Genomic surveillance of SARS-CoV-2 in Belgium

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

Situation update – 2nd of March 2021
(report 2021_15)

Executive summary

Genomic surveillance in Belgium is organised around 3 different arms aiming to monitor the emergence and the further spread of specific viral populations (variants of concern or VOCs) which may impact disease control and/or vaccination strategies.

Through baseline surveillance, an unbiased selection of positive samples from 24 sentinel labs (selected based on magnitude of diagnostic activity, geographical dispersion and diversity of clinical patterns) are currently analysed in 11 sequencing laboratories. Five of these laboratories are linked to university hospitals, and six are linked to other NGS reference centres. Currently, 7.766 Belgian sequences are available on GISAID, among which 4.160 (53%) are from samples collected after 1st of January 2021. Among samples collected during the weeks 6,7 and 8, 1.427 samples have been sequenced as part of the baseline surveillance initiative, among which 658 were 20I/501Y.V1 (46,1%), 102 were 20H/501Y.V2 (7,1%) and 25 were 20J/501Y.V3 (1,7%).

The majority of new infections occurring in Belgium are now caused by a specific VOC. Collectively, these VOCs are now driving the epidemic in Belgium and could be the cause of an upcoming rise in daily infections.

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

Collaborated to this report: *Simon Dellicour (ULB), Tom Wenseleers (KU Leuven), Keith Durkin (U Liège)*

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

Table of content

1. International context

Since the end of the year, 4 variants of concern (VOCs) have arisen independently of one another in the United Kingdom (20I/501Y.V1), South Africa (20H/501Y.V2) and Brazil (20J/501Y.V3 or P.1 and P.2). These variants harbour several mutations and deletions associated with higher infectiousness and immune escape. All variants are spreading internationally, with 4 VOCs having been detected to date in Belgium (2.291 for 20I/501Y.V1, 334 for 20H/501Y.V2, 51 for 20J/501Y.V3 – P.1 and 1 for P.2).

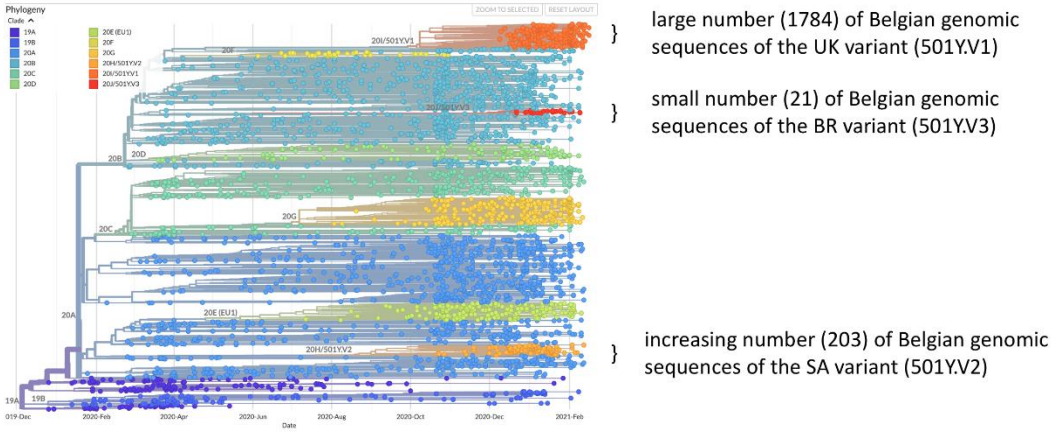

Figure 1: All 3 major VOCs currently described worldwide have recently emerged in Belgium and are actively spreading. Only a representative selection of characterized samples are included in this figure.

2. Baseline surveillance

Since support was offered by the federal government at the end of December 2020, both the temporal coverage (number of sequencing analyses performed per week) and geographical coverage (residence of the patients sampled) have improved significantly. Currently, 7.766 Belgian sequences are available on GISAID.

