

# Virological Surveillance of Influenza in Belgium

Season 2016-2017

## **VIRAL DISEASES**

National Influenza Centre (WHO)

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## **EPIDEMIOLOGY OF INFECTIOUS DISEASES**

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## A. Abstract

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The Influenza activity started early this 2016-2017 season and lasted 7 weeks. In Belgium, the epidemic threshold was exceeded from week 2-2017 (9 - 15 January 2017) to week 9-2017 (27 February-5 March 2017). The epidemic was of medium intensity. The peak of the epidemic was observed in week 5-2017, with an incidence of 745 consultations for influenza-like syndromes per 100,000 inhabitants. After week 6-2017, the number of influenza-like syndromes fell and was below the threshold after week 9.

Based on the surveillance of the sentinel network of general practitioners, we estimate that this season approximately 490000 Belgians visited their GP for influenza-like illness (ILI) and that approximately 280000 Belgians had a clinical infection with the influenza virus. Just as in season 2014-2015, this season relatively more elderly people of 65 years and older were affected than in the other seasons.

Although a peak of excess mortality (all causes) was observed at the onset of the peak of influenza epidemic, the severity indicators (estimated from the surveillance of acute severe respiratory infection by the sentinel network of hospitals) do not indicate that the epidemic was more severe than average this season

The first positive sample was diagnosed in week 47-2016 and increasingly large numbers of positive influenza cases were detected from week 53-2016 onwards, reaching a proportion of 74% positive samples in week 5-2017. These were mainly A(H3N2) viruses. Very few influenza A(H1N1)pdm2009 and influenza B circulated during this season. All respiratory samples were also analysed for other respiratory viruses. In the ILI population, 41% of the influenza negative patients were positive for one or more other respiratory viruses, whereas in the SARI population, this percentage reached 51%. This suggests an important role of other respiratory viruses in hospitalized patients during the flu season.

The preliminary estimation of the vaccine effectiveness was 28% (analysis performed for A(H3N2))

All tested viruses were sensitive to Oseltamivir and Zanamivir.

## B. Background

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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. In 2013, for example, two new highly lethal respiratory viruses emerged (influenza A(H7N9) in Asia and MERS-CoV in the Middle East), demonstrating the importance of the surveillance of respiratory pathogens for the public health.

This report is mainly focusing on the virological results.

## C. Methods

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### 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 were: 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

A subset of 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 2016-2017 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 seven days, fever of  $\geq 38^{\circ}\text{C}$ , 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 2016-2017:

- 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 25 July 2017, 859 human infections with highly pathogenic H5N1 viruses have been reported to WHO by 16 countries (4). About 50% (453) of the laboratory confirmed people died from their illness. Regularly, new cases are reported in different countries, especially in Asia but also in Egypt. Human cases and fatalities due to influenza A(H5N1) virus continue to increase in Egypt, with cases from the country now accounting for the highest number of human cases reported worldwide. In 2017, only 3 cases of A(H5N1) cases were reported in Egypt. 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 (5). From February 2013 until 26 July 2017 1584 confirmed; 612 deaths laboratory-confirmed cases of human infection with the avian A(H7N9) were reported to WHO including. Most of the cases were from China.. Genetic analysis detected diversity in the HA gene showing that influenza A (H7N9) viruses had already started to evolve. 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. 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. As of July 2017, WHO has been notified of more than 2040 laboratory-confirmed cases of infection and at least 693 deaths related to MERS-CoV. MERS-CoV causes severe human infections resulting in high mortality and has demonstrated the ability to transmit between humans (6). So far, the observed human-to-human transmission has occurred mainly in health care settings. To date, 27 countries have reported cases, including countries in the Middle East, North America, Europe and Asia and more recently clusters of cases in Korea and China (7). 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. However the National Influenza Centre has developed real time PCR testing and received samples from suspected cases. 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 (20). Also, a preliminary study (unpublished results) showed that samples from SARI patients are sometimes co-infected by other respiratory viruses. Furthermore, severe influenza cases often seem to be complicated by co-infections with other respiratory viruses (22). We have developed 4 quadruplex 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), human metapneumoviruses (hMPV), paraechoviruses (HPeV), bocaviruses (HBoV), adenoviruses (ADV) and different coronaviruses (CoOC43, CONL63, Co229E, MERS-CoV). During this season, the MERS-CoV was not screened in the multiplex Real time PCR due to absence of evidence of circulation in Europe. A sensitive specific real time PCR for screening and confirmation is available for MERS-coV in cases a suspected cases.

## 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 (10,11); 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 (10); primers and probes are chosen in the hemagglutinine gene.
- RTPCR Influenza A/H3: adapted protocol from RIVM (12); primers and probes in the hemagglutinine gene.

For a subset of samples:

- RT-PCR N1: adapted protocol from RIVM (12); primers and probes in the neuraminidase gene.
- RT-PCR N2: adapted protocol from Pasteur Institute Paris (13); 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 (14).

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 )(10): This test allows to determine if the influenza strain is of animal origin and to continue with complementary tests.



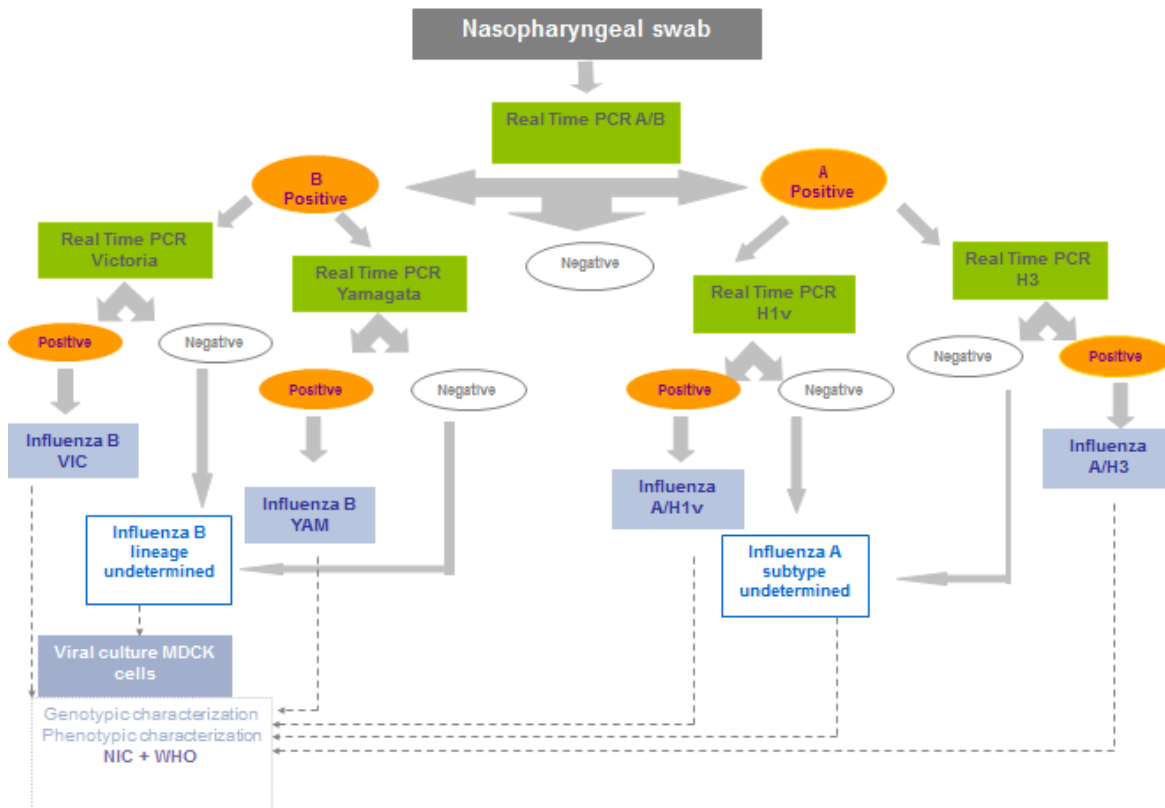


Figure 1. Sequence of the Real time PCR tests used during the 2016-2017 season.

### 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 (15) and adapted protocol from the Health Protection Agency, 2006 (16).

#### **RT-PCR H7N9**

Protocol adapted from WHO (17).

### 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.* (18).

### 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) (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 229F	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		

The protocols have been adapted from those of the Statens Serum institute (19) with some modifications (primers for rhinoviruses as described by Hombrouck *et al.* (20). Rhinoviruses and enteroviruses were considered together as rhinovirus/enterovirus (HRV/ENV).

