Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Short communication

SARS-CoV-2 spread across the Colombian-Venezuelan border

Alberto Paniz-Mondolfi a,b,⁎, Marina Muñoz b, Carolina Florez c, Sergio Gomez c, Angelica Rico c, Lisseth Pardo c, Esther C. Barros c, Carolina Hernández d, Lourdes Delgado b, Jesús E. Jaimes b, Luis Pérez b, Aníbal A. Teherán d, Hala Alejel Alshammary a, Ajay Obla e, Zenab Khan e, Jayeeta Dutta e, Adriana van de Guchte f, Ana S. Gonzalez-Reiche e, Matthew M. Hernandez a, Emilia Mia Sordillo e, Viviana Simon f,g,h, Harm van Bakel a,i, Martin S. Llewellyn i, Juan David Ramírez b

a Department of Pathology, Molecular and Cell Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
b Instituto Nacional de Salud, Bogotá, Colombia
c Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
d The Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
e Instituto Nacional de Salud, Bogotá, Colombia
f Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
The Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
g Instituto Nacional de Salud, Bogotá, Colombia
h Instituto de Biodiversidad, Animal Health & Comparative Medicine, University of Glasgow, Glasgow G12 8QQ, UK

A B S T R A C T

Introduction: Venezuela and Colombia both adopted measures of containment early in response to the COVID-19 pandemic. However, Venezuela’s ongoing humanitarian crisis has decimated its health care system, and forced millions of Venezuelans to flee through its porous border with Colombia. The extensive shared border, and illegal cross-border transit through improvised trails between the two countries are major challenges for public health authorities. We report the first SARS-CoV-2 genomes from Venezuela, and present a snapshot of the SARS-CoV-2 epidemiologic landscape in the Colombian-Venezuelan border region.

Methods: We sequenced and assembled viral genomes from total RNA extracted from nasopharyngeal (NP) clinical specimens using a custom reference-based analysis pipeline. Three assemblies obtained were subjected to comparative genomic analyses. Additionally, the Wuhan-1 strain was used as reference.

Results: We found that two of the SARS-CoV-2 genomes from Venezuela belonged to the B1 lineage, and the third to the B.1.13 lineage. We observed a point mutation in the Spike protein gene (D614G substitution), previously reported to be associated with increased infectivity, in all three Venezuelan genomes. Additionally, three mutations (R203K/G204R substitution) were present in the nucleocapsid (N) gene of one Venezuelan genome.

Conclusions: Genomic sequencing demonstrates similarity between SARS-CoV-2 lineages from Venezuela and viruses collected from patients in bordering areas in Colombia and from Brazil, consistent with cross-border transit despite administrative measures including lockdowns. The presence of mutations associated with increased infectivity in the 3 Venezuelan genomes we report and Colombian SARS-CoV-2 genomes from neighboring borders areas may pose additional challenges for control of SARS-CoV-2 spread in the complex epidemiological landscape in Latin American countries. Public health authorities should carefully follow the progress of the pandemic and its impact on displaced populations within the region.

⁎ Corresponding author.
E-mail address: Alberto.Paniz-mondolfi@mountsinai.org (A. Paniz-Mondolfi).

https://doi.org/10.1016/j.meegid.2020.104616

Received 16 July 2020; Received in revised form 20 October 2020; Accepted 31 October 2020
Available online 4 November 2020
1567-1348/© 2020 Published by Elsevier B.V.
2. Methods

2.1. Ethical statement

The Colombian National Institute of Health (INS) is defined as the reference laboratory in Colombia. When a public health emergency occurs as is the COVID19, the national law 9-1979, decrees 786-1990 and 2323-2006, authorizes the INS to use the biological material and associated epidemiological information without informed consent, including the anonymous disclosure of results. This study was performed following the Declaration of Helsinki and its later amendments. The data of the patients was anonymized which do not represent any risk.

2.2. Patients, sampling and demographic data

Newly arrived migrants from Venezuela meeting case-definition criteria established by the Colombian Ministry of Health and Social Protection were screened for SARS-CoV-2 infection at different hospitals and healthcare centers in Norte de Santander and Bolivar Departments of Colombia between March 31st and May 1st, 2020. Molecular detection of SARS-CoV-2 in nasopharyngeal swab specimens in viral transport media (NP-VTM) was performed using the Berlin Charite protocol. SARS-CoV-2-positive specimens from five individuals who were tested within 24 h of their arrival in Colombia were referred for further characterization. Four of the five individuals already were symptomatic at the time of arrival from Venezuela, and one was a close contact of a confirmed COVID-19 patient. Complete viral genomes were generated from specimens from three of these five cases. The patients, aged 30 (♂) and 56 (♀) years, came from different regions, Cucuta, Norte de Santander, and Bolivar, (Fig. 1A). Sequencing was unsuccessful for the other 2 specimens, most likely due to insufficient target material.

