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CARRERA DE MICROBIOLOGÍA

Relative prevalence of delH69V70 among SARS-CoV-2 infected individuals during the initial Omicron wave in Ecuador.

Disertación previa a la obtención del título de Microbiólogo

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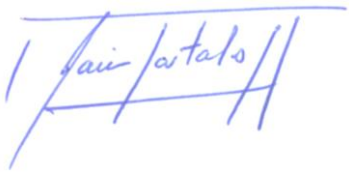
Quito, 2023

CERTIFICACION

Yo Jaime Costales Cordero PhD, certifico que la disertación de Microbiología del estudiante David Alejandro Jaramillo Pérez ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación.



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DEDICATORIA

Para mis padres, mis hermanas, mis cunados y mis sobrinos que son mi fuerza. ¡Los Amo!

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TABLA DE CONTENIDOS

LISTA DE TABLAS

LISTA DE FIGURAS

MANUSCRITO PARA LA PUBLICACION

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Relative prevalence of delH69V70 among SARS-CoV-2 infected individuals during the initial Omicron wave in Ecuador.

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TITLE: Relative prevalence of delH69V70 among SARS-CoV-2 infected individuals during the initial Omicron wave in Ecuador.

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Abstract

Background

The SARS-CoV-2 transmission dynamics in Ecuador was profoundly affected by the arrival of the Omicron variant, whose high transmissibility allowed it to overtake the much more virulent Delta variant. The delH69V70 mutation, which is found in the Omicron 21K variant, allows the virus to spread efficiently in the population due to its capability of increase the tolerance of mutations related to the immune escape and improving the infection.

Methods

Between December, 2021, and May 2022, 44,670 samples were analyzed for SARS-CoV-2 infection at the Pontificia Universidad Católica del Ecuador Molecular Diagnostic Laboratory (MLD-PUCE), in Quito, Ecuador. RNA remnants from 1,693 SARS-CoV-2 positive samples were further tested using sets of primers designed to discriminate the presence absence of five specific SARS-CoV-2 mutations (delH69V70, E484K, K417N, L452R and N501Y), which in turn allow for discrimination of six variants (Delta, Mu, Omicron 21K, Omicron 22A/22B, Omicron 21L and Omicron 22C).

Results

Fifty SARS-CoV-2 positive samples did not display any of the mutations detectable with the qPCR testing scheme employed in the study. One-hundred sixty-two samples carried the Delta (L425R) mutation. Five of them carried the MU/GAMMA (N501Y/E484K) mutation pattern. Most of the studied samples (1,375) carried the delH69V70 mutation, suggestive of Omicron 21K. Eighty-five samples carried the N501Y and K417N mutations. Two samples carried delH69V70 and L452R mutations, and 8 of them carried the N501Y, K417N and L452R mutations.

Discussion

Our results indicate that the arrival of the Omicron variant to Ecuador is associated with a sudden increase in cases between December 2021 and March 2022. Our data suggest Omicron replaced Delta as the predominant variant in the country in just 6 days (4 since it was first detected in MDL-PUCE). The prevalence of the Omicron variant among our dataset correlates well with data form the Ecuadorian Ministry of Health indicating a sharp increase of COVID-19 cases in the country. This investigation showcases the impact of Omicron´s transmissibility on the dynamics of COVID-19 in Ecuador.

Keywords:

Variant, mutation, qRT-PCR, S protein, pandemic

Background

The causative agent of COVID-19 is the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), an enveloped, positive single-stranded RNA (ssRNA) virus [1], which belongs to the Coronaviridae family. Coronaviruses are widespread

among mammals and avian species [2], and most of them cause enteric or mild respiratory infections in their host. There are three viral species within this family capable of infecting humans: namely SARS-CoV, MERS-CoV and SARS-CoV-2. The latter is the most efficient infecting human cells due to their own specific mutations and the greater affinity to the host receptor, the angiotensin-converting enzyme 2 (ACE2) [2].

