Viral RNA in city wastewater as a key indicator of COVID-19 recrudescence and containment measures effectiveness

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ABSTRACT

In recent years, and more specifically at the beginning of the Covid-19 crisis, sewage surveillance has been proposed as a tool to monitor the epidemiology of human viral infections. In the present work, from July to December 2020, we evaluated the number of copies of SARS-CoV-2 RNA in Marseille's wastewater and correlated these data with the number of new positive cases diagnosed in our Institute of Infectious Disease, which tested about 20% of the city's population. It was ob-served that during the great epidemic peak, from October to December 2020, the correlation between the rate of virus in the sewers and the number of positive diagnoses was perfectly cor-related. During the summer period, this correlation was more complex to analyze and subject to many confounding factors that we have discussed. We were also able to correlate the effect of viral circulation in sewage water with containment measures, probably the most impartial correlation on their potential inflection effect of epidemic curves. Not only is this correlation not obvious, but it also clearly appears that the drop in cases as well as the drop in the viral load in the sewers occur before the containment measures. In fact, this suggests that there are factors that initiate the end of the epidemic peak independently of the containment measure. These factors will therefore need to be explored more deeply in the future.

INTRODUCTION

In December 2019, an outbreak of coronavirus disease, further refered to as Covid-19, was detected in Wuhan, China (Al-Tawfiq, 2020; Huang et al., 2020; Rothan and Byrareddy, 2020; Toit, 2020). This epidemic is due to Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-COV-2), which was classified as a new strain of coronavirus. WHO declared on March 11,2020 a worldwide pandemic (World Health Organization, 2020b). To date, more than 54 million cases and more than

1,3 million deaths have been reported worldwide as of November 16,2020 (World Health Organization, 2020a).

This new coronavirus resembles classical respiratory infection with common symptoms, including dry cough, fever, tiredness, myalgia and difficulty in breathing (Petersen et al., 2020).

As with other human coronaviruses such as SARS-CoV and MERS-CoV, SARS-CoV-2 is able to cause gastrointestinal symptoms in addition to respiratory symptoms, in approximately 2-10% of positive cases (Leung et al., 2003; Gao et al., 2020; Memish et al., 2020). Furthermore, previous studies conducted on SARS-CoV and MERS-CoV, showed that viral RNA was found in human feces (Leung et al., 2003; Corman et al., 2016). Several recent studies also reported the presence of SARS-CoV-2 RNA in stool and anal / rectal swabs feces, not only in symptomatic, but also in asymptomatic patients (Gu et al., 2020; Holshue et al., 2020; Song et al., 2020; Tang et al., 2020; Xiao et al., 2020). It has even been shown that virus in stools was still infectious (Dergham et al., 2020).

Setting up monitoring of virus levels in wastewater seemed logical. Indeed, wastewater-based epidemiology approach has already been used to prevent or follow disease outbreak, as previously demonstrated for enteric viruses, such as poliovirus or hepatitis virus (Asghar et al., 2014; Hellmér et al., 2014) and could also been used to monitor SARS-CoV-2 clusters (Carducci et al., 2020; Randazzo et al., 2020). Detection of SARS-CoV-2 RNA in wastewater samples has already been reported in Australia (Ahmed et al., 2020), USA (Wu et al., 2020), China (Wang et al., 2020), Japan (Haramoto et al., 2020), Netherlands (Medema et al., 2020), Spain (Randazzo et al., 2020), Dubai (Albastaki et al., 2020), Emirates (Hasan et al., 2020), Italia (La Rosa et al., 2020), Turkey (Alpaslan Kocamemi et al., 2020), Israel (Bar Or et al., 2020) and Paris, France (Wurtzer et al., 2020b).

In the present work, we evaluated the number of copies of SARS-CoV-2 RNA in Marseille wastewater and correlated these data with the number of new positive cases observed in Marseille at

our Institute of Infectious Diseases since July 1, 2020. Correlation between these two indicators was made on the basis of daily observations and confronted with the effectiveness of the containment measures decreed by the national Ministry of Health throughout the crisis. Our data demonstrate the efficacy of wastewater surveillance as a potential tool for public health monitoring of SARS-CoV-2 circulation, but can be affected by confounding factors, such as touristic flows. Moreover, this work suggests that the effect of containment measures is difficult to assess and that these measures are not the sole to explain case reduction after epidemic peak.

