

# Rapid Identification of SARS-CoV-2 Lineages from Two Major Clusters in Pahang, Malaysia Using **MinION Sequencer**



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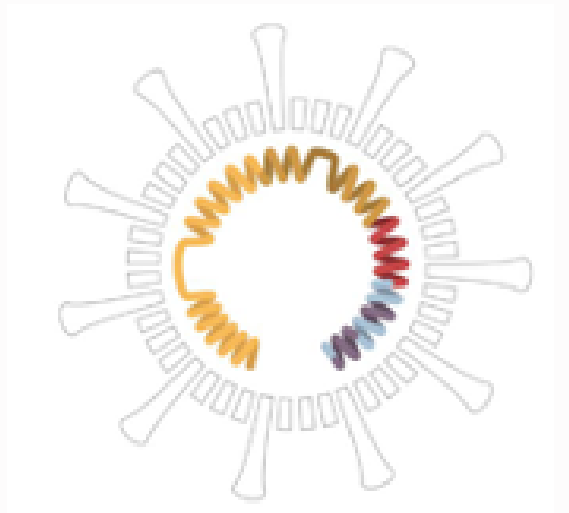
Mushtaq Hussain

Raihanah Haroon

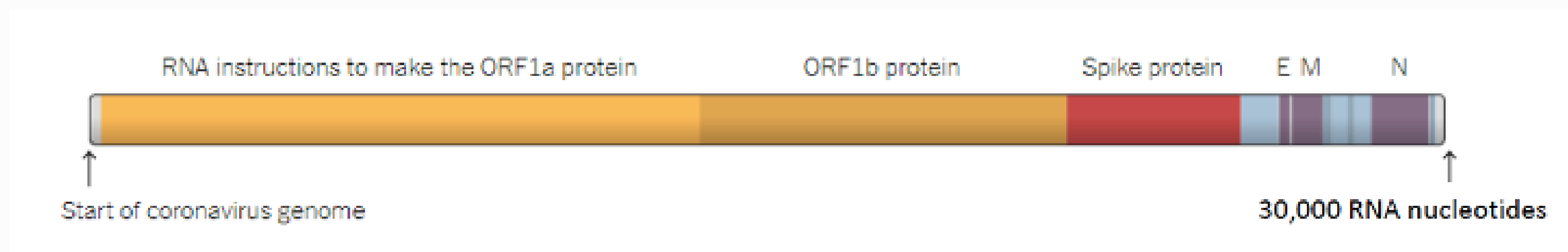
Hajar Fauzan Ahmad



# SARS-CoV-2



- Severe Acute Respiratory Syndrome Coronavirus 2
- Causative agent of Covid-19
- Single stranded positive sense RNA virus of the beta coronavirus genus
- Genome length ~ 30 kb
- First detected in humans late 2019 in Wuhan China
- First genome sequenced and release by Shanghai public health and clinical center and school of public health (Accession: MN908947)
- Targeted sequencing approach required to cover as much of the genome as possible



## **Rationale for sequencing SARS-CoV-2**

- Monitor trends at the national level
  - Monitor emergence of important new strains
  - Monitor trends after interventions such as vaccination
- Better understand epidemiology at the local level
  - Investigate clusters for transmission in a variety of settings (healthcare, workplace, bar, etc.)
  - Provide evidence for or against suspected transmission
  - Reveal important, unsuspected clusters

## **Additional ways to use SARS-CoV-2 sequencing data**

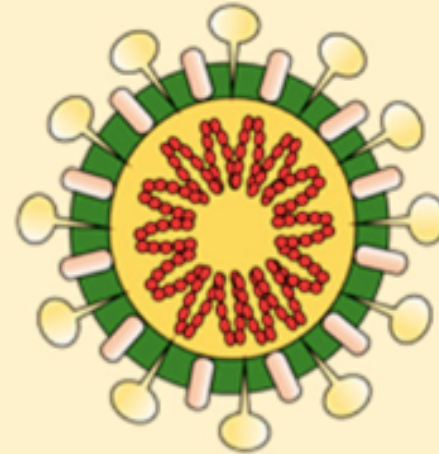
- Identify introductions and sources
- Identify superspreading events
- Predict severity and size of future outbreak seasons



