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SARS-CoV-2 detection in wastewater as an early warning indicator for COVID-19 pandemic. Madrid region case study

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Abstract

This study analyzes the presence and dynamics of SARS-CoV-2 in sewage sanitation systems in Madrid region. Statistical results and data generated are produced and shared daily as a tool for the early detection of SARS-CoV-2 and its spread based on the WBE (wastewater-based epidemiological) approach. The number of sampled points amounts to a total of 289 sampling points in terms of SARS-CoV-2 concentration that collects over six million and a half inhabitants' discharges. The project was developed by Canal de Isabel II, the company responsible for the management of the water cycle in Madrid. Research evaluates the correlation found between SARS-CoV-2 concentration in wastewater and the following public health indicators: incidence rate, reported active cases, and COVID-19 hospitalization data (regular hospitalization and ICU admission cases). SARS-CoV-2 presence and dynamics in wastewater show that there is an association with both 14-day incidence rates with active infection and reported COVID-19 hospitalizations. A lag varying from 3 to 8 days between wastewater presence and hospitalizations is explained because many infected individuals shed the virus in stool before they develop symptoms and thus also before they seek clinical testing. The resulting data are available for consultancy on the company's website (named VIGÍA project) as well as on the regional government's websites. The results have already been useful to anticipate the second and third COVID-19 waves in Madrid. Information is shared daily with health authorities for consultation and decision-making. The results are available as an aggregation for the entire region and for each sewershed.

Keywords: Wastewater-based epidemiology; SARS-CoV-2; COVID-19; Public Health

1. INTRODUCTION

During the first quarter of 2020, due to the evolution of the COVID-19 pandemic, several studies on the validation process for monitoring and detecting traces of SARS-CoV-2 in wastewater were started in multiple countries (Ahmed et al., 2020; La Rosa et al., 2020; Medema et al., 2020; Scherchan et al., 2020). Canal de Isabel II, as the company responsible for water infrastructure management in Madrid region, started to monitor the spread of SARS-CoV-2 in wastewater in March 2020, in the middle of a nationwide lockdown. VIGÍA project was released to the health authorities in early July, just before the second wave started.

The monitoring system has been implemented over the entire Madrid region, with an approximate population of 7 million inhabitants by means of 289 sampling points, the biggest wastewater surveillance system in Europe. VIGíA is based on weekly grab sampling, data validation, statistical analysis, and representation. The results are shared daily with the Madrid Health Department for consultation and decision-making purposes.

2. METHODOLOGY

Due to the novelty of the case study, Canal de Isabel II ran several pilot tests to determine adequate onsite sampling points on the sewer network. The aim is to monitor the full population of the Madrid region in terms of SARS-CoV-2 presence.

2.1 Sampling Locations

Balance must be sought between number of sampling points, laboratory and fieldwork capacity while ensuring sample representativity. Sampling points were established attending to these criteria along with conclusions drawn from a pilot test carried out in May 2020.

Whenever it is possible, a maximum of 25.000 equivalent inhabitants, 3,5 km distance to population centers and 2,5 km distance to last discharging point are preferred to obtain optimal results. Considering the particularities of Madrid region, with high population density in the center of the region and many scattered areas with low density around it, criteria imply a total of 289 sampling points to cover the entire population. Points were selected giving priority to Wastewater Treatment Plants when possible for ease of sampling. Figure 1 shows the final selection of sampling points with corresponding sewersheds.

