Virological Surveillance of Influenza in Belgium

Season 2018-2019

VIRAL DISEASES
National Influenza Centre (WHO)

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EPIDEMIOLOGY OF INFECTIOUS DISEASES
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Reference number : D/2021/14.440/68

Influenza surveillance in Belgium is financed by the Federal Public Service Health, food chain safety and environment, the "Fédération Wallonie Bruxelles " and the "Vlaams Agentschap Zorg en Gezondheid".

A. Abstract

This 2018-2019 season the Influenza epidemic in Belgium lasted 8 weeks and was a flu season of moderate intensity characterized by the co-circulation of A(H1N1)pdm09 and A(H3N2), with the predominance of A(H3N2). The epidemic threshold was crossed from week 4-2019 (January 21 to January 27, 2019) to week 11-2019 (March 11 to March 17, 2019) (Fig. 1). After week 3- 2019, the incidence of ILI consultations increased to reach 761 consultations/100.000 inhabitants in week 7-2019. From week 11-2019, the number of cases decreased to below the threshold (Fig. 1).

It was estimated that 506.000 patients consulted their GP for ILI in Belgium.

Severity was moderate in comparison to the previous season and comparable to previous seasons. Severity was observed mainly (92%) in patients with an underlying condition. The severity was more often associated with A(H1N1) subtype versus A(H3N2).

The first positive sample was diagnosed in week 48-2018 and increasingly large numbers of positive influenza cases were detected from week 52-2018 onwards, reaching a proportion of 82. % in week 10-2019.

All respiratory samples were also analysed for other respiratory viruses. In the ILI population, among the samples negative for influenza viruses, 50% were positive for one or more other respiratory viruses, whereas in the SARI population, this percentage reached 57%. This suggests an important role of other respiratory viruses in hospitalized patients during the flu season.

Preliminary estimates suggest that vaccine effectiveness in the season 2018-19 was very low, for all-type influenza (estimated at 13%) , but varied substantially between subtypes: (71% effectiveness for influenza A(H1N1)pdm09 and only 1% effectiveness for influenza A(H3N2)).

None of the analyzed strains presented mutations known to be associated to resistance to antivirals neuraminidase inhibitors (Oseltamivir et Zanamivir).

B. Background

Influenza virus is a leading cause of human morbidity and mortality worldwide. On average, influenza viruses infect 5 to 15% of the global population, resulting in ~500,000 deaths annually (1). Each year, a flu epidemic occurs usually during the winter period, and three or four times per century a new influenza virus emerges. The type of influenza virus circulating and the vulnerability of the population determine the severity of the epidemic or pandemic. The major objectives of the surveillance are to monitor influenza activity (intensity, duration, severity, …) all over the year, to determine the type and subtypes of circulating strains and their antigenic and genetic characterization, to contribute to the annual determination of influenza vaccine content, to assess the overall vaccine effectiveness, to monitor resistance to antivirals and to detect new potentially pathogenic influenza viruses.

Since 2011, the surveillance has been extended to Severe Acute Respiratory Infection (SARI) cases as a tool to monitor severe diseases caused by influenza to complement surveillance of outpatient monitoring of influenza-like illness (ILI). The main objectives are to measure incidence, risk factors, clinical spectrum and outcomes of SARI caused by influenza virus and other respiratory pathogens and to monitor indicators of severity, season after season.
Furthermore, there is always a risk of emergence of new pathogenic viruses.
This report is mainly focusing on the virological results.

c. Methods

Network of Sentinel General Practices

In Belgium, the influenza surveillance is performed by the National Influenza Centre (NIC), in collaboration with the Unit of Health Services Research and the Unit of Epidemiology of Infectious Diseases of Sciensano in Brussels. A network of sentinel general practices (SGPs) has been involved since 2007 in the clinical and virological influenza surveillance (2). The main purposes of the surveillance are the early detection of an influenza epidemic, the study of the intensity and duration of the epidemic, the identification and characterisation of circulating viruses and the participation to the selection of next-season influenza vaccine strains. The development of capability to detect new emerging viruses, the estimation of vaccine effectiveness and the monitoring of the antiviral susceptibility are also important tasks

Clinical surveillance

The SGPs network is geographically representative of all GPs in Belgium. Besides the number of acute respiratory infections by age group, the GPs reported weekly, on a standardised form, every patient with an influenza-like illness (ILI). The general criteria for ILI are: sudden onset of symptoms, high fever, respiratory (i.e. cough, sore throat) and systemic symptoms (headache, muscular pain). For every patient, age group (<5, 5-14, 15-64, 65-84, 85+), hospitalisation, antiviral treatment, and vaccination status were recorded (3).

Virological surveillance

These GPs are also involved in the virological surveillance and are invited to collect 2 nasopharyngeal swabs/week (each week, the first two patients presenting for ILI belonging to different households).
Sampling kits are sent to all physicians. Each kit contained the materials required to collect nasopharyngeal swabs (2 nostrils + 1 throat) in patients with influenza-like illness. The material consisted of tubes containing 3 ml of transport medium [UTM (COPAN)], swabs [flocked Swabs (COPAN)] and patient registration forms. Samples and forms are returned to the National influenza Centre by mail (postage paid) and new kits are regularly sent depending on the shipment of samples. Patients information, clinical and epidemiological data and laboratory results are encoded in the LIMS system. The lab results of each patient were sent to the physician, after scientific and medical validation.

C.1.1. Sentinel Surveillance of SARI

Network of sentinel hospitals

Following the A(H1N1)2009 pandemic, the WHO and the European Centre for Disease Prevention and Control (ECDC) recommended hospital-based surveillance of severe acute respiratory infections (SARI) as a tool to monitor severe disease caused by influenza to complement outpatient surveillance of influenza like illness (ILI) or acute respiratory illness (ARI) to cover the full spectrum of influenza-related disease. As a result, the Belgian NIC has extended, since 2011, its surveillance to SARI cases. The main objectives were 1) to build a clinical and virological database of hospital cases permitting to rate the severity across seasons and pandemics; 2) to detect signals of severity during the course of an epidemic or a pandemic; 3) to describe genotypic and phenotypic characteristics of influenza viruses associated with severe forms of infection; 4) to test clinical samples for other respiratory viruses.

During the 2018-2019 influenza season, six hospitals located in the three regions of the country participated to the surveillance. The SARI case definition (adapted from the SARI case definition from WHO) is: an acute respiratory illness with onset within the last ten days, fever of ≥ 38°C (or history of fever), cough or dyspnea, and that required hospitalisation (for 24h or more). As we are mostly interested in severe influenza cases, the surveillance is carried out only during the epidemic period of seasonal influenza. Pediatric and adult units collected both clinical data and nasopharyngeal swabs from patients who corresponded to the case definition.

Sampling kits contained the materials required to collect 2 nasopharyngeal swabs (nostrils and throat) per patient responding to the SARI case definition. The material consisted of tubes containing 3 ml of transport medium [UTM (COPAN)], swabs [flocked Swabs (COPAN)] and patient registration forms. Samples and forms are returned to the NIC by mail (postage paid) and new kits are sent regularly to hospitals depending on the shipment of samples.

Patients information, clinical and epidemiological data and laboratory results are encoded in the LIMS system. All the results of one patient are sent to the hospital, after scientific and medical validation, once the results for influenza typing and subtyping and the results for the other respiratory viruses are available.

The following hospitals participated in the SARI surveillance during season 2018-2019:

- Cliniques Universitaires UCL, Mont-Godinne, Belgium
- Centre Hospitalier Universitaire St-Pierre, Brussels, Belgium
- Department of Laboratory Medicine, Medical Microbiology, Algemeen Ziekenhuis Sint-Jan, Brugge, Belgium
- Internal Medicine-Infectious Diseases, Universitair Ziekenhuis Brussel, Brussels, Belgium
- Clinical Laboratory, Jessa Ziekenhuis, Hasselt, Belgium
- Infectiology, Grand Hôpital de Charleroi, Charleroi, Belgium

C.1.2. Non-Sentinel Surveillance

Hospitals and laboratories across the country are encouraged to collect samples from patients presenting with severe acute respiratory diseases in particular specific conditions:

ARDS (acute respiratory distress syndrome), ECMO (extracorporeal membrane oxygenation), death, suspicion of antiviral resistance, returning from abroad. Monitoring of clusters of Influenza cases is also an important task. This surveillance is carried out throughout the year.

C.1.3. Suspected cases of Avian Influenza H5N1 and H7N9

Influenza A (H5N1)

Since 2003, and till 24 June 2019, 861 human infections with highly pathogenic H5N1 viruses have been reported to WHO by 16 countries (4). About 50% (455) of the laboratory confirmed people died from their illness. Since December 2005, an emergency procedure has been developed in Belgium to assure rapid diagnosis in case of suspicion of a human case of influenza A/H5N1. The Belgian NIC at Sciensano was appointed as reference laboratory for testing of the H5N1 suspected cases, which are mainly cases returning from affected countries.

Influenza A (H7N9)

On 31 March 2013, the first human cases of an avian influenza A (H7N9) virus, not previously described as causing disease in humans, were reported in China. As of 8 Augustus 2019 , a total of 1568 laboratory confirmed human cases; 616 deaths laboratory-confirmed cases of human infection with the avian A(H7N9) were reported to WHO (5). Most of the cases were from China. The virus appears to be sensitive to Oseltamivir. The main routes of transmission to humans, and the distribution and prevalence of this virus among people appears to be associated with exposure to infected live poultry or contaminated environments, including markets where live poultry are sold. Information to date does not support sustained human-to-human transmission, although limited human-to-human transmission cannot be excluded in a very few clusters of cases. As the extent of virus circulation in animals is not clear, epidemiological and virological surveillance and follow up of suspected human cases should remain high (6). WHO encourages countries to continue strengthening influenza surveillance, including surveillance for severe acute respiratory infections (SARI) and influenza-like illness (ILI) and to carefully review any unusual patterns, ensure reporting of human infections under the IHR 2005, and continue national health preparedness actions.

The Belgian NIC has developed molecular tests for the detection of A(H7N9) virus in suspected cases. The same surveillance strategy applies as for human infections with highly pathogenic avian influenza A(H5N1) virus.

Influenza A (H3N1)

In 2019, Belgium was confronted with an important H3N1 Avian influenza epidemic in the professional poultry sector. Although this virus was classified as a Low Pathogenic Avian Influenza (LPAI) virus, according to official definitions using the Intravenous Pathogenicity Index (IVPI) or molecular criteria, it was shown to be more pathogenic and transmissible in poultry. No transmission to humans was reported. However, any suspected case showing symptoms and having been in contact with affected poultry should be tested for influenza in order to exclude possible transmission .

C.1.4. Suspected Cases of MERS CoV

The first human cases of Middle East Respiratory Syndrome (MERS) coronavirus (CoV) were identified in April 2012. Since then, WHO has continued to monitor the disease, with more than 2229 laboratory-confirmed cases reported from 27 countries (including countries in the Middle East, North America, Europe and Asia and more recently clusters of cases in Korea and China). Based on the current situation and available information, WHO encourages all Member States to continue their surveillance for severe acute respiratory infections (SARI) and to carefully review any unusual patterns (9).

In Belgium, the National Reference Centre for Mers-CoV is the Microbiology and Immunology Department of UZ Leuven (NRC Respiratory Pathogens). However, the National Influenza Centre has developed real time PCR testing to analyse respiratory samples from suspected cases in the context of differential diagnosis with Influenza. So far there have not been any confirmed cases of MERS-CoV in Belgium.

C.1.5. Surveillance of other respiratory viruses

In addition to flu viruses, several other respiratory viruses can also circulate during the flu season and can cause symptoms and illness similar to those seen with flu infection. Respiratory infections are very common. They may be associated with significant morbidity and even mortality in young children and elderly patients. In about 30-60% of cases with influenza-like symptoms, no influenza virus can be detected, and in at least 20% of influenza-negative ILI cases, other respiratory viruses (such as RSV, rhinovirus, parainfluenza viruses, …) seem to be involved (10). Furthermore, severe influenza cases often seem to be complicated by co-infections with other respiratory viruses (11). We have developed 4 quadriplex Real time PCRs for the detection of 15 different respiratory viruses: respiratory syncytial virus (RSVA and RSVB), parainfluenza viruses (PIV1,2,3, 4), rhinoviruses/enterovirus (HRV/ENV), specific enterovirus D-68 (EV-D68), human metapneumoviruses (hMPV), paraechoviruses (HPeV), bocaviruses (HBoV), adenoviruses (ADV) and different coronaviruses (CoOC43, CONL63, Co229E).

C.2. Laboratory tests

C.2.1. Real-time RT-PCR Influenza

Nasopharyngeal swabs received at the NIC are tested with different real-time RT-PCRs: A/B typing followed by subtyping (for influenza A) or determination of the lineage (for influenza B). The sequence of tests is presented in Figure 1.

Typing A/B

A triplex Real-time qRT-PCR Influenza A/B/RP: adapted protocols (12,13); primers and probes for the matrix gene (influenza A) and hemagglutinine gene (influenza B). The RNase P (RP) primers and probe target the human RNase P gene, which serves as an internal positive control for human nucleic acid.

Subtyping A (H1, H3, N1, N2)

For all influenza A positive samples, the subtype is determined.

- Real-time qRT-PCR Influenza A/H1 sw: adapted protocol from CDC (12); primers and probes are chosen in the hemagglutinine gene.
- Real-time qRT-PCR A/H3: adapted protocol from RIVM (14); primers and probes in the hemagglutinine gene.

For a subset of samples:

- Real-time qRT-PCR N1: adapted protocol from RIVM (14); primers and probes in the neuraminidase gene.
- Real-time qRT-PCR N2: adapted protocol from Pasteur Institute Paris (15); primers and probes in the neuraminidase gene.

Lineage B (Yamagata, Victoria)

For influenza B positive, the lineage (Yamagata or Victoria) is determined.

- Duplex Real-time qRT-PCR B YAM-VIC: adapted protocol from Olav Hungnes (16).

In case of un-subtypable influenza A, if the Ct value is < 36, primers and probe specific for the Nucleoprotein of animal influenza (SWA) are used (protocol CDC)(12): This test allows to determine if the influenza strain is of animal origin and to continue with complementary tests.