### 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.

### C.2.5. Resistance to antivirals

The most commonly used antivirals are neuraminidase inhibitors [oseltamivir (Tamiflu<sup>®</sup>) and zanamivir (Relenza<sup>®</sup>)]. 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

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### D.1 Sentinel surveillance of ILI

#### D.1.1 Clinical surveillance

The Influenza activity started early this 2016-2017 season and lasted 7 weeks. The epidemic threshold was exceeded from week 2-2017 (January 9 - 15 2017) to week 9-2017 (February 27 - March 5 2017). The peak of the epidemic was observed in week 5-2017, with an incidence of 745 GP consultations for influenza-like syndrome per 100,000 inhabitants. After week 6-2016, the number of influenza-like syndromes fell and was below the threshold after week 9. The epidemic was of medium intensity.

Based on the surveillance of the sentinel network of general practitioners, we estimate that this season approximately 490000 Belgians visited their GP for influenza-like illness and that approximately 280000 Belgians had a clinical infection with the influenza virus. Just as in season 2014-2015, this season relatively more elderly people of 65 years and older were affected than in the other seasons.

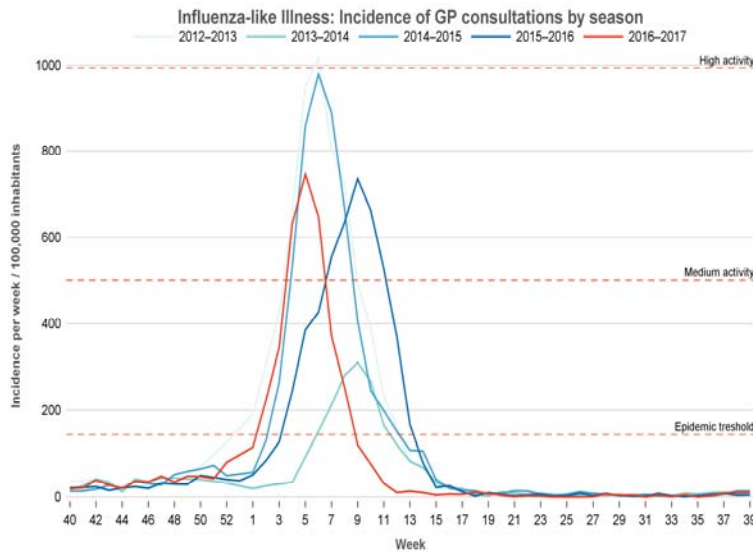


Figure 2. Weekly incidence of influenza-like illness (ILI) 2016-2017 season, Belgium (Source: Sentinel general practices)

### D.1.2 Virological surveillance

The influenza surveillance period started in week 40-2016 and continued to week 20-2017.

#### Origin of samples

A total of 74 general practices (39 for Flanders, 35 for Wallonia-Brussels Federation) took part in the virological surveillance and sent 646 nasopharyngeal swabs to the NIC which were all suitable for analysis.

Number of nasopharyngeal swabs

Flanders : 324 (50.1 %)

Wallonia-Brussels : 322 (49.8 %)

**Total : 646**

#### Typing and subtyping results

The first positive sample was diagnosed in week 47-2016 and increasingly large numbers of positive influenza cases were detected from week 52-2016 onwards, reaching a proportion of 74% in week 50-2016. These were mainly A(H3N2) viruses.

From week 40-2016 to week 20-2017, 646 respiratory samples were sent by the sentinel GPs network and analysed at the National Influenza Centre. Of these samples, 333 (51.5%) were positive for influenza with 332 (51.3%) positive for influenza A and 1 (0.54%) positive for influenza B (Figure. 3a, 3b).

Among the influenza A samples that were subtyped, 97,3% (323/332) were A(H3N2), 0,9% (3/332) were A(H1N1)pdm2009 and 6 samples (1.8%) could not be subtyped due to their low

viral load. Only one influenza B virus was detected in the sentinel surveillance of ILI and belonged to the Yamagata lineage (Table 2).

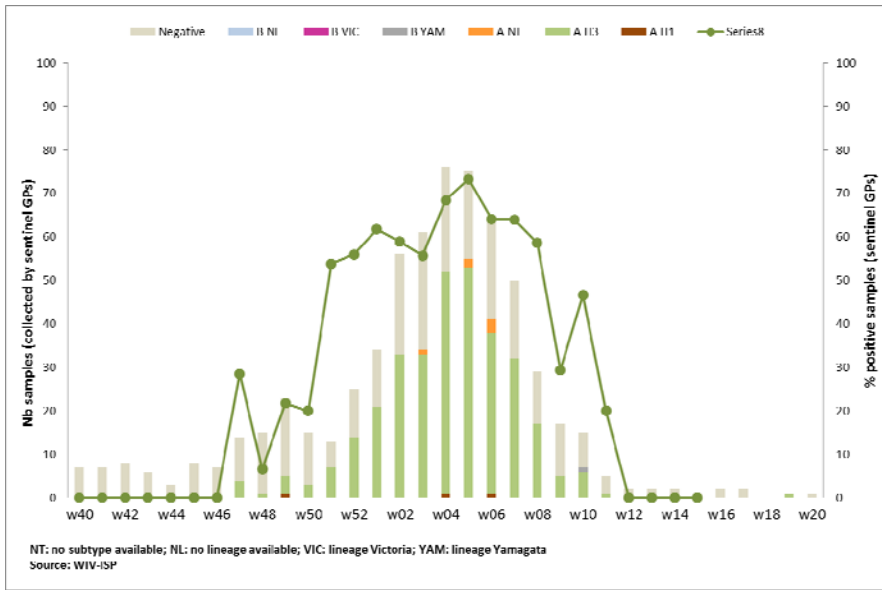


Figure 3a. Weekly detection of influenza viruses in Belgium from week 40-2016 to week 20-2017 in the network of sentinel GPs

Table 2. Numbers and proportion of the different types and subtypes analysed during the 2016-2017 season

	FLU detection/typing			FLU A subtyping			FLU B lineage		
	neg	A	B	A H1*	A H3*	A NT	YAM*	VIC*	B NL
Number of samples with given result	313	332	1	3	323	6	1	0	0
Number of tested samples	646	646	646	332	332	332	1	1	1
Percentage (%)	48,5	51,4	0,2	0,9	97,3	1,8	100,0	0,0	0,0

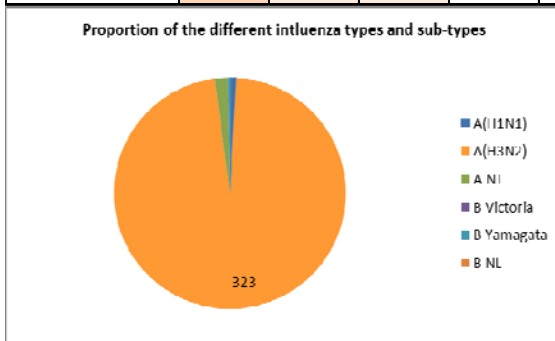


Figure 3b. Repartition of the different types and subtypes during the influenza season 2016-2017 ILI surveillance .

## Influenza viruses according to age group

The age was known for 637 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 (Figure 4). Very few samples were collected from the age group < 5 years old and > 85 years old.

The distribution of influenza types (and subtypes) didn't varied with age during this season as nearly only A(H3N2) circulated.

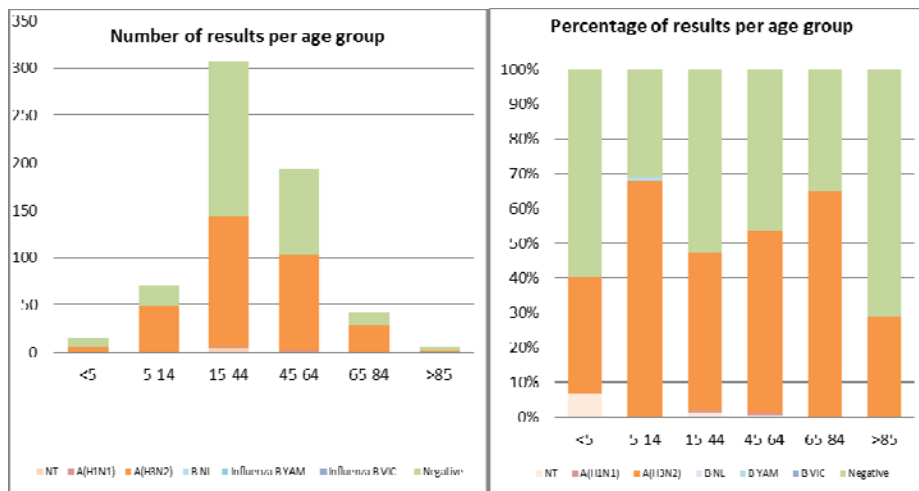


Figure 4 . Influenza virus types and subtypes according to age group (NT= non subtyped)(numbers and percentages)

## D.2 Sentinel Surveillance of SARI

### D.2.1 Virological surveillance

SARI Surveillance started week 1-2017 after the first influenza cases were recorded by the sentinel GPs, and ended week 17-2017, about one month after the end of the epidemic.

