Virological Surveillance of Influenza in Belgium
Season 2012-2013

VIRAL DISEASES
National influenza Centre (WHO)
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**A. Abstract**

Belgium crossed the epidemic threshold for influenza end of December (week 52 -2012), about five weeks earlier than in 2011-2012, and the epidemic ended at week 13-2013 (25 March 2013). The first influenza positive cases were detected during week 45-2012 and since then the percentage of positivity among influenza virus positive ILI cases rapidly increased to reach 85% in week 5-2013. Three influenza viruses A(H1N1)pdm2009, A(H3N2) and B were detected with a predominance of influenza A(H1N1)pdm2009 and B. The epidemic was of high intensity, reaching 1000 ILI consultations/100.000 inhabitants and lasted 12 weeks, quite longer than previous seasons.

In addition to seasonal influenza, 2012-2013 was marked by the circulation of a new respiratory virus in Saudi Arabia, a coronavirus called MERS-coronavirus (Middle Eastern Respiratory Syndrome). So far, worldwide, there have been a total of 150 laboratory-confirmed cases of infection with MERS-CoV, including 64 deaths.

In addition, an outbreak of human infections with a new avian influenza A (H7N9) virus was reported in China by the World Health Organization on April 1st, 2013. More than 130 infections in humans with influenza A H7N9 have been identified, the vast majority during the month of April. Many of the people infected with H7N9 reported contact with poultry, and it is therefore suggested that human infections occurred after exposure to infected poultry or contaminated environments. While some mild illness in human cases has been seen, most patients have severe respiratory illness and 45 people have died. No evidence of sustained person-to-person spread of influenza A H7N9 virus has been found. No cases of H7N9 outside of China have been reported yet.

**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. The emergence this year of two new respiratory viruses in the human community (influenza A (H7N9) and MERS-CoV) has 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 is also an important task (2). Furthermore, the estimation of the vaccine effectiveness is also an important project in development.

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 2012-2013, 112 sentinel physicians were involved in the virological influenza surveillance (9 for the Brussels capital region, 42 for the Walloon region and 61 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 of 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 2012-2013, 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 were 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 2012-2013

- 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 was sent to hospitals and laboratories across the country to encourage them to collect samples of cases of 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)

In 1997, in Asia, an unprecedented epizootic avian influenza A/H5N1 virus that is highly pathogenic crossed the species barrier to cause many human fatalities and since then poses an increasing pandemic threat. H5N1 avian influenza is an infectious disease of birds that can be spread to people, but is difficult to transmit from person to person. Almost all people with
H5N1 infection have had close contact with infected birds or H5N1-contaminated environments. When people do become infected, the mortality rate is about 60% (4). Since 2003, 641 cases have been reported worldwide with 380 fatalities. Some countries are affected annually (e.g., Egypt and Indonesia) with a higher incidence during the winter period, while other countries are more rarely affected, such as China, Thailand, or Pakistan. Since December 2005, an emergency procedure has been developed in Belgium to assure a rapid diagnosis in case of suspicion of a human case of influenza A/H5N1. The National Influenza Center (NIC) of Belgium at the Scientific Institute of Public Health was appointed as reference laboratory for testing of the H5N1 suspect cases, mainly cases returning from affected countries.

**Influenza A (H7N9)**

On 31 March 2013, the first human cases of an avian influenza A (H7N9) reassortant virus, not previously described as causing disease in humans, were reported in China. Since then, the number of reported cases increased to peak in April 2013, from which the number of cases decreased with few cases in May and October 2013. From March 2013 to October 2013, 137 human cases were confirmed, with 45 fatal cases in 10 provinces of China and Taiwan, the latter from an imported case. A few small clusters were detected but almost all cases have occurred sporadically, without obvious epidemiological links. While occasional human-to-human transmission in the clusters cannot be ruled out, there is no confirmed sustained human-to-human transmission. A few mild cases have been detected (5). Phylogenetic analysis of the eight genes indicate that the new influenza A (H7N9) virus resulted from a combination of the eight genomic fragments originating from two avian influenza A (H9N2) viruses previously detected in chickens and domestic and wild birds, i.e., 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 National influenza Centre of Belgium has developed molecular tests for the detection of this 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**

**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. Up until October 31, 2013, about 149 human cases have been identified, with 63 deaths (42% case-fatality rate). Mainly older people, often with weakened immune systems or poor health, are affected by the disease. All cases originate directly or indirectly from the Middle East. Sporadic cases continue to emerge, family or hospital clusters occur, but there is no evidence of sustained transmission in the population. Mild or subclinical infections have been reported. The virus has several virological, epidemiological and clinical characteristics in common with the Severe Acute Respiratory Syndrome (SARS) CoV, which emerged in China in 2002.
The epidemiological pattern of human infections is highly suggestive of a zoonotic infection. The animal vector or reservoir remains unidentified but bats and camelid might be involved.

