Genomic surveillance of SARS-CoV-2 in Belgium

Report of the National Reference Laboratory (UZ Leuven & KU Leuven)

Situation update – 1st of June 2021
(report 2021_29)

Executive summary

22.466 Belgian sequences of SARS-CoV-2 are now publicly available on GISAID.

For baseline surveillance samples collected during the last two weeks (742 sequences collected between 17 May and 30 May),
- B.1.1.7 (*Alpha*) represented 84.6% (compared to 87,6% in the last report) ↘
- P.1 (*Gamma*) represented 10,5% (compared to 7,9% in the last report) ↗
- B.1.617.2 (*Delta*) represented 1,3% ↗
- B.1.351 (*Beta*) represented 0,1% (compared to 0.9% in the last report) ↘

Other points of attention:
- Since last week, the number of Belgian B.1.617.2 sequences deposited on GISAID increased from 58 to 90 sequences
- A new rapid VOC PCR recently implemented in the UZ Leuven/KU Leuven federal platform laboratory highlighted B.1.617.2 in a small set of unselected positive samples (13% : 6 out of 45 samples)
- A new nomenclature has been launched by the WHO on May, 31st for the classification of SARS-CoV-2 VOCs and VOIs

Authors (National Reference Laboratory – UZ Leuven and KU Leuven):
Lize Cuypers, Guy Baele, Piet Maes, Simon Dellicour, Els Keyaerts, Sunita Janssenswillen, Marc Van Ranst, Emmanuel André.

With the collaboration of the laboratories of UCL, ULB, UMons, UNamur, ULiège, UGent, UZA/UAntwerpen, Jessa ZH, AZ Delta, AZ Klina, IPG, AZ St Lucas Gent, OLVZ Aalst, Briant network, ZNA, AZ St Jan Brugge, and UZ Leuven/KU Leuven.

Previous reports can be downloaded using the following link:
https://www.uzleuven.be/nl/laboratoriumgeneeskunde/genomic-surveillance-sars-cov-2-belgium

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1. Monitoring of VoCs in Belgium

Three variants of concern (VoCs) have been introduced in Belgium around the end of the year 2020. The B.1.1.7 variant, which has been introduced through numerous parallel introductions, has since then become the dominant lineage in the country and is considered to be responsible for the latest epidemic resurgence ("third wave").

Over the last month, during which a representative and stable genomic surveillance could be ensured, B.1.1.7, P.1 (together with P.1.1) and B.1.617.2 represented respectively 84%, 8% and 2% of the sequences reported to GISAID from Belgium. All other variants together currently represent 6% of the circulating strains. The evolution of the viral population is thus relatively stable for the moment, but the rise of P.1, and more recently B.1.617.2 should be monitored with caution.

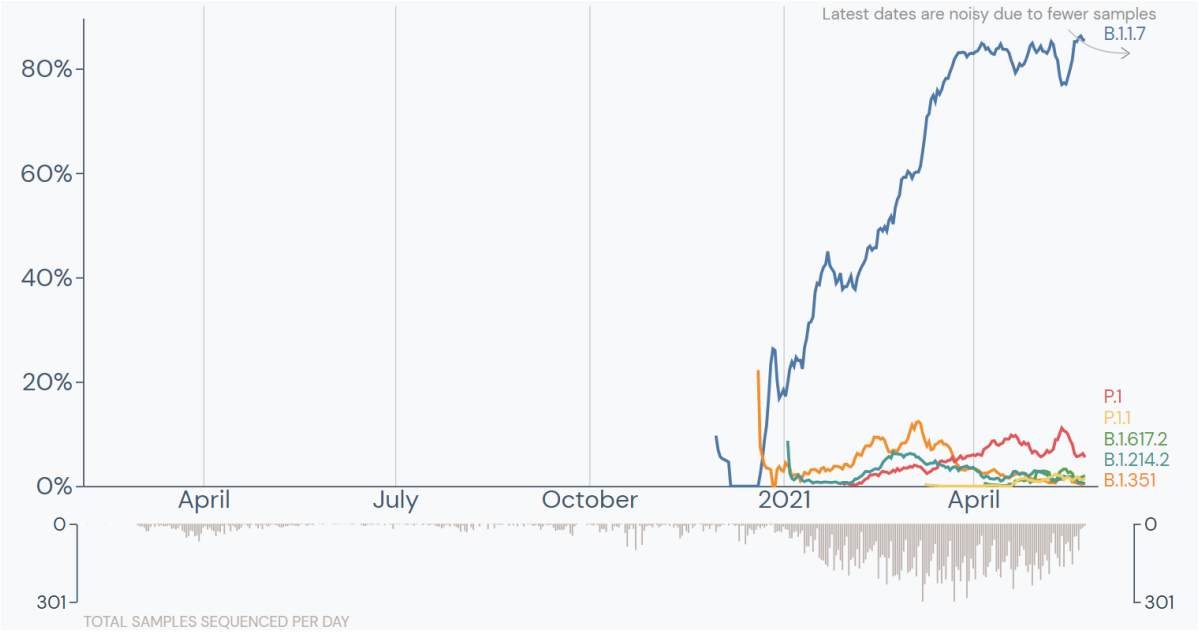

Figure 1: *Lineage prevalence over time in Belgium (combined active and baseline surveillance). B.1.1.7, P.1, B.1.351 and B.1.617.2 are currently classified as VOCs. B.1.617.1 and B.1.214.2 are among the variants of interest actively monitored in the country (source: outbreak.info & GISAID).*