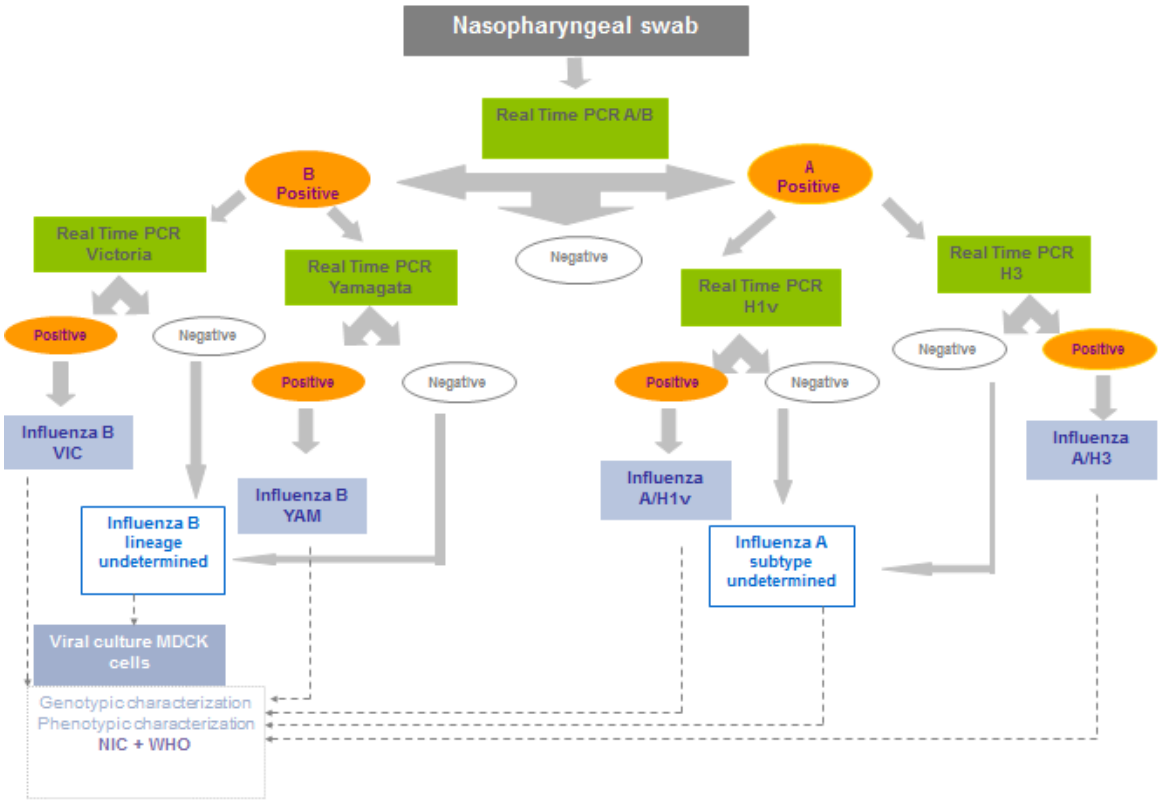

Figure 1. Sequence of the Real time PCR tests used during the 2018-2019 season.

Subtyping (H5, H7, …)

Samples from suspected cases of avian influenza are submitted to real-time RT-PCR A/B for typing and, in case of positivity, to different real-time RT-PCR for subtyping depending on the epidemiological and clinical context.

RT-PCR H5N1

Different sets of primers and probes H5 are used following different protocols: adapted protocol from Spackman et al. 2002 (17) , adapted protocol from the Health Protection Agency, 2006 (18) and from Pasteur Institute (18b).

RT-PCR H7N9

Protocol adapted from WHO (19).

C.2.2 PCR tests for MERS CoV

Samples from suspected cases for MERS-CoV are submitted to specific real-time RT-PCRs for MERS-CoV (screening and confirmation); protocol from Corman *et al.* (20).

C.2.3 PCR tests for other respiratory viruses

Respiratory samples from the different surveillance networks (ILI, SARI, Hospital non-sentinel) were additionally submitted to 4 quadriplex Real-time RT-PCRs detecting 15 other respiratory viruses (Respiratory syncytial virus (RSVA and RSVB), parainfluenza viruses (PIV 1, 2, 3, 4), rhinoviruses/enterovirus (HRV/ENV), human metapneumoviruses (hMPV), paraechoviruses (HPeV), bocaviruses (HBoV), adenoviruses (ADV) and different coronaviruses (CoOC43, CONL63, Co229E). For this season, a new test was included to detect Enterovirus D-68 (EV-D68) (Table 1).

Table 1. Multiplex RT PCR tests for respiratory viruses

MIX 1		MIX 2		MIX 3		MIX 4	
RSV A	HEX	PIV 1	ROX	Co 229E	ROX	Boca	Cy5
RSV B	ROX	PIV 2	HEX	Co OC43	HEX	PIV 4	HEX
hMPV	Cy5	PIV 3	FAM	Co NL63	Cy5	Paraecho	ROX
EV	FAM	Adéno	Cy5	Rhino	FAM	EV-D68	FAM

The protocols have been adapted from those of the Statens Serum institute (21) with some modifications (primers for rhinoviruses as described by Hombrouck et al. (10). Rhinoviruses and enteroviruses were considered together as rhinovirus/enterovirus (HRV/ENV). EV-D68 was tested separately (22).

C.2.4. Genetic characterisation

Since previous season, NGS has been introduced for the routine analysis of circulating strains. Amplification of the 8 segments of Influenza A and B were adapted from published protocols (23, 24). The sequencing of the amplicon was performed with MiSeq Illumina.
Sequence comparison, alignments and phylogenetic trees are realized using MEGA 7 program. Influenza sequences are compared to reference strains and vaccine strains. Based on evolutionary models, influenza strains can be classified in clades characterised by common and specific mutations.

C.2.5. Resistance to antivirals

The most commonly used antivirals are neuraminidase inhibitors [oseltamivir (Tamiflu ®) and zanamivir (Relenza ®)]. Influenza strains may develop resistance to these antivirals, and thus become less susceptible to their inhibitory activity. Resistant strains can be detected by phenotypic tests based on the use of MUNANA and IC50 measurement following the protocol recommended by the WHO reference laboratory (WHO-CC) in London, UK (22). Phenotypic resistance is often associated with mutations, causing reduced binding to the antiviral. For example, the Y275H mutation in N1 is associated with resistance to Oseltamivir. Other mutations associated with resistance to antivirals are also described for A(H3N2) and influenza B. Genotypic tests are based primarily on sequencing of Na gene to highlight potential mutations compared to reference sequences.

C.2.6. Sending of strains to London WHO CC

Each year, representative Belgian strains are sent to the WHO Collaborating Centre for Reference and Research on Influenza, Crick Worldwide Influenza Centre in London to undergo additional tests: antigenic and genetic characterization and monitoring of antiviral resistance. The characterization of circulating strains in Belgium contributes to the determination by WHO of the strains to be included in flu vaccines for the next season.

D. Results

D.1 Sentinel surveillance of ILI

D.1.1 Clinical surveillance

This 2018-2019 season the Influenza epidemic in Belgium lasted 8 weeks. The epidemic threshold was crossed on week 4-2019 (January 21 to January 27, 2019) to week 11-2019 (March 11 to March 17, 2019 (Fig. 1).

The intensity of the epidemic was moderate. After week 3-2019, the incidence of GP visits for influenza-like illness (ILI) rapidly increased to reach 761 GP visits per 100.000 inhabitants in week 7-2018. From week 8-2019, the incidence decreased again and dropped below the epidemic threshold from week 12-2019 on.
An update of the situation was published weekly (3).

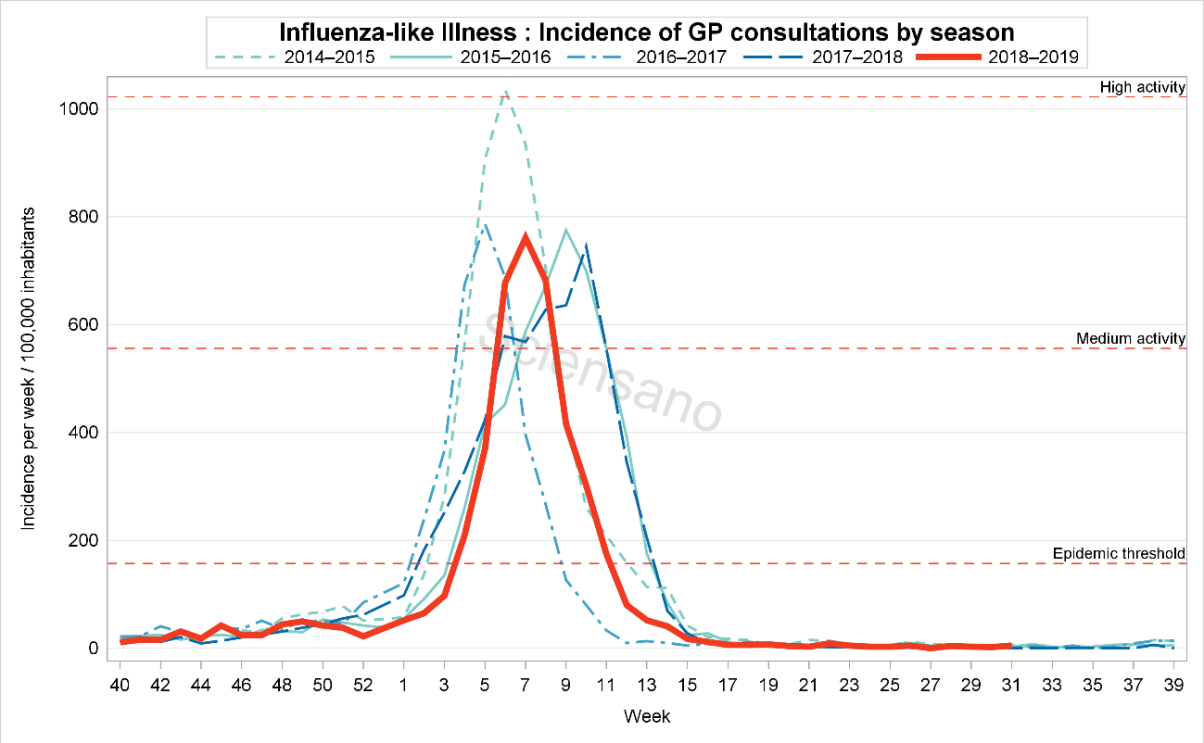

Figure 2a. Weekly incidence of influenza-like illness (ILI), 2018-2019 season, Belgium (Source: Sentinel general practices)

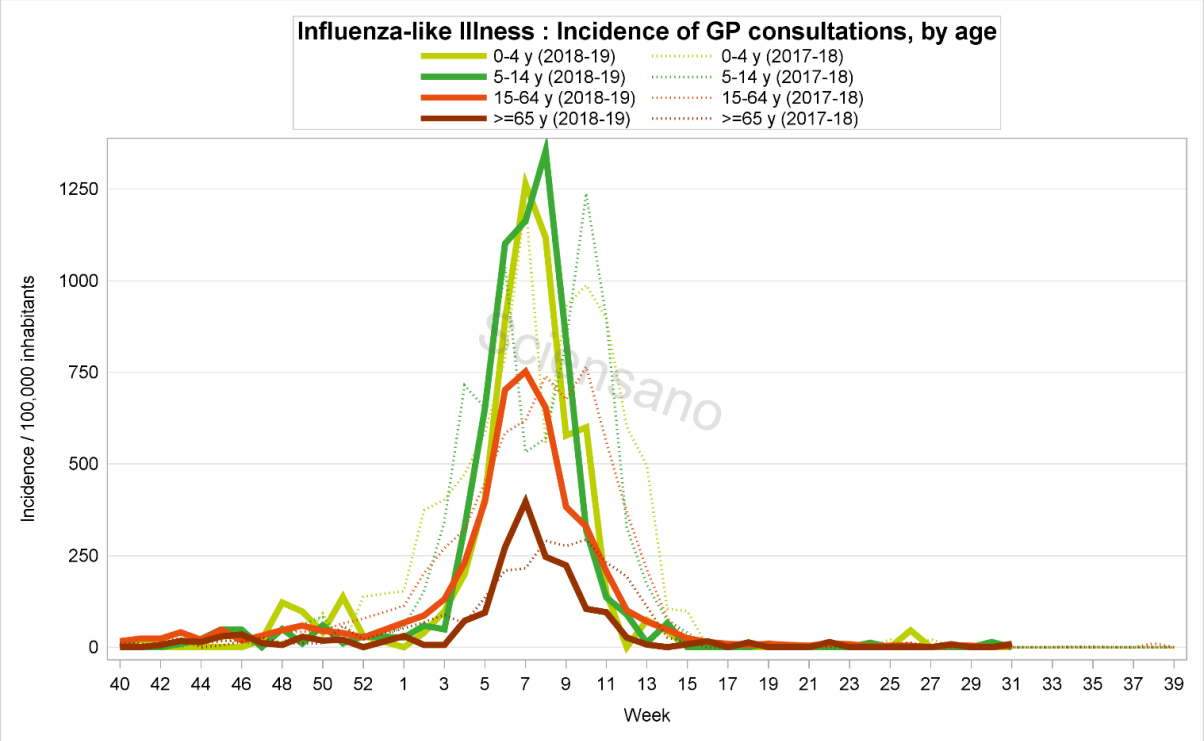

Figure 2b. Weekly incidence of influenza-like illness (ILI) by age groups, 2018-2019 season, Belgium (Source: Sentinel general practices).

D.1.2 Virological surveillance

The influenza surveillance period started in week 40-2018 and continued to week 20-2019.

Origin of samples

A total of 60 general practices (29 for Flanders, 22 for Wallonia and 9 for Brussels) took part in the virological surveillance and sent 658 nasopharyngeal swabs to the NIC.

Number of nasopharyngeal swabs

Flanders :	276
Wallonia :	182
Brussels :	54
Total :	**512**

From these samples **493** were suitable for analyses (sampling date availables).

Influenza Typing and subtyping results

The first positive sample was diagnosed in week 48-2018 and increasingly large numbers of positive influenza cases were detected from week 53-2018 onwards, reaching a proportion of 82% in week 10-2018. These were mainly A(H1N1)pdm09 and A(H3N2) viruses with a predominance of A(H3N2).

From week 40-2018 to week 20-2019, 493 respiratory samples were sent by the sentinel GPs network and analysed at the National Influenza Centre. Of these samples, 260 (52.7 %) were positive for influenza with 258 (52.3%) positive for influenza A and 2 (0.4%) positive for influenza B.

Among the influenza A samples that were subtyped, 23.6% (61/258) were A(H1N1)pdm2009, 72 % (187/258) were A(H3N2) and 3.8% (10/258) could not be subtyped due to low viral load. Both the two influenza B samples belonged to the Victoria lineage (table 2) and (fig.3).