#### Origin of samples

A total of 1497 patients were registered in the database, among which 1422 (95%) corresponded to the case definition and were suitable for analysis.

The age was known for 1410 patients and those were taken into account for the analyses of age group.

#### Typing and subtyping results

From week 1-2017 to week 17-2017, 1422 respiratory samples from the sentinel network of hospitals were analysed by the NIC, among which 563 (39.5%) were positive for influenza, with 556 (39%) influenza A and 7 (0.5%) influenza B. Among the analysed influenza viruses,

526 (93.4%) were A(H3N2), 2 (0.4%) were A(H1)pdm2009, 2 (0.4%) were influenza B/Victoria and 5 (0.9%) were influenza B/Yamagata. Due to the low viral load, 28 (5 %) of the influenza A were not subtypable (Figure 5) (Table 6) . From week 1, the percentage of positivity increased (17%) to reach a peak of 55.5% in week 6. From week 17, no more samples were sent to the NIC.

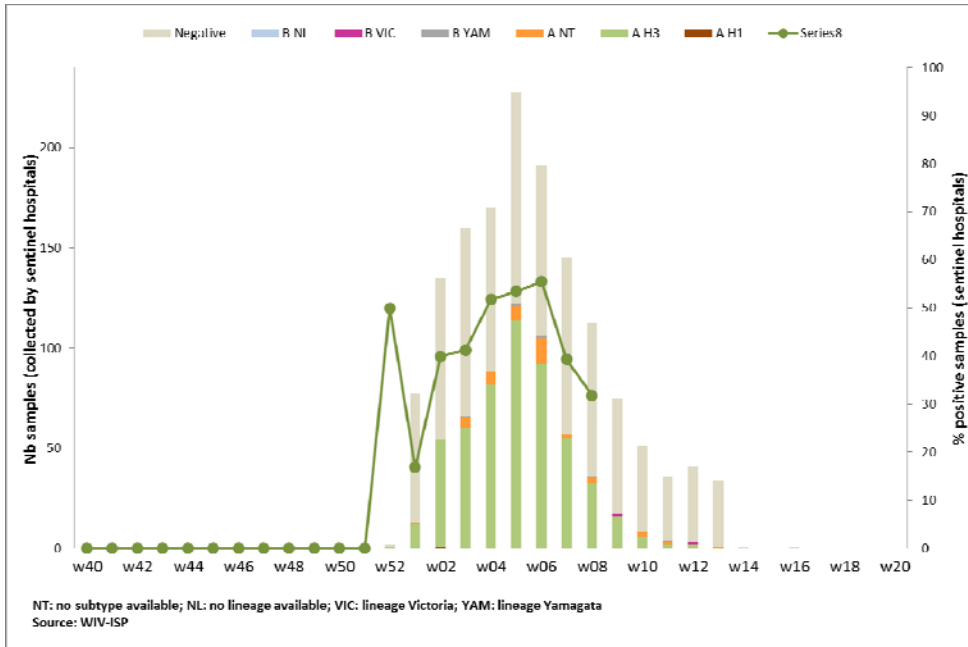


Figure 5. Weekly detection of influenza viruses in Belgium in the SARI network from week 1-2017 to week 17-2017

Table 4. Repartition of the different types and subtypes during the influenza season 2016-2017 SARI surveillance

	Number	%
<b>A(H1N1)</b>	<b>2</b>	<b>0,4</b>
<b>A(H3N2)</b>	<b>526</b>	<b>93,4</b>
<b>A NT</b>	<b>28</b>	<b>5,0</b>
<b>B Victoria</b>	<b>2</b>	<b>0,4</b>
<b>B Yamagata</b>	<b>5</b>	<b>0,9</b>
<b>B NL</b>	<b>0</b>	<b>0,0</b>

### Age distribution of influenza viruses by types and subtypes

The age was known for 1410 patients. A higher number of samples was collected from adults of the 65-84 age group. The percentage of positivity for influenza viruses was higher in the age group ≥65 years old and reached 60% in the elderly. The distribution of influenza types (and subtypes) didn't vary with age this season as nearly only A(H3N2) circulated (Figure 6).

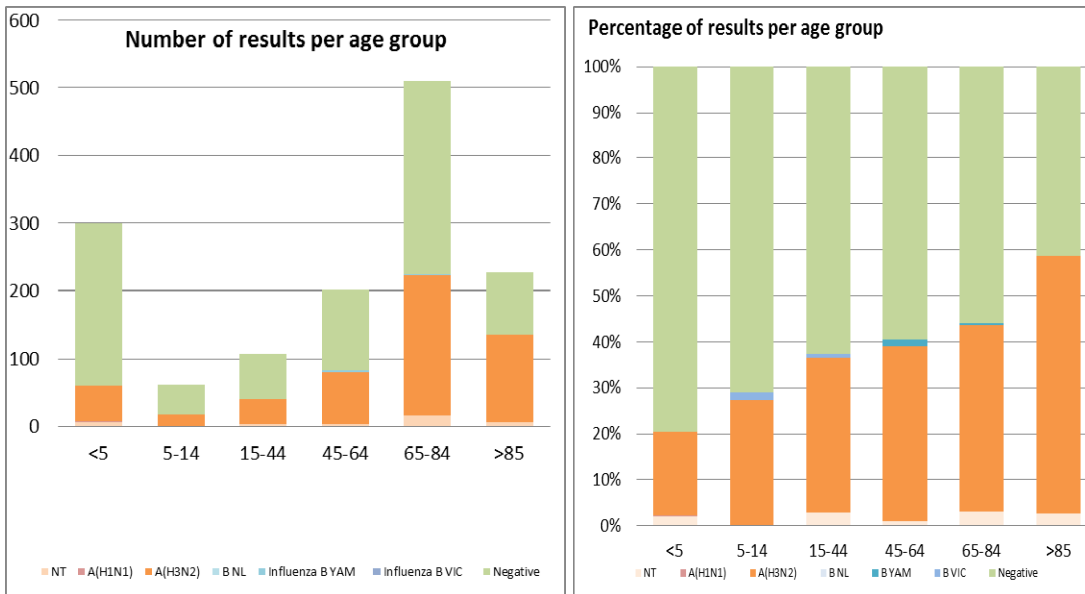


Figure 6. Influenza viruses according to age group SARI surveillance season 2016-2017 (numbers and percentages)

### Positivity and subtype distribution of influenza viruses by surveillance scheme

During the SARI surveillance period (week 1 to week 17 of 2017), the samples from ILI patients (60,2 % positive; were significantly more frequently positive than those from SARI patients (39.5% positive)

During that period, Influenza A(H3N2) circulated in both surveillances.

### D.3 Non sentinel surveillance

Fifty nine respiratory samples from patients with severe influenza were sent from hospitals around the country during the 2016-2017 season and inter-season, and were analysed at the NIC for confirmation and subtyping. Forty two were influenza A positive, among which there were 59 A(H3N2), and 7 non-subtypable due to low viral load. Most of the samples were sent for diagnostic confirmation.

### 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

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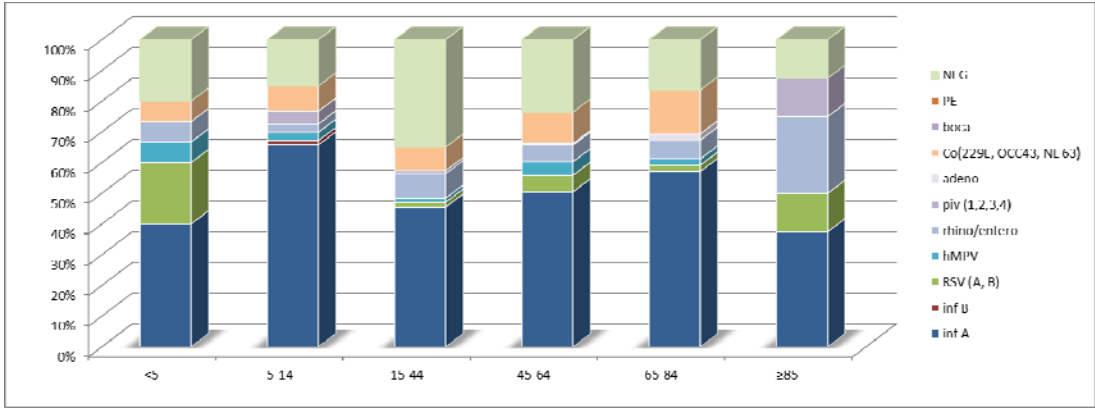


Figure 8 a. Proportion of the different respiratory viruses in the ILI surveillance season 2016-2017 by age group (Percentage).

