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Article

Observations of Respiratory Syncytial Virus (RSV) Nucleic Acids in Wastewater Solids Across the United States in the 2022−2023 Season: Relationships with RSV Infection Positivity and Hospitalization Rates

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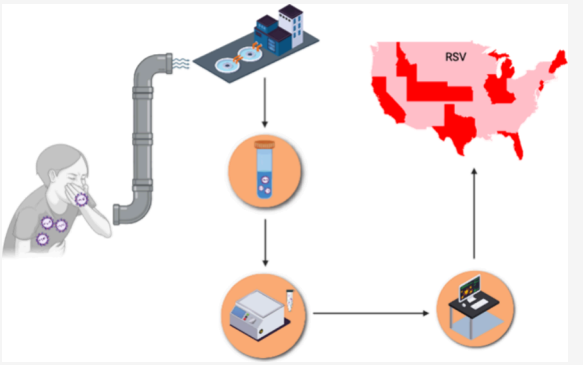
ABSTRACT: Respiratory syncytial virus (RSV) is a leading cause of respiratory illness and hospitalization, but clinical surveillance detects only a minority of cases. Wastewater surveillance could determine the onset and extent of RSV circulation in the absence of sensitive case detection, but to date, studies of RSV in wastewater are few. We measured RSV RNA concentrations in wastewater solids from 176 sites during the 2022−2023 RSV season and compared those to publicly available RSV infection positivity and hospitalization rates. Concentrations ranged from undetectable to 10^7 copies per gram. RSV RNA concentration aggregated at state and national levels correlated with infection positivity and hospitalization rates. RSV season onset was determined using both wastewater and clinical positivity rates using independent algorithms for 14 states where both data were available at the start of the RSV season. In 4 of 14 states, wastewater and clinical surveillance identified RSV season onset during the same week; in 3 states, wastewater onset preceded clinical onset, and in 7 states, wastewater onset occurred after clinical onset. Wastewater concentrations generally peaked in the same week as hospitalization rates but after case positivity rates peaked. Differences in onset and peaks in wastewater versus clinical data may reflect inherent differences in the surveillance approaches.



KEYWORDS: *wastewater-based epidemiology, onset, epidemic pattern, respiratory syncytial virus, RSV, hospitalization rate*

INTRODUCTION

Respiratory syncytial virus (RSV) is a leading cause of pediatric pneumonia and bronchiolitis and is responsible for over 100,000 pediatric deaths worldwide each year.[1−3] In the US, RSV annually accounts for up to 80,000 hospitalizations and over 520,000 emergency department visits in children under 5.[4−6] RSV infects 97% of all children by the age of 2, making it a ubiquitous cause of acute respiratory tract infection in children and a large driver of morbidity and mortality within this cohort.[1,7,8] RSV is also a concern in the aging population, responsible for up to 10,000 recorded yearly deaths in the cohort aged 65 and older.[5,9]

RSV transmission typically follows a predictable seasonal pattern, with peaks in the Northern Hemisphere occurring in the late fall and extending through spring.[10] Pinpointing the onset and severity of the RSV season could enhance efficient allocation of resources to where and when they are needed and signal public health authorities to campaign for immunization. In the US, RSV surveillance is conducted through several networks that track temporal and geographic trends in clinical illness and hospitalizations of patients.[11] The determination of epidemic onset is currently tied to laboratory-based testing of clinical cases—a metric based on voluntary reporting that does not capture the full magnitude of RSV circulation in the community and is prone to reporting time lags.[12] As a result, the annual onset of RSV transmission at the community level may be recognized after it has begun. Although using clinical data is the approach currently available and used to assess the start of the RSV season, the approach is imperfect.

During the COVID-19 pandemic, many countries, including the US, observed drastic reductions in RSV infections the winter of 2020−21 followed by unseasonal RSV activity in the summer of 2021 and atypically early onset of RSV circulation with more severe clinical presentation of disease during the winter of 2022−2023.[10−13] New immunizations became publicly available in the fall of 2023, after the end of our study, and include a vaccine for use during pregnancy, a long-

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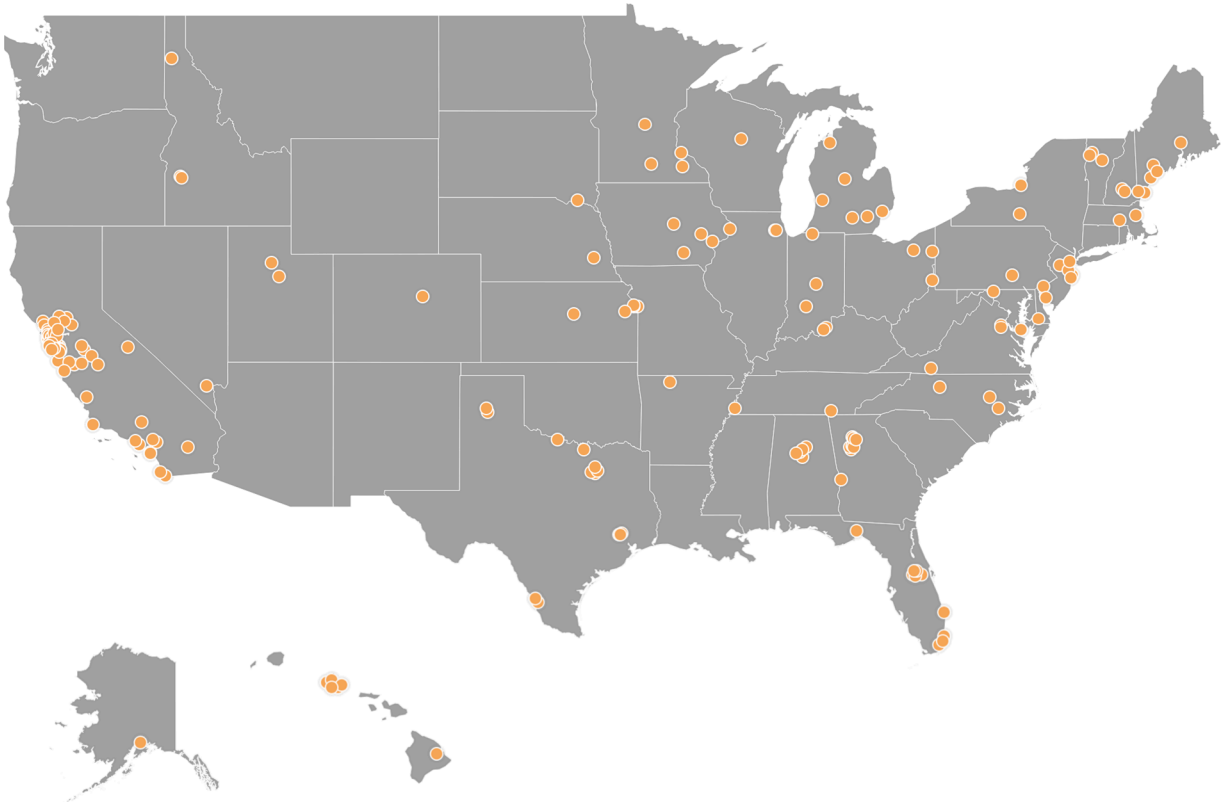