**B.1.1.7**

**Alpha**

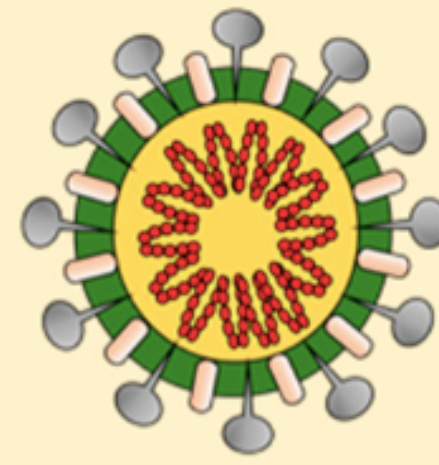
First Detection:  
September 2020  
Country Detected:  
England



**B.1.351**

**Beta**

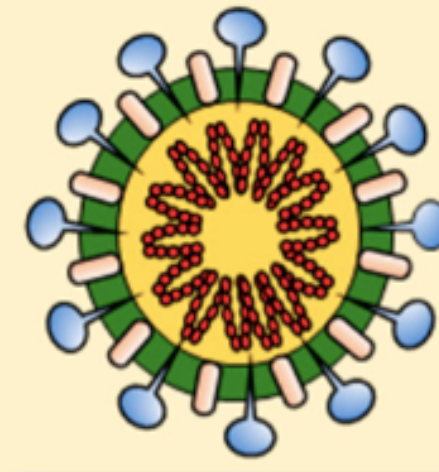
First Detection:  
October 2020  
Country Detected:  
South Africa



**P.1**

**Gamma**

First Detection:  
December 2020  
Country Detected:  
Brazil

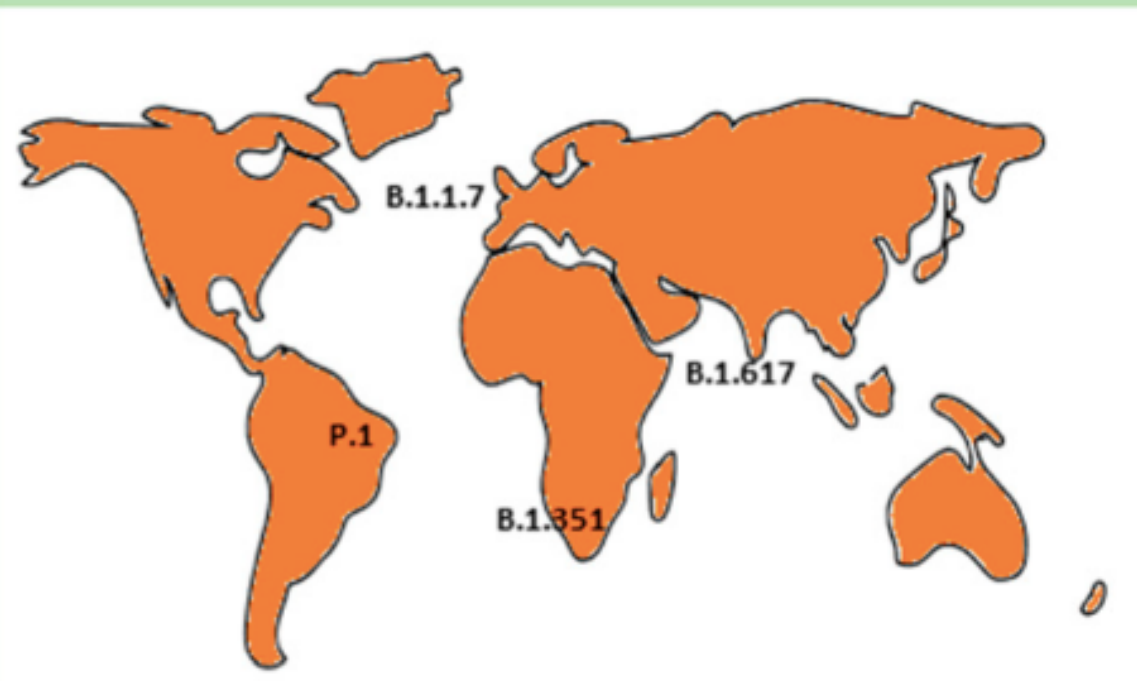


**B.1.617**

**Delta**

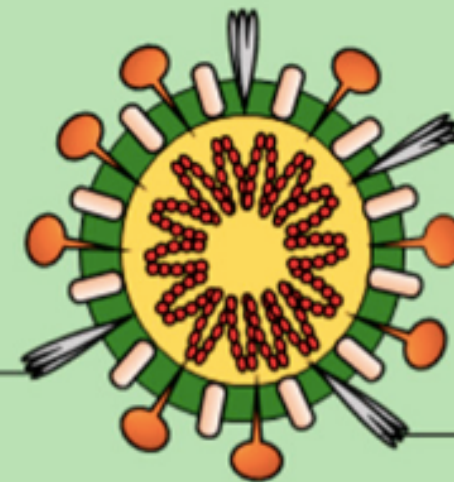
First Detection:  
October 2020  
Country Detected:  
India

