Virological Surveillance of Influenza in Belgium

Season 2017-2018

VIRAL DISEASES
National Influenza Centre (WHO)

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A. Abstract

This 2017-2018 season the Influenza epidemic in Belgium lasted 12 weeks, which is long compared to previous seasons. The epidemic threshold was crossed from week 2-2018 (January 8 to January 14, 2018) to week 13-2018 (March 26 to April 1, 2018) (Fig. 1)
The intensity of the epidemic was moderate. After week 2_2018, the incidence of GP visits for influenza-like illness (ILI) rapidly increased to reach 584 GP visits per 100.000 inhabitants in week 6-2018. The incidence then remained elevated and reached a peak value in week 10-2018 (744 GP visits for ILI / 100.000 inhabitants). During the first phase of the epidemic mainly children were affected, whereas during the second phase all ages were concerned. From week 11-2018, the incidence decreased again and dropped below the epidemic threshold after week 13-2018.

Based on the surveillance of the sentinel network of general practitioners, we estimate that this season approximately 697.000 Belgians visited their GP for influenza-like illness (ILI) and that approximately 470.000 Belgians had a clinical infection with the influenza virus.

The first positive sample was diagnosed in week 47-2017 and increasingly large numbers of positive influenza cases were detected from week 52-2017 onwards, reaching a proportion of 88.7% in week 7-2018. These were mainly B/Yamagata and A(H1N1)pdm09 viruses which have been circulating this season.

All respiratory samples were also analysed for other respiratory viruses. In the ILI population, 35% of the influenza negative patients were positive for one or more other respiratory viruses, whereas in the SARI population, this percentage reached 39%. This suggests an important role of other respiratory viruses in hospitalized patients during the flu season.

Preliminary estimates indicate that this season, the influenza vaccine offered a 41% (95% CI: 22% -55%) reduction of incidence of medically attended influenza infections in vaccinated people.

All tested viruses were sensitive to Oseltamivir and 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 were 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**

C.1. Surveillance

C.1.1. Sentinel Surveillance of ILI

Network of Sentinel General Practices

In Belgium, the influenza surveillance is performed by the NIC, in collaboration with the Unit of Health Services Research and the Unit of Epidemiology of Infectious Diseases of the Scientific Institute of Public Health in Brussels. A network of sentinel general practices (SGPs) has been involved since 2007 in the clinical and virological influenza surveillance. 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 (2).

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 patients 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 were 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 were returned to the National influenza Centre by mail (postage paid) and new kits were regularly sent depending on the shipment of samples. Patients information, clinical and epidemiological data and laboratory results were encoded in the LIMS system. All the results of one patient are sent to the physician, after scientific and medical validation.

C.1.2. Sentinel Surveillance of SARI

Network of sentinel hospitals

Following the A(H1N1)2009 pandemic, 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 2017-2018 influenza season, six hospitals located in the three regions of the country participated to the surveillance. The SARI case definition is: an acute respiratory illness with onset within the last ten days, fever of ≥ 38°C, 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 were returned to the NIC by mail (postage paid) and new kits were sent regularly to hospitals depending on the shipment of samples.

Patients information, clinical and epidemiological data and laboratory results were 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 2017-2018:

- CHU UCL Namur (Godinne)
- CHU Saint-Pierre (Bruxelles)
- AZ Sint Jan (Brugge)
- UZ Brussel (Brussels)
- Jessa Ziekenhuis (Hasselt)
- Grand Hôpital de Charleroi (Charleroi)

C.1.3. 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.1.4. Suspected cases of Avian Influenza H5N1 and H7N9

Influenza A (H5N1)

Since 2003, and till 20 July 2018, 860 human infections with highly pathogenic H5N1 viruses have been reported to WHO by 16 countries (4). About 50% (454) 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 the Scientific Institute of Public Health 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. Most of the cases resulted in severe respiratory illness, with a mortality rate of about 30 percent. As of 5 September 2018, a total of 1625 laboratory confirmed human cases; 623 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 and animals (including the distribution in wild birds) 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.

C.1.5. 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. The case fatality rate is at the moment 35.6% (7). Typical MERS symptoms include fever, cough and shortness of breath. Pneumonia is common, but not always present. Gastrointestinal symptoms, including diarrhea, have also been reported. So far, the

observed human-to-human transmission has occurred mainly in health care settings. Human-to-human transmission is amplified among household contacts and in healthcare settings. The epidemiological pattern of human infections is highly suggestive of a zoonotic infection. The animal vector or reservoir seems to be dromedary camels but infection acquired by exposure to camels represent a minority of all cases. Possible association with bats have also been suggested. We could not find evidence for coronavirus infection in bats (8). 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.1.6. 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 16 different respiratory viruses: respiratory syncytial virus (RSVA and RSVB), parainfluenza viruses (PIV 1, 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 RT-PCR Influenza A/B/RP: adapted protocols (12,13); primers and probes for the matrix gene (influenza A) and hemagglutinine gene (influenza B). The RNaseP (RP) primers and probe target the human RNase P gene and serves as an internal positive control for human nucleic acid.

Subtyping A (H1, H3, N1, N2)

For influenza A positive samples, the subtype is determined.
- RTPCR Influenza A/H1 sw: adapted protocol from CDC (12); primers and probes are chosen in the hemagglutinine gene.
- RTPCR Influenza A/H3: adapted protocol from RIVM (14); primers and probes in the hemagglutinine gene.

For a subset of samples:
- RT-PCR N1: adapted protocol from RIVM (14); primers and probes in the neuraminidase gene.
- RT-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 RT-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.
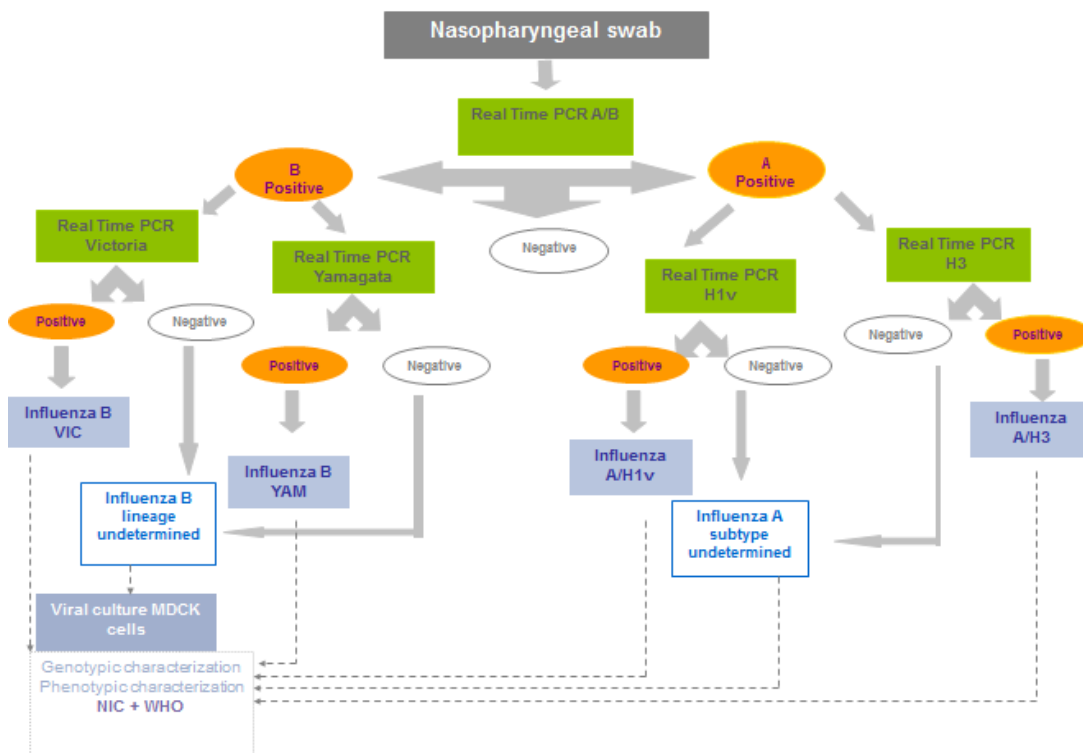
Figure 1. Sequence of the Real time PCR tests used during the 2016-2017 season.

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

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

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) 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).

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

Table 1. Multiplex RT PCR tests for respiratory viruses

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

The nucleotide sequences of the haemagglutinin (HA) and neuraminidase (NA) genes are sequenced directly from clinical specimens, after PCR, with the ABI 3130xl (ABI) using Big Dye Terminator v 3.1 Cycle Sequencing kit. 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 clusters characterised by common and specific mutations. For this season , NGS has been introduced for the routine analysis of circulation strains. Amplification of the 8 segments of Influenza A and B were adapted from the protocols (23, 24). The sequencing of the amplicon was performed with MiSeq Illumina.

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 2017-2018 season the Influenza epidemic in Belgium lasted 12 weeks, which is long compared to previous seasons. The epidemic threshold was crossed from week 2-2018 (January 8 to January 14, 2018) to week 13-2018 (March 26 to April 1, 2018) (Fig. 1)

The intensity of the epidemic was moderate. After week 2_2018, the incidence of GP visits for influenza-like illness (ILI) rapidly increased to reach 584 GP visits per 100.000 inhabitants in week 6-2018. The incidence then remained elevated and reached a peak value in week 10-2018 (744 GP visits for ILI / 100.000 inhabitants). During the first phase of the epidemic mainly children were affected, whereas during the second phase all ages were concerned. From week 11-2018, the incidence decreased again and dropped below the epidemic threshold after week 13-2018.

Based on the surveillance of the sentinel network of general practitioners, we estimate that this season approximately 697.000 Belgians visited their GP for influenza-like illness (ILI) and that approximately 470.000 Belgians had a clinical infection with the influenza virus.

The update situation is published weekly (3).
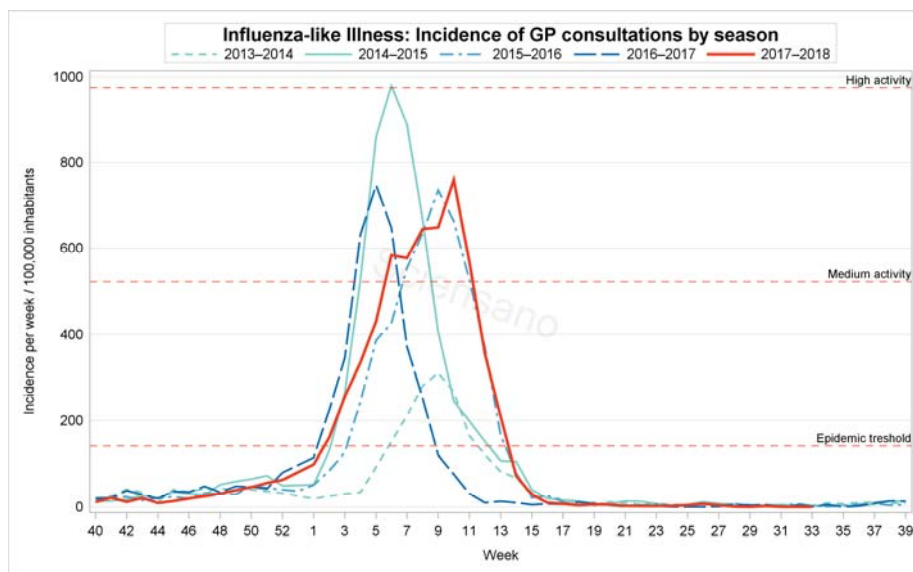
Figure 2a. Weekly incidence of influenza-like illness (ILI) 2017-2018 season, Belgium
(Source: Sentinel general practices)
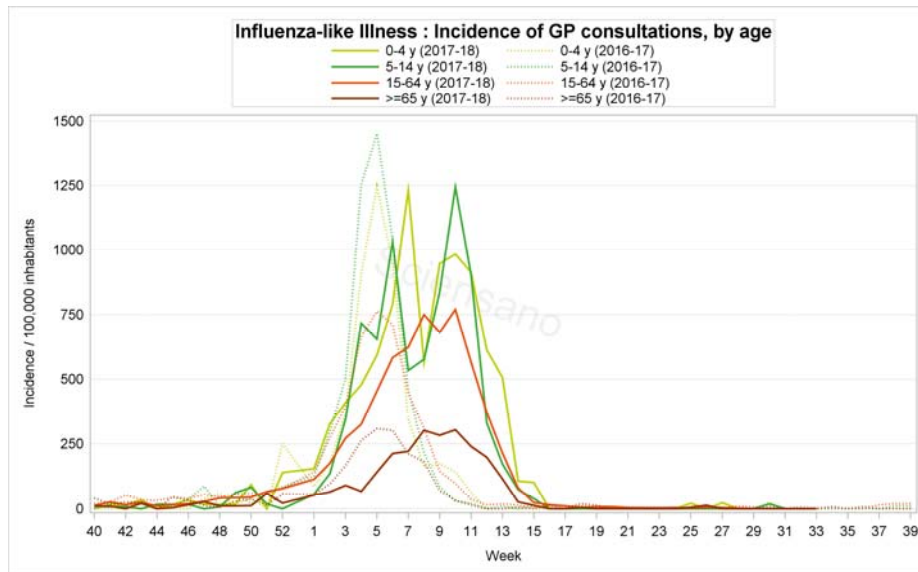
Figure 2b. Weekly incidence of influenza-like illness (ILI) 2017-2018 season, Belgium

D.1.2 Virological surveillance

The influenza surveillance period started in week 40-2017 and continued to week 20-2018.

