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Article

Respiratory Virus Season Surveillance in the United States Using Wastewater Metrics, 2023−2024

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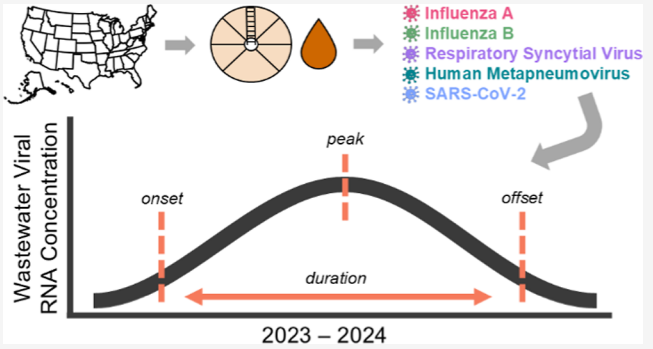
ABSTRACT: Wastewater measurements represent an entire contributing population and can be available within 24 h. Enhanced information about disease occurrence can improve population health through better timing of policies and interventions. We aimed to infer seasonal occurrence patterns for common respiratory viruses alongside transmission dynamics for SARS-CoV-2 across the USA using wastewater samples. We used wastewater RNA concentrations of influenza A and B (IAV/IBV), respiratory syncytial virus (RSV), human metapneumovirus (HMPV), and SARS-CoV-2 from 175 treatment plants (July 2023−June 2024). For IAV, IBV, RSV, and HMPV, we determined epidemic onset, offset, peak, and duration at national and subnational scales. For SARS-CoV-2, we categorized wastewater measurements based on recent wastewater levels and trends. Epidemic onset occurred in November for IAV and RSV which aligned with prepandemic norms. Onset occurred in January for IBV and April for HMPV which were later than expected according to historical data. Duration was longer for IAV and shorter for IBV, RSV, and HMPV than expected based on historical data. Epidemic peak dates were consistent with prepandemic norms for all viruses. Peak dates for influenza and RSV coincided with high, upward trending SARS-CoV-2 RNA concentrations, suggesting potential co-occurrence of SARS-CoV-2 with these viruses.



KEYWORDS: *wastewater-based epidemiological monitoring, influenza A virus, influenza B virus, respiratory syncytial viruses, metapneumovirus, SARS-CoV-2, United States, population health*

INTRODUCTION

Monitoring the timing of annual respiratory virus epidemics is important for clinical and public health awareness.[1,2] In the USA, respiratory virus seasonality is traditionally characterized using clinical metrics such as weekly percentage of positive laboratory-confirmed tests.[3,4] However, disease surveillance is passive and voluntary. The number of specimens tested varies throughout the year and among viruses, and specimens are more likely to come from individuals requiring clinical care.[3] Although the COVID-19 pandemic disrupted the typical seasonality of common respiratory viruses, it brought attention to wastewater monitoring for infectious disease surveillance.[2−6] Wastewater monitoring may represent a less biased surveillance approach for inferring population-level disease dynamics.

A wastewater sample collected from a wastewater treatment plant (WWTP) represents a composite biological sample of the contributing community—irrespective of individuals' socioeconomic conditions, test-seeking behaviors, or symptoms. It is well-established that concentrations of viral RNA in wastewater solids correlate with virus positivity rates from clinical specimens in the same community for a number of respiratory viruses.[7,8] However, only five studies have leveraged wastewater measurements to objectively characterize the timing of annual respiratory virus epidemics (Text S1). Mercier et al.[9] proposed a criterion to infer the date of respiratory syncytial virus (RSV) epidemic onset from wastewater RSV RNA concentrations for two cities in Canada. Fu et al.[10] used criteria to identify the date of influenza A virus (IAV) and SARS-CoV-2 epidemic onset and offset from wastewater RNA concentrations of these viruses for three port cities in China. Schoen et al.[11] and Zulli et al.[12] demonstrated how wastewater RNA concentrations of IAV and RSV, respectively, may be used to infer epidemic onset, offset, and peak for these viruses across the USA. Boehm et al.[13] inferred epidemic onset, offset, and peak for IAV, RSV, and human metapneumovirus (HMPV) across the California Bay Area, USA from wastewater RNA concentrations of these viruses and observed similar patterns for RSV and IAV but more localized patterns for HMPV. No studies have concurrently charac-

