



Virological Surveillance of Influenza in Belgium

Season 2013-2014

VIRAL DISEASES

National influenza Centre (WHO)

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INFECTIOUS DISEASES AMONG THE GENERAL POPULATION

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A. Abstract

The 2013–2014 influenza season was mild and of short duration. In Belgium, the epidemic threshold was crossed at the beginning of February (week 6 -2014), and mid-March (week 11-2014) the epidemic was already over. The peak was observed at week 9-2014, with about 311 ILI (influenza-like-illness) cases per 100.000 inhabitants which is quite lower than previous seasons.

The first influenza positive case was detected in week 46-2013 and an increasing number of influenza positive cases could be detected starting from week 4-2014 to reach a percentage of virus confirmed cases of 60% in week 10-2014. Both Influenza A(H1N1) and A(H3N2) circulated with a slight predominance of A(H3N2).

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 the monitoring of influenza activity (intensity, duration, severity,...) all over the year, the determination of type and subtypes of circulating strains and their antigenic and genetic characterization, the contribution to the annual determination of the influenza vaccine content, the monitoring of resistance to antivirals and the detection of new potentially pathogenic influenza viruses. Furthermore, during the previous season 2012-2013, two new highly lethal respiratory viruses emerged (influenza A (H7N9) and MERS-CoV) which demonstrated the importance of the surveillance of respiratory pathogens for the public health.

C. Methods

C.1. Surveillance

C.1.1. Sentinel Surveillance of ILI

Network of Sentinel General Practitioners

In Belgium, the influenza surveillance is performed by the NIC (National Influenza Centre), in collaboration with the Unit of Health Services Research and the Unit of Infectious Diseases among the General Population of the Scientific Institute of Public Health in Brussels. A network of sentinel general Practitioners (SGPs) is 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 participation to the selection of next-season influenza vaccine strains. The development of capability to detect new emerging viruses and the estimation of vaccine effectiveness are also important tasks (2).



Clinical surveillance

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

Virological surveillance

A subset of these SGPs are also involved in the virological surveillance.

During the season 2013-2014, 94 sentinel physicians were involved in the virological influenza surveillance (44 for the Wallonia-Brussels Federation, 50 for the Flemish region). Physicians were invited to collect 2 nasopharyngeal swabs/week (each week, the two first patients presenting for ILI).

Sampling kits are sent to all physicians. Each kit contains the materials required to collect nasopharyngeal swabs (2 nostrils + 1 throat) in patients with influenza-like illness. The material consists of tubes containing 3 ml of transport medium [UTM (COPAN)], swabs [flocked Swabs (COPAN)] and patient registration forms. Samples and forms are returned to the National influenza Centre by mail (postage paid) and new kits are regularly sent depending on the shipment of samples.

C.1.2. Sentinel Surveillance of SARI

Network of sentinel hospitals

Following the A(H1N1)2009 pandemic, the WHO and the European Centre for Disease Prevention and Control (ECDC) recommended hospital-based surveillance of severe acute respiratory infections (SARI) as a tool to monitor severe disease caused by influenza (3). This can complement surveillance of outpatient monitoring of influenza like illness (ILI) or acute respiratory illness (ARI) to cover the full spectrum of influenza-related disease. As a result, the Belgian National Influenza Center has extended, since 2010, its surveillance to SARI cases. The main objectives were 1) to build a clinical and virological data base 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 influenza season 2013-2014, six hospitals located in the three regions of the country participated to the surveillance. The SARI case definition was: an acute respiratory illness with onset within the last seven days and fever of $\geq 38^{\circ}\text{C}$ and cough or dyspnea, and requiring hospitalisation (24h or more). As we are mostly interested in severe influenza cases, the surveillance was carried out 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 contain the materials required to collect 2 nasopharyngeal swabs (nostrils and throat) in patients responding to the SARI case definition. The material consists of tubes containing 3 ml of transport medium [UTM (COPAN)], swabs [flocked Swabs (COPAN)] and



patient registration forms. Samples and forms are returned to the NIC by mail (postage paid) and new kits were sent regularly to hospitals depending on the shipment of samples. Patients information, clinical data and laboratory results were encoded in a dedicated web based database protected by a login and password.

The following hospitals participated to the SARI surveillance season 2013-2014

- CHU UCL (Mont-Godinne) (Yvoir)
- CHU Saint-Pierre (Brussels)
- AZ St Jan (Brugge)
- UZ Brussel
- Jessa Ziekenhuis (Hasselt)
- Grand hôpital de Charleroi (Charleroi)

C.1.3. Non Sentinel Surveillance

A letter is sent to hospitals and laboratories across the country to encourage them to collect samples from patients presenting with severe influenza in particular specific conditions: ARDS (acute respiratory distress syndrome), ECMO (extracorporeal membrane oxygenation), death, suspicion of antiviral resistance, returning from abroad. This surveillance is planned throughout the year.

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

Influenza A (H5N1)

Since 2003, 650 human infections with highly pathogenic H5N1 viruses have been reported to the World Health Organization (WHO) by 15 countries (4). About 60% of these 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 National Influenza Centre (NIC) of Belgium at the Scientific Institute of Public Health was appointed as reference laboratory for testing of the H5N1 suspected cases, 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). Since June 2013 until 27 June 2014, 317 cases were reported. Phylogenetic analysis of the eight genes indicate that the new influenza A (H7N9) virus is a combination of genomic fragments originating from two avian influenza A (H9N2) viruses previously detected in chickens and domestic and wild birds, influenza A/H7 and A/N7 viruses (6). 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. 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 advises countries to continue strengthening influenza surveillance, and reporting human infections.



The National influenza Centre of Belgium 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 Middle East Respiratory Syndrome (MERS) coronavirus (CoV) is a newly emerging beta-coronavirus that causes a severe acute respiratory infection in the deep airways and lungs. The first human cases were identified in April 2012. Most people who have been confirmed to have MERS-CoV infection developed severe acute respiratory illness. About 50% of people confirmed to have MERS-CoV infection have died. So far, all the cases have been linked to countries in and near the Arabian Peninsula. The epidemiological pattern of human infections is highly suggestive of a zoonotic infection. The animal vector or reservoir remains unidentified (7) but bats and camels might be involved. In Belgium, 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).

C.2. Laboratory tests

C.2.1. Real time RT PCR Influenza

Nasopharyngeal swabs received at the National Influenza Centre are tested with different real-time RT-PCRs : A/B typing, subtyping (influenza A) and determination of the lineage (influenza B). The sequence of tests is presented in figure 1.

Typing A/B

Duplex Real time RT PCR Influenza A/B : adapted protocols (10,11). Primers and probes for the matrix gene (influenza A) and hemagglutinine gene (influenza B).

Subtyping A (H1, H3, N1, N2)

in case of influenza A positive, the subtype is determined.

- RT PCR Influenza A/H1 sw: adapted protocol from CDC (10): primers and probes are chosen in the hemagglutinine gene.
- RT PCR 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)

in case of 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.

