

SARS – CoV-2

Variant surveillance in the Czech Republic

Helena Jirincová

NRL for Influenza and Respiratory Viruses

National Institute of Public Health



Alexander Nagy

Laboratory of Molecular Biology, State Veterinary Institute



Capacity

- 130 laboratories involved in SARS – CoV-2 PCR detection
- Maximal capacity 50 000 samples/day
- Four local producers of PCR kits
 - Diana Biotechnologies (EndoRNase, Spike) 20 % of the market
 - Elisabeth Pharmacon (E, Orf1ab, N)
 - Generi Biotech (E, RdRp)
 - Geneproof (E, Orf1ab, N)

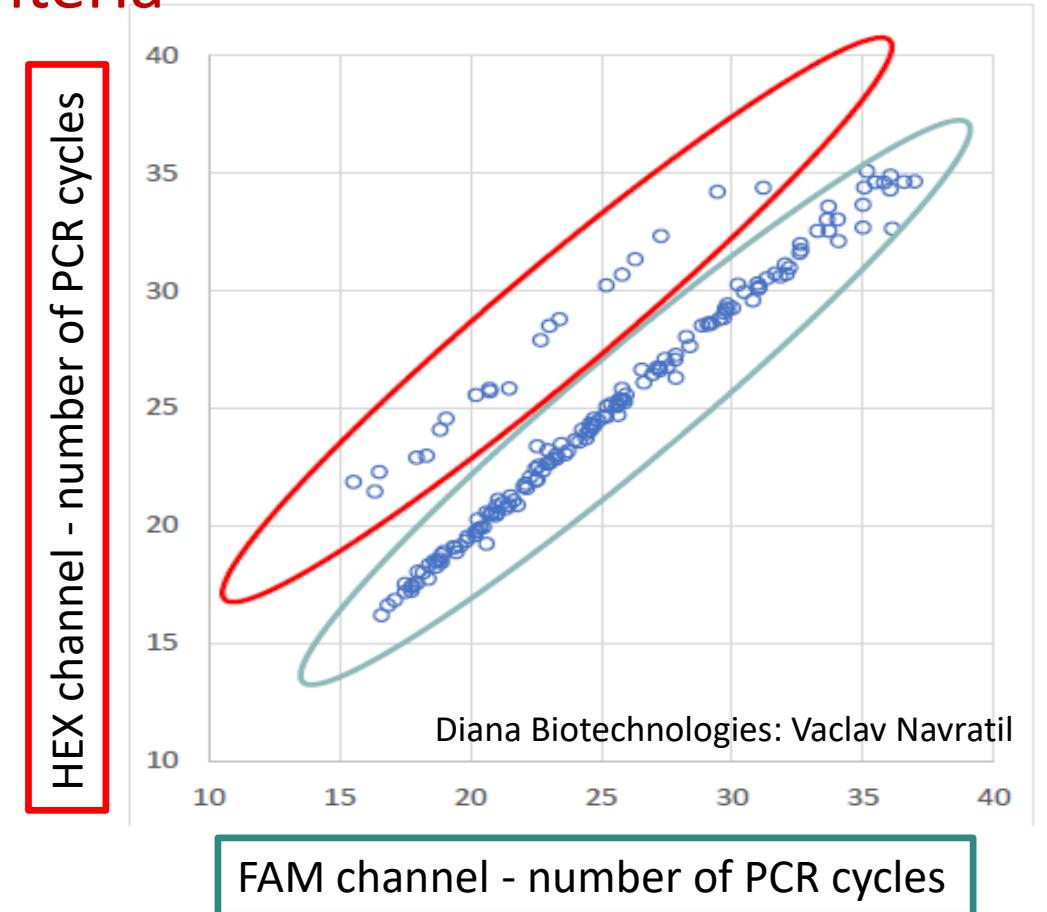
Other oftenly used PCR kits /platform: Liferiver, Seegene / GeneXpert

First detection of B.1.1.7

Based on in 1) silico analysis and 2) WGS criteria

- In silico analyse od RT-qPCR data
 - spike - A570D mismatch primer

- Resulting in the 5 Ct shift



- Retrospective analysis - 5 laboratories – 7th December 2020

PCR based surveillance

B.1.1.526 (South America) S: A701V, D253G, D614G, **E484K**, L5F and T95I

varianta	Pangolin lineage	417N	417T	439K	484K	501Y	570D	del 69,70
Britská	B.1.1.7	-	-	-	-/+	+	+	+
JAR	B.1.351	+	-	-	+	+	-	-
Brazilská	B.1.1.28	-	-	-	+	-	-	-
Brazilská/Manaus	P.1	-	+	-	+	+	-	-
	B.1.525	-	-	-	+	-	-	-
tzv. "česká"	B.1.258	-	-	+	-	-	-	+