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terized the timing of the seasonal epidemic from wastewater measurements for multiple viruses across a large geographic area where the most distant sites are thousands of kilometers apart. Moreover, no studies have distinctly monitored both IAV and influenza B virus (IBV) in wastewater samples collected across a large geographic area. Differentiating influenza A and B types is important because IAV has a higher attack rate and longer incubation period than IBV, and only IAV is known to cause pandemics.[14,15] Also, the influenza vaccine consists of both IAV and IBV components and is updated based on the expected circulating influenza strains.[14]

Here, we use wastewater RNA concentrations of IAV, IBV, RSV, HMPV, and SARS-CoV-2 collected over one year across the USA to infer the timing of the seasonal epidemic for IAV, IBV, RSV, and HMPV at several spatial scales and in relation to SARS-CoV-2 occurrence dynamics. Respiratory diseases have common symptoms, so distinguishing their unique occurrence patterns can improve public health by leading to more targeted administration of vaccines, therapeutics, or policies.[14,16]

METHODS

Wastewater Monitoring. We included 175 WWTPs across 36 states in this study (Table S1). Wastewater samples were collected 2−7 times per week from WWTPs between 1 July 2023 and 30 June 2024, resulting in 27,084 wastewater samples total. The number of WWTPs in states ranged from 1 to 55 WWTPs (median: 2 WWTPs) (Figure 1). The

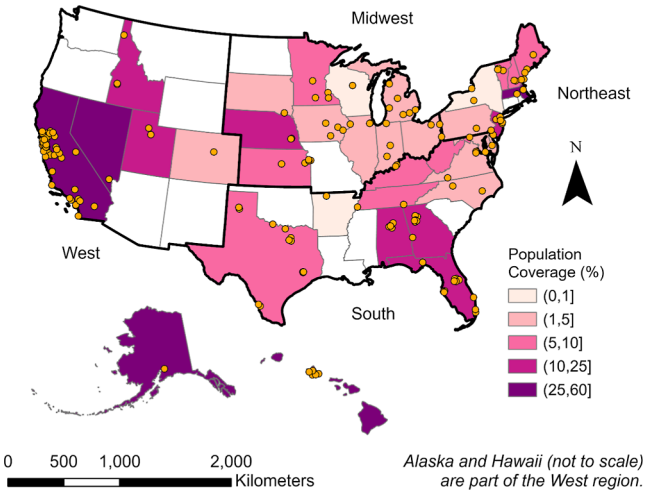

Figure 1. Map of national wastewater monitoring. Locations of wastewater treatment plants are symbolized by circles, and states are colored by the percentage of the total population in the state serviced by a wastewater treatment plant ("population coverage"). Uncolored states did not have any participating wastewater treatment plants. Outlines of the four USA Census regions are bolded. Created in ArcGIS Pro (version 3.1.1) using 2023 state cartographic boundaries (20-m resolution) from the US Census Bureau.[18]

population serviced by WWTPs ranged from 3000 to 4,000,000 persons (median: 90,000 persons). We calculated the population coverage for each state (i.e., the percentage of each state's total population serviced by a participating WWTP) by dividing the population served across all participating WWTPs in the state by the estimated resident population of the state in 2023.[17] Population coverage ranged from 0.49 to 59% (median: 6.5%) (Figure 1).[18] We used RNA concentrations in the settled solids fraction of wastewater samples measured in gene copies (gc) per gram (g) for the following viruses: SARS-CoV-2, IAV, IBV, RSV, HMPV, and pepper mild mottle virus (PMMoV). We used PMMoV RNA concentrations to normalize respiratory virus RNA concentrations. PMMoV is an indigenous wastewater virus which corrects for differences in virus recovery and is of dietary origin which controls for differences in wastewater fecal strength.[19,20] Process-based modeling suggests that PMMoV-normalized concentrations should scale with disease incidence rate.[21] All measurements were made using droplet digital reverse transcription-polymerase chain reaction (ddRT-PCR) following environmental molecular biology best practices. Data and full methodological details are available in a data descriptor by Boehm et al.[22] The data do not carry identifiable private information about individuals; this study is exempt from ethics review and the need for informed consent.

Season Characteristics. We inferred season characteristics (onset, offset, duration, peak) for IAV, IBV, RSV, and HMPV epidemics from wastewater RNA concentrations. Onset and offset describe the start and end of the epidemic. Duration is the time between onset and offset. Peak describes the date of the height of the epidemic. Here, we defined a wastewater event as any period between an onset and offset (or after onset if no offset was reached by 30 June 2024). For each virus, we determined season characteristics at the WWTP, state, and national scales.

