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Research Article

Genomic Surveillance of SARS-CoV-2: Study of Cases Intradomiciliary Infection in Brazilian Amazonia

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Abstract

Studies have shown that the family environment is very favorable for SARS-COV-2 transmission, making family groups susceptible to intra-household infection because they maintain direct contact with an infected person. We screened a cohort of 416 individuals who tested positive for SARS-CoV-2 from June to October 2021 for intradomiciliary infection. Twenty-two families with an average of 2 to 4 members were located in 12 municipalities in the state of Rondônia. Nasopharyngeal samples were collected, extracted using the QIAamp viral RNA mini kit and genomic sequencing was performed using samples with ct<25 by Illumina platform, the sequences were analyzed for phylogeny, mutations, and lineages. Among the samples analyzed 72.72% matched for VOC Delta and 27.27% for Gamma variant. The median viral load for both variants was 6.95. The main symptoms presented were cough, headache, and fever. No hospitalizations or deaths were reported. We observed that among the positive individuals, 24% were immunized with the 1st and 2nd dose, 40% were partially immunized, and 36% were not immunized. For each strain, subvariants were identified, being P.1.4 and P.1.14 for the Gamma variant and AY.43, AY.99.2 and AY.122 for Delta. The mutations mostly behaved as lineage-defining, with only eight families showing point mutations. In conclusion, it was observed that despite the low adherence to immunization, household cases were equally expressed in viral characteristics and in the low rate of hospital evolution for COVID-19, and this may be a factor associated with the permanence of infection in an isolated family environment.

Keywords: SARS-CoV-2; Variant of Concern; Gamma; Delta; Intradomiciliary Infection; Genomic Surveillance

Introduction

SARS-CoV-2 is the virus that causes a respiratory disease denominated COVID-19, it is composed of single-stranded positive sense RNA genetic material, and its transmission occurs through droplets or aerosols, which is a factor that facilitates its dissemination [1]. Furthermore, studies in China have shown that the highest rate of SARS-CoV-2 transmissibility occurred from household contacts [2], even with health prevention being carried out to prevent the spread of the virus, family groups have not managed to avoid contamination, becoming groups susceptible to infection. In the family environment, the infected person has direct contact with their relatives [3], favoring the transmissibility and infectivity of SARS-CoV-2 [4].

The high rate of recurrent infections has caused SARS-CoV-2 to accumulate numerous mutations in its genome, either by gene deletions, silent mutations, or changes in the coding of amino acids, some being responsible for altering the interaction of the Spike (S) protein with the host cell receptor angiotensin-converting enzyme 2 (ACE2). These changes in activity impair the body's immune response against the virus, increasing transmissibility and intensifying its pathogenic potential, thus favoring the emergence of new variants and subvariants [5-7].

The appearance of the first SARS-CoV-2 variants caused a worldwide public health concern, leading the World Health Organization (WHO) to develop proposals for genomic characterization and monitoring. Recently, the Omicron variant (O.1.1.529) was classified as a Variant of Concern (VOC) due to its attributes in increasing transmissibility and potentially reducing vaccine action. Alpha (B.1.1.7), Beta (O.1.351), Gamma (P.1), and Delta (E.1.617.2) variants are also classified as VOCs, but there is no detection or a low level of circulation of some variants in this group. Lambda (C.37), Mu (O.1.621), the latter already detected in Rondônia, Brazil and other variants are classified as Variants of Interest (VOIs), and currently B.1.640 and the recombinant BA.1/AY.4 are classified as Variant Under Monitoring (VUM) [8,9].

Gamma VOC was detected in December 2020 in Manaus, Brazil and Delta VOC was first described in India in October 2020 and was identified in Southern Brazil in April 2021, together these two VOCs favored an exponential increase in the number of infections over the course of 2021 [10-12]. In addition to the circulation of these two VOCs, evaluating the household model of transmission is a point little addressed in the increase in the number of cases, even though we know that intradomiciliary transmission is the key point of most reported infections. This type of infection provides a clear and fixed exposure of the sources of infection, which makes it a viable model to estimate the transmissibility and infectivity of the prevalent virus [13].

