# SARS-CoV-2 has been circulating in northeastern Brazil since February 2020: evidence for antibody detection in asymptomatic patients

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Word count: Abstract: 50 Main text: 849 References: 9

# Highlights

* + COVID-19 clinical cases were reported in Brazil in February 2020.
	+ SARS-CoV-2 may have circulated before the clinical cases were detected.
	+ We tested serum samples collected in Jan-April 2020 for SARS-CoV-2 antibodies
	+ 16/989 (1.6%) had antibodies, suggesting an earlier circulation of the virus.
	+ Patients with undetected and reported infection had similar age, sex and residency.

# Abstract

We screened stored samples collected before COVID-19 had been reported in Brazil. 989 samples were tested for SAR-CoV-2 antibodies using two different methods; 16 (1.6%) were positive (7 (43.8%) IgM, 3 (18.8%) IgG and 6 (37.5%) IgG/IgM positive), suggesting SARS- CoV-2 had circulated before the first reported COVID-19 case in Brazil.

# Main Text

Grall et al (1) reports that asymptomatic patients with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) contaminate their masks and surroundings objects (mobiles, doorbell and bed table) with potential to play a role in transmission. Very few surveys focus on the prevalence of asymptomatic infections and although seroprevalence surveys provide insights of the proportion of the population infected (2, 3) and the intensity of infection, they are unable to distinguished whether a significant proportion of infections are asymptomatic. Our country, Brazil, has the third highest global burden of Coronavirus Disease-19 (COVID- 19), after the USA and India, (4) but does not systematically monitor the proportion of the population with SAR-CoV-2 antibodies. Moreover, the Ministry of Health recommends confirming the presence of the virus only in symptomatic individuals and the total number of infections is likely to be substantially higher. (5)

The first case of COVID-19 in Brazil was reported the 26th February 2020 (4). However, the virus was identified in sewage samples in Santa Catarina State, in the South of Brazil, as early as the 27th November, suggesting the virus had been circulating several months earlier, and, possibly, among asymptomatic infections. (7) This situation is similar to Italy, where the virus was discovered in sewage samples in Milan, in December 2019, even though the first COVID-19 case was reported two months later, the 21st February 2020. (8)

We report here evidence that SARS-CoV-2 may have circulated in the Northeast of Brazil before the first COVID-19 cases were reported in the region. In Aracaju, Sergipe State, in Northeast Brazil, the first case of COVID-19 was documented the 14th March, 2020 (4), but we report here that patients undergoing routine blood examinations for causes unrelated to COVID-19 had SARS-CoV-2 Immunoglobulins (IgM and IgG) before clinical cases were reported in the State.

We obtained 987 anonymized serum samples collected from January 2019 to April 2020. Samples had been collected by a private laboratory from patients undergoing laboratory tests for routine examinations and health checks for causes unrelated to COVID-19. Most of the tests had been paid by health insurance companies, which usually indicate the patients were of upper socioeconomic status. All samples were kept frozen in the laboratory blood bank at - 80◦C and tested for the presence of anti-SARS-CoV-2 IgM and IgG using two in vitro diagnostic tests. The first assay was a lateral flow immunochromatography (Nantong Egens Biotechnology CO, Ltd., China), reported by the manufacturer to have 96.9% sensitivity and 100% specificity. The second assay was a lateral flow sandwich detection immunofluorescence technology (iChroma2™ COVID-19 Antibody), which was used with the Ichroma™ II Reader (Bodytech Med Inc., South Korea). The assay is reported to have 95.8% sensitivity and 97.0% specificity. (3) All samples were thawed the day of testing using a 37°C thermal bath. Samples were considered positive if they were positive by both assays. Assays with only one positive test were repeated. Only samples with two positive assays were considered positive. The study protocol was approved by the research ethics committee Federal University of Sergipe and individual consent was waived (CAAE: 36401320.0.0000.5546).

