SARS-CoV-2 Sequence Analysis during COVID-19 Case Surge, Liberia, 2021

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DOI: https://doi.org/10.3201/eid2712.211818

In June 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases surged in Liberia. SARS-CoV-2 sequences from patients hospitalized during March–July 2021 revealed the Delta variant was in Liberia in early March and was dominant in June, irrespective of geography. Mutations and deletions suggest multiple SARS-CoV-2 Delta variant introductions.

Before May 2021, Liberia reported <10 coronavirus disease (COVID-19) cases per day among its population of ≈5 million (*1*). Thereafter, case numbers, hospitalizations, and deaths rapidly increased and peaked to >200 cases and 10–15 deaths per day in mid-July 2021 (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/27/12/21-1818-App1.pdf). To determine whether the rapid case surge was associated with the introduction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern or newly emerging variants, we collected nasopharyngeal swab samples from 267 hospitalized patients countrywide during March–July 2021 for high-throughput sequencing.

We collected samples in viral transport media from Bomi, Bong, Grand Cape Mount, Lofa, Margibi, Maryland, Montserrado, and Nimba Counties (Appendix Figure 2). We noted sample collection date and site and sex and median age of patients from whom samples were obtained (Table; Appendix Table). We used Buffer AVL (QIAGEN, https://www.qiagen.com) lysis buffer to extract total nucleic acid and performed PCR by using the Triplex-CII-SARS-Cov-2 rRT PCR assay (*2*). We conducted further high-throughput sequencing on 89/267 (33.3%) samples that had cycle threshold values <33 (Appendix Table).

To prepare libraries, we used the Kapa Hyperplus Kit (Roche, https://www.roche.com) on first strand cDNA synthesized from 89 RNA samples (*3*), then we enriched for SARS-CoV-2 by using myBaits Custom RNA-Seq Kit (Daicel Arbor Biosciences, https://arborbiosci.com). We sequenced captured libraries on Nextseq 2000 or Nextseq 550 (Illumina, https://www.illumina.com), which yielded 5–8 million 220-bp reads per sample. We mapped reads to a SARS-CoV-2 reference sequence (GenBank accession no. NC_045512) to determine variants (Table; Appendix Table).

Of the 89 RNA samples, 77 (86.5%) yielded complete coding sequences with a minimum depth of ≈15× (GISAID accession nos. EPI_ISL_3547663–705, EPI_ISL_3560291, and EPI_ISL_4232122–52). Using high-throughput sequencing data, we generated consensus fasta sequences of 77 SARS-CoV-2 genomic sequences and further analyzed sequences by using Geneious R10 (https://www.geneious.com), NextStrain (*4*), and GISAID (*5*).

Among 77 genomes recovered, 4 (5.2%) were Alpha variant (B.1.1.7); 6 (7.8%) were Beta variant (B.1.351); 1 (1.3%) was Iota variant (B.1.526); 6 (7.8%) were Eta variant (B.1.525); and 56 (72.7%) were Delta variant (B.1.617.2) viruses (Table). We identified Delta variant viruses in samples collected in early March and in April and May 2021, from Bong County. Delta variant viruses were co-circulating with Alpha, Beta, Eta, Iota, and other 20B variant viruses in Liberia. All 44 sequences recovered during June–July 2021 were from Delta variant viruses (Table). We used complete polyprotein coding sequences from Liberia, other representative SARS-CoV-2 sequences, and variant reference sequences to create a maximum-likelihood, nucleotide-based phylogenetic tree in MEGA X (*6*) (Figure).

Using reference sequence NC_045512 as a baseline, we found 3 Alpha variant–specific amino acid deletions (H69del, V70del, Y144del) in the surface glycoprotein of all Alpha variant genomes and 3 Beta variant–specific amino acid deletions (L241del, L242del, A243del) in the surface glycoprotein of all Beta variant genomes. All 56 Delta variant genomes had the 2 variant-specific amino acid deletions, F157del and R158del, and 8 of 9 other Delta variant–

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Table. Characteristics of 77 clinical samples collected before and during COVID-19 case surge that yielded complete SARS-CoV-2 coding genomic sequences, Liberia, 2021