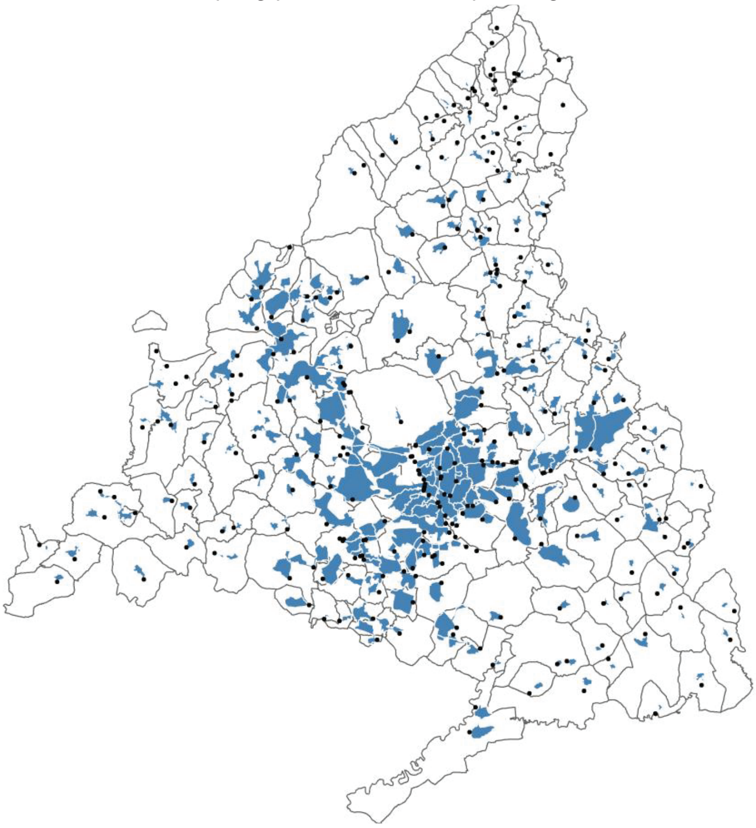

Figure 1. Map of sewersheds (blue) and sampling points

2.2 Sampling campaign: fieldwork and laboratory analysis

The large number of samples as well as its onsite storage requirements, the urgency and impact of the study, and accessibility to the sewer network involved a logistical challenge when defining the sampling campaigns. Three laboratories are currently responsible for sampling SARS-CoV-2 presence. Onsite, they count on a team of one to three field workers each one.

The sampling strategy follows a weekly frequency and samples should be taken at a fixed time for every point to minimize the variability that results show during the day and to better capture the dynamics from week to week (Peccia et al., 2020, The Water Research Foundation, 2020).

At every sample location, 1 liter or half liter (depending on laboratory requirements) of wastewater is collected. The temperature and conductivity of the water are measured onsite, and then the sample is refrigerated. When the collection process from a route is over, samples are sent to the laboratory.

Madrid urban drainage system is mainly combined as described in Lastra et al. (2018). It is unclear how detectable pieces of SARS-CoV-2 virus from infected people interact with stormwater and or industrial effluents. However, to detect unusual dilution that could potentially affect virus detectability, physicochemical parameters are also monitored for outlier detection. COD appears to be most relevant in detecting unusual wastewater composition, given that it is more sensitive to high dilutions due to rainfall episodes and higher than usual pollutant presence from industrial effluents. Chloride levels and electrical conductivity are also monitored as additional criteria to detect unusual composition although they are more stable. Samples with out-of-range values are resampled to verify or rule out qPCR results within 2 to 3 days. Figure 2 shows the observed distribution of the parameters.

