

VIROLOGICAL SURVEILLANCE REPORT OF THE NRC INFLUENZA FOR SEASON 2022-2023

Activity Report

CYRIL BARBEZANGE • SARAH DENAYER • FRANÇOIS DUFRASNE • SOFIE LEEN • ASSIA HAMOUDA • REINOUT VAN EYCKEN • ILHAM FDILLATE • SAIRA YAQOOB • STEVEN VAN GUCHT



WHO WE ARE

Sciensano can count on more than 950 staff members who are committed to health every day.

As our name suggests, science and health are central to our mission. Sciensano's strength and uniqueness lie within the holistic and multidisciplinary approach to health. More particularly we focus on the close and indissoluble interconnection between human and animal health and their environment (the "One health" concept). By combining different research perspectives within this framework, Sciensano contributes in a unique way to everybody's health.

For this, Sciensano builds on the more than 100 years of scientific expertise.

Sciensano

Infectious diseases in humans - Viral diseases Respiratory Viruses

December 2023 • Brussels • Belgium

—

Cyril Barbezange Sarah Denayer François Dufrasne Sofie Leen Assia Hamouda Reinout Van Eycken Ilham Fdillate Saira Yaqoob Steven Van Gucht In collaboration with

Nathalie Bossuyt, Epidemiology of infectious diseases Koen Blot, Epidemiology of infectious diseases Floriane Rouvez, Health services research Robrecht De Schreye, Health services research Milena Callies, Health-associated infections and antimicrobial resistances Boudewijn Catry, Health-associated infections and antimicrobial resistances

With the financial support of



Please cite as: Cyril Barbezange, Sarah Denayer, François Dufrasne, Sofie Leen, Assia Hamouda, Reinout Van Eycken, Ilham Fdillate, Saira Yaqoob, Steven Van Gucht. Virological surveillance report of the NRC influenza for season 2022-2023. Brussels, Belgium : Sciensano ; 2023 38p. Report number: D/2023.14.440/85. Available from: https://doi.org/10.25608/dkjg-bj14

TABLE OF CONTENTS

ABSTRACT	5
BACKGROUND	6
SENTINEL SURVEILLANCE	8
1. Influenza-like illness (ILI)	8
1.1. Network of general practitioners	
1.2. Sample information	8
1.3. Influenza virus	11
1.4. SARS-COV-2 virus	
1.5. Respiratory syncytial virus	
1.6. Other respiratory viruses	
2. Nursing home - ILI	
3. Severe Acute Respiratory Infections (SARI)	
3.1. Network of sentinel hospitals	
3.2. Sample information	
3.3. Influenza virus.	
3.4. SARS-COV-2 virus 3.5. Respiratory syncytial virus	
3.5. Respiratory syncytial virus	
NON-SENTINEL SURVEILLANCE	
1. Hospital laboratories (HOSPI)	
2. Zoonotic influenza	
2.1. Suspected cases of non-seasonal influenza	
2.2. ZOOIS: zoonotic influenza surveillance project	
CHARACTERISATION	27
1. Virus isolation	
2. Sequencing	
2.1. SARS-CoV-2 coronavirus	27
2.2. Influenza viruses	
QUALITY MANAGEMENT	32
1. Quality System	32
2. External Quality Assessment	
ACKNOWLEDGEMENTS	
PUBLICATIONS	
LIST OF TABLES	
LIST OF FIGURES	37

ABSTRACT

Based on the sentinel surveillance networks for influenza-like illness (ILI) and severe acute respiratory infections (SARI), the 2022-2023 season was characterized by the circulation of several respiratory viruses with epidemic waves overlapping each others. Respiratory syncytial virus was responsible for an epidemic wave in November-December 2022, coinciding with a small wave of human metapneumovirus, but it continued to circulate in January 2023. Influenza viruses were responsible for a first epidemic wave at the end of December 2022 - so slightly earlier than during the seasons preceding the COVID-19 pandemic - which was followed by a second small wave in January-February 2023. Seasonal influenza A viruses of the H1N1pdm09 and H3N2 subtypes, and influenza B virus of the Victoria lineage co-circulated without clear dominance. SARS-CoV-2 virus continued to circulate during the whole period but a higher intensity of circulation was detected in March 2023, as well as in August 2023. Parainfluenza viruses were responsible for a small epidemic wave in May 2023. Other respiratory viruses such as seasonal coronaviruses, rhino- and enteroviruses, and adenoviruses were also regularly detected.

Epidemic waves of respiratory syncytial virus and influenza viruses occurred at periods that were close to historical data, indicating that the seasonality of these viruses might be returning to normal following the disruptions due to the COVID-19 pandemic. SARS-CoV-2 coronavirus still circulate without a clear seasonal pattern, but seems to cause smaller epidemic waves than during the pandemic phase. Surveillance all year round with multi-virus testing proves useful to better understand seasonality and interference of the different respiratory viruses.

BACKGROUND

Influenza viruses are a major cause of human morbidity and mortality worldwide. Yearly epidemics during the winter months have also a huge impact on the health care systems and the economic. Surveillance of influenza viruses is coordinated at the global level by WHO (World Health Organisation) through the GISRS network implemented in 1952. The network is organised with reference national laboratories in each country (National Influenza Centre), regional supranational organisations (such as ECDC, European Centre for Disease Control and prevention, in the European Union) and WHO Collaborating Centres, all working together to exchange information and viruses. The main objectives of the surveillance are to monitor the influenza activity (start, intensity, duration) over the whole year, to determine the type and subtype/lineage of influenza viruses circulating, to characterise at the viruses at the antigenic and genetic level, to contribute to the decision process on the yearly influenza vaccine content, to assess the overall vaccine effectiveness, to monitor the susceptibility to antivirals of the circulating viruses, and to detect the appearance of new (non-seasonal) influenza viruses in the human population.

Since the beginning of the COVID-19 pandemic in 2020, it has become clear that an integrated surveillance of several respiratory viruses is needed and such a recommendation has been made by WHO $^{\rm a}$ and ECDC $^{\rm b}$.

Traditionally, an "influenza" season was defined by the period running from week 40 of one year to week 20 of the following year in the Northern Hemisphere. The rest of the year was defined as the interseasonal period.

Since the COVID-19 pandemic, the surveillance in Belgium is officially running all year round and a season is defined from week 40 of the year to week 39 of the following year. The season 2022-2023 thus started on week 40-2022 and ended at the end of week 39-2023.

The surveillance relies on different systems. 'Sentinel' surveillance involves dedicated networks of general practitioners, hospitals, nursing homes, or other settings, who recruit cases based and a precise clinical case definitions. In Belgium, sentinel surveillance included networks of general practitioners (ILI: influenza-like illness), nursing homes (NH-ILI) and hospitals (SARI: severe acute respiratory infection). All other types of surveillance are designated as 'non sentinel' and cover the collection of data from different partners.

For the ILI surveillance (mild cases), several case definitions are available.

- WHO-ILI: sudden onset of symptoms, with fever and cough or dyspnoea.
- ECDC-ARI: sudden onset of symptoms with at least one of the following: cough, sore throat, shortness of breath, coryza.
- ECDC-ILI: sudden onset of symptoms with at least one general symptom among fever, history of fever, malaise, headache or myalgia, and at least one respiratory symptom among cough, sore throat or shortness of breath.

The WHO ILI case definition strictly includes fever, when the ECDC ILI case definition is broader and is not restricted to fever as general symptoms. For an integrated surveillance of influenza, SARS-CoV-2 and RSV, the WHO case definition might have to evolve.

For the SARI surveillance (hospitalised cases), the WHO-SARI case definition is used:

- Onset of symptoms within 10 days of hospitalisation/sampling

^a https://www.who.int/initiatives/mosaic-respiratory-surveillance-framework

^b https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/facts

- Fever or history of fever
- Cough or dysphoea

Since season 2020-2021, the hospitals had the opportunity to enrol cases matching the BE-COVID case definition:

- At least one of the following: fever, history of fever, cough, shortness of breath, fatigue, anosmia, ageusia, diarrhoea, loss of appetite.

The BE-COVID case definition is slightly broader than the ECDC-COVID:

- At least one of the following: fever, history of fever, cough, shortness of breath, anosmia, ageusia.

All samples collected through the sentinel surveillance networks are sent to the NRC (National Reference Centre) influenza for testing. All samples are tested to detect the presence of influenza viruses and determine the type and subtype/lineage for seasonal influenza viruses. Since season 2015-2016, all samples are also tested for other respiratory viruses. This testing algorithm was implemented because only about 30-40% of samples were positive for influenza viruses in the SARI surveillance compared to about 70% in the ILI surveillance in the preceding seasons. In January 2020, testing for SARS-CoV-2 was also included.

For season 2022-2023, samples were tested by multiplex PCRs for: influenza A virus, influenza B virus, SARS-CoV-2, respiratory syncytial virus (RSV), human metapneumovirus, parainfluenza virus (types 1, 2, 3 and 4), seasonal coronavirus (229E, OC43, NL43), rhinovirus, enterovirus, specifically for enterovirus D68, parechovirus, adenovirus, bocavirus. Influenza positive samples were further tested by PCR to determine the subtype of influenza A viruses (H1N1pdm09 or H3N2) and the lineage of influenza B viruses (Victoria or Yamagata). All these PCR tests are following standard operating procedures (SOP) and are accredited according to the ISO 15189 norm.

