DETECTION OF MEASLES VIRUS GENOTYPE A IN A NON-ENDEMIC WASTEWATER SETTING: INSIGHTS FROM MEASLES WASTEWATER AND ENVIRONMENTAL MONITORING IN CANADA'S CAPITAL REGION

Emma Tomalty

A thesis submitted to the University of Ottawa in partial fulfillment of the requirements for the Master's degree of Applied Science in Environmental Engineering

Department of Civil Engineering
Ottawa-Carleton Institute of Environmental Engineering
University of Ottawa

uOttawa

© Emma Tomalty, Ottawa, Canada, 2025

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	**Description**
MeV	Measles Virus
WES	Wastewater and Environmental Surveillance
RT-qPCR	Reverse-Transcriptase quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
SARS-CoV-2	severe acute respiratory syndrome – Coronavirus - 2
RSV	respiratory syncytial virus
WHO	World Health Organization
CDC	Centre for Disease Control
SSPE	subacute sclerosing panencephalitis
MMR	measles/mumps/rubella
MMR-Var	measles/mumps/rubella/varicella
PCR	polymerase chain reaction
MPOX	monkeypox
PMMoV	pepper mild mottle virus
WRRF	water resource recovery facility
ROPEC	Robert O. Pickard Environmental Centre
mL	millilitres
DNA	Deoxyribonucleic acid
N	nucleoprotein
µL	microlitres
g	grams
ALOD	assay limit of detection
ALOQ	assay limit of quantification
cDNA	complementary deoxyribonucleic acid
ng	nanogram
µm	micrometer
NCBI	National Centre for Biotechnology Information

ABSTRACT

The recent global resurgence of measles in 2023-2024, despite vaccine preventability, underscores a critical public health issue, largely due to reduced vaccination coverage during the SARS-CoV-2 pandemic. In response, Ottawa Public Health intensified vaccination efforts in 2023 and 2024. Additionally, a research initiative began in April 2024 to monitor Ottawa wastewater for measles virus (MeV) using established wastewater and environmental surveillance (WES) protocols. Unexpected positive MeV detections through RT-qPCR in Ottawa wastewater – despite no active regional cases – prompted genotypic and retrospective analyses of archived RNA samples dating back to 2020. The genotypic analysis identified positive detection to belong to genotype A, the progenitor strain of the viral vaccines, marking the first report of MeV vaccine RNA in a large catchment area. Linear regression analysis revealed detections aligned with intensified vaccination efforts by Ottawa Public Health. These findings emphasize the importance of integrating genotypic analysis into WES practices to mitigate possible confounding factors, such as vaccine shedding into wastewater. Additionally, this research highlights potential public health applications using MeV WES as a complementary tool. Implementing the findings of this study for MeV WES, and for other re-emerging viruses, could improve public health response and resource allocation.

ACKNOWLEDGEMENTS

Thank you to Dr. Robert Delatolla for your guidance, support, feedback and motivation throughout my studies. I was able to learn a great deal under your supervision and will take the lessons learned with me for the rest of my career.

Thank you to the amazing people in the wastewater and environmental surveillance group at the University of Ottawa. A special thank you to Élizabeth Mercier, who's unwavering support, patience and knowledge took my academic writing to the next level; to Shen Wan, who's faith in my laboratory skills gave me the confidence to perform new techniques at a high degree of difficulty; to Xin Tian, who never wavered in her support, troubleshooting, and problem solving during countless hours performing extractions together; and to Elizabeth Renouf who's dedication, knowledge and expertise provided unparalleled assistance and guidance in the statistical analysis and REB process. Thank you to Lakshmi Pisharody who assisted with the literature review for the published manuscript. Thank you to Tram Nguyen, Md Pervez Kabir, and Chandler Wong for their laboratory support throughout. Thank you to Felix Addo and Nada Hegazy for their academic writing support.

Thank you to Dara Spatz Friedman and the Ottawa Public Health Epidemiology and Evidence Team for providing vaccination data and knowledge about the vaccination programs and practices in the last few years. Their collaboration, assistance, and support are greatly appreciated. Thank you to Dr.

Alessandro Zulli from the Department of Civil and Environmental Engineering, Stanford University who assisted through laboratory knowledge and support. Thank you to the City of Ottawa, Ottawa Public Health, Public Health Ontario and all their employees involved in the project during this study.

I would like to acknowledge funding from the CIHR Applied Public Health Chair in Environment, Climate Change and One Health, awarded to Dr. Robert Delatolla.

Lastly, thank you to my wonderful partner, Guillaume Godin, who provided me with continuous devotion and support during this challenging time.

COPYRIGHTED CONTENTS

This thesis is an expanded version of the published manuscript and supplemental material under the same title. The manuscript was published by the American Chemical Society in Environmental Science and Technology Letters on February 2, 2025. The copyright of this manuscript is owned by the authors who have provided permission to use these materials in the writing of this thesis.

Tomalty, E., Mercier, É. Pisharody, L., Nguyen, T., Tian, X., Kabir, M.P., Wong, C., Addo, F., Hegazy, N., Renouf, E., Friedman, D.S., Wan, S., Delatolla, R. (2025). Detection of measles virus genotype A in a non-endemic wastewater setting: insights from measles wastewater and environmental monitoring in Canada's capital region. *Environmental Science & Technology Letters, 12 (2),* 124-129. https://doi.org/10.1021/acs.estlett.4c00945.

1. INTRODUCTION

1.1 Background

Wastewater and Environmental Surveillance (WES) is a field of study that assesses the health of a community or population by analyzing wastewater for indicators of disease or drug use, both pharmaceutical and otherwise. Although WES has historically been used in the context of public health, it was not a widely implemented tool until the 2019 coronavirus disease pandemic. The viral infection from severe acute respiratory syndrome – coronavirus – 2 (SARS-CoV-2) spread across the globe at an unprecedented rate, quickly overwhelming existing public health measures for many countries, including Canada. WES posed an alternative, non-invasive, unbiased method for monitoring population wide disease burden and distribution. As such, monitoring SARS-CoV-2 using WES during the pandemic became an important complimentary tool to clinical monitoring and fostered a collaborative relationship between public health and researchers in the WES field of study in Ottawa, Canada. This collaborative relationship ultimately led to monitoring the community wastewater for other viral diseases and infections such as respiratory syncytial virus (RSV), influenza A and B, and monkeypox (Mercier et al., 2022; Mercier et al., 2023; Wong et al., 2023).

The pandemic led to other unforeseen challenges in the public setting such as limited access to healthcare and the spreading of misinformation about regularly scheduled immunizations. These challenges led to disruptions in the administration of routine vaccinations, such as the measles vaccine. In 2022, there was a concerning surge in infections, compared to the previous year, with an 18% rise in estimated measles cases and a 43% increase in measles-related deaths globally (World Health Organization [WHO], 2001; WHO, 2015; Minta et al., 2023). The measles vaccine is part of the publicly funded immunization program in Canada with the first dose administered no earlier than a child's first birthday and the second dose usually provided through school-based immunization programs (Public Health Ontario, 2022). In order to halt the transmission of the measles virus, a population wide vaccination rate of 95% or greater is necessary. Despite Canada being a measles non-endemic country, widespread immunity for measles dropped below 95% following the pandemic (Tam, 2023). The drop in widespread immunity increased the risk of introduced infections rapidly spreading in many communities in Canada, as seen in the past with the reemergence of polio when herd immunity falls below the epidemic threshold (Sasaki et al., 2012). Thus, the risk of re-introduced measles infections in Ottawa posed an opportunity to explore WES for the measles virus (MeV).

1.2 Problem Statement

There is presently no standardized approach to implementing MeV WES before the onset of a measles outbreak within a measles non-endemic country. Though the detection of wildtype MeV, that is naturally occurring non-vaccine strain MeV, utilizing WES has been reported in current research, these studies were primarily either reactive, initiating sampling and analysis after an outbreak was identified by clinical surveillance, or by targeting priority populations with known low vaccination coverage (Benschop et al., 2017; Rector et al., 2024; Ndlovu et al., 2024). It remains unclear whether wildtype measles is detectable in large catchment areas. In addition, the administration of live-attenuated measles vaccines in Canada and other countries may confound the detection of MeV in wastewater through RT-qPCR. There is presently no research into confounding effects of vaccine shedding when using WES to quantify measles infections via RT-qPCR in a community (Ndlovu et al., 2024).

1.3 Research Objectives

The intent of this thesis is to advance the current state of knowledge on MeV WES by measuring the signal of MeV in wastewater from a large catchment area in the absence of known active measles cases. The specific objectives of this research are as follows:

1. To validate the standard method developed by the CDC and employed by the Global Measles and Rubella Laboratory Network for the detection of measles RNA via RT-qPCR and the identification of the prevalent genotypes, by assessing its applicability to wastewater samples as an alternative to clinical specimens.
2. To evaluate the relationship between vaccination rates and the presence of vaccine strain measles measurements in wastewater.

The results from this research highlight the importance of molecular characterization of MeV signal in wastewater. It also shows the importance of a collaborative relationship between WES and public health to identify factors contributing to the signal of MeV in wastewater.

2. LITERATURE REVIEW

2.1 History of Measles & the Measles Vaccine

2.1.1 Clinical Manifestation of Measles

Measles, an illness primarily affecting children, results from a viral infection by the measles virus (MeV) of the *Morbillivirus* genus in the family *Paramyxoviridae*. MeV is an enveloped virus with a single-stranded, negative sense RNA genome (Bankamp et al., 2011, Furuse et al., 2010). Measles infections are highly contagious spreading through respiratory droplets, aerosolized transmission, or direct contact with nasal or throat secretions of an infected individual (WHO, 2018). An uncomplicated measles infection consists of four phases: the incubation phase, the prodromal phase, the rash phase, and the convalescent phase (Ontario Ministry of Health, 2024). A typical measles illness consists of a characteristic rash with a fever of 38°C or higher and one or more of the following: cough, coryza, and conjunctivitis (WHO, 2018). Although individuals are considered infectious approximately four days before and after the onset of the rash; in Ontario, Canada, an individual is defined as a suspected measles case only after presenting with fever, rash and at minimum one of cough, coryza or conjunctivitis for at least three days (Ontario Ministry of Health, 2024; WHO, 2018). Measles can have serious complications such as deafness and blindness among immunocompromised individuals as well as severe long-term neurological consequences resulting from acute encephalomyelitis leading to death or permanent impairment for survivors (WHO, 2018). Additionally, measles illness can cause subacute sclerosing panencephalitis (SSPE), a progressive neurodegenerative disease that can manifest 4-10 years after acute infection. The risk of SSPE is higher among children who contracted measles illness at less than five years of age and is invariably fatal (WHO, 2018; Bankamp et al., 2011).

