VIROLOGICAL SURVEILLANCE REPORT OF THE NRC INFLUENZA FOR SEASON 2020-2021

Activity Report

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WHO WE ARE

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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

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ABSTRACT

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Amidst the COVID-19 pandemic, the sentinel surveillance resumed slowly in the season 2020-2021. Only the severe acute respiratory infections (SARI) allowed to properly evaluate the circulation of respiratory viruses during the 2020-2021 season. The season was characterized by the absence of an influenza virus epidemic, and the return of parainfluenza viruses and respiratory syncytial viruses detected mainly in children. SARS-CoV-2 continued to intensively circulate and was mainly detected in adults and older adults. Other respiratory viruses such as metapneumoviruses, seasonal coronaviruses, rhino- and enteroviruses, and adenoviruses were also detected, without clear epidemic wave.

BACKGROUND

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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 [a] and ECDC [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 inter-seasonal 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 2020-2021 thus started on week 40-2020 and ended at the end of week 39-2021.

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:

[a] https://www.who.int/initiatives/mosaic-respiratory-surveillance-framework
[b] https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/facts

- Onset of symptoms within 10 days of hospitalisation/sampling
- Fever or history of fever
- Cough or dyspnoea

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 2020-2021, 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

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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 [c].

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 2020-2021 season, only 8 general practitioners resumed their participation in the virological surveillance and collected samples for the NRC influenza. This represents a drop by more than 85% compared to the last pre-COVID19 season 2019-2020 (Table 1).

Table 1 • Number of ILI/ARI samples and contributing general practitioners per season

Season	n	Nb of GP
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

Only 29 nasopharyngeal swabs were collected during the 2020-2021 season and were sent to the NRC influenza for testing. Regarding the administrative region of origin, the participating GP in Flanders and the Brussels region collected on average 4 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 2020-2021

Region	n	Nb of GP
Brussels	4	1
Flanders	24	6
Wallonia	1	1

n: number of samples; GP: general practitioner

Out of the 29 enrolled cases, 72.4% and 89.7% matched the ECDC case definitions for ILI and ARI, respectively, which is less that during the last pre-COVID19 season 2019-2020 (Table 3). Only 34.5% 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
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 5 days.
The median time between reception date and reporting date (i.e. turnaround time, TAT) was 9 days, well below the target of 15 days.

Samples were sporadically collected during the 2020-2021 season (Figure 1) and almost exclusively from adults (Table 4).
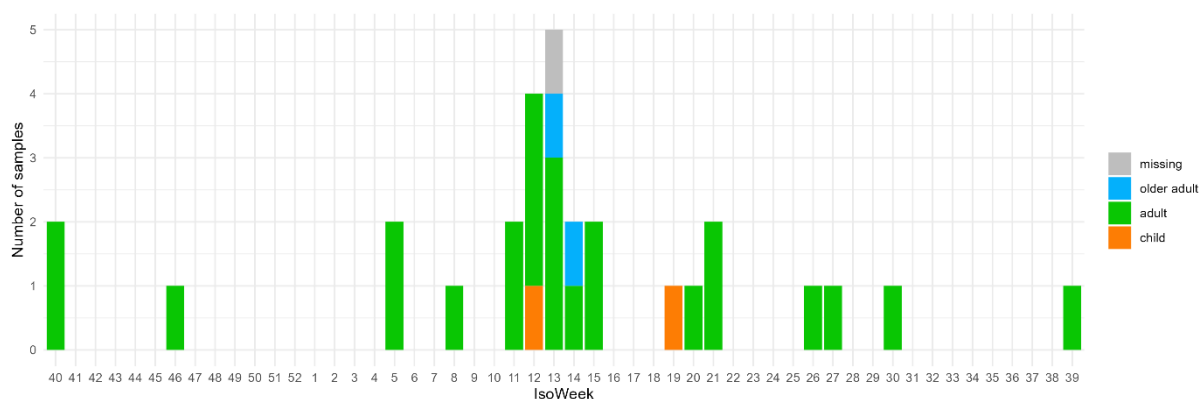
Figure 1 • Age distribution of ILI/ARI samples per week of collection, season 2021-2022

