Mutations Associated with SARS-CoV-2 Variants of Concern, Benin, Early 2021

Anna-Lena Sander,[1] Anges Yadouleton,[1] Edmilson F. de Oliveira Filho, Carine Tchibozo, Gildas Hounkanrin, Yvette Badou, Praise Adewumi, Keke K. René, Dossou Ange, Salifou Sourakatou, Eclou Sedjro, Melchior A. Joël Aïssi, Hinson Fidelia, Mamoudou Harouna Djingarey, Michael Nagel, Wendy Karen Jo, Andres Moreira-Soto, Christian Drosten, Olfert Landt, Victor Max Corman, Benjamin Hounkpatin, Jan Felix Drexler

Intense transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Africa might promote emergence of variants. We describe 10 SARS-CoV-2 lineages in Benin during early 2021 that harbored mutations associated with variants of concern. Benin-derived SARS-CoV-2 strains were more efficiently neutralized by antibodies derived from vaccinees than patients, warranting accelerated vaccination in Africa.

Genomic surveillance is key to elucidate coronavirus disease (COVID-19) transmission chains and to monitor emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants associated with partial or complete immune escape (*1*). Intense transmission likely promotes the emergence of variants, including mutations in the gene encoding the spike (S) protein, which is a major component of all available COVID-19 vaccines (*2*). Genomic surveillance is notoriously weak in sub-Saharan Africa (Appendix Figure, panel A, https://wwwnc.cdc.gov/EID/article/27/11/21-1353-App1.pdf). A total of 55 SARS-CoV-2 lineages were described in West Africa as of May 25, 2021, considerably fewer than the >350 lineages in affluent regions (Appendix Figure, panel B). We previously described 2 diverse lineages (A.4 and B.1) in Benin early in the pandemic (*3*). In this study, we analyzed SARS-CoV-2 genomic diversity in Benin ≈1 year later and assessed the ability of vaccinee-derived and patient-derived serum samples to neutralize SARS-CoV-2 variants.

The Study

We used 378 SARS-CoV-2–positive diagnostic respiratory samples tested at the reference laboratory in Benin during January 30–April 2, 2021, for genomic surveillance. All samples with cycle threshold ≤36 (Sarbeco E-gene assay; TIB Molbiol, https://www.tib-molbiol.de) were used for this study. To enable rapid prescreening of mutations known to affect the viral phenotype, we used 4 reverse transcription PCR (RT-PCR)–based single-nucleotide polymorphism (SNP) assays (VirSNiP; TIB Molbiol) targeting 9 hallmark mutations in 7 S codons of variants of concern (VOCs): B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), and B.1.617.2 (Delta) (Table 1). A total of 374 (98.9%) samples selected for the study tested positive for ≥1 mutation. Of those, ≈67.5% (255/378) showed the

Author affiliations: Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Institute of Virology, Berlin, Germany (A.-L. Sander, E.F. de Oliveira Filho, W.K. Jo, A. Moreira-Soto, C. Drosten, V.M. Corman, J.F. Drexler); Ecole Normale Supérieure de Natitingou, Natitingou, Benin (A. Yadouleton); Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), Cotonou, Benin (A. Yadouleton); Laboratoire des Fièvres Hémorragiques Virales du Benin, Cotonou (A. Yadouleton, C. Tchibozo, G. Hounkanrin, Y. Badou, P. Adewumi); Ministry of Health, Cotonou (K.K. René, D. Ange, S. Sourakatou, B. Hounkpatin); Conseil National de Lutte contre le VIH-Sida, la Tuberculose, le Paludisme, les IST et les Epidémies, Cotonou (E. Sedjro, M.A. Joël Aïssi, H. Fidelia); World Health Organization Regional Office for Africa, Health Emergencies Programme, Brazzaville, Democratic Republic of the Congo (M.H. Djingarey); Deutsche Gesellschaft für Internationale Zusammenarbeit, Bonn, Germany (M. Nagel); German Centre for Infection Research (DZIF), associated partner Charité-Universitätsmedizin Berlin, Berlin (C. Drosten, V.M. Corman, J.F. Drexler); TIB Molbiol Syntheselabor GmbH, Berlin (O. Landt)

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[1]These first authors contributed equally to this article.

