Article

The SARS-CoV-2 Omicron Variant of Concern and its Rapid Spread throughout the Western Brazilian Amazon

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Abstract: Genomic surveillance represents an important strategy for understanding evolutionary mechanisms, transmission profile, and infectivity of different SARS-CoV-2 variants. We assessed the epidemiological profile of 366 individuals who tested positive for SARS-CoV-2 from 29 municipalities in Rondônia between December 2021 to March 2022. Samples were collected, RNA was extracted and screened using RT-qPCR for Alpha, Beta, Gamma, Delta and Omicron VOCs and viral quantification was performed. Sequences were analyzed for phylogeny, mutations and lineages. Of the samples analyzed, 93.71% were positive for the Omicron variant and 6.28% were positive for the Delta variant. The symptoms observed were cough, sore throat, and fever, with a mean duration of 5 days; no hospitalizations or deaths were reported. We noted that among the positive individuals, 51% had been immunized with two doses, 22% received three doses, 13% received one dose, and 13% were not immunized. Just 242 samples were amenable to analysis for alignment and phylogenetic characterization; corresponding to variants BA.1 and BA.1.1; a total of 120 mutations were identified, 36% of which were found in the S gene. In conclusion, there was a high frequency of mutations in the SARS-CoV-2 genome, but no record of clinical severity, demonstrating the positive effect of vaccination.

Keywords: SARS CoV-2; Variant of Concern; Omicron; mutation; genomic surveillance

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1. Introduction

Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) has an RNA genome with a high mutational rate [1,2]. To date, about 12,000 mutations have been reported and some are related to increased infectivity, vaccine-escape and worsening of the clinical presentation, factors that are directly related to the establishment of Variants of Concern (VOC) and Variants of Interest (VOI), as monitored by the World Health Organization (WHO) [1–3].

Genomic surveillance is an approach that has supported the investigation of Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (B.1.1.529) and other (VOI) VOC cases in several countries in order to assist in governmental decision-making and implementation of healthcare measures aimed at infection control [3,4].

The Omicron VOC was identified in November 2021 in South Africa and was detected in over 40 countries during the month of December [2,5]. The new variant has approximately 32 mutations in the receptor-binding domain (RBD) in the Spike (S) protein alone; this quantity of mutations, greater than that found in the Delta variant [2], confers increased transmissibility to this variant [2,5].

In Brazil, the first case of the Omicron VOC was reported in November 2021 in the state of São Paulo, and sequentially, there was an exponential increase of infected individuals; during this period, COVID-19 caused about 70,000 hospitalizations, and approximately 1,300 deaths [6,7]. Genomic surveillance detected that in February, the Omicron variant was dominant in 99.8% of the samples analyzed around the country, 78.2% corresponding to the subvariant BA.1, 21.2% B.A1.1, and 0.4% B.A.2, with no reports of BA.3; current data indicate a significant reduction in Delta VOC-related cases [8].

In the first week of the year 2022 there was an increase in the number of cases of COVID-19, demonstrating an atypical profile in Northern Brazil, where a reduction of infected patients had been previously observed, a fact that could be related to the entry of the Omicron VOC which presents sublineages with high rates of transmissibility and vaccine escape [9,10]. Thus, the aim of this study was to evaluate the epidemiological profile of entry of the new VOC in the Western Brazilian Amazon, as well as to establish the clinical profile and characterization of mutations related to this new variant of SARS-CoV-2.

2. Materials and Methods

2.1 Ethical aspects and study site

This study was conducted at Fiocruz/RO, under the authorization of the FIOCRUZ COVID-19 Genomics Surveillance Network of the Brazilian Ministry of Health and was approved by the Research Ethics Committee of the Centro de Pesquisa em Medicina Tropical de Rondônia-CEPEM/RO 4.000.086.

2.2 Biological samples and epidemiological data

A total of 366 individuals who tested positive for SARS-CoV-2 were selected by convenience from primary care clinics and reference centers in different municipalities of the state of Rondônia during two periods: the first from the second half of December 2021 through January 31, 2022, and the second covering the month of February 2022 through the beginning of March 2022. Diagnosis of SARS-CoV-2 was carried out in Laboratório Central de Saúde Pública de Rondônia (LACEN/RO) by RT-qPCR with One Step/COVID-19 kits (IBMP, Brazil). Epidemiological data and vaccination status were collected from medical records in the GAL/RO, SIVEP-Gripe and E-SUS databases.