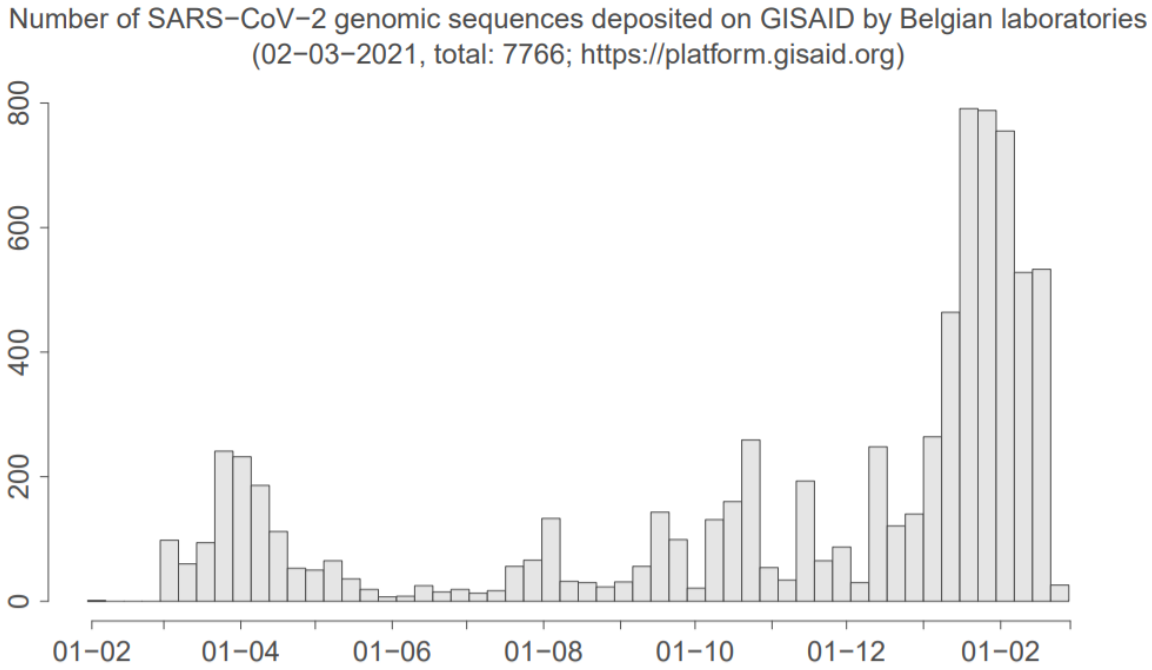

Figure 2: *Number of samples sequenced over time.*

Also the number of sequencing laboratories involved in the sequencing initiative has steadily increased since support was offered by the federal government. This is illustrated by the number of centres uploading data on GISAID. Before the scale-up, 4 out of 5 (80%) sequencing laboratories were linked to a university hospital. Since the start of this scale-up phase, we progressively included additional university hospitals and other reference NGS centres. Today, 6 out of 11 (54%) active sequencing laboratories are not linked to university hospitals. When considering the additional candidates, this proportion may evolve to 62% (13 out of 21) in the coming weeks.