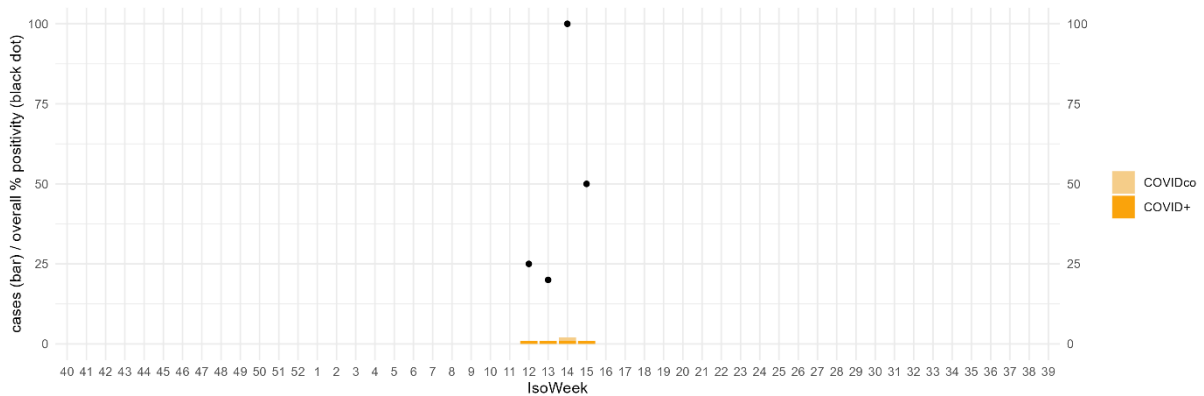**Table 4 • Number of ILI/ARI samples per age group and region, season 2020-2021**

Age group	Brussels	Flanders	Wallonia	Total
child	0	1	1	*2*
adult	3	21	0	*24*
older adult	0	2	0	*2*
missing	1	0	0	*1*
Total	*4*	*24*	*1*	*29*

1.3. INFLUENZA VIRUS

Influenza viruses were not detected during the 2020-2021 season.

1.4. SARS-COV-2 VIRUS

Positive samples were detected during weeks 12-2021 to 15-2021 (Figure 2).

Figure 2 • Weekly number of ILI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2020-2021

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 2020-2021 was 17.2% (Table 5).

Table 5 • Age distribution of the SARS-CoV-2 positive ILI cases, season 2020-2021

SARS-CoV-2 PCR result	child	adult	older adult	missing	Total
SARS-CoV-2 detected alone	0	3	1	0	4
SARS-CoV-2 + another respiratory virus	0	1	0	0	1
SARS-CoV-2 virus not detected	2	20	1	1	24
Total	2	24	2	1	29

1.5. RESPIRATORY SYNCYTIAL VIRUS

RSV was rarely detected during the 2020-2021 season (Table 6).

Table 6 • Age distribution of the RSV positive ILI cases, season 2020-2021

RSV PCR result	child	adult	older adult	missing	Total
RSV type A detected alone	1	1	0	0	2
RSV-A + another respiratory virus	0	0	0	0	0
RSV type B detected alone	0	0	0	0	0
RSV-B + another respiratory virus	0	1	0	0	1
RSV not detected	1	22	2	1	26
Total	2	24	2	1	29

1.6. OTHER RESPIRATORY VIRUSES

Like for RSV, other respiratory viruses were rarely detected.

2. Severe Acute Respiratory Infections (SARI)

2.1. 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 [e]. 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.

2.2. SAMPLE INFORMATION

A total of 1331 nasopharyngeal swabs were collected during the 2020-2021 season and were sent to the NRC influenza for testing, with the hospitals operating in a degraded mode (i.e. not exhaustive recruitment) due to the ongoing COVID-19 pandemic (Table 7).