Month collected	Total no. samples	Patient sex, no.	Average age, y (SD)	County	No. samples/ county	SARS-CoV-2 variant, no. of samples/county/mo					
						Delta B.1.617.2	Alpha B.1.1.7	Beta B.1.351	Eta B.1.525	Iota B.1.526	20B other
Mar	4	2M, 2F	39.25 (6.05)	Montserrado	3			1	1	1	
				Bong	1	1					
Apr	11	10M, 1F	42.54 (11.52)	Montserrado	10	4	1	3	2		
				Grand Cape Mount	1			1			
May	18	9M, 9F	40.11 (16.82)	Bong	1	1					
				Margibi	1						
				Montserrado	14	6	3	2	2		1
				Nimba	2						2
Jun	36	13M, 23F	39.22 (18.36)	Lofa	5	5					
				Margibi	1	1					
				Maryland	1	1					
				Montserrado	29	29					
Jul	8	4M, 4F	51.25 (9.71)	Margibi	1	1					
				Montserrado	5	5					
				Nimba	2	2					

*Liberia experienced a surge in COVID-19 cases during June 2021. Blank cells indicate no variants detected. COVID-19, coronavirus disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

specific amino acid substitutions in the surface glycoprotein (T19R, G142D, E156G, L452R, T478K, D614G, P681R, and D950N). The A222V surface glycoprotein mutation was absent in only 2/56 Delta variant genomes, LIB-0226 and LIB-0217, collected from Monteserrado County in May 2021 (*4*). We observed another mutation in the surface glycoprotein, V367L, in 14 sequences: 1 from Bong, 2 from Margibi, 1 from Maryland, 9 from Montserrado, and 1 from Nimba. No sequences recovered from Lofa County had the V367L mutation. We noted the R724K mutation in the open reading frame 1a region of 2 sequences from Lofa, LIB-0131 and LIB-0133. LIB-0073 and LIB-0093 sequences collected from Montserrado County had 2 amino acid deletions in the open reading frame 8 region (position 120–121).

Recent surges in COVID-19 in many countries have been associated with the emergence of highly transmissible Delta variant viruses (*7*,*8*). In March 2021, the National Public Health Institute of Liberia sequenced 10 random samples from hospitalized COVID-19 patients in Monteserrado; all sequences were Alpha variant viruses (B. Shobayo, unpub. data).

A limitation of our study is the small sample sets used for analysis; nonetheless, our findings suggest that Alpha and other circulating variant viruses were replaced by Delta variant viruses countrywide in Liberia in <3 months. Mutation and phylogenetic analyses further indicate that several Delta variant strains were circulating after March 2021 and suggest multiple separate introductions.

Before June 2021, only a small percentage of the population was vaccinated in Liberia. The infections we report occurred in unvaccinated persons. The Ministry of Health, Liberia, initiated a vaccination drive in August 2021. By September, ≈130,000 persons, >2% of the population, had received a single dose of the Johnson & Johnson/Janssen vaccine (https://www.jnj.com). The COVID-19 vaccination campaign is ramping up as <30 cases/day are reported in Liberia, but the currently circulating Delta variants are a concern because they contain mutations and deletions in the surface glycoprotein that might influence vaccine efficacy (*9*). Liberia should continued surveillance for SARS-CoV-2 variants of concern to determine whether additional vaccination or public health measures are needed to curb severe disease and future case surges in the country.

Acknowledgments

We thank Pryanka Sharma and Gilbert Smith; Melvin Johnson, John Fayiah and Julie Blie and all the members of Partnership for Research on Vaccines and Infectious Diseases in Liberia; Julius Teahton, Francis Jaryan, John Dogba, Fahn Taweh, and Joseph Tahyor; and National Public Health Institute of Liberia research and lab team members for their support in collection and transport of samples.

Financial support was provided by by the Skoll Foundation for the Global Alliance for Pandemic Prevention (GAPP grant no. 20-45017), the Tong Tsung and Wei Fong Chao Foundation (grant no. GT007457), and the Chau Hoi Sheun Foundation (grant no. GT007457).

The National Institute of Allergy and Infectious Diseases, National Institutes of Health, also participated in study design, analysis and interpretation of data, writing of the report, and decision to submit the article for publication.