The 19B with N501Y is now assigned lineage A.27

19B with N501T, assigned as A.28

In France, we are currently Following up such a variant - also has 452R

B.1.525 also linked to import from Nigeria in cases in Norway

Hi Helena, As mentioned in a previous e-mail, we are happy to support the sequencing effort at your end. let me know if we

PCR based surveillance

- Single step PCR for the detection of nt substitutions of concern
 - Single multiplex PCR (SARS – CoV-2 + IC + nt substitution)
- Two step PCR for the detection of nt substitutions of concern
 1. SARS – CoV-2 detection + IC
 2. PCR for the detection of nt within the cohort of positive samples

All positive	Part of positive pool
Same day	Once per week

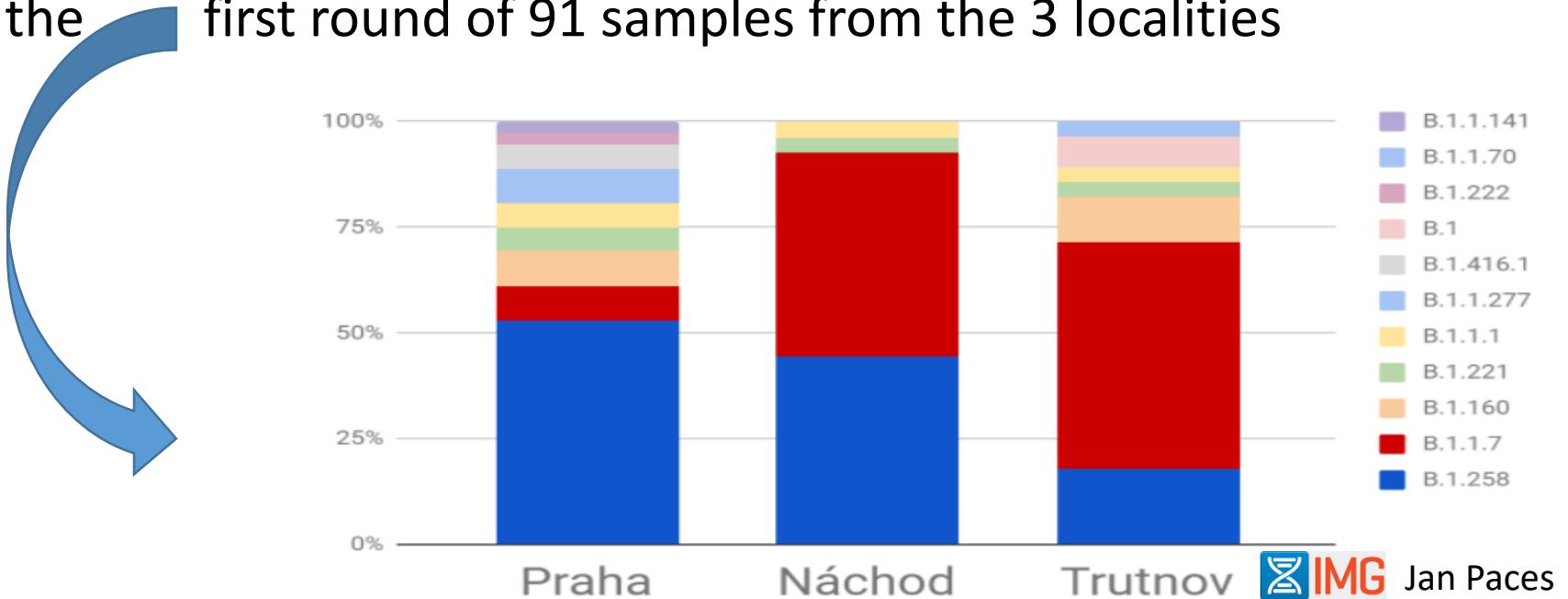
WGS surveillance

NRL & SVU: - Oxford Nanopore Minlon, Artic protocol, 1500 pb

- The only one group has been continuously sequencing from the index case (1. 3 . 2020)

Czech Academy of Science (Swift Protocol, NextSeq)