2.3 Extraction of Viral RNA

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140 µL of samples were collected with combined swabs; then, viral RNA was extracted using QIAamp® Viral RNA Mini Kits (QIAGEN, Germany) according to the manufacturer's instructions. The RNA was eluted in 60 µL of AVE buffer for viral load and inference tests.

2.4 Screening for Alpha, Beta, and Gamma VOCs

In order to screen for the Alpha, Beta and Gamma VOCs, the multiplex RT-qPCR protocol of Vogels et al. was utilized (11). Three targets were included in this multiplex: N1, deletion Δ69/70 and deletion of SGF Δ3675-3677 in the ORF1a gene.

The cycling process used for the reaction was 55 ºC for 10 min for reverse transcription, PCR activation at 95 ºC for 1 min, 39 subsequent cycles of 10 s at 95 ºC and 31 s at 60 ºC. Samples with Ct <35 for the N1 target alone were characterized as the Alpha VOC, and samples with Ct <35 for the N1 target and Δ69/70 deletion were classified as Beta or Gamma.

2.5 Screening for the Delta VOC

The inference test was performed using the primers and probes (Table 1) described by Yaniv et al. with modifications (12). The final reaction volume was 20 µL with a primer concentration of 0.5 µM and a probe concentration of 0.2 µM. The reaction contained 5 µL of RNA sample and the reaction steps were performed according to the factory recommendations using TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems 1, California, USA).

Table 1: RT-qPCR primers and probe for the Delta VOC

Name	Description	Sequence 5'-3'
Delta_CoV	Sense	GTTTATTACCACAAAAACAACAAAAG
Delta_CoV	Antisense	GGCTGAGAGACATATTCAAAAGTG
Delta_CoV	Probe	Cy3- TGGATGGAAAGTGGAG-TTTATTCTAGT- BHQ 2

*Adapted from: Karin Yaniv. Accessed on: July 5th, 2021.

The cycling process used for the reaction was 51 ºC for 10 min for reverse transcription, PCR activation at 95 ºC for 1 min, 40 subsequent cycles of 10 s at 95 ºC and 31 s at 60 ºC; the final step included fluorescence capture.

2.6 Screening for the Omicron VOC

All samples that were negative for Alpha, Beta, Gamma and Delta variants were subjected to RT-qPCR genotyping for the Omicron variant. The reaction was performed using 5 µL of TaqPath™ 1-Step RTqPCR Master Mix (4x), 0.5 µL of TaqMan SARS-CoV-2 Mutation Panel Assay (40X) for the SNP assay S:K417N, 5 µL of extracted RNA and a final volume of 20 µL. The cycling used for the reaction was as follows: pre-reading at 60°C for 30 seconds, Reverse Transcription at 50°C for 10 min, DNA polymerase activation at 95°C for 2 minutes, 45 cycles at 95°C for 3 seconds for denaturation and 60°C for 30 seconds for annealing and extension, ending with a post-reading at 60°C for 30 seconds. The genotyping module of the Design & Analysis Software Version: 2.6.0 (Thermo Fisher Scientific) was used for analysis, with a 95% confidence interval for real-time data. Given that this mutation is described as prevalent only in subvariants of Beta, Delta and Omicron, all samples positive for S:K417N and negative for the other variants tested were classified as Omicron (13–15).

2.7 Quantification of Viral Load

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The viral load of Omicron-positive samples was determined using 5µL of viral RNA extracted using the Multiplex One-Step RT-qPCR assay for detection of SARS-CoV-2 as developed by Queiroz et al, 2021 (16).

2.8 Complete genome sequencing of SARS-CoV-2

Complete genome sequencing of SARS-CoV-2 samples with Ct values <25, based on quantitative assays, were selected to allow for high genomic coverage. Nucleotide sequencing was performed using Illumina MiSeq or NextSeq platforms and the COVIDSEQ Kit (Illumina, San Diego, CA, USA) (17).