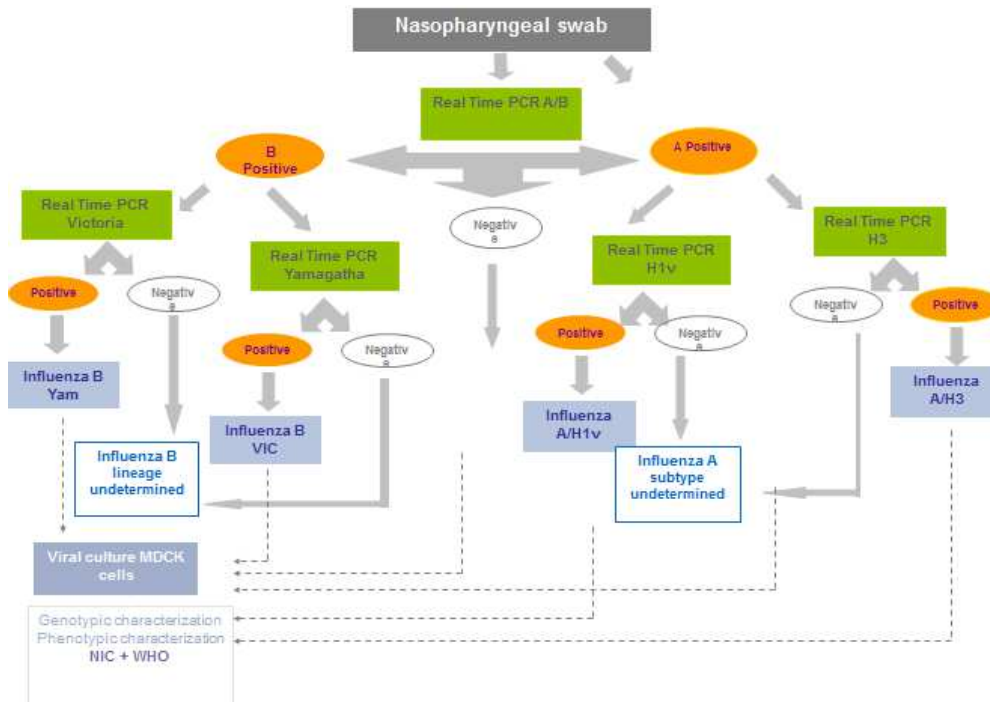


Figure 1. Sequence of the Real time PCR tests used during the 2013-2014 season.

Subtyping (H5, H7, ...)

Samples from suspected cases of avian influenza are submitted to the duplex real time PCR A/B for typing and in case of positivity to different real time PCR for subtyping in function of 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 a specific real time PCR 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 hospital network (SARI samples) can additionally be submitted to 4 quadriplex Real time PCRs detecting 16 other respiratory viruses (RSV A and B, Parainfluenza viruses 1, 2, 3, 4, Rhinoviruses, human Metapneumoviruses, Paraechoviruses, Bocaviruses, Enteroviruses, Adenoviruses and different Coronaviruses including the MERS-CoV) (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	MERS CoV	FAM

The protocols have been adapted from Otsby *et al.* (19) with some modifications (primers for rhinoviruses as described in Hombrouck *et al.*, 2009 (20), and primers for MERS CoV as described by Corman *et al.* (18).

C.2.4. Genetic characterisation

Genetic characterization is performed by sequencing of the HA gene of influenza viruses. Sequencing of PCR products is realized with ABI 3130xl (ABI) using Big Dye Terminator v 3.1 Cycle Sequencing kit. Sequence comparison, alignments and phylogenetic trees are realized using ClustalX, MEGALIGN (DNASTAR) en MEGA 5 programs. 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

An important task of the centre is the monitoring of antiviral resistance of circulating influenza viruses. The most commonly used antivirals are the neuraminidase inhibitors [oseltamivir (Tamiflu[®]) and zanamivir (Relenza[®])]. Influenza strains may develop phenotypic resistance to these antivirals, and thus become less susceptible to their inhibitory activity. Phenotypic resistance is often associated with mutations at the level of the viral target protein of the inhibitor, which causes reduced binding with the antiviral. For example, the Y275H mutation in N1 is associated with resistance to oseltamivir.

Resistant strains can be detected by phenotypic tests based on IC₅₀ measurement or genotypic tests based primarily on sequencing techniques to highlight NA mutations compared to reference sequences.



C.2.6. Sending of strains to London WHO CC

Each year, about fifty representative Belgian strains are sent to the WHO Collaborating 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 of the strains to be included for the next season flu vaccine by the WHO.

D. Results

D.1 Sentinel surveillance of ILI

D.1.1 Clinical surveillance

In Belgium, the epidemic threshold for this season was set at 141 ILI/100.000 inhabitants. The threshold was crossed in week 6-2014, and the epidemic was declared in week 8-2014 (2 consecutive weeks above threshold). Since week 4-2013, the number of consultations for ILI increased to reach the level of 311/100000 inhabitants in week 9-2014. After week 10-2014, the number of ILI decreased and the number was below the threshold after week 11 (Figure 2). The epidemic lasted 6 weeks which was particularly short compared with previous seasons.

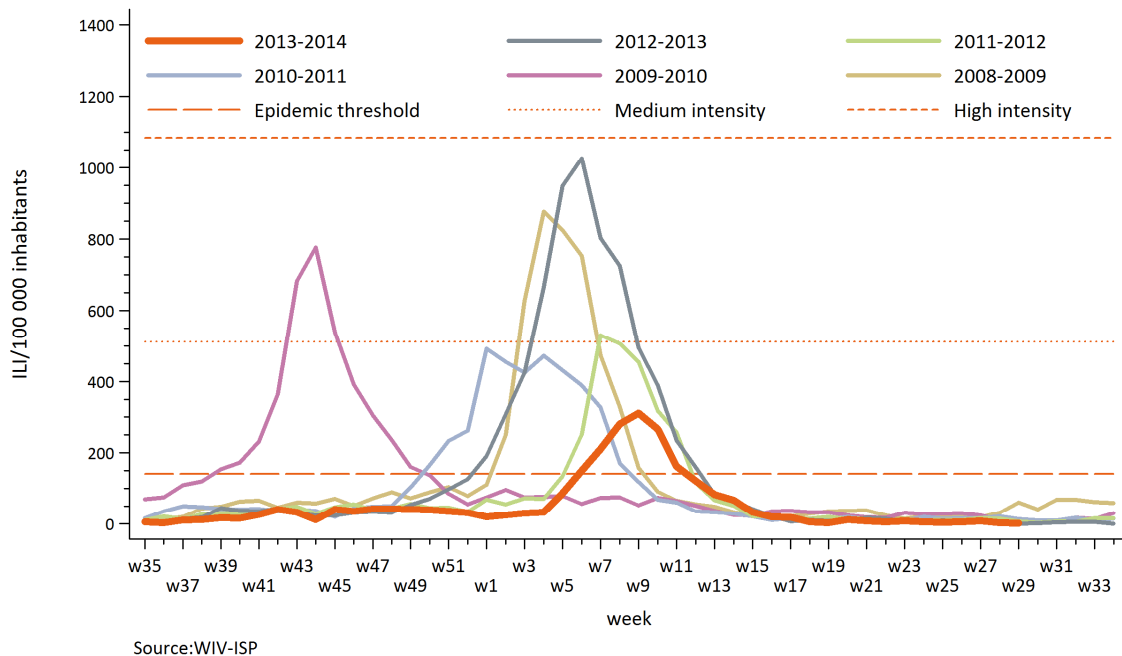


Figure 2. Weekly incidence of ILI for 100.000 inhabitants



D.1.2 Virological surveillance

The influenza surveillance period started in week 40-2012 (September 30, 2013) and continued through week 20-2014 (May 18, 2013). The monitoring period lasted 33 weeks.

