

# SARS-CoV-2 Genome Characterization by Next Generation Sequencing in Bamrasnaradura Infectious Diseases Institute: Implication for vaccine tools



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## Background

The genetic diversity of SARS-CoV-2 has been reported. SARS-CoV-2 strain investigation is important for anti-viral drug design and to assess vaccine efficacy. Nanopore sequencing Technology is included in the WHO guideline (2021) as an accepted method to obtain SARS-CoV-2 genome data

**Objective:** To investigate the SARS-COV-2 variant strains by Nanopore Next Generation Sequencing

## Materials/Methods:

A total of 83 COVID-19 cases from the first (February to April 2020, n=51) and second (January to April 2021, n=32) waves of the COVID-19 outbreak were investigated at BIDI. Viral RNA from nasopharyngeal/throat swabs was extracted by MagNaPure Compact and full-length SARS-CoV-2 genome sequences were determined using Oxford Nanopore Technologies (UK) platform and ARTIC protocol.

## Library preparation & whole-genome sequencing

Extracted RNA was reverse transcribed as described in the PCR tiling of COVID-19 protocol (vPTC\_9096\_v109\_revF\_06Feb2020) published by the ARTIC Network primer V3 (<https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye>). The libraries were prepared using a ligation-based sequencing kit (SQK-LSK109 kit; ONT) and barcoding using NBD104 reagent Kit (ONT), loaded onto a MinION flow cell (106 version), and sequenced with the MinION Mk1B device (ONT).

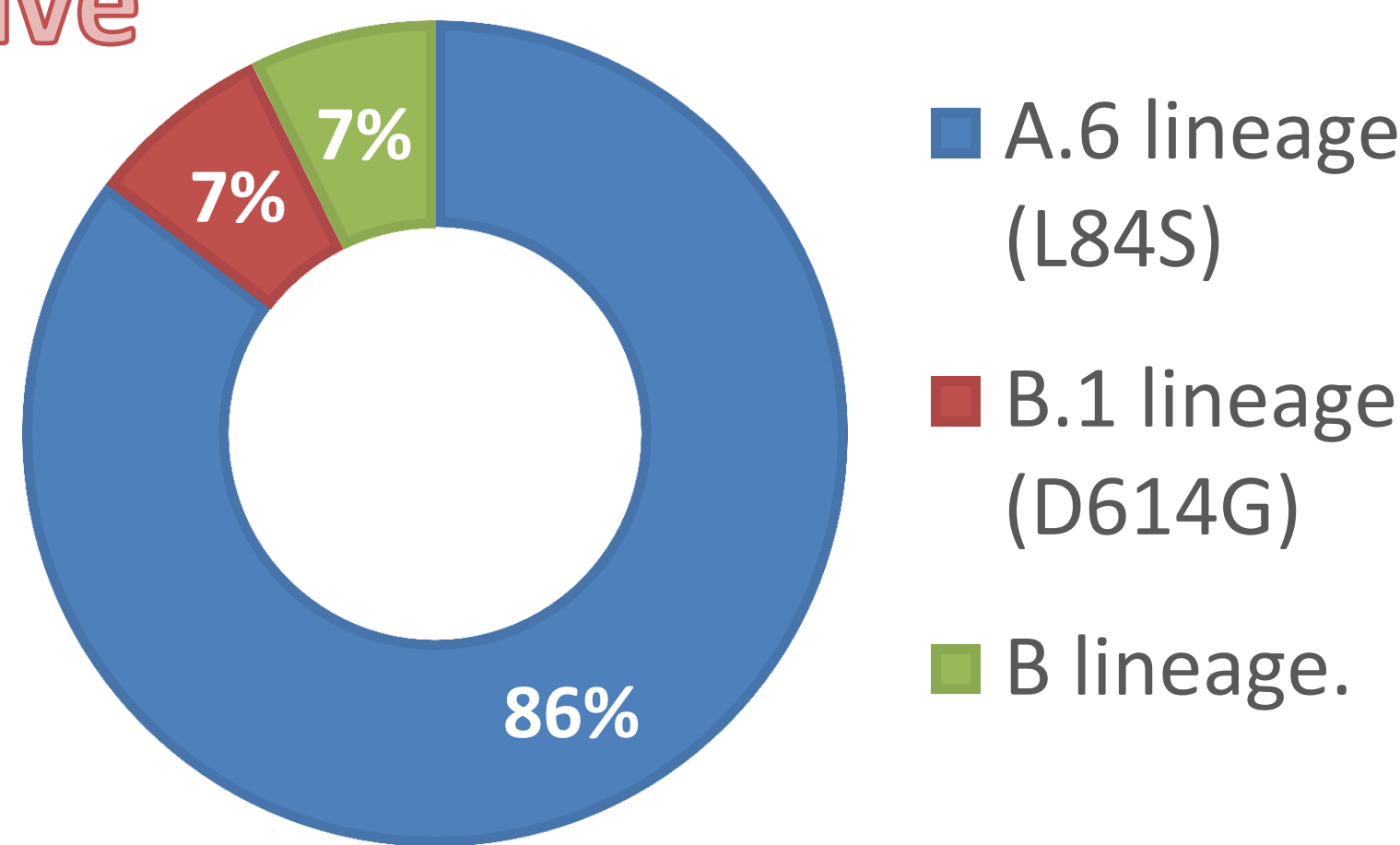
## Bioinformatics

Base calling of the resulting FAST5 files was performed in real time using Guppy (v4.5.2) (1) (ONT) with high accuracy mode (dna\_r9.4.1\_450bps\_hac). RAMPART software (v1.0.5) from the ARTIC Network (<https://github.com/artic-network/rampart>) was used to real-time monitor sequencing. (<https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html>) was used, followed by a reference assembly using the MinION script with Medaka polishing against the sequence of the Wuhan-Hu-1 isolate (GenBank accession number MN908947.3).

