

Retrospective epidemiological analysis of SARS-CoV-2 wastewater surveillance and case notifications data – New South Wales, Australia, 2020

H. S. Camphor ^{a,*}, S. Nielsen ^b, Z. Bradford-Hartke ^a, K. Wall ^a and R. Broome ^a

^a Health Protection New South Wales, NSW Ministry of Health, St Leonards, NSW 2065, Australia

^b NSW Biostatistics Training Program, Centre for Epidemiology and Evidence, NSW Ministry of Health, St Leonards, NSW 2065, Australia

*Corresponding author. E-mail: hendrik.camphor@health.nsw.gov.au

 HSC, 0000-0002-3416-1975; SN, 0000-0001-6855-4227; KW, 0000-0003-3036-1459

ABSTRACT

This epidemiological study analysed SARS-CoV-2 wastewater surveillance and case notifications data to inform evidence-based public health action in NSW. We investigated measures of association between SARS-CoV-2 RNA fragments detected in wastewater samples ($n = 100$) and case notifications ($n = 1,367$, as rates per 100,000 population) within wastewater catchment areas ($n = 6$); and evaluated the performance of wastewater testing as a population-level diagnostic tool. Furthermore, we modelled SARS-CoV-2 RNA fragment detection in wastewater given the case notification rate using logistic regression. The odds of a viral detection in wastewater samples increased by a factor of 5.68 (95% CI: 1.51–32.1, $P = 0.004$) with rates of one or more notified cases within a catchment. The diagnostic specificity of wastewater viral detection results was 0.88 (95% CI: 0.69–0.97); the overall diagnostic sensitivity was 0.44 (95% CI: 0.33–0.56). The probability of a viral detection result in wastewater exceeded 50% (95% CI: 36–64%) once the case rate within a catchment exceeded 10.5. Observed results suggest that in a low prevalence setting, wastewater viral detections are a more reliable indicator of the presence of recent virus shedding cases in a catchment, than non-detect results are of the absence of cases in a catchment.

Key words: case notifications, COVID-19, public health response, SARS-CoV-2 surveillance, viral detection, wastewater epidemiology

HIGHLIGHTS

- Research was undertaken to investigate SARS-CoV-2 wastewater surveillance findings in relation to observed COVID-19 case notifications data in NSW, Australia.
- In a low prevalence setting such as in NSW, wastewater viral detections, particularly in catchments with no recent case notifications should trigger prompt public health action to rule out low-level virus circulation or undetected community transmission.

INTRODUCTION

In New South Wales (NSW), Australia, COVID-19 containment efforts achieved significant early successes in suppressing the epidemic to very low levels of community transmission (McAnulty & Ward 2020). Wastewater collection for SARS-CoV-2 was initiated within 3 months of the first case detection in NSW (January 2020) and has since been utilised as a supplementary surveillance tool to support the state's COVID-19 response efforts (NSW Health Environmental Health Branch 2020). Formal wastewater testing under the NSW Sewage Surveillance Program for SARS-CoV-2 commenced in July 2020 (NSW Health Environmental Health Branch 2020).

In Australia, wastewater-based epidemiological approaches have long been utilised to supplement enterovirus surveillance (Australian Government Department of Health 2020), and its potential has also been recently demonstrated for SARS-CoV-2 (Ahmed *et al.* 2020a). Despite the rapid development of molecular diagnostic assays for wastewater testing, there is still a paucity of evidence to support interpretation and risk-based decision making arising from wastewater-based surveillance for SARS-CoV-2 in metropolitan wastewater catchments in Australia.

This study analysed wastewater surveillance and notifications data collected in the months preceding active commencement of the NSW Sewage Surveillance Program for SARS-CoV-2 and aimed to support the interpretation of initial SARS-CoV-2 wastewater surveillance findings, in relation to observed COVID-19 case notifications data. The study objectives

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were threefold. Firstly, to investigate measures of association between two comparison groups, namely catchments with, or without viral RNA fragment detections in wastewater samples, relative to the presence of notified SARS-CoV-2 shedding cases per 100,000 population allocated to the catchment. Secondly, to investigate the diagnostic performance of wastewater testing, where viral detection results were considered as a population-level diagnostic tool for the presence of notified SARS-CoV-2 shedding cases allocated to a catchment. Finally, to model the predicted probabilities of SARS-CoV-2 RNA fragment detection in wastewater samples by notified cases per 100,000 population in a catchment.