Table 1. Screened mutations, potential effects, and occurrence in severe acute respiratory syndrome coronavirus 2 variants, Benin, 2021

SNP assay	Spike protein variation	Potential effects	SARS-CoV-2 variant						
			B.1.1.7 Alpha†	B.1.525	B.1.351 Beta†	P.1 Gamma†	P.2	P.3	B.1.617.2 Delta†
1	del HV69/70	Immune escape and enhanced viral infectivity (*4*)	x	x					
	E484K	Antibody resistance (*4*)		x	x	x	x	x	
	N501Y	Increased transmission (*4*)	x		x	x		x	
2	V1176F	Higher mortality rates‡				x	x		
3	L452R	Antibody resistance (*4*)							x
4	K417T	No data				x			
	K417N	Immune escape (*5*)			x				
	P681H	No data	x						
	P681R	No data							x

*SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SNP single-nucleotide polymorphism.
†Variants of concern according to the World Health Organization.
‡G. Hahn et al., unpub. data, https://www.biorxiv.org/content/10.1101/2020.11.17.386714v2.

69/70 deletion, 58.9% (223/378) the E484K mutation, 33.9% (128/378) the N501Y mutation, 30.4% (115/378) the P681H mutation, 14.8% (56/378) the L452R mutation, and 0.3% (1/378) the K417N or P681R mutation. The K417T or V1176F mutations associated with the Beta and Gamma VOCs were not detected. Approximately 22.2% (84/378) of samples were typeable to 1 of the lineages covered by the VirSNiP assays. According to SNP-based analyses, 14.8% (56/378) of the overall samples showed the mutation pattern of the Alpha variant, B.1.1.7, and 7.4% (28/378) of the B.1.525 variant. Frequent occurrence of the mutations under study suggests that earlier SARS-CoV-2 lineages not carrying those mutations have been replaced in Benin.

Definite lineage designation relies on the full genome sequence. We selected 68 (9 typeable and 59 nontypeable) samples according to unique mutational patterns covering the complete period of the study for a NimaGen/Illumina-based whole-genome sequencing workflow (Appendix Table 1). All near-full genomes generated within this study were deposited into GISAID (https://www.gisaid.org; accession nos. EPI_ISL_2932532–84 and EPI_ISL_2958658–72). Lineage assignment using the Pangolin COVID-19 Lineage Assigner version 3.0.2 (https://pangolin.cog-uk.io) confirmed SNP-based lineage prediction in all 9 typeable samples selected for whole-genome sequencing (Appendix Table 2). Despite robust lineage prediction based on unambiguous SNP-based results, our data demonstrate the limited use of VirSNiP assays for strain designation; however, these assays can detect relevant mutations of currently circulating variants. The 68 Benin-derived near-complete genomes were designated to 10 unique lineages, suggesting higher genetic diversity in Benin than ≈1 year before (*3*). During early 2021, lineages B.1.1.7 (22%), A.27 (19.1%), B.1.525 (17.6%), and B.1.1.318 (16.2%) were most prominent in Benin (Appendix Table 3). Despite presence of the mutation P681R (associated with the Delta VOC) in 1 sequence, that strain was typed as A.23.1, and no Delta variant was found.

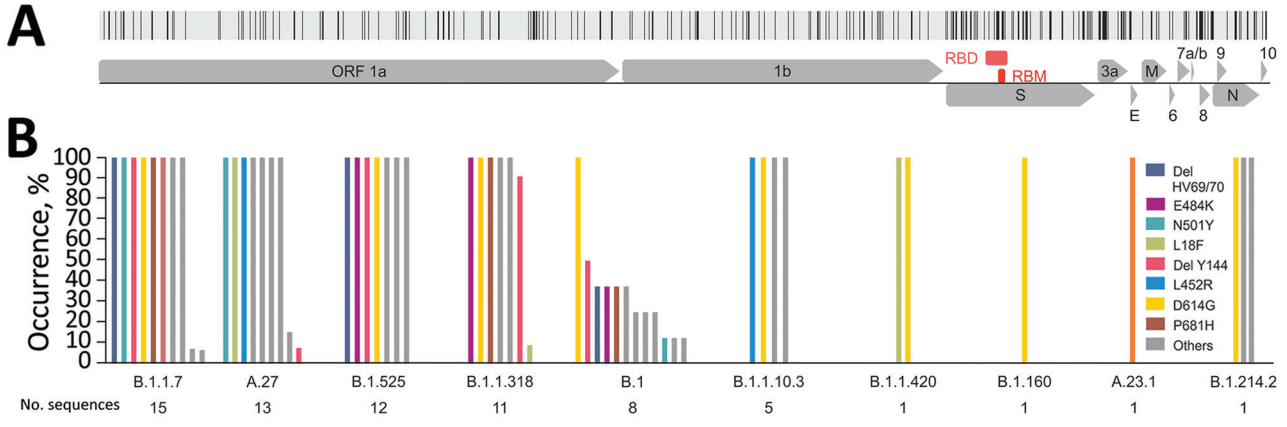

Figure 1. Genomic surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lineages in Benin, 2021. A) Nonsynonymous mutations of Benin-derived SARS-CoV-2 sequences across the full genome. B) Spike mutations occurring in the SARS-CoV-2 lineages circulating in Benin. Hallmark mutations of variants of concern are shown in color. Other mutations occurring in the Benin-derived sequences are depicted in gray and summarized as others. ORF, open reading frame; RBD, receptor-binding domain.