### Worldwide spread of VOC cases



### Key Mutations on their spike proteins

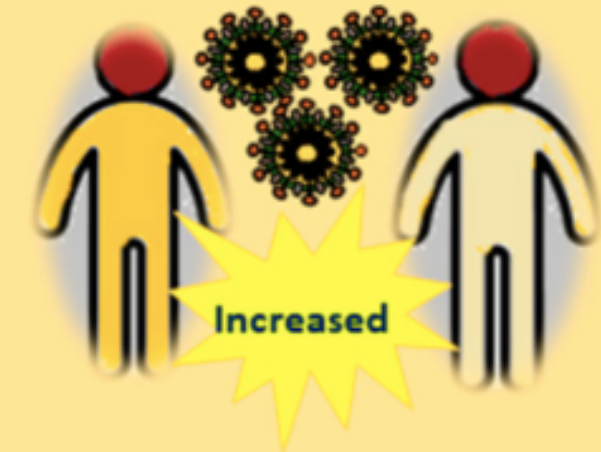
**E484K & K417N**  
Mutations  
seen in  
B.1.351, P.1



**N501Y**  
Mutations  
seen in  
B.1.1.7,  
B.1.351, P.1

**E484Q & L452R**  
Mutations  
seen in  
B.1.617

### Transmissibility



### Severity of illness



Prolong  
hospitalization,  
severe  
symptoms and  
mortality

### Vaccine efficacy

Research is still  
ongoing to determine  
the best vaccine.  
However, it is  
suggested that the  
current vaccines are  
effective against VOC  
strains.





# Objective

**To explore the utility of employing the Oxford Nanopore sequencing in understanding the genomic epidemiology in 2 large clusters in Pahang, Malaysia**



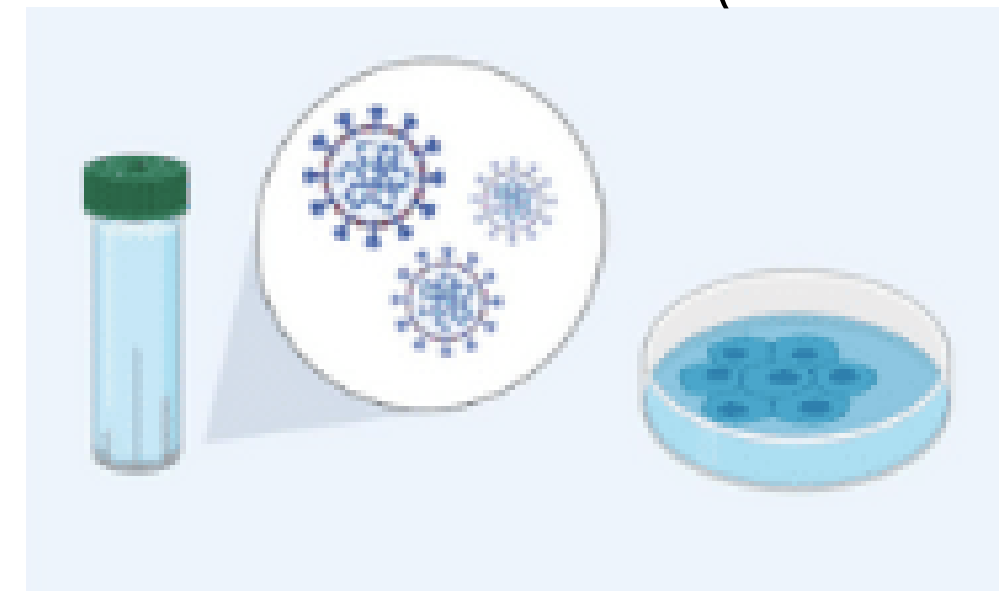
# METHODS

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01

## Sample Collection, Patients Background, and Ethical Approval

Clinical specimens were collected directly from combined oropharyngeal and nasopharyngeal swab specimens from two individuals with diverse clinical presentations representing **Tanah Putih Baru** and **Kemunting** clusters. Both were COVID-19 positive and diagnosed via reverse transcriptase PCR (RT-PCR) with a threshold cycle (CT) value of below 30. The study was approved by the International Islamic University Malaysia Research Ethics Committee (IREC 2021-080).