Origin of samples

A total of 65 general practices (34 for Flanders, 31 for Wallonia-Brussels Federation) took part in the virological surveillance and sent 658 nasopharyngeal swabs to the NIC.

Number of nasopharyngeal swabs
Flanders :   331 (50.3 %)
Wallonia-Brussels :   328 (49.8 %)
Total :   658
From these samples 656 were suitable for analyses

Typing and subtyping results

The first positive sample was diagnosed in week 47-2017 and increasingly large numbers of positive influenza cases were detected from week 52-2017 onwards, reaching a proportion of 88.7% in week 7-2018. These were mainly B/Yamagata and A(H1N1)pdm09 viruses which have been circulating this season.

From week 40-2017 to week 18-2018, 656 respiratory samples were sent by the sentinel GPs network and analysed at the National Influenza Centre. Of these samples, 387 (59 %) were positive for influenza with 134 (20.6%) positive for influenza A and 253 (38.5%) positive for influenza B (**Error! Reference source not found.**3).

Among the influenza A samples that were subtyped, 69.4 % (93/134) were A(H1N1)pdm2009, 25.4 % (34/134) were A(H3N2) and 5.2% (7/134) could not be subtyped due to low viral load. Among the influenza B samples that were subtyped, 92.8% (235/253)

belonged to de Yamagata lineage, 3.2% (8/251) belonged to the Victoria lineage and for 4% (8/253) the lineage could not be determined due to low viral load (table 2).

Overall, among all influenza positive samples, 25% were A(H1N1)pdm09, 9% were A(H3N2), 64% were B Yamagata and 2% were B Victoria (fig. 4).
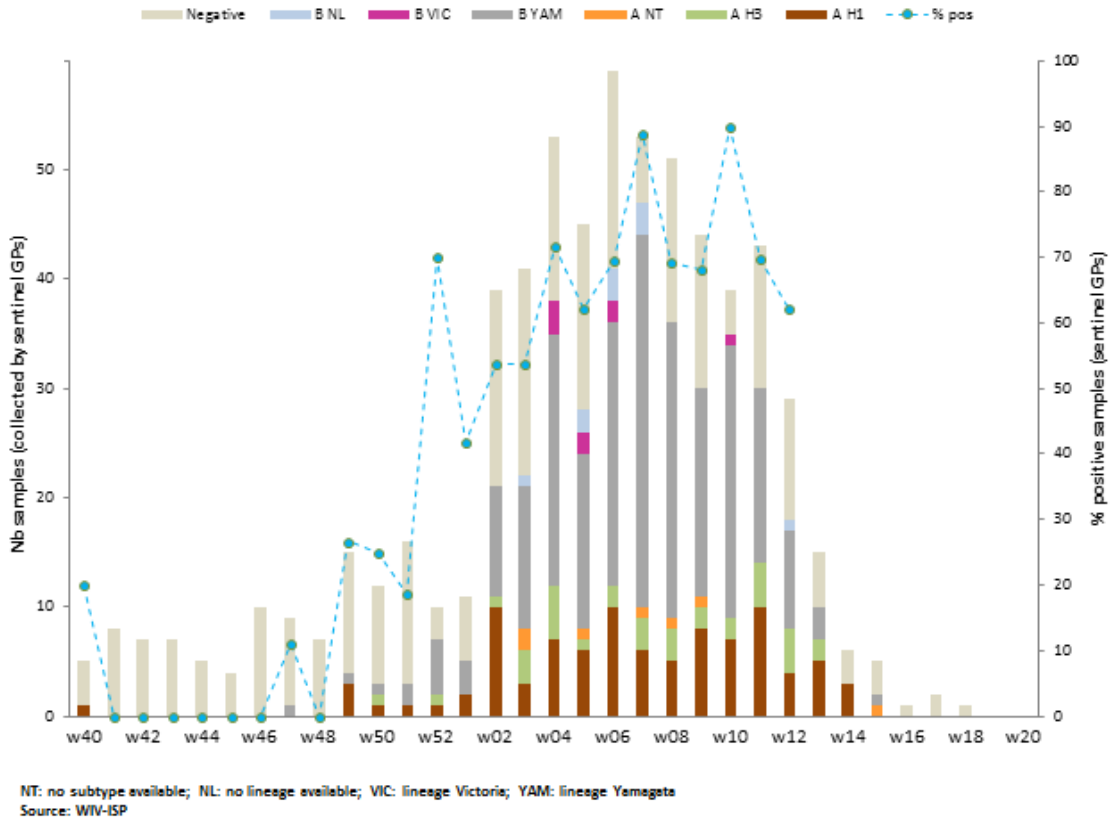
Figure 3. Weekly detection of influenza viruses in Belgium from week 40-2017 to week 20-2018 in the network of sentinel GPs.

		FLU detection/typing			FLU A subtyping			FLU B lineage		
		neg	A	B	A H1	A H3	A NT	YAM	VIC	B NL
Total for season (40/2017 - 18/2018)	Number of samples with given result	269	134	253	93	34	7	235	8	10
	Number of tested samples	656	656	656	134	134	134	253	251	253
	Percentage (%)	41,1	20,5	38,4	69,4	25,4	5,2	92,8	3,2	4,0

Table 2. Numbers and proportion of the different types and subtypes analysed during the 2017-2018 season

Proportion of the different intluenza types and sub-types

- A(H1N1)
- A(H3N2)
- B Victoria
- B Yamagata

25%
9%
2%
64%
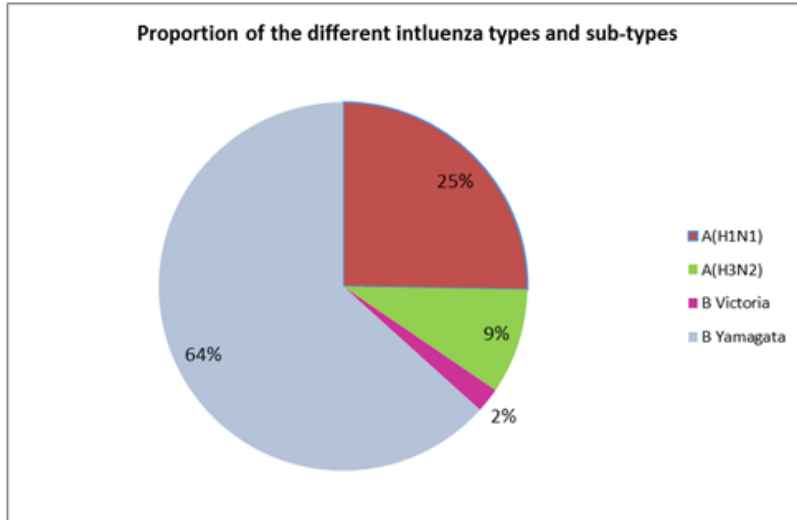
Figure 4. Repartition of the different types and subtypes during the influenza season 2017-2018 ILI surveillance .

Influenza viruses according to age group

The age was known for 656 patients. The NIC received a higher number of samples from the age group 15-44 and 45-64 years old. The higher rate of positivity was observed in the age group 5-14 years old (69%) and 65-84 years (70%) (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. In all age groups, influenza B infections outnumbered influenza A infections. Overall the ratio influenza B infections over influenza A infections was 2: 1, however in children it was 3: 1.
B Yamagata was the predominant virus in all age groups. However, some B| Victoria infections were also observed in persons younger than 65 years, and significantly more so in children (where the fraction of B| Victoria infections amounted to 8% of all influenza B infections)
Among the influenza type A infections, we observed more A(H1N1)pdm09 than A(H3N2) infections in children and adults aged less than 65 years (76% A(H1N1)pdm09), while A(H3N2) was the most frequent subtype in elderly persons aged 65 and older (60 % A(H3N2)).
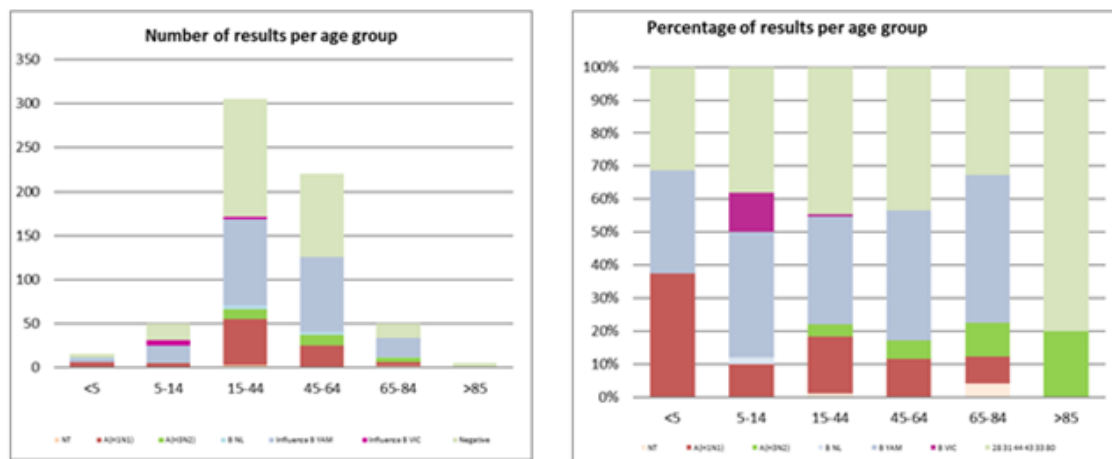
Figure 5. Influenza virus types and subtypes according to age group (NT= non subtyped) (numbers and percentages) influenza season 2017-2018 ILI surveillance .

D.2 Sentinel Surveillance of SARI

D.2.1 Virological surveillance

SARI Surveillance started week 50-2017 after the first influenza cases were recorded by the sentinel GPs, and ended week 18-2018, about two weeks after the end of the epidemic.

Origin of samples

A total of 2470 patients were registered in the database, among which 2180 (88%) 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, ….) and were used for epidemiological analyses.
The age was known for 2160 patients and those were taken into account for the analyses of age group.

Typing and subtyping results

From week 50-2017 to week 18-2018, 2160 SARI (Severe Acute Respiratory Infections) respiratory specimens were sent by the network of hospitals and analyzed by the National Influenza Center. Of these samples, 896 (41.4%) were positive for influenza viruses, of which 351 (16.2%) were positive for influenza A viruses and 546 (25,2 %) positive for influenza B viruses (Fig.6).

Of the subtyped influenza A samples, 67.7% (238/351) were A(H1N1)pdm09 viruses, 27.4% (97/351) were A(H3N2) viruses and 3.9% could not be subtyped due to low viral load. Among influenza B, 96.5% (527/546) belonged to the Yamagata lineage and 1,2% (7/546) to the Victoria lineage. For 1.6% (9/546) the lineage could not be determined due to low viral load. (Table 3).
Overall, among all influenza positive samples, 27% were A(H1N1)pdm09, 11% were A(H3N2), 61% were B Yamagata and 1% were B Victoria (fig. 7).