MATERIALS AND METHODS

Sampling sites and wasterwater collection

Samples were collected by the SERAMM (Marseille Metropole Sanitation Departement) by an automatic sampler "ASP-Station 2000 RPS20B" (Endress Hauser, Huningue, France). This type of sampler allows the filling of a refrigerated flask of 20 L per 24 hours of wastewater collected from 8 am to 8 am. The dates of collection were from July 1st, 2020 to December 15th 2020. Two samples of 250 ml of wastewater were collected from 2 independent vacuum samplers which collect wastewater from two distinct sewer networks. The separate network (noted RS) drains the major surface part of Marseille wastewater and nearly all hospitals of the city, especially Covid-19 dedicated units (zone in red line in Figure 1). The number of inhabitants connected to this network is 614,623. The combined network (noted RU), that contains a mixture of rainwater and wastewater, drains the city center of Marseille (zone in green on the map and dark blue line in Figure 1), a place that concentrates most of the touristic activity of Marseille, including most restaurants and night festive life. The number of inhabitants connected to this network is 359,123. Samples were transferred every day on ice to NRBC's laboratory (NRBC unit – nuclear, radiological, biological, chemical) of the BMPM unit

(Marseille Fire Brigade Battalion) and stored at 4°C before use. Samples to be tested were randomly selected, from 2 to 7 per week. They were treated within 1 hour of collection. SARS-CoV-2 copy number of RU and RS were totalized with adjustment to the respective population of their area when combined. A 8-day moving average was performed and the results were correlated with the SARS-CoV-2 Marseille positive cases.

SARS-CoV-2 virus quantification in wastewater

For detection of SARS-CoV-2 in wastewater, the BioFire®COVID-19 Test (BioFire Defense, Salt Lake City , USA), a nested multiplexed real-time RT-PCR, was used for qualitative detection of the virus according to the manufacturer's intructions, using FilmArray Torch instrument (Biomérieux, Grenoble, France. The limit of detection (LoD) of the BioFire®COVID-19 Test provided by the manufacturer (BioFire Defense, LLC) is 330 genomic copies per milliliter. To control this LoD, serial dilutions of known copies of synthetic SARS-CoV-2 RNA (SARS-CoV-2 Standard COV019, Biorad France, 200,000 copies/ml) were performed, from 2,000 genomic copies/ml to 50 copies/ml. Five technical replicates were performed at each dilution. Interpretation was made according to manufacturer's instructions and based on melt curve analysis as follows: "positive" when at least 2 out of 3 targets were detected, "negative" when no target was detected and "equivocal" when 1 target was detected. For the quantification of SARS-CoV-2 virus in wastewater, several dilutions of the wastewater were performed until no positive results were detected.

Inclusion of Covid-19 patients

Prevalence of Covid-19 was based on the data obtained at the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection (https://www.mediterranee-infection.com/), Assistance Publique-Hôpitaux de Marseille (AP-HM). Since the beginning of Covid-19 epidemic in our city, the IHU laboratory has been the only reference in the city where all people could be tested massively and free of charge,

without any restriction and with results accessible within 24 hours. Since January 29th, we have performed 401,265 SARS-coV-2 RT-PCR, for patients clinically suspected of having COVID-19, contacts of confirmed cases, but also patients simply seeking to know their health status (Lagier et al., 2020). For this study, results are those of patients living in Marseille and tested at the IHU, from July 1st to December 15th 2020. Procedure for RT-PCR targeting SARS-CoV-2 E gene has been detailed elsewhere (Amrane et al., 2020).

Factors of variation analyzed

The different factors that can affect the number of positive SARS-CoV-2 cases or copy numbers were analyzed. Daily mean temperature and amount of rain were found at: https://www.meteo60.fr/stations-releves/station-mois?station_id=07650&mois=11&annee=20.

The different measures implemented by the French government, the date of application and the publication were found as follows:

- Obligation to wear a mask in confined area (https://www.legifrance.gouv.fr/download/file/6jSpA0cWPxkOQ3AuvS7vvBqAan03mhLJC5z3cVMEAsc=/JOE_TEXTE)

- Obligation to wear a mask everywhere in Marseille (https://www.bouches-du-rhone.gouv.fr/content/download/37966/215784/file/Covid-19%C2%A0%20renforcement%20des%20mesures%20pour%20lutter%20contre%20la%20propagation%20du%20virus%20dans%20les%20Bouches-du-Rh%C3%B4ne.pdf)

- Total closure of bars and restaurants in Marseille (http://www.bouches-du-rhone.gouv.fr/content/download/38428/218472/file/Arr%C3%AAt%C3%A9%20n%C2%B0180%20du%2027%20septembre%202020%20OK-.pdf)