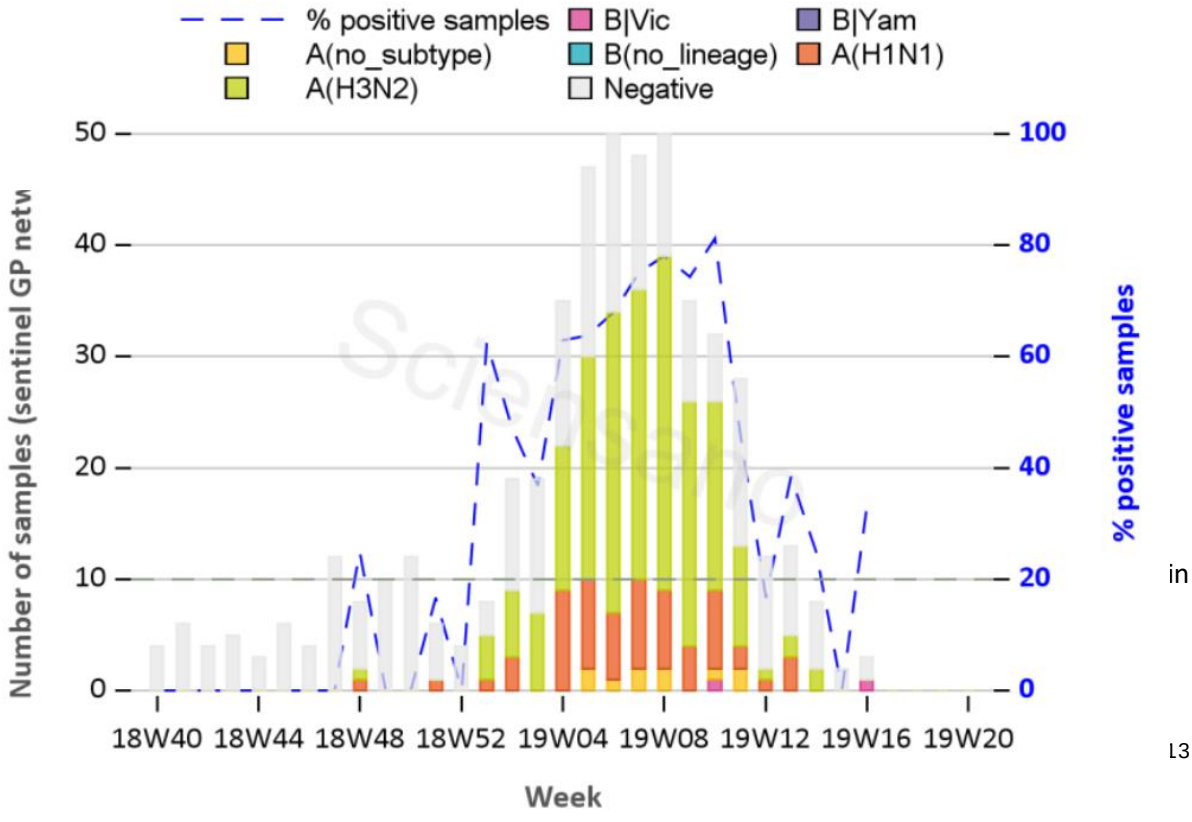

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Table 2. Numbers and proportion of the different types and subtypes analysed during the 2018-2019 season

		Influenza detection / typing			Influenza A subtyping			Influenza B llineage		
		Negative	A	B	A H1	A H3	A NT	B YAM	B VIC	B NL
Total for season (week 40-2018 to 18-2019)	Number of sample with given result	233	258	2	61	187	10	0	2	0
	Number of tested samples	493	493	493	258	258	258	2	2	2
	Percentage (%)	47%	52%	0%	24%	72%	4%	0%	100%	0%

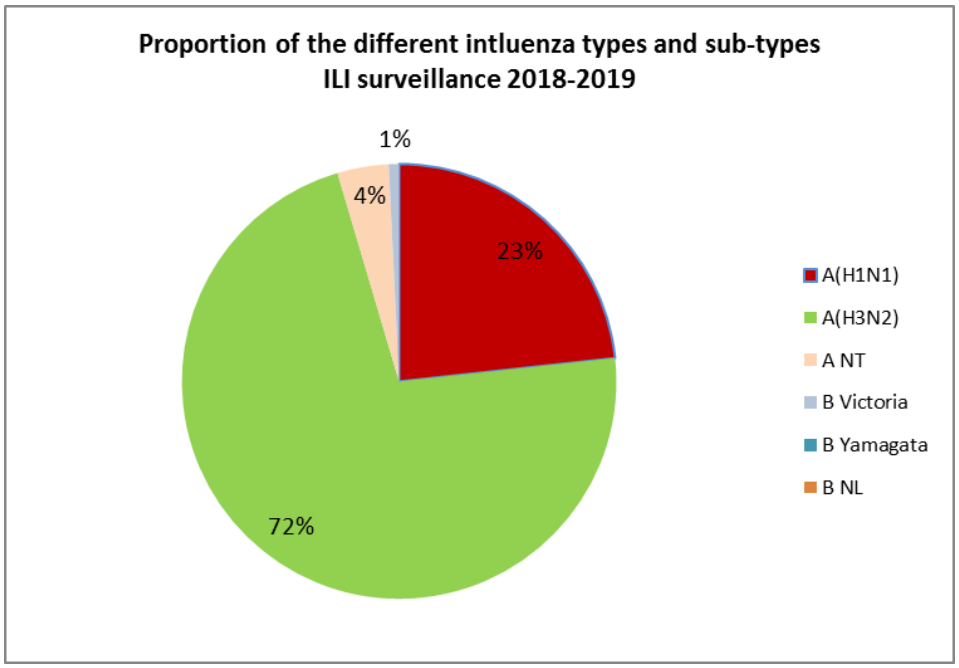
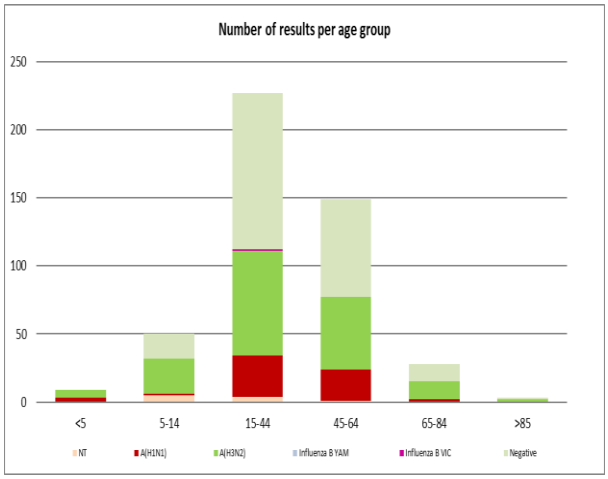


Figure 4. Repartition of the different types and subtypes during the influenza season 2018-2019 (ILI surveillance) .

Influenza viruses according to age group

The age was known for 466 patients. The NIC received a higher number of samples from the age group 15-44 and 45-64 years old. The rate of positivity was similar in all the age groups (Figure 5). Very few samples were collected from the age group < 5 years old and > 85 years old.

The distribution of influenza types (and subtypes) varied with age. A(H3N2) was the predominant virus in all age groups. No A(H1N1)pdm2019 was detected in elderly.



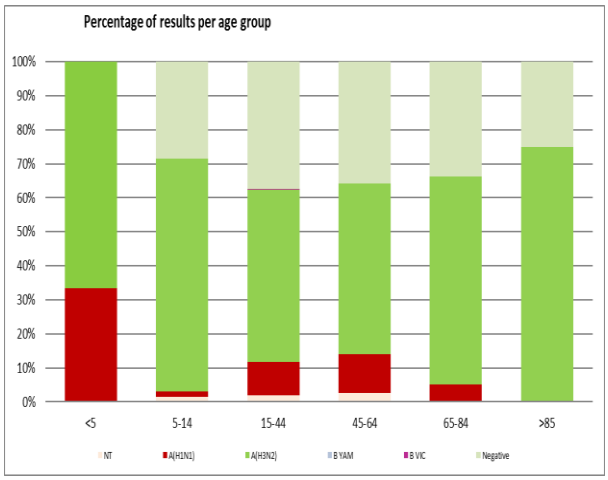


Figure 5. Influenza virus types and subtypes according to age group (NT= non subtyped) (numbers and percentages) influenza season 2018-2019 ILI surveillance .

D.2 Sentinel Surveillance of SARI

D.2.1 Virological surveillance

This season, the SARI Surveillance exceptionally started on week 40-2018 for three of the six hospitals, in order to have a better idea of the epidemiology of RSV and to analyse the possibility to also catch the RSV peak, which usually occurs before the influenza epidemic. The surveillance ended week 14-2018, about two weeks after the end of the influenza epidemic.

Origin of samples

A total of 2681 patients were registered in the database, among which 2669 (99.4%) corresponded to the case definition and were suitable for analysis. Different reasons justified these exclusions (nosocomial infection, undetermined results, hospitalisation for less than 24 hours, ….).

The age was known for 2557 patients and those were taken into account for the analyses of age groups.

Influenza Typing and subtyping results

From week 40-2018 to week 18-2019, 2669 SARI (Severe Acute Respiratory Infections) respiratory specimens were sent by the network of hospitals and analyzed by the National Influenza Center. Of these samples, 796 (29.7%) were positive for influenza viruses, of which most 794 (29.7%) were positive for influenza A viruses and very few 2 positive for influenza B viruses (Fig.6).

Of the subtyped influenza A samples, 18,2% (144/794) were A(H1N1)pdm09 viruses, 79.5% (632/794) were A(H3N2) viruses and 2.2% could not be subtyped due to low viral load. Among the two influenza B viruses, one belonged to the Yamagata lineage and the other to the Victoria lineage (Table 3 and fig. 7).

Table 3. Repartition of the different types and subtypes during the influenza season 2018-2019 (SARI surveillance).

		Influenza detection / typing			Influenza A subtyping			Influenza B lineage		
		Negative	A	B	A H1	A H3	A NT	B YAM	B VIC	B NL
Total for season (week 40-2018 to 18-2019)	Number of sample with given result	1873	794	2	144	632	18	1	1	0
	Number of tested samples	**2669**	**2669**	**2669**	**794**	**794**	**794**	**2**	**2**	**2**
	Percentage (%)	70%	29.7%	0.1%	18.2%	79.5%	2.2%	50%	50%	0%

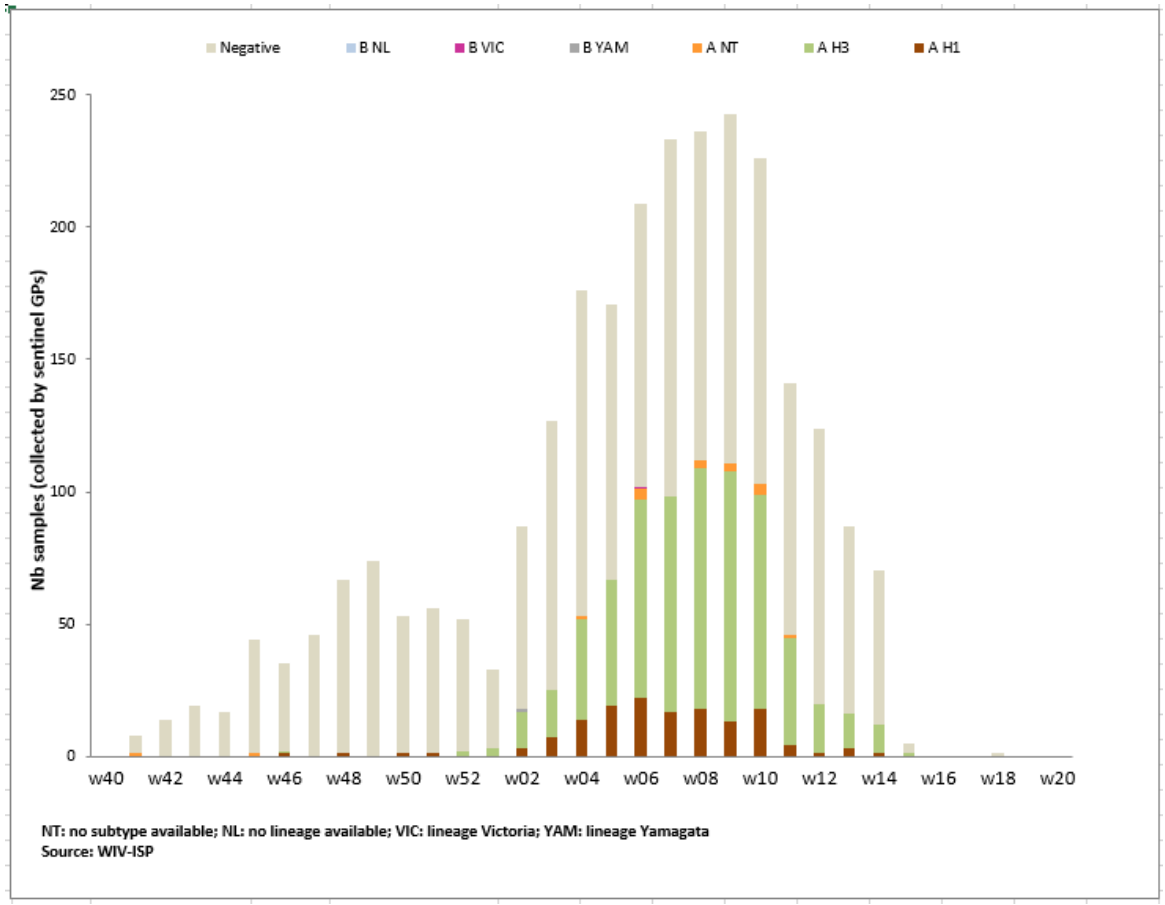


Figure 6. Weekly detection of influenza viruses in Belgium in the SARI surveillance from week 40-2018 to week 18-2019 .

