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Short Communication

Assessment of rapid wastewater surveillance for determination of communicable disease spread in municipalities

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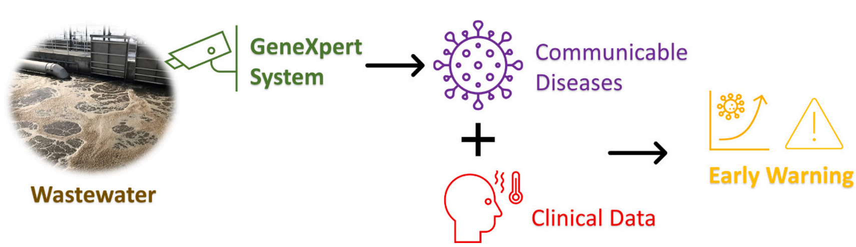
HIGHLIGHTS

- GeneXpert is useful in resource-limited communities for wastewater surveillance.
- GeneXpert results are correlated to clinical data for influenza and SARS-CoV-2.
- Raw wastewater sample analysis can indicate the trend of viral disease spreads.
- Simultaneous transmissibility of diseases can be detected by GeneXpert wastewater surveillance.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater surveillance (WS) helps to improve the understanding of the spread of communicable diseases in communities. WS can assist public health decision-makers in the design and implementation of timely mitigation measures. There is an increased need to use reliable, cost-effective, simple, and rapid WS systems, given traditional analytical (or 'gold-standard') programs are instrument/time-intensive, and dependent on highly skilled personnel. This study investigated the application of the portable GeneXpert platform for WS of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus (IAV), influenza B virus (IBV), and respiratory syncytial virus (RSV). The GeneXpert system with the Xpert Xpress-SARS-CoV-2/Flu/RSV test kit uses reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to analyze wastewater samples. From September 2022 through January 2023, wastewater samples were collected from the influents of municipal wastewater treatment plants (MWTPs) of Saskatoon, Prince Albert, and North Battleford in the province of

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Saskatchewan, Canada. Both raw and concentrated wastewater samples were subjected to the GeneXpert analysis. Results showed that the Saskatoon wastewater viral loads were significantly correlated to Saskatchewan's influenza and COVID-19 clinical cases, with a lead time of 10 days for IAV and a lag time of 4 days for SARS-CoV-2. Additionally, the GeneXpert analysis of the three cities' wastewater samples showed that the raw WS could capture the dynamics of SARS-CoV-2 and IAV due to their correlation with concentrated WS. Interestingly, IBV loads were not detected in any wastewater samples, while the Saskatoon and Prince Albert wastewater samples collected following the 2023 holiday season (end of December and beginning of January) were positive for RSV. This study indicates that the GeneXpert has excellent potential for use in the development of an early warning system for transmissible disease in municipalities and limited-resource communities while simultaneously providing stakeholders with an efficient WS methodology.

1. Introduction

Communicable diseases are of high concern to public health, given the potential emergence of pandemics caused by the development and quick spread of new infectious disease strains (World Health Organization, 2019). For example, the recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the coronavirus disease 2019 (COVID-19) pandemic leading to widespread loss of human life accompanied by significant economic and social disruptions worldwide. Thus, accurate prediction of outbreak/pandemic timing, location, and magnitude are of great importance to public health decision-makers. This will allow stakeholders to better understand disease spread in a community while informing the design and implementation of effective and timely mitigation measures. Typical outbreak estimation methods are based on clinical data such as sentinel surveillance, outpatient cases, hospitalizations, and human biomonitoring (Sims and Kasprzyk-Hordern, 2020). However, the collection of these metrics is highly resource-intensive and is not always a leading indicator of outbreaks (Thacker et al., 2006; Mercier et al., 2022). Wastewater surveillance (WS) can be a complementary, inexpensive, and quick method to augment clinical surveillance programs (Perez-Zabaleta et al., 2023).

