High SARS-CoV-2 IgG/IGM seroprevalence in asymptomatic Congolese adults in Brazzaville, the Republic of Congo

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Introduction (1)

- Asymptomatic cases of SARS-CoV-2 ==> PUBLIC HEALTH PROBLEM[1].
- Most countries, including those in Central Africa resort to virus detection +++ symptomatic subjects
- None have systems in place to systematically detect mild and asymptomatic cases.
- Obviously, more testing will allow the identification of more asymptomatic individuals, but for low resources countries like many in sub-Saharan Africa, more testing is a daily challenge.

Introduction (2)

• Republic of Congo

  - Slight growth in testing capacities since the beginning of the outbreak 14th March 2020 (first case detected in the country).
  - Limited capacities [2]
  - Difficulty to evaluate the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus within the population and referring only on symptomatic cases will not reflect the true situation.

• Brazzaville and Pointe-Noire = Most affected cities in the country (6180 and 2375 respectively, SITREP 133).

Introduction (3)

• *World Health Organization* Recommendations for diagnosis
  – Reverse transcription polymerase chain reaction (RT-PCR) [3].
    → Expensive++ Facilities++ Trained human resources ++ Electricity.
  – However, rapid diagnostic tests (RDT) ==> antibodies anti-SARS-CoV-2 detection
    – Practical alternative for the identification of individuals that have been exposed to the infection a minimum of 7 to 14 days before.
    – Relevant to determine the course of the infection +++ seroconversion when PCR sensitivity has decreased.
    – Useful to inform public health stakeholders on the development of herd immunity within the population [1].

Objective: to evaluate the incidence of SARS-CoV-2 within the Congolese population residing in Brazzaville, by conducting a seroprevalence pilot study.
Materials and Methods:

Study area

• The study was conducted in Brazzaville, capital of the republic of the Congo.

• Individuals were recruited at the Health center of the Fondation Congolaise pour la Recherche Médicale located in Massissia (district of Madibou), southern part of Brazzaville

• Period April to July 2020.

• The municipality of Madibou is located in the south of the city of Brazzaville with an area of 80 km². The population of Madibou is 100,000 (Arrondissement 8 Madibou).
Materials and Methods:

Study participants and sample collection

• Cross-sectional study by open-invitation screening.
• RNA and serum samples from asymptomatic individuals.
• Personal data (age, sex, occupation, place of residence)
• Nasopharyngeal swab and blood samples were collected and stored at -80°C until analysis performance.
Materials and Methods: 

**SARS-CoV-2 detection** 

- RNA was extracted using QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA) according to instructions. 

- Qualitative RT-PCR assays to detect SARS-CoV-2 were performed using a clinically validated kit approved by the Chinese National Medical Products Administration (Liferiver, Shanghai, China) 

- High-performance, high-throughput PCR platform (96 well plates) LightCycler® 480 RealTime PCR System (Roche Diagnostics) according to the instructions, with **Ct values below 40 considered positive**.
Results:

IgM/IgG detection

• Qualitative detection
• Two COVID-19 IgG/IgM rapid test cassettes: Immunochromatographic assay
  – Sienna™ from Salofa Oy manufacturer, Finland (Under license of T&D Canada Pvt. Ltd.)
  – UNsiences® from Wuhan UNniscience Biotechnology manufacturer (Under license of Wellkang Ltd. England, UK).
• Positive result: control line region (C) and one and/or two colored lines appeared in test line region G and/or M.
• Negative result: colored line in the control line region (C) appeared and no line appeared in test line regions G or M.
• Invalid result: control line failed to appear.
Ethics statement

• The study was submitted to and approved by the institutional ethics committee of the Fondation Congolaise pour la Recherche Médicale, Brazzaville, Republic of Congo. Before enrollment, an informed, signed consent has been obtained from the participants and the confidentiality of data ensured.
Statistical Analysis

• Data entry: CSpro (version 7.4.0).
• Data processing: SPSS version 24.
• Figures: GraphPad (version 8.0.4)
• Maps: QGIS
• Descriptive statistics were done for all variables.
• Prevalence and proportions were calculated, adjusted for clustering effect of RT-PCR results.
• Chi square tests and Fisher’s Exact test were used to evaluated the dependance between results of RT-PCR, IgG, IgM, gender, age, month of enrolment, et location of all study participants.
• $P.value<0.05$ was considerate statistically significant.
Results: Sociodemographic findings

• A total of 754 individuals (463 male and 291 female) were recruited from 01 April to 31 July and were resident from all districts of Brazzaville. The average age was 39.69 ± 13.07 with values ranging from 2 to 90 years old.

• Out of 754 samples of asymptomatic subjects, 56 were found to have active SARS-COV2 infection (7.4%); including 9.3% males and 4.5% females.

• This prevalence was associated with the gender (P=0.014).

• The infection was not associated with age.

