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Detection of the Omicron variant of SARS-CoV-2 in international travelers returning to Venezuela

Rossana C. Jaspe^a, Yoneira Sulbaran^a, Carmen L. Loureiro^a, Zoila C. Moros^b, Ernestina Marulanda^c, Francis Bracho^c, Nieves A. Ramírez^d, Yeilis Canonico^d, Pierina D'Angelo^e, Lieska Rodríguez^e, Julio Castro^f, Ferdinando Liprandi^b, Héctor R. Rangel^a, Flor H. Pujol^{a,*}

^a Laboratorio de Virología Molecular, Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas, Caracas, Miranda, Venezuela

^b Laboratorio de Biología de Virus, Centro de Microbiología y Biología Celular, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela

^c Laboratorio de Biología Molecular Casa Lab 2020, Caracas, Venezuela

^d Laboratorio de Biología Molecular Tu Buen Doctor, Caracas, Venezuela

^e Instituto Nacional de Higiene "Rafael Rangel", Caracas, Miranda, Venezuela

^f Policlínica Metropolitana, Caracas, Venezuela

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ABSTRACT

Background: By the end of 2021, the SARS-CoV-2 Variant of Concern (VOC) Delta was predominant in most of the world. At the end of November, the Omicron variant was first detected in South Africa. This variant was immediately classified as VOC, due to the explosive increase of cases in South Africa, and the great number of mutations exhibited by this new lineage. Since then, Omicron VOC displaced Delta one in almost every country. Venezuela implemented in May 2021 molecular testing of all the passengers arriving at Venezuelan airports.

Methods: In this study, we analyzed the presence of variants of SARS-CoV-2 in those positive samples, by sequencing a small fragment of the Spike genomic region.

Results: The Omicron variant was found in passengers arriving to Venezuela from the beginning of December. Complete genome analysis confirmed the presence of the Omicron VOC. The detection of this VOC coincided with an unprecedented increase in the frequency of passengers with positive nucleic acid testing.

Conclusions: Genomic surveillance of samples for international travelers returning to Venezuela allowed us to rapidly detect the introduction of the Omicron variant in the country.

1. Introduction

SARS-CoV-2 infection, responsible for the COVID-19 pandemic, has caused more than 370 million cases and more than 5.6 million deaths worldwide until January 2022. In these two years of a high rate of replication, this virus has accumulated several mutations, which allows its classification in more than 1700 lineages by January 2022 [1,2]. Some of these lineages are denominated variants by WHO [3]. These variants (lineages of viruses sharing particular types of mutations) emerged since the end of 2020, and have been defined as Variants of Interest (VOI) and Variants of Concern (VOC), when any of different phenotypic traits, such as increase transmissibility or immune evasion, among others, have been confirmed [3]. By December 2021, 5 VOCs have been described: variant Alpha which emerged in the UK (lineage

B.1.1.7), variant Beta in South Africa (B.1.351), variant Gamma in Brazil (P1), variant Delta in India (B.1.617.2), and variant Omicron, which might have emerged in South Africa (B.1.1529) [4]. This last VOC was included in the list very soon after its identification [3]. This variant caused immediate concern, due to the explosive increase in cases in South Africa, and the large number of mutations exhibited by this new lineage [4].

Venezuela implemented since March 2021 SARS-CoV-2 molecular testing to all international travelers arriving at Venezuelan Airports (<http://www.inac.gob.ve/wp-content/uploads/2020/11/PDF-BIOSEGURIDAD.pdf>). The aim of this study is to describe the detection of the Omicron VOC among the international travelers returning to Venezuela.

* Corresponding author. Laboratorio de Virología Molecular, IVIC, CMBC, Apdo 21623, Caracas, 1020A, Venezuela.

E-mail address: fhpujol@gmail.com (F.H. Pujol).

