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WASTEWATER AS AN INDICATOR OF COVID-19: MONITORING OF MASS GATHERINGS IN A CITY OF LEGAL AMAZON ON THE NORTH OF BRAZIL

ÁGUAS RESIDUAIS COMO UM INDICADOR DE ALERTA PARA COVID-19: MONITORAMENTO DE EVENTO DE MASSA EM UMA CIDADE DA AMAZÔNIA LEGAL NO NORTE DO BRASIL

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ABSTRACT

One of the biggest challenges on health surveillance during COVID-19 pandemic was surveillance and detection of new cases of infection with the objective of stopping fast disease propagation. Mass gatherings are receiving attention from public authorities, considering their roles in circulating COVID-19 virus on an extensive contingent of people. Considering the limitation for mass laboratory tests, high rates of sub-notification and asymptomatic infections, some complementary surveillance strategies were implemented in many countries, such as sewage epidemiological surveillance. This study aimed to detect the presence of SARS-CoV-2 on sewage of the Araguaína-TO, Brazil, community during a mass gathering; a traditional agricultural exposition that gathered many people in a few weeks. Quantification of SARS-Cov-2 through sewage

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was performed by RT-qPCR and the detected viral loads were normalized using the population served by the studied sewage points. Data generated were compared to the city's epidemiological data of clinical and laboratory trials of COVID-19 displaying a sub-notification of infections. Amidst vaccination progression, reduction of symptomatic infections and return of events, sewage monitoring presented itself as a fast alert system, exposing virus circulation in the city before and after an increase in case notifications. Results demonstrated a tool to aid surveillance, indirectly testing hundreds of people through a single sample, therefore, enhancing the health system to give assistance to new cases.

Keywords: SARS-CoV-2; Environmental surveillance; Sewage-based epidemiology; People gathering.

RESUMO

Um dos maiores desafios da vigilância sanitária na pandemia da COVID-19 foi o monitoramento e a detecção de novos casos de infecção, na tentativa de impedir rapidamente a propagação da doença. Eventos de Massa têm merecido crescente atenção das autoridades públicas, por favorecer a circulação do vírus da COVID-19 em um grande contingente de pessoas. Considerando a limitação da testagem laboratorial em massa, as elevadas taxas de subnotificação e as infecções assintomáticas, foram implementadas estratégias complementares de vigilância em vários países, como a vigilância epidemiológica baseada no esgoto. O presente estudo objetivou detectar a presença de SARS-CoV-2 na rede esgoto, em escala comunitária no município de Araguaína-TO, durante um evento de massa, uma tradicional exposição agropecuária, que mobilizou em poucas semanas uma grande circulação de pessoas. A quantificação do SARS-CoV-2 via esgoto foi realizada por RT-qPCR e a carga viral detectada foi normalizada pela população atendida pelo ponto de ligação à rede de esgoto estudada. Os dados gerados foram comparados aos dados epidemiológicos municipais notificados da testagem clínica e laboratorial da COVID-19 revelando a subnotificação das infecções. Com a progressão da vacinação, redução de casos sintomáticos e retomada de eventos, o monitoramento do esgoto se apresentou como um sistema de alerta rápido, revelando a circulação do vírus na cidade antes e durante o aumento de casos notificados. Os resultados demonstraram uma ferramenta de apoio à vigilância, testando indiretamente centenas de pessoas por meio de uma única amostra, assim potencializando o sistema de saúde para assistência dos novos casos.

Palavras–chave: SARS-Cov-2; Vigilância Ambiental; Epidemiologia baseada em esgoto; Aglomeração de pessoas.

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INTRODUCTION

Among various impacts created by the COVID-19 pandemic, one that can be highlighted is mass gatherings. Mass gatherings are meetings of a considerable contingent of people, generally motivated by work, politics, sports, religions or entertainment activities, which may or may not be previously organized and that can have consequences to society, including the public health sector[1,2].