Table 7 • Number of SARI samples per province, season 2020-2021

Region	n
Brussels	290
Flanders	685
Wallonia	356

n: number of samples

Out of the 1331 enrolled cases, 73.8% matched the case definition defined in the protocol and following the WHO SARI case definition (Table 8). When considering broader case definitions, 87.1% and 94.1% of the enrolled cases matched the ECDC-COVID and BE-COVID case definitions, respectively.

Table 8 • Number of SARI samples responding to the different clinical case definitions (with percentages)

Season	n	WHO-SARI	ECDC-COVID	BE-COVID	p_WHO-SARI	p_ECDC-COVID	p_BE-COVID
2020-2021	1331	982	1231	1253	73.8	87.1	94.1

n: number of samples; p: percentage

The median time between sampling date and reception date was 9 days, but it varied by region (50 days for Flanders, 4 days for Wallonia, and 9 days for Brussels). For convenience, samples collected for the SARI surveillance were often sent by batch covering a few weeks of collection.

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[e] https://www.sciensano.be/en/projects/severe-acute-respiratory-infection-surveillance-a-sentinel-network-hospitals

Age was missing or not yet communicated for 2.9% of the samples. Overall, when age was known, 40.6% (525/1293), 35.1% (454/1293) and 24.3% (314/1293) of the patients were children (below the age of 15), older adults (above 65 years old) and adults (between 15 and 65 years old), respectively (Table 9). As requested in the protocol for this season, sample collection took place each week during the season, but the SARI surveillance only started on week 04-2021 following the disruption caused by the ongoing COVID-19 pandemic (Figure 3).
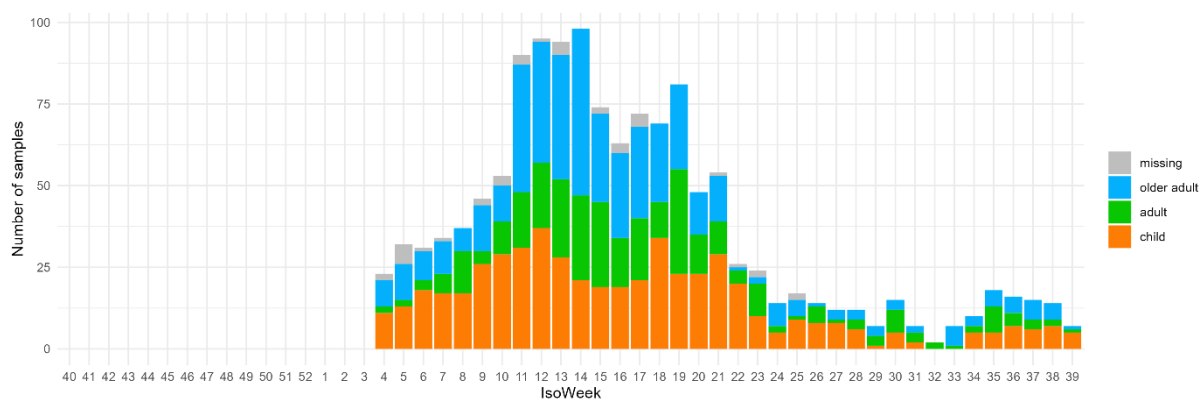
Figure 3 • **Age distribution of SARI samples per week of collection, season 2020-2021**

Table 9 • **Number of SARI samples per age group and region, season 2020-2021**

Age group	Brussels	Flanders	Wallonia	Total
child	156	337	32	525
adult	53	157	104	314
older adult	47	191	216	454
missing	34	0	4	38
Total	290	685	356	1331

When looking by case definition, more than 90% of child cases matched the WHO-SARI case definition, but less than 70% for adult and older adult cases (Table 10). More than 90% of cases matched the broader ECDC-COVID or BE-COVID case definitions.

Table 10 • **Number of SARI samples per age group and case definition, season 2020-2021**

Case definition	Child	Adult	Older adult	Missing
WHO-SARI	486	218	261	17
ECDC-COVID	523	294	393	21
BE-COVID	523	298	411	21
Total	525	314	454	38

2.3. INFLUENZA VIRUS

No influenza viruses were detected during the 2020-2021 season.

