Virological Surveillance of Influenza in Belgium
Season 2014-2015

VIRAL DISEASES
National influenza Centre (WHO)
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EPIDEMIOLOGY OF INFECTIOUS DISEASES
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A. Abstract

The 2014–2015 influenza season was of high intensity and long duration. In Belgium, the epidemic threshold was crossed in the mid of January (week 2-2015), and mid-March (week 13-2015) the epidemic was over. The peak was observed at week 6-2015, with 979 ILI (influenza-like-illness) cases per 100,000 inhabitants, which is very to the 2012-2013 epidemic which was qualified as intense.

The first influenza positive case was detected in week 40-2014 and an increasing number of influenza positive cases could be detected starting from week 50-2014 to reach a percentage of virus confirmed cases of 74% in week 5-2015. Three viruses A(H1N1)pdm2009, A(H3N2) and B circulated during this season. While influenza A viruses have dominated since the start of the season with a predominance of A(H3N2), influenza B viruses have done so since week 10/2015. Antigenic drift in A(H3N2) viruses was observed in the 2014–2015 influenza season, therefore the northern hemisphere vaccine did not provide broad protection against A(H3N2) viruses. Despite some antigenic drift among B/Yamagata viruses, the A(H1N1)pdm09 and B/Yamagata components in the vaccine are likely to have protected against circulating viruses.

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. Since the (H1N1)2009 pandemic, special attention has been given to closer monitoring of the severity of influenza cases. Following the WHO and ECDC recommendations, the Belgian National Influenza Center has extended, since 2010, its surveillance to SARI (Severe Acute Respiratory Infection) 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 tests clinical samples for other respiratory viruses. 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) and MERS-CoV) which demonstrated the importance of
the surveillance of respiratory pathogens for the public health.

This report is mainly focused on virological results.

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, 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. They are 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 2014-2015, 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 and fever of ≥ 38°C and cough or dyspnea, and requiring hospitalisation (24h or more). As we are mostly interested in severe influenza cases, the surveillance is 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 are encoded in a dedicated web based database protected by a login and password.

The following hospitals participated to the SARI surveillance season 2014-2015

- 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 carried out throughout the year.

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

**Influenza A (H5N1)**

Since 2003, and till 17 of July 2015, 844 human infections with highly pathogenic H5N1 viruses have been reported to the World Health Organization (WHO) by 16 countries (4). About 50%
(449) of the contaminated 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. 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). From June 2013 until April 10th 2015, 631 laboratory-confirmed cases of human infection with the avian A(H7N9) were reported to the WHO including 212 deaths. Most of the cases were from China. One case in a Chinese traveler was reported by Malaysia and 2 in travelers returning from China were reported by Canada. 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. The main exposures and 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 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. As of 19 June 2015, 1338 laboratory-confirmed cases of human infection with Middle East respiratory syndrome coronavirus (MERS-CoV) have been reported to WHO since 2012, including at least 475 deaths. To date, 25 countries have reported cases, including countries in the Middle East: Egypt, Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia (KSA), United Arab Emirates (UAE) and Yemen; in Africa: Algeria, and Tunisia; in Europe: Austria, France, Germany, Greece, Italy, the Netherlands, Turkey and the United Kingdom; in Asia: China, the Republic of Korea, Malaysia
and Philippines; and in North America: the United States of America (USA). The majority of cases (>85%) have been reported from KSA. Since May, 2015 two new countries have been affected (China and Republic of Korea). From 20 May 2015 until 21st of July, 185 laboratory-confirmed cases including 36 deaths have been identified in the Republic of Korea. There is evidence of tertiary transmission. This is the largest outbreak of MERS-CoV outside the Middle East (7).