On the context of COVID-19 pandemic, mass gatherings are receiving more attention from public authorities regarding concerns with the health of people involved and the capacity of the health sector to respond to new cases[3,4]. Certainly, displacement and concentration of a contingent of people on mass gatherings represent and increased potential of disease transmission and outbreak[5,3,4].

Numerous measures were performed globally to deal with the COVID-19 pandemic, among them social distancing, ban on gatherings and agglomeration, mask use, closure of schools and non-essential establishments. In determined countries, these measures were implemented through the public health authority and a non-conformity with the established rules were penalized with fines or jail sentences[6,7].

COVID-19 created a circumstance where the national public health systems must validate their surveillance system and health care regarding early detection[8]. On the SARS-CoV-2 situation, considering the practical and economic limitations of mass clinical tests, researchers around the world and in Brazil suggested the use of sewage epidemiology as a strategy to aid monitoring of the pandemic propagation. Epidemiology based on analysis of wastewater and sewage has been used as an alternative and complementary strategy to estimate exposure of communities and populations towards determined chemical substances and pathogens. This strategy was also adjusted to identify exposure of populations to chemical products, pesticides, mycotoxins, diets and infectious diseases[9].

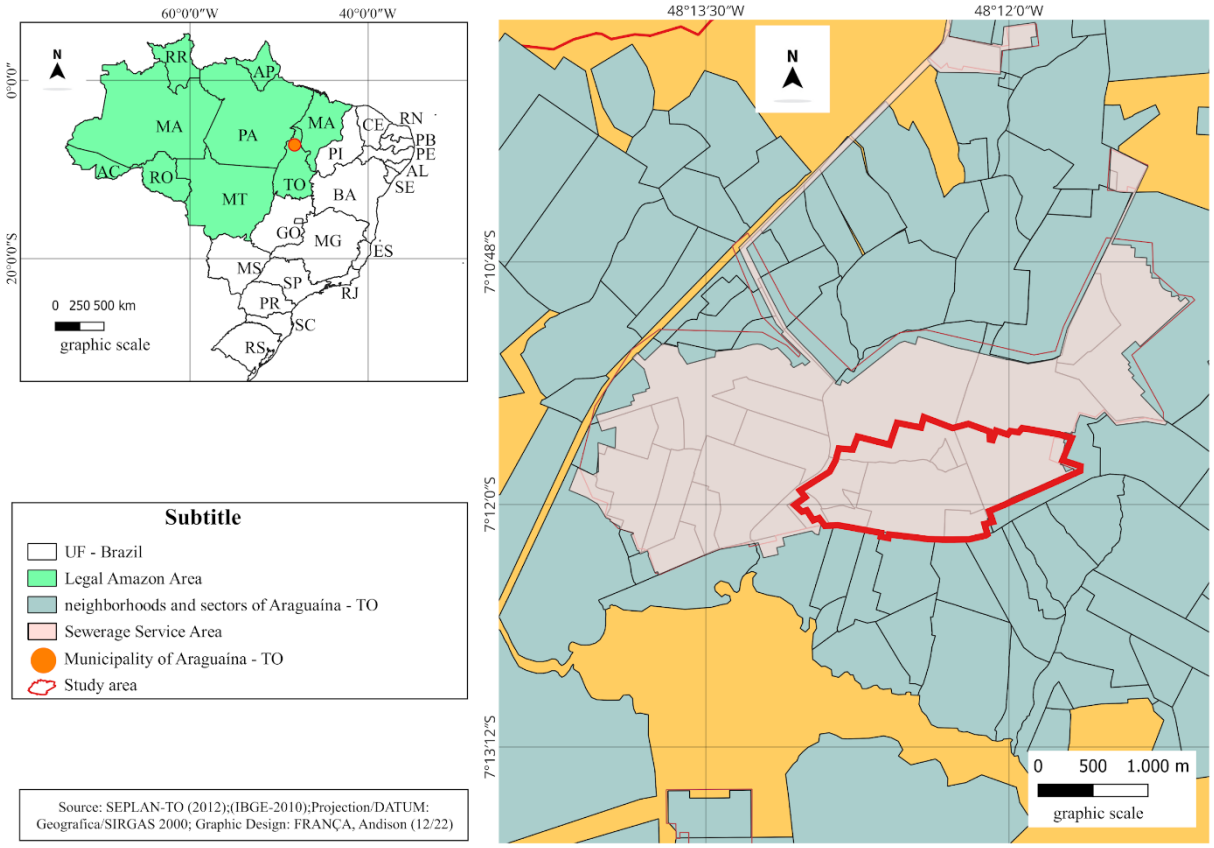
Detection and quantification of viral ribonucleic acid (RNA) of the new coronavirus on human waste identified on sewage samples allows the association of viral presence on these by-products and progression of COVID-19 pandemic. Quantification and mapping of the SARS-CoV-2 through sewage samples has been performed in different parts of the world, such as Netherlands[10], United States[11], Australia[12] and Brazil[13,14]. In Brazil, seven cities used sewage samples to monitor SARS-CoV-2, six of them located in the southeast, south and northeast regions and one in the north of Brazil[15,16].