2.1.2 The Measles Vaccine

According to WHO, wild-type measles can be found classified into 24 genotypes distributed across 8 clades (A-H) with all vaccine strains belonging to genotype A (WHO, 2018). Since MeV derives from one genus and species with relatively stable antigens, this means that it is potentially eradicable by immunization (Barrero et al., 2001). Research into the development of the measles vaccine began in the 1950s when the Edmonston strain, considered the prototype, was first isolated from a child with measles in a primary culture of human kidney cells, subsequently giving rise to several of the currently used highly attenuated, live measles vaccines (Bankamp et al., 2011). The Edmonston isolate is not the only wild-type progenitor strain. Others were isolated independently in Russia, Japan and China, giving rise to diverse geographic origins and attenuation procedures (Bellini & Rota, 1998). Despite these differences, the sequences of the different vaccines demonstrate remarkable similarities (Parks et al, 2001; Bankamp et al., 2011).

Even though measles infections are highly contagious, measles outbreaks can be prevented through a population-wide vaccination rate of 95%, leading to herd immunity (WHO, 2018). Protective immunity is 89.6% from a single dose at 9 months of age and is the recommended age of first administration for measles endemic areas with ongoing transmission. The recommended age of first administration for areas without ongoing transmission is 12 months. This is due to the waning of maternal antibodies leading to an increase in protective immunity to 99% (WHO, 2018). In Canada, the measles/mumps/rubella (MMR) and measles/mumps/rubella/varicella (MMR-Var) vaccines are part of the publicly funded immunization programs for people born in Canada as well as for new Canadians entering the school, childcare, and healthcare settings (Public Health Ontario, 2022; Public Health Ontario, 2012). All measles vaccines administered in Canada use the Schwarz and Moraten strains, both deriving from the Edmonston isolate, and belonging to genotype A.

2.1.3 Measles Elimination

Measles elimination is defined as the absence of endemic measles transmission in a defined geographical area for a period of 12-months or more in the presence of an adequate surveillance system (WHO, 2015). The introduction of immunization programs has greatly reduced the incidence of measles worldwide, however, immunization coverage greater than 95% is required to sustain elimination (WHO, 2018). For areas that have already achieved good control of measles infections through immunization efforts, molecular epidemiological studies provide a means to describe outbreaks and cases (Bellini & Rota, 1998). The WHO recommends molecular surveillance via genotypic analysis to track endemic measles transmission, global genotypic distribution, and verification of elimination of wild-type MeV (WHO, 2015; WHO, 2018). In Canada, isolation of MeV via PCR detection is strongly recommended to allow for downstream amplification and genotypic analysis (Ontario Ministry of Health, 2024). Additionally, using molecular epidemiology to compare the variability of measles infections before and after a vaccine campaign has been demonstrated to be an effective strategy for monitoring the efficacy of vaccination campaigns (Mulder et al., 2001). While Canada maintains no ongoing transmission of MeV and is therefore measles non-endemic, the risk persists due to incoming travelers from measles endemic regions where the virus is prevalent (Government of Canada, 2024). Consequently, susceptible populations, such as under-immunized communities or individuals with fading immunity, remain vulnerable to introduced infections (WHO, 2018). Therefore, early detection, genotypic analysis, and containment of circulating infections within the community are crucial to preventing widespread transmission.

2.1.4 Measles Vaccine Shedding

Despite being a respiratory virus, the MeV has been detected in the urine samples of infected individuals. Previous research has shown that isolation of MeV from urine specimens of infected individuals is possible for up to 10 days after the rash onset (Rota et al., 1994). Additionally, in a fourteen-year study measuring morning urine specimens from suspected measles patients admitted to hospital from 1965-1979, the highest positive rate of MeV detection was from urine before the rash onset and second highest at the onset of the rash (Boyd, 1983). Furthermore, MeV has been detected in urine of asymptomatic close contacts of infected individuals (Boyd, 1983; Vardas & Kreis, 1999). In Canada, urine is considered an appropriate clinical specimen for use in laboratory confirmation of infection with clinically compatible signs and symptoms (Ontario Ministry of Health, 2024).

As previously mentioned in section 2.1.2, the measles vaccine is derived from a live-attenuated wild-type strain of MeV. Past research has demonstrated the occurrence of viral vaccine shedding with live-attenuated vaccines (Armas et al., 2023). Research examining the shedding and persistence of MeV in vaccinated individuals has demonstrated the presence of viral RNA in urine samples 7 to 15 days post-vaccination (Eckerle et al., 2013; Kaic et al., 2010). Evidence from a 2019 study indicates a slow clearance of the virus from the body, with positive detections in urine persisting for up to 100 days post-vaccination (McMahon et al., 2019). Furthermore, it has been suggested that the virus or viral antigens from a measles vaccine can be detected in urine as little as one day post-vaccination (Rota et al., 1995).

2.2 Wastewater and Environmental Surveillance

Wastewater and Environmental Surveillance (WES) is an anonymous approach for monitoring drug use, disease prevalence, and infection rates of a community through molecular analysis of wastewater. In recent decades, wastewater has been used to monitor consumption patterns of both regulated and unregulated substances (Metcalfe et al., 2010; Erickson et al., 2021). In addition, WES has been employed to monitor community infection rates of non-enveloped viruses transmitted via the fecal-oral route, such as poliovirus, hepatitis virus, and norovirus (Brouwer et al., 2018; Petrinca et al., 2009). WES of polio has been part of the United Nations Polio Eradication Program since 1988 and has played a key role in documenting the eradication of polio in India and Egypt as well as documenting re-emerging infections in polio free countries such as the Netherlands (Medema et al., 2020a). Lessons learned from the use of WES for polio supports the implementation of WES for other respiratory viruses. During the pandemic, clinical surveillance was quickly overwhelmed by the massive influx of infected individuals leading to the necessity of alternative surveillance methods, such as WES. Implementation of WES for the surveillance of SARS-CoV-2 was found to be a useful complementary tool to clinical surveillance, with many programs initiated across the world (Ahmed et al., 2020; D'Aoust et al., 2021a; Graham et al., 2020; Naughton et al., 2023). Research out of Canada has contributed considerably to the WES field of study. For instance, SARS-CoV-2 research out of Ottawa, Canada found a significant correlation between normalized SARS-CoV-2 concentrations with regional public health metrics, indicating viral signals precede laboratory confirmed cases by 5 days, hospitalizations by 10 days, and deaths by 19 days (D'Aoust et al., 2021a; Hegazy et al., 2022). Parallel findings of early detection of SARS-CoV-2 have also been reported in other developed countries (Ahmed et al., 2021; Medema et al., 2020b). Across the world, wastewater monitoring for SARS-CoV-2 has shown to be an effective predictive tool with online dashboards displaying the latest data occurring in 72 countries (Naughton et al., 2023).

WES has broadened to include the analysis of other respiratory viruses including RSV, influenza, monkeypox, norovirus, and rotavirus (Mercier et al., 2023; Mercier et al., 2022; Wong et al., 2023; Bucardo et al., 2011; Hughes et al., 2022; Boehm et al., 2023). It has been found that concentrations of RSV and influenza in the wastewater mirrors clinical surveillance data (Boehm et al., 2023). A finding that has been corroborated through research out of Ottawa, Canada which demonstrated the feasibility of WES for RSV and influenza by identifying lead-times of 12 and 17-days, respectively (Mercier et al., 2023; Mercier et al., 2022). Wastewater surveillance of the re-introduction of monkeypox has been demonstrated by researchers through detection of MPOX viral signal in wastewaters with the ability to track outbreaks and/or resurgences (de Jonge et al., 2022; Wolfe et al., 2022; Wong et al., 2023). Although the research agenda for pathogen WES is region specific, advancements and innovation in WES are contributed by research institutions across the world (Han et al., 2023; Berry et al., 2022; Shaw et al., 2023; Benedetti et al., 2024). However, the demonstrated use of different methodological approaches for WES across research facilities, and therefore lack of reliable and timely data generation, limits its future application (Chik et al., 2021; Kumblathan et al., 2021). Research is ongoing to improve WES systems globally with the ultimate goal of improving the understanding of the viral prevalence of infectious diseases in wastewater.

Traditional clinical surveillance of respiratory viruses generally relies on individual testing, hospitalization and mortality data (Gracia-Lor et al., 2018). Often, there are testing restrictions in place, such as testing only symptomatic individuals, to avoid overwhelming the public health system. Other clinical testing biases, such as unequal access to healthcare and testing avoidance due to stigma, may lead to an underestimation of the disease prevalence within the community. WES is beneficial in that everyone in the population uses bathroom facilities, allowing viral shedding to make its way into the sewer system thereby allowing detection through wastewater analysis. Consequently, WES is a more comprehensive representation of true disease burden in the community through overcoming individual, behavioral and systemic biases within the clinical testing system by providing a non-invasive, anonymous, cost-effective, and adaptable population-level monitoring tool (Medema et al., 2020a). The systematic collection, analysis and interpretation of disease specific data using WES has been found to be valuable for public health decision making and health action plans (Benedetti et al., 2024). It has been suggested that a dynamic surveillance system, including WES, would efficiently monitor trends in respiratory diseases, allowing for the ability to rapidly detect emergence, re-emergence, and resurgences of viral variants (Berry et al., 2022). The emergence and re-emergence of viral outbreaks are becoming more frequent for various reasons, such as increased international travel and suspected vaccine hesitancy, posing serious public health threats and increasing the need for pandemic preparedness in the future (Han et al., 2023). Continual monitoring of wastewater and timely sharing of data with public health can be valuable for public health decision making and the overall community health.

2.3 Measles Wastewater and Environmental Surveillance

MeV RNA has been detected in the urine of infected individuals, enabling its detection in wastewater (Riddell et al., 2007; Moss & Griffin, 2012). Measles WES has been presented in research conducted in the Netherlands, Belgium, and South Africa. In 2013, a Dutch study detected the MeV D8 strain in wastewater samples from sewage pits located in residential communities and schools within the

Netherlands' 'Dutch Bible Belt' (Benschop et al., 2017). This study demonstrates the employment of targeted surveillance of priority populations by choosing wastewater sampling sites with a small catchment area in a region with known low vaccination rates. More recently, a Belgian study utilized WES during an active outbreak to identify MeV hotspots and the circulating genotype D8 strain by testing wastewater samples from three large regional wastewater treatment plants (Rector et al., 2024). This study highlights the feasibility of measles WES by demonstrating successful detections from large catchment areas during an active outbreak. However, both European studies did not detect the MeV vaccine strain.

In 2024, a South African study utilized WES to co-detect both MeV genotype A (the measles vaccine) and MeV genotype non-A through targeted surveillance during an active outbreak by in-line sewer sampling within two large municipalities following supplemental immunization activity (Ndlovu et al., 2024). This study demonstrates the potential of MeV WES for tracking the efficacy of supplemental immunization activity through targeted surveillance of a small catchment area in a measles endemic country. Genotype A and genotype non-A were identified in wastewater by employing a technique deemed 'PCR genotyping' using primers specific for genotype A and the wildtype genotypes dominant in the region (B3, D8 and H1) to differentiate between vaccine and wildtype MeV. However, they lacked sufficient material to sequence the wastewater samples positive for genotype non-A and since their 'PCR genotyping' method identifies three dominant genotypes in the region, they were unable to identify the circulating genotype. According to the World

Health Organization, molecular characterization of the virus is a vital component of verification of measles elimination, and particularly important when the source of the infection is unknown (WHO, 2018). Moreover, performing sequencing on positive genotype non-A wastewater samples would provide confirmation testing, a common practice in microbiology. Despite this limitation, sequencing or PCR genotyping are both good practice for routine testing of wastewater samples for MeV.