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. National influenza Centres have been encouraged to develop or adapt molecular diagnostic tests for MERS-CoV. Increasing evidence suggests that nasopharyngeal (NP) swabs are not as sensitive as lower respiratory specimens for detecting MERS-CoV infections. NP swabs have been negative in patients who were close contacts of confirmed cases and who developed pneumonia following contact. In addition, a number of cases have now had negative tests on NP swabs but positive tests of lower respiratory track specimens.

Case definition from WHO for MERS-CoV

**The case definition for Probable case**

Three combinations of clinical, epidemiological and laboratory criteria can define a probable case:

- A person with a febrile acute respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome)
  AND
  Testing for MERS-CoV is unavailable or negative on a single inadequate specimen
  AND
  The patient has a direct epidemiologic-link with a confirmed MERS-CoV case.
- A person with a febrile acute respiratory illness with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome)
  AND
  An inconclusive MERS-CoV laboratory test (that is, a positive screening test without confirmation)
  AND
  A resident of or traveler to Middle Eastern countries where MERS-CoV virus is believed to be circulating in the 14 days before onset of illness.
- A person with an acute febrile respiratory illness of any severity
  AND
  An inconclusive MERS-CoV laboratory test (that is, a positive screening test without confirmation)
  AND
  The patient has a direct epidemiologic-link with a confirmed MERS-CoV case.

**Confirmed case**

A person with laboratory confirmation of MERS-CoV infection.
C.2. Laboratory tests

C.2.1. Real time RT PCR Influenza

Nasopharyngeal swabs received at the National Influenza Centre are submitted to 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 (8,9). 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 (8): primers and probes are chosen in the hemagglutinine gene.
- RT PCR Influenza A/H3 : adapted protocol from RIVM (10): primers and probes in the hemagglutinine gene.

For a subset of samples
- RT PCR N1: adapted protocol from RIVM (10): primers and probes in the neuraminidase gene.
- RT PCR N2 : adapted protocol from Pasteur Institute Paris (11): 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 (12).

In case of un-subtypable influenza A, if the Ct is < 36, primers and probe specific for the Nucleoprotein of animal influenza (SWA) are used (protocol CDC (8)): This test allows to determine if the influenza strain is of animal origin and to continue with complementary tests.
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 (13) and adapted protocol from the Health Protection Agency, 2006 (14).

RT PCR H7N9
Adapted protocol from WHO (15).

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. 2012 (16).

C.2.3 PCR tests for other respiratory viruses
Respiratory samples from the hospital network (SARI samples) are additionally 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

<table>
<thead>
<tr>
<th>MIX 1</th>
<th>MIX 2</th>
<th>MIX 3</th>
<th>MIX 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV A</td>
<td>HEX</td>
<td>PIV 1</td>
<td>ROX</td>
</tr>
<tr>
<td>RSV B</td>
<td>ROX</td>
<td>PIV 2</td>
<td>HEX</td>
</tr>
<tr>
<td>hMPV</td>
<td>Cy5</td>
<td>PIV 3</td>
<td>FAM</td>
</tr>
<tr>
<td>EV</td>
<td>FAM</td>
<td>Adeno</td>
<td>Cy5</td>
</tr>
</tbody>
</table>

The protocols have been adapted from Otsby et al. (17) with some modifications (primers for rhinoviruses as described in Hombrouck et al., 2009 (18), and primers for MERS CoV as described by Corman et al. (16).

**C.2.4. Genetic characterisation**

Genetic characterization is performed by sequencing of 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 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 different 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 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 139 ILI/100.000 inhabitants. The threshold was crossed in week 52-2012, and the epidemic was declared in week 1-2013 (2 consecutive weeks with crossed threshold). Since week 1-2013, the number of consultations for ILI increased spectacularly to reach the level of 1000/100000 inhabitants in week 6-2013. After week 7-2013, the number of ILI decreased and the number was below the threshold after week 12 (Figure 2). The epidemic lasted 12 weeks which was particularly long as compared with previous seasons.