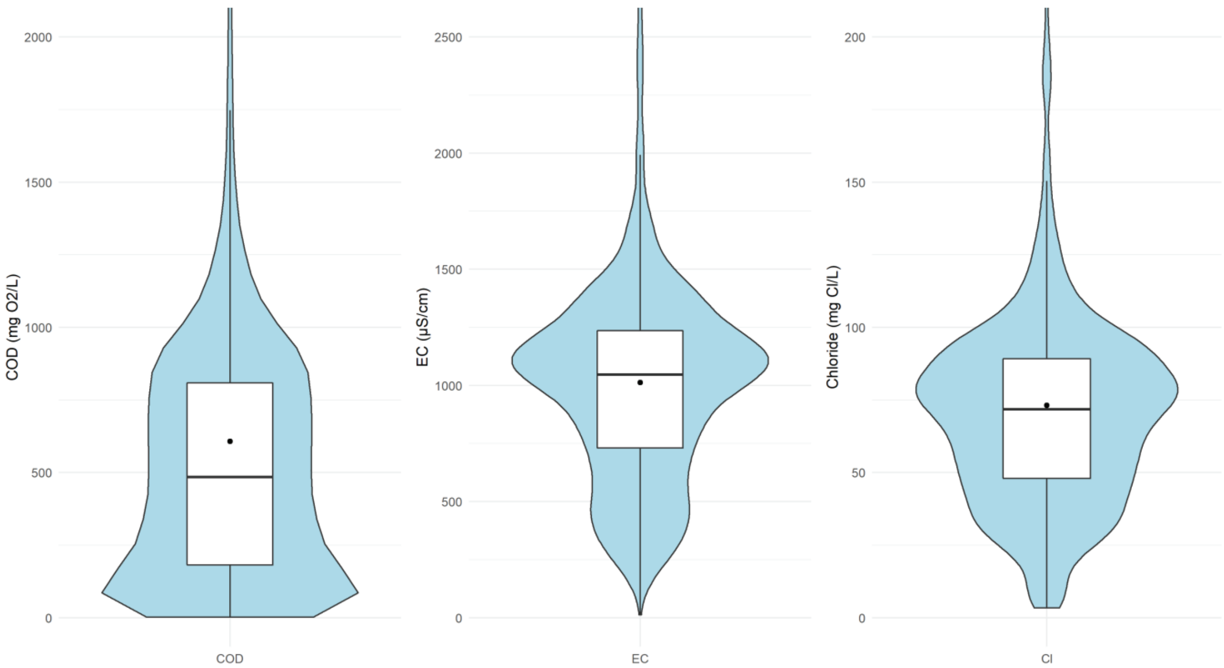

Figure 2. Observed distribution of different physicochemical parameters

3. RESULTS AND DISCUSSION

3.3 SARS-CoV-2 signal

More than 25,000 samples have been analyzed since the start of the project. Highly diluted samples are often related to inconsistent SARS-CoV-2 concentrations; however, this does not mean concentrations are necessarily low. In fact, some results showed high SARS-CoV-2 concentrations along with low COD in samples taken on rainy days; this could be due to first flush or resuspension phenomena. Figure 3 shows multiple ruled out test at one sampling point.

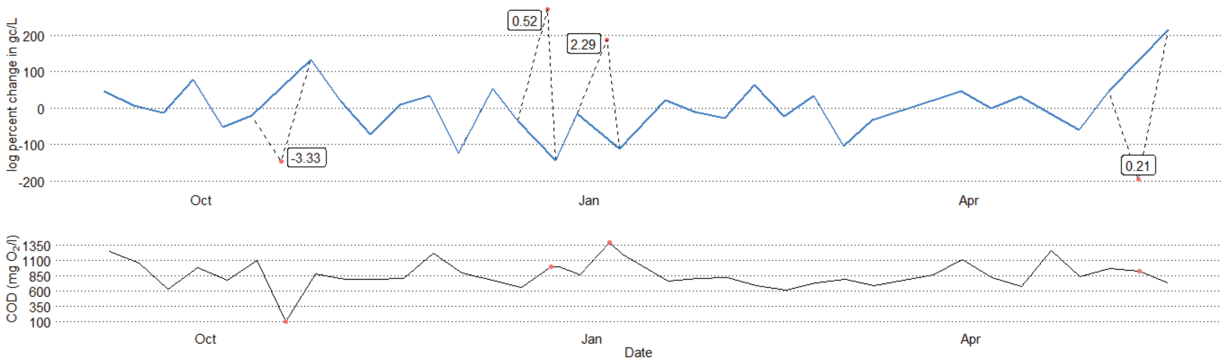

Figure 3. Outlier detection based on COD. Log percent change of SARS-CoV-2 concentration in one sampling point (blue). Labels represent z scores of COD for resampled and ruled out tests.

Since a subset of points was sampled each day of the week, results were extended to a daily frequency with two considerations:

(i) The signal was estimated constant from one sample to the next,
(ii) When the interval between two samples exceeds the 7-day period (due to re-sampling or any fieldwork difficulties), the previous results were extrapolated to fill in the missing information by applying the slope of the moving average series of the two previous results.

To show the weekly presence of SARS-CoV-2 in wastewater in the Madrid region, all sample points are aggregated into one curve. The results are normalized by 100,000 inhabitants to be aligned with the health department and local statistics.