These data are consistent with recent online sequence reports from West Africa (A.E. Augustin, unpub. data, https://www.medrxiv.org/content/10.1101/2021.05.06.21256282v1; E.A. Ozer et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.04.09.21255206v3). A 100% consensus sequence of all 68 Benin-derived sequences showed 229 nonsynonymous nucleotide substitutions across the whole genome; 57 (24.9%) occurred in the S protein (Figure 1, panel A). Of note, variants with mutations in the S protein might alter the transmissibility and antigenicity of the virus (4). Internationally recognized VOCs to date share 16 S mutations in unique combinations (https://covariants.org/shared-mutations). The Benin-derived SARS-CoV-2 strains shared 10 unique S mutations reported in VOCs, although most of those strains were not defined as any VOC other than Alpha (Figure 1, panel B), suggesting convergent evolution of key mutations across different lineages (D.P. Martin et al., unpub. data, https://www.medrxiv.org/content/10.1101/2021.02.23.21252268v3; S. Cherian, unpub. data, https://www.biorxiv.org/content/10.1101/2021.04.22.440932v2). Putative higher fitness mediated by genomic change was consistent with more mutations in predominant lineages than in lineages found at lower frequencies (Figure 1, panel B).

Because S mutations, individually or in combination, have been shown to afford viral escape to antibody-mediated immune responses, the high prevalence of variants with large numbers of these mutations circulating in Benin was cause for concern. To investigate whether and to what extent

