# Genomic surveillance of SARS-CoV-2 in Belgium

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

**Situation update – 4th of May 2021**
**(report 2021_25)**


**Executive summary**

17,986 Belgian sequences of SARS-CoV-2 are publicly available on GISAID.

For baseline surveillance samples collected during the last two weeks,
- B.1.1.7 (20/501Y.V1) represented 87.3% (compared to 81.4% in the last report). There are currently 4 Belgian sequences of B.1.1.7 with the S:E484K mutation deposited on GISAID.
- P.1 (20J/501Y.V3) represented 5.3% (compared to 8.0% in the last report).
- B.1.351 (20H/501Y.V2) represented 2.3% (compared to 2.8% in the last report)
- There are currently 5 sequences of B.1.617.1 and 4 sequences of B.1.617.2 deposited on GISAID.

In this report, we discuss
1) The intrinsic risk of transmission associated with mass international migrations such as holidays.
2) The first 100+ post-vaccination infections and the system put in place with Sciensano to automatically identify such cases so that they can be sent to the NRC for further investigation
3) The emergence and significance of additional mutations of concern among variants of concern



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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, OLV 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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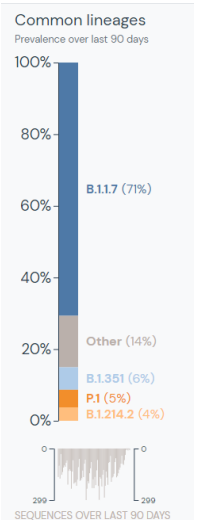
## 1. Baseline surveillance

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 as responsible for the latest epidemic resurgence ("third wave").




Over the last 3 months, during which a representative and stable genomic surveillance could be ensured, VOCs (B.1.1.7, P.1, and B.1.351) represented 82% of the circulating strains in Belgium (increasing trend: currently 95%). The lineage B.1.214.2, represents for the same period 5% of the circulating strains in Belgium (decreasing trend).

The P.1 lineage, which emerged in Brazil, continues to increase in frequency, with 557 sequences uploaded since the first case was reported on January 29, 2021. Its evolution remains of concern (see also our section below for a summary on the current state of the knowledge on that particular variant).

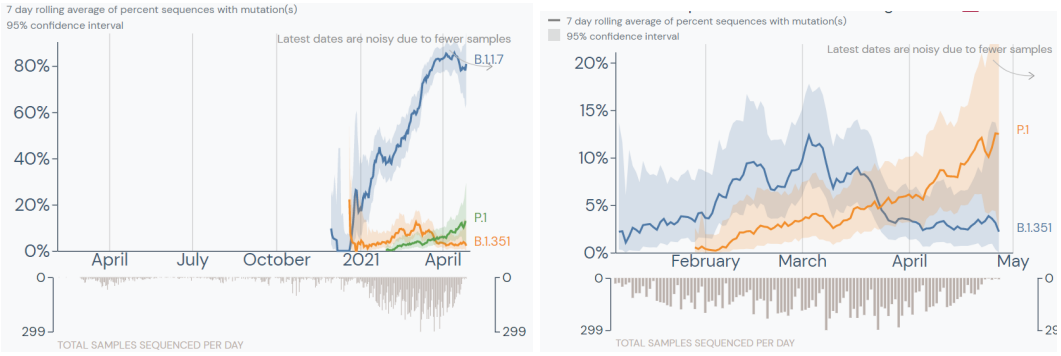



***Figure 1:*** *Lineage prevalence over time (left) and zoom (right) on the recent evolution of P.1 (originally described in Brazil) and B.1.351 (originally described in South-Africa) (source: outbreak.info & GISAID).*

## 2. Risk evaluation criteria for European travels

Although the epidemiological situation varies from one country to the other, we do not currently observe major differences among European countries with regard to the distribution of variants of concern, as they are in general those reported in Belgium (B.1.1.7, B.1.351 and P.1).