<u>Particular attention to be given to B.1.617.2 (Delta) originally described in India</u>

The increasing number of B.1.617.2 strains reported in Belgium is of concern, and this lineage is now the 3rd most frequent variant in Belgium. Although targeted active case finding interventions tend to overrepresent the current incidence of this new VOC, this variant is now also detected by the baseline surveillance system, which illustrates active community transmission.

The UZ Leuven/KU Leuven federal platform lab has now adapted its VOC PCR to be able to detect the B.1.617.2 and B.1.617.1 variants. This test is constantly adapted, and is currently based on 4 independent PCR reactions targeting mutations present in the Spike gene of the different variants of concern. The combination of the 8 results (501N or Y; 484E or K; 417K or T and 452L or R) allows a presumptive positive identification of the different VOCs circulating in Belgium.

This updated VOC PCR design was used today on 45 unselected positive samples, and showed among these 11 *Gamma* VOCs (25%) and 6 *delta* VOCs (13%). These proportions are higher than those

observed through the genomic surveillance system. This higher proportion can be due to the (currently low) sample size, but might also partly be associated with the fact that these two VOCs continue to rise (a VOC PCR is typically representative of the past days, while sequencing-based surveillance is more representative of the past weeks).

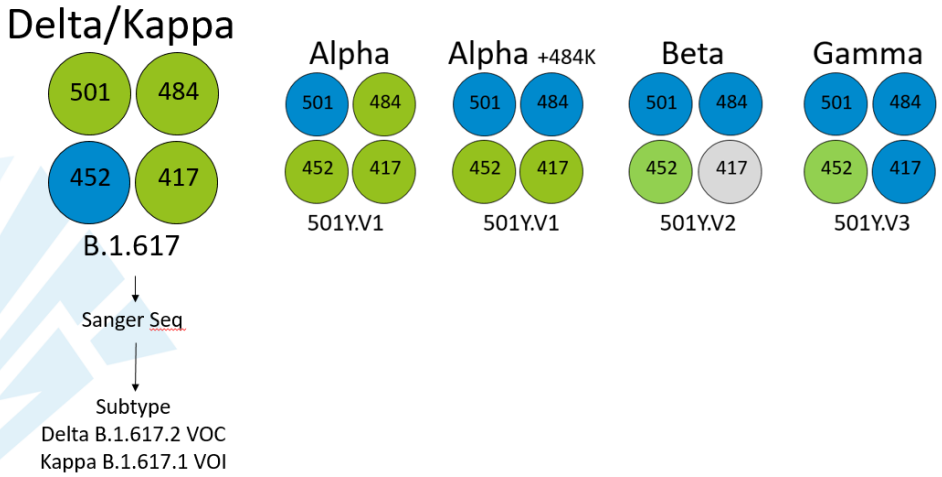

Figure 2: Interim design of the VOC PCR at the UZ Leuven/KU Leuven federal platform laboratory. The four PCRs can give a wildtype (green), mutated (blue) or indeterminate (grey) result at positions 501, 484, 417 and 452 of the Spike protein.

Lineage	Number of Belgian cases reported on GISAID	First reported
B.1.1.7 (alpha)	12.934	30/11/2020
B.1.351 (beta)	873	20/12/2020
P.1 (gamma)	886	29/1/2021
B.1.617.2 (delta)	90	6/4/2021
B.1.1.7 +S:E484K B.1.1.7 +S:S477R	20 15	31/3/2021 15/3/2021
B.1.525 (eta)	54	30/1/2021
B.1.620	16	31/3/2021
A.27	9	11/1/2021
B.1.617.1 (kappa)	8	25/3/2021

Table 1: List of VOCs (red) and VOIs (orange) identified in Belgium to date on number of sequences on GISAID

2. Evolution of variants of concern in the United Kingdom

The last wave in the UK (September - March 2021) has been the consequence of a rapid rise of B.1.1.7. This phenomenon in the UK has had an important impact for Belgium, as it led to an important number of imported cases, which were followed by the so-called “third wave” (March - May 2021).

Currently, the UK is observing a similar phenomenon of rapid viral population replacement, with B.1.617.2 now being the most prevalent variant. In the last few days, this phenomenon has been associated with a slight increase in the number of reported cases. The situation should be followed-up carefully for two reasons:

- A rapid rise of cases in the UK could lead to an uncontrolled number of imported cases in Belgium;
- If a rapid rise of cases would be observed in the UK, this would mean that Belgium and the rest of Europe would be exposed to the same risk in the coming weeks and months.