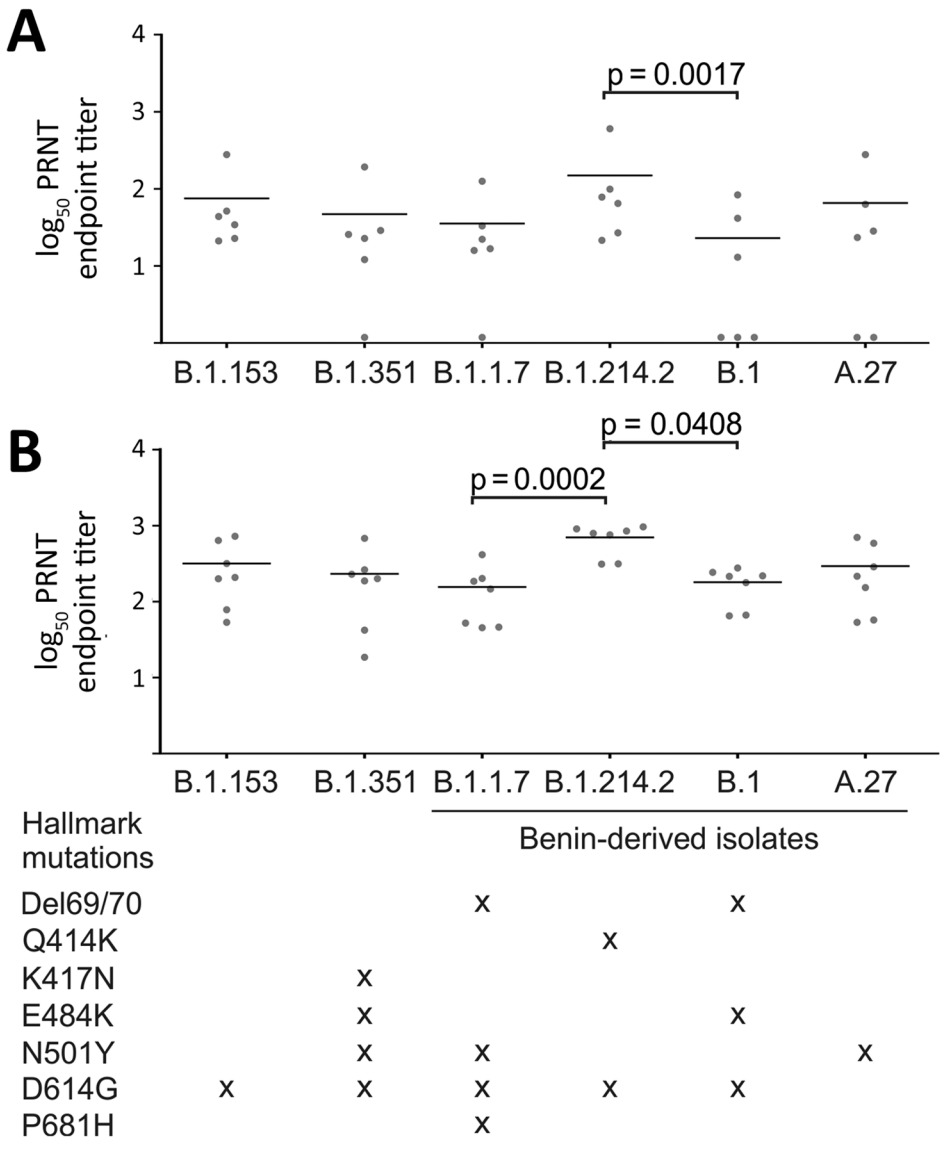

Figure 2. PRNT results of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants from Benin, 2021. Graphs compare results of neutralization tests for naturally infected persons (A) and persons who received the Pfizer-BioNTech vaccine (BNT162b2; https://www.pfizer.com) (B) against the B.1.153 lineage from January 2020 (Munich/ChVir929/2020 strain; GISAID [http://www.gisaid.org] accession no. EPI_ISL_406862; Pangolin version 2021–05–19), the Beta strain (Baden-Wuertemberg/ChVir22131/2021; accession no. EPI_ISL_862149; B.1.351; Pangolin version 2021–05–19) and the B.1.1.7, B.1.214.2, B.1, and A.27 lineages isolated from patients from Benin. Lines denote the mean $PRNT_{50}$ endpoint titer. Statistical significance was determined by the Dunn's multiple comparisons test. Nonsignificant values are not shown for clarity of presentation. $PRNT_{50}$, 50% plaque reduction neutralization test.

Table 2. Hallmark mutations and $PRNT_{50}$ results of Benin-derived severe acute respiratory syndrome coronavirus 2 lineages, Benin, 2021

Sample no.	251307	314235	251455	312541
Lineage	B.1	B.1.1.7	A.27	B.1.214.2
Mutations	Q52R, Del HV69/70, Del Y144, E484K, D614G, Q677H, F888L	Del HV69/70, Del Y144, F490S, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	L18F, L452R, N501Y, A653V, H655Y, D796Y, G1219V	Ins R214TDR, Q414K, D614G, T716I
Patient-derived samples				
Mean titer (95% CI)	23 (–12.4 to 58.4)	35.5 (–12 to 83)	65.6 (–46.6 to 177.7)	148.9 (–86.59 to 384.3)
No. (%) neutralized	3/6 (50)	5/6 (83.3)	4/6 (66.7)	6/6 (100)
Titer difference†	52.2 (1.5-fold)	39.7	9.7	–73.6‡
Vaccinee-derived samples				
Mean titer (95% CI)	180.5 (102.8–258.1)	156.2 (33.6–278.7)	293.7 (57.1–530.2)	698.3 (446.8–949.9)
No. (%) neutralized	7/7 (100)	7/7 (100)	7/7 (100)	7/7 (100)
Titer difference†	136.7	161	23.5	–381.1‡

*$PRNT_{50}$, 50% plaque reduction neutralization test.
†Compared to variant B.1.153.
‡Lower titers against the early isolate compared with this Benin-derived isolate.

SARS-CoV-2 variants circulating in Benin and West Africa (*5*) evade neutralizing antibody responses, we isolated 4 lineages with unique mutational patterns (Table 2): an A.27 lineage isolate harboring the N501Y mutation; a B.1 isolate harboring the 69/70 deletion and the E484K and D614G mutations; a B.1.1.7 lineage isolate harboring the 69/70 deletion and the N501Y, D614G, and P681H mutations; and a B.1.214.2 lineage harboring the Q414K and D614G mutations (Figure 2). Additional isolation attempts of strains belonging to the frequently detected B.1.525 and B.1.318 lineages failed, likely because of degradation after repeated freeze-thaw cycles under tropical conditions. We tested neutralization potency of 6 serum samples from patients in Benin taken ≈8 days after RT-PCR–confirmed SARS-CoV-2 infection during early 2020 (*6*) and another 7 serum samples from persons in Europe 4 weeks after receiving the second dose of the Pfizer/BioNTech vaccine (BNT162b2; https://www.pfizer.com) (Appendix Table 4). Sampling was approved by the ethics committee of the Benin Ministry of Health (approval no. 030/MS/DC/SGM/DNSP/CJ/SA/027SGG2020) and of Charité-Universitätsmedizin Berlin (approval nos. EA1/068/20 and EA4/245/20). We compared neutralization titers with a SARS-CoV-2 strain (B.1.153) from January 2020 and the Beta strain (B.1.351) known to evade antibody-mediated neutralization (*7*). Despite the early sampling time after RT-PCR confirmation of SARS-CoV-2 infection, all 6 serum specimens from patients in Benin efficiently neutralized the early SARS-CoV-2 isolate carrying only the D614G mutation. In contrast, only 3 of those 6 serum specimens neutralized the B.1 isolate, the only isolate with the E484K mutation (Figure 2, panel A). Among the serum specimens from vaccinated persons, all neutralized the B.1 isolate, albeit at 1.5-fold lower titers than the early lineage B.1.153 isolate (by Friedman test and Dunn's multiple comparisons test; $p>0.99$) (Figure 2, panel B). Those data were consistent with a recent report describing efficient neutralization of a B.1.525 strain from Nigeria by vaccinee-derived serum specimens (*8*). Of note, another strain classified as B.1.214.2 was neutralized more efficiently than all other tested lineages (Figure 2), highlighting that not every mutation in circulating lineages affords reduced antibody-mediated neutralization. Other hypothetically present fitness advantages of such strains will require detailed virologic investigation.

