

## FINAL REPORT OF THE SENTINEL SURVEILLANCE OF RESPIRATORY VIRUSES INCLUDING SARS-COV-2

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### Background - description of the situation

The sentinel surveillance system for acute respiratory infections (ARI) including influenza-like illness (ILI) in the Czech Republic serves to ensure a uniform procedure for surveillance of ARI (including COVID-19). The ARI surveillance system includes two main components, namely 1) Morbidity reporting (numbers of new cases of ARI/ILI in primary care) and 2) Virological surveillance of influenza, RSV, SARS-CoV-2 and other respiratory viruses.

1. The numbers of ARI/ILI patients are reported throughout the year. This part of the surveillance system works across the whole area of the Czech Republic but each region has its specificity in reporting, size and population density, so sophisticated statistics have been applied to obtain standardized data. This part of surveillance has been organized by the Department of Infectious Diseases Epidemiology, NIPH.

2. Virological surveillance has been organized by the NIC/NRL for Influenza and Respiratory Viruses (NRL) during the respiratory season, typically from calendar week (CW) 37 till CW 22 (September to May, 37 CWs) based on the requirements of the Ministry of Health. The aim is to examine the representative portion of samples for the presence of respiratory viruses by area, gender and age. There are 14 regions in the Czech Republic, from each region a defined number of samples from primary care was collected on a weekly basis. These samples have been sent to NRL for the detection of the whole panel of respiratory viruses including influenza virus A/B and subtypes H1pdm, H3 resp. B-lineages Victoria, Yamagata, RSV, MPV, ADV, EV, PEV, HRV, Bocavirus, seasonal CoVs and PIVs 1 - 4. Until the 2020/21 season two samples per week were collected from each region. From the season 2021/22 onward the attempt to integrate SARS-CoV-2 into the ARI/ILI virological surveillance started and thus the number of tested samples from each region was increased to 5 samples per week as a compromise for the high throughput national COVID testing and sequencing capacity of the NRL. The increased number of samples should also ensure the higher reliability of virological surveillance data. While the regions differ in their area and population density we have decided to set the same amount of samples, five, from each region to simplify the procedure. The samples were collected by GPs from pediatric primary care facilities (2 samples) and from primary care facilities for adults (2 samples from patients under 65 YoA, a sample from patients older than 65 YoA). The positive samples with the appropriate viral load (estimated according to C<sub>q</sub> obtained in PCR) were selected for virus isolation (influenza viruses, RSV, SARS-CoV-2 and ADV), and sequencing (WGS of SARS-CoV-2, influenza A, partial for genotyping of ADVs).

### Objectives of surveillance

The virological surveillance of acute respiratory infections and influenza like infections (ARI/ILI) gives the picture of the actual situation of circulating respiratory viruses and unifies the epidemiological morbidity data with the causative virus.

**The Goal of the project was to ensure collection and testing of sufficient amount of adequate biological samples from the upper respiratory tract from patients with ARI/ILI from each region of the Czech Republic for the systematic monitoring of the occurrence of influenza viruses and the whole spectrum of non-influenza respiratory viruses, including SARS-CoV-2, in the population of the Czech Republic as part of the integrated sentinel surveillance of respiratory viruses.**

The existing model that was set up in the season 2021/22 continues to be considered the basis of integrated ARI/ILI virological surveillance in the 2022/2023 season and also in the future seasons. The typical season has been recognized from 37 calendary week (CW) till the 22 CW of the next year that means 37 CWs. According to the guidelines of ECDC and WHO, the virological surveillance should cover the whole year. Because of a substantial delay of this project the decision should be made if this support is going to cover the costs of surveillance 2022/23 or surveillance 2023/24.

This specific project included the period 1<sup>st</sup> March till 30<sup>th</sup> April, but the report cover the period of time from the 12<sup>th</sup> September (37CW) till 30<sup>th</sup> April (17 CW)

#### Specific objectives were:

1. Ensure the ARI/ILI primary care sample collection based on the codes ICD-10 within the 37 CWs of the 2022/23 respiratory season to cover the whole year of surveillance (beginning the 23 CW of 2023 till the 22 CW of 2024).

#### **Inclusion criteria for sampling: ARI/ILI diagnoses included the following codes:**

- J00-J06 Acute upper respiratory tract infection, including: J06.8 Other acute multisite upper respiratory tract infection
  - J09-J18 Influenza and inflammation of the lungs (pneumonia)
  - J20-J22 Other acute lower respiratory tract infections
  - B34.1, B4.2, B34.8 – infectious viral diseases of undetermined localization
2. Ensure the representative selection and sufficient amount of sample for the virus isolation : Influenza A/B, RSV, ADV, SARS-CoV-2
  3. Ensure the representative selection sufficient amount of sample for the genetic characterization by WGS (influenza A/H1pdm/H3, RSV and SARS-CoV-2)
  4. To ensure the collection and archiving the representative selection of samples or isolated viral strains.
  5. To ensure representative selection of clinical samples, respective virus isolates respective or WGS inserted in GISAID to support of vaccination strategy of WHO/ECDC (influenza vaccine strains selection, SARS-CoV-2 vaccine composition changes, etc.).

In order to achieve the objectives, the implementing partner (regional epidemiology department or co-operating GP) will organize the following during of Project implementation (either 37 CWs of 2022/23 season, or whole year – 52 CWs of 2023/24 season):

- It was planned to test 5 samples per week from 14 regions of Czechia, so that samples per week should have been tested for the presence of the panel of respiratory viruses: Influenza virus A/B and subtypes H1pdm, H3 resp. variants Victoria, Yamagata, RSV/A, RSV/B, MPV, ADV, EV, PEV, HRV, BocaV, seasonal CoVs (OC43, 229E, NL63, HKU1) and PIVs (PIV1-4) during the period of 37 CWs. Total amount of planned samples was 2 240.

