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Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases

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Comments/Letter

Since many SARS-CoV-2 carriers are assumed to exhibit no or few non-specific symptoms (1–3), SARS-CoV-2 circulation among human populations may be detected too lately and only when massive human testing is available or when clinal COVID-19 cases are reported. This is obviously a major pitfall for evaluating and possibly controlling the current epidemic. Due to the presence of SARS-CoV-2 in stool samples (4), qualitative detection of SARS-CoV-2 in wastewaters has recently been proposed as a complementary tool to investigate the virus circulation in human populations (5). If this assumption is correct, SARS-CoV-2 relative amounts in wastewaters should correlate with the number of confirmed COVID-19 cases. To test this hypothesis, we performed a time-course quantitative analysis of SARS-CoV2 by RT-qPCR in 23 raw and 8 treated wastewater samples collected from 3 major wastewater treatment plant (WWTP) of the Parisian area collecting inhabitants reject. This study was conducted from 5 March to 7 April 2020. Viral particles were concentrated by ultracentrifugation from 11ml samples, and viral genomes were extracted by using an optimized protocol (PowerFecal Pro kit on a QIAsymphony extractor, QIAGEN). Environmental inhibitors were removed using PCR inhibitor removal resins (Zymoresearch). The RT-qPCR primers have been previously described within viral E gene (6). Quantification was performed using a standard curve based on synthetic oligonucleotide corresponding to

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the full-length amplicon on the gene E (113 bases). Positive results were confirmed by amplification of a region from the viral RNA polymerase-RNA dependent (6). The quantification limit was 10^3 equivalent viral genomes per liter.

All raw wastewater samples scored positive for SARS-CoV2. Additionally, 6 out of 8 samples from treated wastewater scored positive by RT-qPCR. Treated wastewater effluents showed a 100 times reduction in the viral load compared to the corresponding raw wastewater samples, which is in agreement with previous work on enteric viruses (7). We next compared the average level of SARS-CoV2 genomes in wastewater samples over time with the number of confirmed fatal cases of COVID19 in Paris area and in France (fig 1). As expected, we confirmed that the increase of genome units in raw wastewaters accurately followed the increase in the number of fatal cases observed at the regional and national level (Data obtained from "Santé Publique France"). Therefore, our study demonstrates that the contamination of wastewater and the detection of viral genome occurred before the beginning of the exponential growth of the epidemic. Our research group is currently evaluating the presence of infectious viruses in wastewater samples.

As a conclusion, this work demonstrated that a quantitative monitoring of SARS-CoV2 genomes in wastewaters should bring important and additional information for better survey of SARS-CoV2 circulation at the local or regional scale. This would notably argue for the long-time conservation of wastewater samples in dedicated local wastewater-bank, which would allow a retrospective investigation of pathogens circulation. Additionally, wastewater survey may provide an alternative and possibly early tool to detect pathogens in populations when investigations in humans is difficult for logistic, ethical or economic reasons.

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Conflict of interest.

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The authors declare no conflict of interest

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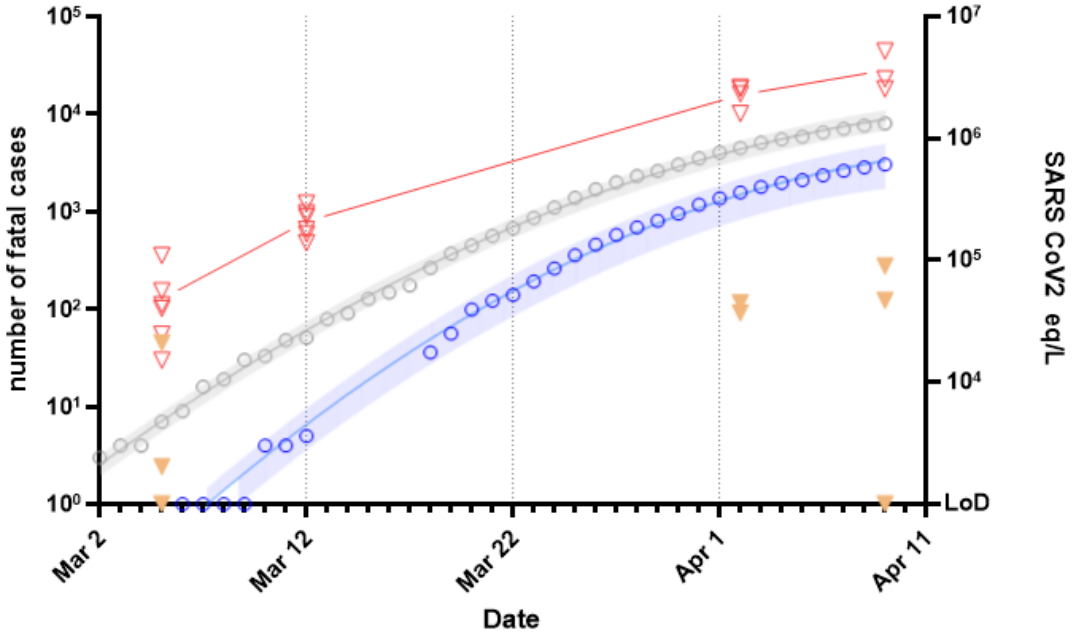

Figure 1: Quantitative time-course monitoring of SARS-CoV2 in wastewater samples from Paris area

Quantification of SARS-CoV2 genomes in raw (open inverted red triangles) or treated wastewater (full inverted red triangles) from 3 WWTP of the Parisian area. The numbers of COVID-19 fatal cases in France (in grey) or in the Parisian area (open circle in dark blue) are indicated. Gaussian curves were fitted on data (r^2> 0.98) for modeling the Parisian area where information was lacking.