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Dr. Shobayo is public health and medical research scientist and deputy director at National Public Health Institute of Liberia, Monrovia, Liberia, and member of Partnership for Research on Infectious Diseases in Liberia. His research interests include survillence and investigation of emerging and re-emerging viruses and their impacts on public health.

Figure. Phylogenetic analysis of 77 nasopharyngeal swab samples collected during coronavirus disease case surge, Liberia, March–July 2021, and reference sequences. We created a maximum-likelihood nucleotide phylogenetic tree of the complete polyprotein coding region by using MEGA X (https://www.megasoftware.net), with a bootstrap value of 100 and and used Tamura-Nei 93 (TN93) as a substitution model with a discrete gamma distribution (+G) for evolutionary rate; the rate variation model allowed some sites to be evolutionarily invariable (+I). Numbers along the branches are bootstrap values of 100 bootstrap resamplings. Teal indicates samples collected in March 2021; purple indicates samples collected in April 2021; pink indicates samples collected in May 2021; blue indicates samples collected in June 2021; orange indicates samples collected in July 2021; brown indicates variants of concern or variants of interest; black indicates other circulating variants; green indicates severe acute respiratory syndrome coronavirus 2 reference sequence and other early parental sequences from 2020.

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Real-Time Projections of SARS-CoV-2 B.1.1.7 Variant in a University Setting, Texas, USA

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DOI: https://doi.org/10.3201/eid2712.210652

We used the incidence of spike gene target failures identified during PCR testing to provide an early projection of the prevalence of severe acute respiratory syndrome coronavirus 2 variant B.1.1.7 in a university setting in Texas, USA, before sequencing results were available. Findings from a more recent evaluation validated those early projections.

Identification of the highly transmissible novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant B.1.1.7 (Alpha variant) in the United Kingdom raised concerns for renewed pandemic surges worldwide (*1*,*2*). B.1.1.7 likely arrived in the United States by October 2020 (*1*); it was first detected in December 2020 and declared the dominant strain in April 2021, as projected in January 2021 (*3*). However, the regional prevalence of B.1.1.7 was largely unknown in early 2021 because of limited molecular surveillance for SARS-CoV-2 (*4*). To provide local situational awareness at that pivotal moment in the coronavirus disease (COVID-19) pandemic, we estimated the prevalence of B.1.1.7 on the basis of 17,003 student SARS-CoV-2 PCR test results reported through the Proactive Community Testing Program at the University of Texas (UT; Austin, Texas, USA), a large public university located in a metropolitan area with a population >2 million, during January 16–February 12, 2021 (K.E. Johnson et al., unpub. data, https://doi.org/10.1101/2021.03.05.21252541. Those early estimates were subsequently validated by using PCR data through April 9, 2021.

Mutations in the B.1.1.7 spike protein result in a failure to detect the spike gene probe in standard SARS-CoV-2 quantitative reverse transcription PCR (qRT-PCR). In estimating the prevalence of B.1.1.7 from local quantitative PCR data, we initially assumed US estimates for the proportion of spike gene target failures (SGTF) attributable to B.1.1.7 (*4*) and, in our retrospective analysis, update that proportion on the basis of local sequencing data. We used a Bayesian model to estimate the local growth rate of B.1.1.7 among all SARS-CoV-2 infections and applied a compartmental susceptible-exposed-infected-recovered model of SARS-CoV-2 transmission to project the effect of B.1.1.7 on future COVID-19 prevalence.

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Article DOI: https://doi.org/10.3201/eid2712.211818

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Appendix

Appendix Table. Dates, collection sites, sex, age, quantitative PCR cycle threshold values, and sequence coverage for 89 samples from COVID-19 patients, Liberia, 2021