Origin of samples

A total of 94 GPs (50 for Flanders, 44 for Wallonia-Brussels Federation) participated to the virological surveillance and sent 734 nasopharyngeal swabs to the NIC.

Number of Nasopharyngeal swabs

Flanders : 387 (52.5 %)

Wallonia-Brussels : 347 (47.2 %)

Total : 734

Typing and subtyping results

The first influenza virus-positive case was detected in week 46-2013 and an increasing number of influenza virus-positive cases could be detected starting from week 4-2014 to reach a percentage of virus-confirmed cases of 60% in week 10-2014.

From week 40-2013 to week 20-2014, a total of 734 respiratory samples were collected by sentinel GPs and tested at the Belgium National influenza Centre, among which 249 (33.9%) were positive for influenza A and only 4 (0.54%) were positive for influenza B viruses. Among the subtyped influenza A viruses, 146/242 (60.3%) were A(H3N2) and 96/242 (39.7%) were A(H1N1)pdm2009. Seven samples were non-subtypable due to low viral load. Of the 4 analysed influenza B viruses, 2 were of the B/Yamagata lineage and 1 was from the B/Victoria lineage. For one sample the lineage could not be determined due to low viral load (Figure 3).

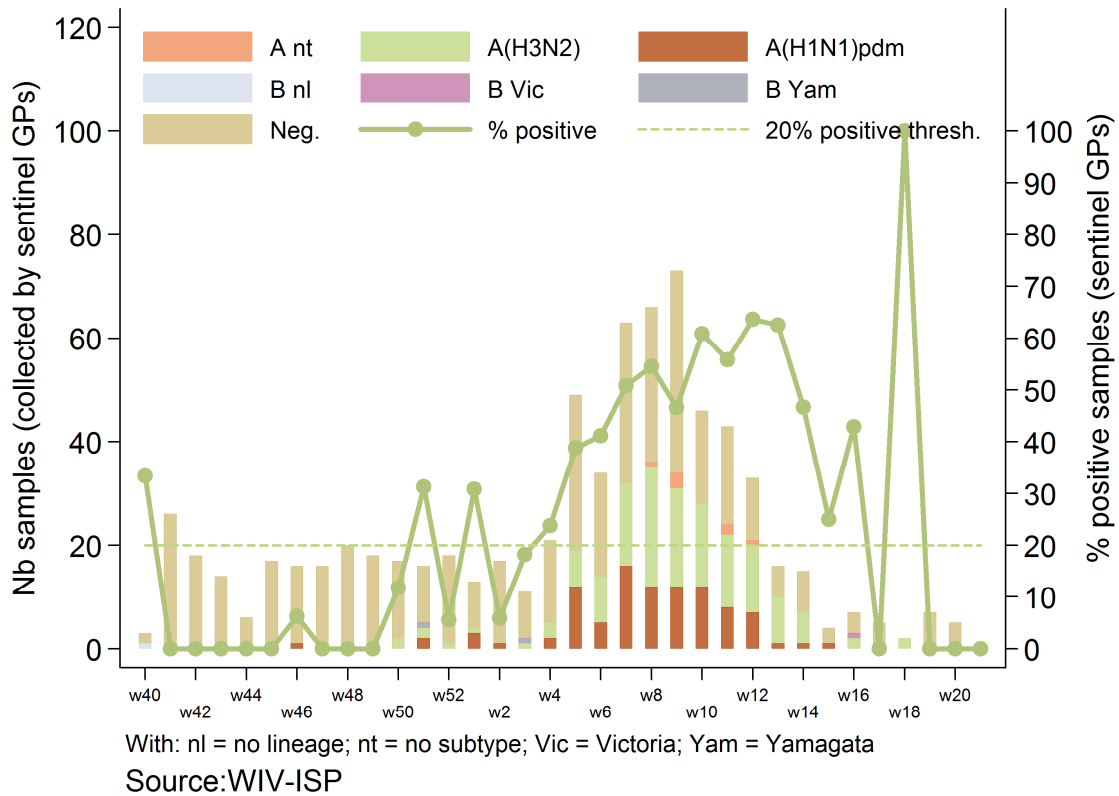


Figure 3. Weekly detection of influenza viruses in Belgium from week 40-2013 to week 20-2014 in the network of sentinel GPs

Influenza viruses according to age group

We received a higher number of samples from the age group 15 to 44 year old. The percentage of positivity for influenza did not vary between the different age groups (Fig 4).

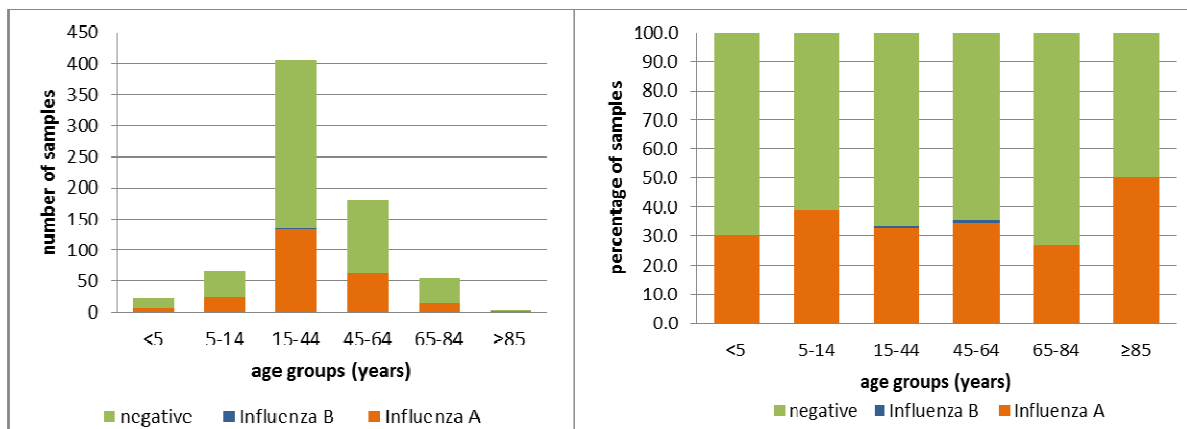


Figure 4. Influenza viruses according to age group



The distribution of influenza A subtypes does not vary with age as shown in figure 5. Influenza A(H3N2) was the predominant virus detected in samples from children and adults.

