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Article

Variability of Clinical Metrics in Small Population Communities Drive Perceived Wastewater and Environmental Surveillance Data Quality: Ontario, Canada-Wide Study

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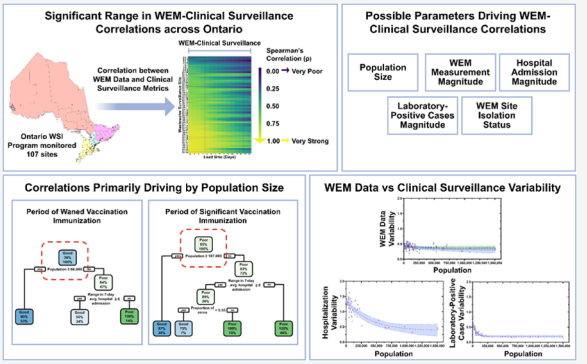
ABSTRACT: The emergence of COVID-19 in Canada has led to over 4.9 million cases and 59,000 deaths by May 2024. Traditional clinical surveillance metrics (hospital admissions and clinical laboratory-positive cases) were complemented with wastewater and environmental monitoring (WEM) to monitor SARS-CoV-2 incidence. However, challenges in public health integration of WEM persist due to perceived limitations of WEM data quality, potentially driving inconsistent correlations variability and lead times. This study investigates how factors like population size, WEM measurement magnitude, site isolation status, hospital admissions, and clinical laboratory-positive cases affect WEM data correlations and variability in Ontario. The analysis uncovers a direct relationship between clinical surveillance data and the population size of the surveyed sewersheds, while WEM measurement magnitude was not directly impacted by population size. Higher variability in clinical surveillance data was observed in smaller sewersheds, likely reducing correlation strength for inferring COVID-19 incidence. Population size significantly influenced correlation quality, with thresholds identified at ∼66,000 inhabitants for strong WEM-hospital admissions correlations and ∼68,000 inhabitants for WEM-laboratory-positive cases during waned vaccination periods in Ontario (the Omicron BA.1 wave). During significant vaccination immunization (the Omicron BA.2 wave), these thresholds increased to ∼187,000 and 238,000, respectively. These findings highlight the benefit of WEM for strategic public health monitoring and interventions, especially in smaller communities. This study provides insights for enhancing public health decision making and disease monitoring through WEM, applicable to COVID-19 and potentially other diseases.



KEYWORDS: *WEM data, population, hospital admissions, laboratory-positive cases, small communities, variability*

1. INTRODUCTION AND BACKGROUND

The emergence of the novel 2019 coronavirus (COVID-19) in Canada on January 25th, 2020[1] has resulted in over 4.9 million confirmed cases and more than 59,000 COVID-19 deaths by May, 2024.[2] In response to this public health crisis, population-wide SARS-CoV-2 diagnosis using polymerase chain reaction (PCR) has proven effective in identifying outbreaks and informing public health decisions.[3] Utilizing similar analytical methods, wastewater and environmental monitoring (WEM) has emerged as a valuable population-wide tool for monitoring and providing an early indication of COVID-19 incidence.[4−8] By the time of writing this manuscript (May 2024), the global landscape of WEM for COVID-19 has spanned across more than 72 countries, encompassing over 4600 monitoring sites.[9]

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WEM not only plays a significant role in real-time monitoring of SARS-CoV-2 infection rates,[6,8,10] but also extends its reach to the surveillance of other diseases at a population level, including influenza,[11−13] respiratory syncytial virus (RSV),[14−16] and Mpox.[11,17]

The Ontario Wastewater Surveillance Initiative (Ontario WSI) is a Canadian wastewater surveillance network that oversaw up to 107 sites at its peak.[18,19] The Ontario WSI began initial surveillance targeting SARS-CoV-2, and eventually expanded testing to include Influenza subtypes A and B, and RSV, with all data being accessible to public health agencies across the province. The initiative was inaugurated on January first, 2021, by the Government of Ontario, led by the Ministry of the Environment, Conservation and Parks (MECP) and supported by the Ontario Wastewater Surveillance Consortium (OWSC). Following a strategic sampling plan initiated on April first, 2023, resources were focused on community sites that reliably represent unique populations and geographical areas across Ontario, resulting in 59 sites being monitored across the province, covering 60% of Ontario's residents. The initiative was officially closed on July 31st, 2024. The Ontario WSI monitored the province of Ontario, which has a population size of approximately 15.6 million people, representing 38.5% of the nation's residents.[20] The province has reported 34.0% of Canada's total reported COVID-19 infections, with more than 1.6 million confirmed COVID-19 cases,[21] necessitating extensive sample processing facilitated through collaborations with 13 academic institutions across the province.[18] Notably, Ontario encompasses 19 census metropolitan areas.[22] Of the total 107 sampling locations, 25 sites were used to survey populations exceeding 100,000 inhabitants, while the remaining 82 sites were dedicated to serving areas with less than 100,000 inhabitants.

Assessment of the relationship between wastewater SARS-CoV-2 viral signal and clinical surveillance metrics, particularly COVID-19-caused hospital admissions and laboratory-confirmed positive cases, was critical to interpret and validate the WEM results. This assessment is typically achieved by evaluating Spearman's rank correlation coefficient (ρ) between the WEM data and clinical surveillance metrics (COVID-19-caused hospital admissions and laboratory-confirmed positive cases) and determining the lead time of the wastewater surveillance data. As the COVID-19 pandemic evolved, many countries reduced population-wide PCR testing with the widespread availability of at-home COVID-19 rapid antigen testing, leading to a reduced correlation between WEM data and laboratory-confirmed positive cases.[23−27] Effective December 31st, 2021, Ontario's PCR testing eligibility shifted from population-wide access to focus on symptomatic and high-risk individuals.[28] Consequently, the Ontario WSI program progressively integrated hospital admissions data, geospatially linked to the specific sewersheds (areas draining via a sanitary sewer network to the sampling point) under surveillance, as an essential comparator for validating the trends observed in WEM data measurements through the WEM-hospital admission correlation.

Despite the rapid expansion of WEM over the past four years, there remains a significant gap in its integration and actionable use by public health officials and policymakers in Canada and worldwide.[4,29,30] This gap is primarily due to concerns about the quality of WEM data. Additionally, the inherent variability of the WEM data and inconsistencies in the lead times between WEM data and clinical surveillance metrics further complicate its perceived reliability.[31−35] During the surge of the two prominent waves in Ontario caused by the B.1.1.529.1 Variant of Concern (VOC) (first Omicron BA.1 sublineage, hereafter called Omicron BA.1, predominant in Canada from December 2021 to March 2022) and the B.1.1.529.2 VOC (second Omicron BA.2 sublineage, hereafter called Omicron BA.2, dominant in Canada from March 2022 to July 2022), inconsistencies in the Spearman's rank correlation coefficient (ρ) between WEM data, both in copies per liter of wastewater sample collected (hereafter referred to as cp/L) and in copies per copies of pepper mild mottle virus (PMMoV) (hereafter referred to as cp/cp) and clinical surveillance metrics (hospital admissions and laboratory-positive cases), were revealed within the Ontario WSI program. Furthermore, a wastewater surveillance study across 55 sampling locations in the U.S. from April 2020 to May 2021 also displayed similarly inconsistent Spearman's correlations between WEM data and laboratory-positive cases, as ref. no. 32 reported. WEM data is also perceived to be highly variable due to the differences in WEM site characteristics or methodologies, such as population mobility, sampling location in the network, methods of sample collection, reporting standards, and high standard deviations in viral signal concentration measurements.[31,35] Across Canada and worldwide, WEM was found to lead clinical surveillance data by 3 to 14 days. This range in lead time is attributed to various factors, such as wastewater to laboratory travel time, population immunity dynamics, VOC onset, and climatic conditions.[6,24,33,34,36−38] This lack of consistency in the correlation, perceived high variability of WEM data, and inconsistent lead times between WEM data and clinical metrics across sites has largely been attributed to assumed limitations of WEM. This assumption in turn has led to the mistrust or delayed trust of WEM viral signal data by health decision-makers and has directly limited the actionability of WEM data in Canada and globally,[39,40] which continues to pose a significant challenge in the field of WEM and public health. Inconsistencies in correlations between WEM and traditional clinical surveillance metrics may not, however, be entirely attributable to WEM data sets and instead may be due to disparities in clinical data reporting, along with the absence of consistent standards of reporting of traditional clinical surveillance data.[1,41]

The specific factors driving the observed range of correlations between WEM data and clinical surveillance metrics in Ontario, and in WEM programs around the world, remain unclear, particularly during periods of high transmission like the Omicron BA.1 and BA.2 waves. Understanding these factors is crucial, particularly in regions with limited healthcare infrastructure, and where the need for WEM extends beyond COVID-19 to monitor other viruses such as influenza, RSV, Mpox, and other diseases. This study hypothesizes that population size, the magnitude of WEM measurements, site isolation status (proximity to metropolitan centers), and limitations in clinical surveillance metrics, including daily range and the proportion of zeros, influence the quality of correlation between WEM viral signal data (in cp/L and cp/cp) and clinical surveillance data (hospital admissions and laboratory-positive cases). Furthermore, this study investigates the variability of both WEM viral signal data (in cp/L and cp/cp) and clinical surveillance data to determine their role in shaping correlation qualities. It is noted that the laboratory-positive cases are likely to be underreported during the period between the Omicron BA.1 and BA.2 waves due to the change in testing eligibility in Ontario on December 31st, 2021.[28] By examining these factors, the study aims to enhance the

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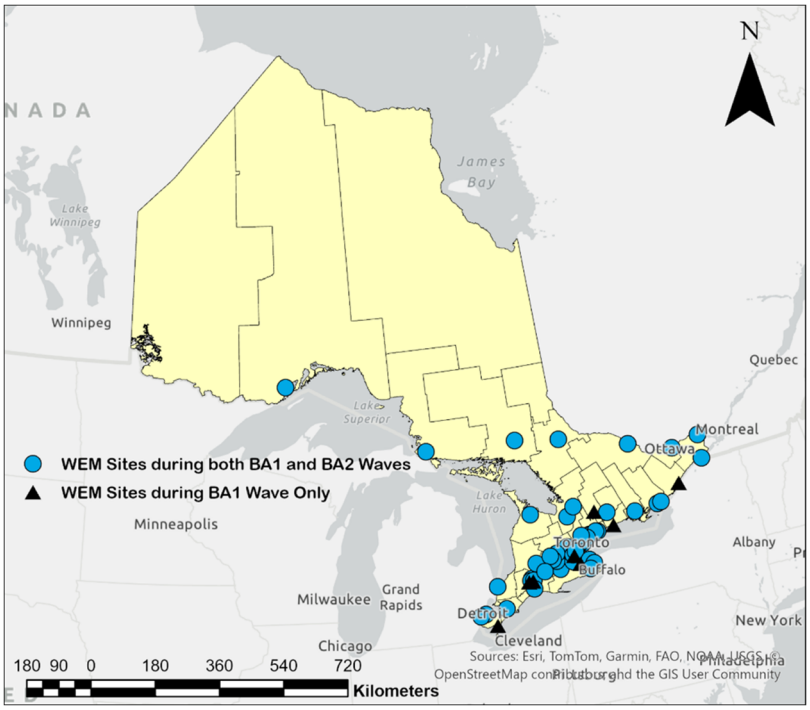

Figure 1. Geographic overview of Ontario and the study locations. Blue points represent locations analyzed during both the Omicron BA.1 and BA.2 waves, while black triangles indicate locations analyzed only during the Omicron BA.1 wave. WEM, hospital admissions, and laboratory-positive case data were collected at these locations with all data sets linked to the population of the respective surveyed wastewater sewershed, ensuring a comprehensive representation of the interconnected epidemiological metrics across the same geospatial locations.

understanding of the dynamics influencing the quality of WEM-clinical metrics correlations and to contribute to a more effective integration of WEM in public health surveillance, thereby enhancing its actionability in future disease monitoring efforts.

