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Mutations in Spike Gene of SARS-CoV-2 that Are Associated with a Higher Viral Load: A Clinical Case Study

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Abstract

Background: Quantitative PCR (qPCR) can be used to detect and quantify a load of a pathogen. It is a good indicator of the degree of transmissibility. While performing routine qPCR, we observed an unusually short cycle threshold (Ct) value of SARS-CoV-2 for a clinical specimen obtained in Bamako, Mali. This prompted us to sequence the short-cycle SARS-CoV-2 sample to identify potential mutations in the Spike gene (S gene) gene. Methods: Post-infection, Quantitative Reverse Transcription (qRT-PCR) was performed over a defined time course to estimate the Ct of the SARS-CoV-2 specimen collected from the patient. Sanger sequencing was done on the entire fragment of the S gene to identify mutations. Findings: Sanger sequencing revealed mutations in the lineage of interest, designated B.1.525 by Pango, and also known as "Eta" using the nomenclature defined by WHO. This variant was originally found in Nigeria and Italy. The four novel mutations identified in Eta (D228N, Y451N, I1172M, and C1250F) were otherwise observed with a low frequency worldwide. Although the initial Ct was 10 in the case study patient, he did not exhibit severe symptoms of SARS-CoV-2, for example, pneumonia. However, we observed a longer viral clearance period than usual, of 3 weeks. We note that as compared to SARS-CoV-2 samples obtained during the first peaks of SARS-CoV-2 infection in Mali, when the

infection was at its peak in March 2020 (Ct = 30.4), circulating strains evaluated at the time the Eta sample was obtained demonstrated a lower mean Ct (Ct = 24). Conclusions: The short cycle threshold associated with this variant, and the temporal association with a decrease in the mean Ct in the region of Bamako, may indicate higher levels of transmissibility due to a circulating variant. This variant is a lineage of interest designated B.1.525 by Pango or Eta by WHO.

Keywords

SARS-CoV-2, Variant Eta, Ct Value, Mali

1. Introduction

The new SARS-CoV-2 coronavirus disease (COVID-19) outbreak initiated a global health emergency on January 30, 2020. Since then, the virus has evolved and a range of mutants have been identified globally [1] [2]. Currently, the delta (originated from India) [3] and omicron (discovered in South Africa) [4] variants have largely replaced other SARS-CoV-2 viruses. However, a number of other variants have been described in England and Brazil that contain N501Y mutation and 69 - 70 deletions [1] [5]. These specific mutations have been observed to be associated with higher viral load and transmissibility in clinical studies [6] [7]. In addition, the mutations may pose a diagnostic challenge and could potentially adversely affect the effectiveness of immunization programs and health preventive measures.

Mali registered four peaks (waves) of infection from March 2020 to May 2021 after the SARS-CoV-2 was introduced (on March 25, 2020). The first, second, and third waves were observed from March 2020 to July 2020, November 2020 to January 2021, and February 2021 and May 2021, respectively, each with a strong peak. The fourth wave of COVID-19 occurred from December 2021 to February 2022 (http://covid19-ml.org/, May 30, 2021). As was observed in many other countries, the fourth peak was driven by the omicron variant that was discovered in South Africa in November 2021 [4].

The spread of COVID-19 can be prevented by identifying and isolating infected people. In this context, real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) is helpful as it can help detect the presence of SARS-CoV-2 in the clinical samples and quantify the viral load at the time of measurement. The standard measure used for viral load is the cycle threshold (Ct) value. The Ct value is the PCR cycle number at which the generated fluorescence crosses a fluorescence signal threshold at which a detectable amount of target nucleic material has been generated during the amplification process. The lower the number of PCR cycles that are required to cross the threshold, the greater the total amount of nucleic material that is present in the sample, indicating a greater burden of infection. Thus, lower threshold (Ct) values reflect higher viral loads

and higher threshold (Ct) values indicate lower viral loads.

The relation between the spread of the SARS-CoV-2 variant and the third COVID-19 peak has been a concern in Mali. In this report, we have described a potential association of mutations in the S gene of SARS-CoV-2 with high viral load and prolonged recovery time in a COVID-19 patient who was apparently infected in Bamako, as he did not have any history of travel outside Mali.

2. Materials and Methods

2.1. Patient and Nasopharyngeal Swab Samples

The patient (ID ZH0001256X) is a 68-year-old male who was examined at the National COVID-19 Care Center (Hôpital du Mali) on April 16, 2021, after reporting persistent cough and muscle pains, during the third wave of SARS-CoV-2 in Mali. The Laboratoire de Biologie Moléculaire Appliquée at the University of Science, Techniques and Technologies of Bamako received five sequential nasopharyngeal specimens collected from the patient for qRT-PCR analysis (Figure 1).

2.2. Clinical Follow-Up

The patient was followed for 20 days. Following the diagnosis of COVID-19, the patient underwent clinical examination and specimens for qRT-PCR were obtained on day 0, day 8, day 10, day 13, day 16, and day 19. In addition, computational tomography of the chest (CCT) was conducted on days 8, 16, and 19 post-COVID-19 diagnoses (Figure 1).

