

1 **Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome**
2 **quantification in Paris wastewaters**

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4 **Wurtzer S¹, Marechal V²⁺, Mouchel JM³, Maday Y⁴⁺, Teyssou R⁵, Richard E¹, Almayrac JL⁶ & Moulin**
5 **L^{1*}.**

6 ¹ Eau de Paris, R&D Laboratory, DRDQE 33 Avenue Jean Jaurès 94200 Ivry/Seine

7 ² Sorbonne Université, INSERM, Centre de Recherche Saint-Antoine, F-75012, Paris, France

8 ³ Sorbonne Université, CNRS, EPHE, UMR 7619 Metis, 75005 PARIS, e-LTER Zone Atelier

9 Seine, France⁴ Sorbonne Université, CNRS, Université de Paris, Laboratoire Jacques-Louis Lions
10 (LJLL), F-75005 Paris, France et Institut Universitaire de France

11 ⁵ Department of Virology, Institut de Recherche Biomédicale des Armées. 1Place du Général
12 Valérie André. BP73. 91223 BRETIGNY-SUR-ORGE

13 ⁶ SIAAP, Service process-laboratoire SIAAP site Seine Amont

14 ⁺ VM and YM are co-founders of the COVID-IA initiative

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16 **Corresponding author: * laurent.moulin@eaudeparis.fr**

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18 **Keywords: SARS-CoV-2, COVID-19, lockdown, quantification, wastewater**

19

20 **Summary**

21 SARS-CoV-2 is the etiological agent of COVID-19. Most of SARS-CoV-2 carriers are assumed to exhibit
22 no or mild non-specific symptoms. Thus, they may contribute to the rapid and mostly silent
23 circulation of the virus among humans. Since SARS-CoV-2 can be detected in stool samples it has
24 recently been proposed to monitor SARS-CoV-2 in wastewaters (WW) as a complementary tool to
25 investigate virus circulation in human populations. In the present work we assumed that the
26 quantification of SARS-CoV-2 genomes in wastewaters should correlate with the number of
27 symptomatic or non-symptomatic carriers. To test this hypothesis, we performed a time-course
28 quantitative analysis of SARS-CoV-2 by RT-qPCR in raw wastewater samples collected from several
29 major wastewater treatment plant (WWTP) of the Parisian area. The study was conducted from 5
30 March to 23 April 2020, therefore including the lockdown period in France (since 17 March 2020).
31 We confirmed that the increase of genome units in raw wastewaters accurately followed the
32 increase of human COVID-19 cases observed at the regional level. Of note, the viral genomes could
33 be detected before the beginning of the exponential growth of the epidemic. As importantly, a
34 marked decrease in the quantities of genomes units was observed concomitantly with the reduction
35 in the number of new COVID-19 cases which was an expected consequence of the lockdown. As a
36 conclusion, this work suggests that a quantitative monitoring of SARS-CoV-2 genomes in wastewaters
37 should bring important and additional information for an improved survey of SARS-CoV-2 circulation
38 at the local or regional scale.

39

40 Introduction

41 SARS-Cov-2 is a positive-sense single-stranded RNA virus of the *Coronaviridae* family and the etiologic
42 agent of COVID-19, a globalized infection affecting more than 2.5 million people worldwide and
43 causing more than 180 000 deaths in total, including more than 160 000 cases in France on April 22,
44 2020. Virus transmission is mainly associated with the projection of respiratory droplets although a
45 possible contamination through aerosols, contaminated hands and inert surfaces is likely. SARS-Cov-
46 2 causes severe complications mostly in elderly or people suffering from comorbidity factors (such as
47 diabetes, hypertension, obesity, acquired or iatrogenic immunosuppression).

48 The viral infection may initiate in the upper respiratory and/or the lower respiratory tracts. Similarly
49 to SARS-CoV-1 (1) and MERS-CoV (2), SARS-CoV-2 genome was also detected in blood and stools (3–
50 5). This argue for a possible enteric phase of the infection although isolation of infectious virus from
51 feces seems difficult (6). Of note diarrhea have been reported in some cases of COVID-19 (6).
52 Importantly, SARS-CoV-2 genomes could be detected in feces several weeks after it could not be
53 detected anymore in oral swabs, suggesting that viral excretion in stools may be longer than oral
54 secretion (7). The presence of viral genomes in stools may open new perspective in the survey of
55 SARS-CoV-2 carriers. It would notably suggest that the virus could possibly be transmitted by a feco-
56 oral route, an hypothesis that should likely deserve a careful examination (8).

