Státní zdravotní ústav

Sentinel surveillance of respiratory viruses including SARS-CoV-2 during the season 2023/2024 in the Czech republic

Investigator: NRL for Influenza and Non-Influenza Respiratory Viral Diseases of the State Institute of Health in Prague (hereinafter referred to as NRL)

The aim of the project was to ensure integrated virological surveillance of ARI/ILI and SARS-CoV-2 without the possibility of ensuring a sufficient number of adequate biological samples from each region of the Czech Republic.

The integrated system enables systematic monitoring of the occurrence of influenza viruses and non-influenza respiratory viruses, including SARS-CoV-2, in the population of the Czech Republic as part of sentinel surveillance of respiratory viruses.

Description of the current situation

The basis for ARI surveillance in 2023/2024 was the existing model, within which each primary KHS ensures the collection of materials from the upper respiratory tract in the outpatient clinic, including transport to the NRL. These are swabs from pediatric patients in the pediatrician GP office and swabs from adult patients (at least a third of whom are in the 18-49 age group and at least a third in the 50+ age group) in the GP office.

Sampling period

- At least from the 39th calendar week of 2022 to the 22nd calendar week of 2023, ECDC and WHO recommend year-round

Expected sample size

- Minimum 5 samples/week/region; i.e. 2,240 per season including extraordinary samples from outbreaks

Respiratory agents tested

Nasopharyngeal samples from patients with ARI are tested using multiplex PCR at the NRL after nucleic acid isolation

Spectrum of respiratory agents tested:

- Influenza A (including subtypes H1, H3, H5, H7)
- Influenza B (Yamagata and Victoria lineages)
- Influenza A typing A/H1N1 pdm; A/H3N2
- adenoviruses
- RSV (includes A/B typisation)
- metapneumoviruses ((includes A/B typisation)
- rhinovirus (types A, B, C)
- enterovirus (types A, B, C, D)

- bocavirus (human bocavirus 1)
- seasonal coronaviruses (OC43, 229E, HKU1, NL63)
- parainfluenza viruses (types 1–4)
- parechovirus (types 1–8)
- adenoviruses
- SARS-CoV-2

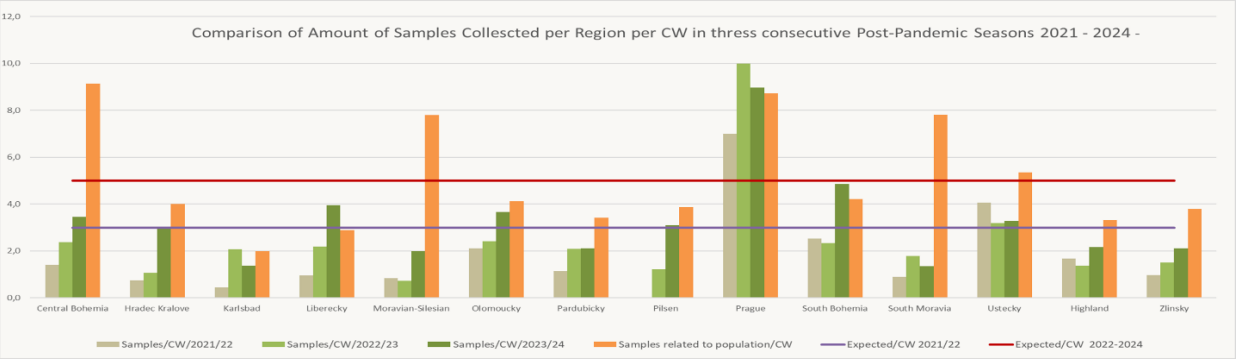
Results:

As part of the project, all samples sent to the NRL in 22 KTs were examined, i.e. 1588 samples. An average of 3 samples were sent from each KT from the regions, the least from the South Moravian Region - 1.3 samples/KT, the most from Prague - 9 samples/CW - .see Tab 1

Tab 1: Comparison of sent and examined samples in 3 seasons

Season	Number of samples examined	Number of Calendary Weeks	Expected number of samples	Proportion of negative samples	Average number of samples/CT
2021/22	891	36	3	51%	1,8
2022/23	1171	35	5	33%	2,5
2023/24	1588	35	5	38%	3