**The expected outcomes of the activities should be:**

1. Integration of SARS-CoV-2 in virological ARI/ILI sentinel surveillance
2. National report of virological surveillance made on weekly bases during the whole year as recommended by WHO and ECDC <https://szu.cz/centra/centrum-epidemiologie-a-mikrobiologie/celogenomova-sekvenace-v-cr-tydenni-zpravy/>. This report summarizes not only ARI/ILI sentinel surveillance, but also summarized the data from non-sentinel surveillance and the genetic surveillance of SARS-CoV-2 with respect to the actual situation of circulating variants based on the whole genome sequencing (WGS).
3. WHO/ECDC report to TESSY. EPI Pulse on weekly base
4. Upload the WGS to GISAID

**Methodology**

1. Collecting the samples:

The sample collection was ensured by the regional epidemiologist and by regional GPs and pediatrician GPs. NRL recommended collecting each week 3 samples from a GP ambulance and 2 samples from a pediatrician GP ambulance. The virological transport media (VTM) were prepared in the National Institute of Public Health. VTMs including the set of swabs were distributed to the regional epidemiology departments or to the ambulance of co-operating GP (ambulances in Prague, one pediatrician GP). The nasopharyngeal or oropharyngeal plus nasal swabs were taken by GP, collected, and shipped via the regional epidemiology departments to the NRL. The samples were kept at +2 to +8 °C to ensure the virus stability.

The other source of samples was the National Institute of Public Health (NIPH), as the part of the preventive care the employees with the signs of ARI/ILI were offered the PCR test.

2. Virus detection:

RNA/DNA was isolated on magnetic beads (MGV) using Zymio EXM 3000 and Zymio Nucleic Acid Extraction Kit. The RNA/DNA was amplified using nested RT-PCR in real time using AUS diagnostic system for processing 24 samples per run and Tandem Plex Respiratory panel detecting the required spectrum of respiratory viruses.

3. Virus characterization

The representative part of Influenza A/H1pdm2009 and A/H3pdm were characterized by WGS using amplicon sequencing based on in-house method and Minlon Oxford Nanopore sequencing technology.

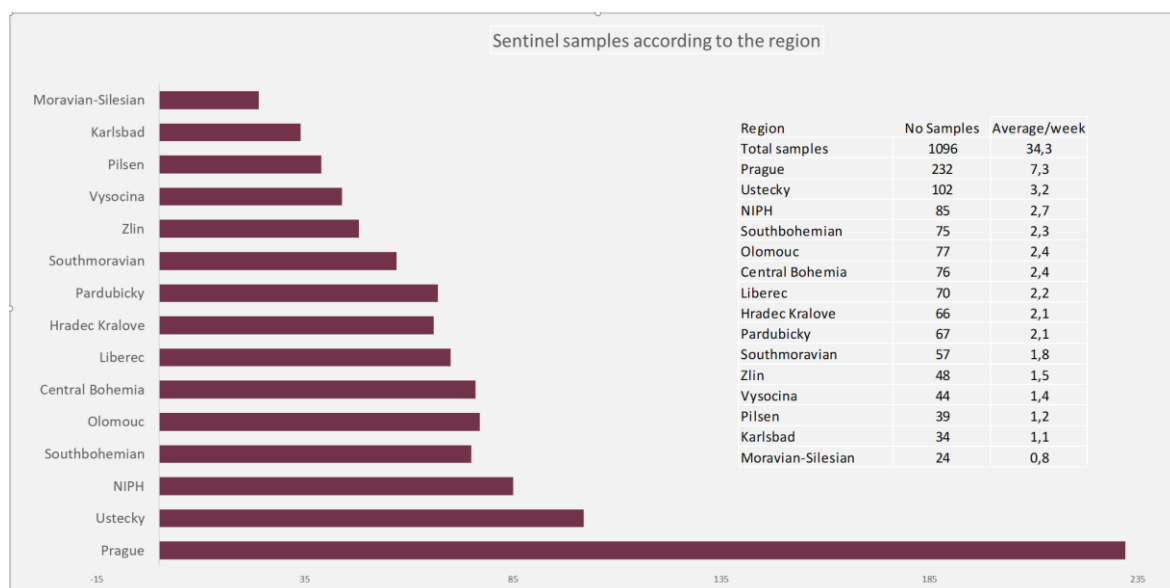
All SARS-CoV-2 positive samples were characterized by WGS using amplicon sequencing based using IonTorrent technology (Genexus Integrated Sequencer, Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay GX ) or Illumina MySeq (NEBNext® ARTIC SARS-CoV-2 Library Prep Kit (Illumina®), V3).

## Results

### Overview of collected samples and surveillance performance

During the surveillance regular period starting 37 CW 2022 and ending 17 CW 2023 1097 samples were collected and analyzed. One sample was withdrawn from the analysis as non-valid. Most of the samples were collected in Prague and in the National Institute of Public Health. The lowest amount of samples were sent from Moravian-Silesian Region, even thus this region is highly populated. Any region did not meet the requirements of NRL (5 samples per week). Requirements of MoH, three respective four samples per week, met except for Prague, Ustecky region. The data are presented in the Table 1.

Table 1: Number of collected samples from regions

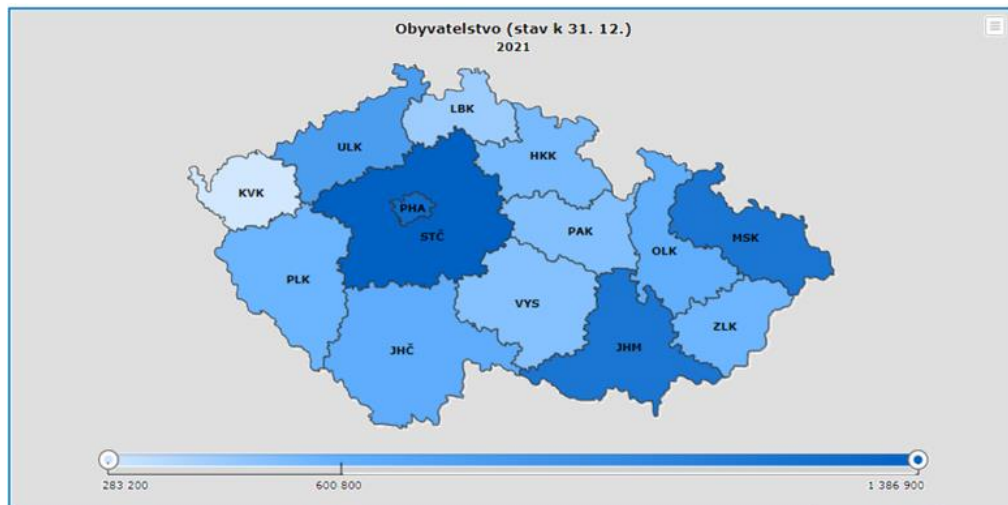


The regions of the Czech Republic are not equal concerning the number of inhabitants. Once we calculated the proportion of analyzed samples with respect to relative regional population, some regions collected sufficient amounts of samples as shown in table 2 and Graph 2 and Graph 3.

