SARS-CoV-2 in human sewage in Santa Catalina, Brazil, November 2019

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Abstract

We analysed human sewage located in Florianópolis (Santa Catalina, Brazil) from late October until the Brazil lockdown on early March. We detected SARS-CoV-2 in two samples collected independently on 27th November 2019 (5.49±0.02 log genome copies/L). Subsequent samplings were positive until 4th March 2020 (coinciding with the first COVID-19 case reported in Santa Catalina), with a SARS-CoV-2 RNA increase of one log (6.68±0.02 log genome copies/L). Our results show that SARS-CoV-2 has been circulating in Brazil since late November 2019, much earlier than the first reported case in the Americas (21st January 2020, USA).

Keywords:

SARS-CoV-2, COVID-19, human sewage, Brazil, epidemiology, surveillance

The first cases of atypical pneumonia related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were described in Wuhan City, Hubei Province (China) on December, 2019 [1]. However, some indirect evidences could suggest that the virus had already been circulating a few months previously [2]. After the first reports, the number of cases increased exponentially around the world, and the WHO declared the status of pandemic on 11th March 2020. As of 24th June, 2020, SARS-CoV-2 has caused more than 9,1 M COVID-19 cases worldwide resulting in more than 473,000 deaths in 216 countries. The first diagnosed COVID-19 case in the Americas was reported on 21st January 2020 in the USA (on 25th February in Brazil) [1]. Since then, almost half of the diagnosed cases (49.4%) and deaths (47.8%) worldwide have been reported in that continent [1].

SARS-CoV-2 is a respiratory virus and the transmission route is mainly through respiratory droplets or contact with contaminated surfaces [3]. However, gastrointestinal and liver manifestations with high and prolonged faecal shedding of up to 10^8 SARS-CoV-2 genome copies per g of stool have reported [4,5]. Consequently, the faecal-oral transmission of SARS-CoV-2 cannot be ruled out, although its role in COVID-19 epidemiology has not been yet determined [4].

The analysis of human and animal sewage has been successfully applied in monitoring the presence of biological risks [6], including enteric viruses [7]. A similar monitoring strategy has been applied for the detection of SARS-CoV-2 [8-15], demonstrating its value as a non-invasive early-warning tool for monitoring the status and trend of COVID-19 infection [12].

Sampling urban sewage in Florianopolis, Santa Catalina, Brazil

We collected urban sewage samples in Florianopolis, Santa Catalina, Brazil (Figure 1) in 6 independent sampling events since 30th October, 2019 until 4th March, 2020. Raw sewage samples were collected from a separate collection sewage system in central Florianopolis, serving a population of approximately 5,000 inhabitants. The sampling was conducted using a submersible pump inside a well, used for inspection and cleaning of the network. The collection system receives wastewater exclusively, with a linear infiltration rate varying from 0.05 – 1 L / s Km, according to the Brazilian regulation for sanitation projects (ABNT – Associação Brasileira de Normas Técnicas

n.9646/1986). In all the sampling events, 200 ml of urban sewage was collected and immediately transferred to the Applied Virology and Environmental Engineering Laboratory of the Federal University of Santa Catarina (Brazil), and stored at -80ºC until its use. The concentration of the viral particles was performed using 25 mL of each sample as previously described [16]. Murine Norovirus (MNV-1) was artificially inoculated as sample process control virus to estimate the efficiency of extraction and the final viral load [17]. Viral RNA was extracted from concentrates using the QIAamp® Viral RNA Mini kit (QIAGEN, CA, USA) according to the manufacturer's instructions, and eluted in 200 µL of RNAse free water. Viral RNA was detected by real-time RT-PCR (RT-qPCR) on a 7500 Real-Time PCR instrument (Applied Biosystems, USA). Sample process control virus, MNV-1, was detected as previously published [18]. SARS-CoV-2 was detected by OneStep qPCR Quantinova kit (QIAGEN, Germany) using oligonucleotides and probe previously published targeting the N1 [19], S and RdRp [20] regions of the SARS-CoV-2 genome. Oligonucleotides and a commercial SARS-CoV-2 RNA (2019-nCoV_N_Positive Control, 2×10^5 genome copies/µL) as positive quantitative control were purchased from IDT (Integrated DNA Technologies, Belgium). Samples were considered as positive when the Cq values were ≤ 38.00. All RT-qPCRs were performed in duplicate in two independent experiments. All the positive samples were confirmed using the Seegene Allplex™ 2019-nCoV commercial kit in an independent laboratory (Laboratório de Biologia Molecular, Microbiologia e Sorologia, UFSC University Hospital, Florianopolis, Brazil).

