High Number of RNA Copies in Asymptomatic Individuals Infected with SARS-CoV-2 in an Area of the Colombian Caribbean

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Short report

Keywords: Asymptomatic infections, COVID-19, Coronavirus infections, pre symptomatic disease, environment and public health, Communicable Disease Control

DOI: https://doi.org/10.21203/rs.3.rs-32302/v1

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Abstract

Background: Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) is an emerging pandemical virus that has caused millions of reported cases and hundreds of thousands of deaths in less than six months. South America has suffered the pandemic because it lacks the hospital and economic capacities needed to contain the pandemic's advance. Public health implications of transmission, while asymptomatic is a critical concern at the current pandemic.

Objective: Describe the socio-demographic, clinical, and viral kinetics features of a cohort of SARS-CoV-2 infected individuals from the Colombian Caribbean.

Methods: Six hundred eighty-six clinical samples from several hospital centers in the province were received between April 9th and May 16th, 2020. RNA was extracted using lysis buffers and spin columns. The samples were tested for SARS-CoV-2 by RT-qPCR (Reverse transcription real-time polymerase chain reaction) using commercially available multiplex real-time PCR assay for simultaneous detection of 3 target genes of SARS-CoV-2 (AllplexTM, 2019-nCoV assay, Korea). Viral copies quantification was done using a standard curve constructed from seriated dilutions of a SARS-CoV-2 positive control.

Results: Thirty-five nasopharyngeal samples were positive for SARS-CoV-2 infection; the average age was 43 (range, 1-95 years). Seventeen of 35 (49%) of the patients showed symptoms. Most of them had cough, fever, and odynophagia, 3 of the patients reported having arthralgia. Only two patients required hospitalization. None of the patients had known co-morbidities. RT-qPCR results show that two of the symptomatic patients had significantly higher RNA copies than the rest of the patients. Eighteen of 35 (51%) individuals were asymptomatic, the average age was 30 (range, 6-61 years. Four individuals showed a higher copy than some symptomatic patients. Nonetheless, the average of RNA copies 8.26 x10+10 was lower than the symptomatic.

Conclusions: the population studied was young with an average of 43 years in symptomatic and 30 years of asymptomatic; this is important because of the high impact in the economy, and probably it is the cause of the reduced lethality observed in the department. Because a large proportion of infections probably result from transmission from asymptomatic or pre-symptomatic persons, the usefulness of public health interventions in Colombian provinces should be based in the molecular screening in a vast conglomerate's population and to quantify the viral load.

Background

Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) is an emerging pandemical virus that has caused millions of reported cases and hundreds of thousands of deaths in less than six months. South America has suffered the pandemic because it lacks the hospital and economic capacities needed to contain the pandemic's advance. South America has 433,497 infected people. The top five countries' distribution is as follows: Brazil has 241,080 cases, Peru 92,273, Chile 43,781, and Ecuador 33,182, and Colombia 15,574 cases [1]. The mortality per million people in Ecuador, Peru, Brazil, Chile, Bolivia, and
Colombia are 155, 80, 74, 24, 14, and 11, respectively, with a total of 23,117 people who died in the continent [1]. Meanwhile, Colombia's first case was on March 6th, 2020, and has been shocked by the number of cases with an infection rate of 294/million [2].

In the department of Cordoba, Colombia Caribbean area, the first case was reported on March 26th. Fortunately, only 10% of 78 cases have not required hospitalization; as of today, the mortality rate reaches 0.22/100,000 population [2].

Public health implications of transmission, while asymptomatic is a critical concern at the current pandemic [3, 4]. This study aims to describe the socio-demographic, clinical, and viral kinetics features of a cohort of SARS-CoV-2 infected individuals from the Colombian Caribbean.

**Methods**

**Type of study, geographic area and sample collection**

The present work is a prospective, descriptive study carried out in the department of Cordoba, located in the northwest of Colombia, and is a part of the Caribbean savanna (Fig. 1A). The mean annual temperature is 28 °C, occurring a dry and a rainy season. The department's population is 1,828,947 inhabitants, and Monteria, Cordoba's capital city, has a population of 505,334. This study was conducted in the laboratory of Universidad de Cordoba, which is licensed by Colombia's National Health Institute for the molecular diagnostic of SARS-CoV-2 human infection. Six hundred eighty-six clinical samples from several hospital centers in the province were received and processed by RT-qPCR between April 9th and May 16th, 2020, with 35 positive results for SARS-CoV-2 infection. The ethical standards of the Ministry of Health of Colombia Resolution No. 8430 of 1993 were followed. The data of the present study correspond to patients coded under strict anonymity with an internal laboratory number.