Given this, aware of the high rate of transmissibility of the SARS-

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CoV-2 virus, the main objective of this study was to perform an epidemiological description of cases of intradomiciliary infection within a cohort of 416 samples sequenced from June to October 2021 in Western Amazonia, bringing the state of Rondônia as the main study site.

Materials and Methods

Ethical aspects and study site

The study was carried out at the Laboratory of Molecular Virology FIOCRUZ/RO in partnership with the Central Public Health Laboratory of Rondônia LACEN and the Laboratory of Virology of the Instituto Leônidas e Maria Deane, FIOCRUZ/AM. The proposal was submitted and approved by the Research Ethics Committee of the Centro de PesquisaemMedicina Tropical de Rondônia-CEP/CEPEM with opinion No. 4.637.465.

Biological samples and epidemiological data

A cohort of 416 individuals positive for SARS-CoV-2 were selected in the period from June to October 2021. Biological samples were collected in primary health care units and reference centers in different municipalities of the state of Rondônia. Laboratory diagnosis was performed by RT-qPCR at the Laboratório Central de SaúdePública de Rondônia (LACEN/RO) using the One Step/COVID-19 kit (IBMP, Brazil).

Biological samples were collected by nasopharyngeal swab technique in health care units, hospitals and reference centers located in different municipalities of the state of Rondônia. Epidemiological data were collected from electronic medical records available in the following platforms: the Laboratory Environment Manager (GAL) of Rondônia, E-SUS notifica, and SIVEP-Gripe.Screening of cases of intradomiciliary infection occurred through electronic bank tracking and home address location.

Nucleic acid isolation and RT-qPCR

Sample quantification was performed in the Molecular Virology Laboratory (FIOCRUZ/RO) where viral RNA was extracted from 140 µL of nasopharyngeal Swab samples using QIAamp® viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. For viral load was determined using 5µL of this extracted viral RNA using Multiplex One-Step RT-qPCR assay for detection of SARS-CoV-2, developed by Queiroz and colleagues, 2021 [14].

Complete genome sequencing of SARS-CoV-2

Samples with Ct values <25 was selected based on quantitative assays for SARS-CoV-2. Nucleotide sequencing was performed using the Illumina MiSeq or NextSeq platforms and the COVIDSEQ Kit (Illumina, San Diego, CA, USA) [15].

Mutation analysis and lineage determination

The Nextclade v.1.5.4 software [16] and Pangolin COVID-19 Lineage Assigner v2.1.7 [17] were used to evaluate mutations and determine lineages.

Phylogenetic analysis

Complete genomes (n=230) of high quality (>99% N's) sampled in Brazil and of each lineage identified were downloaded from the GISAID platform [18]. MAFFT v.7.487 was used to align the sequences [19].The data set was used to perform a maximum likelihood based phylogenetic analysis using IQ-TREE v.2.1.3 [20]. The GTR+G+I substitution model was measured by the ModelFinder tool [21]. Branching support values were obtained using 1,000 Ultrafast Bootstrap replicates. FigTree v.1.4.4 was used to visualize the maximum likelihood tree [22].

Analysis of the Secondary Attack Rate among households

The secondary attack rate was estimated from the number of infected household contacts with the overall number of cases per household. Age group, vaccination, and other criteria were not taken into consideration [23].

Statistical analysis

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

Results

A total of 416 individuals with molecular diagnosis for COVID-19 were analyzed from June to October 2021 in the state of Rondônia, in which 22 families with an average of 2 to 4 members were identified, with a total of 50 (50/416) individuals. The household infections are distributed in 12 municipalities in the state of Rondônia (Figure 1) with Porto Velho (n=6) and Jaru (n=3) showing the highest frequency of cases, as they are some of the most populous municipalities in the state of Rondônia, and in a smaller proportion the cases registered in AlvoradaD'Oeste (n=1), Ariquemes (n=2), São Miguel do Guaporé (n=1), Teixeirópolis (n=1), Cacoal (n=2), Cerejeiras (n=1), Ji-paraná (n=2), MachadinhoD'Oeste (n=1), Seringueiras (n=1) and Vilhena (n=1).