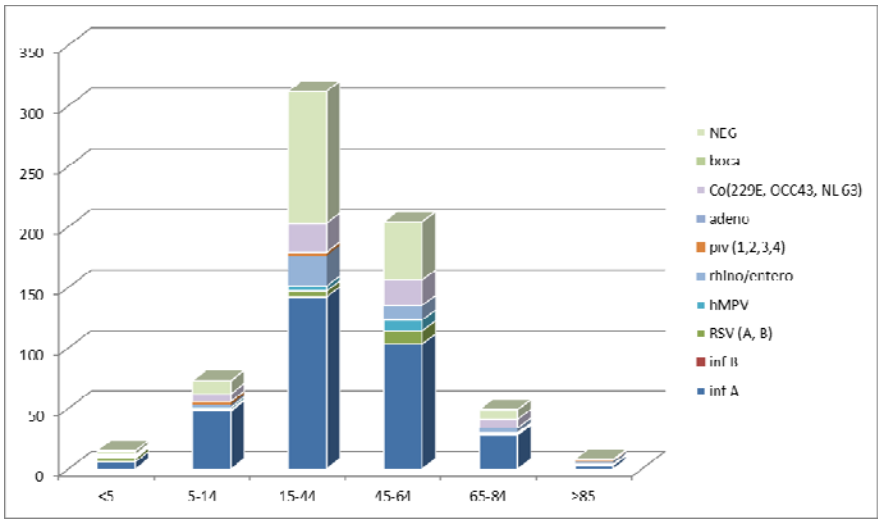


Figure 8b. Proportion of the different respiratory viruses in the ILI surveillance season 2016-2017 by age group (numbers)

**Co-infections**

The percentage of co-infection was 6.2 % (40/636). Among influenza positive samples, co-infection with other viruses was observed in 28/332 (8.5%). Regarding co-infection of respiratory viruses other than influenza, the percentage of co-infection was 4.2% (13/305) and no particular combination of viruses was dominant.

**Weekly evolution**

Figure 9 shows the weekly proportion of respiratory viruses that were laboratory-confirmed during the 2016-2017 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 45 to week 7, at percentages varying from 0% to 15.4% positive samples per week (week 51). Coronaviruses were detected from week 45 at percentages varying from 0% to 20% positive samples per week. The hMPV, and ADV viruses were detected more sporadically. Neither HPeV nor HBoV were detected in the ILI surveillance this season.

We did not observe the RSV peak that usually appears around week 51. This is probably 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.

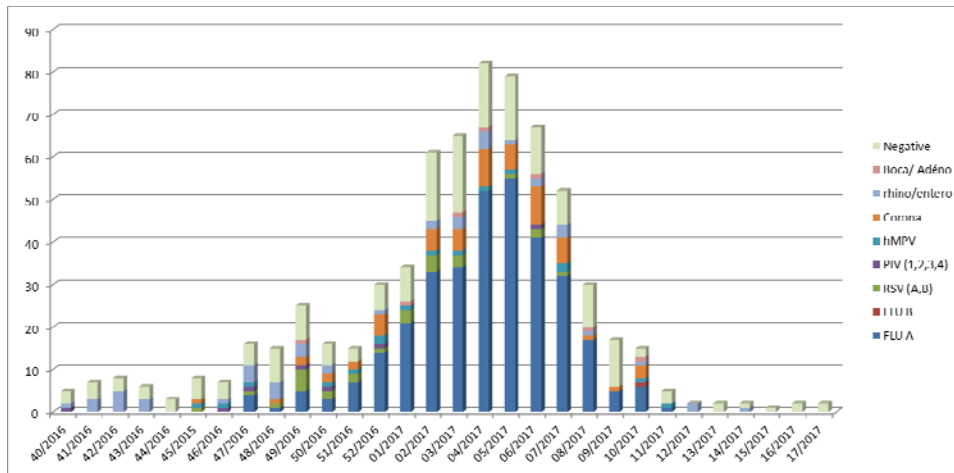


Figure 9. Weekly proportion of respiratory viruses during the 2016-2017 ILI surveillance

### D.6.2 SARI surveillance

From week 1 to week 17-2016, the respiratory samples analysed for influenza were also submitted to the diagnosis for the other respiratory viruses. The age groups were known for 1396 patients and the analyses were performed on those samples. Among the influenza viruses negative samples, 295/574 (51%) were positive for at least one other respiratory viruses.

For 388/1396 (27.8%) patients no respiratory viruses were detected. Overall, 1007/1396 (72.2%) of the patients were positive for at least one respiratory viruses (including influenza alone or in co-infections). This percentage reached 82% (260/317) in children below the age of 5 years old, and 80.2% (162/202) in elderly > 85 years. The most prevalent respiratory viruses were Influenza A (39.7%), RSVB (8.81%), HRV/ENV viruses (7.73%), and hMPV (7.23%), CoOC43 (4.65%), ADV (4.08%). Influenza B was nearly not detected (0.6%) during this season. For the other viruses, the percentages were lower and varied from 2.7% for Co229E to 0.1% for HPeV and PIV 1 (Figure 10 ).

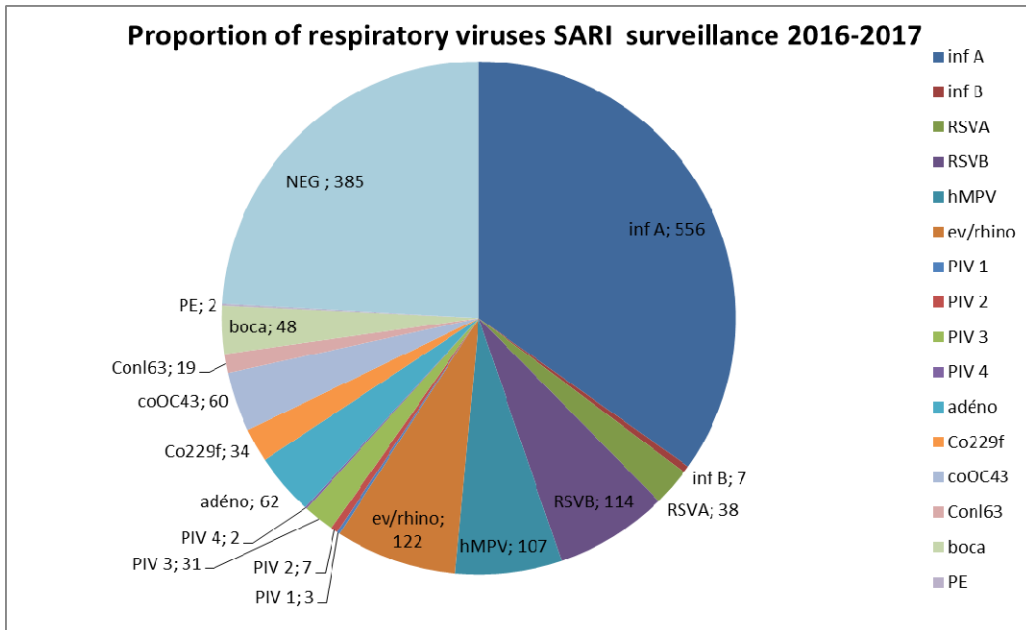


Figure 10. Proportion of the different respiratory viruses in the SARI surveillance season 2016-2017

### Proportion of the different Respiratory viruses according to age group

The proportion of the different viruses varied between age groups. For all viruses except influenza A and coronaviruses, the percentage was higher in children below 15 years old in comparison with adults (Figure 11) ( Figure 12a, 12b).