Other PCR tests are available at the NRC influenza to determine the subtype of non-seasonal influenza A viruses that have already been responsible for severe cases of human infection (H5, H7 and H9) and for MERS coronavirus.

Samples positive for influenza viruses by PCR and with a good viral load are selected to attempt viral isolation and further characterisation by sequencing and phenotypic tests to evaluate the susceptibility to neuraminidase inhibitor antivirals. Representative samples are sent to the WHO Collaborating Center at the Crick Institute (United-Kingdom) for detailed characterisation, according to the terms of reference of the WHO-recognised National Influenza Centres.

Samples positive for SARS-CoV-2 by PCR and with a good viral load are selected for whole genome sequencing using the ARTIC protocol for Oxford Nanopore MinION technology. There is currently no official mechanism in place to exchange SARS-CoV-2 viruses at the global level.

SENTINEL SURVEILLANCE

1. Influenza-like illness (ILI)

1.1. NETWORK OF GENERAL PRACTITIONERS

The surveillance of influenza-like illness (ILI) is organised through a network of general practitioners spread all over Belgium. The network is involved in the surveillance of many diseases, reporting weekly information to the Department of Infectious Diseases at Sciensano. More information on the network can be found on Sciensano's website °.

Regarding the surveillance of respiratory infections, the general practitioners are requested to weekly report the total number of consultations they had during the previous week, and the specific number of consultations for influenza-like illness (ILI) and for acute respiratory infection (ARI). These numbers are used to calculate incidence rates that allow to follow the epidemic situation throughout the year and that are presented in the weekly bulletin for acute respiratory infections ^d.

A subset of the general practitioners are also taking part in an active virological surveillance for influenza viruses and other respiratory viruses. They are requested to take a nasopharyngeal swab from the first 3 ILI and first 2 ARI cases of the week belonging to different households. The NRC influenza provides the sampling kits (nasopharyngeal swab and UTM universal transport medium) and the packaging for sending the samples (prepaid envelops). All the samples are sent to the NRC influenza for testing.

1.2. SAMPLE INFORMATION

During the 2022-2023 season, 41 general practitioners took part in the virological surveillance and collected samples for the NRC influenza. This represents a drop by about 40% compared to the last pre-COVID19 season 2019-2020 (Table 1).

Table 1	Number of I	II/ADI complet	s and contributing	acharal	prostitionara pa	
	Number of L	LI/ART samples	s and contributing	General	practitioners pe	season

Season	n	Nb of GP
2022-2023	562	41
2021-2022	394	38
2020-2021	29	8
2019-2020	698	69
2018-2019	512	59
2017-2018	677	65
2016-2017	651	72
2015-2016	752	78

n: number of samples; GP: general practitioner

A total of 562 nasopharyngeal swabs were collected during the 2022-2023 season and were sent to the NRC influenza for testing. Regarding the administrative region of origin, the participating GP in Flanders and Wallonia collected on average 13 samples each, when participating GP in the Brussels region collected on average 24 samples each (Table 2).

^c https://www.sciensano.be/en/network-general-practitioners

^d https://www.sciensano.be/en/health-topics/acute-respiratory-tract-infection/numbers

 Table 2 • Number of ILI/ARI samples and contributing general practitioners per province, season

 2022-2023

Region	n	Nb of GP
Brussels	97	4
Flanders	305	24
Wallonia	160	13

n: number of samples; GP: general practitioner

Out of the 562 enrolled cases, 89% and 94.7% matched the ECDC case definitions for ILI and ARI, respectively, which is similar to the percentages obtained during the last pre-COVID19 season 2019-2020 (Table 3). On the contrary, only 49.1% of the enrolled cases matched the very narrow WHO case definition for ILI, which is much lower than during the last pre-COVID19 season 2019-2020.

Table 3 • Number of ILI/ARI samples responding to the different clinical case definitions (with percentages)

Season	n	ECDC- ILI	ECDC- ARI	WHO- ILI	p_ECDC- ILI	p_ECDC- ARI	p_WHO- ILI
2022-2023	562	500	532	276	89.0	94.7	49.1
2021-2022	394	342	365	200	86.8	92.6	50.8
2020-2021	29	21	26	10	72.4	89.7	34.5
2019-2020	698	663	668	555	95.0	95.7	79.5
2018-2019	512	381	413	381	74.4	80.7	74.4
2017-2018	677	528	575	528	78.0	84.9	78.0
2016-2017	651	473	534	473	72.7	82.0	72.7
2015-2016	752	415	599	415	55.2	79.6	55.2

n: number of samples; p: percentage

The median time between sampling date and reception date was 4 days, but it varied by region (3 days for Flanders, 4 days for Wallonia, and 5 days for Brussels).

The median time between reception date and reporting date (i.e. turnaround time, TAT) was 7 days, well below the target of 15 days.

The age distribution showed a predominance of adult (between 15 and 65 years old) patients within the ILI/ARI surveillance (Figure 1). Overall, 81.8% of the patients were adults (Table 4). Children (below the age of 15) and older adults (above the age of 65) are structurally not well covered by the network of general practitioners. No samples were collected between week 21-2023 and 28-2023.

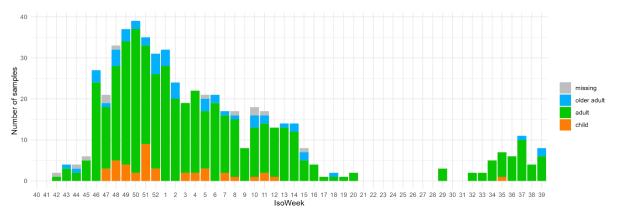


Figure 1 • Age distribution of ILI/ARI samples per week of collection, season 2022-2023

Age group	Brussels	Flanders	Wallonia	Total
child	9	18	14	41
adult	74	253	131	458
older adult	10	27	14	51
missing	4	7	1	12
Total	97	305	160	562

Table 4 • Number of ILI/ARI samples per age group and region, season 2022-2023

When looking by case definition (ECDC-ILI, ECDC-ARI, WHO-ARI), similar weekly age group distributions were observed (Figure 2).

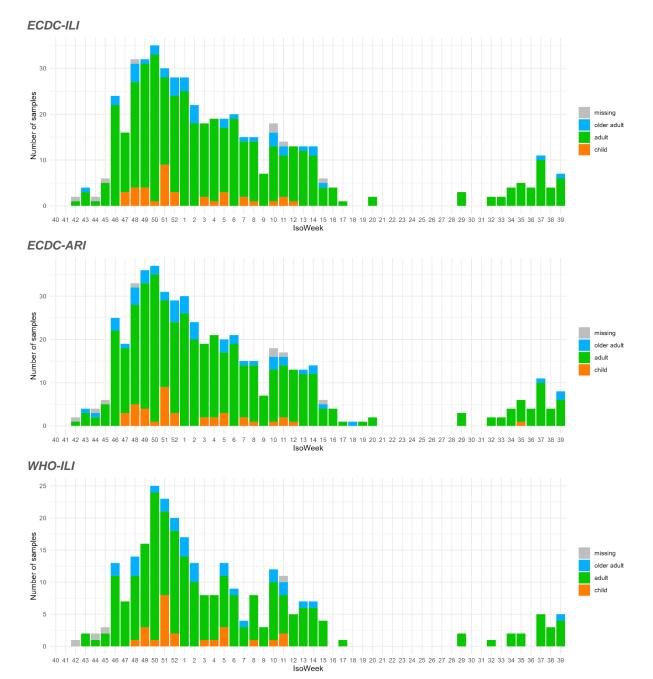


Figure 2 • Weekly age distribution of ILI cases matching the different case definitions, season 2022-2023

1.3. INFLUENZA VIRUS

The result for the influenza virus typing PCR was not available for one sample of bad quality, which was reported as 'undetermined' and was not tested for the other respiratory viruses.

Out of the 561 samples with a result for the influenza virus typing PCR, the first positive samples was detected in week 43-2022 and the last in week 14-2023 (Figure 3). The active period of circulation of influenza viruses truly started in week 46-2022. The highest proportion of positive samples was reached in week 51-2022.

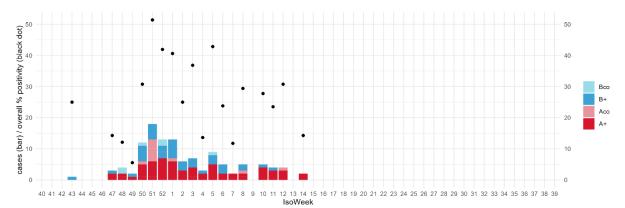


Figure 3 • Weekly number of ILI samples positive for influenza viruses A or B and percentage of influenza positivity, season 2022-2023

A+: influenza A virus detected alone; Aco: co-detection of influenza A virus and another respiratory virus; B+: influenza B virus detected alone; Bco: co-detection of influenza B virus and another respiratory virus

Overall the percentage of positivity for the season 2022-2023 was 21% (Table 5), with two third (61%, 72/118) positive for influenza type A and one third (32%, 46/118) for type B. Since adults represent the majority of cases, the percentage of positivity and the distribution between types A and B among adults were similar to the overall values. On the other hand, the percentage of positivity was higher among children (below 15 years old; 45%; 18/40) and lower among older adults (above 65 years old; 5.9%; 3/51).

