



Virological Surveillance of Influenza in Belgium

Season 2011-2012

VIRAL DISEASES

National influenza Centre (WHO)

Isabelle Thomas, Anneleen Hombrouck, Florence Naze, Steven Van Gucht
Jeannine Weyckmans, Daniëlle Bauwens, Karim El Kadaani, Mona Abady, Amal Toufik

T +32 2 642 50 74

F +32 2 642 56 92

E-mail: isabelle.thomas@wiv-isp.be

HEALTHCARE SERVICES RESEARCH

Viviane Van Casteren, Yolande Pirson

INFECTIOUS DISEASES AMONG THE GENERAL POPULATION

Françoise Wuillaume

Scientific Institute of Public Health
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A. Abstract

In Belgium, the influenza epidemic of 2011-2012 was late and moderate as compared with previous seasons. The epidemic threshold was crossed in week 5 -2012 and a consultation rate of 531 per 100.000 inhabitants was reached in week 7-2012. The first influenza positive cases were detected since week 46 and peaked during week 7-2012 with a positive detection rate of 71 %. In week 12-2012 (mid-March), the epidemic ended but the rate of positivity among sentinel collected samples still remained high. The season was characterised by the predominance of Influenza A (H3N2) and influenza B. Influenza A (H1N1)pdm09 viruses were detected at a very low rate. The majority of B viruses belonged to the B/Yamagata lineage which was not included in the vaccine. The majority of A(H3N2) viruses which circulated during this season were not homologous to the vaccine strain A/Perth/16/2009 and were more close to the reference strain A/Victoria/361/2011.

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. After the (H1N1)2009 pandemic, special attention has come to better monitor 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.



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).

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. 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 2011-2012, six hospitals located in the three regions of the country participated to the surveillance.

The SARI case definition, based on the WHO recommendation was : an acute respiratory illness with onset within the last seven days and fever of $\geq 38^{\circ}\text{C}$ and cough and 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.

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 continuous throughout the year.

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 (4,5). 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/H1sw: adapted protocol from CDC (4): primers and probes are chosen in the hemagglutinine gene.
- RT PCR Influenza A/H3 : adapted protocol from RIVM (6): primers and probes in the hemagglutinine gene.



For a subset of samples

- RT PCR N1: adapted protocol from RIVM (6): primers and probes in the neuraminidase gene.
- RT PCR N2 : adapted protocol from Pasteur Institute Paris (7): 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 (8).