Figure 1. Location of WWTPs with RSV RNA data is included in the paper. Each orange dot represents the location of each WWTP.

acting monoclonal antibody (nirsevimab) recommended for immunization of infants 8 months old and younger, and a new vaccine available for people 60 and older. These recent changes in RSV circulation and tools for response make the determination of the onset of annual RSV epidemics an especially important, yet difficult, task.

Wastewater provides a composite biological sample for a community that can be used to monitor the local occurrence of infectious diseases. Previous studies have demonstrated that wastewater concentrations of RSV RNA correlate with disease occurrence within the communities contributing to wastewater;[14−21] however, these studies have been conducted at small geographic scales. We have previously demonstrated that wastewater-based measurements of influenza can be related to traditional surveillance metrics across the US.[22] In this study, we use data from across the US to investigate how RSV RNA concentrations in the solids from wastewater relate to traditional disease metrics during the 2022−2023 RSV season. We also test the feasibility of using wastewater-based estimations to determine the onset of the RSV season.

METHODS

We conducted a retrospective observational analysis using data on RSV RNA concentrations in wastewater collected as part of this study along with publicly available clinical and laboratory data on RSV test positivity and hospital admissions. Data between 1 January 1, 2022 to 31 July 31, 2023 were used. This study analyzed publicly available clinical data that do not contain protected health information and was therefore exempt from ethics review and the need for informed consent as per Common Rule 45 CFR46.102.

Clinical Surveillance Data. The CDC National Respiratory and Enteric Virus Surveillance System (NREVSS) is a laboratory-based surveillance system that records voluntarily reported data on laboratory testing, including confirmed RSV infections, on a weekly basis from participating laboratories nationwide.[23] The number of PCR tests performed and the proportion of positive PCR results for RSV were extracted by the state and at a national level directly from the website and are available for each morbidity and mortality weekly report (MMWR) week as 3 week moving averages; this proportion is referred to as "positivity rate". We did not utilize results from the antigen testing. No universal recommendations or eligibility criteria exist for access to RSV clinical testing, and there are frequently geographic and financial barriers to healthcare in the US. As a result, testing practices can vary considerably among states, counties, and healthcare providers participating in NREVSS.

We obtained RSV hospitalization rates from the CDC Respiratory Syncytial Virus Hospitalization Surveillance Network (RSV-Net), a platform that conducts surveillance for laboratory-confirmed hospital admissions associated with RSV in 12 states.[24] Hospitalization rate data are available on a weekly basis. Clinical data are available at the Stanford Digital Repository (https://purl.stanford.edu/hd388xb1982).

Wastewater Data: Sample Collection. Between 1 January 2022 and 31 July 2023, wastewater samples (either 24 h composited influent or grab samples from the primary clarifier, Table S1) were collected by wastewater treatment plant (WWTP) staff using sterile containers. Samples were typically obtained three times per week and shipped overnight to the laboratory at 4 °C where they were processed immediately. During the time between sample collection and transport (0−3 days), we expect minimal losses of the RNA target based on results of viral RNA persistence studies.[25] Samples were collected from WWTPs in a total of 34 states

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over the course of the study period and 176 distinct WWTPs (Figure 1). The date when sample collection commenced varied by WWTP, depending on when the sample was enrolled in the study (Table S1). A total of 22,809 samples were collected and analyzed as part of this study.

Previous work has indicated that RSV RNA in wastewater strongly adsorbs to wastewater solids, so in this study, we make measurements in the solid phase of wastewater.[26] Details of the isolation of solids from the samples are provided in other peer-reviewed publications.[27] In short, samples were centrifuged to dewater the solids, and an aliquot was used for nucleic-acid extractions, and another was used to determine the dry weight of the solids.