NT: no subtype available; NL: no lineage available; VIC: lineage Victoria; YAM: lineage Yamagata
Source: WIV-ISP
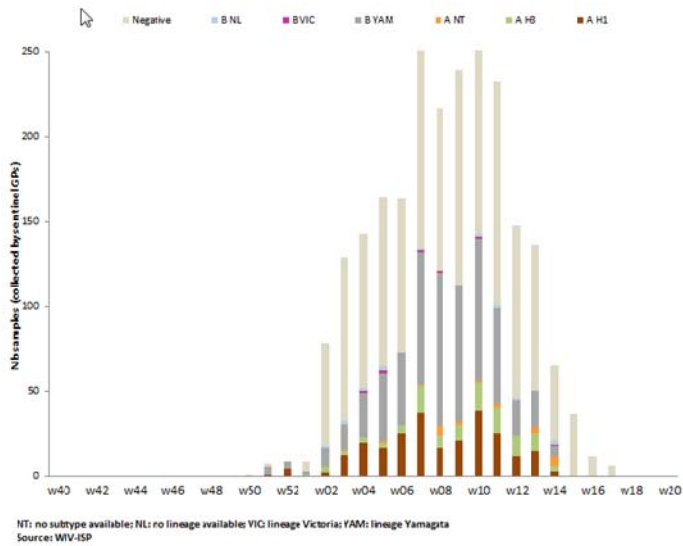
Figure 6. Weekly detection of influenza viruses in Belgium in the SARI surveillance from week 50-2017 to week 18-201 .

		FLU detection/typing			FLU A subtyping			FLU B lineage		
		neg	A	B	A H1	A H3	A NT	YAM	VIC	B NL
Total for season (50/2017 - 18/2018)	Number of samples with given result	1264	351	545	238	97	14	527	6	9
	Number of tested samples	2160	2160	2160	351	351	351	546	546	546
	Percentage (%)	58.1	16.2	25.2	67.7	27,6	3.9	96.5	1,1	1.6

Table 3. Repartition of the different types and subtypes during the influenza season 2017-2018 SARI surveillance.
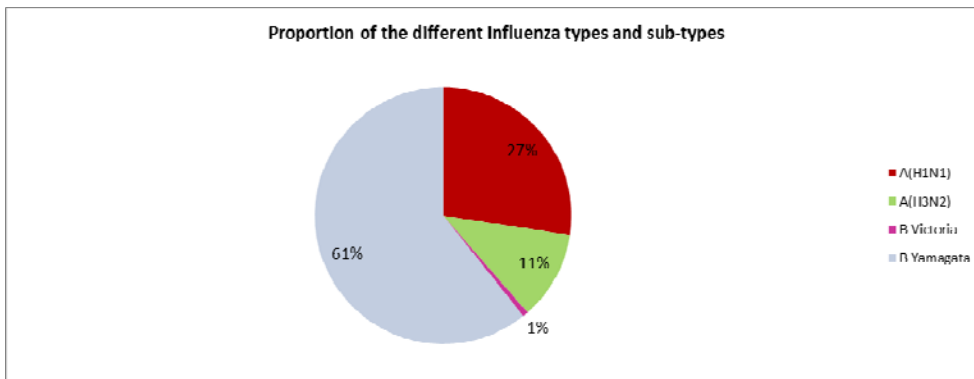
Figure 7. Repartition of the different types and subtypes during the influenza season 2017-2018 SARI surveillance.

Age distribution of influenza viruses by types and subtypes

The age was known for 2160 patients. A higher number of samples was collected from children and adults of the 65-84 age group. The percentage of positivity for influenza viruses was higher in the age group 5-14 years old and in the elderly. 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. The virus B Yamagata was represented in every age group (Figure 8).
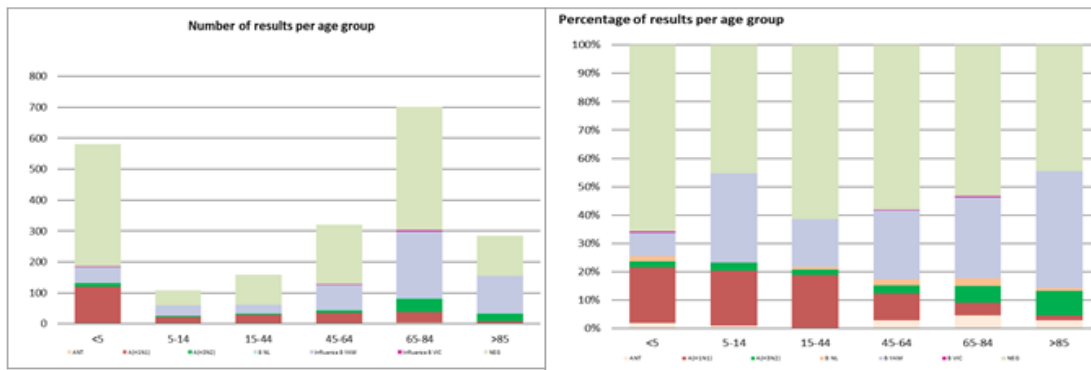
Figure 8. Influenza viruses according to age group SARI surveillance season 2017-2018 (numbers and percentages).

Positivity and subtype distribution of influenza viruses by surveillance scheme

During the SARI surveillance period (week 50-2017 to week 18 of 2018), the samples from ILI patients were significantly more frequently positive (66 % positive) than those from SARI patients (41.4% positive).

During that period, Influenza A(H1N1)pdm09 and B/Yamagata circulated in both surveillances. There was a slightly but significantly higher percentage of influenza B viruses (66% influenza B infections) among the flu patients in primary care compared to the hospitalised flu patients (60% influenza B infections). Among the confirmed influenza B infections, the vast majority of infections were of the Yamagata lineage, but the fraction B/Victoria infections was significantly higher among the patients from primary care (3%) than among the hospitalised influenza B patients (1%). 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

One hundred and fifty-four respiratory samples from patients with severe influenza were sent from hospitals around the country during the 2017-2018 season and inter-season, and were analysed at the NIC for confirmation and subtyping. Thirty five were influenza A positive, among which there were 23 A(H1N1)pdm09, and 12 A(H3N2). Twenty five samples were influenza B with nearly all samples B/Yamagata.

D.4 Suspected cases of Avian Influenza

No sample was sent for diagnosis of Avian flu during this season.

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 40-2017 and 20-2018, the 656 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 59 %, which means that 41% 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, 72. % of the patients were positive for at least one respiratory virus (including Influenza and co-infections). Among the samples negative for influenza viruses, 96/275 (35%) were positive for one or more other respiratory viruses. The most prevalent other respiratory viruses were HRV/ENV (4.7%), CoOC43 (2.43%), hMPV (1.82%), adenovirus (1.67%), RSVA (1.52%). For the other viruses, the percentages were lower and varied from 1.06% for RSVB to 0% for PIV 3 and PIV 4 (Figure 9).

Figure 9. Proportion of the different respiratory viruses in the ILI surveillance in Belgium season 2017-2018

Proportion of the different respiratory viruses according to age group

The age group was known for 657 patients; the analyses were performed on these samples.

The prevalence of the different respiratory viruses varies with 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 2017-2018 age group (Percentage).

Figure 10b. Proportion of the different respiratory viruses in the ILI surveillance season 2017-2018 by age group (numbers)

Co-infections

Overall, the percentage of co-infection was low 2.4 % (16/657). Among influenza positive samples, co-infection with other viruses was observed in 12/373 (3.2%). Regarding co-infection of respiratory viruses other than influenza, the percentage of co-infection was 1.4% (4/284) 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 2017-2018 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.
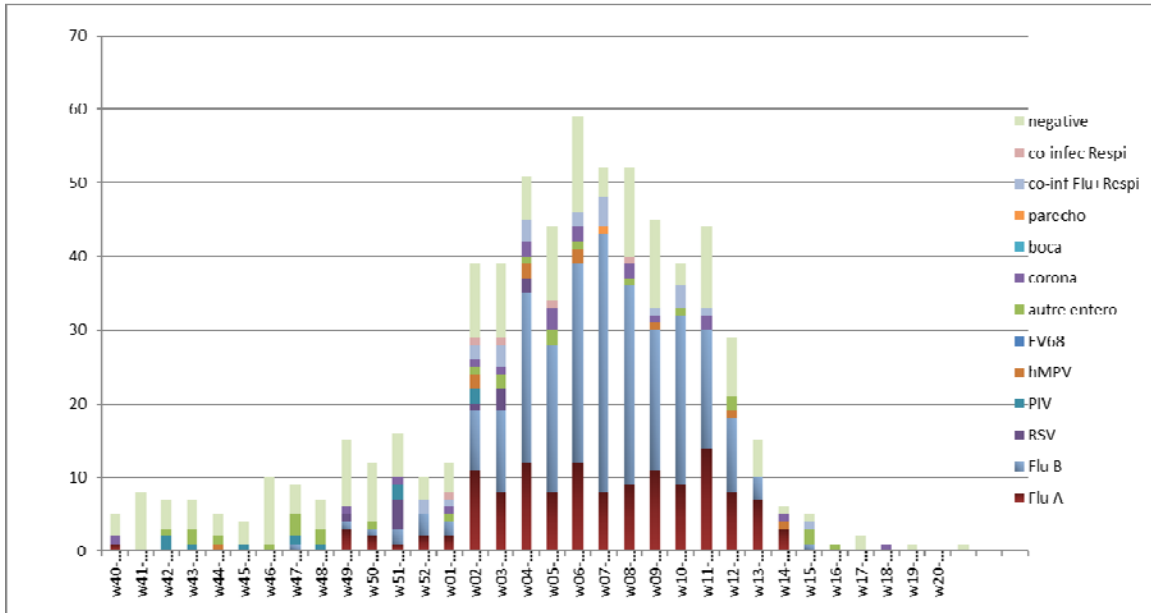
Figure 11. Weekly proportion of respiratory viruses during the 2017-2018 ILI surveillance

D.6.2 SARI surveillance

From week 1 to week 18-2018, the respiratory samples analysed for influenza were also submitted to the diagnosis for the other respiratory viruses. The age groups were known for 2160 patients and the analyses were performed on those samples. Among the influenza viruses negative samples, 493/1264 (39%) were positive for at least one other respiratory viruses.