WS has been successfully developed to predict SARS-CoV-2 outbreaks during the COVID-19 pandemic in small, large, and high-risk municipalities and communities. The consensus is that WS provides a 'lead time' of 5–14 days to peaks in clinical cases (Xie et al., 2022; Oloye et al., 2022; Wurtzer et al., 2020; Maal-Bared et al., 2023). WS can also be used for monitoring other viral diseases in wastewater, such as influenza A virus (IAV), influenza B virus (IBV), and respiratory syncytial virus (RSV) (Ahmed et al., 2023; Hughes et al., 2022). For example, detections of IAV, IBV, and RSV in college and city wastewaters showed correlations between viral loads and clinical data (Ahmed et al., 2022; Hughes et al., 2022). WS can lead to the quantification, typing, and subtyping of these viruses in wastewater (Mercier et al., 2022). This is particularly the case for IAV given the potential for pandemics caused by the virus' ability to cross species (World Health Organization, 2019) and the estimation of 12,200 hospitalizations and 3500 deaths annually in Canada alone (Mercier et al., 2022).

The WS programs using 'gold-standard' methods (the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and recently digital PCR) are instrument and time-intensive while also being dependent on highly skilled personnel (Zeng et al., 2020). This makes their rapid implementation challenging, especially for high-sampling-frequency WS programs and for communities with limited resources (Holst et al., 2022; Daigle et al., 2022). Therefore, there is an increased need to use cost-effective, simple, and rapid test systems. Recently, RT-qPCR-based systems such as GeneXpert, LuminUltra GeneCount®, and qualitative loop-mediated isothermal amplification (LAMP)-based assays have been developed and used for WS of SARS-COV-2 in small communities (Daigle et al., 2022; Guardado et al., 2020; Jain et al., 2022; Amoah et al., 2021). Among these fast and easy-to-use systems, the GeneXpert has been reported to have the ability to quantify SARS-CoV-2 viral load with high sensitivity (Daigle et al., 2022), making it a viable option for the WS of other viral diseases. Thus, the objective of this study was to investigate the GeneXpert system use for WS of SARS-CoV-2, IAV, IBV, and RSV in the cities of Saskatoon, Prince Albert, and North Battleford, in the Province of Saskatchewan, Canada. The GeneXpert results were then compared with province-wide clinical data published by the Saskatchewan Health Authority (SHA) (Government of Saskatchewan, 2023). This study follows the prior literature (Daigle et al., 2022) and is unique given that: (1) it tested and analyzed both raw wastewater samples (without 10 % Tween 80 addition and centrifuging) and processed wastewater samples (with 10 % Tween 80 addition and centrifuging) collected from Saskatoon, Prince Albert, and North Battleford with similar climatic conditions and various population sizes and wastewater quality parameters, and (2) its time period was from September 2022 through January 2023 which coincided with the spread of various infectious diseases, caused by SARS-CoV-2, Influenza viruses, and RSV. The results could be used to develop an early warning system for the emergence and spread of various infectious diseases in municipalities and limited-resource communities.

2. Materials and methods

2.1. Study areas and wastewater sample collection

The study period was from September 10, 2022, through January 4, 2023, and included the three cities of Saskatoon (~270,000 people), Prince Albert (~43,000 people), and North Battleford (~19,300 people) in the province of Saskatchewan, Canada, which has a total population of about 1.2 million. Each city municipal wastewater treatment plant (MWTP) collected three 24-h composite raw wastewater samples weekly from the facility's influent and shipped the samples on ice to the University of Saskatchewan laboratory. Overall, 155 samples were used for this study including 50 wastewater samples from Saskatoon from September 10, 2022 – January 4, 2023; 52 samples from Prince Albert from August 17, 2022 – January 1, 2023; and 53 samples from North Battleford from August 15, 2022 – December 16, 2022. Publicly available clinical data, including the Saskatchewan cases for influenza, COVID-19, and RSV, was downloaded from the SHA's Dashboard including community respiratory illness surveillance program (CRISP) reports (Government of Saskatchewan, 2023). The CRISP was released bi-weekly during the study period. Data was collected from provincial laboratories and Point-of-Care tests conducted in the SHA facilities.