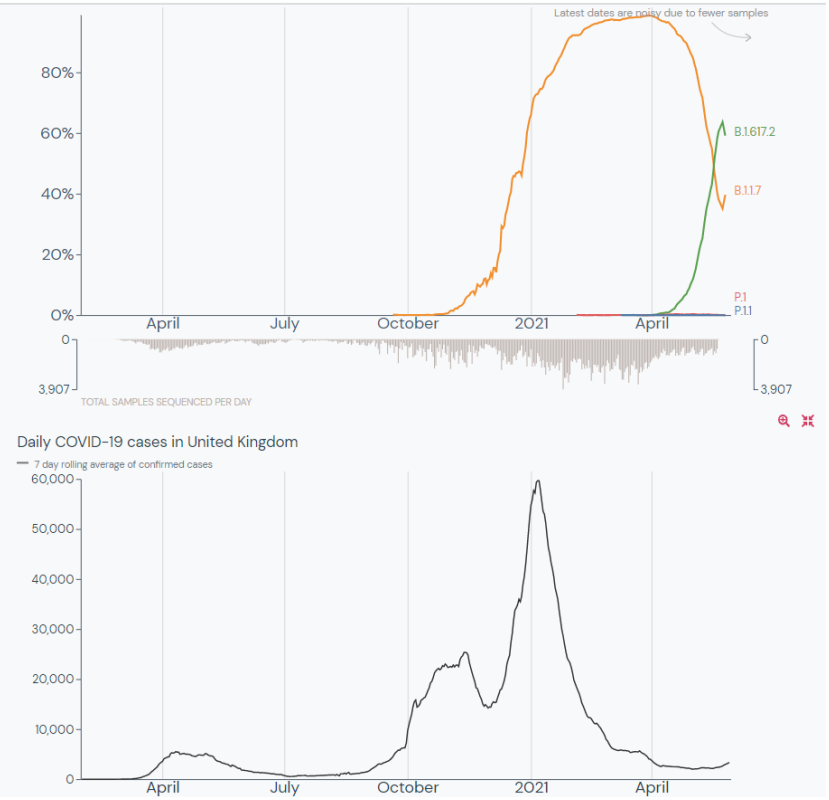

Figure 3: *Evolution of the share of viral populations and epidemiological situation in the United Kingdom (source: outbreak.info & GISAID).*

3. Update on P.1 (Gamma) in Belgium

The overall situation regarding P.1 in Belgium remains essentially unaltered with a small number of large clades being made up of entirely Belgian P.1 genomes clearly visible in the Nextstrain phylogeny. A small cluster can be seen distinct from the majority of infections (middle of the phylogeny), with genomes determined by multiple labs, pointing to a possible separate introduction event into Belgium.

