RECOMMENDED CITATION
Prado T, Fumian TM, Mannarino CF, Maranhão AG, Siqueira MM, Miagostovich MP. Preliminary results of SARS-Cov-2 detection in sewerage system in Niterói municipality, Rio de Janeiro, Brazil [Submitted]. Mem Inst Oswaldo Cruz E-pub: 28 Apr 2020.doi: 10.1590/0074-02760200196.

PRELIMINARY RESULTS OF SARS-CoV-2 DETECTION IN SEWERAGE SYSTEM IN NITERÓI MUNICIPALITY, RIO DE JANEIRO, BRAZIL

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Financial support: This study was supported by Instituto Oswaldo Cruz (PAEF/2), Fundação de Amparo à Pesquisa do Rio de Janeiro (Faperj-E-26/202.821/2018) and, CNPq (306655/2018-7).

Abstract

This study presents preliminary results from a sewage-based surveillance to monitor the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the municipality of Niterói, State of Rio de Janeiro, Brazil. By using ultracentrifugation method associated to RT-qPCR we detected SARS-CoV-2 in 41.6% (5/12) of raw sewage samples obtained from sewage treatment plants and sewers network in the city. This pioneer study carried out in Brazil aims to subsidize information for health surveillance concerning the viral circulation in different areas of the city and, revealed the insertion and importance of Environmental Virology in Health Public Policies.

Keywords: SARS-CoV-2, sewage, Niterói.

Similar to many other viruses-causing respiratory syndromes, the main transmission route of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is through respiratory droplets generated by coughing and/or sneezing, although the route of contamination by fomites is also considered [(1)]. However, diarrhoea has been described in a significant number of cases (incidences varying from 2% to 50% of cases), with viral loads ranging up to 1 x 10^6 genome copies per g of faecal material [(2,3)]. Consequently, the presence of SARS-CoV-2 has been also detected in sewage samples from different countries, such as the Netherlands, Australia, France and China [(4,5,6,7)]. These studies have demonstrated the importance of sewage-based surveillance, for an early detection of news

cases, as recently found in the shedding/excretion of coronavirus by infected people two to three days before the onset of symptoms [8].

In this context, a pilot project was underway to investigate the dissemination of SARS-CoV-2 in sewage system in areas of interest for health surveillance in the city of Niterói, in order to monitor the transmission pattern over the period of the epidemic. Niterói notified the first COVID-19 case on 12th March 2020, and by now, seventeen fatal cases associated with COVID-19 were reported [9]. Until April, 2020 (17th epidemiological week), the city had 254 confirmed cases of SARS-CoV-2, distributed in 32 of its 52 neighbourhoods [9]. The maximum lethality rate was of 14.29%, in the second week after the beginning of the registered cases, decreasing to 6.69% in the last epidemiological week [9].

On April 15th, raw sewage samples were obtained at 12 different sampling points in the city of Niteroi, including sewage treatment plants (STPs), hospital wastewater and sewers network (Figure). Ten-hour composite sewage samples were collected in sterile polypropylene bottles and pasteurized at 60°C for 90 minutes to inactivate the virus [6]. The concentration of the viral particles was performed using ultracentrifugation method, as previously described [10]. For detection of SARS-CoV-2, 140 µl of suspended viral concentrates were extracted using the QIAamp® Viral RNA Mini kit (QIAGEN, CA, USA) and a QIAcube® automated system (QIAGEN) and coronavirus RNA was detected by RT-qPCR. Primers and probe previously published by the CDC (Centers for Disease Control and Prevention, 2020) [11] targeting the N2 region of SARS-CoV-2 genome was used and RT-qPCR was performed in a 15 µl final volume reactions, according to manufacturer's instructions (Biomanguinhos, Fiocruz, Rio de Janeiro, Brazil). The reactions were carried out in duplicate on undiluted and diluted (1:10) RNA samples.

Reactions were considered positive for samples showing cycle threshold (Ct) values below 40 cycles recorded for at least two of the four wells tested for each sample.