Our study is limited by patient-derived samples taken an average of 8 days after infection (*7*), which could imply incomplete maturation of antibodies. However, similar neutralization patterns between patient-derived and vaccinee-derived serum specimens suggest robustness of our data. Another limitation is that vaccinee-derived serum samples originated exclusively from Europe. Vaccine responses vary between populations, possibly influenced by genetic background and immune-modulating diseases (e.g., malaria or HIV) (*9*), highlighting the importance of testing serum samples from vaccinees in Africa for future studies. Of note, the efficacy trial of the Pfizer/BioNTech vaccine enrolled ≈40,000 participants, only ≈800 of whom were from Africa, and all of those from South Africa (*10*).

Conclusions

Our data highlight the importance of ongoing monitoring of population immunity to emerging SARS-CoV-2 variants in Africa and of using serum specimens from local settings for phenotypic characterizations. Vaccination programs in Africa should be accelerated urgently, emphasizing the importance of global access to vaccines.

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O.L. is the owner of TIB Molbiol, the company developing and marketing SARS VirSNiP assays.

About the Author

Ms. Sander is a PhD student at the Institute of Virology at Charité-Universitätsmedizin, Berlin, Germany; her main research interest is the evolution of newly emerging viruses. Dr. Yadouleton is a medical entomologist in the Centre de Recherche Entomologique de Cotonou, Benin, head of the Laboratoire des Fièvres Hémorragiques in Cotonou, and a teacher at the University of Natitingou, Benin; his research interests include mosquito control and the diagnosis of viral hemorrhagic fevers.

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Address for correspondence: Jan Felix Drexler, Helmut-Ruska-Haus, Institute of Virology, Campus Charité Mitte, Charitéplatz 1, 10098 Berlin, Germany; email: felix.drexler@charite.de

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Appendix

Virus Isolation

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolation was performed by using 100µl of the reverse transcription PCR (RT-PCR)–positive nasal swab specimens diluted at 1:1 in OptiPro medium (ThermoFisher Scientific, https://www.thermofisher.com/us) and inoculated onto 1.5×10^5 Vero E6 cells, seeded in 24-well plates, maintained at 37°C, and cultivated in DMEM. After 1 h incubation, the inoculum was replaced by 500µl DMEM medium supplemented with 2% fetal calf serum (FCS), 1% nonessential amino acids, 1% Natrium Pyruvate, 1% Amphotericin B, 1% penicillin/Streptomycin (100 U/mL) (ThermoFisher Scientific). Infected cells were controlled daily for cytopathic effect (CPE) and passaged after 3 days. SARS-CoV-2 stocks were produced by propagation in Vero E6 cells. Cells and supernatant were harvested 3 days post-infection, centrifuged at 2500 × g for 10 min, and the supernatant titrated by plaque assay in Vero E6 cells.

Plaque Reduction Neutralization Test

Neutralizing antibodies against the different SARS-CoV-2 variants were detected by using a plaque reduction neutralization test ($PRNT_{50}$) conducted in monolayers of 1.6×10^5 Vero E6 cells, seeded in 12-well plates 1 day before the infection. Fifty plaque-forming units (PFUs) were incubated with serum dilutions of 1:20, 1:80, 1:320, and 1:1280 for 1 h, added onto the cell monolayer, and incubated again for 1 h before adding an overlayer containing DMEM with 1% FCS and 2% Avicell. After 2 days for B.1.153, B.1.351, and 3 days for the Benin isolates (B.1.1.7, B.1.214.2, B.1, and A.27), the overlayer medium was removed, and cells were fixated with 6% paraformaldehyde and stained with crystal violet. $PRNT_{50}$ endpoint titers were calculated by using a logistic regression function in GraphPad prism 6 (GraphPad Software. https://www.graphpad.com).