2. METHODOLOGY

2.1. Epidemiological WEM and Clinical Data. The Ontario-wide epidemiological information, including WEM data, daily hospital admissions, and daily laboratory-positive cases, was collected and shared internally from the Ontario WSI Data and Visualization Hub. This Hub, operated by the Government of Ontario, was accessible only to municipalities, public health units, and academic and research institutions participating in the Ontario WSI program through the Ontario Wastewater Surveillance Consortium (OWSC). The WEM data generated from the Ontario WSI program, covering the period from January first, 2021 to March 31st, 2023, is described in further detail in ref. no. 18, is available for download in CSV format from Zenodo[42] and GitHub repository (https://github.com/OntarioWastewaterSurveillanceConsortium/sars-cov-2-data).

The WEM data used in this study consisted of SARS-CoV-2 N1 and N2 genomic copies, both as concentration, non-normalized (in cp/L of sample collected) and normalized by the biomarker PMMoV (in cp/cp of PMMoV) for each WEM site within the Ontario WSI program from January first, 2021, to March 31st, 2023. Wastewater samples were collected and transported on ice for all sites under the Ontario WSI program, where SARS-CoV-2 RNA enrichment and extraction were performed within 48−72 h ref. no. 18. Most participating institutions enriched the solids fraction of wastewater prior to nucleic acid extraction of the SARS-CoV-2 and PMMoV targets ref. no. 18. Extracted nucleic acids were stored under controlled conditions and analyzed through RT-qPCR within 72 h of sample enrichment ref. no. 18. All participating testing institutions analyzed for two gene regions of the SARS-CoV-2 genome, particularly the N1 and N2 gene regions, as well as the PMMoV biomarker for normalization of the SARS-CoV-2 genome ref. no. 18. Detailed methods, including sample collection, enrichment, concentration, extraction, and RT-qPCR quantification of SARS-CoV-2 and PMMoV employed by each laboratory are extensively described by ref. no. 18 Hospital admissions data acquired in this study from the Hub (internally accessible to participating institutions under the Ontario WSI program) specifically pertains to patients whose primary diagnosis was COVID-19 (patients admitted to the hospital primarily due to COVID-19 related symptoms or complications). Laboratory-positive case data acquired from the Hub are based on the date reported (i.e., case by reported date). These clinical surveillance metric data sets (hospital admissions and laboratory-positive cases) were geospatially matched with the WEM data set by Ontario's Ministry of the Environment, Conservation and Parks (MECP). This process involved linking patients' postal codes from the clinical data sets to the corresponding WES sewershed catchments, ensuring that the data sets reflect the same population. While sewershed boundaries are not identical to clinical reporting regions, this method allowed for alignment between the data sets. This ensured that the analyses of the WEM data and the clinical surveillance metrics reflect the actual impact of COVID-19 within the same populations. The population size of each surveyed sewershed was also acquired from the Hub. Vaccination information for the province of Ontario was obtained from Public Health Ontario's Respiratory Virus Tool for each study location.[43]

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2.2. Geospatial Locations. This study collected and compared epidemiological data sets, including WEM data, hospital admission data, and laboratory-positive case data, for the Omicron BA.1 and BA.2 waves at specific geospatial locations across Ontario. Out of the 107 WEM sites surveyed under the program at its peak, data from a total of 58 sites were investigated during the Omicron BA.1 wave (Figure 1), and from 50 sites were investigated during the Omicron BA.2 wave (Figure 1), following an exclusion process using criteria detailed in Section 2.2.1.

2.2.1. Exclusion of Locations Prior to Analysis. Prior to the analysis of the Ontario WSI data set, several criteria were established to ensure the robustness and reliability of the findings. These considerations aimed to filter out sites and data segments that might introduce bias or limit the statistical power of the analysis.

2.2.1.1. Minimal Clinical Surveillance Metrics. To ensure robust statistical analyses of the study's three epidemiological data sets, this research included only study locations with significant population-COVID-19 activity. Specifically, locations were excluded if they reported no more than one hospital admission or had fewer than 3 days with daily admissions exceeding one hospital admission during the Omicron BA.1 or BA.2 waves. Although this specific criterium has not been previously discussed in the literature, its implementation was necessary to address the frequent discrete values in the hospital admissions, a factor that has been found to underestimate the true correlation between variables, in this case WEM data and clinical surveillance data.[44,45] These exclusions are intended to ensure sufficient data quality for meaningful WEM-clinical surveillance correlation analyses. This resulted in the exclusion of up to 57 (out of 107) sites with populations ranging from 279 to 47,868 inhabitants for the WEM-hospital admission correlation analyses of both Omicron BA.1 and BA.2 waves, maintaining the clinical relevance of the analysis by reflecting substantial COVID-19 transmission. Specifically, for the Omicron BA.1 wave, 49 sites were excluded, leaving 58 sites in the WEM-hospital admission correlation analysis. For the Omicron BA.2 wave, 57 sites were excluded under these criteria, resulting in 50 sites for WEM-hospital admission analysis. In addition, study locations were excluded from the data set used in this study if no more than one laboratory-positive case was reported during the BA.1 or BA.2 wave. One site met this exclusion criterion but was already excluded by the hospital admission criteria during the Omicron BA.1 and BA.2 waves.

2.2.1.2. Low Wastewater Sampling Frequency. Adequate wastewater sampling frequency is crucial for capturing fluctuations in WEM data and ensuring robust statistical power when determining Spearman's rank correlation coefficient (ρ) between the respective WEM data and hospital admissions across Ontario. Thus, study locations where less than three samples per week were analyzed were excluded from this analysis.[46] Eighteen sites (279 to 47,868 inhabitants) were identified with this criterion; however, those sites were previously excluded from the analysis by the hospital admissions criteria outlined in Section 2.2.1.1.

2.3. Analysis Period of Omicron BA.1 and Omicron BA.2 Waves across the Study Locations. The onset of the Omicron BA.1 and Omicron BA.2 waves were estimated from the WEM data set for each respective Ontario WSI site due to limitation in obtaining population-wide VOC sequencing data for each individual site. The analysis period for the Omicron BA.1 and BA.2 waves was set to 80 days (~11 weeks),[47] encompassing 40 days before and 40 days after the identified peaks for each WEM site. To account for potential asynchronous spread of the virus across the province, the peaks were identified through visualization of the WEM data and the clinical surveillance data for each individual WEM site.

2.4. Data Analysis. *2.4.1. Spearman's Coefficient between WEM Data and Clinical Metrics.* Spearman's rank correlation coefficient (ρ) was calculated for all the Ontario WSI sites between the 7-day midpoint average of WEM data, both non-normalized (in cp/L) and normalized (in cp/cp), and the 7-day midpoint average of hospital admissions, and the 7-day midpoint average of laboratory-positive cases. The data sets were analyzed during the Omicron BA.1 and Omicron BA.2 waves. The forward time step (lead time of the epidemiological metric) of 1−14 days with the strongest correlation between the data sets, along with the visual alignment in recorded peaks, was considered when selecting the optimal lead time between wastewater measurements and hospital admission data, providing the "maximum Spearman's coefficient (ρ)" used throughout this study.[24] A value of $p < 0.05$ was used to indicate a statistically significant correlation. As the analyses were conducted independently for each site to characterize localized relationships, no corrections for multiple comparisons were applied. The Spearman's rank correlation analysis was chosen because of the absence of linearity in hospital admission data, WEM data, and laboratory-positive cases.[48] The analysis was performed in RStudio and accounts for ties in rankings by assigning average ranks to tied values during the calculation process.

Throughout this study, the maximum Spearman's coefficient (ρ) within the 1−14-day time step was used as an index to define correlation quality at the Ontario WSI sites. Correlations were evaluated based on their magnitude rather than their direction, with both strong positive and strong negative correlations suggesting good correlation quality between two metrics. Conversely, a weak or near zero Spearman's coefficient (ρ) might indicate discrepancies in the rankings,[48,49] suggesting potential problems with data consistency and accuracy or reporting limitations in either the WEM data or clinical surveillance metrics.

2.4.2. Variability of WEM Data and Clinical Metrics. To provide a measure of fluctuation/variability in WEM data and clinical surveillance metrics, specifically hospital admissions and laboratory-positive cases, and to quantitatively assess whether it affects the WEM-clinical metric correlation quality of the surveillance data, the variability of both parameters was calculated longitudinally for each study location using eq 1. The mean variability across the range of the Omicron BA.1 and BA.2 VOCs for each site was then calculated.

$$\text{variability} = \frac{1}{n}\sum_{i=1}^{n}\frac{|x_i - 7\text{ day midpoint avg.}|}{7\text{ day midpoint avg.}} \quad (1)$$

Where:

- x_i is the daily data point for WEM signal or clinical surveillance metrics.
- 7-day midpoint avg. is the moving average of the 7-day window centered around x_i for each date.
- n is the total number of data points for the given WEM site.