- Re-opening of bars and restaurants in Marseille (https://www.bouches-du-rhone.gouv.fr/content/download/38563/219477/file/C.P.%20Covid-19%C2%A0%20r%C3%A9ouverture%20des%20restaurants%20%C3%A0%20Aix-en-Provence%20et%20Marseille.pdf)

- Reduction to 50% of the presence of student in universities (https://services.dgesip.fr/fichiers/Circulaire_Renforcement_des_consignes_sanitaires_en_zones_d_alerte_renforcee_et_maximale.pdf)

- Implementation of the curfew (https://medias.amf.asso.fr/upload/files/D%C3%A9cret_17_octobre.pdf)

- Implementation of the lockdown (https://www.legifrance.gouv.fr/download/file/YTXQyL3I14RgMkscchJ4EWWUgvYvfJ3GciREwkWtl3E=/JOE_TEXTE)

RESULTS

Verification of LoD showed that at 6 and 1.8 LoD, all replicates were positive with 2 or 3 genes detected (Table 1). At dilution just above the LoD (LoD 1.2), 1 was positive for all 3 targets, whereas 3 replicates were positive for targets and one was equivocal. For 0.9 LoD, 3 replicates were positive for two targets and 2 replicates were equivocal. Below 0.9 LoD, all sample tested were equivocal or negative. Thus, the LoD where all samples are detected is 0.9LoD, corresponding to 300 genomic copies / ml in perfect agreement with manufacturer's data. This value was used as our reference for further analyses.

From July 1st to September 1st, the amount of virus in the sewer increases to reach a mean of almost 6,000 copies / ml. Then, the amount of virus in the sewer dropped to an average of approximately 1,000 copies / ml from September 1 to September 23. From September 24, the level of virus increased rapidly with a peak on October 22, with a quantity of 9,000 copies / ml on that day. Subsequently, a decrease in the amount of virus was observed in wastewater reaching 0 copy / ml.

Variations in mean outdoor temperature (ranging between 4.1 and between 29.5) had no effect on the number of SARS-CoV-2 copy numbers in wastewater (Figure 2). The effect of the 2 episodes of rain that occurred from September 19 to 22 and November 7 to 8 are difficult to correlate with virus concentration. The quantity of viruses did not drop during the first episode, but possibly with the second.

The daily number of new cases of SARS-CoV-2 in Marseille detected at the IHU using an 8-day moving average was analyzed and is represented on Figure 3. Since July 1st, the number of positive cases has been slowly increasing until reaching a plateau in September 2020 with an average number of positive cases of about 100 per day. This plateau is grossly observable during the first 3 weeks of September. This period corresponds to the end of the summer holidays with the end of the touristic season and the beginning of the academic year. Then, for one week, the number of positive cases decreased with a minimum average number of positive cases of about 60. From September 28, a rapid increase in the number of positive cases was observed, peaking on October 26 with a maximum of 303 positive cases on that day. From this date, the number of positive cases decreases considerably, reaching an average of 20 positive cases in the first weeks of December.

When looking at the evolution between the number of SARS-CoV-2 copies in wastewater and the number of SARS-CoV-2 positive cases, from July 1st to September 1st, the amount of virus in the sewer increases as does the number of positive cases. Then, while the number of positive patients

stagnates, the amount of virus in the sewer drops from September 1st to September 23rd. In this phase, a discrepancy between the number of SARS-CoV-2 positive cases and the amount of virus in the wastewater was observed. From September 24, a perfect correlation was observed between the number of positive cases and the amount of virus observed in the sewers, with a peak observed on October 22. Then, a decrease in the amount of virus was observed in wastewater, correlating with the decrease in the number of SARS-CoV-2 positive cases.

The different measures implemented by the French government with precise dates were correlated with the evolution of the cases and the concentration of the virus in sewage (Figure 3), and their effect was tentatively assessed between 5 and 10 days after their implementation (in grey). The first measure implemented during summer was the mandatory wearing of a mask in confined areas on July 20. Five to 10 days after this was introduced, the number of SARS-CoV-2 positive cases continues to increase slowly, as does the number of SARS-Co-2 copies in sewage. The 25th of august, to wear a mask became mandatory everywhere in Marseille. Five to 10 days later, the number of SARS-CoV-2 positive cases continued to increase, while the number of SARS-Co-2 copies stagnated and began to decline. This decrease is also precisely associated with the end of the touristic season and the beginning of the academic year when Marseille's autochthons returned to the city and the children go back to school. The 27th of September, bars and restaurants in Marseille were ordered to close. Five to 10 days later, both level of positive cases and copy number of SARS CoV-2 first slowly decreased, and then rapidly increased. Finally, the bars and restaurants reopened on October 5 and on October 6, and universities reduced their capacity by 50%. The curve for positive cases and wastewater SARS-CoV-2 copy numbers continued to increase rapidly. The 17th of October, a curfew was implemented from 9 p.m. to 6 a.m. This curfew corresponded to the fall vacations for the children and therefore the closure of schools for two weeks. Five to 10 days later, the number of positive cases reached a peak and the copy number of SARS-CoV-2 started to decrease. Just after this