2.2. Wastewater sample preparation for GeneXpert analysis

Raw and concentrated wastewater samples were subjected to GeneXpert testing (Cepheid, Sunnyvale, CA). Raw wastewater samples were undisturbed for 30 min to allow large particles to settle prior to testing while the concentrated wastewater samples were subjected to ultrafiltration using Amicon. To prepare a concentrated wastewater sample, 40 μL of 10 % Tween 80 was added to 40 mL of raw wastewater. Samples were then vortexed and centrifuged at 6000 rpm (3710 rcf) for 20 min to pellet solids. An Amicon Ultra15 10-kDa centrifugal filter unit (Millipore Sigma, Burlington, MA) was filled with 15 mL supernatant and centrifuged at 6000 rpm for 35 min (Daigle et al., 2022). Xpress-SARS-CoV-2/Flu/RSV cartridges (Cepheid, Sunnyvale, CA) were then individually loaded with 300 μL aliquots of raw and concentrated wastewater samples and analyzed according to the manufacturer's protocol. This assay uses RT-qPCR and targets genes for instantaneous detection of SARS-CoV-2 (through the envelope (E gene) and nucleocapsid (N2 gene) regions of the SARS-CoV-2 genome), IAV (through genes encoding matrix protein (M1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA)), IBV (through genes to encode the M1 and non-structural protein (NS1)), and RSV (through genes to encode the nucleocapsids of RSV A and RSV B) (Leung et al., 2021; Daigle et al., 2022). Each test took around 37 min (Johnson et al., 2021) and provided a cycle threshold (C_T) value that through the utilization of a relevant standard curve, enabled the wastewater viral load estimations (Daigle et al., 2022). The reported Xpert Xpress SARS-CoV-2/Flu/RSV limit of detections (LODs) were based on the clinical samples (Cepheid, 2021; Johnson et al., 2021, 2023) in which 131 gc/mL for SARS-CoV-2; 0.004–0.087 TCID50/mL (50%tissue culture infectious dose) for IAV TCID50/mL; 0.04 TCID50/mL for IBV; 0.43 TCID50/mL for RSV A; and 0.22 TCID50/mL for RSV B (Cepheid, 2021). However, the literature shows the SARS-CoV-2 LOD of 64 gc/mL (Johnson et al., 2021).

In addition to routine internal quality control (through the sample processing control) for all samples and positive/negative controls for the verification of the GeneXpert system, 18 random technical replicates were taken to assess the GeneXpert measurement variations.

2.3. Statistical analysis

Statistical analysis and visualization were conducted via the custom scripts developed in Python 3.8.8 using scip and matplotlib libraries (https://github.com/mohsen-asadi/Asadi_et_al_GX_Flu_COVID?search=1). The GeneXpert results and province-wide clinical data were subjected to a normality test using the Lilliefors test at a 5 % decision level and did

