*Epidemiology and Infection*

Application of joinpoint regression to SARS-CoV-2 wastewater-based epidemiology in Las Vegas, Nevada, USA

Casey A. Barber 1, 2, Lung-Chang Chien 1, Brian Labus 1, Katherine Crank 2, Katerina Papp 2, Daniel Gerrity 2, Cheryl Collins 3, Edwin C. Oh 4, Lei Zhang 5, Anil T. Mangla 1, 5, Cassius Lockett 5, Lung-Wen Antony Chen 1\*

1 School of Public Health, University of Nevada, Las Vegas, USA

2 Applied Research and Development Center, Southern Nevada Water Authority, USA

3 Desert Research Institute, USA

4 College of Sciences; School of Integrated Health Sciences; Kirk Kerkorian School of Medicine at UNLV, University of Nevada, Las Vegas, USA

5 Southern Nevada Health District, USA

\*Corresponding Author. Email: antony.chen@unlv.edu

SUPPLEMENTARY MATERIAL

## **Supplementary Text S1.** Materials and calculations used in wastewater sample processing and analysis.

**Sample Preparation and qPCR Assays**

Wastewater sample supernatants were concentrated by centrifugal ultrafiltration (Centricon Plus70, 30 kDa or 100 kDa, Millipore Sigma, Burlington, MA, USA) or hollow-fiber ultrafiltration (HFUF; REXEED-25S dialysis filters, Asahi Kasei Medical Co., Tokyo, Japan) upon arrival to the study laboratory following sample collection. Resulting concentrates were stored at -20oC) until further processing the following day. Nucleic acid extraction (PureLink Viral 52 RNA/DNA Mini Kit, Thermo Fisher Scientific, Waltham, MA) was followed by cDNA synthesis (iScript™Select cDNA Synthesis Kit, Bio-Rad Laboratories, Hercules, CA, or Maxima First Strand cDNA Synthesis Kit, Thermo Fisher Scientific) and quantitative PCR (qPCR; CFX384 Touch Real-Time PCR Detection System, Bio-Rad Laboratories). qPCR assays were performed in duplicate for nucleocapsid proteins N1 and N2 [1], envelope protein *E\_Sarbeco* [2], and non-structural proteins *orf1a* [3] to yield the raw SARS-CoV-2 concentration, calculated as an average of these assays and duplicates. Raw concentrations were converted to concentration in the sample volume by dividing by the equivalent sample volume as described by Crank et al., which also facilitated comparisons between the HFUF and centricon concentration methods that differed in starting sample volume [4]. Analyses were monitored with negative controls and the spiked recovery control bovine coronavirus (BCoV; Calf-Guard, Zoetis, Parsippany, NJ, USA); pepper mild mottle virus (PMMoV; SYBR-based qPCR assay) was also monitored as a human faecal indicator and used as a normalization factor. Sample preparation and all sample analyses for this study were conducted at the same Southern Nevada Water Authority study laboratory.

**Data Processing**

 To obtain the BCoV-corrected concentration, the raw SARS-CoV-2 RNA concentration was corrected to reflect observed losses of the known initial spiked quantity of BCoV throughout processing. This value was then log10-transformed to yield log10 gene copies per litre (gc/L). As recovery correction was not performed for PMMoV in the sample workflow, the PMMoV-normalized concentration was calculated by dividing the raw SARS-CoV-2 RNA concentration by the raw PMMoV concentration, resulting in a unitless ratio. Given the known abundance of PMMoV in human faecal material [5], its measured concentrations were generally much greater than those of SARS-CoV-2, resulting in small normalized values. Accordingly, these values were multiplied by one million [6], and subsequently log10-transformed for analysis.

Limits of quantification (LoQs) for the SARS-CoV-2 RNA qPCR assays were established previously [7]; only seven (<1%) of 971 total samples were below the LoQ, while only three were non-detect (no amplification). The LoQ for the qPCR assay (20 gene copies) was imputed for both <LoQ and non-detect samples using each sample-specific equivalent sample volume [4,8]. No PMMoV results were <LoQ or non-detect. There were 0-2 missing weeks for a given sewershed (*i.e.,* no sample collected) and only a total of 9 missing wastewater concentrations across the entire dataset (<1% of all data; see Table S1). For these missing values, the average of the values from the previous and subsequent weeks was imputed, as there were no sequentially missing weeks for any sewershed. Vo et al. previously identified a Sewershed 7 wastewater concentration in December 2020 to be a non-representative spike and adjusted it down to the following week’s concentration (from 8.73 log10 gc/L down to 6.37 log10 gc/L) when estimating wastewater-derived infection incidence [9]; this adjustment was also repeated here for consistency.

**Text S1 References**

1. **Centers for Disease Control and Prevention**. *2019-novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes*. 2020 Jan.

2. **Corman V, *et al.*** *Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR*. Berlin, Germany, 2020 Jan.

3. **Lu R, *et al.*** Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* 2020; **395**: 565–574.

4. **Crank K, *et al.*** Correspondence on “The Environmental Microbiology Minimum Information (EMMI) Guidelines: qPCR and dPCR quality and reporting for environmental microbiology”. *Environmental Science & Technology* American Chemical Society, 2023; **57**: 20448–20449.

5. **Zhang T, *et al.*** RNA viral community in human feces: prevalence of plant pathogenic viruses. *PLoS Biology* United States: United States: Public Library of Science, 2006; **4**: e3–e3.

6. **WastewaterSCAN**. *WastewaterSCAN Dashboard*. 2024(data.wastewaterscan.org).

7. **Gerrity D, *et al.*** Early-pandemic wastewater surveillance of SARS-CoV-2 in Southern Nevada: Methodology, occurrence, and incidence/prevalence considerations. *Water Research X* Elsevier, 2021; **10**: 100086.