Influenza virus typing PCR result	child	adult	older adult	missing	Total
Influenza A virus detected alone	4	53	2	2	61
Influenza A + another respiratory virus	6	5	0	0	11
Influenza B virus detected alone	8	31	1	0	40
Influenza B + another respiratory virus	0	6	0	0	6
Influenza virus not detected	22	363	48	10	443
Total	40	458	51	12	561

Table 5 • Age distribution of the influenza positive ILI cases, season 2022-2023

Although only 49% (275/561) of the cases matched the WHO ILI case definition, compared to 88.9% (499/561) or 94.7% (531/561) matching the ECDC ILI or ARI case definition, respectively, the percentage of positivity was higher. Out of the 275 cases matching the WHO ILI case definition, 50 (18.2%) and 33 (12%) were positive for influenza A and B, respectively. Out of the 499 cases matching the ECDC ILI case definition, 66 (13.2%) and 44 (8.8%) were positive for influenza A and B, respectively (Table 6).

Case	number	negative for	positive	positive
definition		influenza	for type A	for type B
WHO-ILI	275	192	50	33
ECDC-ILI	499	389	66	44
ECDC-ARI	531	420	66	45

Table 6 • Influenza typing PCR results for each ILI/ARI case definition, season 2022-2023

Among the 72 influenza A positive samples, the subtypes could be determined for 66 samples. The viral load of the remaining six samples was too low to allow subtype determination. H3N2 and H1N1dpm09 subtypes were identified in 41 and 25 samples, respectively, without a clear pattern in time (Figure 4).

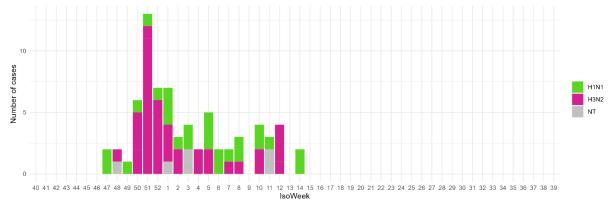


Figure 4 • Weekly distribution of influenza A viruses per subtype among ILI samples, season 2022-2023

NT: no subtype available; H1N1: subtype H1N1pdm09; H3N2: subtype H3N2

All 46 influenza B positive samples were of the Victoria lineage. No Yamagata lineage viruses were detected in 2022-2023 (Figure 5).

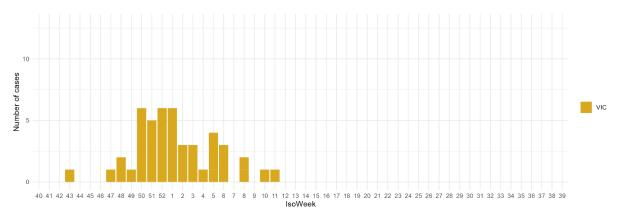


Figure 5 • Weekly distribution of influenza B viruses per lineage among ILI samples, season 2022-2023

VIC: Victoria lineage

1.4. SARS-COV-2 VIRUS

Positive samples were detected during almost the entire surveillance period when samples were collected (Figure 6). The highest proportion for positive samples were found outside the period of intense circulation of influenza viruses (weeks 50-2022 to 05-2023).

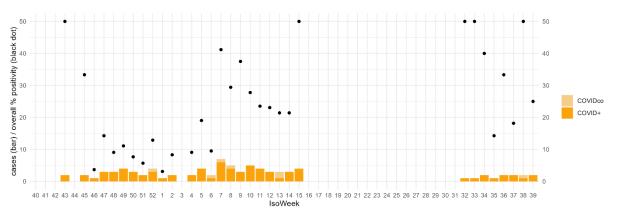


Figure 6 • Weekly number of ILI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2022-2023

COVID+: SARS-CoV-2 detected alone; COVIDco: co-detection of SARS-CoV-2 and another respiratory virus

Overall the percentage of positivity for the season 2022-2023 was 17.9% (Table 7), but reaching 25.5% (13/51) among older adults (above 65 years old).

SARS-CoV-2 PCR result	child	adult	older adult	missing	Total
SARS-CoV-2 detected alone	1	61	12	4	78
SARS-CoV-2 + another respiratory virus	1	5	1	0	7
SARS-CoV-2 virus not detected	38	392	38	8	476
Total	40	458	51	12	561

The differences in percentage of positivity were less important between case definitions than for influenza viruses. Out of the 275 cases matching the WHO ILI case definition, 33 (12%) were positive SARS-CoV-2. Out of the 499 and 531 cases matching the ECDC ILI and ARI case definitions, 79 (15.8%) and 80 (15.1%) were positive for SARS-CoV-2, respectively (Table 8). The WHO ILI case definition appears to be less adapted to properly capture the circulation of SARS-CoV-2 in the general population.

Table 8 • SARS-CoV-2 PCR results for each ILI/ARI case definition, season 20	22-2023
--	---------

Case definition	number	negative for SARS-CoV-2	SARS-CoV-2
WHO-ILI	275	242	33
ECDC-ILI	499	420	79
ECDC-ARI	531	451	80

1.5. RESPIRATORY SYNCYTIAL VIRUS

Positive samples were detected between week 44-2022 and 7-2023 (Figure 7). The highest proportions for positive samples were found just before the period of intense circulation of influenza viruses (weeks 50-2022 to 05-2023).

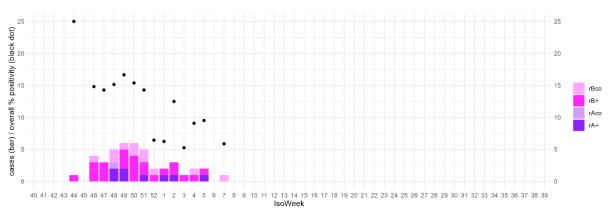


Figure 7 • Weekly number of ILI samples positive for respiratory syncytial viruses A or B and percentage of RSV positivity, season 2022-2023

rA+: RSV type A virus detected alone; rAco: co-detection of RSV type A virus and another respiratory virus; rB+: RSV B virus detected alone; rBco: co-detection of RSV B virus and another respiratory virus

Overall the percentage of positivity for the season 2022-2023 was 7.7% (Table 9), with type A virus dominating (79%, 34/43) over type B (21%, 9/43). The percentage of positivity and the distribution between types A and B among adults were similar to the overall values. The number of positive samples are too low among children (below 15 years old) and older adults (above 65 years old) to draw conclusion.

RSV PCR result	child	adult	older adult	missing	Total
RSV type A detected alone	2	5	1	0	8
RSV-A + another respiratory virus	1	0	0	0	1
RSV type B detected alone	0	16	7	0	23
RSV-B + another respiratory virus	1	10	0	0	11
RSV not detected	36	427	43	12	518
Total	40	458	51	12	561

Table 9 • Age distribution of the RSV positive ILI cases, season 2022-2023

The percentage of positivity were very similar between case definitions. Out of the 275 cases matching the WHO ILI case definition, 5 (1.8%) and 19 (6.9%) were positive for RSV A and B, respectively. Out of the 499 and 531 cases matching the ECDC ILI and ARI case definitions, 6 (1.2%) and 28 (5.6%), and, 8 (1.5%) and 34 (6.4%) were positive for RSV A and B, respectively (Table 10).

Table 10 • RSV PCR results for each ILI/ARI case definition, season 2022-2023

			,	
Case definition	number	negative for RSV	positive for type A	positive for type B
WHO-ILI	275	251	5	19
ECDC-ILI	499	465	6	28
ECDC-ARI	531	489	8	34

1.6. OTHER RESPIRATORY VIRUSES

Beside for human metapneumoviruses, for which an epidemic wave was detected (Figure 8), other respiratory viruses were only sporadically but regularly detected throughout the season.

Rhinoviruses and enteroviruses were detected in 15.6% of the samples, followed by human metapneumoviruses in 5.7%, seasonal coronaviruses in 3.8%, parainfluenzaviruses in 2.1% and adenoviruses in 1.4%.

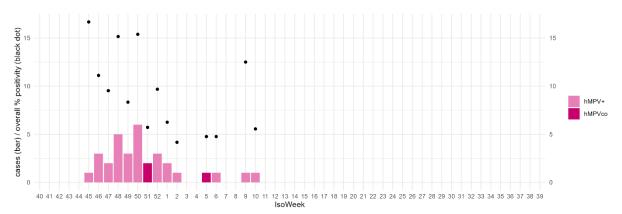


Figure 8 • Weekly number of ILI samples positive for human metapneumoviruses and percentage of positivity, season 2022-2023

hMPV+: human metapneumovirus detected alone; hMPVco: co-detection of human metapneumovirus and another respiratory virus

2. Nursing home - ILI

The surveillance of influenza-like illness (ILI) in nursing homes started in October 2022, but sampling only started in December 2022. The network comprised 37 nursing homes in the country, that weekly report to the Department of Infectious Diseases at Sciensano the total number of influenza-like infection identified in the nursing home ^e.

In addition, they were requested to take a nasopharyngeal swab from the first 2 ILI cases of the week. The NRC influenza provided the sampling kits (nasopharyngeal swab and UTM transport medium) and the packaging for sending the samples (prepaid envelops). All the samples were sent to the NRC influenza for testing.

In total, 15 nursing homes submitted 58 samples between week 51-2022 and week 18-2023. Two samples were collected outside this period, one in week 24-2023 and one in week 35-2023. The nursing homes participating to the virological surveillance were predominantly from the Flemish region (Table 11).