2.3. Phylogenetic analysis

We sequenced and assembled viral genomes from total RNA extracted from NP viral transport medium (VTM) clinical specimens. Sample preparation for sequencing was done using whole-genome amplification with custom designed tiling primers and the Artic Consortium protocol (https://artic.network/ncov-2019) with a read length of 150, and the modifications previously described (Gonzalez-Reiche et al., 2020). Amplicon libraries were prepared using the Nextera XT DNA Sample Preparation kit (Illumina, cat. FC-131-1096), as recommended by the manufacturer that then were sequenced by Illumina sequencing.

The initial bioinformatic analysis was previously described (Gonzalez-Reiche et al., 2020). Briefly, the primer sequences were trimmed using cutadapt version 2.8, and were aligned to SARS-CoV-2 genome MN908947.3 using minmap2 version 2.17-r941, to call a consensus sequence using Pilon v1.23, allowing for all variant types (single nucleotide variants, small insertions/deletions, large insertions/deletions or block substitution events, and local mis-assemblies). Regions with less than 10 fold-coverage were masked and unamplified regions at the end of the viral genome were also removed. Finally, consensus sequences were annotated using Prokka v1.14.6 and a custom SARS-CoV-2 reference annotation file. The complete genomes were typed using the Phylogenetic Assignment of Named Global Outbreak Lineages ‘Pangolin’ tool (Rambaut et al., 2020).

A total of 376 publicly available SARS-CoV-2 genomes encompassing the lineage diversity from South America were downloaded from the GISAID EpiCoV™ database (https://www.gisaid.org/) for comparative genomic analyses (Table S1). These sequences were aligned in MAFFT (Katoh et al., 2018), using the Wuhan-1 strain (NC_045512.2) as reference. The complete data set was subjected to the same typing scheme using Pangolin tool. Thus far, 28 pangolin lineages have been reported for SARS-CoV2 strains circulating in South American countries (Fig. 1B), with B1 as the predominant lineage representing 62.9% of the total reported genomes. Four other lineages include B.1.5 (12.5%), A.5 (4.5%), A.2 and B (3.4% each) account for an additional 23.8%. Each of the remaining 15 lineages accounted for fewer than 10 genomes, with several having only a single representative. A time-scaled maximum likelihood (ML) phylogeny based on TreeTime built in IQTREE (Rambaut et al., 2016; Sagulenko et al., 2018) revealed that there was no clustering by originating country in the reconstruction, although a general clustering by pangolin lineages was observed (Fig. 1C). These analyses are in agreement with the simultaneous circulation of SARS-CoV-2 lineages from different geographical origins.

A detailed screening of single-nucleotide polymorphisms (SNPs) in important open reading frames (ORFs) of SARS-CoV-2 was then conducted, and substitutions in Spike and Nucleocapsid sequences were evaluated. The alignment was inspected using Ugene (http://ugene.net/). The regions of interest were exported considering the ORFs described for the reference strain Wuhan-1 (NC_045512.2), as previously described in NCBI: https://www.ncbi.nlm.nih.gov/nuccore?term = Severe + acute + respiratory + syndrome + coronavirus +2 + isolate + Wuhan-Hu-1.