8. **Borchardt MA, *et al.*** The Environmental Microbiology Minimum Information (EMMI) Guidelines: qPCR and dPCR quality and reporting for environmental microbiology. *Environmental Science & Technology* American Chemical Society, 2021; **55**: 10210–10223.

9. **Vo V, *et al.*** Use of wastewater surveillance for early detection of Alpha and Epsilon SARS-CoV-2 variants of concern and estimation of overall COVID-19 infection burden. *Science of The Total Environment* 2022; **835**: 155410.

## **Supplementary Figure S1.** Map of study sewershed boundaries.



Note: The boundary for Sewershed 7 reflects city limits.

## **Supplementary Figure S2.** Flow chart of inclusion and exclusion criteria for aggregated COVID-19 confirmed case data. Included cases were confirmed (PCR-positive) initial or reinfection cases in the Las Vegas area, with a specimen collection date between Sunday, 31 May 2020, and Saturday, 15 April 2023. Cases within census blocks with zero residential population were excluded, along with cases occurring outside of the sewershed boundaries and cases associated with a nearby military base, given differences in case reporting workflows. Finally, confirmed cases reported prior to the sewershed-specific monitoring periods were also excluded.

**Included cases:** Confirmed, initial or reinfection, in the Las Vegas area, with a residential address with 100% geocoding matching, with a specimen collection date 31 May 2020-15 Apr. 2023: **564,408** (total)

Cases in non-zero-population census blocks, not military base, and within study sewersheds, 31 May 2020-15 Apr. 2023: **556,920**

**Excluded cases: 7,488** (1.33% of total)

2,127 from zero-population census blocks

672 from military base

4,689 from outside of sewersheds

Cases reported during study wastewater monitoring periods\* across all sites: **531,277** (94.13% of total)

Cases reported prior to study monitoring periods\* across all sites: **25,643** (4.54% of total)

\*As described in the main text, SARS-CoV-2 wastewater surveillance began on different dates in June, August, or December 2020, depending on when the site was onboarded.

## **Supplementary Table S1.** Inventory of study-specific wastewater sampling and SARS-CoV-2 qPCR outcomes by sewershed.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sewershed | Study sampling start date(DD/MM/YYYY) | Study sampling end date(DD/MM/YYYY) | Total sampling weeks (T) | Missing samples (M)\* | Non-missingsamples (T-M) | Of non-missing samples: |
| <LoQ | Non-detect |
| 1 | 1/6/2020 | 10/4/2023 | 150 | 1 | 149 | 0 | 0 |
| 2 | 24/8/2020 | 10/4/2023 | 138 | 0 | 138 | 0 | 0 |
| 3 | 31/8/2020 | 10/4/2023 | 137 | 2 | 135 | 4 | 1 |
| 4 | 1/6/2020 | 10/4/2023 | 150 | 2 | 148 | 0 | 0 |
| 5 | 31/8/2020 | 10/4/2023 | 137 | 1 | 136 | 2 | 0 |
| 6 | 31/8/2020 | 10/4/2023 | 137 | 2 | 135 | 1 | 0 |
| 7 | 14/12/2020 | 10/4/2023 | 122 | 1 | 121 | 0 | 2 |
|  |  | Totals: | 971 | 9 | 962 | 7 | 3 |

\*One Sewershed 6 sample (23 November 2020) was treated as missing due to lack of BCoV amplification indicating a compromised sample; one Sewershed 3 sample (9 May 2022) also demonstrated inhibition and was treated as missing.

## **Supplementary Table S2.** Weighted Bayesian information criteria (WBIC) used in joinpoint regression model selection by sewershed and study phase for (A) log10 BCoV-corrected SARS-CoV-2 RNA wastewater concentration, (B) log10 PMMoV-normalized SARS-CoV-2 RNA wastewater concentration (×1 million), and (C) weekly COVID-19 confirmed case totals per 100K sewershed population. The smallest WBIC value (bolded) indicates each final selected model for each sewershed in each phase.