2.3. Quantitative Reverse Transcription-PCR (qRT-PCR)

RNA was extracted from nasopharyngeal specimens using QIAamp Viral RNA Mini Kit (Qiagen, Germany). Next, 10 µl of the extracted RNA samples were mixed with 15 µl of the 2019-nCoV-PCR Master Mix (Sansure Biotech Inc, China). The viral RNA amplification program was performed using the Step-one plus™ Real-Time PCR System, using the ORF1ab (FAM) and N-gene (ROX) as gene targets: 50˚C for 30 min; 95˚C for 1 min, 1 cycle; and 95˚C for 15 sec, 60˚C for 31 sec, 45 cycles [8].

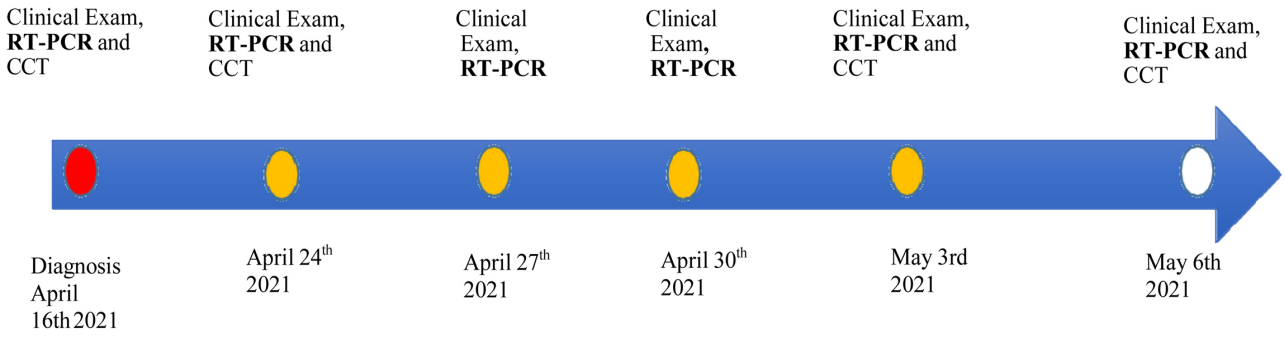

Figure 1. Timelines of the case from day of treatment initiation to complete cure.

2.4. Sequencing of Specimen

Prior to initiation of treatment, Sanger sequencing was performed using the CEQ™ 8000 Genetic Analysis System (Beckman Coulter) from the nasopharyngeal specimen. First, qRT-PCR was performed in two steps. Eight microliters of the extracted RNA were converted to cDNA using the OneTaq® RT-PCR Kit (New England Biolabs® Inc) and seven pairs of primers covering the entire domain of the S gene were used for amplification [8]. Following amplification, the RT-PCR products were purified and re-amplified using the GenomeLab DTCS Quick Start Kit (Beckman Coulter, USA) as per the manufacturer's instructions. Finally, cycle sequencing products were purified by ethanol precipitation and then separated using a CEQ™ 8000 Genetic Analyzer Sequencer.

2.5. RT-PCR and Sequencing Data

Positive PCR samples normally have S-shaped amplification curves with ORF1ab (FAM) or N-gene (ROX) and Ct ≤ 40. The StepOne Plus™ (Applied Biosystem) was used to perform real-time PCR. The obtained sequences were aligned and analyzed using Geneious Prime 2021. 1 software for mutation research. The sequence was submitted to GenBank under the accession number MZ798310. The phylogenetic tree was constructed using the MEGA 6 software [9] and the Clustal W algorithm for alignment [10]. The tree was annotated and modified in iTOL [11].

2.6. Data Analysis

Comparative analysis was done with 724 and 83 specimens of SARS-CoV-2 obtained during the first and third peaks of COVID-19 infection, respectively. The mean Ct values during the first wave (March 2020 to July 2020) and the third wave (February 2021 to May 2021) of SARS-CoV-2 were estimated based on the samples and the two-sample t-test used for the comparison.

3. Results

3.1. Clinical Presentation of the Patient

The patient (ID ZH0001256X) was examined in National COVID-19 Care Center (Hôpital du Mali) on April 16, 2021, owing to complaints of cough and muscle pains. At the time of admission, the patient, a 68-year-old male individual, had been taking 75 mg of aspirin (once a day for several years) as home medication and had a tobacco history dating back several years. His condition was stable and febrile with a body temperature of 38˚C and normal blood pressure and pulse rates (130/70 mm Hg and 80/min, respectively) with respiratory frequency of 16 cycles per min. The qRT-PCR analysis revealed SARS-CoV-2 infection. The CCT scan was normal. The CCT scan showed inflammation of the bronchus with the absence of diffuse bilateral patches of ground-glass opacities during the follow-up. The patient received treatment according to the national protocol for COVID-19 therapy in Mali [12] and was followed up for three weeks

(Figure 1).

3.2. CCT during the Clinical Follow-Up

After initiation of the treatment protocol the patient underwent CCT scan on April 24, 2021. The CCT scan showed minor diffuse bilateral patches of ground-glass opacities, which were associated with severe cough, exacerbated muscle pains, and asthenia. A follow-up CCT scan three days later revealed a normal radiology profile (Figure not available) and the patient's oxygen saturation was >95%.