![Figure 2. Weekly incidence of ILI for 100.000 inhabitants](image)

D.1.2 Virological surveillance
The influenza surveillance period started in week 40-2012 (September 26, 2012) and continued through week 20-2013 (May 19, 2013). The monitoring period lasted 33 weeks.

Origin of samples
A total of 112 GPs (61 for Flanders, 42 for Wallonia and 9 for Brussels) participated to the virological surveillance and sent 1419 nasopharyngeal swabs to the NIC.

| Number of Nasopharyngeal swabs |
|-------------------------------|---|
| Flanders: 694 (48.9 %)        |
| Wallonia: 689 (48.5 %)        |
| Brussels: 36 (2.5 %)          |
| **Total:** 1419               |
Typing and subtyping results

The first positive influenza specimens were detected in week 45/2012 and an increasing number of influenza viruses have been detected starting from week 52-2012 to reach a percentage of positivity of 85% in week 5-2013. From week 40-2012 to week 20-2013, a total of 1419 respiratory samples were collected by sentinel GPs and tested at the NIC, among which 845 (59%) were positive for influenza: 376 (44.4%) influenza A viruses and 468 (55.3%) influenza B viruses. One sample showed a co-infection of Influenza A and B. Among the subtyped influenza A viruses, 252/336 (75%) were A(H1)pdm2009, 68/336(20.2%) were A(H3) and one was a co-infection by A(H1) and A(H3). Fifteen samples (4.4%) were non-subtyable due to low viral load. Of the 411 analysed influenza B viruses, 392(95.4%) were B/Yamagata lineage and 17 (4.1%) were B/Victoria lineage. One sample was positive for the two lineages Yamagata and Victoria. For two samples the lineage could not be determined due to low viral load (Figure 3).

Figure 3. Weekly detection of influenza viruses in Belgium from week 40-2012 to week 20-2013 in the network of sentinel GPs

Influenza viruses according to age group

The distribution of influenza types varies with age as shown in figure 4. The proportion of influenza positive samples was highest in children attending school (5-14 years) (74%), and lowest in the elderly (>65 years) (42%). In school attending children, a clear predominance of influenza B infections was observed whereas in all other age groups influenza A and B co-circulated.
The distribution of influenza A subtypes varies with age as shown in figure 5. 36% of influenza A infections in children was caused by A(H3), whereas 16% of influenza A infections in adults was caused by A(H3).

D.2 Sentinel Surveillance of SARI

D.2.1 Virological surveillance

Surveillance of SARI started week 51/2012, after the first influenza cases were recorded by the SGP sentinel GPs network and ended week 19-2013, about one month after the end of the epidemic.

Origin of samples
A total of 1034 samples were collected and sent by the hospital network, among which 990 were accompanied of clinical data and were analysed.

Typing and subtyping results
From week 51-2012 to week 19-2013, 990 respiratory samples from de sentinel network of hospitals were sent and analysed by the National Centre for Influenza among which 427 (43.1%) were positive for influenza with 224 (22.6%) influenza A and 201 (20.3%) influenza B. Two samples were co-infected by influenza A and B. Among the analysed influenza A viruses,
148 (66%) were A(H1)pdm2009 and 65 (29.529%) were A(H3). Thirteen samples (5.8%) could not be subtyped due to low viral load (Ct>36). Among analysed influenza B viruses, 187 (93%) were from the Yamagata lineage and 5 (2.5%) were from the Victoria lineage. For 9 influenza B samples (4.4%), the lineage could not be determined due to low viral load (Ct>36) (Figure 6).

![Influenza tests in SARI patients](image)

Figure 6. Weekly detection of influenza viruses in Belgium in the SARI network from week 51-2012 to week 17-2013

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

The distribution of influenza types varied with age as shown in figure 7, with the lowest positivity rate in children under five. Similar to the results of the SGPs network, the positivity rate was highest in children aged 5-14 years with a clear predominance of influenza B infections.
The distribution of influenza subtypes varied with age as shown in figure 8. Only 6% of influenza infections in children was caused by A(H3), whereas 20% of influenza infections in adults was caused by A(H3).