Table 11 • Number of samples and contributing nursing homes per province, season 2022-2023

	ve gion		The of frailoning normed
В	russels	9	2
F	landers	38	11
W	/allonia	13	2

n: number of samples; Nb: number

https://www.sciensano.be/fr/biblio/influenza-illness-including-covid-19-sentinel-surveillance-belgian-nursing-homesamended-protocol-v2

Thirty-three samples (55%) were positive for respiratory viruses. SARS-CoV-2 was detected in 11 samples, followed by RSV in 7 and seasonal coronaviruses in 8. Influenza viruses were detected in only 3 samples. The remaining 4 samples were positive for entero- or rhinoviruses or human pneumoviruses.

3. Severe Acute Respiratory Infections (SARI)

3.1. NETWORK OF SENTINEL HOSPITALS

The surveillance of severe acute respiratory infections (SARI) is organised through a network of 6 hospitals in Belgium, 2 in each region (Flanders, Wallonia, Brussels). The network was implemented in 2012, following the recommendations of WHO after the 2009 H1N1 pandemic to reinforce the surveillance of severe cases. More information on the network can be found on Sciensano's website ¹. The hospitals are requested to recruit all cases matching the case definition and to take a nasopharyngeal swab. The NRC influenza can provide the sampling kits (nasopharyngeal swab and UTM transport medium) and the packaging for sending the samples (prepaid envelops), but following the COVID-19 pandemic, less hospitals require the sampling kits. All the samples are sent to the NRC influenza for testing, even if they already have been tested in the hospital microbiological laboratory.

3.2. SAMPLE INFORMATION

A total of 3450 nasopharyngeal swabs were collected during the 2022-2023 season and were sent to the NRC influenza for testing, with very similar numbers from the three regions (Table 12).

Region	n
Brussels	1284
Flanders	1135
Wallonia	1031

n: number of samples

Out of the 3450 enrolled cases, 64.3% matched the case definition defined in the protocol and following the WHO SARI case definition (Table 13). When considering broader case definitions, 87.1% and 88.4% of the enrolled cases matched the ECDC-COVID and BE-COVID case definitions, respectively. These proportions remained relatively stable over the seasons within the SARS-CoV-2 pandemic area.

Table 13 • Number	of S/	ARI :	samples	responding	to t	he	different	clinical	case	definitions	(with
percentages)											

Season	n	WHO- SARI	ECDC- COVID	BE- COVID	p_WHO- SARI	p_ECDC- COVID	p_BE- COVID
2022-2023	3450	2220	3004	3051	64.4	87.1	88.4
2021-2022	2621	1806	2370	2387	68.9	90.4	91.1
2020-2021	1331	982	1231	1253	73.8	87.1	94.1

n: number of samples; p: percentage

^f https://www.sciensano.be/en/projects/severe-acute-respiratory-infection-surveillance-a-sentinel-network-hospitals

The median time between sampling date and reception date was 17 days, but it varied by region (38 days for Flanders, 11 days for Wallonia, and 22 days for Brussels). For convenience, samples collected for the SARI surveillance were often sent by batch covering a few weeks of collection.

Age was missing or not yet communicated for 9.6% of the samples. The age distribution showed a predominance of older adult (above the age of 65) patients within the SARI surveillance (Figure 9). Overall, when age was known, 50.3% (1570/3120) of the patients were older adults (Table 14). Children (below the age of 15) and adults (between 15 and 65 years old) were nonetheless well covered too, representing 28.3% (884/3120) and 21.3% (666/3120), respectively. As requested in the protocol for this season, sample collection took place each week during the season.

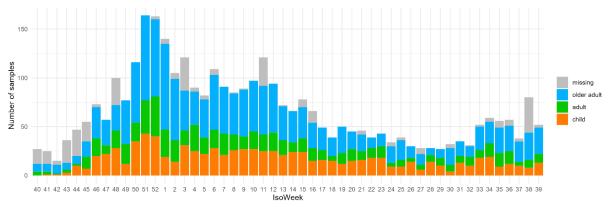


Figure 9 • Age distribution of SARI samples per week of collection, season 2022-2023

Age group	Brussels	Flanders	Wallonia	Total
child	373	427	84	884
adult	213	182	271	666
older adult	371	526	673	1570
missing	327	0	3	330
Total	1284	1135	1031	3450

Table 14 • Number of SARI samples per age group and region, season 2022-2023

When looking by case definition, more than 90% of child cases matched the WHO-SARI case definition, but only around 60% for adult and older adult cases (Table 15). Nearly all cases matched the broader ECDC-COVID and BE-COVID case definitions.

Table 15 • Number of SARI samples per age group and case definition, season 2022-2023

Case definition	Child	Adult	Older adult	Missing
WHO-SARI	811	439	959	11
ECDC-COVID	884	656	1449	15
BE-COVID	884	658	1494	15
Total	884	666	1570	330

3.3. INFLUENZA VIRUS

The result for the influenza virus typing PCR was not available for 11 samples of bad quality, which were reported as 'undetermined' and were not tested for the other respiratory viruses.

Out of the 3439 samples with a result for the influenza virus typing PCR, the first positive sample was detected in week 44-2022 and the last in week 14-2023 (Figure 10). Sporadic positive samples were detected afterwards. The highest proportion of positive samples was reached in week 52-2022.

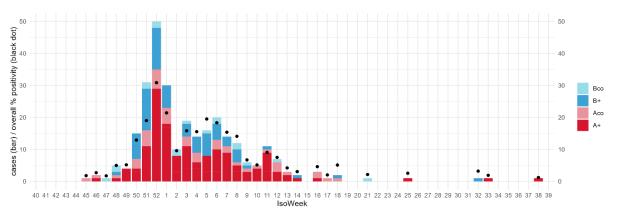


Figure 10 • Weekly number of SARI samples positive for influenza viruses A or B and percentage of influenza positivity, season 2022-2023

A+: influenza A virus detected alone; Aco: co-detection of influenza A virus and another respiratory virus; B+: influenza B virus detected alone; Bco: co-detection of influenza B virus and another respiratory virus

Overall, 289 samples were positive for an influenza virus and the percentage of positivity for the season 2022-2023 was 8.4% (Table 16), with two third (67.8%, 196/289) positive for influenza type A and one third (32.2%, 93/289) for type B. The percentage of positivity decreased with age, ranging from 12.9% (114/881) for children (below 15 years old), to 8.8% (58/663) for adults (between 15 and 65 years old) and 6.4% (100/1569) for older adults (above 65 years old). The proportion of influenza type B was higher among children (55.3%), while influenza type A was more frequent among adults (65.5%) and almost exclusive among older adults (93%).

Influenza virus typing PCR result	child	adult	older adult	missing	Total
Influenza A virus detected alone	24	33	83	11	151
Influenza A + another respiratory virus	27	5	10	3	45
Influenza B virus detected alone	50	17	6	2	75
Influenza B + another respiratory virus	13	3	1	1	18
Influenza virus not detected	767	605	1469	309	3150
Total	881	663	1569	326	3439

Table 16 • Age distribution of the influenza positive SARI cases, season 2022-2023

Among the 196 influenza A positive samples, the subtypes could be determined for 185 samples. The viral load of the remaining 11 samples was too low to allow subtype determination. H3N2 and H1N1pdm09 subtypes were identified in 101 and 84 samples, respectively, without a clear pattern in time (Figure 11).

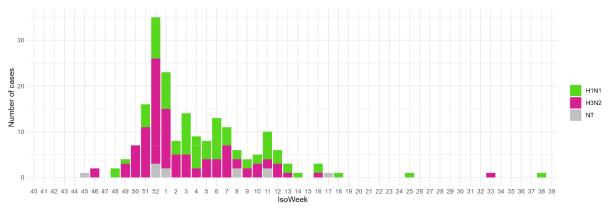


Figure 11 • Weekly distribution of influenza A viruses per subtype among SARI samples, season 2022-2023

NT: no subtype available; H1N1: subtype H1N1pdm09; H3N2: subtype H3N2

All 93 influenza B positive samples were of the Victoria lineage. No Yamagata lineage viruses were detected in 2022-2023 (Figure 12).

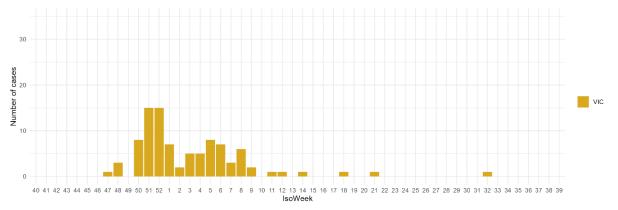


Figure 12 • Weekly distribution of influenza B viruses per lineage among SARI samples, season 2022-2023

VIC: Victoria lineage

3.4. SARS-COV-2 VIRUS

Positive samples were detected during almost the entire surveillance period when samples were collected (Figure 13). The highest proportion for positive samples were found after the period of intense circulation of influenza viruses (weeks 51-2022 to 08-2023, Figure 10).

