable signal over time of gene copies, Adenovirus, CrAssphage, and PMMV are more representative targets for conducting comparisons with *E. coli*. The gene copy values were transformed into $\log_{10}$ form. These values ran a Generalized Linear Model Tukey-Kramer analysis with 95% level of confidence (p-level = 0.05). The means of each wastewater sample stage for each enteric virus targeted were analyzed. Events 1 and 2 refer to the time of collection of samples in the fall season (October 22nd and November 28th, 2019 respectively). Events 3 and 4 refer to the time of collection of samples in the winter season (December 18th, 2019 and February 6th, 2020 respectively). The gene copy numbers were expressed in terms of biomass and volume (except for sludge cake; it was expressed in grams) because it allows one to analyze if a selection process is occurring within the wastewater treatment facility.

Conclusion

The average gene copies assessed in the raw sewage were not significantly different than the average gene copies assessed in the effluents for the DNA enteric viruses (Adenovirus and CrAssphage), PMMV, and *uidA* in terms of both volume and biomass. However, the average gene copies assessed in the activated sludge were significantly lower compared to raw sewage and effluents. Although a significant reduction of enteric viruses and *uidA* was observed consistently in activated sludge samples, the higher copy numbers of viruses and *E. coli* observed in the effluents may be associated with the hydraulic retention time (12 hours) in the facility. Samples were collected within a 2-hour period from raw sewage, activated sludge, and effluents consecutively. In this context, the community quantified in effluents may not completely reflect the changes occurring in the activated sludge. Other variables to consider are the overflow of sewage from rainy events and fluctuations in mixed liquor-suspended solids (Pérez, et al. 2019). To discard the possibility of inhibitors or contaminants such as humic acids, additional qPCR tests using bovine serum albumin (data not shown) were conducted with environmental samples (including activated sludge). No significant differences were observed in samples with and without the enzyme.

Moreover, the duration of anaerobic sludge digestion is 25 days. In this context, gene copy numbers of virions and *uidA* in the sludge cake were significantly reduced by anaerobic digestion (the by-product was used as compost and landfill). This may explain why the genes copies of *uidA* were lower in sludge cake compared to all treatments. The average gene copies across all wastewater stages (raw sewage, activated sludge, and effluents) for *uidA* are not significantly different in terms of both volume and biomass. This may indicate that enteric viruses can survive during the wastewater treatment process and therefore suggest that viral-like particles are being released into the aquatic environment. When compared to *uidA*, enteric viruses were found at least one (biomass) to two (volume) orders of magnitude higher than the *E. coli* marker. In regards to the sludge cake, the gene copies of CrAssphage and PMMV detected were high in terms of biomass. Since sludge cake is the by-product of activated sludge and raw sewage using anaerobic digestion, this may mean that the presence of CrAssphage and PMMV may have been lower in the wastewater being treated in the activated sludge, but higher in the solids. However, the gene copies of Adenovirus in terms of biomass were not significantly different between the activated sludge and sludge cake samples. This reduction of viral gene copies of Adenovirus may be due to efficient digestion in the activated sludge and the anaerobic digestion process (sludge cake). Meanwhile, plant viruses such as PMMV remain more stable (in terms of biomass) during these digestion processes (Jumat et al. 2017). Furthermore, the low detection of RNA viruses (Rotavirus, Astrovirus, GI & GII Norovirus, and Sapovirus) across all of the wastewater treatment stages may be due to seasonal variability (Pérez, et al. 2019) and efficiency of the wastewater facility to remove these viruses during the process.

Selected References

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