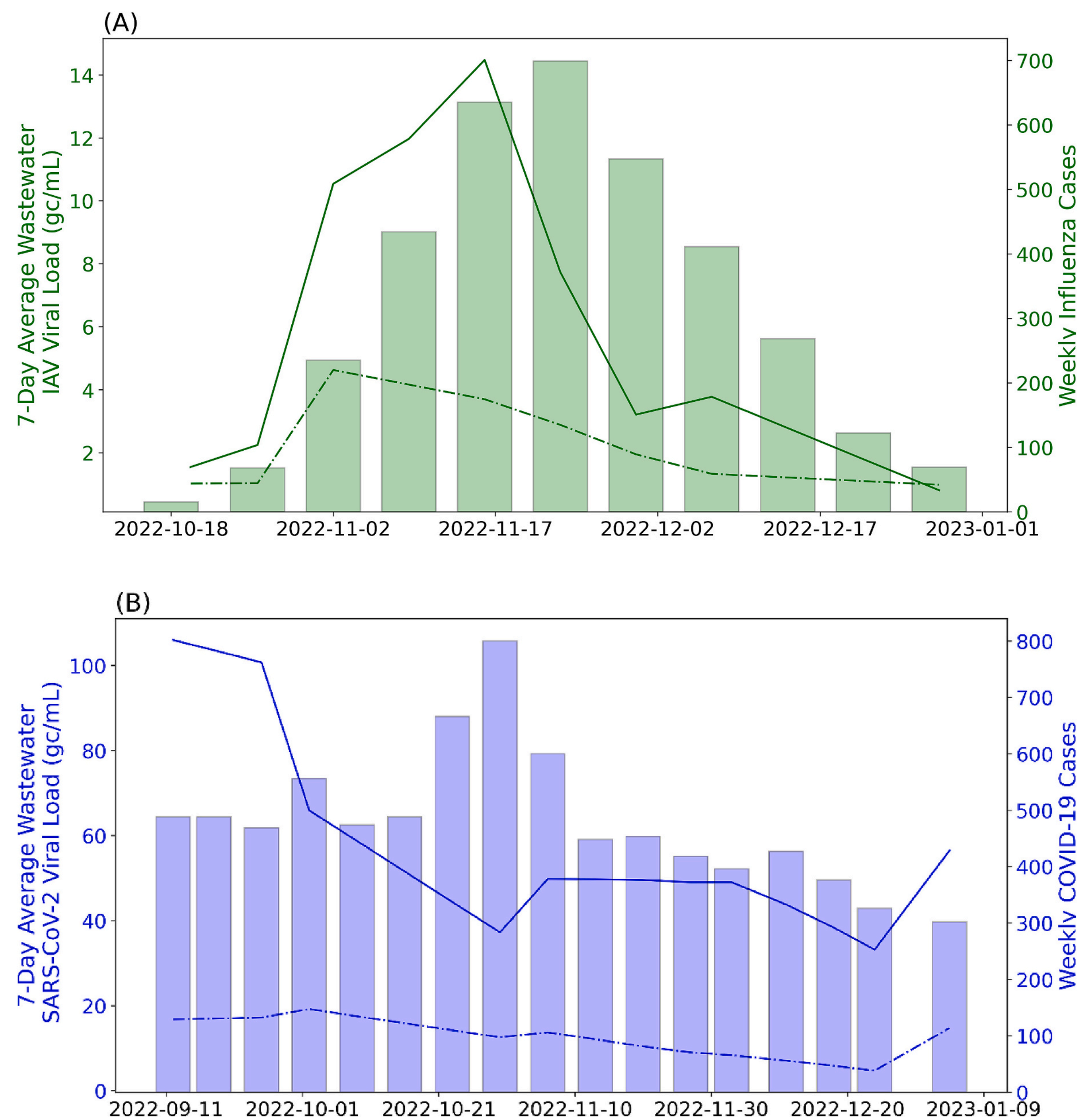

Fig. 1. (A) Time series of the 7-day average wastewater Saskatoon influenza A virus (IAV) signal (gc/mL; green lines) and smoothed Saskatchewan weekly influenza case counts (ppl; green bars); and (B) Time series of the 7-day average wastewater Saskatoon SARS-CoV-2 virus (gc/mL; blue lines) and smoothed Saskatchewan weekly COVID-19 case counts (ppl; blue bars). Note: Solid lines indicate concentrated sample analysis and dashed lines indicate raw sample analysis. See text for further information.

not follow normal distributions. Therefore, the Spearman correlation test was used for the non-normal datasets for the statistical analysis (Ai et al., 2021). Additionally, the Locally Weighted Scatterplot Smoothing (LOWESS) function was applied to the viral loads and clinical data to smooth the time-series datasets and remove irregular data.