3.4 Incidence rates and hospitalizations

In order to assess wastewater capabilities as an early warning indicator, aggregation of SARS-CoV-2 concentration is compared to new reported cases and new COVID-19 hospitalizations. These daily series present strong weekly seasonality, so a 7-day moving average is preferred as a more robust indicator.

Following Figure 4 shows similarities between series, with wastewater SARS-CoV-2 concentration in wastewater ahead of both series.

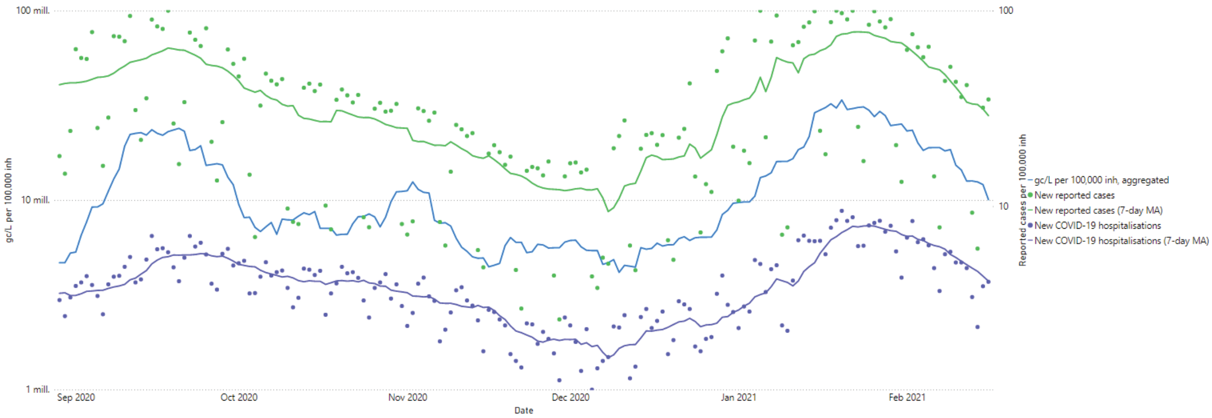

Figure 4. Second and third waves in Madrid - SARS-CoV-2 concentration in wastewater (blue) and public health indicators.

The mismatch that can be seen in the graph above between the presence of SARS-CoV-2 in sewage and hospitalizations is because the virus is already present in feces some days before symptom onset (Olesen et al., 2021; Zhu et al., 2021). Anticipation of wastewater tests may differ depending on locations, sewershed size and population, sampling strategies or temperature among many other factors as wastewater is in general a very heterogeneous environment. Results range between 3 to 11 days of anticipation among the studied sewersheds for this project.

4. CONCLUSIONS

This study has demonstrated the ability to use wastewater epidemiology as an early warning tool for the current COVID-19 pandemic in the Madrid region. Sampling points selection process was critical to this aim: a total of 289 sample points which represent the same amount of sewersheds are systematically tested for SARS-CoV-2 concentration. A pilot test to define sampling point selection criteria concluded that whenever possible, a maximum of 25.000 equivalent inhabitants, 3,5 km distance to population centers and 2,5 km distance to last discharging point are preferred to obtain optimal results.

It has been found that a weekly sampling strategy offers adequate quantification with fixed sampling hours for every point to reduce the effect of daily variations. However, laboratory results must be validated with physicochemical parameters to detect unusual compositions.

SARS-CoV-2 presence and evolution in wastewater show a strong connection with incidence rates and reported COVID-19 hospitalisations. Information is daily shared with Health authorities for consultancy and decision-making. Results are available as an aggregation for the entire region and for each sewershed.

Future work will include a permanent epidemiological surveillance system where a subset of 87 out of the current 289 sampling points will be monitored. In the event of virus detection, more detailed surveillance points will be activated in that sewershed to pinpoint infection hotspots.

A study SARS-CoV-2 decay in raw wastewater is also under development. It is based on a 1D sewer network model for water quality where a set of new theoretical pollutants are declared to model its evolution along the sewer network.

There is also an ongoing pilot test to assess relationships between PCR results for grab and composite samples with automatic refrigerated samplers. The goal is to determine if composite samples can offer a more detailed analysis of virus loads where power and security requirements are met for automatic sampler installation, mainly in wastewater treatment plants.

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