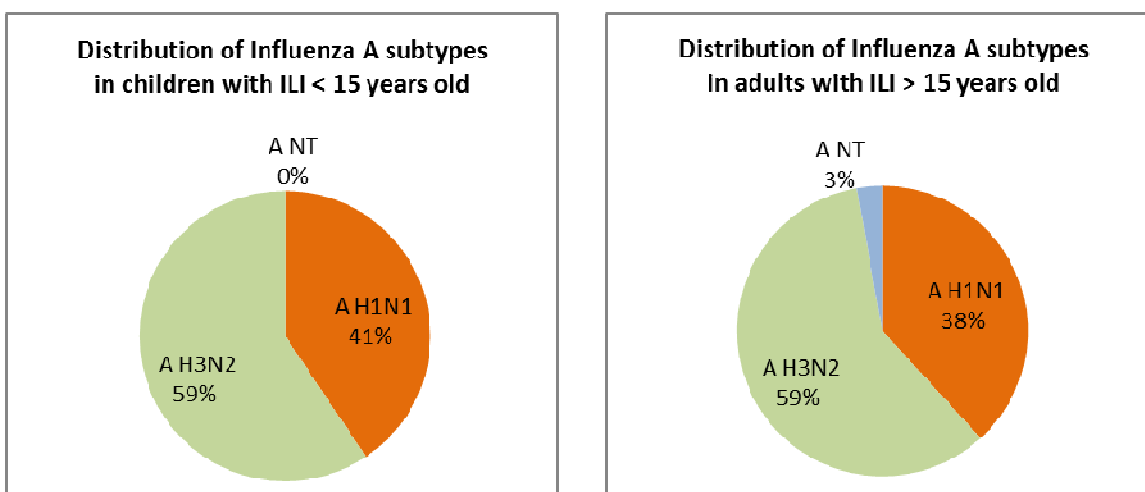


Figure 5. Influenza A virus subtypes according to age group (NT= non subtyped)

D.2 Sentinel Surveillance of SARI

D.2.1 Virological surveillance

Surveillance of SARI started week 6/2014, after the first influenza cases were recorded by the SGP sentinel GPs and ended week 16-2014, about one month after the end of the epidemic.

Origin of samples

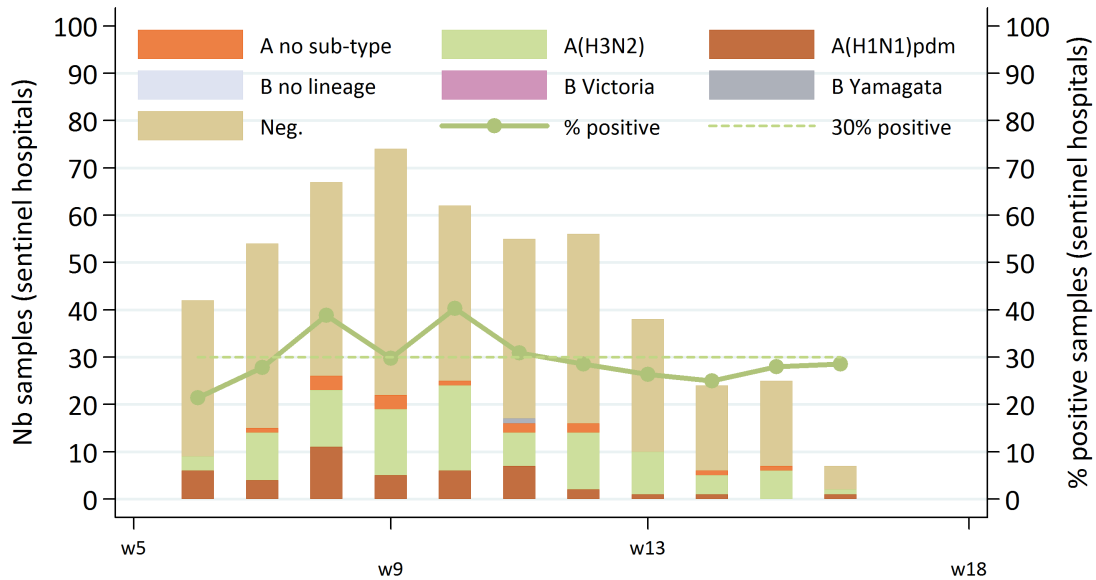
A total of 522 samples were collected and sent by the hospital network, among which 498 corresponded to case definition and were analysed.

Typing and subtyping results

From week 6-2014 to week 16-2014, 498 respiratory samples from the sentinel network of hospitals were analysed by the National Centre for Influenza among which 152 (30.7%) were positive for influenza with 151 (99.3%) influenza A and 1 (0.6%) influenza B. Six samples could not be analysed due to the low quality of the sample. Among the analysed influenza A viruses, 47 (31%) were A(H1N1)pdm2009 and 91 (60.2%) were A(H3N2). Thirteen samples (8.6%) could not be subtyped due to low viral load (Ct>35). The influenza B virus was of the Yamagata lineage (Figure 6).



Influenza tests in SARI patients



Source:WIV-ISP - nl = no lineage; nt = no subtype
 Comment: 31% positive samples.
 Among SARI = 31% influenza A and 0% influenza B.
 Among influenza A: 96/154 are A(H3N2) and 44/154 are A(H1N1)09pdm.
 Among influenza B: 1/ 1 are Yamagata lineage and 0/ 1 are Victoria lineage
 0 co-infections Influenza A + B in adults.

Figure 6. Weekly detection of influenza viruses in Belgium in the SARI network from week 6-2014 to week 16-2014

Age distribution of influenza viruses by types and subtypes

A higher number of samples was collected from children under five years old and in this age group we observed a significantly lower positivity rate for influenza (21%). For the other age groups, the positivity rates were similar (around 30%-40%) (Fig.7)

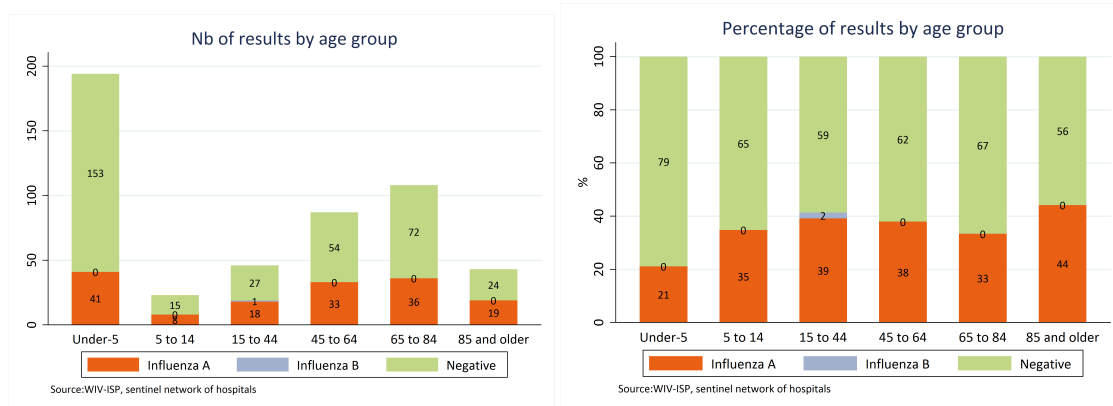


Figure 7. Influenza viruses according to age group



The distribution of influenza subtypes doesn't vary with age as shown in figure 8. In children below 15 years old 52% of influenza infection was caused by A(H3N2), whereas in adults, 64% of influenza infections was caused by A(H3N2).