METHODS

Wastewater sampling period and testing

Sydney's major water utility (Sydney Water) collected one hundred 24-hour composite samples from seven wastewater treatment plant inlet points servicing six catchment areas in greater metropolitan Sydney over a 14-week sampling period, between late-March and July 2020. To support internal validation of wastewater testing protocols, samples were collected across a range of locations and catchment populations on dates where, based on COVID-19 case notifications data received, testing was anticipated to return both negative and positive results (i.e. including areas from zero to large case notification numbers). The largest wastewater catchment by population size (Malabar) included samples collected from two inlet points. These inlet points have a common upstream catchment, but also distinct catchment areas near the inlet. The inlets were considered separately as one inlet includes an industrial and port zone, with potential inputs from foreign vessels and interference from trade waste. Samples were stored frozen until tested for SARS-CoV-2 RNA fragments. Samples were filtered using electronegative membranes and SARS-CoV-2 RNA, or ribonucleic acid extracted using the RNeasy Power Water kit (Qiagen) as per manufacturer's instructions (Griffiths *et al.* 2021). Samples were analysed using an RT-qPCR assay targeting the N1 and N2 regions of the viral genome (US Centers for Disease Control and Prevention 2020; Griffiths *et al.* 2021; Payyappat 2021). For this study, results were expressed as a binary viral RNA target gene fragment detection (detection or non-detection).

Case notification reference period

Peak virus shedding into wastewater was assumed to occur during a 9-day period, starting 2 days prior and lasting until the 7th day from the calculated onset date (Cevik *et al.* 2021). The study, therefore, aimed to account for all notified (i.e. known) cases located in sampled catchments, with an onset corresponding with the wastewater sampling date (day 1), or ranging from 6 days prior (day 2–7 since onset), to 2 days after the wastewater sampling date (day –2, –1 before onset). Notifications data for laboratory-confirmed SARS-CoV-2 cases were extracted from the NSW Ministry of Health's Notifiable Conditions Information Management System (NCIMS) in November 2020. All notified cases with an onset ranging from 6 days prior to the first wastewater sample date, to 2 days beyond the last sample date were included, to correspond with the wastewater sampling period. These data were collated with additional data relevant to cases managed in the NSW hotel quarantine program (NSW Government 2020a), including accommodation location and dates of arrival, onset, testing and notification.

Allocating case location to wastewater catchments

Case locations were determined through a manual review of NCIMS case records. Unless found to have spent time in hotel quarantine and/or a healthcare facility, cases were assumed to have been residing at their primary residential address for the entirety of their 9-day viral shedding period. Quarantine hotel and healthcare facilities were manually assigned point coordinates using facility address and Google Maps (Google Maps 2020). Residential locations were geocoded using the Geocoded National Address File (GNAF) database and the NSW Health GOPHER server to obtain point coordinates. Cases were allocated to one or more catchments (one catchment, per day) by determining whether their point coordinates and 9-day period fell within sampled catchment boundaries and sampling dates, respectively. Geographic dispersal of case locations from one another within catchments by sampling date were analysed by calculating pairwise Euclidean distances between point coordinates and then case location distance to the group median centroid.

Data integration and analysis

Data cleaning were performed in R software (version 4.0.3) (R Core Team 2020), using methods from the packages 'base', 'stats' (R Core Team 2020), 'tidyverse' (Wickham *et al.* 2019), 'sf' (Pebesma 2018) and 'epitools' (Aragon 2020). Categorical data were compared using Fisher's exact test, with two-tailed *P*-values < 0.05 considered statistically significant. For

univariable analysis, a case–control study approach was used to investigate associations between wastewater detection results (binary outcome), and the presence of notified cases allocated to catchments during their 9-day viral shedding period (categorical exposure). Contingency tables calculated odds ratios (OR) and 95% confidence intervals (CIs). Wastewater detection results were also considered as a population-level diagnostic tool for the presence of notified cases allocated to a catchment during their 9-day viral shedding period, to calculate diagnostic test performance statistics. Logistic regression was employed to model the log odds that either of two viral RNA gene fragments (N1 or N2) were detected in a wastewater sample, given case numbers and catchment population size. The log odds were converted to probabilities and graphed to facilitate interpretation.

Ethics statement

This study was conducted as part of public health surveillance under the *NSW Public Health Act (2010)*, so ethical approval was not required.

RESULTS

Descriptive epidemiology

There were $n = 1,367$ notified cases with an onset falling within the case notification reference period (24 March–11 July 2020) allocated to catchments from where wastewater samples were collected, within which reside a cumulative estimated population of 3,793,038 (period prevalence: 0.04%) (Table 1). There were $n = 1,450$ unique case locations identified (residence, quarantine accommodation or healthcare facility). There were $n = 2,277$ cases counted on wastewater sampling dates, noting that during their 9-day viral shedding period, cases could be counted more than once across wastewater samples. Over 90% of case counts were allocated to three catchments: Malabar (39.79%), Bondi (36.5%) and North Head (14.8%) (Table 1).

