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

Posted Date: August 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-57254/v1

Version of Record: A version of this preprint was published on December 7th, 2020. See the published version at https://doi.org/10.1186/s12941-020-00397-5.

Abstract

Background. Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) is an emerging pandemical virus. The virus 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. Statics descriptive methods were used.

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 them. 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. It is probably the cause of the reduced lethality observed in the department. Because a large proportion of infections probably result from transmission from asymptomatic, pre-symptomatic persons. The usefulness of public health interventions in Colombian provinces should be based on molecular screening in a vast conglomerate population and quantify the viral load.

Introduction

Coronavirus 2 (SARS-CoV-2) is an emerging 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 4,700,853 infected people. The top five countries' distribution is as follows: Brazil has 3,013,369 cases, Peru 471,012, Chile 371,023, and Ecuador 93,572, and Colombia 376,870 cases [1]. The mortality per million people in Peru, Chile, Brazil, Ecuador, Bolivia, and Colombia are 631, 523, 473, 335, 307, and 246, respectively, with a total of 153,441 people who died representing the 96% of the total deaths in South America [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 7,398/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; 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 (Figure 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 (AllplexTM, 2019-nCoV assay, Korea). The test 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 $\leq$ 40 were considered positive. If a tested sample had a Ct between 40-45, the test was repeated, and the sample was deemed to be positive if the curve showed a trend to arise. 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 4x9+11 (Table 1).

Individual codes 585, 600, 613, and 612 showed a higher copy than some symptomatic patients (Table 1, Figure 1C). Nonetheless, the average of RNA copies 8.26 x10+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 (Figure 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 (Figure 1C). We do not know whether asymptomatic individuals of the present study develop a COVID-19 disease after taking the sample. Forty-three of the health staff were 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 males are more affected by coronaviruses than females. However, in the present study, women were more affected than men (53% symptomatic and 56% asymptomatic). This trend, 52,56%, continues throughout Cordoba's department and is opposed to national behavior (44,25%). The present study strengthens the concern about the public health implications of pre-symptomatic 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 symptomatic patients (Figure 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].

Conclusion

If a considerable percentage of infections are asymptomatic, increased testing approaches may be needed to detect these persons [6, 7]. Colombia has tested 36,855 per million people in a country with 50 million people, Brazil, with 330 million people have tested 62,202 subjects, Peru and Chile are the countries with more tested people in South America, exceeding the 171,931 tests. Because a large proportion of infections probably result from transmission from asymptomatic-pre-symptomatic persons, the usefulness of public health interventions in Colombian provinces should be based on two steps. The first should be 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.

Declarations

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 paper and approved it before submit it.

Funding. This study was supported by the University of Cordoba.

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

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.

Competing interests. The authors declare no competing interests.

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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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Tables

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	RNA Copies/mL
505a	9/05/2020	Symptomatic (30-apr) Fe, Od	Sahagun	65/M/No	No	24,00	8,33E+12
556	11/05/2020	Symptomatic (9-May) Co, Ar, Od	Sahagun	32/F/Yes	Yes	36,94	2,96E+04
557	12/05/2020	Symptomatic (10-May) Fe, Ar, Od	Sahagun	95/M/No	No	39,27	1,70E+02
568a	12/05/2020	Symptomatic (9-May) Od	Sahagun	52/M/No	Yes	24,03	8,26E+09
593	13/05/2020	Symptomatic (6-May) Co, Od	Sahagun	28/F/Yes	Yes	38,64	7,97E+03
594	13/05/2020	Symptomatic (11-May) Co, Od	Sahagun	31/F/Yes	Yes	37,42	1,82E+04
595	13/05/2020	Symptomatic (11-May) Co	Sahagun	56/M/Yes	Yes	38,04	1,27E+04
615	13/05/2020	Symptomatic (10-May) Fe	Sahagun	24/F/No	Yes	36,91	3,05E+04
517b	9/05/2020	Symptomatic (2-May) Co, Od	Monteria	26/M/No	No	37,12	3,59E+03
602	12/05/2020	Symptomatic (10-May) Co, Od	Monteria	41/F/Yes	Yes	40,37	2,15E+02
605b	13/05/2020	Symptomatic (4-May) Co	Monteria	23/F/No	Yes	34,71	3,43E+04
606b	13/05/2020	Symptomatic (10-May) Co	Monteria	60/M/No	Yes	38,13	1,18E+04
608b	13/05/2020	Symptomatic (11-May) Co	Monteria	43/F/No	Yes	37,67	1,68E+04
609b	13/05/2020	Symptomatic (10-May) Od	Monteria	1/M/No	Yes	37,27	2,30E+04
610b	13/05/2020	Symptomatic (12-May) Fe	Monteria	6/M/No	Yes	41,18	1,24E+03
684	15/05/2020	Symptomatic (1-May) Ar	Monteria	72/F/No	Yes	37,75	2,04E+03
559	11/05/2020	Symptomatic (7-May) Od	Cerete	74/F/No	No	36,62	3,82E+04
558a	11/05/2020	Asymptomatic	Sahagun	46/F/No	Yes	36,62	2,76E+04
583	13/05/2020	Asymptomatic	Sahagun	32/F/Yes	Yes	37,63	1,43E+03
585	13/05/2020	Asymptomatic	Sahagun	30/F/Yes	Yes	24,84	1,49E+12
592	13/05/2020	Asymptomatic	Sahagun	21/M/Yes	Yes	38,96	6,26E+03