We detected SARS-CoV-2 RNA in five of 12 (41.7%) samples that presented mean cycle threshold (Ct) values ranging from 36.3 to 39.8 (Table). All positive samples had at least two positive reactions, from diluted or undiluted samples. The majority of positive samples were detected in samples collected from Icaraí neighbourhood, and reflects the number of reported COVID-19 cases (70) until the collection date. We also detected SARS-CoV-2 RNA in one sample at Camboinhas STP, demonstrating the expansion of the outbreak to other areas of the city (Figure).

Since there is still no consensus on the use of a set of more effective primers to detect SARS-Cov-2 in environmental samples [(4)], we believe that new studies using primers targeting different genome regions should be conducted to compare the effectiveness of the method.

To the best of our knowledge, there is no direct evidence to prove that the SARS-CoV-2 detected in the sewage system is infectious and contagious. Therefore, it is early to state that wastewater could be considered an important route of transmission. However, with the increase circulation of the virus in the population, the concentration of coronavirus particles in sewage waters could reach significant levels. Thus, it is important to monitor the occurrence and dissemination of SARS-CoV-2 in the sewerage system, identifying COVID-19 hotspots areas, as well as those areas with underreported cases in the health system. Through assessment of viral load and its distribution in sewers network in different areas of the city, the monitoring of the coronavirus circulation during the epidemic will subsidize information for health surveillance, allowing optimizing the use of available resources and strengthening measures for prophylaxis in the area. This study confirms

wastewater-based surveillance as a promising approach to understand the prevalence of the virus in a given community and the insertion of Environmental Virology in Health Public Policies [12].

Acknowledgements

The authors thanks teams from Niterói Municipality and Águas de Niterói in support of sample collections and to Sérgio de Silva e Mouta Júnior for help with sample processing. This research study is under the scope of the activities of the Oswaldo Cruz Foundation (Fiocruz) as a Collaborating Centre of PAHO/WHO of Public and Environmental Health.

The authors declare that they have no conflict of interest related to this work.

Authors' Contribution

TP and TMF contributed to the dissertation of the manuscript and evaluation and execution of methodologies, with evaluation and sample collection, AGM performed virus concentration, MMS, editing and revising the te MPM coordinating the study, supervising and revising the manuscript.

References

1 - Sohrabia C, Alsafib Z, O'Neilla N, Khanb M, Kerwanc A, Al-Jabirc A, Iosifidisa C, Aghad R. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surgery. 2020; 76: 71-76. https://doi.org/10.1016/j.ijsu.2020.02.034

2 - D'Amico F, Baumgart DC, Danese S, Peyrin-Biroulet L. Diarrhea during COVID-19 infection: pathogenesis, epidemiology, prevention and management. Clin Gastroenterol Hepatol. 2020 Apr 8. pii: S1542-3565(20)30481-X. doi: 10.1016/j.cgh.2020.04.001.

3 - Zhang N, Gong Y, Meng F, Bi Y, Yang P, Wang F. Virus shedding patterns in nasopharyngeal and faecal specimens of COVID-19 patients. medRxiv, 2020; in press, doi:10.1101/2020.03.28.20043059.

4 - Ahmed W, Angel N, Edson J, Bibby K, Bivins A, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Environ. 2020; https://doi.org/10.1016/j.scitotenv.2020.138764

5 - Medema G, Heijnen L, Elsinga G, Italiaander R. Presence of SARS-Coronavirus-2 in sewage. 2020; https://doi.org/10.1101/2020.03.29.20045880 doi: medRxiv preprint.

6 - Wu FQ, Xiau A, Zhang JB, Gu XQ, Lee WL, Kauffman K, Hanage WP, et al. SARS-Cov-2 titers in wastewater are higher than expected from clinically confirmed cases. 2020; doi: https://doi.org/10.1101/2020.04.05.20051540.