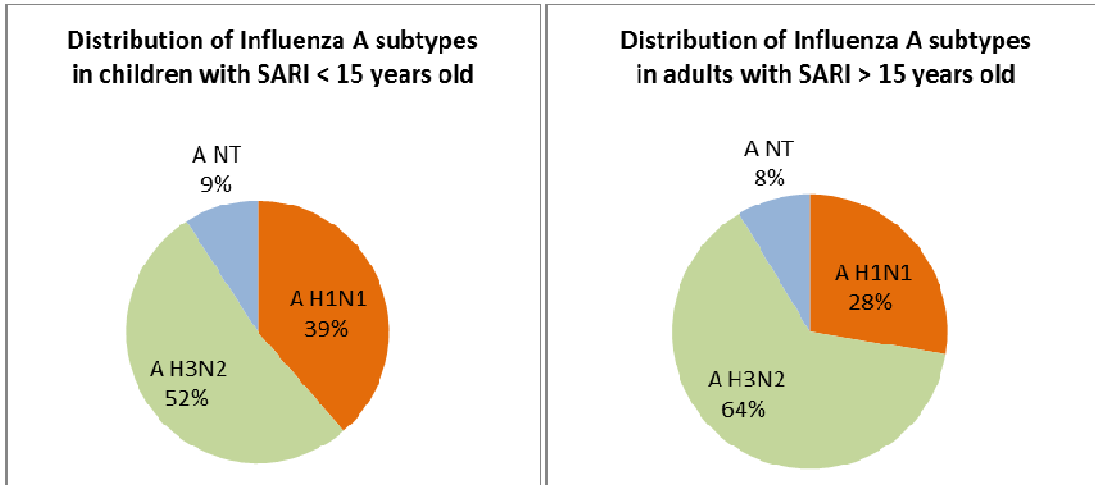


Figure 8. Proportion of the different types and subtypes of influenza viruses among positive samples by age group

D.3 Non sentinel surveillance

Fifty nine respiratory samples from patients with severe influenza were sent from hospitals around the country during the 2013-2014 season and inter season and analysed at the NIC for confirmation and subtyping. Twenty eight samples were influenza A positive among which 4 were A(H1N1)pdm2009, 23 were A(H3N2), and 1 was non-subtypable due to low viral load. None of the samples was positive for influenza B.

Among those respiratory samples, 35 were from an influenza outbreak which occurred in April in a nursing home in Vlaams-Brabant. Swabs were taken from 32 residents and 3 staff members, among which 15/32 and 2/3 were influenza positive. Nearly all the residents were vaccinated against influenza at the beginning of the flu season. The virus was characterized to be an A(H3N2) virus, belonging to subgroup 3C. The strain was sensitive to oseltamivir and zanamivir. Serological analyses of patient sera taken during the acute phase, showed non-protective HI titers against the vaccine strain A/Texas/50/2012, despite previous vaccination circa 4 months earlier.

D.4 Suspected cases of Avian Influenza

One sample from a patient returning from China was sent for Influenza A(H7N9) analysis. The patient presented mild symptoms and was negative for Influenza.

D.5 Suspected cases of MERS CoV

Six samples from suspected cases were analysed for MERS-CoV and other respiratory viruses. The first case was a patient returning from Saudi Arabia, which did not present serious symptoms, the sample was negative for influenza and MERS CoV. The second case was a patient returning from Saudi Arabia, the swab was negative for MERS CoV and positive for



Influenza A (H1N1pdm). The third one was from a patient which had been in transit in Abu-Dabi, also this sample was negative for influenza and MERS CoV. Neither of these three patients responded to the case definition.

The last three cases were two sisters which had returned from Dubai to Belgium already 5 weeks before, and had mild respiratory symptoms which became more severe after 4 weeks. In addition, a sample from the mother (as close contact, with mild respiratory symptoms) was analysed. Both, nose, throat and serum samples of all three cases were negative for MERS-CoV RNA. No antibodies against MERS-CoV could be detected (serology performed at Erasmus MC Hospital, Rotterdam).

D.6. Characterisation of the viruses

D.6.1 A(H1N1)pdm2009

Genetic characterisation

Since the emergence of A(H1N1)pdm2009, eight genetic groups, defined by specific amino acid substitutions in HA1, have emerged and circulated to various extents. In recent months viruses from two genetic groups have predominated:

Group 6 represented by **A/St Petersburg/27/2011** carries a number of HA gene mutations and is characterised by K283E, D97N and S185T substitutions.

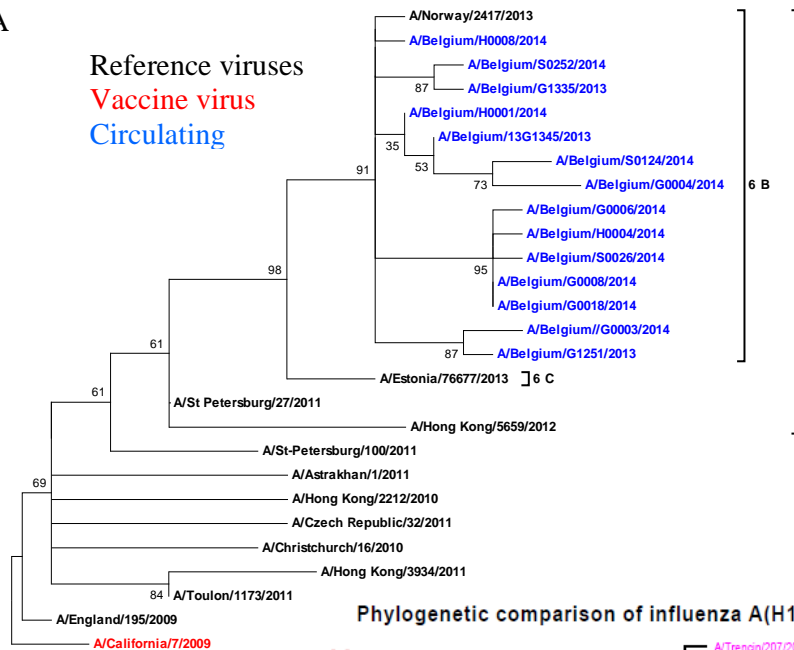
Group 7 represented by **A/St Petersburg/100/2011** carries a number of HA gene mutations and is characterised by S84G, S143G, S185T and A197T substitutions, with recently circulating viruses carrying additional substitutions at one or more positions.