2.4.3. Recursive Partitioning and Regression Trees. To explore the relationships between the factors possibly influencing the epidemiological metric correlations, regression

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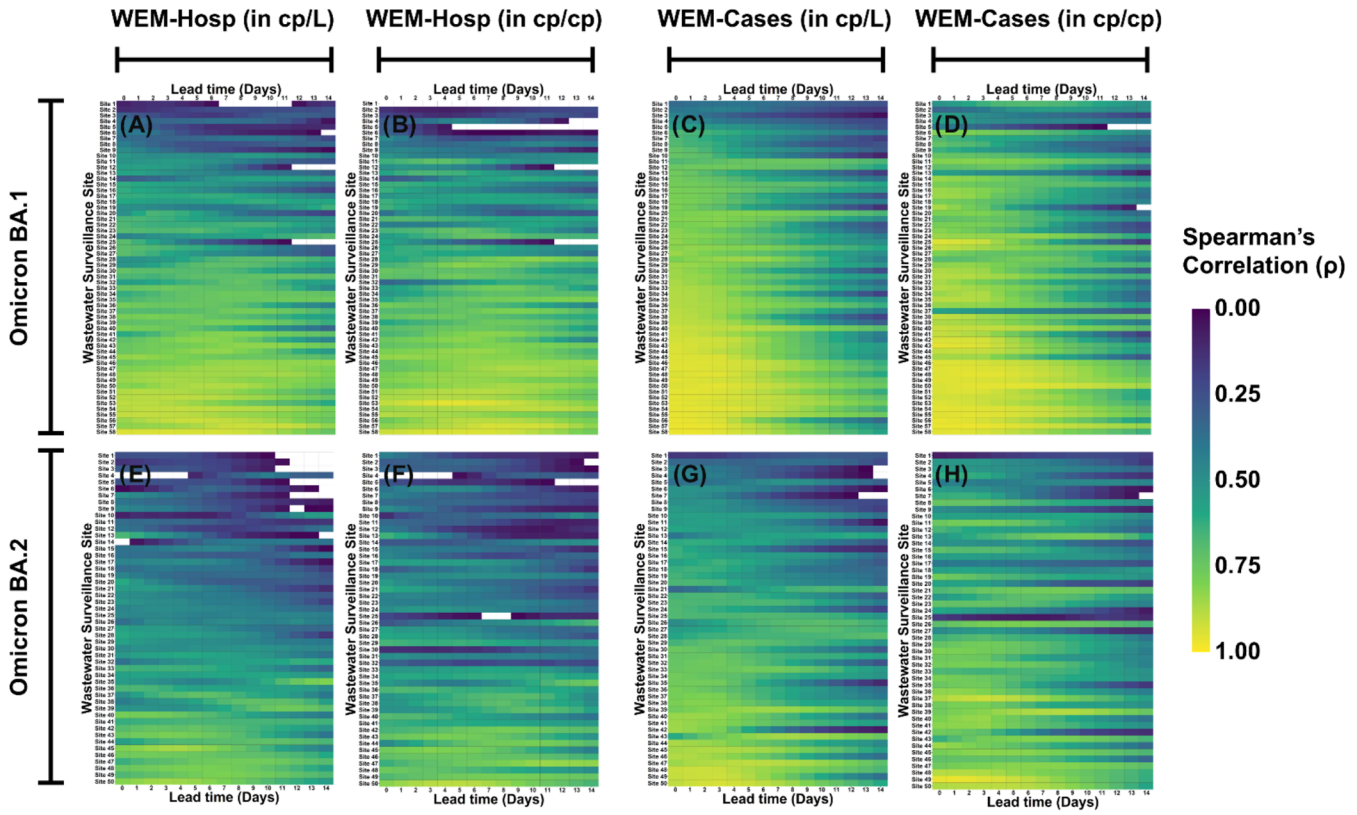

Figure 2. Heatmap illustrating the range in correlations between wastewater signals (WEM data) and clinical metrics (hospital admissions and laboratory-positive cases data) during the Omicron BA.1 and BA.2 waves. (A, E) Correlations between WEM (in cp/L) and hospital admissions, (B, F) correlations between WEM (in cp/cp) and hospital admissions. (C, E) Correlations between WEM (in cp/L) and laboratory-positive cases, and (D, H) correlations between WEM data (in cp/cp) and laboratory-positive cases. The *x*-axis represents the lead time (0−14 days), and the *y*-axis represents the wastewater surveillance study locations, ordered from weakest to strongest average Spearman's Correlation (ρ). Negative and nonsignificant correlations ($p > 0.05$) are shown as blank areas. Color intensity indicates the strength of correlation, with purple denoting very weak or nonexistent correlation, and yellow indicating very strong correlation. Statistically nonsignificant correlations (ρ near 0.00 and $p > 0.05$) are denoted in purple.

trees were constructed. Explanatory variables included population size of the surveyed sewersheds, range in the 7-day average hospital admissions, proportion of zeros in the clinical metrics data, and isolation status of the sampling site (isolated vs not isolated). In this study, isolated study locations are described as those at least 350 km away from a major city center that offers essential government services and is accessible by roads throughout the year.[50] The range in the 7-day midpoint average hospital admissions reflects the occurrence of unique values in the daily hospital admissions over a week. This is important because fewer unique values (i.e., many similar daily hospital admissions) can result in similar 7-day midpoint averages, leading to ties in the Spearman's rank correlation between WEM-clinical surveillance metric correlations. The proportion of zeros in the clinical metrics data was calculated by determining the percentage of days with zero reported hospital admissions or laboratory-positive cases over the duration of the studied VOC. The R statistical environment (version 4.3.3) was used for the analysis with the "rpart" and "rpart.plot" packages supporting tree creation and visualization.[51]

3. RESULTS AND DISCUSSION

3.1. Large Range of Correlations and Lead Time between WEM and Clinical Metrics across Ontario, Canada. A large range of correlations was observed between the 7-day midpoint average wastewater viral signal (cp/L and cp/cp) and both 7-day midpoint average hospital admissions and 7-day midpoint average laboratory-positive cases during the Omicron BA.1 and BA.2 waves (Figure 2). For hospital admissions, the Spearman's rank correlation coefficient (ρ) between WEM data and hospital admissions ranged from nonexistent ($\rho = -0.188$ in cp/L and $\rho = 0.026$ in cp/cp) to very strong ($\rho = 0.859$ in cp/L and $\rho = 0.955$ in cp/cp) across both the Omicron BA.1 and BA.2 waves (Figure 2A,B,E,F). Similarly, for laboratory-positive cases, ρ ranged from nonexistent ($\rho = -0.261$ in cp/L and $\rho = 0.026$ in cp/cp) to very strong ($\rho = 0.970$ in cp/L and $\rho = 0.971$ in cp/cp) (Figure 2C,D,G,H). While the analytical methods used in the Ontario WSI data set were largely based on or similar to the analytical protocols of ref. no. 52, it is noted that across the 13 institutions, a variety of specific and distinct steps and protocols were used by laboratories for sample collection, viral RNA concentration, extraction and RT-qPCR quantification of SARS-CoV-2 and PMMoV, which may introduce confounding effects in this analysis. Since differing analytical methods were used to compile the Ontario WSI data set, the potential confounding effect of various methods on the quality of WEM-clinical metrics correlations was first determined in this study. This range in correlation was found to not vary based on the specific methods used by the 13 participating academic institutions under the

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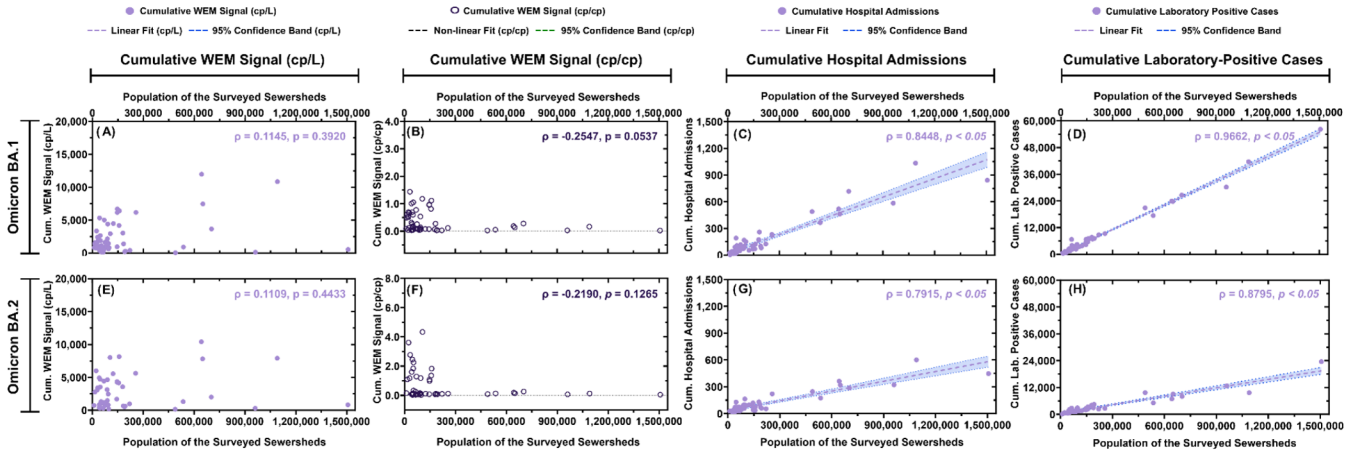

Figure 3. Relation between population size of the surveyed sewershed (x-axis) and cumulative, concentration non-normalized WEM data (cp/L) (A, E), cumulative normalized WEM data (cp/cp) (B, F), cumulative hospital admissions (C, G), and cumulative laboratory-positive cases (D, H) against population size of the surveyed wastewater sewershed during the Omicron BA.1 (top row) and Omicron BA.2 (bottom row) waves. ρ exclusively refers to Spearman's rank correlation coefficient. *Laboratory-positive cases in all analyzed sites are underreported during the peak of the Omicron BA.1 wave and throughout the Omicron BA.2 wave due to updated PCR eligibility in Ontario as of December 31st, 2021.