period, a lockdown was implemented (October 30). First, immediately after the implementation of the lockdown, the decline in Covid cases and in the copy number of SARS-CoV-2 stopped abruptly and, paradoxically, increased before the downtrend resume. Following this period, the drop in the number of positive cases and the amount of virus in sewers continued, reaching a level roughly equivalent to that of early August for cases and early July for sewage.

DISCUSSION

Recently, several studies have explored the detection and quantification of SARS-CoV-2 in wastewater around the world, such as in the Netherlands (Medema et al., 2020), France(Wurtzer et al., 2020a), USA (Wu et al., 2020), Australia (Ahmed et al., 2020), Italy (La Rosa et al., 2020) and Germany (Westhaus et al., 2021). However, few have attempted to establish a correlation between viral load in the wastewater and the number of infected patients (Ahmed et al., 2020; Chavarria-Miró et al., 2020; Medema et al., 2020; Randazzo et al., 2020; Trottier et al., 2020; Vallejo et al., 2020; Wu et al., 2020; Wurtzer et al., 2020a).

In the present work, we were able to make the correlation between the quantitative detection of SARS-CoV-2 in wastewater and the number of cases diagnosed in our institute. The Biofire system, which is not usually used in this application, appeared to be effective and the LoD supplied by the manufacturer was in agreement both with our titration controls and with the previous studies (Liotti et al., 2020). This does not mean that the values we found on the number of copies were absolutely correct, because there are possible inhibitors or interfering substances in the wastewater (Shieh et al., 1995; Haramoto et al., 2020), but it still allowed us to study the kinetics of viral circulation. As for Covid-19 cases, the number was based on people attending our institute, which represents 20% of of all SARS-Cov -2 tests carried out for the whole city during the period of the present study

(geodes.santepubliquefrance.fr). While other private sites, even during the summer, had deadlines for making appointments and reporting results ranging from 2 to 5 days, by modifying our organization (Fenollar et al, Drancourt et al., submitted data), all people arriving at the institute without an appointment between 7 am and 7 pm obtained their results in less than 12 hours, on their mobile phone if they have one, or by picking it up at the institute. Thus, the rates observed in our institute were representative of the evolution of the epidemic in real time.

We observed that throughout the study period, i.e. from July to mid-December, three types of curves and correlations between the levels of SARS-CoV-2 in wastewater and the number of cases could be observed. During the third part of the period, roughly from the end of September to the end of November, there was a perfect correlation in the kinetics of the two curves with a variable shift on the accelerations or decelerations remaining very moderate (Figure 3). This trend of correlation between SARS-CoV-2 rate in wastewater and number of positive patients has been observed in other studies (Medema et al., 2020; D'Aoust et al., 2021). It was only at the beginning of December that there was a real dissociation with the disappearance of SARS-CoV-2 below the LoD while the number of cases remained relatively high. We interpret this, by recurrent information from our health authorities who have made an important publicity for the French to be tested massively. Indeed, when looking at the number of tests performed in France, particularly before Christmas, we see that the number of tests has increased enormously and that the number of positive cases has also (see supplementary Figurer S1, (Roser et al., 2020)). The aim was to detect as many cases as possible in order to avoid an explosion of cases after the holiday season for fear of a rebound like the one observed after Thanksgiving in California (Fernandez et al., 2020; Mehta et al., 2020).

During the first period, the picture was clearly different. From the beginning of July to the beginning of September, i.e. exactly at the peak of the tourist season, the correlation was also observed, but in a very different way. The copy number of SARS-CoV-2 and the number of cases increased in a linear