Wastewater Data: Nucleic-Acid Extraction. The methods for nucleic acid extraction and purification are provided on protocols.io.[28] In brief, dewatered solids were suspended in a DNA/RNA shield (Zymo, Irvine, CA) containing bovine coronavirus (BCoV, an extraction control). The concentration of solids in the DNA/RNA shield approximately 75 mg/mL; we found that there is limited inhibition of downstream analytical measurements using this concentration of solids.[27] The suspension was homogenized and then centrifuged, and nucleic acids were extracted from 6 to 10 aliquots of the supernatant using a commercially available kit (Chemagic Viral DNA/RNA 300 Kit H96, PerkinElmer, Shelton, CT). The resultant extracts were subjected to inhibitor removal using a Zymo OneStep PCR Inhibitor Removal Kit (Zymo, Irvine, CA). A total of 300 μL entered each extraction resulting in 50 μL of nucleic-acid extract for each of the replicates. The number of replicates varied by WWTP as indicated in Figure S1.

Wastewater Data: Analytical Methods. Concentrations of RSV, pepper mild mottle virus (PMMoV), and BCoV RNA in each extract were measured using droplet digital RT-PCR. Primers for RSV were previously developed and detect both RSV A and RSV B.[15] PMMoV is used as a whole process endogenous control and a fecal strength control; it is a highly abundant plant virus present in wastewater globally.[29,30] BCoV served as an extraction control. Primers and probes for all of the assays were purchased from IDT (Coralville, Iowa) and are presented in Table S2.

PMMoV and BCoV were measured using a duplex assay previously described using a 1:100 dilution of the nucleic-acid extracts as template.[27] Two replicate wells were run for the PMMoV/BCoV assays, with 10 replicate wells run for a small subset of the WWTPs (Figure S1). Each of the 6−10 replicate nucleic-acid extractions obtained from each sample was run neat as a template in its own RT-PCR well to measure RSV RNA concentrations. The RSV assay was run in a multiplex reaction with assays for other targets; the precise other targets included varied over the course of the prospective study, as public health needs and interests changed with emerging outbreaks and science to support the use of wastewater for a broad range of infectious disease targets. The precise assays that the RSV assay was multiplexed with are presented in Figure S1. Additional details of the RT-PCR assays (cycling conditions, thresholding) are presented in the SI.[27] Extraction and PCR positive and negative controls were run on each 96-well plate, as described elsewhere.[27]

Concentrations of the targets are presented as copies per gram of dry weight. For a sample to be scored as a positive, there had to be at least three positive droplets. The lower detection limit for RSV is approximately 1000 copies/g of dry weight. Errors are reported as standard deviations on the measurements as obtained from the instrument software, QuantaSoft Analysis Pro Software (Bio-Rad, version 1.0.596) and QX Manager Software (Bio-Rad, version 2.0). Wastewater data are publicly available through the Stanford Digital Repository (https://purl.stanford.edu/hd388xb1982).

Spatial-Aggregation of Wastewater Data. We aggregated data across states and across the nation using the following methods. First, state-aggregated population weighted averages were calculated for each state with at least one WWTP participating in the project using the following equation:

$$\mathrm{RSV/PMMoV}_S(d) = \left(\sum_{i=1}^{X} pop_i^{*}\,\mathrm{trimmedRSV/PMMoV}_i(d)\right) \Big/ \left(\sum_{i=1}^{X} \mathrm{pop}_i\right) \quad (1)$$

where $\mathrm{RSV/PMMoV}_S(d)$ is the state-aggregated population weighted average of RSV normalized by PMMoV for state s on day d, pop_i is the population served by WWTP i of X total in the state, and trimmed $\mathrm{RSV/PMMoV}_i(d)$ is the 5-adjacent sample centered, trimmed average of RSV/PMMoV for WWTP i on day d. Prior to calculating $\mathrm{RSV/PMMoV}_S(d)$, nondetect values were set to 500 cp/g (approximately half the limit of detection) divided by the PMMoV value for the plant that day d, and values for trimmed $\mathrm{RSV/PMMoV}_i(d)$ days without data were interpolated using a linear interpolation from adjacent data points. We also calculated $\mathrm{RSV}_S(d)$, which is analogous to $\mathrm{RSV/PMMoV}_S(d)$ but uses trimmed $\mathrm{RSV}(d)$ rather than trimmed RSV/PMMoV in eq 1.

We aggregated data across the nation using the following equation:

$$\mathrm{RSV/PMMoV}_N(d) = \left(\sum_{s=1}^{S} \mathrm{pop}_s^{*}\mathrm{RSV/PMMoV}_s(d)\right) \Big/ \left(\sum_{s=1}^{S} \mathrm{pop}_s\right) \quad (2)$$

where $\mathrm{RSV/PMMoV}_N(d)$ is the nationally aggregated average of wastewater concentrations of RSV/PMMoV as a function of day d, pop_s is the population of state s of S states included in the study, and $\mathrm{RSV/PMMoV}_S(d)$ is defined above in eq 1. We also calculated $RSV_N(\mathrm{d})$ which is analogous to $\mathrm{RSV/PMMoV}_N(d)$ but uses $\mathrm{RSV}_S(d)$ rather than $\mathrm{RSV/PMMoV}_S(d)$ in eq 2.

In cases where data from a specific WWTP had significant gaps between observations (21 or more days of no observation), the data from that WWTP were not included in the state level aggregation for those periods but reincluded once observations resumed.