|  |  | Model WBICs |
| --- | --- | --- |
| Study phase | Sewershed | 0joinpoints | 1joinpoints | 2joinpoints | 3joinpoints | 4joinpoints | 5joinpoints | 6joinpoints |
| (A) log10 BCoV-corrected SARS-CoV-2 RNA wastewater concentration |
| 1 | 1 | -4.90 | -5.62 | -5.64 | **-5.78** | -5.65 | -5.43 | - |
|  | 2 | -5.77 | **-6.02** | -5.84 | -5.49 | - | - | - |
| Weeks | 3 | **-6.22** | -6.07 | -6.00 | -5.65 | - | - | - |
| 1-31 | 4 | -4.54 | -4.45 | **-5.16** | -4.97 | -4.76 | -4.53 | - |
|  | 5 | -5.09 | **-5.34** | -5.04 | -4.65 | - | - | - |
|  | 6 | **-4.20** | -3.92 | -3.69 | -3.22 | - | - | - |
|  | 7 | - | - | - | - | - | - | - |
| 2 | 1 | -5.61 | -5.76 | -5.80 | -5.73 | -5.91 | **-6.18** | -6.16 |
|  | 2 | -5.35 | -5.53 | -5.84 | -5.80 | **-5.88** | -5.70 | -5.54 |
| Weeks | 3 | -4.91 | -4.99 | -5.24 | -5.13 | **-5.35** | -5.21 | -5.18 |
| 32-79 | 4 | -4.72 | -5.10 | -5.07 | -5.23 | **-5.36** | -5.20 | -5.07 |
|  | 5 | -4.58 | -4.52 | -4.50 | -4.47 | -4.41 | **-4.59** | -4.44 |
|  | 6 | -4.76 | **-4.95** | -4.83 | -4.88 | -4.80 | -4.73 | -4.66 |
|  | 7 | -4.18 | -4.53 | **-4.79** | -4.63 | -4.56 | -4.25 | -4.17 |
| 3 | 1 | -4.85 | -5.11 | -5.41 | **-5.63** | -5.49 | -5.42 | -5.27 |
|  | 2 | -5.53 | -5.54 | -5.62 | **-5.66** | -5.56 | -5.57 | -5.44 |
| Weeks | 3 | -4.42 | -4.51 | -4.60 | -5.02 | -4.87 | -4.96 | **-5.12** |
| 80-150 | 4 | -4.73 | -5.06 | -5.16 | -5.48 | -5.39 | **-5.60** | -5.49 |
|  | 5 | -4.35 | -4.36 | -4.69 | **-4.99** | -4.88 | -4.81 | -4.73 |
|  | 6 | -4.08 | -4.09 | -4.19 | **-4.50** | -4.40 | -4.41 | -4.30 |
|  | 7 | -4.60 | -4.72 | **-4.91** | -4.88 | -4.80 | -4.69 | -4.67 |
| (B) log10 PMMoV-normalized SARS-CoV-2 RNA wastewater concentration (×1 million) |
| 1 | 1 | -3.33 | -3.83 | -3.94 | **-4.48** | -4.33 | -4.22 | - |
|  | 2 | -3.94 | -4.00 | **-4.20** | -3.79 | - | - | - |
| Weeks | 3 | -3.80 | -3.91 | **-4.09** | -3.83 | - | - | - |
| 1-31 | 4 | -2.92 | -2.96 | **-3.45** | -3.45 | -3.37 | -3.17 | - |
|  | 5 | -3.52 | **-3.91** | -3.57 | -3.36 | - | - | - |
|  | 6 | **-2.55** | -2.29 | -2.02 | -1.63 | - | - | - |
|  | 7 | - | - | **-** | - | - | - | - |
| 2 | 1 | -3.84 | -3.95 | -4.02 | -4.41 | -4.51 | -4.52 | **-4.61** |
|  | 2 | **-3.81** | -3.72 | -3.71 | -3.60 | -3.50 | -3.34 | -3.16 |
| Weeks | 3 | **-3.73** | -3.71 | -3.65 | -3.51 | -3.33 | -3.19 | -3.02 |
| 32-79 | 4 | -3.35 | -3.64 | -3.82 | **-3.85** | -3.74 | -3.72 | -3.67 |
|  | 5 | -3.15 | **-3.25** | -3.13 | -3.07 | -2.96 | -2.95 | -2.80 |
|  | 6 | -3.55 | **-3.88** | -3.76 | -3.65 | -3.46 | -3.33 | -3.19 |
|  | 7 | -2.79 | **-3.04** | -3.00 | -2.85 | -2.70 | -2.56 | -2.48 |
| 3 | 1 | -3.45 | -3.61 | -3.54 | -3.81 | -3.90 | **-3.90** | -3.80 |
|  | 2 | -2.99 | -3.02 | -3.01 | -3.01 | -3.12 | **-3.12** | -3.03 |
| Weeks | 3 | -2.62 | -2.63 | -2.59 | -2.80 | **-3.06** | -3.04 | -2.99 |
| 80-150 | 4 | -3.70 | -3.78 | -3.73 | -3.86 | **-4.09** | -4.03 | -4.05 |
|  | 5 | -2.93 | -2.94 | -3.06 | -3.39 | **-3.63** | -3.51 | -3.42 |
|  | 6 | -2.72 | -2.66 | -2.65 | -2.73 | -2.83 | **-2.90** | -2.78 |
|  | 7 | -2.43 | -2.39 | -2.36 | -2.44 | **-2.46** | -2.34 | -2.29 |
| (C) Weekly COVID-19 confirmed case totals per 100K sewershed population |
| 1 | 1 | -0.91 | -1.08 | -2.65 | **-3.52** | -3.38 | -3.21 | - |
|  | 2 | -2.53 | -2.70 | -3.27 | **-3.45** | - | - | - |
| Weeks | 3 | -2.23 | -3.29 | -3.39 | **-3.41** | - | - | - |
| 1-31 | 4 | -0.05 | -0.11 | -1.64 | -2.34 | -2.43 | **-2.50** | - |
|  | 5 | -1.64 | -2.86 | **-3.09** | -2.91 | - | - | - |
|  | 6 | -1.63 | -2.78 | **-3.45** | -3.11 | - | - | - |
|  | 7 | - | - | **-** | - | - | - | - |
| 2 | 1 | -0.42 | -1.01 | -1.68 | -2.59 | -2.92 | -3.72 | **-3.76** |
|  | 2 | -0.53 | -1.18 | -1.81 | -2.76 | -3.10 | -3.53 | **-3.64** |
| Weeks | 3 | -0.55 | -1.17 | -1.93 | -2.52 | -3.28 | **-3.51** | -3.46 |
| 32-79 | 4 | -0.10 | -0.62 | -1.16 | -1.84 | -1.96 | -2.31 | **-2.38** |
|  | 5 | -0.65 | -1.21 | -1.68 | -2.43 | -2.75 | **-3.34** | -3.33 |
|  | 6 | -0.42 | -1.09 | -1.45 | -1.97 | -2.16 | -2.38 | **-2.63** |
|  | 7 | 0.67 | 0.29 | -0.18 | -0.16 | **-0.30** | -0.18 | -0.06 |
| 3 | 1 | -0.74 | -0.71 | -0.97 | -1.63 | -1.77 | **-2.48** | -2.43 |
|  | 2 | -0.76 | -0.75 | -1.00 | -1.67 | -1.80 | **-2.57** | -2.53 |
| Weeks | 3 | -0.61 | -0.61 | -0.85 | -1.55 | -1.58 | **-2.10** | -2.05 |
| 80-150 | 4 | -0.97 | -0.90 | -1.14 | -1.66 | -1.76 | **-1.97** | -1.93 |
|  | 5 | -0.69 | -0.66 | -0.92 | -1.47 | -1.60 | **-2.10** | -2.02 |
|  | 6 | -0.50 | -0.49 | -0.72 | -1.22 | -1.26 | **-2.08** | -1.99 |
|  | 7 | 0.01 | 0.04 | -0.31 | -0.74 | -0.70 | **-1.13** | -1.01 |