57 Management of an epidemic, such as lockdown decision, requires a careful monitoring of the
58 infected population by detecting the virus in carriers through massive or targeted testing.
59 Investigating the proportion of people that have been infected through sero-epidemiological surveys
60 is equally important but antibodies against SARS-CoV-2 will appear only weeks after initial infection
61 (9,10). In the case of COVID-19, due to the lack of systematic and repeated screening of the
62 population, the precise number of infected people is difficult to assess, especially because of the high
63 proportion of infected people that exhibit only few or no symptoms but could secrete and silently
64 transmit the virus (11–14). Depending on screening kit availability and public health policy, testing
65 strategy varies between countries which may explain some discrepancy between worldwide data.
66 Estimating the effective proportion of infected individuals is essential for monitoring the epidemic
67 spread and to propose adapted and efficient control procedures, such as partial or total lockdown.
68 France went into lockdown on the March 17 2020, a decision that was expected to have a major
69 impact on virus circulation especially when asymptomatic carriers are considered to have a strong
70 impact on virus transmission. This decision was motivated by the urgent need to limit exposure of so-
71 called fragile people who are at highest risk to develop the most severe forms of the disease
72 (11,13,15).

73 Analysis of raw wastewaters collected at the inlet of wastewater treatment plants (WWTP) may
74 provide essential information on the health of the human population that is connected to the WWTP.
75 It may notably allow measurement and identification of pathogens or drugs that may be difficult to
76 assess otherwise. Using this method, the European Monitoring Center for Drugs and Drug Addiction
77 follows drugs and their metabolites in the wastewater of several European cities (16). In addition
78 previous works on human enteric viruses in urban river and in raw and treated wastewaters
79 demonstrated that the presence of these viruses was directly linked to epidemic state in the
80 population (17,18). This strongly argue for a close monitoring of fecal viruses in wastewater as a new
81 and complementary tool for investigating human epidemics.

82 Enveloped viruses like coronaviruses are expected to be less resistant than naked viruses that are
83 usually tracked in waste and environmental waters. There is still little information on the persistence
84 of coronaviruses in waters and most of our current knowledge has been inferred from experiments
85 made on surrogate viruses. Recent data suggested that infectious SARS-CoV-2 is particularly resistant
86 in environmental conditions: 3.5 half-life days in the air, 7 days on some surfaces, any reduction at
87 pH between 3 and 10 (6,19). Previous studies on SARS-CoV-1 indicated a significant persistence at 4
88 °C even in wastewater (more than 20 days), or a persistence of at least 1 or 2 days at summer
89 temperatures (1,20,21).

90 Altogether these results led us and others to suggest that the detection of SARS-CoV-2 genomes in
91 wastewater could provide an early and global tool to monitor virus circulation in addition to human
92 epidemiological data (22–24).

93 A first publication underlined the putative benefit of a qualitative approach for monitoring SARS-CoV-
94 2 in wastewaters (24). Other studies used quantitative measurements of viral genomes but the
95 survey only started at the apex of the epidemic (25). Here, we used a specific reverse-transcription
96 quantitative PCR (RT-qPCR) method to precisely quantify SARS-CoV-2 genome equivalents in raw
97 wastewaters of the Parisian area. A 2-months survey covering the lockdown period allowed to
98 observe an increase and a decrease in the total quantities of viral genomes that paralleled the
99 number of new COVID-19 cases in the same region. To our knowledge, this is the first real-time
100 indirect survey of SARS-CoV-2 circulation during a lockdown period.

101

102 **Methods**

103 **Sample collection**

104 Three WWTP (more than 100 000 inhabitants linked to the station from the Parisian area were
105 sampled since the start of the epidemic (March 5th, 2020). Samples were kept at 4°C and processed
106 less than 24 hours after sampling.