For 771/2160 (35.7%) patients no respiratory viruses were detected. Overall, 1398/2160 (64.3%) of the patients were positive for at least one respiratory viruses (including influenza alone or in co-infections). This percentage reached 79% (460/581) in children below the age of 5 years old, and 67% (194/288) in elderly > 85 years. The most prevalent respiratory viruses were Influenza (41.5%), HRV/ENV viruses (7.53%), hMPV (6.94%), RSV (5.55%), Boca (2.5%). For the other viruses, the percentages were lower (Figure 12).
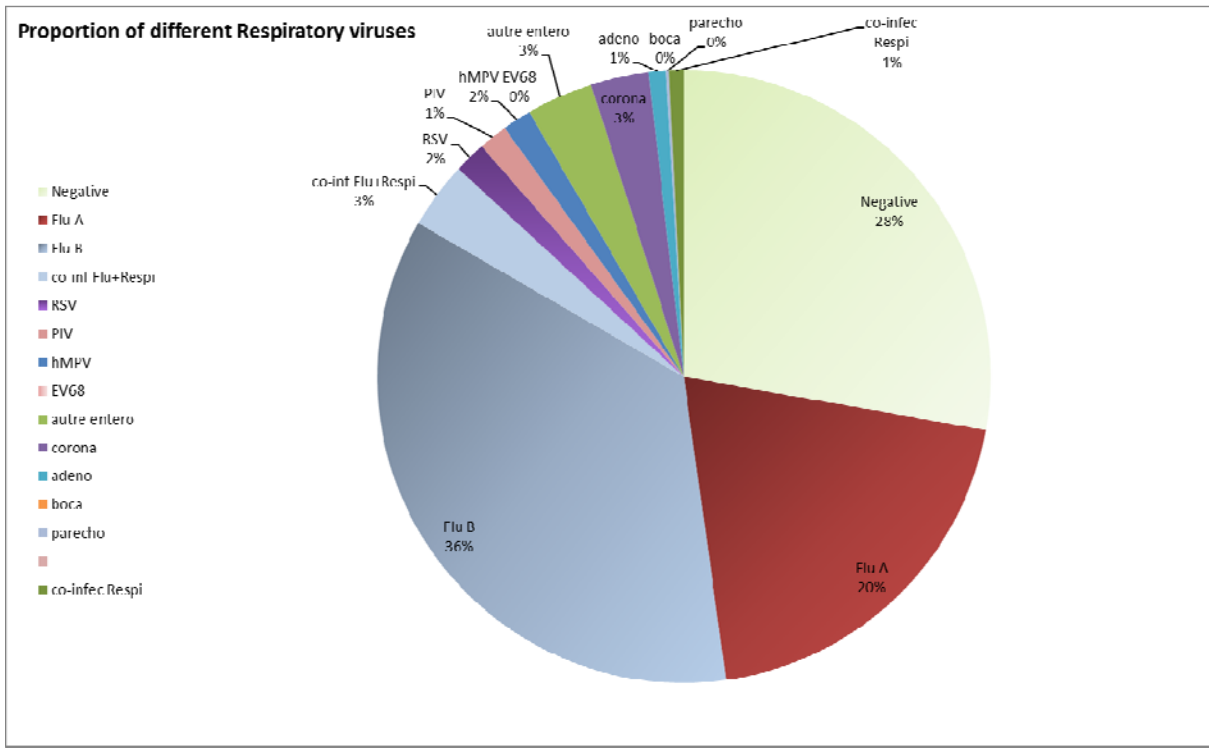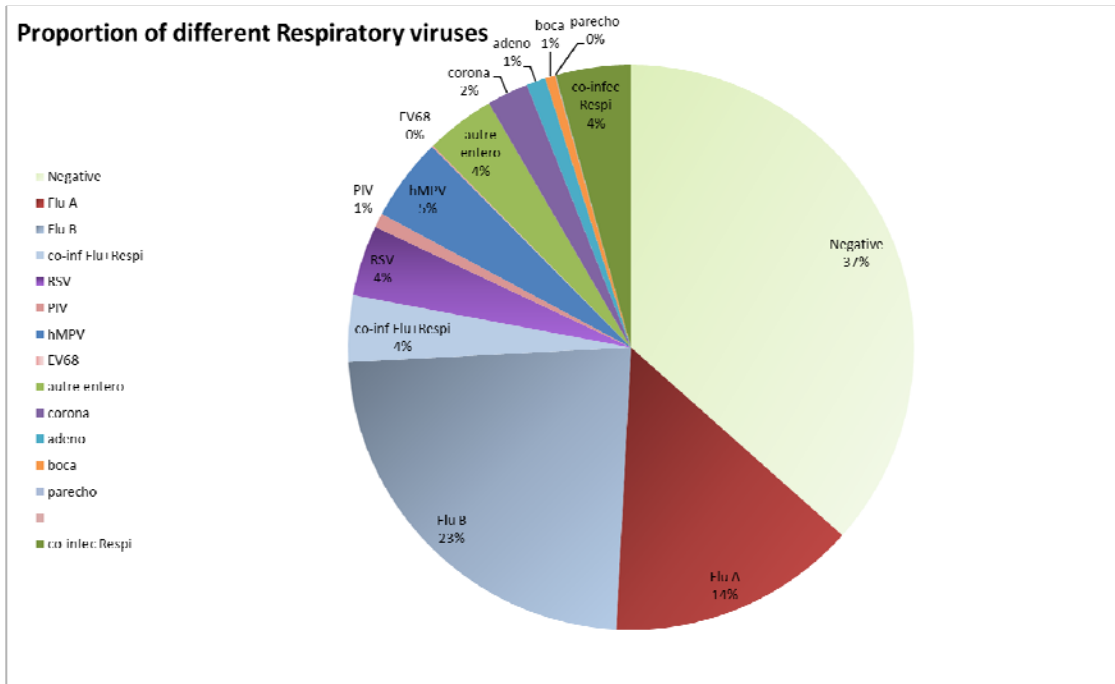
Figure 12. Proportion of the different respiratory viruses in the SARI surveillance season 2016-2017

Proportion of the different respiratory viruses according to age group

The age group was known for 2160 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 79%. All tested respiratory viruses were detected in this age group with the most prevalent virus being influenza A (22.7%), Rhino/enterovirus (19.8%), RSV (12.4%), and hMPV (10.7%) and bocavirus (8.4%). In patients aged more than 85 years old, influenza B was far the most prevalent (42.4%), followed influenza A (12.5% and hMPV(8.3%) (Figure 13a and 13b).
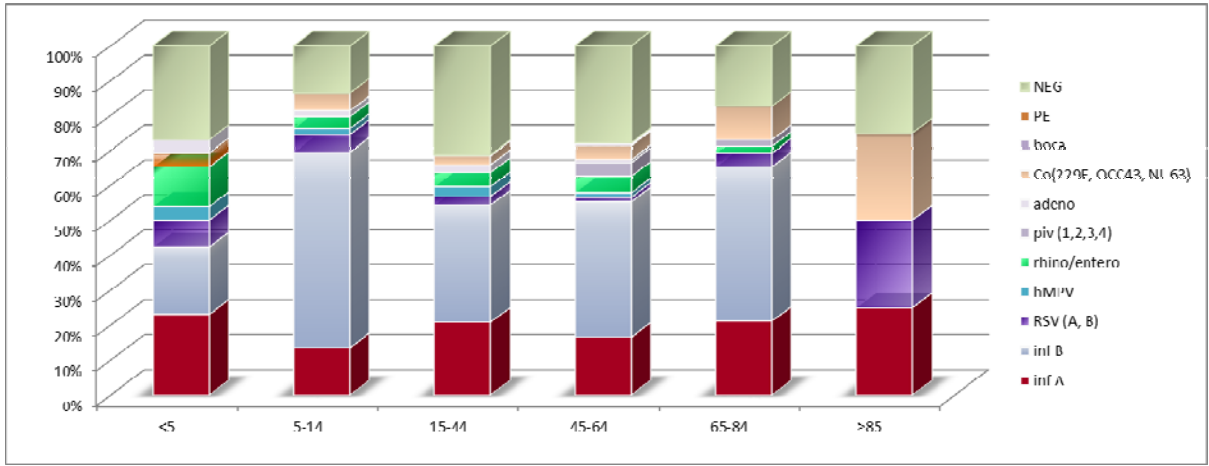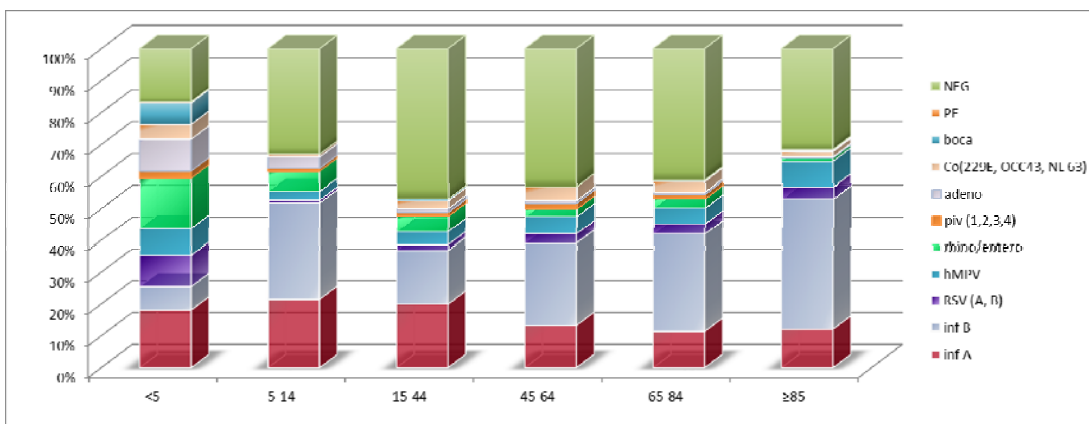

Figure 13a. Proportion of the different respiratory viruses in the SARI surveillance season 2017-2018 by age group (percentage)
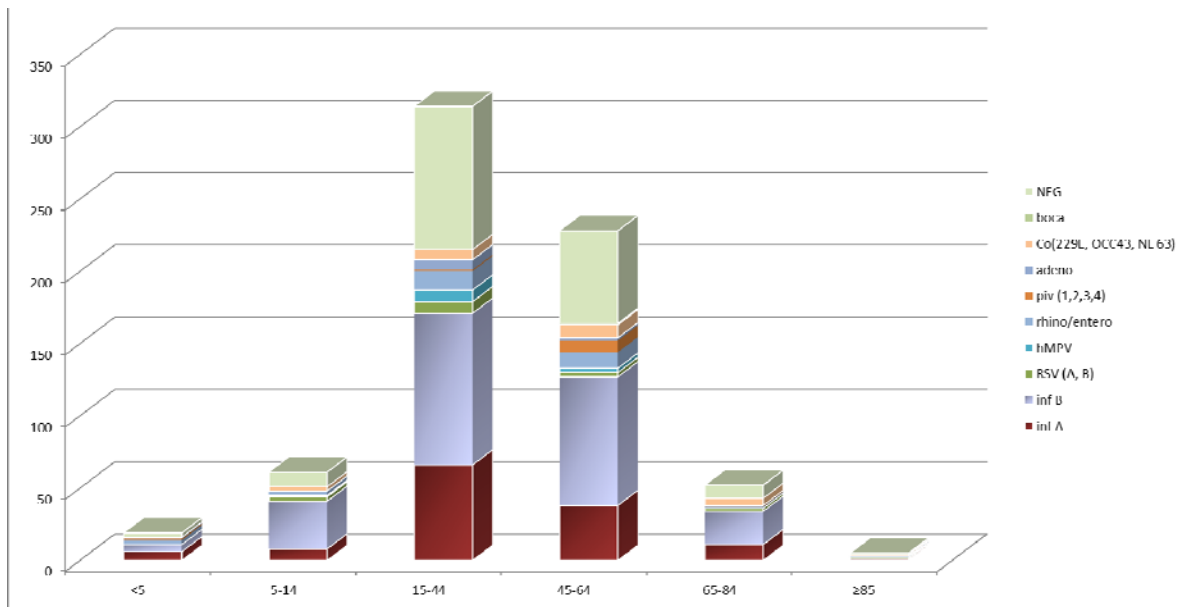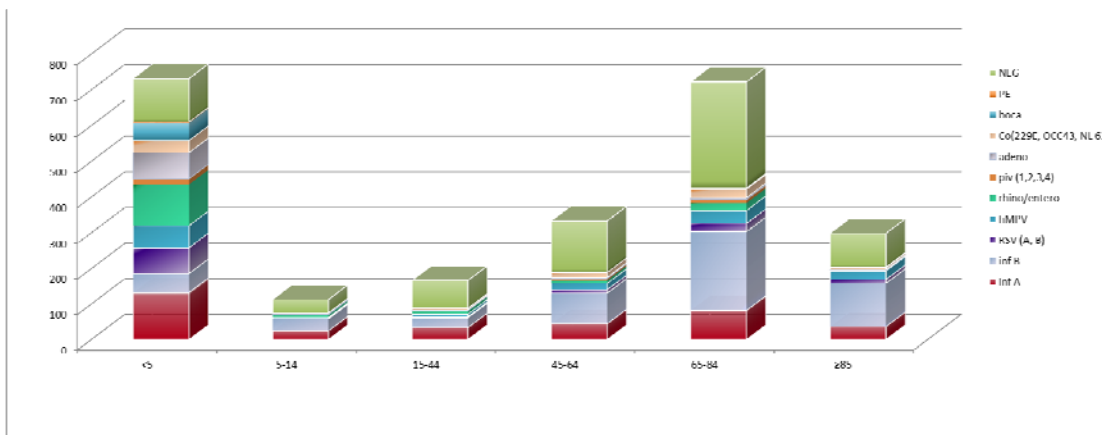
Figure 13b. Proportion of the different Respiratory viruses in the SARI surveillance season 2017-2018 by age group (numbers)

Coinfections

Overall, the percentage of co-infection (two to 5 viruses) was high, 9.1% (198/2160). In patients below the age of 5 years old this percentage reached 24% (144/581). Among influenza positive samples, co-infection with other viruses were observed in 82/896 (9.1%) with the most frequent association being Influenza/HRV found in 18/82 (21%), Influenza/hMPV and Influenza/Boca in 16/82 (19%). Regarding co-infections by respiratory viruses other than influenza, the percentage was also 9.1% (116/1264) with the most common viral co-infections being with HRV/ENV 27% (32/116) and with hMPV 19% (23/116) . Co-infection with 3 or more virus were observed in 18 patients, below 5 years old.