## Taman Tanah Putih Baru cluster

- was previously under Enhanced Movement Control Order (EMCO) by the National Security Council (NSC) of Malaysia since 5th May 2021 after 11 residents were tested positive for COVID-19
- state government had to 'appeal' for free screening test after 200 residents ran away from their house prior the EMCO
- Total 4795 surveillance test for COVID-19 was done and total 385 individual was reported positive from this cluster.

**Pahang state sec: Several 'missing' Taman Tanah Putih Baru residents under EMCO have returned home**



# Pasar Kemunting cluster

- a workplace cluster linked to a market
- started with one case reported on 21 June 2021 in Kuantan District
- name of the cluster refers to the locality where the outbreak is suspected to have occurred at the workplace located at Jalan Seri Kemunting 2, Kemunting, Kuantan.
- index case for this cluster is a Malaysian citizen working there - started experiencing symptoms of fever and sore throat on 14 June 2021
- results of the investigation found that there were 5 other colleagues who tested positive for COVID-19.
- the transmission of the infection is suspected to have occurred in the workplace due to the occurrence of social gatherings and there were violations of SOPs.
- as of 20th August 2021, a total of 5135 individuals had been screened in this cluster and 1123 cases were detected positive for COVID-19 and 16 deaths had been reported from this cluster.

## 'Peniaga Pasar Besar Kuantan sila tampil buat saringan Covid-19'

NORAWAZNI YUSOF | 09 Julai 2021

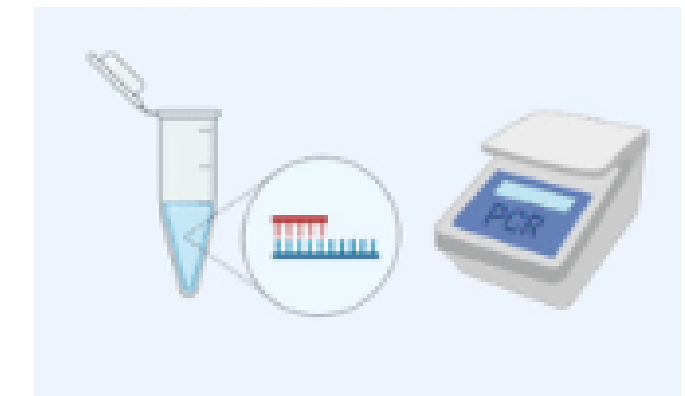
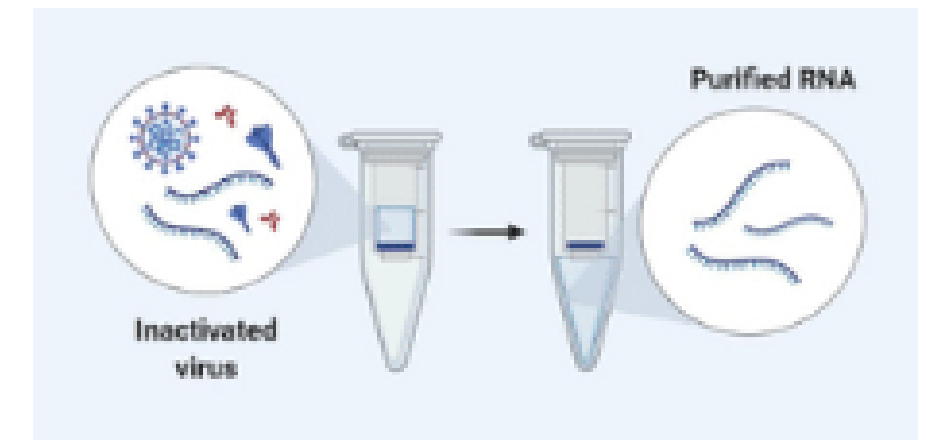
Listen f t w e c Chat