• The most affected district of Brazzaville was Mounagali with 17 cases (18.1%). There was significant difference (P=0.004).
## Results: Serology testings (1)

### Table 1: Serological responses of participants to SARS-COV2 detection through the RT-PCR.

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>All participants</th>
<th>SARS-COV2 RT-PCR</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=754</td>
<td>Negative N=698</td>
<td>Positive N=56</td>
</tr>
<tr>
<td>IgG+</td>
<td>81 (10.7)</td>
<td>64 (9.2)</td>
<td>17 (30.4)</td>
</tr>
<tr>
<td>IgG-</td>
<td>673 (89.3)</td>
<td>634 (90.8)</td>
<td>39 (69.6)</td>
</tr>
<tr>
<td>IgM+</td>
<td>103 (13.7)</td>
<td>80 (11.5)</td>
<td>23 (41.1)</td>
</tr>
<tr>
<td>IgM-</td>
<td>651 (86.3)</td>
<td>618 (88.5)</td>
<td>33 (58.9)</td>
</tr>
<tr>
<td>IgM+ and IgG+</td>
<td>70 (9.28)</td>
<td>54 (7.74)</td>
<td>16 (28.6)</td>
</tr>
<tr>
<td>IgM+ and/or IgG+</td>
<td>149 (19.7)</td>
<td>117 (16.8)</td>
<td>32 (57.14)</td>
</tr>
</tbody>
</table>
Results: Serology testings (2)

Figure 1: IgM and IgG prevalence of SARS-COV2 in asymptomatic Congolese individuals by period
Results: IgG/IgM and gender

**IgG**

- **A** All tested by RT-PCR
  - Female: $P=0.081$
  - Male: $P=0.002$

- **B** Negative RT-PCR
  - Female: $P=0.012$
  - Male: $P=0.010$

**IgM**

- **C** All tested by RT-PCR
  - Female: $P=0.143$
  - Male: $P<0.01$

- **D** Negative RT-PCR
  - Female: $P=0.045$
  - Male: $P<0.01$
Discussion (1)

• First reporting on seroprevalence to SARS-COV2 in Congolese population and to our knowledge for a Central African population.

• Pilot investigation using two serologic rapid diagnostic tests
  – conducted during the lockdown in the country
  – no reagents were able to be purchased and shipped to the country.

• Cross-sectional study was carried out as part of a massive screening campaign for the detection of SARS-COV2 within the general Congolese population residing in Brazzaville, the capital which is the city with the highest incidence of COVID-19 (SITREP, Congo)
Discussion (2)

- Sub-Saharan Africa:
  - Kenya, July 2020: **5.6%**
  - Malawi, August 2020: **12.3%**
  - **Congo: 19.7%** (All participants) and **15%** (RT-negative individuals)
    - → *High circulation of the virus*
  - **USA: 31.5%** (nearly two-fold) +++ Most affected country so far
• Our objective here was to estimate the proportion of the Congolese population who have been in contact with the virus SARS-CoV-2 along the course of the infection including the lockdown (period of the lockdown in Congo).

• Therefore, all the participants did not present any symptom of the disease. The findings showed that a significant part of the community harbored asymptomatic infection.

• Here 7.4% had active detectable infection and about 15% were seroconverting meaning that in July 22% of the study population have been infected without any symptom.
Discussion (4)

• Our results present a high prevalence of SARS-COV2 antibodies, much higher than those reported by Ruijie Ling in Wuhan, the epicenter of COVID-19 in China, who revealed a seroprevalence of 3.35% among the general population despite being in Wuhan, the epicenter of COVID-19.

• Congolese population = VERY EXPOSED TO THE VIRUS.

• With an estimated population of 1,838,348 in Brazzaville, an overall seroprevalence of 19.7% would mean 362,154 inhabitants of seropositive subjects to SARS-COV2 ie 1/5 inhabitant.
Discussion (5)

• In addition, although Moungali was the most affected district as confirmed by the national screening of symptomatic cases (SITREP CONGO)

• No significant difference in the geographical distribution of the disease.

• Nevertheless, the high seroprevalence of 18.1% in this district would be partly due to non compliance with mitigation of containment measures in this urban area with intensive business activities.

• Such observations have also been made in a business and economic center of Brazil [10]
In most cases, seroprevalence studies are conducted with the data are important to understand the scale and spread of the pandemic and predict the probability and timing of future waves of recrudescence [11].

Can address public health questions, such as city lockdown, churches and school closings, travel restrictions and social distancing [11].

Furthermore, performing diagnostic tests on individuals based on clinical suspicion can induce selection biases in some cases. Indeed, some authors have shown the importance of considering asymptomatic patients in order to assess with more certainty the prevalence of a disease [12] [13].
Discussion (7)

• Although some controversial studies suggested poor reliability and accuracy due to high risk for false-positive and false-negative cases, we performed simultaneously two serologic testing to minimize biases [14].

• Those biases are common when a serological testing is considered at individual level [14] [15]. However, when used at population level, it can help estimate reliable average seroprevalence even with moderate sensitivity and specificity [11]
• 149 who had detectable anti-SARS-COV-2 antibodies (IgG+ and/or IgM+), 117 had negative PCR performed on nasopharyngeal swabs.

• This is equal to 78.5% (n=117/149) of asymptomatic individuals having being infected by the virus but presenting zero viral carriage.

• **Comment:** nasopharyngeal swabs would present false negatives because of the instability of the nasopharyngeal carriage as previously reported [16] [17].

• Also, this helps us to notice that since the start of the pandemic, the infection has sometimes remained underdiagnosed in some patients, and therefore less treated, thus explaining this high seroprevalence in the country.
The seroprevalence of SARS-COV2 remained almost higher among women than among men throughout the study. These results are almost identical to those reported by A Shields et al. (26.3% women vs 18.8% men) [18].

Limitation: Selection bias = subjects enrolled were basically people who felt themselves as being in contact with symptomatic individuals meaning that they tend to minimize healthy restrictions measures.

Also, immunofluorescence need to be performed for better sensitivity for serological testing.
Conclusion

• The proportion of the population who seroconvert over the course of the first wave will contribute to better evaluation of the risk of future waves and will facilitate the efficient use of limited resources.

• It also calls into question the hypothesis that the virus is transmitted only by symptomatic subjects.

• Thus, these data suggest that airborne transmission through droplets aerosols is also involved while talking [8] [9]. Hence the need for the compulsory and appropriate wearing of the mask as long as the pandemic plagues.