The risk associated with European travels can be considered as limited with regard to the importation of new variants of concern. There are nevertheless two major attention points: first, the level of this risk may evolve over a few weeks, and second, the risk cannot only be assessed based on local circulation of variants. We suggest to regularly re-evaluate this risk based on 3 questions and 5 main indicators listed below:

- **What is the likelihood a traveller would be infected during travel?**

    - Main indicator 1: current national incidence and effective reproduction number (R(t)) in the country. Trusted source of information: https://graphics.reuters.com/world-coronavirus-tracker-and-maps/fr/

      *Travel should be discouraged if the incidence is close to the maximal historical peak reported by this country (this indicator allows to compensate for unequal test coverage, and assumes that testing capacity in a defined country will increase or be stable over the time) and if this trend is increasing over the last two weeks. These indicators are not applicable if the travel mainly takes place in a highly touristic place (seasonal crowding, international tourism).*

      In Europe, all countries are below their maximal historical peak today.
        - The countries that currently have more than 60% of their maximal level are Sweden (71%), Germany (69%), Greece (66%), Belarus (60%) and The Netherlands (60%). Belgium is currently at 17% of its maximal level;
        - The countries where the number of cases has increased in the last two weeks are Latvia, Lithuania and Ireland. Belgium has currently a decreasing trend;
        - NB: Turkey and Morocco are both currently “green” with regard to these indicators.

- **What is the likelihood a traveller would experience a severe health condition if infected during travel?**

    - Main indicator 2: vaccination status of the traveller

      *Travel in highly touristic places (seasonal crowding, international tourism) should be discouraged for unvaccinated people presenting a higher risk of developing a severe disease.*

    - Main indicator 3: current national mortality rate in the country. Trusted source of information: https://www.ecdc.europa.eu/en/cases-2019-ncov-eueea

      *Travel should be discouraged if the mortality rate among recently notified cases is above 2%. The current mortality rate in Belgium is 1.12%.*

| | |
|---|---|
| Malta | 2.08% |
| Italy | 2.58% |
| Czechia | 2.78% |
| Greece | 2.98% |
| Poland | 3.96% |
| Romania | 5.80% |
| Bulgaria | 5.84% |
| Hungary | 6.69% |
| Slovakia | 10.19% |

**Table 1:** Current list of European countries with a mortality rate among recently notified cases above 2% (source: ECDC)

- Main indicator 4: high level of circulation of variants of concern
  Trusted source of information: https://outbreak.info/location-reports

  *Travel should be discouraged if the P.1, B.1.351, B.1.617.1, B.1.617.2 and B.1.1.7 with S:E484K or any emerging variant represents more than 10% of the recent circulating viruses.*

  *Travel should be discouraged in countries which do not have a minimal genome surveillance program in place (for example more than 1,000 strains available on GISAID for the last 3 months).*

- **What is the likelihood a traveller will be exposed to an unexpected exposure of variants of concern or emerging variants of unknown severity?**

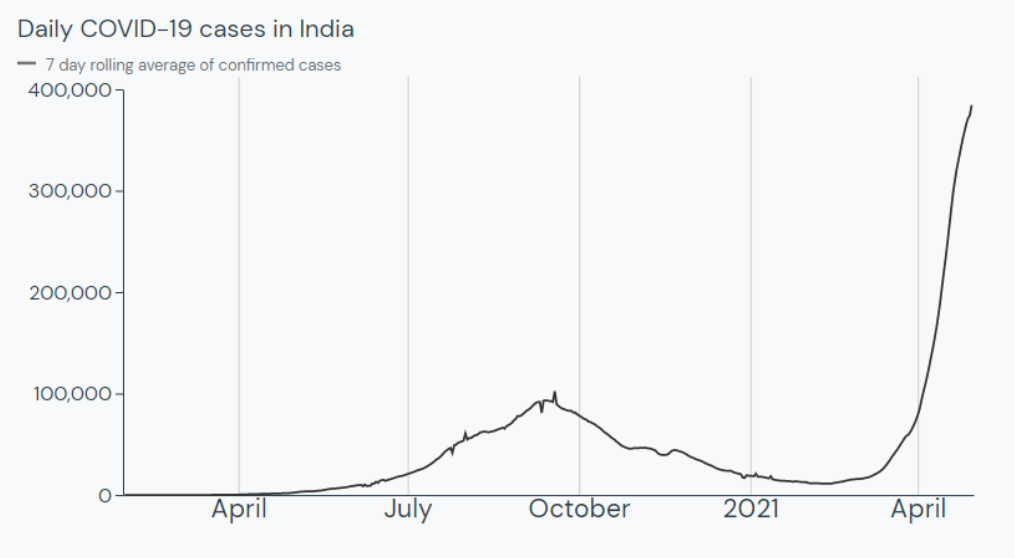
  - Main indicator 5: travel restrictions of the host country (mainly applicable for highly touristic places). Trusted source of information:
    https://www.iatatravelcentre.com/world.php

    *Travel should be discouraged in countries with no travel restriction or not implementing strict testing & isolation policies for travellers originating from countries for which Belgium discourages travels (see points above).*

## 3. Evolution of variants of concern in India, Brazil and South-Africa

India

The current epidemiological situation in India is alarming and appears to be related to the simultaneous increase of three variants of concern: B.1.1.7, B.1.617.1, and B.1.617.2. Currently, about 2% of the Indian population is fully vaccinated, while 9% has received at least one dose of vaccine.