Weekly evolution

Figure 14 shows the weekly proportion of respiratory viruses that were laboratory-confirmed during the 2017-2018 SARI surveillance period. All tested respiratory viruses were detected during the surveillance period (week 51-2017 to 18-2018). Influenza viruses were the most prevalent with a percentage of 41% (0% to 54%) positive samples per week, followed by RSV (11.1% positive samples per week). The other viruses were detected more sporadically. Although the number of young children is high in the SARI population, the number of RSV were quite low and we did not observe the RSV peak which usually occurs before the start of the SARI surveillance. It is possible that the SARI case definition is also more specific for influenza. It is known that RSV infection has a different clinical presentation, age distribution, risk factors, and seasonality compared to influenza infection. It was thus reported that a case definition requiring fever like that used for ILI and SARI may underestimate the incidence of RSV by 50-80% (25).
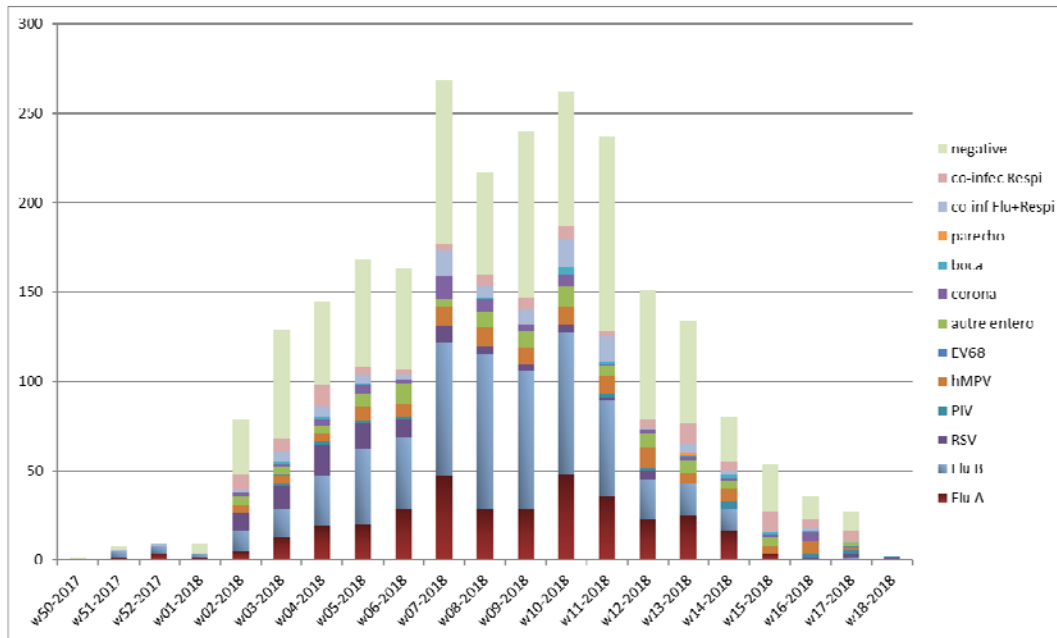
Figure 14. Weekly evolution of respiratory viruses during the SARI surveillance season 2017-2018

D.7. Characterisation of the influenza viruses

D.7.1 A(H1N1)pdm2009

Genetic characterisation

A high number of A(H1N1)pdm09 viruses were detected during this season. All sequenced strains belonged to group 6B.1, represented by the vaccine strain A/Michigan/45/2015 characterized by the substitutions **S74R, S164T and I295V** in HA1. These viruses have circulated through most of the world. The main characteristics of viruses in the 6B.1 group are that the viruses carry the amino acid substitutions **S84N**, **S162N** and **I216T** (e.g. A/Slovenia/2903/2015) (Figure 15a/b). Furthermore, a higher number of samples have been proceed with wole genome sequencing amplification of all eight segments. All of these sequences have been submitted to GISAID.

Vaccine virus
Reference virus
Circulating virus Belgium

A/Belgium/G0008/2018
A/Belgium/S0644/2018
A/Belgium/G0047/2018
A/Belgium/G0543/2017
A/Belgium/S0017/2018
A/Belgium/H0080/2018
A/Belgium/G0157/2018
A/Belgium/G0244/2018
A/Belgium/G0003/2018
A/Belgium/S0886/2018
A/Belgium/S1761/2018
A/Belgium/G0039/2018
A/Belgium/G0043/2018
A/Belgium/G0218/2018
A/Belgium/G0620/2017
A/Belgium/G0034/2018
A/Belgium/H0078/2018
A/Belgium/H0112/2018
A/Belgium/H0076/2018
A/Belgium/G0559/2018
A/Belgium/H0074/2018
A/Belgium/G0021/2018
A/Belgium/G0616/2017
A/Belgium/S1662/2018
A/Belgium/S0874/2018
A/Belgium/S0890/2018
A/Belgium/G0014/2018
A/Belgium/G0238/2018
A/Belgium/S0945/2018
A/Belgium/G0604/2017
A/Paris/1289/2017
A/Belgium/S0451/2018
A/Belgium/H0005/2018
A/Hong Kong/2214/2017
A/Hong Kong/2245/2017
A/Norway/2146/2017
A/Torino/93/2017
A/Hong-Kong/15587/2015
A/Slovenia/2903/2015
A/Bayern/81/2017
A/Dakar/09/2017
A/Thuringen/130/2017
A/Hong Kong/2111/2017
A/Michigan/45/2015
A/Stockholm/41/2015
A/OSAKA/170/2017
A/Hong Kong/2199/2017
A/Yokohama/98/2015
A/Israel/Q/504/2015
A/Jilin-Ningjiang/SWL1297/2017
A/Norway/2227/2015
A/South Africa/3626/2013
A/Dakar/04/2014
A/Hong-Kong/5659/2012
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/2212/2010
A/Hong/Kong/3934/2011
A/England/195/2009
A/California/07/2009

6B.1
6B
6
6B.2
6C
6 A

0,0050

Phylogenetic comparison of A(H1N1)pdm09 HA genes

Vaccine virus
Reference viruses

Collection date
Jan 2018
Feb 2018
Mar 2018
Apr 2018

A/Athens.GR/767/2018 Mar
A/Bremen/21/2018 Apr
A/Thuringen/7/2018 Jan
A/Thuringen/170/2017 Dec
A/Belgium/G0014/2018 Jan
A/Rheinland-Pfalz/52/2017 Dec
A/Rheinland-Pfalz/6/2018 Jan
A/Greece/58/2018 Jan
A/Sachsen/41/2018 Feb
A/Nordrhein-Westfalen/11/2018 Jan
A/Belgium/G0616/2017 Dec
A/Malta/33600/2018 Jan
A/Baden-Wurttemberg/77/2018 Feb
A/Rheinland-Pfalz/34/2018 Mar
A/Berlin/8/2018 Jan
A/Belgium/S0020/2018 Dec
A/Greece/50/2018 Jan
A/Greece/49/2018 Jan
A/Bayern/95/2017 Dec
A/Malta/33680/2018 Jan
A/Baden-Wurttemberg/252/2017 Nov
A/Belgium/G0603/2017 Dec
A/Belgium/G0502/2017 Dec
A/Bayern/96/2017 Dec
A/Athens.GR/39/2018 Jan
A/Belgium/G0134/2018 Jan
A/Thessaloniki/15/2018 Jan
A/Thessaloniki/3/2017 Dec
A/Brandenburg/19/2018 Mar
A/Belgium/G0047/2018 Jan
A/Belgium/G0003/2018 Dec
A/Belgium/G0543/2017 Oct
A/Belgium/G0008/2018 Dec
A/Belgium/G0053/2018 Jan
A/Belgium/G0039/2018 Jan
A/Tunis/13189/2017 Dec
A/Tunis/418/2018 Jan
A/Tunis/527/2018 Jan
A/Tunis/595/2018 Jan
A/Athens.GR/2680/2017 Dec
A/Cyprus/F93/2018 Jan
A/Cyprus/F38/2018 Jan
A/Ireland/54308/2017 Oct
A/Ireland/54260/2017 Oct
A/Ireland/71503/2017 Dec
A/Ireland/64110/2017 Nov
A/Hessen/5/2018 Jan
A/Saarland/25/2017 Dec
A/Athens.GR/521/2018 Feb
A/Cyprus/F11/2018 Jan
A/Hessen/14/2018 Feb
A/Sachsen-Anhalt/101/2017 Nov
A/Tunis/539/2018 Jan
A/Tunis/303/2018 Jan
A/Paris/1447/2017 Oct
A/Greece/32/2018 Jan
A/Cyprus/F166/2018 Feb
A/Cameroon/9498/2017 Sep
A/Cameroon/9503/2017 Sep
A/Cameroon/9405/2017 Sep
A/Cameroon/9782/2017 Oct
A/Cameroon/9662/2017 Oct
A/Cameroon/9514/2017 Oct
A/Cameroon/9513/2017 Oct
A/Cameroon/9525/2017 Oct
A/Cameroon/9538/2017 Sep
A/Cameroon/8776/2017 Sep
A/Cameroon/9150/2017 Sep
A/Cameroon/9660/2017 Oct
A/Cameroon/9883/2017 Oct
A/Cameroon/9653/2017 Oct
A/Cameroon/9404/2017 Sep
A/Cameroon/9535/2017 Sep
A/Cameroon/9149/2017 Sep
A/Cameroon/9536/2017 Sep
A/Cameroon/9880/2017 Oct
A/Cameroon/9544/2017 Sep
A/Cameroon/9884/2017 Oct
A/Cameroon/9543/2017 Sep
A/Cameroon/9511/2017 Oct
A/Cameroon/9524/2017 Oct
A/Cameroon/9504/2017 Sep
A/Cameroon/9505/2017 Sep
A/Cameroon/9517/2017 Sep
A/Cameroon/9766/2017 Oct
A/Cameroon/9755/2017 Oct
A/Cameroon/9412/2017 Sep
A/Cameroon/9687/2017 Oct
A/Slovenia/2903/2015 Oct
A/Cameroon/9147/2017 Sep
A/Cameroon/9408/2017 Sep
A/Cameroon/9415/2017 Sep
A/Cameroon/9416/2017 Sep
A/Cameroon/9500/2017 Sep
A/Cameroon/9533/2017 Oct
A/Cameroon/9657/2017 Oct
A/Cameroon/9882/2017 Oct
A/Michigan/45/2015 Sep
A/Israel/Q-504/2015 Dec
A/South Africa/3626/2013 Jun
A/Hong Kong/5659/2012 May
A/St Petersburg/27/2011 Feb
A/St Petersburg/100/2011 Mar
A/Astrakhan/1/2011 Feb
A/Lviv/N6/2009 Oct
A/Bayern/69/2009 Jul
A/California/7/2009 Apr