## **Supplementary Table S3.** Average weekly per cent change (AWPC) values from joinpoint regressions by sewershed and study phase for (A) log10 BCoV-corrected SARS-CoV-2 RNA wastewater concentration, (B) log10 PMMoV-normalized SARS-CoV-2 RNA wastewater concentration (×1 million), and (C) weekly COVID-19 confirmed case totals per 100K sewershed population. Values that are statistically significantly different from zero are shown in bold.

|  |  |  |  |
| --- | --- | --- | --- |
|  | (A) log10 BCoV-corrected wastewater concentration | (B) log10 PMMoV-normalized wastewater concentration(×1 million) | (C) Weekly COVID-19 confirmed case totals per 100K sewershed population |
| Sewershed | AWPC(95% CI) | *p*-value | AWPC(95% CI) | *p*-value | AWPC(95% CI) | *p*-value |
| Phase 1 (Weeks 1-31) |
| 1 | 0.21 (-0.45, 0.87) | 0.5368 | 0.32 (-0.78, 1.43) | 0.5754 | 8.29 (6.15, 10.5) | <0.0001 |
| 2 | 0.97 (0.45, 1.50) | 0.0002 | 1.55 (-2.12, 5.35) | 0.4138 | 11.2 (5.43, 17.2) | 0.0001 |
| 3 | 0.96 (0.72, 1.19) | <0.0001 | -0.02 (-2.40, 2.42) | 0.9852 | 12.6 (4.74, 21.0) | 0.0013 |
| 4 | 1.22 (0.15, 2.31) | 0.0255 | 2.43 (0.10, 4.82) | 0.0407 | 13.4 (6.57, 20.6) | 0.0001 |
| 5 | 1.46 (0.73, 2.20) | 0.0001 | 2.69 (0.94, 4.47) | 0.0025 | 14.6 (8.81, 20.6) | <0.0001 |
| 6 | 1.30 (0.58, 2.03) | 0.0015 | 2.85 (1.26, 4.46) | 0.0015 | 15.6 (12.5, 18.8) | <0.0001 |
| 7 | - | - | - | - | - | - |
| Phase 2 (Weeks 32-79) |
| 1 | 0.02 (-0.53, 0.57) | 0.9500 | 0.16 (-1.03, 1.37) | 0.7917 | -3.17 (-4.90, -1.41) | 0.0005 |
| 2 | -0.18 (-0.72, 0.36) | 0.5198 | 0.37 (0.13, 0.62) | 0.0039 | -3.18 (-4.85, -1.48) | 0.0003 |
| 3 | -0.22 (-1.13, 0.70) | 0.6434 | 0.24 (-0.05, 0.54) | 0.1057 | -2.95 (-4.25, -1.62) | <0.0001 |
| 4 | -0.11 (-0.53, 0.31) | 0.6037 | 0.28 (-0.55, 1.12) | 0.5090 | -2.63 (-6.18, 1.06) | 0.1603 |
| 5 | 0.02 (-1.20, 1.24) | 0.9805 | -0.05 (-0.85, 0.76) | 0.9019 | -2.56 (-4.00, -1.10) | 0.0007 |
| 6 | -0.14 (-0.44, 0.17) | 0.3780 | -0.16 (-0.75, 0.44) | 0.6036 | -2.13 (-5.17, 1.01) | 0.1822 |
| 7 | -0.16 (-1.50, 1.20) | 0.8199 | 0.02 (-0.70, 0.75) | 0.9565 | -2.66 (-15.4, 12.0) | 0.7071 |
| Phase 3 (Weeks 80-150) |
| 1 | -0.02 (-0.65, 0.62) | 0.9474 | -0.30 (-1.23, 0.64) | 0.5343 | -2.97 (-4.66, -1.25) | 0.0008 |
| 2 | 0.06 (-0.31, 0.44) | 0.7432 | -0.52 (-2.39, 1.39) | 0.5937 | -2.96 (-4.55, -1.35) | 0.0003 |
| 3 | 0.08 (-0.41, 0.56) | 0.7522 | -0.33 (-1.50, 0.86) | 0.5835 | -2.93 (-5.08, -0.72) | 0.0095 |
| 4 | -0.06 (-0.34, 0.21) | 0.6534 | -0.22 (-1.04, 0.61) | 0.6019 | -2.38 (-4.82, 0.12) | 0.0614 |
| 5 | 0.07 (-0.34, 0.48) | 0.7503 | 0.02 (-0.81, 0.86) | 0.9541 | -2.73 (-4.86, -0.55) | 0.0143 |
| 6 | 0.15 (-0.28, 0.58) | 0.4989 | -0.12 (-1.27, 1.04) | 0.8344 | -3.42 (-5.47, -1.33) | 0.0014 |
| 7 | -0.30 (-1.19, 0.61) | 0.5209 | -1.08 (-4.08, 2.00) | 0.4865 | -2.83 (-5.84, 0.28) | 0.0739 |

## **Supplementary Table S4.** Joinpoint regression segments and weekly per cent change (WPC) values by sewershed and study phase for (A) log10 BCoV-corrected SARS-CoV-2 RNA wastewater concentration, (B) log10 PMMoV-normalized SARS-CoV-2 RNA wastewater concentration (×1 million), and (C) weekly COVID-19 confirmed case totals per 100K sewershed population. Bolded values are statistically significant. (\* = Sewershed).