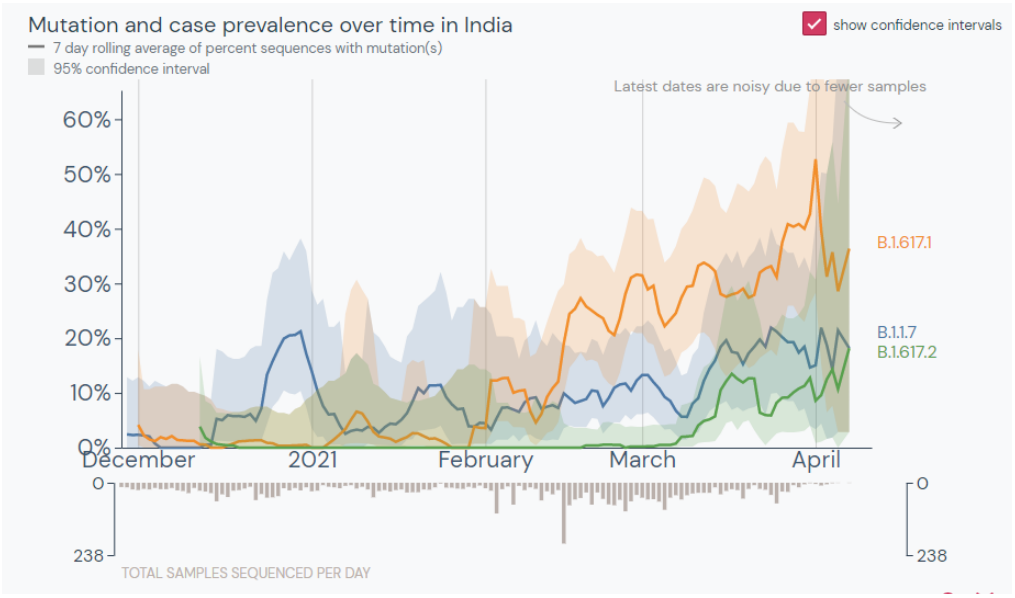




**Figure 2:** Epidemiological evolution and share of viral populations in India

Brazil

The current epidemiological situation in Brazil is still unfavorable and at this stage appears to be solely associated with the spread of the variant of concern P.1. Currently, 6.6% of the Brazilian population is fully vaccinated, while 14% has received at least one dose of vaccine.

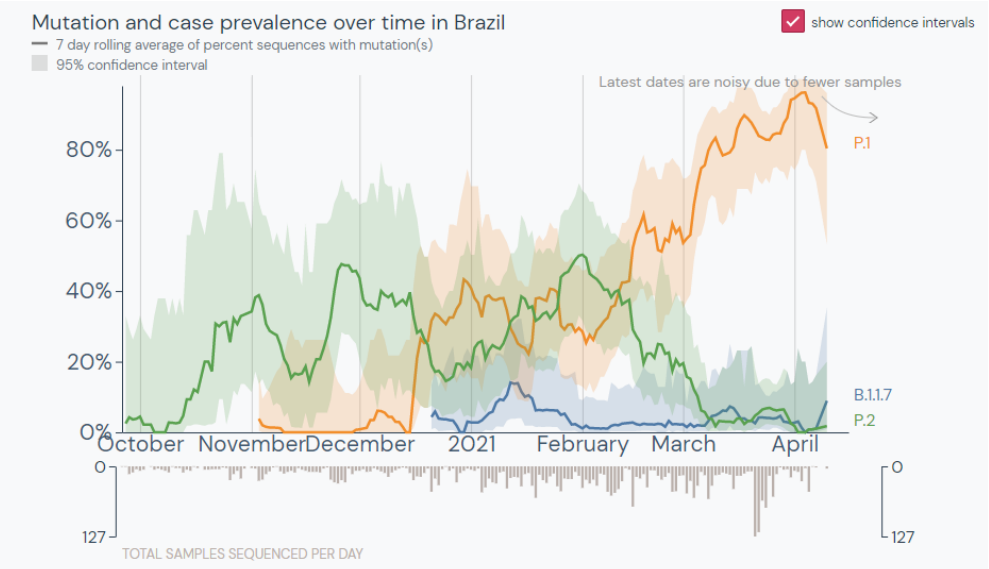




**Figure 3:** Epidemiological evolution and share of viral populations in Brazil

South Africa

The current epidemiological situation in South Africa is currently stable after a recent resurgence associated with B.1.351. Currently, only 0.55% of the South African population is fully vaccinated.