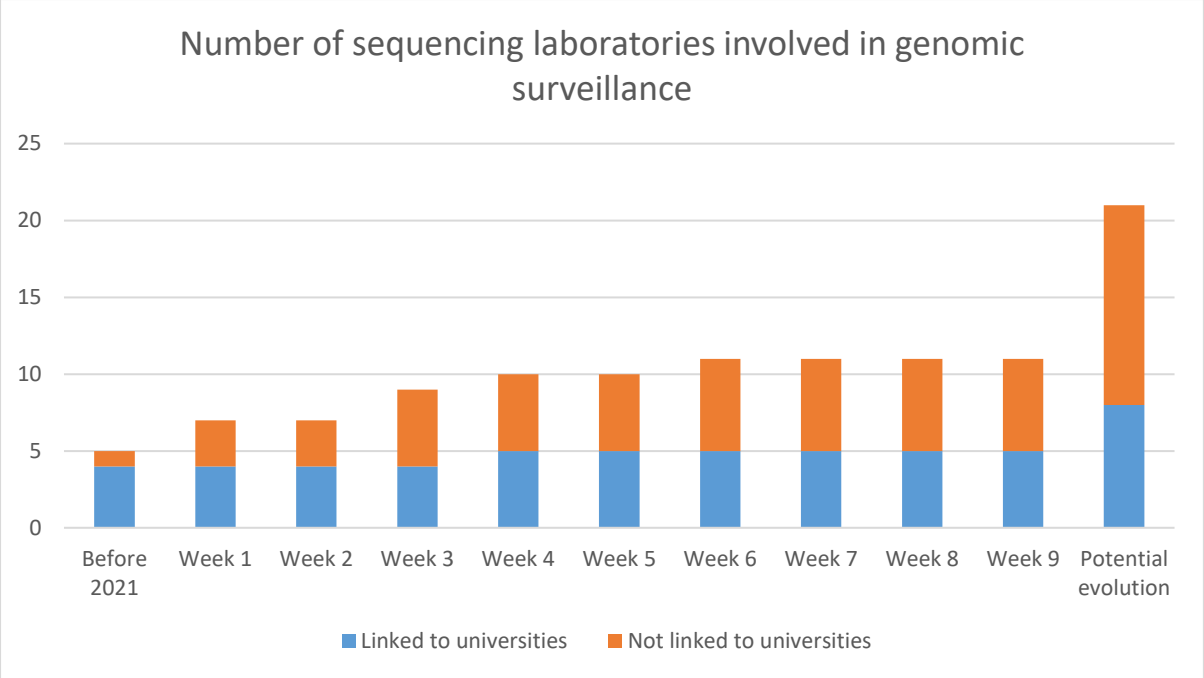

Figure 3: Evolution of participating sequencing laboratories.

As most of the new sequencing laboratories are in a start-up phase, 97% of the recent sequencing activity (informative information based on data available on GISAID since 1 January 2021) is still taking place in 6 major sequencing platforms, namely KU /UZ Leuven Reference Laboratory (39,5%), U Liège (27,3%), Jessa (10.3%), UZ Gent (9,2%), AZ Delta (8,3%) and UZA (5,5%).

Overall since the first positive case was diagnosed on Belgium, 1% of all positive samples have been sequenced, with a different, but still contained level of coverage between provinces (0,2% - 2,3%).