Of the wastewater samples collected between 30 March and 9 July 2020, 36/100 (36%) returned a detection of either the N1 or N2 viral RNA fragment (the ‘case’ to ‘control’ ratio of 1:1.8) (Table 2). All sampled catchments returned at least two wastewater results with viral detection, with 27/36 (75%) detections occurring during the first month of sampling in April, and 33/36 (92%) during the first half of the sampling period (Figure 1). Of the 100 wastewater samples tested, 42% returned a non-detect result despite having one or more cases allocated to the catchment; while 3% returned viral detections with no notified cases allocated to the catchment (Table 2).

Of the 75% of wastewater samples with one or more cases allocated to the catchment at the time of sampling, the median ratio of allocated cases per 100,000 population in these catchments was 1.8 (range: 0.05–43.6). Of the 42% of samples that returned non-detect results but with one or more cases allocated to the catchment, the median ratio of cases per 100,000 population in these catchments was 0.5 (range: 0.05–29.5). Of note, seven consecutive non-detect samples collected from the Bondi catchment in the latter half of the sampling period (June–July) had a median ratio of 14.25 allocated cases per 100,000 population (range: 7.2–29.5). Of the 3/100 (3%) wastewater samples which returned viral detections with no notified cases allocated to the catchment, two were from Brooklyn catchment in April, and one from St Mary’s catchment in May (Figure 1).

Table 1 | Cumulative case counts on wastewater sampling days and estimated resident population of sampled wastewater catchments in greater metropolitan Sydney

Wastewater catchments	Cumulative case count on wastewater sampling days (%)		Estimated resident population (%)	
Brooklyn	0	0	1,241	(0.03)
Penrith	70	(3.10)	110,114	(2.90)
St Marys	133	(5.84)	163,147	(4.30)
Bondi	831	(36.50)	318,810	(8.41)
North Head	337	(14.80)	1,341,986	(35.38)
Malabar	906	(39.79)	1,857,740	(48.98)
Total	2,277	(100.0)	3,793,038	(100.0)

Table 2 | Contingency tables comparing viral detection results in wastewater with COVID-19 case notification rates

		'Case' Catchment day with viral detection	'Control' Catchment day without viral detection	Crude odds ratio (95% CI)	P-value	
Case notifications allocated to wastewater catchment ('Exposure' presence or absence)	All catchments	<i>n</i> = 36	<i>n</i> = 64			
	Yes	33	42	5.68 (1.51–32.1)	0.004	
	No	3	22			
	Catchments with under 1 notified case per 100,000 population					
		<i>n</i> = 8	<i>n</i> = 51			
	Yes	5	29	1.26 (0.22–8.99)	0.79	
	No	3	22			
	Catchments with 1–9 notified cases per 100,000 population					
		<i>n</i> = 20	<i>n</i> = 28			
	Yes	17	6	19.04 (3.84–136)	<0.001	
	No	3	22			
	Catchments with 10–50 notified cases per 100,000 population					
	<i>n</i> = 14	<i>n</i> = 29				
Yes	11	7	10.74 (2.08–77.6)	0.001		
No	3	22				

Case notification rates were further compared with viral RNA detection upon stratification at <1, 1–9 and 10–50 notified cases per 100,000 population.