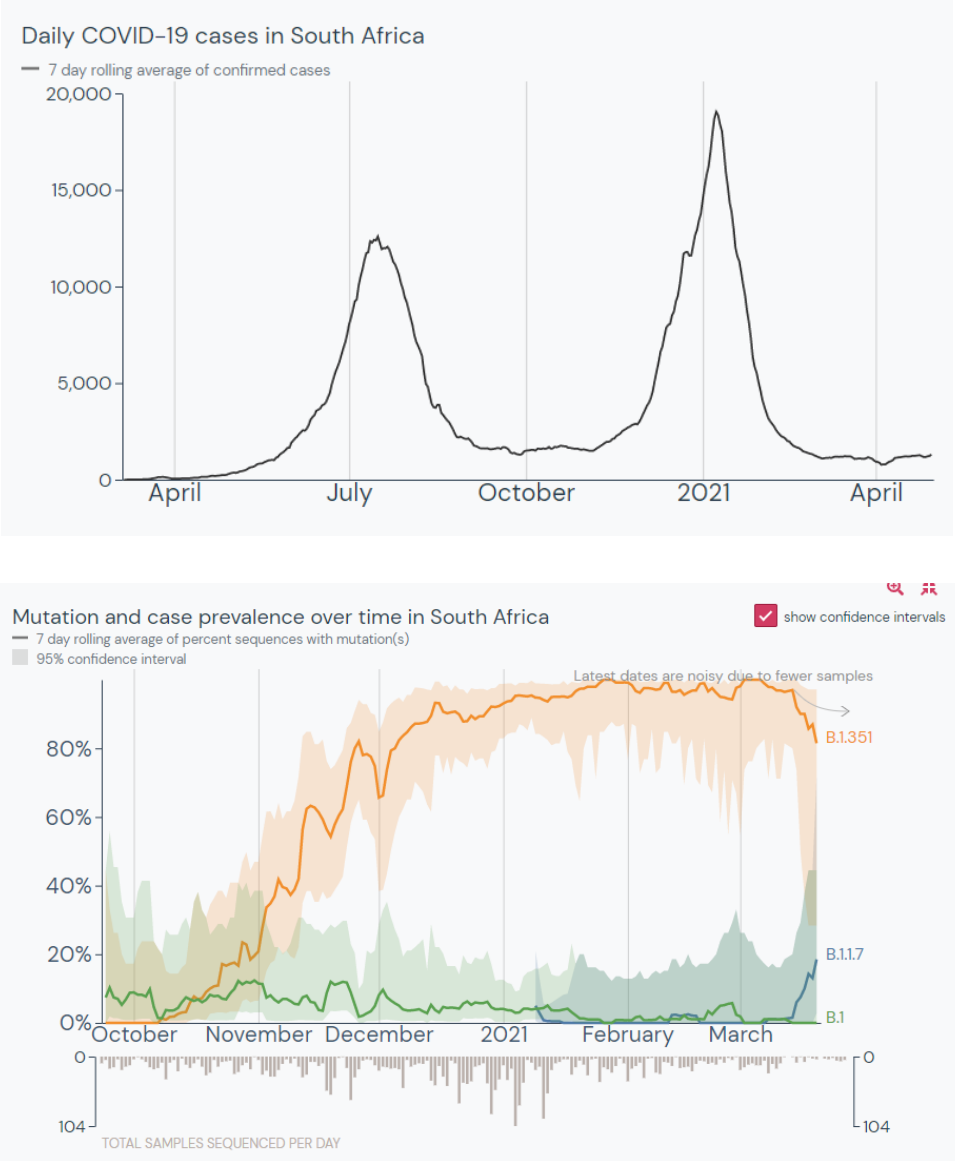



**Figure 4:** Epidemiological evolution and share of viral populations in South Africa

The United Kingdom

The situation in the United Kingdom is closely monitored as this country has the largest genomic surveillance program, has a more advanced vaccination coverage compared to Belgium (22,8% fully vaccinated, 50% partially vaccinated), had B.1.1.7 as the dominant lineage before the effect of vaccination could take place.