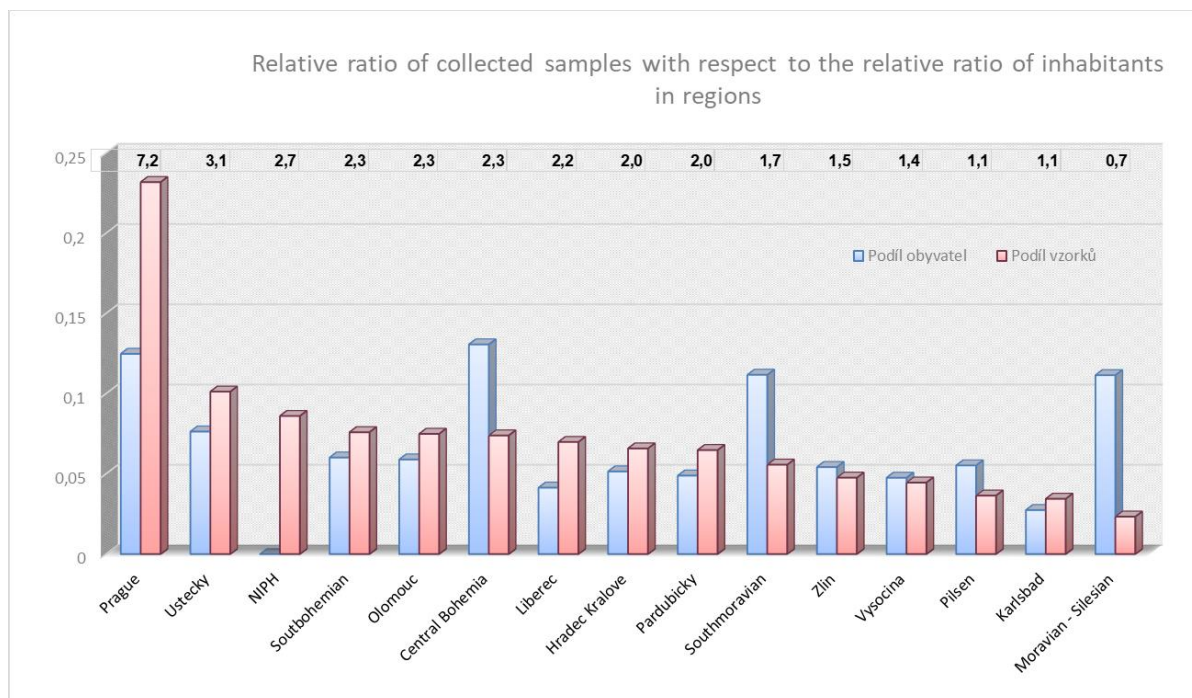
Table 2: Overview of collected samples with the proposed amount of samples and with relative proportion to the regional population

	Average samples		Required Samples		Relative proportion	
Region	collected/week	No Inhabitants	MoH	NRL	Inhabitant	Samples
Average amount per week	34,3					
Prague	7,3	1 335 084	4	5	0,125	0,23
Ustecky	3,2	817 004	3	5	0,076	0,10
SZÚ	2,7		3	5	0,000	0,08
Southbohemian	2,3	643 551	3	5	0,060	0,07
Olomouc	2,4	630 522	3	5	0,059	0,08
Central Bohemia	2,4	1 397 997	4	5	0,131	0,08
Liberec	2,2	442 476	3	5	0,041	0,07
Hrade Kralove	2,1	550 803	3	5	0,051	0,07
Pardubicky	2,1	522 856	3	5	0,049	0,07
Southmoravian	1,8	1 195 327	4	5	0,112	0,06
Zlin	1,5	580 119	3	5	0,054	0,05
Vysocina	1,4	508 852	3	5	0,048	0,04
Pilsen	1,2	591 041	3	5	0,055	0,04
Karlsbad	1,1	293 311	3	5	0,027	0,03
Moravian - Silesian	0,8	1 192 834	4	5	0,111	0,02