3.3. qRT-PCR

The Laboratoire de Biologie Moléculaire Appliquée at the University of Science, Techniques and Technologies of Bamako received five nasopharyngeal specimens collected from the patient for the RT-PCR analysis during the third wave of SARS-CoV-2 in Mali (Figure 1). The first nasopharyngeal specimen collected on April 16, 2021, before the commencement of the treatment tested positive with a cycle threshold (Ct) of 10 over 40 cycles (Figure 2(a)). The second specimen was collected on April 24, 2021, a week after the initiation of the treatment. The test remained positive with a Ct of 22 (Figure 2(b)). To monitor the progression of disease, three additional nasopharyngeal specimens were tested until negative results were obtained.

3.4. Sequencing of Specimen Collected before Treatment Commencement

Sanger sequencing approach was performed using the CEQ XL 8000 (Beckman

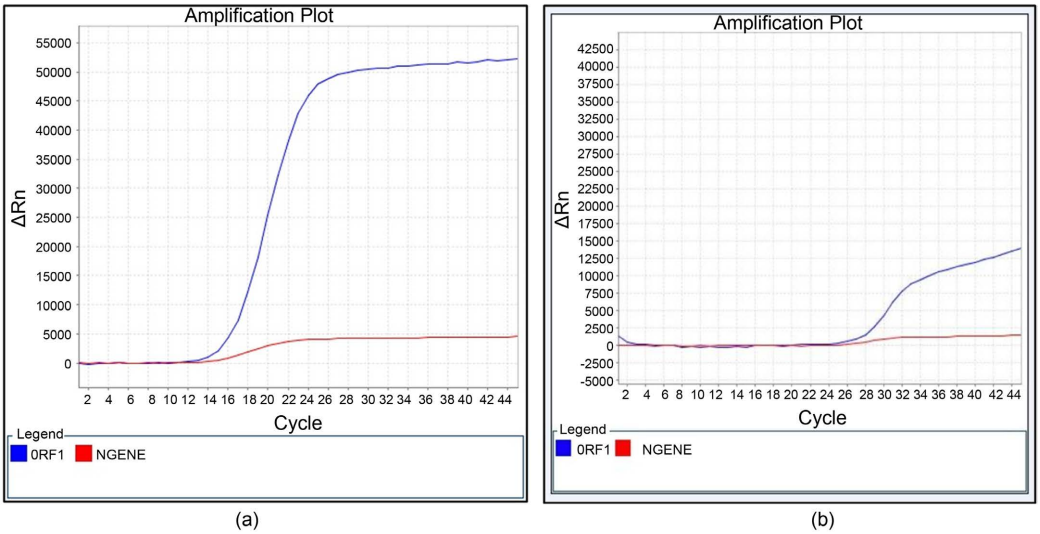

Figure 2. (a) qRT-PCR amplification profile of the specimen of SARS-CoV-2 collected on April 16, 2021. The Orf1 gene marker (confirmatory marker) appears at cycle 10 out of 44, while the N gene (screening marker) at the cycle 14 out of 44. (b) qRT-PCR amplification profile of the specimen of SARS-CoV-2 collected on April 24, 2021. The Orf1 gene marker (confirmatory marker) appears at cycle 26 out of 44, while the N gene (screening marker) at the cycle 28 out of 44.

Coulter) from the first nasopharyngeal specimen that was obtained before the initiation of the treatment. Seven primers covering the entire domain of the S gene were used to examine a 3822 bp long fragment [8]. Using the Geneious Prime 2021.1 software, the obtained sequence was aligned with the Wuhan strain (Accession number: S – 43740568) as sequence reference (**Figure 3**). The alignment approach revealed the presence of the following amino acid substitutions and deletions in the target sequence:

Substitutions: 52 (Q- > R), 67 (A- > V), 451 (Y- > N), 484 (E- > K), 614 (D- > G), 677 (Q- > H), 733 (K- > Q), 888 (F- > L), 1172 (I- > M), and 1250 (C- > F)

Deletions: 68 (del), 69 (del), 70 (del), and 144 (del).

No changes were observed at amino acid position 501, which is an unusual finding for this strain of SARS-CoV-2.