**RNA extraction and SARS-CoV-2 detection**

Briefly, RNA was extracted using lysis buffers and spin columns. After RNA extraction, the samples were tested for SARS-CoV-2 by RT-qPCR using commercially available multiplex real-time PCR assay for simultaneous detection of 3 target genes of SARS-CoV-2 (Allplex™, 2019-nCoV assay, Korea). The assay is designed to detect RdRP (RNA dependent RNA polymerase) and N genes specific for SARS-CoV-2, and E gene for all of Sarbecovirus, including SARS-CoV-2. Viral copies quantification was done using a standard curve constructed from seriated dilutions of a SARS-CoV-2 positive control. This control includes synthetic RNA target sequences for the three genes (RdRP, S, and N). Samples tested with a Ct value < 40 were considered positive. If a tested sample had a Ct between 40, and 45 the test was repeated, and the sample was considered positive if the curve showed a trend to arise, and the individual had an epidemiological link with an infected patient.
Results And Discussion

Thirty-five nasopharyngeal samples were positive for SARS-CoV-2 infection. Seventeen of 35 (49%) of the patients showed symptoms, 9/17 (53%) of them were female, eight patients were from the urban area of Monteria (505,334 inhabitants), eight from Sahagun (107,636 inhabitants), and one from Cerete (105,815 inhabitants). Most of them had cough, fever, and odynophagia, 3 of the patients reported having arthralgia (Table 1). Only two patients required hospitalization. So far, all of the symptomatic patients are alive and have recovered from the infection. The average age was 43 (range, 1–95 years). Both pediatric patients were symptomatic (Table 1). None of the patients had known co-morbidities. Only four symptomatic patients reported not having expositional contact with an infected person. RT-qPCR results show that two of the symptomatic patients (codes 505, 568) had significantly higher RNA copies than the rest of the patients. The average RNA copies were very high $4 \times 9^{11}$ (Table 1).
Table 1
Epidemiological, clinical, demographic information, and RNA virus copies.

| Anonymous code | Date of sample | Symptomatic (onset date) / Asymptomatic | Municipality | Age/Gender /Health staff | Contact | Ct            |
|----------------|---------------|----------------------------------------|--------------|--------------------------|---------|---------------|
| a 505          | 09/05/2020    | Symptomatic (30-apr) Fe, Od            | Sahagun      | 65/M/No                  | No      | 24,000890 21  |
| 556            | 11/05/2020    | Symptomatic (9-May) Co, Ar, Od         | Sahagun      | 32/F/Yes                 | Yes     | 36,942015 0638368 |
| 557            | 12/05/2020    | Symptomatic (10-May) Fe, Ar, Od        | Sahagun      | 95/M/No                  | No      | 39,265640 0322052 |
| a 568          | 12/05/2020    | Symptomatic (9-May) Od                 | Sahagun      | 52/M/No                  | Yes     | 24,029534 0854369 |
| 593            | 13/05/2020    | Symptomatic (6-May) Co, Od             | Sahagun      | 28/F/Yes                 | Yes     | 38,643151 9592528 |
| 594            | 13/05/2020    | Symptomatic (11-May) Co, Od           | Sahagun      | 31/F/Yes                 | Yes     | 37,417420 2687062 |
| 595            | 13/05/2020    | Symptomatic (11-May) Co               | Sahagun      | 56/M/Yes                 | Yes     | 38,035314 8721936 |
| 615            | 13/05/2020    | Symptomatic (10-May) Fe               | Sahagun      | 24/F/No                  | Yes     | 36,905455 4946246 |
| b 09/05/2020   |               | Symptomatic (2-May) Co, Od            | Monteria     | 26/M/No                  | No      | 37,120751 845905 |
| 602            | 12/05/2020    | Symptomatic (10-May) Co, Od           | Monteria     | 41/F/Yes                 | Yes     | 40,371039 5393544 |
| b 605          | 13/05/2020    | Symptomatic (4-May) Co               | Monteria     | 23/F/No                  | Yes     | 34,706884 1210098 |