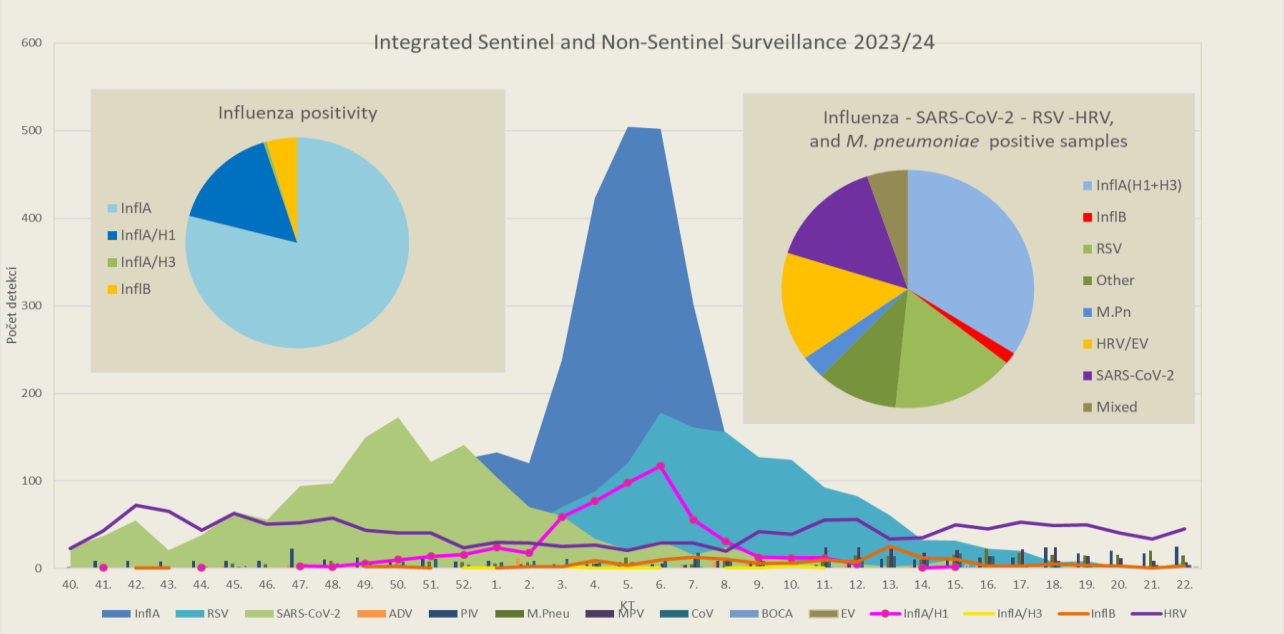
Graph 1: Comparison of the number of expected and examined samples in the three seasons .



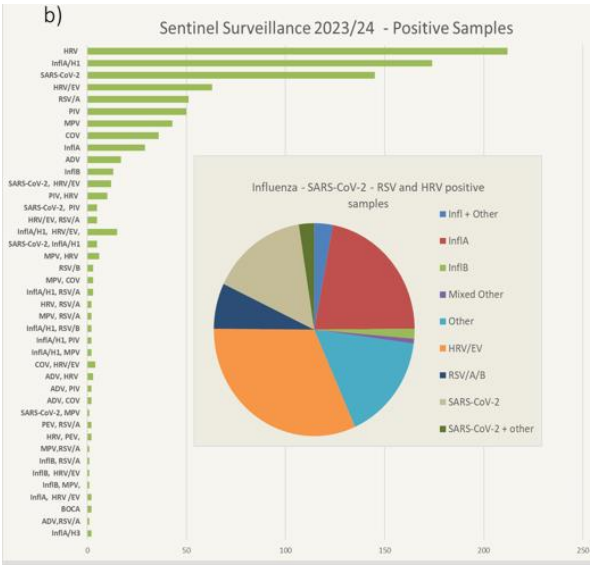
Graph 1: Legenda graf 1: The numbers (green) should correspond to at least the expected number, or in the case of less populated regions, reach the level of the orange column) theoretical number of samples in a distribution taking into account the population

The 2023-24 respiratory season was characterized by two major overlapping waves of dominance of three respiratory viruses. The first wave began in 40–42 CWs 2023 and ended in 5th CW 2024 and was characterized by the dominance of SARS-CoV-2 and the beginning of the influenza epidemic A/H1N1pdm in 50th CW. The second wave, 50th CW to 18th CW, represented an influenza epidemic with dominance of A/H1N1pdm and co-circulation of RSV. A/H3N2 viruses also appeared to a small extent and the end of the wave would be characterized by the circulation of influenza B/Victoria. B/Yamagata was declared a deescalated variant, and has not been detected worldwide since 2019. Rhinoviruses circulated for almost the entire season. Other respiratory viruses were detected in low numbers, peaks were almost undetectable during the epidemic and their maxima varied throughout the season, the end of the season was characterized by a slight increase in parainfluenza and metapneumo viruses and seasonal coronaviruses. (see Chart 2)