2.9 Data acquisition and Maximum-Likelihood (ML) phylogeny

Available high quality (>29 kb) whole genomes (<1% of N) of BA.* sampled in Brazil (n = 70) were downloaded from the GISAID EpiCoV database on March 3, 2022. The sequences were aligned using MAFFT v.7.487 (18). The best model of nucleotide substitution was measured (GTR+G+I) using ModelFinder (19) and the phylogenetic tree was reconstructed using the maximum likelihood method in the program IQ-TREE v.2.1.3 (20). Branch support values were obtained using Ultrafast Bootstrap with 1,000 replicates. The tree was visualized and edited with FigTree v.1.4.4 (21). SARS-CoV-2 genomes were classified into lineages using the available software Pangolin (22) and mutations were analyzed with Nextclade Beta (23).

2.10 Statistical analysis

Descriptive analyses were represented through central tendency and dispersion measurements. A Chi-square test was used for statistical inference with a significance level of 5% ($p<0.05$). Statistical analysis was performed and graphics were generated using the software R v4.0.3.

2. Results

In the cohort of 366 SARS-CoV-2 positive samples, 93.71% (343/366) corresponded to the Omicron variant and 6.28% (23/366) to the Delta VOC, with no detection of either Gamma, Alpha or Beta VOCs; the data were confirmed by three RT-qPCR screening assays for VOCs.

These cases were identified beginning on December 20, 2021 from 29 municipalities in the state of Rondônia (55.77%), demonstrating a wide distribution of the variant (Figure 1). In the first sample collection period we observed a higher proportion of cases distributed in all regions of the state with prevalence in the North, West, and South regions; sequentially, in the second collection period, the notifications came from the most populous municipalities of the state, such as Porto Velho, Ji-paraná, and Ariquemes, which accounted for the rates of cases reported by the Primary Care Units in the state.

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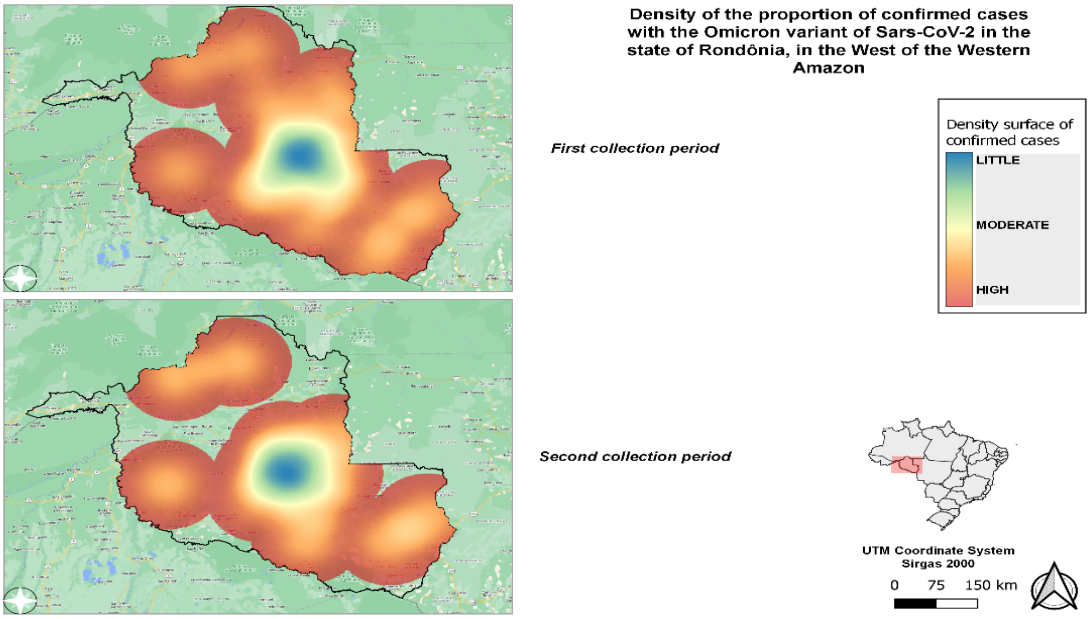

Figure 1: Kernel map demonstrating the density distribution of confirmed SARS-CoV-2 cases. The proportion of infected individuals can be visualized from December through February, divided into two distinct periods. The color intensity provides the identification of the municipalities with higher numbers of infections.

The median age of the population analyzed was 37 years old (SD 15.29), with ages ranging from 1 to 81 years old; 57% (196/343) were female and 43% (147/343) were male. The main symptoms reported by patients included cough in 48% of cases (165/343), sore throat in 45% (153/343), and fever in 45% of cases (153/343). Less frequent symptoms included dyspnea, smell disturbance and taste disturbance with 8% (28/343), 3% (9/343) and 2% of patients (6/343), respectively. Only 13% (45/343) of patients were asymptomatic.