- Early January 2021, the first round of 91 samples from the 3 localities



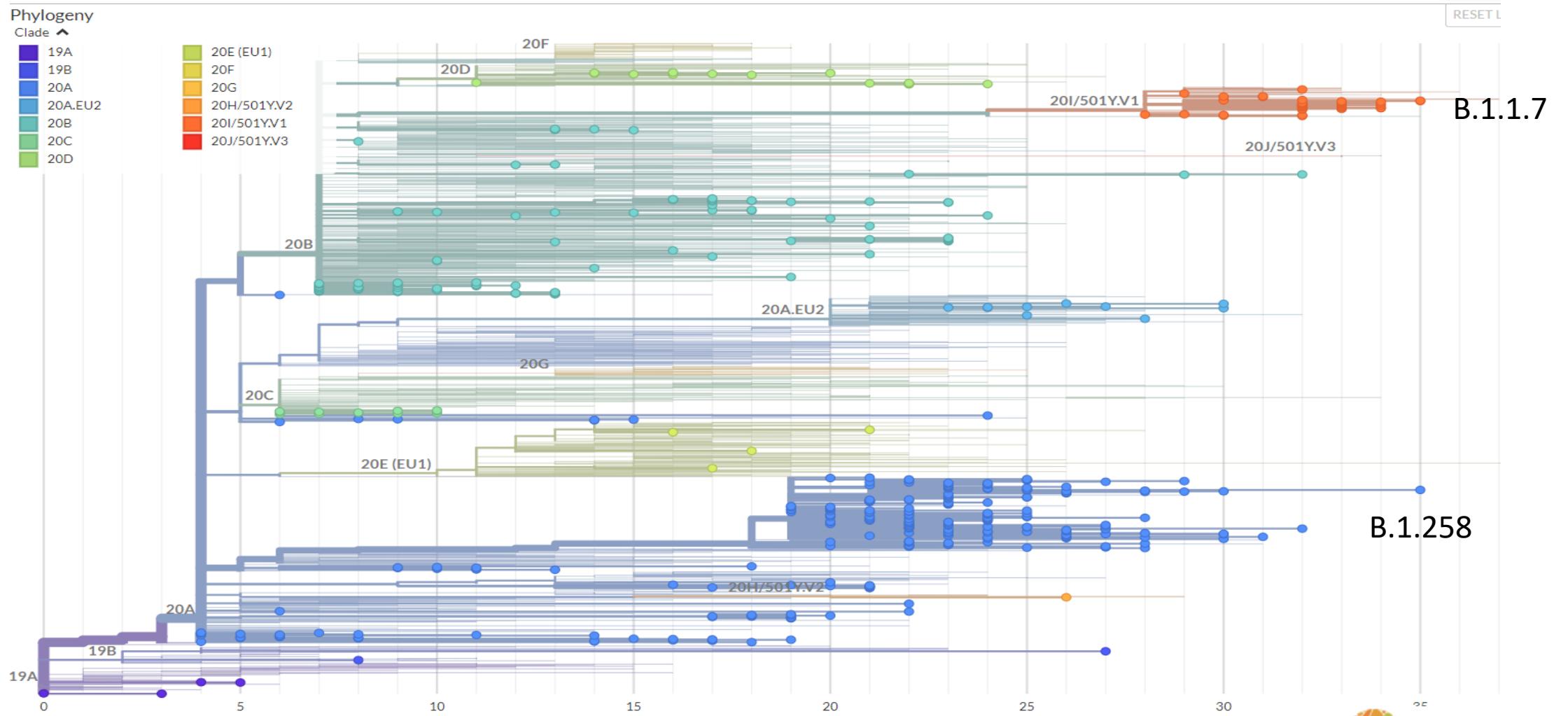
- PHE- ECDC sequencing support, the first 9 of samples sent 12.2. 2020

Samples intended for the WGS – WGS criteria

- Increased R in local outbreaks
- Accumulation of low Ct values in the local outbreaks ($Ct < 16$)
- Increasing proportion of inapparent cases with $Ct < 26$
- Atypical clinical manifestation / atypical positive clinical material (liquor)
- Hospitalised severe cases under 50 YoA without comorbidities
- Hospitalised under 16 YoA
- Airport arrivals
- Positive cases after vaccination $Ct < 30$