The hemagglutinine gene (HA 1 fragment) of 15 Influenza A(H1N1)pdm2009 samples was sequenced. A phylogenetic tree showing the vaccine strain, reference strains and A(H1N1)pdm2009 isolates is presented in figure 9A/B. All the sequenced viruses belonged to group 6.



9A

Reference viruses
 Vaccine virus
 Circulating



Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes

Vaccine virus
 Reference viruses

Collection date
 Feb 2014
 Mar 2014
 Apr 2014
 Jun 2014

9B

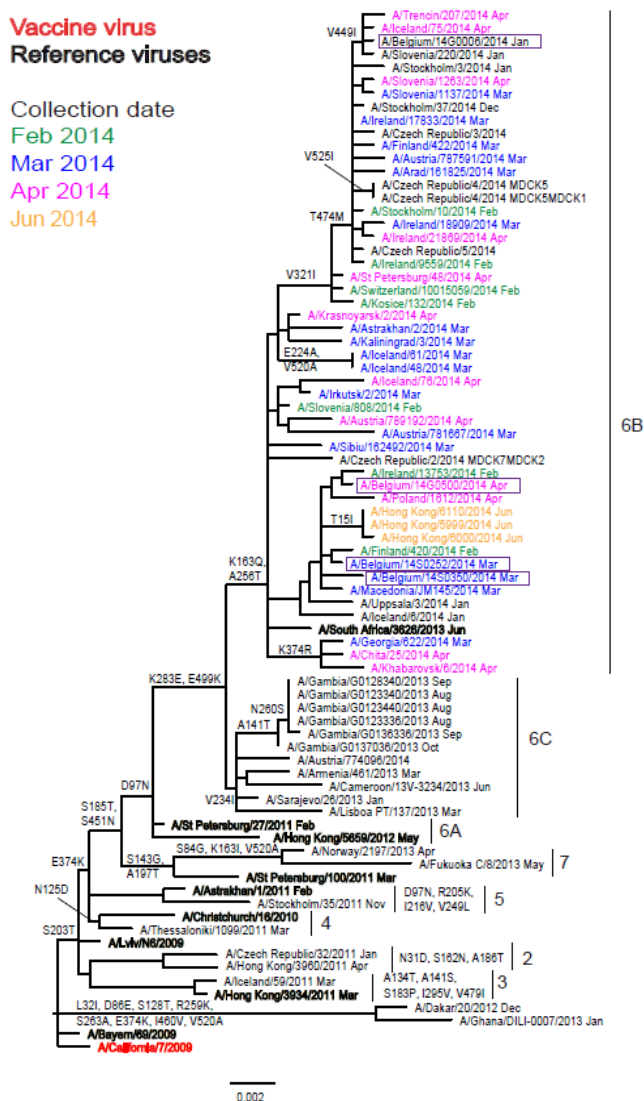


Figure 9A/B. Phylogenetic analysis of influenza A(H1N1)pdm09 strains



Antigenic characterisation

Eight of the fifteen A(H1N1) viruses that were sent to WHO CC-London were successfully propagated in cell culture. Hemagglutination-inhibition (HI) analysis showed that they reacted well with the antiserum raised against the vaccine virus A/California/07/2009, being recognised at the same titre as A/California/07/2009. The viruses were also well recognised by antisera raised against most other reference viruses (Table 2).

Table 2a/b. Antigenic analyses of influenza H1N1pdm09 viruses

Antigenic analyses of influenza A(H1N1)pdm09 viruses												
Haemagglutination inhibition titre ¹												
Viruses	Collection date	Passage History	Post infection ferret antisera									
			A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/09/34	A/Chch 16/10 F30/10	A/HK 3934/11 F21/11	A/Astrak 1/11 F22/11	A/St. P 27/11 F23/11	A/St. P 100/11 F24/11	A/HK 5659/12 F30/12	
Genetic group												
REFERENCE VIRUSES												
A/California/7/2009	2009-04-09	E1/E2	1280	640	1280	640	320	320	320	320	320	320
A/Bayern/69/2009	2009-07-01	MDCK5/MDCK1	320	640	160	40	40	<	<	40	80	80
A/Lviv/N6/2009	2009-10-27	MDCK4/S1/MDCK3	640	1280	1280	160	80	<	320	160	320	320
A/Christchurch/16/2010	2010-07-12	E1/E3	1280	1280	1280	5120	1280	1280	1280	2560	2560	2560
A/Hong Kong/3934/2011	2011-03-29	MDCK2/MDCK4	640	320	640	320	1280	1280	1280	2560	1280	1280
A/Astrakhan/1/2011	2011-02-28	MDCK1/MDCK5	1280	640	1280	1280	1280	2560	2560	5120	5120	5120
A/St. Petersburg/27/2011	2011-02-14	E1/E3	1280	640	1280	1280	1280	2560	1280	5120	2560	2560
A/St. Petersburg/100/2011	2011-03-14	E1/E3	640	640	1280	640	1280	1280	1280	2560	2560	2560
A/Hong Kong/5659/2012	2012-05-21	MDCK4/MDCK2	320	160	320	160	640	640	640	1280	1280	1280
TEST VIRUSES												
A/Belgium/13G1251/2013	2013-11-16	MDCK2	1280	1280	1280	1280	2560	2560	2560	5120	5120	5120
A/Belgium/14G0003/2014	2014-01-02	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	5120	5120
A/Belgium/14G0004/2014	2014-01-03	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	5120	2560

1. <= <40

Vaccine

Antigenic analyses of influenza A(H1N1)pdm09 viruses (2014-06-11)

Antigenic analyses of influenza A(H1N1)pdm09 viruses (2014-06-11)												
Haemagglutination inhibition titre ¹												
Viruses	Collection date	Passage History	Post infection ferret antisera									
			A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/09/34	A/Chch 16/10 F30/10	A/HK 3934/11 F21/11	A/Astrak 1/11 F22/11	A/St. P 27/11 F23/11	A/St. P 100/11 F24/11	A/HK 5659/12 F30/12	A/SA 3626/13 F3/14
Genetic group												
REFERENCE VIRUSES												
A/California/7/2009	2009-04-09	E1/E2	640	640	640	160	160	320	320	320	320	320
A/Bayern/69/2009	2009-07-01	MDCK5/MDCK2	160	320	160	40	40	40	40	80	80	80
A/Lviv/N6/2009	2009-10-27	MDCK4/S1/MDCK3	320	1280	640	160	80	80	160	320	640	160
A/Christchurch/16/2010	2010-07-12	E1/E3	1280	1280	1280	5120	640	1280	2560	2560	5120	1280
A/Hong Kong/3934/2011	2011-03-29	MDCK2/MDCK3	320	160	640	320	640	640	640	1280	1280	640
A/Astrakhan/1/2011	2011-02-28	MDCK4/MDCK1	640	320	320	320	640	640	640	1280	2560	640
A/St. Petersburg/27/2011	2011-02-14	E1/E3	640	640	640	640	640	1280	1280	2560	2560	1280
A/St. Petersburg/100/2011	2011-03-14	E1/E3	640	640	1280	640	640	1280	1280	1280	2560	1280
A/Hong Kong/5659/2012	2012-05-21	MDCK4/MDCK2	320	160	640	640	640	1280	1280	2560	5120	640
A/South Africa/3626/2013	2013-06-06	E1/E2	640	320	640	640	640	640	640	1280	1280	1280
TEST VIRUSES												
A/Belgium/14G0500/2014	2014-04-10	MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560	2560
A/Belgium/14S0252/2014	2014-03-04	MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560	2560
A/Belgium/14S0350/2014	2014-03-13	MDCK2	1280	640	1280	1280	5120	2560	1280	2560	2560	1280
A/Belgium/14H0004/2014	2014-02-04	MDCK1	1280	640	1280	1280	2560	2560	1280	5120	2560	2560
A/Belgium/14G0006/2014	2014-01-04	MDCK1	1280	640	1280	2560	2560	2560	2560	5120	2560	2560