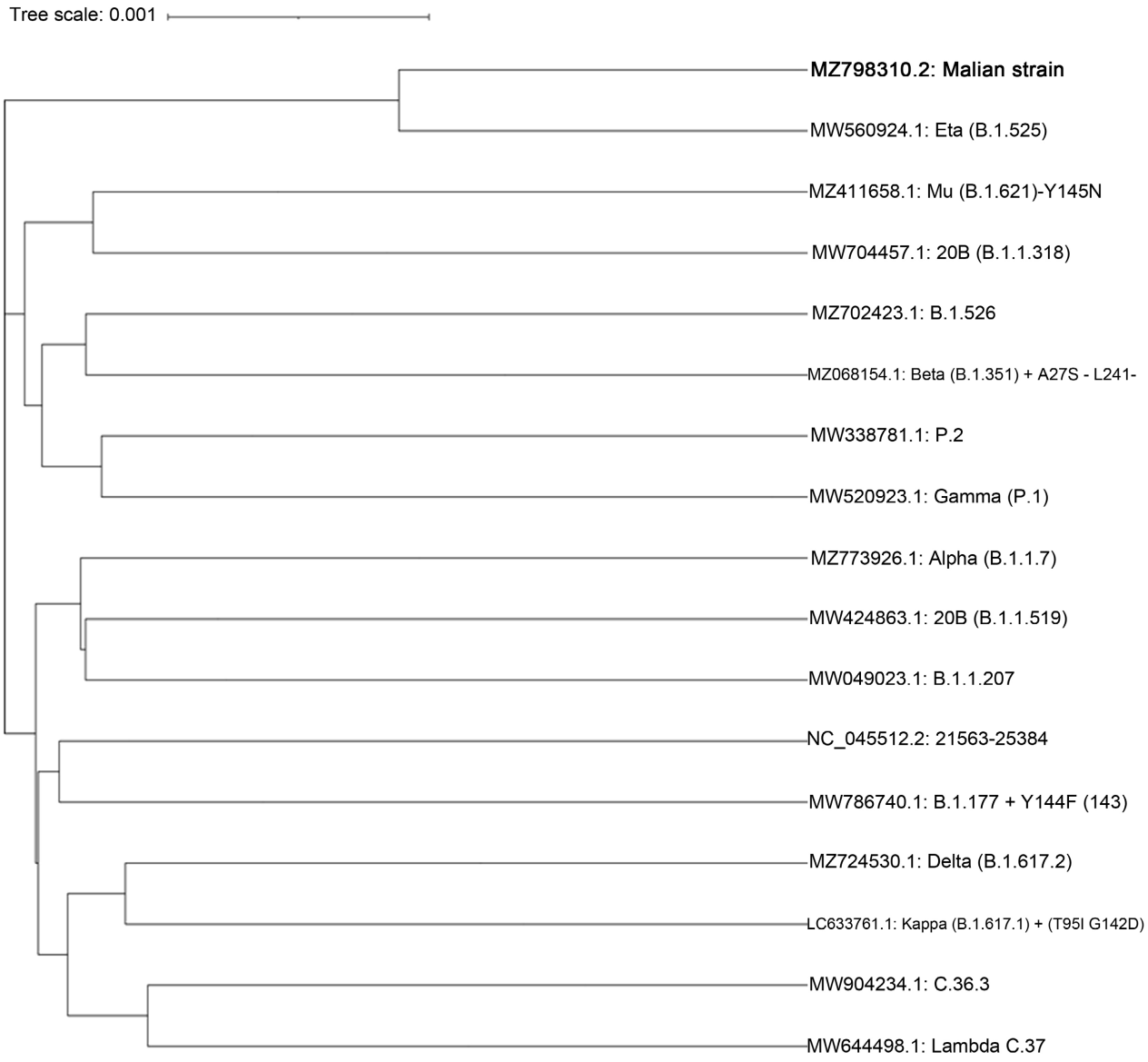

Figure 3. Phylogenetic tree of the Malian strain.

3.5. Relationship between Mutations on S Gene and the Viral Load (Mean Ct)

To determine whether the amino acid substitutions Q52R, A67V, Y451N, E484K, D614G, Q677H, F888L, I1172M, and C1250F and deletions at the positions 68, 69, 70, 144, and 833 were associated with changes to the overall viral load, the mean Ct values were compared to the Ct values of samples of other strains that had been circulating in Mali. The mean Ct values during the first wave of SARS-CoV-2 (March 2020 to July 2020) and the third wave (February 2021 to May 2021) were estimated for comparison using the two-sample t-test. We hypothesized that there could be a significant difference in terms of viral load between the two peaks if wild SARS-CoV-2 strains were associated with the first peak, while the new variant and other strains would be associated with the third peak.

The data showed that the mean Ct value of patients from the third wave of the infection (24.6 ± 5.8) was significantly lower than the mean Ct-value from the first peak (30.4 ± 5.5) (p-value < 0.00001). The mean difference between Wave 1 and Wave 3 is about six cycles (Table 1). To confirm this finding, the Ct value obtained from the specimen with mutated S gene was compared with the estimated mean Ct value from the first peak of the infection. The data showed that the patient Ct value (10) was significantly lower than the estimated mean Ct value of the first peak (30.4 ± 5.5) (p-value < 0.00001).

A computational analysis was performed to determine if the variant had different T cell epitopes from the originator strain of SARS-CoV-2 (Wuhan 2019) that could explain a more rapid reproductive rate (due to the absence of immune pressure [13]. The iVAX toolkit developed by EpiVax [14] was used to compare the potential immunogenicity of the sequence to the Wuhan.

(NC_045512.2:21563-25384) strain. The results are summarized in Table 2 below. The variant sequence had a slightly higher predicted immunogenicity [14] (EpiMatrix Score) than the reference spike sequence. The difference in tolerogenic potential (JanusMatrix Human Homology Score) was also negligible. These differences are minimal and do not explain the high viral load observed in

Table 1. Relation between Ct and waves of SARS-CoV-2 infection in Mali.