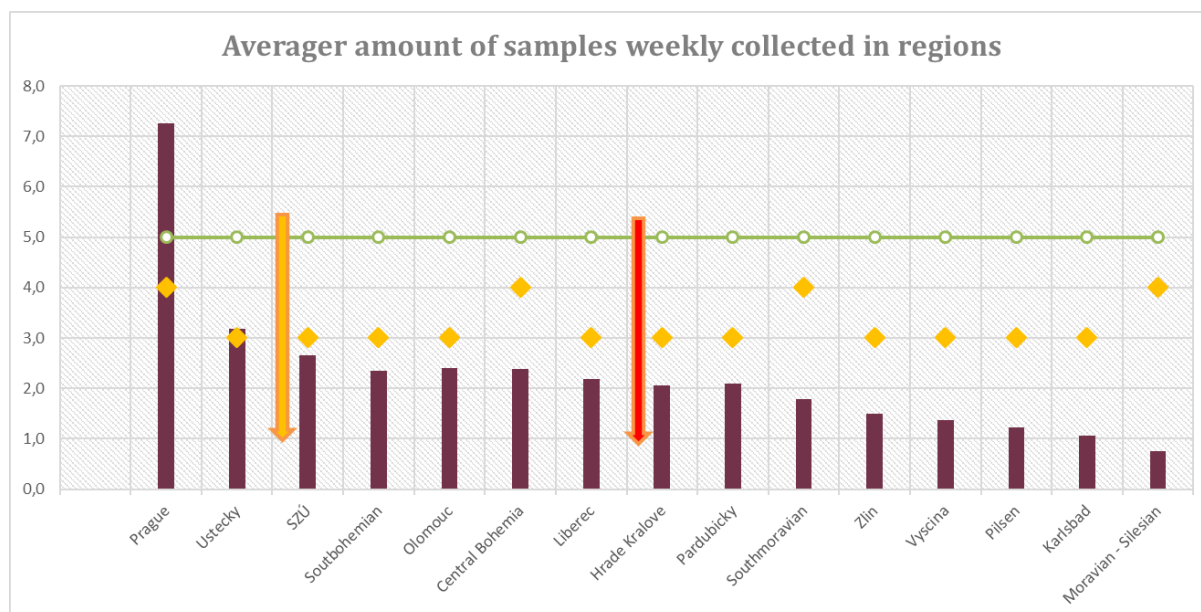
Pict. 1: The population density of regions of the Czech Republic



Graph 2: Relative ratio of collected samples with respect to the relative ratio of inhabitants in regions



Graph 3: Average amount of weekly collected samples in regions

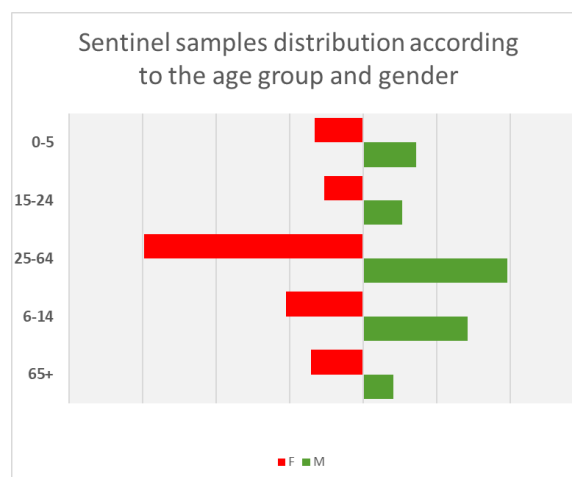


Graph 3: Left of the yellow arrow presents the regions that met MoH requirements, left of the orange arrow presents the regions where the number of collected samples are sufficient with respect to the low population density. The green line represents NRL recommended amounts of samples per week, the yellow symbols represent MoH requirements.

The gender proportion was nearly equal, an exception was the age group 25 – 64 YoA, with a higher proportion of female samples (298 versus 196) and 65+ YoA. The data are presented in table 3 and graph 4.

Table 3 and Graph 4: Sentinel sample distribution according to age group and gender

Age group	No samples	F	M
0-5	138	66	72
6-14	246	53	53
15-24	106	298	196
25-64	494	105	142
65+	112	71	41