1. <= <40

Vaccine

G155E
G155E-G, D222G

D.6.2 A(H3N2)

Genetic characterisation

A phylogenetic tree showing the vaccine strain, reference strains and 26 A(H3N2) isolates is presented in figure 10A. Figure 10B shows circulating strains in Belgium and other European countries.

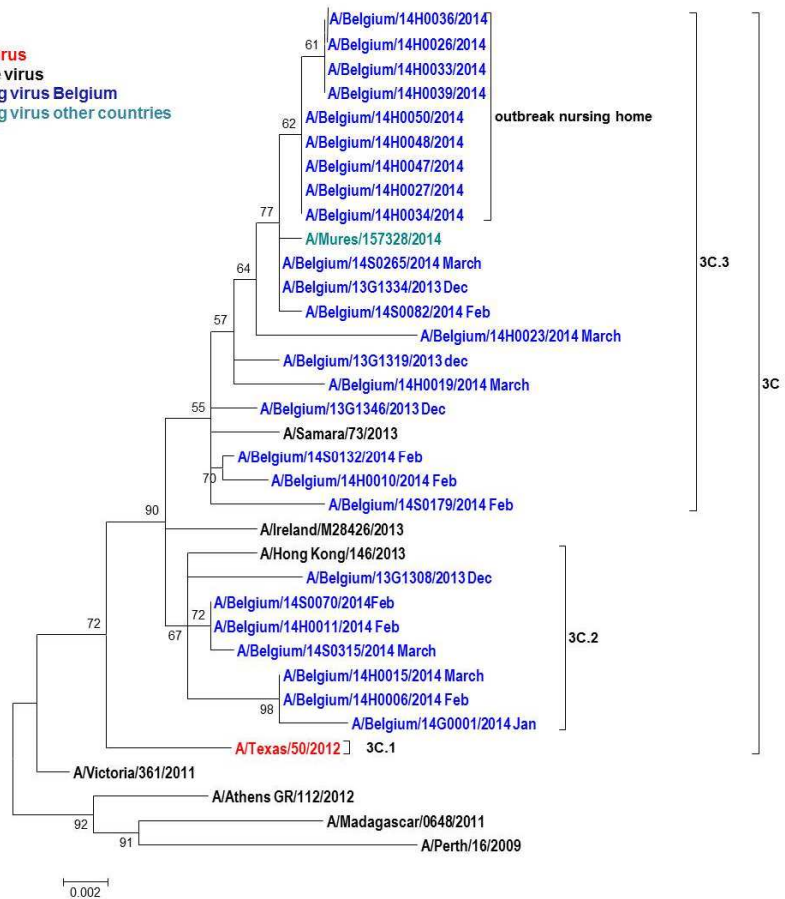
All sequenced A(H3N2) viruses belonged to subgroup 3C, and could be subdivided into clade 3C.2 and 3C.3, represented by respectively A/Hong Kong/146/2013 and A/Samara/73/2013.



Viruses in both genetic clades are antigenically indistinguishable from each other as well as from viruses in clade 3C.1 (includes A/Texas/50/2012 virus) in HI and neutralization assays.

10A

Vaccine virus
 Reference virus
 Circulating virus Belgium
 Circulating virus other countries





However, test viruses reacted well with ferret antisera raised against reference viruses that are genetically close to A/Victoria/361/2011 but propagated in cells. These data indicate that significant antigenic drift has not occurred, but the cell-grown test viruses are antigenically distinguishable from egg-propagated viruses, which is probably due to the induction of mutations in the H3N2 strain during the (vaccine) production on eggs.

Table 3A/B. Antigenic analyses of influenza A H3N2 viruses

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir)													
Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre ¹									
				Post infection ferret antisera									
				A/Perth 16/09 F35/11	A/Stock 18/11 F28/11	A/Iowa 19/10 F15/11	A/Vic 361/11 T/C F11/13	A/Athens 112/12 Egg F42/13	A/Texas 50/12 F24/13	A/Samara 73/13 F24/13	A/Serbia NS-210/13 F39/13	A/HK 146/13 F40/13	NIB-85 146/13 F45/13
REFERENCE VIRUSES													
A/Perth/16/2009		2009-07-04	E3/E3	640	160	160	160	320	160	160	80	160	160
A/Stockholm/18/2011	3A	2011-03-28	SIAT4	80	640	320	320	640	320	1280	320	320	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	1280	1280	1280	2560	1280	1280	640	1280	640
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT6	80	320	160	640	640	320	640	320	320	320
A/Athens/112/2012	3B	2012-02-01	SIAT4	80	320	160	640	640	320	640	320	320	320
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	640	1280	1280	1280	1280	1280	1280	1280	1280	1280
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT2	160	640	320	1280	1280	320	1280	640	1280	640
A/Serbia/NS-210/2013	3C.3	2013-01-18	E5/E1	320	1280	640	1280	1280	1280	1280	1280	1280	1280
A/Hong Kong/146/2013	3C.2	2013-01-11	E5/E1	320	2560	1280	640	1280	640	1280	640	2560	640
NIB-85 (A/Almaty/2958/2013)	3C.3	2013-01-27	E5/E1	640	1280	1280	1280	1280	1280	1280	1280	1280	1280
TEST VIRUSES													
A/Belgium/13G1319/2013	3C.3	2013-12-12	SIAT2	<	160	160	320	640	160	640	320	320	320
A/Belgium/13G1334/2013	3C.3	2013-12-16	SIAT2	<	80	40	320	640	80	640	320	160	320
A/Belgium/13G1326/2013	3C.2	2013-12-19	SIAT2	40	160	80	320	320	80	640	160	320	160
A/Belgium/14G0001/2014	3C.2	2014-01-03	SIAT2	40	160	160	320	640	80	640	160	320	320
A/Belgium/13G1348/2013	3C.3	2013-12-27	SIAT3	<	160	80	320	640	80	640	160	160	160
A/Belgium/13G1308/2013	3C.2	2013-12-10	SIAT4	80	320	160	320	640	160	640	320	320	320
										Vaccine			