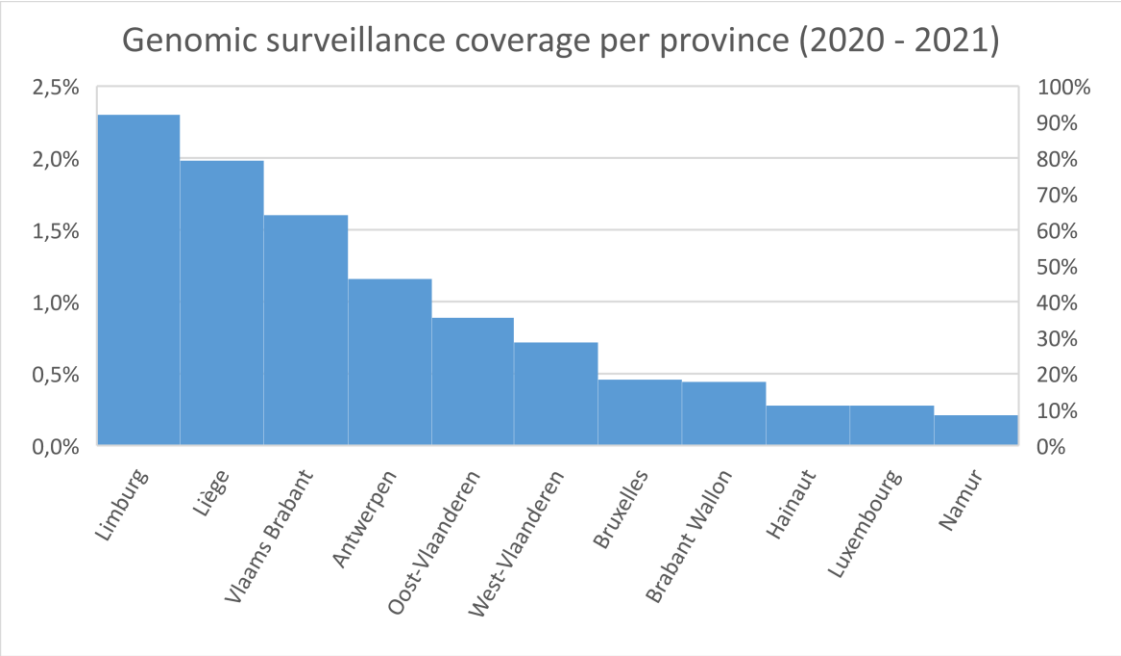

Figure 4: Genomic surveillance coverage per province since the start of the epidemic in Belgium.

Unfortunately, the scale-up of sequencing initiated since 1/1/2021 has to date unequally impacted the coverage at provincial level. When looking at the first 3 weeks of February 2021 and the initial scale-up objective of a 2-5% coverage, 3 provinces are now above this objective, 3 provinces are meeting the objective, and 5 provinces are below the objective. The National Reference Centre is actively collaborating with laboratories of the current sentinel network and beyond in each of the provinces not yet meeting the objectives and has developed an adapted follow-up plan for these. Awaiting a clear signal from the government for scale-up beyond the initial objective, a more uniform coverage of all provinces, and in order to avoid surveillance biases due to over-sampling, we do not at this stage of the scale-up encourage provinces exceeding the demanded coverage to further increase their activity.

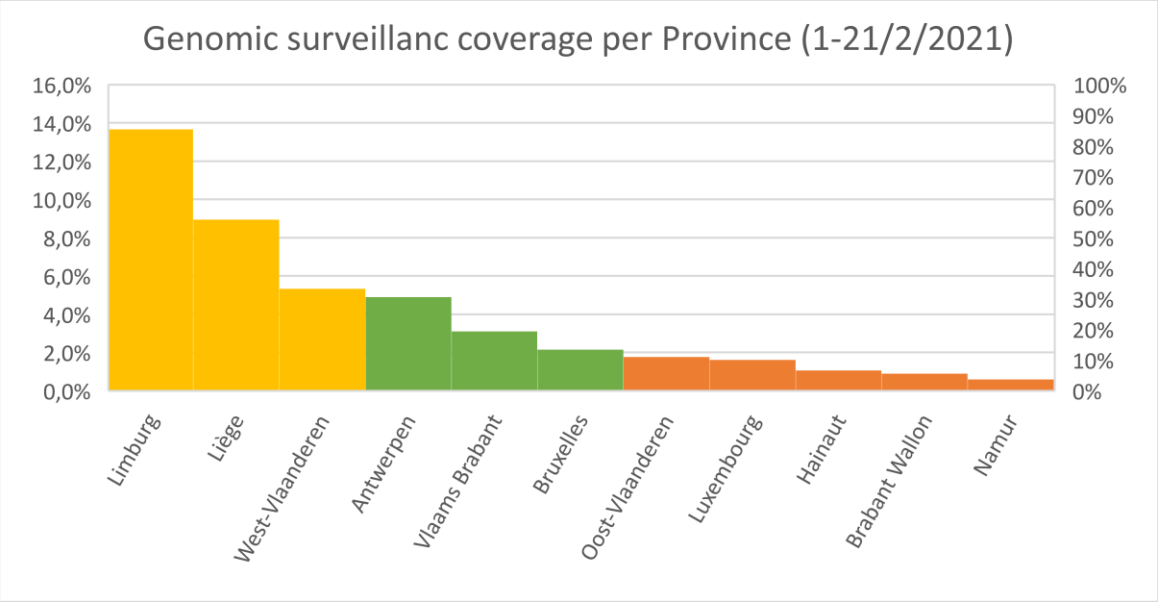

Figure 5: Genomic surveillance coverage per province for the first 3 weeks of February 2021.