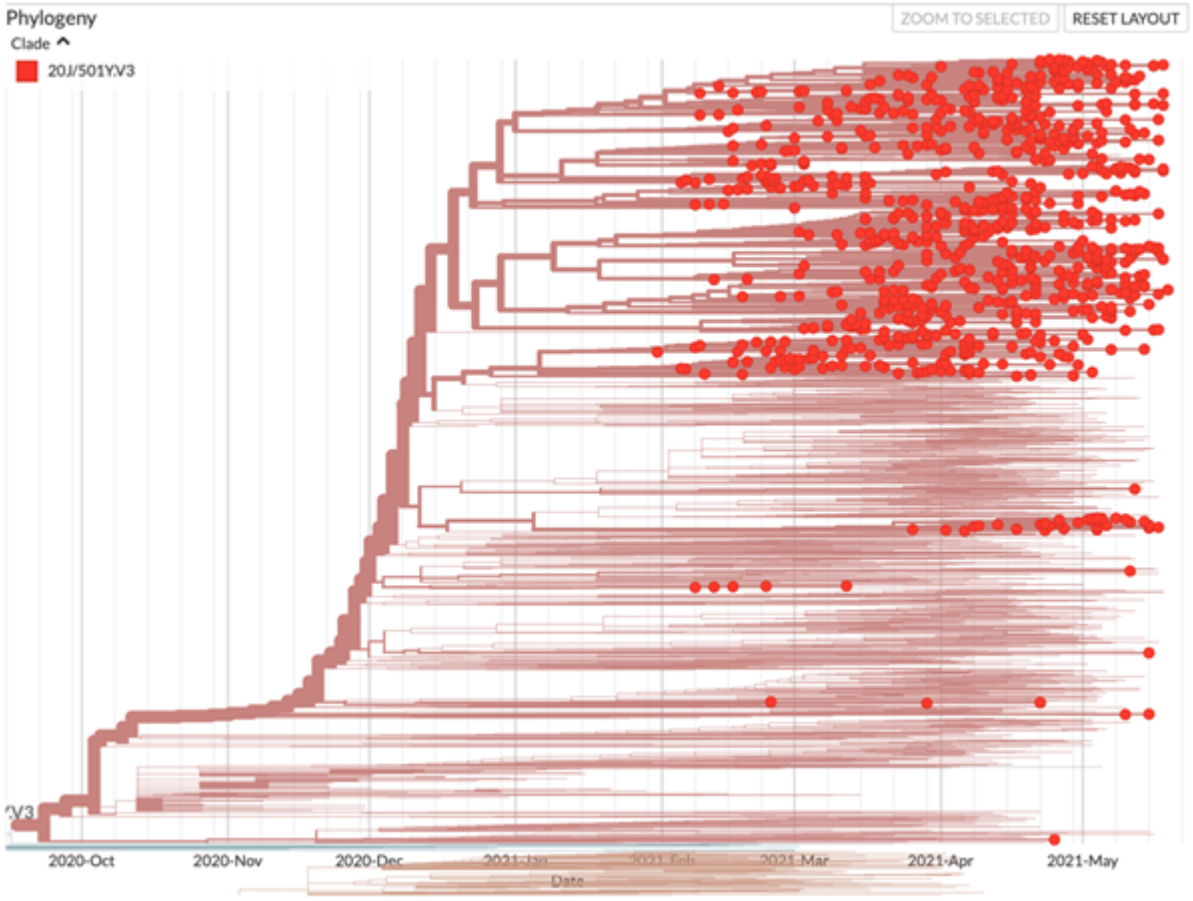

Figure 4: *Overview of the global P.1 phylogeny including all currently available Belgian genomes which are indicated as dots in the tree. All Belgian genomes were included while for other countries a representative set is part of the tree.*

What about P.1.1?

There seems to be some confusion regarding sublineages of the P.1 / Gamma variant first identified in Brazil. There is however no major genomic difference between P.1.1 and P.1 in terms of key mutations that can easily be identified at the moment. The classification (of P.1.1) was done based on the outcome of a large-scale phylogenetic inference exercise, which showed that a novel distinct P.1 clade has emerged containing genomes from mostly Italy and Germany. This can be seen in Figure 5 (dark yellow clade in the overall P.1 phylogeny), with this specific clade being visualized in more detail in Figure 6.