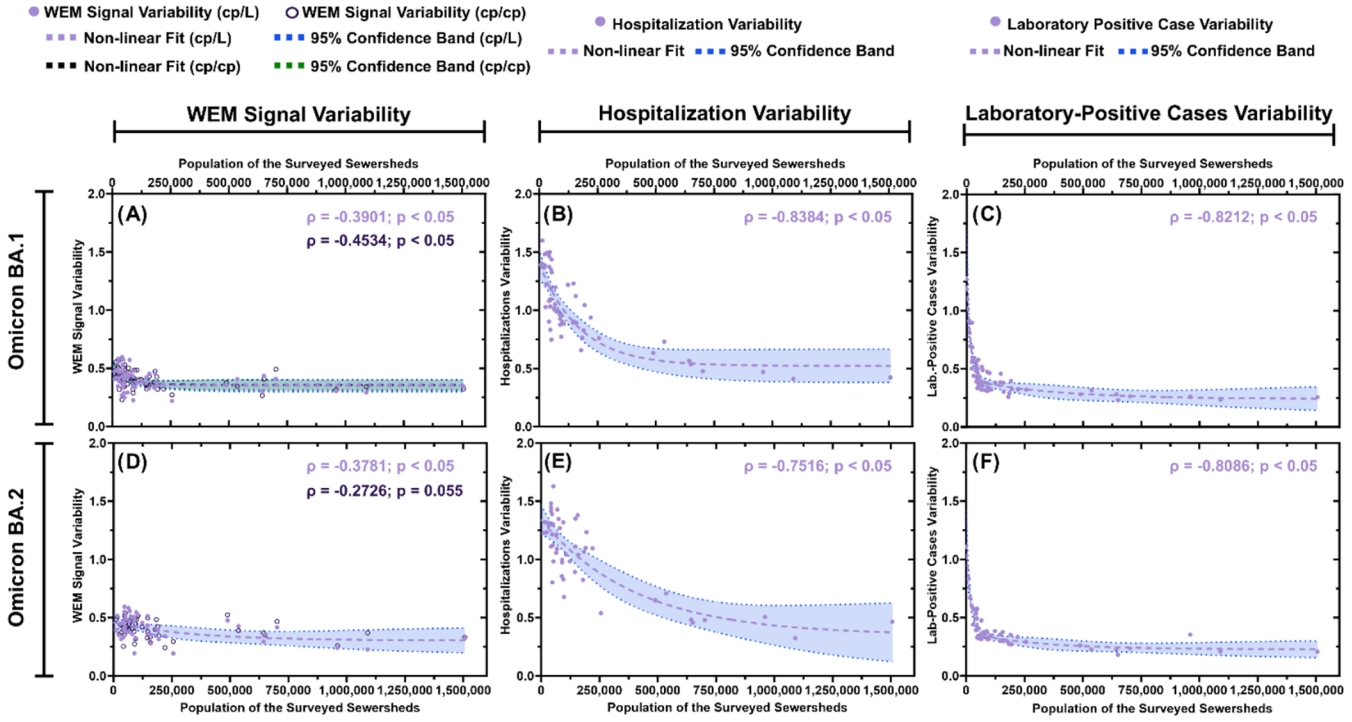

Figure 4. Relation between population size of the surveyed sewersheds (x-axis) and WEM data variability (y-axis) (A, D), hospital admissions variability (y-axis) (B, E), and laboratory-positive case variability (y-axis) (C, F) the Omicron BA.1 (top row) and BA.2 (bottom row) waves. *Laboratory-positive cases in all analyzed sites are underreported halfway during the Omicron BA.2 wave and throughout the Omicron BA.2 wave due to updated PCR eligibility in Ontario as of December 31st, 2021.

Ontario WSI program, and thus was not included as a possible confounder for WEM and clinical surveillance correlations in this study.

3.2. Effect of Population Size on WEM and Clinical Metrics. The relationships between the population size of the surveyed sewersheds (the 58 sites during the Omicron BA.1 wave and the 50 sites during the Omicron BA.2 wave) and the three major clinical indicators of COVID-19: WEM, hospital admissions, and laboratory-positive cases were first evaluated. Specifically, the cumulative WEM concentration, non-normalized WEM viral signal (cp/L), normalized WEM viral signal (cp/cp) and the cumulative clinical surveillance metrics for each of the sites were assessed.

Population size of the surveyed sewersheds considered in this study following the exclusion criteria detailed in Section 2.2.1 (n = 58 during the Omicron BA.1 wave, and n = 50 during the Omicron BA.2 wave) and the cumulative non-normalized WEM signal (cp/L) and normalized WEM signal (cp/cp) per each WEM surveyed site displayed no significant correlation during both the Omicron BA.1 wave (in cp/L: ρ = 0.1145, p = 0.3920 and in cp/cp: ρ = −0.2547, p = 0.0537, respectively) (Figure 3A,B) and the Omicron BA.2 wave (in cp/L: ρ = 0.1109, p =

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0.4433 and in cp/cp: $\rho = -0.2190$, $p = 0.1265$, respectively) (Figure 3E,F). At population sizes below 300,000 inhabitants which represented the majority of sites (50/58 during Omicron BA.1 and 42/50 during Omicron BA.2), cumulative WEM signal trends (in cp/L and cp/cp) remain consistent (Figure 3A,B,E,F). The slight elevation observed with the normalized WEM (cp/cp) in sites with populations below 300,000 is more likely attributed to the differences in the quantification of the PMMoV normalization biomarker among the 13 participating academic institutions within the Ontario WSI program (Figure 3B,F). Population size and the cumulative hospital admissions displayed a strong and significant correlation, during both the Omicron BA.1 wave ($\rho = 0.8448$, $p < 0.05$) (Figure 3C) and the Omicron BA.2 wave ($\rho = 0.7915$, $p < 0.05$) (Figure 3G). Population size of the surveyed sewersheds and the cumulative laboratory-positive cases displayed a similar observation during both the Omicron BA.1 wave ($\rho = 0.9662$, $p < 0.05$) (Figure 3D) and the Omicron BA.2 wave ($\rho = 0.8795$, $p < 0.05$) (Figure 3H). These outcomes align with the expectation that WEM data differs fundamentally from hospital admissions and laboratory-positive cases in its measurement approach. Within the context of this study and the viral quantification methods used by the Ontario WSI program, the WEM data is a measurement performed on a representative volume or mass of wastewater that may be further normalized by the mass of fecal material within the wastewater, inherently reducing its dependence on population size of the surveyed sewersheds and thus may be further capable of providing clinical insights into transmission dynamics in smaller population sizes (Figures S1 and S2 in Supporting Information). In contrast, COVID-19 caused hospital admission and laboratory-positive cases are measured as discrete counts of illness measures within a population. Consequently, larger populations inherently experience higher occurrences of COVID-19-caused hospital admission and higher incidence of laboratory-positive cases, reflecting both the larger number of individuals at risk and the concentration of healthcare resources in urban centers. In addition to the location population, these healthcare facilities may also serve patients from smaller communities.

3.3. Effect of Population Size on Variability of WEM and Clinical Metrics. The relationship between the population size of the surveyed sewersheds and variability of WEM data, hospital admissions, and laboratory-positive cases were investigated in this study during the Omicron BA.1 and BA.2 waves. Variability in WEM data (non-normalized and normalized) exhibited a moderate and significant negative correlation with population size during the Omicron BA.1 wave (in cp/L: $\rho = -0.3901$; $p < 0.05$, and in cp/cp: $\rho = -0.4534$; $p < 0.05$) (Figure 4A). During the Omicron BA.2 wave, a weak to moderate negative correlation was observed between the WEM data variability and population size of the surveyed sewershed (in cp/L: $\rho = -0.3781$; $p < 0.05$, and in cp/cp: $\rho = -0.2726$; $p = 0.055$) (Figure 4D). At population sizes below 300,000 inhabitants which represented the majority of sites (50/58 during Omicron BA.1 and 42/50 during Omicron BA.2), cumulative WEM signal trends (in cp/L and cp/cp) remain consistent (Figure 4A,D). A strong and significant negative correlation was observed between the variability of hospital admissions and population size during both the Omicron BA.1 wave ($\rho = -0.8384$; $p < 0.05$) (Figure 4B) and the Omicron BA.2 wave ($\rho = -0.7516$; $p < 0.05$) (Figure 4E). A strong and significant negative correlation was similarly observed between the variability of laboratory-positive cases and population size during the Omicron BA.1 wave ($\rho = -0.8212$; $p < 0.05$) with a mean variability of 0.423 ± 0.152 (Figure 4C) and the Omicron BA.2 wave ($\rho = -0.8086$; $p < 0.05$) (Figure 4F). Visual observation indicates that both hospital admissions and laboratory-positive cases exhibit higher variability in smaller populations, as indicated by the higher data points at lower populations in Figure 4B−F. This higher variability in hospital admissions and laboratory-positive cases at lower populations decreases significantly as population size increases. In contrast, the variability of WEM data across different population sizes is shown to be more consistent across all population sizes (Figure 4).

WEM data exhibited lower mean variability compared to hospital admissions and laboratory-positive cases. During the Omicron BA.1 wave, the mean variability of WEM data was 0.399 ± 0.086 (in cp/L) and 0.408 ± 0.078 (in cp/cp). During the Omicron BA.2 wave, the mean variability of the WEM data was 0.408 ± 0.098 (in cp/L) and 0.404 ± 0.077 (in cp/cp). As for hospital admissions, the mean variability was 1.027 ± 0.301 during the Omicron BA.1 wave, and 1.048 ± 0.321 during the Omicron BA.2 wave, indicating significant fluctuations in daily hospital admissions, exceeding the 7-day moving average by more than 100%. This level of fluctuation is particularly prevalent at study locations with limited range of discrete hospital admission counts, often between 0 and 3 daily admissions, and at locations with zero-heavy count data for hospital admissions (proportion of zero hospital admission exceeding 0.50 in 39 out of the 58 sites during the Omicron BA.1 wave, and in 33 out of the 50 sites during the Omicron BA.2 wave) (Figures S1 and S2, respectively, in Supporting Information). The significant variability in hospital admissions reported by regions with smaller populations is attributed to the narrow daily count range (e.g., 0, 1, or 2 admissions), which results in a larger relative difference between the daily hospital admission counts and the 7-day average. This is in contrast to the lower variability (smaller relative differences from the 7-day average) measured in larger populations, despite the wider range of data points, consistent with the premise that larger populations with a higher frequency of events produce more consistent hospital admissions data. As for laboratory-positive cases, the mean variability is 0.423 ± 0.152 during the BA.1 wave and 0.369 ± 0.126 during the BA.2 wave. Thus, while WEM data do demonstrate some variability with population size, which is likely attributed to higher sensitivity to individual shedding rates,[53] WEM data are markedly more stable across different population sizes compared to hospital admissions and laboratory-positive cases. The effect of the variability of the WEM data (in cp/L and cp/cp), hospital admissions, and laboratory-positive cases on the strength of the maximum Spearman's coefficient (ρ) between WEM-hospital admission and WEM-laboratory-positive cases during the Omicron BA.1 and BA.2 waves are discussed in more detail in Sections S1 and S2 of the Supporting Information.