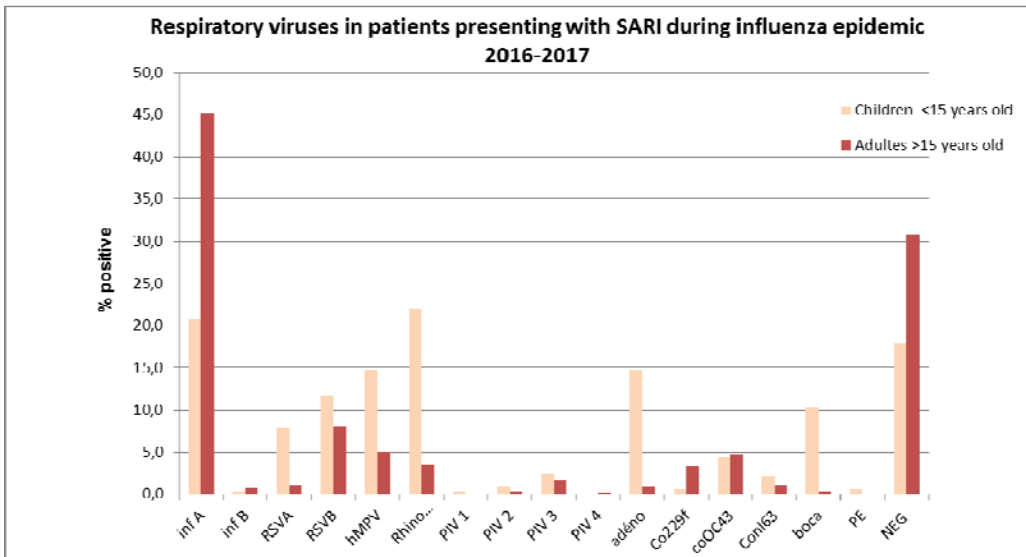


Figure 11. Percentage of the different Respiratory viruses in the SARI surveillance season 2016-2017 according to age group (children <15 years old and adults)

In children below the age of 5 years old, the percentage of positivity for at least one respiratory virus reached 82%. All tested respiratory viruses were detected in this age group

with the most prevalent virus being Rhino/enterovirus (25%), influenza A (20%), RSV (18.7%), and hMPV (16.3%). In patients aged more than 85 years old, influenza A was far the most prevalent (59.1%), followed by RSV (10.9%) and coronavirus (10.4%) (Figure 12a and 12b).

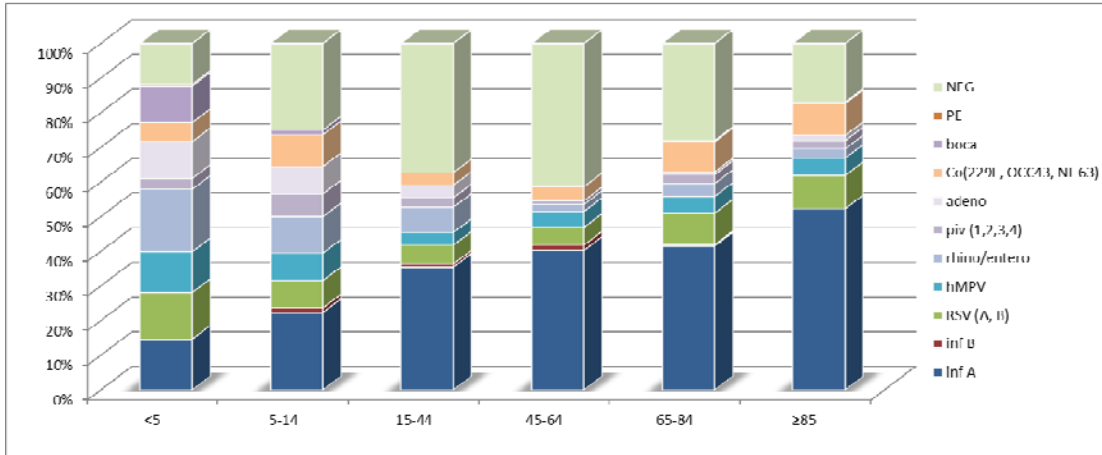


Figure 12a. Proportion of the different respiratory viruses in the SARI surveillance season 2016-2017 by age group (percentage)

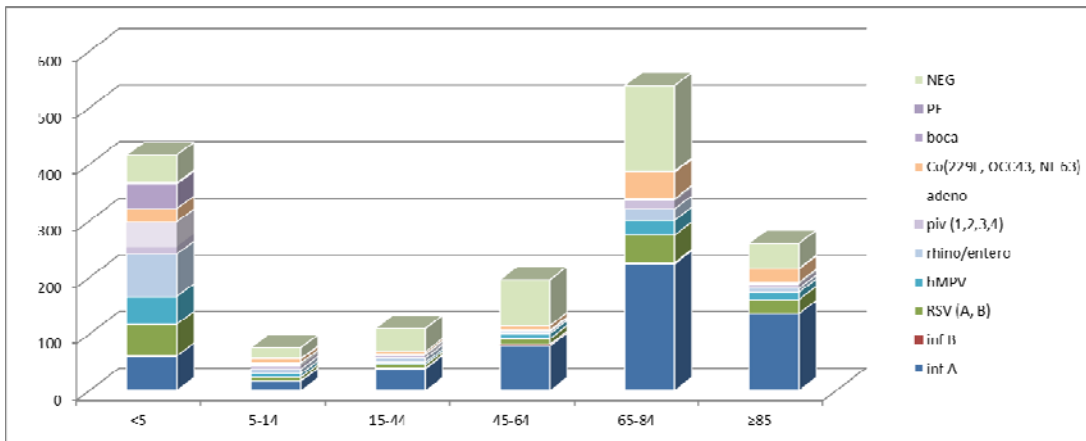


Figure 12b. Proportion of the different Respiratory viruses in the SARI surveillance season 2016-2017 by age group (numbers)

### Coinfections

Overall, the percentage of co-infection (two to 5 viruses) was high, 10.9% (152/1396). Co-infection of respiratory viruses except influenza accounted for 66/391 (16.9%). Most of these co-infections 48/66 (72%) were observed in patients below the age of 5 years old. Among influenza positive samples, co-infection with other viruses were observed in 86/562 (14%) with the most frequent association being A/Coronavirus found in 27/86 (32%), A/ADV and A/HRV/ENV in 15/86 (17.4%) samples. Regarding co-infections by respiratory viruses other

than influenza, the most common viral co-infections were HRV/ENV with RSV 16/66 (24%) followed by HRV/ENV with ADV 14/66 (21%). Co-infection with 3 or more virus were observed in 13 patients, 12 of with were below 5 years old.

### Weekly evolution

Figure 13 shows the weekly proportion of respiratory viruses that were laboratory-confirmed during the 2016-2017 SARI surveillance period. All tested respiratory viruses were detected during the surveillance period (week 1-2017 to 17-2016). Influenza viruses were the most prevalent with percentage of 40% (0% to 55%) positive samples per week, followed by RSV 11.1% (17.8% in week 2), CoV 8.7% (0% to 11%), and ENT/RHV(8.3%) positive samples per week.

The second more frequently detected virus was RSV B. The other viruses were detected more sporadically however, 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 peak of influenza, but this was not surprising since the surveillance began only in week 1-2017, so too late to detect the peak. 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% (21).

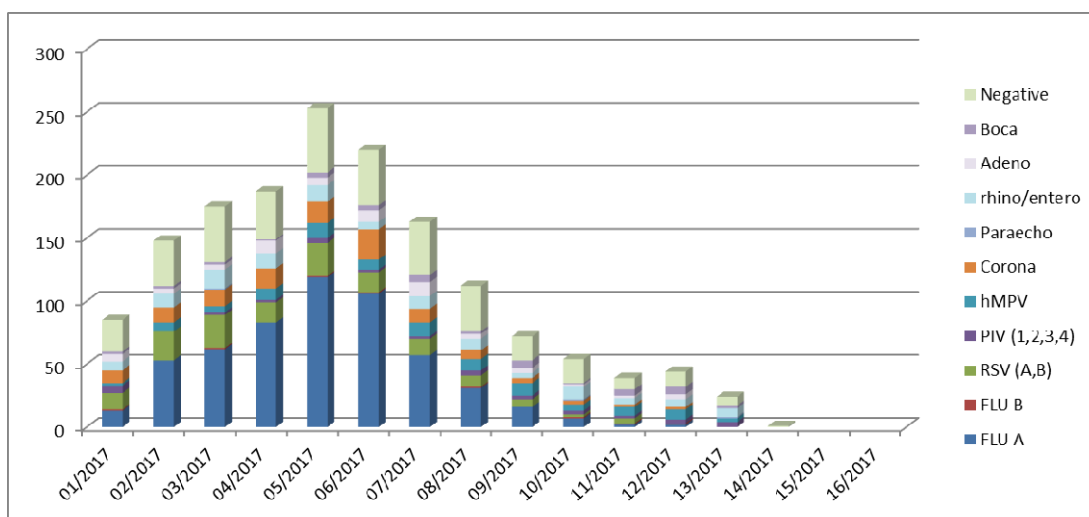


Figure 13. Weekly evolution of respiratory viruses during the SARI surveillance season 2016-2017

## D.7. Characterisation of the influenza viruses

### D.7.1 A(H1N1)pdm2009

#### Genetic characterisation

Very few A(H1N1)pdm09 viruses were detected during this season. Four strains were sequenced and belonged to group 6B.1, represented by the reference strain A/Michigan/45/2015 characterized by

the substitutions S84N, S162N and I216T. All of these sequences have been submitted to GISAID. These viruses are antigenically homologous and similar to the vaccine strain A/California/7/2009 (Figure 14).