3. MATERIALS AND METHODS

3.1 Wastewater Sampling

Twenty-four-hour composite primary clarified sludge wastewater samples were collected from the City of Ottawa's only water resource recovery facility (WRRF), Robert O. Pickard Environmental Centre (ROPEC). ROPEC serves approximately 91% of the population (910, 000 of 1, 000, 000) with an average daily capacity of 545 million liters. The wastewater samples, comprised of four grab samples collected every six hours and combined into a 500 mL bottle, were collected by process technicians at ROPEC and stored at 4°C before being transported to the laboratory on ice. The samples were collected daily as part of an ongoing wastewater surveillance program since 2020.

3.2 Sample Concentration and Nucleic Acid Extraction

Upon arrival at the laboratory, samples were processed within 48 hours for nucleic acid extraction. Forty millilitres (mL) of well-homogenized wastewater samples were concentrated by centrifugation at 10, 000 x g for 45 minutes at 4°C. The supernatant was discarded, and 0.250 ± 0.01 grams (g) of the resulting pellet was immediately processed for RNA extraction using Qiagen's AllPrep PowerViral DNA/RNA kit with a combination of a manual and automated methodology. The manual and automated methodology was created in conjunction with Qiagen application specialists and engineers where the cell lysis and inhibitor removal steps were performed manually before the sample was transferred to a microcentrifuge tube then loaded into the QIAcube Connect instrument where all subsequent wash, DNase addition and elution steps were automated (Qiagen, 2018; D'Aoust et al., 2021a, 2021b).

3.3 RT-qPCR

3.3.1 Sample Analysis

Daily samples collected between April 6, and May 31, 2024, were quantified for viral MeV immediately following concentration and extraction procedures. Retrospective analysis of a subset of archived previously extracted RNA from wastewater samples were quantified for viral MeV after being stored at -80°C for periods ranging from 1 to 45 months. The frequency of samples tested for retrospective analysis is detailed in Table 3.1. Missing dates were due to lack of archived RNA. The archived RNA samples from the year 2020 underwent a maximum of two freeze- thaw cycles and the archived RNA samples from year 2021 to 2024 underwent a maximum of one freeze-thaw cycle. Viral MeV was quantified using singleplex, probe-based, one-step RT-qPCR (CFX96, Biorad) using nucleoprotein (N) specific primers and probes. Primers, probes and cycling conditions are summarized in Table 3.2. Reactions were prepared using TaqMan™ Fast Step Master Mix (Thermo Scientific) according to the manufacturer's guidelines for a final reaction volume of 10 microlitres (μl). All daily samples were run in eight technical replicates, while retrospective samples were run in triplicate, each with non-template controls and a five-point standard curve, prepared with a MeV G-block (Integrated DNA Technologies). The retrospective samples were run in triplicate instead of eight technical replicates because of limited RNA of archived samples.

Table 3.1: Frequency of retrospective analysis of archived wastewater RNA from July 2020 to March 2024.

Year	Number of Samples Analysed	Frequency
2020	5	Starting July, once per month, missing December
2021	11	Once per month, missing December
2022	8	Once per month, missing January, August, September and December
2023	40	Once per month January to June. Starting July, once per week
2024	17	Once per week, ending March

Table 3.2: Amplification target regions, Primers/Probes and Cycling conditions

Amplicon length	Primers/Probes	Cycling Conditions	Reference
75 bp	MVN 1139F 5' TGGCATCTGAACTCGGTATCAC 3’ MVN 1213R 5' TGTCCTCAGTAGTATCGATTGCAA 3’ MVNP 1136 5’ FAM-CCCCGAGGATGCAAGGCTTGTTTCAGA-BHQ1 3’	**RT:** 50°C for 5 min (1 cycle) **RT Inactivation/ Initial Denaturation:** 95°C for 20s (1 cycle) **Denature:** 95°C for 30s (44 cycles) **Anneal/Extend:** 60°C for 30s (44 cycles)	Hummel et al. 2006 RT-qPCR for quantification
439 bp	MVN 3 5' GGATGAGGCGGACCAATACT 3' MVN 6.1 5' TGACCATGCTGCCATAGCTT 3'	**Initial Denaturation:** 98°C for 30s (1 cycle) **Denaturation:** 98°C for 10s (35 cycles) **Annealing:** 55°C for 30s (35 cycles) **Extension:** 72°C for 60s (35 cycles) **Final Extension:** 72°C for 120s 4°C, hold	Brzovic et al., 2022 Nested PCR for amplification
695 bp	MVN 5 5' GGAGTAGGAGTGGAACTTG 3' MVN 6 5' TCTGCCATCGGCTCCAATCG 3'	**Initial Denaturation:** 98°C for 30s (1 cycle) **Denaturation:** 98°C for 10s (35 cycles) **Annealing:** 55°C for 30s (35 cycles) **Extension:** 72°C for 30s (35 cycles) **Final Extension:** 72°C for 120s 4°C, hold	

3.3.2. Quality Control Measures

The limit of detection of the quantification workflow was previously determined using spiked viruses and was found to be 9% (D'Aoust et al., 2021a). However, caution is advised in extrapolating these results, as spiked surrogates have been found to not be representative of endogenous targets in wastewaters (Kantor et al., 2021). The assays limit of detection (ALOD) and limit of quantification (ALOQ) for MeV's N gene region were approximately 3.66 and 3.74 copies/reaction respectively following recommended MIQE guidelines (Bustin et al., 2009). PCR efficiency ranged from 90 – 110% and R^2 measurements were greater than 0.95. All samples were analysed for pepper mild mottle virus (PMMoV) to assess possible inhibition using previously described methods (D'Aoust et al., 2021a). It was determined that inhibition was not evident in the wastewater samples and therefore the results did not require normalization to the PMMoV faecal indicator. Despite these quality control measures, the potential for false positive detections remains, particularly at low target concentrations near the assays limit of detection. To mitigate this, all reactions included non-template controls (NTCs) to monitor for contamination, and results were only considered positive if amplification was observed in a minimum of 2 replicates with appropriate amplification curves.

3.4 Sample Preparation for Sanger Sequencing

3.4.1 cDNA Synthesis, Nested PCR, Gel Electrophoresis and Purification

Select wastewater samples showing a positive detection for MeV through RT-qPCR were further analysed to identify the strain circulating in the community via

Sanger sequencing. The 450 nucleotides encoding the carboxylterminal 150 amino acids of the N were sequenced according to the WHO guidelines as the minimum amount of sequence data required for determining the genotype of MeV (WHO, 2018; CDC, 2018). For these samples, a nested-PCR approach was used to increase the sensitivity; two sequential amplification reactions were performed using two different sets of primers. Complementary DNA (cDNA) was first synthesized using SuperScript™ IV First-Strand cDNA Synthesis Reaction (Invitrogen) following the manufacturer guidelines. The primary and secondary PCR of the nested PCR were prepared using Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs) according to the user guide instructions in a final reaction volume of 50 µL. Primers and cycling conditions are summarized in Table 3.2. The PCR product from the primary PCR and the secondary PCR were visualized through gel electrophoresis followed by gel purification using the QIAquick Gel Extraction Kit (Qiagen) as per manufacturer's instructions.

3.4.2 Sample Concentration, Sanger Sequencing & Analysis of Sequences

The concentration of the purified amplicon product was quantified using a Nanodrop (Thermo Scientific) followed by dilution to a final concentration of 1 nanograms per microlitre (ng/µL). The samples were then prepared for sequencing by combining 1 ng/µL of concentrated sample with 2 micrometer (µm) concentration of each primer listed in Table 3.3 for a total volume of 50 µL. Sanger sequencing was performed at the Ottawa Hospital's Research Institute StemCore Sequencing Facility using an ABI Prism 3730 DNA Sequencer (Applied Biosystems). The sequences were compiled and edited using Snapgene Viewer version 7.2.0. Genotype and clade were determined by comparing the sequences with the NCBI Blast database of sequences.

Table 3.3: Amplification Targets and Primers used for Sanger Sequencing

Amplicon Length	Primers	Reference
695 bp	MVN 5 5' GGAGTAGGAGTGGAACTTG 3' MVN 6 5' TCTGCCATCGGCTCCAATCG 3'	Brzovic et al., 2022
634 bp	MeV 216 5’ TTGAGCTATGCCATGGGAGT 3’ MeV 214 5’ TAACAATGATGGAGGGTAGG 3’	CDC, 2018

3.5 Analysis of Vaccination Data

3.5.1 Vaccine Administration Data

Analysis of the vaccine administration data was performed with permission from the University of Ottawa Review Ethics Board (see appendix). The de-identified aggregated data was collected and anonymized by Ottawa Public Health representing the number of MMR and MMR-Var vaccinations administered in the Ottawa region from January 2017 to July 2024. The data was organized in a way to exclude individuals under the age of 4 receiving the vaccination as it was assumed that most people at that age would be wearing a diaper and therefore not be contributing to the wastewater. Only data collected between the years of 2020 to 2024 were used.

3.5.2 Vaccine Distribution Data

Due to a discrepancy between the date of administration and the date of reporting in the vaccine administration data, the vaccine distribution data was used as a proxy. Since the vaccine distributions have consistent zeros on weekends, the data was smoothed with a 7-day endpoint moving average. This better aligns with the practical context, as weekends represent no activity, and avoids smoothing across periods with known structural zeros. The MeV wastewater data has many zeros and no clear periodicity; therefore, it was smoothed with a 7-day centered moving average to maintain symmetry and better capture true trends amidst the noise. For the correlation analysis, neither dataset was normally distributed, thus the Spearman's method was used over the Pearson's method to identify the optimal lag between vaccine distribution and MeV vaccine detections in Ottawa wastewater. Linear regression analysis was carried out using MeV detections in wastewater as a function of lagged distributions to determine whether the number of vaccine distributions had an effect on the detection of MeV vaccine strain in wastewater samples. Another linear regression analysis was performed to determine whether the vaccine distributions changed significantly from 2020 to 2024, using monthly total distributions for each month and year.

4. RESULTS AND DISCUSSION

4.1 MeV Vaccine Shedding: Detection of Measles Genotype A

Positive MeV signal was detected in 8% of wastewater samples (11/135) (**Figure 4.1**). The first positive detection was on August 4, 2023, and was the only detection in 2023. All subsequent positive detections were in 2024. Nine of the positive detections were from the daily samples and only two positive detections were from the retrospective analysis of archived RNA samples. The samples exhibited low concentrations, and detections were at the limit of detection corresponding to the 80% confidence interval.