The spike (S) protein is crucial for SARS-CoV-2 interaction with the host. This protein binds the ACE2 in the surface of the host cells, including cells in the lungs, vessels, brain, heart, and kidneys [3]. The S protein is cleaved by a host protease (furin-like protease), separating its two domains, namely S1 and S2. S1 binds the receptor, while S2 is responsible for the membrane fusion with the host and provides structural support as the stalk of the S protein [4]. The S gene, encoding the S protein, has a high mutation rate, which allows for evolution of viral variants displaying increasing infectivity [4]. The mutation ability of SARS-CoV-2 is one of the most important factors that helps it to spread so efficiently [5].

A variant is described as a virus displaying multiple mutations in its genome. If the variant displays different biological properties, such as the capacity to bind strongly to a receptor or more efficient transmission, then it is designated as a new strain [6]. To designate variants, mutations within their genomes are studied and compared. This classification is carried out via phylogenetic analysis, which demonstrates the differences existing between virus sharing a common ancestor [7].

In the U.S., the organization in charge of creating a SARS-CoV-2-variant classification and tracking system is the Department of Health and Human Services (HHS), through its SARS-CoV-2 Interagency Group (SIG). SIG is a consortium created to strengthen coordination among CDC, National Institutes of Health (NHI) Food and Drug Administration (FDA), Biomedical Advanced Research and Development Authority (BARDA), and Department of Defense (DoD). SIG designates four categories of variants, namely, variants of high consequence (VOHC), variants of concern (VOC), variants of interest (VOI) and variants being monitored (VBM) [8].

A VOHC has significantly increased the resistance against the preventive measures or medical countermeasures (i.e. medical supplies used to diagnose, prevent or treat diseases), on top of the attributes of a VOC, such as reduction in vaccine effectiveness, failure of diagnostic test targets or more severe clinical disease. These variants must be reported to WHO under the International Health Regulations. Presently, no SARS-CoV-2 variants are assigned to this group [8].

A VOC represents a huge impact on public health due to enhanced transmissibility, escape from antibody-mediated neutralization, decreased effectiveness of therapeutics or vaccination and the ability to evade detection. Among the VOCs, Alpha (B.1.1.7) was first discovered in the United Kingdom in late December 2020. This variant carries eight mutations in its S protein. Its most significant mutation is N501Y, which increase spike protein affinity for the ACE2 receptor [9]. Beta (B.1.351) was first discovered in South Africa in October 2020. This variant carries nine mutations in the S protein. Three of them, namely, K417N, E484K, and N501Y, increase affinity for ACE

2 receptor [9]. Delta (B.1.617.2), carrying ten mutations in the S gene, was first discovered in India, December 2020, and caused the second wave of COVID-19, in April 2021 [9]. Additionally, Omicron (B.1.1.529), first discovered in South Africa in November 2021, was recognized as a VOC after an unexpected rise in the number of positive cases. Omicron became the dominant variant in many countries, and subvariants such as 21K, 21L, 21M, 22A, 22B, 22C, 22D, were identified [9]. The principal mutation found in this variant is the delH69V70 which allows the virus to elude immune response and enforce the infection [10]. Currently, there are no variants that meet the requirements to be considered VOC [8].

A VOI possesses genetic markers that have been associated with changes that may cause the same features as the VOC [9]. The World Health Organization (WHO) describes eight VOI: Epsilon (B.1.427 and B.1.429), Eta (B.1.525), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37), Mu (B.1.621) Theta (P.3) and Zeta (P.2) [9].

VBMs include previous VOI, VOC or VOHC currently circulating at low levels, as well as variants with multiple antigenic mutations in various countries with collection dates within 4 weeks. Based on genetic analysis, they also represent a potential impact on medical countermeasures [8].

