Identification of Human Enteric Viruses Present in Waterbodies Receiving Wastewater Discharges in Urban Manitoba

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Background

Raw-sewage is purified at Winnipeg's WWTPs and discharged as effluents into Red and Assiniboine river. Traditional methods for monitoring the microbial quality of wastewater focus on the detection of fecal indicator bacteria as current gold-standards of aquatic-health. This is problematic as a negative fecal bacterial culture test disregards other pathogens such as viruses. 10,000 Finnish people were hospitalized due to surface-water contamination by waterborne-norovirus. Environmental samples from urban-waterbodies will undergo filtration and skimmed-milk flocculation procedures to be processed via quantification and sequencing methods. This allows for characterization of baseline viral aquatic-community structures. Since viruses are more pathogenic than bacteria, knowledge of these in "pure" effluents is imperative to aquatic health and environment.

Objectives

1. To implement a reliable method of concentrating aquatic human enteric viruses from environmental surface water samples.
2. To characterize viral DNA and RNA baseline community structures using a culture independent approach followed by a sequence-based metagenomic approach.
3. To evaluate dispersion and drift of aquatic human enteric viruses as a result of WWTPs outfalls.

Significance

- Identification of human enteric-viruses present in "pure" effluents sheds insight into the effectiveness of current wastewater treatment processes at Winnipeg's WWTPS. The characterized viral-community structures leads to the development of novel viral markers of fecal contamination over time, through continuous sequencing and validation methods. This may encourage Manitoban health policy-makers to implement wastewater treatment policies removing human enteric-viruses.
- Results obtained from this study is limited to a one-year period to align with the length of a Master degree. To ascertain accurate and reliable results, this project will be continued over the next few years.
- Results from this study account for seasonal variability as sampling will be conducted three times in one year.

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Methodology

- **Study Population:**

Environmental sampling will be conducted in 10 locations along Red and Assiniboine River. **Method to concentrate human enteric viruses from environmental surface water samples:** Separation of viruses from bacteria first involves vacuum filtration to remove contaminants from effluents. The filtrate undergoes skimmed-milk flocculation where skimmed-milk particulates target viruses to clump together into "flocs" through sedimentation. Flocs are treated with DNase and RNAse to get rid of free DNA and RNA present. A microbiome kit will extract total nucleic-acids. A Qubit Fluorometer will assess nucleic concentration and purity.

- **Data Analysis:**

After nucleic extraction, two methods to establish a baseline of viral community structure are: Culture independent approach: Targeted q-PCR will quantify viral DNA and RNA. Metagenomic approach: Illumina-technology will sequence the samples. Geneious workflow software will process the samples. Viral metadata will be uploaded to M-G-RAST viral database to identify viral DNA and RNA.

- **Statistical Analysis:**

R and RStudio® statistical software will evaluate dispersion and drift of human enteric-viruses over time using predictive modelling and data visualization approaches.

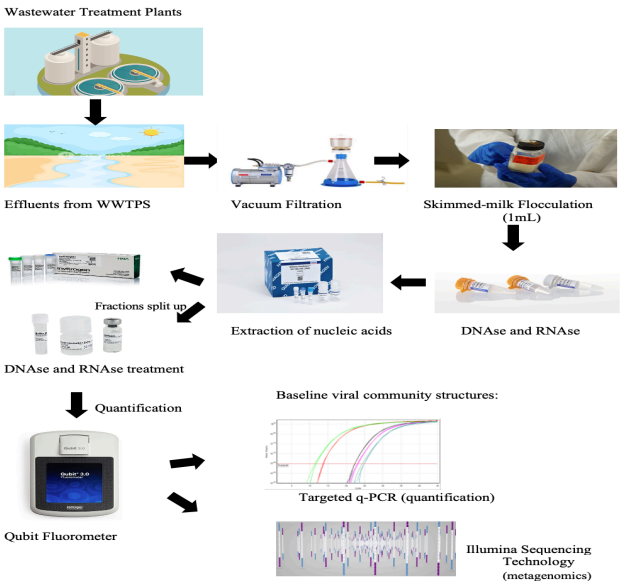

Figure 1:. Flow Diagram describing steps taken to conduct proposed research.

References

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