Phylogeny treee SARS –CoV-2 Czechia

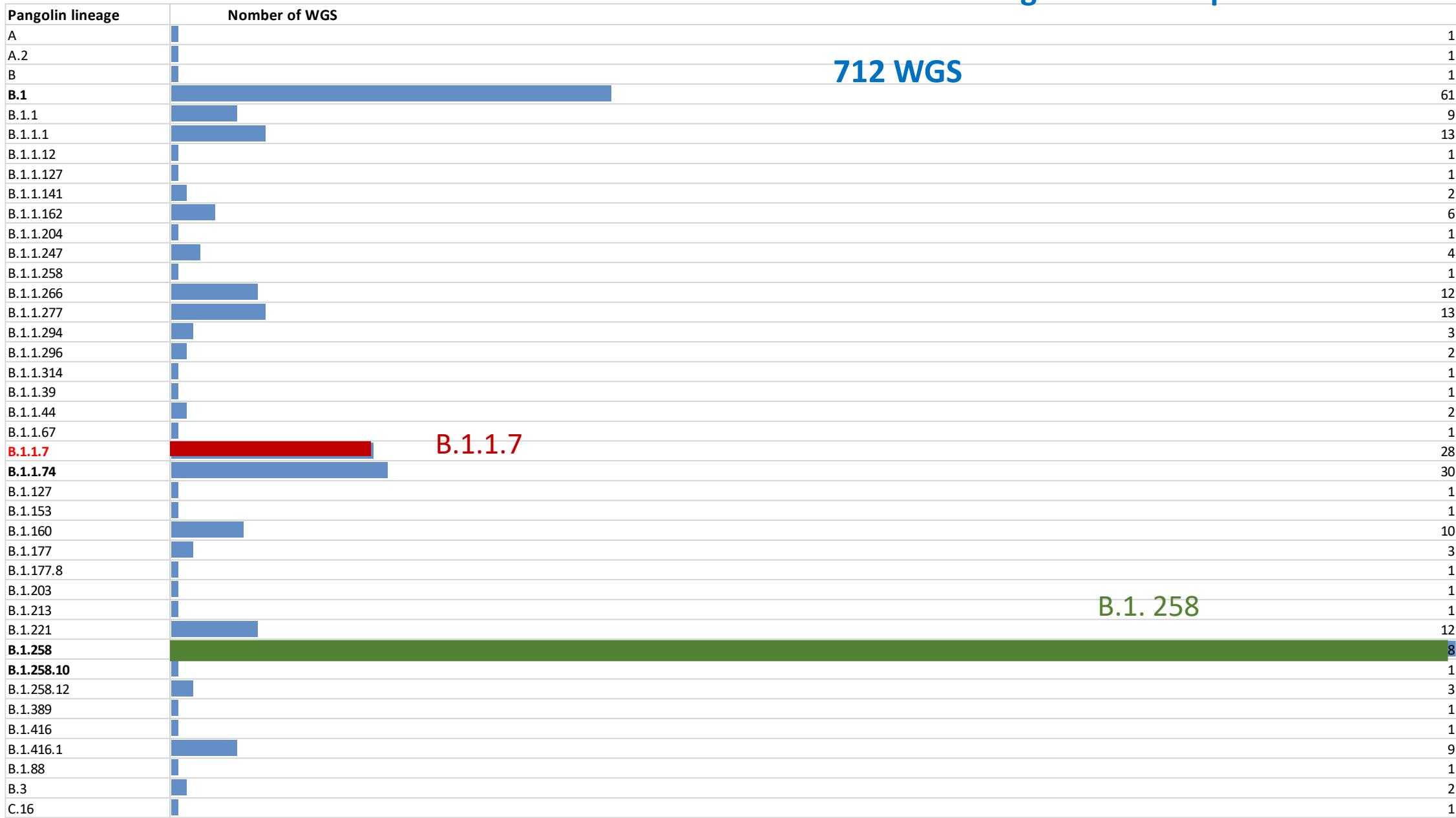
423 of consensus fasta – NRL and State Veterinary Institute