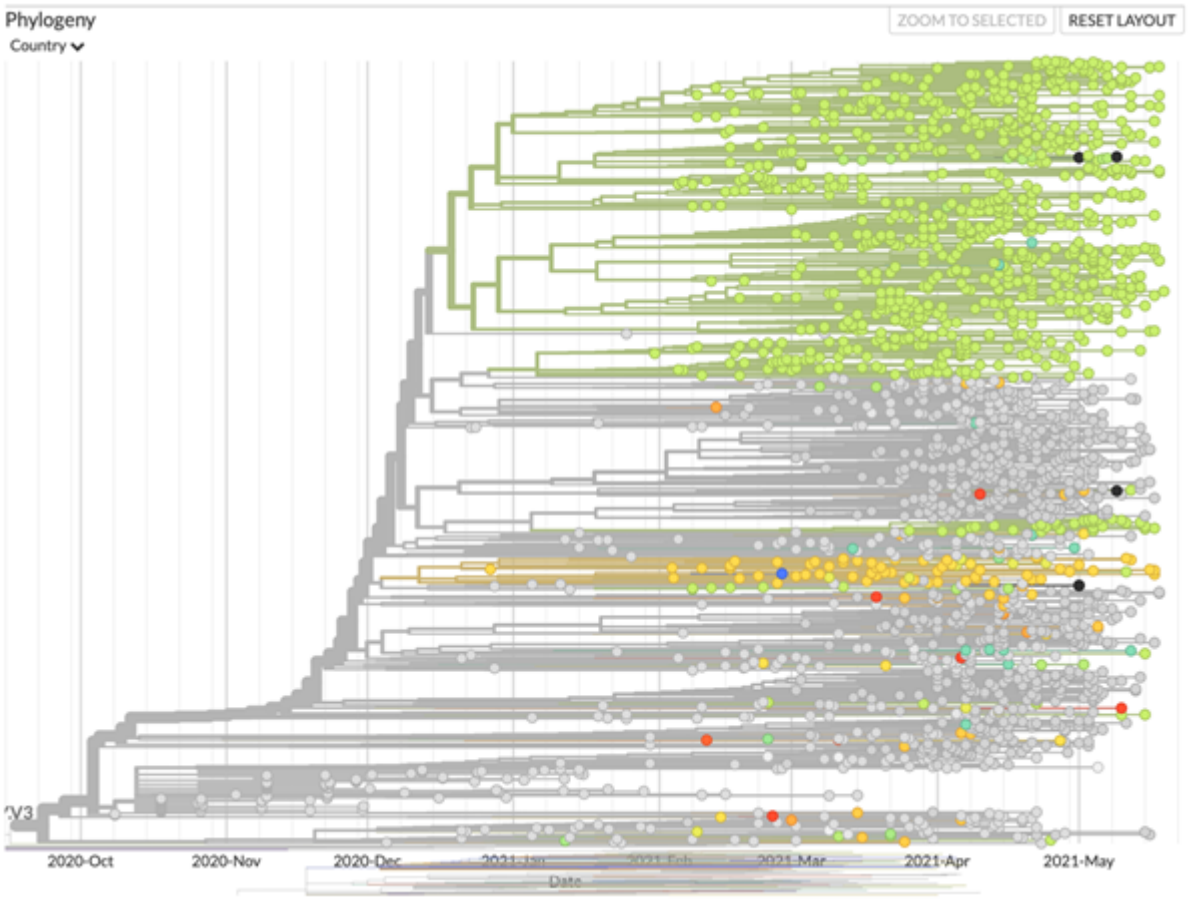

Figure 5: *The P.1 lineage coloured according to the country of sampling. The dark yellow clade consists mostly of Italian and German SARS-CoV-2 genomes and has been classified as P.1.1. There is a cluster of Belgian genomes (in green) relatively close to it, but these are likely not actual P.1.1 that stem from the Italian and German genomes.*

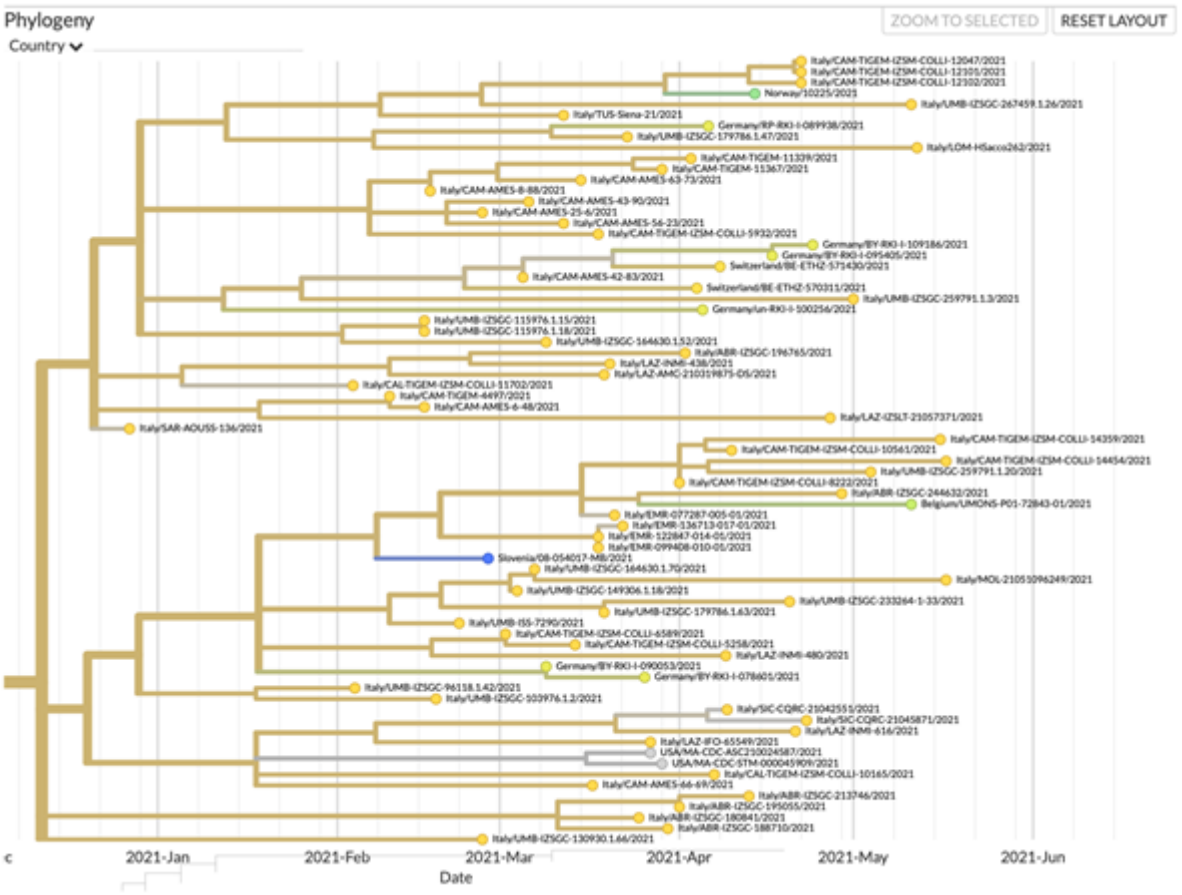

Figure 6: *Close-up view of the P.1.1 lineage. One single Belgian genome can be identified (just below the middle - coloured in light green) in this lineage, possibly a travel case returning from Italy, among mostly Italian and German genomes that make up this cluster.*