107 **Concentration methods**

108 Samples were homogenized, then 11 ml were centrifugated at 200 000 x g for 1 hour at +4°C. Viral
109 pellets were resuspended in 400 µL of PBS 1X buffer.

110 200µL of viral concentrate were lysed and extracted using PowerFecal Pro kit (QIAGEN) on a
111 QIASymphony automated extractor (QIAGEN) according to a modified manufacturer's protocol.
112 Extracted nucleic acids were filtered through OneStep PCR inhibitor removal kit (Zymoresearch)
113 according to the manufacturer's instructions.

114 **Molecular detection method**

115 The RT-qPCR primers and PCR conditions used herein have been previously described (26). The
116 amplification was done using Fast virus 1-step Master mix 4x (Lifetechnologies) with oligonucleotide
117 concentrations recommended previously. Detection and quantification were carried on the E gene by
118 RT-qPCR. Positive results were confirmed by amplification of a region located within the gene
119 encoding for the viral RNA-dependent RNA polymerase (RdRp). An internal positive control (IPC) was
120 added to evaluate the presence of residual inhibitors. The detection limit was estimated to be
121 around 10³ genome units per liter of raw wastewater.

122 The quantification was performed using a standard curve based on synthetic oligonucleotide
123 corresponding to the full-length amplicon on the E gene (SARS-Cov2 Wuhan-Hu isolate sequence
124 NC_045512.2). Amplification reaction and fluorescence detection were performed on ViiA7 Real
125 Time PCR system (Lifetechnologies).

126 **Modelization of Viral RNA excretion**

127 Even if the real number of infected people is unknown, we attempted to compute the expected
128 amount of viral RNA shed in stools, using available information. Since the very beginning of the
129 epidemic, the daily number of patients consulting at the emergency department of greater Paris
130 hospitals and diagnosed with COVID symptoms was published
131 (<https://www.santepubliquefrance.fr/>). Wölfel et al. measured the daily amount of vRNA in swab
132 samples for few patients (27). This data was used as an estimation of viral expression for all infected

133 people and we also assume that people with symptoms would consult 2 days after the onset of the
134 illness, and that at any moment the number of patients with strong symptoms was proportional to
135 the total number of infected people. Accepting the above hypothesis, the convolution of two dataset
136 (i.e. number of consulting patient and model of excretion) is an emission-proxy, proportional to the
137 total amount of viral RNA shed in stools in a given population.

138 **Results**

139 Three major wastewater treatment plants (WWTP) managing over 75 000 cubic meters per day, were
140 sampled at the inlet of the plant from March 5th to April 23th 2020. All processed samples scored
141 positive for the presence of SARS-CoV-2 genomes as assessed by RT-qPCR on the viral E gene. All
142 positive samples were confirmed by RT-qPCR on the viral RdRp gene (Figure 1). The COVID-19
143 epidemic in the same region was illustrated by various indicators such as the total number of COVID-
144 19 cases treated in the regional hospitals, the accumulation of hospitalized patients or the daily
145 death toll linked to COVID 19. Based on these epidemiologic statistics and on published data on virus
146 shedding quantity and delay, an estimated indicator of the viral excretion in the region was
147 calculated and compared to the viral load in wastewaters.

148 Briefly, the concentration of vRNA in raw wastewater was around $5 \cdot 10^4$ genome units /L on the 5th of
149 March 2020. At the same date, less than 10 COVID-19 confirmed patients were reported and only
150 404 individuals were tested positive in France. For the Parisian area more specifically, 91 confirmed
151 cases were reported at that time (on a total number of more than 12 million inhabitants) and no
152 death was recorded. Altogether this information indicated that the COVID-19 epidemic was at an
153 early stage in the Parisian area.

154 The time-course monitoring of viral load in WW displayed an exponential increase (from $5 \cdot 10^4$ GU/L
155 on March 5th to $3 \cdot 10^6$ GU/L, a 2-log increase in average). A peak was observed on the 9th of April,
156 followed by a marked decrease (1-log reduction in average). The shape of the concentration curve
157 was reminiscent of the disease dynamics at the regional level, with an 8-day temporal shift.