3.4. Driving Factors of WEM Correlation with Clinical Metrics. *3.4.1. Driving Factors during Period of Waned Vaccination Immunization in Ontario.* The Omicron BA.1 wave exhibited a significant surge in all three epidemiological metrics: WEM data, hospital admissions, and laboratory-positive cases across all the sites analyzed in this study (Figure S2 in Supporting Information). This surge is likely attributed to a combination of factors, including the waning of vaccine effectiveness against symptomatic disease caused by highly infectious Omicron BA.1 VOC, its vaccine escape proper-

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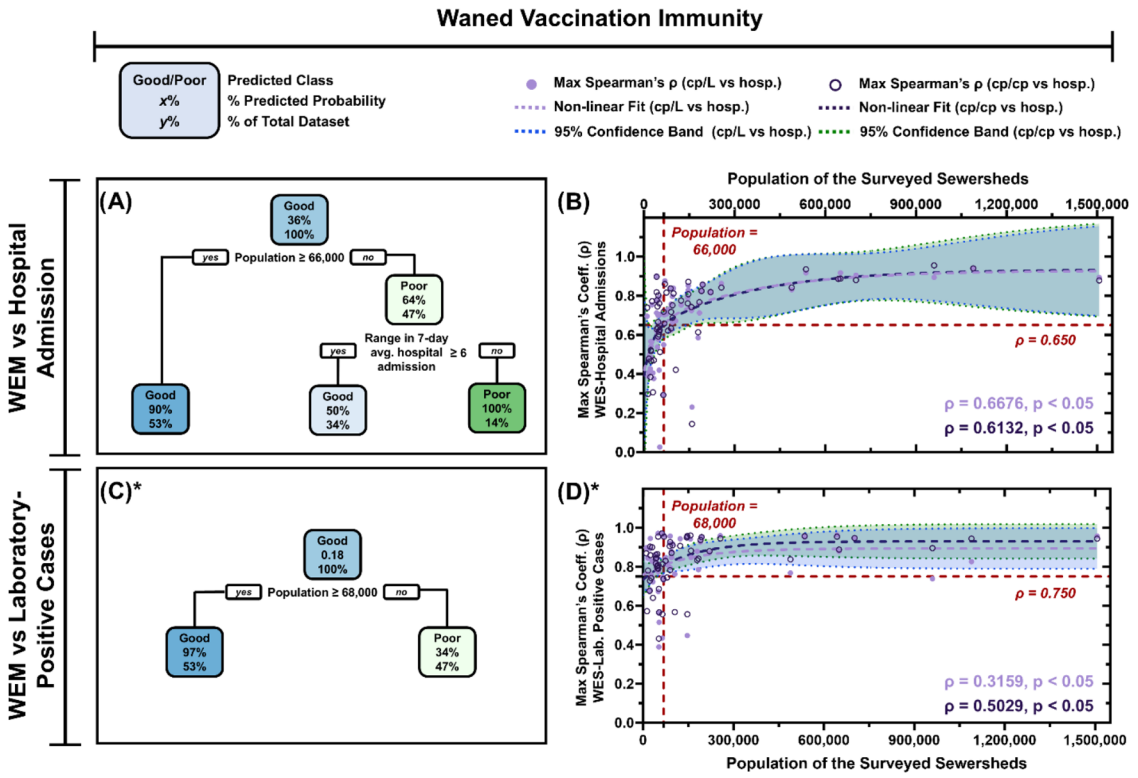

Figure 5. Regression trees representing classification models for evaluating the maximum WEM-clinical metric quality during the Omicron BA.1 wave—coinciding with a period of waned vaccination immunity: (A, C) regression trees for WEM-hospital admissions and WEM-laboratory-positive cases; (B) relation between maximum WEM-hospital admission Spearman's coefficient (ρ) and population size; (D) relation between maximum WEM-laboratory-positive case Spearman's coefficient (ρ) and population size. Each node shows the predicted class ("Good" WEM-hospital admissions and WEM-laboratory-positive cases correlation, $\rho > 0.650$ or "Poor" WEM-hospital admissions and WEM-laboratory-positive cases correlation, $\rho < 0.650$), the predicted probability (between 0% and 100%) of a "Poor" outcome, and the percent of observations in the node. *"Good" laboratory-positive cases-WEM correlation classified as $\rho > 0.750$ due to insufficient observations under "Poor" outcome classification with $\rho < 0.650$.

ties,[24,54] and its emergence during the winter months (November 2021 to March 2022), during which rise in symptomatic illness from respiratory viruses, including SARS-CoV-2, were previously observed.[55,56] At the onset of the Omicron BA.1 wave across Ontario, approximately 10 weeks had passed since 70% of Ontario's total population had achieved full vaccination immunization (two doses of COVID-19 vaccine) by October third, 2021,[43] thus subjecting the province to waned vaccine immunity.[24] In the analysis of the factors driving the quality of the maximum WEM-hospital admissions Spearman's coefficient (ρ), and the maximum WEM-laboratory-positive cases Spearman's coefficient (ρ) during this period of the COVID-19 pandemic, a regression tree model was applied (detailed methodology is provided in Section 2.4.3). This model explored the following parameters hypothesized to contribute to undermining the Spearman's coefficient (ρ): population size of the surveyed sewersheds, 7-day average hospital admissions range, 7-day average laboratory-positive case range, the proportion of zero admissions, the proportion of zero reported case count, and site isolation status (Figure 5). The regression tree analysis, stratified by the two Omicron subvariants, revealed distinct population thresholds at which the maximum WEM-hospitalization Spearman's coefficient (ρ) and the WEM-laboratory-positive case Spearman's coefficient (ρ) quality diverge.

During this period of waned vaccination immunization, the regression tree analysis suggested an approximate population threshold of 66,000 inhabitants (Figure 5A). Beyond this threshold, there is a 90% probability of resulting in a strong WEM-hospital admissions Spearman's coefficient (ρ) ($\rho > 0.650$) indicating a consistent relationship between WEM data and hospital admissions (Figure 5A). This threshold can be further visually illustrated by the moderate to strong and significant relation observed between the population size of the surveyed sewersheds and the maximum Spearman's coefficient (ρ) of WEM-hospital admissions (in cp/L: $\rho = 0.6676$, $p < 0.05$ and in cp/cp: $\rho = 0.6132$, $p < 0.05$, respectively) (Figure 5B). For surveyed sewershed with population sizes below or equal to 66,000 inhabitants, the limited range in the 7-day average hospital admissions data (fewer than 3 or 4 daily values) likely contributed to undermining the WEM-hospital admissions Spearman's coefficient (ρ), with a range lower than six unique 7-day average hospital admissions exhibited a 1.00 (100%) probability of resulting in a "poor" "Spearman's coefficient (ρ) ($\rho < 0.650$) (Figure 5A). This is consistent with previous observations that limited daily occurrence of clinical surveillance metrics resulted in a poor Spearman's coefficient with WEM data.[57] Site isolation status of the Ontario WSI sites was found not to drive the maximum WEM-hospital admissions Spearman's coefficient (ρ).

Similarly, the regression tree analysis identified a population threshold of approximately 68,000 for WEM-laboratory-positive case correlations, with a 97% probability of strong correlations ($\rho > 0.750$) in populations exceeding this threshold (Figure 5C). This threshold can be further visually illustrated where a weak to moderate relation existed between the population size of the

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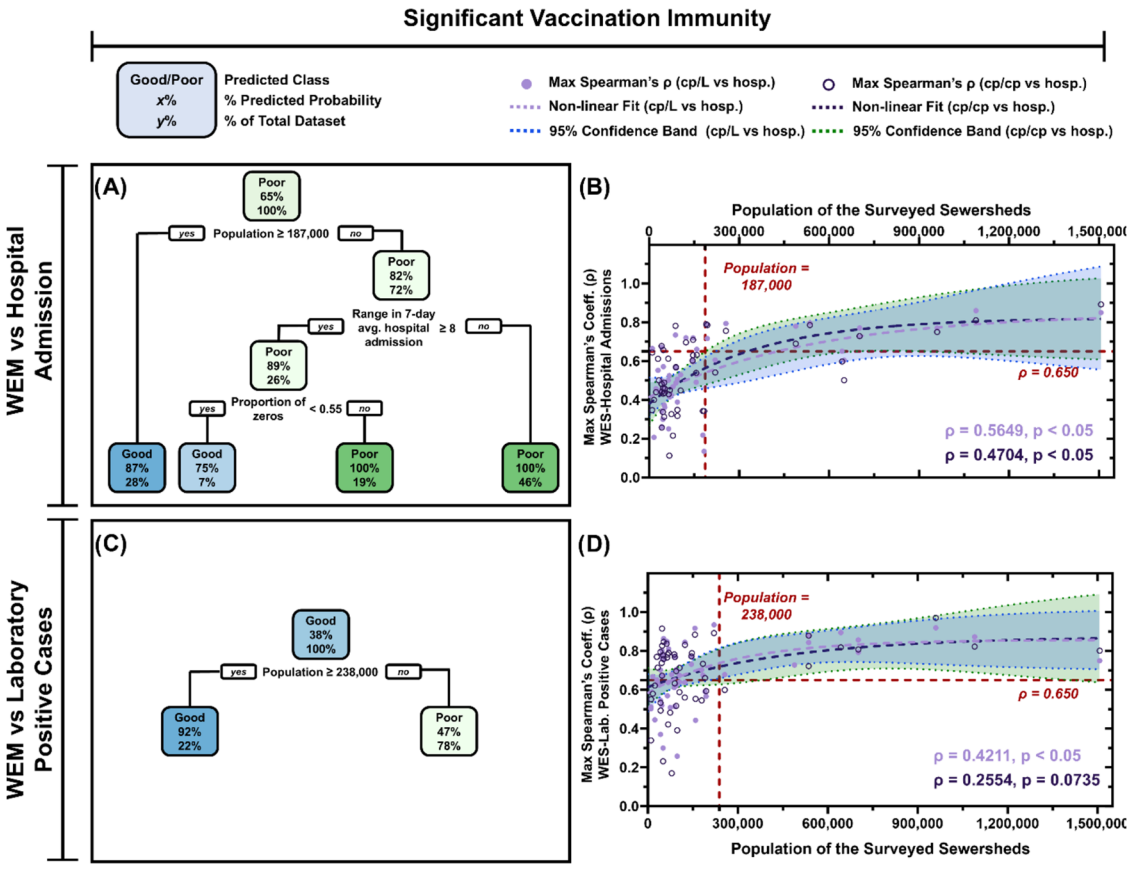

Figure 6. Regression trees representing classification models for evaluating the maximum WEM-clinical surveillance quality during the Omicron BA.2 wave—coinciding with a period of significant vaccination immunity: (A, C) regression tress for WEM-hospital admissions and WEM-laboratory-positive cases; (B) relation between maximum WEM-hospital admission Spearman's coefficient (ρ) and population size (D) relation between maximum WEM-laboratory-positive case Spearman's coefficient (ρ) and population size. Each node shows the predicted class ("Good" WEM-hospital admissions and WEM-laboratory-positive cases correlation, $\rho > 0.650$ or "Poor" WEM-hospital admissions and WEM-laboratory-positive cases correlation, $\rho < 0.650$), the predicted probability (between 0 and 100%) of a "Poor" outcome, and the percent of observations in the node.

surveyed sewersheds and the maximum WEM-laboratory-positive case correlation (in cp/L: $\rho = 0.3159$, $p < 0.05$ and in cp/cp: $\rho = 0.5029$, $p < 0.05$) (Figure 5D). The poorer WEM-laboratory-positive case correlations at populations under 68,000 are likely attributed to the policy change of population-wide PCR eligibility in Ontario on December 31st, 2021, halfway through the Omicron BA.1 wave, which consequently resulted in underreporting of laboratory-positive cases and lack of true representation of disease incidence.[28] This policy driven underreporting affected the resolution of laboratory-positive case data but did not influence the quality of WEM data.[27,58] As the population size of the surveyed sewersheds decreases, the implication of these changes on the relationship between WEM data and laboratory-positive cases becomes more apparent.