WGS of unbiased samples (baseline surveillance) currently represents 60% (3.235/5.374) of samples which have been characterized since 1/1/2021 through the different molecular techniques currently used in the surveillance network. The different techniques include WGS, Sanger sequencing and reflex PCR targeting at least the 501Y and 484K Spike mutations.

3. Quality monitoring

The multiplication of additional sequencing laboratories will potentially increase turn-around time in some instances (e.g. less samples per centre increasing the time for a complete batch), but have a positive impact on the overall surveillance level. Nevertheless, this multiplication of sequencing centres has a potential impact on the cost per sample (as large numbers are a key determinant for NGS cost per sample) and on the burden with regard to quality supervision.

A number of initiatives are currently taken to monitor the quality of the results provided by the different sequencing platforms, including a first external quality assessment that was initiated last week. Once all laboratories have returned the results of this panel to the NRC UZ/KU Leuven, a summary will be provided in one of these follow-up reports. To properly evaluate quality assurance, we further need to take into account the fact that all platforms do not use the same sequencing technology and analytical pipelines. All sequencing laboratories have been asked to share their wet- and dry-lab standard operating procedures and they are currently evaluated and summarized at the NRC. Numerous quality metrics are being considered for the different steps of the sequencing process (e.g. to process and interpret the sequence data: e.g. minimal or median coverage and correct mapping of mutations, deletions and insertions). Overall, we obtain a good quality, although some outlier laboratories have been identified as illustrated in Figure 6, based on the quality parameter of missing information. Using WGS we expect the full genome of SARS-CoV-2 to be covered to a large extent, aiming for as much as possible sequences with a length of >90% of the SARS-CoV-2 whole genome. As shown in Figure 6, there currently exists a large difference in the number of sequences not meeting this criterion between the 6 labs that are currently contributing >97% of the data to GISAID.

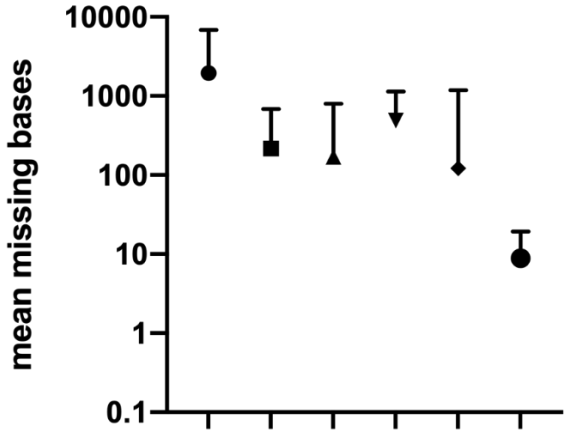

Figure 6: Mean missing bases (log scale) among sequences uploaded on GISAID per laboratory. The first laboratory has a significantly lower coverage of the viral genomes, and the entire sequence of the genome is often not available for further analysis. Names of laboratories are not provided in this report. An individual feedback will be provided to all participating laboratories.

4. Monitoring of VOCs in Belgium

Among samples collected during the weeks 6,7 and 8, 1.307 samples have been sequenced as part of the baseline surveillance, among which 605 were 20I/501Y.V1 (46,3%), 99 were 20H/501Y.V2 (7,6%) and 24 were 20J/501Y.V3 (1,8%).