We defined onset and offset for WWTPs using criteria presented by Boehm et al.[13] Onset was the first date that all wastewater samples collected in the past 14 days were ≥2000 gc/g (approximately twice the assay detection limit); offset was the first date after onset that ≤50% of wastewater samples in the past 14 days were ≥2000 gc/g. WWTPs did not have an onset or offset designation for the first 14 days of data availability. We defined peak as the date during a wastewater event on which the maximum moving average wastewater RNA concentration occurred. We calculated moving average wastewater RNA concentrations akin to Schoen et al.[11] and Zulli et al.[12] (Text S2).

We determined season characteristics for states by aggregating wastewater data across all WWTPs in the state. We only determined season characteristics for states with ≥2 WWTPs or >1% population coverage (n = 34). For states with 1 WWTP, season characteristics at the state level were identical to season characteristics at the WWTP level. For states with ≥2 WWTPs, we defined onset as the first date that ≥50% of WWTPs in the state were in onset and offset as the first date after onset that <50% of WWTPs in the state were in onset.[12] To determine peak, we used moving average wastewater RNA concentrations averaged across all WWTPs in the state and weighted by WWTP service population (Text S2).

We determined national season characteristics by aggregating wastewater data across states with ≥2 WWTPs or >1% population coverage. We aggregated wastewater data across states rather than WWTPs because the number of WWTPs in states was not representative of a state's total population.[11] We defined onset as the first date that ≥50% of states were in onset and offset as the first date after onset that <50% of states were in onset. To determine peak, we used moving average wastewater RNA concentrations averaged across all states with data availability and weighted by state population (Text S2).[17]

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For each spatial scale, we identified all wastewater events. If more than one wastewater event occurred, we determined the magnitude of the peak moving average wastewater RNA concentration for each event and defined the "seasonal wastewater event" as the event associated with the maximum peak moving average value. For IAV, the seasonal wastewater event also had to onset before 1 March 2024 to avoid designating any anomalous wastewater events likely related to animal-associated IAV (H5N1) contributions as the seasonal wastewater event.[23] For the statistical analyses, we used onset, offset, and peak dates associated only with the seasonal wastewater event for instances when more than one wastewater event occurred. If no offset for the seasonal wastewater event occurred, we used 30 June 2024 as an offset date to calculate duration. Duration may be underestimated in such cases.

Wastewater Categories. SARS-CoV-2 RNA is routinely detected in wastewater, so we categorized its concentrations as high, medium, or low based on recent wastewater levels and trends. Wastewater levels describe whether wastewater RNA concentrations are relatively low or high; wastewater trends describe how wastewater RNA concentrations are changing.

We assigned wastewater levels using moving average SARS-CoV-2 RNA concentrations (Text S2). For each WWTP, we calculated threshold values for the bottom, middle, and top third of all moving average SARS-CoV-2 RNA concentrations during the analysis period and then categorized the moving average SARS-CoV-2 RNA concentration each day based on these thresholds. We assigned wastewater trends using measured PMMoV-normalized SARS-CoV-2 RNA concentrations in the past 21 days and the percent change method described by Chan et al.[24] (Text S2). We assigned an upward trend if the 90% confidence interval (CI) of the percent change estimate was above 0; we assigned a downward trend if the 90% CI of the percent change estimate was below 0. We determined wastewater categories each day according to the wastewater level and trend (Table 1).

Table 1. Wastewater Categories as Determined by Wastewater Level and Trend

wastewater level	21-day wastewater trend	wastewater category
lower third	downward	low
lower third	no trend	low
lower third	upward	medium
middle third	downward	medium
middle third	no trend	medium
middle third	upward	high
upper third	downward	high
upper third	no trend	high
upper third	upward	high

We determined wastewater categories for SARS-CoV-2 at the state and national level by identifying the most frequent wastewater category each day among all WWTPs in the state or all states, respectively. Only states with ≥2 WWTPs or >1% population coverage were considered when determining state and national level wastewater categories. If there was a tie for the most frequent category, we selected the elevated wastewater category.

Statistical Analysis. We determined wastewater categories for SARS-CoV-2 as ancillary information to present alongside season characteristics for the seasonal viruses. The COVID-19 pandemic impacted the seasonality of common respiratory viruses;[3,6] it remains yet to be seen how occurrence patterns of SARS-CoV-2 might coincide with other respiratory viruses.[25] The remaining analyses focus solely on season characteristics (onset, offset, duration, peak) inferred from wastewater for the seasonal viruses (IAV, IBV, RSV, HMPV). We represented dates as days since 1 July 2023 when conducting statistical tests. All analyses were conducted in R (version 4.1.3).