Appendix Table 1. Characteristics of severe acute respiratory syndrome coronavirus 2–positive samples from which full genomes were generated

ID	Collection date	C_t E-gene	Del HV69/70	E484K	N501Y	L452R	V1176F	K417N/T	P681H/R	Indicated variant	Pango lineage	Genome completeness, %
			Assay 1			Assay 2	Assay 3		Assay 4			
256146	2021 Feb 4	35.74	+	wt‡	+	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1	91
315551	2021 Mar 3	17.88	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	99
314235	2021 Mar 2	15.76	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	100
255701	2021 Feb 4	17.85	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	100
255138	2021 Feb 3	26.62	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	99
253094	2021 Feb 2	18.84	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	100
251917	2021 Feb 1	20.57	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	100
251354	2021 Feb 1	19.88	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	100
251326	2021 Feb 1	15.97	+	wt‡	+	wt‡	wt‡	wt‡	+	B.1.1.7	B.1.1.7	100
311929	2021 Mar 2	23.94	+	wt‡	+	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.1.7	98
256208	2021 Feb 4	25.25	+	wt‡	+	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.1.7	100
256046	2021 Feb 4	24.86	+	wt‡	+	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.1.7	98
249234	2021 Jan 30	33.83	+	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1	97
251307	2021 Feb 1	17.21	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1	99
250990	2021 Feb 1	19.01	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1	96
312964	2021 Mar 2	31.63	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	96
312950	2021 Mar 2	29.43	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	98
312266	2021 Mar 1	17.89	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	99
312198	2021 Mar 1	19.66	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	99
254242	2021 Feb 3	17.73	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	99
253408	2021 Feb 2	16.49	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	99
250541	2021 Jan 31	21.35	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	99
315465	2021 Mar 3	15.61	+	+	wt‡	wt‡	wt‡	wt‡	wt‡	B.1.525	B.1.525	99
254128	2021 Feb 3	18.45	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	681R	not typeable	A.23.1	99
253832	2021 Feb 2	24.05	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1	100
249868	2021 Jan 31	25.14	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1	100
249713	2021 Jan 31	25.52	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1	95
250814	2021 Feb 1	22.26	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	no mutation	B.1	100
250323	2021 Jan 31	31.74	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	no mutation	B.1.1.420	94
250412	2021 Feb 1	32.49	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	wt‡	no mutation	B.1.160	97
254278	2021 Feb 3	29.65	wt‡	wt‡	wt‡	+	wt‡	wt‡	wt‡	not typeable	L.3	99
250924	2021 Feb 1	21.61	wt‡	wt‡	wt‡	+	wt‡	wt‡	wt‡	not typeable	L.3	100
250772	2021 Feb 1	28.08	wt‡	wt‡	wt‡	+	wt‡	wt‡	wt‡	not typeable	L.3	99
249964	2021 Jan 31	31.88	wt‡	wt‡	wt‡	+	wt‡	wt‡	wt‡	not typeable	L.3	95
249110	2021 Jan 30	29.33	wt‡	wt‡	wt‡	+	wt‡	wt‡	wt‡	not typeable	L.3	98
250699	2021 Feb 1	22.88	wt‡	wt‡	+	+	n.e.	wt‡	wt‡	not typeable	A.27	100
312648	2021 Mar 1	28.07	wt‡	+	wt‡	wt‡	wt‡	n.e.	+	not typeable	B.1.1.318	97
312572	2021 Mar 1	21.97	+	wt‡	+	wt‡	wt‡	n.e.	+	not typeable	B.1.1.7	100
311995	2021 Mar 2	25.31	+	wt‡	+	wt‡	wt‡	neg	+	not typeable	B.1.1.7	99
314058	2021 Mar 2	21.58	n.e.	n.e.	+	wt‡	wt‡	wt‡	+	not typeable	B.1.1.7	100
312541	2021 Mar 1	23.00	wt‡	wt‡	wt‡	wt‡	wt‡	417N	wt‡	not typeable	B.1.214.2	98
248661	2021 Jan 30	32.81	+	+	wt‡	wt‡	n.e.	n.e.	missing peak†	not typeable	B.1.525	92
314176	2021 Mar 2	28.31	+	+	wt‡	wt‡	n.e.	n.e.	wt‡	not typeable	B.1.525	98
253620	2021 Feb 2	21.22	+	+	wt‡	wt‡	wt‡	wt‡	missing peak†	not typeable	B.1.525	98
254286	2021 Feb 3	23.32	+	+	wt‡	wt‡	n.e.	wt‡	missing peak†	not typeable	B.1.525	99
315530	2021 Mar 3	18.83	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99