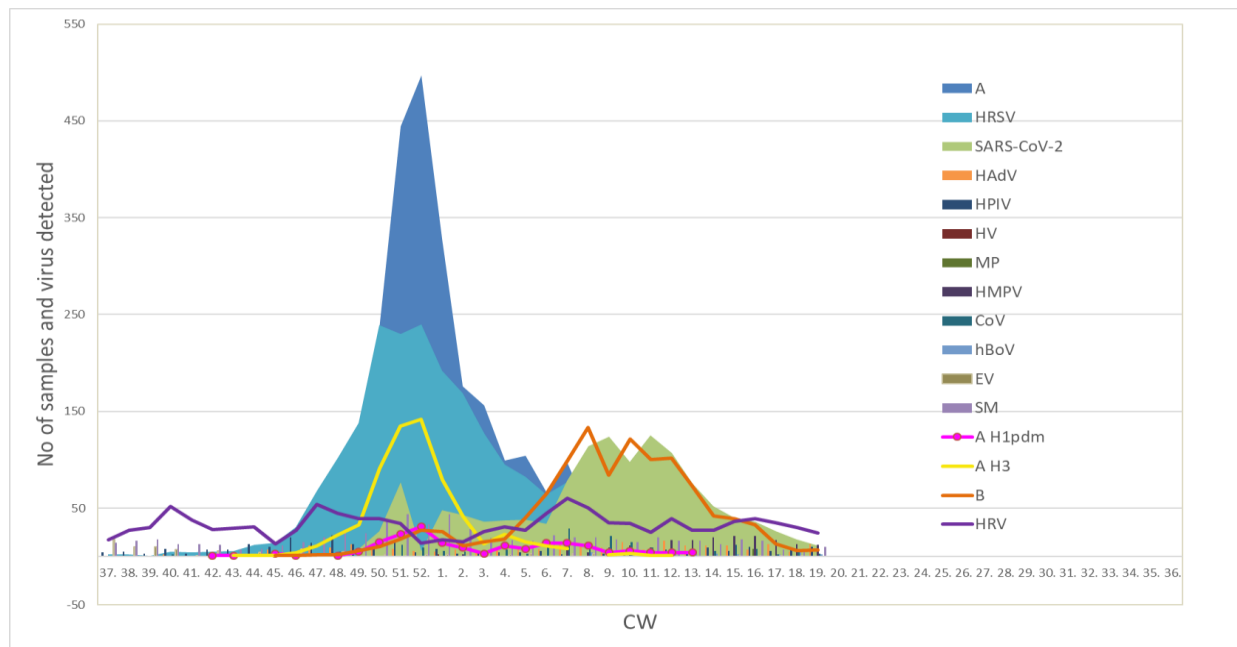


### Overview of virological surveillance:

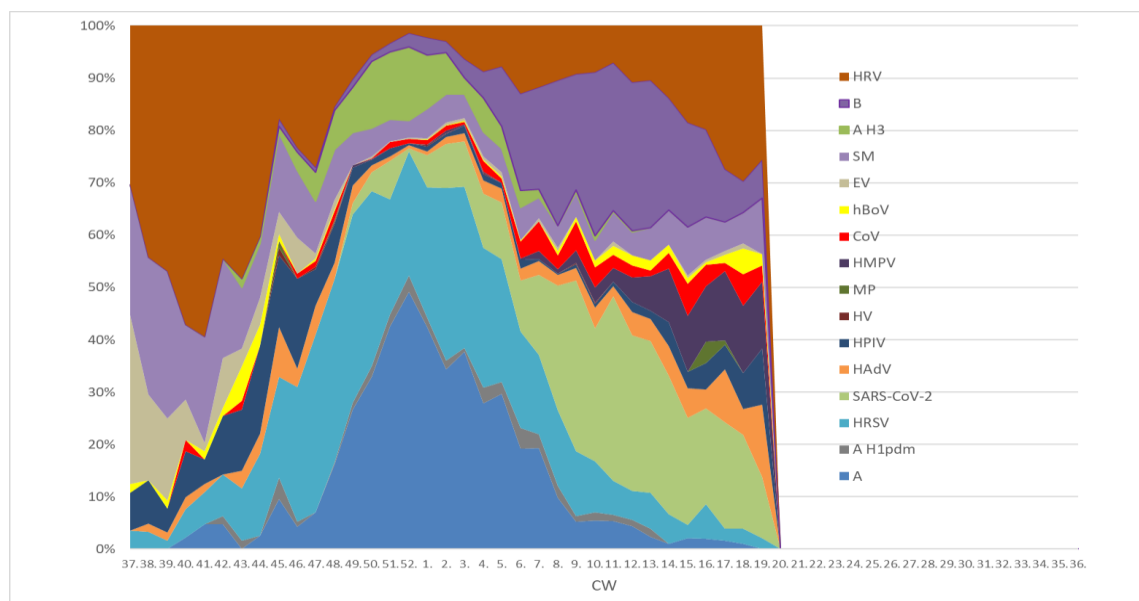
The respiratory season 2022-23 was characterized by two main overlapping waves of the dominance of four respiratory viruses. The first wave started at 44 CW 2022 and ended at 10 CW 2023 and it was characterized by co-dominance of influenza A/H3N2 and RSV virus. The second wave started at 4 CW and ended at 16 CW 2023 and it was characterized by codominance of influenza B virus and SARS-CoV-2. Influenza A/H1N1pdm circulated in low proportion from 49 CW 2022 till 16 CW 2023. Rhinoviruses

circulated for nearly the whole season. Other respiratory viruses were detected in low numbers, during the epidemic peaks were nearly undetectable and their maxima differ throughout the season. The data presented in graphs 5 and 6 summarized both sentinel and non-sentinel surveillance data.

Graph 5: Overview of the sentinel and non-sentinel virological surveillance (data in absolute numbers)



Graph 6: Overview of the sentinel and non-sentinel virological surveillance in relative proportion



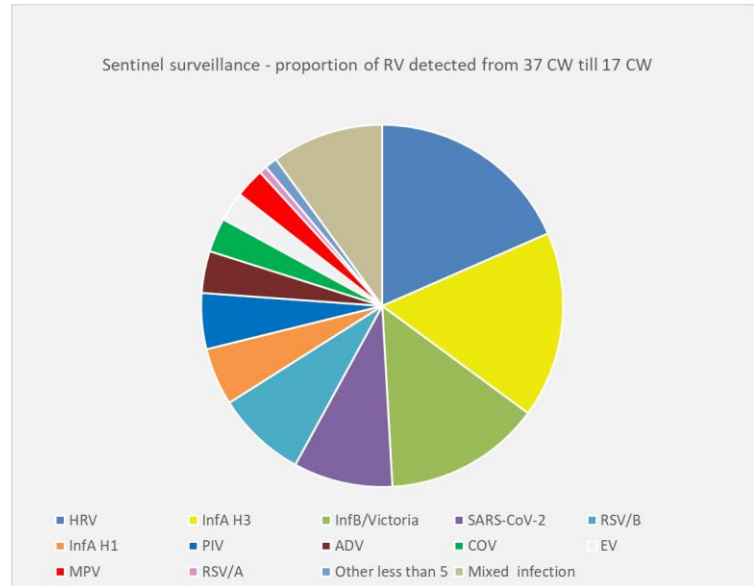


The spectrum of detected viruses during the respiratory season is presented in table 4 and graphs 7 and 8.