Chart 2a): Summary of the cumulative virus detected in sentinel and non-sentinel surveillance,



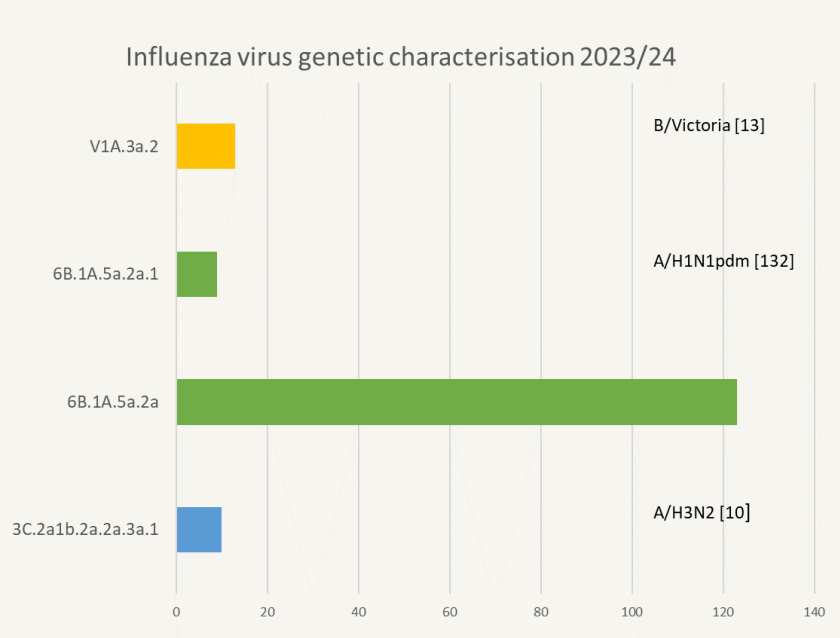
2b) Proportion of respiratory viruses detected in sentinel surveillance



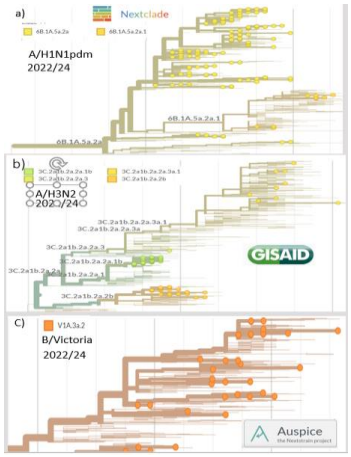
Molecular surveillance of influenza viruses

As part of virological/molecular surveillance, 155 influenza viruses A/H1N1 pdm, A/H3N2 and B were sequenced by whole-genome sequencing (Tab: 1). This number proved to be not entirely sufficient for the characterization of circulating variants in the Czech Republic due to the small representation of A/H3N2, given the evolutionary dynamics of H3N2.

Chart 3 A/H1N1pdm, A/H3N2 and B/Victoria positive samples characterized by WGS



Clade	2023/24
3C.2a1b.2a.2a.3a.1	10
6B.1A.5a.2a	123
6B.1A.5a.2a.1	9
V1A.3a.2	13
Celkový součet	155



Molecular surveillance of SARSCoV-2

All samples positive for SARS-CoV-2 (a total of 899 in the period September 23 - May 24), June-July (35) and August - September /24 (36) were sequenced using the NGS (WGS) method, see Table 3. However, compared with global data and data from the currently ongoing molecular surveillance within the non-sentinel cohort of SARS-CoV-2 samples, the number of samples from sentinel surveillance is insufficient for the needs of molecular surveillance and therefore genetic characterization and does not allow determining the dynamics of circulating variants in the Czech Republic.

The results of the whole-genome sequencing of SARS-CoV-2 are divided into 3 periods, the respiratory season (September 23 - May/24), the summer period - June - July 24, and the period of the onset of the KP.3.1.1 variant in August 2024

Tab 3: SARS-CoV-2 Variant of SARS – CoV – 2 ba WGS

09/23 – 05/24

Varianta	Počet
JN.1.4 (co	128
JN.1	103
JG.3	44
JN.1.4	44
EG.5.1.1	33
GS.4.1	29
HK.3	28
FL.1.5.1	26
HV.1	26
JD.1.1	26
EG.5.1.3	23
GS.4	21
XBB.1.16.1	21
XBB.1.16.1	21
JN.1.1 (co	20
BA.2.86.1	16
JN.1.22 (c	15
XBB.1.16	12
EG.5	11
GJ.1.2	10
JN.1.1	10
JN.1.22	10
EG.5.1.4	8
HK.3.2	8
HK.6	8
JN.1.2 (co	8
XBB.1.16.6	8
XBB.2.3.11	8
HF.1	7
JN.2	7
FU.2.1	6
XBB.1.16.1	6
JN.18 (con	5
JN.5	5
XCP	5
Varianty <	133
Celkem	899

