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Early Circulation of SARS–CoV–2, Congo, 2020

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To determine when severe acute respiratory syndrome coronavirus 2 arrived in Congo, we retrospectively antibody tested 937 blood samples collected during September 2019–February 2020. Seropositivity significantly increased from 1% in December 2019 to 5.3% in February 2020, before the first officially reported case in March 2020, suggesting unexpected early virus circulation.

After coronavirus disease (COVID-19) was reported in China in December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread around the world; most countries officially reported their first cases within the first 3 months of 2020. However, reports from China show a possible earlier first case on November 17, 2019, detected retrospectively in Hubei Province (*1*). Furthermore, phylogenetic analysis places the date of emergence as sometime during October–December 2019 (*2*). These data suggest possible virus spread outside China before the first officially reported case in December 2019. Indeed, several retrospective studies that analyzed stored respiratory samples and wastewater for RNA detection, as well as serologic studies, suggest that SARS-CoV-2 may have been circulating in France, Spain, and Italy (*3–7*) before December 2019, months before the first official cases were reported.

In central Africa, the first cases were officially reported during March 6–April 6, 2020; in Congo, the first case was reported on March 14, 2020. However, a

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serologic study in Kenya suggested that the virus was present in January 2020, two months before the first official case was reported (*8*). Similar retrospective studies have not been conducted in Central Africa, meaning that the time of SARS-CoV-2 introduction in this region remains unknown.

To provide a more accurate date for the arrival of SARS-CoV-2 in Congo, we retrospectively examined serum samples collected from persons with HIV (PWH) as a part of the national HIV program. These samples were collected during July 2019–February 2020 in Brazzaville and Pointe-Noire, the 2 biggest cities in Congo (Appendix, https://wwwnc.cdc.gov/EID/article/28/4/21-2476-App1.pdf). The study was conducted with approval of the Comité Technique de la Riposte à la Maladie à Coronavirus COVID-19, of which F.R.N. is president of the commission laboratory and research, and the Programme National de Lutte Contre le SIDA, led by the National Public Health laboratory of Congo, of which F.R.N. is director.

We tested 1,212 plasma samples for SARS-CoV-2 IgG by using a microsphere immunoassay with beads coupled with receptor-binding domain antigen. We used 275 samples collected during July–August 2019 as negative controls and to establish the seropositivity cutoff value of our test (Appendix). The remaining 937 samples were collected September 2019–February 2020. Overall, 28/937 (3.0%) samples were positive: 22/655 (3.3%) from women, 5/241 (2.1%) from men, and 1/41 (2.4%) from a patient for whom sex was not reported. SARS-CoV-2 seropositivity rate was 1.7% (10/563) in Brazzaville and 4.8% (18/374) in Pointe-Noire. However, the Pointe-Noire samples were all collected in 2020 and compared with those from Brazzaville from the same period (5.4%; 6/110) did not differ significantly (p = 0.8). Although seropositivity was very low from September through November, seropositivity subsequently increased linearly, reaching 5.3% by February 2020 (Figure). Furthermore, sero-
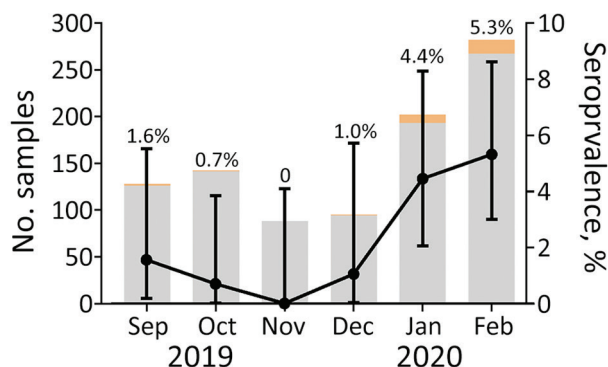

Figure. Number of plasma samples tested each month for severe acute respiratory syndrome coronavirus antibodies by using a microsphere immunoassay with beads coupled with receptor-binding domain antigen, Congo, September 2019–February 2020. Gray shading indicates the number of seronegative samples; orange, seropositive samples. Seropositivity is represented by black dots; error bars indicate 95% binomial CIs.

positivity was significantly higher in January–February 2020 (p = 0.0002) than in the preceding 4 months of 2019 (Table). We also observed a significant increase between samples collected in Brazzaville in 2019 and those collected in Brazzaville in 2020 (p = 0.0052).