We assessed spatial trends in seasonal occurrence patterns. For each virus, we generated cumulative fraction curves to examine the spatial progression of onset, offset, and peak among states. Because only 34 states had ≥2 WWTPs or >1% population coverage, and two of these states were located outside the contiguous USA, we did not have sufficient observations to adequately test for measures of spatial autocorrelation among states. Instead, we grouped states by Census regions (Figure 1) and used the nonparametric Kruskal–Wallis test to evaluate the null hypothesis that the median value among states is equal across regions for each virus and season characteristic pair as values were not normally distributed within regions for all pairs (Shapiro–Wilk test, $p < 0.05$). When the Kruskal–Wallis null hypothesis was rejected ($p < 0.05$), we used the Conover–Iman posthoc test with a Bonferroni adjustment for multiple comparisons to identify which region(s) significantly differed.

We compared seasonal occurrence patterns across viruses. For each season characteristic, we compared values among states grouped by virus. Values were not normally distributed when grouped by virus for all season characteristics (Shapiro–Wilk test, $p < 0.05$), so we used the nonparametric Kruskal–Wallis test to evaluate the null hypothesis that the median value among states is equal across viruses for each season characteristic. When the Kruskal–Wallis null hypothesis was rejected ($p < 0.05$), we used the Conover–Iman posthoc test with a Bonferroni adjustment for multiple comparisons to identify which virus(es) significantly differed.

RESULTS

PMMoV RNA was detected in all wastewater samples for each WWTP (Figure S1), suggesting acceptable viral recovery throughout the study. The median and interquartile range (IQR) of respiratory virus wastewater RNA concentrations for each WWTP are in the Supporting Information (Table S1). Moving average PMMoV-normalized wastewater RNA concentrations displayed increases and decreases over time, and a seasonal wastewater event occurred at the national scale for IAV, IBV, RSV, and HMPV (Figure 2).

Onset occurred nationally in November for RSV and IAV, January for IBV, and April for HMPV (Table 2). Visually, national IAV and RSV onset aligned with the start of the wintertime high SARS-CoV-2 national wastewater category (Figure 2). Onset dates for individual states are in the Supporting Information (Figure S2 and Table S2). All states onsetted for IAV and IBV, one state did not onset for RSV, and seven states did not onset for HMPV. The median onset date among states (Table 3) was not equal across viruses ($\chi^2 = 85.2$, $p < 0.001$), with significant differences between all pairs of viruses except IAV and RSV. The progression of onset across states occurred relatively quickly for IAV, IBV, and RSV (Figure 3A); the IQRs of onset dates among states were <35 days (Table 3). Onset progressed less quickly across states for HMPV (Figure 3A); the IQR of onset dates among states was 49 days (Table 3). Onset dates differed across regions for IAV

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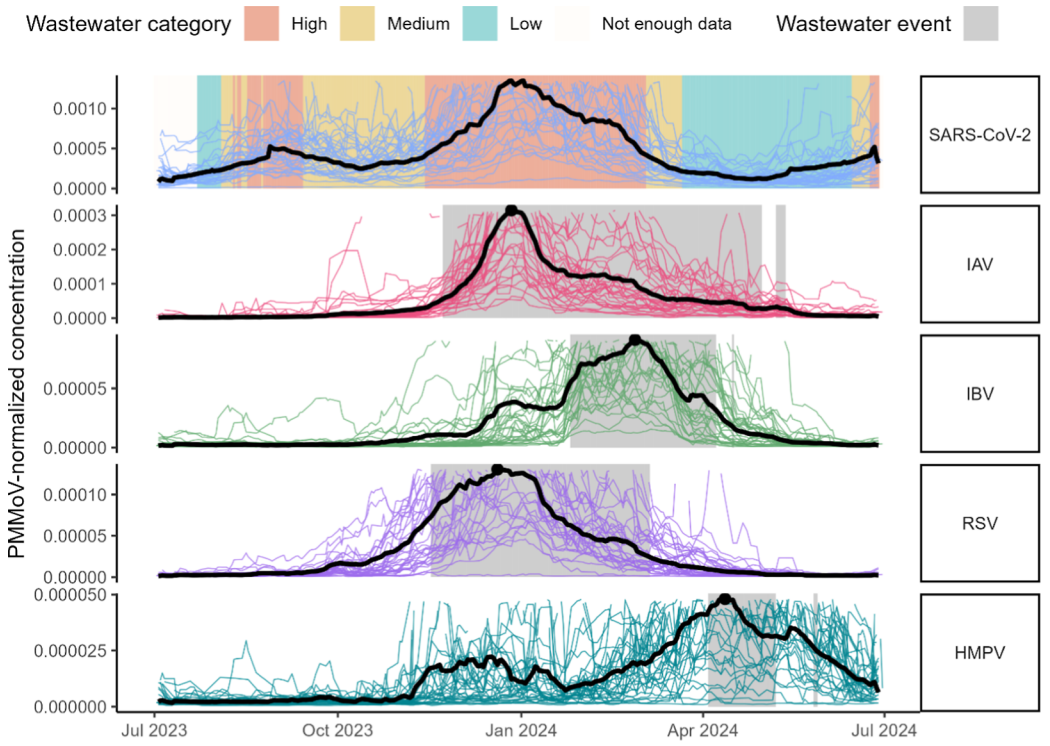