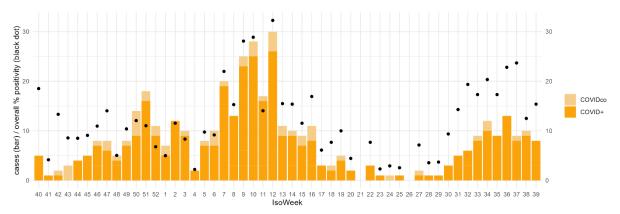


Figure 13 • Weekly number of SARI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2022-2023

COVID+: SARS-CoV-2 detected alone; COVIDco: co-detection of SARS-CoV-2 and another respiratory virus

Overall, 422 samples were positive for SARS-CoV-2 and the percentage of positivity for the season 2022-2023 was 12.3% (Table 17), but reaching 17.7% (278/1569) among older adults (above 65 years old) and only 5.6% (49/881) among children (below 15 years old).

Table 17 • Age distribution of the SARS-CoV-2 positive				
SARS-CoV-2 PCR result child a	adult	older adult	missing	Тс

SARS-COV-2 PCR result	child	adult	older adult	missing	Iotal
SARS-CoV-2 detected alone	28	63	263	15	369
SARS-CoV-2 + another respiratory virus	21	12	15	5	53
SARS-CoV-2 virus not detected	832	588	1291	302	3013
Total	881	663	1569	322	3435

.

3.5. RESPIRATORY SYNCYTIAL VIRUS

Positive samples were detected during almost the entire surveillance period when samples were collected (Figure 14). The highest proportions for positive samples were found just before the period of intense circulation of influenza viruses (weeks 51-2022 to 08-2023, Figure 10), although they remained quite high until week 03-2023.

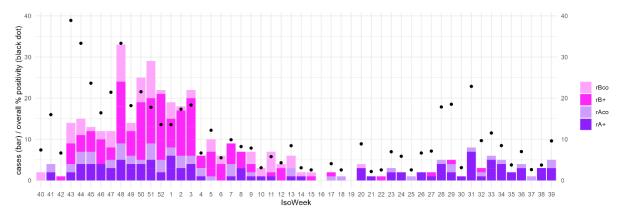


Figure 14 • Weekly number of SARI samples positive for respiratory syncytial viruses A or B and percentage of RSV positivity, season 2022-2023

rA+: RSV type A virus detected alone; rAco: co-detection of RSV type A virus and another respiratory virus; rB+: RSV B virus detected alone; rBco: co-detection of RSV B virus and another respiratory virus

Overall, 391 samples were positive for a respiratory syncytial virus and the percentage of positivity for the season 2022-2023 was 11.4% (Table 18), with RSV type B (57.8%, 226/391) being slightly more detected than type A viruses (42.2%, 165/391). However, RSV type A dominated during the epidemic wave in winter (week 43-2022 to 13-2023), while RSV type B continued to circulate in spring and summer (Figure 14). The percentage of positivity reached 21.9% (193/880) for children (below 15 years old), with RSV type A slightly dominating over type B (56.5%, 109/193 vs 43.5%, 84/193). Among adults (between 15 and 65 years old) and older adults (above 65 years old), the proportion of RSV positivity was only 4.5% (30/663) and 5.7% (90/1569), respectively, with RSV type B clearly dominating (more than 70% among the positives).

RSV PCR result	child	adult	older adult	missing	Total
RSV type A detected alone	68	6	17	17	108
RSV-A + another respiratory virus	41	2	2	12	57
RSV type B detected alone	43	18	62	29	152
RSV-B + another respiratory virus	41	4	9	20	74
RSV not detected	687	633	1479	245	3044
Total	880	663	1569	323	3435

Table 18 • Age distribution of the RSV positive SARI cases, season 2022-2023

3.6. OTHER RESPIRATORY VIRUSES

3.6.1. Picornavirus (rhinovirus, enterovirus, parechovirus)

Positive samples were detected at relatively high proportion during the entire surveillance period when samples were collected (Figure 15). Viruses were mostly entero- and rhinoviruses. Very few parechoviruses were detected. The specific enterovirus D68 was detected in 4 samples. Overall, picornaviruses were frequently co-detected with another respiratory viruses (Table 19).

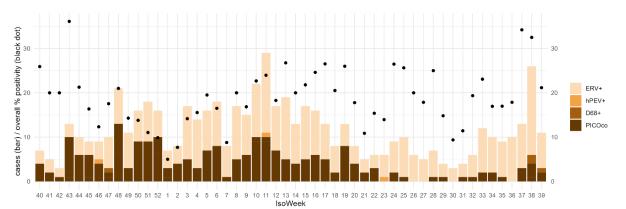


Figure 15 • Weekly number of SARI samples positive for rhino-, entero- and parechoviruses and percentage of positivity, season 2022-2023

ERV+: entero- or rhinovirus detected alone; hPEV+: parechovirus detected alone; D68+: enterovirus D68 detected alone; PICOco: co-detection of entero-, rhino- or parechovirus and another respiratory virus

PCR result	child	adult	older adult	missing	Total
Rhino- / enterovirus detected alone	173	66	109	37	385
Enterovirus D68 detected alone	1	2	0	1	4
Parechovirus detected alone	3	0	0	0	3
Picornavirus + another respiratory virus	149	6	19	39	213
Picornavirus not detected	554	589	1441	249	2833
Total	880	663	1569	326	3438

Table 19 • Age distribution of the picornavirus positive SARI cases, season 2022-2023

3.6.2. Human metapneumovirus

Positive samples were mainly detected as an epidemic wave concomitant with respiratory syncytial virus (Figure 16). All age groups were concerned, but co-detection with another respiratory virus was more frequent among children (Table 20).

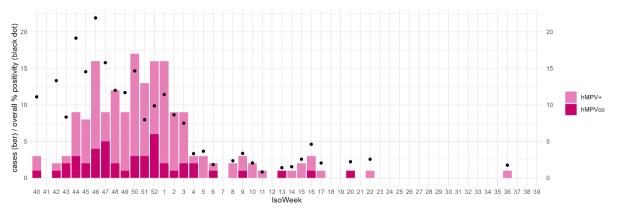


Figure 16 • Weekly number of SARI samples positive for human metapneumoviruses and percentage of positivity, season 2022-2023

hMPV+: human metapneumovirus detected alone; hMPVco: co-detection of human metapneumovirus and another respiratory virus

Table 20 • Age distribution of the metapneumovirus	positive SARI cases, season 2022-2023
--	---------------------------------------

PCR result	child	adult	older adult	missing	Total
Metapneumovirus detected alone	25	25	69	14	133
Metapneumovirus + another resp. virus	23	1	9	12	45
Metapneumovirus not detected	833	637	1491	300	3261
Total	881	663	1569	326	3439

3.6.3. Parainfluenzavirus

Positive samples were detected throughout the entire surveillance period when samples were collected, with a small epidemic wave following that of SARS-CoV-2 (Figure 17). Parainfluenzavirus type 3 dominated with all age groups being concerned (Table 21). Co-detection with another respiratory virus was more frequent among children.

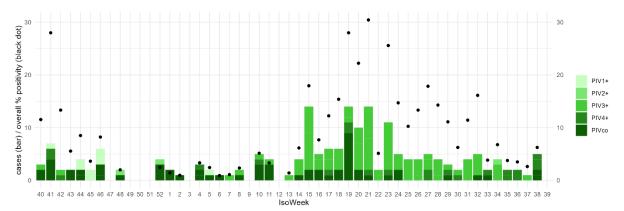


Figure 17 • Weekly number of SARI samples positive for human parainfluenzaviruses and percentage of positivity, season 2022-2023

PIV1+: parainfluenzavirus type 1 detected alone; PIV2+: parainfluenzavirus type 2 detected alone; PIV3+: parainfluenzavirus type 3 detected alone; PIV4+: parainfluenzavirus type 4 detected alone; PIVco: co-detection of parainfluenzavirus and another respiratory virus

PCR result	child	adult	older adult	missing	Total
Parainfluenzavirus type 1 detected alone	1	1	4	3	9
Parainfluenzavirus type 2 detected alone	1	2	0	0	3
Parainfluenzavirus type 3 detected alone	27	19	47	6	99
Parainfluenzavirus type 4 detected alone	4	11	4	3	22
PIV + another respiratory virus	36	5	6	12	59
Parainfluenzavirus not detected	812	625	1508	301	3246
Total	881	663	1569	325	3438

PIV: parainfluenzavirus

3.6.4. Seasonal coronavirus

Positive samples were detected at relatively low proportion during the winter months (Figure 18). Human coronavirus OC43 was the most frequently detected, with all age groups being concerned (Table 22). Co-detection with another respiratory virus was more frequent among children.

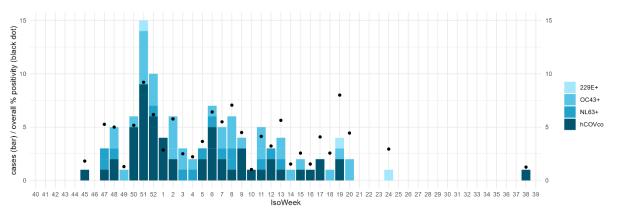


Figure 18 • Weekly number of SARI samples positive for seasonal coronaviruses and percentage of positivity, season 2022-2023

229E+: coronavirus 229E detected alone; OC43+: coronavirus OC43 detected alone; NL63+: coronavirus NL63 detected alone; hCOVco: co-detection of seasonal coronavirus and another respiratory virus

PCR result	child	adult	older adult	missing	Total
Coronavirus 229E detected alone	0	1	2	0	3
Coronavirus NL63 detected alone	8	2	3	1	14
Coronavirus OC43 detected alone	11	9	16	1	37
hCOV + another respiratory virus	40	3	9	3	55
Seasonal coronavirus not detected	822	648	1539	321	3330
Total	881	663	1569	326	3439

Table 22 • Age distribution of the seasonal coronavirus positive SARI cases, season 2022-2023

3.6.5. Adenovirus

Positive samples were detected at relatively low proportion during the entire surveillance period when samples were collected (Figure 19). Adenoviruses were frequently co-detected with another respiratory viruses, and almost exclusively among children (Table 23).