The cohort was consisted age-range individuals from 7 to 78 years, with a mean age of 39 years, 5 children from 7 to 12 years, 5 adolescents from 13 to 18 years, 35 adults from 20 to 64 years, and 5 elderly from 65 to 78 years, where 54% (27/50) were men and 46% (23/50) were women. The most prevalent symptoms reported were cough with 76% (38/50), headache and fever with 56% (28/50) of cases, respectively. Other symptoms had a lower proportion, such as sore throat with 40% (20/50), runny nose with 32% (16/50), taste disturbance with 14% (7/50), smell disturbance with 14% (7/50), and dyspnea with 4% (2/50). Among all cases, only 4% (2/50) were asymptomatic.

The viral load was evaluated in relation to the days of reported symptoms, where most individuals were detectable until the first seven days of infection and only 8% (4/50) remained detectable after the eighth day of symptoms. The viral load had a median of Log 6.95 (Figure 2).

A secondary attack rate was defined from the contact assessment among all infected individuals, stratifying for family groups identified among those analyzed. The individuals described with household contact (50/416) were evaluated from the onset of symptoms, with no definition of age group, kinship level, and number of individuals for each family, resulting in a secondary attack rate of 12.01%.

Among the 50 individuals, the vaccination was divided into three groups: 24% (12/50) of the individuals had completed their vaccination schedule with two doses of the immunizer, 40% (20/50) of