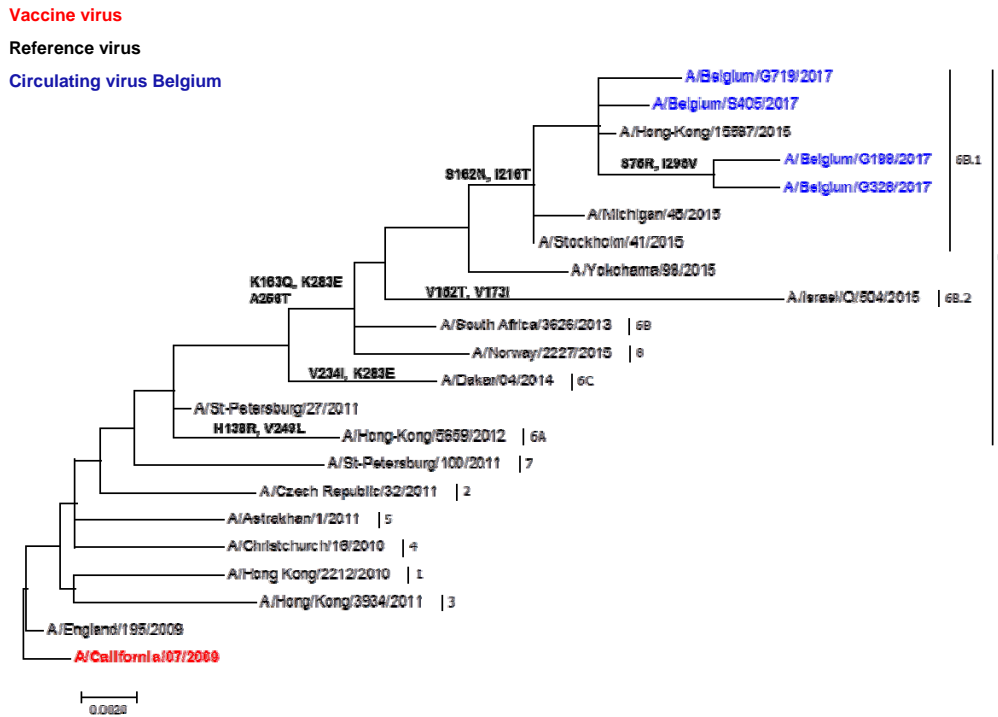


Figure 14. Phylogenetic analysis of influenza A(H1N1)pdm09 strains in Belgium, season 2016-2017

### Antigenic characterisation

No A(H1N1)pdm2009 viruses were available for characterisation during this season. In the European countries, the A(H1N1)pdm09 viruses characterized antigenically were similar to the vaccine virus A/California/7/2009 but showed better reactivity with antiserum raised against the A/Michigan/45/2015 which will be included in the 2017-2018 vaccine.

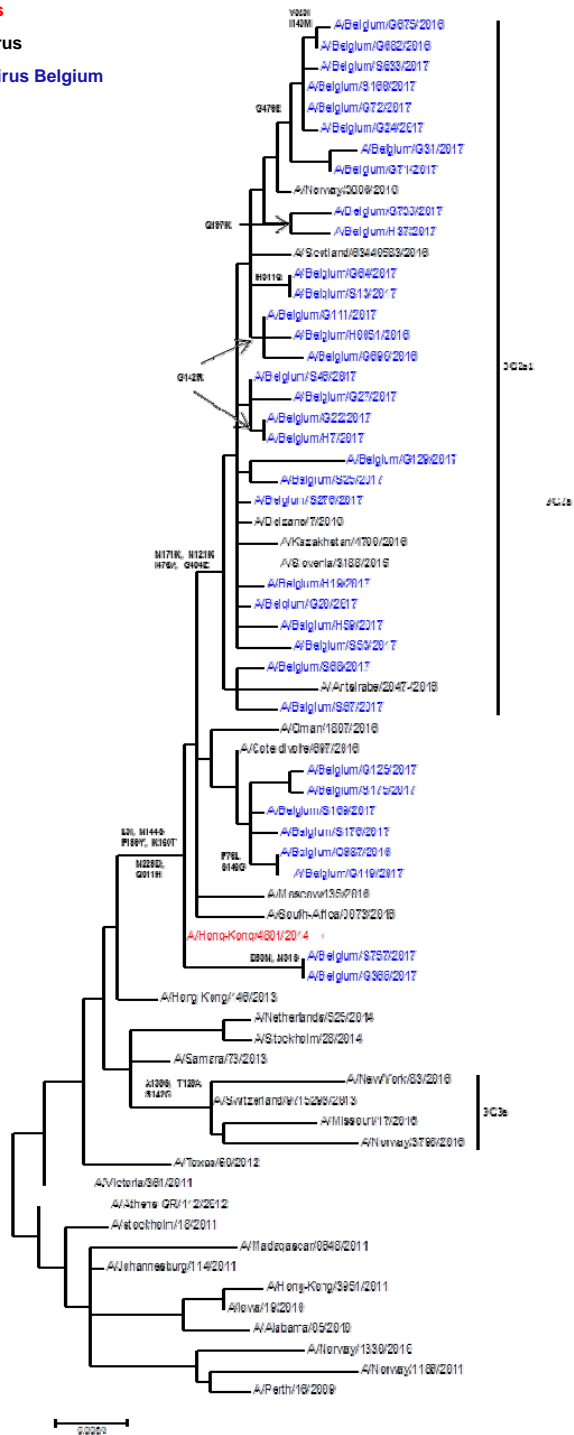
### D.7.2 A(H3N2)

#### Genetic characterisation

In Belgium, the majority of the viruses which circulated during this season were A(H3N2). Thirty six strains (HA gene) were sequenced among which 22 belonged to the newly emerging subclade 3C.2a1, represented by the reference strain A/Bolzano/7/2016 defined by the mutations N171K and N121K and 8 belonged to the 3C.2a clade represented by the vaccine strain A/Hong-Kong/4801/2014. All of these sequences have been submitted to GISAID. The subclade 3C.2a1 was reported to be antigenically close to the vaccine strain A/Hong Kong/4801/2014 (Figure 15a, 15b).

Viruses in these two clades have been antigenically similar, but both clades 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