Our results suggest increased SARS-CoV-2 circulation during January–February 2020 in Congo, indicating that the virus arrived in the country in December 2019. Our findings align with those of a serologic study of an asymptomatic general population in Congo, conducted in April 2020, which found 1.7% seropositivity for IgG and 2.5% for IgM (*9*). The higher seropositivity found before April in our study may result from the higher sensitivity of the microsphere immunoassay assay compared with that of rapid tests (*9*). Moreover, the PWH in our study may be more exposed to the virus than the randomized general population tested by Batchi-Bouyou et al. because PWH must regularly visit healthcare centers as part of their treatment. A recent study of participants with and without HIV tested during January–March 2020 in Kenya reported 3%–4%

Table. Seropositivity of SARS-CoV-2 IgG among persons with HIV, Congo, July 2019–February 2020*		
Sample	No. tested/no positive (% positive)	p value
Sex		
F	655/22 (3.3)	0.38
M	241/5 (2.1)	
Unknown	41/1 (2.4)	NA
Location		0.0052 for Brazzaville
Brazzaville 2019	453/4 (0.8)	2020 vs. 2019; 0.8 for
Brazzaville 2020	110/6 (5.4)	Brazzaville 2020 vs.
Pointe-Noire 2020	374/18 (4.8)	Pointe-Noire 2020
Date		
2019 September–December	453/4 (0.9)	0.0002
2020 January–February	484/24 (4.9)	
Total	937/28 (3.0)	NA

*NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

seropositivity, which did not differ between these populations (*8*). Early circulation of SARS-CoV-2 has also been found in France, Spain, and Italy; seropositivity estimates in France increased from 1.3% in November 2019 to 6.7% in February 2020 (*6*).

There is some concern that seropositive samples may reflect possible cross-reactions with other coronaviruses that infect humans (human coronaviruses NL63, 229E, OC43, and HKU1 and Middle East respiratory system coronavirus) (*10*). Although cross-reaction may explain the very low SARS-CoV-2 seropositivity in September–October 2019, the significant increase in seropositivity from the end of 2019 to the beginning of 2020 argues in favor of actual detection of antibodies directed against SARS-CoV-2. The early introduction of SARS-CoV-2 in Congo, and more generally in Africa, probably results from the intense trade activities that link Africa to China, leading to frequent exchange of persons between these countries.

Determining early circulation patterns of SARS-CoV-2 in Africa or other countries requires retrospective testing of as many samples as possible from existing national sample repositories. Such studies will help enrich knowledge of the propagation of pathogens in the context of globalization of human and material exchange. To better evaluate the epidemiology of future pandemics, international organizations should help reinforce and develop repositories in low- and middle-income countries.

About the Author

Mr. Bobouaka Bonguili is a master's student under the direction of co-authors F.R.N., E.M.L., and M.F. He is a member of the SARS-CoV-2 response team established by the National Laboratory of Public Health. Dr. Fritz is a postdoctoral researcher in the unit Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle at the Institute for sustainable development. He develops serologic techniques to detect zoonotic viruses.

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Serologic Evidence for SARS-CoV-2 Circulation in Early 2020, Congo

Appendix

Material and methods

Origin of sample

Plasma samples were gathered from the PWH library of the National Public Health Laboratory in Brazzaville. The library contains samples collected in the various outpatient treatment centers in the Congo and samples from PWH received at the LNSP from July 2019 to February 2020. Only patients receiving ARV treatment and from Brazzaville and Pointe-Noire were choose for this study. No other inclusion criteria has been applied for this study. The average age of the patients was 46 years (range: 17 to 80), 72% were women, 24.5% were men, and for 3.5% the information on gender was unavailable.

Microsphere immunoassay

The plasma samples were tested using a Microsphere immunoassay (MIA). 10µg of Spike receptor binding domain (RBD) recombinant SARS-CoV-2 antigen (The Native Antigen Company) was used to capture specific plasma antibodies. MagPlex microsphere (Luminex Corp) was coupled to the viral antigen using the amine coupling kit (Bio-Rad Laboratories) according to manufacturers' instructions. The MIA procedure was performed by incubating the plasma samples (50 µl), diluted 1:400 in assay buffer (PBS-1% BSA-0.05% Tween 20), with the antigen-coated beads (1250 beads) protected from the light on an orbital shaker at 700 rpm for 30 min. After washing, 50 µl of biotinylated conjugated Fc Fragment goat anti-Human IgG (Jackson ImmunoResearch) at 4 µg/ml each in assay buffer were transferred to each well and incubated on an orbital shaker for 30 min at 700 rpm in the dark. After washing, the beads were incubated for 10min at 700 rpm in the dark with 50 µl of Streptavidin-R-Phycoerythrin (Life technologies) and diluted to 4 µg/mL in assay buffer. After washing, beads were resuspended in 125 µl of assay

buffer. Measurements were performed using a Magpix instrument (Luminex), with at least 50 events were read. Binding events were displayed as median fluorescence intensities (MFI).

Calculation of the cutoff, specificity and sensitivity

To take into account antigen specificity, seropositivity cutoff values was set at three standard deviations above the mean MFI of 275 plasma samples available from this cohort farthest from the beginning of the epidemic, i.e., in July and August 2019. Based on this population, MIA specificity was set at 97,5%. The sensitivity of our test was set up using samples (12) from COVID-19 PCR+ confirmed non-HIV participant sampled within 2 weeks after the PCR test. Based on this population, MIA sensitivity was set at 100%.

Statistical analyses

Fisher exact test and binomial confidence intervals was calculated using R software (*1*).

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