The vaccination profiles observed were: 51% (176/343) of individuals immunized (1st and 2nd dose), 22% (77/343) immunized with a booster dose (1st, 2nd and 3nd dose), 13% (44/343) partially immunized (1st dose) and 13% (46/343) not immunized. There were no deaths or hospitalizations in any of the groups analyzed. In symptomatic individuals, the mean number of days of symptoms at diagnosis was 5 days (SD 3.6), with a maximum time of 19 days in only one individual. The viral load had an interquartile median of 7.08 Log10 copies/mL, and less than 1% (3/312) of individuals had a quantifiable viral load after 14 days of symptoms (Figure 2).

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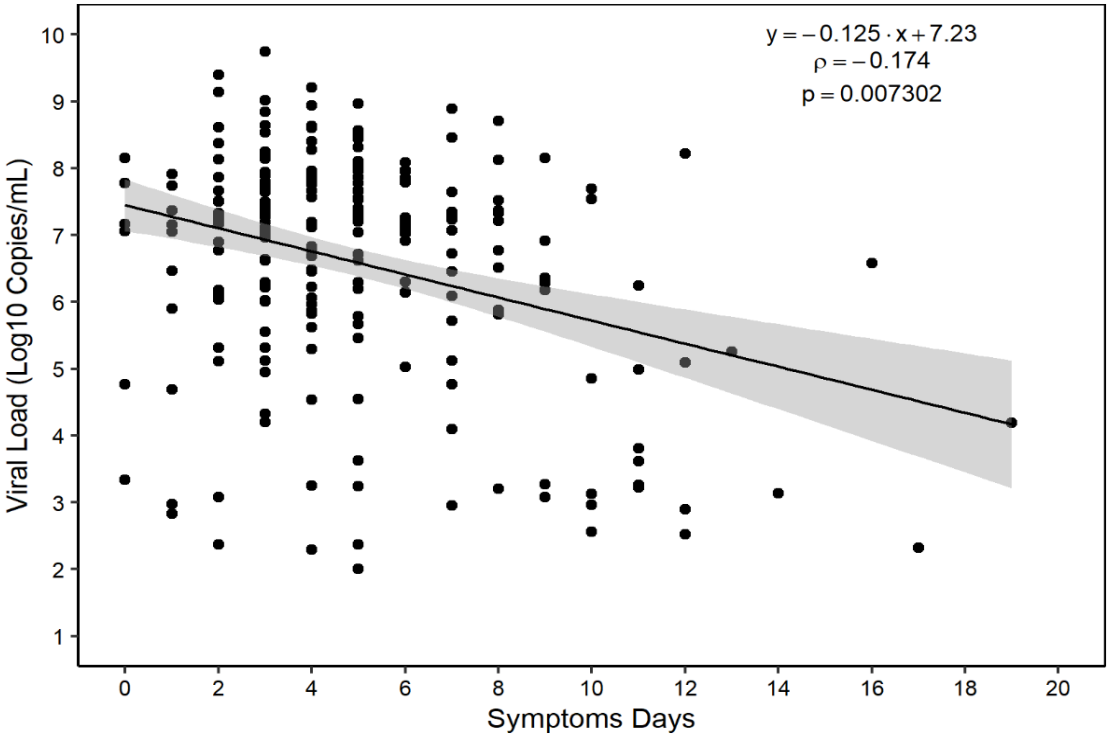

Figure 2: Viral load measured by the number of days after onset of symptoms.

Among the 343 samples tested by RT-qPCR, 242 samples were selected for sequencing, taking into consideration the viral load and multiplex tests for exclusion of other VOCS. Figure 3 demonstrated the maximum likelihood phylogeny in relation to the clade classification of the Omicron variant. The analyses of the sequenced samples corresponded to 78.51% (190/242) BA.1 and 21.48% (52/242) BA.1.1, located throughout 28 cities in Rondônia.