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 seems to be dromedary camel but infection acquired by exposure to camels represent a minority of all cases. Human-to-human transmission is amplified among household contacts and in healthcare settings. 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

A triplex Real time RT PCR Influenza A/B/RP: adapted protocols (10,11). Primers and probes for the matrix gene (influenza A) and hemagglutinin 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 hemagglutinin gene.
- RT PCR Influenza A/H3: adapted protocol from RIVM (12): primers and probes in the hemagglutinin 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).
Influenza 2014-2015

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 2014-2015 season.

**Subtyping (H5, H7, …)**

Samples from suspected cases of avian influenza are submitted to 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.2.4. Genetic characterisation

Genetic characterization is performed by sequencing of the HA gene and NA 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. Other mutations associated with resistance to antivirals have also been described for A(H3N2) and influenza B. Resistant strains can be detected by phenotypic tests based on IC\textsubscript{50} 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 140 ILI/100.000 inhabitants. The threshold was crossed in the mid of January (week 3-2015), and the epidemic was declared in week 4-2015 (2 consecutive weeks above threshold). Since week 3-2015, the number of consultations for ILI increased to reach the level of 979 ILI/100000 inhabitants in week 6-
2015. After week 8-2015, the number of ILI decreased and the number was below the threshold after week 12 (Figure 2). The epidemic lasted 10 weeks.

Figure 2. Weekly incidence of ILI for 100,000 inhabitants

D.1.2 Virological surveillance

The influenza surveillance period started in week 40-2014 (September 29, 2014) and continued through week 20-2015 (May 17, 2015).

Origin of samples
A total of 90 GPs (48 for Flanders, 42 for Wallonia-Brussels Federation) participated to the virological surveillance and sent 934 nasopharyngeal swabs to the NIC among which 932 samples were suitable for analyse.

Number of Nasopharyngeal swabs
Flanders : 518 (55.5 %)
Wallonia-Brussels : 413 (44.2 %)
Total : 932
**Typing and subtyping results**

The first influenza virus-positive case was detected in week 40-2014 and an increasing number of influenza virus-positive cases could be detected starting from week 50-2014 to reach a percentage of virus-confirmed cases of 74% in week 5-2015 (Fig 3).

From week 40-2013 to week 20-2014, a total of 932 respiratory samples were collected by sentinel GPs and tested at the Belgium National influenza Centre, among which 405 (43%) were positive for influenza A and 79 (8%) were positive for influenza B viruses. Among the subtyped influenza A viruses, 60/385 (16%) were A(H1N1)pdm2009 and 325/385 (84%) were A(H3N2). Two influenza A samples were co-infected by A(H1N1)/A(H3N2). Ninety samples were non-subtypable due to low viral load. Of the 79 analysed influenza B viruses, 73 (92%) were of the B/Yamagata lineage and 6 (8%) were from the B/Victoria lineage (Table 1). As shown in figure 3, influenza A viruses have dominated since the start of the season with a predominance of A(H3N2), and influenza B viruses have done so since week 10/2015.

![Figure 3. Weekly detection of influenza viruses in Belgium from week 40-2014 to week 20-2015 in the network of sentinel GPs](image-url)
Table 1. Numbers and proportion of the different types and subtypes analysed during the 2014-2015 season

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<tr>
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<th>FLU B</th>
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<tr>
<td></td>
<td>total</td>
<td>A H1</td>
</tr>
<tr>
<td>Number of positive samples</td>
<td>485</td>
<td>405</td>
</tr>
<tr>
<td>Number of tested samples</td>
<td>932</td>
<td>932</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>52</td>
<td>43</td>
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NT= not subtyped/non subtypable
NL = no lineage determined

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 varied significantly between the different age groups. in the age group 15-44 years, there were significantly less samples that were positive for influenza than in the age groups 5-14 years and 45-65 years. (Fig 4).