The main physical-chemical features of urban sewages sampled are shown in Table 1. Viral RNA extraction from the sewage samples worked adequately considering the nature of the matrix, showing process efficiency values ranging from 1.6 % to 2.6 %, with an overall value of 2.1% ± 0.2% (M ± ES). Notably, while samples were negative in the first two sampling events (30th October and 6th November, 2019), all samples in subsequent events were positive (since 27th November, 2019 until 4th March, 2020) (Figure 2). The overall load was 5.83 ± 0.12 $\log_{10}$ SARS-CoV-2 genome copies L^{-1}, ranging from 5.49 ± 0.02 (27th November, 2019) to 6.68 ± 0.02 (4th March, 2020) (Figure 2). Those SARS-CoV-2 RNA loads are similar to those found in studies performed in France [15], Spain [12], and USA [11,14]. Remarkably, SARS-CoV-2 RNA

was detected as early as 27th November, 2019, 66 days in advance of the first COVID-19 confirmed case in the Americas (in the USA), 91 days in advance of the first case in Brazil, and 97 days in advance of the first confirmed case in Santa Catalina Region. This demonstrates that SARS-CoV-2 was being shed within the community for several months prior to the first cases being reported by regional, national or Pan-American authorities. Few data are available on retrospective studies of SARS-CoV-2 RNA detection in sewage prior to onset of COVID-19 clinical cases in the region of the study. Randazzo et al. [12] conducted a retrospective study of wastewater process plants in the Murcia Region, Spain, and compared the observed data to declared COVID-19 cases at municipality level: the presence of SARS-CoV-2 RNA in sewage was also earlier than the first reported cases.

Interestingly, while the SARS-CoV-2 RNA loads were stable until late February (5.49 ± 0.02, 5.82 ± 0.01 and 5.65 ± 0.10 $\log_{10}$ SARS-CoV-2 genome copies L^{-1} in 30th November, 2019, 11th December, 2019 and 20th February, 2020, respectively), an increase of approximately 1 $\log_{10}$ was observed on 4th March 2020 (6.68 ± 0.03 $\log_{10}$ SARS-CoV-2 genome copies L^{-1}), coinciding with the first COVID-19 case diagnosed in the region. Unfortunately, 10 days after the last sampling, the Federal Brazilian government ordered a national lockdown which impaired the continuation of the sampling and therefore the confirmation of that increasing tendency.

In conclusion, we have confirmed the presence of SARS-CoV-2 in the Americas as early as 27th November, 60 days ahead of the reports of COVID-19 cases in the continent and more than 90 days in the case of Brazil. Therefore, our findings demonstrate that SARS-CoV-2 was circulating unnoticed in the community for some months before pandemic status was declared. Our results also show that the SARS-CoV-2 load remained constant until early March, then rose coinciding with the onset of COVID-19 cases in Santa Catalina region. Consequently, this study demonstrates that monitoring of SARS-CoV-2 in wastewater or urban sewage is an excellent tool for anticipating potential epidemiological outbreaks, and would be highly valuable in helping Public Health authorities to define protection measures.