1. < = <40

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2014-06-10

Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre ¹									
				Post infection ferret antisera									
				A/Perth 16/09 F35/11	A/Vic 361/11 T/C F09/12	A/Texas 50/12 Egg F42/13	A/Samara 73/13 F24/13	A/Serbia NS-210/13 F39/13	A/HK 146/13 F40/13	A/Sth Africa/Stockholm 4655/13 1/13	A/St'holm 1/13 6/14	F12/14	F14/14
REFERENCE VIRUSES													
A/Perth/16/2009		2009-07-04	E3/E3	640	160	160	160	160	160	40	80	40	40
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT3	80	320	320	640	320	320	80	160	320	320
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	320	1280	1280	1280	1280	1280	80	160	320	320
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT2	80	320	320	640	640	640	80	160	640	640
A/Serbia/NS-210/2013	3C.3	2013-01-18	E5/E1	320	1280	1280	1280	1280	1280	80	160	320	320
A/Hong Kong/146/2013	3C.2	2013-01-11	E5/E1	320	640	640	1280	1280	640	2560	80	160	160
A/South Africa/4655/2013	3C.3	2013-06-25	E7 clone 101-60	80	80	160	320	160	320	320	160	80	80
A/Stockholm/1/2013	3C.2	2013-01-13	E7 clone 36-18	40	80	160	320	160	320	320	320	160	160
A/Stockholm/6/2014	3C.3a	2014-02-06	SIAT2/SIAT1	<	40	40	160	160	80	40	80	320	320
TEST VIRUSES													
A/Belgium/14S0265/2014	3C.3	2014-03-07	SIAT1	<	160	80	320	320	160	80	80	80	320
A/Belgium/14S0070/2014	3C.2	2014-02-16	SIAT1	40	160	160	640	160	160	80	80	80	320
A/Belgium/14H0010/2014	3C.3	2014-02-13	SIAT1	<	160	80	320	160	160	80	80	80	320
A/Belgium/14G0508/2014	3C.3	2014-04-16	SIAT1	80	320	160	640	320	320	80	160	640	640
A/Belgium/14G0510/2014	3C.3	2014-04-16	SIAT1	80	160	160	320	320	160	40	80	320	320
A/Belgium/14H0034/2014	3C.3	2014-04-10	SIAT1	80	320	160	640	320	320	160	160	160	640
A/Belgium/14G0496/2014	3C.3	2014-04-03	SIAT2	160	320	320	640	320	320	80	160	160	640

1. < = <40

Vaccine
2013-14

D.6.3 B Yamagata

Genetic characterisation

Very few influenza B viruses have circulated this year. The influenza B virus of the Yamagata lineage that was sequenced, belonged to clade 3 represented by the reference strain B/Wisconsin/1/2010.



D.6.4 B Victoria

One Influenza virus of Victoria lineage was sequenced this season, the virus belonged to clade 1A, the B/Brisbane/60/2008 clade.

D.7. Antiviral Monitoring

We performed the sequencing of the neuraminidase gene of a subset of viruses (8 N1 and 12 N2) in order to detect any mutations known to be associated with resistance to neuraminidase inhibitors, however none were identified.

Furthermore, phenotypic in vitro testing for antiviral resistance was performed in house (5 A(H3N2) and 7 A(H1N1)) and in the WHO CC London (1 B Vic, 7 A(H1N1) and 14 A(H3N2)). They were all sensitive to Oseltamivir and Zanamivir except for one A(H1N1) strain who had reduced susceptibility to Oseltamivir and Zanamivir and carried an I223R change in the neuraminidase gene. It was isolated from a 33-year old patient within the ILI surveillance network.

D.8 Composition of influenza virus vaccines for use in the 2014-2015 northern hemisphere influenza season

- A/California/7/2009 (H1N1)pdm09-like virus;
- A/Texas/50/2012, A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011;
- B/Massachusetts/2/2012-like virus.

The composition of the vaccine remains the same

Saison	A(H1N1)	H3N2	B
2000-2001	A/New Caldedonia/20/99	A/Panama/2007/99	B/Yamanashi/116/98
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	B/Malaysia/2506/2004 (VIC)
2006-2007	"	A/Wisconsin/6/2005	"
2007-2008	A/Solomon Island/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
2014-2015	"	"	"

Figure 12. Evolution of the composition of the trivalent influenza vaccine 2000 – 2015



D.9 Vaccine effectiveness

Vaccination status with the seasonal influenza vaccine was known for 113 SARI patients who tested positive for influenza and for 215 SARI patients who tested negative. Analysis indicates that 29% of influenza negative patients and 28% of influenza positive patients had been vaccinated against influenza this season.

A logistic regression that models influenza positivity in function of vaccination status (adjusted for age group, sex and month of sampling) shows that vaccine effectiveness was 55% (3%-79%). More detailed analyses are in process.

E. Conclusion

The influenza season 2013-2014 was characterized by a low intensity and a short duration as compared to previous seasons. A lower number of samples was collected during the whole season. Influenza A(H1N1) and A(H3N2) co-circulated with a predominance of A(H3N2). These observations were confirmed in other European countries. The distribution of the different viruses A(H1N1), A(H3N2) and B was similar for the ILI surveillance and the SARI surveillance. Phenotypic, genetic and antigenic characterisation of a subset of isolated strains have been performed. All but one of the tested viruses were sensitive to Oseltamivir and Zanamivir. All sequenced A(H1N1) viruses belonged to group 6 B. All A(H1N1) samples showed good reactivity with sera raised against the vaccine strain A/California/7/2009. All sequenced A(H3N2) viruses belonged to subgroup 3C. Antigenically, the analysed A(H3N2) viruses showed poor reactivity with antisera raised against the egg-propagated vaccine virus A/Texas/50/2012. Conversely, test viruses reacted well with antisera raised against reference viruses that are genetically similar to A/Victoria/361/2011 but propagated in cells. These data indicate that there was no significant antigenic drift between A(H3N2) viruses in the field and the original A(H3N2) strain which was chosen as vaccine strain. However, the cell-grown test viruses are antigenically distinguishable from egg-propagated viruses, which is probably due to the induction of mutations in the H3N2 strain during the (vaccine) production on eggs. This might have an impact on the vaccine effectiveness of the H3N2 component in the 2013/2014 vaccine. Of notice, an A(H3N2) influenza outbreak occurred in April in a nursing home in Vlaams-Brabant, although nearly all the residents were vaccinated against influenza at the beginning of the flu season. Serological analyses of patient sera taken during the acute phase, showed non-protective HI titers (<40) against the vaccine strain A/Texas/50/2012, despite previous vaccination circa 4 months earlier. A number of factors could have lied on the basis of this outbreak, such as virus/vaccine mismatch, immunosenescence and waning immunity (21) among others.

In Belgium, overall vaccine effectiveness for the season 2013-2014 was estimated to be 53%, further analyses are underway to estimate VE per subtype.



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