ID	Collection date	C_t E-gene	Del HV69/70	E484K	N501Y	L452R	V1176F	K417N/T	P681H/R	Indicated variant	Pango lineage	Genome completeness, %
				Assay 1		Assay 2	Assay 3		Assay 4			
312262	2021 Mar 1	18.44	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
312182	2021 Mar 1	16.95	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
311979	2021 Mar 2	18.25	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	94
254375	2021 Feb 3	20.15	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
253228	2021 Feb 2	21.04	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
249971	2021 Jan 31	20.47	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
249944	2021 Jan 31	22.11	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
248922	2021 Jan 30	21.60	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	99
251411	2021 Feb 1	24.71	wt‡	+	wt‡	wt‡	wt‡	wt‡	+	not typeable	B.1.1.318	96
255062	2021 Feb 3	17.53	wt‡	wt‡	+	+	wt‡	wt‡	missing peak†	not typeable	A.27	100
252348	2021 Feb 2	21.44	wt‡	wt‡	+	+	wt‡	wt‡	missing peak†	not typeable	A.27	98
312239	2021 Mar 1	24.61	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
255170	2021 Feb 3	25.66	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
254323	2021 Feb 3	19.68	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
253312	2021 Feb 2	19.71	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
251455	2021 Feb 1	19.76	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	99
251296	2021 Feb 1	20.07	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
250498	2021 Jan 31	20.67	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
250471	2021 Jan 31	31.86	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	98
249839	2021 Jan 31	22.66	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	100
248651	2021 Jan 30	23.22	wt‡	wt‡	+	+	wt‡	wt‡	wt‡	not typeable	A.27	98
314080	2021 Mar 2	21.90	wt‡	wt‡	+	wt‡	wt‡	wt‡	+	not typeable	B.1.1.7	100

*n.e., not evaluable; neg, negative; wt, wildtype.
†According to the manufacturer's instructions, missing peaks could be due to a drop out of the primer binding site
‡Sample does not harbor the tested mutation.

Appendix Table 2. Single-nucleotide polymorphism assay typing

Category	Typing based on SNP assay	No. samples typed	No. samples sequenced (%)	No. samples typing confirmed
Typeable	B.1.1.7	56	8 (14.3)	8
	B.1.525	28	1 (3.6)	1
	Total	84	9 (10.7)	9
Nontypeable	No mutation	4	3 (75)	n.a.
	Other variant†	337	56 (16.6)	n.a.
	Total	341	59	
Total		425	68	

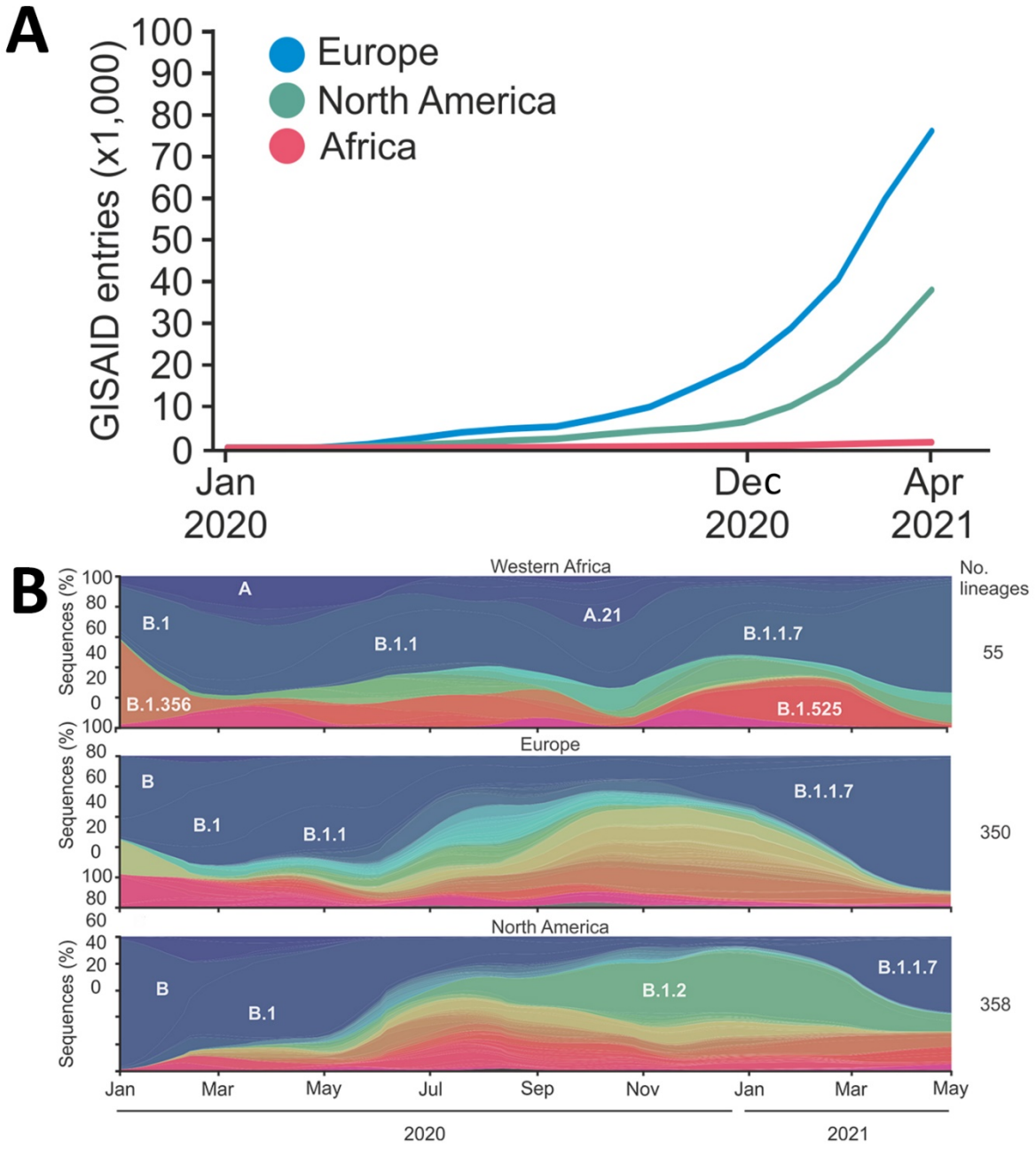
*SNP, single-nucleotide polymorphism.
†Samples were categorized as other variant if any of the assays had negative or unclear results or if the mutational pattern did not enable typing to a specific lineage.