Czechia: in total 651 WGS in GISAID + 61 waiting for the complete metadata

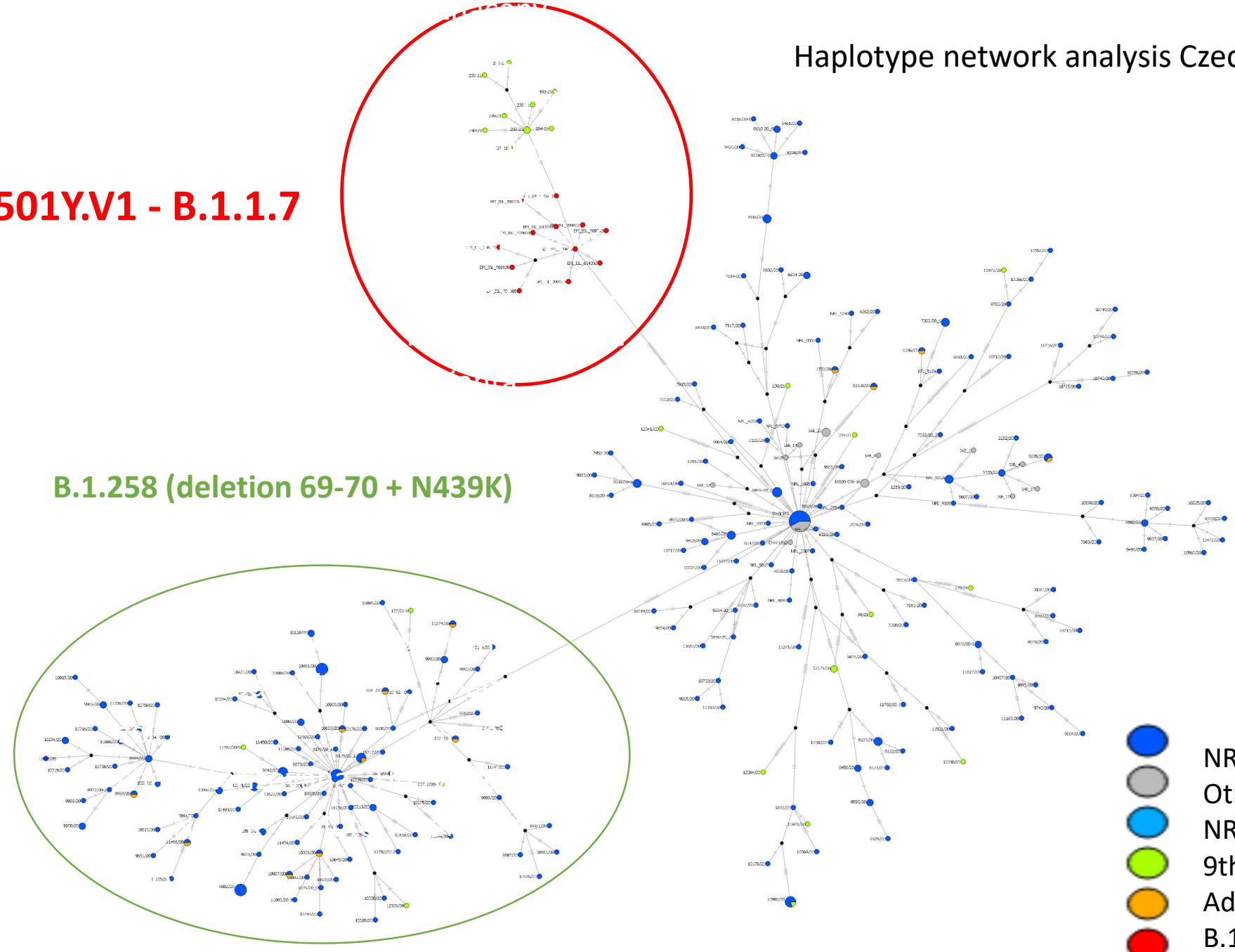
WGS

423 WGS – NRL and State veterinary Institute