2.4. SARS-COV-2 VIRUS

Positive samples were detected during the entire surveillance period when samples were collected (Figure 4).
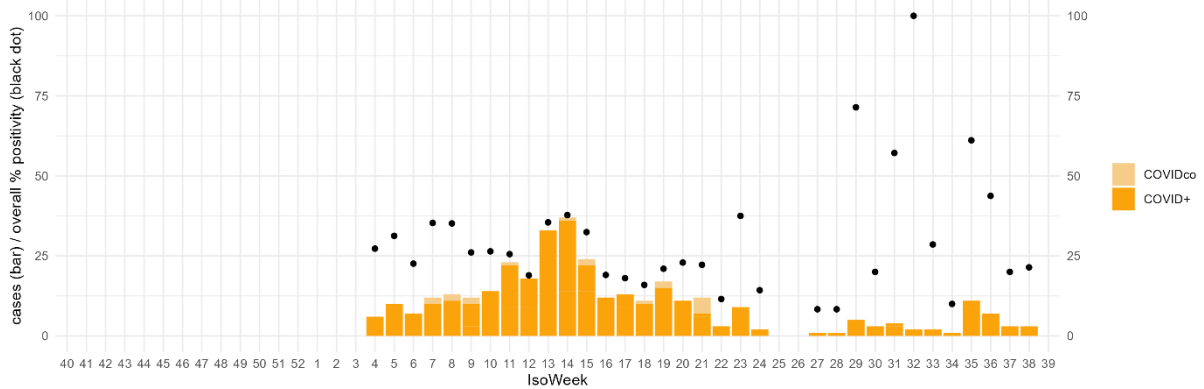
Figure 4 • Weekly number of SARI samples positive for SARS-CoV-2 coronavirus and percentage of positivity, season 2020-2021

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

Overall, 342 samples were positive for SARS-CoV-2 and the percentage of positivity for the season 2020-2021 was 25.7% (Table 11), reaching 51.9% (163/314) among adults (between 15 and 65 years old) and 34.0% (154/453) among older adults (above 65 years old), but only 3.1% (16/524) among children (below 15 years old).

Table 11 • Age distribution of the SARS-CoV-2 positive SARI cases, season 2020-2021

SARS-CoV-2 PCR result	child	adult	older adult	missing	Total
SARS-CoV-2 detected alone	9	159	147	9	324
SARS-CoV-2 + another respiratory virus	7	4	7	0	18
SARS-CoV-2 virus not detected	508	151	299	29	987
Total	524	314	453	38	1329

2.5. RESPIRATORY SYNCYTIAL VIRUS

Positive samples were detected as an epidemic wave between weeks 11-2021 and 22-2021 (Figure 5).
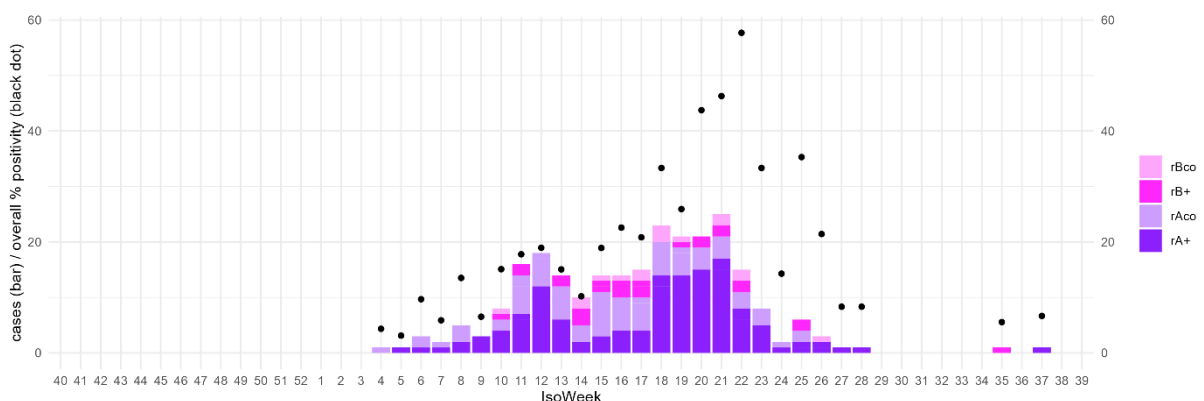
Figure 5 • Weekly number of SARI samples positive for respiratory syncytial viruses A or B and percentage of RSV positivity, season 2020-2021