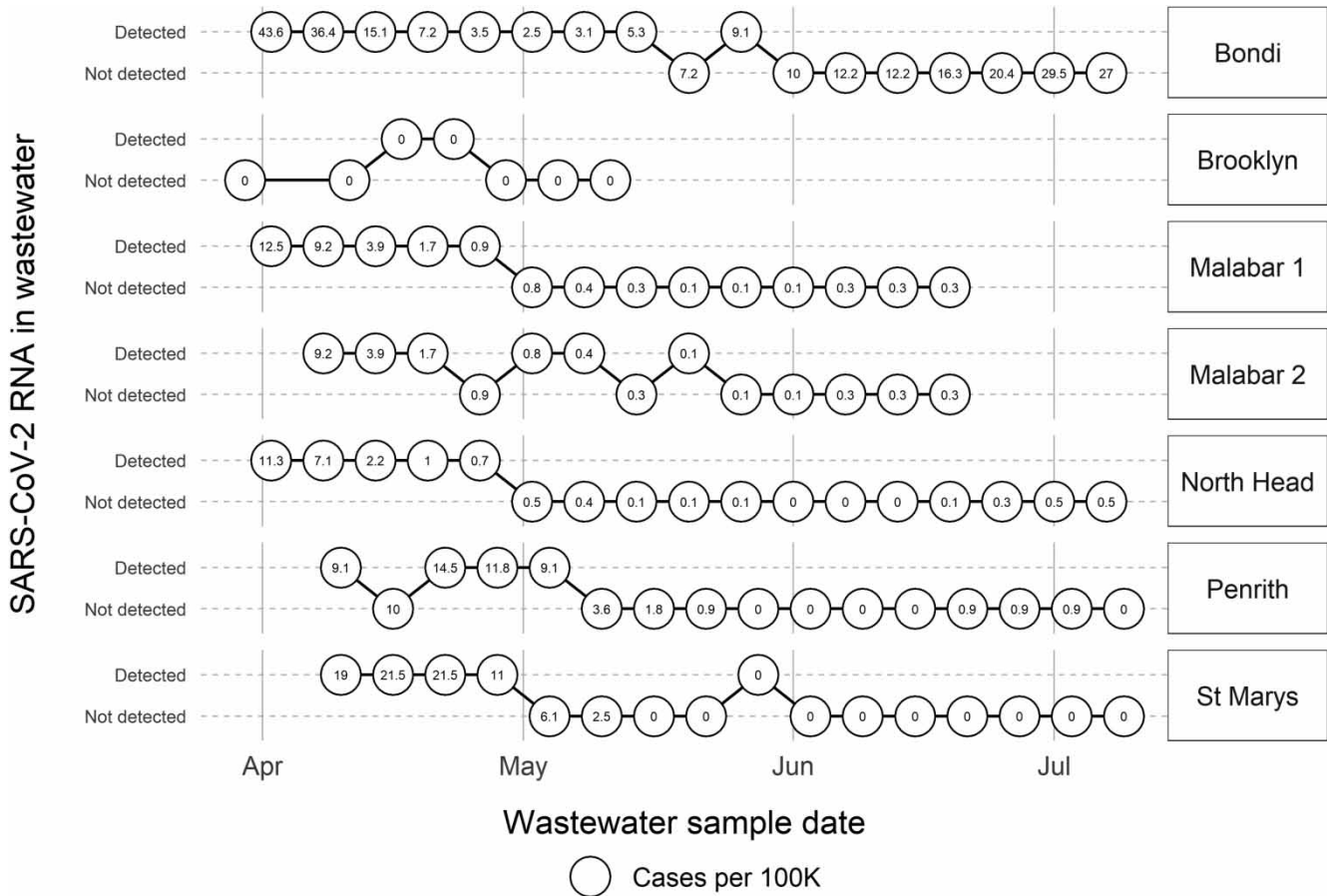


Figure 1 | SARS-CoV-2 RNA fragment detection results and corresponding case notification rates per 100,000 population (within white circles) for 100 samples collected from Sydney wastewater treatment plant inlet points between 30 March and 9 July 2020. Viral detections were either N1 or N2 positive, or both.

A quantitative comparison of the distance between notified case locations per sample date and catchment revealed that wastewater viral detections were observed with wider geographic dispersal of case locations from one another within a catchment, compared with non-detect wastewater results where cases were geographically more clustered. This effect was particularly evident in the Bondi catchment (Figure 2).

Analytical epidemiology

Univariable analysis

Overall, 92% of catchments with viral detection results had one or more cases allocated to the catchment at the time of wastewater sampling, compared with 66% of catchments with non-detect results. The odds of a viral detection result on a given day at a given sampling site were increased in wastewater catchments with one or more cases allocated to the catchment by a factor of 5.68 (95% CI: 1.51–32.1, $P = 0.004$). The OR increased to 19.04 (95% CI: 3.84–136, $P < 0.001$) and 10.74 (95% CI: 2.08–77.6, $P = 0.001$) in wastewater catchments with 1–9 cases, and 10–50 cases per 100,000 population, respectively. In catchments with fewer than 1 case per 100,000 population, there was no statistically significant association between viral detection results and case allocation to the catchment, compared with catchments with no cases (OR = 1.26, 95% CI: 0.22–8.99, $P = 0.79$) (Table 2).

Wastewater viral detection results were also considered as a population-level diagnostic tool for the presence of one or more notified cases allocated to a catchment during their 9-day viral shedding period. The proportion of catchments without cases allocated and corroborated by a non-detect result (specificity) was 0.88 (95% CI: 0.69–0.97). Overall, the proportion of catchments with one or more cases allocated and corroborated by a viral detection result (sensitivity) was 0.44 (95% CI: 0.33–0.56). Sensitivity ranged from 0.15 (95% CI: 0.05–0.31) in catchments with under one case, to 0.74 (95% CI: 0.52–0.9) in catchments with 1–9 cases per 100,000 population (Table 3).