Mass clinical trials for COVID-19 diagnosis are commonly chosen; however, environmental modeling through sewage represents a complement which strengthens health surveillance and epidemiological and environmental systems. Sewage monitoring can detect asymptomatic people infected with the new coronavirus, considering these people excrete viral particles even though they do not present symptoms[17]. Overall, the aim of this study was to quantify SARS-CoV-2 viral load in the sewage system on the areas attended with sanitary sewer of Araguaína's (Brazil, TO) county fair park, aiming to estimate new cases caused by agglomeration in the city, creating a fast strategy of detection to control dissemination of COVID-19.

MATERIALS AND METHODS

A delimitation of interest points for viral load survey of SARS-Cov 2 was achieved through mapping of strategic points of the sewage system. The sample collection point was the park Exhibition Park Dair José Lourenço (Figure 1). The event occurred between June 02 and 12 of 2022, with the estimation of 145,000 people circulating on the event. The sample point was monitored through the epidemiological weeks 23, 24, 25, 26 and 27[18], among the dates June 08 and July 07 of 2022 (Figure 2A). The methods and procedures used in this present study were based on the methods of INCT ETEs Sustentáveis (National Institute of Science and Technology on Sustainable Sewage Treatment Plants, INCT 2022) of Federal University of Minas Gerais (UFMG)[19].

Figure 1 – Delimitation of a strategic visit point (VP) for monitoring of viral load of Araguaína sewage, highlighting the neighborhood of the sewage system. The red part in evidence is the area of coverage connected to Parque de Exposições Dair José Lourenço.



The materials analyzed were weekly samples with a 3 L volume of sewage in aliquots collected for 3 hours regular period. Samples were stored between 4ºC and 7ºC until it was transported to the laboratory. The material was pasteurized at 65ºC for 90 minutes, homogenized and concentrated through filtration, from 40 to 70 ml, using a HAWP04700-Millipore- 0, 45 µm[13]. The samples were filtered as duplicates and stored at -20ºC, resuspended in buffer I of the extraction kit (described below), until the following step. RNA extraction was performed following the protocol established by

the manufacturer of the AllPrep PowerViral DNA/RNA kit (Qiagen®, Hilden, Alemanha). At the end of each extraction, RNA was stored at -80ºC until the real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) quantification was performed. Highlights about the protocol: (1) primers and probes (fluorescently labeled oligonucleotides) used to detect SARS-CoV-2 amplified the virus gene parts of Nucleocapsid (N) - whereas two parts named N1 and N2 were targeted (Table 1); (2) this technique goes from the reverse transcriptase of RNA isolated from sewage samples to cDNA, followed by amplification of genetic material by Real time PCR[19] and (3) RT-qPCR was recommended by World Health Organization (WHO) for SARS-CoV-2 detection[19].