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, 252 samples were positive for a respiratory syncytial virus and the percentage of positivity for the season 2020-2021 was 19.0% (Table 12), with RSV type A (83.3%, 210/252) dominating over type

B viruses (16.7%, 38/252). Eighty-six percent (216/252) of the positive samples were from children (below 15 years old).

Table 12 • Age distribution of the RSV positive SARI cases, season 2020-2021

RSV PCR result	child	adult	older adult	missing	Total
RSV type A detected alone	116	1	8	6	131
RSV-A + another respiratory virus	72	1	2	4	79
RSV type B detected alone	16	3	4	3	26
RSV-B + another respiratory virus	12	1	2	1	16
RSV not detected	309	308	436	24	1077
Total	525	314	452	38	1329

2.6. OTHER RESPIRATORY VIRUSES

2.6.1. Picornavirus (rhinovirus, enterovirus, parechovirus)

Positive samples were detected at relatively high proportion during the entire surveillance period when samples were collected (Figure 6). Viruses were mostly entero- and rhinoviruses. Very few parechoviruses and the specific enterovirus D68 were detected. Overall, picornaviruses were frequently co-detected with another respiratory viruses (Table 13).
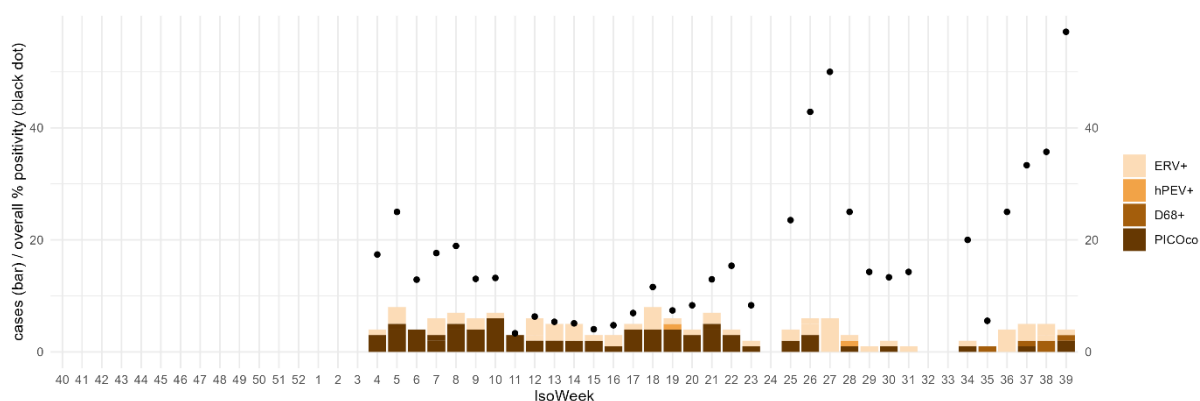
Figure 6 • Weekly number of SARI samples positive for rhino-, entero- and parechoviruses and percentage of positivity, season 2020-2021