In the context of a stable and low-level circulation of the virus, the UK observes an increasing trend (when sufficient numbers were available for a variant to estimate this) related to the "Indian" variants (B.1.617.2 and B.1.617.2), the "Brazilian" variant (P.1), the "South-African" (B.1.351), the "UK variant with E484K" (B.1.1.7 with S:E484K) and the lineage B.1.1.318.

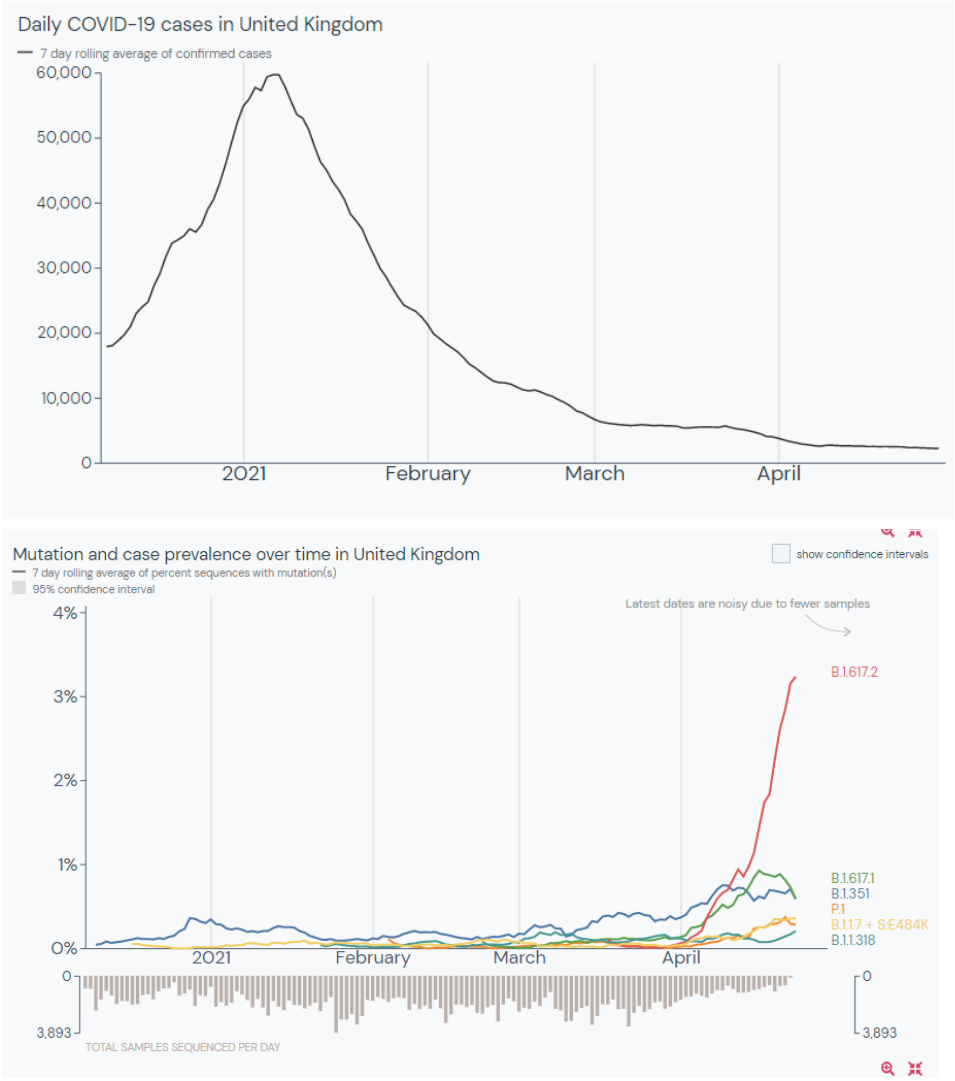



**Figure 5:** Epidemiological evolution and share of viral populations in the United Kingdom

## 4. 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). Samples that meet this definition are to be sent to the national reference center at UZ/KU Leuven for sequencing, as agreed in the latest convention of RIZIV/INAMI.

To facilitate the transfer of samples of post-vaccination breakthrough cases 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. The linkage of positive test results to vaccination data will occur on the level of HealthData. 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

Between January 28th and April 26th, the NRC was notified of 106 infections meeting the criteria of - post-vaccination infections. A number of these samples were identified before the INAMI/RIZIV convention and were therefore referred to the different sequencing laboratories. Below is a compilation of sequencing information kindly provided by the sequencing laboratories of UZA / UA, Jessa, and AZ Delta, in addition to the UZ / KU Leuven sequencing platform. As sequencing laboratories could not yet share their early results, and because the numbers are still limited, the distribution of lineages may still evolve importantly in the coming weeks. The situation will be weekly reported.