|  |
| --- |
| Phase 1 (Weeks 1-31) |
|  | (A) log10 BCoV-corrected wastewater concentration | (B) log10 PMMoV-normalized wastewater concentration(x 1 million) | (C) Weekly COVID-19 confirmed case totals per 100K sewershed population |
| \* | Weeks | WPC(95% CI) | *p*-value | Weeks | WPC(95% CI) | *p*-value | Weeks | WPC(95% CI) | *p*-value |
| 1 | 1-7 | 0.95 (-0.75, 2.68) | 0.2585 | 1-12 | 0.14 (-0.86, 1.15) | 0.7770 | 1-6 | 55.0 (44.9, 65.8) | <0.0001 |
| 7-18 | -1.86 (-2.61, -1.11) | 0.0001 | 12-18 | -5.25 (-8.56, -1.83) | 0.0048 | 6-15 | -18.3 (-20.9, -15.5) | <0.0001 |
| 18-24 | 3.38 (1.06, 5.74) | 0.0062 | 18-24 | 8.36 (4.59, 12.3) | 0.0001 | 15-27 | 20.0 (17.6, 22.5) | <0.0001 |
| 24-31 | 0.19 (-1.14, 1.53) | 0.7701 | 24-31 | -1.12 (-3.14, 0.94) | 0.2677 | 27-31 | -4.25 (-12.9, 5.27) | 0.3504 |
| 2 | Start of monitoring: Week 13 |  |  |  |  |  |  |
| 13-25 | 1.59 (1.12, 2.06) | <0.0001 | 13-20 | 0.23 (-2.54, 3.09) | 0.8587 | 13-15 | -8.12 (-36.3, 32.3) | 0.6080 |
| 25-31 | -0.24 (-1.65, 1.19) | 0.7228 | 20-23 | 14.2 (-9.38, 43.9) | 0.2328 | 15-21 | 14.9 (7.02, 23.3) | 0.0020 |
|  |  |  | 23-31 | -1.71 (-3.93, 0.56) | 0.1243 | 21-25 | 32.9 (12.4, 57.1) | 0.0044 |
|  |  |  |  |  |  | 25-31 | 1.81 (-3.44, 7.34) | 0.4575 |
| 3 | Start of monitoring: Week 14 |  |  |  |  |  |  |
| 14-31 | 0.96 (0.72, 1.19) | <0.0001 | 14-19 | -5.93 (-9.65, -2.06) | 0.0070 | 14-21 | 14.2 (10.9, 17.7) | <0.0001 |
|  |  |  | 19-23 | 8.01 (-2.07, 19.1) | 0.1103 | 21-24 | 28.4 (-8.41, 79.9) | 0.1238 |
|  |  |  | 23-31 | -0.08 (-1.93, 1.81) | 0.9258 | 24-27 | 14.6 (-18.2, 60.6) | 0.3715 |
|  |  |  |  |  |  | 27-31 | -1.88 (-9.27, 6.12) | 0.5853 |
| 4 | 1-6 | 7.69 (4.45, 11.03) | <0.0001 | 1-6 | 16.4 (9.07, 24.1) | 0.0001 | 1-4 | 141.8 (100.7, 191.2) | <0.0001 |
| 6-10 | -5.69 (-12.36, 1.49) | 0.1123 | 6-10 | -9.33 (-22.7, 6.38) | 0.2175 | 4-7 | 7.33 (-30.4, 65.6) | 0.7315 |
| 10-31 | 1.09 (0.77, 1.42) | <0.0001 | 10-31 | 1.71 (1.05, 2.37) | <0.0001 | 7-14 | -18.1 (-22.4, -13.6) | <0.0001 |
|  |  |  |  |  |  | 14-20 | 12.8 (4.88, 21.3) | 0.0032 |
|  |  |  |  |  |  | 20-23 | 50.2 (-2.63, 131.7) | 0.0638 |
|  |  |  |  |  |  | 23-31 | 4.49 (0.98, 8.11) | 0.0153 |
| 5 | Start of monitoring: Week 14 |  |  |  |  |  |  |
| 14-24 | 2.53 (1.66, 3.41) | <0.0001 | 14-26 | 4.74 (3.37, 6.14) | <0.0001 | 14-16 | 0.28 (-36.2, 57.7) | 0.9892 |
| 24-31 | -0.05 (1.55, 1.47) | 0.9414 | 26-31 | -2.08 (-7.43, 3.57) | 0.4327 | 16-25 | 28.0 (24.1, 32.0) | <0.0001 |
|  |  |  |  |  |  | 25-31 | 1.43 (-3.55, 6.68) | 0.5437 |
| 6 | Start of monitoring: Week 14 |  |  |  |  |  |  |
| 14-31 | 1.30 (0.58, 2.03) | 0.0015 | 14-31 | 2.85 (1.26, 4.46) | 0.0015 | 14-20 | 17.2 (12.6, 22.0) | <0.0001 |
|  |  |  |  |  |  | 20-25 | 30.5 (20.5, 41.2) | <0.0001 |
|  |  |  |  |  |  | 25-31 | 3.15 (-0.92, 7.39) | 0.1172 |
| 7 |  | Not applicable |  |  | Not applicable |  |  | Not applicable |  |