Table 4: The spectrum of detected viruses from 37 CW 2022 till 17 CW 2023

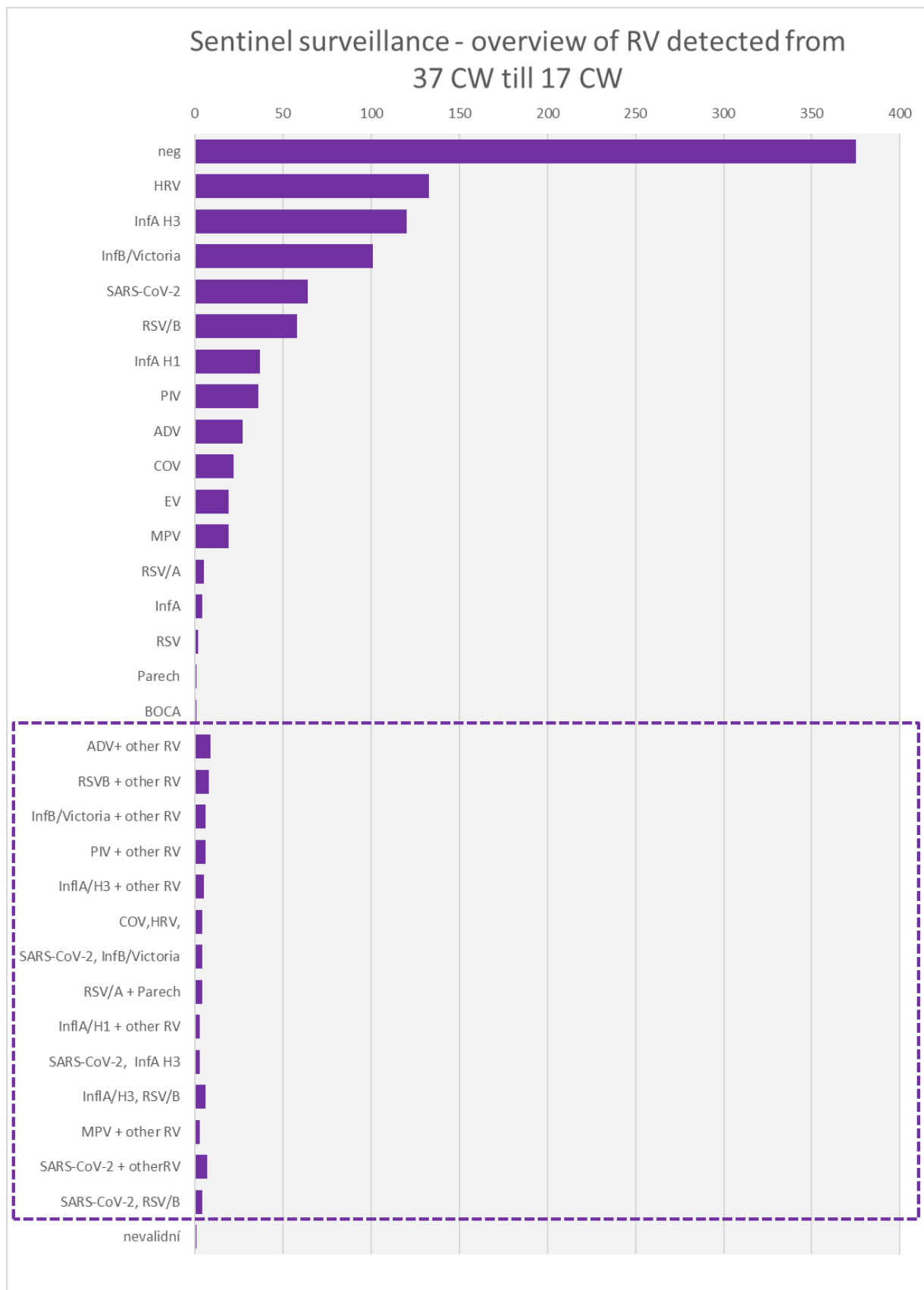
Detected virus	Amount
Negative	375
HRV	133
InfA H3	120
InfB/Victoria	101
SARS-CoV-2	64
RSV/B	58
InfA H1	37
PIV	36
ADV	27
COV	22
EV	19
MPV	19
RSV/A	5
InfA	4
RSV	2
Parech	1
BOCA	1
ADV+ other RV	9
RSVB + other RV	8
InfB/Victoria + other RV	6
PIV + other RV	6
InfA/H3 + other RV	5
COV,HRV,	4
SARS-CoV-2, InfB/Victoria	4
RSV/A + Parech	4
InfA/H1 + other RV	3
SARS-CoV-2, InfA H3	3
InfA/H3, RSV/B	6
MPV + other RV	3
SARS-CoV-2 + otherRV	7
SARS-CoV-2, RSV/B	4
non-valid	1

Graph 7: The proportion of detected respiratory viruses





Graph 8: The spectrum of detected viruses from 37 CW 2022 till 17 CW 2023, the area bordered with the dashed purple line contents mixed infections



## Overview of molecular surveillance - genetic characterization by WGS:

### Molecular characterization of Influenza A virus:

Only influenza viruses H1N1pdm and H3N2 were characterized by WGS. The Influenza B viruses were characterized by PCR discriminating between the lines Victoria and Yamagata. The situation is similar to that seen globally; in the first wave influenza virus A/H3N2 dominated and four clades were detected (see table 5). Influenza virus A/H1N1pdm circulated in low proportion from 49 CW 2022 till 16 CW 2023 and all viruses belong to two clades. Only B Victoria lineage was detected.

Table 6: Influenza samples characterized by WGS

Clades of Infl A H1pdm	No WGS	Clades of A/H3N2	No WGS
6B.1A.5a.2a	43	3C.2a1b.2a.2a.1b	53
6B.1A.5a.2a.1	4	3C.2a1b.2a.2a.3	1
Total amount of WGS	47	3C.2a1b.2a.2a.3a.1	1
		3C.2a1b.2a.2b	41
		Total amount of WGS	96

**AH1N1pdm:** 47 samples were sequenced (6 sentinel samples and 41 SARI samples), none of the samples belongs to the same clade as vaccine strains proposed for the season 2022-23 and four samples belong to the same clade as vaccine strain proposed for the season 2023-24 (highlighted by red frame).

A/H3N2: 96 samples were sequenced (81 sentinel samples, 5 post-mortem samples, and 10 SARI cases) none of the samples belongs to the same clade as vaccine strains proposed for the season 2022-23 and also none of the samples belongs to the same clade as vaccine strains proposed for the season 2023/24.

Table 7: Vaccine strains 2022-23 and 2023-24 seasons:

#### 2022-23

A/Victoria/2570/2019	(H1N1)pdm09-like	6B.1A.5a.2	EPI_ISL_417210
A/Wisconsin/588/2019			
A/Darwin/9/2021	(H3N2)-like	3C.2a1b.2a.2a	EPI_ISL_2233240
A/Darwin/6/2021			
B/Austria/1359417/2021	(B/Victoria lineage)-like	V1A.3a.2	EPI_ISL_1519459
B/PHUKET/3073/2013	(B/Yamagata lineage)-like		EPI_ISL_168822

#### 2023-24

A/Victoria/4897/2022	(H1N1)pdm09-like	6B.1A.5a.2a.1	EPI_ISL_417210
A/Darwin/9/2021	(H3N2)-like	3C.2a1b.2a.2a	EPI_ISL_2233240
A/Darwin/6/2021			
B/Austria/1359417/2021	(B/Victoria lineage)-like	V1A.3a.2	EPI_ISL_1519459
B/PHUKET/3073/2013	(B/Yamagata lineage)-like		EPI_ISL_168822

Only a proportion of sequenced viruses originated in samples from sentinel surveillance. Many sequenced samples were delivered to NRL from hospitals as SARI (severe acute respiratory infection). In spite of the serious clinical condition of patients no unique molecular markers of clinical severity were detected.