Peak (Period)	Number of patients	Mean Ct	Std. Dev.	95% confidence interval	*P*-value
Wave One (March 2020-July 2020)	724	30.4	5.5	29.9 - 30.8	<0.00001
Patient (April 2021)	1	10	-	-	
Wave One (March 2020-July 2020)	724	30.4	5.5	29.9 - 30.8	<0.00001
Wave Three (Feb. 2021-May 2021)	83	24.6	5.8	23.3 - 25.9	

Table 2. Predicted immunogenicity of the Malian variant sequence using the EpiMatrix score.

Sequence	9-mers analyzed	EpiMatrix Hits	EpiMatrix Score
Spike reference	1035	568	11.96
Mali Spike (GenBank id: MZ798310)	1033	575	13.44

the patient. In addition, more than 90% of class II predicted T cell epitopes in the novel sequence match the T cell epitopes identified in representative sequences of alpha, beta, delta, and gamma variants and the reference sequence. Memory T cells induced by prior exposure to these strains of SARS-CoV-2 or to any of the available COVID-19 vaccines would be able to respond to 90% of the putative class II T cell epitopes identified in novel variant from Mali.

4. Discussion

Several viral lineages are circulating in the world and at least four variants were tagged as variants of concern (VoC) [15] [16]. The variant observed in this study displayed six non-synonymous mutations (Q52R, A67V, E484K, D614G, Q677H, and F888L) as well as two in-frame deletions at positions 69 - 70, which are similar to those found in the variant of interest (VOI) belonging to the lineage B.1.525 or variant eta identified in Central Italy and Nigeria [17]. However, we also observed four novel mutations, which are Y451N, D228, I1172M, and C1250F. These mutations are rarely observed. In contrast, D614G (aspartic acid to glycine amino acid substitution), which is observed across several countries, was also observed in our sequence. This mutation is well established in several variants identified across various countries, including Alpha (B.1.1.7), Beta (B.1351), Gamma (P.1), Delta (B.1617.2) and Omicron (B.1.5.529). All these variants have displayed higher transmissibility, which suggests that D614G substitution is associated with higher transmissibility of the virus residing in our specimen as well [17]-[25]. Mutations such as the 69 - 70 del (deletion of six bases coding for histidine and valine at positions 60 and 70, respectively, in the S gene), observed previously in both Alpha and omicron variants [19] [26] were also present in our viral sequence. In addition, E484K mutation observed in Beta and Gamma variants [17] [19] has been found to be present with the N501Y mutation. In contrast, the N501Y was absent in our sequence, though it carried the E484K mutation.

Q677H is located close to the furin cleavage site at the S1-S2 junction [27]. This substitution has the potential to affect the dynamics of the Spike cleavage, which is a necessary step required for cell entry [28] [29] [30] [31]. This mutation was first detected at the beginning of the epidemic and is now found across 164 countries (GISAID, March 16, 2022). The higher transmissibility of our variant may also be attributed to the role of Q677H in the cell entry process [27]

[30]. We also observed F888L to be present in our sequence as well as in the mutants belonging to the lineage B.1.525. This mutation was detected for the first time in the USA 2 years ago (hCoV-19/USA/MD-HP27792-PIDVEQVXMT/2020; GISAID, March 16, 2022) and continued to spread with a recent appearance in Denmark (hCoV-19/Denmark/DCGC- 412780/2022; GISAID, March 16, 2022).

We found eight common mutations in our specimen sequence as compared to SARS-CoV-2 lineage B.1.525 and Eta Variant according to WHO [32]. This finding indicates that the four novel mutations along our S gene sequence, that is, D228N, Y451N, I1172M, and C1250F, could be the result of a distancing evolutionary process (**Figure 3**). The D228N emerged in England 2 years ago (GISAID, March 16, 2022), while Y451N is a rare mutation first identified in our sequence in April 2021 before its appearance in Turkey (hCoV-19/Turkey/HSGM-E1255/2021) in August 2021. This mutant sequence was submitted in GenBank (Accession number: MZ798310). Until now, this mutation has been found only in 11 countries (GISAID, March 16, 2022).

I1172M and C1250F are in the C-terminus region of the S gene upstream of the junction S1-S2. It is noteworthy that they have been detected in 9 and 77 countries, respectively, but they do not occur in the lineage B.1.525 or Eta variant. The two mutations were found in the C-terminus domain of the S gene and downstream of L1114R and are believed to be associated with the CoV tropisms [33] [34]. The presence of these mutations in our variant, along with the mutations D228 and Y451, support the notion that the SARS-CoV-2 genome is highly flexible.

Of all reported sequences between January 1, 2021, and February 14, 2021, in Nigeria, the authors observed that 68% of the sequences belonged to the B.1.1.7 lineage while 17% belonged to the B.1.525 lineage.

No T cell epitope changes were identified that could justify a determination of "immune escape" or "immune camouflage" as the reason for the higher viral load, as determined by PCR Ct [35].