Fe: fever, Co: cough, Ar: arthralgia, Od: odynophagia, M: male, F: female.
| Code  | Date of sample | Symptomatic (onset date) | Municipality | Age/Gender | Contact | Ct         |
|-------|----------------|--------------------------|--------------|------------|---------|------------|
| b 606 | 13/05/2020     | Symptomatic (10-May) Co  | Monteria     | 60/M/No    | Yes     | 38,128815 0448141 |
| b 608 | 13/05/2020     | Symptomatic (11-May) Co  | Monteria     | 43/F/No    | Yes     | 37,669618 5948042 |
| b 609 | 13/05/2020     | Symptomatic (10-May) Od  | Monteria     | 1/M/No     | Yes     | 37,265560 3597797 |
| b 610 | 13/05/2020     | Symptomatic (12-May) Fe  | Monteria     | 6/M/No     | Yes     | 41,184726 8770343 |
| 684   | 15/05/2020     | Symptomatic (1-May) Ar   | Monteria     | 72/F/No    | Yes     | 37,751737 3406786 |
| 559   | 11/05/2020     | Symptomatic (7-May) Od   | Cerete       | 74/F/No    | No      | 36,619628 2425225 |
| a 558 | 11/05/2020     | Asymptomatic             | Sahagun      | 46/F/No    | Yes     | 36,619628 2425225 |
| 583   | 13/05/2020     | Asymptomatic             | Sahagun      | 32/F/Yes   | Yes     | 37,628085 1364822 |
| 585   | 13/05/2020     | Asymptomatic             | Sahagun      | 30/F/Yes   | Yes     | 24,841946 0495114 |
| 592   | 13/05/2020     | Asymptomatic             | Sahagun      | 21/M/Yes   | Yes     | 38,964734 3140328 |
| c 666 | 15/05/2020     | Asymptomatic             | Sahagun      | 34/F/No    | Yes     | 42,944099 7130024 |
| c 674 | 15/05/2020     | Asymptomatic             | Sahagun      | 27/F/Yes   | Yes     | 39,734051 4680728 |
| c 677 | 15/05/2020     | Asymptomatic             | Sahagun      | 34/F/Yes   | Yes     | 44,773722 37901  |
| c 678 | 14/05/2020     | Asymptomatic             | Sahagun      | 30/F/Yes   | Yes     | 36,631190 7471569 |

Fe: fever, Co: cough, Ar: arthralgia, Od: odynophagia, M: male, F: female.
| Anonymous code | Date of sample | Symptomatic (onset date) / Asymptomatic | Municipality | Age/Gender /Health staff | Contact | Ct             |
|----------------|---------------|----------------------------------------|--------------|--------------------------|---------|----------------|
| c 682          | 15/05/2020    | Asymptomatic                           | Sahagun      | 50/M/Yes Yes             | Yes     | 36,8407657818134 |
| d 574          | 12/05/2020    | Asymptomatic                           | Monteria     | 26/M/No Yes              | Yes     | 38,5063617495545  |
| d 575          | 12/05/2020    | Asymptomatic                           | Monteria     | 17/F/No Yes              | Yes     | 37,1084176398085  |
| 599            | 13/05/2020    | Asymptomatic                           | Monteria     | 35/M/No Yes              | Yes     | 39,3886493111317  |
| 600            | 12/05/2020    | Asymptomatic                           | Monteria     | 26/M/Yes Yes             | Yes     | 33,6632756072637  |
| 601            | 12/05/2020    | Asymptomatic                           | Monteria     | 25/M/Yes Yes             | Yes     | 39,0785098708386  |
| 604            | 12/05/2020    | Asymptomatic                           | Monteria     | 61/M/Yes Yes             | Yes     | 36,0769730910986  |
| b 607          | 13/05/2020    | Asymptomatic                           | Monteria     | 17/M/No Yes              | Yes     | 38,2068660785118  |
| d 612          | 13/05/2020    | Asymptomatic                           | Monteria     | 24/F/No Yes              | Yes     | 37,6687265771356  |
| d 613          | 13/05/2020    | Asymptomatic                           | Monteria     | 6/F/No Yes               | Yes     | 35,5706197133347  |

Fe: fever, Co: cough, Ar: arthralgia, Od: odynophagia, M: male, F: female.