Figure 2. State and national moving average wastewater concentrations of respiratory viruses. Colored lines represent moving average wastewater RNA concentrations for individual states (breaks in lines indicate value is beyond the range displayed); black lines represent national moving average wastewater RNA concentrations. Wastewater RNA concentrations are normalized by PMMoV RNA concentrations in wastewater. For SARS-CoV-2, national wastewater categories are shaded (red: high, yellow: medium, blue: low, white: not enough data). For IAV, IBV, RSV, and HMPV, periods between onset and offset dates ("wastewater events") are shaded with the peak date of the seasonal wastewater event marked by a black dot. Abbreviations: PMMoV, pepper mild mottle virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IAV, influenza A virus; IBV, influenza B virus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus.

Table 2. National Seasonal Wastewater Event Characteristics[a]

	onset date[b]	offset date[b]	duration	peak date[b]
IAV	2023-11-23	2024-05-01	160 days	2023-12-27
IBV	2024-01-26	2024-04-08	73 days	2024-02-27
RSV	2023-11-17	2024-03-06	110 days	2023-12-20
HMPV	2024-04-04	2024-05-08	34 days	2024-04-12

[a]Abbreviations: IAV, influenza A virus; IBV, influenza B virus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus. [b]Dates are formatted as year-month-day.

(χ^2 = 13.7, p = 0.003); IAV onsetted in the West before all other regions (Table S3).

Peak occurred nationally in December for RSV and IAV, February for IBV, and April for HMPV (Table 2). Visually, the national RSV and IAV peak aligned with the wintertime SARS-CoV-2 peak (Figure 2). Peak dates for each state are in the Supporting Information (Figure S2 and Table S2). The median peak date among states (Table 3) was not equal across viruses (χ^2 = 86.4, p < 0.001), with significant differences between all pairs of viruses except RSV and IAV. The progression of peak across states occurred relatively quickly for all viruses (Figure 3B); the IQRs of peak dates among states were <35 days (Table 3). Peak dates differed across regions for IBV (χ^2 = 8.5, p = 0.037) and RSV (χ^2 = 13.7, p = 0.003). IBV peaked in the South before the Midwest; RSV peaked in the South before the West and Midwest (Table S3).

Offset occurred nationally in March for RSV, April for IBV, and May for IAV and HMPV (Table 2). Visually, national RSV offset aligned with the end of the wintertime high SARS-CoV-2 national wastewater category (Figure 2). Offset dates for individual states are in the Supporting Information (Figure S2 and Table S2). Two states that onsetted for IAV never

Table 3. Median and IQR of Seasonal Wastewater Event Characteristics Among States (n = 34)[a]

	onset date[e], median (IQR)	offset date[e], median (IQR)	duration[f], median (IQR)	peak date[e], median (IQR)
IAV[b]	2023-11-23 (2023-11-14, 2023-12-01)	2024-04-02 (2024-03-02, 2024-05-07)	126.5 days (103.8, 166.8)	2023-12-27 (2023-12-26, 2024-01-02)
IBV	2024-01-27 (2024-01-02, 2024-02-05)	2024-04-04 (2024-03-25, 2024-04-29)	80.0 days (48.5, 98.8)	2024-02-28 (2024-02-05, 2024-03-05)
RSV[c]	2023-11-17 (2023-11-10, 2023-11-23)	2024-02-28 (2024-01-29, 2024-03-19)	102.0 days (86.0, 125.0)	2023-12-27 (2023-12-11, 2024-01-05)
HMPV[d]	2024-03-29 (2024-03-01, 2024-04-19)	2024-05-16 (2024-04-22, 2024-06-05)	47.0 days (18.0, 69.5)	2024-04-10 (2024-04-05, 2024-04-26)

[a]Abbreviations: IQR, interquartile range; IAV, influenza A virus; IBV, influenza B virus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus. [b]Two states did not reach offset. [c]One state did not reach onset. States had no offset, duration, or peak by default if no onset. [d]Seven states did not reach onset. States had no offset, duration, or peak by default if no onset. [e]Dates are formatted as year-month-day. [f]For states reaching onset but not offset, we used 30 June 2024 as an offset date to calculate duration. Duration may be underestimated for these states.