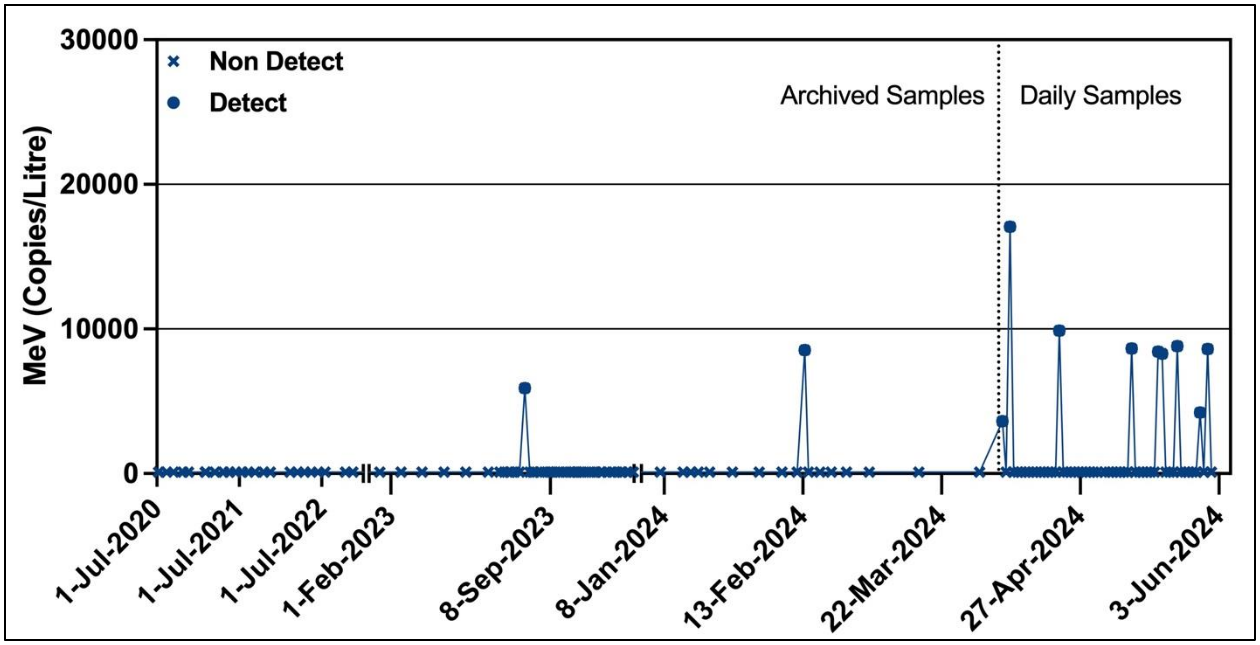


Figure 4.1: Genome copies of MeV per litre of wastewater sample from July 2020 to May 31, 2024. The crosses (X) indicate non detects and closed circles indicate detects. The dotted line indicates the distinction between the archived and daily samples.

The detection of MeV in wastewater was a public health concern in the city of Ottawa, particularly as there were no known active measles cases in the population at the time (Public Health Ontario, 2024). As such, in accordance with WHO framework, genetic characterization of MeV upon detection in wastewater was carried out to distinguish the virulent strain and to confirm the reliability of our MeV results at low concentrations (Kaic et al., 2010; WHO, 2015). Two of the wastewater samples with positive MeV detection were Sanger sequenced for genomic analysis: wastewater samples collected on April 6, 2024, and May 22, 2024. The selection of these two samples was based on first detection of the daily samples and strength of the signal. Sequence comparison with NCBI Blast database revealed a 100% homology with the Moraten vaccine strain and the Schwarz vaccine strain (NCBI Accension # AF266287 and AB591381, respectively). Phylogenetic analysis using the measles typing tool on the Genome Detective website confirmed identification of measles genotype A (**Figure 4.2**). Therefore, the positive detections of MeV were the vaccine strain since all wildtype strains of genotype A are extinct, indicative of vaccine shedding of the live-attenuated MeV (WHO, 2018). This outcome was unexpected, as most reports of MeV detections in other regions involved circulating wildtype strains during an active measles outbreak or, where MeV vaccine strain was detected, resulted from sampling a smaller catchment area (Rector et al., 2024; Ndlovu et al., 2024; Benschop et al., 2017).

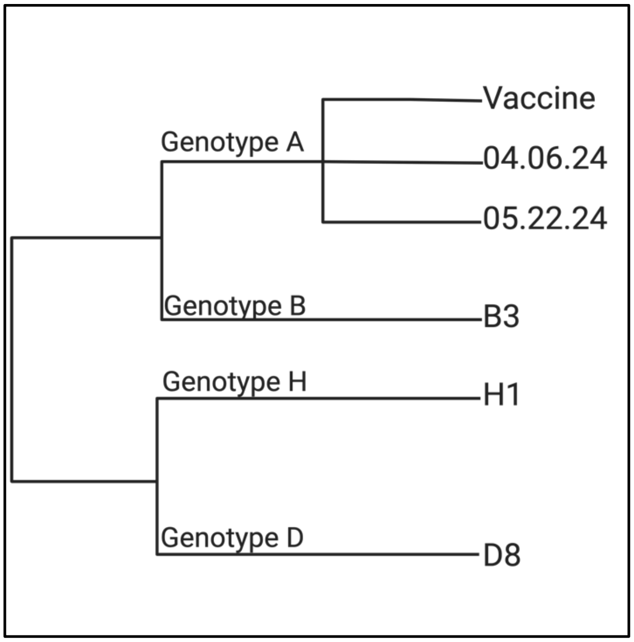


Figure 4.2: Phylogenetic analysis of Sanger sequencing results confirmed identification of genotype A indicating MeV vaccine strain. The figure is adapted from measles typing tool results from the Genome Detective website (genomedetective.com).

Low recovery of MeV from RNA samples stored at -80°C, (July 2020 – March 2024) may have produced non-detects due to lower RNA volume limiting the number of replicates to three. Hence, the archived samples in this study were analyzed in triplicate instead of eight technical replicates that was used for the daily samples. Additionally, it may be due to viral loss during storage as samples collected between April 6, and May 31, 2024, were not archived and instead were processed and analyzed within 48 hours of collection. Research on the effects of storage at -80°C of SARS-CoV-2 RNA from wastewater samples found no significant difference in recovery when re-quantified after 16 months storage (Williams et al., 2024). Research has been done to understand the stability of MeV spiked raw wastewater samples stored at room temperature and 4°C which showed limited decay to the virus after 28 days storage (Wu et al., 2024). However, the effects of long-term storage of naturally occurring MeV RNA, as opposed to spiked MeV RNA, in raw wastewater has not yet been studied nor has the effects of long-term storage of previously extracted MeV RNA from wastewater samples. Additionally, the viral signal from fresh samples was already low, consequently it is stipulated that even a small amount of decay could lead to loss of viral signal in the archived samples.

4.2. Public Health Measles Vaccine Initiatives Associated with MeV Measurements in Wastewater

The measles vaccines available in Ontario, Canada, are Priorix (MMR), Proquad (MMR-Var), and MMR II (MMR), all manufactured with either the Schwarz or Moraten strain, both derived from the Edmonston isolate. Analysis of these strains shows identical MeV genotype A coding sequences (Bankamp et al., 2011; Rota et al., 1994; GlaxoSmithKline, 2022; Merck Sharp & Dohme, 2005). Research has shown that live-attenuated vaccines can result in viral shedding, a phenomenon demonstrated through the detection of live-attenuated viral vaccines for polio and rotavirus in wastewater (Armas et al., 2023; Bucardo et al., 2011; Ito et al., 2021; Pavlov, 2006). Previous studies examining the shedding and persistence of MeV in vaccinated individuals has demonstrated the presence of viral RNA in urine samples 1 to 15 days post vaccination with the possibility of slow viral clearance from the body persisting for up to 100 days post vaccination (Eckerle et al., 2013; McMahon et al., 2019; Rota et al., 1995). In Canada, vaccines are disposed of by incineration;

therefore, the viral signal detected in this study is likely from recently vaccinated individuals and those continuing to shed the vaccine over extended periods, rather than from disposal down the drain (Public Health Unit, 2018).

Reduced vaccination rates during the pandemic in combination with a global rise in measles cases and reduced travel restrictions has led to an increase in the number of measles cases seen in Ontario (Public Health Ontario, 2024). As of May 31, 2024, there were 23 confirmed cases of measles reported in Ontario occurring in nine different public health units; no measles cases were reported in Ottawa to date. Ottawa Public Health increased vaccination initiatives in 2023 and 2024 by sending automated messages via text, email or phone call to households with one or more children overdue for vaccinations. These messages, sent periodically between June and October 2023 and January and March 2024, were intended to drive families to the school clinics provided by Ottawa Public Health's Immunization Program or to primary care providers (Day, V., 2023). Additionally, due to increased risk of exposure, health care workers often receive routine immunization booster shots, including the MMR vaccine (Public Health Ontario, 2012).

Given that 91% (10/11) of MeV detections occurred in 2024, and none were observed before 2023, the authors investigated potentially contributing factors by analyzing vaccine administration and distribution data for Ottawa. Initial analysis of the data revealed a notable delay in reporting vaccine administrations before and after the SARS-CoV-2 pandemic years (**Figure 4.3**). For instance, on average, only about 60% of vaccinations were reported within the same year they were administered, with remaining administrations being reported periodically in subsequent years. Due to this delay in reporting, Ottawa Public Health's data may not reflect up-to-date totals. As a result, the distribution data was used as a proxy for statistical analysis. Spearman's rho correlation analysis identified the optimal lag between vaccine distribution and MeV vaccine detections in Ottawa wastewater at 8 days (rho = 0.37, p = 0.008). However, the true correlation is likely under-estimated due to the high number of zeros in the MeV detection data, which caused ties in the ranks.

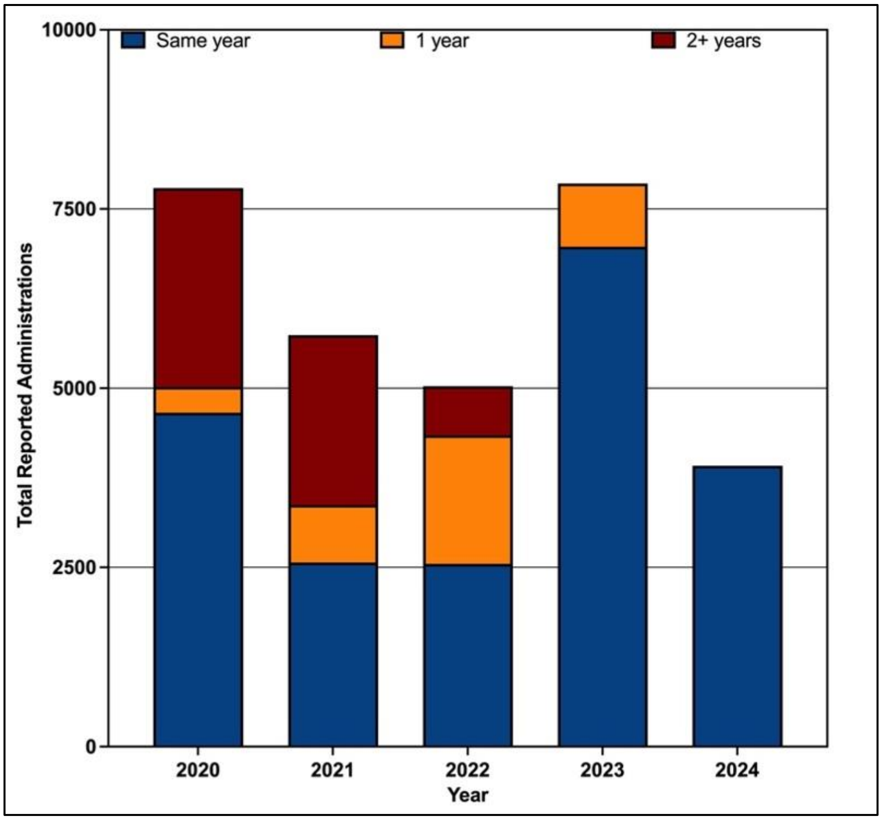


Figure 4.3: A representation of the delay in reporting vaccine administrations. The bar graph is comprised of three sections: blue represents the vaccine administrations reported within the same year they were administered, orange represents vaccine administrations reported within the year following the year they were administered, and red represents vaccine administrations reported two or more years after they

were administered. The total reported vaccine administrations for 2020 to 2023 are from January to December, while total reported vaccine administrations for 2024 are from January to June.