Our variant was associated with a significantly lower Ct value (equal to 10) and longer viral clearance duration of 3 weeks with a p-value < 0.00001 (**Table 1** and **Figure 1**). Ong *et al*. (2021) observed a median duration for delta variant and wild type variant as 18 and 13 days, respectively [36]. Upon analyzing a total of 724 SARS-CoV-2-positive nasopharyngeal samples, we observed a higher mean Ct value (Ct = 30.4) during the first peak of infection driven by the wild type compared to the mean Ct value during the third peak during which our variant was identified (Ct = 24). The low Ct values observed in our variant were consistent with those of the beta and alpha variants [37].

5. Conclusions

Our findings suggested that a strain that is very similar to the Eta variant was present in Mali during the third wave of SARS-CoV-2 infection and this strain is associated with a low Ct value which can be a good indicator of transmissibility. The individual identified in this case study recovered quickly but demonstrated a

persistently high viral load.

It is worth noting that access to COVID-19 testing is not widespread in Mali. Thus numerous cases of COVID-19 may have gone undetected during the pandemic [38]. A serosurvey of healthcare personnel performed in 2021 indicated that up to 70% of providers were positive for SARS-CoV-2 spike antibodies by June 2021 and that the rate of infection was lower among personnel treating COVID-19 patients. The infection rate among healthcare workers suggests that SARS-CoV-2 may have been more widespread than reported in the Malian population. This was confirmed in a rural study that demonstrated up to 45% seropositivity in community members over the age of 60 in the same time period [39]. Viral strains such as the one reported here, which have the capacity for rapid reproductive rates, may have contributed to the wide dissemination of the SARS-CoV-2 virus in the population.

Ethics Approval

This case report was conducted in accordance with the declaration of Helsinki. The collection and analysis of the data were performed in a compliant manner.

Consent for Publication

All the authors have given their consent for this submission.

Authorship Contribution

Ousmane Aliou Koita: Writing, Analysis, Study Design. Ibrahim Keita: Sequencing. Youssouf Diarra: Sequencing, Analysis. Mariam Traoré: Analysis. Garan Dabo: Investigation. Yacouba Toloba: Investigation. Aliou Sissako: Investigation. Mahamadou Alpha Diallo: Investigation; Lassina Doumbia: Processing. Djeneba Sy: Processing. Ibrahim Guindo: Processing. Mohamed Diallo: Processing. AM: Processing. SB: Processing. Akory Ag Iknane: Processing. Lansana Sangaré: Analysis. Ibrahim Traoré: Analysis. Andres H. Gutierrez: Analysis. Mounkaila Abdou Billo: Processing. Anne S. De Groot: Analysis.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or

personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Tang, J.W., Tambyah, P.A. and Hui, D.S. (2021) Emergence of a New SARS-CoV-2 Variant in the UK. *Journal of Infection*, **82**, e27-e28. https://doi.org/10.1016/j.jinf.2020.12.024

[2] Cherian, S., Potdar, V., Jadhav, S., Yadav, P., Gupta, N., Das, M., *et al.* (2021) SARS-CoV-2 Spike Mutations, L452R, T478K, E484Q and P681R, in the Second Wave of COVID-19 in Maharashtra, India. *Microorganisms*, **9**, Article No. 1542. https://doi.org/10.3390/microorganisms9071542

[3] Dougherty, K., Mannell, M., Naqvi, O., Matson, D. and Stone, J. (2021) SARS-CoV-2 B.1.617.2 (Delta) Variant COVID-19 Outbreak Associated with a Gymnastics Facility—Oklahoma, April-May 2021. *Morbidity and Mortality Weekly Report*, **70**, 1004-1007. https://doi.org/10.15585/mmwr.mm7028e2

[4] WHO (2021) Update on Omicron. https://www.who.int/news/item/28-11-2021-update-on-omicron

[5] Imai, M., Halfmann, P.J., Yamayoshi, S., Iwatsuki-Horimoto, K., Chiba, S., Watanabe, T., *et al.* (2021) Characterization of a New SARS-CoV-2 Variant That Emerged in Brazil. *Proceedings of the National Academy of Sciences of the United States of America*, **118**, e2106535118. https://doi.org/10.1073/pnas.2106535118

[6] Kidd, M., Richter, A., Best, A., Cumley, N., Mirza, J., Percival, B., *et al.* (2021) S-Variant SARS-CoV-2 Lineage B1.1.7 Is Associated with Significantly Higher Viral Load in Samples Tested by TaqPath Polymerase Chain Reaction. *The Journal of Infectious Diseases*, **223**, 1666-1670. https://doi.org/10.1093/infdis/jiab082

[7] Calistri, P., Amato, L., Puglia, I., Cito, F., Di Giuseppe, A., Danzetta, M.L., *et al.* (2021) Infection Sustained by Lineage B.1.1.7 of SARS-CoV-2 Is Characterised by Longer Persistence and Higher Viral RNA Loads in Nasopharyngeal Swabs. *International Journal of Infectious Diseases*, **105**, 753-755. https://doi.org/10.1016/j.ijid.2021.03.005

[8] Gudbjartsson, D.F., Helgason, A., Jonsson, H., Magnusson, O.T., Melsted, P., Norddahl, G.L., *et al.* (2020) Spread of SARS-CoV-2 in the Icelandic Population. *The New England Journal of Medicine*, **382**, 2302-2315. https://doi.org/10.1056/NEJMoa2006100