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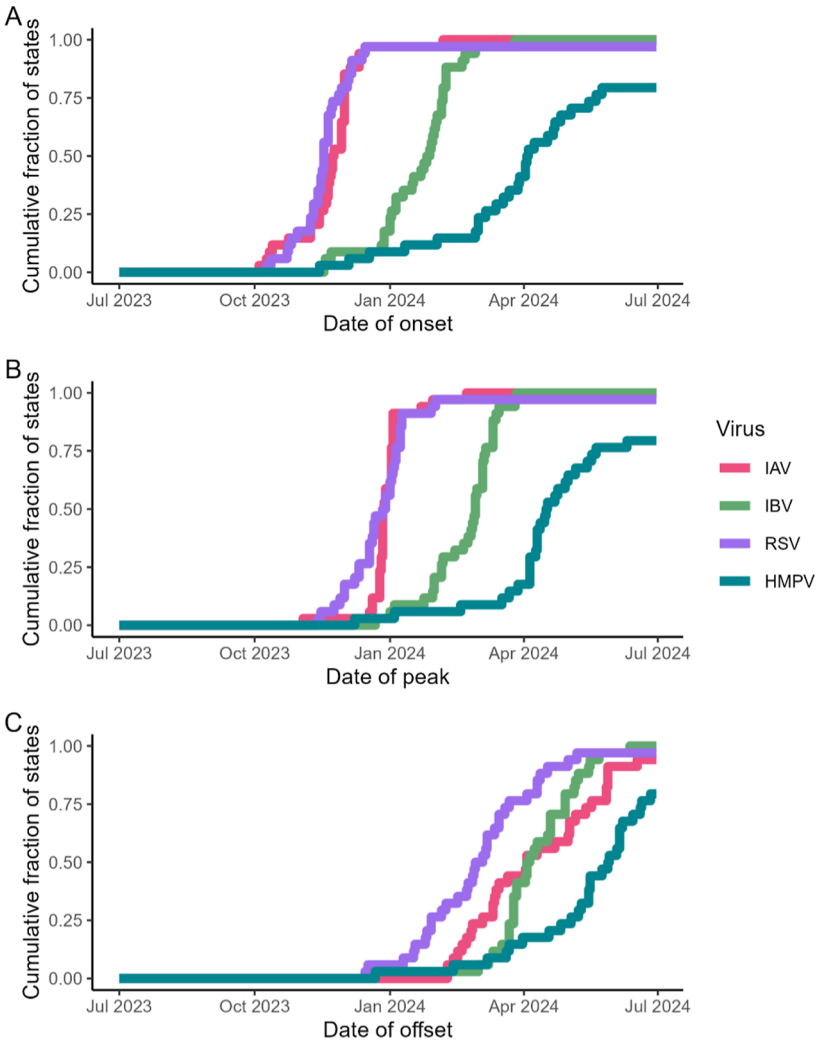

Figure 3. Progression of onset, peak, and offset of the seasonal wastewater event across states. Cumulative curves of the fraction of states (n = 34) reaching (A) onset, (B) peak, and (C) offset for the seasonal wastewater event over time. Refer to Figures S3−S9 in the Supporting Information for the specific order of states reaching onset, peak, and offset for each virus. States did not have an offset or peak date by default if no onset was reached. Abbreviations: IAV, influenza A virus; IBV, influenza B virus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus.

offsetted by the study end date (30 June 2024); all other states that onsetted for a virus offsetted for the same virus. The median offset date among states (Table 3) was not equal across viruses (χ^2 = 38.8, p < 0.001), with significant differences between all pairs of viruses except IAV and IBV. The progression of offset across states occurred relatively slowly for all viruses (Figure 3C); the IQRs of offset dates among states were ≥35 days (Table 3). Offset dates differed across regions for IAV (χ^2 = 11.8, p = 0.008) and RSV (χ^2 = 9.4, p = 0.025). IAV offsetted in the South before the Midwest and Northeast; RSV offsetted in the South before the Midwest (Table S3).