To date, 61/106 samples have been sequenced and of those 57 samples could be typed. All 57 samples were sampled between January 28 and April 16, 2021.

- Vaccines involved:
  The majority of these patients have been fully vaccinated with COMIRNATY (Biontech/Pfizer), but this over-representation of this vaccine at an early stage of the surveillance has to be looked at acknowledging that this vaccine was the first to be largely deployed in Belgium. This surveillance is ongoing, and further reports will include more details per vaccine and with regard to disease severity.

- Variants involved:
  The distribution of lineages and variants identified in the first 57 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 in the general population during the last 3 months | Share among notified post-vaccination infections | Comment |
|---|---|---|---|
| B.1.1.7 | 71% | 42.1% | Post vaccination outbreaks in nursing homes not yet included in this table |
| P.1 | 5% | 10.5% | |
| B.1.351 | 6% | 7% | Post vaccination outbreaks in nursing homes not yet included in this table |
| B.1.214.2 | 4% | 22.8% | A large part (>70%) of the samples associated with a unique outbreak in a nursing home |
| Others | 14% | 17.7%<br>• B.1.221 (8,8%)<br>• B.1.160 (5,3%)<br>• B.1.1.10 (1,8%)<br>• B.1.214.3 (1,8%) | |

**Table 2:** 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.

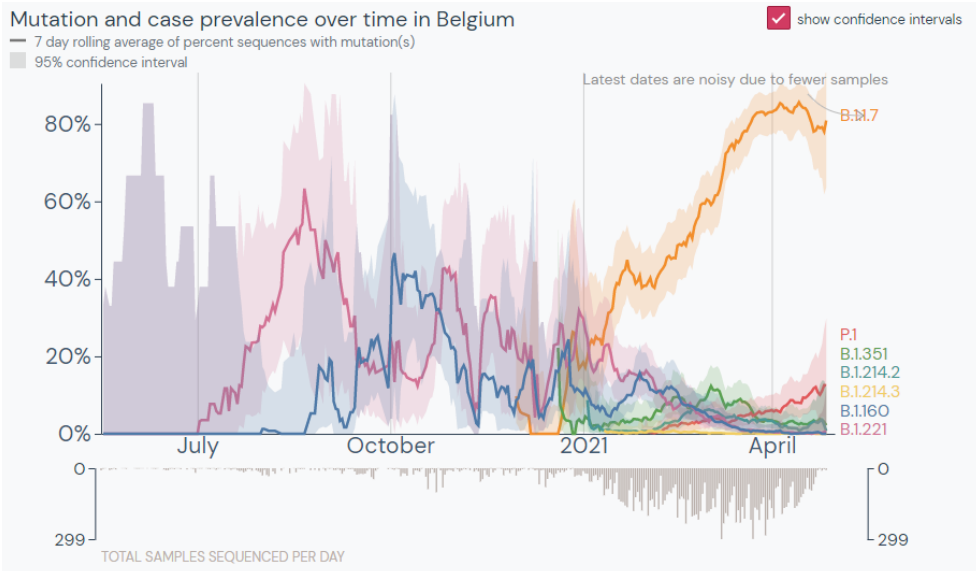



***Figure 6:*** *Distribution observed through baseline surveillance of the different lineages and variants involved in post-vaccination infections.*

## 5. Monitoring of VOCs in Belgium

After a constant rise in proportion starting from January 2021, 95% new SARS-CoV-2 infections in Belgium are currently associated with a variant of concern (VOC), mostly B.1.1.7 (20I/501Y.V1).

For baseline surveillance samples collected during the last two weeks,
- B.1.1.7 (20/501Y.V1) represented 87,3% (compared to 81,4% in the last report). There are currently 4 sequences of B.1.1.7 with the S:E484K mutation deposited on GISAID.
- P.1 (20J/501Y.V3, originally from Brazil) represented 5,3% (compared to 8,0% in the last report).
- B.1.351 (20H/501Y.V2) represented 2,3% (compared to 2,8% in the last report)
- There are currently 5 sequences of B.1.617.1 and 4 sequences of B.1.617.2 deposited on GISAID.

We recently observed the emergence of strains harboring additional mutations compared to the set of mutations initially described for the lineage B.1.1.7. The significance of these mutations is discussed in the next section.