158 Altogether these results underline that essential information could be obtain from wastewater
159 epidemical monitoring, such as early starting of the epidemics, evolution of the infections, and
160 impact of the lockdown procedures.

161

162 Discussion

163 It is demonstrated here for the first time that a quantitative detection of SARS-CoV-2 in wastewaters
164 could reflect the circulation of the virus in human populations in the Parisian area, a region called Ile-
165 de-France. Since similar results were obtained from three independent and distant WWTP around
166 Paris with striking similarities, the time-course survey that has been done is likely to be a direct
167 reflect of SARS-CoV-2 dynamic in Parisian inhabitants that are connected to these WWTP. It is to note
168 that at home lockdown is effective since March 17th 2020, therefore limiting daily transport.
169 Importantly no significant rain fall was recorded in the Parisian area that could have had an impact
170 on virus concentration in wastewaters. More surprisingly this decrease stopped after 7 days, and the
171 virus concentration has been stable since. This plateau is intriguing, although the emission-proxy
172 suggests that it can partly be explained by the duration of the virus shedding period, and several
173 hypotheses can be made. First, one may suggest that many infected people are still secreting viruses
174 in their feces whereas the virus is not present anymore in the ORL region. This hypothesis has
175 recently been confirmed in Chinese patients with longer periods of excretion than reported by Wölfel
176 (7). Second, lockdown has been partial since some specific workers have been allowed to pursue
177 essential activities that were not compatible with homeworking. These people are usually not
178 considered at risk of severe infections (i.e. more pauci-symptomatic case), but they may promote
179 virus circulation at a low level notably in their family if they do not strictly respect hands cleaning and
180 mask wearing. Third, one may suggest that lockdown is not respected by few people that maintain
181 virus circulation at a low but significant level. Virus survey in the same WWTP will likely provide some
182 answers in the following weeks.

183 The observed delay between epidemiological curves in humans compared to virus quantification in
184 wastewaters is probably due to several parameters. This may include the effective number of
185 infected people, the timing and temporal kinetics of viral shredding in feces and other causes that
186 are still to be investigated. Nevertheless, our data are in very good agreement with epidemiological
187 parameters such as the number of confirmed COVID-19 patients or our excretion model. To that
188 respect, let us note that our study provides a strong indirect evidence for a significant reduction of
189 virus transmission in response to lockdown. According to our results, the number of people
190 producing SARS-CoV-2 is likely underestimated when based on individual testing, especially during a
191 pandemic where a limited quantity of virological tests did not allow for extensive testing so far. As a
192 comparison the quantity of human enteric virus concentration in raw wastewater is around 10^6 per
193 liter (17).

194 Epidemiological investigations that have been conducted on the Diamond Princess cruise ship
195 suggested that less than 20% of infected people were asymptomatic (12). Most of the infected
196 people were reported to exhibit moderate nonspecific symptoms including fever, headache, body
197 aches, intense tiredness and/or dry cough. However infected people can produce SARS-CoV-2 for a
198 few days before the onset of symptoms and up to several days after recovery (2,7,28). Another
199 extensive study based on Iceland population shows that 43% of SARS-CoV-2 positive patients did not
200 report any symptoms (29). In this context, a clear majority of infected carriers may silently
201 contaminate sensitive people. This led us to suggest that the contamination of raw wastewaters may
202 occur before the significant appearance of clinical cases. The evolution of SARS-CoV-2 viral load in
203 wastewater was in good agreement with the dynamics of pandemic during the first wave of infection
204 in urbanized area, which is also in agreement with the excretion model that is proposed here. To our
205 knowledge this is the first report demonstrating that the quantitative monitoring of SARS-CoV-2 in
206 raw wastewater is a time-related relevant indicator of the evolution of the health status of a
207 population linked to a sewage network. This quantitative approach was especially useful to
208 unveil the dynamics of the pandemic and follow impact of government measures such as
209 containment.

210 To finish, this data, if carefully utilized, could help to describe the proportion of SARS-CoV-2 excretors
211 during all the monitored pandemic event and allow to calculate the immunity of the population,
212 especially at the local level.