and perfectly parallel manner. In contrast, unlike in the third period, the rate of viruses in wastewater was comparatively higher than in the last period. We can try to make hypotheses to explain this difference. This discrepancy could be due to the fact that the increase in wastewater precedes the appearance of signs in patients for a longer period of time during the summer period. Indeed, in other studies, it has been observed that the increase in wastewater rates precedes the increase in cases by 4 days to several weeks (Ahmed et al., 2020; La Rosa et al., 2020; Medema et al., 2020; Randazzo et al., 2020; Trottier et al., 2020). The particularity during the summer was also that the tourists invested in masse the city during the day and part of the night for visits, then the restaurants and nightclubs (some received up to 3000 people simultaneously), but they did not stay there permanently. They could therefore emit the virus in the toilets in quantity but were mostly not tested in Marseille. This type of discrepancy would certainly be avoidable by continuously measuring the effluent flow rate, whereas in our study, we assume that this flow rate is always the same. This is true for a constant population, but it can change during periods of high tourist activity. Another hypothesis could be that patients were less symptomatic in summer and therefore less tested. This has been verified in other works and other viral diseases (Shaman et al., 2018; Jones et al., 2020) and may be partly related to the fact that those infected during the summer were on average much younger. Indeed, younger subjects are both less symptomatic and less inclined to be tested with minor symptoms (Kronbichler et al., 2020; Gautret et al., 2021). The last period of interest we have been able to identify is intermediate spanning approximately the month of September. It corresponds to the time of the departure of the tourists from the city, the return of the inhabitants and the beginning of the school year. During this period, we observe a real dissociation between the rates of virus in the sewers, which drop sharply before gradually increasing, while the number of positive cases remains stable. We checked whether this shift was due to interfering factors, such as temperature or precipitation, but found no correlation (Figure 2). There is a time lag between case and virus in the sewers, which is comparable to that observed in the first phase but in reverse. In

total, we can therefore conclude that there are two really different episodes, the epidemic from early July to early September, where the difference between the wastewater virus and the case of covid-19 is significant, then the period from the beginning of October to the end of December, where a real correlation exists. Besides the period (great summer heat) and the different habits (nightlife and more visitors), the last thing that was different over these periods is the distribution of the majority strains. While during the summer the Marseille 1 genotype was predominant, it was the Marseille 4 genotype that predominated over the second period (Colson et al., 2020a, 2020b). The lower severity of the infections linked to this Marseille 1 variant is the last possible hypothesis that could explain this difference (Colson et al., 2020a).

In a second part of this work, we correlated the levels of SARS-CoV-2 RNA in wastewater, the number of newly diagnosed COVID-19 patients and the different measures implemented by the French government, their effect being evaluated between 5 and 10 days after their application. This delay corresponds to the Covid-19 mean incubation period, with a median of 4-5 days from exposure to symptoms, but can be extended to 14 days (Lauer et al., 2020). This incubation period is also similar with that of other known human coronaviruses, including SARS (Varia et al., 2003), MERS (Virlogeux et al., 2016) and non-SARS human coronavirus (Lessler et al., 2009). The first measure implemented was the obligation to wear a surgical mask, first in confined areas and then, one month later, everywhere in Marseille. Systematic reviews have reported that mask use reduces the risk of SARS, Middle East respiratory syndrome and COVID-19 by 66% overall, 70% in health care workers and 44% in the community (Chu et al., 2020), and that the evidence for the effectiveness of masks in preventing respiratory infections is stronger in the health care sector than in the general community (Qaseem et al., 2020; Xiao et al., 2020). However, another study showed that wearing a surgical mask did not reduce the rate of SARS-CoV-2 infection by more than 50% in a community where infection rates are modest, where social distance prevails, and where the general use of masks

294 is uncommon (Bundgaard et al., 2020). In addition, in its guidance on mask use in the context of
295 COVID-19, WHO acknowledges that there is insufficient evidence that wearing a mask protects
296 healthy people from SARS-CoV-2 in June 2020 (WHO, 2020a). Despite several studies, WHO
297 continues to evaluate the evidence on the effectiveness of the use of different masks and their
298 potential harms, risks and disadvantages in December 2020 (WHO, 2020b). In our study, we observe
299 that the number of new Covid-19 infected persons continued to increase despite wearing a mask. This
300 measure seems to be not effective against the spread of Covid-19, but it is also obvious that the
301 wearing of a mask when the outside temperature exceeds 30°C is not bearable and is therefore not
302 worn properly.

303 The second measure implemented was the closure of bars and restaurants at the end of September,
304 mostly on the basis of two American studies that pointed the role of restaurants in contamination.
305 The first study consisted in tracing the movements of 98 million inhabitants of 10 metropolises using
306 data from their telephone during the first epidemic. This study showed that restaurants, gymnasiums,
307 bars and cafés, hotels, limited-service restaurants as well as religious gatherings are the places where
308 the contamination is most prevalent(Chang et al., 2021). The second study, based on a case-control
309 survey of symptomatic outpatients from 11 U.S. health care facilities reported that close contact
310 individuals known to have COVID-19 was associated positivity. Infected adults were about twice as
311 likely to have reported eating out in restaurant as those whose result for SARS-CoV-2 was negative
312 (Fisher et al., 2020). In France, the ComCor study was conducted last October on the places and
313 circumstances of new contaminations. They concluded that the highest risk of transmission of the
314 virus occurs during meals, whether they take place in the private sphere (family, friends) or in public
315 places (cafes, restaurants...) (Galmiche et al., 2020). However, it is to note that this study was
316 conducted during a period of curfew and the beginning of lockdown, where most establishments
317 were partially or completely closed. It is therefore difficult to determine the role that restaurants have