3. Results and discussion

3.1. Influenza A virus (IAV) results

The GeneXpert analysis of Saskatoon wastewater showed the first detection of IAV RNA on October 21, 2022 (Supporting Information (SI), Tables S1 and S2), with average viral loads of 1.42gc/mL and 1.03 gc/mL for the concentrated and raw samples, respectively (Fig. 1A). IAV loads in wastewater markedly increased in November 2022 with values above 10.0 gc/mL and 3.00 gc/mL, respectively, before decreasing for the remainder of the reporting period. GeneXpert results were correlated to the Saskatchewan weekly influenza case counts with a 3 to 20-day lead time of clinical data (Fig. S1). Higher Spearman correlation roughly corresponded to 10-day lead time ($R = 0.811$, and $p = 0.000$ for concentrated samples, and $R = 0.865$ and $p = 0.000$ for raw samples; Table 1). These higher correlations may be attributed to decreased Saskatoon and Saskatchewan vaccination rates of around 25 % of the population until January 2023, which is markedly lower than the previous year's 37 % at the same time (Government of Saskatchewan, 2023). Typically, lower vaccination rates result in more serious influenza infections leading to clinical visits, thus, resulting in good agreement between WS results and clinical data.

Similarly, Mercier et al. (2022) quantified influenza loads in the City of Ottawa's MWTP influents, reporting that the analysis of suburban wastewater samples (covering 9 % of the city's population) could be helpful for the whole city to understand the dynamics of influenza spread. Additionally, previous studies used 'gold standard' WS techniques and reported the WS association with influenza cases making it a potential leading indicator of influenza diagnosis able to supplement routine public health surveillance techniques (Mercier et al., 2022; Wolfe et al., 2022; Ahmed et al., 2023). For instance, Mercier et al. (2022) predicted an IAV outbreak with a 17-day lead time in the City of Ottawa. Ahmed et al. (2022) conducted IAV WS using collected wastewater samples from four MWTPs in Australia and reported lead times of 12–17 days for two MWTPs.

Wastewater IAV RNA loads were also detected between October 21–24, 2022, in Prince Albert and North Battleford (Tables S3–S6), with average 7-day viral loads of 2.37 gc/mL and 1.00 gc/mL in Prince Albert and 1.86 gc/mL and 0.67 gc/mL in North Battleford for concentrated and raw samples, respectively (Figs. S3A and S4A). In Prince Albert, wastewater IAV RNA loads reached maximum values of 10.2 gc/mL and 3.71 gc/mL in concentrated and raw samples, respectively, in early November 2022. Following these maximums, a similar decrease in loading occurred as for Saskatoon. In contrast to the other two municipalities, the wastewater loads in North Battleford were more stable during the study period and varied between 1.15 and 3.77 gc/mL and 0.67–1.57 gc/mL for concentrated and raw samples, respectively (Fig. S4A).

Table 1
Spearman correlations between GeneXpert Saskatoon wastewater load (gc/mL) and Saskatchewan case numbers (ppl). Sample analysis includes both concentrated and raw wastewater samples processed using the GeneXpert. Note: R = correlation coefficient, and p = *p*-value.

Sample	Test	Influenza (ppl)	COVID-19 (ppl)
Concentrated (gc/mL)	R	0.811	0.281
	p	0.000	0.048
Raw (gc/mL)	R	0.865	0.561
	p	0.000	0.001

3.2. Influenza B virus (IBV) and respiratory syncytial virus (RSV) results

IBV was not detected during the surveillance campaign (Tables S1–S6) which was expected given the significantly lower detection of IBV cases, as compared to IAV-positive cases, across Canada in 2022–2023. The report from the Public Health Agency of Canada showed 2818 IAV cases versus only 23 IBV cases during the reporting period (Public Health Agency of Canada, 2023).

The GeneXpert detected weak RSV signals (observed C_T values above 38.1 equivalent to negative RSV samples with estimated viral load below 50 gc/mL) leading to negative samples from mid-November 2022 in Saskatoon wastewater, and late December in Prince Albert (Tables S1-S6). Generally, RSV was not positive in wastewater samples during the study period, except for samples from Saskatoon collected between January 1–4, 2023, in which the wastewater RSV loads were 62–81 gc/mL for concentrated samples and 23–42 gc/mL for raw samples. It corresponded to the increased RSV cases from 176 to 199 across the Province of Saskatchewan in which at least 50 % of cases were aged 0–4 years (Fig. S5, Government of Saskatchewan, 2023). Similar to IBV, these results were somewhat expected given that the Saskatchewan RSV cases were markedly lower than influenza and COVID-19 cases until mid-December (Fig. S5). The clinical RSV cases were first observed in early October 2022, reached fewer than 100 cases until early December 2022, and then totalled 200 cases in early January 2023 (Fig. S5), which might lead to increased fecal shredding rates into sewage systems as evidenced by the detection during the January 1–4, 2023 period. Around 65–75 % of RSV cases, hospitalizations, and intensive care unit (ICU) admissions after early December were people aged <19 years old (Government of Saskatchewan, 2023), indicating that monitoring wastewater from public schools can be more practical for early detection of RSV outbreaks using the rapid GeneXpert system.