The mean (SD) age of the 987 participants was 38.9 (22.2) years, ranging from birth to 90 years and 683 (69.2%) were women (Table). Sixteen (1.6%) samples tested positive for both

assays, 968 (98.1%) were negative and three (0.3%) were indeterminate. Thirteen of the participants with positive samples were women and three men. Seven (43.8%) samples were IgM positive, three (18.8%) IgG positive and six (37.5%) were positive for both (Table). These sero-positive individuals had similar age, gender and residence than individuals reported within the first 30 days after the first COVID-19 case in the State (3). These included 46 RT-PCR positive cases, of which 17 were men (37%) and 29 women (63%); of

which 34 (74%) were adults aged between 20 and 60 years and 37 (80.4%) lived within in Aracaju City.

Aracaju has reported the highest number of COVID-19 cases in Sergipe (5). Initial COVID- 19 cases occurred in the Southern districts of the city, which are inhabited by high income populations who frequently travel to the South and Southeast regions of the country, where the pandemic was first reported. Our study has the limitations that participants were not screened for the presence of SAR-CoV-2 antigens and that the assays may have had false positive results resulting from cross-reactions with endemic infections. However, we used assays based on different technologies, and it is likely their combination may have provided higher specificity than the individual assays. (9)

Our data suggest that SARS-CoV-2 may have circulated in Northeast Brazil before the first COVID-19 case reported. As samples were collected for routine screening of pre-operative procedures or to monitor other morbidities, it is possible that most of these infections were asymptomatic at the time of testing. It is possible that large popular events occurring in February in Brazil, such as the traditional Carnival (21st-25th), may have accelerated the spread of the virus throughout the country. Our findings are in agreement with sewage-based studies in Santa Catarina (7) and Italy (8), suggesting that clinical cases were preceded by asymptomatic infections several weeks earlier.

# Acknowledgments

We thank the Santa Helena Laboratory who donated the blood samples. We also thank the staff at the Department of Medicine and Pharmacy (LaBiC) of UFS, and acknowledge financial support received from the Ministério Público do Trabalho, Ministério Público Federal and Ministério Público Estadual.

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**Figure 1.** Analysis of the timeline of events, with the evolution of SARS-CoV-2 in the world, Brazil and Sergipe.

**Table 1 –** Positive tests participants characteristics´ (Age, Sex, and district of residence) and of IgM and IgG analysis for SARS-CoV-2, Aracaju/SE, Brazil

Sample Sample date IgM IgG Sex Age District Economic

|  |  |
| --- | --- |
|  | range\* |
| 49 | 16/02/2020 | 1.8 | 7.7 | F | 36 | Atalaia | A |
| 121 | 18/02/2020 | 2.4 | 3.3 | F | 30 | Jardins | A |
| 123 | 18/02/2020 | 7.9 | 14.8 | F | 63 | Treze de Julho | A |
| 124 | 18/02/2020 | 3.4 | 6.1 | F | 45 | Farolândia | B |
| 271 | 25/02/2020 | 4.4 | 0.0 | M | 32 | Suissa | B |
| 309 | 26/02/2020 | 3.9 | 0.0 | F | 34 | Farolândia | B |
| 305 | 26/02/2020 | 3.0 | 8.7 | M | 73 | Grageru | B |
| 306 | 26/02/2020 | 1.7 | 7.4 | F | 29 | Jardins | A |
| 468 | 29/02/2020 | 4.4 | 0.0 | F | 41 | Centro | C |
| 483 | 29/02/2020 | 0.2 | 2.5 | F | 36 | Farolândia | B |
| 477 | 29/02/2020 | 4.4 | 0.0 | F | 26 | Atalaia | A |
| 475 | 29/02/2020 | 1.8 | 0.0 | M | 48 | Treze de Julho | A |
| 472 | 29/02/2020 | 1.7 | 0.0 | F | 63 | Jabotiana | B |
| 454 | 29/02/2020 | 1.7 | 0.0 | F | 73 | Ponto Novo | B |
| 486 | 01/03/2020 | 0.0 | 2.4 | F | 39 | Centro | C |
| 938 | 03/04/2020 | 0.1 | 2.4 | F | 47 | Coroa do Meio | B |

Legends: \* A- Neighborhoods with residents with salaries above $ 2,000; B- Neighborhoods with residents with salaries $ 300 to $ 1,900; C- neighborhoods with residents with salaries below $ 300.