666c	15/05/2020	Asymptomatic	Sahagun	34/F/No	Yes	42,94	1,93E+00
674c	15/05/2020	Asymptomatic	Sahagun	27/F/Yes	Yes	39,73	9,41E+01
678c	14/05/2020	Asymptomatic	Sahagun	30/F/Yes	Yes	36,63	5,49E+03
682c	15/05/2020	Asymptomatic	Sahagun	50/M/Yes	Yes	36,84	4,12E+03
574d	12/05/2020	Asymptomatic	Monteria	26/M/No	Yes	38,51	4,52E+02
575d	12/05/2020	Asymptomatic	Monteria	17/F/No	Yes	37,11	2,87E+03
599	13/05/2020	Asymptomatic	Monteria	35/M/No	Yes	39,39	4,57E+03
600	12/05/2020	Asymptomatic	Monteria	26/M/Yes	Yes	33,66	9,54E+04
601	12/05/2020	Asymptomatic	Monteria	25/M/Yes	Yes	39,08	5,75E+03
604	12/05/2020	Asymptomatic	Monteria	61/M/Yes	Yes	36,08	9,34E+03
607d	13/05/2020	Asymptomatic	Monteria	17/M/No	Yes	38,21	1,11E+04
612d	13/05/2020	Asymptomatic	Monteria	24/F/No	Yes	37,67	1,68E+04
613d	13/05/2020	Asymptomatic	Monteria	6/F/No	Yes	35,57	2,38E+04

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

Figures

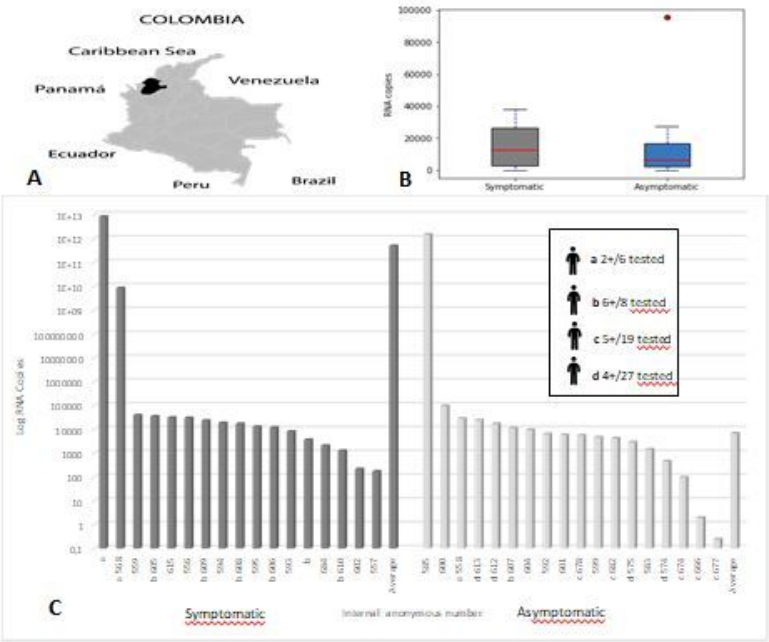

Figure 1

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). RNA copies of symptomatic patients and asymptomatic subjects, and map of Colombia with its neighborhood countries. Figure. Map of Colombia with its neighborhood countries, boxes of the median of RNA copies and bars of individual symptomatic patients and asymptomatic subjects with RNA copies. 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, 601,604). Figure A. Map of Colombia with its neighborhood countries. Figure B. 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). Figure C. Symptomatic patient "a" had contact with "a" 568 and asymptomatic patient "a" 558, so it is presumed that "a" was the source of infection for 568 and 558. Patient "b" was presumably the source of infection for symptomatic 605, 606, 608, 609, 610; it was also a source of infection for the asymptomatic individual 607. The asymptomatic persons c666, c674, c678, and c677 were in contact with each other, but it is unknown who started the infection. Asymptomatic people d574, d575, d612, and d613, were in contact with each other, but it is unknown who started the infection. X-axis internal patient's code number, Y-axis Log by RNA copies/ml of symptomatic and asymptomatic patients, the average of symptomatic was higher than asymptomatic patients, but some of asymptomatic show important high RNA copies. Patient 32 a health worker resulted with the higher RNA copies of the study, 3 contacts of the patients were negative to RT-PCR; patient 127 showed a moderate RNA copies, but 3 of 13 analyzed contacts of the patient were positive to RT-PCR. A confirmed COVID-19 patient from a small village near Lorica, resulted with 38 contacts which were analyzed, asymptomatic individuals 353*, 354*, and 355* were positive with important RNA copies. Subject 178 had 3 contacts, one of them resulted positive by RT-PCR. Average of asymptomatic individuals was lower than symptomatic.