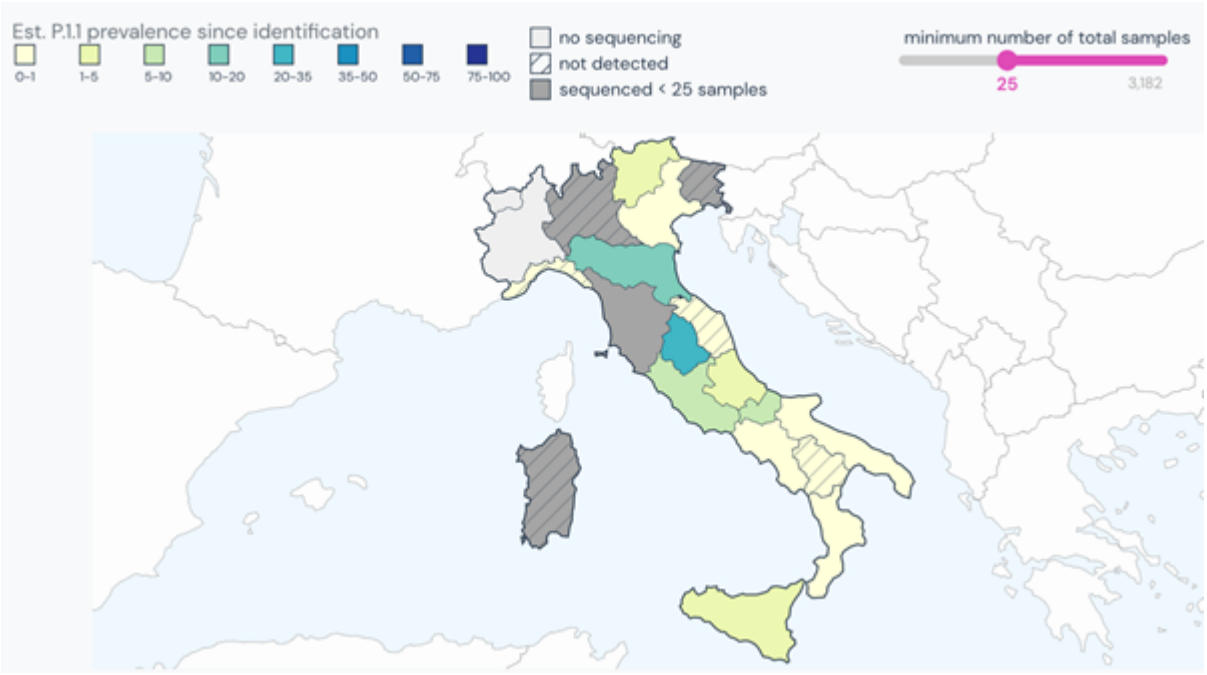

Figure 7: *According to outbreak.info, and as can be seen in Figure 5, P.1.1 is predominantly circulating in Italy.*

4. Update on B.1.617.2 (delta) and B.1.617.1 (kappa) in Belgium

Our custom B.1.617 Nextstrain build that focuses on the situation in Belgium clearly indicates the two major variants (B.1.617.1 and 2) that were first identified in India in Figure 8. Both variants are characterized by the occurrence of the amino acid Arginine (R) on position 452 in the S-gene or the so-called mutation L452R. While variant B.1.617.2 did not harbour an amino acid change on position 484, variant B.1.617.1 is characterized by the presence of mutation E484Q.