The study analyzed trends in vaccine distributions since 2020 and their impact on detecting the MeV vaccine strain in Ottawa wastewater (**Figure 4.4**). Linear regression revealed a significant year-over-year increase in vaccine distributions ($p = 0.0006$), likely reflecting expanded vaccination efforts or demand. A significant positive relationship was observed between vaccine distributions and wastewater detection ($p = 0.0055$), with every 100 vaccines distributed corresponding to a 2, 000 copies/litre increase in the 7-day moving average of MeV detections. This supports the hypothesis that vaccine distributions contribute to detectable increases in wastewater MeV levels. However, the model accounts for only 15% of the variance ($R^2 = 0.1469$), suggesting other influential factors. Future studies should consider longer observation periods, real-time vaccine administration data, and demographic variables to better understand these effects.

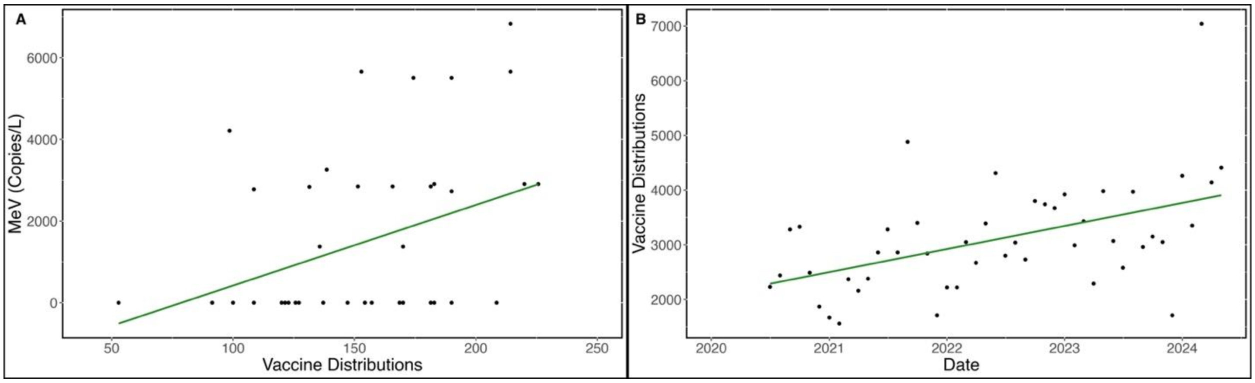


Figure 4.4: The relationship between MeV vaccine strain detection in wastewater and vaccine distributions (A) and the year over year increase in vaccines distributions (B). The linear regression analysis in graph A represents MeV vaccine detections as

a function of lagged distributions (lag = 8 days). The linear regression analysis in graph B represents monthly total distributions for each month and year.

4.3 Measles WES and Public Health Applications

WES has the potential to serve as an important public health tool by detecting MeV RNA in wastewater and hence identifying risk of measles transmission in communities prior to clinical cases presenting within the community. The time from exposure to rash onset of clinical measles cases averages 14 days with patients considered infectious during the prodromal phase approximately four days before rash onset during the rash phase (WHO, 2018). In Ontario, individuals presenting with fever, maculopapular rash for at least three days, and at least one of: cough, coryza or conjunctivitis are suspected measles cases requiring confirmation via virus isolation (culture) or RNA detection (PCR) from a collected clinical specimen (Ontario Ministry of Health, 2024). Therefore, the earliest possible detection using clinical surveillance is during the rash phase while the earliest possible detection using measles WES is during the prodromal phase, potentially allowing for early warning to public health.

In the event of a detected measles signal in wastewaters, public health authorities would be notified and in turn swiftly mobilize resources, initiating urgent clinical measures, including enhanced surveillance and rapid laboratory confirmation via PCR or serology, leading to prompt isolation of suspected cases and immediate contact tracing (Ontario Ministry of Health, 2024). These actions could be accompanied by expedited vaccination efforts to prevent further transmission due to the highly contagious nature of measles and the risk of large-scale outbreaks (Ramsay et al., 2019). However, accurately interpreting a MeV signal in wastewater requires identifying the genotype because vaccination rates and recent immunization efforts can confound results. As such, routine genotypic analysis of MeV in wastewater, a process that spans approximately 7 days, is essential to ensure that signals from the vaccine strain are correctly identified, allowing public health resources to be directed appropriately. Advancements have been made to streamline the process of distinguishing wildtype and vaccine strain MeV by using genotype specific primers/probes (Ndlovu et al., 2024; Wu et al., 2024). However, when possible, these results should be corroborated with sequencing and phenotypic analysis.

The detection of MeV signal in wastewater offers valuable insights, extending beyond its potential use as an early warning system for measles re-emergence. It could also provide real-time information on the presence of vaccine-virus in wastewater following vaccine campaigns. Similar applications have been successfully demonstrated for the live-attenuated poliovirus vaccine in Finland and Cuba following a vaccine campaign, and more recently for the MeV vaccine in South Africa following supplemental immunization activity during an active measles outbreak (Ndlovu et al., 2024; Lago et al., 2003; Poyry et al., 1988). In Canada, maintaining the elimination of endemic measles transmission relies heavily on strategies such as effective surveillance and high immunization coverage. However, delays in reporting vaccine administrations may render the clinical data outdated, particularly during periods of intensified vaccination efforts. The observed positive relationship between vaccine distributions and MeV vaccine strain detections in wastewater highlights the potential of WES as a complementary public health tool. Further research, including more comprehensive modeling approaches, is needed to fully understand and refine its applications.

5. CONCLUSIONS & RECOMMENDATIONS

5.1 Conclusion

This thesis investigated positive MeV signal in wastewater from a large contributing population without any known active measles cases in the region. The results of molecular analysis revealed that the positive signal was due to the MeV vaccine type rather than the wildtype virus. This marked the first report of MeV vaccine type identified in wastewater from a large catchment area in a measles non-endemic country.

One of the main conclusions drawn from this research is that vaccine shedding is a confounding factor of WES. This highlights the importance of genotypic analysis within WES practices to distinguish between vaccine type and wildtype viruses. Routine genotypic analysis can prevent the misallocation of public health resources in response to MeV WES signals that do not pose a transmission risk, as is also the case with clinical surveillance.

Additionally, this research highlights potential complementary public health applications of MeV WES. By ensuring accurate interpretation of a positive MeV signal in wastewater, routine MeV WES has the potential to be used as a complementary tool for public health officials such as an early warning system for measles re-emergence, or to provide insights into the presence of MeV vaccine type in wastewater following vaccine campaigns. The findings in this study can be applied when investigating other re-emergent viruses using WES to enhance outbreak preparedness, prevention, and response.

5.2 Recommendations for Further Research

The conclusions drawn from this research has led to the following recommendations for future research to assist in optimizing WES for surveillance of measles and other pathogenic diseases:

5.2.1 Advancement of MeV WES genotyping methods

As demonstrated through this research, the MeV vaccine may confound RT-qPCR results necessitating genotypic analysis to differentiate between wildtype and vaccine type. However, the process for genotypic analysis of MeV in wastewater is lengthy and expensive, potentially requiring up to 7 days to process from sample collection to sequencing. Rapid confirmation of vaccine type or wildtype signal in wastewater is important to avoid unnecessary public health response. 'PCR genotyping' is a process of designing primers and probes to differentiate between the dominant wildtypes of a region and the vaccine type. The use of PCR genotyping has been demonstrated in research by Wu et al. (2024) to be effective at distinguishing between the vaccine type (genotype A) and two dominant wildtypes (genotypes D8 and B3) of spiked wastewater samples, which was then validated using raw wastewater collected outside a facility with a known measles outbreak (targeted surveillance). Additionally, research by Ndlovu et al. (2024), demonstrated the usefulness of PCR genotyping by distinguishing between the vaccine type and the wildtype strains B3, D8, and H1. Both studies validated their methods using spiked wastewater samples and then using wastewater samples from targeted surveillance, usually during a known outbreak.

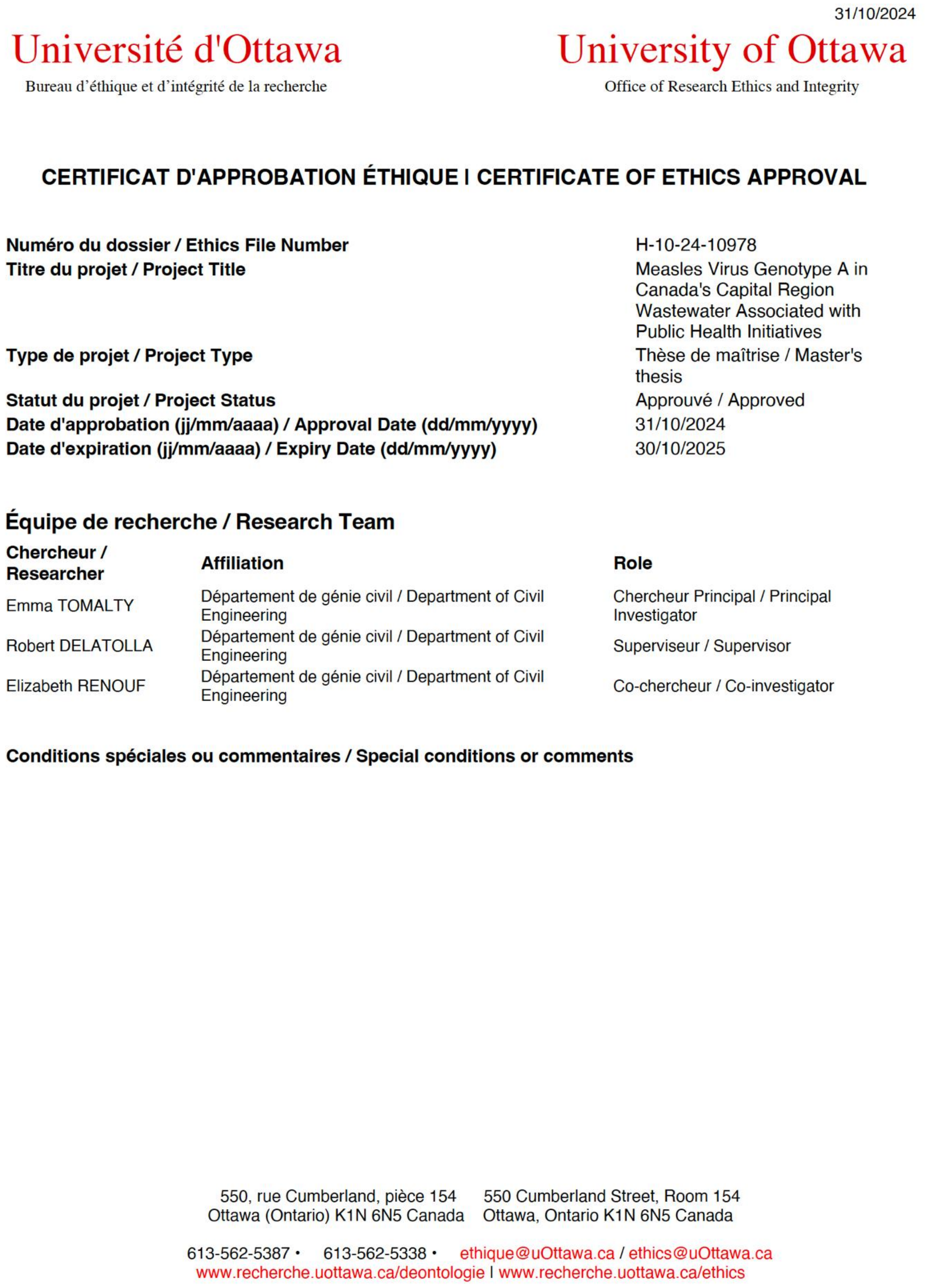
Investigating the usefulness of PCR genotyping in measles non-endemic settings, from large-scale wastewater treatment plants in the absence of a measles outbreak may save valuable resources by avoiding unnecessary genotyping of the MeV vaccine strain. However, this approach is not without limitations since without molecular epidemiology, clades that are considered inactive may resurface unknowingly and could lead to false negatives in a PCR genotyping approach (Kremer et al., 2004). Further research on this approach could be useful to enhance MeV WES.