- Worldwide, 620 B.1.1.7 with the E484K spike mutation have been reported. All Western European countries with a consistent genomic surveillance program have reported such strains, and these currently represent less than 1% of the circulating strains.
- Worldwide, 57 B.1.1.7 with the E484Q spike mutation have been reported. All Western European countries with a consistent genomic surveillance program have reported such strains, and these currently represent less than 1% of the circulating strains.
- Worldwide, 243 B.1.1.7 with the S477R spike mutation have been reported. 6 Western European countries with a consistent genomic surveillance program have reported such strains, and these currently represent less than 1% of the circulating strains.

| | | | | |
|---|---|---|---|---|
| B.1.1.7 + S:S477R | 9 | < 0.5% | 15 Mar 2021 | 9 Apr 2021 |
| B.1.1.7 + S:E484K | 4 | < 0.5% | 31 Mar 2021 | 14 Apr 2021 |
| B.1.1.7 + S:E484Q | 1 | < 0.5% | 2 Mar 2021 | 2 Mar 2021 |

**Table 3:** Emergence of mutations of concern among B.1.1.7 strains from Belgium, with date of first and last notification

## 6. The emergence of additional mutations of concern among variants of concern

E484K is a mutation of concern because it generates an antigenic drift in the receptor binding domain of the Spike protein, which plays two important roles: (1) it is one of the preferred targets of the human antibodies and (2) it it the region of the virus which allows it to enter the human cells.

1) This mutation arose in experimental conditions when the virus was put under selective pressure by the presence of convalescent or vaccine-derived antisera. These laboratory experiments illustrate the fact that the virus "naturally" choses this genetic evolution to escape human immunity.

   Collier et al. (Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. Nature. 2021) assessed the immune responses of individuals after vaccination with the mRNA-based vaccine BNT162b22. The authors measured neutralizing antibody responses after the first and second immunizations using pseudoviruses that expressed the wild-type spike protein or a mutated spike protein that contained the eight amino acid changes found in the B.1.1.7 variant. Introduction of the mutation that encodes the E484K substitution in the B.1.1.7 background to reflect a newly emerged variant of concern (VOC 202102/02) led to a more-substantial loss of neutralizing activity by vaccine-elicited antibodies and monoclonal antibodies (19 out of 31) compared with the loss of neutralizing activity conferred by the mutations in B.1.1.7 alone. The emergence of the E484K substitution in a B.1.1.7 background represents a threat to the efficacy of the BNT162b2 vaccine.

2) E484K is associated with increased binding to the human ACE2 (the cell receptor for SARS-COV-2)

According to the sequencing data available on GISAID, the first B.1.1.7 strain harboring this additional mutation was reported on 17/12/2020, two months after the start of the global emergence of B.1.1.7.

In its latest report (22/4/2021), Public Health England, reported a strong positive growth rate for B.1.617.1 - which carries the E484Q mutation and for B.1.1.318, a lineage which carries the S:E484K and the S:P681H mutations.