|  |
| --- |
| Phase 2 (Weeks 32-79) |
|  | (A) log10 BCoV-corrected wastewater concentration | (B) log10 PMMoV-normalized wastewater concentration(x 1 million) | (C) Weekly COVID-19 confirmed case totals per 100K sewershed population |
| \* | Weeks | WPC(95% CI) | *p*-value | Weeks | WPC(95% CI) | *p*-value | Weeks | WPC(95% CI) | *p*-value |
| 1 | 32-41 | -1.60 (-2.20, -1.01) | <0.0001 | 32-41 | -3.26 (-4.36, -2.16) | <0.0001 | 32-40 | -25.9 (-27.4, -24.4) | <0.0001 |
| 41-48 | 1.65 (0.51, 2.81) | 0.0061 | 41-46 | 2.58 (-1.59, 6.94) | 0.2187 | 40-47 | 4.93 (1.59, 8.38) | 0.0050 |
| 48-51 | -4.81 (-11.8, 2.68) | 0.1940 | 46-51 | -3.84 (-7.75, 0.24) | 0.0638 | 47-53 | -15.5 (-19.1, -11.8) | <0.0001 |
| 51-63 | 1.67 (1.22, 2.12) | <0.0001 | 51-57 | 5.57 (2.58, 8.65) | 0.0006 | 53-56 | 57.7 (27.1, 95.7) | 0.0002 |
| 63-72 | -1.14 (-1.85, -0.43) | 0.0028 | 57-67 | 0.61 (-0.52, 1.76) | 0.2809 | 56-60 | 27.9 (15.6, 41.5) | <0.0001 |
| 72-79 | 1.32 (0.42, 2.23) | 0.0052 | 67-70 | -10.6 (-22.9, 3.62) | 0.1308 | 60-72 | -8.14 (-9.29, -6.98) | <0.0001 |
|  |  |  | 70-79 | 4.47 (3.29, 5.67) | <0.0001 | 72-79 | 3.30 (0.71, 5.96) | 0.0141 |
| 2 | 32-41 | -1.38 (-2.17, -0.58) | 0.0013 | 32-79 | 0.37 (0.13, 0.62) | 0.0039 | 32-38 | -27.6 (-30.4, -24.8) | <0.0001 |
| 41-49 | 0.67 (-0.52, 1.88) | 0.2595 |  |  |  | 38-42 | -15.3 (-24.6, -4.83) | 0.0068 |
| 49-53 | -4.27 (-8.82, 0.51) | 0.0776 |  |  |  | 42-46 | 13.6 (1.18, 27.6) | 0.0322 |
| 53-59 | 3.33 (1.23, 5.46) | 0.0026 |  |  |  | 46-53 | -13.1 (-16.4, -9.68) | <0.0001 |
| 59-79 | -0.17 (-0.41, 0.07) | 0.1510 |  |  |  | 53-59 | 47.0 (39.7, 54.8) | <0.0001 |
|  |  |  |  |  |  | 59-72 | -6.76 (-8.01, -5.49) | <0.0001 |
|  |  |  |  |  |  | 72-79 | 2.19 (-0.89, 5.36) | 0.1583 |
| 3 | 32-38 | -2.58 (-4.26, -0.87) | 0.0044 | 32-79 | 0.24 (-0.05, 0.54) | 0.1057 | 32-41 | -24.6 (-26.2, -22.9) | <0.0001 |
| 38-53 | -0.35 (-0.80, 0.09) | 0.1136 |  |  |  | 41-47 | 10.0 (4.29, 16.0) | 0.0010 |
| 53-62 | 1.89 (0.80, 2.98) | 0.0012 |  |  |  | 47-52 | -22.4 (-28.0, -16.3) | <0.0001 |
| 62-71 | -1.40 (-2.45, -0.34) | 0.0113 |  |  |  | 52-60 | 38.2 (33.9, 42.7) | <0.0001 |
| 71-79 | 1.47 (0.38, 2.58) | 0.0098 |  |  |  | 60-72 | -7.37 (-8.86, -5.85) | <0.0001 |
|  |  |  |  |  |  | 72-79 | 2.22 (-1.00, 5.55) | 0.1719 |
| 4 | 32-43 | -1.67 (-2.34, -1.00) | <0.0001 | 32-49 | -2.26 (-3.09, -1.41) | <0.0001 | 32-41 | -23.7 (-25.9, -21.5) | <0.0001 |
| 43-46 | 5.03 (-8.21, 20.2) | 0.4641 | 49-61 | 3.86 (2.13, 5.61) | 0.0001 | 41-45 | 16.3 (-3.69, 40.3) | 0.1124 |
| 46-54 | -2.27 (-3.61, -0.91) | 0.0018 | 61-71 | -2.01 (-4.23, 0.26) | 0.0810 | 45-53 | -9.88 (-13.7, -5.90) | <0.0001 |
| 54-61 | 2.95 (1.15, 4.79) | 0.0020 | 71-79 | 3.42 (0.59, 6.33) | 0.0189 | 53-56 | 59.7 (1.58, 151.1) | 0.0430 |
| 61-79 | -0.46 (-0.78, -0.15) | 0.0053 |  |  |  | 56-60 | 17.4 (-2.77, 41.7) | 0.0923 |
|  |  |  |  |  |  | 60-73 | -7.75 (-9.43, -6.04) | <0.0001 |
|  |  |  |  |  |  | 73-79 | 7.26 (1.37, 13.5) | 0.0168 |
| 5 | 32-43 | -1.44 (-2.31, -0.56) | 0.0022 | 32-41 | -3.63 (-7.34, 0.23) | 0.0646 | 32-41 | -24.3 (-26.0, -22.5) | <0.0001 |
| 43-49 | 3.26 (0.10, 6.51) | 0.0436 | 41-79 | 0.82 (0.38, 1.25) | 0.0005 | 41-47 | 11.8 (5.45, 18.5) | 0.0005 |