### Phylogenetic comparison of A(H3N2) HA genes

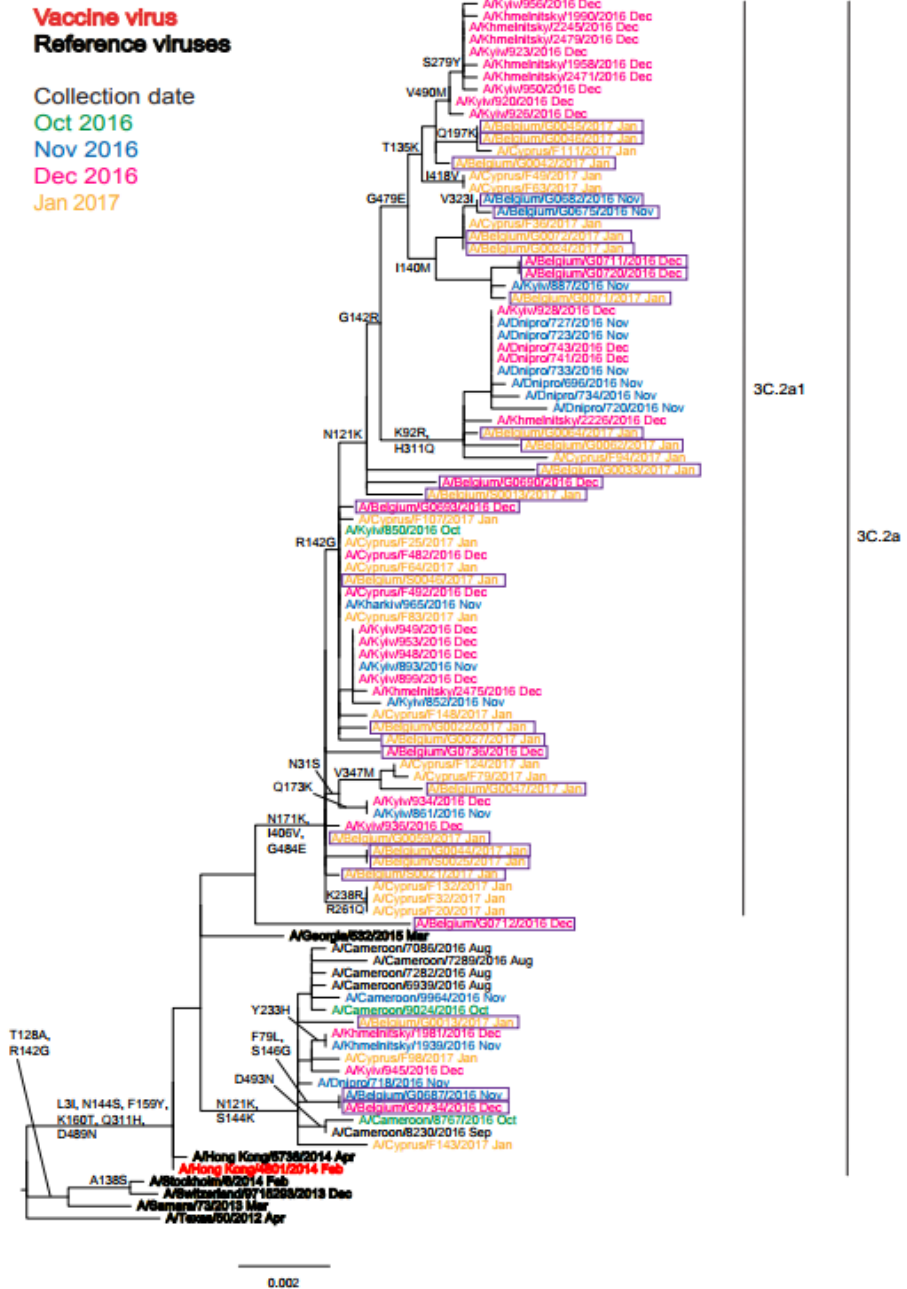


Figure 15a/b Phylogenetic analysis of the HA sequences of the A(H3N2) viruses analysed from Belgium and other European countries during the 2016-2017 season in comparison with the vaccine strain and the reference strains.

### Antigenic characterisation

Antigenic characterisation of three A(H3N2) viruses was carried out. All three viruses were recognized by the antiserum raised against the egg-propagated vaccine virus A/Hong Kong/4801/2014 (2 fold). Two of the three viruses were also recognized by an antiserum raised against the cell culture-propagated A/Hong Kong/4801/2014 (2-fold). An antiserum raised against another 3C.2a virus

propagated in cell culture A/Hong Kong/5738/2014 recognized all three test viruses at a titer equal to the titer of the antiserum for the homologous virus.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2017-05-12															
			Haemagglutination inhibition titre												
WIC number			Post-infection ferret antisera												
Viruses	Other information	Passage history	A/Texas 5012	A/Samara 73/13	A/Stockholm 6/14	A/Stockholm 9715293/13	A/Switzerland 73/15	A/Switzerland 9715293/13	A/HK 5738/14	A/HK 4801/14	A/HK 4801/14	A/Georgia 532/15	A/Oman 2585/16	A/Hong Kong 5738/14	
	Passage history		Egg	SIAT	SIAT	Egg	SIAT	Egg	MDCK	MDCK	Egg	SIAT	SIAT	SIAT	
	Ferret number		F08/14 <sup>1</sup>	F3/15 <sup>1</sup>	F14/14 <sup>1</sup>	F20/14 <sup>1</sup>	F18/15 <sup>1</sup>	F73/15 <sup>1</sup>	F30/14 <sup>1</sup>	F43/15 <sup>1</sup>	F12/15 <sup>1</sup>	F33/15 <sup>1</sup>	NB F50/16 <sup>1</sup>	F03/17 <sup>1</sup>	
	Genetic group		3C.1	3C.3	3C.3a	3C.3a	3C.3a	3C.3a	3C.2a	3C.2a	3C.2a	3C.2a	3C.2a1	3C.2a1	
<b>REFERENCE VIRUSES</b>															
A/Texas/50/2012		3C.1	E5/E2	2560	640	160	640	80	320	160	320	80	320	160	640
A/Samara/73/2013		3C.3	C1/SIAT4	640	640	320	320	160	320	320	640	160	640	320	640
A/Stockholm/6/2014		3C.3a	SIAT1/SIAT3	40	40	320	160	160	80	160	80	160	80	160	160
A/Stockholm/6/2014	isolate 2	3C.3a	E4/E1	160	160	160	320	80	320	160	320	40	80	160	320
A/Switzerland/9715293/2013		3C.3a	SIAT1/SIAT3	<	40	160	80	160	80	80	80	40	80	160	160
A/Switzerland/9715293/2013	clone 123	3C.3a	E4/E1	80	160	160	320	80	640	160	320	40	160	160	320
A/Hong Kong/5738/2014		3C.2a	MDCK1/MDCK2/SIAT3	80	40	160	160	80	40	160	640	160	320	320	320
A/Hong Kong/4801/2014		3C.2a	MDCK1/MDCK1	80	80	160	160	80	80	160	640	160	320	320	320
A/Hong Kong/4801/2014	isolate 1	3C.2a	E6/E2	40	80	80	80	40	<	320	640	160	640	640	320
A/Georgia/532/2015	plaq 20	3C.2a	SIAT1/SIAT5	80	80	160	160	80	80	160	320	80	640	320	320
<b>TEST VIRUSES</b>															
A/Belgium/G004/2017		3C.2a1	SIAT1	40	<	160	80	40	40	160	160	80	80	160	160
A/Belgium/G004/2017		3C.2a1	SIAT1	40	40	160	80	80	40	160	320	80	160	160	160
A/Belgium/G0687/2017		3C.2a	SIAT1	40	40	160	80	40	40	160	160	80	80	320	320
Assay	H8 (Guinea Pig RBC with 20nM Oseltamivir)							Vaccine NI 2015-16			Vaccine SH 2016 NI 2016-17				
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

#### Genetic characterisation

Very few influenza B viruses were circulating in Belgium during the 2016-2017 season. The four viruses that were genetically characterised belonged to clade 3 represented by the vaccine strain B/Phuket/3073/2013 contained in the quadrivalent vaccine. All of these sequences have been submitted to GISAID.

Vaccine virus

Reference virus

Circulating virus Belgium

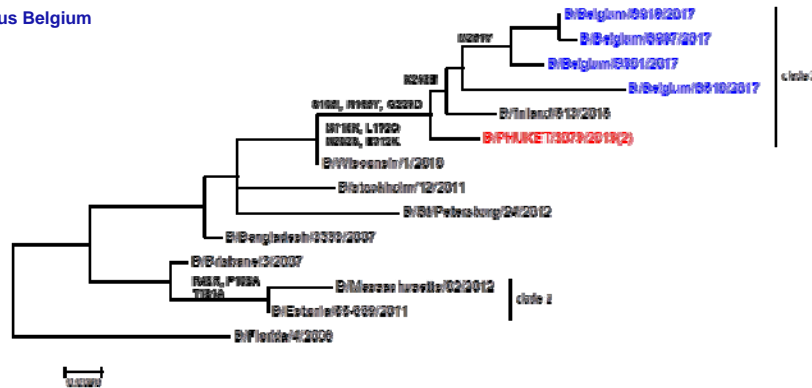


Figure 16. Phylogenetic analysis of the HA sequences of the influenza B/Yamagata viruses detected in Belgium during 2016-2017 season.

#### Antigenic characterisation

No influenza B virus was antigenically characterized during this season

## D.7.4 B Victoria

### Genetic characterisation

Only one influenza B virus from the Victoria lineage was detected during this season and belonged to clade 1A, the B/Brisbane/60/2008 clade which is contained in the trivalent vaccine.

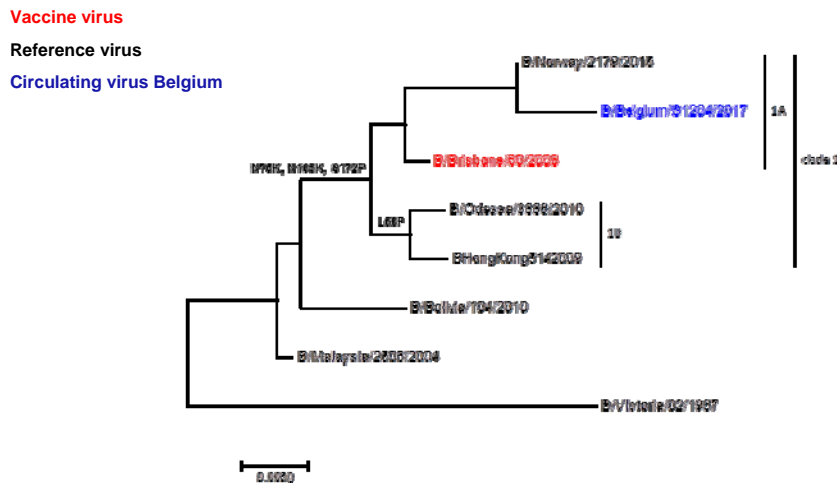


Figure 17. Phylogenetic analysis of circulating influenza B/Victoria viruses detected in Belgium during the 2016-2017.

### Antigenic characterisation

No influenza B viruses were antigenically characterized.

## D.8. Antiviral monitoring

All the strains (45) analysed were sensitive to neuraminidase inhibitors : Oseltamivir and Zanamivir.