S183P,
E235D,
N260D,
V520A

N496S

T120A

K22R, I372V,
G394S, N451T

P137S,
N451T

S183P

S164T

V250A

S74R, I295V

I372V

A186T

S84N,
S162N,
I216T

V525I

I404L, I418V,
S534G

K163Q, A256T,
K283E, E499K

S185T,
S451N

D97N

E374K

S203T

6B.1

0.003

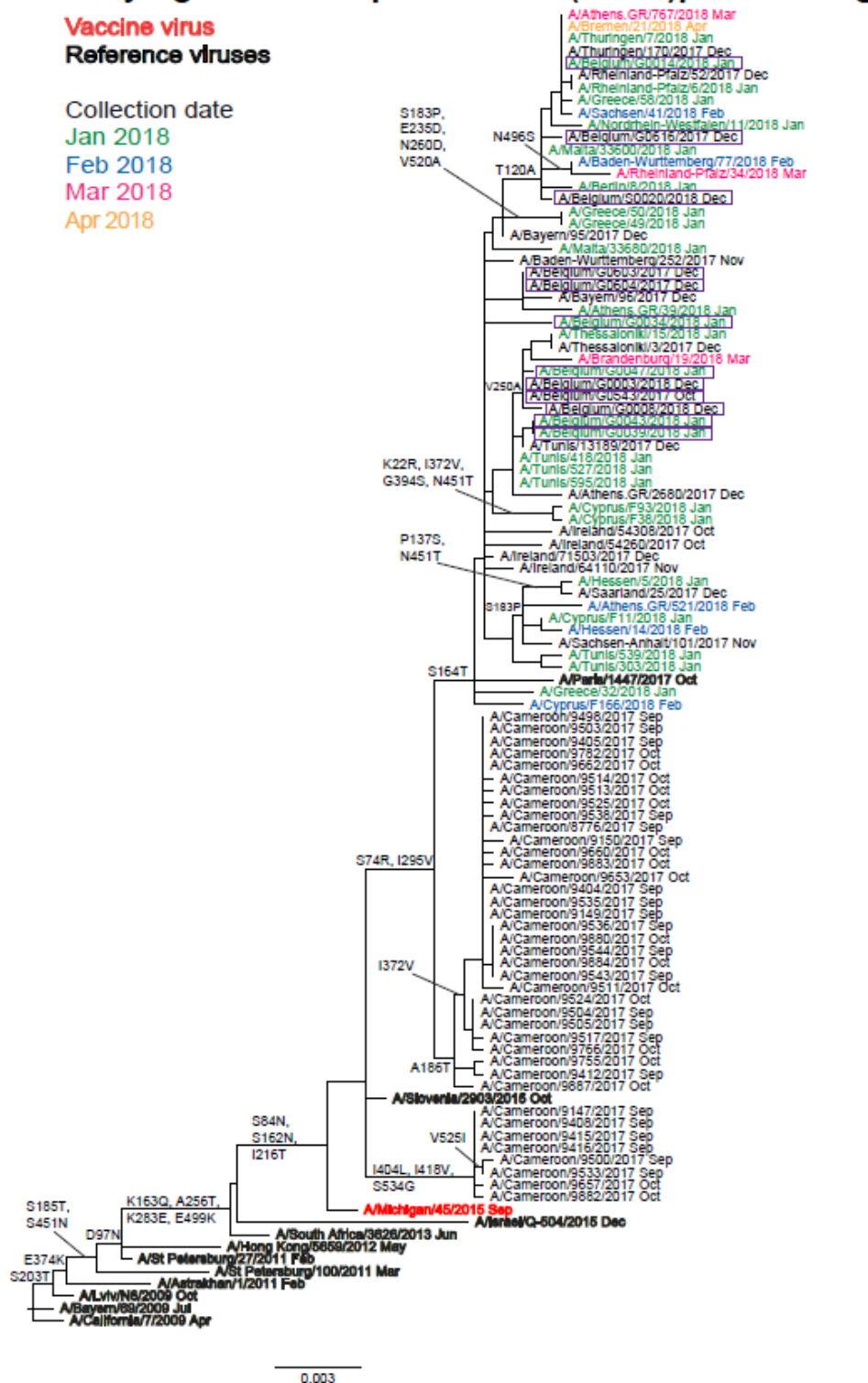Figure 15a/b. Phylogenetic analysis of influenza A(H1N1)pdm09 strains in Belgium, season 2017-2018

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 analyses of influenza A(H1N1)pdm09 viruses (2018-05-02)

VIC number / Viruses	Other information	Collection date	Passage history	A/Mich 45/15 Egg NIB F42/16[-1] 6B.1	A/Cal 7/09 Egg F06/16[-1]	A/Bayern 69/09 MDCK F09/15[-1]	A/Lviv N6/09 MDCK F14/13[-1]	A/Astrak 1/11 MDCK F22/13[-1]	A/St. P 27/11 Egg F26/14[-1] 5	A/HK 5659/12 MDCK F30/12[-1] 6	A/Sth Afr 3626/13 Egg F03/14[-1] 6A	A/Slov 2903/2015 Egg F02/16[-1] 6B	A/Israel Q-504/15 MDCK F08/16[-1] 6B.1	A/Paris 1447/17 MDCK F03/18[-2] 6B.2
REFERENCE VIRUSES														
A/Michigan/45/2015	6B.1	2015-09-07	E3/E3	1280	1280	640	320	640	640	1280	640	1280	1280	2560
A/California/7/2009	Clone38-32	2009-04-09	E3/E3	640	640	640	640	1280	640	640	640	1280	1280	2560
A/Bayern/69/2009	G155E	2009-07-01	MDCK5/MDCK1	40	80	320	320	80	40	40	80	80	40	320
A/Lviv/N6/2009	G155E, D222G	2009-10-27	CK4/SIAT1/MDCK2	80	80	640	640	80	160	160	80	160	80	640
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	1280	640	320	1280	640	1280	1280	640	2560	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E4	640	640	640	320	1280	640	640	640	1280	1280	2560
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	320	320	160	160	640	320	640	320	640	640	1280
A/South Africa/3626/2013	6B	2013-06-06	E1/E8	640	640	640	640	640	640	640	640	1280	640	2560
A/Slovenia/2903/2015	clone 37 6B.1	2015-10-26	E4/E2	640	640	320	320	640	320	640	640	1280	1280	2560
A/Israel/Q-504/2015	6B.2	2015-12-15	C1/MDCK2	640	640	320	320	640	320	640	640	1280	2560	2560
A/Paris/1447/2017	6B.1		MDCK1/MDCK3	640	320	320	160	640	320	320	640	1280	1280	2560
TEST VIRUSES														
A/Belgium/G0047/2018	6B.1	2018-01-09	MDCK1	1280	640	640	320	1280	640	1280	640	2560	1280	2560
A/Belgium/G0039/2018		2018-01-09	MDCK1	2560	1280	640	640	1280	640	1280	1280	2560	2560	5120
A/Belgium/G0034/2018	6B.1	2018-01-08	MDCK1	1280	640	640	320	1280	640	1280	1280	2560	1280	2560
A/Belgium/S0020/2018	6B.1	2017-12-25	MDCK1	1280	640	640	320	1280	320	640	640	2560	1280	2560
A/Belgium/G0616/2018	6B.1	2017-12-11	SIAT1	1280	1280	640	320	1280	640	1280	1280	2560	1280	5120
A/Belgium/G0604/2017	6B.1	2017-12-06	MDCK1	2560	2560	1280	1280	2560	1280	1280	2560	2560	5120	5120
A/Belgium/G0603/2017	6B.1	2017-12-06	MDCK1/MDCK1	2560	1280	1280	1280	2560	1280	1280	2560	5120	2560	5120
A/Belgium/G0543/2017	6B.1	2017-10-06	MDCK1/MDCK1	1280	640	320	160	640	640	640	640	1280	1280	2560
A/Belgium/G0043/2018	6B.1	2018-01-08	MDCK3	320	320	160	160	320	320	320	320	640	640	1280

Assay: HI (Turkey RBC) — Vaccine
RBC: Turkey
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

Antigenic analyses of influenza A(H1N1)pdm09 viruses (2018-02-07)

VIC number / Viruses	Other information	Collection date	Passage history	A/Mich 45/15 Egg NIB F42/16[-1] 6B.1	A/Cal 7/09 Egg F06/16[-1]	A/Bayern 69/09 MDCK F09/15[-1]	A/Lviv N6/09 MDCK F14/13[-1]	A/Astrak 1/11 MDCK F22/13[-1]	A/St. P 27/11 Egg F26/14[-1] 5	A/HK 5659/12 MDCK F30/12[-1] 6	A/Sth Afr 3626/13 Egg F03/14[-1] 6A	A/Slov 2903/2015 Egg F02/16[-1] 6B	A/Israel Q-504/15 MDCK F08/16[-2] 6B.1	A/Paris 1447/17 MDCK F03/18[-2] 6B.2
REFERENCE VIRUSES														
	6B.1	2015-09-07	E3/E3	640	1280	320	320	1280	320	1280	640	1280	1280	2560
A/California/7/2009	Clone38-32	2009-04-09	E3/E3	640	640	640	320	640	320	640	640	1280	640	2560
A/Bayern/69/2009	G155E	2009-07-01	MDCK5/MDCK1	<	40	640	320	40	<	40	40	80	40	160
A/Lviv/N6/2009	G155E, D222G	2009-10-27	CK4/SIAT1/MDCK3	40	160	1280	640	80	80	160	80	160	80	640
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK4	640	1280	1280	640	1280	640	1280	640	1280	640	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E4	640	640	640	640	640	320	640	640	2560	640	2560
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	160	160	160	80	320	80	320	160	320	160	640
A/South Africa/3626/2013	6B	2013-06-06	E1/E3	640	640	640	640	640	320	640	640	1280	640	2560
A/Slovenia/2903/2015	clone 37 6B.1	2015-10-26	E4/E1	640	1280	640	320	1280	640	1280	640	1280	1280	2560
A/Israel/Q-504/2015	6B.2	2015-12-15	C1/MDCK2	640	640	320	160	640	320	640	320	1280	640	2560
A/Paris/1447/2017	6B.1		MDCK1/MDCK2	640	640	320	160	640	160	640	640	1280	640	2560
TEST VIRUSES														
A/Belgium/G0014/2018	6B.1	2018-01-02	SIAT1/MDCK1	640	1280	320	320	640	320	640	1280	2560	1280	2560
A/Belgium/G0008/2018	6B.1	2017-12-28	MDCK1/MDCK1	640	640	320	160	640	320	640	640	1280	640	2560
A/Belgium/G0003/2018	6B.1	2017-12-22	SIAT1/MDCK1	640	640	320	320	640	320	640	640	1280	640	2560

Assay: HI (Turkey RBC) — Vaccine
RBC: Turkey
Virus: A(H1N1)pdm09
Date:
* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
[-1] < = <40
ND: Not Done
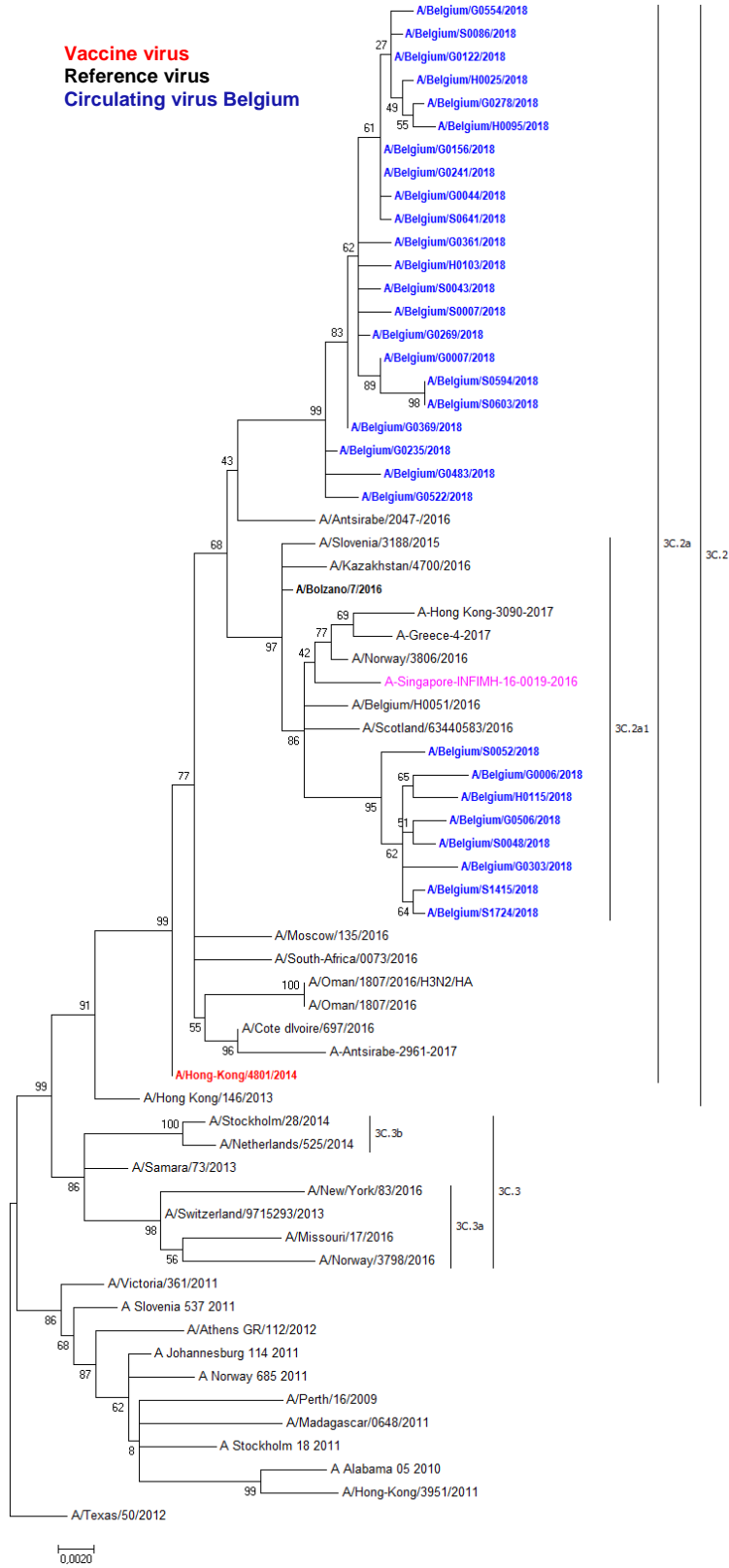Sequences in phylogenetic trees

D.7.2 A(H3N2)

Genetic characterisation

In Belgium, about 9% of the positive influenza viruses were A(H3N2). All sequenced viruses belonged to the subclade 3C.2a, represented by the vaccine strain A/Hong-Kong/4801/2014. Four of those viruses fell into subclade 3C.2a1 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 (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 haemagglutinin, thereby emphasizing the need for continued monitoring of antigenic characteristics.