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
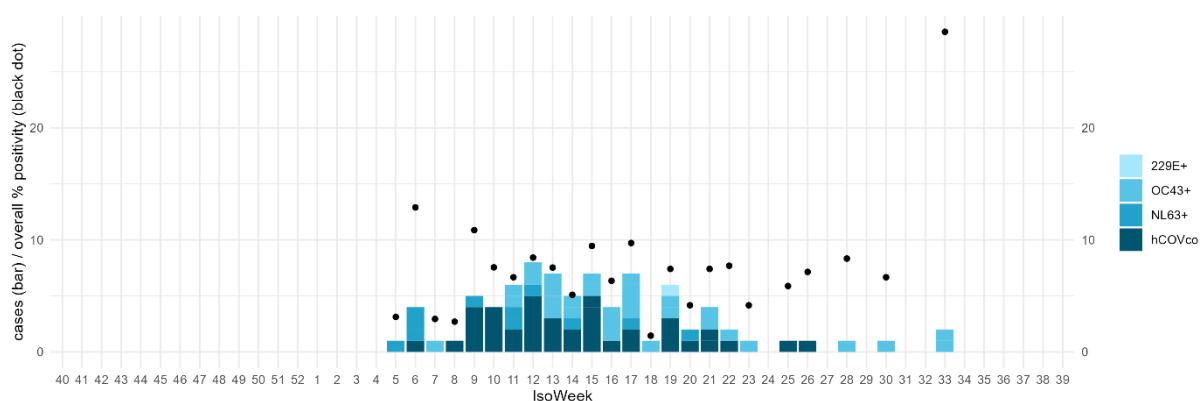
Table 13 • Age distribution of the picornavirus positive SARI cases, season 2020-2021

PCR result	child	adult	older adult	missing	Total
Rhino- / enterovirus detected alone	43	4	16	0	63
Enterovirus D68 detected alone	5	0	0	0	5
Parechovirus detected alone	1	1	0	0	2
Picornavirus + another respiratory virus	72	2	2	1	77
Picornavirus not detected	404	307	435	37	1183
Total	525	314	453	38	1330

2.6.2. Human metapneumovirus

Positive samples were sporadically detected throughout the season, but without clear epidemic wave (Figure 7). Mainly children (below the age of 15) were concerned (Table 14).
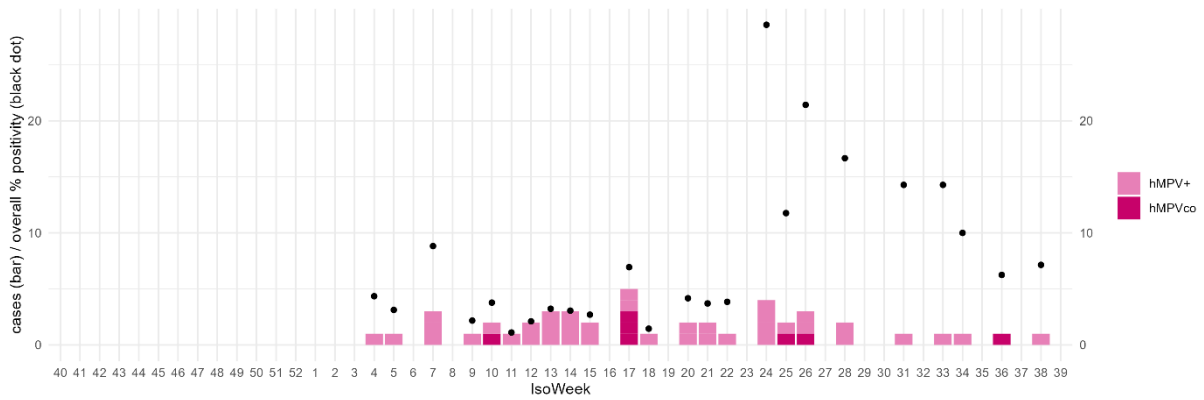
Figure 7 • Weekly number of SARI samples positive for human metapneumoviruses and percentage of positivity, season 2020-2021