[9] Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, **30**, 2725-2729. https://doi.org/10.1093/molbev/mst197

[10] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Clustal W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Research*, **22**, 4673-4680. https://doi.org/10.1093/nar/22.22.4673

[11] Letunic, I. and Bork, P. (2021) Interactive Tree of Life (iTOL) v5: An Online Tool for Phylogenetic Tree Display and Annotation. *Nucleic Acids Research*, **49**, W293-W296. https://doi.org/10.1093/nar/gkab301

[12] Ministère de la santé et des affaires socials (2020) Directives de prise en charge et de protection du personnel de sante dans le cadre de la maladie a COVID-19. http://www.sante.gov.ml/index.php/2014-11-10-17-29-36/documents-standards/item/3572-directives-de-prise-en-charge-et-de-protection-du-personnel-de-sante-dans-le-cadre-de-la-maladie-a-covid-19

[13] Rousseau, C.M., Daniels, M.G., Carlson, J.M., Kadie, C., Crawford, H., Prendergast, A., *et al*. (2008) HLA Class I-Driven Evolution of Human Immunodeficiency Virus Type 1 Subtype C Proteome: Immune Escape and Viral Load. *Journal of Virology*, **82**, 6434-6446. https://doi.org/10.1128/JVI.02455-07

[14] De Groot, A.S., Moise, L., Terry, F., Gutierrez, A.H., Hindocha, P., Richard, G., *et al*. (2020) Better Epitope Discovery, Precision Immune Engineering, and Accelerated Vaccine Design Using Immunoinformatics Tools. *Frontiers in Immunology*, **11**, Article No. 442. https://doi.org/10.3389/fimmu.2020.00442

[15] da Silva, S.J.R., de Lima, S.C., da Silva, R.C., Kohl, A. and Pena, L. (2021) Viral Load in COVID-19 Patients: Implications for Prognosis and Vaccine Efficacy in the Context of Emerging SARS-CoV-2 Variants. *Frontiers of Medicine*, **8**, Article ID: 836826. https://doi.org/10.3389/fmed.2021.836826

[16] CDC (2020) Coronavirus Disease 2019 (COVID-19)|2020 Interim Case Definition. https://stacks.cdc.gov/view/cdc/103898

[17] Mukherjee, R. and Satardekar, R. (2021) Why Are Some Coronavirus Variants More Infectious? *Journal of Biosciences*, **46**, Article No. 101. https://doi.org/10.1007/s12038-021-00221-y

[18] Hou, Y.J., Chiba, S., Halfmann, P., Ehre, C., Kuroda, M., Dinnon, K.H., *et al*. (2020) SARS-CoV-2 D614G Variant Exhibits Efficient Replication *ex Vivo* and Transmission *in Vivo*. *Science*, **370**, 1464-1468. https://doi.org/10.1126/science.abe8499

[19] Davies, N.G., Abbott, S., Barnard, R.C., Jarvis, C.I., Kucharski, A.J., Munday, J.D., *et al*. (2021) Estimated Transmissibility and Impact of SARS-CoV-2 Lineage B.1.1.7 in England. *Science*, **372**, eabg3055. https://doi.org/10.1126/science.abg3055

[20] Mishra, S.K. and Tripathi, T. (2021) One Year Update on the COVID-19 Pandemic: Where Are We Now? *Acta Tropica*, **214**, Article ID: 105778. https://doi.org/10.1016/j.actatropica.2020.105778

[21] Souza, W.M., Amorim, M.R., Sesti-Costa, R., Coimbra, L.D., Brunetti, N.S., Toledo-Teixeira, D.A., *et al*. (2021) Neutralisation of SARS-CoV-2 Lineage P.1 by Antibodies Elicited through Natural SARS-CoV-2 Infection or Vaccination with an Inactivated SARS-CoV-2 Vaccine: An Immunological Study. *The Lancet Microbe*, **2**, e527-e535. https://doi.org/10.1016/S2666-5247(21)00129-4

[22] Gómez, C.E., Perdiguero, B. and Esteban, M. (2021) Emerging SARS-CoV-2 Variants and Impact in Global Vaccination Programs against SARS-CoV-2/COVID-19. *Vaccines*, **9**, Article No. 243. https://doi.org/10.3390/vaccines9030243

[23] Deng, X., Garcia-Knight, M.A., Khalid, M.M., Servellita, V., Wang, C., Morris, M.K., *et al*. (2021) Transmission, Infectivity, and Neutralization of a Spike L452R SARS-CoV-2 Variant. *Cell*, **184**, 3426-3437.e8. https://doi.org/10.1016/j.cell.2021.04.025

[24] Chen, J., Wang, R., Gilby, N.B. and Wei, G.W. (2022) Omicron Variant (B.1.1.529) Infectivity, Vaccine Breakthrough, and Antibody Resistance. *Journal of Chemical Information and Modeling*, **62**, 412-422. https://doi.org/10.1021/acs.jcim.1c01451