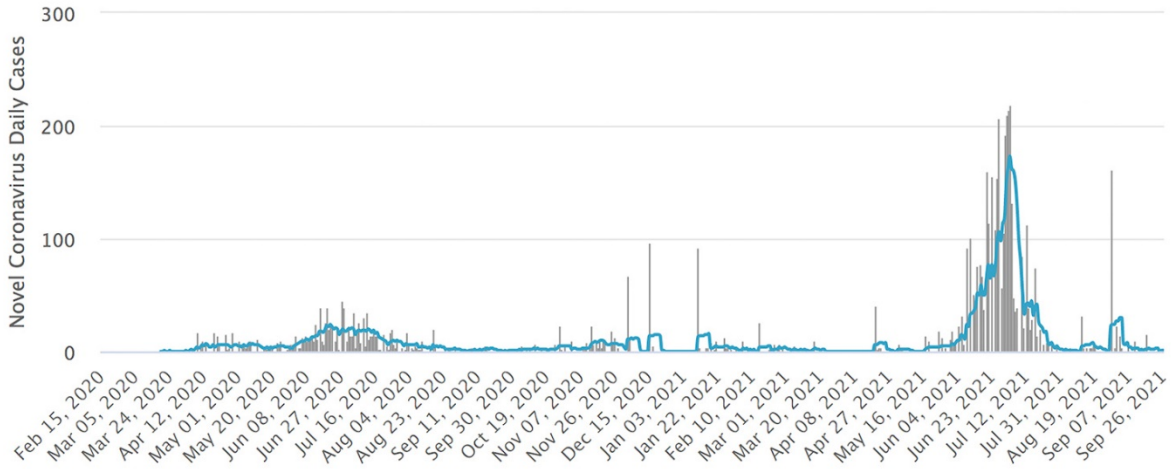
Sample ID	Collection date	County	Age, y/sex	C_t value	% Genome recovered	Avg. depth/nucleotide	Nextclade	Variant†	GISAID clade
LIB-0233	9 Mar	Bong	47/M	32.39	99.82	53.35	21A	VOC Delta B.1.617.2	G
LIB-0193	10 Mar	Montserrado	30/F	32.70	99.73	29.48	21D	VOI Eta B.1.525	G
LIB-0166	31 Mar	Montserrado	40/F	26.94	99.94	1534.63	20H	VOC Beta B.1.351	GH
LIB-0200	31 Mar	Montserrado	40/M	23.43	99.94	14633.94	21F	VOI Iota B.1.526	GH
LIB-0170	2 Apr	Montserrado	58/M	24.77	99.97	24611.29	20H	VOC Beta B.1.351	GH
LIB-0171	4 Apr	Montserrado	64/F	25.04	99.86	185.61	21D	VOI Eta B.1.525	G
LIB-0174	4 Apr	Montserrado	35/M	24.63	99.98	27842.07	20I	VOC Alpha B.1.1.7	GR
LIB-0192	4 Apr	Montserrado	30/M	22.07	100.00	69811.5	20H	VOC Beta B.1.351	GH
LIB-0197	6 Apr	Montserrado	54/M	23.87	99.96	16087.41	21A	VOC Delta B.1.617.2	G
LIB-0152	8 Apr	Montserrado	41/M	24.83	99.91	681.27	20B	Other	GR
LIB-0173	8 Apr	Grand Cape Mount	41/M	27.23	99.94	2708	20H	VOC Beta B.1.351	GH
LIB-0196	10 Apr	Montserrado	30/M	19.29	100.00	93864.59	21A	VOC Delta B.1.617.2	G
LIB-0198	10 Apr	Montserrado	27/M	24.05	99.98	11960.47	21A	VOC Delta B.1.617.2	G
LIB-0201	13 Apr	Montserrado	46/M	26.42	99.94	2851.87	21D	VOI Eta B.1.525	G
LIB-0182	17 Apr	Montserrado	42/M	23.72	99.99	22901.55	21A	VOC Delta B.1.617.2	G
LIB-0204	3 May	Montserrado	20/M	32.16	43.18	1.62	NA	NA	NA
LIB-0220	3 May	Montserrado	34/M	29.37	99.92	215.57	20I	VOC Alpha B.1.1.7	GR
LIB-0248	4 May	Montserrado	30/M	31.31	99.85	100.86	21A	VOC Delta B.1.617.2	G
LIB-0206	15 May	Montserrado	46/M	28.51	57.74	1.99	NA	NA	NA
LIB-0162	17 May	Margibi	77/M	32.11	99.85	434.73	21D	VOI Eta B.1.525	G
LIB-0236	17 May	Nimba	62/F	23.46	99.94	4894.08	20B	Other	GR
LIB-0203	20 May	Nimba	34/M	32.15	99.71	39.59	20B	Other	GR
LIB-0205	20 May	Montserrado	64/F	31.14	99.88	64.74	20I	VOC Alpha B.1.1.7	GR
LIB-0217	20 May	Montserrado	37/M	30.01	99.89	175.39	21A	VOC Delta B.1.617.2	G
LIB-0218	20 May	Montserrado	24/F	32.81	99.81	18.65	21A	VOC Delta B.1.617.2	G
LIB-0221	20 May	Montserrado	10/F	31.91	99.71	14.87	20I	VOC Alpha B.1.1.7	GR
LIB-0225	20 May	Montserrado	68/M	32.97	85.36	2.78	NA	NA	NA
LIB-0226	20 May	Montserrado	63/M	32.64	99.80	34.1	21A	VOC Delta B.1.617.2	G