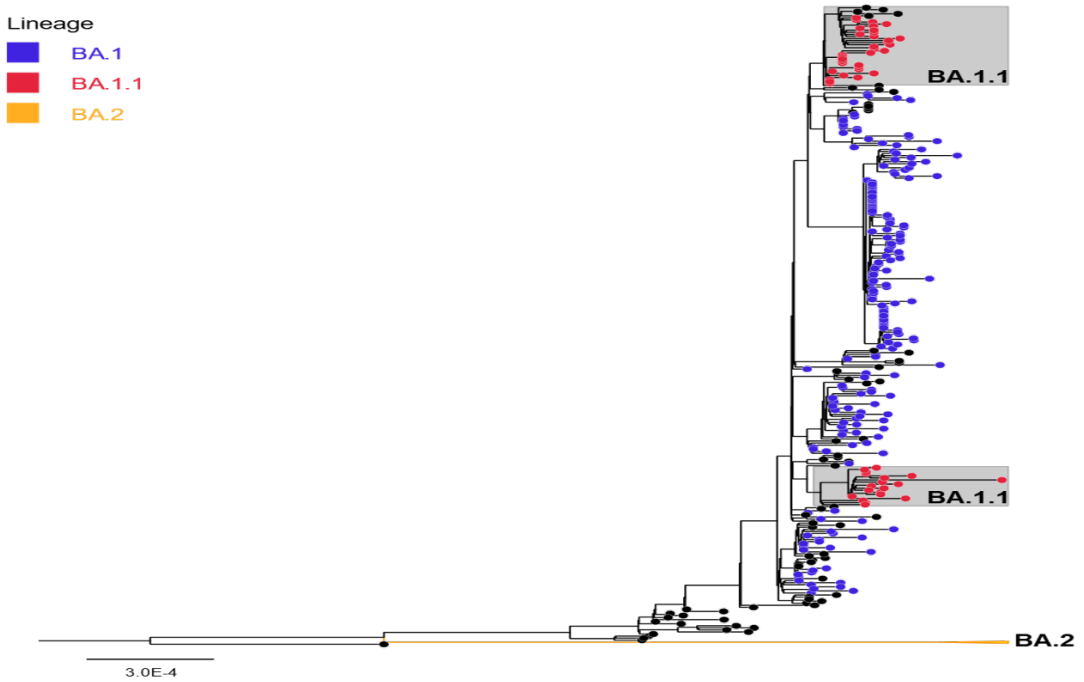

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Figure 3: Maximum likelihood phylogenetic tree showing 242 sequences obtained in this study and 70 genomes retrieved from GISAID. The tree was rooted with the most ancestral sequence (EPI_ISL_402123). The BA.1, BA.1.1 and BA.2 strains are indicated as blue, red and orange circles, respectively.

A total number of 120 mutations were identified (figure 4), with the highest proportion, 36% (43/120), located in the S gene, followed by 32% (38/120) in ORF1a, 13% (15/120) in ORF1b, 6% (7/120) in N, and 14% (17/120) distributed among the other genes (ORF3a; M; ORF9b; E; ORF7a; ORF8).

Mutations previously identified as signatures for VOC Omicron genomes were analyzed. The substitutions N679K and P681H, considered definitive over other variants showed a frequency of 100%, along with a substitution at N501Y present in 97% of sequences, all of which were characterized as alterations in the Spike protein.