213 **Conclusions**

214 Our results strongly argue for the use of a quantitative monitoring of SARS-CoV-2 genomes in urban
215 wastewaters. This would also argue for a long-time conservation of wastewater samples in dedicated
216 local i.e. wastewater-bank, which would allow a retrospective investigation of pathogens circulation.
217 Additionally, wastewaters survey may provide an alternative and possibly early tool to detect
218 pathogens in populations when investigations in humans are difficult to conduct for logistic, ethical
219 or economic reasons, notably in poor countries that are strongly exposed to COVID-19 epidemic.

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221

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299

300 **Acknowledgment**

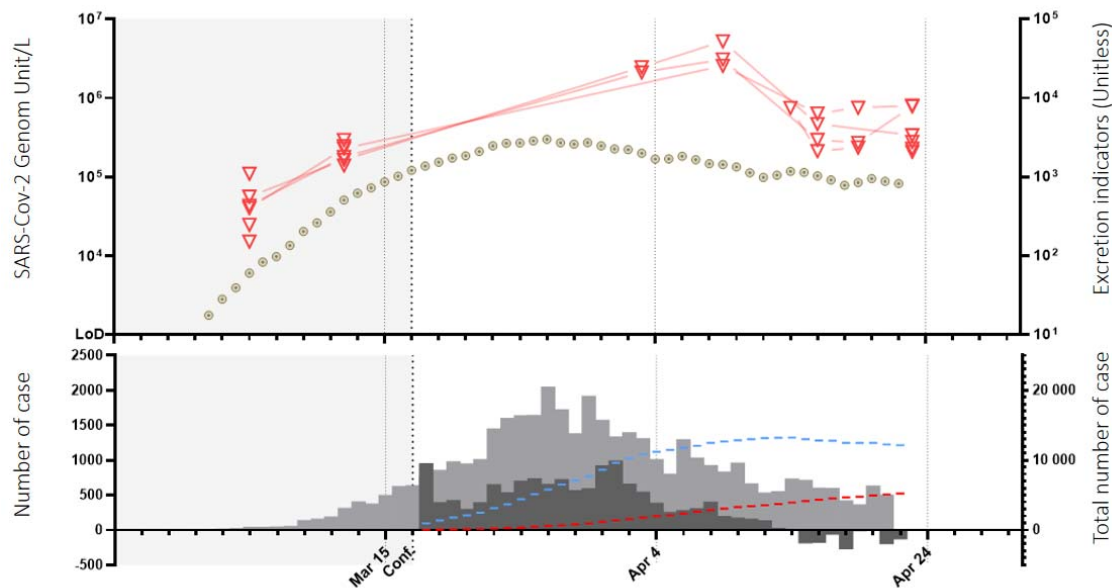
301 Olivier Rousselot (director of “laboratoires et de l'environnement”, SIAAP) is acknowledged for his
302 support and critical reading of the manuscript. Vincent Rocher for management of sampling at the
303 SIAAP Innovation department. Technical assistance from the R&D Eau de Paris Laboratory members,
304 and Alban Robin for helpful support.

305 **Contribution**

306 SW, ER performed the virus measurements; SW, LM started the project; JLA, SW provided samples;
307 LM, VM, JMM, SW for the redaction of the manuscript; YM, RT, JLA technical and theoretical discussion
308 .

309 **Funding**

310 Analysis were carried on the Eau de Paris 2020 Research grant. Samples were provided by SIAAP.



311

312 **Figure 1: Upper panel: Quantification of SARS-Cov-2 in wastewater samples in Parisian Area in**
313 **different WWTP (open inverted red triangles for important WWTP, purple open inverted triangles**
314 **for smaller WWTP) in circle estimators of the viral excretion.**

315 **Lower panel : In Light grey area, daily number of consultation for COVID 19 Symptoms in hospital**
316 **of the Parisian area. Dark grey daily growth of hospitalized patient. Blue bar total hospitalized**
317 **patient in the Parisian area, red bar cumulative deaths each day, in Parisian area.**

318 **Both panel : grey background, pre-lockdown period.**

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322 **Supplementary data**

323 None

324