

Partial Sanger Sequence Analysis of SARS-CoV-2 Spike (S) Gene Detects the Delta SARS-CoV-2 Variant of Concern in Baringo, Migori and Nakuru Counties and the Alpha VOC in Nyamira county

This is a preliminary report prepared by CDC Kenya's Diagnostic Laboratory Systems Program and the KEMRI Centre for Global Health Research on June 17, 2021.

Summary of Findings

- The Sanger partial sequencing method provides a quick, low-cost pre-screening approach to rapidly identify variants of concern (VOC) or interest (VOI).
- Twenty-three (23) SARS-CoV-2 RT-PCR positive samples from five counties were sequenced including: Baringo (4), Kisumu (3), Migori (7), Nyamira (5) and Nakuru (4). These samples were collected between June 4 - June 11, 2021 from patients aged 1-80 years; 10 (43.5%) were female and 13 (56.5%) male. All cases were Kenyan nationals, and none of the 23 cases indicated a recent travel history (i.e., travel within the past 14 days) on their case investigation form (CIF).
- The following VOC were identified:
 - **13 samples (56.5%) aligned with the Delta SARS-CoV-2 VOC (B.1.617.2 first identified in India);** and
 - 10 samples (43.5%) aligned with the Alpha SARS-CoV-2 VOC (B.1.1.7 first identified in the United Kingdom).
- **This is the first time we have detected the Delta SARS-CoV-2 VOC in Baringo, Migori and Nakuru counties. The Delta VOC was again detected in Kisumu county. Nyamira County only had the Alpha SARS-CoV-2 VOC detected in the samples sequenced.**
- Representatives of the identified variants will undergo whole genome sequencing to confirm the assigned lineages. In the meantime, these findings may inform public health action in these counties to prevent further spread of the Delta VOC.

Background

There is increasing need for genomic surveillance of SARS-CoV-2 to determine new introductions and current circulation of variants of concern (VOC) and variants of interest (VOI) in the local population. Timely release of sequencing data is important for initiating mitigation measures and linking variants to clinical manifestation and vaccine effectiveness in our population.

Methods

Here we describe findings from partial S-gene sequencing at the CDC-supported KEMRI laboratory in Kisumu of 23 SARS-CoV-2 samples collected from Baringo, Kisumu, Migori, Nyamira and Nakuru counties. These samples were collected between June 4 through June 11, 2021.

Portions of spike (S) gene at nucleotide positions 21358 to 23847 of SARS-CoV-2 reference sequence (NC_045512) genome were amplified using Qiagen Onestep RT-PCR kit, and nested PCR was performed using New England BioLabs Taq DNA polymerase. The PCR products were purified using ExoSAP-IT (ThermoFisher) and cycle sequencing was performed using BigDye sequencing kit v3.1. Excess BigDye removal was done using DyeEx 96 kit (Qiagen) and resultant purified products read-out in ABI 3730xl genetic sequence analyser. Multiple sequence alignment (MSA) was generated using BioEdit vr 7.2. Maximum likelihood (ML) tree was constructed in MEGA vr 7.

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Results - Sequence and Phylogenetic Analysis

All the 13 samples (56.5%) that clustered with the sub-lineage Delta SARS CoV-2 (B.1.617.2) contained amino acid changes at L452, T478K and D614G. Among these samples, eleven (84.6%) had amino acid changes at G142D and deletions at amino acid positions 156 and 157. All the ten samples that aligned with Alpha SARS CoV-2 (B.1.1.7) had deletions at amino acid 69/70 and 144. Amino acid changes at N501Y, A570D and D614G found in this lineage were also identified in these samples. Of the samples that aligned with Alpha VOC, six (60%) had amino acid changes D138H and F490S.

Information including age, gender, location and cluster are shown in the table below.

Conclusions

- This report provides preliminary sequencing results that identified the Delta VOC (B.1.617.2) in Baringo, Migori and Vihiga Counties for the first time. These findings may inform public health action in these counties.
- Using Sanger partial sequencing of the SARS-CoV-2 S gene, we identified thirteen cases (56.5%) with the Delta VOC (B.1.617.2, first identified in India) in Baringo, Migori and Nakuru counties between June 4 - June 11, 2021. All the cases were Kenyan nationals with no history of travel reported on their CIF.
- We also identified ten cases (43.5%) with the Alpha VOC (B.1.1.7, first identified in the United Kingdom) in Baringo, Migori, Nyamira and Nakuru counties.
- Unlike the other four counties in which the Delta VOC is most prevalent, Nyamira had the Alpha VOC as the only variant detected in the samples sequenced during this time period.
- All results from this report require further confirmation with whole genome sequencing to confirm the lineages identified by partial sequencing.

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Table. Case information of sequences identified five counties

Age (Years)	Gender	County	Symptoms	Date of sample collection	Pango lineage	WHO lineage
64	M	Baringo	NA	6/11/2021	B.1.1.7	Alpha
57	M		NA	6/11/2021	B.1.1.7	Alpha
1	F		NA	6/7/2021	B.1.1.7	Alpha
59	F		NA	6/11/2021	B.1.617.2	Delta
36	M	Kisumu	Yes	6/11/2021	B.1.617.2	Delta
75	M		Yes	6/11/2021	B.1.617.2	Delta
36	F		NA	6/11/2021	B.1.617.2	Delta
43	M	Migori	NA	6/10/2021	B.1.617.2	Delta
44	M		NA	6/10/2021	B.1.617.2	Delta
38	M		NA	6/10/2021	B.1.617.2	Delta
65	F		NA	6/10/2021	B.1.1.7	Alpha
61	F		NA	6/10/2021	B.1.617.2	Delta
52	M		NA	6/8/2021	B.1.617.2	Delta
74	M		NA	6/8/2021	B.1.617.2	Delta
33	M	Nyamira	Yes	6/9/2021	B.1.1.7	Alpha
62	F		NA	6/9/2021	B.1.1.7	Alpha
80	F		NA	6/10/2021	B.1.1.7	Alpha
50	F		NA	6/10/2021	B.1.1.7	Alpha
53	F		NA	6/10/2021	B.1.1.7	Alpha
40	M	Nakuru	Yes	6/4/2021	B.1.617.2	Delta
26	M		Yes	6/8/2021	B.1.1.7	Alpha
32	F		Yes	6/10/2021	B.1.617.2	Delta
9	M		Yes	6/10/2021	B.1.617.2	Delta

Note: No cases reported a travel history within the last 14 days from the date of sample collection; NA = not available