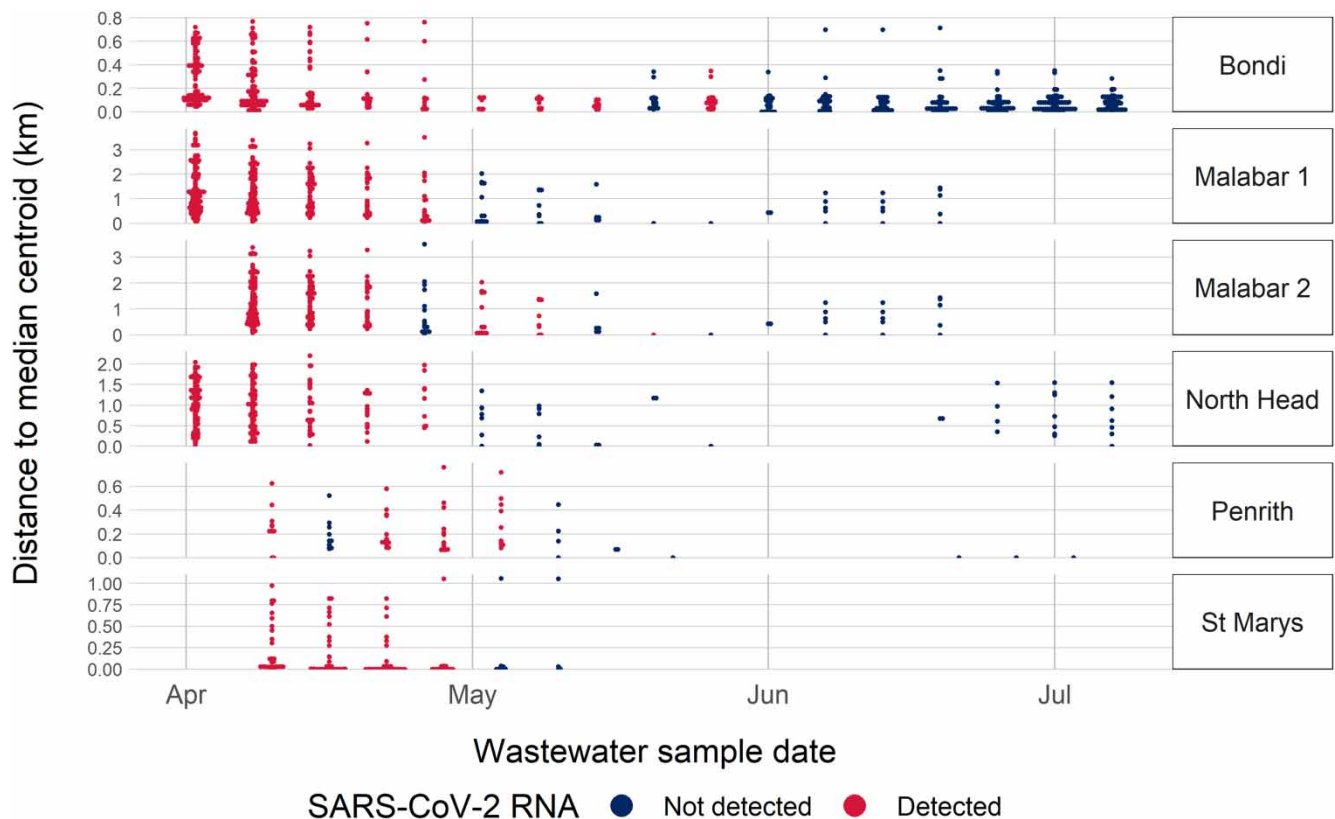


Figure 2 | Dispersion of notified COVID-19 case locations within wastewater catchments on sampling dates, by SARS-CoV-2 RNA fragment detection result. Points represent the case location distance to the group median centroid, calculated by the pairwise Euclidean distances of latitude and longitude of case locations within a catchment and sampling date.

Table 3 | Contingency tables and metrics for the performance of wastewater testing as a population-level diagnostic tool for case allocation to a catchment

		'Test' positive Catchment day with viral detection	'Test' negative Catchment day without viral detection	Diagnostic sensitivity (95% CI)	Diagnostic specificity (95% CI)	Predictive value of a positive (95% CI)	Predictive value of a negative (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Case notifications allocated to wastewater catchment ('Disease' presence or absence)	All catchments	<i>n</i> = 36	<i>n</i> = 64						
	Yes	33	42	0.44 (0.33–0.56)	0.88 (0.69–0.97)	0.92 (0.78–0.98)	0.34 (0.24–0.47)	3.67 (1.72–7.83)	0.64 (0.47–0.86)
	No	3	22						
	Catchments with under 1 notified case per 100,000 population	<i>n</i> = 8	<i>n</i> = 51						
	Yes	5	29	0.15 (0.05–0.31)	0.88 (0.69–0.97)	0.62 (0.24–0.91)	0.43 (0.29–0.58)	1.23 (0.32–4.66)	0.97 (0.79–1.19)
	No	3	22						
	Catchments with 1–9 notified cases per 100,000 population	<i>n</i> = 20	<i>n</i> = 28						
	Yes	17	6	0.74 (0.52–0.90)	0.88 (0.69–0.97)	0.85 (0.64–0.95)	0.79 (0.60–0.90)	6.16 (2.19– 17.36)	0.30 (0.14–0.63)
	No	3	22						
	Catchments with 10–50 notified cases per 100,000 population	<i>n</i> = 14	<i>n</i> = 29						
	Yes	11	7	0.61 (0.36–0.85)	0.88 (0.69–0.97)	0.79 (0.52–0.92)	0.76 (0.58–0.88)	5.09 (1.75–14.85)	0.44 (0.23–0.86)
	No	3	22						