Data Analysis: Correlations. To test associations between wastewater and clinical measures, we calculated spearman's rho correlations between wastewater concentrations and positivity rates and between wastewater concentrations and hospitalizations for states where both data sets were available. Data normality was assessed using Shapiro−Wilk's method for $\mathrm{RSV/PMMoV}_S$ and $\mathrm{RSV/PMMoV}_N$ and were found to not be normally distributed (all $p < 10^{-10}$). Therefore, a non-parametric method was chosen to assess correlation. As clinical data are available on a weekly basis, we used the weekly median $\mathrm{RSV/PMMoV}_S$ or $\mathrm{RSV/PMMoV}_N$ depending on whether the correlations were done at the state or national

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Table 1. Spearman's Rho and *P* Values for Correlations Between State-aggregated Wastewater Concentrations and Positivity Rate and Hospitalization Rate[a]

state	rho between WW and positivity rate	*P* value of rho between WW and positivity rate	rho between WW and hospitalization rate	*P* value of rho between WW and hospitalization rate	*N*
Alabama*	0.75	<0.001			49
Alaska*	0.51#	0.164#			9
Arkansas*	NA	NA			13
California	0.89	<0.001	0.90	<0.001	81
Colorado	0.8	<0.001	0.80	<0.001	62
Delaware*	NA	NA			24
Florida	0.81	<0.001			68
Georgia*	0.82	<0.001	0.86	<0.001	56
Hawaii*	0.09#	0.783#			11
Idaho	0.75	<0.001			73
Illinois	0.8	<0.001			51
Indiana	0.71	<0.001			49
Iowa*	0.93	<0.001			32
Kansas	0.81	<0.001			51
Kentucky	0.46	<0.001			73
Maine	0.8	<0.001			46
Maryland*	0.83	<0.001	0.93	<0.001	32
Massachusetts*	0.82	<0.001			32
Michigan	0.71	<0.001	0.89	<0.001	66
Minnesota*	0.26#	0.0757#	0.31	0.14#	47
Nevada*	0.36#	0.154#			17
New Hampshire	0.77	<0.001			41
New Jersey	0.74	<0.001			50
North Carolina*	0.86	<0.001			48
Ohio*	0.8	<0.001			32
Pennsylvania*	0.8	<0.001			51
South Dakota*	NA	NA			13
Tennessee*	NA	NA	−0.5#	0.66#	6
Texas	0.42	<0.001			73
Utah	0.7	<0.001	0.93	<0.001	44
Vermont*	0.56#	0.00968#			20
Virginia*	0.3#	0.0969#			32
West Virginia*	NA	NA			15
Wisconsin*	NA	NA			5

[a]Hospitalization rate is only available for a small subset of states with wastewater data, and a blank cell indicates there was no hospitalization data available for that state. NA indicates that a rho could not be calculated due to a lack of variation in one of the two variables. *N* is the number of data points used in the correlations. *P* values greater than or equal to 0.001 indicate a non-significant correlation and in these cases, both rho and the *P* value are marked with a #. An asterisk appears next to each state for which wastewater data were not available before the state of the RSV season as determined by clinical positivity rate data.

level, respectively. Medians were chosen as data were not-normal, but similar results were obtained using weekly means. Weeks were defined as by the CDC morbidity and mortality weekly report (MMWR). We conducted a total of 44 correlations and to account for multiple correlations used a *p* value of 0.001 to identify statistically significant correlations for alpha <0.05 (0.05/44 = 0.001). This study did not consider a time series regression analysis, due to autocorrelation in the time series and the low resolution clinical data streams. Future work with higher resolution clinical data and advanced time series methods would be useful to better understand the temporal relationship between the two indicators of RSV.

Data Analysis: RSV Season Characteristics. The onset and offset of the RSV season were determined using both clinical metrics and wastewater. States for which wastewater data did not exist prior to the clinical onset date of the 2022−2023 RSV season, as determined by positivity rate data, were excluded from this analysis (Figure S2). This left 14 states included in the analysis (CA, CO, FL, ID, IL, IN, KS, KY, MI, ME, NH, NJ, UT, and TX).

Epidemic onset and offset were determined at state-level based on the first and last of two consecutive weeks when the weekly percentage of positive PCR tests for RSV was greater than or equal to 3%.[31] This definition is commonly used by researchers and public health agencies. From here on, these weeks will be referred to as the clinical onset and offset and defined by the last day of the week. It should be noted that the dates of clinical onset and offset are determined retrospectively after the 2 weeks of ≥3% (Figure S3). The duration of the epidemic was defined as the number of weeks between onset and offset.We recorded the median of RSV/PMMoV$_S$ during the weeks of clinical onset and offset, for each state.

The algorithm for determining dates of wastewater onset and offset was developed independently of clinical data and was informed by our previous work observing RSV concentrations in wastewater.[21,32] First, we used the criteria described by Boehm et al. to identify onset and offset for each

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Table 2. Clinical and Wastewater RSV Onset, Offset, and Peak in 14 States[a]

state	clinical onset,offset	WW median at onset ($\times 10^{-6}$)	WW median at offset ($\times 10^{-6}$)	WW onset,offset	date of peak hosp. rate	date of peak positivity rate	date of peak WW
California	9/17/2022, 2/4/2023	5.5	12.8	9/25/2022, 4/6/2023	12/3/2022	11/12/2022	11/27/2022
Colorado	10/1/2022, 2/18/2023	10.5	17.5	10/4/2022, 4/9/2023	11/12/2022	11/19/2022	11/09/2022
Florida	5/7/2022, 2/4/2023; 7/22/2023, ?	8.7	8.6	**6/21/2022,** 7/10/2022; 7/19/2022, 8/9/2022; 8/19/2022, **1/31/2023**		10/1/2022	6/11/2022
Idaho	10/29/2022, 2/11/2023	8.3	47.3	10/29/2022, 4/11/2023		11/26/2022	12/14/2022
Illinois	9/3/2022, 12/24/2022	2.2	1.9	9/20/2022, 4/25/2023		10/22/2022	10/30/2022
Indiana	8/27/2022, 1/21/2023	8.0	3.0	9/27/2022, 4/7/2023		10/15/2022	11/8/2022
Kansas	9/17/2022, 1/21/2023	7.2	31.6	8/17/2022, 3/15/2023		10/22/2022	12/2/2022
Kentucky	6/11/2022, 6/25/2022; 7/23/2022, 7/30/2022; 8/06/2022, 1/7/2023	1.8	16.5	9/26/2022, 2/21/2023		10/22/2022	11/15/2022
Maine	9/10/2022, 3/4/2023	3.0	12.2	9/28/2022, 4/10/2023		10/22/2022	11/12/2022
Michigan	9/10/2022, 1/14/2023	2.0	92.2	8/20/2022, 3/12/2023	11/12/2022	11/5/2022	11/17/2022
New Hampshire	10/29/2022, 2/4/2023	85	58.6	10/28/2022, 3/17/2023		11/5/2022	12/13/2022
New Jersey	9/17/2022, 12/24/2022	4.6	71.4	10/13/2022, 4/2/2023		11/5/2022	12/17/2022
Texas	4/30/2022, 1/28/2023	1.6	13.6	**6/2/2022,** 8/16/2022; 9/7/2022, **3/5/2023**		10/15/2022	12/10/2022
Utah	4/02/2022, 4/23/2022; 5/21/2022, 6/11/2022; 11/12/2022, 2/25/2023; 4/08/2023, 4/22/2023; 6/17/2023, 7/01/2023	27	7.9	10/26/2022, 4/5/2023	11/26/2022	12/10/2022	12/4/2022