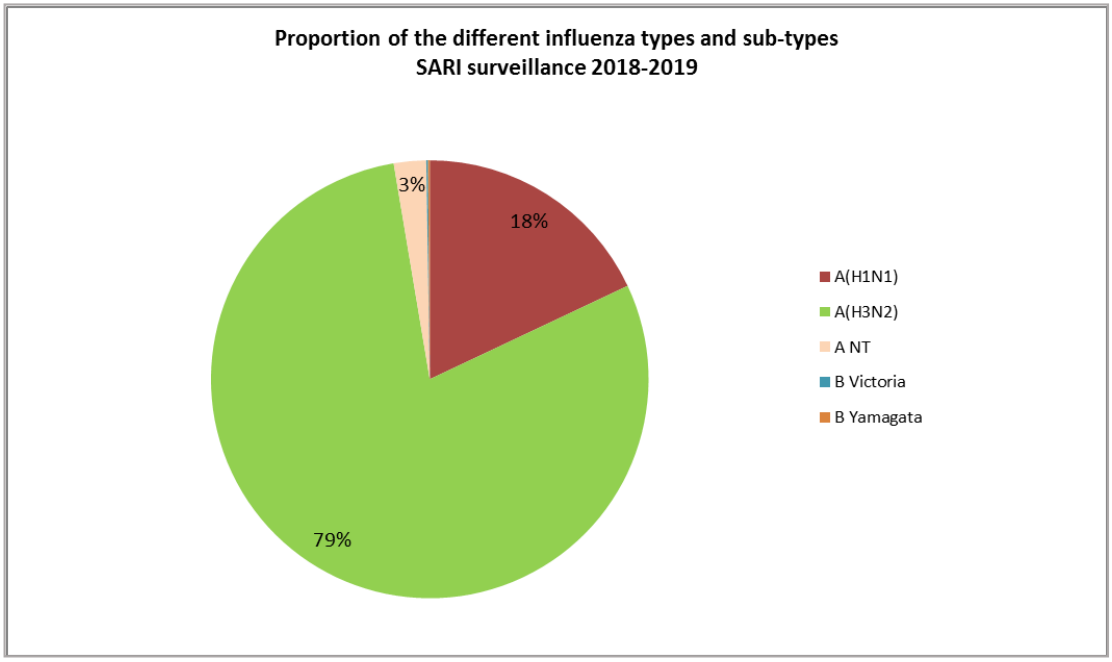
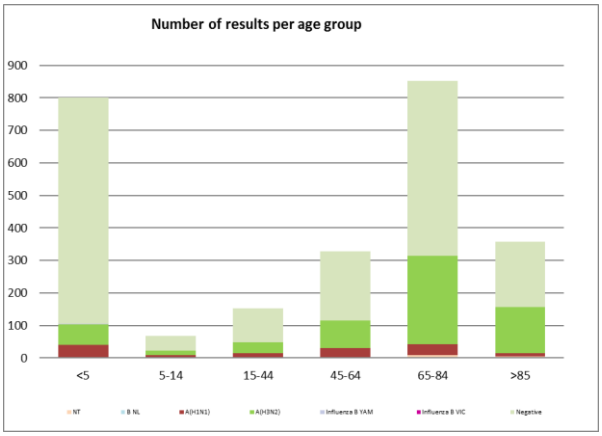


Figure 7. Repartition of the different types and subtypes during the influenza season 2018-2019 (SARI surveillance).

Age distribution of influenza viruses by types and subtypes

The age was known for 2557 patients. A higher number of samples was collected from children below 5 years old and adults of the 65-84 age group. The percentage of positivity for influenza viruses was lower in children below 5 years. The distribution of influenza types (and subtypes) varied with age with higher percentage of A(H1N1)pdm09 in children and young adults and a higher percentage of A(H3N2) in the group age 65-84 years old and elderly. (Figure 8).



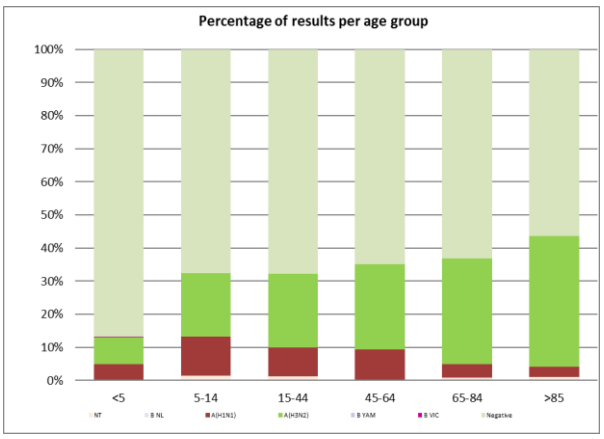


Figure 8. Influenza viruses according to age group SARI surveillance season 2018-2019 (numbers and percentages).

Positivity and subtype distribution of influenza viruses by surveillance scheme

During the usual SARI surveillance period (week 50-2018 to week 18 of 2019), the samples from ILI patients were significantly more positive 258/431 (59% positive) than those from SARI patients 787/2356 (33% positive).

During this period, Influenza A(H1N1)pdm09 and A(H3N2) circulated in both surveillances.

There was no significant difference between both surveillances in the ratio of influenza A(H1N1)pdm09 to A(H3N2) infections among confirmed influenza A patients.

D.3 Non sentinel surveillance

Eighty-one respiratory samples from patients with severe influenza were sent from hospitals around the country during the 2018-2019 season and inter-season, and were analysed at the NIC for confirmation and subtyping. Twenty-one were A(H1N1)pdm09, 34 were A(H3N2), 11 were negative and 11 were contaminated by one or more other respiratory viruses.

D.4 Suspected cases of Avian Influenza

In 2019, Belgium was confronted with an important H3N1 Avian influenza epidemic in the professional poultry sector. No transmission to humans was confirmed. However, any suspected case showing symptoms and having been in contact with affected poultry should be tested for influenza. Thirty-three samples from patients suspected of H3N1 infection were sent to the national influenza Centre and tested for Influenza . All tested samples were negative.

D.5 Suspected cases of MERS CoV

No sample was sent to the NIC for diagnosis of MERS CoV during this season.

D.6 Other Respiratory viruses

All respiratory samples submitted to influenza diagnosis were also analysed for 16 other respiratory viruses: RSV-A and -B, PIV 1-2-3-4, HRV/ENV, EV-D68, hMPV, HPeV, HBoV, ADV and different Coronaviruses (Co229E, CoOC43, CoNL63).

D.6.1 ILI surveillance

Between weeks 40-2018 and 20-2019, the 493 respiratory samples analysed for influenza were also submitted to the diagnosis of the other respiratory viruses. Overall, the positivity rate for influenza in the ILI surveillance was 52 %, which means that 48% of the samples were negative for influenza viruses. The analyses of positive and negative samples for the other respiratory viruses showed that, during the flu epidemic season, other respiratory viruses were also circulating in varying proportions. Overall, 76 % of the patients were positive for at least one respiratory virus (including Influenza and co-infections). Among the samples negative for influenza viruses, 117/233 (50%) were positive for one or more other respiratory viruses. The most prevalent other respiratory viruses were HRV/ENV (7.1%), RSVB (4.3%), hMPV (3.4%), CoOC43 (3.2%). For the other viruses, the percentages were lower (Figure 9).

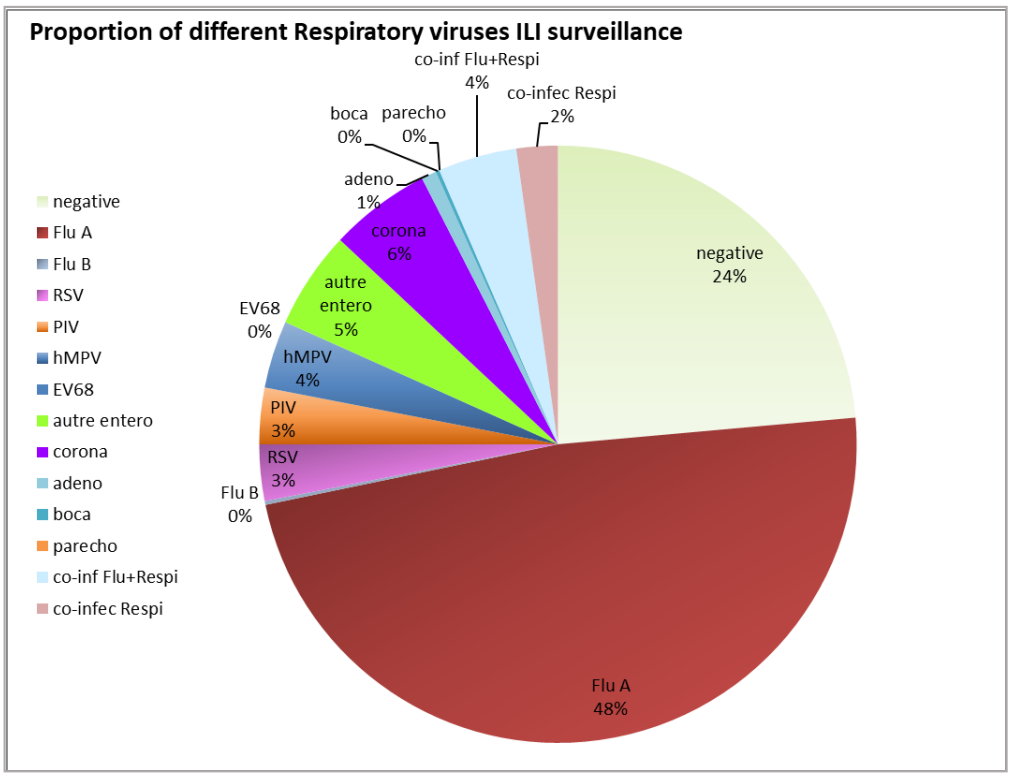


Figure 9. Proportion of the different respiratory viruses in the ILI surveillance in Belgium season 2018-2019

Proportion of the different respiratory viruses according to age group

The age group was known for 466 patients; the analyses were performed on these samples.

The prevalence of the different respiratory viruses varies by age group with a higher percentage of other respiratory viruses in patients below the age of 5 years old. However, the numbers were very low in this age group (Figure 10a, 10b).

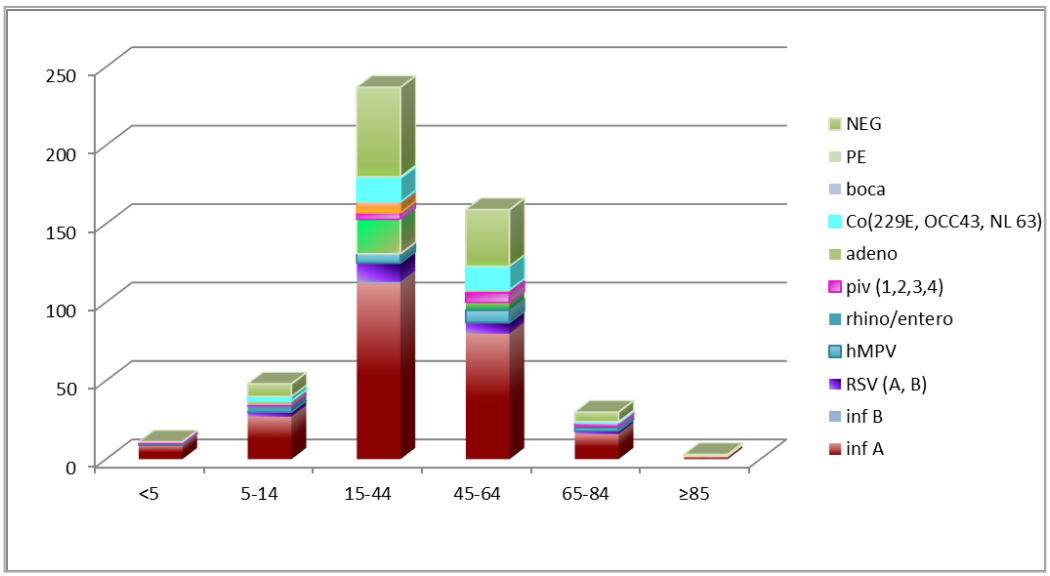


Figure 10a. Proportion of the different respiratory viruses in the ILI surveillance season 2018-2019 age group (Numbers).

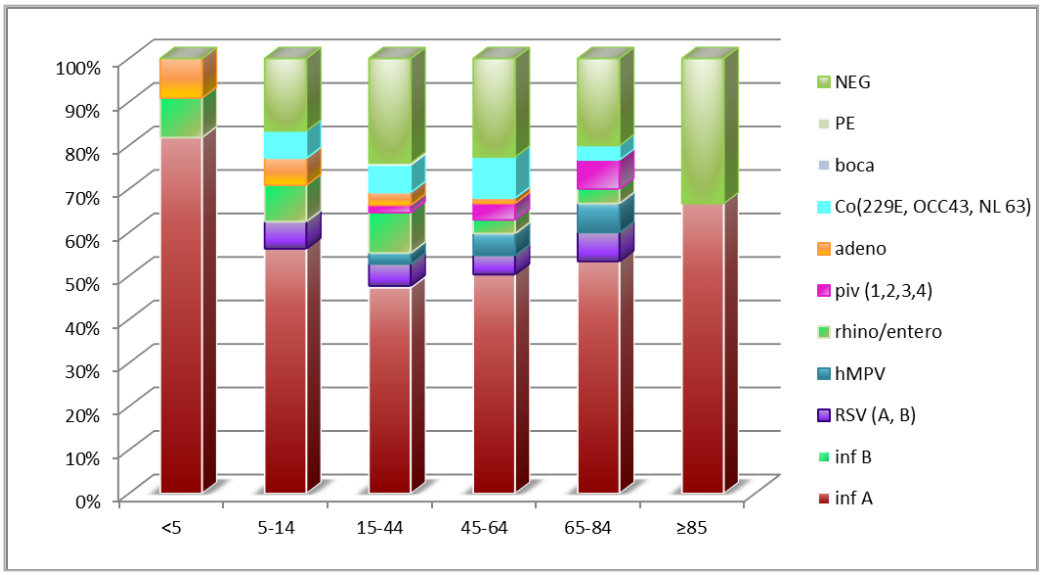


Figure 10b. Proportion of the different respiratory viruses in the ILI surveillance season 2018-2019 by age group (percentages)

Co-infections

The percentage of co-infection was 6.5 % (32/493). Among influenza positive samples, co-infection with other viruses was observed in 21/258 samples (4.6%). Regarding co-infection of respiratory viruses other than influenza, the percentage of co-infection was 2.2% (11/493) and no particular combination of viruses was dominant.

Weekly evolution

Figure 11 shows the weekly proportion of respiratory viruses that were laboratory-confirmed during the 2018-2019 flu season. From week 51 to week 11, most of the respiratory infections were caused by influenza. HRV/ENV were the following most prevalent viruses detected during all the surveillance period with percentages varying from 0% to 100% positive samples per week. RSV (A and B) were detected from week 50 to week 4, at percentages varying from 0.2% to 25% positive samples per week (week 51). Coronaviruses were detected from week 45 at percentages varying from 0% to 20% positive samples per week. The other respiratory viruses were detected more sporadically. No HBoV was detected in the ILI surveillance this season.

We did not observe the RSV peak that usually appears around week 51. This is due to the fact that the case definition used for ILI cases is very specific for influenza, but also mainly because the proportion of respiratory samples from young children and elderly, who are more susceptible to RSV, is very low in the ILI population.