Diagnostic performance was compared with case notification rates stratified at <1, 1–9 and 10–50 notified cases per 100,000 population.

Regression analysis

The number of notified cases per 100,000 population was a statistically significant predictor of wastewater viral detection ($\chi^2 = 12.5$, $df = 1$, $P < 0.001$). Each additional case per 100,000 population resulted in an increase in the odds of viral detection by a factor 1.11 (95% CI: 1.04–1.19). The predicted probabilities were graphed to aid in interpretation of the model, noting that the relationship is non-linear on the probability scale (Figure 3). At zero, 10.5 and 21.3 notified cases per 100,000 population, the predicted probability of wastewater viral detection was 25, 50 and 75%, respectively. Wide confidence intervals were observed and regression deletion diagnostics (large DFFIT values) associated this with the seven consecutive non-detect results in wastewater samples collected from the Bondi catchment during June and July (median of 14.25 cases per 100,000) (Figure 1).

DISCUSSION

This study showed that during the 14-week wastewater sampling period, SARS-CoV-2 viral RNA fragment detections in wastewater samples showed a positive association at over 1 case per 100,000 population, and provided good predictive power at increasing case notification rates. The probability of a viral detection result exceeded 50% at over 10 cases per 100,000 population. As a population-level diagnostic tool for the presence of recent cases allocated to a catchment, wastewater testing results showed consistently higher specificity than sensitivity at various case notification rates; however, various factors including geographic clustering and distance between case location and wastewater sampling points could have attributed to the lower observed sensitivities.

In 2020, the daily rate of SARS-CoV-2 case notifications in NSW reached its highest peak some 10–14 days prior to the first wastewater sample collection on 30 March (NSW Health Public Health Response Branch 2020). Case notification rates then declined rapidly and remained comparatively much lower during the 14-week wastewater sampling period. This decline was largely attributable to Australia's external border closures on 20 March (Australian Government Media Release 2020), and mandatory hotel quarantine policy for all returning residents and citizens from 27 March onwards (NSW Government 2020a). 75% of wastewater viral detections occurred within the first month (April), and 92% during the first half of the sampling period. This can be explained by the initially much higher case numbers, and subsequent larger volume of residual viral shedding into wastewater from cases with onsets of 7 days and longer prior to the first sampling date. This finding is consistent with the results of an earlier European study which found a significant correlation between the strength of viral assay detection results and COVID-19 case prevalence (Medema *et al.* 2020).

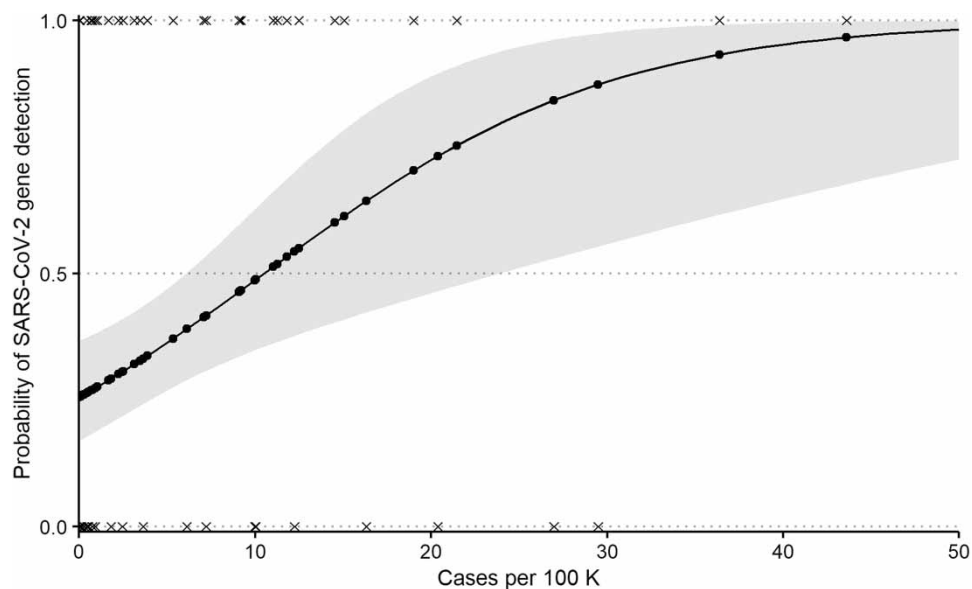


Figure 3 | Predicted probabilities from a logistic regression model of SARS-CoV-2 RNA fragment detection by cases per 100,000 population. The black points are fitted values of the samples used to create the model, and the line and shaded area are the fitted model and 95% confidence interval, respectively. The crosses are the observed viral detections and cases per 100,000 population.