Table 1 – Polymerase Chain Reaction (PCR) Primers for N gene- SARS-CoV-2

Institution	Forward Primers (5´-3´)	Reverse Primers (5´-3´)	Probe (5´-3´)
U.S. CDC	N1: GACCCCAAAATCAGCGAAAT	N1: TCTGGTTACTGCCAGTTGAATCTG	N1: FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1
U.S. CDC	N2: TTACAAACATTGGCCGCAAA	N2: GCGCGACATTCCGAAGAA	N2: FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1

CDC = Center for Disease Control and Prevention[20]

RESULTS AND DISCUSSION

Araguaína, the second most populated city of the state of Tocantins, which is part the of the Legal Amazon region, was affected by the pandemic with 130,428 notified cases, 50,890 confirmed cases and 628 deaths[21]. The expressive number of infected compared to other cities on the same region may be related with its localization in an urban and road junction with important interconnection with other local and sub-regional centers[22,23].

Table 2 shows viral load detected on sewage samples through the epidemiological weeks analyzed, considering N1 and N2 genes of SARS-Cov-2 and the average streamflow of the connected service. The estimates of the infected population was carried out by dividing the load virus detected in the sewage by the average per capita viral load. The calculation to obtain an estimation is a technical and scientific resource and was performed following the suggestion of the Epidemiological report no. 29 and 31 of INCT[19] and considers: a) Daily load of feces per capita with an average of $2x10^7$ copies of RNA per person; b) Estimation of infected people for each week whereas it is divided by 6 (adjusted estimation) that stands for the approximate number of weeks that an infected person may excrete viral parts on their lees. Therefore, population estimates using this methodology might be interpreted as an estimation of weekly new infections. In this work, we adopted the equations based on standards cited international references[24].

Even though these results were specific to the geographical location of the service connected to the exposition, it indicated an early warning of increase of infections related to virus circulation in the city, based on epidemiological data from the

municipal health department. We have collected and evaluated data that do not depend on COVID-19 testing, including the number of people seeking health care with COVID-19 symptoms [21]. Likewise, our results demonstrated that the estimates of the infected population based on the sewage followed the increase in cases related to the mass event, even if indirectly.

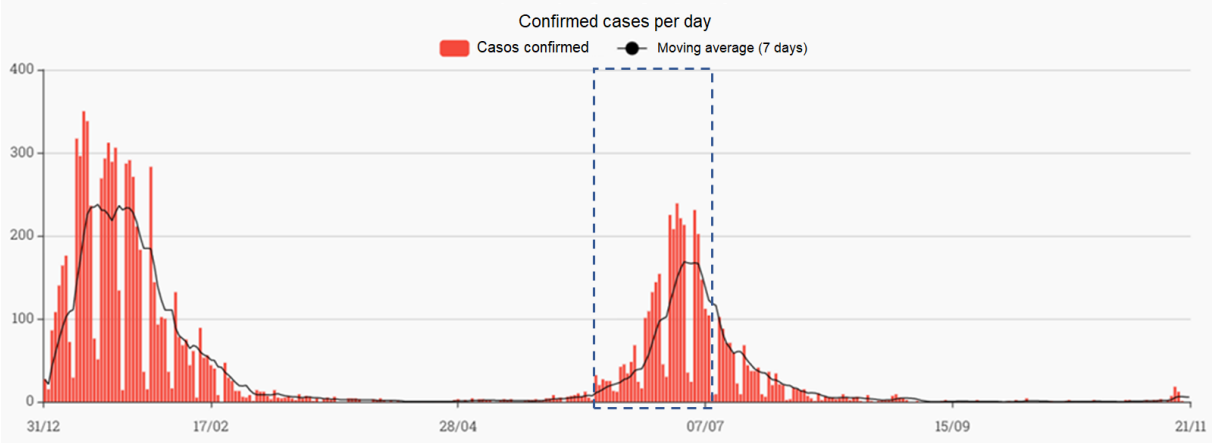
Table 2 – Concentration distribution of viral copies on positive samples for SARS-Cov-2 on sewage through five epidemiological weeks of monitoring during the EXPOARA 2022 through the sampling point at Rua Dom Bosco/Florêncio.