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2. Materials and methods

Five international commercial airports were available in Venezuela in 2021: Maiquetia (close to Caracas, the capital of the country), Maracaibo, Valencia, Barcelona, and Porlamar (Margarita Island, touristic destination, receiving only international flights arriving from Russia). Positive samples from 4 out of these 5 airports (excepting Maracaibo) were analyzed in this study. Samples from Valencia airport are evaluated by qRT-PCR and the samples from the 3 other airports studied are evaluated by LAMP. Positive samples from November 28 to December 30 were sent to Instituto Venezolano de Investigaciones Científicas (IVIC) for sequence analysis. This study was approved by the Human Bioethical Committee of IVIC.

RNA from clinical samples positive by qRT-PCR or LAMP was amplified with primers 75L (Artic primer) and 76.8R to generate an amplicon of 614 nt, with the PCR conditions previously described [5,6]. PCR purified fragments were sent to Macrogen Sequencing Service (Macrogen, Korea). This fragment allows us to analyze amino acids 345 to 533 of the Spike gene, which includes two important mutations of the Delta VOC (L452R and K478T) and several mutations of the Omicron VOC, including K417 N, N440K, T478K, E484A, N501Y, and D405N R408S, these two ones absent in BA.1, but present in of BA.2 (both) and BA.3 (only D405N).

Complete genome sequencing was performed in some samples classified as Delta or Omicron by Sanger sequencing, to confirm the identification of the two VOCs found: Delta and Omicron. Next generation sequencing was performed as previously described [5]. Nucleotide sequences of complete genomes have been deposited into the GISAID database with the accession numbers described in Table 1.

Statistical differences were evaluated by the Student *t*-test. P values less than 0.05 were considered significant.

3. Results

Fig. 1A shows the significant increase in the number of positive SARS-CoV-2 cases observed in the travelers from Maiquetia airport during the second half of December. This situation was also observed in Valencia and Barcelona airports (data not shown), but not in Margarita airport (Fig. 1B), where this increase began to be observed later, during the second half of January 2022.

The sequence of 257 samples could be obtained during this period. A rapid displacement of Delta VOC by Omicron one was observed in the second half of December (Fig. 2). No significant difference was found in the Ct values between Omicron and Delta VOCs samples (data not shown).

Complete genome confirmed the identification of the Omicron VOC in Venezuela (Table 1). The two samples belonged to sub lineage BA.1.1. One of the Omicron samples for which sequence was available was from a passenger whose sample was negative when she arrived at Maiquetia airport, but developed symptoms one day later, suggesting that she

Table 1
Complete genome sequences of selected samples from international travelers.

| Accession number | Date of arrival | Provenance | VOC | Lineage |
|------------------------------|-----------------|----------------------|---------|---------|
| EPI_ISL_8804532 | Dec 1, 2021 | N/A | Delta | AY.3 |
| EPI_ISL_8804697 | Dec 1, 2021 | N/A | Delta | AY.122 |
| EPI_ISL_8805061 | Dec 1, 2021 | N/A | Delta | AY.33 |
| EPI_ISL_8063663 | Dec 13, 2021 | North America | Omicron | BA.1.1 |
| EPI_ISL_8063574 ^a | Dec 14, 2021 | North America | Omicron | BA.1.1 |
| EPI_ISL_9345977 | Dec 15, 2021 | Dominican Republic** | Delta | AY.44 |

N/A: information not available.

^a The sample of this passenger was negative on molecular testing at the airport (December 13, 2021) but developed symptoms one day after her arrival. **May have visited other countries before Dominican Republic.

acquired the infection before her travel. The 4 samples of Delta VOC belonged each one to a different sub lineage of Delta (Table 1).

4. Discussion

Venezuela implemented molecular testing of SARS-CoV-2 in all international passengers arriving at the commercial airports of the country since March 2021 (<http://www.inac.gob.ve/wp-content/uploads/2020/11/PDF-BIOSEGURIDAD.pdf>). A significant increase of positive samples was observed since the second half of December in most of these airports. This increase was coincident with the detection of the Omicron VOC: most of the positive samples during this period belonged to this lineage. The explosive number of Omicron VOC cases throughout the world might be associated with the observed increase in cases detected at the Venezuelan airports. In Russia, the peak of Omicron VOC, and the corresponding peak in COVID-19 cases, was only after January 15, 2022 (<https://www.worldometers.info/coronavirus/country/russia/>). Indeed, an increase in the rate of positive passengers was observed for this airport later, since the end of January 2022 (Fig. 1B). This may have contributed to the delay observed in the peak of positive samples in this airport, compared to the other Venezuelan airports analyzed.