WEM-laboratory-positive case correlations were generally stronger (mean ρ: 0.821 ± 0.138) compared to WEM-hospital admissions correlations (mean ρ: 0.682 ± 0.194), which is likely due to the greater occurrence of daily laboratory-positive cases and lower proportion of zeros (3.0% ± 7.0% compared to 57.2% ± 22.7% with hospital admissions data). Despite this, a population size threshold of approximately 68,000 inhabitants was again identified. This indicates that for WEM sites servicing populations below this threshold, WEM could potentially provide a better understanding of disease burden. It is important to note that these thresholds are not hard boundaries but instead reflect trends within the context of this specific data set. These thresholds are best understood as guides to understanding relationships between population size, WEM data, and clinical surveillance metrics, under the conditions of limited vaccination immunity during the Omicron BA.1 wave.

3.4.2. Driving Factors during Period of Significant Vaccination Immunization in Ontario. The Omicron BA.2 wave coincided with a period of significant vaccination immunization following a significant rise in booster dose administration across Ontario.[24,43] During this period, the regression tree analysis identified an approximate population threshold of 187,000 inhabitants. Areas exceeding this threshold had a high probability (87%) to exhibit a strong Spearman's coefficient (ρ) ($\rho > 0.650$) (Figure 6A). This threshold is further visually illustrated by the moderate to strong and significant relationships between the population size of the surveyed sewersheds and the maximum Spearman's coefficient (ρ) between WEM data (in cp/L and cp/cp) and hospital admissions during the Omicron BA.2 wave ($\rho = 0.5649$, $p < 0.05$ and $\rho = 0.4704$, $p < 0.05$) (Figure 6B). When the population size of the surveyed sewershed is below or equal to 187,000 inhabitants, the limited range in hospital admissions data further contributed to undermining the WEM-hospital admissions Spearman's coefficient (ρ), with a 7-day average hospital admissions range below eight admissions having a 100% predicted probability of poor correlation between WEM and hospital admissions ($\rho < 0.650$) (Figure 6A). Additionally, for

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sites with greater hospital admission ranges, a high proportion of zeros in hospital admissions greater than 0.55 had a 100% predicted probability of a weaker WEM-hospital admissions Spearman's coefficient (ρ) ($\rho < 0.650$) (Figure 6A). Site isolation status of the Ontario WSI sites was found not to drive the maximum WEM-hospital admissions Spearman's coefficient (ρ).

For the WEM-laboratory-positive case Spearman's coefficient (ρ), the regression tree analysis suggested an approximate population threshold of 238,000 was identified, above which there was a 92% probability of strong correlations ($\rho > 0.650$) (Figure 6C). This threshold can be visually illustrated where a weak to moderate relation existed between the population size of the surveyed sewersheds and the maximum Spearman's coefficient (ρ) between WEM data (in cp/L and cp/cp) and laboratory-positive cases during the Omicron BA.2 wave ($\rho = 0.4211$, $p < 0.05$ and $\rho = 0.2554$, $p = 0.0735$, respectively) (Figure 6D). In populations below this threshold, the relationship between WEM and laboratory-positive cases were weaker ($\rho > 0.650$), likely attributed to the reduced reporting accuracy due to the policy changes of population-wide PCR eligibility,[28] and reduced incidence of symptomatic disease due to significant vaccination immunization. This suggests that the maximum obtainable association between WEM and clinical metrics is influenced by population size, data variability, and the completeness of clinical surveillance metrics.

Differences in the population thresholds of 66,000−68,000 during a period of waned vaccination immunization (BA.1) and 187,000−238,000 a period of significant vaccination immunization (BA.2) wave are likely attributed to the differences in the population immunity dynamics between the two waves, which influenced the relation between WEM data, hospital admissions, and laboratory-positive cases.[24] The rapid increase in booster dose administration (third mRNA COVID-19 vaccine) across Ontario between January 2022 and February 2022, combined with natural immunity among those who acquired COVID-19 during the BA.1 wave, the Omicron BA.2 wave displayed an overall significant decrease (18.90% ± 39.90) in the cumulative hospital admissions and cumulative laboratory-positive case compared to the BA.1 wave. Booster vaccination, which provides up to 90% protection against severe or fatal disease from the Omicron BA.2 subvariant at 7 weeks postvaccination,[59] were administered to over 45% of Ontario's population by March 13th, 2022.[43] Additionally, widespread transmission of the Omicron BA.1 VOC contributed to further natural immunity, as evidenced by the rise in anti-N antibodies (indicative of infection) from a seroprevalence of 4.5% on December seventh, 2021 to 27.8% on March 15th, 2022.[60] These high level of vaccination coverage, combined with the increase in anti-N antibodies prior to the peak of the Omicron BA.2 wave in mid-March 2022, likely contributed to a decrease in hospital admissions (Figure 3G) and laboratory-positive cases (Figure 3H) compared to the BA.1 wave. This reduced severity of COVID-19-caused symptoms undermined the WEM-hospital admissions Spearman's coefficient (ρ) during this period, particularly in populations below 187,000 and 238,000. As such, a rank-based method may be untenable in the interpretation of the relationship between WEM data, hospital admissions, and laboratory-positive cases under certain conditions. Despite this reduced severity, WEM was demonstrated to more accurately reflect the viral circulation within a community.[57] WEM data, hospital admissions, and laboratory-positive cases are all reliable indicators of COVID-19 disease burden when applied to populations above approximately 238,000 inhabitants, during a period of significant vaccination immunization, while WEM data might be the only reliable indicator of disease burden in smaller populations. It is important to note that the population thresholds identified by the regression tree analysis are not definitive boundaries but instead reflect trends within the context of this data set. They highlight the general relationship between population size, WEM data, and clinical surveillance metrics, particularly under the conditions of significant vaccination immunization during the Omicron BA.2 wave.

4. CONCLUSIONS AND RECOMMENDATIONS

This study represents the first comprehensive, province-wide investigation of geospatially linked wastewater viral signal levels and their association with hospital admissions and laboratory-positive cases during two distinct stages of the COVID-19 pandemic. It was found that population size significantly influenced the quality of correlations between WEM data and clinical surveillance metrics, specifically hospital admissions and laboratory-positive cases, during the Omicron BA.1 and BA.2 waves. WEM data was more stable across different population sizes than clinical surveillance metrics at both waves. Smaller populations exhibited higher variability in hospital admissions and laboratory-positive cases, contributing to poorer correlation quality with WEM data. During the Omicron BA.1 wave, study location populations with over 66,000 inhabitants had a higher probability (90%) of achieving strong correlation between WEM and hospital admissions ($\rho > 0.650$). Similarly, a threshold of approximately 68,000 inhabitants indicated a high probability (97%) of strong correlations ($\rho > 0.750$) for laboratory-positive cases. For the Omicron BA.2 wave, these thresholds increased to approximately 187,000 for hospital admissions and 238,000 for laboratory-positive cases, reflecting the enhanced vaccine coverage and natural immunity. Below these thresholds, when clinical surveillance metrics are limited, WEM can be a reliable tool for reflecting viral circulation in smaller communities. Inconsistent lead times were not found to be driven by population size and are more likely attributed to complex disease transmission dynamics and clinical data reporting schedules within each community.

The results underscore a pivotal distinction: as population size increases, the relation between hospital admissions, laboratory-positive cases, and wastewater data becomes more pronounced and consistent. Simultaneously, the population size of the surveyed sewershed demonstrated no influence on the WEM data across the Ontario WSI. The bifurcation of the population into two categories—those with no more than one hospital admission and those where incidence trends are more discernible through wastewater data—becomes particularly apparent at regions servicing lower population areas. With 49 sites (during Omicron BA.1) and 57 sites (during Omicron BA.2) in the Ontario WSI program lacking sufficient clinical surveillance data, the ability of wastewater surveillance to measure disease burden efficiently and effectively is highlighted especially in smaller populations, regardless of the current state of population immunity.

A limitation of this study is that hospital admission data included in this analysis specifically pertains only to patients with a primary diagnosis of COVID-19. Expanding this to include secondary and tertiary diagnoses, as well as considering emergency department visits with a COVID-19 diagnosis, could potentially improve Spearman's correlation (ρ) by

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increasing the incidence of nonzero hospitalization days in smaller regions, and provide a more comprehensive understanding of disease health burden across the study locations. Additionally, differences in sewerage systems (e.g., network length, type of sewer system—mixed or separate channels) or physicochemical properties could potentially influence the wastewater signals and introduce variability in the WEM data. Further studies should consider these factors to refine and interpret WEM as a complementary surveillance metric.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.4c00958.

Details on the time series relationship between WEM data and hospital admissions from November 2021 to June 2022, covering the Omicron BA.1 and BA.2 waves for each site considered in this study (the 58 sites during the Omicron BA.1 wave and 50 sites during the Omicron BA.2 wave). Further analysis examining the impact of WEM and clinical surveillance data (both hospital admissions and laboratory-positive cases) variability on correlation quality, including Spearman's correlation coefficients and statistical significance testing, assessing how fluctuations in reported laboratory-positive cases influence WEM-laboratory-positive case correlations during both Omicron waves. Details on the relationship between population size and lead times between WEM data and clinical surveillance data (both hospital admissions and laboratory-positive cases), presenting statistical analysis and visualization (PDF)

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Author Contributions

N.H. led the formal analysis and manuscript writing process. All authors contributed to the development of the RNA extraction and RT-qPCR protocols presented in this study. All members of the WSI Consortium contributed to the production of the SARS-CoV-2 N1 and N2 gene and PMMoV gene quantification in wastewater throughout the study period. P.M.D'A., E.M., and R.D. developed the first high-sensitivity methodologies to detect SARS-CoV-2 N1 and N2 in wastewater in Canada. P.M.D'A., E.M., R.D., M.S., H.D., and R.M.M. further developed the first three methodologies for the surveillance of SARS-CoV-2, which led to the roll-out of the Ontario WSI program. E.A.E., M.H., L.G., A.P., E.A., S.B., S.J.P., A.K., D.S., J.-P.D., G.Y., B.O., C.O., D.B., M.S., T.C., R.M.M., K.G., C.O., H.P., C.DeG., E.R., and R.D. supervised the implementation and generation of the data and figures in this manuscript, as well as its review and editing. K.K.P., L.P., J.H., C.D., and E.R. contributed to the statistical analysis, validation of methodology, and manuscript review and editing. P.M.D'A., E.M., N.T.R., M.P.K., T.B.N., E.T., F.A., C.H.W., and S.W. contributed to the review and editing of this manuscript. CRediT: **Nada Hegazy** formal analysis, investigation, methodology, writing - original draft; **K Ken Peng** formal analysis, writing - review & editing; **Patrick M. D'Aoust** data curation, writing - review & editing; **Lakshmi Pisharody** writing - review & editing; **Elisabeth Mercier** data curation, writing - review & editing; **Nathan Thomas Ramsay** writing - review & editing; **Md Pervez Kabir** writing - review & editing; **Tram Bich Nguyen** writing - review & editing; **Emma Tomalty** writing - review & editing; **Felix Addo** writing - review & editing; **Chandler Hayying Wong** writing - review & editing; **Shen Wan** writing - review & editing; **Joan Hu** methodology, writing - review & editing; **Charmaine Dean** methodology, writing - review & editing; **Minqing Ivy Yang** data curation, writing - review & editing; **Hadi Dhiyebi** data curation, writing - review & editing; **Elizabeth A. Edwards** data curation, writing - review & editing; **Mark R. Servos** data curation, writing - review & editing; **Gustavo Ybazeta** data curation, writing - review & editing; **Marc Habash** data curation, writing - review & editing; **Lawrence Goodridge** data curation, writing - review & editing; **Art F. Y. Poon** data curation, writing - review & editing; **Eric J. Arts** data curation, writing - review & editing; **Stephen Brown** data curation, writing - review & editing; **Sarah Jane Payne** data curation, writing - review & editing; **Andrea Kirkwood** data curation, writing - review & editing; **Denina Bobbie Dawn Simmons** data curation, writing - review & editing; **Jean-Paul Desaulniers** data curation, writing - review & editing; **Banu Ormeci** data curation, writing - review & editing; **Christopher Kyle** data curation, writing - review & editing; **David Bulir** data curation, writing - review & editing; **Trevor Charles** data curation, writing - review & editing; **R. Michael McKay** data curation, writing - review & editing; **K. A. Gilbride** data curation, writing - review & editing; **Claire Jocelyn Oswald** data curation, writing - review & editing; **Hui Peng** data curation, writing - review & editing; **Christopher DeGroot** data curation, writing - review & editing; **Elizabeth Renouf** formal analysis, methodology, validation, writing - review & editing; **Robert Delatolla** funding acquisition, methodology, supervision, validation, writing - review & editing.