| 49-52 | -8.21 (-22.7, 9.02) | 0.3176 |  |  |  | 47-52 | -16.0 (-22.7, -8.74) | 0.0002 |
| 52-63 | 2.46 (1.41, 3.51) | <0.0001 |  |  |  | 52-59 | 37.8 (31.9, 44.0) | <0.0001 |
| 63-72 | -1.95 (-3.37, -0.52) | 0.0095 |  |  |  | 59-72 | -7.56 (-8.97, -6.14) | <0.0001 |
| 72-79 | 2.05 (0.22, 3.91) | 0.0292 |  |  |  | 72-79 | 3.79 (0.25, 7.46) | 0.0364 |
| 6 | 32-40 | -1.89 (-3.53, -0.23) | 0.0266 | 32-40 | -4.56 (-7.66, -1.35) | 0.0067 | 32-43 | -22.8 (-24.6, -21.0) | <0.0001 |
| 40-79 | 0.23 (0.09, 0.37) | 0.0024 | 40-79 | 0.77 (0.48, 1.06) | <0.0001 | 43-47 | 26.4 (6.04, 50.7) | 0.0108 |
|  |  |  |  |  |  | 47-53 | -17.0 (-23.3, -10.3) | <0.0001 |
|  |  |  |  |  |  | 53-56 | 79.9 (26.3, 156.2) | 0.0021 |
|  |  |  |  |  |  | 56-61 | 15.4 (3.32, 28.9) | 0.0130 |
|  |  |  |  |  |  | 61-72 | -8.34 (-10.8, -5.83) | <0.0001 |
|  |  |  |  |  |  | 72-79 | 7.44 (2.55, 12.6) | 0.0038 |
| 7 | 32-55 | -1.17 (-1.50, -0.84) | <0.0001 | 32-49 | -2.37 (-3.97, -0.74) | 0.0055 | 32-43 | -22.8 (-28.9, -16.1) | <0.0001 |
| 55-58 | 6.72 (-14.0, 32.4) | 0.5457 | 49-79 | 1.40 (0.69, 2.11) | 0.0002 | 43-49 | 12.6 (-15.6, 50.2) | 0.4095 |
| 58-79 | 0.01 (-0.37, 0.39) | 0.9557 |  |  |  | 49-52 | -67.7 (-93.1, 50.7) | 0.1452 |
|  |  |  |  |  |  | 52-55 | 303.7 (-13.4, 1780.7) | 0.0741 |
|  |  |  |  |  |  | 55-79 | 0.27 (-2.21, 2.82) | 0.8263 |
| Phase 3 (Weeks 80-150) |
|  | (A) log10 BCoV-corrected wastewater concentration | (B) log10 PMMoV-normalized wastewater concentration(x 1 million) | (C) Weekly COVID-19 confirmed case totals per 100K sewershed population |
| \* | Weeks | WPC(95% CI) | *p*-value | Weeks | WPC(95% CI) | *p*-value | Weeks | WPC(95% CI) | *p*-value |
| 1 | 80-84 | 3.86 (0.04, 7.83) | 0.0476 | 80-84 | 8.00 (-1.02, 17.9) | 0.0826 | 80-84 | 107.6 (76.5, 144.2) | <0.0001 |
| 84-98 | -2.49 (-3.04, -1.95) | <0.0001 | 84-98 | -4.73 (-6.03, -3.41) | <0.0001 | 84-93 | -41.3 (-44.8, -37.4) | <0.0001 |
| 98-101 | 6.65 (-7.28, 22.7) | 0.3612 | 98-103 | 8.96 (-0.13, 18.9) | 0.0533 | 93-106 | 26.8 (22.3, 31.5) | <0.0001 |
| 101-150 | -0.01 (-0.09, 0.06) | 0.7353 | 103-124 | -1.82 (-2.52, -1.11) | <0.0001 | 106-120 | -16.8 (-19.4, -14.0) | <0.0001 |
|  |  |  | 124-142 | 1.63 (0.70, 2.56) | 0.0009 | 120-130 | 11.4 (5.54, 17.5) | 0.0002 |
|  |  |  | 142-150 | -2.13 (-4.94, 0.76) | 0.1437 | 130-150 | -8.79 (-10.4, -7.15) | <0.0001 |
| 2 | 80-85 | 3.59 (0.47, 6.81) | 0.0247 | 80-86 | 5.01 (-0.99, 11.4) | 0.1015 | 80-84 | 109.0 (79.5, 143.5) | <0.0001 |
| 85-91 | -4.63 (-7.50, -1.67) | 0.0029 | 86-95 | -6.88 (-10.3, -3.35) | 0.0003 | 84-93 | -41.7 (-45.1, -38.2) | <0.0001 |
| 91-103 | 1.29 (0.32, 2.27) | 0.0096 | 95-105 | 4.26 (1.08, 7.55) | 0.0093 | 93-106 | 26.2 (22.0, 30.6) | <0.0001 |
| 103-150 | -0.00 (-0.12, 0.11) | 0.9425 | 105-129 | -2.75 (-3.46, -2.03) | <0.0001 | 106-120 | -16.2 (-18.8, -13.6) | <0.0001 |
|  |  |  | 129-132 | 14.6 (-23.3, 71.2) | 0.4992 | 120-131 | 10.1 (5.33, 15.0) | 0.0001 |
|  |  |  | 132-150 | -0.94 (-2.00, 0.12) | 0.0816 | 131-150 | -9.04 (-10.7, -7.36) | <0.0001 |
| 3 | 80-85 | 4.75 (2.05, 7.51) | 0.0008 | 80-86 | 7.96 (0.49, 16.0) | 0.0368 | 80-84 | 121.9 (81.3, 171.6) | <0.0001 |
| 85-94 | -4.59 (-5.74, -3.43) | <0.0001 | 86-93 | -12.0 (-18.1, -5.52) | 0.0007 | 84-93 | -43.0 (-47.3, -38.4) | <0.0001 |