Sample ID	Collection date	County	Age, y/sex	C_t value	% Genome recovered	Avg. depth/nucleotide	Nextclade	Variant†	GISAID clade
LIB-0247	20 May	Montserrado	43/F	18.86	100.00	303638.25	20H	VOC Beta B.1.351	GH
LIB-0250	20 May	Montserrado	38/F	22.72	99.98	14212.06	20B	Other	GR
LIB-0251	20 May	Montserrado	33/F	26.09	100.00	7626.71	21A	VOC Delta B.1.617.2	G
LIB-0255	20 May	Montserrado	31/M	17.25	100.00	504226.47	20H	VOC Beta B.1.351	GH
LIB-0253	27 May	Montserrado	27/F	18.58	100.00	223902.08	21D	VOI Eta B.1.525	G
LIB-0240	29 May	Bong	54/M	16.01	100.00	193424.09	21A	VOC Delta B.1.617.2	G
LIB-0242	29 May	Montserrado	23/F	22.93	99.99	18571.69	21D	VOI Eta B.1.525	G
LIB-0244	29 May	Montserrado	38/M	19.90	100.00	15944.35	21A	VOC Delta B.1.617.2	G
LIB-0004	5 Jun	Montserrado	57/M	21	100.00	132105.37	21A	VOC Delta B.1.617.2	G
LIB-0007	5 Jun	Montserrado	67/F	21	100.00	24117.24	21A	VOC Delta B.1.617.2	G
LIB-0071	5 Jun	Montserrado	28/M	28	99.98	748.95	21A	VOC Delta B.1.617.2	G
LIB-0017	6 Jun	Montserrado	62/F	28	99.94	302.55	21A	VOC Delta B.1.617.2	G
LIB-0018	6 Jun	Montserrado	41/M	24	99.30	106.19	21A	VOC Delta B.1.617.2	G
LIB-0019	6 Jun	Montserrado	34/M	17	99.90	359124.54	21A	VOC Delta B.1.617.2	G
LIB-0021	6 Jun	Montserrado	56/F	22	100.00	24466.47	21A	VOC Delta B.1.617.2	G
LIB-0022	6 Jun	Montserrado	37/F	25	100.00	1307.05	21A	VOC Delta B.1.617.2	G
LIB-0026	6 Jun	Montserrado	38/M	19	99.90	366820.93	21A	VOC Delta B.1.617.2	G
LIB-0028	6 Jun	Montserrado	27/F	27	99.94	797.2	21A	VOC Delta B.1.617.2	G
LIB-0032	6 Jun	Margibi	46/F	23	100.00	30961.5	21A	VOC Delta B.1.617.2	G
LIB-0064	8 Jun	Montserrado	40/F	28	99.95	399.9	21A	VOC Delta B.1.617.2	G
LIB-0012	12 Jun	Montserrado	45/F	19	100.00	12751.38	21A	VOC Delta B.1.617.2	G
LIB-0029	12 Jun	Montserrado	30/M	23	100.00	6319.4	21A	VOC Delta B.1.617.2	G
LIB-0030	12 Jun	Maryland	32/F	25	100.00	7395.49	21A	VOC Delta B.1.617.2	G
LIB-0037	12 Jun	Montserrado	35/M	20	100.00	2987.68	21A	VOC Delta B.1.617.2	G
LIB-0040	12 Jun	Montserrado	38/M	22	100.00	40117.32	21A	VOC Delta B.1.617.2	G
LIB-0042	12 Jun	Montserrado	29/M	22	100.00	23365.37	21A	VOC Delta B.1.617.2	G
LIB-0045	12 Jun	Montserrado	32/F	20	100.00	10116.23	21A	VOC Delta B.1.617.2	G
LIB-0047	12 Jun	Montserrado	32/F	22	100.00	46601.79	21A	VOC Delta B.1.617.2	G
LIB-0128	15 Jun	Lofa	1/M	21	100.00	29163.85	21A	VOC Delta B.1.617.2	G
LIB-0010	16 Jun	Montserrado	26/F	24	100.00	9787.07	21A	VOC Delta B.1.617.2	G
LIB-0131	16 Jun	Lofa	56/F	25	100.00	1864.44	21A	VOC Delta B.1.617.2	G
LIB-0133	16 Jun	Lofa	47/F	26	99.99	65698.71	21A	VOC Delta B.1.617.2	G
LIB-0136	16 Jun	Lofa	35/M	22	99.98	1442.69	21A	VOC Delta B.1.617.2	G
LIB-0137	16 Jun	Bomi	40/F	25	0	NA	NA	NA	NA
LIB-0143	16 Jun	Lofa	30/F	23	99.95	5513	21A	VOC Delta B.1.617.2	G