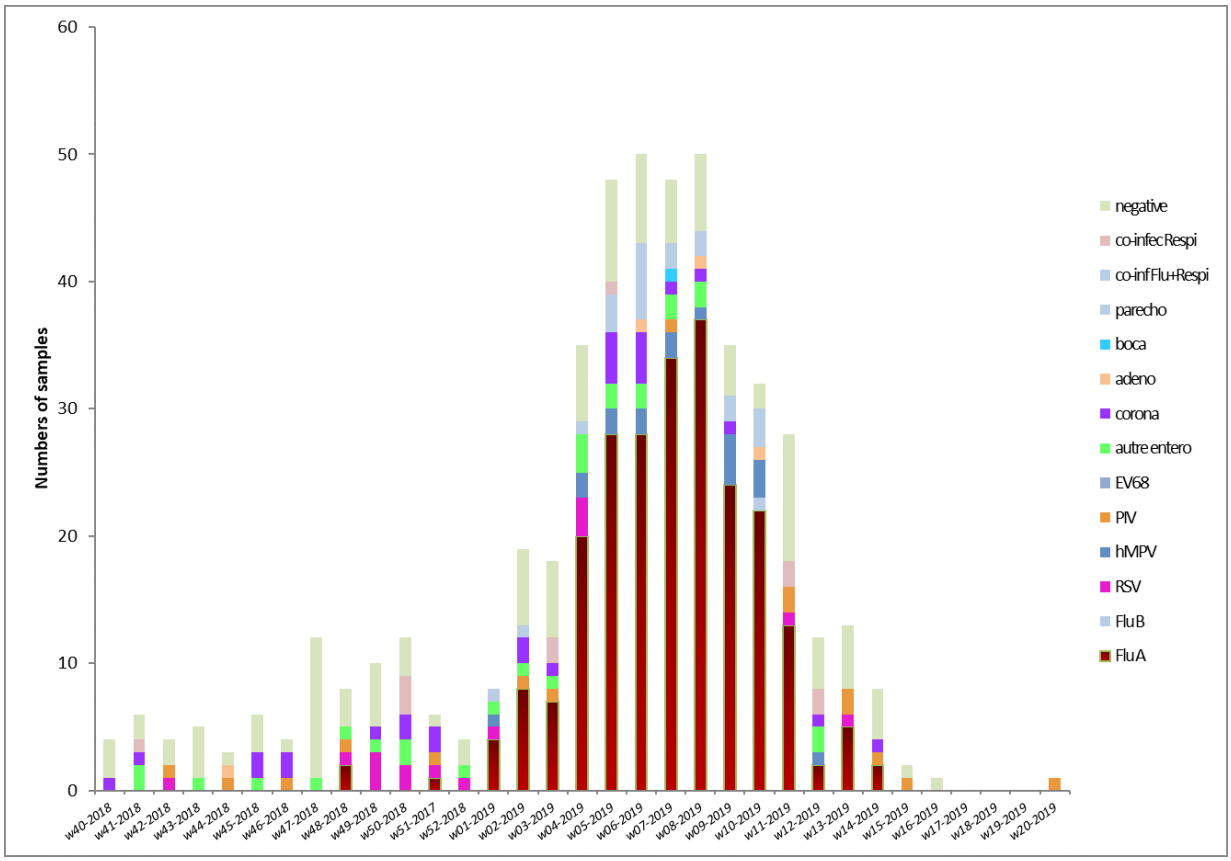


Figure 11. Weekly proportion of respiratory viruses during the 2018-2019 ILI surveillance

D.6.2 SARI surveillance

From week 40-2018 to week 18-2019, all SARI samples were also submitted to the diagnosis for the other respiratory viruses. For 784/2669 (30 %) patients no respiratory viruses were detected. Overall, 1669/2669 (70%) of the patients were positive for at least one respiratory viruses (including influenza alone or in co-infections). This percentage reached 90 % (717/801) in children below the age of 5 years old, and 68% (244/357) in elderly > 85 years. The most prevalent respiratory viruses were Influenza (27%), RSV (13%) HRV/ENV viruses (5%), hMPV (5%). For the other viruses, the percentages were lower (Figure 12).
The age groups were known for 2557 patients and the analyses were performed on those samples.

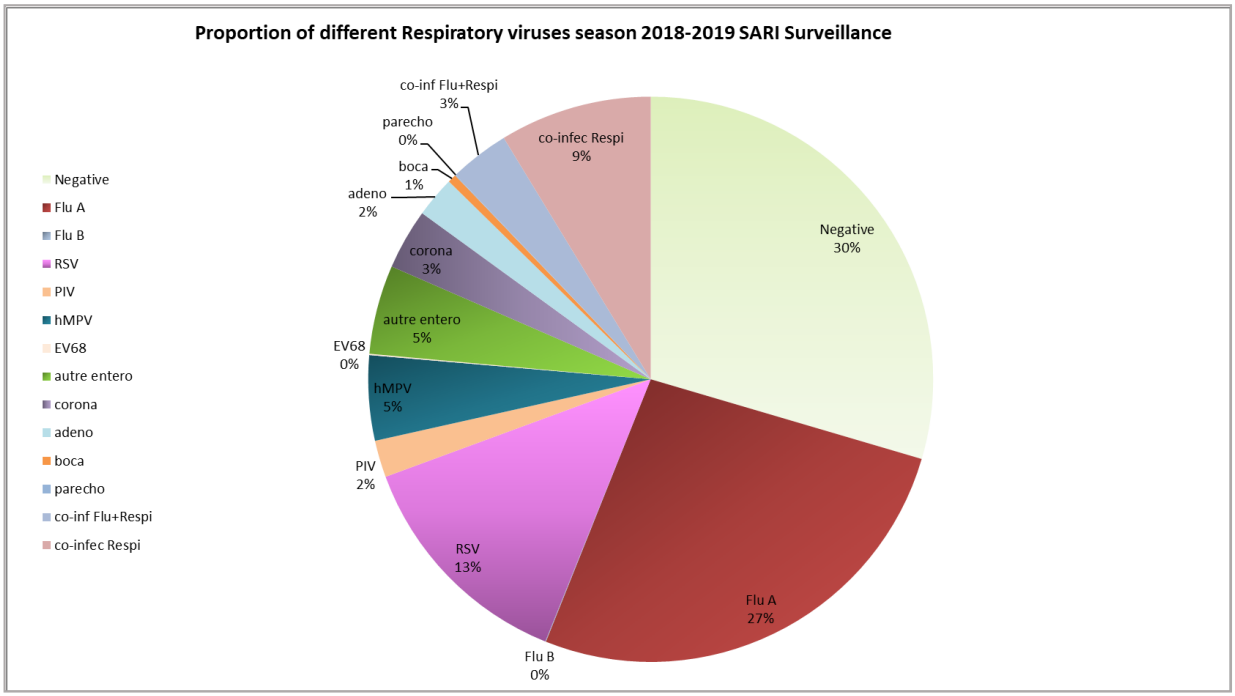


Figure 12. Proportion of the different respiratory viruses in the SARI surveillance season 2018-2019

Proportion of the different respiratory viruses according to age group

The age group was known for 2557 patients. The proportion of the different viruses varied between age groups.
In children below the age of 5 years old, the percentage of positivity for at least one respiratory virus reached 90%. All tested respiratory viruses were detected in this age group with the most prevalent virus being RSV (41.2%), rhino/enterovirus (22.8%), adenovirus (16.6%), influenza A (13%) and hMPV (8.9%). In patients aged more than 85 years old, influenza A was far the most prevalent (43.7%), followed by RSV (11.5%) (Figure 13a and 13b).

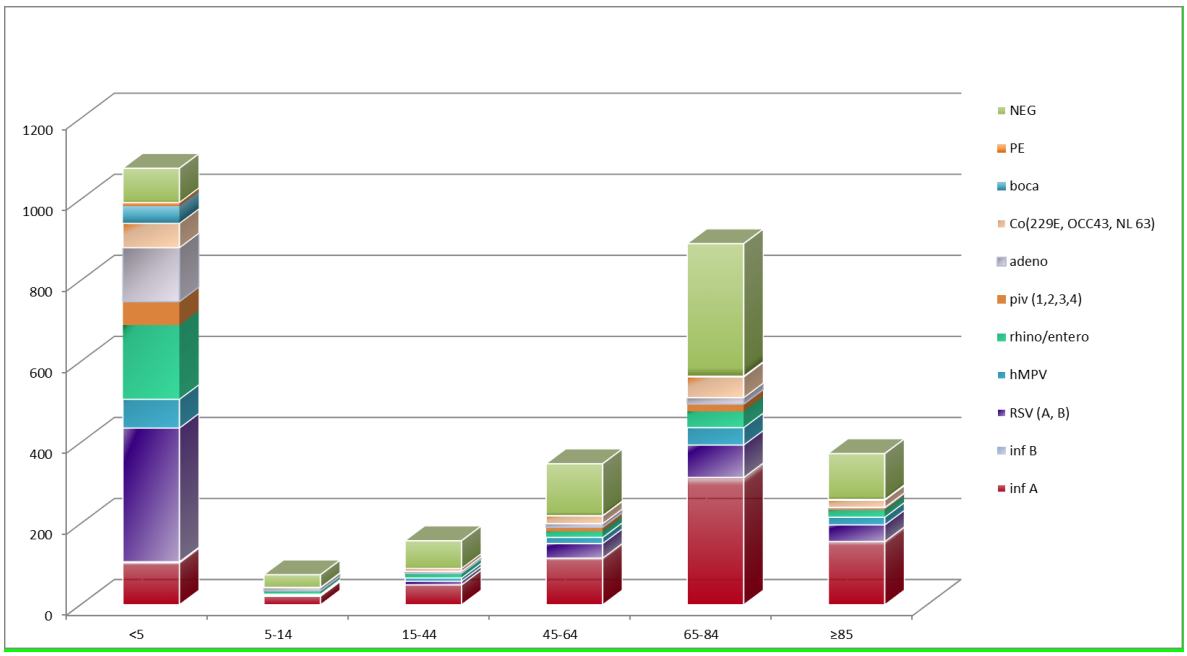


Figure 13a. Proportion of the different respiratory viruses in the SARI surveillance season 2018-2019 by age group (numbers)

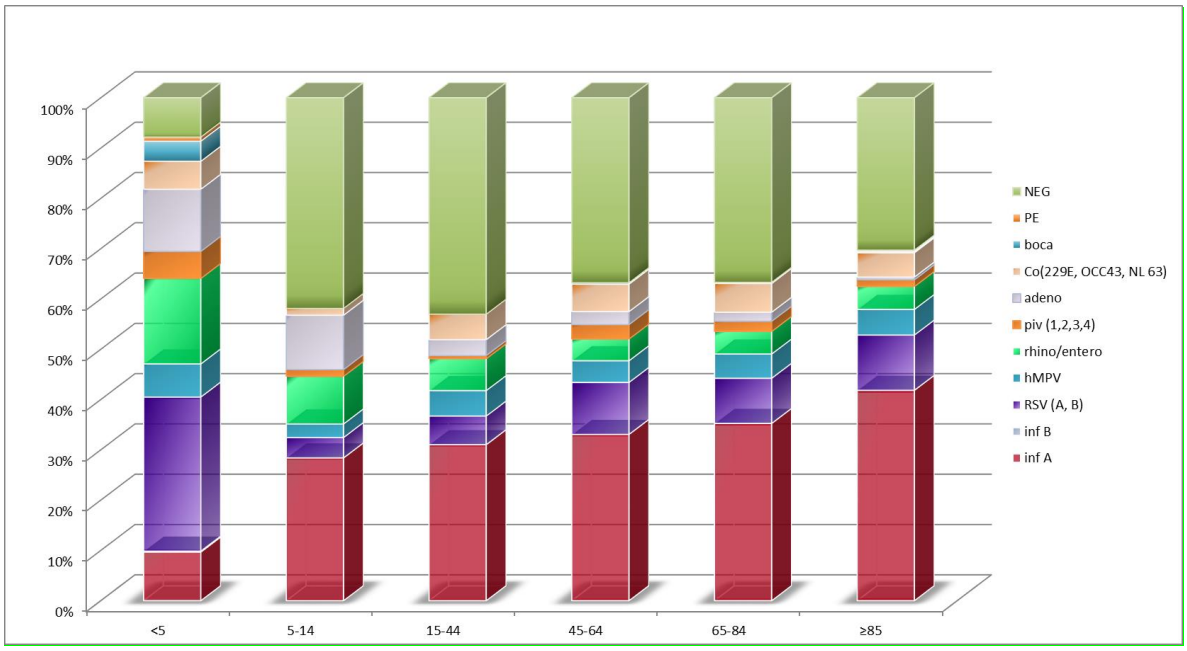


Figure 13b. Proportion of the different Respiratory viruses in the SARI surveillance season 2018-2019 by age group (percentages)

Coinfections

Overall, the percentage of co-infection (two to 5 viruses) was high (12%, 322/2669). In patients below the age of 5 years old this percentage reached 27% (230/840). Among influenza-positive samples, co-infection with other viruses were observed in 91/781 (10%) with the most frequent associations being Influenza/HRV found in 10/91 (10%) and Influenza/Adeno in 12/91 (13%). Regarding co-infections by respiratory viruses other than influenza, the percentage was 8.2% (221/2669) with the most common viral co-infections being with HRV/ENV and RSV 54% (120/221). Co-infection with 3 or more virus were observed in 50 patients, all below 5 years old.

Weekly evolution

Figure 14 shows the weekly proportion of respiratory viruses that were laboratory-confirmed during the 2018-2019 SARI surveillance period. All tested respiratory viruses were detected during the surveillance period (week 40-2018 to 18-2019).

As previously explained, for this season, we extended the severe acute respiratory infection (SARI) surveillance implemented in Belgium for influenza virus to capture the RSV season. The usual SARI case definition was used. Between weeks 40-2018 and 2-2019, we received 508 samples from SARI patients. The overall detection rate for RSV was 62.4% (317/508), with differences depending on the age group: 79.8% in <5 years old children (253/326) and 13.9% in >65 years old adults (44/128). Over 90% of the samples positive for both RSV and another virus were from under-5-year-old children (80/85). The peak was reached at week 49 with 55/64 RSV-positive samples. RSV-B dominated over RSV-A during the 2018-2019 season. From Week 2-2019 to week 18-2019, we received 2136 samples and Influenza A viruses were the most prevalent with a percentage of 36.6% . This percentage varied from week to week with a maximum of 48,8% during week 6-2019. The other viruses were detected more sporadically. This pilot study showed that the Belgian SARI network built to assess the severity of influenza virus can also be used to evaluate the severity of RSV without changing the case definition and shows that it can capture the RSV peak usually seen before the influenza peak.