Forty eight respiratory samples from patients with severe influenza were sent from hospitals around the country during the 2012-2013 season but also interseason and analysed at the NIC for confirmation and subtyping. Twenty five samples were influenza A positive among which 11 were A(H1)pdm2009, 6 were A(H3), one was co infected by A(H1)pdm2009 and A(H3), and 7 were non-subtypable due to low viral load. Nine were influenza B positive, all belonging to the Yamagata lineage.
D.4 Suspected cases of Avian Influenza

Five samples were sent for Influenza A(H7N9) analysis. Four of the 5 patients reported recent travel to China and one travel to Dubai and all of them presented mild symptoms. All samples tested negative for A(H7N9), four being negative for influenza A and one positive for A(H3N2).

D.5 Suspected cases of MERS CoV

Two samples were sent for MERS-CoV analysis. The first one was from a patient returning from Saudi Arabia, which did not present serious symptoms. The second one was from a patient returning from Bangladesh. Neither of these two patients responded to the case definition. The two patients were negative for MERS-CoV, but the second was positive for A(H3N2) influenza Virus.

In addition, all respiratory samples from the SARI surveillance were tested for 16 different respiratory viruses including MERS-CoV using the Multiplex PCR for respiratory viruses. All 988 SARI 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 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.

We sequenced the hemagglutinin gene (HA 1 fragment) of 21 Influenza A(H1N1)pdm2009 samples. A phylogenetic tree showing the vaccine strain, reference strains and A(H1N1)pdm2009 isolates is presented in figure 9. All the sequenced viruses belonged to group 6 or 7.

One of the A/H1N1pdm strains harboured a mixed population at the 222 codon position of the hemagglutinin gene: D222D/G with a higher proportion of the mutated virus. Several reports have suggested that this mutation is more frequently detected in patients with severe morbidity and mortality than in patients with mild outcomes (19). In this case, the mutation was detected in a sample from a patient admitted to the ICU with acute respiratory distress syndrome.
Antigenic characterisation

From the twenty two clinical Influenza A(H1N1)pdm2009 samples sent to WHO CC London, 17 were able to be recovered in cell culture and were analysed by hemagglutination-inhibition (HI) tests with post-infection ferret antisera. All samples showed good reactivity with sera raised against the vaccine strain A/California/7/2009 and all the reference strains with the exception of the antiserum raised against A/Christchurch/16/2010 (genetic group 4, a genetic group that does not seem to be currently in circulation) (Table 2).

Isolates all belonged to the clade represented by the strain Influenza 2012-2013.

### Genetic characterisation

A phylogenetic tree showing the vaccine strain, reference strains and 12 A(H3N2) isolates is presented in figure 10. Isolates all belonged to the clade represented by the strain A/Victoria/208/2009 – this clade is defined by amino acid substitution T212A relative to A/Perth/16/2009.

Among them 11 belonged to group 3C represented by A/Victoria/361/2011 characterised by S45N, T48I, A198S and N312S mutations and 1 belonged to the group 3A represented by A/Stockholm/18/2011 and characterised by substitution N144D.

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### Table 2. Antigenic analyses of influenza H1N1pdm09 viruses

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Collection date</th>
<th>Passage History</th>
<th>Haemagglutination inhibition titre</th>
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<td><strong>MDCK</strong>/MDCK</td>
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<tr>
<td>A/Belgium/G932/2012</td>
<td>2009-04-09</td>
<td>E1/E2</td>
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<td>A/Belgium/G917/2012</td>
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<td>MDCK5/MDCK1</td>
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</tr>
<tr>
<td>A/Belgium/G916/2012</td>
<td>2009-10-27</td>
<td>MDCK4/MDCK3</td>
<td>640 1280 640 320 60 160 320 160 320</td>
</tr>
<tr>
<td>A/Christchurch/16/2010</td>
<td>2010-07-12</td>
<td>E2/E2</td>
<td>1280 1280 2560 5120 2560 2560 2560 5120 5120</td>
</tr>
<tr>
<td>A/Hong Kong/3934/2011</td>
<td>2011-03-29</td>
<td>MDCK2/MDCK3</td>
<td>320 160 320 320 640 1280 640 1280 1280</td>
</tr>
<tr>
<td>A/Astrakhan/1/2011</td>
<td>2011-02-28</td>
<td>MDCK1/MDCK5</td>
<td>1280 640 640 1280 1280 1280 1280 1280 2560</td>
</tr>
<tr>
<td>A/St. Petersburg/27/2011</td>
<td>2011-02-14</td>
<td>E1/E2</td>
<td>1280 1280 1280 1280 2560 2560 2560 5120 5120</td>
</tr>
<tr>
<td>A/St. Petersburg/100/2011</td>
<td>2011-05-14</td>
<td>E1/E2</td>
<td>1280 1280 1280 1280 2560 2560 2560 5120 5120</td>
</tr>
<tr>
<td>A/Hong Kong/5659/2012</td>
<td>2012-05-21</td>
<td>MDCK4/MDCK1</td>
<td>320 160 320 640 640 1280 640 1280 2560</td>
</tr>
</tbody>
</table>