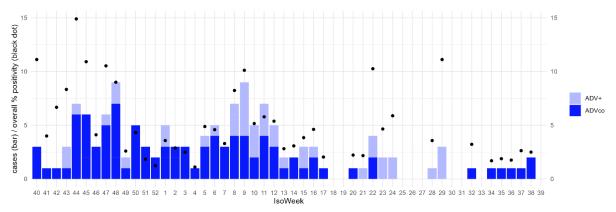


Figure 19 • Weekly number of SARI samples positive for adenoviruses and percentage of positivity, season 2022-2023

ADV+: adenovirus detected alone; ADVco: co-detection of adenovirus and another respiratory virus

PCR result	child	adult	older adult	missing	Total
Adenovirus detected alone	26	5	7	4	42
Adenovirus + another resp. virus	68	1	2	27	98
Adenovirus not detected	787	657	1560	295	3299
Total	881	663	1569	326	3439

3.6.6. Bocavirus

Positive samples were detected at very low proportion throughout the entire surveillance period when samples were collected (Figure 20). Bocaviruses were almost exclusively detected among children and in co-detection with another respiratory virus (Table 24).

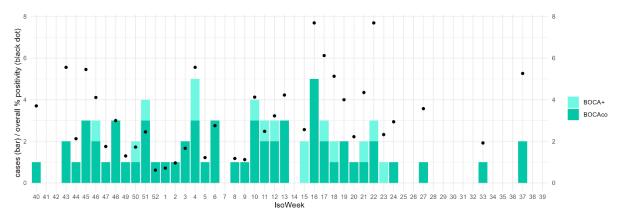


Figure 20 • Weekly number of SARI samples positive for bocaviruses and percentage of positivity, season 2022-2023

BOCA+: bocavirus detected alone; BOCAco: co-detection of bocavirus and another respiratory virus

Table 24 • Age distribution of the bocavirus positive SARI cases, season 2022-2023

PCR result	child	adult	older adult	missing	Total
Bocavirus detected alone	14	0	1	0	15
Bocavirus + another resp. virus	44	4	1	12	61
Bocavirus not detected	823	659	1567	314	3363
Total	881	663	1569	326	3439

NON-SENTINEL SURVEILLANCE

1. Hospital laboratories (HOSPI)

The laboratories from all the hospitals in Belgium can send samples to the NRC influenza for confirmation of influenza positive cases or for the determination of the subtype (influenza A) or the lineage (influenza B) or for a differential diagnostic with other respiratory viruses in particular MERS-CoV (very specific cases).

During the season 2022-2023, 19 hospitals submitted 94 samples to the NRC influenza. The hospitals sending samples for characterisation were predominantly from the Flemish region (Table 25).

Table 25 • Number of HOSPI samples and contributing hospitals per province, season 2022-2023

Region	n	Nb of hospitals
Brussels	22	2
Flanders	67	14
Wallonia	5	3

n: number of samples; Nb: number

Thirteen samples were confirmed as positive for influenza type B virus: all belonged to the Victoria lineage. Among the 50 samples confirmed positive for influenza type A virus, 21 H1N1pdm09 and 28 H3N2 were detected. The viral load in one influenza type A virus did not allow the subtype determination. The remaining samples were negative for influenza viruses: they were either not confirmed by the NRC influenza or sent for another purpose (tested for other respiratory viruses).

2. Zoonotic influenza

2.1. SUSPECTED CASES OF NON-SEASONAL INFLUENZA

The NRC influenza did not receive any samples to test from a true suspicion of a human case of infection with a non-seasonal influenza virus (infection with an influenza virus of animal origin).

However, with the epizootic situation of highly pathogenic H5N1 viral infection in birds, and following ECDC's recommendations to increase surveillance in humans during the summer months, the NRC influenza received additional samples for subtyping in the HOSPI surveillance.

2.2. ZOOIS: ZOONOTIC INFLUENZA SURVEILLANCE PROJECT

The NRC influenza, in collaboration with Sciensano's services Epidemiology of Infectious Diseases and Avian Virology and Immunology, worked with the Flemish Regional Health Authorities (AZG) to develop and implement a research project aiming at implementing an active surveillance of zoonotic transmission events in exposed populations.

The project focuses on three worker categories who are at higher risk of exposure to influenza viruses of animal influenza viruses: workers in poultry farms, workers and volunteers in wild life rehabilitation centres, veterinarians in the pig sectors.

The first months of the project were dedicated to the preparation of the documents to obtain approval from an ethical committee, as the research project is assimilated to a clinical study.

Enrolment of participants started in September 2023.

The project was included in the United4Surveillance ^g project funded by the European Union, as a pilot study within work package 4. It was presented during the workshop organised in the framework of the United4Surveillance project, when all the stakeholders involved in zoonotic influenza surveillance were invited to discuss on how to improve the surveillance of zoonotic transmission events ^h.

⁹ https://united4surveillance.eu/

h https://www.sciensano.be/en/events/u4s-workshop-one-health-surveillance

CHARACTERISATION

1. Virus isolation

Virus isolation is routinely performed only for influenza viruses at the NRC influenza. SARS-CoV-2 virus isolation are not currently performed routinely.

In total, 109 ILI and 258 SARI samples positive for influenza viruses were used to attempt influenza virus isolation (Table 26 and Table 27). H3N2 virus positive samples were inoculated in MDCK-SIAT cells, and H1N1 and Victoria virus positive samples were inoculated in MDCK cells. After inoculation, cells were observed daily over a period of 72 hours post infection to check for the appearance of a characteristic cytopathic effect.

Table 26 • Virus isolation from ILI samples, season 2022-2023

Subtype or lineage	isolated	negative	contaminated	Total
A/H3N2	40	1	0	41
A/H1N1pdm09	17	2	3	22
B/VIC	37	5	4	46
Total	94	8	7	109

Table 27 • Virus isolation from SARI samples, season 2022-2023

Subtype or lineage	isolated	negative	contaminated	Total
A/H3N2	71	9	14	94
A/H1N1pdm09	44	17	15	76
B/VIC	74	4	10	88
Total	189	30	39	258

A representative selection of these isolated influenza viruses were sent to the WHO Collaborating Centre at the Crick Institute in London (United-Kingdom), as part of the WHO-recognised National Influenza Centre's terms of reference, to be further characterised and compared with isolates from other countries in preparation of the Vaccine Composition Meetings to select the vaccine strains.

There is currently no similar system of systematic exchange in place for SARS-CoV-2 viruses.

2. Sequencing

Whole genome sequencing using NGS Oxford Nanopore MinION technology is performed at the NRC influenza for SARS-CoV-2 and influenza viruses.

2.1. SARS-COV-2 CORONAVIRUS

Sequencing was attempted on all SARI samples positive for SARS-CoV-2 with a sufficient viral load, as part of a specifically-funded project.

A total of 257 samples positive for SARS-CoV-2 coronavirus were processed for sequencing. Two systems are used for further subdivision to classify SARS-CoV-2 based on genomic analysis: Nextclade (Table 28) and Pangolin lineage (Table 29). Figure 21 presents a summary of the evolution of SARS-

CoV-2 viruses with a correspondence between the Nextclade system and the main representant in the Pangolin lineage (taken from ¹).

During the 2022-2023 season, only viruses belonging to the Omicron group were detected, all descending from clade 21L (BA.2). Clades 22B (BA.5) and its progeny 22E (BQ.1), and 22F (XBB) and its progeny 23A (XBB.1.5) were the most prominent groups.