[a]For each of 14 states with both wastewater and clinical data for the duration of the 2022-2023 RSV season, we show the dates of clinical onset and offset; if there are more than one, they are all given separated by a semicolon and a bolded date is the one used for Figure 3 and in the text to describe the start of RSV season. The weekly median RSV/PMMoV$_S$ during the MMWR week of clinical onset and offset is provided; the value is unitless. "WW onset,offset" provides the dates of wastewater event onset and offset; if more than one occurred, then the dates are separated by semicolons. The date of onset of the first wastewater event is used in Figure 3 and in the text. Date of the peak value for hospitalization (hospitalization) rate, positivity rate, and wastewater (RSV/PMMoV$_S$) are provided. All dates are month/day/year format. The "?" in the FL clinical onset/offset column indicates that the offset date for the second onset event in FL occurred after the end of this study and is unknown.

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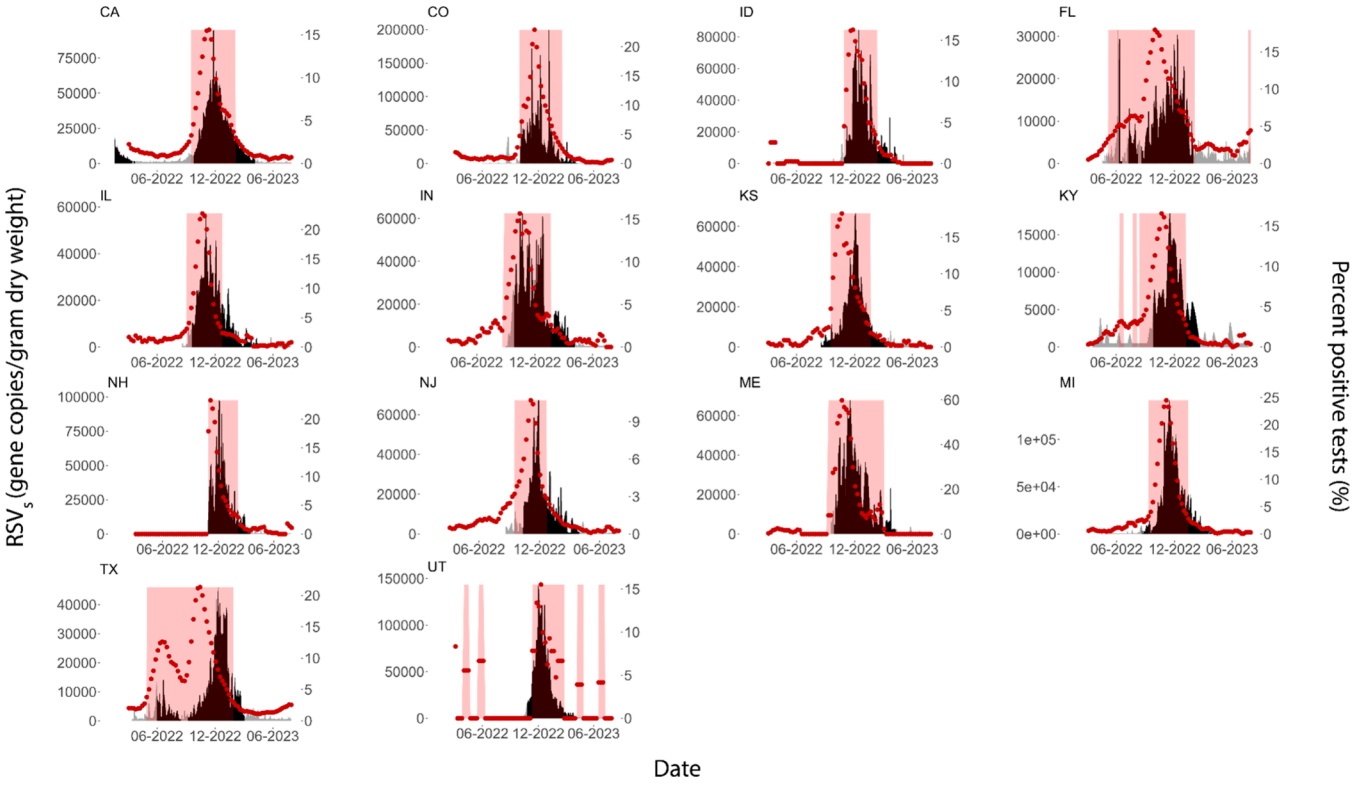