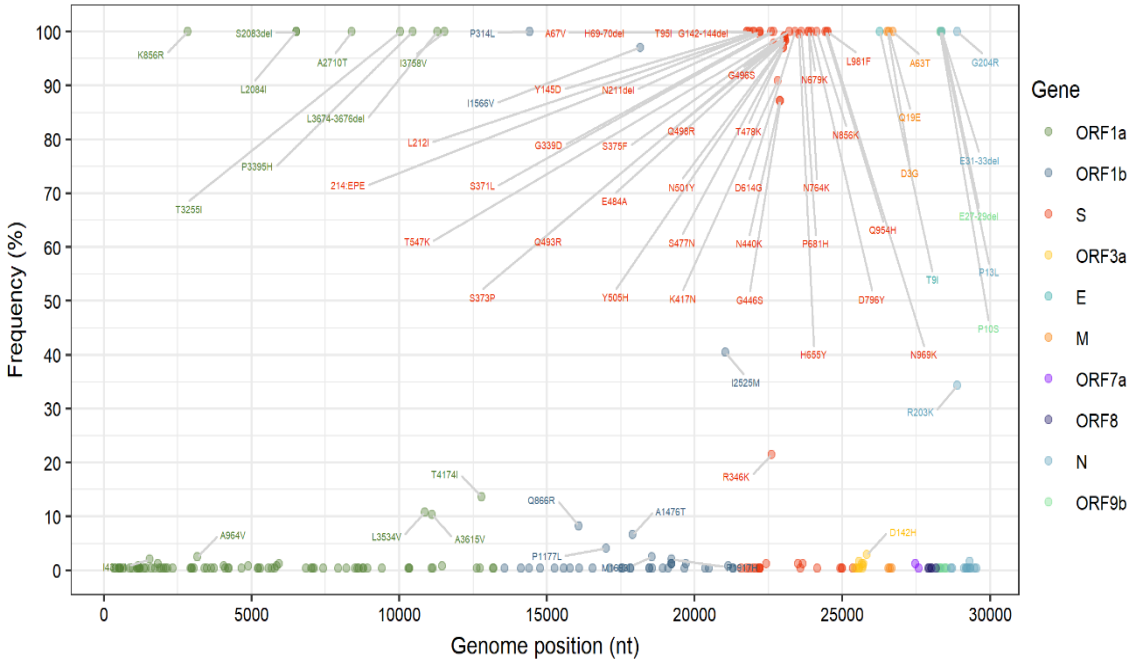

Figure 4: Dot plot demonstrating the frequency of mutations identified in the set of 242 samples analyzed by NGS. The labels of mutations with frequencies <2% have been hidden.

4. Discussion

From 343 samples we were able to gather epidemiological, viral load, sequencing, and mutational profile data from the first samples characterized with the new VOC of SARS-CoV-2 in the state of Rondônia, located in the northern region of Brazil.

The emergence of the Omicron VOC with its potential impact through increased infectiousness and transmissibility has become worrisome; since 2021, there has been an increase in the number of cases in young populations and our results verified this trend with cases in individuals <12 years old, corresponding to 3% of total cases [24]. In Brazil, vaccination for children between 5 and 11 years of age was not authorized until December 2021. Since then, there have been several vaccination campaigns in this age group in order to boost adherence [25]. In February, Rondônia still had a low number of vaccinated individuals <12 years old, with only 8.76% of this population partially immunized [26].

In parallel with the emergence of the Omicron VOC, many questions have arisen regarding the frequency of symptoms reported during the period of infection. In this study, the most commonly described symptoms were cough, sore throat, and fever, similar to

previous reports in the scientific literature on cases of the Gamma and Delta VOCs [27]. Unlike the first cases of SARS-CoV-2 reported in early 2020, a low frequency of individuals infected with the Omicron VOC presented taste and smell disturbances [28,29].

In this cohort, 87% of the individuals had received at least 1 dose of the COVID-19 vaccine; there were no hospitalizations or deaths, and there is evidence that immunization substantially reduced the progression to more severe cases, especially in individuals with comorbidities and advanced age [30]. However, it is worth noting that the Omicron variant is capable of vaccine escape and a booster dose is strongly recommended [31–33].

The viral profile has observed that individuals infected with the Omicron variant presented high viral titers up to 9 days after the onset of symptoms, with values declining sharply after 10 days [34,35]. Similarly, the cohort in this study showed high levels and maintenance of viral load for up to 10 days after symptom onset, with a mean of 5 days.

The Omicron variant was first detected in the state of Rondônia in December 2021 while the Delta variant was still predominant. After the introduction of Omicron, it subsequently became predominant, accounting for approximately 100% of the samples analyzed in February 2022. A previously published review article showed that Omicron's average basic and effective reproduction numbers were 8.2 and 3.6, respectively, meaning a 2.5- to 3.8-fold higher transmissibility than the Delta variant, which may be partially explained by the higher number of mutations, higher transmissibility and greater capability for immune escape [36].

Our findings showed the presence of the BA.1 and BA.1.1 subvariants, with no detection of BA.2 present in other regions of Brazil [37]. BA.2 is an important subvariant due to its high rate of transmissibility, although it was not identified in this cohort. It is important to note that genomic surveillance of subvariants represents the Achilles heel for understanding the circulation of SARS-CoV-2 [38–41].