Vaccine virus
Reference virus
Circulating virus Belgium

A/Belgium/G0554/2018
A/Belgium/S0086/2018
A/Belgium/G0122/2018
A/Belgium/H0025/2018
A/Belgium/G0278/2018
A/Belgium/H0095/2018
A/Belgium/G0156/2018
A/Belgium/G0241/2018
A/Belgium/G0044/2018
A/Belgium/S0641/2018
A/Belgium/G0361/2018
A/Belgium/H0103/2018
A/Belgium/S0043/2018
A/Belgium/S0007/2018
A/Belgium/G0269/2018
A/Belgium/G0007/2018
A/Belgium/S0594/2018
A/Belgium/S0603/2018
A/Belgium/G0369/2018
A/Belgium/G0235/2018
A/Belgium/G0483/2018
A/Belgium/G0522/2018
A/Antsirabe/2047-/2016
A/Slovenia/3188/2015
A/Kazakhstan/4700/2016
A/Bolzano/7/2016
A-Hong Kong-3090-2017
A-Greece-4-2017
A/Norway/3806/2016
A-Singapore-INFIMH-16-0019-2016
A/Belgium/H0051/2016
A/Scotland/63440583/2016
A/Belgium/S0052/2018
A/Belgium/G0006/2018
A/Belgium/H0115/2018
A/Belgium/G0506/2018
A/Belgium/S0048/2018
A/Belgium/G0303/2018
A/Belgium/S1415/2018
A/Belgium/S1724/2018
A/Moscow/135/2016
A/South-Africa/0073/2016
A/Oman/1807/2016/H3N2/HA
A/Oman/1807/2016
A/Cote dIvoire/697/2016
A-Antsirabe-2961-2017
A/Hong-Kong/4801/2014
A/Hong Kong/146/2013
A/Stockholm/28/2014
A/Netherlands/525/2014
A/Samara/73/2013
A/New/York/83/2016
A/Switzerland/9715293/2013
A/Missouri/17/2016
A/Norway/3798/2016
A/Victoria/361/2011
A Slovenia 537 2011
A/Athens GR/112/2012
A Johannesburg 114 2011
A Norway 685 2011
A/Perth/16/2009
A/Madagascar/0648/2011
A Stockholm 18 2011
A Alabama 05 2010
A/Hong-Kong/3951/2011
A/Texas/50/2012

3C.2a
3C.2
3C.2a1
3C.3b
3C.3
3C.3a

0,0020

Vaccine virus
Reference viruses

Collection date
Oct 2017
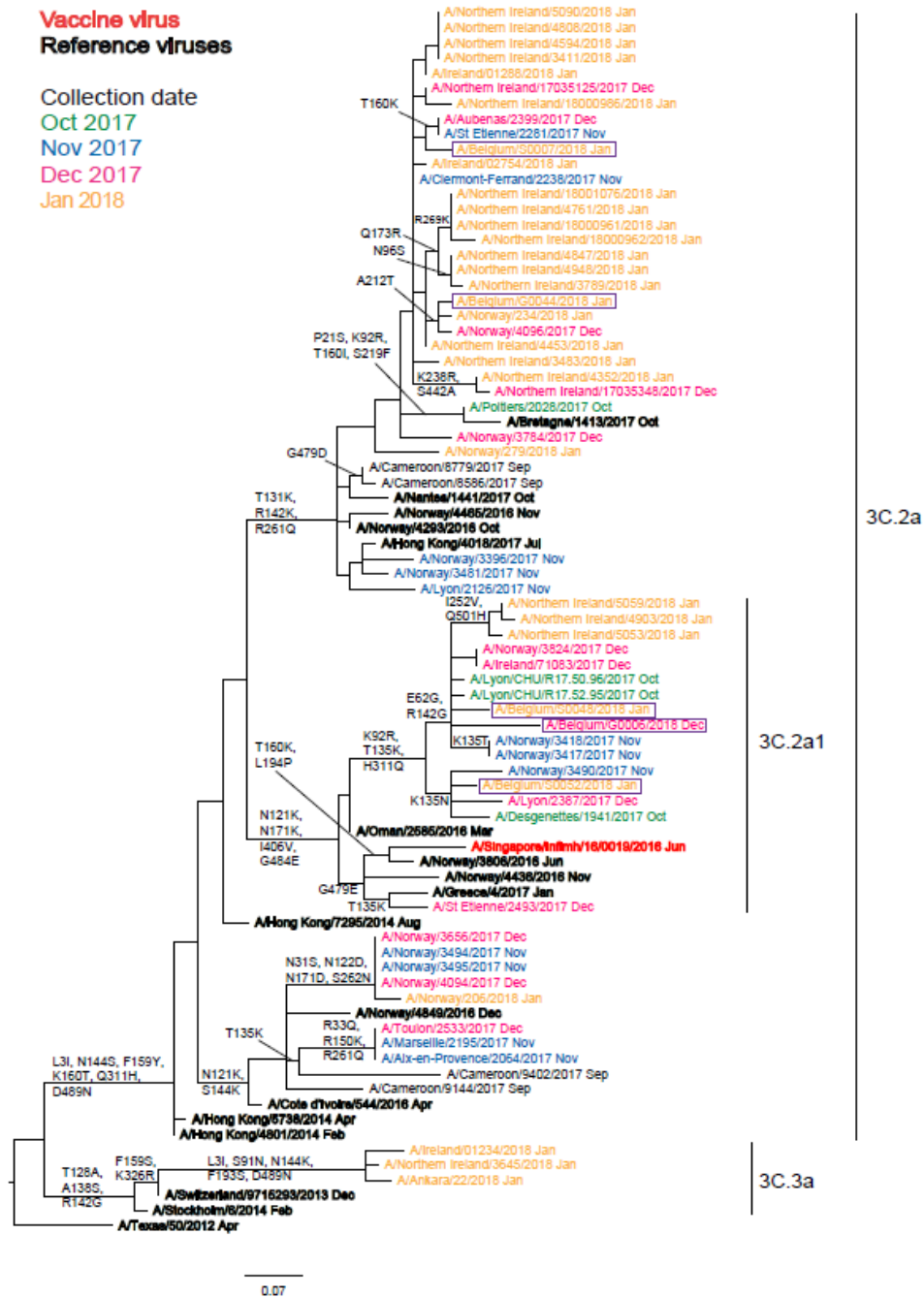Nov 2017
Dec 2017
Jan 2018

Figure 16 a/b Phylogenetic analysis of the HA sequences of the A(H3N2) viruses analysed from Belgium and other European countries during the 2017-2018 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

D.7.3 B Yamagata

Genetic characterisation

The majority of circulating strains during 2017-2018 season were influenza B Yamagata. Sequence analysis of the HA gene on 21 samples has been carried out. All had HA genes in clade 3, the B/Phuket/3073/2013 clade. The Yamagata lineage virus was not included in the trivalent vaccine but well in the quadrivalent vaccine. Furthermore, a higher number of samples have been proceed with wole genome sequencing amplification of all eight segments. All of these sequences have been submitted to GISAID.
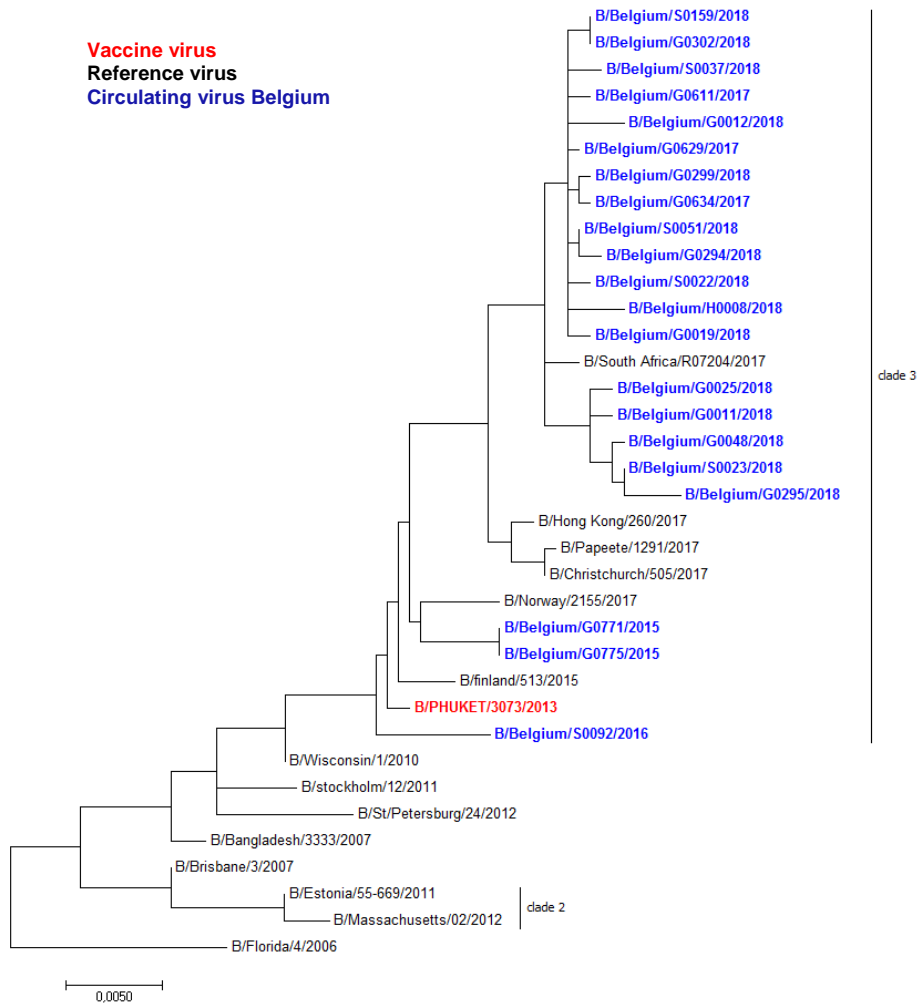

Figure 17. Phylogenetic analysis of the HA sequences of the influenza B /Yamagata viruses detected in Belgium during 2017-2018 season.

In the HI tests the majority of the test viruses were recognised by the antiserum raised against the current vaccine virus, egg-propagated B/Phuket/3073/2013, at titres within 4-fold of the titre of the antiserum for the homologous virus

The antisera raised against viruses in clade 2, the B/Massachusetts/02/2012 clade (clade 2), recognised the test viruses generally less well.