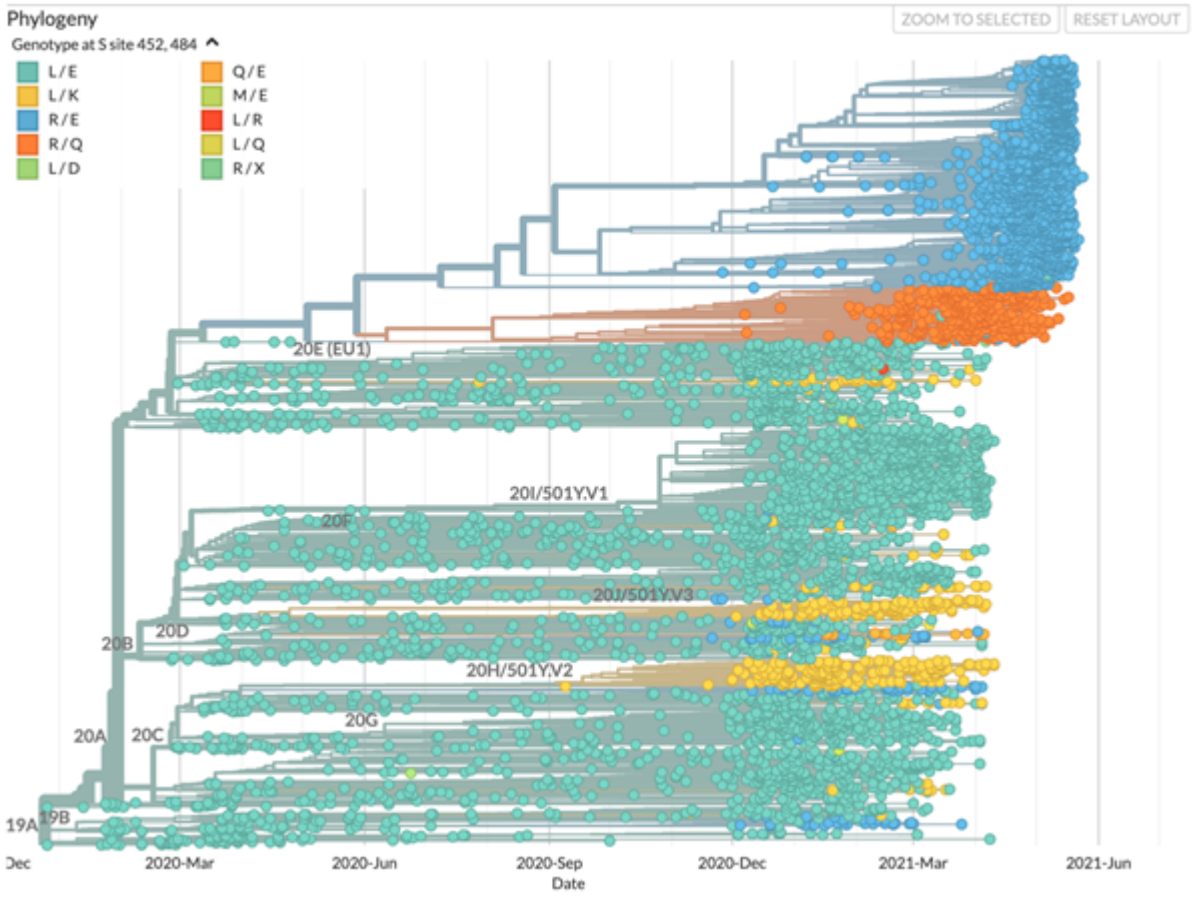

Figure 8. *A large clade of B.1.617.2 genomes (coloured in light blue) and a smaller clade of B.1.617.1 (coloured in dark orange) are shown on the top of the global SARS-CoV-2 phylogeny and shown in more detail in Figure 9.*

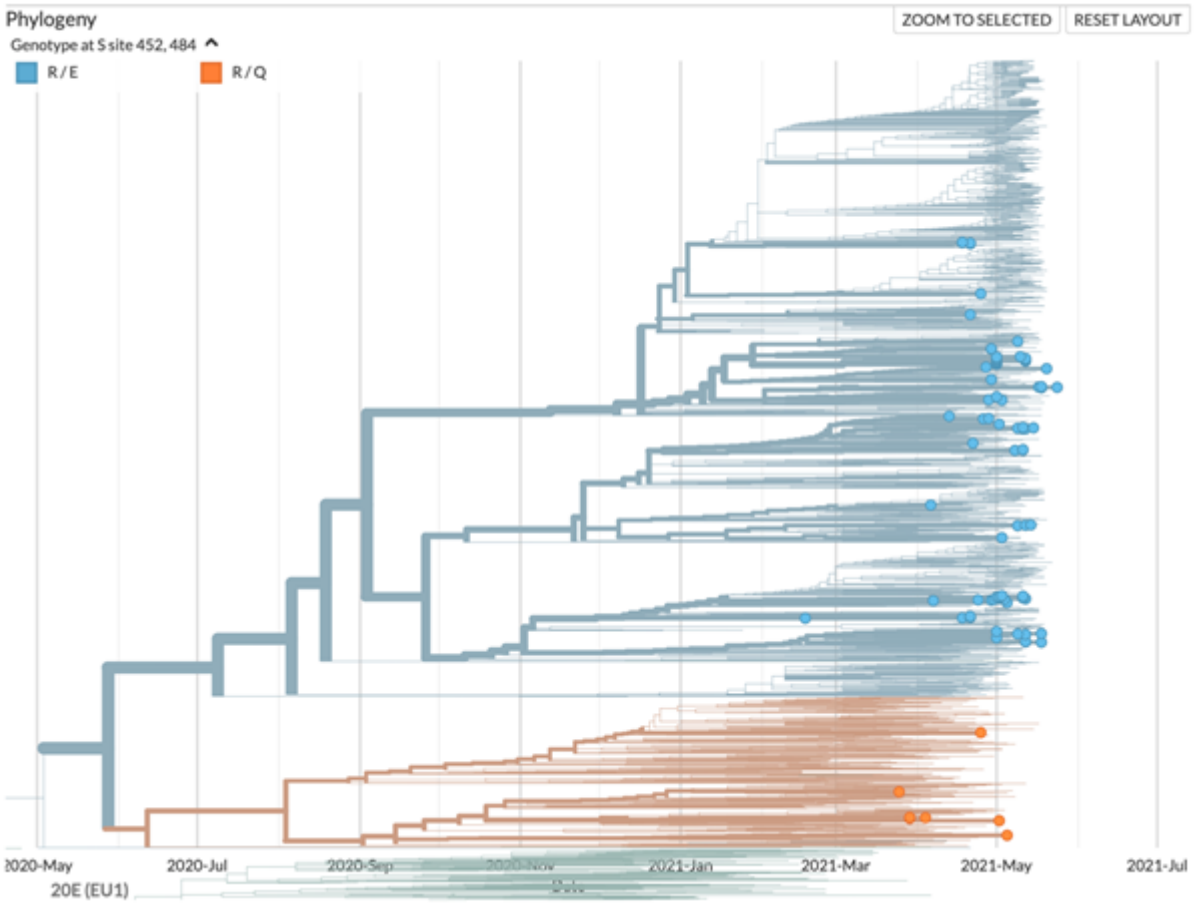

Figure 9: *The currently available Belgian B.1.617.1 (orange) and B.1.617.2 (blue) genomes on GISAID are highlighted in the tree, showing many introductions into Belgium (Belgian genomes indicated as dots in the tree) and are definitely not the result of a single source of origin. This illustrates the importance of screening incoming travelers and performing contact tracing.*