[25] Zhang, L., Jackson, C.B., Mou, H., Ojha, A., Peng, H., Quinlan, B.D., *et al*. (2020) SARS-CoV-2 Spike-Protein D614G Mutation Increases Virion Spike Density and Infectivity. *Nature Communications*, **11**, Article No. 6013. https://doi.org/10.1038/s41467-020-19808-4

[26] Gao, S.J., Guo, H. and Luo, G. (2022) Omicron Variant (B.1.1.529) of SARS-CoV-2, a Global Urgent Public Health Alert! *Journal of Medical Virology*, **94**, 1255-1256. https://doi.org/10.1002/jmv.27491

[27] Hodcroft, E.B., Domman, D.B., Snyder, D.J., Oguntuyo, K.Y., Diest, M.V., Densmore, K.H., *et al*. (2021) Emergence in Late 2020 of Multiple Lineages of SARS-

CoV-2 Spike Protein Variants Affecting Amino Acid Position 677. https://www.medrxiv.org/content/10.1101/2021.02.12.21251658v3 https://doi.org/10.1101/2021.02.12.21251658

[28] Xie, Y., Du, D., Karki, C.B., Guo, W., Lopez-Hernandez, A.E., Sun, S., *et al.* (2020) Revealing the Mechanism of SARS-CoV-2 Spike Protein Binding with ACE2. *Computing in Science & Engineering*, **22**, 21-29. https://doi.org/10.1109/MCSE.2020.3015511

[29] Starr, T.N., Greaney, A.J., Hilton, S.K., Ellis, D., Crawford, K.H.D., Dingens, A.S., *et al.* (2020) Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell*, **182**, 1295-1310.e20. https://doi.org/10.1016/j.cell.2020.08.012

[30] Hoffmann, M., Kleine-Weber, H. and Pöhlmann, S. (2020) A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Molecular Cell*, **78**, 779-784.e5. https://doi.org/10.1016/j.molcel.2020.04.022

[31] Peacock, T.P., Goldhill, D.H., Zhou, J., Baillon, L., Frise, R., Swann, O.C., *et al.* (2021) The Furin Cleavage Site in the SARS-CoV-2 Spike Protein Is Required for Transmission in Ferrets. *Nature Microbiology*, **6**, 899-909. https://doi.org/10.1038/s41564-021-00908-w

[32] WHO (2021) Tracking SARS-CoV-2 Variants. https://www.who.int/activities/tracking-SARS-CoV-2-variants

[33] Tsai, J.C., de Groot, L., Pinon, J.D., Iacono, K.T., Phillips, J.J., Seo, S., *et al.* (2003) Amino Acid Substitutions within the Heptad Repeat Domain 1 of Murine Coronavirus Spike Protein Restrict Viral Antigen Spread in the Central Nervous System. *Virology*, **312**, 369-380. https://doi.org/10.1016/S0042-6822(03)00248-4

[34] Yamada, Y., Liu, X.B., Fang, S.G., Tay, F.P.L. and Liu, D.X. (2009) Acquisition of Cell-Cell Fusion Activity by Amino Acid Substitutions in Spike Protein Determines the Infectivity of a Coronavirus in Cultured Cells. *PLOS ONE*, **4**, e6130. https://doi.org/10.1371/journal.pone.0006130

[35] De Groot, A.S., Moise, L., Liu, R., Gutierrez, A.H., Tassone, R., Bailey-Kellogg, C., *et al.* (2014) Immune Camouflage: Relevance to Vaccines and Human Immunology. *Human Vaccines & Immunotherapeutics*, **10**, 3570-3575. https://doi.org/10.4161/hv.36134

[36] Ong, S.W.X., Chiew, C.J., Ang, L.W., Mak, T.M., Cui, L., Toh, M.P.H.S., *et al.* (2021) Clinical and Virological Features of SARS-CoV-2 Variants of Concern: A Retrospective Cohort Study Comparing B.1.1.7 (Alpha), B.1.315 (Beta), and B.1.617.2 (Delta). *Clinical Infectious Diseases*, ciab721. https://doi.org/10.1093/cid/ciab721

[37] Teyssou, E., Soulie, C., Visseaux, B., Lambert-Niclot, S., Ferre, V., Marot, S., *et al.* (2021) The 501Y.V2 SARS-CoV-2 Variant Has an Intermediate Viral Load between the 501Y.V1 and the Historical Variants in Nasopharyngeal Samples from Newly Diagnosed COVID-19 Patients. *Journal of Infection*, **83**, 119-145. https://doi.org/10.1016/j.jinf.2021.04.023

[38] Somboro, A.M., Cissoko, Y., Camara, I., Kodio, O., Tolofoudie, M., Dembele, E., *et al.* (2022) High SARS-CoV-2 Seroprevalence among Healthcare Workers in Bamako, Mali. *Viruses*, **14**, Article No. 102. https://doi.org/10.3390/v14010102

[39] Alber, D., Haidara, F.C., Luoma, J., Adubra, L., Ashorn, P., Ashorn, U., *et al.* (2022) SARS-CoV-2 Infection and Antibody Seroprevalence in Routine Surveillance Patients, Healthcare Workers and General Population in Kita Region, Mali: An Observational Study 2020-2021. *BMJ Open*, **12**, e060367. https://doi.org/10.1136/bmjopen-2021-060367