Differences in viral levels between Omicron and Delta VOCs may also affect the detection of positive samples. RNA levels have been reported to be lower for the Omicron VOC when compared to Delta VOC [7,8], but higher in another more recent study [9], or similar in another one [10]. In the present study, the same Ct values were observed for Delta and Omicron VOCs, probably due to the type of sample analyzed (from travelers originally negative, retested 1–3 days later). The number of Delta positive samples was also low (only 39 samples, compared to 216 of the Omicron samples).

The explosive number of cases observed in international travelers might also be related to other factors, such as the higher transmissibility and the shorter incubation period of the Omicron VOC: 3 days, instead of 4 days for Delta VOC [11].

The significant increase in positive cases in international passengers arriving to Venezuela was not observed previously since March 2021, when this molecular testing was implemented, not even during the months of June to September (data not shown), corresponding to the third epidemic wave of COVID-19, which was mainly associated with the Delta VOC.

The strategy adopted in this study (sequencing of a small genomic fragment of the RBD of SARS-CoV2), allowed us to analyze many samples in a short period of time, with a good correlation (99% in more than 200 complete genome already analyzed) in variant assignment between this Sanger sequencing and complete genome sequencing (Jaspe, RC, and Pujol; FH, personal communication).

Complete genome sequencing allows the identification of sub lineages in each isolate. Of note, the 4 samples of Delta VOC for which the complete genome was available belonged to different sub lineages, confirming that international travelers might be contributing to the diversity of the variants circulating in the countries.

5. Conclusions

Genomic surveillance of samples for international travelers returning to Venezuela allowed us to rapidly detect the introduction of the Omicron variant in the country, taking advantage of the availability of the samples detected by the molecular testing implemented in the international airports.

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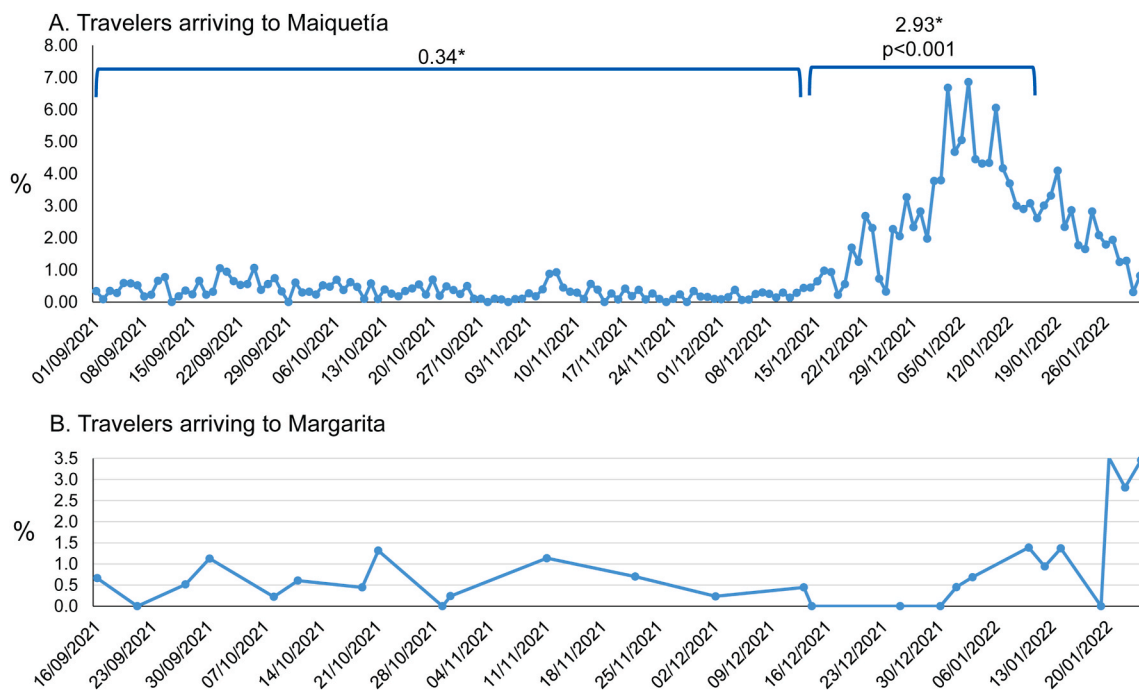