Figure 2. State-aggregated concentrations of RSV RNA (RSV_S, black/grey shaded area, left axis) and positivity rates (red dots, right axis). Red background shading indicates periods between the clinical onset and offset. Black fill below the wastewater concentrations indicates periods of onset of wastewater events, whereas gray fill indicates time periods when wastewater events are not in onset. The gray fill is barely visible in most panels as the concentrations prior to and after onset are low.

individual WWTP as follows.[21] For each individual WWTP, the wastewater onset date was identified as the date on which all samples in a 14 day look back period had measured concentrations of RSV RNA that exceeded or were equal to 2000 cp/g dry weight. The wastewater offset date was identified as the first date after wastewater onset for which only 50% of samples during a 14 day look back period had concentrations over or equal to 2,000 cp/g. 2000 cp/g was chosen as it represents approximately two times the detection limit, as described previously.[21] Wastewater event onset and offset dates were identified for the entire state as follows. The date of wastewater onset was determined as a date when 50% or more of the individual WWTPs in the state were in onset, as described above for the individual plants, and offset as the date after onset for which less than 50% of the sites in the state were in onset. For this analysis, an individual plant was not given an onset or offset designation for the first 14 days of data availability.

We compared epidemic peaks, as defined by diagnostic and hospital data, to the state-aggregated peaks in RSV wastewater concentrations. Peaks of clinical data were determined as the week with the highest values (last date of MMWR week) whereas wastewater peaks were identified as the day with highest value of RSV/PMMoV$_S$.

RESULTS

QA/QC. All wastewater measurements positive and negative controls were positive and negative, respectively, indicating acceptable assay performance and no contamination across the entire study. Median (IQR) BCoV recoveries across all samples were 1.1 (0.83, 1.2), indicating good recovery across the samples. Recoveries greater than 1 are likely a result of uncertainties in the measurement of BCoV added to the buffer matrix. Additional details of reporting outlined in the Environmental Microbiology Minimal Information (EMMI) reporting guidelines are provided elsewhere.[27]

RSV Measurements and Correlations with Clinical RSV Data. Over the study period of January 1, 2022 to July 31, 2023, wastewater data were available from 176 WWTPs in 34 states (Table S1). Population coverage of the wastewater catchment areas varied from 0.5 to 59.5% with a median (interquartile range, IQR) state-aggregated WWTP population of 324,045 (119,000, 693,000). RSV measurements at individual WWTPs varied from nondetect to 9.4×10^6 cp/g dry weight (median across all measurements was 1.1×10^3 cp/g). Time series plots of RSV measurements from all WWTPs are provided in the Supporting Information (SI) (Figure S4). We generally found undetectable levels in the late spring and summer, with peak values in the winter in line with general expectations for seasonality of RSV in the Northern hemisphere.[10]

Wastewater concentrations paralleled clinical surveillance data. State-aggregated population-weighted average RSV/PMMoV (RSV/PMMoV$_S$) was calculated for each state for which wastewater data were available (N = 34), and Spearman's rho was determined between RSV/PMMoV$_S$ and the state-aggregated positivity rate. Rho for six states could not be calculated because available wastewater values were constant; and rho was not statistically significant for five states (Table 1). The vast majority of the states for which rho was not statistically significant or could not be calculated had RSV wastewater data available only after late spring/early summer

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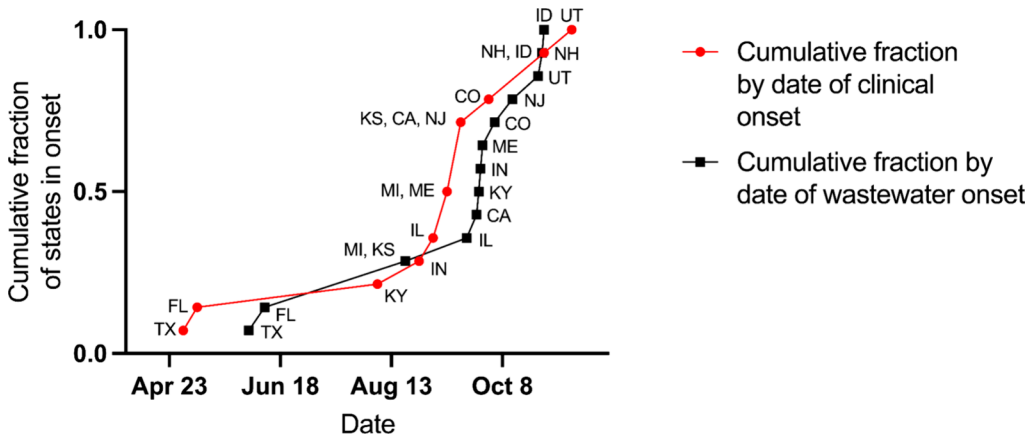

Figure 3. Cumulative fraction of 14 states (y-axis) in RSV onset, as determined by clinical positivity rates in red and by wastewater concentrations in black. The date of clinical onset (red) or wastewater event onset (black) is shown on the x-axis. Each symbol is labeled with the state.

of 2023, after the RSV season had passed (Figures S2 and S4, Table S1). Rhos for the remaining states were statistically significant (rho between 0.42 and 0.93, $p < 0.001$). Both wastewater and hospitalization rate data were available for 8 states, and so rho was calculated to assess their associations (Table 1). Rho varied between 0.80 and 0.93 ($p < 0.001$) for six states. Rho was insignificant for the other two states (wastewater data collection started in June 2023, after RSV season). Nationally aggregated RSV/PMMoV (RSV/$PMMoV_N$) was positively associated with nationally aggregated positivity and hospitalization rates (rho = 0.73, $p < 0.001$ and rho = 0.84, $p < 0.001$, respectively).