Nationally, the seasonal wastewater event lasted about 5.3 months for IAV, 3.7 months for RSV, 2.4 months for IBV, and 1.1 month for HMPV (Table 2). Visually, the national RSV seasonal wastewater event coincided with the wintertime high SARS-CoV-2 national wastewater category; the national HMPV seasonal wastewater event only occurred during the springtime low SARS-CoV-2 national wastewater category. The national seasonal wastewater events for IAV and IBV occurred during low, medium, and high SARS-CoV-2 national wastewater categories (Figure 2). Durations for each state are in the Supporting Information (Figure S2 and Table S2). The median duration among states (Table 3) was not equal across viruses (χ^2 = 52.6, p < 0.001), with significant differences between all pairs of viruses. Durations differed across regions for IAV (χ^2 = 15.6, p = 0.001) and RSV (χ^2 = 9.9, p = 0.019). IAV duration was shorter in the South than all other regions; RSV duration was shorter in the South than the Midwest (Table S3).

DISCUSSION

Epidemiologic study of respiratory infections is essential for clinical and public health awareness−especially considering the COVID-19 pandemic disrupted typical infection patterns.[3,4,6] Atypical infection patterns may affect other health outcomes, as seen by increased rates of sudden unexpected infant death associated with off-season RSV resurgences.[26] We used wastewater RNA concentrations for IAV, IBV, RSV, and HMPV across the USA for prospective surveillance of the 2023−2024 respiratory virus season and observed some discrepancies with prepandemic patterns of infection caused by these viruses according to historical data.

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Influenza activity is typically dominated by influenza A,[14] and this was the case in the USA during the 2023−2024 season according to clinical specimens.[27] Influenza and RSV activity generally begin in November in the USA, with RSV epidemics occurring 0.3 months before influenza epidemics in temperate zones.[28] Generally, IBV onsets 0.6 months after IAV and HMPV onsets 1.7 months after RSV in temperate areas.[28] We inferred onset for IAV and RSV that aligned with prepandemic patterns; however, we inferred later onset for IBV and HMPV than expected. Nationally, IBV onsetted 2.1 months after IAV and HMPV onsetted 4.6 months after RSV. Unlike clinical data, which are subject to reporting delays, wastewater measurements can be available within 24 h of sample collection. Communicating real-time increases in viral activity to clinicians is critical for informing the timing of immunoprophylaxis and vaccination campaigns (for influenza and RSV) as there may be variation across seasons.[4,29] We conducted the analysis for the 2022−2023 season and observed no national wastewater event for IBV and earlier onset for IAV, RSV, and HMPV than in 2023−2024 (Text S3 and Figures S10 and S11 and Table S4).

In the USA, viral activity generally peaks in January/February for influenza, December/January for RSV, and March/April for HMPV based on historical clinical data.[3] We inferred peaks that were overall consistent with prepandemic tendencies for all viruses. Furthermore, we observed peak wastewater levels for IAV, IBV, and RSV when SARS-CoV-2 was in the high wastewater category; we observed peak wastewater levels for HMPV when SARS-CoV-2 was in the low wastewater category. Although future seasonality of SARS-CoV-2 is uncertain, our findings suggest potential cocirculation of SARS-CoV-2 with influenza and RSV and interference of SARS-CoV-2 with HMPV. Townsend et al.[25] similarly project peak SARS-CoV-2 activity in late fall/winter in the temperate northern hemisphere which aligns with typical peaks for influenza and RSV. Additionally, Arimura et al.[30] retrospectively reviewed clinical tests between November 2020 and March 2023 in Japan and observed reduced likelihood of SARS-CoV-2 codetection with HMPV. Interestingly, reduced likelihood of SARS-CoV-2 codetection was also observed with RSV,[30] but it is possible codetection likelihood may differ among SARS-CoV-2 variants which are continually emerging. Wastewater measurements represent an entire contributing population in a localized area where not enough of the population may be seeking clinical testing (e.g., during holiday seasons) to accurately assess localized disease dynamics. Objectively identifying the timing of epidemic peaks and preparing for coinciding epidemics is important for surge planning (e.g., allocating hospital staffing, supplies, bed capacity) and informing the public so individuals can choose to modify their behaviors.[2] Moreover, the heightened spatial granularity in wastewater monitoring data can improve health equity and social justice by identifying where efforts, such as testing or public messaging, should be prioritized and where health disparities may occur.[2]