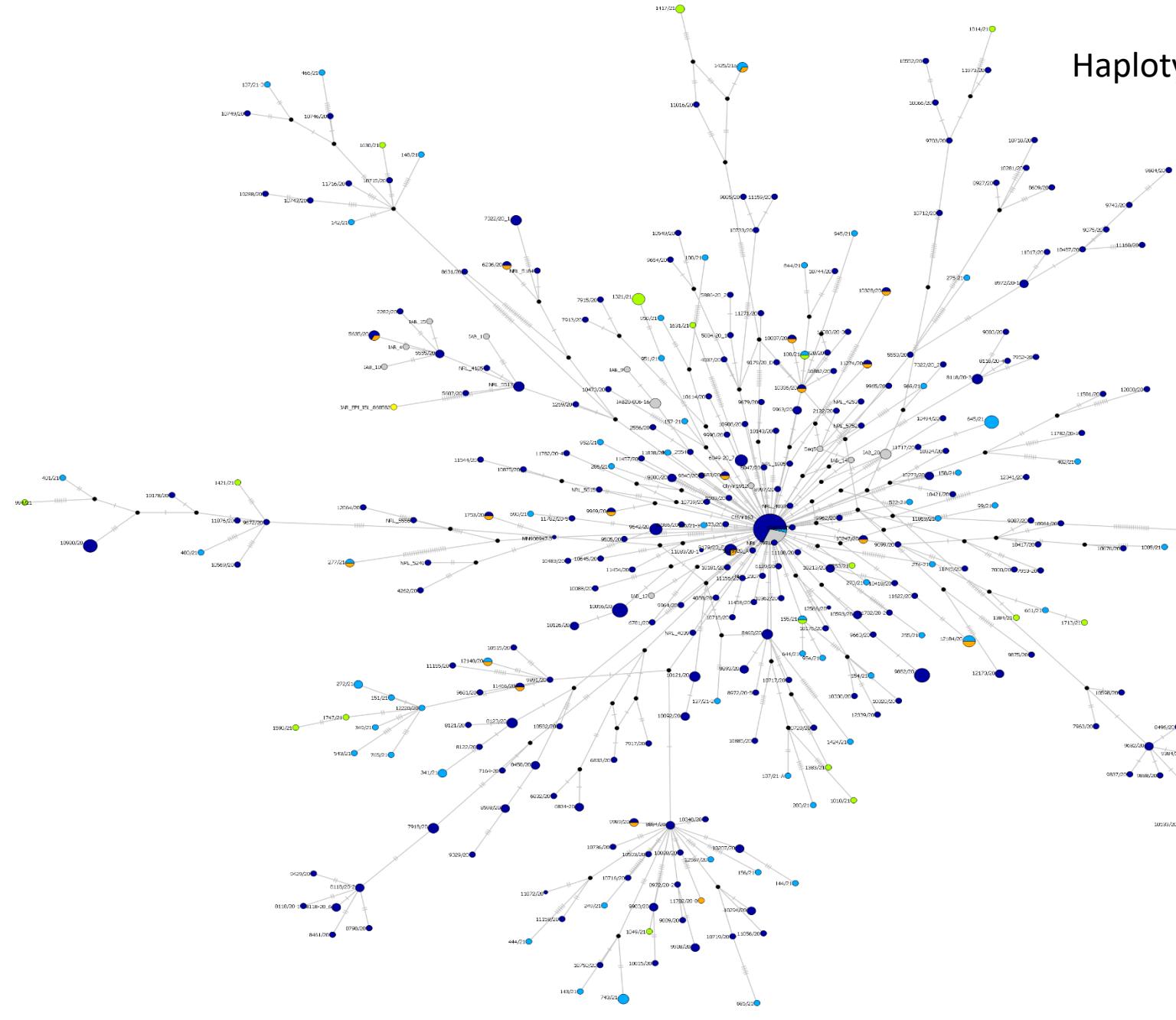


Haplotype network analysis Czechia - 19-01-2021

20I/501Y.V1 - B.1.1.7



Haplotype network analysis Czechia - 19-02-2021

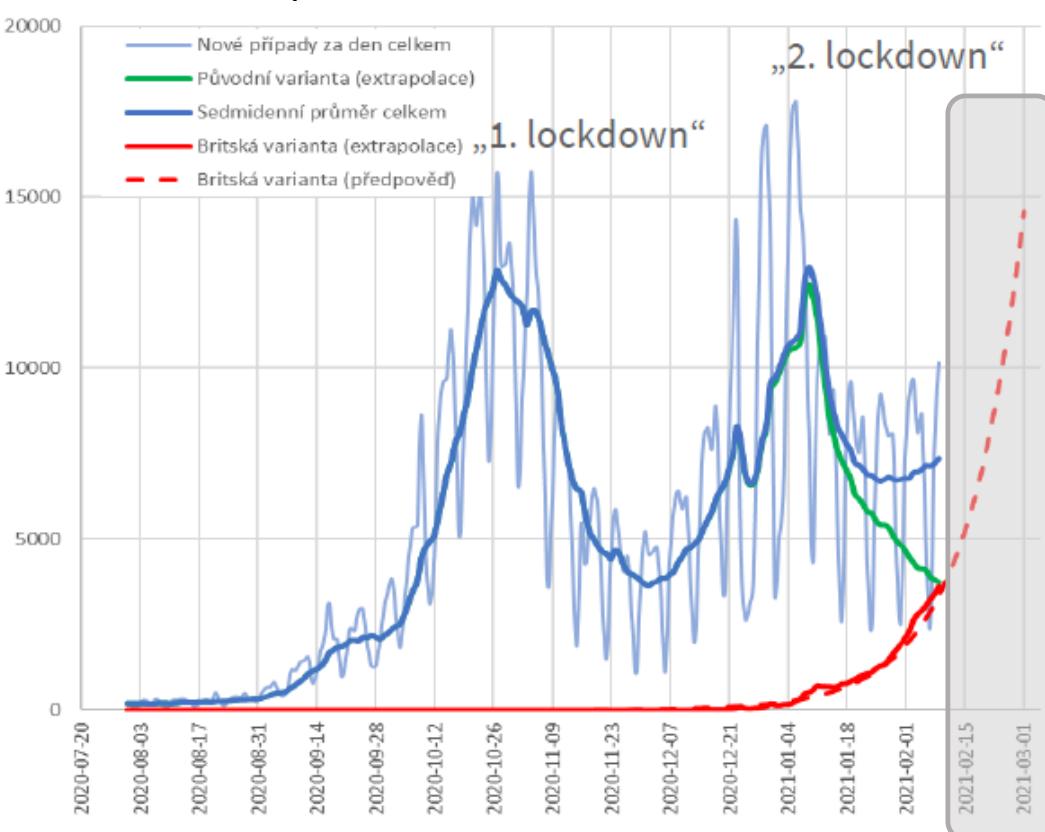