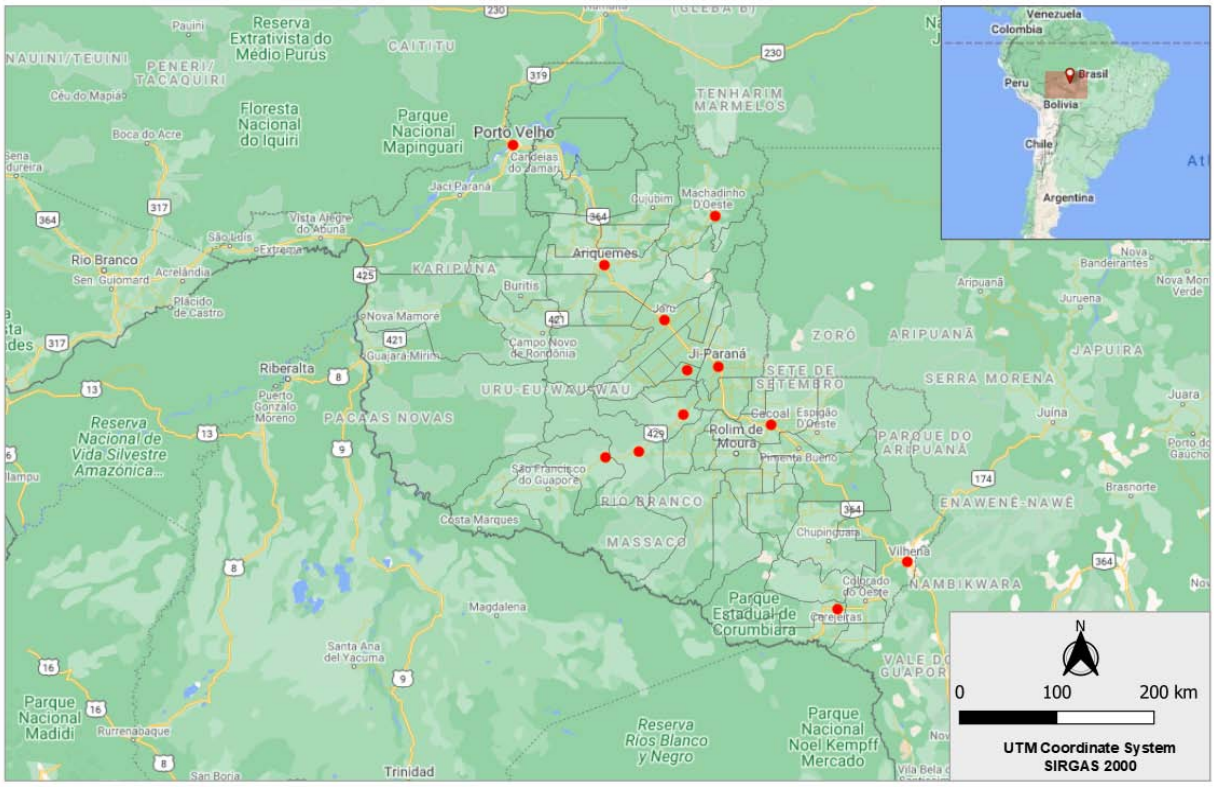

Figure 1: Scattered cases of intradomiciliary infections in the State of Rondônia.
Legend: Distribution of cases in 12 municipalities of the state of Rondônia.

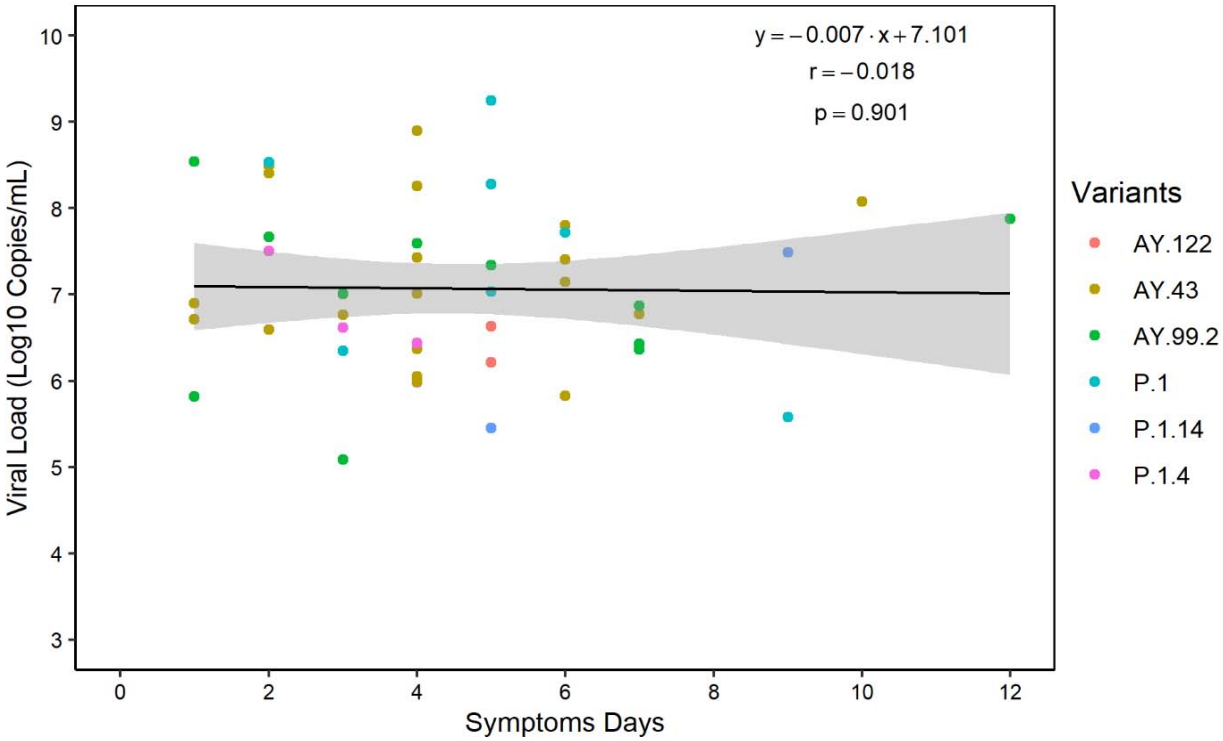

Figure 2: Graph plot showing the relationship of viral load to days of symptoms.
Legend: Statistically non-significant correlation (r = 0.018; p = 0.901, Pearson correlation).