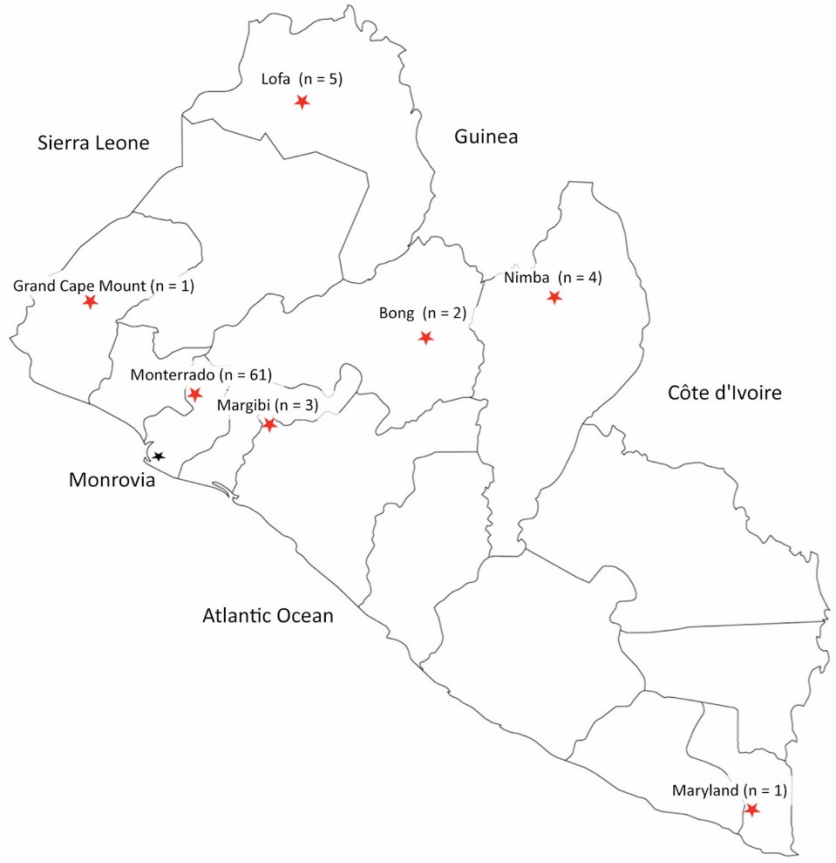
Sample ID	Collection date	County	Age, y/sex	C_t value	% Genome recovered	Avg. depth/nucleotide	Nextclade	Variant†	GISAID clade
LIB-0118	17 Jun	Montserrado	41/M	19	100.00	74762.42	21A	VOC Delta B.1.617.2	G
LIB-0121	17 Jun	Montserrado	82/F	30	99.66	40	21A	VOC Delta B.1.617.2	G
LIB-0123	17 Jun	Montserrado	25/F	21	100.00	10448.21	21A	VOC Delta B.1.617.2	G
LIB-0135	17 Jun	Montserrado	55/F	23	99.97	598.15	21A	VOC Delta B.1.617.2	G
LIB-0099	18 Jun	Montserrado	2/F	21	100.00	12031.71	21A	VOC Delta B.1.617.2	G
LIB-0124	18 Jun	Montserrado	25/F	29	99.88	117.63	21A	VOC Delta B.1.617.2	G
LIB-0125	18 Jun	Montserrado	89/F	22	100.00	13008.5	21A	VOC Delta B.1.617.2	G
LIB-0102	19 Jun	Montserrado	8/F	20	100.00	10170.05	21A	VOC Delta B.1.617.2	G
LIB-0130	24 Jun	Montserrado	37/M	30	71.32	NA	NA	NA	NA
LIB-0134	24 Jun	Montserrado	62/F	28	12.74	NA	NA	NA	NA
LIB-0142	26 Jun	Montserrado	42/F	27	5.53	NA	NA	NA	NA
LIB-0147	26 Jun	Montserrado	36/M	30	99.97	1970.45	21A	VOC Delta B.1.617.2	G
LIB-0069	27 Jun	Montserrado	48/F	19	100.00	86159.99	21A	VOC Delta B.1.617.2	G
LIB-0111	7 Jul	Montserrado	11/F	29	0.65	0.9	NA	NA	NA
LIB-0113	7 Jul	Montserrado	48/M	26	1.65	0.86	NA	NA	NA
LIB-0073	8 Jul	Montserrado	31/F	29	99.47	117.79	21A	VOC Delta B.1.617.2	G
LIB-0074	8 Jul	Montserrado	47/M	24	100.00	4168.52	21A	VOC Delta B.1.617.2	G
LIB-0076	8 Jul	Montserrado	51/F	22	100.00	14232.36	21A	VOC Delta B.1.617.2	G