For the follow-up of 501Y.V1 (B.1.1.7), we complement baseline surveillance (weekly evolution) with the daily follow-up of the "S dropout" signal detected among positive COVID-19 PCRs reported by the 8 federal platform laboratories. In order to obtain the best view on the number of recent infections actively contributing to transmission, we consider for the daily follow-up only positive samples for which the N gene has a Cq value under 25. By excluding for this analysis, the samples with a Cq value between 25 and 30, we avoid including possibly older infections and possible false positive S dropout signals that can occur when the signal is close to the limit of detection. Over 50% of the COVID-19 infections diagnosed during the last days are actively infectious and harbour the 501Y.V1.

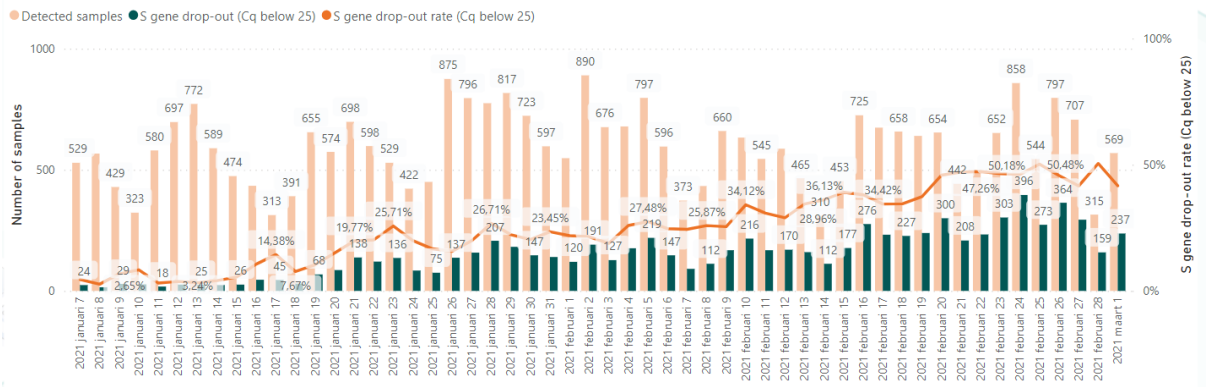

Figure 7: *Daily evolution of the proportion of infectious samples detected among all positive tests diagnosed in the federal platform laboratories (Presence of the S dropout signal and Cq <25).*

5. Impact of VOCs on the dynamic of the epidemic in Belgium

The ongoing replacement of previous circulating strains by 501Y.V1 has a measurable impact on the evolution of the epidemic, as it has "pulled" the reproduction rate above 1 despite the current measures in place, a phenomenon that would not have occurred otherwise. The impact of this more transmissible VOC is nevertheless currently partially controlled in Belgium, and the shift in viral populations did not – at this stage - translate into a major increase of cases.

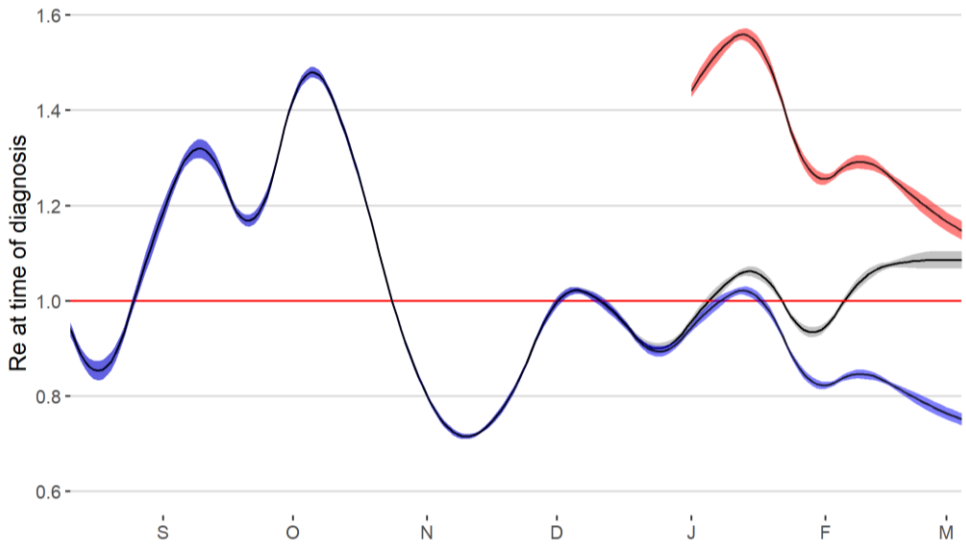

Figure 8: Evolution of Re of 501Y.V1 (red) and other circulating strains (blue) in Belgium based on new confirmed cases and estimated 501Y.V1 transmission advantage.