Also the distribution of influenza A subtypes slightly varied with age as shown in figure 4. Influenza A(H3N2) was the predominant virus detected in samples from children and adults. However, there was relatively more influenza A(H1N1) than influenza A(H3N2) among the adults aged 15-64 years than among the children and elderly, but the differences were not significant. No relative age differences could be observed in the frequency of either influenza B from the Yamagata lineage or influenza B from the Victoria lineage compared to influenza A(H3N2).
D.2 Sentinel Surveillance of SARI

D.2.1 Virological surveillance

Surveillance of SARI started week 52/2014, after the first influenza cases were recorded by the sentinel GPs and ended week 17-2015, about one month after the end of the epidemic.

Origin of samples
A total of 1263 patients were registered in the data base, among which 1248 (99%) corresponded to the case definition and of which samples were analysed. For 13 samples, the influenza diagnostic could not be realized due to non-conformity of the sample, therefore a total of 1235 samples were taken into account for the analyses.

Typing and subtyping results
From week 6-2014 to week 16-2014, 1235 respiratory samples from the sentinel network of hospitals were analysed by the National Centre for Influenza among which 571 (46%) were positive for influenza with 504 (88%) influenza A and 67 (12%) influenza B. Among the analysed influenza A viruses, 58 (12%) were A(H1N1)pdm2009 and 417 (83%) were A(H3N2). Most of the influenza B viruses were from the Yamagata lineage except 5 that could not be subtyped due to the low viral load (Figure 5 &6). From week 3, the percentage of positivity increased (15%) to reach a peak of 43% in week 6. From week 16 on, no more samples were sent to the NRC.
Figure 5. Weekly detection of influenza viruses in Belgium in the SARI network from week 52-2014 to week 17-2015.

Figure 6. Repartition of the different influenza types and subtypes in the SARI surveillance during the season 2014-2015.

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

A higher number of samples was collected from children under five years old and from adults from the 65-84 age group. The samples of young children (0-4 years) were less frequently positive than those of patients in all elder age groups. A higher percentage of positivity was observed in patient older than 85 years old (70%).

In all age groups, A(H3N2) viruses were predominant (Fig.7). There were differences by age however. The proportion of patients that were positive for A(H1N1) compared to patients that were positive for A(H3N2) was significantly lower among the influenza positive patients aged 85 years and older than among younger influenza positive patients.
Influenza 2014-2015

Figure 7. Influenza viruses according to age group

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

During the period of SARI surveillance (week 52 of 2014 to week 17 of 2015), the samples of the ILI patients (62% positive; 95% CI: 58% - 65%) were significantly more frequently positive than the samples of the SARI patients (46% positive; 95% CI: 43% - 49%).

During that period, Influenza A(H3N2) was the predominant virus in both surveillances. Of the subtypable samples, the proportion of influenza A(H3N2) viruses was higher among SARI patients (77%) than among ILI patients (70%) and the proportions of influenza B viruses (10.8% vs 11.4%) and of influenza A(H1N1) (12% vs 18%) were both lower among the SARI patients. These differences were not significant.

**D.3 Non sentinel surveillance**

Fifty six respiratory samples from patients with severe influenza were sent from hospitals around the country during the 2014-2015 season and interseason and analysed at the NIC for confirmation and subtyping. Thirty nine were influenza A positive among which 7 were A(H1N1)pdm2009, 28 were A(H3N2), and 2 were non-subtypable due to low viral load. Five samples were positive for influenza B (all from the Yamagata lineage).

**D.4 Suspected cases of Avian Influenza**

Two samples (nasopharyngeal (NP) washes and bronchial alveolar lavage (BAL)) from one patient returning from China were sent for Influenza A(H7N9) and A(H5N1) analysis. The patient presented mild symptoms and was positive for Influenza B.
D.5 Suspected cases of MERS CoV

Five samples from suspected cases returning from Saudi Arabia, which did not present serious symptoms were analysed for MERS-CoV and influenza viruses. Two of them were positive for influenza A, one A(H1N1) and one A(H3N2) and the 3 other were influenza negative. All samples were negative for MERS-CoV.

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 genetic group 6 B have predominated.