hMPV+: human metapneumovirus detected alone; hMPVco: co-detection of human metapneumovirus and another respiratory virus
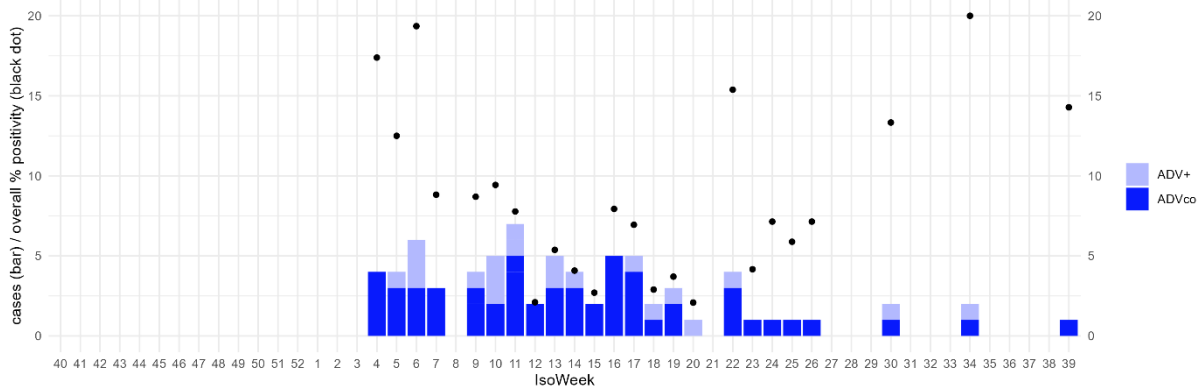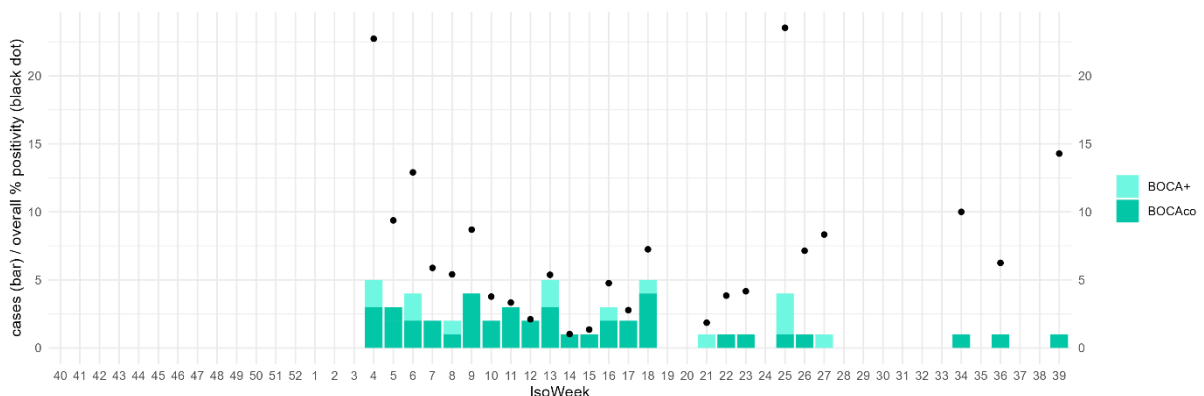
Table 14 • Age distribution of the metapneumovirus positive SARI cases, season 2020-2021

PCR result	child	adult	older adult	missing	Total
Metapneumovirus detected alone	27	4	6	2	39
Metapneumovirus + another resp. virus	5	0	1	1	7
Metapneumovirus not detected	493	310	446	35	1284
Total	525	314	453	38	1330

2.6.3. Parainfluenzavirus

Positive samples were detected as an epidemic wave between weeks 04-2021 and 13-2021 (Figure 8). Parainfluenzavirus type 3 dominated with mainly children being concerned (Table 15). Co-detection with another respiratory virus was very frequent.
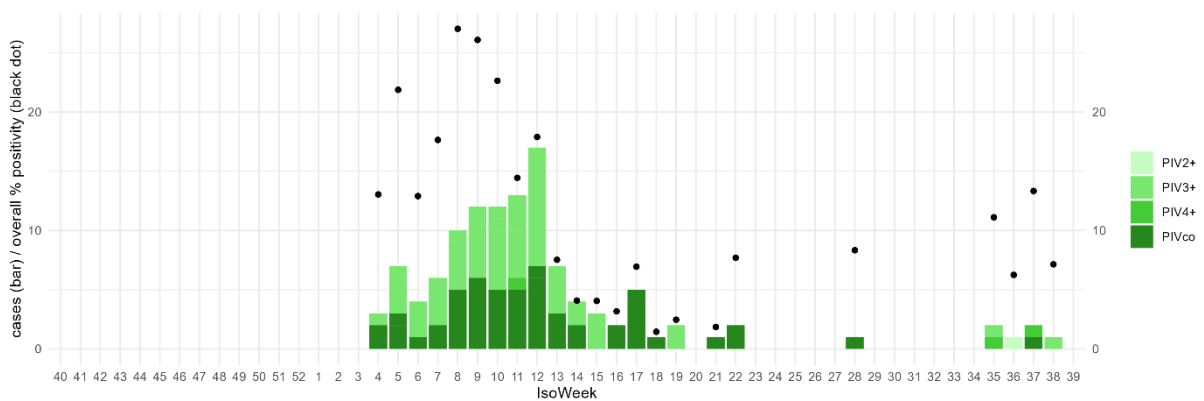
Figure 8 • Weekly number of SARI samples positive for human parainfluenzaviruses and percentage of positivity, season 2020-2021