Epidemiological week	Viral copies/mL of sewage (N1)	Viral copies/mL of sewage (N2)	Copies on average streamflow (mL) (3h of sampling)	Infected estimation	Infected adjusted estimation
23	30.65	--	596.49 x 10^7 N1	596.49	99.42
24	181.08	489.08	3524.11 x 10^7 N1 9518.28 x10^7 N2	3524.11 9518.28	587.35 1586.38
25	--	--	--	--	--
26	42.15	--	820.3064 x 10^7 N1	820.30	136.7
27	--	187.88	3656.445 x 10^7 N2	3656.4	609.4

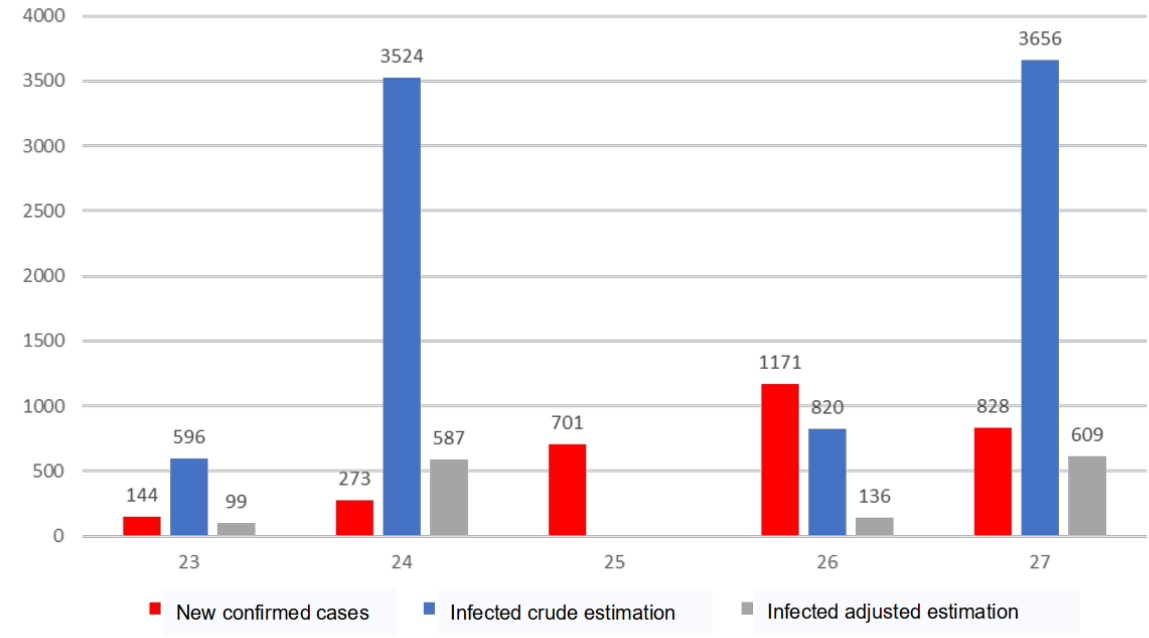
Even though virus detection occurred on a narrow interval, aiming to relate large events to increase of viral transmission in the city, 4 of 5 collected samples had virus, demonstrating that the number of COVID-19 cases in Araguaína were greater than the ones followed by epidemiological surveillance through clinical cases on the same period of time[21]. We emphasize that the sewage quantifications demonstrate an estimation, not a quantification of SARS-Cov-2 positive cases. Figure 2B shows the comparison of data referring to notified clinical cases in the city of Araguaína[21] and crude and adjusted estimates of infection based on SARS-CoV-2 detection on sewage. The results of the infected estimation through recovered viral loads highlight epidemiological weeks 24 and 27 as the ones with the major viral recovery (sampling performed on 6/17/2022, five days after the last day of the event and 7/7/2022, twenty-five days after the last day of the event). In this moment, the infection of the ones releasing the virus might have occurred in the weeks before or during the event, which would be consistent with the clinical data followed by epidemiological reports that indicated an increase of cases in June and July of 2022[21].