The hemagglutinine genes (HA 1 fragment) of 8 Influenza A(H1N1)pdm2009 samples were sequenced. A phylogenetic tree showing the vaccine strain, reference strains and A(H1N1)pdm2009 isolates is presented in figure 8A/B. All the sequenced viruses belonged to group 6 B.

Vaccine virus
Reference virus
Circulating virus Belgium

![Phylogenetic tree](image-url)
Figure 8A/B. Phylogenetic analysis of influenza A(H1N1)pdm09 strains

Antigenic characterisation

Three isolated A(H1N1) viruses that were sent to WHO CC-London were successfully propagated in cell culture. All three isolates were recognised well by the antiserum raised against the current vaccine virus A/California/7/2009 and were also recognised well by antisera against most of the other reference viruses. The exception was that two of the three test viruses were recognised less well by the antiserum raised against A/Christchurch/16/2010 (Table 2).
Table 2. Antigenic analyses of influenza H1N1pdm09 viruses (2015-01-14)

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<tr>
<td></td>
<td>Case%</td>
<td></td>
<td>7/09 69/09 N6/09 16/10 10394/11 1/11 27/11 100/11 5659/12 3626/13</td>
</tr>
<tr>
<td>REFERENCE VIRUSES</td>
<td></td>
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<td>F30/11 F11/13 F30/10 F21/11 F22/13 F31/11 F24/11 F30/12 F3/14</td>
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<tr>
<td>A/Bayern/69/2009</td>
<td>2009-07-01</td>
<td>MDCK5/MDCK2</td>
<td>160 320 320 40 40 40 80 40 40 80 80 320 160 160</td>
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<tr>
<td>A/Christchurch/16/2010</td>
<td>2010-07-12</td>
<td>E1/E3</td>
<td>1280 1280 2560 1280 1280 2560 1280 2560</td>
</tr>
<tr>
<td>A/Hong Kong/3934/2011</td>
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<td>A/Astrakhan/1/2011</td>
<td>2011-02-28</td>
<td>MDCK4/MDCK1</td>
<td>640 320 320 320 640 640 640 1280 1280 1280 1280 640 640</td>
</tr>
<tr>
<td>A/St. Petersburg/7/2011</td>
<td>2011-03-14</td>
<td>E1/E3</td>
<td>1280 1280 2560 1280 1280 2560 1280 1280 1280 1280</td>
</tr>
<tr>
<td>A/Hong Kong/5659/2012</td>
<td>2012-05-21</td>
<td>MDCK4/MDCK1</td>
<td>320 160 160 160 160 160 160 160</td>
</tr>
<tr>
<td>A/South Africa/366/2013</td>
<td>2013-06-06</td>
<td>E1/E2</td>
<td>320 1280 1280 1280 1280 1280 1280 1280 1280 1280</td>
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<tr>
<td>TEST VIRUSES</td>
<td></td>
<td></td>
<td>6B 1280 1280 1280 1280 1280 1280 1280</td>
</tr>
</tbody>
</table>

Vaccine

D.6.2 A(H3N2)

Genetic characterisation

A phylogenetic tree showing the vaccine strain, reference strains and 30 A(H3N2) isolates is presented in figure 9A. Figure 9B shows circulating strains in Belgium and other European countries. All the sequenced HA fell within genetic group 3C. This group has three subdivisions: 3C.1, 3C.2 and 3C.3. The A/Texas/50/2012 strain used in the 2014-2015 northern hemisphere vaccine belongs to genetic subgroup 3C.1. Three genetic subgroups have emerged since last season, one in subdivision 3C.2, 3C.2a, and two in subdivision 3C.3, 3C.3a and 3C.3b. In Belgium, the 2014-2015 season was dominated by the circulation of influenza A(H3N2) viruses belonging to subgroup 3C.2a and 3C.3b. Viruses in subgroup 3C.3b remain closely related to the vaccine strain A/Texas/50/2012 but viruses in subgroup 3C.2a and 3C.3a are considered antigenically distinct. Viruses belonging to clade 3C.2a and 3C.3b, are represented by respectively A/Hong Kong/146/2013 and A/Ireland/14MO3520/2014.