Onset, Offset, and Peak of RSV Season. Wastewater data were available before clinical onset of the RSV season in 14 states (CA, CO, FL, ID, IL, IN, KS, KY, MI, ME, NH, NJ, UT, TX, Table 2, Figure S2). In these states, clinical onset of RSV season occurred between April 20, 2022, and November 12, 2022, and clinical offset occurred between December 24, 2022, and March 4, 2023, depending on state (Table 2). Duration of RSV epidemic ranged from 14 to 39 weeks across states; longer duration was generally observed in states with earlier onset. Most (11 of 14) states had one RSV onset during this time period; FL, UT, and KY had multiple clinical onset events (Figure 2, Table 2) where positivity rates increased over 3% for two consecutive weeks and then decreased again before again meeting the onset threshold. For these three states, we considered the RSV seasonal clinical onset date to be that which occurred prior to the peak in the positivity rates.

State weekly median PMMoV-normalized wastewater concentrations (RSV/$PMMoV_S$) during the week of clinical onset ranged from 2.17×10^{-6} to 8.50×10^{-5}, while the weekly median concentrations during the week of clinical offset ranged from 1.88×10^{-6} to 9.22×10^{-5}, depending on the state (Table 2). Values tended to be higher during offset compared to onset (one-sided Wilcoxon signed rank test, $p = 0.04$, median difference = 8.25×10^{-6}).

For the 14 states, the date of wastewater onset varied between June 21 to October 29, 2022, and the date of offset varied between January 31 and April 25, 2023 (Table 2), with the duration of the event between 20 and 39 weeks. Most (12 of 14) of the state wastewater data showed one RSV wastewater event over the study period (Figure 2, Table 2). Two states (FL and TX) had multiple RSV wastewater events. TX had two, one between early June and mid August 2022, and the other between early September 2022 and early May 2023. FL had three RSV wastewater events: one between late June and mid-July 2022, one between late July and early August 2022, and one between mid August and late January 2023. The date used for the onset of wastewater for TX and FL was the onset date of the first wastewater event.

For four states, wastewater event onset occurred the same week as clinical onset; for three states, wastewater event onset occurred 2 to 4 weeks before clinical onset; and for seven states, wastewater event onset commenced 2 to 7 weeks after clinical onset.

Peaks in state-aggregated population weighted average RSV/PMMoV ($RSV/PMMoV_S$) across the 14 states ranged from October 30 to December 17, 2022, except for FL where the peak was in mid-June (Table 2). Peaks in hospitalization rates, for the 4 of 14 states with available hospitalization rate data, were within the same week as the peak in wastewater data (Table 2). Peaks in positivity rate data were generally earlier than the peaks in the wastewater variable. For three states (FL, CO, and UT), wastewater peaked 1−16 weeks earlier than the positivity rate data. In the remaining 11 states, positivity rates peaked before wastewater (median 3 weeks earlier, range 1−8 weeks earlier).

The 2022−2023 RSV season demonstrated a consistent geotemporal pattern of wastewater onset (Figure 3). Wastewater onset occurred earliest in the south in Texas and Florida. Then, wastewater onset moved north and west almost uniformly, with the midwest (Kansas and Michigan) first followed closely by the remaining states where wastewater onset occurred nearly in coherence. Clinical onset followed a similar pattern with clinical onset occurring in Texas and Florida first, followed by a move of onset both west and north.

DISCUSSION

RSV RNA concentrations in wastewater collected throughout the United States correlate to both positivity rates and hospitalizations nationally and at the state level, as shown for single or small groups of wastewater treatment plants in previous studies.[15−17,19−21] Sites varied from urban to rural areas, spanned climates from tropical to continental,[33] and represented populations served from 3000 to 4 million per site with overall coverage of 0.05 to 59.5% of the state population. Despite significant diversity in site characteristics and coverage from state to state, correlations to clinical positivity rates and hospitalizations were positive and significant. Hospitalization

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peaks largely matched wastewater peaks temporally, suggesting the value of wastewater monitoring for informing hospital staff and public health notifications. For the four states with sufficient hospitalization and wastewater data, the peaks of both wastewater and hospitalizations all fell within an 8 day range of each other.

Despite the coherence between wastewater and positivity rate data, the peaks and onset determined using the two data sources were not always aligned. The lack of alignment is a result of the inherent differences and biases associated with the two different surveillance approaches. The two surveillance sources capture information about different populations, those who are sick and receive testing and those who are shedding RSV RNA. Wastewater concentrations are controlled by inputs to the system from every individual shedding RSV RNA, including those with severe or mild symptoms and those who are both symptomatic and asymptomatic. There is no quantitative data on longitudinal RSV shedding in human excretions, and it is possible prolonged shedding in convalescing or recovered individuals occurs.[34] Shedding magnitude and duration, while unknown, are key drivers of wastewater RSV concentrations.[35] The positivity rate is not a measure of disease incidence or prevalence but is often used as a proxy for disease occurrence. It measures the fraction of individuals who seek and obtain medical care and for whom a medical professional orders and administers a RSV test that obtains a positive test result. These individuals likely suffer from severe illness or come from at-risk populations. Some of these may include infants in diapers who are less likely to contribute to wastewater. Therefore, the differences in onset and peaks in the two measures could reflect different patterns of incident symptomatic, moderate to severe RSV of those with access to medical care (reflected by the positivity rate data), and community level viral shedding by all those infected with RSV (reflected by the wastewater data).