In Ecuador, the first SARS-CoV-2 case was reported on February 29th, 2020, confirmed by the National Institute of Public Health (INSPI) using reverse transcriptase polymerase chain reaction (RT-PCR) [11]. By December, 2022, 2,504,312 positive COVID-19 cases and 35,934 deaths were reported in the country [12]. Between January

2021 to January 2022, 4 VOCs SARS-CoV-2 strains were reported in Ecuador: Alfa 20I, on January 4th, 2021; Gamma 20J, on March 29th, 2021; Delta 21A, on June 21st, 2021; and Omicron 21K (BA.1) on December 6th, 2021, [13].

qRT-PCR-based assays can identify SARS-CoV-2 variants in function of the alleles they display [14]. This method assesses their quantitative differences in gene expression discriminating the presence or absence of a specific mutation [15]. The simplicity of the assay allows for processing a much larger number of samples rapidly, for a fraction of the cost necessary for genomic sequencing.

In this study, commercial primers were employed to discriminate SARS-CoV-2 variants present in Quito, Ecuador, during the period when the Omicron 21K variant was present. We aimed to obtain a greater resolution picture of the incidence of variants during said time frame.

Methods

Sample panel

A total of 1,693 SARS-CoV-2-positive RNA remnants, originally collected for qRT-PCR diagnostic purposes in the Molecular Diagnosis Laboratory from Pontificia Universidad Católica del Ecuador (MDL-PUCE) in the period comprised between

December 12th, 2021, and May 17th, 2022, were included in the study. This period includes the Omicron variant peak in Ecuador [16]. MDL-PUCE analyzed almost 200, 000 samples during the pandemic, corresponding to roughly 6,5% SARS-CoV-2 qRT-PCR diagnostic tests performed in Ecuador during the pandemic and therefore, provides good representativity for Ecuador, especially Quito.

For routine diagnosis, MDL-PUCE received nasal swab samples collected in RNA/DNA shield medium. Automated nucleic acid extraction was performed using the ANDiS Viral RNA Auto Extraction & Purification kit in The ANDiS®350 Nucleic Acid Extraction System. The study included remnants from RNA which yielded positive results in routine diagnostic qRT-PCR assays performed employing the Thermo Fisher Scientific qRT-PCR. Cycle threshold (Ct) values were between 18 and 30. RNA was stored at -80ºC until use.

RT-qPCR for specific mutations

Reverse transcription, coupled with qRT-PCR amplification was performed on 2.5 µL of RNA, using 5 µL of enzyme, with 0.4 µL of forward and 0.4 µL of reverse primers and temperature conditions following a PCR guide from Thermo Fisher Scientific Inc. [17]. Eighty samples, along with negative (ddH_2O) and positive RNA controls were included in each run, using a Bio-Rad CFX96 Touch Real-Time PCR thermal cycler.

RNA remnants were tested employing a specific primers/probe set, designed to discriminate the presence/absence of specific mutations in the SARS-CoV-2 genome, which allow to identify the different SARS-CoV-2 variants present in Ecuador during the study period (Table 1).

Table 1 Mutations analyzed in the study allow for identification of variants reported to be present in Ecuador during the study period.

Variant	Mutations			
ALPHA	N501Y			
DELTA 21A	L452R			
DELTA 21I	L452R			
DELTA 21J	L452R			
EU2	E484K			
GAMMA	N501Y	E484K		
MU	N501Y	E484K		
OMICRON 21K (BA.1)	delH69V70 GenS	N501Y	K417N	
OMICRON 21L (BA.2)		N501Y	K417N	
OMICRON 22A (BA.4) / 22B (BA.5)	delH69V70 GenS	N501Y	K417N	L452R
OMICRON 22C (BA.2.12.1)		N501Y	K417N	L452R

Results

The total number of SARS-CoV-2 analysis carried-out by MDL-PUCE between December 1st, 2021, and May 17th, 2022, was 44,670. Among these, 1,692 yielded positive results. Between December 1st and December 15th, 2021, the proportion of positive cases (78 reactive samples, 5%) was relatively low compared with the following weeks (95%). Between mid-December 2021, and the beginning of March 2022, there was notable increase in the percentage of positive samples, and a total of 1,340 positive

samples were reported. From March to May 2022 the laboratory reported a decrease on the number of positive cases, from 1340 to 274 (81% reduction) (Figure 1)