3. Results

An average of 113,821.3 reads were obtained per sample reaching 960.52× of average depth of coverage in the genomes analyzed. We found that two of the SARS-CoV-2 genomes obtained (VEN-89312 and VEN-95072) were identical and belonged to the B1 lineage, while the third (VEN-95070) belonged to the B.1.13 lineage, suggesting two separate introductions (da Candido et al., 2020; Ramirez et al., 2020). Phylogenetic analysis revealed that two of the Venezuelan SARS-CoV-2
Fig. 1. Regional comparative genomic analysis of SARS-CoV-2. A. Geographical distribution of the sequences from Venezuelan genomes and the available Colombian genomes analyzed in this study. The pink symbols indicate the Venezuelan patients identified in Colombia with their respective Venezuelan state origin. B. Stacked bar plot of the number of genomes per lineage determined using Phylogenetic Assignment of Named Global Outbreak LINeages ‘Pangolin’ tool. Three SARS-CoV-2 genomes from Venezuela were compared with 376 assemblies from other 7 South American countries (Argentina, Brazil, Chile, Colombia, Ecuador, Peru and Uruguay) using the publicly available GISAID EpiCoV™ database (https://www.gisaid.org/). Frequencies are discriminated by country of origin. The Venezuelan lineages are identified by black arrows. C. Maximum likelihood tree built in IQtree shows the phylogenetic relationships between genomes from Venezuela (pink dots) and the closest Colombian regions (yellow dots) with other South American genomes. The branches were colored according to the country of origin, using the color code of A panel. The clustering of the most frequent pangolin lineages (n > 10) is represented on the right side. The black dots represent highly supported nodes. D. Multiple alignment of the Nucleocapsid gene showing the substitutions found using the Wuhan-1 sequence as reference.
The concurrent humanitarian crisis has forced millions of Venezuelans to flee to neighboring countries—mainly Colombia—seeking economic and social stability (Torres and Castro, 2019; Tuite et al., 2018). Venezuelans with ongoing health issues also travel to Colombia to obtain high-quality healthcare such as surgical procedures and hemodialysis (Daniels, 2020). However, massive Venezuelan migration has resulted in an unprecedented infectious disease exodus, representing one of the most concerning public health threats in the region (Grillet et al., 2019; Torres and Castro, 2019). COVID-19 has deepened the situation and has prompted xenophobia and further marginalization of Venezuelan migrants and refugees at the Venezuelan-Colombian border (Daniels, 2020).

The shared presence of B lineages in SARS-CoV-2 from Venezuela and Colombia reinforces the close interactions of persons living in border regions and the difficulty of containment across a porous border. Similarly, the detection of the B.1.13 lineage, only previously described in cases in Spain, England and Australia (Batty et al., 2020) further underlines the rapid global spread of SARS-CoV-2 through interconnected populations. Additionally, the presence of substitution D614G in the spike protein of the three viruses from patients residing in the current hotspots of COVID-19 in Venezuela may correlate with the reported increased infectivity (Korber et al., 2020) observed in SARS-CoV-2-infected patients in the state of Zulia (“Zulia suma 15 muertes confirmadas por coronavirus y seis no reportadas, Efecto Cocuy, 2020”).

An important limitation to our study is that the small number of genomes currently available from Venezuela could potentially result in sampling bias. Given the difficulty in obtaining samples, the extent to which our findings truly reflect Venezuela’s overall phylogenetic landscape remains to be determined. Future studies are needed to expand the SARS-CoV-2 genome repertoire in Venezuela and related areas, and to enable better understanding of the interplay between genotype and phenotype, and their relevance for disease surveillance and containment. Additionally, collection and sequencing of SARS-CoV-2 from all geographical regions (Colombia and Venezuela) will be needed to understand the true spread between these two neighbors.

5. Conclusion

The Venezuelan humanitarian and refugee crisis, coupled with uncontrolled migration across the Colombian-Venezuelan border, is a devastating reminder of the potential effects of infectious disease spill-over on the already vulnerable public health systems of neighboring countries. As SARS-CoV-2 continues to spread across Latin America, public health authorities and the international community should carefully follow the impact of the pandemic on displaced populations. Intensive efforts are urgently needed to help minimize the impact of Venezuela’s crisis on the COVID-19 pandemic crisis. Future efforts to obtain additional viral genomes will be essential to fully uncover the genomic epidemiology of SARS-CoV-2 in the Colombian-Venezuelan border.

Funding

This work was supported by the University of Glasgow, Scottish Funding Council and the Global Challenges Research Fund (GCRF) and GCRF Research Network EP/T003782/1.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2020.104616.

References

Andrus, J.K., Evans-Gilbert, T., Santos, J.L., Guzman, M.G., Rosenthal, P.J., Toscano, C., Yolenzuela, M.T., Siqueira, M., Eitenne, C., Breman, J.G., 2020. Perspectives on battling COVID-19 in countries of Latin America and the Caribbean. Am. J. Trop. Med. Hyg. 1–4 https://doi.org/10.4269/ajtmh.20-0571.

Batty, E.M., Kochakarn, T., Panthian, B., Kumpornsin, K., Jararanai, P., Wangwirawat, A., Kotanan, N., Jaruampornpan, P., Wattanachockchai, T., Raksanee, K., Sensorn, I., Daniels, L., Evans-Gilbert, T., Andrus, J.K., Santos, J.I., Guzman, M.G., Rosenthal, P.J., Toscano, C., Yolenzuela, M.T., Siqueira, M., Eitenne, C., Breman, J.G., 2020. Perspectives on battling COVID-19 in countries of Latin America and the Caribbean. Am. J. Trop. Med. Hyg. 1–4 https://doi.org/10.4269/ajtmh.20-0571.