played in the transmission of the virus, since this period did not correspond to their normal functioning. Moreover, the risk even increases during the lockdown than during the curfew, which seems paradoxical since the establishments were then supposed to be completely closed. In our study, the number of Covid-19 positive cases continued to increase despite the total closure of bars and restaurants. Indeed, a recent study (Luo et al., 2020) showed that contact transmission through environmental contamination, probably due to self-inoculation via mouth, nose or eyes through hands, leads to a high risk of infection with SARS-CoV-2, particularly at home and in the toilet. Similar findings were made during SARS-CoV and MERS-CoV epidemics, where the environment in patients's rooms (refrigerator door, bed table, television remote control) was found to be positive (Booth et al., 2005; Bin et al., 2016). In other sites, such as restaurants and marketplaces, no environmental samples tested positive for SARS-CoV-2 (Luo et al., 2020). Another French study confirmed this hypothesis, stating that the measures implemented since September 23rd (in particular the closure of bars and restaurants) had no effect in the weeks that followed (Spaccaferri et al., 2020).

The third measure implemented was the curfew from 9 pm to 6 am. There is little data on the effectiveness of the curfew. As mentioned by the Scientific Council of the French government, the control of the COVID-19 outbreak in Guyana in June-July 2020 was achieved through a combination of different measures implemented (Andronico et al., 2020), including a curfew. However, it is not possible to distinguish the direct impact of the curfew from that of other measures. A French study compared the evolution of SARS-CoV-2 epidemic (second episode) in departments where the curfew was implemented and not (Spaccaferri et al., 2020). The study reported that the departments that have not been subject to curfew are those that have been only slightly or not affected by this epidemic phase. However, the passage to the peak is done at the same time, within 4 days. The authors remain cautious about the interpretation of their results and only raise the question of effectiveness of this measure. In our study, we found a slight decrease in the number of positive cases, but just after the

number of positive cases had peaked. Curfew may also have a small impact on the number of positive cases.

The last measure implemented by the French government was the lockdown on October 30. Numerous studies analyzing French and international epidemiological data have estimated that the first lockdown resulted in a very significant reduction in the rate of transmission of SARS-CoV-2 (Davies et al., 2020; Di Domenico et al., 2020; Flaxman et al., 2020; Hyafil and Moriña, 2020; Prague et al., 2020; Salje et al., 2020). In France, this reduction has been estimated at almost 80% (Di Domenico et al., 2020; Prague et al., 2020; Salje et al., 2020). On the other hand, Spaccaferri *et al.* showed that the increase in the number of daily infections ceased on October 28 in the group of the most affected departments (Spaccaferri et al., 2020). Only one study evaluates the effect of the lockdown on SARS CoV-2 viral RNA in wastewater (Wurtzer et al., 2020a). They noted that their study provides strong indirect evidence for a relevant reduction of virus transmission in response to a lockdown. However, when looking at their results, our conclusion is that the lockdown did not lead to the reduction of SARS-CoV-2 cases, since the virus peak detected in wastewater was observed more than 3 weeks after the implementation of the lockdown. Our study shows that, in our city, lockdown does not play a role in slowing down the rate of contamination. Nor is it associated with death rates in all countries affected by the pandemic, and no one has, to date, provided scientific proof of its long-term benefits (De Larochelambert et al., 2020) . In our study, the number of positive cases began to clearly decrease before the lockdown was implemented. Therefore, although this measurement may have had an effect in our country on the rate of reduction or elsewhere on peak height, it was not the cause. Furthermore, given the very low level of seroprevalence, for which it is inconceivable that this reduction is the effect of herd immunity (Sisay and Tolessa, 2020), there are clearly other factors to be investigated. In 2002, we witnessed the appearance of the SARS-CoV epidemic which lasted about a year and a half, infecting at least 8,000 people and killing 10% of

them (Davis, 2020). Although it mainly affected East Asian countries, by its end, SARS had spread throughout the world. It is accepted that the epidemic was contained by strict quarantine measures in front of a symptomatic viral disease that allowed for the rapid identification and isolation of cases. In this widespread self-satisfaction over our ability to end a pandemic, no one has tried to understand whether this disappearance could be linked to factors other than strict quarantine measures. The results that we report here on the fact that the epidemic linked to the majority clone of the period begins to decrease independently of these measures suggest that for SARS-CoV-2, other factors than containment measures, may play a role. Identifying them could have a major effect on the control of the current pandemic and should avoid the most restrictive that were not proven to be efficient as compared to less restrictive (Bendavid et al.).