Table 28 • Clade distribution of the SARS-CoV-2 positive samples based on Nextclade system, season 2022-2023

Nextclade	ILI	SARI	Total
21L (Omicron)	0	1	1
22B (Omicron)	5	30	35
22D (Omicron)	8	21	29
22E (Omicron)	12	73	85
22F (Omicron)	5	25	30
23A (Omicron)	20	41	61
23C (Omicron)	0	5	5
23F (Omicron)	6	0	6
Recombinant	1	5	6
Total	56	201	257

Table 29 • Clade distribution of the SARS-CoV-2 positive samples based on Pangolin lineage system, season 2022-2023

Pangolin	ILI	SARI	Total
BA.2		1	1
BA.5		1	1
BA.5.1		3	3
BA.5.1.8		1	1
BA.5.1.9		1	1
BA.5.2		3	3
BA.5.2.1		2	2
BA.5.2.20		1	1
BA.5.2.27		1	1
BA.5.2.6	1		1
BA.5.2.7		1	1
BA.5.6.3		1	1
BA.5.9	1		1
BE.1	1		1
BE.1.1		1	1
BE.9	1		1
BF.10		1	1
BF.11		1	1
BF.14	1		1
BF.7		1	1
BF.7.21		1	1
BF.7.4		2	2
BF.7.5		1	1
BF.7.6		1	1
BN.1.4	1		1
BN.1.4.3		1	1
BN.1.5		1	1
BQ.1	1	7	8
BQ.1.1	4	30	34

PO 1 1 1		2	2
BQ.1.1.1		3	3
BQ.1.1.14			1
BQ.1.1.15	0	1	1
BQ.1.1.18	2	6	8
BQ.1.1.23	4	1	1
BQ.1.1.3	1	4	5
BQ.1.1.35	1	1	2
BQ.1.1.38	1		1
BQ.1.1.4		1	1
BQ.1.1.45	1	3	4
BQ.1.1.5		2	2
BQ.1.1.7		2	2
BQ.1.10		3	3
BQ.1.11.1		1	1
BQ.1.13.1		1	1
BQ.1.18		1	1
BQ.1.2		1	1
BQ.1.8		1	1
CH.1.1	7	14	21
CH.1.1.1		2	2
CH.1.1.11		4	4
CH.1.1.14		1	1
CH.1.1.2		1	1
CK.1		1	1
CK.2.1.1		1	1
CL.1.3		1	1
CY.1		1	1
DB.3		1	1
EA.1		1	1
EF.1		1	1
EF.1.1		2	2
EG.1	2	7	9
EG.5.1	1		1
EG.5.1.1	1		1
EG.5.1.3	4		4
FD.4.1		2	2
FL.2.1		1	1
FH.1	1		1
GK.1	2		2
GK.1.1	1		1
XBA		1	1
XBB	1	1	2
XBB.1		2	2
XBB.1.10		1	1
XBB.1.4		2	2
XBB.1.5	14	31	45
XBB.1.5.1	1	01	1
XBB.1.5.13		1	1
XBB.1.5.14		1	1
XBB.1.5.63		1	1
XBB.1.5.7	1	3	4
XBB.1.9.1	1	5	5
XBB.1.9.2	2	8	10
XBB.2.3	۷	1	1
XBF	1	2	3
XBF.7	I	1	1
XBK.1		2	2
Total	56	201	257
TOLAT	50	201	201

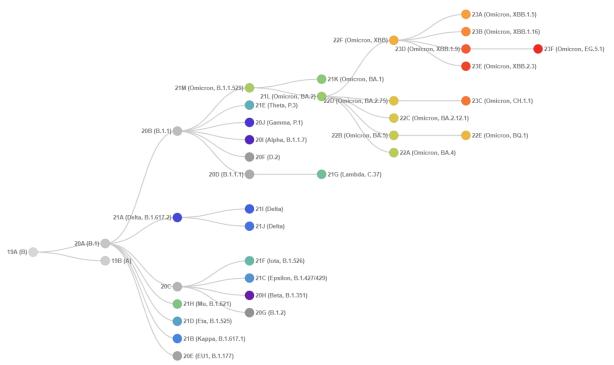


Figure 21 • Simplified evolution tree of SARS-CoV-2 viruses indicating the correspondence between the different classification system.

2.2. INFLUENZA VIRUSES

Sequencing was attempted on only a subset of the ILI and SARI samples positive for influenza viruses with a sufficient viral load. Sequence analysis allowed to classified the viruses in clades, as defined by WHO Euro / ECDC for reporting into TESSy (The European Surveillance System).

A total of 80 samples positive for H3N2 viruses were processed for sequencing (Table 30). Similar distributions among clades were found in the ILI and SARI surveillance systems. Several clades circulated during the 2022-2023 but two clearly dominated: 3C.2a1b.2a.2a.1b and 3C.2a1b.2a.2b.

Clade assignment	ILI	SARI	Total
3C.2a	0	1	1
3C.2a1b.2a.2a	1	1	2
3C.2a1b.2a.2a.1b	13	17	30
3C.2a1b.2a.2a.3a.1	4	1	5
3C.2a1b.2a.2a.3b	0	2	2
3C.2a1b.2a.2b	13	19	32
3C.3a	0	1	1
unassigned	1	6	7
Total	32	48	80

Table 30 • Clade distribution of the sequenced A/H3N2 viruses, season 2022-2023

Sixty-one samples positive for H1N1pdm09 viruses were processed for sequencing (Table 31). Less variability was observed for H1N1pdm09 viruses, with clade 6B.1A.5a.2a dominating, including its subpopulation 6B.1A.5a.2a.1.

Clade assignment	ILI	SARI	Total
6B.1A.5a.1	1	0	1
6B.1A.5a.2a	10	37	47
6B.1A.5a.2a.1	2	5	7
unassigned	4	2	6
Total	17	44	61

 Table 31 • Clade distribution of the sequenced A/H1N1pdm09 viruses, season 2022-2023

Seventeen samples positive for B/Victoria viruses were processed for sequencing (Table 32). Viruses mostly belonged to the V1A.3a.2 clade.

 Table 32 • Clade distribution of the sequenced B/Victoria viruses, season 2022-2023

Clade assignment	ILI	SARI	Total
V1A	1	0	1
V1A.1	2	0	2
V1A.3a.2	10	4	7
unassigned	0	0	0
Total	13	4	17

QUALITY MANAGEMENT

1. Quality System

The NRC influenza is accredited by BELAC (Belgian Accreditation Body) according to the ISO 15189 norm. In 2023, BELAC conducted a surveillance audit of all the NRCs hosted within the Viral Diseases service, including the NRC influenza. No comments were raised for the NRC influenza.

All the SOP (Standard Operating Procedures) for the accredited RT-PCR tests of the NRC influenza have been revised during the season. A new test for the simultaneous detection of SARS-CoV-2, influenza A and influenza B viruses was validated and the SOP and validation dossier were officialised in the Quality System (SOP 13/3/135).

In addition SOP about cell culture, virus isolation and titration were also officialised in the Quality System (SOP 13/3/131, 13/3/132, 13/3/133 and 13/3/134).

2. External Quality Assessment

As part of its requirements to maintain ISO 15189 accreditation and its recognition as National Influenza Centre by WHO, the NRC influenza successfully took part in several Proficiency Tests / External Quality Assessments (EQA) during the 2022-2023 season.

For influenza viruses:

The NRC influenza took part in the annual EQA organised by WHO during the summer to control the capacities of National Influenza Centres to detect influenza viruses and to correctly identify seasonal influenza viruses (influenza A virus subtype H1N1pdm09, seasonal influenza A virus subtype H3N2, seasonal influenza B virus), and non-seasonal influenza A viruses (subtype H5, H7 and H9). The EQA also includes some samples for sequencing characterisation to detect potential mutations associated with increased resistance to antivirals.

The NRC influenza also registered to commercial EQAs organised by QCMD: INFRNA22S_QAV54134 (only seasonal influenza viruses), INFTP22S_QAV064138 (also including non-seasonal influenza viruses).

Finally, the NRC influenza took part in the EQA organised by ECDC, coordinated by NIVM and distributed through QCMD.

For SARS-CoV-2 coronavirus:

WHO is now recommending an integrated surveillance of respiratory viruses through the ILI/ARI and SARI systems. In this context, capacity to detect SARS-CoV-2 is also now included in the annual EQA in summer.

The NRC influenza also registered to commercial EQAs organised by QCMD: SCV2_22C1D_QAC20415, SCV2_23C1B_QAV204215.

In addition, the NRC influenza took part in the EQA organised by ECDC, coordinated by RIVM (The Netherlands) and distributed through QCMD.

Finally, the NRC influenza took part in the Bioinformatic EQA organised by ECDC and coordinated by RIVM (The Netherlands) and Institut Pasteur (France).

For other respiratory viruses:

The NRC influenza registered to several commercial EQAs organised by QCMD covering the different viruses that are targeted by the PCRs routinely used for the surveillance:

- Respiratory syncytial viruses: RSV22S_QAV054142
- Human metapneumoviruses: MPV23S_QAV054135
- Parainfluenza viruses: PINFRNA23S_QAV064136
- Seasonal coronaviruses: CVRNA23S_QAV064137
- MERS coronavirus: MERS23S_QAV154181
- Picornaviruses: RVRNA23S_QAV064143, EVRNA23S_QAV984104, PeVRNA23S_QAV114145
- Adenoviruses: ADVDNA22S_QAV054133

ACKNOWLEDGEMENTS

The NRC influenza would like to thank:

- The general practitioners of the ILI network
- The coordinators in the nursing homes of the NH-ILI network
- The coordinators in the hospitals of the SARI network:
 - o AZ Sint-Jan, Brugge-Ostende: Marijke Reynders, Evelyn Petit
 - Hasselt: Koen Magerman, Door Jour, Marieke Bleyven
 - o UZB, Brussels: Lucie Seyler, Thomas Demuyser, Arne Witdouck, Els Van Nedervelde
 - Hôpital Universitaire St-Pierre: Nicolas Dauby, Marc Hainaut
 - o Grand Hôpital de Charleroi: Xavier Holemans, Bénédicte Lissoir
 - o Mont-Godinne: Marc Bourgeois, Bénédicte Delaere

The sentinel surveillance of respiratory viruses and the activities of the NRC influenza are funded by:

- The institute for health and social security RIZIV-INAMI
- The federal public service Public Health
- The regional health agencies of the federated entities:
 - o AZG
 - o AVIQ
 - COCOM

PUBLICATIONS

Scientific articles published during the 2022-2023 season:

Denayer S, Dufrasne FE, Monsieurs B, van Eycken R, Houben S, Seyler L, Demuyser T, van Nedervelde E, Bourgeois M, Delaere B, Magerman K, Jouck D, Lissoir B, Sion C, Reynders M, Petit E, Dauby N, Hainaut M, Laenen L, Maes P, Baele G, Dellicour S, Cuypers L, André E, Couvreur S, Brondeel R, Barbezange C, Bossuyt N, van Gucht S. Genomic monitoring of SARS-CoV-2 variants using sentinel SARI hospital surveillance. Influenza Other Respir Viruses. 2023 Oct 13;17(10):e13202.