# METHODS

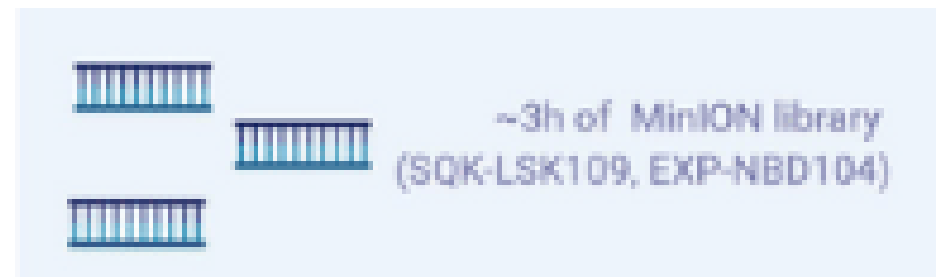
02

DNA Extraction, Nanopore Library Preparation and Sequencing



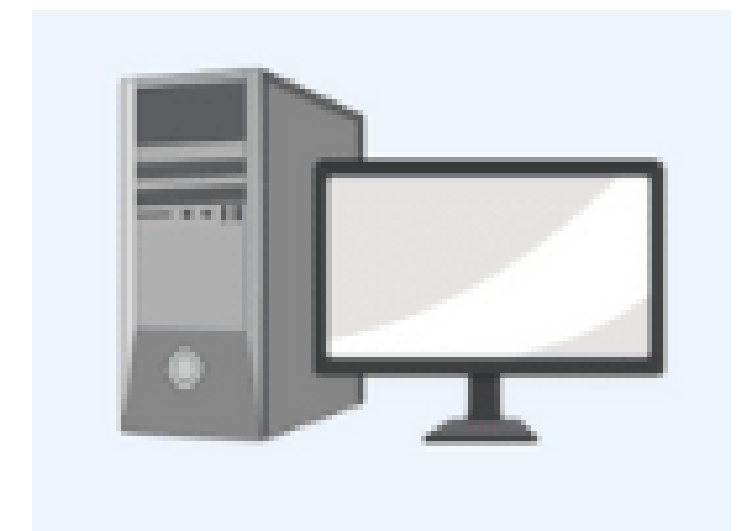
03

Nanopore Basecalling and Data Analysis



04

Whole-genome Sequencing and Phylogenetic Tree Analysis

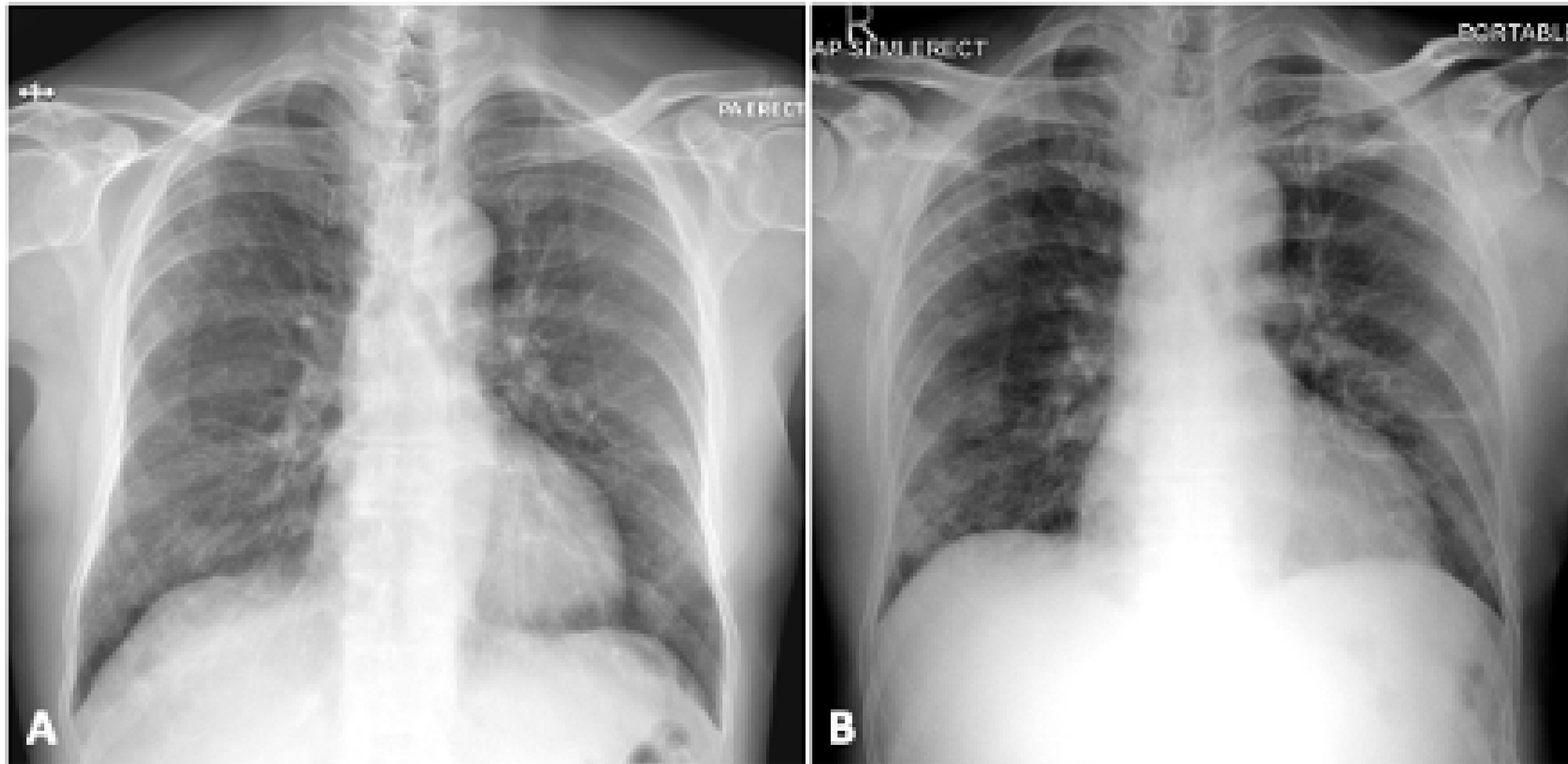


# Results

## Case 1

A 72-year-old man, active smoker with underlying hypertension was treated for Covid-19 Category 4. He showed marked clinical improvement subsequently and discharge on day 12 