Figure 2 – Distribution of confirmed clinical cases of COVID-19 in Araguaína city in 2022. Dotted area delimits period of monitoring (6/5/2022 to 7/9/2022) which included the days that occurred the mass gathering (EXPOARA- 6/2/2022 to 6/12/2022) on the city Araguaína, Tocantins, Brazil (A) and data distributions referring to notified clinical cases in the city of Araguaína and crude and adjusted estimates of infection based on SARS-Cov-2 detection on sewage during epidemiological weeks 23 and 27 of 2022 (B).

A



B



Source: SESAU-TO (2022)

On Table 2 mentioned above, we showed the quantifications of the target N1 and N2 of the viral genome. Most of the samples were positive for region N1; however, on Week 24 both regions were detected and when the calculation was performed based on copies of region N2, we verified a crude and adjusted estimate, respectively, with approximate values 30 and 5 times bigger of notified infections (Figure 2).

Differences of N1 and N2 assays in wastewater samples were also reported in other investigations[9,25].

The difference among the results of N1 and N2 targets probably occurs because different RT-qPCR assays are not equally susceptible to reaction inhibition by substances that are co-purified on nucleic acid extracts, because it depends on conditions such as sewage composition and quality of the extracted genetic material. Therefore, even though there is no consensus among researchers about which target region would be more adequate to determine the presence and quantification of genetic material of SARS-Cov-2, the results of this study indicate greater sensibility to the target N1. However, these results still demonstrate more sensibility to the N2 gene on the epidemiological weeks 24 and 27, highlighting the importance of including more than one gene in the analysis for qualitative and quantitative essay.

Even though we did not detect a viral load on the epidemiological week 25, we cannot reject the presence of virus on the sewage system. This result is probably associated with limited recovery of viral load on sewage in a 3-hour period for collection caused by sampling technical difficulties in the field, such as a short period of 3 hours (time of technical support conceded by BRK-Ambiental enterprise). Hence, samples that were negative for viral load suggest aspects of eventuality of low contribution of feces by the population especially during sampling. Epidemiological surveillance studies of SARS-CoV-2 virus recently published are indicating considerable recovering through automatic samplers, which allows collection of composite samples over a period of 24 hours [13,14].

Araguaína is a city from the north of Brazil, which is a region of precarious sanitation, around 30% of its population has access to sanitary sewer (BRK-Ambiental), which represents twice more access than the average of this location[26]. Despite this, in the area where the sewer network extends, there are also the most populous neighborhoods. Therefore, this study monitored a considerable part of the urban area covered by sewage and the results highlight that, on the epidemiological weeks 24 and 27, occurred an increase of viral concentration that followed the increase of confirmed and notified cases on the epidemiological reports of the Municipal Office of Health.

Estimates for viral load associated with SARS-Cov-2 overcame the quantification of epidemiological data of the health surveillance, which suggests virus circulation with 3 to 10 times more infected people when the crude estimation was used. A sewage investigation on Belo Horizonte metropolitan area indicated that on the evaluated epidemiological weeks, there were 2 to 20 times more cases of infections on the epidemiological reports in comparison to the same corresponding period[13].