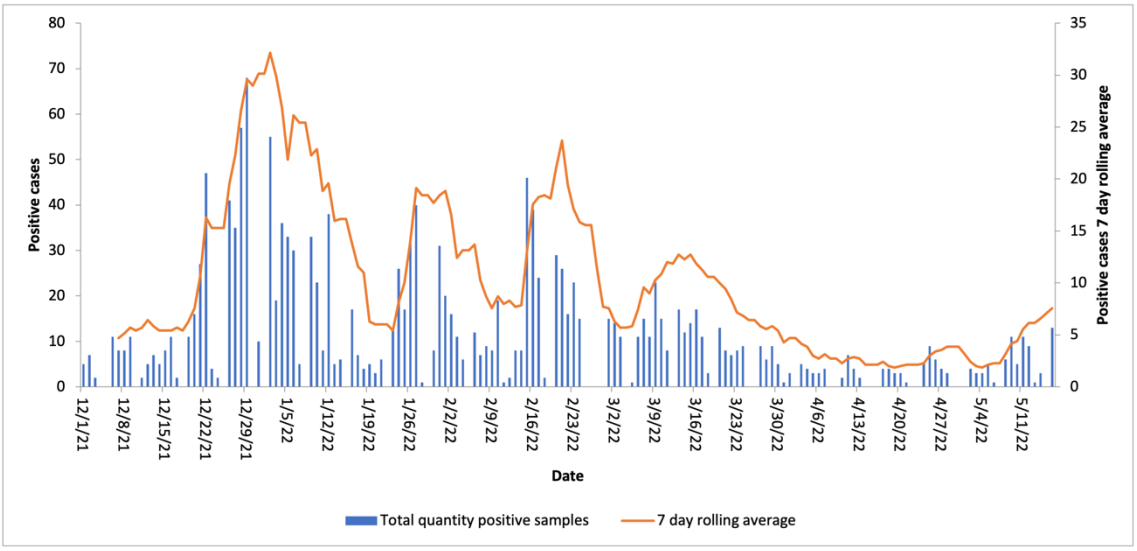

Fig. 1 SARS-COV-2 positive cases detected at MDL-PUCE between December 2021, and May 2022. The 7-day Rolling average of SARS-CoV-2 cases per day is depicted by the orange line and the blue lines represent the total number of positive cases registered.

The data collected by the Ecuadorian Ministry of Public Health (MoH) during this wave shows a rapid growth in the number of infections (Figure 2). The first sign of increase was reported by December 27th, 2021. A total of 19,476 COVID-19 cases by symptoms (based on the date where the symptoms started), 15,575 positive cases by medical attention (based on the date where the patient was attended on a clinic/hospital) and 8,779 positives for test notification (based on the date where the patient tested positive) were reported. The peak number of cases, according to the MoH was reached on January 17th (66,011 positive cases by symptoms, 64,852 positive cases by medical attention and 51,741 positive cases by test result notification, were reported). From that

point, the number of cases decreased. By February 28th, 2022, only 2,704 positive cases by symptoms, 3,358 positive cases by attention and 8,780 positive cases by notification were reported (Figure 2).