Analysis of the performance of SARS-CoV-2 wastewater surveillance as a population-level diagnostic tool for the presence of cases in a catchment provides invaluable evidence to inform COVID-19 containment efforts. In this study, 56% of catchments with cases allocated on the sampling date returned non-detect wastewater results (false negatives), whereas only 12% of catchments without cases allocated returned viral detections (false positives). Combined with interpretation of the odds ratios (ORs), these findings suggest that SARS-CoV-2 wastewater surveillance may be a highly specific modality for verifying the presence, but not a highly sensitive modality for verifying the absence of recent cases within the metropolitan wastewater catchments sampled. The lower sensitivity suggests that a non-detect wastewater result should not provide a high degree of reassurance that viral shedding cases are indeed not present within the catchment. Conversely, a higher specificity may provide actionable public health intelligence, particularly where wastewater viral detections occur in catchments with few or no notified (i.e. known) cases. Such a finding may point to suboptimal clinical surveillance sensitivity or undetected, low-level virus circulation in the population serviced by the wastewater catchment. This may justify prompt public health action, e.g. issuing public health alerts to maintain high community testing rates, or risk communication targeting vulnerable subpopulations residing in the catchment, e.g. residential aged-care facilities or culturally diverse communities. The influence of disease prevalence on the interpretation of diagnostic test performance metrics is well described (Leeflang *et al.* 2013; Baeyens *et al.* 2019). Considering the very low estimate of SARS-CoV-2 prevalence in sampled catchments during the sampling period (0.04%), the individual observed estimates for sensitivity, specificity, predictive values for detect or non-detect wastewater results, and likelihood ratios should be interpreted with caution. Rather, the inference that wastewater viral detections are a more reliable indicator of the presence of recent virus shedding cases in a catchment, than non-detect results are of the absence of cases in a catchment is based on the consistent trend observed in these estimates across varying case rates per catchment population size, and supported by our interpretation of the positive odds of viral detections in wastewater samples collected from catchments with one or more cases allocated to the catchment.

Study limitations

There are various assumptions and limitations inherent to the methodologies applied in this study. Data captured in NCIMS are dynamic in nature; therefore, the presented data presents a point-in-time analysis of SARS-CoV-2 case notifications and may differ from other reports covering the same period.

We utilised a case-control study approach to investigate measures of association between wastewater viral detection results and case allocation to a catchment. There was an equal probability of incorrect allocation or non-allocation of cases to a catchment (non-differential misclassification). This would be expected to bias the measures of association towards no observable effect. However, the ORs showed a statistically significant and strongly positive association between wastewater viral detections and case allocation to a catchment compared with catchments without allocated case notifications, except where there were fewer than 1 case per 100,000 catchment population. This suggests that the observed results are a true effect. Wide confidence intervals for the observed estimates are likely due to the small sample size; therefore, the trend observed across the variable catchment population sizes is more informative than any one result. Case-control studies require control selection to be independent of exposure status. To validate the molecular diagnostic assay protocol used, historical samples were selected for analysis with knowledge of notifications data. Selection bias may have occurred for those catchments that returned non-detect wastewater results, if these were more likely to have been selected because there were no case notifications reported in the catchment at the time of sampling.

The various possible reasons for misclassification bias (i.e. incorrect allocation or non-allocation of cases to catchments on sampling days) deserve attention. Possible explanations for false negatives observed (i.e. a non-detect wastewater result, despite having one or more cases allocated to the catchment) are many and varied. Upon confirmation of their positive test results, Public Health Order (No 4) 2020 under the *NSW Public Health Act (2010)* required all cases to immediately self-isolate for a period no less than 10 days from onset (NSW Government 2020b). Despite health authorities urging persons to self-isolate upon any symptom development and following SARS-CoV-2 testing, there was no legal requirement to do so until being informed of a positive test result. Unless cases were verified to have been in a quarantine hotel or healthcare facility during their 9-day viral shedding period of interest, we assumed that upon notification of a positive test result, cases would have been self-isolating at their primary residential address recorded in NCIMS. However, we had no readily accessible way of verifying whether the address was correctly recorded, or whether cases were indeed located there during their 9-day viral shedding period of interest. Therefore, cases may have been allocated to a catchment, when in fact they were not present.