Another investigation performed on Niterói, a metropolitan area of Rio de Janeiro, indicated that in overall, SARS-Cov-2 RNA load detected on sewage samples corresponded to the period that the city registered its greatest number of cases and deaths by COVID-19[14]. Sewage monitoring results of the city Goiânia indicated increased viral concentrations on the most critical moments of the pandemic (greatest number of COVID-19 notifications), and lower concentrations on the less critical moments, with viral load detection on sewage anticipating clinical notifications on certain weeks[27]. Even though our investigation's monitoring occurred in only 5

epidemiological weeks, sewage data showed an apparent agreement with the notified clinical cases when compared to other studies discussed previously. Clearly, the increase of virus circulation in the population potentially increases the viral load on the city's sewage systems, therefore, knowledge about the city's sanitary system allow the identification of areas with greater virus occupation, which can anticipate possible outbreaks and contribute to responses on each neighborhood[28], and with that, anticipate the impact of the disease dissemination caused by mass gatherings.

Virus quantification is the main objective of sewage-based epidemiology; however, according to recommendation of the U.S Centers for Disease Control and Prevention[20] estimates of prevalence should not be used as the only point to endorse actions, since there are still several uncertainties about the calculations[25]. In addition, viral load quantified on wastewater samples varies accordingly with the sampling methods, pre-concentration, extraction and viral RNA quantification selected by different research groups, and by the methods limitations.

Epidemiological investigations indicate distribution of diseases over time, presenting information for comprehension, prediction, prevention and impact assessment of health interventions[28]. Therefore, it is clear that records and monitoring of disease's evolution over time is necessary to recognize patterns and tendencies of diseases through time (eg., days, weeks and months) and determine the limits to periodic variations of an event, enabling identification of increase of incidence or prevalence of a disease beyond the expected from a determined period of time. Moreover, considering the COVID-19 pandemic situation, which is a challenge to strategies of control for disease dissemination, the sewage system is being used as a tool to an effective epidemiological surveillance as was indicated on studies of considerable areas management and people circulation such as the Olympic and Paralympic Games of 2020 and 2021, respectively, in Tokyo, Japan[29]. Sewage surveys were used by the Olympic committee as an indicator of an early incidence of COVID-19 that aided preventive measures of infections, which allowed revision of periodicity of clinical tests application. Considering that, we suggest this strategy to be a fast alert as a low-cost alternative, even for locations with a limited sewage system as demonstrated in this study of Araguaína, a mid-sized city located in the north of Brazil, where the sewage system reaches only one third of urban area.

CONCLUSION

Estimates based on sewage monitoring indicates that the infected population by the new coronavirus in Araguaína, a mid-sized city in the north of Brazil, are superior than the number of confirmed cases on the studied areas, and possible extrapolation to other areas, considering that there was a considerable circulation of people during the EXPOARA event.

Wastewater monitoring is a considerable tool for surveillance on epidemiology, which allows follow-up of virus circulation, considering it works as an indirect mass testing of the population through evaluation of SARS-Cov-2 genomic load, originated by both symptomatic and asymptomatic individuals.

Due to the diversity of mass gatherings, we believe that this type of surveillance presented may aid organizers and local authorities in taking decisions in regards of events planning, including calculus of target-audience, restriction and distancing measures, vaccination certification, acquisition of laboratory tests and opening of more hospital beds, therefore preparing the health system to deal with new clinic cases. We highlight that this is the first work of epidemiological control through sewage in the state of Tocantins, whereas with others states of Legal Amazon presents a lack of studies of COVID-19 surveillance and where agribusiness events have considerable socio-economic importance.

A limitation of the present study is the limited sewage system of Araguaína city which does not permit establishing a causal relationship between the outcome and epidemiological variables to each city district. However, the present study corresponds to an important and fast warning to health system. Future studies would benefit from a longitudinal approach that captures the time and the relationship between different mass gatherings in the city and the occurrence of new cases of the disease.

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