Fig. 1. Frequency of international travelers arriving to Venezuela with positive nucleic acid SARS-CoV-2 testing. **A:** Passengers arriving to Maiquetia. This airport received during the period evaluated, on average, 1219 international passengers/day. International flights arrive mainly from Cuba, Dominican Republic, Mexico, Panama, and Turkey. The numbers above the brackets describe the average frequency of positive samples during the two periods of time compared (September 2021 to December 14, 2021, vs. December 15, 2021, to January 14, 2022). **B:** Passengers arriving to Margarita. This airport received during the period evaluated, on average, 2072 international passengers/month. International flights arrive only from Russia.

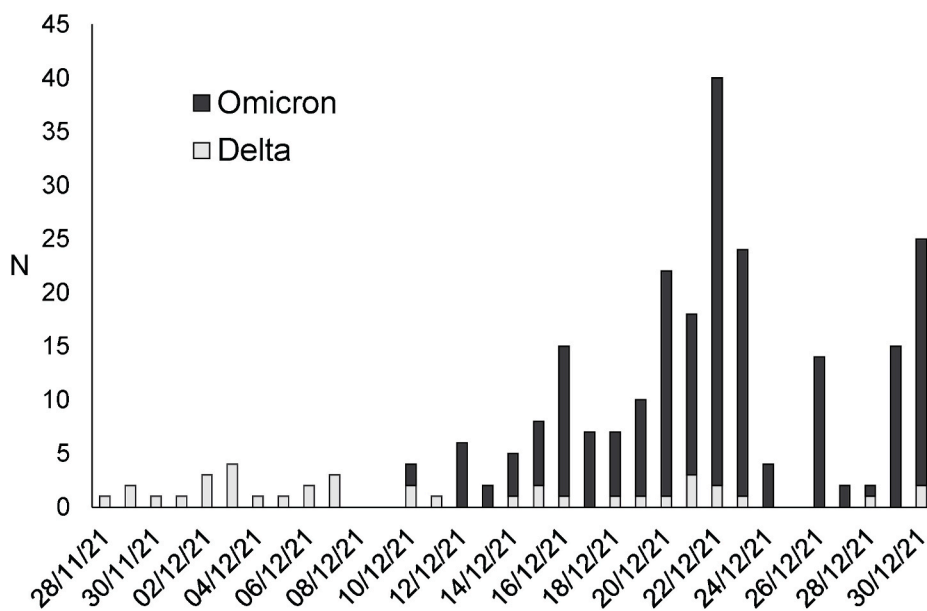


Fig. 2. Number of Delta and Omicron VOCs detected among international passengers positive on nucleic acid testing. For each day, the number of the samples that were successfully amplified and for which sequence was obtained, is reported. Samples were mainly from Maiquetia airport, the one receiving most passengers.

CRedit authorship contribution statement

Rossana C. Jaspe: Investigation, Writing – original draft, All authors have read and approved the final version of the manuscript. **Yoneira Sulbaran:** Investigation, All authors have read and approved the final version of the manuscript. **Carmen L. Loureiro:** Investigation, All authors have read and approved the final version of the manuscript. **Zoila C. Moros:** Investigation, All authors have read and approved the final

version of the manuscript. **Ernestina Marulanda:** data acquisition, All authors have read and approved the final version of the manuscript. **Francis Bracho:** data acquisition, All authors have read and approved the final version of the manuscript. **Nieves A. Ramírez:** data acquisition, All authors have read and approved the final version of the manuscript. **Yeilis Canonico:** data acquisition, All authors have read and approved the final version of the manuscript. **Pierina D’Angelo:** data acquisition, All authors have read and approved the final version of

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Declaration of competing interest

The authors declare that they have no competing interest.

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