Based on historical clinical data, IAV epidemics generally have a shorter duration (4 months) than IBV epidemics (4.5 months), and the typical epidemic duration for RSV (4.6 months) and HMPV (4.8 months) are both longer than that of influenza viruses.[28] Nationally, we inferred a longer duration than expected for IAV (5.3 months) and shorter durations than expected for IBV (2.4 months), RSV (3.7 months), and HMPV (1.1 month). Epidemic duration is important for determining how long nonpharmaceutical interventions should be implemented and how long elevated healthcare staffing and resources need to be sustained.[2,31] It is possible we inferred shorter durations than expected for IBV, RSV, and HMPV because wastewater samples capture an inherently different population than clinical specimens. Wastewater measurements reflect infections in individuals with any symptom presentation (including those with no symptoms) whereas clinical data are more likely to reflect infections in individuals requiring clinical care. It is possible that the national seasonal wastewater event for IAV was prolonged by the avian influenza (H5N1) outbreak in the USA starting in March 2024.[23] During this outbreak, Wolfe et al.[23] demonstrated how wastewater detected abnormal IAV activity in real-time resulting from industrial waste inputs not associated with human disease. The IAV assay used herein does not distinguish between human and animal wastewater contributions, so it is likely the IAV epidemic duration among humans was shorter than the duration we inferred from wastewater monitoring.

LIMITATIONS

Limited knowledge exists about fecal shedding load and duration for influenza viruses, RSV, and HMPV,[32] making it challenging to compare wastewater concentrations across viruses. Relative wastewater concentrations may not necessarily reflect relative disease incidence if viruses are shed in differing quantities. Second, we observed that onset and offset were most variable across states for HMPV, similar to how Boehm et al.[13] observed that occurrence patterns were least spatially coherent across the California Bay Area for HMPV. Observations of more localized occurrence patterns lacking national coherency for HMPV might suggest the concept of a national season for HMPV is not appropriate. HMPV was only discovered in 2001,[16] so further research is needed to understand its epidemiology. Third, influenza, RSV, and HMPV are not nationally notifiable diseases in the USA.[33] Given that clinical testing results are not notifiable—and that reported results are associated with biases and delays arising from variation in test-seeking and test-reporting patterns—the historical clinical data we compare our wastewater-based inferences to may not represent true occurrence patterns of these viruses. Lastly, there are inequities in household sewer connectivity in the USA which may have implications for the representativeness of our observations.[34] For instance, it is possible our study population misrepresents the true proportion of high-income households in the USA given that sewer connectivity is lower for households with higher income at the national scale.[34] The national epidemic onset, peak, and offset dates we identified could therefore be biased if household income is a key factor impacting the progression of respiratory virus epidemics. Although we did not compare the characteristics of populations represented in this study to those of the general USA population, modeling work suggests that wastewater monitoring in one community may be representative of disease dynamics in a neighboring community without wastewater monitoring—even when interaction between the communities is weak.[34] Therefore, there are likely many populations indirectly represented in our study through interactions with communities with wastewater monitoring in our study, thereby bolstering the representativeness of our observations.

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CONCLUSIONS

The COVID-19 pandemic underscored the need for improved infectious disease surveillance as clinical data are inherently passive and biased. This is the first study to demonstrate how wastewater measurements may be interpreted to concurrently monitor occurrence patterns of respiratory viruses in real-time across a geographic area spanning thousands of kilometers. Seasonal occurrence patterns for influenza, RSV, and HMPV have partially returned to pre-COVID-19 pandemic tendencies and influenza and RSV may co-occur with SARS-CoV-2 in the future. Better knowledge about respiratory virus seasonality will improve population health outcomes in the present and strengthen preparedness against public health threats in the future.

ASSOCIATED CONTENT

Data Availability Statement

Measurements of viral nucleic acids in wastewater solids underlying this study are publicly available through the Stanford Digital Repository (https://purl.stanford.edu/hj801ns5929). Anyone seeking to use the data for a specified purpose is required to contact aboehm@stanford.edu. When the data are used in any format, the following attribution should be made: "These data were collected as part of the WastewaterSCAN/SCAN project, a partnership between Stanford University, Emory University, and Verily funded philanthropically through a gift to Stanford University."

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.4c01013.

Additional methodological details (Text S1 and Table S1 and Text S2), wastewater PMMoV RNA concentrations (Figure S1), state-level results (Figure S2 and Table S2), annotated cumulative curves and maps (Figures S3−S9), statistical output (Table S3), and supplementary analysis for the 2022−2023 season (Text S3 and Figures S10 and S11 and Table S4) (PDF)

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Author Contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. CRediT: **Elana M. G. Chan** conceptualization, formal analysis, investigation, methodology, software, visualization, writing - original draft, writing - review & editing; **Alexandria B Boehm** conceptualization, data curation, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing - original draft, writing - review & editing.

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Notes

The authors declare no competing financial interest.

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