of admission. His genome surveillance showed that he contracted with B.1.351 (beta) variant. He lived at Taman Tanah Putih Baru and visited Tanah Putih Mosque where one of the attendees was positive for COVID-19.





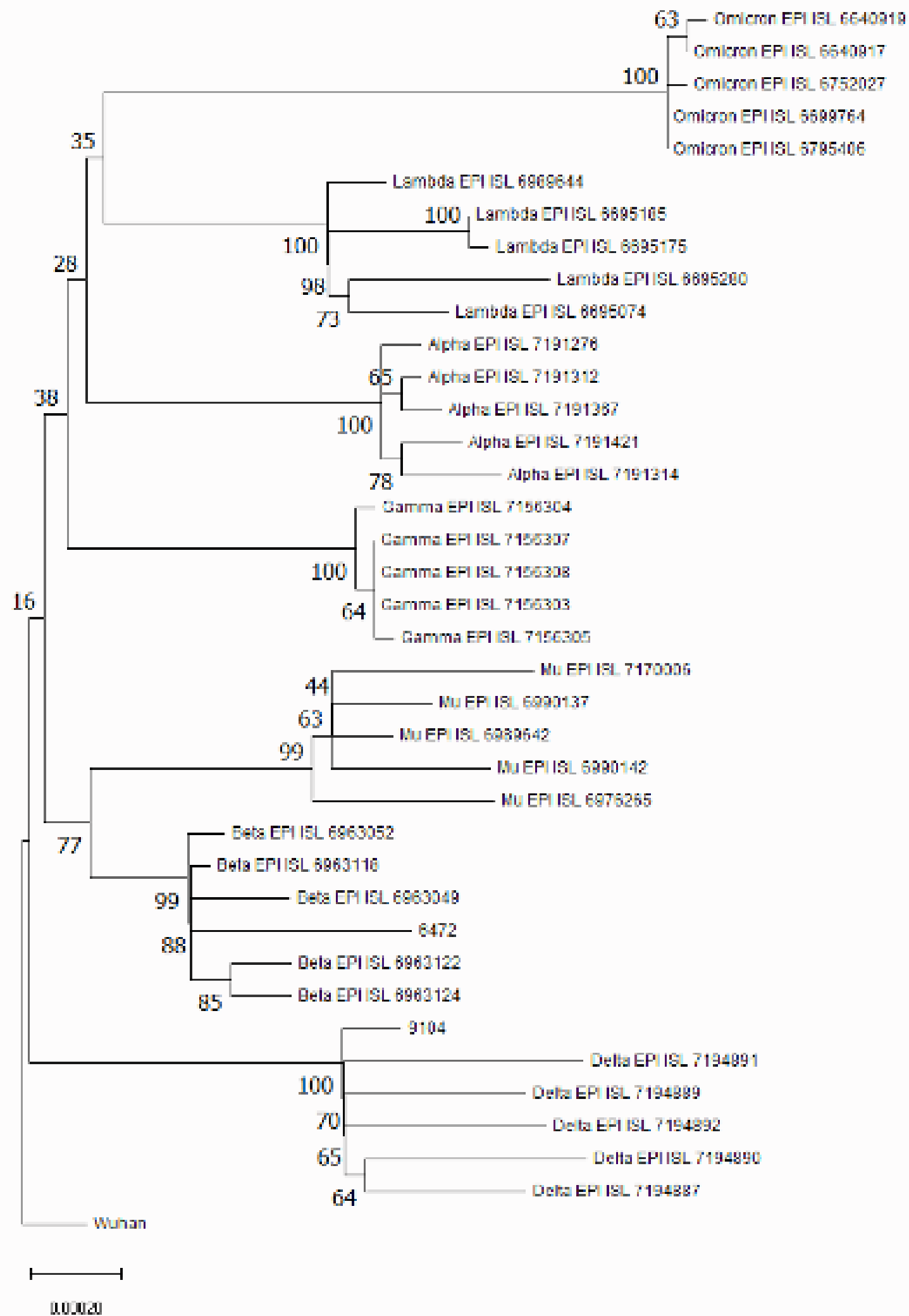
**Figure 1:** Two serial frontal chest radiographs. (A) – Acquired on the day of admission and (B) – Acquired 4 days afterwards showing worsening bilateral, almost symmetrical subpleural air space opacities which are peripherally distributed.