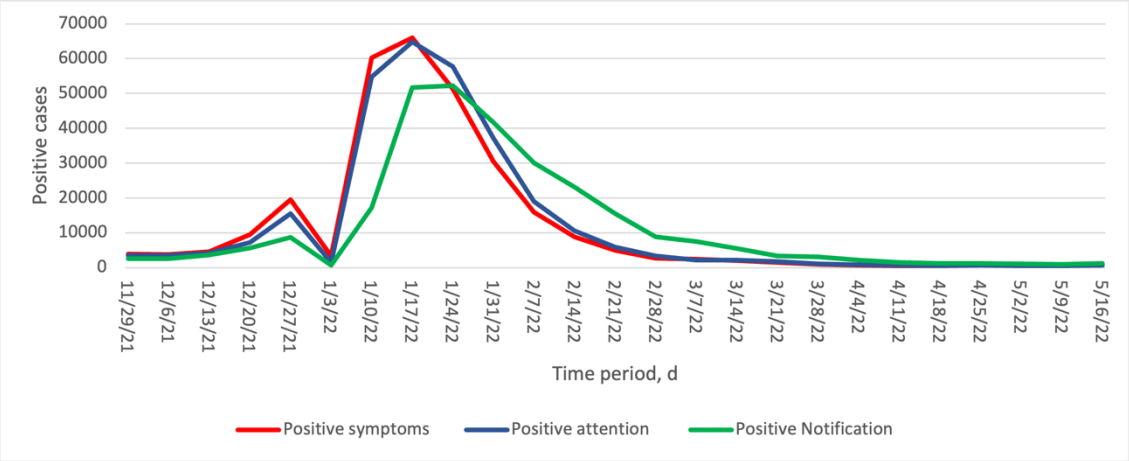

Fig. 2 COVID-19 cases reported to the Ministry of Public Health of Ecuador during from October 2021 – April 2022. The red line corresponds to COVID-19 cases according to symptoms. The blue line depicts COVID-19 cases by medical attention in hospitals or clinics. The green line represents the number of COVID-19 cases according to laboratory test result notification [18].

From December 2021 to May 2022, mutations suggestive of six different variants were detected among the 1,692 positive samples collected at MDL-PUCE. Table 2 indicates the number of samples displaying a particular mutation or set of mutations. Fifty samples were positive for SARS-CoV-2 but not reactive for any of the variants our qPCR testing scheme was able to detect.

Table 2 Frequency of the mutations N501Y, L452R, E484K, K417N and delH69V70 among the SARS-CoV-2 positive samples studied. The number of samples in which a given mutation is present (*) or absent (-) is indicated.

# of SAMPLES	MUTATIONS				
	N501Y	**L452R**	**E484K**	**K417N**	**delH69V70**
50	-	-	-	-	-
162	-	*	-	-	-
5	*	-	*	-	-
1,375	-	-	-	-	*
85	*	-	-	*	-
2	-	*	-	-	*
8	*	*	-	*	-

Figure 3 displays the prevalence of the six variants analyzed in our dataset across the study period. Delta (green) predominated from December 1st to mid-December, when Omicron 21K (purple) first appears. By the end of December, Omicron predominates, and Delta slowly disappears, with a few Delta cases still lingering until late January. Two cases of the MU/GAMMA variant (yellow) were detected in the third week of December. Additionally, 50 samples for which the variant could not be identified with the primers used for this investigation (gray) were detected throughout December. Omicron 21K was first detected in our dataset on December 16th, 2021, and had not completely disappeared by the end of May 2022, On January 28th and January 31st, we detected 2 samples carrying the MU/GAMMA variant. By March 16th, the first case of Omicron 21L was detected (light purple). This variant displaced Omicron 21K as the main circulating variant by

April 11th. At the end of April, beginning of May, we detected the first positive samples for Omicron 22C. This variant was circulating at the same time as Omicron 21L.