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Table 1. Sewage system and physical-chemical characterization

	Sewer at collection point			Sewage physic-chemical characterization				
Samples collected	Average Flow ($L.s^{-1}$)	Diameter (mm)	Population served (inhabitants)	COD [a] ($mg\ L^{-1}$)	BOD [b] ($mg\ L^{-1}$)	pH	Alkalinity ($mgCaCO_3\ L^{-1}$)	TSS [c] ($mg\ L^{-1}$)
	1.5	100 mm	5.000	703	338	7.9	320	142

[a] COD: Chemical Oxygen Demand
[b] BOD: 5 days Biochemical Oxygen Demand
[c] Total Suspended Solids

Figure 1: Maps of the sampling location in Florianópolis city, Santa Catarina State, Brazil.

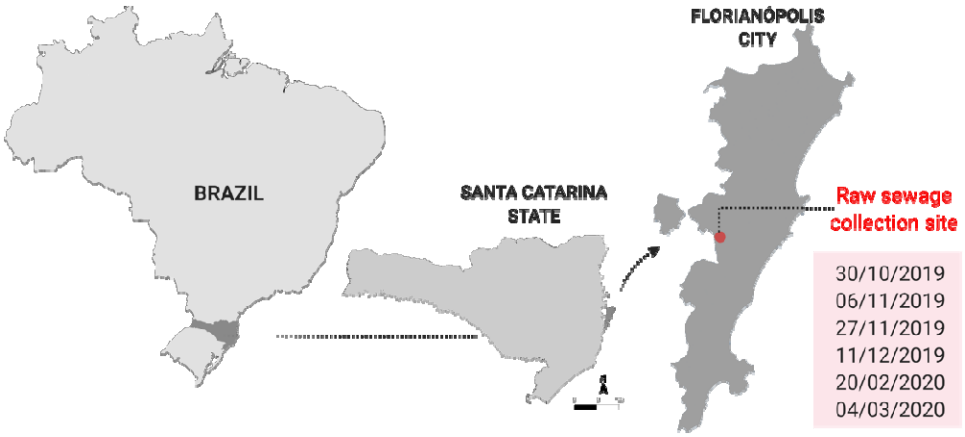
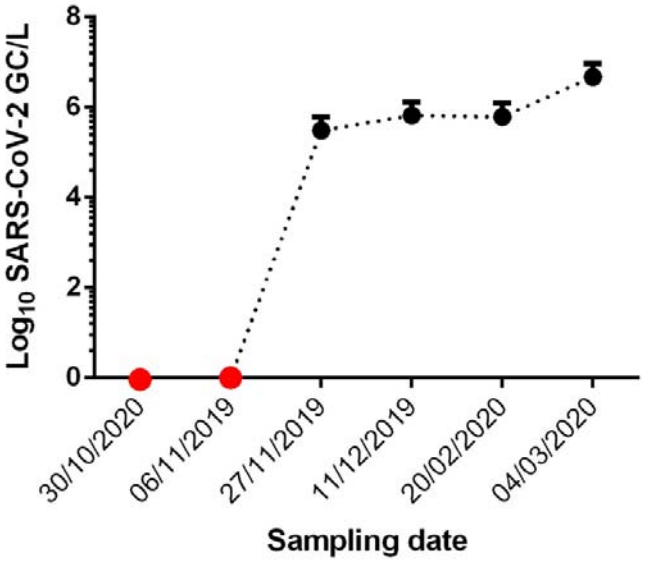
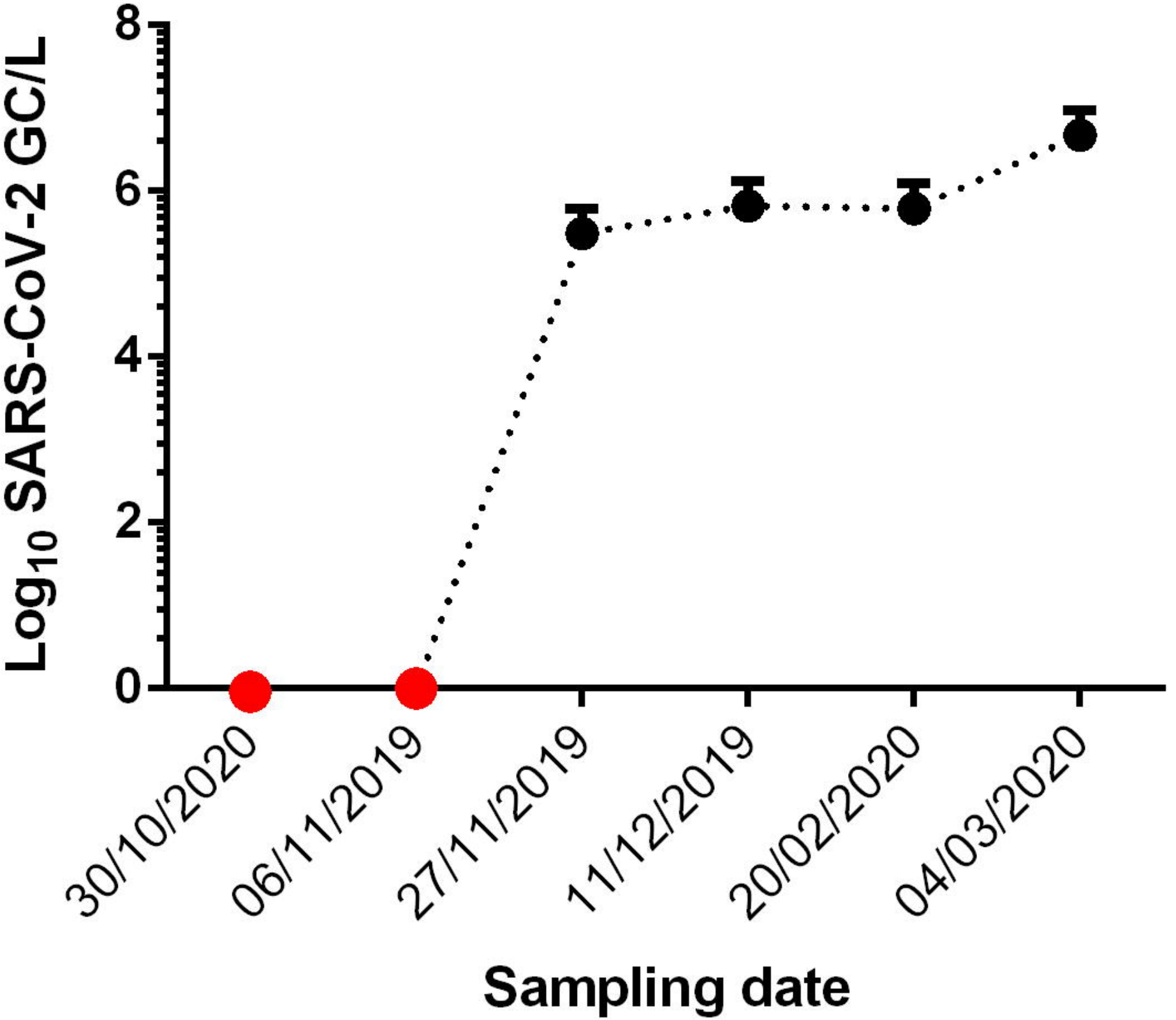


Figure 2: Evolution of the presence of SARS-COV-2 RNA in urban sewage, Florianopolis, Brazil. Red dots represent negative results. Black dots represent log_{10} SARS-CoV-2 genome copy per litre of urban sewage (M ± ES)




FLORIANÓPOLIS
CITY
BRAZIL
SANTA CATARINA
STATE
Raw swage
collection site
30/10/2019
06/11/2019
27/11/2019
11/12/2019
20/02/2020
04/03/2020


Log10 SARS-CoV-2 GC/L
8
6
4
2
0
30/10/2020
06/11/2019
27/11/2019
11/12/2019
20/02/2020
04/03/2020
Sampling date