Notes

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REFERENCES

(1) Canadian Public Health Association Review of Canada's Initial Response to the COVID-19 Pandemic|Canadian Public Health Association, 2023 https://www.cpha.ca/review-canadas-initial-response-covid-19-pandemic. (accessed October 29, 2023).

(2) Health Infobase COVID-19 Epidemiology Update: Summary—Canada.ca 2024. https://health-infobase.canada.ca/covid-19/. (accessed May 24, 2024).

(3) Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N. Engl. J. Med.* **2020**, *382* (8), 727–733.

(4) Keshaviah, A.; Diamond, M. B.; Wade, M. J.; Scarpino, S. V.; Ahmed, W.; Amman, F.; Aruna, O.; Badilla-Aguilar, A.; Bar-Or, I.; Bergthaler, A.; et al. Wastewater Monitoring Can Anchor Global

https://doi.org/10.1021/acsestwater.4c00958
ACS EST Water 2025, 5, 1605–1619

Disease Surveillance Systems. *Lancet Global Health* **2023**, *11* (6), e976−e981.
(5) Kilaru, P.; Hill, D.; Anderson, K.; Collins, M. B.; Green, H.; Kmush, B. L.; Larsen, D. A. Wastewater Surveillance for Infectious Disease: A Systematic Review. *Am. J. Epidemiol.* **2023**, *192* (2), 305−322.
(6) Ahmed, W.; Angel, N.; Edson, J.; Bibby, K.; Bivins, A.; O'Brien, J. W.; Choi, P. M.; Kitajima, M.; Simpson, S. L.; Li, J.; et al. 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. *Sci. Total Environ.* **2020**, *728*, No. 138764.
(7) La Rosa, G.; Mancini, P.; Bonanno Ferraro, G.; Veneri, C.; Iaconelli, M.; Bonadonna, L.; Lucentini, L.; Suffredini, E. SARS-CoV-2 Has Been Circulating in Northern Italy since December 2019: Evidence from Environmental Monitoring. *Sci. Total Environ.* **2021**, *750*, No. 141711.
(8) Wu, F.; Xiao, A.; Zhang, J.; Moniz, K.; Endo, N.; Armas, F.; Bushman, M.; Chai, P. R.; Duvallet, C.; Erickson, T. B.; et al. Wastewater Surveillance of SARS-CoV-2 across 40 U.S. States from February to June 2020. *Water Res.* **2021**, *202*, No. 117400.
(9) Naughton, C. C.; Roman, F. A.; Alvarado, A. G. F.; Tariqi, 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. Show Us the Data: Global COVID-19 Wastewater Monitoring Efforts, Equity, and Gaps. *FEMS Microbes* **2023**, *4*, No. xtad003.
(10) Zhang, Y.; Cen, M.; Hu, M.; Du, L.; Hu, W.; Kim, J. J.; Dai, N. Prevalence and Persistent Shedding of Fecal SARS-CoV-2 RNA in Patients With COVID-19 Infection: A Systematic Review and Meta-Analysis. *Clin. Transl. Gastroenterol.* **2021**, *12* (4), No. e00343.
(11) Wolfe, M. K.; Yu, A. T.; Duong, D.; Rane, M. S.; Hughes, B.; Chan-Herur, V.; Donnelly, M.; Chai, S.; White, B. J.; Vugia, D. J.; Boehm, A. B. Wastewater Surveillance for Monkeypox Virus in Nine California Communities *medRxiv* 2022 DOI: 10.1101/2022.09.06.22279312.
(12) 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.; et al. Municipal and Neighbourhood Level Wastewater Surveillance and Subtyping of an Influenza Virus Outbreak. *Sci. Rep.* **2022**, *12* (1), No. 15777.
(13) de Melo, T.; Islam, G.; Simmons, D. B. D.; Desaulniers, J. P.; Kirkwood, A. E. An Alternative Method for Monitoring and Interpreting Influenza A in Communities Using Wastewater Surveillance. *Front. Public Health* **2023**, *11*, No. 1141136, DOI: 10.3389/fpubh.2023.1141136.
(14) Thampi, N.; Mercier, E.; Paes, B.; Edwards, J. O.; Rodgers-Gray, B.; Delatolla, R. Perspective: The Potential of Wastewater-Based Surveillance as an Economically Feasible Game Changer in Reducing the Global Burden of Pediatric Respiratory Syncytial Virus Infection. *Front. Public Health* **2024**, *11*, No. 1316531.
(15) Boehm, A. B.; Hughes, B.; Duong, D.; Chan-Herur, V.; Buchman, A.; Wolfe, M. K.; White, B. J. Wastewater Concentrations of Human Influenza, Metapneumovirus, Parainfluenza, Respiratory Syncytial Virus, Rhinovirus, and Seasonal Coronavirus Nucleic-Acids during the COVID-19 Pandemic: A Surveillance Study. *Lancet Microbe* **2023**, *4* (5), e340−e348.
(16) Mercier, E.; Pisharody, L.; Guy, F.; Wan, S.; Hegazy, N.; D'Aoust, P. M.; Kabir, M. P.; Nguyen, T. B.; Eid, W.; Harvey, B.; et al. Wastewater-Based Surveillance Identifies Start to the Pediatric Respiratory Syncytial Virus Season in Two Cities in Ontario, Canada. *Front. Public Health* **2023**, *11*, No. 1261165, DOI: 10.3389/fpubh.2023.1261165.
(17) Wong, C. H.; Zhang, Z.; Eid, W.; Plaza-Diaz, J.; Kabir, P.; Wan, S.; Jia, J.-J.; Mercier, E.; Thakali, O.; Pisharody, L.; et al. Rapidly Developed, Optimized, and Applied Wastewater Surveillance System for Real-Time Monitoring of Low-Incidence, High-Impact MPOX Outbreak. *J. Water Health* **2023**, *21*, 1264−1276.
(18) D'Aoust, P. M.; Hegazy, N.; Ramsay, N. T.; Yang, M. I.; Dhiyebi, H. A.; Edwards, E.; Servos, M. R.; Ybazeta, G.; Habash, M.; Goodridge, L.; et al. SARS-CoV-2 Viral Titer Measurements in Ontario, Canada Wastewaters throughout the COVID-19 Pandemic. *Sci. Data* **2024**, *11* (1), No. 656.
(19) Lawal, O. U.; Zhang, L.; Parreira, V. R.; Brown, R. S.; Chettleburgh, C.; Dannah, N.; Delatolla, R.; Gilbride, K. A.; Graber, T. E.; Islam, G.; et al. Metagenomics of Wastewater Influent from Wastewater Treatment Facilities across Ontario in the Era of Emerging SARS-CoV-2 Variants of Concern. *Microbiol. Resour. Announce.* **2022**, *11* (7), No. e00362-22.
(20) Statistics Canada Population and Dwelling Counts: Canada, Provinces, and Territories, 2023. https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=9810000101. (accessed October 29, 2023).
(21) Health Infobase COVID-19 Epidemiology Update: Current Situation− Canada.ca, 2023. https://health-infobase.canada.ca/covid-19/current-situation.html. (accessed October 30, 2023).
(22) Statistics Canada Statistical Area Classification by Province and Territory - Variant of SGC 2016—35A— Census Metropolitan Areas (Ontario), 2023 https://www23.statcan.gc.ca/imdb/p3VD.pl?Function=getVD&TVD=317043&CVD=317046&CPV=35A&CST=01012016&CLV=2&MLV=5.
(23) Boehm, A. B.; Wolfe, M. K.; White, B.; Hughes, B.; Duong, D. Divergence of Wastewater SARS-CoV-2 and Reported Laboratory-Confirmed COVID-19 Incident Case Data Coincident with Wide-Spread Availability of at-Home COVID-19 Antigen Tests. *PeerJ.* **2023**, *11*, No. e15631.
(24) Hegazy, N.; Cowan, A.; D'Aoust, P. M.; Mercier, É.; 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. Understanding the Dynamic Relation between Wastewater SARS-CoV-2 Signal and Clinical Metrics throughout the Pandemic. *Sci. Total Environ.* **2022**, *853*, No. 158458.
(25) D'Aoust, P. M.; Tian, X.; Towhid, S. T.; Xiao, A.; Mercier, E.; Hegazy, N.; Jia, J.-J.; Wan, S.; Kabir, P.; Fuzzen, M.; Hasing, M.; Yang, M. I.; Sun, J.; Plaza-Diaz, J.; Zhang, Z.; Cowan, A.; Eid, W.; Stephenson, S.; Servos, M. R.; Wade, M. J.; Mackenzie, A. E.; Peng, H.; Edwards, E. A.; Pang, X.-L.; Alm, E. J.; Graber, T. E.; Delatolla, R. Wastewater to Clinical Case (WC) Ratio of COVID-19 Identifies Insufficient Clinical Testing, Onset of New Variants of Concern and Population Immunity in Urban Communities. *Sci. Total Environ.* **2022**, *853*, No. 158547, DOI: 10.1016/j.scitotenv.2022.158547.
(26) Nattino, G.; Castiglioni, S.; Cereda, D.; Della Valle, P. G.; Pellegrinelli, L.; Bertolini, G.; Pariani, E. Association Between SARS-CoV-2 Viral Load in Wastewater and Reported Cases, Hospitalizations, and Vaccinations in Milan, March 2020 to November 2021. *JAMA* **2022**, *327* (19), 1922−1924.
(27) Xiao, A.; Wu, F.; Bushman, M.; Zhang, J.; Imakaev, M.; Chai, P. R.; Duvallet, C.; Endo, N.; Erickson, T. B.; Armas, F.; et al. Metrics to Relate COVID-19 Wastewater Data to Clinical Testing Dynamics. *Water Res.* **2022**, *212*, No. 118070.
(28) Government of Ontario Updated Eligibility for PCR Testing and Case and Contact Management Guidance in Ontario|Ontario Newsroom, 2022 https://news.ontario.ca/en/backgrounder/1001387/updated-eligibility-for-pcr-testing-and-case-and-contact-management-guidance-in-ontario. (accessed January 16, 2022).
(29) Kasprzyk-Hordern, B.; Sims, N.; Farkas, K.; Jagadeesan, K.; Proctor, K.; Wade, M. J.; Jones, D. L. Wastewater-Based Epidemiology for Comprehensive Community Health Diagnostics in a National Surveillance Study: Mining Biochemical Markers in Wastewater. *J. Hazard. Mater.* **2023**, *450*, No. 130989.
(30) Hrudey, S. E.; Conant, B. The Devil Is in the Details: Emerging Insights on the Relevance of Wastewater Surveillance for SARS-CoV-2 to Public Health. *J. Water Health* **2022**, *20* (1), 246−270.
(31) Bivins, A.; Kaya, D.; Bibby, K.; Simpson, S. L.; Bustin, S. A.; Shanks, O. C.; Ahmed, W. Variability in RT-QPCR Assay Parameters Indicates Unreliable SARS-CoV-2 RNA Quantification for Wastewater Surveillance. *Water Res.* **2021**, *203*, No. 117516.
(32) Duvallet, C.; Wu, F.; Mcelroy, K. A.; Imakaev, M.; Endo, N.; Xiao, A.; Zhang, J.; Floyd-O'sullivan, R.; Powell, M. M.; Mendola, S.; et al. Nationwide Trends in COVID-19 Cases and SARS-CoV-2 RNA