Fischer N, Moreels S, Dauby N, Reynders M, Petit E, Gérard M, Lacor P, Daelemans S, Lissoir B, Holemans X, Magerman K, Jouck D, Bourgeois M, Delaere B, Quoilin S, Van Gucht S, Thomas I, Bossuyt N, Barbezange C. Influenza versus other respiratory viruses - assessing severity among hospitalised children, Belgium, 2011 to 2020. Euro Surveill. 2023 Jul;28(29):2300056. doi: 10.2807/1560-7917.ES.2023.28.29.2300056.

Cuypers L, Keyaerts E, Hong SL, Gorissen S, Menezes SM, Starick M, Van Elslande J, Weemaes M, Wawina-Bokalanga T, Marti-Carreras J, Vanmechelen B, Van Holm B, Bloemen M, Dogne JM, Dufrasne F, Durkin K, Ruelle J, De Mendonca R, Wollants E, Vermeersch P; COVID-19 Genomics Belgium Consortium; Boulouffe C, Djiena A, Broucke C, Catry B, Lagrou K, Van Ranst M, Neyts J, Baele G, Maes P, André E, Dellicour S, Van Weyenbergh J. Immunovirological and environmental screening reveals actionable risk factors for fatal COVID-19 during post-vaccination nursing home outbreaks. Nat Aging. 2023 Jun;3(6):722-733. doi: 10.1038/s43587-023-00421-1.

Kissling E, Maurel M, Emborg HD, Whitaker H, McMenamin J, Howard J, Trebbien R, Watson C, Findlay B, Pozo F, Bolt Botnen A, Harvey C, Rose A; European IVE group; Members of the European IVE group. Interim 2022/23 influenza vaccine effectiveness: six European studies, October 2022 to January 2023. Euro Surveill. 2023 May;28(21):2300116. doi: 10.2807/1560-7917.ES.2023.28.21.2300116.

Van Poelvoorde LAE, Dufrasne FE, Van Gucht S, Saelens X, Roosens NHC. Development of Digital Droplet PCR Targeting the Influenza H3N2 Oseltamivir-Resistant E119V Mutation and Its Performance through the Use of Reverse Genetics Mutants. Curr Issues Mol Biol. 2023 Mar 17;45(3):2521-2532. doi: 10.3390/cimb45030165.

Stobbelaar K, Mangodt TC, Van der Gucht W, Delhaise L, Andries J, Gille V, Barbezange C, Smet A, De Winter BY, De Dooy JJ, Schepens T, Duval ELIM, Cos P, Jorens PG, Verhulst S, Delputte PL. Risk Factors Associated with Severe RSV Infection in Infants: What Is the Role of Viral Co-Infections? Microbiol Spectr. 2023 Jun 15;11(3):e0436822. doi: 10.1128/spectrum.04368-22. Epub 2023 May 22.

LIST OF TABLES

Table 1 • Number of ILI/ARI samples and contributing general practitioners per season	8
Table 2 • Number of ILI/ARI samples and contributing general practitioners per province, season 2022-2023	
	9
Table 3 • Number of ILI/ARI samples responding to the different clinical case definitions (with percentages)	9
Table 4 • Number of ILI/ARI samples per age group and region, season 2022-2023	. 10
Table 5 • Age distribution of the influenza positive ILI cases, season 2022-2023	. 11
Table 6 • Influenza typing PCR results for each ILI/ARI case definition, season 2022-2023	. 12
Table 7 • Age distribution of the SARS-CoV-2 positive ILI cases, season 2022-2023	. 13
Table 8 • SARS-CoV-2 PCR results for each ILI/ARI case definition, season 2022-2023	. 13
Table 9 • Age distribution of the RSV positive ILI cases, season 2022-2023	. 14
Table 10 • RSV PCR results for each ILI/ARI case definition, season 2022-2023	. 14
Table 11 • Number of samples and contributing nursing homes per province, season 2022-2023	. 15
Table 12 • Number of SARI samples per province, season 2022-2023	. 16
Table 13 • Number of SARI samples responding to the different clinical case definitions (with percentages)	
Table 14 • Number of SARI samples per age group and region, season 2022-2023	. 17
Table 15 • Number of SARI samples per age group and case definition, season 2022-2023	. 17
Table 16 • Age distribution of the influenza positive SARI cases, season 2022-2023	
Table 17 • Age distribution of the SARS-CoV-2 positive SARI cases, season 2022-2023	. 19
Table 18 • Age distribution of the RSV positive SARI cases, season 2022-2023	
Table 19 • Age distribution of the picornavirus positive SARI cases, season 2022-2023	
Table 20 • Age distribution of the metapneumovirus positive SARI cases, season 2022-2023	
Table 21 • Age distribution of the parainfluenzavirus positive SARI cases, season 2022-2023	. 22
Table 22 • Age distribution of the seasonal coronavirus positive SARI cases, season 2022-2023	. 23
Table 23 • Age distribution of the adenovirus positive SARI cases, season 2022-2023	. 23
Table 24 • Age distribution of the bocavirus positive SARI cases, season 2022-2023	
Table 25 • Number of HOSPI samples and contributing hospitals per province, season 2022-2023	
Table 26 • Virus isolation from ILI samples, season 2022-2023	
Table 27 • Virus isolation from SARI samples, season 2022-2023	. 27
Table 28 • Clade distribution of the SARS-CoV-2 positive samples based on Nextclade system, season	
2022-2023	. 28
Table 29 · Clade distribution of the SARS-CoV-2 positive samples based on Pangolin lineage system,	
season 2022-2023	
Table 30 • Clade distribution of the sequenced A/H3N2 viruses, season 2022-2023	
Table 31 • Clade distribution of the sequenced A/H1N1pdm09 viruses, season 2022-2023	
Table 32 • Clade distribution of the sequenced B/Victoria viruses, season 2022-2023	. 31

LIST OF FIGURES

Figure 1 • Age distribution of ILI/ARI samples per week of collection, season 2022-2023 Figure 2 • Weekly age distribution of ILI cases matching the different case definitions, season 2022-2023 Figure 3 • Weekly number of ILI samples positive for influenza viruses A or B and percentage of influenza	
positivity, season 2022-2023	. 11
Figure 4 • Weekly distribution of influenza A viruses per subtype among ILI samples, season 2022-2023 Figure 5 • Weekly distribution of influenza B viruses per lineage among ILI samples, season 2022-2023 Figure 6 • Weekly number of ILI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2022-2023	12 12
Figure 7 • Weekly number of ILI samples positive for respiratory syncytial viruses A or B and percentage of	. 15
RSV positivity, season 2022-2023	14
Figure 8 • Weekly number of ILI samples positive for human metapneumoviruses and percentage of	
positivity, season 2022-2023	15
Figure 9 • Age distribution of SARI samples per week of collection, season 2022-2023	
Figure 10 • Weekly number of SARI samples positive for influenza viruses A or B and percentage of influenza	
positivity, season 2022-2023	. 18
Figure 11 • Weekly distribution of influenza A viruses per subtype among SARI samples, season 2022-2023	
	. 18
Figure 12 • Weekly distribution of influenza B viruses per lineage among SARI samples, season 2022-2023	
	. 19
Figure 13 • Weekly number of SARI samples positive for SARS-CoV-2 coronavirus and percentage of	
positivity, season 2022-2023	. 19
Figure 14 • Weekly number of SARI samples positive for respiratory syncytial viruses A or B and percentage	
of RSV positivity, season 2022-2023	. 20
Figure 15 • Weekly number of SARI samples positive for rhino-, entero- and parechoviruses and percentage	04
of positivity, season 2022-2023 Figure 16 • Weekly number of SARI samples positive for human metapneumoviruses and percentage of	. 21
positivity, season 2022-2023	21
Figure 17 • Weekly number of SARI samples positive for human parainfluenzaviruses and percentage of	. 21
positivity, season 2022-2023	22
Figure 18 • Weekly number of SARI samples positive for seasonal coronaviruses and percentage of	. 22
positivity, season 2022-2023	23
Figure 19 • Weekly number of SARI samples positive for adenoviruses and percentage of positivity, season	. 20
2022-2023	. 23
Figure 20 • Weekly number of SARI samples positive for bocaviruses and percentage of positivity, season	
2022-2023	. 24
Figure 21 • Simplified evolution tree of SARS-CoV-2 viruses indicating the correspondence between the	
different classification system.	. 30

CONTACT: respirvir@sciensano.be

MORE INFORMATION

Visit our website >www.sciensano.be o contact us at

Sciensano • Rue Juliette Wytsmanstraat 14 • Brussels • Belgium • T + 32 2 642 51 11 • T press + 32 2 642 54 20 • info@sciensano.be • www.sciensano.be

Responsible editor: C. Léonard, Managing director • Rue Juliette Wytsmanstraat 14 • Brussels • Belgium • D/2023.14.440/85