The Federal Platform laboratories are finalizing the validation of a VOC PCR test that will be performed as a reflex test on all positive samples. Based on the validation results presented by the NRC, federal platform laboratories have jointly decided to use the TF kit, as it offers the possibility to detect and discriminate all VOCs currently circulating in Belgium. Further, this approach will allow some flexibility in the design of the test as further mutations of concern may emerge in the future. Federal Platform laboratories will perform this test in complement to baseline WGS surveillance as long as it will be asked by regional public health agencies.

This initiative will allow monitoring over time – and throughout the vaccination rollout - the evolution of VOCs harbouring immune escape mechanisms. 501Y.V3 is still limited to two clusters for the moment (figure 9).

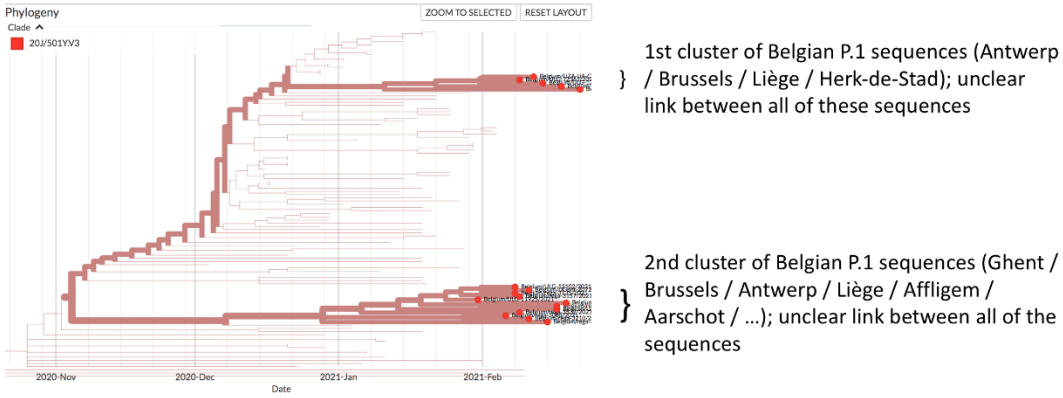

Figure 9: Currently, all 501Y.V3 strains are assigned to 2 clusters, which spread over several provinces.

6. Positivity rate in federal platform laboratories

The proportion of positive samples detected among all samples tested is an indicator used to monitor throughout the different phases of the epidemic if the number of tests performed is sufficient to support disease-control interventions. Under 5%, we estimate that the situation is under control, while a positivity rate above 10% is usually the sign that testing should be leveraged to efficiently support disease-control interventions. A positivity rate above 15% is usually the sign that the situation is out of control and that a consistent proportion of infected patients are left untested.

This rate has increased from January to February (5,9% to 7,8%), and it currently at 8,9% for March. Increasing testing and/or enlarging testing criteria should therefore be considered.