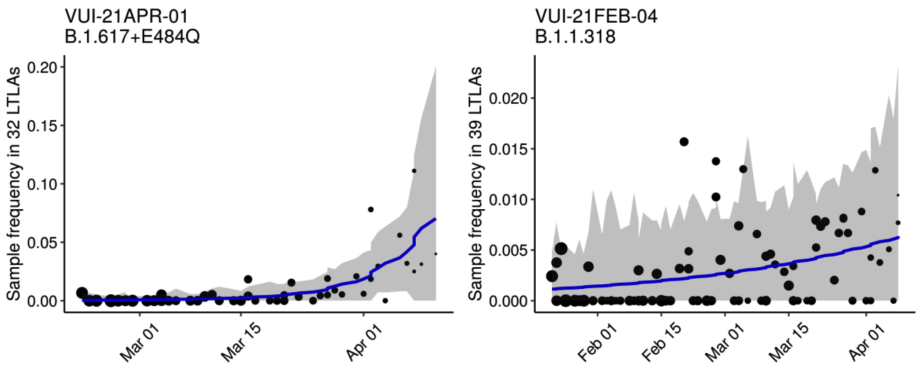



**Figure 7:** Evolution of B.1.617.2 and B.1.1.318 in the United Kingdom

## 7. 501Y.V3 “Brazilian” variant (lineage P.1): what do we know so far?

First detected in Japan in four people who contracted it on a trip to Brazil, SARS-CoV-2 lineage P.1 likely emerged late 2020 in the region of Manaus, Brazil (Sabino *et al*. 2021, *The Lancet*; Faria *et al*. 2021, *Science*), a region that had been notably impacted by the early phase of the epidemic. Indeed, a study of blood donors conducted in Manaus indicated that 76% (95% CI 67–98) of the population had been infected with SARS-CoV-2 by October 2020 (Buss *et al*. 2020, *Science*), which would be above the theoretical herd immunity threshold (67%), given a basic case reproduction number ($R_0$) of 3.4 (Fontanet & Cauchemez 2020, *Nat Rev Immunol*). Despite this estimate, Manaus saw an abrupt and thus unexpected increase in the number of COVID-19 hospital admissions during January 2021 (Sabino *et al*. 2021). In their study, Sabino *et al*. (2021) lists at least four (non-mutually exclusive) possible explanations for the resurgence in Manaus: (i) an overestimation of the attack rate during the first wave (judged unlikely by the authors regarding the study of blood donors), (ii) a decrease in immunity against infections (could potentially contribute but judged unlikely by the authors to fully explain the recent resurgence), (iii) new lineage(s) evading immunity generated in response to previous infections, (iv) new lineage(s) associated with a higher transmissibility.

Assumptions (iii) and (iv) could therefore be related to the emergence of P.1 in the region. While the ability of that variant to escape previously acquired immunity (following the infection of wild type variants) has still to be thoroughly investigated, P.1 contains the spike protein mutation E484K associated with in-vitro evidence of reduced neutralisation by polyclonal antibodies in convalescent sera (Greaney *et al*. 2021, *preprint*). Consistently, in their study, Faria *et al*. (2021) estimated that P.1 could evade 21–46% of protective immunity elicited by previous infection with non-P.1 lineages. Regarding a potentially increased transmissibility, Faria *et al*. (2021) estimated that P.1 may be 1.7 to 2.4 times more transmissible. In this study, the authors report a small yet significant association between P.1 infections and lower Ct values (indicating higher viral loads), which would be coherent with a higher transmissibility for P.1.

Regarding the potentially increased virulence of P.1, a recent study published in the ECDC journal *Eurosurveillance* (Funk *et al*. 2021) aimed to compare the disease severity associated with VOC and non-VOC SARS-CoV-2. The authors concluded to a higher risk of hospitalisation: 2.6 higher (95% CI [1.4-4.8]) when adjusting for age, sex, week of reporting and country. However, this study is based on European countries (mostly Portugal) and a period during which the circulation of P.1 lineages was limited in those countries. As a result, the number of cases included in the study and associated with those variants is relatively low: only 436 (1.9%) and 352 (1.5%) cases, respectively. In conclusion, further investigations on the severity of P.1 infections are needed.

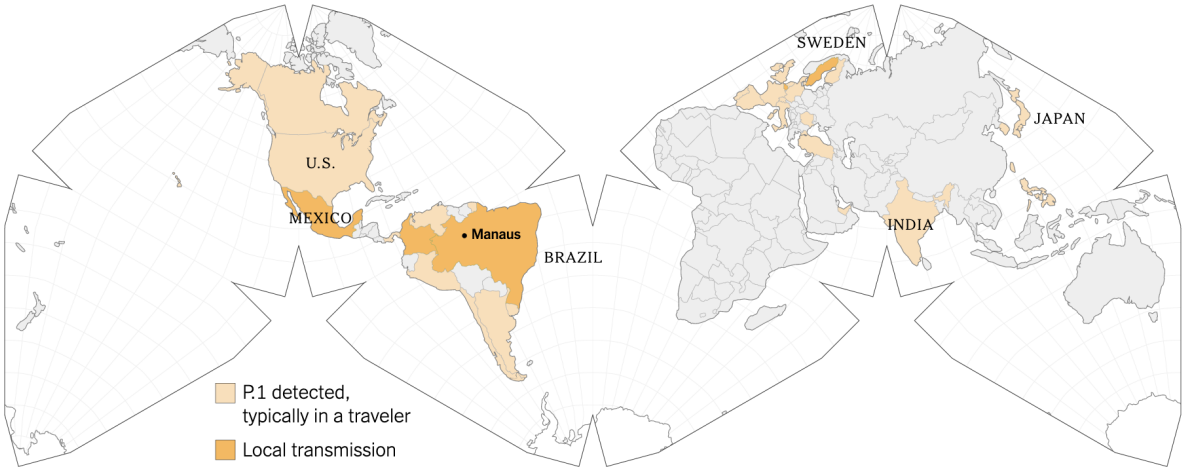



**Figure 8:** Current worldwide distribution of VOC 501Y.V3 (lineage P.1). Source: https://www.nytimes.com/interactive/2021/health/coronavirus-variant-tracker.html