Appendix Table 3. Severe acute respiratory syndrome coronavirus 2 lineages to which the 68 Benin-derived full genomes were designated and the frequency of sequences within each lineage

Lineage	Number of sequences (%)
B.1.1.7	15 (22.0)
A.27	13 (19.1)
B.1.525	12 (17.6)
B.1.1.318	11 (16.2)
B.1	8 (11.8)
B.1.1.10.3	5 (7.4)
B.1.1.420	1 (1.5)
B.1.160	1 (1.5)
A.23.1	1 (1.5)
B.1.214.2	1 (1.5)

Appendix Table 4. Severe acute respiratory syndrome coronavirus 2 plaque reduction neutralization test titers ($PRNT_{50}$) of individual vaccinee- and patient-derived serum samples

Source	B.1.153	B.1.351	B.1.1.7	B.1.214.2	A.27	B.1
Patients						
Benin 1	34.3	22.8	33.0	78.1	23.4	13.0
Benin 2	278.1	192.0	125.4	602.8	278.5	83.4
Benin 3	43.7	12.1	16.7	64.8	28.4	neg
Benin 4	51.6	25.6	22.2	99.1	63.1	41.5
Benin 5	21.1	28.9	neg	21.5	neg	neg
Benin 6	22.8	neg	15.9	26.9	neg	neg
Mean	75.3	46.9	35.5	148.9	65.6	23.0
Vaccinees						
13684	200.4	198.0	185.3	790.5	588.2	215.2
13685	637.3	185.1	415.1	962.5	286.9	243.3
13686	53.4	18.1	52.1	311.4	57.3	66.6
13702	317.1	259.9	202.2	756.4	152.8	218.5
13703	78.3	41.3	45.4	314.4	53.4	65.0
13704	208.3	226.3	146.8	848.2	215.7	177.4
13812	725.7	677.0	46.2	905.0	701.3	277.4
Mean	317.2	229.4	156.2	698.3	293.7	180.5



Appendix Figure. Globally available severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomic data. A) SARS-CoV-2 sequence entries from Africa and affluent settings in GISAID on April 24, 2021. B) SARS-CoV-2 genomic epidemiology in West Africa and affluent settings during January 2020–May 2021. Frequency plots were attained from Nextstrain (https://nextstrain.org/ncov/global) on May 25, 2021. The global dataset was filtered for the 3 different regions and their associated countries, including 11 countries for West Africa, 45 countries for Europe, and the United States and Canada for North America. Frequencies are colored by PANGO lineages and normalized to 100% at each time point for 705 sequences from West Africa, 2,400 sequences for Europe, and 1,515 sequences for North America.