Antigenic analyses of influenza B viruses (Yamagata lineage) 2018-05-01

Viruses	Other information		Collection date	Passage history	Haemagglutination inhibition titre								
VIC number					B/Phuket 3073/13 Egg	B/Estonia 55669/11 MDCK	B/Mass 02/12 MDCK	B/Mass 02/12 MDCK	B/Vis 1/10 Egg	B/Stock 12/11 Egg	B/Phuket 3073/13 MDCK	B/Phuket 3073/13 Egg	B/Maur 1791/17 MDCK
	Passage history				SH614$^{-1,1}$	F27/13^{-2}	F10/16^{-2}	F16/14^{-2}	F36/15^{-2}	F06/15^{-2}	F27/15^{-2}	F25/17^{-1}	F04/18^{-2}
	Ferret number												
	Genetic Group				3	2	2	2	3	3	3	3	
REFERENCE VIRUSES													
B/Estonia/55669/2011	196-198 NKT	2	2011-03-14	MDCK2/MDCK3	1280	640	80	160	160	40	80	80	40
B/Massachusetts/02/2012	196-198 NK(T=	2	2012-03-13	MDCK1/C2/MDCK3	1280	640	160	640	320	160	80	320	40
B/Massachusetts/02/2012	196-198 DKT	2	2012-03-13	E3/E3	1280	160	20	640	160	160	20	160	10
B/Visconsin/1/2010	196-198 (D>N)I	3	2010-02-20	E3/E2	2560	80	20	320	160	160	80	320	80
B/Stockholm/12/2011		3	2011-03-28	E4/E1	1280	40	10	160	160	160	40	160	40
B/Phuket/3073/2013	196-198 NKT	3	2013-11-21	MDCK2/MDCK3	2560	160	80	160	160	80	160	160	320
B/Phuket/3073/2013	196-198 DKT	3	2013-11-21	E4/E3	1280	40	10	160	160	80	40	160	40
B/Mauritius/1791/2017				MDCK1/MDCK3	5120	320	320	320	320	160	320	320	320
TEST VIRUSES													
B/Belgium/H0008/2018		3	2018-01-11	MDCK1	2560	80	10	80	80	40	80	80	80
B/Belgium/S0037/2018		3	2018-01-08	MDCK1	1280	80	10	80	80	40	40	80	80
B/Belgium/G0011/2018		3	2017-12-28	SIAT1/MDCK1	1280	80	<	40	40	20	40	40	40
B/Belgium/S0023/2018		3	2017-12-26	MDCK1	2560	80	40	160	80	80	80	160	160
B/Belgium/G0634/2017		3	2017-12-19	MDCK1/MDCK1	2560	80	20	160	80	40	80	80	80
B/Belgium/G0629/2017		3	2017-12-18	SIAT1/MDCK1	2560	160	40	160	160	80	80	160	160
B/Belgium/G0593/2017		3	2017-11-21	MDCK1/MDCK1	2560	80	20	320	160	40	80	80	80
B/Belgium/G0019/2018		3	2018-01-02	MDCK1/MDCK1	2560	80	40	80	80	20	80	80	160
B/Belgium/G0013/2018		3	2017-12-29	MDCK1/MDCK1	2560	80	40	80	80	20	80	80	80
B/Belgium/G0012/2018		3	2017-12-29	SIAT1/MDCK1	2560	80	40	80	80	40	80	80	80

Vaccine NH 2015-16 SH2016*

Assay   HI (Turkey RBC)

RBC   Turkey

D.7.4 B Victoria

Genetic characterisation

Very few influenza B virus from the Victoria lineage were detected during this season and belonged to clade 1A, the B/Brisbane/60/2008 clade which is contained in the trivalent vaccine. However, all the sequenced strains have several amino acid changes, including two amino acid deletions in the HA gene at position 162 and 163 (referred to as the "B/Victoria deletion variant subgroup") (Fig. 18, fig.19). This deletion in the HA resulted in these viruses being antigenically different from the vaccine virus.

A higher number of samples have been proceed with wole genome sequencing amplification of all eight segments. All of these sequences have been submitted to GISAID.

Figure 18. Phylogenetic analysis of circulating influenza B/Victoria viruses detected in Belgium during the 2017-2018.

Figure 19. Part of the alignment of circulating influenza B/Victoria viruses detected in Belgium during the 2017-2018.

Antigenic characterisation

No influenza B Victoria viruses were antigenically characterized.

D.8. Antiviral monitoring

All the strains 155 analysed phenotypically were sensitive to neuraminidase inhibitors: Oseltamivir and Zanamivir.
Of the 18 NA sequenced of A(H1N1)pdm09, no mutation (H275Y) was detected .

D.9 Composition of influenza virus vaccines for use in the 2018-2019 northern hemisphere influenza season

The WHO has published its recommendation for the vaccine composition that should be used in the 2018-2019 season in the Northern hemisphere.

It is recommended that vaccines for use in the 2018-2019 northern hemisphere influenza season contain the following:

Trivalent vaccine

- A/Michigan/45/2015 (H1N1)pdm09-like virus (no change);
- A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus (change);
- B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)(change)

Quadrivalent vaccine

- A/Michigan/45/2015 (H1N1)pdm09-like virus(no change);
- A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus(change);
- B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage) (change); and
- B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage(no change)).

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	"	B/Brisbane/60/2008 VIC	*
2018-2019	"	A/Singapore/INFIMH-16-0019/2016	B/Colorado/06/2017-like virus VIC	*

Figure 20. Evolution of the composition of the trivalent influenza vaccine 2000 – 2019

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 offered a 41% % (95% CI: 22% -55%) reduction of incidence of medically attended influenza infections in vaccinated people (.

D.11 Severity

Based on the indicators of severity among hospitalized flu patient (SARI surveillance), the severity of the current epidemic appeared to be moderate this season. (Figure)
11% of the hospitalised confirmed flu patients had a severe complication, which is rather low in comparison with the past seasons. 89% of the affected patients had an underlying condition/risk factor. We observed few severe complications in children this season. There were relatively more patients with ARDS than the two previous seasons.

We observed 6% fatal infections (of which 90% had an underlying condition/risk factor) among the confirmed flu patients. This season, the odds on mortality were mainly associated with age ≥ 85y and with A(H3N2) (vs B|Yamagata) subtype. The median time between onset of symptoms and death was 14 days. In comparison with the previous seasons, there were slightly more fatal cases in the age group 15-44y in season 2017-2018.

The average length of stay for a confirmed flu infection was 8.8 days (95% CI: 8.2 – 9.4 days), which was shorter than in seasons 2014-15 and 2016-17, but higher than in the other seasons. The length of stay was positively associated with age, male sex and (although not statistically significant) the A(H3N2) subtype.
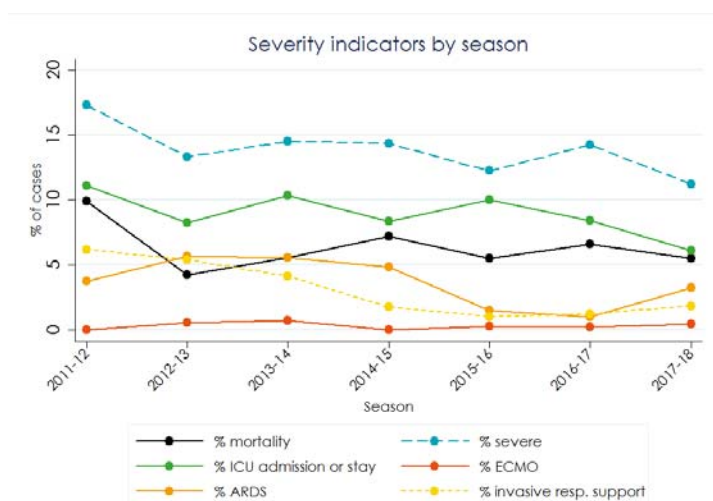

Figure 21. Evolution of indicators of severity in patients with confirmed influenza infection during the past 7 seasons in Belgium (Source: WIV-ISP: SARI surveillance by the network of sentinel hospitals)

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

From week 41-2017 until week 19-2018, on top of the 68 938 deaths that were expected in that time period, 3100 excess deaths (4.5%) have been observed in Belgium. The excess mortality was most pronounced in the age group 65-84 years (5.5%) (26).

Significant excess mortality started in week 7-2018 in Flanders (among men aged 65-84 years) and in week 8 in Wallonia (among women aged 65-84). There were overall 3 weeks with significant excesses of mortality in the whole country (weeks 9-2018 to 11-2018). We observed a peak in mortality in week 10-2018, more specifically on March 7th 2018 (465 deaths).

The observed excess mortality coincided with peak of the flu epidemic, a cold spell (from February 18th 2018 until March 4th 2018) and increased PM10 and PM2.5 levels (on February 20th - 21th and March 2nd – 3rd).
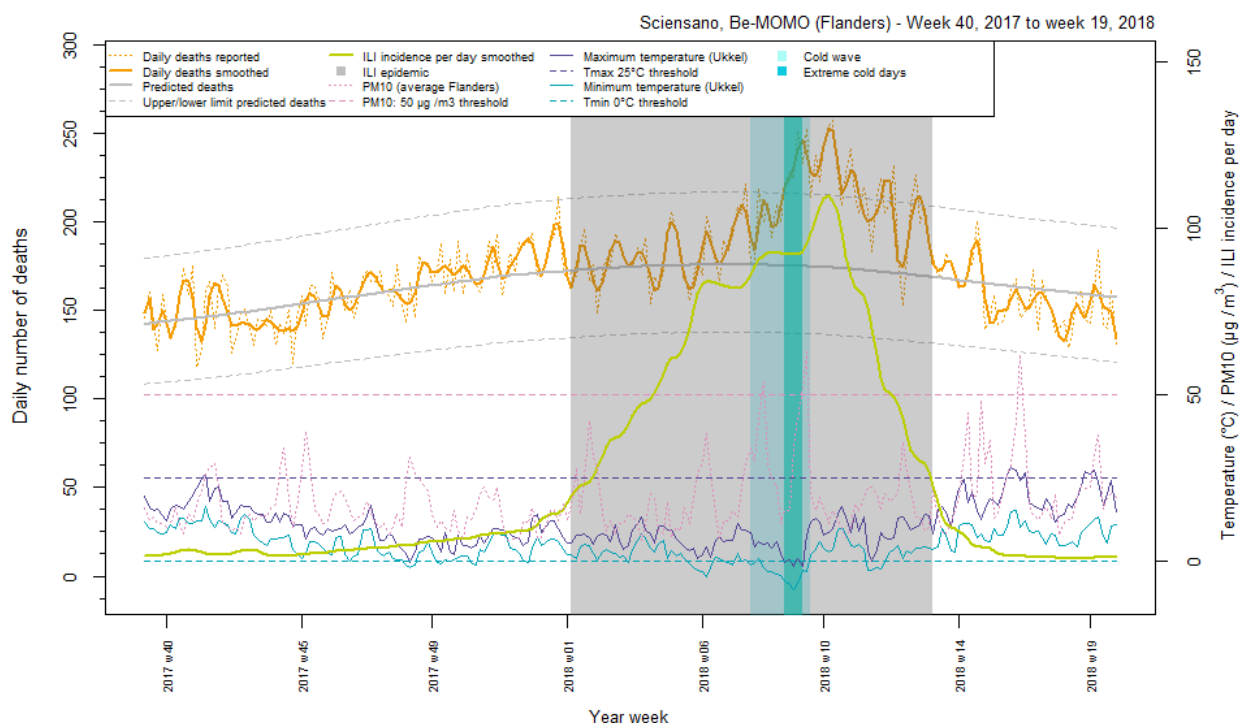
Figure 22. Predicted and observed daily mortality and risk factors in the winter of 2017-2018 in Belgium (Source: Be-MOMO surveillance, Sciensano).

E. Conclusion

The 2017–2018 influenza season was of medium intensity and lasted for 13 weeks. During the epidemic peak, a total number of 721 ILI per 100.000 inhabitants was reached. A(H1N1)pdm09 and B/Yamagata viruses predominated during this season. The overall percentage of positivity for influenza was higher in the ILI surveillance (59%) as compared to the SARI surveillance (41.4%), which is probably due to a better specificity of the case definition for influenza in the ILI surveillance.

Sequencing of a subset of the different viruses showed that the strains belonged to groups that were close to the corresponding vaccine strains except for B/Victoria. 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 either the subclade 3C.2a1b, represented by the reference strain A/Bolzano/7/2016 or the 3C.2a2. 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 characteristic

All the sequenced **B Yamagata** viruses belonged to clade 3 represented by the vaccine strain B/Phuket/3073/2013 present in the quadrivalent vaccine.

All the sequenced **B Victoria** virus belonged to the B/Victoria deletion variant subgroup which is antigenically different from the vaccine virus.

All tested viruses were sensitive to Oseltamivir and Zanamivir.

During this season, all respiratory samples were also analysed for other respiratory viruses. In the ILI population, 35% of the influenza negative patients were positive for one or more other respiratory viruses, whereas in the SARI population, this percentage reached 39%. This suggest an important role of other respiratory viruses in hospitalized patients during the flu season with symptoms relevant to the case definition. It will be important to continue this surveillance over several seasons to be able to analyse the impact of the different respiratory viruses on public health (correlation with severity, …).

F. Acknowledgements

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