06/24 – 07/24

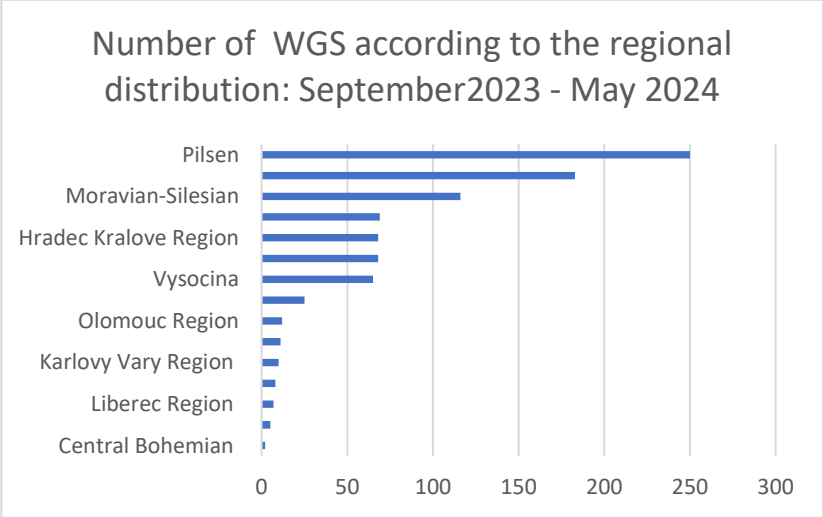
Varianta	Počet
KP.3.1.1	10
KP.3.3	4
KP.2	3
JN.1.50	2
KP.1.1.3	2
XDV.1	2
JN.1.1	1
JN.1.18.2	1
JN.1.39.1	1
KP.2.3	1
KP.3	1
KP.3.1	1
KP.3.1.4	1
KP.3.2.1	1
KS.1	1
LB.1.3	1
MC.1	1
MD.1.1	1
Celkem	35

08/24 – 16/9/24

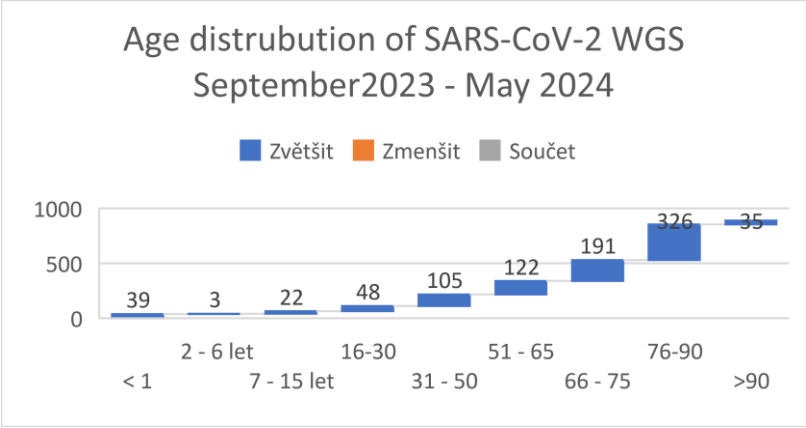
Varianta	Počet
KP.3.1.1	25
KP.2.3	2
BQ.1.1.32	1
JN.1.18	1
KP.1.1	1
KP.1.1.5	1
KP.2.2	1
KP.3.1	1
LF.1.1.1	1
MD.1	1
XDV.1	1
Celkem	36

Number of SARS-CoV-2 WGS according to the regional distribution: September2023 - May 2024

The number of SARS-CoV-2 positive samples sent from individual regions is not uniform (Chart 4), given the fact that this is a voluntary activity on both sides (sending laboratory and NRL), the number of samples examined can be considered adequate. Most samples were analyzed from senior patients, which is related to their higher rate of hospitalization. The results in children under 1 year of age should be paid attention to with regard to the possibility of a more serious clinical impact of c19 disease in this age cohort (Chart 5)



Graf 5: Distribution of SARS-CoV-2 sequenced samples by age:

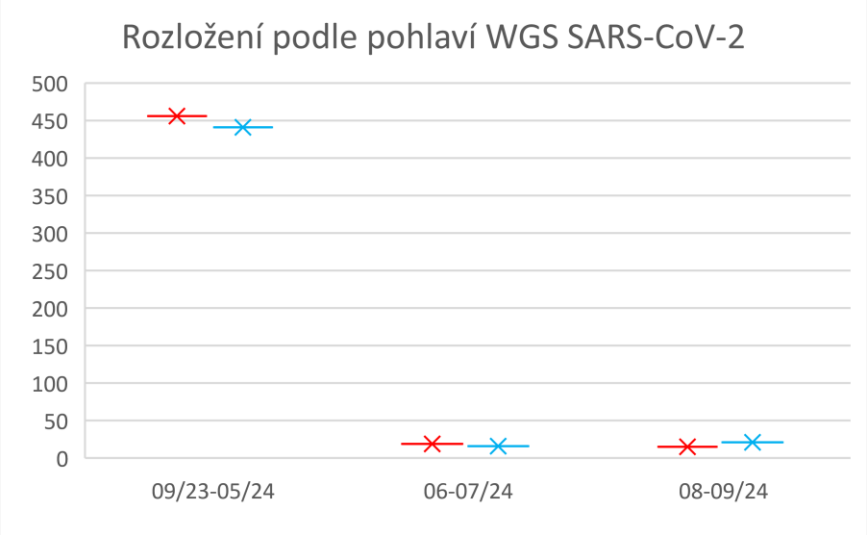


Věk	Počet WGS září 2023 - květen 2024
< 1	39
2 - 6 let	3
7 - 15 let	22
16-30	48
31 - 50	105
51 - 65	122
66 - 75	191
76-90	326
>90	35

Most samples were sequenced from senior patients, which is related to the rate of hospitalization. The number of samples from children under one year of age should be a warning sign. We do not have data on the health status of these infants,

The distribution of sequenced samples by gender is even, with a slightly higher number of samples from women. (Chart 6).

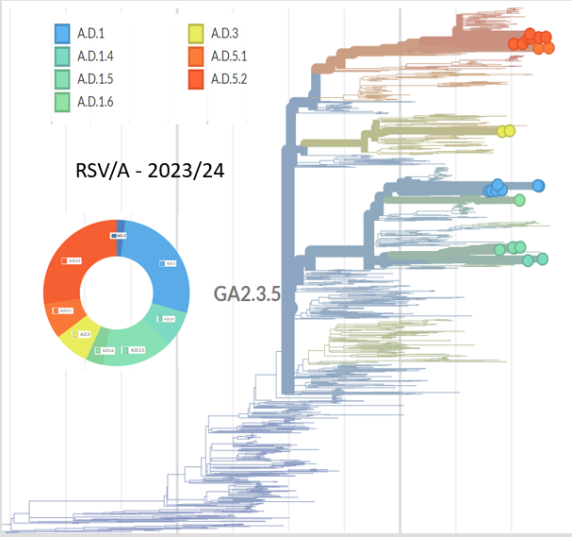
Graf 6: Distribution of SARS-CoV-2 sequenced samples by gender



Molecular surveillance of RSV -

In the 2023/24 season, molecular surveillance of RSV was introduced. All sequences are obtained as part of sentinel surveillance. Unfortunately, RSV is a virus very susceptible to degradation, so there are only a few samples suitable for whole-genome sequencing. Therefore, the distribution in the Czech Republic is very uneven. RSV positive samples must be frozen at least -20 °C within 24 hours of collection, which is not a feasible process within virological surveillance. Therefore, molecular surveillance will have to rely on samples sent from hospital laboratories rather than on samples from the sentinel. In this case, this is not a representative proportion. Most samples fall into the GA.2.3.5 clade, the relationship of molecular data and more detailed taxonomic classification with clinical impact is the subject of research.

Chart 7: Genetic characterization of RSV



Region	WGS
Central Bohemia	3
Hradec Kralove	3
Moravian-Silesian Region	2
Pilsen	13
South Moravia	5

Conclusion:
The number of samples 5/region/week should be sufficient for the integration of SARS-CoV-2 into the virological surveillance of ARI/ILI, unfortunately we still do not reach this number. For the genetic characterization of SARS-CoV-2 (molecular surveillance), it is necessary to ensure at least 10 times the number of samples, for the genetic characterization of the influenza virus this number seems adequate, RSV genetic surveillance, due to the instability of the virus, must rely on the analysis of samples from hospital laboratories that have the ability to freeze samples and send them frozen to the NRL. Given that this is SARI surveillance, this process should not bring greater complexity to the existing system.

RNDr. Helena Jiřincová
Prague dne 24th September 2024