An alternate explanation for the lack of agreement in peaks and onset according to wastewater and positivity rate data streams is the limitations and biases associated with each. The majority of RSV infections are unrecognized and are not captured by clinical surveillance systems. In a prospective surveillance study of more than 5000 children in the U.S. with an acute respiratory infection, only 45% of inpatient RSV infections—and a mere 3% of outpatient infections—received the diagnosis RSV-associated illness.[6] Publicly available state-aggregated positivity rate data can also be affected by the number of tests administered. In our data series, some states reported as few as 1 performed test per week, which limits the ability to determine a reliable estimate of the positivity rate representing a community. In addition, clinical testing rates are not temporally uniform and tend to fluctuate according to the clinical landscape and clinicians' awareness of ongoing RSV circulation. For example, on the date of clinical onset in IL, 321 weekly tests were performed, while on the date of offset, 1370 weekly tests were performed. Additionally, publicly available positivity rate data was only available weekly, as 3 week averages, limiting the resolution at which changes in positivity rates can be identified. Positivity rate data are generated by clinical laboratories participating in NREVSS, which is a passive, voluntary program. The number of laboratories participating in NREVSS varies by state (N = 1 to 29 per state), and the exact laboratories contributing positivity rate data at any time are not publicly available and may change over time.[36] Similarly, the number of WWTP participating in wastewater surveillance during the study period differed across states (N = 1 to 57 per state). The spatial coverage of the participating laboratories reporting RSV test results to NREVSS likely does not exactly match the spatial coverage of the WWTP in the state (Figure S5), which means that the two surveillance methods are capturing distinct populations geographically as well. Finally, there may be fate processes such as decay and partitioning that affect the concentrations of RSV as it moves through the sewage network between drain and sampling location at the WWTPs; however, we have observed very limited decay of RSV RNA in wastewater solids over these time scales (unpublished data).

The algorithm for identifying RSV season onset based on positivity rate data is one commonly used;[31] the algorithm for identifying wastewater onset was developed independently of the positivity rate data. The wastewater algorithm represents an example approach for identifying significant emergence of RSV RNA in wastewater, but different wastewater event onset and offset thresholds or algorithms that are fit for specific public health or other stakeholder use-cases can be developed. For example, statistical or machine learning approaches could be applied to wastewater and positivity rate data to derive an algorithm to predict the RSV clinical onset from wastewater data. However, such an approach would require an assumption that the positivity rate data used herein represent the gold standard metrics for RSV disease occurrence, when in fact, they have serious limitations and biases. The PMMoV-normalized RSV RNA concentrations in wastewater during clinical onset and offset were of a similar magnitude across states and the country, suggesting that a simple concentration threshold could be considered for identifying dates of clinical onset and offset using wastewater.

We posit that wastewater measurements of RSV provide important information about population-level RSV infections, which can augment traditional RSV season indicators like hospitalization and positivity rates. Wastewater data are available in real time (as soon as 24 h after sample collection) and are not subjected to delays associated with clinical data reporting.[15,37,38] In addition, wastewater measurements are not subject to any clinical testing biases typically present due to socioeconomic factors or testing availability.[38,39] Importantly, wastewater data can be available at locations where or times when traditional disease metrics like positivity and hospitalization rates are not available offering the ability to provide public health resources at appropriate spatial and temporal scales and providing insights into disease epidemiology uniquely available through wastewater.

Limitations and Recommendations. There are limitations associated with this study. Importantly, the timing and spatial distribution of wastewater and clinical data do not match. Moreover, RSV positivity rates are biased by inconsistent test seeking, availability, and reporting. To more precisely determine the relationship between wastewater and disease, studies designed a priori will require better enumeration of infections occurring in well-defined sewersheds. Expanding clinical RSV testing would improve RSV positivity rate data and possibly make estimates of RSV incidence and prevalence available at different spatial and temporal resolutions. Until then, however, the clinical data used herein represent the best available publicly accessible RSV data. Lack of complete, reliable population-level clinical data is a challenge for a wide range of important infectious diseases that are being studied using wastewater surveillance.[40,41]

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Scientists in the field of wastewater-based epidemiology will need to continue to develop creative approaches for validating wastewater data when high-quality population-level clinical data do not exist. Collaborations with infectious disease scientists to carry out coupled wastewater-disease surveillance studies in small geographic regions may be one solution.

Additionally, we are unable to link a specific wastewater concentration of RSV to population-level RSV incidence or prevalence rates. More work is needed to better understand the shedding of RSV in human excretions to provide a linkage between wastewater concentrations and disease occurrence in the contributing population. Until such data are available, the data provided herein suggest a strong relationship between RSV RNA in wastewater solids across the United States and more traditional RSV indicators. Future work can use wastewater to explore the relative importance of RSV A and RSV B.[42] While most clinical tests do not distinguish between the two, some evidence suggests there may be differences in disease severity for the different subtypes.[43]

CONCLUSIONS

Wastewater concentrations of RSV RNA can complement traditional clinical indicators of disease and inform public health and clinical decision making. RSV wastewater concentrations correlate well with traditional disease metrics, such as positivity and hospitalization rates. Onset dates of RSV season derived from the positivity rate and wastewater data sometimes agreed and sometimes differed; differences are likely driven by the two data sources sampling different groups of individuals with different illness severity. Peaks in wastewater and hospitalization rates generally occurred at the same time, while peaks in wastewater and positivity rates were not aligned, with wastewater peaks generally occurring after positivity rate peaks. We suspect that the difference in timing of peaks is controlled by both complexities associated with magnitude and duration of RSV shedding by infected individuals and biases and limitations associated with the positivity rate data. The lack of complete agreement between the two data sources highlights the advantages and disadvantages associated with each.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.3c00725.

Additional methodological details as well as Tables S1 and S2 and Figures S1−S5 (PDF)

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