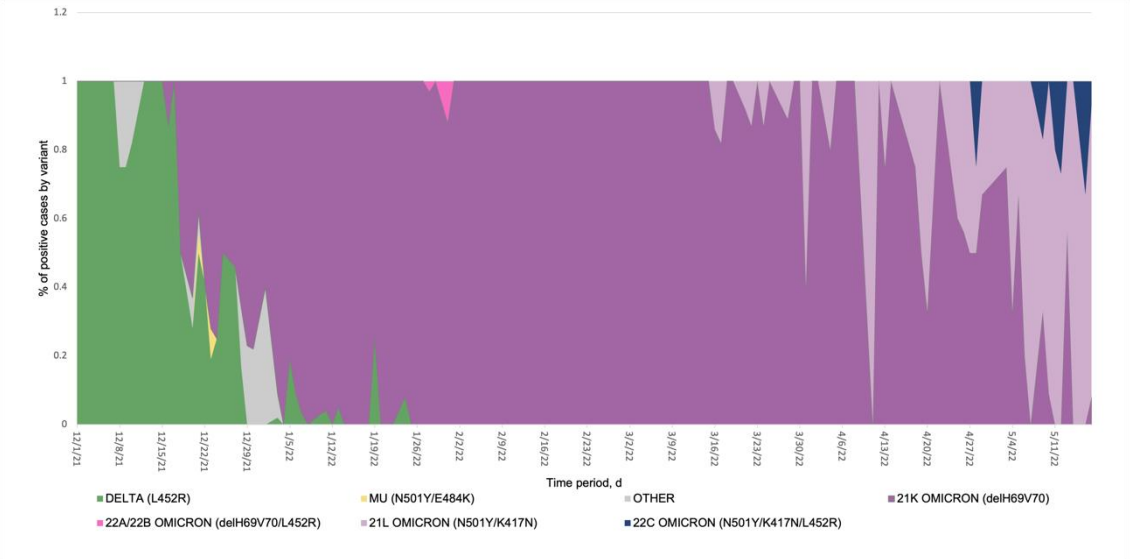

Fig. 3 Distribution of SARS-CoV-2 variants detected in MDL-PUCE during the December 1st, 2021- April 6th, 2022, period. Variants were assigned according to the mutations as indicated in Methods (Table 1). Each variant is represented with a different color (see legend).

Discussion

In this study, we analyzed the dynamic of the SARS-CoV2 variants during the Omicron wave in Ecuador, via qRT-PCR assays for detection of relevant mutations. Our data shows the Delta variant, which carries the L452R mutation [19], was promptly replaced by Omicron 21K, which carries the delV68H70 mutation [20]. The first report of Omicron 21K in Ecuador took place on December 14th, 2021, [13]. In agreement with

this, the first samples bearing the delH69V70 were detected on December 16th in our dataset. Our results suggest Omicron 21K became predominant in Ecuador in just 4 days. By December 20th, 2021, ~60% of SARS-CoV-2 samples in our dataset carried delH69V70, and 100% carried this mutation by January 4th, 2022, the few remaining samples carrying the L542R mutation disappeared by January 24th, 2022. The arrival of Omicron 21K during the December holidays (Christmas and New Year's) might have also facilitated by the increase in frequency of SARS-CoV-2 cases because of the large gatherings taking place during this time of the year.

Various studies suggest a similar epidemiological pattern for Omicron around the world. In England, Omicron was first reported on November 27th, 2021, and it took over a month to almost completely replace Delta, becoming the predominant variant of the region [21]. In Puerto Rico, Omicron was first reported on November 29th, 2021, and, within a week, Delta variant was replaced as the main variant in the country [22]. This pattern is attributable Omicron´s high transmissibility due to the large number of mutations present in the RBD region compared with Delta [23]. The Reproduction Number or R0 (average number of new cases caused by an infectious individual in a healthy population, if R0>1, the number of positive cases increases, and if R<1 the number of positive cases is likely to decrease) for the Omicron variant has been estimated to be between 3.8 and 2.5 higher than the delta variant, respectively [24].

Samples displaying both the N501Y and E484K mutations simultaneously, indicative of the Mu/GAMMA variant [25][26], were also present, with 5 cases encountered (0.28% of the total samples quantity) mid to late December. Omicron

22A/22B cases were briefly detected on January 28th and January 31st, 2022. Two positive samples carried delH69V70 and L452R mutations (0.1% of the total samples quantity) [27]. Other variants, which could not be properly identified with the primers/probes used, were also present between December 8, 2021, and January 3, 2022 (a total of 50 cases, corresponding to the 2.9% of the samples analyzed).

By February 16th, 2022, the laboratory detected the first case suggestive of Omicron 21L. It was identified by using delH69V70, N501Y and K417N primers. The sample was positive for N501Y and K417 primers and negative for delH69V70 which was a primary indicative for this variant [28]. Omicron 21L replaced Omicron 21K as the main variant in the country in 38 days after its first appearance. Omicron 21K is capable of infecting patients previously infected with Omicron 21K and displays greater neutralization escape [29]. The last variant detected during the investigation period was Omicron 22C. Eight samples were found in our dataset between April 28th and May 17th, 2022 (0.5% of the total samples quantity). These samples carried the N501Y, K417N and L452R mutations[30].