Physicochemical and environmental factors, as well as case clustering within a catchment, and cases' geographic location relative to, or distance from the wastewater sampling point could also be important determinants of sensitivity. A combination of these factors could have contributed towards the observed sensitivity of wastewater viral detection results in this study. As enveloped virions, the infectivity of coronaviruses are known to be adversely affected by factors within the sewer network such as elevated temperature, pH, solids, other microorganisms and degradation by detergents or pollutants (Gundy *et al.* 2008; Foladori *et al.* 2020). However, data suggest that the SARS-CoV-2 RNA is likely to remain detectable in wastewater for days to weeks, if temperature is controlled to limit viral degradation once sampled (Ahmed *et al.* 2020b; Bivins *et al.* 2020). Detection in wastewater samples can also be limited by infiltration and ingress of stormwater during high rainfall events causing dilution, inadequate mixing in the network and inhibitory substances in trade waste which can interfere with sample analysis. The impact of such limiting factors may become more pronounced where there is geographic clustering of cases; and further influenced by the distance between case location and the wastewater sampling point. This was particularly evident in the Bondi catchment, where seven consecutive wastewater samples collected during June and July returned non-detect results, despite case allocations on relevant sampling dates ranging from 7 to 29 cases per 100,000 catchment population. At this stage of the NSW response, most cases allocated to the catchment were confined to hotel quarantine and tightly geographically clustered within the inner-city limits. This effect may be less likely to occur where virus shedding cases are more geographically dispersed across a wastewater catchment area.

There are also various possible explanations for false positives (i.e. a wastewater viral detection, despite having no cases allocated to the catchment). The same uncertainty surrounding cases' true location during their period of mandatory self-isolation that may result in incorrect allocation to a catchment, could also result in cases being non-allocated to a catchment, when they were in fact present. Furthermore, despite very high clinical testing rates in NSW by international comparison (Hasell *et al.* 2020; NSW Health Public Health Response Branch 2020) and an effective contact tracing capability, it is accepted that case notifications represent an undercount of true case numbers. SARS-CoV-2 shedding cases, therefore, may have been present in one or more catchments without the knowledge of health authorities, and therefore not allocated to said catchments. Furthermore, we assumed peak viral shedding occurs during a 9-day period (−2 to 7 days from onset) and restricted our study period of interest. However, it is well-described that SARS-CoV-2 shedding into wastewater may continue for many weeks after clinical recovery (Cevik *et al.* 2021), and well beyond the first week from symptom onset. Therefore, wastewater viral detections could be attributable not only to known cases within their 9-day viral shedding period allocated to the catchment, but also earlier, clinically recovered cases with prolonged virus shedding, or asymptomatic infected persons living, working or visiting a catchment. Of the false-positive wastewater samples, 2/3 (66%) were collected during the NSW school holidays from the Brooklyn catchment, which had the smallest catchment population in this study by far. It is possible that these viral detections were due to one or more non-notified cases, or cases non-allocated for reasons described earlier (e.g. visitors). These findings raise questions whether the sensitivity of wastewater viral detections as an indicator of viral shedding cases may be higher with smaller catchment populations, and/or in higher prevalence settings. This should be an area of further research, particularly considering the potential for wastewater viral detections to provide early warning of undetected cases in non-urban catchment populations, or in vulnerable communities.

CONCLUSION

Field epidemiological approaches are an important tool to integrate and analyse SARS-CoV-2 wastewater surveillance results and case notifications data to inform evidence-based public health action. The results of this study suggest that wastewater testing for SARS-CoV-2 is a valuable supplementary surveillance component to support the public health response in NSW. Considered together, the observed results suggest that in a low prevalence setting, wastewater viral detections are a more reliable indicator of the presence of recent virus shedding cases in a catchment, than non-detect results are of the absence of cases in a wastewater catchment. Wastewater viral detections, particularly in catchments servicing populations with no recent case notifications, should trigger prompt public health investigations to rule out low-level virus circulation or undetected community transmission. This may be supported by the development of upstream sampling methodologies to better localise areas from which wastewater viral detection signals are emanating, thereby allowing health authorities to target public health resources to the highest risk areas or communities. Further research is required to better understand the relationship between prolonged virus shedding and wastewater viral detections. Further research is also needed to explore alternative sampling strategies and testing methodologies to better characterise the sensitivity of wastewater viral detection in

low prevalence settings, and in and catchments servicing variable populations sizes. Considering the study assumptions and limitations, the observed results do not exclude the potential of wastewater surveillance as a sensitive modality in certain settings, particularly to provide early warning of undetected cases. This may be particularly useful in smaller or non-urban catchments, vulnerable subpopulations, or in communities that are otherwise less inclined to engage with health authorities or present for clinical testing.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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