5.2.3. Integration of MeV WES within public health

There has been a reduction in routine immunizations in Canada and many countries, likely due to changes in access to healthcare caused by the SARS-CoV-2 pandemic and potentially due to the growing misinformation spread on the internet regarding immunizations (Tam, 2023). This study detected and identified the MeV vaccine strain (genotype A) within wastewater from a large catchment area. The findings of this research reinforce previously published achievements of MeV WES illustrating the potential of an alternate surveillance tool for public health officials. Research by Ndlovu et al. (2024) also detected the MeV vaccine strain, but from targeted surveillance in an area following supplemental immunization activity, thus demonstrating the potential for MeV WES to be used to monitory efficacy of vaccine campaigns. This phenomenon has been demonstrated with the detection of live-attenuated poliovirus vaccine in wastewater in Finland and Cuba following vaccine campaigns (Lago, et al., 2003; Poyry, et al., 1988). A similar approach in monitoring Ottawa wastewater for MeV could provide valuable insights, allowing researchers and public health officials to track trends and fluctuations in the signal. For instance, early detection of a wildtype MeV signal could trigger a vaccination campaigns to halt the transmission.

Alternatively, there is a potential of WES to reflect the impact of vaccination campaigns if, for instance, the dominating wildtype signal is no longer detected following a vaccine campaign. Such an approach would require a thorough experimental plan and cooperation of Ottawa Public Health as well as an active measles outbreak in the Ottawa community.

APPENDIX



31/10/2024

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-10-24-10978
Titre du projet / Project Title	Measles Virus Genotype A in Canada's Capital Region Wastewater Associated with Public Health Initiatives
Type de projet / Project Type	Thèse de maîtrise / Master's thesis
Statut du projet / Project Status	Approuvé / Approved
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	31/10/2024
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	30/10/2025

Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Emma TOMALTY	Département de génie civil / Department of Civil Engineering	Chercheur Principal / Principal Investigator
Robert DELATOLLA	Département de génie civil / Department of Civil Engineering	Superviseur / Supervisor
Elizabeth RENOUF	Département de génie civil / Department of Civil Engineering	Co-chercheur / Co-investigator

Conditions spéciales ou commentaires / Special conditions or comments

550, rue Cumberland, pièce 154
Ottawa (Ontario) K1N 6N5 Canada

550 Cumberland Street, Room 154
Ottawa, Ontario K1N 6N5 Canada

613-562-5387 • 613-562-5338 • ethique@uOttawa.ca / ethics@uOttawa.ca
www.recherche.uottawa.ca/deontologie I www.recherche.uottawa.ca/ethics

31/10/2024

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Le Comité d'éthique de la recherche (CÉR) de l'Université d'Ottawa, opérant conformément à l'*Énoncé de politique des Trois conseils* (2014) et toutes autres lois et tous règlements applicables, a examiné et approuvé la demande d'éthique du projet de recherche ci-nommé.

L'approbation est valide pour la durée indiquée plus haut et est sujette aux conditions énumérées dans la section intitulée "Conditions Spéciales ou Commentaires". Le formulaire « Renouvellement ou Fermeture de Projet » doit être complété quatre semaines avant la date d'échéance indiquée ci-haut afin de demander un renouvellement de cette approbation éthique ou afin de fermer le dossier.

Toutes modifications apportées au projet doivent être approuvées par le CÉR avant leur mise en place, sauf si le participant doit être retiré en raison d'un danger immédiat ou s'il s'agit d'un changement ayant trait à des éléments administratifs ou logistiques du projet. Les chercheurs doivent aviser le CÉR dans les plus brefs délais de tout changement pouvant augmenter le niveau de risque aux participants ou pouvant affecter considérablement le déroulement du projet, rapporter tout évènement imprévu ou indésirable et soumettre toute nouvelle information pouvant nuire à la conduite du projet ou à la sécurité des participants.

The University of Ottawa Research Ethics Board, which operates in accordance with the *Tri-Council Policy Statement* (2014) and other applicable laws and regulations, has examined and approved the ethics application for the above-named research project.

Ethics approval is valid for the period indicated above and is subject to the conditions listed in the section entitled "Special Conditions or Comments". The "Renewal/Project Closure" form must be completedfour weeks before the above-referenced expiry date to request a renewal of this ethics approval or closure of the file.

Any changes made to the project must be approved by the REB before being implemented, except when necessary to remove participants from immediate endangerment or when the modification(s) only pertain to administrative or logistical components of the project. Investigators must also promptly alert the REB of any changes that increase the risk to participant(s), any changes that considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project or the safety of the participant(s).

Mathieu LAFLAMME
Responsable d'éthique en recherche / Protocol Officer
Pour/For **Daniel LAGAREC** Président(e) du/ Chair of the **Comité d'éthique de la recherche en sciences de la santé et sciences / Health Sciences and Sciences Research Ethics Board**

550, rue Cumberland, pièce 154 550 Cumberland Street, Room 154
Ottawa (Ontario) K1N 6N5 Canada Ottawa, Ontario K1N 6N5 Canada

613-562-5387 • 613-562-5338 • ethique@uOttawa.ca / ethics@uOttawa.ca
www.recherche.uottawa.ca/deontologie I www.recherche.uottawa.ca/ethics

BIBLIOGRAPHY

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K., Mueller, J. F. (2020). First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment, 728,* 138764. https://doi.org/10.1016/j.scitotenv.2020.138764.

Ahmed, W., Tscharke, B., Bertsch, P.M., Bibby, K., Bivins, A., Choi, P., Clarke, L., Dwyer, J., Edson, J., Nguyen, T.M.H., O'Brien, J.W., Simpson, S.L., Sherman, P., Thomas, K.V., Verhagen, R., Zaugg, J., Mueller, J.F. (2021). SARS-CoV-2 RNA monitoring in wastewater as a potential early warning system for COVID-19 transmission in the community: A temporal case study. *Science and the Total Environment, 761,* 144216. https://doi.org/10.1016/j.scitotenv.2020.144216.

Armas, F., Chandra, F., Lee, W.L., Gu, X., Chen, H., Xiao, A., Leifels, M., Wuertz, S., Alm, E.J. (2023). Contextualizing wastewater-based surveillance in the COVID-19 vaccination era. *Environment International, 171,* 107718. https://doi.org/10.1016/j.envint.2022.107718.

Bankamp, B., Takeda, M., Zhang, Y., Xu, W., Rota, P.A. (2011). Genetic characterization of measles vaccine strains. *The Journal of Infectious Diseases, 204,* S533-S548. https://doi.org/10.1093/infdis/jir097.

Barrero, P.R., Zandomeni, R.O., and Mistchenko, A.S. (2001). Measles virus circulation in Argentina: 1991-1999. *Archives of Virology, 146,* 815-823. https://doi.org/10.1007/s007050170150.

Bellini, W.J. & Rota, P.A. (1998). Genetic diversity of wild-type measles viruses: Implications for global measles elimination programs. *Emerging Infectious Diseases, 4(1),* 29-35. https://doi.org/10.3201/eid0401.980105.

Benedetti, G., Krogsgaard, L.W., Maritschnik, S., Stuger, H.P., Hutse, V., Janssens, R., Blomqvist, S., Pitkanen, T., Koutsolioutsou, A., Roka, E., Vargha, M., La Rosa, G., Suffredini, E., Cauchie, H.M., Ogorzaly, L., van der Beek, R.F.H.J., Lodder, W.J., Madslien, E.H., Lomba, J.A.B, Ethelberg, S. (2024). A survey of the representativeness and usefulness of wastewater-based surveillance systems in 10 countries across Europe in 2023. *Eurosurveillance, 29(33),* 1-11. https://doi.org/10.2807/1560-7917.ES.2024.29.33.2400096.

Benschop, K.S.M., van der Avoort, H.G., Jusic, E., Vennema, H., van Binnendijk, R., Duizer, E. (2017). Polio and measles down the drain: environmental enterovirus surveillance in the Netherlands, 2005 to 2015. *Applied and Environmental Microbiology, 83*, e00558-17. https://doi.org/10.1128/AEM.00558-17.

Berry, I., Brown, K.A., Buchan, S.A., Hohenadel, K., Kwong, J.C., Paten, S., Rosella, L.C., Mishra, S., Sander, B. (2022). A better normal in Canada will need a better detection system for emerging and re-emerging respiratory pathogens. *Canadian Medical Association Journal, 194(36),* E1250-E1254. https://doi.org/ 10.1503/cmaj.220577.

Boehm, A.B., Hughes, B., Duong, D., Chan-Herur, V., Buchman, A., Wolfe, M.K., White, B.J. (2023). Wastewater concentrations of human influenza, metaphneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. *The Lancet, 4(5),* E340-E348. https://doi.org/10.1016/ S2666-5247(22)00386-X.

Boyd, J.F., (1983). A fourteen-year study to identify measles antigen in urine specimens by fluorescent-antibody methods. *Journal of Infection, 6,* 163-170. https://doi.org/10.1016/S0163-4453(83)92818-9.