LIB-0078	8 Jul	Margibi	54/F	23	100.00	12901.34	21A	VOC Delta B.1.617.2	G
LIB-0093	8 Jul	Montserrado	48/M	27	99.31	84.44	21A	VOC Delta B.1.617.2	G
LIB-0103	8 Jul	Montserrado	41/F	29	0	NA	NA	NA	NA
LIB-0112	8 Jul	Montserrado	32/F	29	0.67	1.67	NA	NA	NA
LIB-0084	9 Jul	Nimba	67/M	27	99.91	1228.28	21A	VOC Delta B.1.617.2	G
LIB-0085	9 Jul	Nimba	59/M	22	100.00	63134.43	21A	VOC Delta B.1.617.2	G
LIB-0094	10 Jul	Montserrado	52/F	32	99.93	1979.92	21A	VOC Delta B.1.617.2	G
LIB-0101	20 Jul	Montserrado	64/M	31	26.44	NA	NA	NA	NA

*Twelve samples had insufficient genome coverage of average 15× depth/nucleotide.C_t, cycle threshold; NA, not applicable; VOC, variant of concern; VOI, variant of interest. Sequencing data can be accesed using GISAID (https://www.gisaid.org) accession numbers EPI_ISL_3547663–705, EPI_ISL_3560291, and EPI_ISL_4232122–52).

†According to Phylogenetic Assignment of Named Global Outbreak Lineages (https://cov-lineages.org) software tool.



Appendix Figure 1. Daily coronavirus disease cases, Liberia, February 2020–September 2021. Blue lines indicate 7 day moving average of daily new cases. Data were derived from Worldometer (https://www.worldometers.info/coronavirus/country/liberia).



Appendix Figure 2. Sites from which nasopharyngeal swab samples were collected during a coronavirus disease case surge, Liberia, 2021. Red stars indicate locations inside counties; black star indicates the capital, Monrovia. Numbers of cases per county are indicated in parentheses.