#### Molecular characterization of SARS-CoV-2

During the respiratory season 2022/23 (17 CW -37 CW) 7 234 viruses were characterized by WGS. There were 75 SARS-CoV-2 positive samples collected in sentinel surveillance (64 SARS-CoV-2 positive and 11 SARS CoV-2 positive in samples with mixed infection). 72 sentinel samples were sequenced. The results of sequencing is presented in the table 8. No dominant variant was detected, except for XBB.1.5.12.

Table 8: SARS-CoV-2 positive samples characterized by WGS. Results are shown as counts of detected variants.

Variants	No WGS	Variants	No WGS
BA.2	1	BQ.1.2.1	1
BA.5.1	2	BQ.1.22	1
BA.5.2	3	BQ.1.23	1
BA.5.2.1	4	BQ.1.5	1
BA.5.2.20	1	CK.1	1
BA.5.2.34	2	CV.1	1
BA.5.2.44	1	XBB.1	1
BE.1.1	1	XBB.1.13	1
BF.14	3	XBB.1.28	1
BF.7	4	XBB.1.5	7
BF.7.22	1	XBB.1.5.1	1
BF.7.3	1	XBB.1.5.12	11
BN.1	1	XBB.1.5.37	1
BN.1.2	1	XBB.1.9.1	1
BN.1.3	3	XBB.1.9.2	1
BN.1.3.5	1	XBB.2.3	1
BN.1.5	1	XBK	1
BQ.1	3		
BQ.1.1	5	Total	72

#### Overview of the period covered by the project (March and April):

During March and April, 381 samples were collected, this number represents 326 cases. In sentinel surveillance 297 samples were tested.

Non sentinel:

The non-sentinel samples included a SARI case and 90 post-mortem samples from 30 fatal cases deceased outside the hospital without the medical care. The section material tested includes trachea, lungs, and myocardial excisions. In the tested section material SARS-CoV-2 was detected in 6 cases as a causal agent, RSV B was detected in one case. The 23 post mortem materials were negative for any respiratory virus. The influenza virus A/H1N1pdm was proved as a causal agent of a SARI case.

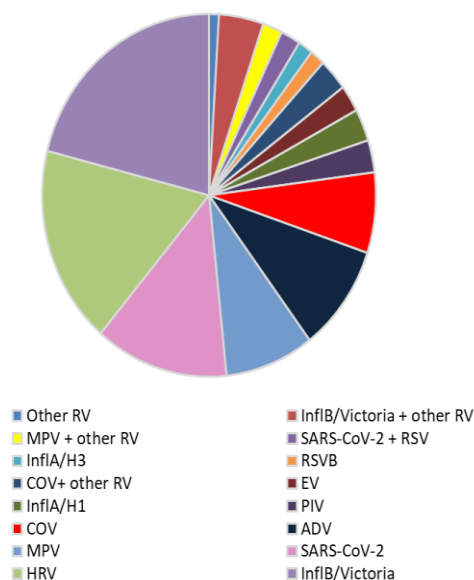
Sentinel surveillance March-April:

The dominant viruses detected in more than 10 samples in sentinel surveillance were: Influenza B Victoria lineage, rhinoviruses (HRV), SARS-CoV-2, metapneumoviruses (MPV) and seasonal coronaviruses (CoV), see the Table 9, Graphs 9 and 10

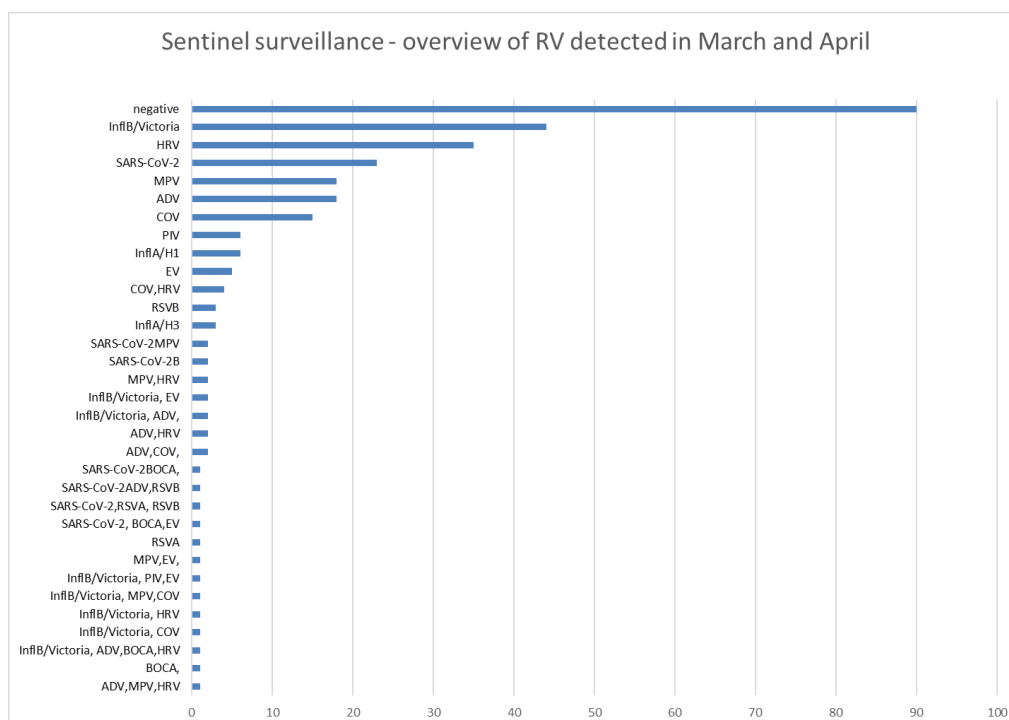
Tab 9 and graph 9: Overview of virological sentinel surveillance in March and April