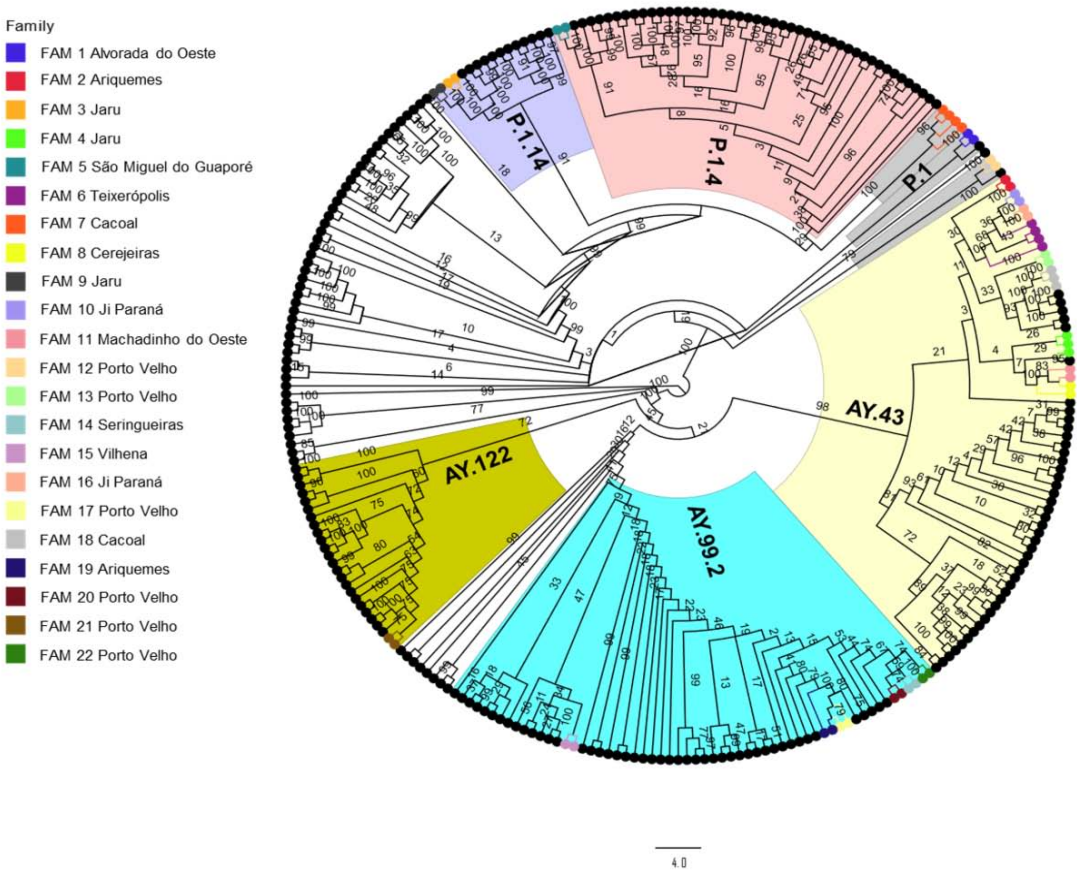

Figure 3: Phylogeny demonstrating the distribution of the intradomiciliary cases and their respective families (FAM)9 (22/36) distributed for AY.43, 6 (12/36) for AY.99.2 and 1 family infected by AY.122.
Legend: Maximum Likelihood phylogenetic tree containing sequences downloaded from GISAID and study samples. The colors of the circles represent the families and the colors of the clades represent the subvariants found. Support (bootstrap) values are present in the branches.