The Omicron variant has a highly transmissible profile due to a greater number of mutations when compared to the other variants [42]; however, studies have shown that there are mutations and phenotypic aspects that have a neutral function related to [43,44]. Other primary substitutions maintain classification stability within the set of variants throughout the viral evolutionary process, resulting in the ability of SARS-CoV-2 to maintain its genomic tracking throughout propagation in the population [45,46].

Mutations in the Spike protein that play a signature role in other VOCs, such as N679K, P681H, and N501Y were visualized in all sequences, and this persistence of mutations is directly linked to increased infectivity rates, high transmission capacity, and the rapid dispersal potential of this variant [47–50].

5. Conclusions

In conclusion, the data demonstrated the rapid dissemination of the Omicron VOC throughout Rondônia, in the Western Amazon region in the first few weeks of the year 2022, including the subvariants BA.1 and BA.1.1. Although the sequences analyzed showed a high frequency of mutations, the infected population did not present a severe clinical profile, demonstrating that vaccination had a positive effect in these cases.

Supplementary Materials: The list of accession IDs may be found in the attached file in the supplemental materials.

Author Contributions: Conceptualization: G.S., J.Q., R.C.P.R., D.V.; Methodology: G.S., J.Q., T.P.R., R.C.P.R., F.G.N., D.V.; software: G.S., J.Q., T.P.R.; validation: G.S., J.Q., R.C.P.R., D.V.; Formal analysis: G.S., J.Q., T.P.R., R.C.P.R., D.V.; Investigation: G.S., J.Q.; resources: J.M.V.S., V.R.S., F.M.O., F.G.N., L.G.M., F.K.M., D.V.; Data curation: G.S.O., J.A.S.Q., T.P.R., N.W.G., K.S.T., A.A.S., P.R.F.S., E.S.S., A.C.S.M.; C.C.S., C.F.G.A. writing—original draft preparation: G.S.O., J.Q., A.M.P., N.W.G., K.S.T., A.A.S., P.R.F.S., E.S.S., R.C.P.R., D.V.; writing—review and editing: S.S.P., F.S.B., J.M.V.S., L.G.M., F.K.M., R.C.P.R., F.G.N., D.V.; visualization: G.S., J.Q., R.C.P.R., D.V.; Supervision: R.C.P.R., D.V.; Project Administration: D.V.; Funding Acquisition: J.M.S., L.G.M., F.K.M., F.G.N., D.V.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The project was evaluated and approved by the Research Ethics Committee of the Research Center for Tropical Medicine - CEPEM - Rondônia under protocol no. 4,000,086 and carried out in accordance with the ethical principles stipulated by the 1975 World Medical Assembly and the Ministry of Health (Resolution 466).

Informed Consent Statement: Patients' consent was waived for reasons of urgency in monitoring the Omicron variant in the State of Rondônia, with due approval by the local ethics committee. The samples were previously collected for diagnosis and used in this study, thus having minimal risk for the research subject. the generated data does not contain information about the research subject, do not generate information for the treatment of patients; and are very important for collective health if processed quickly.

Data Availability Statement: All the SARS-CoV-2 genomes generated and analyzed in this study are available in the EpiCov database in GISAID [51] under the following ID numbers: EPI_ISL_11112675-11112679, EPI_ISL_11112681-11112700, EPI_ISL_11112702-11112704, EPI_ISL_11112706-11112719, EPI_ISL_11112721-11112725, EPI_ISL_11112727-11112746, EPI_ISL_11112748-11112760, EPI_ISL_11112762, EPI_ISL_11112764-11112768, EPI_ISL_11112770, EPI_ISL_11112772-11112776, EPI_ISL_11112778-11112789, EPI_ISL_11112791-11112798, EPI_ISL_11622642-11622700, EPI_ISL_11622702-11622725, EPI_ISL_9414761-9414772, EPI_ISL_9636793-9636797, EPI_ISL_9636803-9636804, EPI_ISL_9636811, EPI_ISL_9636819, EPI_ISL_9636821-9636822, EPI_ISL_9636842-9636843, EPI_ISL_9636849-9636857, EPI_ISL_9636860-9636861, EPI_ISL_9636863-9636864, EPI_ISL_9636867, EPI_ISL_9636869, EPI_ISL_9636873, EPI_ISL_9636875-9636876, EPI_ISL_9636878.

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Conflicts of Interest: At the time of submission, RCPR, LGM and FKM were employees at IBMP, which manufactures and commercializes the test described in this study.

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