Wastewater Concentrations in the United States. *ACS ES&T Water* **2022**, *2* (11), 1899−1909.
(33) Olesen, S. W.; Imakaev, M.; Duvallet, C. Making Waves: Defining the Lead Time of Wastewater-Based Epidemiology for COVID-19. *Water Res.* **2021**, *202*, No. 117433.
(34) Kumar, M.; Jiang, G.; Thakur, A. K.; Chatterjee, S.; Bhattacharya, T.; Mohapatra, S.; Chaminda, T.; Tyagi, V. K.; Vithanage, M.; Bhattacharya, P.; Nghiem, L. D.; Sarkar, D.; Sonne, C.; Mahlknecht, J. Lead Time of Early Warning by Wastewater Surveillance for COVID-19: Geographical Variations and Impacting Factors. *Chem. Eng. J.* **2022**, *441*, No. 135936.
(35) Wade, M. J.; Lo Jacomo, A.; Armenise, E.; Brown, M. R.; Bunce, J. T.; Cameron, G. J.; Fang, Z.; Farkas, K.; Gilpin, D. F.; Graham, D. W.; et al. Understanding and Managing Uncertainty and Variability for Wastewater Monitoring beyond the Pandemic: Lessons Learned from the United Kingdom National COVID-19 Surveillance Programmes. *J. Hazard. Mater.* **2022**, *424*, No. 127456.
(36) Clark, M.; Severn, M. Wastewater Surveillance for Communicable Diseases *Can. J. Health Technol.* 2023, *32* DOI: 10.51731/cjht.2023.575.
(37) Bibby, K.; Bivins, A.; Wu, Z.; North, D. Making Waves: Plausible Lead Time for Wastewater Based Epidemiology as an Early Warning System for COVID-19. *Water Res.* **2021**, *202*, No. 117438.
(38) Miura, F.; Kitajima, M.; Omori, R. Duration of SARS-CoV-2 Viral Shedding in Faeces as a Parameter for Wastewater-Based Epidemiology: Re-Analysis of Patient Data Using a Shedding Dynamics Model. *Sci. Total Environ.* **2021**, *769*, No. 144549.
(39) McClary-Gutierrez, J. S.; Mattioli, M. C.; Marcenac, P.; Silverman, A. I.; Boehm, A. B.; Bibby, K.; Balliet, M.; De Los Reyes, F. L.; Gerrity, D.; Griffith, J. F.; et al. SARS-CoV-2 Wastewater Surveillance for Public Health Action. *Emerging Infect. Dis.* **2021**, *27* (9), No. e210753.
(40) Hrudey, S. E.; Bischel, H. N.; Charrois, J.; Chik, A. H. S.; Conant, B.; Delatolla, R.; Dorner, S.; Graber, T. E.; Hubert, C.; Isaac-Renton, J.; Pons, W.; Safford, H.; Servos, M.; Sikora, C. Wastewater Surveillance for SARS-CoV-2 RNA in Canada. *FACETS* **2022**, *7*, 1493−1597.
(41) Alvarez, E.; Bielska, I. A.; Hopkins, S.; Belal, A. A.; Goldstein, D. M.; Slick, J.; Pavalagantharajah, S.; Wynfield, A.; Dakey, S.; Gedeon, M. C.; Alam, E.; Bouzanis, K. Limitations of COVID-19 Testing and Case Data for Evidence-Informed Health Policy and Practice. *Health Res. Policy Syst.* **2023**, *21* (1), No. 11.
(42) DeGroot, C. Ontario Wastewater Surveillance Consortium SARS-CoV-2 Data—v0.1.0 *Zenodo* 2024, *45*.
(43) Public Health Ontario Ontario Respiratory Virus Tool|Public Health Ontario 2024. https://www.publichealthontario.ca/en/Data-and-Analysis/Infectious-Disease/Respiratory-Virus-Tool. (accessed April 09, 2024).
(44) Liu, Y. A Short Note on Spearman Correlation: Impact of Tied Observations. *SSRN Electron. J.* **2017**, 1−12.
(45) Huson, L. W. Performance of Some Correlation Coefficients When Applied to Zero-Clustered Data. *J. Mod. Appl. Stat. Methods* **2007**, *6* (2), 530−536.
(46) Huisman, J. S.; Scire, J.; Caduff, L.; Fernandez-Cassi, X.; Ganesanandamoorthy, P.; Kull, A.; Scheidegger, A.; Stachler, E.; Boehm, A. B.; Hughes, B.; et al. Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2. *Environ. Health Perspect.* **2022**, *130* (5), No. 051304, DOI: 10.1289/EHP10050.
(47) Ionescu, I. G.; Skowronski, D. M.; Sauvageau, C.; Chuang, E.; Ouakki, M.; Kim, S.; De Serres, G. BNT162b2 Effectiveness Against Delta and Omicron Variants of Severe Acute Respiratory Syndrome Coronavirus 2 in Adolescents Aged 12−17 Years, by Dosing Interval and Duration. *J. Infect. Dis.* **2023**, *227* (9), 1073−1083.
(48) Moore, D. S.; Notz, W.; Fligner, M. A. *The Basic Practice of Statistics*, 6th ed.; W.H. Freeman and Co.: New York, 2013.
(49) Piaw, C. *Mastering Research Statistics*; McGraw-Hill Education: Malaysia, 2013.
(50) Crown-Indigenous Relations and Northern Affairs Canada First Nation Profiles, 2024. https://fnp-ppn.aadnc-aandc.gc.ca/fnp/Main/Definitions.aspx?lang=eng. (accessed May 03, 2024).
(51) Therneau, T. M.; Mayo, E. J. A. An Introduction to Recursive Partitioning Using the RPART Routines. 1997, *61*.
(52) 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. Quantitative Analysis of SARS-CoV-2 RNA from Wastewater Solids in Communities with Low COVID-19 Incidence and Prevalence. *Water Res.* **2021**, *188*, No. 116560.
(53) Fitzgerald, S. F.; Rossi, G.; Low, A. S.; Mcateer, S. P.; O'keefe, B.; Findlay, D.; Cameron, G. J.; Pollard, P.; Singleton, P. T. R.; Ponton, G.; et al. Site Specific Relationships between COVID-19 Cases and SARS-CoV-2 Viral Load in Wastewater Treatment Plant Influent. *Environ. Sci. Technol.* **2021**, *55*, 15276−15286.
(54) Sanyaolu, A.; Marinkovic, A.; Prakash, S.; Haider, N.; Williams, M.; Okorie, C.; Badaru, O.; Smith, S. SARS-CoV-2 Omicron Variant (B.1.1.529): A Concern with Immune Escape. *World J. Virol.* **2022**, *11* (3), No. 137.
(55) Wiemken, T. L.; Khan, F.; Puzniak, L.; Yang, W.; Simmering, J.; Polgreen, P.; Nguyen, J. L.; Jodar, L.; McLaughlin, J. M. Seasonal Trends in COVID-19 Cases, Hospitalizations, and Mortality in the United States and Europe. *Sci. Rep.* **2023**, *13* (1), No. 3886.
(56) Moriyama, M.; Hugentobler, W. J.; Iwasaki, A. Seasonality of Respiratory Viral Infections. *Annu. Rev. Virol.* **2020**, *7*, 83−101.
(57) Carducci, A.; Federigi, I.; Lauretani, G.; Muzio, S.; Pagani, A.; Atomsa, N. T.; Verani, M. Critical Needs for Integrated Surveillance: Wastewater-Based and Clinical Epidemiology in Evolving Scenarios with Lessons Learned from SARS-CoV-2. *Food Environ. Virol.* **2024**, *16* (1), 38−49.
(58) Zhan, Q.; Babler, K. M.; Sharkey, M. E.; Amirali, A.; Beaver, C. C.; Boone, M. M.; Comerford, S.; Cooper, D.; Cortizas, E. M.; Currall, B. B.; et al. Relationships between SARS-CoV-2 in Wastewater and COVID-19 Clinical Cases and Hospitalizations, with and without Normalization against Indicators of Human Waste. *ACS ES&T Water* **2022**, *2* (11), 1992−2003.
(59) Iorio, A.; Little, J.; Linkins, L.; Abdelkader, W.; Bennett, D.; Lavis, J. N. COVID-19 Living Evidence Synthesis 6.31: What is the Efficacy and Effectiveness of Available COVID-19 Vaccines in General and Specifically for Variants of Concern?, 2022. https://www.mcmasterforum.org/docs/default-source/product-documents/living-evidence-syntheses/covid-19-living-evidence-synthesis-6.31---what-is-the-efficacy-and-effectiveness-of-available-covid-19-vaccines-in-general-and-specifically-for-variants-of-conce. (accessed June 15, 2022).
(60) COVID-19 Immunity Task Force Seroprevalence in Canada—COVID-19 Immunity Task Force, 2024. https://www.covid19immunitytaskforce.ca/seroprevalence-in-canada/. (accessed April 15, 2024).

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