3.3. SARS-CoV-2 results

Saskatoon wastewater SARS-CoV-2 loads in concentrated samples were at the highest level of approximately 105 gc/mL in early September 2022, coinciding the opening of schools and universities across the city (Fig. 1B). A negative trend in viral loads was then observed, with 65 gc/mL in early October and declining further to 40 gc/mL in late October 2022. The viral loads were stable in November 2022 at around 50 gc/mL, reduced in December 2022 at 25 gc/mL, and then markedly increased to 56 gc/mL in early January 2023. Increases in early 2023 can be attributed to increased gatherings during the holiday season (Christmas and New Year's). Similar to the concentrated sample trends, the raw wastewater sample SARS-CoV-2 loads were 17–19 gc/mL in September 2022 and had a slightly decreasing trend until late December 2022, reaching a minimum value of 3.80 gc/mL before increasing similarly to the concentrated samples in early January 2023 to 14.7 gc/mL.

As for the IAV results, the GeneXpert SARS-CoV-2 WS were correlated to provincial-wide COVID-19 positive cases ($R = 0.281$, $p = 0.048$ for concentrated wastewater samples and $R = 0.561$, $p = 0.001$ for raw wastewater samples; Table 1 and Fig. S2) with a 4-day lag which is in contrast with previous studies reporting lead times varying 2–14 days (Maal-Bared et al., 2023; Xie et al., 2022; Oloye et al., 2022; D'Aoust et al., 2021). However, this 4-day lag time was somewhat expected given the COVID-19 vaccination rates were 46 % in Saskatchewan and 49 % in Saskatoon (Government of Saskatchewan, 2023). Similarly, previous studies have shown that the SARS-CoV-2 variant mutations, increased vaccination rates, and natural immunity evolving could affect the SARS-CoV-2 fecal shedding rates and its WS (Prasek et al., 2022; Siedner et al., 2022; Hopkins et al., 2023). For instance, Hopkins et al. (2023) investigated wastewater SARS-CoV-2 levels over Alpha, Delta, and Omicron waves; and reported strong correlations ($R = 0.91$) between the WS results and COVID-19 positivity rates, and emergency department visits. However, Alpha WS results led positivity rate data by up to 6 days, and, interestingly, Delta and Omicron WS results followed positivity rate data with a 4-day lag (as in the current study), which was attributed to variabilities of testing availability, health-seeking behavior, and viral variants (Hopkins et al., 2023).

The highest wastewater SARS-CoV-2 loads in Prince Albert and North Battleford were detected in September 2022, following a similar trend as Saskatoon (Fig. S1B and S2B). The average concentrated and raw wastewater SARS-CoV-2 loads reached 68 and 22 gc/mL in Prince Albert and 40 and 19 gc/mL in North Battleford, respectively. Both Prince Albert concentrated and raw wastewater loads had decreasing trends and reached steady levels in December 2022 with 35 gc/mL and 5.9 gc/mL, respectively. Similarly, North Battleford wastewater loads decreased to 22 gc/mL and 7.2 gc/mL, respectively. Unlike Saskatoon, Prince Albert data showed no increases around the holiday season. However, it should be noted that samples were not collected throughout the holiday season in Prince Albert and North Battleford due to limited personnel availabilities in these smaller municipalities.