# Results

## Case 2

A 17-year-old man worked at Pasar Kemunting market presented with headache and fever for 1 week associated with anosmia and loose. He was treated as COVID-19 Category 2 and quarantined at PKRC Gambang for nearly 11 days. He was discharged well. No blood or radiological investigation were done. His genome surveillance showed that he contracted with B.1.617 (delta variant).





**Omicron**

**Lambda**

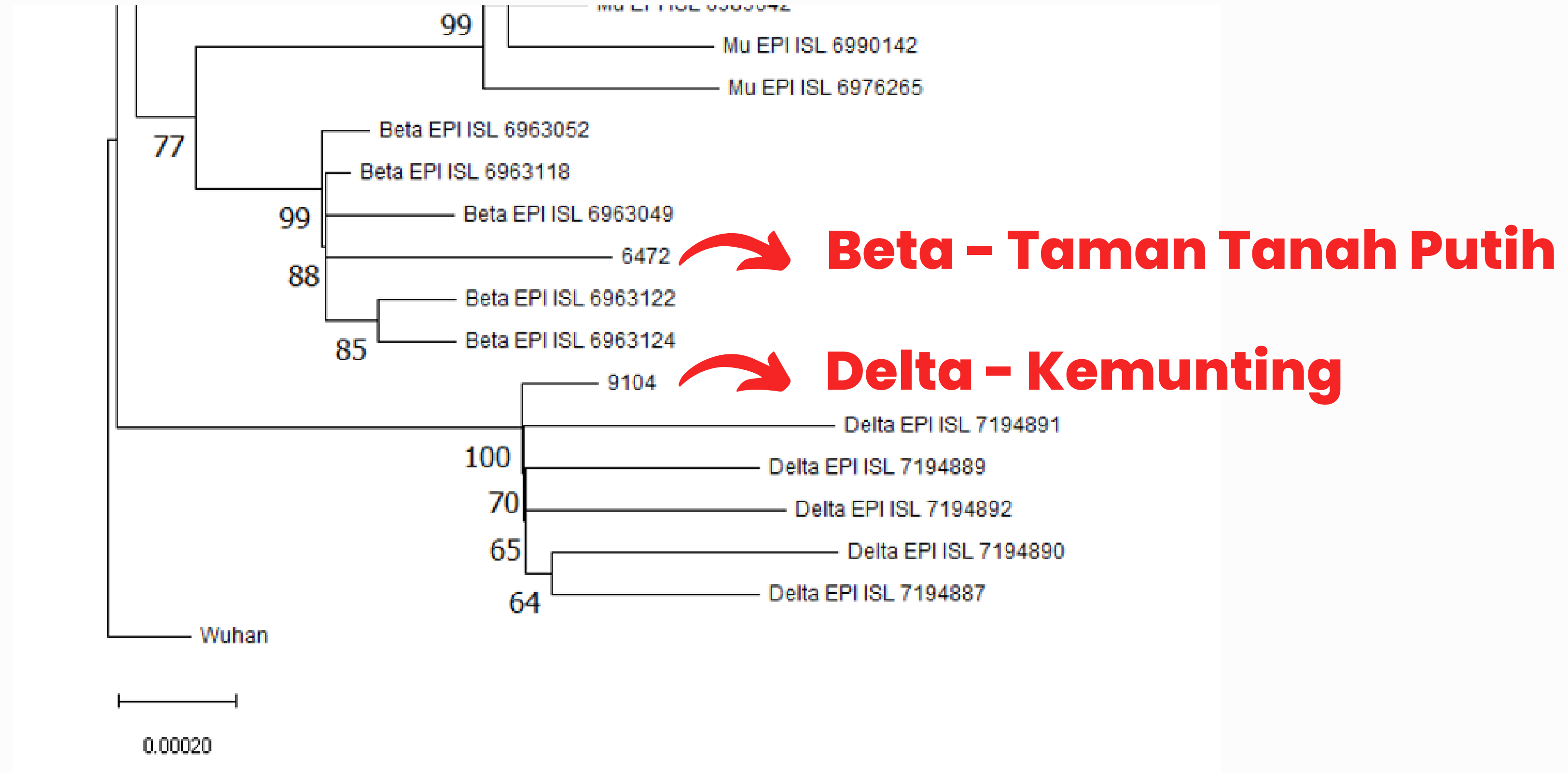
**Alpha**

**Gamma**

**Mu**

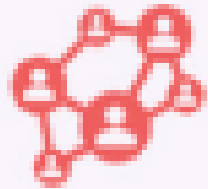
**Beta**

**Delta**





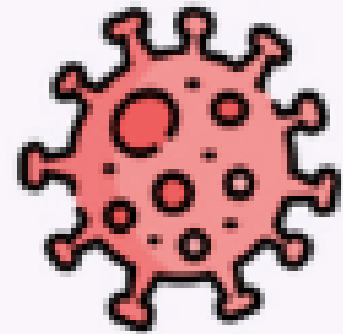
**First Identified:** United Kingdom



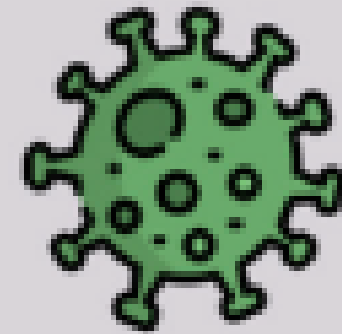
**Spread:** Spreads much faster than other variants



**Severe Effects:** May potentially cause more sickness and death



**Alpha B.1.1.7**

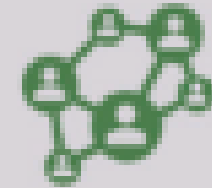


**Beta B.1.351**

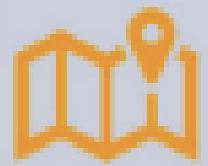
**First Identified:** South Africa



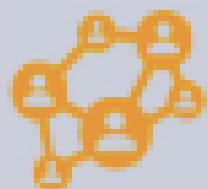
**Spread:** May spread faster than other variants



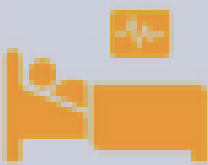
**Severe Effects:** Current data do not indicate more severe illness or death comparatively



**First Identified:** Japan & Brazil

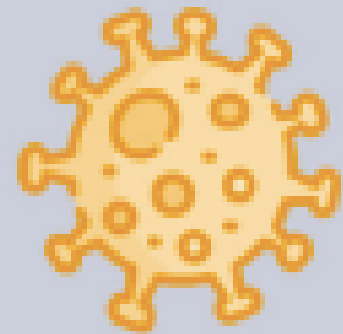