**Results.** Fifty-one COVID-19 cases included 35 males and 16 females with median age of 48 (range 3-79 years) were studied in the first wave. The majority of SARS-CoV-2 genomes analyzed (35 cases, 68.6%) were classified as type S, A.6 lineage (with L84S) followed by three cases (5.9%) classified as type G, B.1 lineage (with D614G) and three cases (5.9%) of type L, B lineage. During the second wave, 32 COVID 19 cases included 20 males and 12 females (median age of 33, range 6-88 years); 14 cases (43.8%) were classified as type GH, B.1.36.16 lineage followed by 12 cases (37.5%) classified as type GRY, B.1.1.7 lineage which is the UK variant related to the Thong Lor cluster.

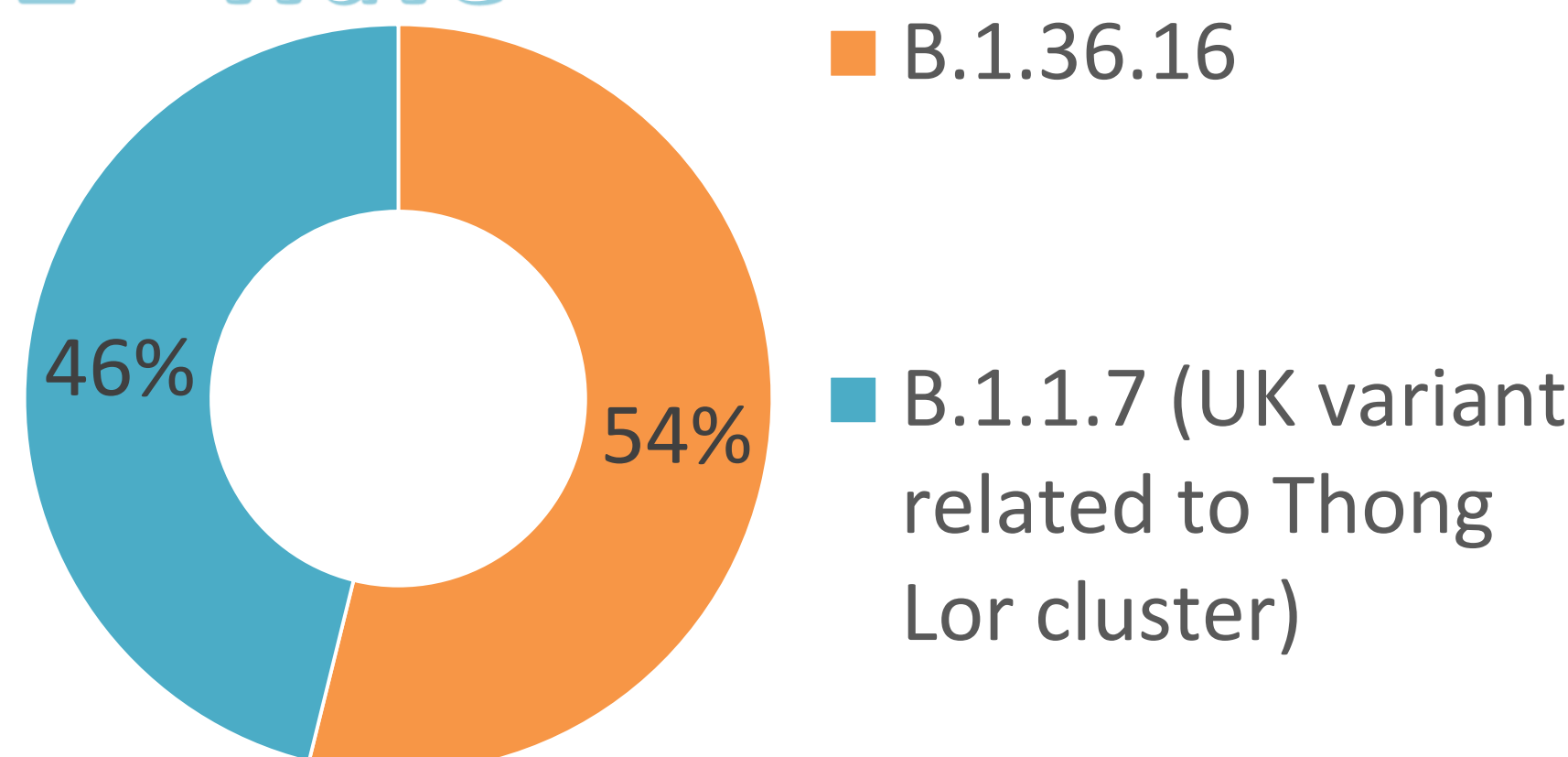
### 1<sup>st</sup> Wave

35 Males & 16 Females median age: 48 (range 3-79 years)  
35 cases (68.6%) : type S, A.6 lineage (L84S)  
3 cases (5.9%) : type G, B.1 lineage (D614G)  
3 cases (5.9%) : type L, B lineage.



### 2<sup>nd</sup> Wave

20 males & 12 females median age: 33 (range 6-88 years)  
14 cases (43.8%) : type GH, B.1.36.16  
12 cases (37.5%) : type GRY, B.1.1.7 (UK variant related to Thong Lor cluster)



## Conclusion

The most prevalent SARS-CoV-2 strain in the first wave was S type, A.6 lineage which related to a boxing stadium cluster. The variant in the most recent outbreak was GH type, B.1.36.16 lineage. This information could inform COVID-19 vaccine efficacy monitoring.

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