Overall, the association of raw wastewater SARS-CoV-2 loads and the Saskatchewan clinical data ($R = 0.561$, $p = 0.001$) was markedly higher than the concentrated wastewater samples ($R = 0.281$, $p = 0.048$), which is indicative of the potential of raw WS to capture the dynamics of SARS-CoV-2 (Table 1). On the other hand, it has been suggested previously that the use of GeneXpert in raw wastewater WS should only be used for indication of the positive or negative presence of SARS-CoV-2 without quantification (Daigle et al., 2022). Therefore, these results for the use of raw wastewater in the current study indicate a promise potential for this sample type to be used for monitoring instead of the more technical requirements of concentrating wastewater.

3.4. Overall

The IAV loads in concentrated samples were highly correlated to raw sample viral loads in Saskatoon ($R = 0.739$, $p = 0.000$; Table 2), as well as in Prince Albert ($R = 0.811$, p = 0.000; Table 3) and North Battleford ($R = 0.541$, $p = 0.011$; Tables 4). This correlation indicates that the GeneXpert raw sample analysis has the potential to describe the dynamics of IAV loads in various-sized cities. This is important as it would allow for the elimination of the concentration steps, which could be challenging for remote communities lacking the resources and personnel to perform this process prior to analysis.

Similar to IAV, the concentrated SARS-CoV-2 loads were correlated, however less so, to raw sample viral loads in Saskatoon ($R = 0.341$, $p = 0.069$; Table 2). A higher correlation was also found for Prince Albert ($R = 0.691$, p = 0.000, Table 3) and North Battleford ($R = 0.472$, $p = 0.031$, Table 4), which might be attributed to the lower vaccination rates in Prince Albert (40.6 %) and North Battleford (35.4 %), as compared to Saskatoon (48.9 %) leading to higher fecal shredding rates and resultant WS viral loads. Variability of wastewater pH has also been shown to impact SARS-CoV-2 loads with a peak pH of 7.1–7.4 (Bertels et al., 2022; Morvan et al., 2022; Amoah et al., 2022; Hopkins et al., 2023). In this study, Prince Albert and North Battleford wastewater had an average pH value of 7.5, while Saskatoon wastewater had an average pH of 7.9. Thus, the higher pH of Saskatoon wastewater would likely have resulted in lower SARS-CoV-2 loadings than the other two municipalities.

Correlations between WS IAV and WS SARS-CoV-2 in Saskatoon ($R = 0.424$, $p = 0.022$, Table 2), Prince Albert ($R = 0.526$, $p = 0.007$, Table 3), and North Battleford ($R = 0.434$, $p = 0.046$, Table 4) indicate the simultaneous transmissibility's of both diseases in each city. It is important to note that both influenza and COVID-19 have similar symptoms, and simultaneous WS of seasonal respiratory viruses may provide a beneficial understanding of respiratory disease circulation dynamics. Additionally, the chronological order of the emergence of each disease wave was initiated by SARS-CoV-2 wave, followed by IAV wave, and a minor RSV wave (Fig. S5) which might be attributed to competitive exclusion. However, further research would be needed to determine the precise mechanisms underlying this observed pattern.

GeneXpert WS targets genetic material of the wastewater dissolved fraction while a marked number of WS studies have analyzed the solid fraction of wastewater, specifically for SARS-CoV-2 (Duvallet et al., 2022). Previous studies have used both dissolved and particulate-based extraction methods for SARS-CoV-2 and have reported that the overall interpretation of collected data from both methods were similar (Duvallet et al., 2022). It indicates that the wastewater dissolved fraction includes sufficient colloidal material (containing the virus) allowing for a marked viral nucleic acid extraction. Similarly, the dissolved-based extraction have been successfully developed and used in conducting SARS-CoV-2 WS (Hubert et al., 2022; BC Centre for Disease Control, 2023). Typically, the successful detection of virus genetic materials in wastewater depends on its fecal shredding and stability in wastewater, and virus compatibility with dissolved and particulate-based extraction methods. In light of the vital role that virus characteristics play in wastewater surveillance, it is imperative that future studies focus on evaluating dissolved and particulate-based extraction methods for other

Table 2
Spearman correlations between the Saskatoon wastewater load (gc/mL) in both concentrated and raw samples analyzed by GeneXpert for influenza A virus (IAV) and SARS-CoV-2. The italicized indicates significance at $p < 0.05$. Note: R = correlation coefficient, and p = p-value.