### D.6.2 A(H3N2)

A phylogenetic tree showing the vaccine strain, reference strains and 12 A(H3N2) isolates is presented in figure 10. Isolates all belonged to the clade represented by the strain A/Victoria/208/2009 – this clade is defined by amino acid substitution T212A relative to A/Perth/16/2009.

Among them 11 belonged to group 3C represented by A/Victoria/361/2011 characterised by S45N, T48I, A198S and N312S mutations and 1 belonged to the group 3A represented by A/Stockholm/18/2011 and characterised by substitution N144D.
Antigenic characterisation

From the 5 A(H3N2) samples sent to CC WHO London, 4 recovered well in cell culture. All samples showed good reactivity against sera raised against cell-propagated reference strain A/Victoria/361/2011, however they showed lower reactivity against the egg-propagated vaccine strain A/Victoria/361/2011 (Table 3) which was the egg produced strain included in the seasonal influenza vaccine.

Table 3. Antigenic analyses of influenza A H3N2 viruses

D.6.3 B Yamagata

Genetic characterisation
Twenty two samples positive for influenza B Yamagata lineage were sequenced. Two of them belonged to B(Yam)-lineage clade 3, represented by the vaccine strain B/Wisconsin1/2010, and characterised by amino acid substitutions of S150I, N165Y and G229D. The strains were closely related to the strain B/Stockholm/12/2011 containing a number of additional substitutions V29A, L172Q and M251V (figure 11).

The remaining 20 viruses belonged to B(Yam) lineage clade 2, representative: B/Estonia/55669/2011 – viruses within this clade have characteristic amino acid substitutions of R48K, P108A and T181A.

Figure 11. Phylogenetic analysis of circulating influenza B Yamagata strains

Antigenic characterisation
Twenty isolates of B Yamagata clinical samples were cultured and assayed by HI. As shown in table 4, only one virus reacted well with antiserum raised against the vaccine virus B/Wisconsin1/2010 with titres within 4-fold of that of the homologous virus. All but two viruses showed good reactivity with antisera raised against E/Estonia/55669/2011 and all
showed good reactivity with antiserum raised against the cell-propagated B/Massachusetts/02/2012.

Table 4. Antigenic analyses of influenza B (yamagata lineage) viruses

<table>
<thead>
<tr>
<th>Viruses Collection date</th>
<th>Passage History</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/Victoria 2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/Brisbane/3/2007</td>
<td>2007-09-03</td>
<td>E2/E1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/Wisconsin/1/2010</td>
<td>2007-08-07</td>
<td>E3/E2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/Essexia/S5669/2011</td>
<td>2011-03-14</td>
<td>MDCK1/MDCK1</td>
<td>40 40 160 160 80 320 1280</td>
<td>1280 320 320</td>
</tr>
<tr>
<td>B/Northern/1/2012</td>
<td>2012-02-14</td>
<td>C2/MDCK2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/Hong Kong/357/2012</td>
<td>2012-06-13</td>
<td>MDCK2/MDCK3</td>
<td>160 160 160 160 160 160 160</td>
<td>160 160 160</td>
</tr>
<tr>
<td>B/Massachusetts/02/2012</td>
<td>2012-03-13</td>
<td>E3/E2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B/Massachusetts/02/2012</td>
<td>2012-03-13</td>
<td>MDCK1/2/MDCK2</td>
<td>80 320 80 80 1280 1280 640</td>
<td>320 320 320</td>
</tr>
</tbody>
</table>

**D.6.4 B Victoria**

The one sequenced Influenza B Victoria sample was homologous to the reference strain B/Brisbane/60/2008.