7 - Wurtzer S, Marechal V, Mouchel J-M, Moulin L. Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. medRxiv 2020.04.12.20062679; doi:https://doi.org/10.1101/2020.04.12.20062679

8 - He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F,

Cowling BJ, Li F, Leung GM. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med. 2020; doi: 10.1038/s41591-020-0869-5.

9 - ICICT – Instituto de Comunicação e Informação Científica e Tecnológica em Saúde. 2020. MonitoraCovid19. [updated 2020 April 12]. Available from: https://bigdata-covid19.icict.fiocruz.br/.

10 - Pina S, Jofre J, Emerson SU, Purcell RH, Girones R. Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. Appl Environ Microbiol. 1998; 64(11): 4485-8.

11 - US Centers for Disease Control and Prevention. 2019-Novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. [Updated 2020 April]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

12 - Machado CJS, Miagostovich MP, Leite JPG, Vilane RM. Promoção da relação saúde–saneamento–cidade por meio da Virologia Ambiental. Revista de Informação Legislativa. Ano 50 Número 199 jul./set. 2013.

Table: Detection of SARS-CoV-2 according to collection sites at Niterói.

Sampling Points	Collection Points Description (Neighbourhood – Address)	Cycle Treshold*
P1	Centro - Icaraí Hospital – 233 Marquês de Paraná St.	-
P2	Fátima – Carlos Tortelly Municipal Hospital, 266 Ataíde Parreiras St.	-
P3	Ingá - Morro do Palácio slum, 11 de agosto St.	-
P4	Icaraí – 95 Dr. Paulo César St.	38,7
P5	Icaraí – 245 Cinco de Julho St.	36,3
P6	Icaraí - 17 Sete de Setembro Ave.	-
P7	Icaraí - Icaraí STP, 510 Lemos Cunha St.	36,8
P8	Icaraí - 504 Ary Parreiras Ave.	38,5
P9	São Francisco - Morro do Cavalão slum, Leopoldo Fróes Rd.	-
P10	Charitas – Preventório slum, Sílvio Picanço Ave./14 de Abril St.	-
P11	Jurujuba – Eugênio José Bernardes St./Matos St.	-
P12	Camboinhas – Camboinhas STP, Projetada St.	39,8

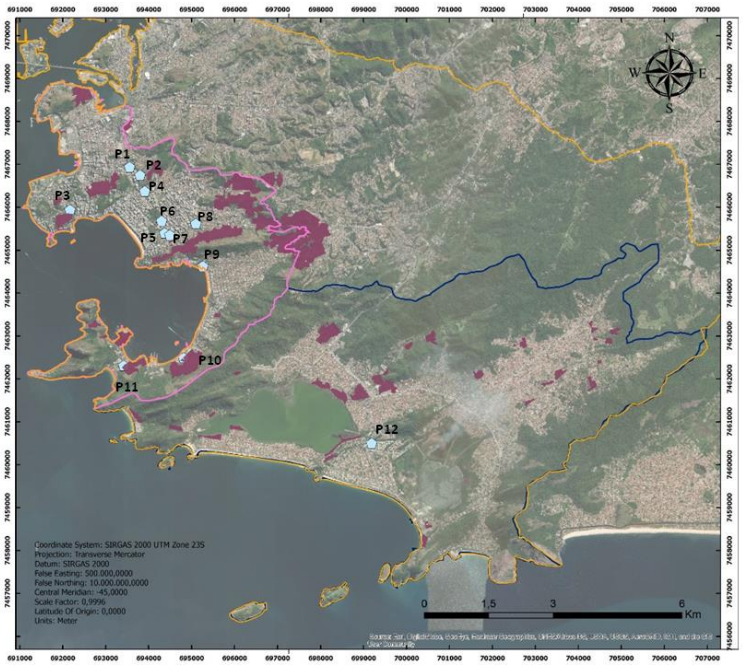

Figure: Map of the municipality of Niterói with sampling points.

Source: SIGEO - Sistema de Gestão da Geoinformação. Secretaria de Planejamento, Orçamento e Modernização da Gestão. Prefeitura de Niterói.