Wastewater sample viral load		Test	IAV		SARS-CoV-2	
			Conc.	Raw	Conc.	Raw
IAV	Conc.	R	*1.000*	*0.739*	*0.532*	*0.424*
		p	*0.000*	*0.000*	*0.003*	*0.022*
	Raw	R	*0.739*	*1.000*	*0.419*	0.357
		p	*0.000*	*0.000*	*0.023*	0.056
SARS-CoV-2	Conc.	R	*0.532*	*0.419*	*1.000*	0.341
		p	*0.003*	*0.023*	*0.000*	0.069
	Raw	R	*0.424*	0.357	0.341	*1.000*
		p	*0.022*	0.057	0.069	*0.000*

Table 3
Correlation tests for the Prince Albert wastewater viral loads in concentrated and raw samples analyzed by GeneXpert for IAV and SARS-CoV-2. The italicized indicates significance at $p < 0.05$. Note: R = correlation coefficient, and p = p-value.

Wastewater sample viral load		Correlation test	IAV		SARS-CoV-2	
			Conc.	Raw	Conc.	Raw
IAV	Conc.	R	*1.000*	*0.811*	*0.605*	*0.526*
		p	*0.000*	*0.000*	*0.001*	*0.007*
	Raw	R	*0.811*	*1.000*	*0.581*	*0.529*
		p	*0.000*	*0.000*	*0.002*	*0.007*
SARS-CoV-2	Conc.	R	*0.605*	*0.581*	*1.000*	*0.691*
		p	*0.001*	*0.002*	*0.000*	*0.000*
	Raw	R	*0.526*	*0.529*	*0.691*	*1.000*
		p	*0.007*	*0.007*	*0.000*	*0.000*

Table 4
Correlation tests for the North Battleford wastewater viral loads in concentrated and raw samples analyzed by GeneXpert for IAV and SARS-CoV-2. The italicized indicates significance at $p < 0.05$. Note: R = correlation coefficient, and p = p-value.

Wastewater sample viral load		Correlation test	IAV		SARS-CoV-2	
			Conc.	Raw	Conc.	Raw
IAV	Conc.	R	*1.000*	*0.541*	*0.434*	0.302
		p	*0.000*	*0.011*	*0.046*	0.183
	Raw	R	*0.541*	*1.000*	0.305	0.231
		p	*0.011*	*0.000*	0.179	0.314
SARS-CoV-2	Conc.	R	*0.434*	0.305	*1.000*	*0.472*
		p	*0.046*	0.179	*0.000*	*0.031*
	Raw	R	0.302	0.231	*0.472*	*1.000*
		p	0.183	0.314	*0.031*	*0.000*

viruses.

4. Conclusions

- GeneXpert Saskatoon wastewater analysis results were highly correlated to influenza cases with a 10-day lead time and COVID-19 cases with a 4-day lag time, while the city vaccination rates were around 25 % for influenza and 49 % for COVID-19.
- IBV loads were not detected, and RSV loads were only detected in Saskatoon and Prince Albert wastewater samples collected during/after the new year holiday season, accompanied by the marked RSV cases across the province.
- The direct association of WS IAV and SARS-CoV-2 indicated the simultaneous transmissibility of both diseases in Saskatoon, Prince Albert, and North Battleford.
- GeneXpert WS using raw wastewater samples could capture the dynamics of SARS-CoV-2 and IAV, which is indicative of the GeneXpert potential for the development of an early warning system for transmissible disease in limited-resource communities.

CRediT authorship contribution statement

KNM, MB, JPG, PDJ: project directors, funding of all research and stipend, project conceptualization, informed method development and data collection, and manuscript revisions.

MA: project management, data collection and curation, first draft of manuscript including figures and tables, and manuscript revisions.

MGB, EM, CM: method development, final manuscript revisions.

DH, CC, CDL: sample collection, data collection and curation, manuscript revisions.

FFO, XP, CO: manuscript revisions.

CC, CDL, JC, SE: sample collection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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