Brouwer, A.F., Eisenberg, J.N.S., Pomeroy, C.D., Shulman, L.M., Hindiyeh, M., Manor, Y., Grotto, I., Koopman, J.S., Eisenberg, M.C. (2018). Epidemiology of the silent polio outbreak in Rahat, Israel, based on modelling of environmental surveillance data. *Proceedings of the National Academy of Sciences of the United States of America, 115(45)*, E10625 – E10633. https://doi.org/10.1073/pnas.1808798115.

Brzovic, M., Juretic, K.B., Jurcev-Savicevic, A., Mihojevic, L., Nonkovic, D., Rizvan, P., Petrovic, M.V., Tonkic, M., Kaic, B., Babic-Erceg, A., Vilibic-Cavlek, T., Ivancic-Jelecki, J. (2022). Measles cases in Split-Dalmatia County (a Croatian tourist region), in May-July 2019: outbreak report and lessons learnt. *European Journal of Public Health, 32(6),* 948-954. https://doi.org/10.1093/eurpub/ckac162.

Bucardo, F., Lindgren, P.E., Svensson, L., Nordgren, J. (2011). Low prevalence of rotavirus and high prevalence of norovirus in hospital and community wastewater after introduction of rotavirus vaccine in Nicaragua. *PLOS One, 6(10),* e25962. https://doi.org/10.1371/journal.pone.0025962

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T. (2009). The MIQE Guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry, 55(4)*, 611-622. https://doi.org/10.1373/clinchem.2008.112797.

Centre for Disease Control (CDC). (2018). *Annex C1: RT-PCR Protocol for Measles genotyping*. WHO International Publishing.

Chik, A.H.S., Glier, M.B., Servos, M., Mangat, C.S., Pang, X.L., Qiu, Y., D'Aoust, P.M., Burnet, J.B., Delatolla, R., Dorner, S., Geng, Q., Giesy, J.P., McKay, R.M., Mulvey, M.R., Prystajecky, N., Srikanthan, N., Xie, Y., Conant, B., Hrudey, S.E., Canadian SARS-CoV-2 Inter-Laboratory Consortium (2021). Comparison of approaches to quantify SARS-CoV-2 in wastewater using RT-qPCR: Results and implications from a collaborative inter-laboratory study in Canada. *Journal of Environmental Sciences, 107*, 218-229. https://doi.org/10.1016/j.jes.2021.01.029.

D'Aoust, P. M., Mercier, E.,Montpetit, D., Jia, J.J., Alexandrov, I., Neault, N., Baig, A.T., Mayne, J., Zhang, X., Alain, T., Langlois, M.A., Servos, M.R., MacKenzie, M., Figeys, D., MacKenzie, A.E., Graber, T.E., Delatolla, R. (2021a). Quantitative analysis of SARS-COV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. *Water Research, 188,* 116560. https://doi.org/10.1016/j.watres.2020.116560.

D'Aoust, P.M., Graber, T.E., Mercier, E., Montpetit, D., Alexandrov, I., Neault, N., Baig, A.T., Mayne, J., Zhang, X., Alain, T., Servos, M.R., Srikanthan, N., MacKenzie, M., Figeys, D., Manuel, D., Juni, P., MacKenzie, A.E., Delatolla, R. (2021b). Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified in wastewater 48 h before COVID-19 clinical tests and 96 h before hospitalizations. *Science of the Total Environment, 770,* 145319. https://doi.org/10.1016/j.scitotenv.2021.145319.

Day, V. *Kids Come First to help families catch up on immunizations with innovative, seamless solution.* (2023). Cheo.on.ca. https://www.cheo.on.ca/en/news/kids-come-first-to-help-families-catch-up-on-immunizations-with-innovative-seamless-solution.aspx.

Eckerle, I., KellerStanislawski, B., Santibanez, S., Buderus, S., Hillmann, M., Drost en, C., Eis-Hübinger, A.M. (2013). Nonfebrile Seizures after Mumps, Measles, Rubella, and Varicella-Zoster Virus Combination Vaccination with Detection of Measles Virus RNA in Serum, Throat, and Urine. *Clinical and Vaccine Immunology, 20(7),* 1094-1096. https://doi.org/10.1128/CVI.00084-13

Erickson, T.B., Endo, N., Duvallet, C., Ghaeli, N., Hess, K., Alm, E.J., Matus, M., Chai, P.R. (2021). "Waste not, want not" – Leveraging sewer systems and wastewater-based epidemiology for drug use trends and pharmaceutical monitoring. *Journal of Medical Toxicology, 17*, 397-410. https://doi.org/10.1007/s13181-021-00853-4.

Furuse, Y., Suzuki, A., & Oshitani, H. (2010). Origin of measles virus: divergence from rinderpest virus between the 11th and 12th centuries. *Virology Journal, 7,* 2-5. https://doi.org/10.1186/1743-422X-7-52.

GlaxoSmithKline. (2022). PRIORIX (Measles, Mumps, and Rubella Vaccine, Live [package insert]. www.vaers.hhs.gov.

Government of Canada. (2024). *Measles: For health professionals*. Canada.ca. https://www.canada.ca/en/public-health/services/diseases/measles/health-professionals-measles.html.

Gracia-Lor, E., Rousis, N.I., Herna, F., Zuccato, E., Castiglioni, S., 2018. Wastewater-based epidemiology as a novel biomonitoring tool to evaluate human exposure to pollutants. *Environmental Science and Technology, 52*, 10224‑10226. https://doi.org/10.1021/acs.est.8b01403.

Graham, K.E., Loeb, S.K., Wolfe, M.K., Catoe, D., Sinnott-Armstrong, N., Kim, S., Yamahara, K.M., Sassoubre, L.M., Mendoza Grijalva, L.M., Roldan-Hernandez, L., Langenfield, K., Wigginton, K.R., Boehm, A.B. (2020). SARS-CoV-2 RNA in wastewater settled solids is associated with COVID-19 cases in a large urban sewershed. *Environmental Science and Technology, 55(1),* 488-498. https://doi.org/10.1021/acs.est.0c06191.

Han J.J., Song, H.A., Pierson, S.L., Gunther, J.S., Xia, Q. (2023). Emerging infectious diseases are virulent viruses – Are we prepared? An overview. *Microorganisms, 11(11)*, 1-17. https://doi.org/10.3390/microorganisms11112618.

Hegazy, N., Cowan, A., D'Aoust, P.M., Mercier, E., Towhid, S.T., Jia, J.J., Wan, S., Zhang, Z., Kabir, M.P., Fang, W., Graber, T.E., MacKenzie, A.E., Guilherme, S., Delatolla, R. (2022). Understanding the dynamic relationship between wastewater SARS-CoV-2 signal and clinical metrics throughout the pandemic. *Science and the Total Environment, 853,* 158458. https://doi.org/10.1016/j.scitotenv.2022.158458.

Hughes, B., Duong, D., White, B.J., Wigginton, K.R., Chan, E.M.G., Wolfe, M.K., Boehm, A.B. (2022). Respiratory Syncytial Virus (RSV) RNA in wastewater settled solids reflects RSV clinical positivity rates. *Environmental Science and Technology Letters, 9(2),* 173-178. https://doi.org/10.1021/acs.estlett.1c00963.

Hummel, K.B., Lowe, L., Bellini, W.J., Rota, P.A. (2006). Development of quantitative gene-specific real-time RT-PCR assays for the detection of measles virus in clinical specimens. *Journal of Virological Methods, 132 (1-2),* 166-173. https://doi.org/10.1016/j.jviromet.2005.10.006.

Ito, E., Pu, J., Miura, T., Kazama, S., Nishiyama, M., Ito, H., Konta, Y., Omura, T., Watanabe, T. (2021). Detection of rotavirus vaccine strains in oysters and sewage and their relationship with the gastroenteritis epidemic. *Applied and Environmental Microbiology, 87(10),* 1-9. https:// doi.org/10.1128/AEM.02547-20.

de Jonge, E.F., Peterse, C.M., Koelewijn, J.M., van der Drift, A.M.R., van der Beek, R.F.H.J., Nagelkerke, E., Lodder, W.J. (2022). The detection of monkeypox virus DNA in wastewater samples in the Netherlands. *Science and the Total Environment, 852,* 158265. https://doi.org/10.1016/j.scitotenv.2022.158265.

Kaic, B., Gjener-Margan, I., Aleraj, B., Vilibic-Cavlek, T., Santak, M., Cvitkovic, A., Nameth-Blazic, T., Ivic Hofman, I. (2010). Spotlight on measles 2010: Excretion of vaccine strain measles virus in urine and pharyngeal secretions of a child with vaccine associated febrile rash illness, Croatia, March 2010. *Eurosurveillance, 15(35),* 1-2. https://doi.org/10.2807/ese.15.35.19652-en.

Kantor, R.S., Nelson, K.L., Greenwald., H.D., Kennedy, L.C. (2021). Challenges in measuring the recovery of SARS-CoV-2 from wastewater. *Environmental Science and Technology, 55(6),* 3514-3519. https://doi.org/10.1021/acs.est.0c08210.

Kremer, J. R., Fack, F., Olinger, C.M., Mulders, M.N., Muller, C.P. (2004). Measles virus genotyping by nucleotide-specific multiplex PCR. *Journal of Clinical Microbiology, 42(7),* 3017-3022. https://doi.org.10.1128/JCM.42.7.3017-3022.2004.

Kumblathan, T., Liu, Y., Uppal, G.K., Hrudey, S.E., Li, X.F. (2021). Wastewater-based epidemiology for community monitoring of SARS-CoV-2: Progress and challenges. *ACS Environmental Au, 1(1),* 18-31. https://doi.org/10.1021/acsenvironau.1c00015.

Lago, P.M., Gary, H.E., Perez, L.S., Caceres, V., Olivera, J.B., Puentes, R.P., Corredor, M.B., Jimenez, P., Pallansch, M.A., Cruz, R.G. (2003). Poliovirus detection in wastewater and stools following an immunization campaign in Havana, Cuba. *International Journal of Epidemiology, 32(5),* 772-777. https://dx.doi.org/10.1093/ije/dyg185.

McMahon, J., Mackay, I.M., & Lambert, S.B. (2019). Measles vaccine virus RNA in children more than 100 days after vaccination. *Viruses, 11(7),* 636. https://doi.org/10.3390/v11070636.

Medema, G., Been, F., Heijnen, L., Petterson, S. (2020a). Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: Opportunities and challenges. *Environmental Science and Health*, *17,* 49-71. https://doi.org/10.1016/j.coesh.2020.09.006.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A. (2020b). Presence of SARS-CoV-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. *Environmental Science and Technology Letters, 7(7),* 511-516. https://doi.org/10.1021/acs.estlett.0c00357.

Metcalf, C., Tindale, K., Li, H., Rodayan, A., Yargeau, V. (2010). Illicit drugs in Canadian munifipal wastewater and estimates of community drug use. *Environmental Pollution, 158(10),* 3179 – 3185. https://doi.org/10.1016/j.envpol.2010.07.002.