In agreement with our data, the variants identified in Ecuador via sequencing, which were reported in Covariant (https://covariants.org) during the investigation period, were Mu, Delta, Omicron 21K, Omicron 21L and Omicron 22C [30]. All these variants were detected in our dataset, including two Omicron 22A/22B samples, detected on January 28th and January 31st. This suggests that the mentioned variants could have been around the population in small numbers until they started spreading more widely by the end of May.

From December 2021 to May 2022, both the MDL-PUCE and MoH datasets show a wave of positive cases related to the first appearance of the Omicron 21K variant. The capacity to perform molecular diagnosis at MDL-PUCE during the second part of December 2021, early January 2022 was affected by the lack of materials and reagents, which were unavailable due to the increased country-wide demand imposed by the Omicron wave. This resulted in a hampered overall processing capacity and therefore, a reduced number of positive samples available for this study. Therefore, in terms of absolute number of samples, the Omicron "wave" in our dataset displays three different peaks, according to the availability of testing supplies (Figure 1). The MoH data (Figure 2), built in base of its Integrated epidemiological surveillance system COVID-19 and the reports of all testing laboratories in the country, shows a clearer shaped peak.

Nonetheless, the transition from Delta to Omicron 21K and from Omicron 21K to 21L are very well defined in our dataset and it consistent with the pattern observed via full-genome sequencing reported in Covariants. During the study period, Covariants reported 1,698 genomes for Ecuador [30]. Our dataset comprised by 1,692 shows the value of testing mutations via qRT-PCR to rapidly provide the opportunity to analyze larger datasets for a fraction of the cost.

Conclusions

This study shows the prevalence of the delH69V70 mutation, related to the Omicron 21K variant, among the population in Ecuador. We demonstrate how this variant influence the increase of number of positive cases during the peak between December 2021 and February 2022 due to its high ability to evade the immune response. This investigation is a reference of the behavior of the SARS-CoV-2 variants in the country and how they transition through time.

Abbreviations

ACE2: Angiotensin-converting enzyme 2

BARDA: Biomedical advanced research and development authority

CDC: Centers for disease control and prevention

CEISH-PUCE: Pontificia universidad católica del ecuador´s committee for ethics on human research

COVID-19: Coronavirus induces disease 2019

Ct: Cycle threshold

ddH2O: Double distilled water

DNA: Deoxyribonucleic acid

DoD: Department of defense

FDA: Food and drug administration

INSPI: National institute of public health

MDL-PUCE: Molecular diagnosis laboratory from pontificia universidad católica del ecuador

MERS-CoV: Middle East respiratory syndrome coronavirus

MoH: Ecuadorian ministry of public health

NHI: National institutes of health

P: Present

qRT-PCR: Real-time quantitative reverse transcription PCR

R0: The Reproduction Number

RBD: Receptor-binding domain

RNA: Ribonucleic acid

RT-PCR: Reverse transcription-polymerase chain reaction

S protein: Spike protein

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

SARS-CoV: Severe acute respiratory syndrome coronavirus

SIG: SARS-CoV-2 Interagency group

ssRNA: Positive-sense single-stranded RNA

U.S.: United states

VBM: Variants being monitored

VOC: Variants of concern

VOHC: Variants of high consequence

VOI: Variants of interest

WHO: World health organization

Declarations

Ethical approvals

The use of diagnostic sample remnants, disconnected from personal information, is considered exempt under Ecuadorian law (regulation 00038 - 2021 MSP). The study was reviewed by and deemed exempt by Pontificia Universidad Católica del Ecuador´s Committee for Ethics on Human Research (CEISH-PUCE, by its initials in Spanish), evaluation code # EO-44-2021.

Availability of data and materials

The datasets analyzed during the current study are available in the MoH repository, [https://www.salud.gob.ec/coronavirus-covid19-ecuador/], Covariants repository, [https://covariants.org] and from the corresponding author on reasonable request.

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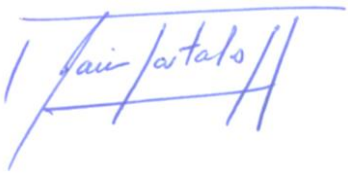

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