20I/501Y.V1 - B.1.1.7

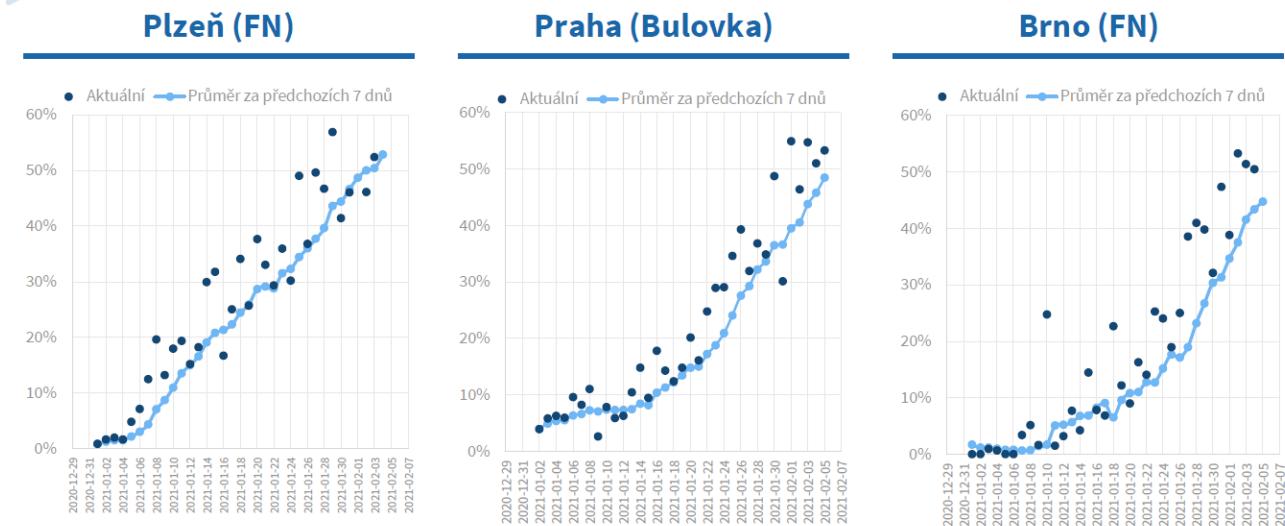
- NRL 2020
- Other labs 2020
- NRL 2021
- 9th January – 19th February
- Additional nt deletion
- B.1.1.7 UK strains