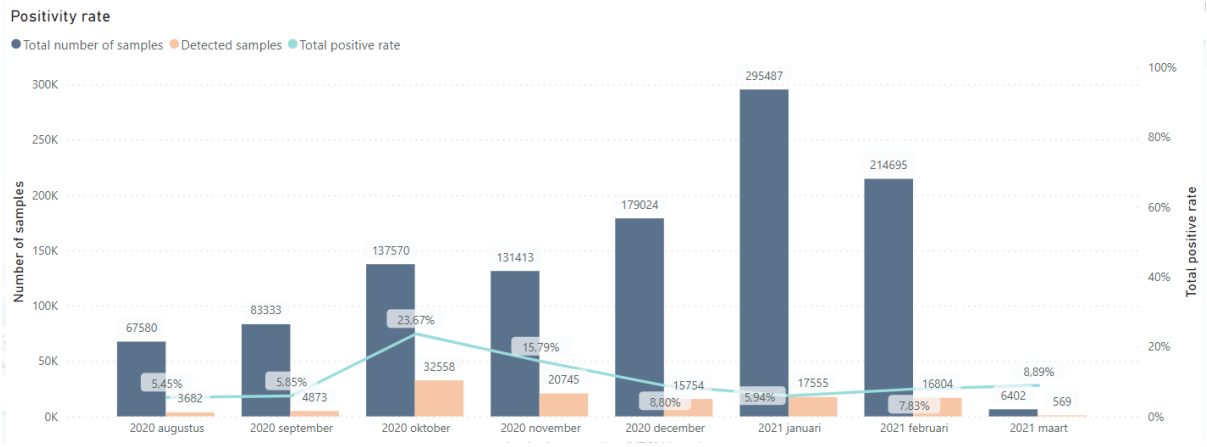

Figure 3: *Monthly (figure above) evolution of the proportion of infectious samples detected among all tests performed in the federal platform laboratories*

7. Proportion highly infectious samples among positive samples detected

The proportion of positive samples presenting a very high viral load (Cq < 15) can be seen as the number of patients diagnosed during the first days of infection, when they are highly infectious. This proportion tends to increase when the tracing is efficient in identifying recent transmissions but can also be observed in the early weeks of a resurgence.

This rate has increased significantly from January to February (21% to 30% ; please note that a new version of the software generated changes compared to the last report), and is currently 28% for March. The risk of super-spreading events is currently important, and we therefore discourage large events, in particular when transmission cannot be prevented efficiently.

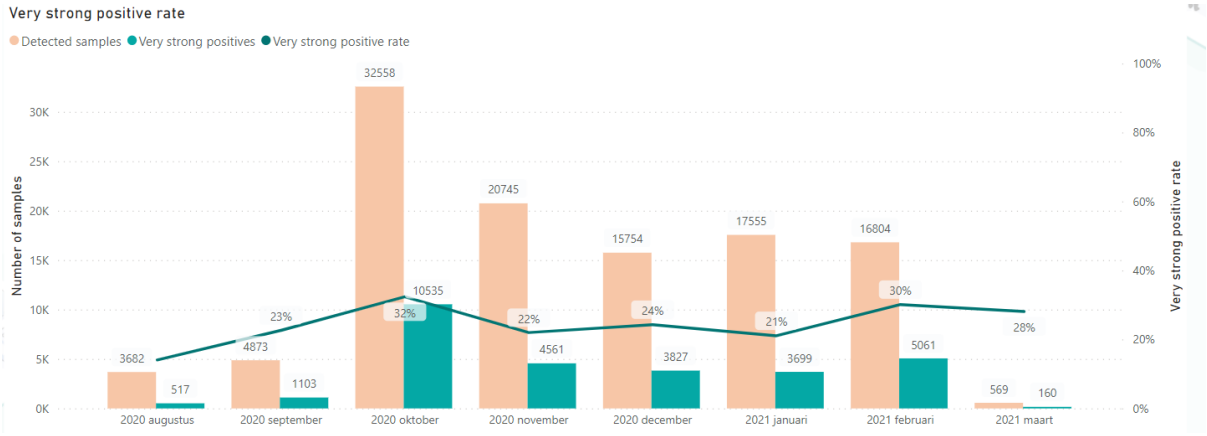

Figure 10: *Monthly (figure above) and daily (figure below) evolution of the proportion of highly infectious samples detected among all positive tests diagnosed in the federal platform laboratories (Cq <15).*