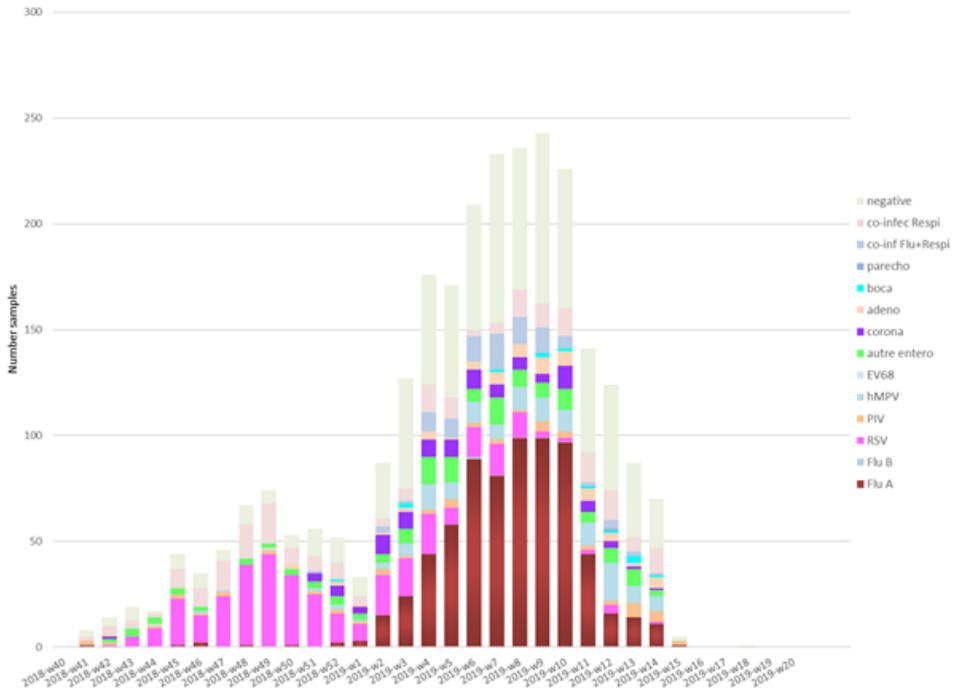


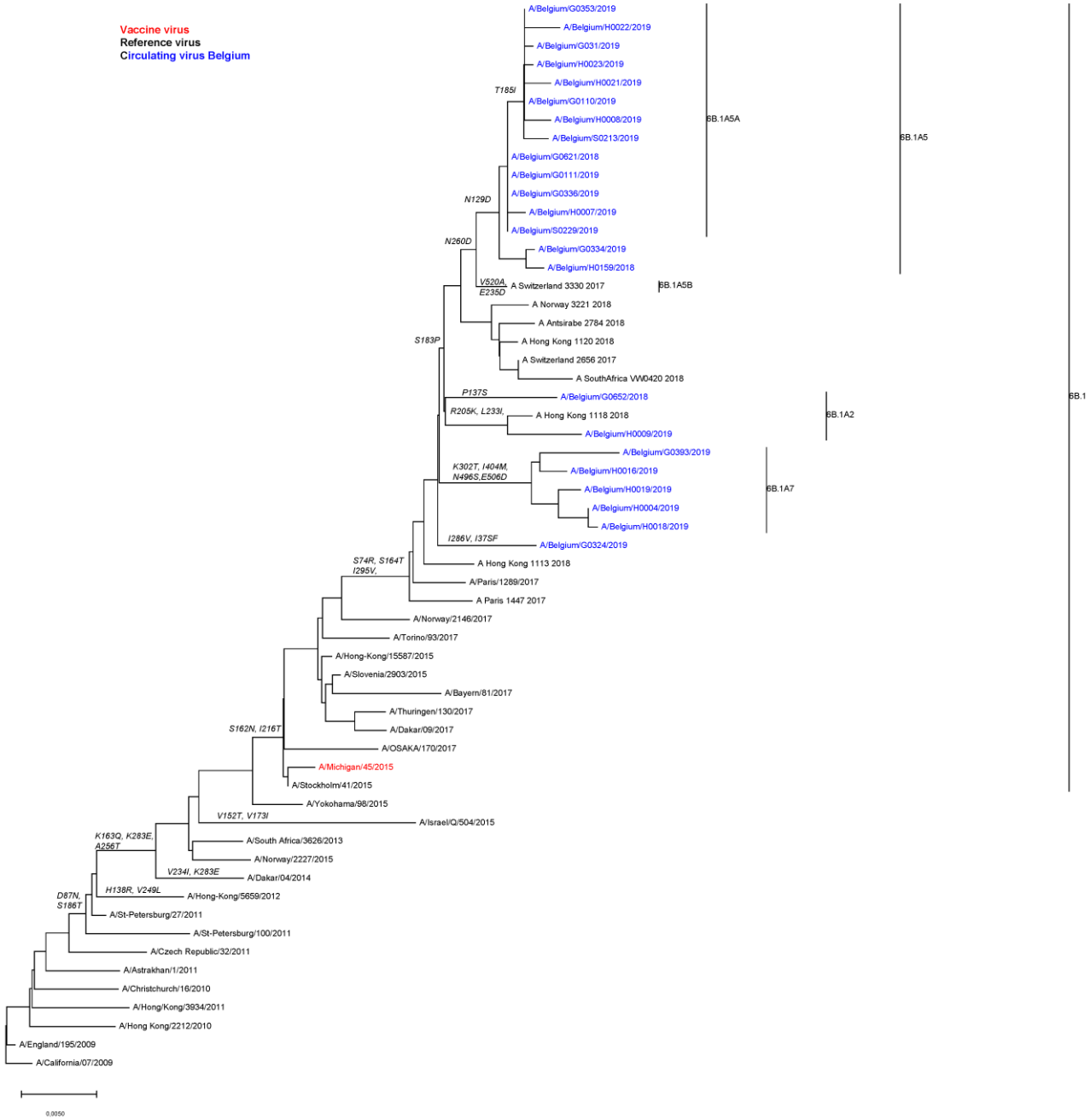
Figure 14. Weekly evolution of respiratory viruses during the SARI surveillance season 2018-2019

D.7. Characterisation of the influenza viruses

D.7.1 A(H1N1)pdm2009

Genetic characterisation

Genetic characteristics by whole-genome sequencing (WGS) of 29 A(H1N1)pdm09 were analyzed and some of them are presented in figure 15a/b. All H1N1pdm09 viruses belonged to clade 6B.1, represented by the vaccine strain A/Michigan/45/2015 and falling mainly in genetic group 6B.1A, all showing the substitutions **S74R, S164T and I295V**. More precisely, all the sequenced viruses fell into different genetic subgroups (6B.1A5A, 6B.1A2, 6B.1A7,..). All these sequences have been submitted to GISAID.


Vaccine virus
Reference virus
Circulating virus Belgium
A/Belgium/G0353/2019
A/Belgium/H0022/2019
A/Belgium/G031/2019
A/Belgium/H0023/2019
A/Belgium/H0021/2019
T185I
A/Belgium/G0110/2019
A/Belgium/H0008/2019
A/Belgium/S0213/2019
6B.1A5A
6B.1A5
A/Belgium/G0621/2018
A/Belgium/G0111/2019
N129D
A/Belgium/G0336/2019
A/Belgium/H0007/2019
A/Belgium/S0229/2019
N260D
A/Belgium/G0334/2019
A/Belgium/H0159/2018
V520A, E235D
A Switzerland 3330 2017
6B.1A5B
A Norway 3221 2018
A Antsirabe 2784 2018
S183P
A Hong Kong 1120 2018
A Switzerland 2656 2017
A SouthAfrica VW0420 2018
P137S
A/Belgium/G0652/2018
6B.1
R205K, L233I,
A Hong Kong 1118 2018
6B.1A2
A/Belgium/H0009/2019
A/Belgium/G0393/2019
K302T, I404M, N496S,E506D
A/Belgium/H0016/2019
A/Belgium/H0019/2019
6B.1A7
A/Belgium/H0004/2019
A/Belgium/H0018/2019
I286V, I37SF
A/Belgium/G0324/2019
S74R, S164T I295V,
A Hong Kong 1113 2018
A/Paris/1289/2017
A Paris 1447 2017
A/Norway/2146/2017
A/Torino/93/2017
A/Hong-Kong/15587/2015
A/Slovenia/2903/2015
A/Bayern/81/2017
A/Thuringen/130/2017
S162N, I216T
A/Dakar/09/2017
A/OSAKA/170/2017
A/Michigan/45/2015
A/Stockholm/41/2015
A/Yokohama/98/2015
V152T, V173I
A/Israel/Q/504/2015
K163Q, K283E, A256T
A/South Africa/3626/2013
A/Norway/2227/2015
V234I, K283E
A/Dakar/04/2014
H138R, V249L
A/Hong-Kong/5659/2012
D87N, S186T
A/St-Petersburg/27/2011
A/St-Petersburg/100/2011
A/Czech Republic/32/2011
A/Astrakhan/1/2011
A/Christchurch/16/2010
A/Hong/Kong/3934/2011
A/Hong Kong/2212/2010
A/England/195/2009
A/California/07/2009
0.0050

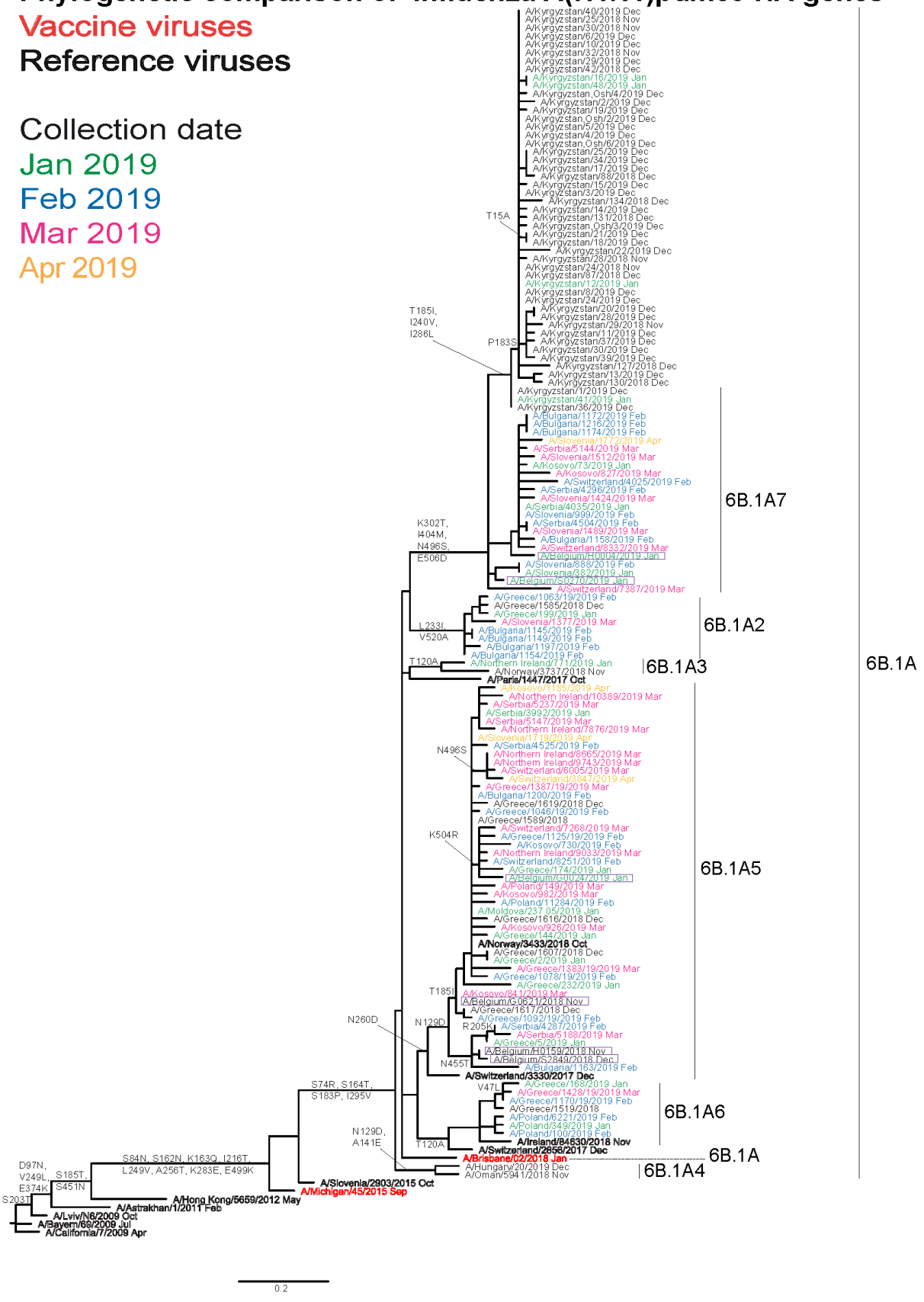


Figure 15a/b. Phylogenetic analysis of influenza A(H1N1)pdm09 strains in Belgium, season 2018-2019

Antigenic characterisation

The majority of tested viruses were recognized well by the antiserum raised against the currently used vaccine virus, A/Michigan/45/2015, and were generally recognized well by the other antisera in the panel. Antigenic data indicated that circulating A(H1N1)pdm09 viruses were similar to the 2018/2019 vaccine virus (Table 4)

Table 4. Antigenic analyses of influenza A(H1N1) viruses from Belgium 2018-2019 (WHO CC-London)

Antigenic analyses of influenza A(H1N1)pdm09 viruses (2019-02-12)