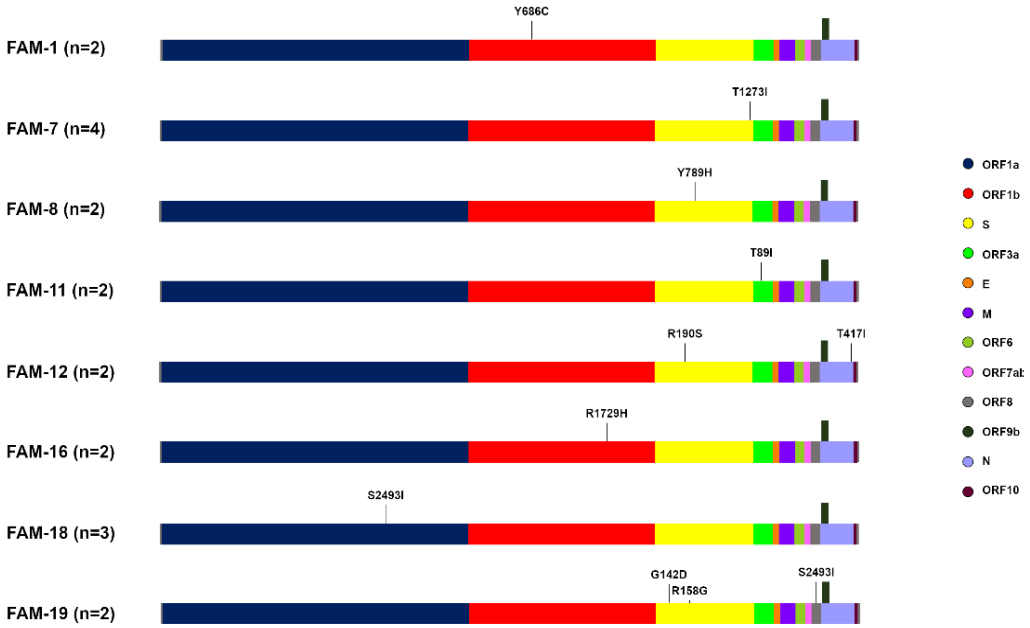

Figure 4: Divergent point mutations between sequences of households.
Legend: Representation of divergent substitutions found among 8 families, being specific to only 1 representative per household.

the individuals had taken only one dose of the vaccine, being classified as partially immunized, and 36% (18/50) of the individuals were not immunized. Within this cohort, there were no hospitalizations or deaths.

In the 22 families analyzed, 72.72% (16/22) were characterized by VOC Delta and 27.27% (6/22) by VOC Gamma. For each lineage, two subvariants were identified, being P.1.4 and P.1.12 for VOC Gamma and AY.43, AY.99.2 and AY.122 for VOC Delta (Figure 3). The dispersion of variants among family groups (FAM) was equal and unique among members, preserving unique virological identification among some family groups. The distribution of intradomiciliary infections remained 6 families totaling 14 individuals infected by VOC Gamma, distributed between it and its two subvariants, 3 for P.1 (8/14), 2 (4/14) for P.1.14 and 1 (2/14) for P.1.4, for the variant Delta, 16 families totaling 36 individuals, which presented intradomiciliary infection being 9 (22/36) distributed for AY.43, 6 (12/36) for AY.99.2 and 1 family infected by AY.122.

By analyzing the signature mutational substitutions between sequences, not much divergence was visualized among the evaluated households. It was observed that a small proportion of family members (8/50) (Figure 4) had point mutations, with characteristics presented for only one representative from each family.

Discussion

This study showed that 54% of the 22 participating families were male and 46% were female, with a proximal profile of uniformity in the parameters of gender differentiation, which is reported by the World Health Organization (WHO) worldwide [24]. Studies report that there is a verification of the profile of higher incidence in regions that have higher population density [24,25], which was verified in the study where the highest number of infected families is found in the city of Porto Velho, capital of the state of Rondônia, which has approximately 548,952 inhabitants and that since the beginning of the pandemic showed high rates of infection compared to other municipalities in the state [26,27].

Consistent with the results of other recent studies, the most common symptoms in the families analyzed were cough, headache, and fever, not differing from one variant or from one family to another [28,29]. Other symptoms were less frequently reported such as taste and smell disturbances and dyspnea, corroborating with studies that show a variation in the frequency of these symptoms in recent times, differing from those at the beginning of the pandemic when these symptoms were more frequent in the population [30-32].