PCR surveillance

Number of positive cases 20-7-20 till 10-02-21

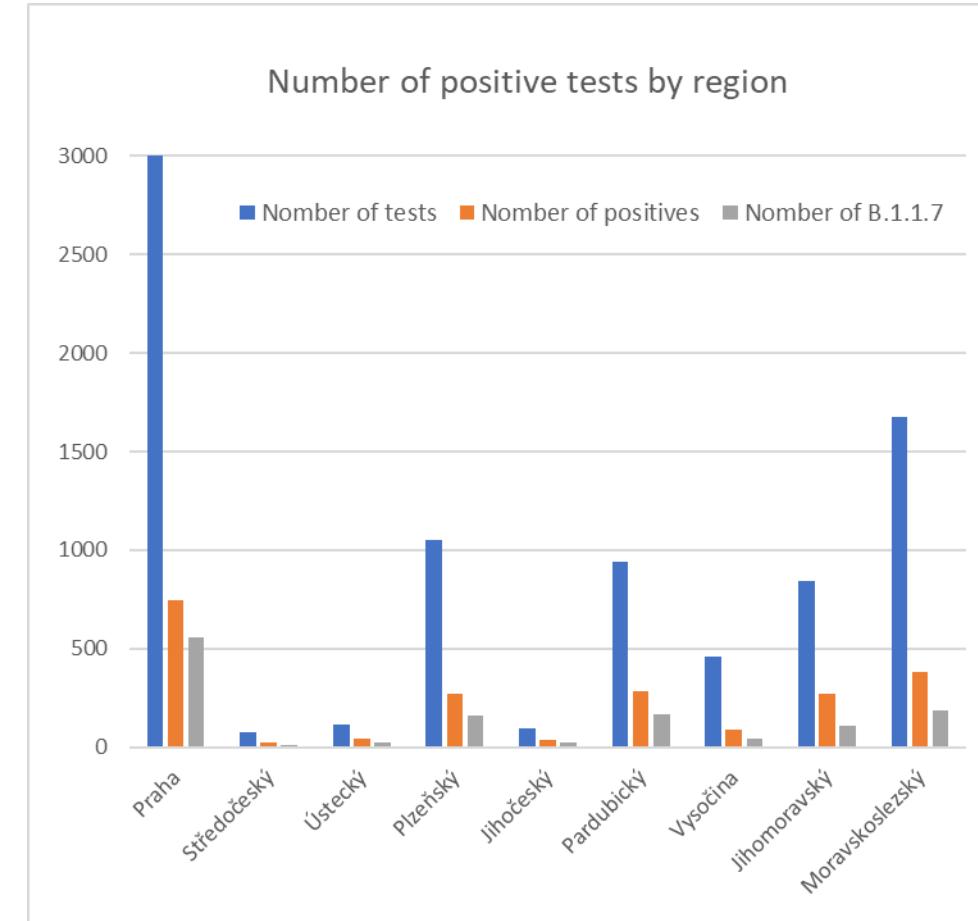
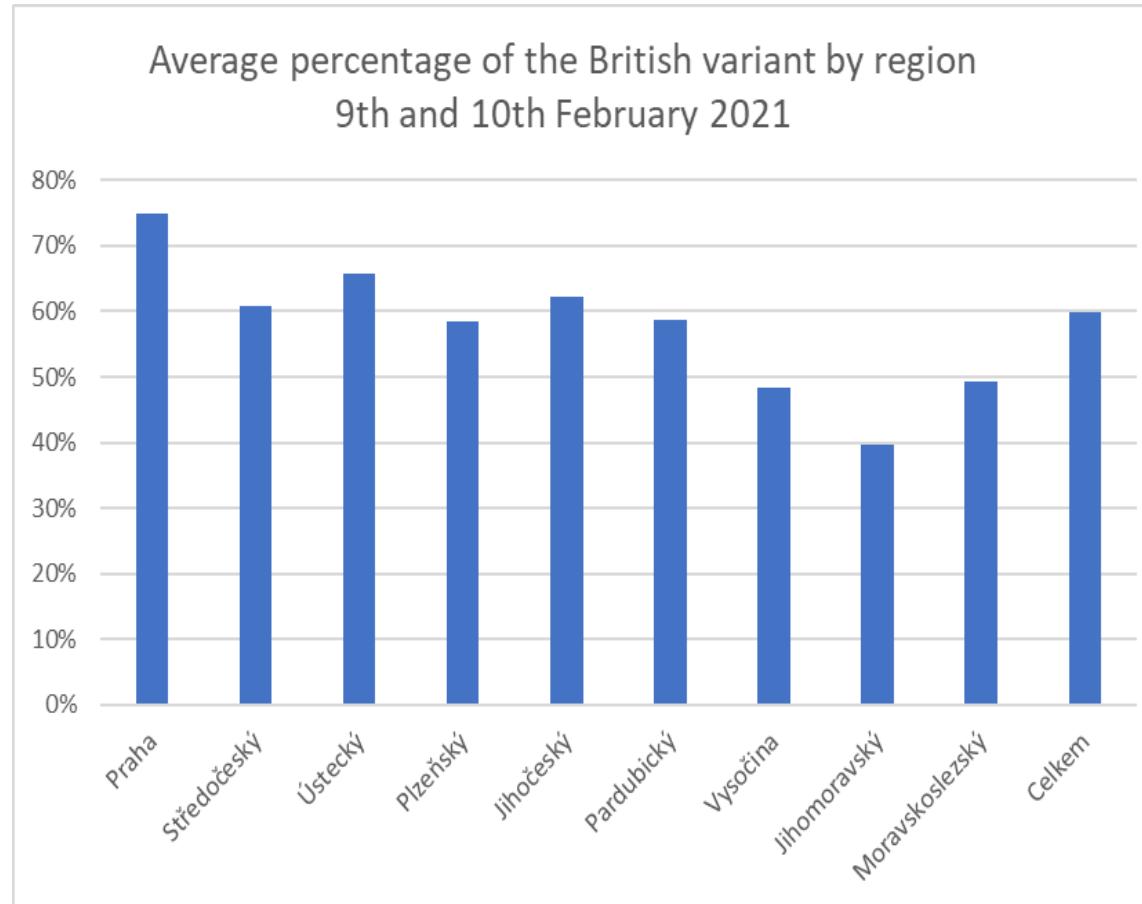


Proportion of the detected B.1.1.7 by RT-q-PCR
Results of 3 laboratories, period 29-12-20 till 7-2-21



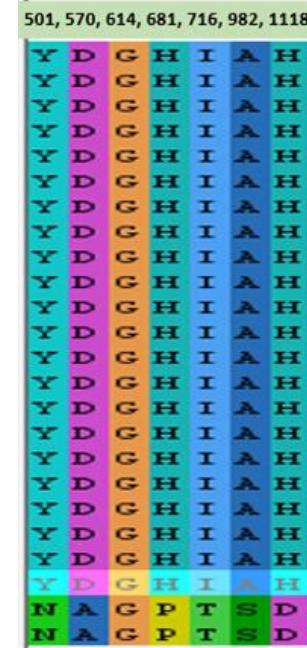
Approximation by 1. 3 .2021

PCR surveillance (20 % of tests done on regular basis in the Czechia):
The proportion of the "British variant" has been growing rapidly since
the beginning of January



Ongoing activity - future prospective

WGS consortium (NRL and the Czech Academy of Science)



- NRL for influenza and Respiratory Viruses, NIPH
Jaromira Vecerova, Marcela Trinklova, Helena Jirincova
- Laboratory of Molecular Biology, SVU:
Lenka Vernikova, Alexander Nagy
- Diana Biotechnologies: Vaclav Navratil
- Institute of Molecular Genetics of the Czech Academy of Sciences:
Michal Kolar, Jan Paces, Miluse Hradilova