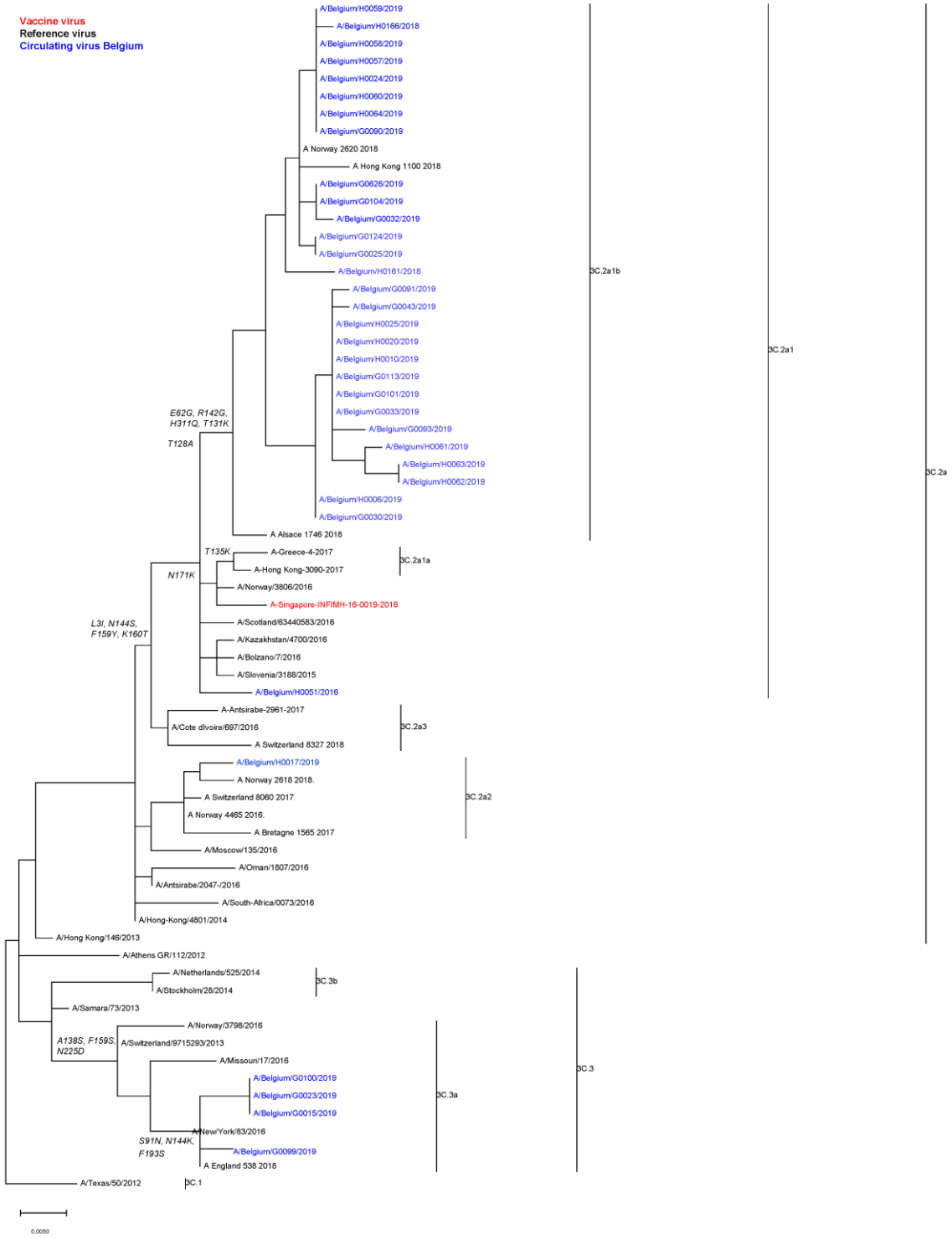
						Haemagglutination inhibition titre										
WIC number						Post-infection ferret antisera										
	Viruses	Other information		Collection date	Passage history	A/Mich 45/15	A/Cal 7/09	A/Bayern 69/09	A/Lviv N6/09	A/Astrak 1/11	A/HK 5659/12	A/Slov 2903/2015	A/Paris 1447/17	A/Swit 2656/17	A/Swit 3330/17	A/Norway 3433/18
			Passage history			Egg	Egg	MDCK	MDCK	MDCK	MDCK	Egg	MDCK	Egg	Egg	MDCK
			Ferret number			NIB F42/16*1	F07/16*1	F09/15*1	F13/18*1	F22/13*1	F17/15*1	NIB F48/16*1	F03/18*2	F20/18*1	F23/18*1	F04/19*1
			Genetic group			6B.1				5	6A	6B.1	6B.1	6B.1	6B.1	6B.1
	A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	640	640	320	320	320	640	1280	2560	1280	320	1280
	A/California/7/2009	clone 38-32		2009-04-09	E3/E3	320	640	320	320	320	320	640	1280	640	320	1280
	A/Bayern/69/2009	G155E		2009-07-01	MDCK5/MDCK1	40	<	320	320	<	<	40	320	<	40	320
	A/Lviv/N6/2009	G155E, D222G		2009-10-27	MDCK4/SIAT1/MDCK3	80	80	1280	1280	80	80	160	640	320	320	1280
	A/Astrakhan/1/2011		5	2011-02-28	MDCK1/MDCK7	640	640	640	640	320	640	1280	2560	640	640	2560
	A/Hong Kong/5659/2012		6A	2012-05-21	MDCK4/MDCK2	320	160	160	80	160	320	320	640	320	160	1280
	A/Slovenia/2903/2015	clone 37	6B.1	2015-10-26	E4/E2	640	640	320	320	640	640	1280	2560	1280	640	2560
	A/Paris/1447/2017		6B.1A	2017-10-20	MDCK1/MDCK3	640	320	320	80	320	320	1280	2560	640	320	2560
	A/Switzerland/2656/2017		6B.1A	2017-12-21	E5/E3	640	640	640	320	640	640	1280	2560	1280	640	2560
	A/Switzerland/3330/2017	clone 35	6B.1A5	2017-12-20	E6/E2	320	640	320	160	320	320	640	2560	1280	640	2560
	A/Norway/3433/2018		6B.1A5	2018-10-30	MDCK3	640	160	160	80	160	160	640	1280	640	320	2560
	A/Ireland/84630/2018		6B.1A6	2018-11-28	MDCK1/MDCK2	320	ND	160	80	ND	ND	640	1280	320	160	1280
	A/Brisbane/02/2018		6B.1A1		E3/E1	640	ND	320	320	ND	ND	1280	2560	640	320	1280
	IVR-190(A/Brisbane/02/2018)		6B.1A1		E3/D8/E1	1280	ND	640	320	ND	ND	2560	5120	1280	640	2560
	TEST VIRUSES															
	A/Belgium/H0004/2019		6B.1A7	2019-01-16	MDCK1	640	640	640	80	320	640	1280	5120	1280	640	5120
	A/Belgium/S2849/2018		6B.1A5	2018-12-01	C1/MDCK1	640	320	640	320	320	640	1280	2560	640	640	2560
	A/Belgium/G0621/2018		6B.1A5	2018-11-26	C1/MDCK1	640	320	640	320	320	640	1280	2560	640	640	2560
	A/Belgium/H0159/2018		6B.1A5	2018-11-02	C1/MDCK1	640	640	640	160	320	320	1280	2560	1280	640	2560
	A/Belgium/S0270/2019		6B.1A7	2019-01-15	MDCK2	320	ND	160	80	ND	ND	640	1280	320	160	1280
	A/Belgium/G0024/2019		6B.1A5	2019-01-07	MDCK2	320	ND	160	80	ND	ND	320	1280	160	160	2560
Virus	A(H1N1)pdm09															
Date																
*	Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)															
1	< = <40															
2	< = <80															
ND	Not Done															
	Sequences in phylogenetic trees															

D.7.2 A(H3N2)

Genetic characterisation

In Belgium, about 80% of the positive influenza viruses were A(H3N2). Twenty six A(H3N2) were completely sequenced by next-generation sequencing (NGS) and are presented in figure 16a/b. Most of the sequenced viruses belonged to the subclade 3C.2a. Twenty one of those viruses fell into subclade 3C.2a1b represented by the reference strain A/Bolzano/7/2016 defined by the mutations N171K and N121K and ten had HA genes in subclade 3C.2a1b and one fell in subclade 3C.2a2. Four viruses fell the subclade 3C.3a. represented by the reference strain (A/England/538/2018). This group viruses, characterized by the amino acid substitutions T128A, R142G, A138S, F159S and N225D in the HA, is antigenically distinct from the vaccine virus (Fig. 16a/b). All of these sequences have been submitted to GISAID.

As noticed during previous seasons, A(H3N2) viruses are evolving rapidly with emergence of several virus clusters defined by additional amino acid substitutions in the hemagglutinin, thereby emphasizing the need for continued monitoring of antigenic characteristics.


Vaccine virus
Reference virus
Circulating virus Belgium
A/Belgium/H0059/2019
A/Belgium/H0166/2018
A/Belgium/H0058/2019
A/Belgium/H0057/2019
A/Belgium/H0024/2019
A/Belgium/H0060/2019
A/Belgium/H0064/2019
A/Belgium/G0090/2019
A Norway 2620 2018
A Hong Kong 1100 2018
A/Belgium/G0626/2019
A/Belgium/G0104/2019
A/Belgium/G0032/2019
A/Belgium/G0124/2019
A/Belgium/G0025/2019
A/Belgium/H0161/2018
A/Belgium/G0091/2019
A/Belgium/G0043/2019
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A/Belgium/G0101/2019
A/Belgium/G0033/2019
A/Belgium/G0093/2019
A/Belgium/H0061/2019
A/Belgium/H0063/2019
A/Belgium/H0062/2019
A/Belgium/H0006/2019
A/Belgium/G0030/2019
E62G, R142G, H311Q, T131K
T128A
A Alsace 1746 2018
T135K
A-Greece-4-2017
A-Hong Kong-3090-2017
N171K
A/Norway/3806/2016
A-Singapore-INFIMH-16-0019-2016
L3I, N144S, F159Y, K160T
A/Scotland/63440583/2016
A/Kazakhstan/4700/2016
A/Bolzano/7/2016
A/Slovenia/3188/2015
A/Belgium/H0051/2016
A-Antsirabe-2961-2017
A/Cote dIvoire/697/2016
A Switzerland 8327 2018
A/Belgium/H0017/2019
A Norway 2618 2018.
A Switzerland 8060 2017
A Norway 4465 2016.
A Bretagne 1565 2017
A/Moscow/135/2016
A/Oman/1807/2016
A/Antsirabe/2047-/2016
A/South-Africa/0073/2016
A/Hong-Kong/4801/2014
A/Hong Kong/146/2013
A/Athens GR/112/2012
A/Netherlands/525/2014
A/Stockholm/28/2014
A/Samara/73/2013
A/Norway/3798/2016
A138S, F159S, N225D
A/Switzerland/9715293/2013
A/Missouri/17/2016
A/Belgium/G0100/2019
A/Belgium/G0023/2019
A/Belgium/G0015/2019
A/New/York/83/2016
S91N, N144K, F193S
A/Belgium/G0099/2019
A England 538 2018
A/Texas/50/2012
3C.2a1b
3C.2a1
3C.2a
3C.2a1a
3C.2a3
3C.2a2
3C.3b
3C.3
3C.3a
3C.1
0.0050

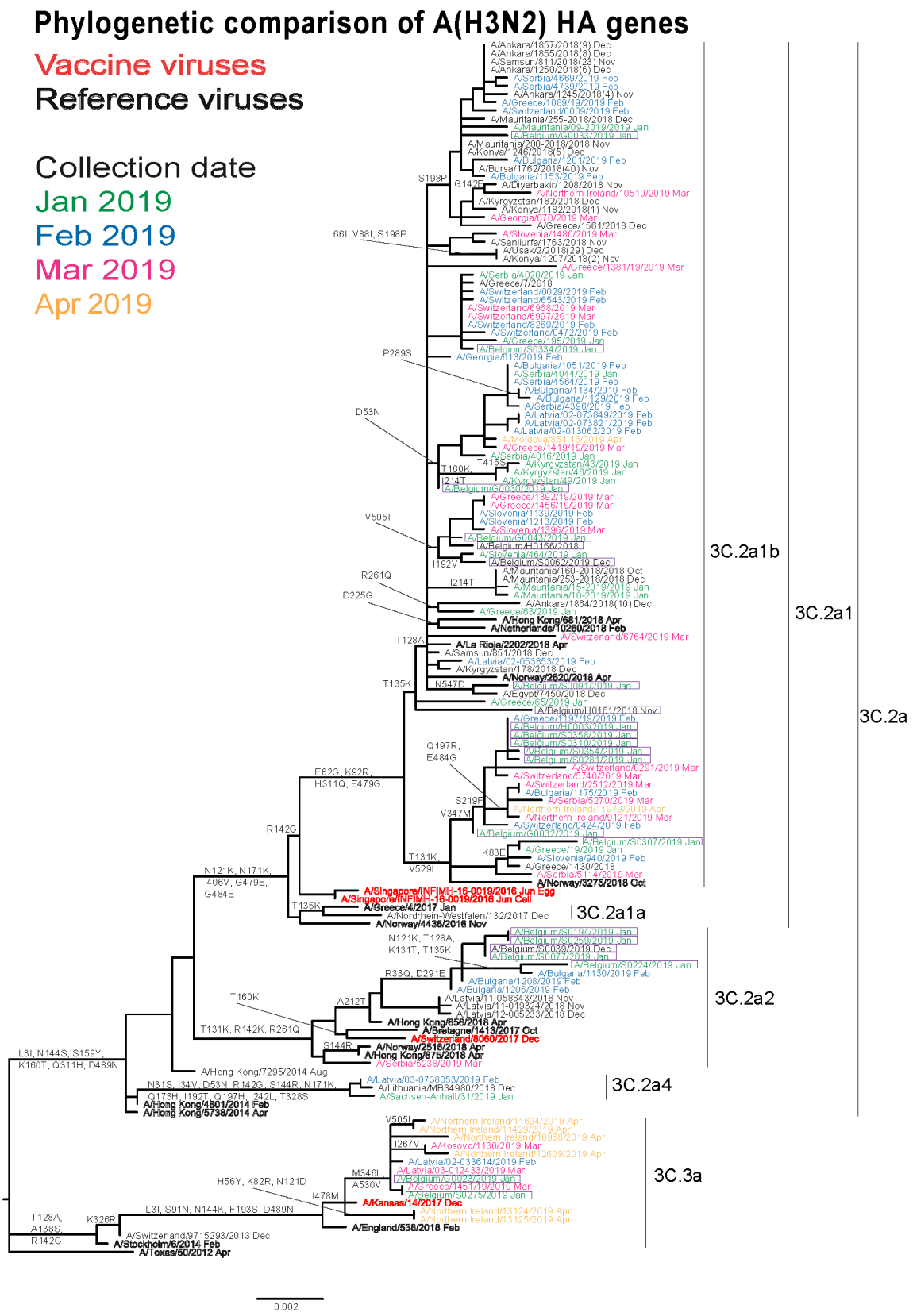

Figure 16 a/b Phylogenetic analysis of the HA sequences of the A(H3N2) viruses analysed from Belgium and other European countries during the 2018-2019 season in comparison with the vaccine strain and the reference strains.

Antigenic characterisation

Antigenic characterisation of A(H3N2) viruses was very difficult by HI assay due to variable agglutination of red blood cells. Only two strains sent to WHO CC-London were antigenically characterized (table 5).