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624 **Table 1.** Results of the BioFire COVID-19 using serial dilutions of synthetic SARS-CoV-2 RNA.

Biofire LoD	**Genomic copies / ml**	**Number of replicates**	**Number of target detected**			
			3/3 positive	2/3 positive	1/3 equivocal	0/3 negative
0.15	50	5	0	0	0	5
0.3	100	5	0	0	2	3
0.6	200	5	0	0	4	1
0.9	300	5	0	3	2	0
1.2	400	5	1	3	1	0
1.8	600	5	3	2	0	0
6	2000	5	5	0	0	0

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627 **Figure 1.** Wastewater networks in Marseille. The separate network (red lines) drains the major
628 surface part of Marseille wastewater. The combined network (green lines), that contains a mixture of
629 rainwater and wastewater, drains the city center. The blue circle represents the sampling point

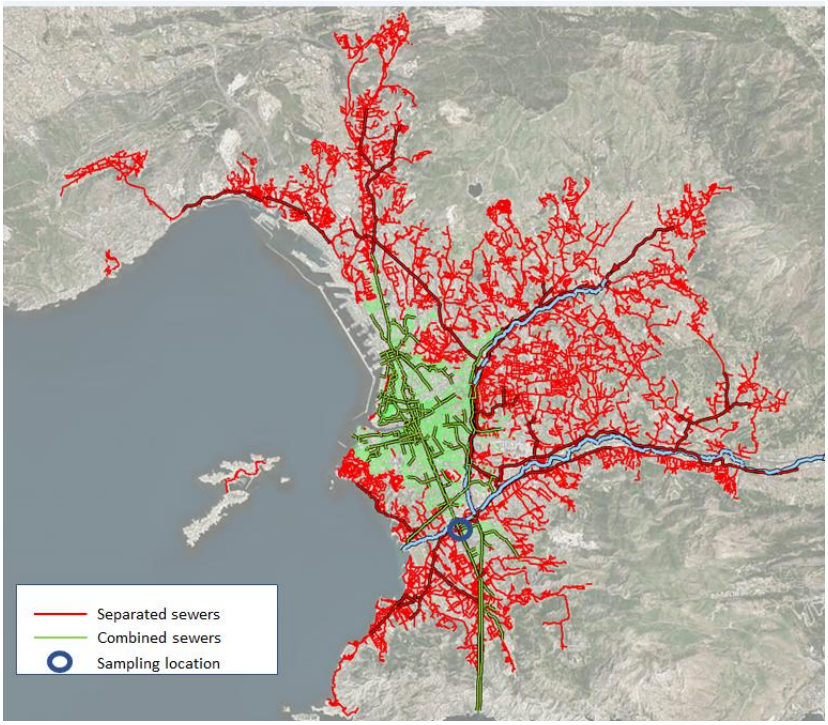

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Figure 2. Variations in mean outdoor temperature (dark grey) and rain fall (light grey) during the period of study (from July 1 to December 15). Copy number of SARS-CoV-2 in RU (green) and RS (red) wastewater networks were represented.