5. Update on post-vaccination breakthrough cases

Surveillance methodology

A breakthrough infection is defined as a positive SARS-CoV-2 test at least 7 days after the full completion of a vaccination scheme (e.g. 2 doses). To facilitate the transfer of samples that meet the definition to the sequencing lab in Leuven, laboratories that submit RT-PCR test results to HealthData, will receive an automatic message from HealthData notifying them that a particular sample meets the criteria of a post-vaccination breakthrough case. It remains the responsibility of each lab to verify whether the sample meets the criteria for sequencing (viral load is sufficiently high, corresponding to a Ct value <25) and if so, to send the sample accompanied with the completed application form (see the website of Sciensano) to the NRC UZ/KU Leuven.

Intermediate results

The NRC is actively collecting information on post-vaccination infections, of which to date, 347 samples could be typed. All 347 samples were sampled between January 28th and May 17th, 2021.

- Vaccines involved in post-vaccination infections

  Information on the type of vaccine received is currently available for only 183 out of 347 (52.7%) documented post-vaccination infections. These limited numbers do not allow any conclusion, but it does not appear at this stage that specific vaccines would be particularly over- or under-represented in this group of patients, particularly because we consider here only people which were fully vaccinated.

	Vaccines received by persons with a post-vaccination infection
Pfizer	88.5%
Moderna	6.0%
J&J	-
AZ	5.5%
Total people	183 available to date out of 347

Table 2: *Distribution of vaccines among post-vaccination infections.*

- Variants involved in post-vaccination infections

  The distribution of lineages and variants identified in these 347 sequenced breakthrough infections is not entirely similar to the distribution of lineages and variants reported by baseline surveillance during this same period of time. These differences can be explained by immune escape mechanisms, but may also result from sampling bias or specific epidemic circumstances. These differences should therefore be interpreted with caution and will need to be further consolidated.

Variant involved	Share among notified post-vaccination infections
B.1.1.7	76.1%
P.1	8.4%
B.1.351	4.3%
B.1.214.2	4.3%
B.1.617.1	0.6%
B.1.617.2	1.2%
Others	5.2%
Total sequences	347

Table 3: *Distribution of post-vaccination infections reported to date in Belgium compared to the distribution reported for the same period of time through the baseline surveillance.*

6. WHO new SARS-CoV-2 variant Classification and Definitions

Variant of Concern (VOC)
A SARS-CoV-2 variant that meets the definition of a VOI (Variant Of Interest) and, through a comparative assessment, has been demonstrated to be associated with one or more of the following changes at a degree of global public significance:

- Increase in transmissibility or detrimental change in COVID-19 epidemiology;
- Increase in virulence or change in clinical disease presentation;
- Decrease in effectiveness of public health and social measures or available diagnostic, vaccines or therapeutics.

Variant of Interest (VOI)
A SARS-CoV-2 variant is considered a VOI if it is phenotypically changed compared to a reference isolate or has a genome with mutations that lead to amino acid changes associated with established or suspected phenotypic implications, and either:

- has been identified to cause community transmission/multiple COVID-19 cases or clusters, or has been detected in multiple countries;
- is otherwise assessed to be a VOI by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group.

The established nomenclature systems for naming and tracking SARS-CoV-2 genetic lineages by Nextstrain, Pangolin and GISAID are currently in use and will remain in use by scientists and in scientific research. To assist with public discussions of variants, WHO has recommended the use of letters of the Greek alphabet to label VOC/VOI to make it easier and more practical for discussions by non-scientific audiences:

WHO label	***Nextstrain Clade***	***Pangolin Lineage***	***GISAID Clade***	***VOC/VOI***	***Detected in Belgium***
Alpha	20I/501Y.V1	B.1.1.7	GRY	VOC	Yes
Beta	20H/501Y.V2	B.1.351	GH/501Y.V2	VOC	Yes
Gamma	20J/501Y.V3	P.1	GR/501Y.V3	VOC	Yes
Delta	21A/478K	B.1.617.2	G/452R.V3	VOC	Yes
Epsilon	20C/452R	B.1.427 / B.1.429	GH/452R.V1	VOI	Yes (B.1.427)
Zeta	20B/484K	P.2	GR	VOI	Yes
Eta	20A/484K	B.1.525	G/484K.V3	VOI	Yes
Theta	20B/265C	P.3	GR	VOI	No
Iota	20C/484K	B.1.526	GH	VOI	No
Kappa	21A/154K	B.1.617.1	G/452R.V3	VOI	Yes