Amino acid substitutions that define subgroups 3C.2 and 3C.3 are:

- **3C.2** N145S in HA1, and D160N in HA2, e.g. A/Hong Kong/146/2013;
- **3C.2a** also carries N1445 K160T, N225D, Q311H in HA1 and some carry F159Y in HA1.
- **3C.3** T128A, R142G and N145S in HA1, e.g. A/Samara/73/2013;
- **3C3a** also carries A138S, F159S and N225D in HA1. Some carry K326R in HA1.
Antigenic characterisation of A(H3N2) viruses continues to be difficult by HI assay due to variable agglutination of red blood cells from guinea pig, turkey and humans, and NA mediated agglutination of red blood cells.
Eight viruses sent to WHO CC-London were recovered but HI could only be realized on three of the eight, the other five had no HA titre but were recovered as indicated by NA activity in the supernatant and CPE.

The majority of recent A(H3N2) viruses were poorly inhibited by ferret antisera raised against egg-propagated reference vaccine A/Texas/50/2012 (clade 3C.1) virus. The HI results show that the three 3C.3 viruses were recognised generally well with antisera raised against cell culture-propagated viruses of A/Victoria/361/2011 (genetic group 3C.1), A/Stockholm/6/2014 (group 3C.3a), A/Switzerland/9715293/2013 (group 3C.3a) and A/Hong Kong/5738/2014 (group 3C.2a) but less well with antiserum raised against A/Samara/73/2013 (genetic group 3C.3) which recognised only one of the three viruses (A/Belgium/H80/2014) at a titre within four-fold of the titre of the antiserum for the homologous virus.

Antiseras raised against all but one of the egg-propagated viruses (in genetic groups 3C.1, 3C.2, 3C.2a, 3C.3a) did not recognise the test viruses well with an exception. The antiserum raised against the egg-propagated A/Stockholm/6/2014 recognised the 3C.3 viruses at titres within four-fold of the titres of the homologous antiserum. This generally poor recognition by antiseras raised against egg-propagated H3N2 viruses is usual.

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

The two samples antigenically characterized were poorly recognised by antisera raised against cell culture-propagated viruses in clade 2 (B/Estonia/55669/2011, B/Hong Kong/3577/2012 and B/Massachusetts/02/2012). Only one (B/Belgium/G668/2014) of the two viruses was recognised by the antiserum raised against the cell culture-propagated viruses B/Phuket/3073/2013 at a titre within 4-fold of the titre of the antiserum for the homologous virus. Both viruses were recognised by antiserum raised against the egg-propagated viruses of clade 3 viruses (B/Wisconsin/1/2010, B/Stockholm/12/2011, B/Phuket/3073/2013 at titres within 4-fold of the titre of the antiserum for the homologous viruses.

Antigenic analyses of influenza B viruses (Yamagata lineage) 2015-01-14
D.6.4 B Victoria

One Influenza B virus of the 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 (1 N1 and 4 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 and in the WHO CC London for 8 A(H3N2), 3 A(H1N1) and 2 influenza B viruses. They were all sensitive to Oseltamivir and Zanamivir...

D.8 Composition of influenza virus vaccines for use in the 2015-2016 northern hemisphere influenza season

- A/California/7/2009 (H1N1)pdm09-like virus
- A/Switzerland/9715293/2013 (H3N2)-like virus
- B/Phuket/3073/2013-like virus

- It is recommended that quadrivalent vaccines containing two influenza B viruses contain the above three viruses and a B/Brisbane/60/2008-like virus.