Individual codes 585, 600, 613, and 612 showed a higher copy than some symptomatic patients (Table 1, Fig. 1C). Nonetheless, the average of RNA copies $8.26 \times 10^{10}$ was lower than the symptomatic. The Wilcoxon test for independent samples showed that there is no significant difference ($p > 0.05$) between the viral RNA copy number of symptomatic and asymptomatic patients (Fig. 1B). Nine of 18 individuals were from Monteria, the remaining nine from Sahagun. All asymptomatic individuals had a known infected contact (Table 1); the figure shows the number of infected contacts (Fig. 1C). We do not know if the asymptomatic individuals of the present study develop a COVID-19 disease after taken their nasopharyngeal sample, but we believe that they are pre-symptomatic or asymptomatic. 43% of the health staff was infected, an important issue because they are in the front line facing the pandemic (Table 1).
Eighteen of 35 (51%) individuals were asymptomatic, 10/18 (56%) were female, the average age was 30 (range, 6–61 years. Most studies report that male is more affected by coronaviruses than female; however, in the present study, women were more affected than men (53% symptomatic and 56% asymptomatic). This trend, 52.56%, continues throughout the department of Cordoba and is opposing to national behavior (44.25%). The present study strengthens the concern about public health implications of pre-symptomatic or asymptomatic SARS-CoV-2 infection [3, 5]. Our results show a 51%) of asymptomatic infected individuals, of which 78% (14/18) presented a significative viral copy, some of them higher than several of symptomatic patients (Fig. 1C). Moreover, RT-PCR Ct lower than 34 of some of them presumes the possibility to isolate infectious SARS-CoV-2 to demonstrate viral viability [3, 5, 6].

**Conclusions**

If a considerable percentage of infections are asymptomatic, increased testing approaches may be needed to detect these persons [6, 7]. Colombia has tested 3,741 per million people in a country with 50 million people, Brazil, with 330 million people have tested 3,462 subjects, Peru and Chile are the countries with more tested people in South America, exceeding the 25,000 tests. Because a large proportion of infections probably result from transmission from asymptomatic or pre-symptomatic persons, the usefulness of public health interventions in Colombian provinces should be based in two steps, first the molecular screening in a vast conglomerate's population and second to quantify the viral load. Finally, a remarkable issue is that the population studied is very young with an average of 43 years in symptomatic and 30 years of asymptomatic; this data is essential because produce a high impact in the economy and probably it is the cause of the reduced lethality observed in the country and the department.

**Abbreviations**

SARS-CoV-2: Severe acute respiratory syndrome Coronavirus 2; RNA: ribonucleic acid; RT-qPCR: Reverse transcription real-time polymerase chain reaction; RdRP: RNA dependent RNA polymerase; Ct: threshold cycle; COVID-19: Coronavirus diseases 2019.

**Declarations**

**Ethical approval and consent to participate.**

The study follows the ethical standards of the Ministry of Health of Colombia Resolution No. 8430 of 1993. The data of the present study correspond to patients coded under strict anonymity with an internal laboratory number. This study is the results of a research project that was approved by the Comité de ética del Instituto de Investigaciones Biológicas del Trópico de la Universidad de Cordoba, with the number N° 0410-2020.

**Consent to publication.**
Availability of data and material.

Our results are preliminary, and we do not wish to share this preliminary data for the moment.

Competing interests.

The authors declare no competing interests.

Funding.

This study was supported by the University of Cordoba.

Authors' contributions.

All authors participated for equal in the conception of the manuscript, design of the study, collection, analysis, and interpretation of the data. Besides, all authors did review the manuscript and approved it before submit it.

Acknowledgments.

To the department of Cordoba, Secretary of Health. To the Rectory of the University of Cordoba for their determined commitment to face coronavirus's pandemic and financial support. To Paola Diaz for the elaboration of the Figure, and Instituto Nacional de Salud of Colombia.

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Figures
Figure 1

A: Map of Colombia with its neighborhood countries. B: boxes of the median of RNA copies and bars of individual symptomatic patients and asymptomatic subjects with RNA copies. Asymptomatic data. Median = 6003,985; P25 = 2152.519; P75 = 16780.655. Symptomatic data: Median = 12652,686; P25 = 2813,048; P75 = 26288.189. Extreme values were excluded, red spot outlayer extreme. The median RNA copies for symptomatic was 12,652.6 (IQR 2,813.0 - 26,288.1) and for asymptomatic 6,003.9 (IQR 2,152.5 -16,780.6). C: X-axis internal patient's code number, Y-axis Log by RNA copies/ml of symptomatic and asymptomatic individuals, the average of symptomatic was higher than asymptomatic patients. Several asymptomatic patients show higher RNA copies than some symptomatic patients. Confirmed patients a and b resulted in 2 and 6 infected contacts, respectively. Asymptomatic individuals c and d resulted in 5 and 4 asymptomatic individuals, some of them with important RNA copies. The average of symptomatic was higher than asymptomatic. A remarkable data that 15 health workers resulted infected in order according to viral load (symptomatic=556, 593, 594, 595, 602; asymptomatic=583, 585, 592, 674, 677, 678, 682, 600, 601 and 604).
Supplementary Files

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