RV detected	Amount
ADV,MPV,HRV	1
BOCA,	1
InflB/Victoria, ADV,BOCA,HRV	1
InflB/Victoria, COV	1
InflB/Victoria, HRV	1
InflB/Victoria, MPV,COV	1
InflB/Victoria, PIV,EV	1
MPV,EV,	1
RSVA	1
SARS-CoV-2, BOCA,EV	1
SARS-CoV-2,RSVA, RSVB	1
SARS-CoV-2ADV,RSVB	1
SARS-CoV-2BOCA,	1
ADV,COV,	2
ADV,HRV	2
InflB/Victoria, ADV,	2
InflB/Victoria, EV	2
MPV,HRV	2
SARS-CoV-2B	2
SARS-CoV-2MPV	2
InflA/H3	3
RSVB	3
COV,HRV	4
EV	5
InflA/H1	6
PIV	6
COV	15
ADV	18
MPV	18
SARS-CoV-2	23
HRV	35
InflB/Victoria	44
negative	90
Total amount	297

Sentinel surveillance - proportion of RV detected in March and April



Graph 10: Virological sentinel surveillance – the list of respiratory viruses (RV) detected in March and April



#### The expected outcomes:

1. Integration of SARS-CoV-2 in virological ARI/ILI sentinel surveillance was met.
2. NRL presented on the weekly bases the report summarized the virological sentinel surveillance and molecular surveillance (<https://szu.cz/centra/centrum-epidemiologie-a-mikrobiologie/celogenomova-sekvenace-v-cr-tydenni-zpravy/>.) During the March April 9 reports were published and during the whole period of surveillance (37 CW – 17 CW) 33 reports were published,-
3. WHO/ECDC report to TESSY. EPI Pulse on weekly base – NRL report directly to TESSY the metadatasets: INFLVIRWAGGR, through the GISAID database the following metadatasets are reported: INFLANTIVIR, NCOVVARIANT.
4. Upload the WGS to GISAID:
  - 7 234 WGS of SARS-CoV.2 were uploaded
  - 47 WGS of influenza A/H1N1pdm were uploaded
  - 96 WGS of influenza A/H3N2 were uploaded

#### Evaluation of the achievements of the specific objectives

1. The whole year surveillance has not been achieved as a standard procedure. The cooperation of MoH and funding of all stakeholders is necessary.
2. Inclusion criteria for sampling concerning clinical diagnosis were met, gender proportionality was slightly weighted in favor of women.
3. The SARS-CoV-2 material for virus isolation was obtained in the relevant amount, the influenza virus isolation was negatively influenced by the personnel capacity in NRL and NIPH. The

4. Ensure the representative selection sufficient amount of samples for the genetic characterization by WGS (influenza A/H1pdm/H3, RSV and SARS-CoV-2): the obtained result clearly prove that the sampling is adequate for the routine virological surveillance but for the molecular surveillance of SARS-CoV-2 at least ten time higher amount of samples should be collected. It is clearly visible in SARS-CoV-2 WGS results, no dominant variants were detected in sentinel surveillance in contrast with the non-sentinel WGS data (<https://szu.cz/centra/centrum-epidemiologie-a-mikrobiologie/celogenomova-sekvenace-v-cr-tydenni-zpravy/>).
5. The isolated SARS-CoV-2 variants (BA.5.2, BF.7, BQ.1.1 and XBB.1.5.12) were added to the archive, the influenza strains isolation is ongoing.
6. The uploaded WGS data can support the attempt of WHO/ECDC to choose the appropriate variant of vaccine strains.

## **Conclusion**

While the expected results were met, some of the specific goals were not completely fulfilled. The agreement on the establishment of the national virological surveillance of ARI/ILI/SARI/SARS between the stakeholders (NIPH, MOH, GPs, and epidemiologists) was not concluded and negotiations were not initiated. Funding for virological or molecular surveillance was not established and data flow settings were not clearly defined. The proposed amount of tested samples was not met.

## **The justification of the funding of the project despite the lower amount of samples analyzed**

The WHO project was established for 2 months surveillance from 1st March 2023 till 30th April 2023 but it should cover the cost of the surveillance of the whole season. The typical respiratory season includes 37 weeks but the recommendation of WHO/ECDC is to include whole year surveillance. This report summarizes the data from the 12th September 2022 (37 CW) till the 30th April 2023 (17CW). According to the schedule proposed by the NRL, during the 32 CW 2 240 samples should have been tested, 5 samples per week per region, as was announced in the season 2021-2022. The MoH requirement (MZDR29069/2022-1/OVZ Metodický pokyn k sentinelové surveillance respiračních virů pro epidemickou sezónu 2022-2023 v ČR) has not reflected the recommendation of NRL and required only 3 samples per week from each region with recommendation to collect 4 samples per week in regions where the populations counts exceeds 1 million. Moreover the start of the season was 8 weeks delayed (according this document the season has commenced on 6<sup>th</sup> November in CW 45). This late decision negatively influenced the number of samples collected in the period starting from CW 37 till CW 44. In spite of the official requirements Prague provided 5 samples or more per week and only one region meet the MoH requirements, Ustecký kraj, with an average of 3 samples collected per week.

Due to the end of the 2022/2023 season and due to the development of the epidemic situation in the occurrence of respiratory diseases and an atypically short flu epidemic, a lower number of samples was sent than proposed. The remaining funds and acquired material (regents, kits and disposable plastics) from WHO financial support will be used purposefully for the virological surveillance of ARI/ILI in the next respiratory season 2023/2024 to ensure the continuity of the project.

## **Related document**

MZDR29069/2022-1/OVZ Metodický pokyn k sentinelové surveillance respiračních virů pro epidemickou sezónu 2022-2023 v ČR

The study was part of the project „Genomic surveillance of selected infectious diseases in the Czech Republic“ (HERA2CZ) and it was cofunded by the European Union EU4H-2022-DGA-MS-IBA-01-02 and it was partially funded by WHO Czech Office