Mercier, E., D'Aoust, P.M., Thakali, O., Hegazy, N., Jia, J.J., Zhang, Z., Eid, W., Plaza-Diaz, J., Kabir, M.P., Fang, W., Cowan, A., Stephenson, S.E., Pisharody, L., MacKenzie, A.E., Graber, T.E., Wan, S., Delatolla, R. (2022). Municipal and neighbourhood level wastewater surveillance and subtyping of an influenza virus outbreak. *Scientific Reports, 12*, 15777. https://doi.org/10.1038/s41598-022-20076-z.

Mercier, E., Pisharody, L., Guy, F., Wan, S., Hegazy, N., D'Aoust, P.M., Kabir, M.P., Nguyen, T.B., Eid, W., Harvey, B., Rodenburg, E., Rutherford, C., MacKenzie, A.E., Willmore, J., Hui, C., Paes, B., Delatolla, R., Thampi, N. (2023). Wastewater-based surveillance identifies start to the paediatric respiratory syncytial virus season in two cities in Ontario, Canada. *Frontiers in Public Health, 11,* 1261165. https://doi.org/10.3389/fpubh.2023.1261165.

Merck Sharp & Dohme. (2005). ProQuad (Measles, Mumps, Rubella and Varicella Virus Vaccine, Live [package insert]. www.vaers.hhs.gov.

Minta, A.A., Ferrari, M., Antoni, S., Portnoy, A., Sbarra, A., Lambert, B., Hatcher, C., Hsu, C.H., Ho, L.L., Steulet, C., Gacic-Dobo, M., Rota, P.A., Mulders, M.N., Bose, A.S., Caro, W.P., O'Connor, P., Crowcroft, N.S. (2023). *MMWR. Morbidity and Mortality Weekly Report, 72(46),* 1262-1268. https://doi.org/10.15585/mmwr.mm7246a3.

Moss, W.J. & Griffin, D.E. (2012). Measles. *The Lancet, 379,* 153-164. https://doi.org/10.1016/S0140-6736(10)62352-5.

Mulders, M.N., Truong, A.T., & Muller, C.P. (2001). Monitoring of measles elimination using molecular epidemiology. *Vaccine, 19,* 2245-2249. https://doi.org/10.1016/S0264-410X(00)00453-9.

Naughton, C.C., Roman, F.A., Alvarado, A.G.F., Tariq, A.Q., Deeming, M.A., Kadonsky, K.F., Bibby, K., Bivins, A., Medema, G., Ahmed, W., Katsivelis, P., Allan, V., Sinclair, R., Rose, J.B. (2023). Show us the data: global COVID-19 wastewater monitoring efforts, equity, and gaps. *FEMS Microbes, 4,* 1-8. https://doi.org/10.1093/femsmc/xtad003.

Ndlovu, N., Mabasa, V., Sankar, C., Msomi, N. & Phalane, E. (2024). *Wastewater testing during the South African 2022-2023 measles outbreak demonstrates the potential of environmental surveillance to support measles elimination*. medRxiv. https://doi.org/10.1101/2024.09.01.24312904.

Ontario Ministry of Health (2024). *Appendix 1: Case definitions and disease specific information, Disease: Measles.* Retrieved from: Ontario Public Health Standards: Requirements for Programs, Services and Accountability Infectious Disease Protocol. https://www.ontario.ca/files/2024-03/moh-measles-appendix-en-2024-03-19.pdf.

Pavlov, D.N. (2006). Poliovirus vaccine strains in sewage and river water in South Africa. *Canadian Journal of Microbiology, 52(8),* 717-723. https://doi.org/10.1139/W06-026.

Parks, C.L., Lerch, R.A., Walpita, P., Wang, H., Sidhu, M.S., Udem, S.A.(2001).Comparison of Predicted Amino Acid Sequences of Measles Virus Strains in the Edmonston Vaccine Lineage. *Journal of Virolology, 75*(2), 910-920. https://doi.org/10.1128/jvi.75.2.910-920.2001.

Petrinca, A.R., Donia, D., Pierangeli, A., Gabrieli, R., Degener, A.M., Bonanni, E., Diaco, L., Cocchini, G., Anastasi, P., Divizia, M. (2009). Presence of environmental circulation of enteric viruses in three different wastewater treatment plants. *Journal of Appleid Microbiology, 106(5),* 1608-1617. https://doi.org/10.1111/j.1365-2672.2008.04128.x.

Poyry, T., Stenvik, M. & Hovi, T. (1988). Viruses in sewage waters during and after a poliomyelitis outbreak and subsequent nationwide oral poliovirus vaccination campaign in Finland. *Applied and Environmental Microbiology, 54(2),* 371-374. https://doi.org/10.1128/AEM.54.2.371-374.1988.

Public Health Ontario. *Routine Practices and Additional Precautions in All Health Care Settings*. (2012). Publichealthontario.ca. https://www.publichealthontario.ca/en/Health-Topics/Infection-Prevention-Control/Routine-Practices-Additional-Precautions

Public Health Ontario. *Publicly Funded Immunization Schedules for Ontario – June 2022*. (2022). Ontario.ca. https://www.ontario.ca/files/2024-01/moh-publicly-funded-immunization-schedule-en-2024-01-23.pdf

Public Health Ontario. *Enhanced epidemiological summary measles in Ontario*. (2024). Publichealthontario.ca ca https://www.publichealthontario.ca/-/media/Documents/M/24/measles-ontario-epi-summary.pdf.

Public Health Unit. *Vaccine Storage and Handling Guidelines*. (2018). Health.gov.on.ca. https://files.ontario.ca/moh-ophs-ref-vaccine-storage-handling-guidelines-2021-en.pdf.

Qiagen. (2018). *AllPrep® PowerViral® DNA/RNA Kit Handbook*. Qiagen Press. https://www.qiagen.com/us/resources/resourcedetail?id=41a1323f-581c-4e65-8d66-2491be4c625e&lang=en.

Ramsay, L.C., Crowcroft, N.S., Thomas, S., Aruffo, E., Teslya, A., Heffernan, J.M., Gournis, E., Hiebert, J., Jaeger, V., Jiaravuthisan, M., Sharron, J., Severini, A., Deeks, S.L., Gubbay, J., Mazzulli, T., Sander, B. (2019). Cost-effectiveness of measles control during elimination in Ontario, Canada, 2015. *Eurosurveillance, 24(11),* 1-11. https://doi.org/10.2807/1560-7917.ES.2019.24.11.1800370.

Rector, A., Bloemen, M., Hoorelbeke, B., Van Ranst, M. & Wollants, E. (2024). *Detection of measles virus genotype D8 in wastewater of Brussels capital region, Belgium, March 2024*. medRxiv. https://doi.org/10.1101/2024.04.08.24305478.

Riddell, M.A., Moss, W.J., Hauer, D., Monze, M., Griffin, D.E. (2007). Slow clearance of measles virus RNA after acute infection. *Journal of Clinical Virology, 39(4),* 312-317. https://doi.org/10.1016/j.jcv.2007.05.006.

Rota, P.A., Bloom, A.E., Vanchiere, J.A., Bellini, W.J. (1994). Evolution of the nucleoprotein and matrix genes of wildtype strains of measles virus isolated from recent epidemics. *Virology, 198(2),* 724-730. https://doi.org/10.1006/viro.1994.1086.

Rota, P. A., Khan, A.S., Durigon, E., Yuran, T., Villamarzo, Y.S., Bellini, W.J. (1995). Detection of measles virus RNA in urine specimens from vaccine recipients. *Journal of Clinical Microbiology, 33,* 2485–2488. https://doi.org/10.1128/jcm.33.9.2485-2488.1995.

Sasaki, A., Haraguchi, Y., & Yoshida, H. (2012). Estimating the risk of re-emergence after stopping polio vaccination. *Frontiers in Microbiology, 3*. https://doi.org/10.3389/fmicb.2012.00178.

Shaw, A.G., Troman, C., Akello, J.O., O'Reilly, K.M., Gauld, J., Grow, S., Grassly, N., Steele, D., Blazes, D., Kumar, S., The Environmental Surveillance Working Group. (2023). Defining a research agenda for environmental wastewater surveillance of pathogens. *Nature Medicine, 29(9),* 2155-2157. https://doi.org/10.1038/s41591-023-02457-7.

Tam, T. (2023). *Vaccines in Canada: Why meeting our routine vaccination goals matters.* The Science of Health Blog. https://science.gc.ca/site/science/en/blogs/science-health/vaccines-canada-why-meeting-our-routine-vaccination-goals-matters.

Vardas, E. & Kreis, S. (1999). Isolation of measles virus from a naturally immune, asymptomatically re-infected individual. *Journal of Clinical Virology, 13,* 173-179. https://doi.org/10.1016/s1386-6532(99)00026-8.

Williams, R.C., Perry, W.B., Lambert-Slosarska, K., Futcher, B., Pellet, C., Richardson-O'Neill, I., Paterson, S., Grimsley, J.M.S., Wade, M.J., Weightman, A.J., Farkas, K., Jones, D.L. (2024). Examining the stability of viral RNA and DNA in wastewater: Effects of storage time, temperature, and freeze-thaw cycles. *Water Research, 259,* 121879. https://doi.org/10.1016/j.watres.2024.121879.

Wolfe, M.K., Duong, D., Hughes, B., Chan-Herur, V., White, B.J., Boehm, A.B. (2022). *Detection of monkeypox viral DNA in a routine wastewater monitoring program. MedRXiv.* https://doi.org/10.1101/2022.07.25.22278043.

Wong, C.H., Zhang, Z., Eid, W., Plaza-Diaz, J., Kabir, M.P., Wan, S., Jia, J.J., Mercier, E., Thakali, O., Pisharody, L., Hegazy, N., Stephenson, S.E., Fang, W., Nguyen, T.B., Ramsay, N.T., Mckay, R.M., Corchis-Scott, R., MacKenzie, A.E., Graber, T.E., D'Aoust, P.M., Delatolla, R. (2023). Rapidly developed, optimized, and applied wastewater surveillance system for real-time monitoring of low-incidence, high-impact MPOX outbreak. *Journal of Water and Health, 21(9),* 1264-1276. https://doi.org/10.2166/wh.2023.145.

World Health Organization & United Nations Children's Fund (UNICEF). (2001). *WHO-UNICEF joint statement on strategies to reduce measles mortality worldwide.* Who.int. https://iris.who.int/handle/10665/67257.

World Health Organization (2015). Genetic diversity of wildtype measles viruses and the global measles nucleotide surveillance database (MeaNS). *Weekly Epidemiological Report, 90(30),* 373-380. https://iris.who.int/handle/10665/242393.

World Health Organization. (2018). *Manual for the laboratory-based surveillance of measles, rubella, and congenital rubella syndrome.* World Health Organization: Geneva, Switzerland.

Wu, J., Wang, M.X., Kalvapalle, P., Nute, M., Treangen, T.J., Ensor, K., Hopkins, L., Poretsky, R., Stadler, L.B. (2024). Multiplexed detection, partitioning, and persistence of wild-type and vaccine strains of measles, mumps, and rubella viruses in wastewater. *Environmental Science and Technology, 58(50),* 21930-21941. https://doi.org/10.1021/acs.est.4c05344