Of the study participants, about 40% had already taken the first dose of COVID-19 vaccine by the time of diagnosis, consistent with the advance of vaccination in this period. Even in fully vaccinated individuals, SARS-CoV-2 infection is a reality, mainly due to the emergence of new variants and mutations that contribute to immune escape. However, vaccinated individuals are less likely to develop the severe form of the disease or die [31-33]. In this study, there were no hospitalizations or deaths.

When comparing the secondary attack rate data for the study group with the data presented in the literature, there is a lower incidence of cases within the study population, but it still represents a substantial portion of the primary attack rates for SARS-CoV-2 [33]. The susceptibility to infection by age group resembles the profile found in studies conducted, showing children less susceptible and adults between 26 and 64 years more susceptible [34-36].

The viral load of groups positive for Gamma and Delta variants has been correlated in other studies to Alpha and Beta variants, which showed a significant difference [37,38]. In this study, no pattern was observed in the viral levels of the variants and subvariants identified, as well as no significance in kinetics over days of symptoms, possibly due to the limited number of individuals analyzed. However, it is possible to observe that most individuals presented high viral load until 7 days of symptoms, and only a small number exceeded this period, corroborating with studies that state that 10 days are sufficient for a drastic reduction in viral levels [39,40].

The estimated secondary attack rate in this study was 12.01%. Authors of a meta-analysis pooled data from 54 relevant studies reporting secondary home transmission, where the estimated attack rate was 16.6%, with higher values identified in households with symptomatic index cases [41]. In another study, the estimated rate was 17.1% suggesting that testing household contacts of COVID-19 cases with more than one test and at different times may help identify secondary cases [42]. The findings show that home isolation makes the environment conducive to the spread of the virus among contacts.

The persistence of a high viral load consists of findings in this study that demonstrated minimal mutational variation among individuals in each family. Researchers in a study aimed at understanding the diversity of SARS-CoV-2 infection within the host by iSNVs (Intrahost Single Nucleotide Variants) found that the higher the viral load, the less variation there is in substitutions in the virus genome [43].

In this context, the results showed no infection by different strains among individuals of the same family, revealing concrete evidence of intradomiciliary infection. Corresponding to 72.72% of infections, VOC delta was already prevalent in the state of Rondônia during the study period [44] Research shows that Delta has a higher transmissibility profile compared to Alpha, Beta and Gamma variants [45,46] In addition, the most common underlining found in the study (AY.43), possibly showed a greater spreading effect than the other subvariants in the state of Rondônia.

Conclusion

The high transmissibility of the Gamma and Delta variants and subvariants of SARS-CoV-2 was demonstrated in the intra-household groups of this study. The signature mutation profile divergence was not observed, despite low adherence to the vaccine. However, although the secondary attack rate was high, the evolution of aggravated infectious conditions was not identified, and this may be a factor associated with viral lineage compatibility. These data highlight the importance of genomic surveillance in monitoring variants and subvariants, as well as mapping specific cases in the home environment and their impact on the population in relation to the spread and impact of viral severity on the clinic of infected individuals.

Limitations

This study had a considerable limitation for tracing the families,

because the only way to locate the cases of intradomiciliary infection using the analysis of the electronic home addresses made available by the families in the banks during the screening [47].

Data Availability

The list of accession IDs may be found in the attached supplemental material file.

Acknowledgments

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Author Contributions

Conceptualization:K.S.T., G.S., A.M.S.P, D.V.; Data curation: K.S.T., A.M.S.P, G.S., J.A.S.Q., T.P.R., N.W.F.G., A.A.S., A.C.S.M.; C.C.S., C.F.G.A. Formal analysis: K.S.T., A.M.S.P, G.S., T.P.R., D.V.; Funding Acquisition: D.V.; Investigation:K.S.T, A.M.S.P., G.S.; Methodology: K.S.T, G.S., A.M.S.P, J.A.S.Q., T.P.R.,D.V.; Project Administration: D.V.; Supervision: D.V.; Writing–original draft:K.S.T.,G.S., A.M.S.P., J.A.S.Q, N.W.F.G., A.A.S.,T.P.R, D.V.; Writing–review & editing: 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).

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