## D.9 Composition of influenza virus vaccines for use in the 2016-2017 northern hemisphere influenza season

The WHO has published its recommendation for the vaccine composition that should be used in the 2017-2018 season in the Northern hemisphere.

### Trivalent vaccine

- A/Michigan/45/2015 (H1N1)pdm9 (new strain)
- A/Hong Kong/4801/2017 (H3N2) (no change)
- B/Brisbane/60/2008 (Victoria lineage) (no change )

## Quadrivalent vaccine

A/Michigan/45/2015 (H1N1)pdm9 (new strain)  
 A/Hong Kong/4801/2017 (H3N2) (no change)  
 B/Brisbane/60/2008 (Victoria lineage) (no change)  
 B / Phuket / 3073/2013-like virus (Yamagata 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	A/Hong-Kong/4801/2017	B/Brisbane/60/2008 VIC		B/Phuket/3073/2013 YAM

Figure 18. Evolution of the composition of the trivalent influenza vaccine 2000 – 2018

### D.10 Vaccine effectiveness

The preliminary estimation of the vaccine effectiveness was 28% (analysis performed for A(H3N2))(23).

### D.11 Severity

Although excess mortality (all causes) was observed at the onset of the influenza epidemic, the severity indicators (estimated from the surveillance of acute severe respiratory infections (SARI) by the sentinel network of hospitals) do not indicate that the epidemic was more severe than average this season. (Figure 1)

In season 2016-2017, fourteen percent of hospitalized patients with confirmed influenza developed severe complications, mainly patients over 65 years of age who had respiratory comorbidity. Six percent of patients under surveillance died during this hospital stay. Deaths were almost exclusively observed among patients over the age of 65 years (23).

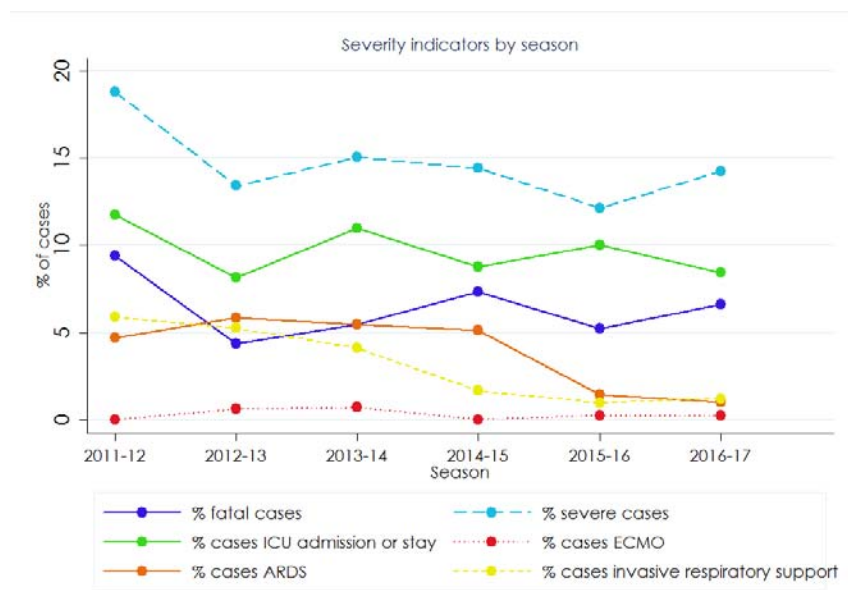


Figure 1 Evolution of indicators of severity in patients with confirmed influenza infection during the past 5 seasons in Belgium (Source: WIV-ISP: SARI surveillance by the network of sentinel hospitals)

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

From week 40-2016 (October 3) to week 20-2017 (May 21), there were 73,562 deaths within the total population, which amounts to 4,117 more deaths than expected. The significant excess mortality began in week 51 (December 21) and ended in week 7 (February 17). There were 2 peaks of mortality during the winter: The first peak occurred in week 2 (January 9) and the second peak in week 5 (January 30)(24).

Among persons over the age of 85 years, 31,672 deaths were observed, which means 2,757 deaths more than expected. The excess mortality this winter mainly affected this age group. In the same period, among the 65-84 years old, there were 31,045 deaths, corresponding to 1,195 excess deaths. There was no significant excess mortality in the less than 65 years. (Figure )

Within this period (and mainly at the beginning of the winter), there were 41 days when the minimum temperatures were below 0 degrees.

Hence, the first peak of excess mortality (all causes) occurred at the onset of the influenza epidemic, while the second peak coincided with the peak in the incidence of influenza-like illness.

### Mortality winter 2016 - 2017

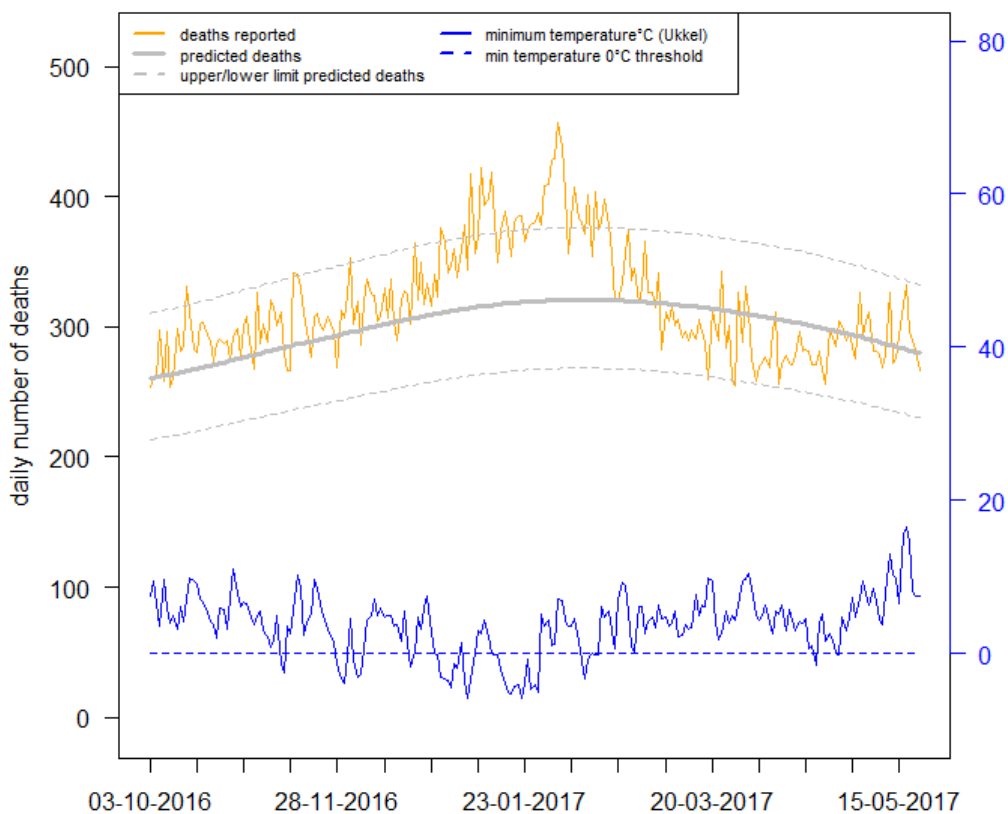


Figure 20 Excess (all causes) and minimum temperature in season 2016-2017 (Source : WIV-ISP: BE-MOMO surveillance excess mortality)

## E. Conclusion

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The 2016–2017 influenza season was of medium intensity and lasted for 7 weeks. During the epidemic peak, a total number of 745 ILI per 100.000 inhabitants was reached. A(H3N2) viruses predominated during this season. The overall percentage of positivity for influenza was higher in the ILI surveillance (51.5%) as compared to the SARI surveillance (39.5%), which was 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. A few **A(H1N1)pdm2009** were sequenced and belonged to group 6B.1, represented by the reference strain A/Michigan/45/2015. These viruses are antigenically homologous and similar to the vaccine strain A/California/7/2009.

The majority of the **A(H3N2)** strains belonged to the newly emerging subclade 3C.2a1, represented by the reference strain A/Bolzano/7/2016 which is antigenically close to the vaccine strain A/Hong Kong/4801/2014.

Independently of this surveillance, three influenza **B Yamagata** viruses which were genetically characterized belonged to clade 3 represented by the vaccine strain B/Phuket/3073/2013 present in the quadrivalent vaccine.

Only one influenza **B Victoria** virus was detected during this season and belonged to clade 1A, the B/Brisbane/60/2008 clade which is present in the trivalent vaccine.

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, 41% of the influenza negative patients were positive for one or more other respiratory viruses, whereas in the SARI population, this percentage reached 51%. This suggests 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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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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