**Spread:** Spreads faster than other variants

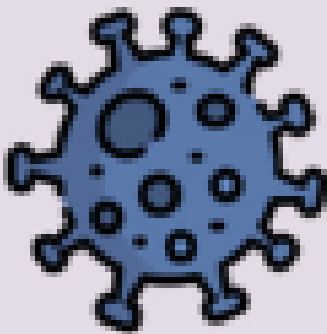


**Severe Effects:** Current data do not indicate more severe illness or death comparatively

**Gamma P.1**



**Delta B.1.617.2**



**First Identified:** India



**Spread:** Spreads much faster than other variants



**Severe Effects:** May cause more severe cases comparatively



# Why use Oxford Nanopore to sequence SARS-CoV-2?

- Decentralized sequencing
  - Everyone can help
  - Quick set up
  - Flexible – multiple some or loads or none
  - Portable – take your sequencer to your samples
  - Scalable – sequence as samples arrive on GridION
  - Low instrument cost
  - Used in other outbreak situations (Ebola, Zika)
- Short time to result
  - Sequence until you have enough data
  - Monitor during run
  - End experiment and start anew based on results not time

**Quick, J., Loman, N., Duraffour, S. et al. Real-time, portable genome sequencing for Ebola surveillance. Nature 530, 228–232 (2016). <https://doi.org/10.1038/nature16996>**

**Quick, J., Grubaugh, N., Pullan, S. et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc 12, 1261–1276 (2017). <https://doi.org/10.1038/nprot.2017.066>**