One virus was isolated that belonged to the B/Victoria lineage. In HI assays the virus showed low reactivity with antisera raised against the egg-propagated previous vaccine virus B/Brisbane/60/2008 but a good reactivity with antisera raised against cell-propagated virus B/Brisbane/60/2008 (Table 5).
Table 5. Antigenic analyses of influenza B (victoria lineage) viruses

Antigenic analyses of influenza B viruses (Victoria lineage) 05/02/2013

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Collection date</th>
<th>Passage History</th>
<th>HA titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>B/Bris*</td>
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<tr>
<td>Reference Viruses</td>
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<td></td>
</tr>
<tr>
<td>B/Malaysia/256/2004</td>
<td>2004-12-06</td>
<td>E3/E5</td>
<td>1280</td>
</tr>
<tr>
<td>B/Brisbane/60/2008</td>
<td>2004-08-04</td>
<td>E4/E3</td>
<td>1280</td>
</tr>
<tr>
<td>B/Paris/1762/2008</td>
<td>2004-02-09</td>
<td>C2/MDCK2</td>
<td>1280</td>
</tr>
<tr>
<td>B/Hong Kong/514/2009</td>
<td>2004-10-11</td>
<td>MDCK4</td>
<td>2560</td>
</tr>
<tr>
<td>B/Odessa/3988/2010</td>
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<td>C2/MDCK2</td>
<td>1280</td>
</tr>
<tr>
<td>B/Malta/38714/2011</td>
<td>2011-03-07</td>
<td>E4</td>
<td>1280</td>
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<tr>
<td>B/Johannesburg/3964/2012</td>
<td>2012-06-03</td>
<td>E1/E1</td>
<td>1280</td>
</tr>
<tr>
<td>B/Formosa/V2367/2012</td>
<td>2012-08-06</td>
<td>MDCK1/MDCK2</td>
<td>2560</td>
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<tr>
<td>Test Viruses</td>
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<td></td>
</tr>
<tr>
<td>B/Belgium/G886/2012</td>
<td>12/11/2012</td>
<td>MDCK2</td>
<td>2560</td>
</tr>
</tbody>
</table>

D.7. Antiviral Monitoring

We performed the sequencing of the neuraminidase gene of a subset of viruses (9 N1, 3 N2, and 4 NB) 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 WHO CC London on 31 samples (15 influenza B, 12 influenza A(H1N1) and 4 A(H3N2). They were all sensitive to Oseltamivir and Zanamivir.

D.8 Recommended composition of influenza virus vaccines for use in the 2013-2014 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.
<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>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>
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<tr>
<td>2010-2011</td>
<td>A/California/7/2009</td>
<td>A/Perth/16/2009</td>
<td>&quot;</td>
</tr>
<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>
</tbody>
</table>

Figure 12. Evolution of the composition of the trivalent influenza vaccin 2000 – 2014

D.9 Vaccine effectiveness

The vaccine status against influenza was known for 330 SARI patients testing positive for influenza and 381 SARI patients testing negative. Analysis indicates that 28% of the influenza-positive patients and 35% of the influenza-negative patients reported to be vaccinated against influenza. The difference between both proportions is significantly different, with an OR of 0.53 [0.36-0.79], adjusted for age and weeks. The vaccine effectiveness was 47% (1 – OR) and appeared better in children (5-15) and in younger adults (15-45).

E. Conclusion

The influenza season 2012-2013 was characterized by a very high intensity and duration as compared to previous seasons. Three viruses have co-circulated A(H1N1), A(H3N2) and B of Yamagata lineage, with a predominance of A(H1N1) and B. The surveillance of severe influenza cases by the network of hospitals has permitted to monitor the severity of the epidemic. Data from the SARI surveillance and analyses of severity indicators suggest that the severity of this season was moderate. Genetic and antigenic characterisation of a subset of isolated strains have been performed. All the sequenced A(H1N1) viruses belonged to group 6 or 7. All A(H1N1) samples showed good reactivity with sera raised against the vaccine strain A/California/7/2009. All A(H3N2) isolates belonged to the clade represented by the strain A/Victoria/208/2009 but showed lower reactivity against egg-propagated vaccine strain A/Victoria/361/2011. Most of the Influenza B Yamagata viruses analysed belonged to clade 2 and showed lower reactivity with antisera raised against the vaccine virus B/Wisconsin/1/2010. So the vaccine match for this season was not ideal except for A(H1N1). All tested viruses were sensitive to Oseltamivir and Zanamivir. The emergence in 2013 of two new respiratory viruses A(H7N9) and MERS CoV has demonstrated the importance of the surveillance of respiratory viruses in the community.
F. Acknowledgements

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G. References


Influenza 2012-2013