| 94-103 | 3.13 (1.89, 4.39) | <0.0001 | 93-108 | 4.40 (2.40, 6.43) | <0.0001 | 93-107 | 23.6 (18.7, 28.6) | <0.0001 |
| 103-119 | -0.67 (-1.12, -0.22) | 0.0046 | 108-117 | -7.05 (-11.2, -2.72) | 0.0022 | 107-122 | -15.7 (-18.6, -12.6) | <0.0001 |
| 119-135 | 0.76 (0.30, 1.22) | 0.0015 | 117-150 | 0.67 (0.14, 1.20) | 0.0138 | 122-129 | 20.2 (7.17, 34.9) | 0.0022 |
| 135-139 | -4.31 (-10.1, 1.88) | 0.1644 |  |  |  | 129-150 | -8.66 (-10.5, -6.77) | <0.0001 |
| 139-150 | 1.19 (0.45, 1.94) | 0.0022 |  |  |  |  |  |  |
| 4 | 80-84 | 4.41 (1.30, 7.61) | 0.0059 | 80-86 | 5.45 (0.96, 10.13) | 0.0177 | 80-84 | 95.6 (55.5, 146.2) | <0.0001 |
| 84-92 | -4.16 (-5.26, -3.05) | <0.0001 | 86-91 | -10.7 (-17.1, -2.99) | 0.0081 | 84-93 | -36.7 (-42.2, -30.8) | <0.0001 |
| 92-102 | 1.80 (1.02, 2.59) | <0.0001 | 91-109 | 1.74 (0.84, 2.65) | 0.0003 | 93-106 | 23.4 (17.2, 30.0) | <0.0001 |
| 102-121 | -0.34 (-0.60, -0.07) | 0.0128 | 109-117 | -4.61 (-7.82, -1.29) | 0.0078 | 106-121 | -14.9 (-18.4, -11.2) | <0.0001 |
| 121-131 | 0.94 (0.17, 1.72) | 0.0183 | 117-150 | 0.48 (0.15, 0.81) | 0.0047 | 121-130 | 10.7 (1.17, 21.1) | 0.0276 |
| 131-150 | -0.45 (-0.69, -0.20) | 0.0005 |  |  |  | 130-150 | -7.16 (-9.53, -4.72) | <0.0001 |
| 5 | 80-86 | 3.39 (1.00, 5.84) | 0.0059 | 80-87 | 5.55 (2.53, 8.66) | 0.0005 | 80-84 | 97.5 (60.0, 143.7) | <0.0001 |
| 86-91 | -7.43 (-11.6, -3.09) | 0.0013 | 87-91 | -17.1 (-26.9, -5.97) | 0.0042 | 84-93 | -39.4 (-44.0, -34.4) | <0.0001 |
| 91-102 | 2.41 (1.37, 3.45) | <0.0001 | 91-104 | 4.55 (3.28, 5.83) | <0.0001 | 93-106 | 26.2 (20.7, 32.0) | <0.0001 |
| 102-150 | -0.06 (-0.15, 0.03) | 0.1966 | 104-116 | -2.37 (-3.72, -1.00) | 0.0010 | 106-120 | -16.7 (-20.0, -13.3) | <0.0001 |
|  |  |  | 116-150 | 0.29 (0.04, 0.54) | 0.0217 | 120-130 | 11.0 (3.75, 18.7) | 0.0031 |
|  |  |  |  |  |  | 130-150 | -8.00 (-9.98, -5.98) | <0.0001 |
| 6 | 80-84 | 8.20 (2.49, 14.23) | 0.0051 | 80-83 | 18.4 (-3.36, 45.1) | 0.1010 | 80-85 | 65.2 (41.7, 92.7) | <0.0001 |
| 84-91 | -5.05 (-7.47, -2.57) | 0.0002 | 83-96 | -4.59 (-6.32, -2.84) | <0.0001 | 85-93 | -43.3 (-48.4, -37.7) | <0.0001 |
| 91-104 | 1.43 (0.57, 2.29) | 0.0014 | 96-104 | 5.80 (1.33, 10.5) | 0.0114 | 93-106 | 25.2 (20.0, 30.7) | <0.0001 |
| 104-150 | -0.07 (-0.18, 0.04) | 0.1910 | 104-123 | -2.17 (-3.12, -1.21) | <0.0001 | 106-120 | -16.6 (-19.7, -13.4) | <0.0001 |
|  |  |  | 123-135 | 2.09 (-0.03, 4.24) | 0.0531 | 120-130 | 13.3 (6.11, 20.9) | 0.0003 |
|  |  |  | 135-150 | -1.75 (-3.02, -0.46) | 0.0088 | 130-150 | -9.71 (-11.5, -7.86) | <0.0001 |
| 7 | 80-100 | -1.78 (-2.33, -1.24) | <0.0001 | 80-101 | -3.30 (-4.65, -1.93) | <0.0001 | 80-85 | 66.2 (31.7, 109.7) | 0.0001 |
| 100-103 | 7.78 (-12.7, 33.1) | 0.4804 | 101-104 | 21.9 (-35.7, 131.2) | 0.5380 | 85-92 | -47.8 (-56.1, -37.8) | <0.0001 |
| 103-150 | -0.15 (-0.31, -0.00) | 0.0496 | 104-128 | -3.56 (-4.73, -2.37) | <0.0001 | 92-105 | 28.9 (21.4, 36.9) | <0.0001 |
|  |  |  | 128-133 | 11.6 (-7.30, 34.35) | 0.2413 | 105-119 | -14.5 (-19.0, -9.85) | <0.0001 |
|  |  |  | 133-150 | -1.94 (-3.82, -0.01) | 0.0484 | 119-130 | 9.59 (1.23, 18.7) | 0.0245 |
|  |  |  |  |  |  |  | 130-150 | -10.0 (-12.5, -7.52) | <0.0001 |