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

Table 15 • **Age distribution of the parainfluenzavirus positive SARI cases, season 2020-2021**

PCR result	child	adult	older adult	missing	Total
Parainfluenzavirus type 1 detected alone	0	0	0	0	0
Parainfluenzavirus type 2 detected alone	0	1	0	0	1
Parainfluenzavirus type 3 detected alone	45	9	5	1	60
Parainfluenzavirus type 4 detected alone	1	2	0	0	3
PIV + another respiratory virus	46	1	3	4	54
Parainfluenzavirus not detected	433	301	445	33	1212
Total	525	314	453	38	1330

PIV: parainfluenzavirus

2.6.4. Seasonal coronavirus

Positive samples were detected at relatively low proportion throughout the surveillance period (Figure 9). Human coronavirus OC43 was the most frequently detected, with all age groups being concerned (Table 16). Co-detection with another respiratory virus was very frequent, especially for children.

Figure 9 • **Weekly number of SARI samples positive for seasonal coronaviruses and percentage of positivity, season 2020-2021**

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

Table 16 • **Age distribution of the seasonal coronavirus positive SARI cases, season 2020-2021**

PCR result	child	adult	older adult	missing	Total
Coronavirus 229E detected alone	0	0	1	0	1
Coronavirus NL63 detected alone	7	2	2	0	11
Coronavirus OC43 detected alone	11	11	9	0	31
hCOV + another respiratory virus	36	2	0	1	39
Seasonal coronavirus not detected	471	299	441	37	1248
Total	525	314	453	38	1330

2.6.5. Adenovirus

Positive samples were sporadically detected during the surveillance period when samples were collected (Figure 10). Adenoviruses were frequently co-detected with another respiratory virus, and almost exclusively among children (Table 17).

Figure 10 • Weekly number of SARI samples positive for adenoviruses and percentage of positivity, season 2020-2021

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

Table 17 • Age distribution of the adenovirus positive SARI cases, season 2020-2021

PCR result	child	adult	older adult	missing	Total
Adenovirus detected alone	19	0	1	0	20
Adenovirus + another resp. virus	50	1	0	4	55
Adenovirus not detected	456	313	452	34	1255
Total	525	314	453	38	1330

2.6.6. Bocavirus

Positive samples were sporadically detected throughout the surveillance period when samples were collected (Figure 11). Bocaviruses were almost exclusively detected among children and in co-detection with another respiratory virus (Table 18).

Figure 11 • Weekly number of SARI samples positive for bocaviruses and percentage of positivity, season 2020-2021

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

Table 18 • Age distribution of the bocavirus positive SARI cases, season 2020-2021

PCR result	child	adult	older adult	missing	Total
Bocavirus detected alone	14	0	0	0	14
Bocavirus + another resp. virus	41	0	0	1	42
Bocavirus not detected	469	314	453	37	1273
Total	524	314	453	38	1329

NON-SENTINEL SURVEILLANCE

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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 2020-2021, in the middle of the ongoing COVID-19 pandemic, hospitals submitted only 6 samples to the NRC influenza.
Only 3 samples were confirmed as positive for influenza type A virus of the H3N2 subtype and 1 sample was confirmed as positive for influenza type B virus of the Victoria lineage.

2. Zoonotic 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).

ACKNOWLEDGEMENTS

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PUBLICATIONS

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