Table 5. Antigenic analyses of influenza A(H3N2) viruses from Belgium 2018-2019 (WHO CC-London)

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2019-03-22 — NEW NEW

WIC number	Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection ferret antisera									
						A/Stock	A/HK	A/Bretagne	A/Singapore	A/La Rioja	A/Switz	A/Eng	A/Norw	A/Kansas	A/Kansas
			Passage history			6/14	5738/14	1413/17	0019/16	2202/18	8060/17	538/18	3275/18	14/17	14/17
			Ferret number			SIAT	MDCK	SIAT	Egg 10-4	SIAT	Egg	SIAT	SIAT	Egg	Egg
						F14/14[-1]	St Judes F60/17[-1]	F01/18*[-1]	F46/17*[-1]	F26/18[-1]	F27/18[-1]	F31/18[-1]	F03/19[-1]	F11/19[-1]	F12/19[-1]
			Genetic group			3C.3a	3C.2a	3C.2a2	3C.2a1	3C.2a1b	3C.2a2	3C.3a	3C.2a1b	3C.3a	3C.3a
	REFERENCE VIRUSES														
	A/Stockholm/6/2014		3C.3a	2014-02-06	SIAT1/SIAT3	320	160	160	160	160	320	160	160	160	160
	A/Hong Kong/5738/2014		3C.2a	2014-04-30	MDCK1/MDCK2/SIAT2	160	160	160	160	80	320	160	320	160	160
	A/Bretagne/1413/2017		3C.2a2	2017-10-09	MDCK1/SIAT4	160	160	640	160	80	640	160	320	160	160
	A/Singapore/INFIMH-16-0019/2016		3C.2a1	2016-04-14	E5/E2	80	160	80	640	320	160	80	80	40	40
	A/Switzerland/8060/2017	Clone 57	3C.2a2	2017-12-12	E7/E1	80	160	1280	640	160	1280	80	80	40	80
	A/England/538/2018		3C.3a	2018-02-26	MDCK1/SIAT3	80	80	80	80	40	80	640	40	320	320
	A/Kansas/14/2017		3C.3a		E7/E2	40	<	40	40	<	<	320	<	1280	1280
	TEST VIRUSES														
	A/Belgium/S0275/2019		3C.3a	2019-01-15	SIAT1	160	80	80	80	40	80	640	40	320	320
	A/Belgium/G0023/2019		3C.3a	2019-01-03	SIAT1	160	80	80	80	40	80	640	40	320	320
											Vaccine			Vaccine	
											SH 2019			NH 2019	

Assay HI (Guinea Pig RBC with 20nM Oseltamivir)
RBC Guinea Pig
Virus Influenza A(H3N2)
Date
* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
† < = <40
ND Not Done
Sequences in phylogenetic trees

D.7.3 B Yamagata

Very few influenza B Yamagata circulated during this season, none of them were sequenced nor submitted to antigenic characterisation

D.7.4 B Victoria

Genetic characterisation

Very few influenza B viruses from the Victoria lineage were detected during this season, two of them were sequenced and belonged to clade 1A. However, these viruses showed three amino acid deletions in the HA gene at positions 162 and 164 (in comparison, the strains isolated during the previous season possessed the deletion 162-163) (Fig. 17, Fig. 18). This deletion in the HA resulted in these viruses being antigenically different from the vaccine virus.
The sequences have been submitted to GISAID.

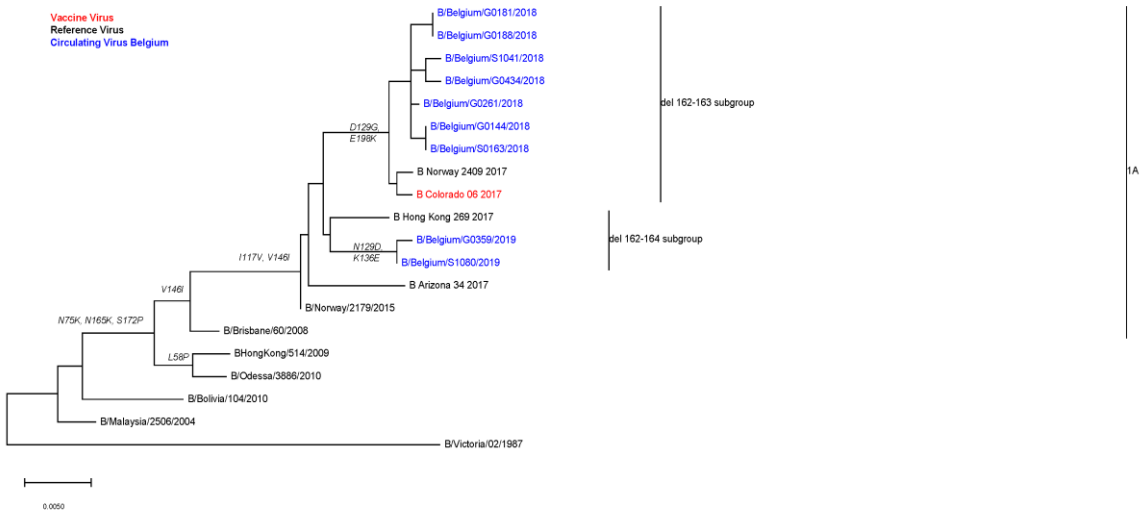

Figure 17. Phylogenetic analysis of circulating influenza B/Victoria viruses detected in Belgium during the 2018-2019 season.

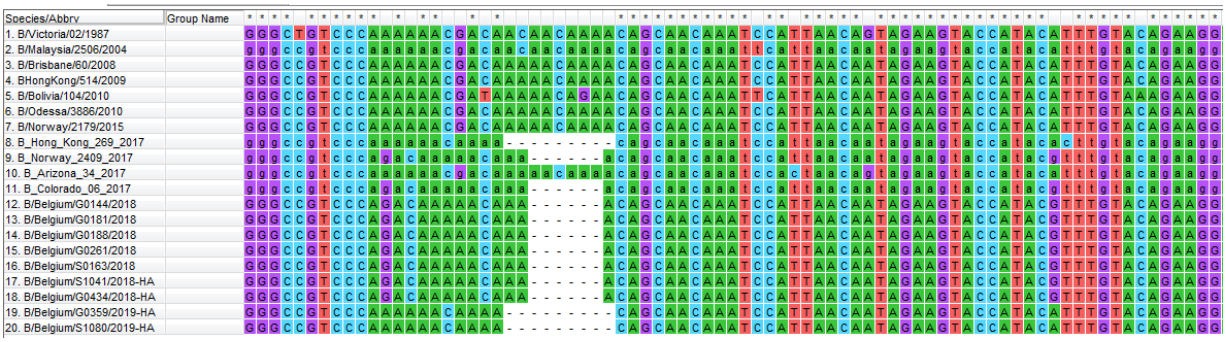

Figure 18. Part of the alignment of circulating influenza B/Victoria viruses detected in Belgium during the 2018-2019 season.

Antigenic characterisation

No influenza B Victoria viruses were antigenically characterized.

D.8. Antiviral monitoring

Among the 139 strains analysed phenotypically, all except one were sensitive to neuraminidase inhibitors: Oseltamivir and Zanamivir. The only strain that showed a reduced sensitivity will be further sequenced.

D.9 Composition of influenza virus vaccines

It is recommended that quadrivalent vaccines for use in the 2019-2020 northern hemisphere influenza season contain the following strains:

- an A/Brisbane/02/2018 (H1N1)pdm09-like virus;
- an A/Kansas/14/2017 (H3N2)-like virus;
- a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage); and
- a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage).

Saison	A/H1N1	A/H3N2	B	Quadrivalent
2000-2001	A/New Caledonia/20/99	A/Panama/2007/99)	B/Yamanashi/166/98	
2001-2002	"	"	B/Sichuan/379/2000	
2002-2003	"	"	B/Hong Kong/330/2001	
2003-2004	"	"	"	
2004-2005	"	A/Fujian/411/2002	B/Shanghai/361/2002	
2005-2006	"	A/California/7/2004	"	
2006-2007	"	A/Wisconsin/67/2005	B/Malaysia/2506/2004 VIC	
2007-2008	A/Solomon Islands/3/2006	"	"	
2008-2009	A/Brisbane/59/2007	A/Brisbane/10/2007	B/Florida/4/2006 YAM	
2009-2010	"	"	B/Brisbane/60/2008 VIC	
2010-2011	A/California/7/2009	A/Perth/16/2009	"	
2011-2012	"	"	"	
2012-2013	"	A/Victoria/361/2011	B/Wisconsin/1/2010 YAM	
2013-2014	"	A/Texas/50/2012	B/Massachusetts/2/2012 YAM	
2014-2015	"	"	"	
2015-2016	"	A/Switzerland/971529/2013	B/Phuket/3073/2013 YAM	B/Brisbane/60/2008 VIC
2016-2017	"	A/Hong-Kong/4801/2017	B/Brisbane/60/2008 VIC	B/Phuket/3073/2013 YAM
2017-2018	A/Michigan/45/2015	"	"	*
2018-2019	"	A/Singapore/INFIMH-16-0019/2016	B/Colorado/06/2017-like virus VIC	*
2019-2020	A/Brisbane/02/2018	A/Kansas/14/2017	B/Colorado/06/2017-like virus VIC	*

Figure 19. Evolution of the composition of the trivalent influenza vaccine 2000 – 2020

D.10 Vaccine effectiveness

Estimates of vaccine effectiveness are obtained by using the data from both the ILI and SARI surveillance, by means of a test-negative design case-control study, adjusting for age, sex, month of sampling, chronic disease and surveillance scheme. These estimates indicate that this season, the influenza vaccine had a vaccine effectiveness of 13% for all-type influenza (75% for influenza A(H1N1)pdm09 and 1% for influenza A(H3N2)).

D.11 Severity

The SARI surveillance by the sentinel hospital network showed a fairly high number of hospitalizations due to an acute severe respiratory infection during the flu epidemic this winter, of which 37% was a confirmed influenza infection.

Although the number of hospitalizations for influenza infection was high this season, in relative terms, the severity indicators (13% severe complications and 6% deaths during the hospital stay) suggest that severity was moderate in comparison to the previous season and comparable to the seasons before.

Severe complications were mainly (92%) observed in patients with an underlying condition (notably chronic respiratory disease). The odds on severity was also associated with A(H1N1) subtype versus A(H3N2).

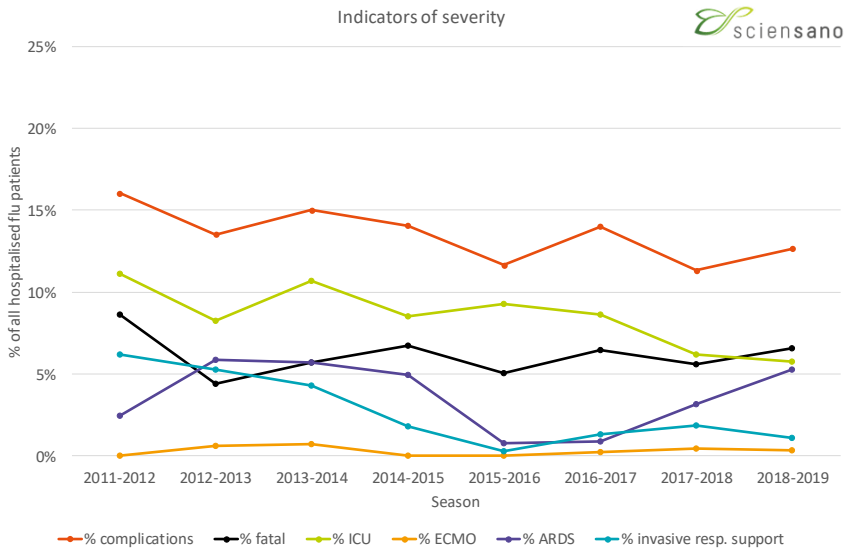

Figure 20. Evolution of indicators of severity in patients with confirmed influenza virus infection during the past 7 seasons in Belgium (Source: Sciensano: SARI surveillance by the network of sentinel hospitals)

The average length of hospitalization for confirmed influenza infection the surveillance network was 8.8 days (ranging with age from 3.6 days in 0-4 years to 13.3 days in people aged 85 and over). This duration was comparable to other seasons.

D12. Surveillance of all-cause mortality (BE-MOMO : Belgian Mortality Monitoring)

No significant all-cause mortality was observed during the flu epidemic this season (see https://epistat.wiv-isp.be/momo/)

E. Conclusion

The 2018–2019 influenza epidemic was of medium intensity and lasted 8 weeks. During the epidemic peak, a total number of 761 ILI consultations per 100.000 inhabitants was reached. The season was characterized by the mixed circulation of A(H1N1)pdm09 and A(H3N2) with a predominance of A(H3N2).

All sequenced **A(H1N1)pdm2009** belonged to group 6B.1, represented by the vaccine strain A/Michigan/45/2015.

The majority of the sequenced **A(H3N2)** strains belonged to the subclade 3C.2a1b, represented by the reference strain A/Bolzano/7/2016 , and the rest were from the 3C.2a2 and 3C.3a clades. Vaccine effectiveness against A(H3N2) very low during this season, this is likely due to several factors including a suboptimal match between the circulating A(H3N2), especially from clade 3C.3a and the vaccine but also because of egg propagation of the vaccine seed virus witch can lead to mutations. A(H3N2) viruses are evolving rapidly with emergence of several virus clusters defined by additional amino acid substitutions in the

haemagglutinin, thereby emphasizing the need for continued monitoring of antigenic characteristics.
The few sequenced **B Victoria** viruses belonged to the B/Victoria deletion variant subgroup (del 162-164 subgroup) which is antigenically different from the vaccine virus.

All respiratory samples were also analysed for other respiratory viruses. In the ILI population, 50% of the influenza virus-negative patients were positive for one or more other respiratory viruses, whereas in the SARI population, this percentage reached 57%. This suggest an important role of other respiratory viruses in hospitalized patients during the flu season with symptoms relevant to the case definition. Co-infections of influenza virus and other respiratory viruses were frequently detected in hospitalized patients (9%) .

F. Acknowledgements

The influenza surveillance in Belgium is financially supported by the Federal Public Service Health, Food Chain Safety and Environment, the “Fédération Wallonie Bruxelles” and the “Agentschap Zorg en Gezondheid“. The National Influenza Centre (national reference centre) is partly financially supported by the Federal Institute for Health Insurance (RIZIV). The SARI surveillance is supported by the Federal Public Service Health, Food Chain Safety and Environment, DG1.
We would like to acknowledge all our partners of the different surveillance networks (the sentinel GPs and the different sentinel hospitals involved in the SARI surveillance). We also want to acknowledge the WHO Collaborating Centre for Reference and Research on Influenza, Crick Worldwide Influenza Centre in London.

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