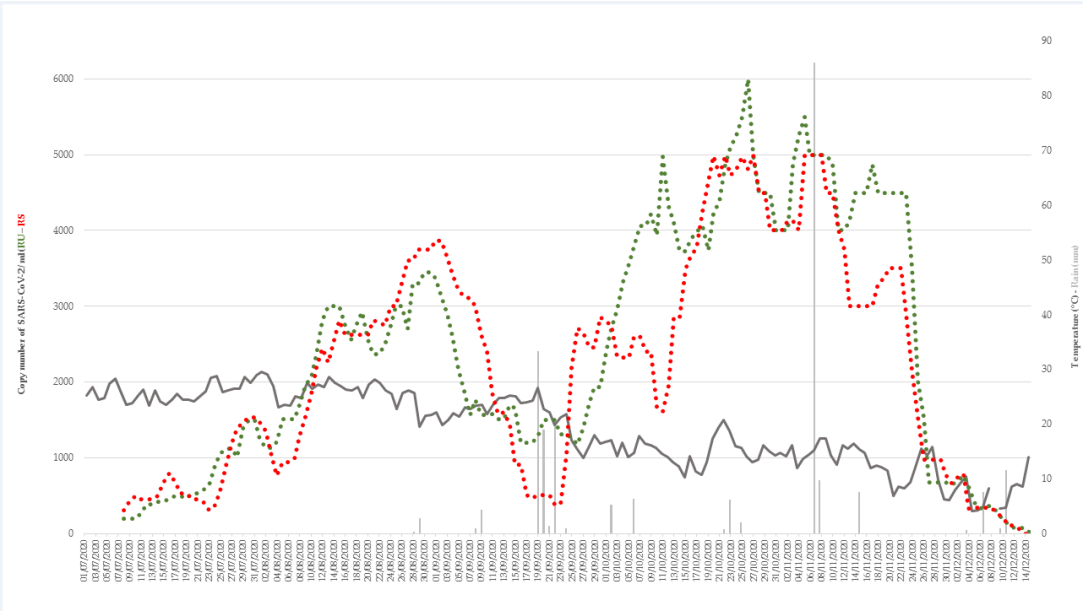
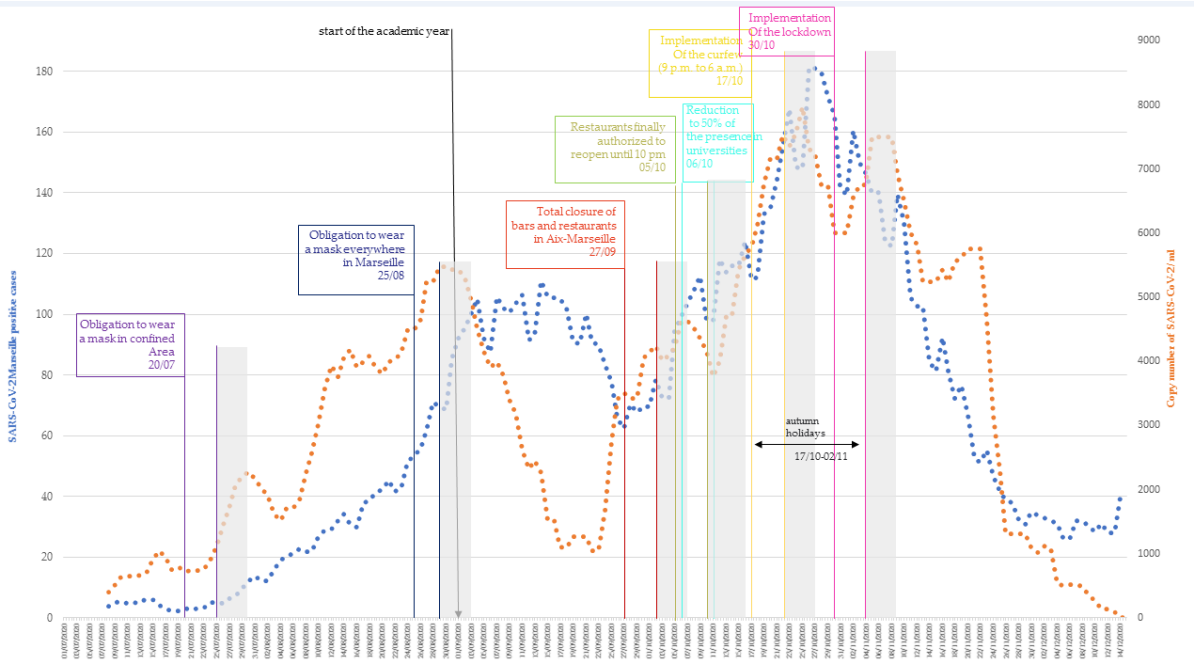


Figure 3. Correlation of SARS-CoV-2 Marseille new positives cases (blue curve) and copy number of SARS-CoV-2 in RU+RS wastewater networks (orange curve). 8-days moving average was represented. The measures implemented by government were positioned at the day of application. In grey, the period where efficiency of the measures can be observed.



CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, AL, PA,BL, and AD; methodology, NW and AL; validation, BL and PA; formal analysis, NW, AL and AGG; investigation, NW, AL, PJ, XF, MV and AA; writing—original draft preparation, NW and AL; writing—review and editing, BL and PEF; project administration, PA and BL; funding acquisition, BL and PA. All authors have read and agreed to the published version of the manuscript.

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STATEMENTS

Nasopharyngeal samples were done at the IHU Mediterranean infection as part of Covid-19 diagnosis and follow-up of patients. The study was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (N°: 2020-029). Informed consent was obtained from all subjects involved in the study.