<table>
<thead>
<tr>
<th>Saison</th>
<th>A(H1N1)</th>
<th>H3N2</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002-2003</td>
<td>&quot;</td>
<td>&quot;</td>
<td>B/Hong Kong/330/2001</td>
</tr>
<tr>
<td>2003-2004</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>2006-2007</td>
<td>&quot;</td>
<td>B/Wisconsin/6/2005</td>
<td>&quot;</td>
</tr>
<tr>
<td>2007-2008</td>
<td>A/Solomon Island/3/2006</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>2009-2010</td>
<td>&quot;</td>
<td>&quot;</td>
<td>B/Brisbane/60/2008(VIC)</td>
</tr>
<tr>
<td>2010-2011</td>
<td>A/California/7/2009</td>
<td>A/Perth/16/2009</td>
<td>&quot;</td>
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<tr>
<td>2011-2012</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>2013-2014</td>
<td>&quot;</td>
<td>A/Texas/50/2012</td>
<td>B/Massachusetts/2/2012</td>
</tr>
<tr>
<td>2014-2015</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>2015-2016</td>
<td>&quot;</td>
<td>A/Switzerland/9715293/2013</td>
<td>B/Phuket/3073/2013</td>
</tr>
</tbody>
</table>

Figure 12. Evolution of the composition of the trivalent influenza vaccine 2000 – 2016
D.9 Vaccine effectiveness

By pooling data of ILI and SARI patients, we estimated vaccine effectiveness using a logistic regression model that models influenza positivity in function of vaccination status (adjusted for age group, sex and month of sampling). Trivalent vaccine effectiveness during the 2014-2015 season was estimated to be 19% (-16%-43%). It is likely that the dominant circulation of the H3 drift variant in Belgium affected the effectiveness of this year’s vaccine. More detailed analyses are in process.

E. Conclusion

The 2014–2015 influenza season was of high intensity, lasted 10 weeks and was comparable to the 2012-2013 season. During the epidemic peak, a total number of 979 ILI per 100.000 inhabitant was reached. Three viruses A(H1N1)pdm2009, A(H3N2) and B circulated during this season, with a large predominance of A(H3N2). This predominance was observed in every age group. The percentage of positivity was higher in the ILI surveillance as compared to the SARI surveillance which is probably due to a better specificity of the case definition in the ILI surveillance. The distribution of the different viruses A(H1N1), A(H3N2) and B was similar for the ILI surveillance and the SARI surveillance.

All sequenced A(H1N1)pdm2009 viruses were from group 6B and were antigenically similar to the vaccine strain A/California/7/2009. Most of influenza B viruses that have circulated this season were from the Yamagata lineage, and all the sequenced influenza B viruses belonged to clad 3 represented by the reference strain B/Phuket/3073/2013. The 2014-2015 chosen vaccine strain B/Massachussetts/02/2012 belongs to clad 2 and most of B viruses were not well recognised by the antisera raised against the vaccine strain Massachussetts/02/2015. The same situation was observed in other countries in Europe and in the USA.

In Belgium, the 2014-2015 season was dominated by the circulation of influenza A(H3N2) viruses belonging to subgroup 3C.2a and 3C.3b, with viruses in subgroups 3C.3b remaining closely related to the vaccine strain A/Texas/50/2012 but viruses in subgroup 3C.2a and 3C.3a being considered antigenically distinct. About 60% of the A(H3N2) viruses in circulation in Belgium (belonging to subgroup 3C2.a) were antigenically dissimilar to the vaccine strain A/Texas/50/2012. As such, the H3 component of the vaccine most likely did not provide broad protection against the majority of circulating A(H3N2) viruses. This was reflected in our vaccine effectiveness estimation which revealed that the efficiency was quite low (19%). The same situation was described in other European countries. All tested viruses were sensitive to Oseltamivir and Zanamivir.
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 of London for their analyses and their support.

G. References


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5. WHO risk Assessment of Human infection with avian influenza A (H7N9) virus, 23 February 2015