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

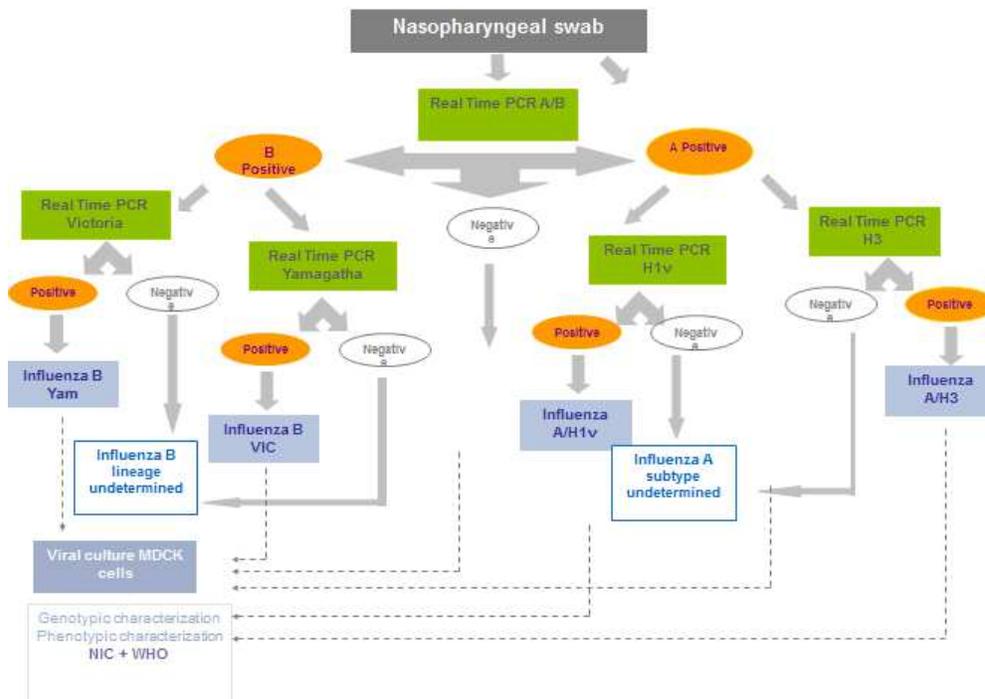


Figure 1. Sequence of the Real time PCR tests used during the 2011-2012 season.

C.2.3 Real Time RT PCR for other respiratory viruses

- RT PCR RSV-A-PIV-2 Duplex: (9)
- RT PCR RSV-B-hMPV duplex : (9)
- RT PCR PIV 1-3 : (9)
- RT PCR Rhinovirus : (9)



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. Sending of strains to London WHO CC

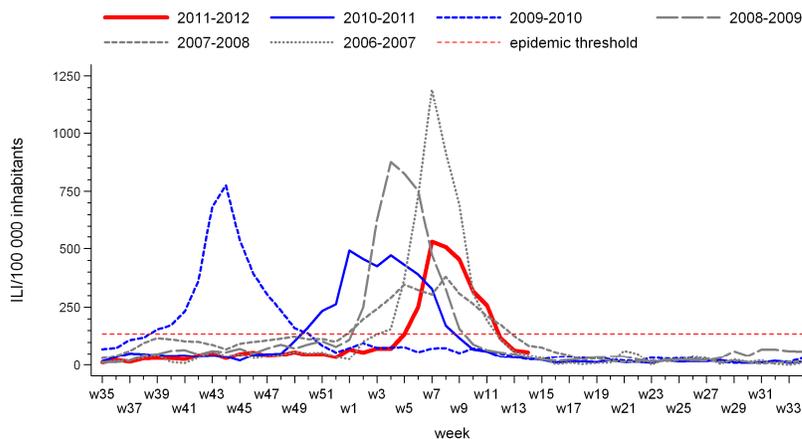
Each year, about fifty representative Belgian strains are sent to the WHO Collaborating Centre in London to undergo different tests: antigenic and genetic characterization and monitoring of antiviral resistance. The characterization of circulating strains in Belgium contributes to the determination of 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

The flu epidemic lasted 6 weeks, from the week of the 6th of February (week 6-2012) until the week of the 12th March (week 11-2012). The peak was observed in the second week of the epidemic (week 7-2012) with a consultation rate of 531 consultations per 100,000 inhabitants (Figure 1). Children under 5 years old were the age group most frequently affected. In comparison with former seasons, the intensity of the epidemic was moderate.



Source:WIV-ISP

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 26, 2012) and continued through week 20-2013 (May 19, 2013). The monitoring period lasted 33 weeks.

Origin of samples

A total of 109 GPs (58 for Flanders, 39 for Wallonia and 12 for Brussels) participated to the virological surveillance and sent 995 nasopharyngeal swabs to the NIC.

Number of Nasopharyngeal swabs

Flanders : 544(54.6 %)

Wallonia : 356 (35.7 %)

Brussels : 95 (9.5 %)

Total : 995

Typing and subtyping results

The first positive influenza specimens were detected in week 46/2011 and an increasing number of influenza viruses have been detected starting from week 3-2012 to reach a percentage of positivity of 73 % in week 7-2012. From week 40-2012 to week 20-2013, a total of 995 respiratory samples were collected by sentinel GPs and tested at the NIC, among which 397 (39.8%) were positive for influenza A viruses and 28 (2.8%) for influenza B viruses. Among the subtyped influenza A viruses, 2/225 (0.09%) were A(H1N1)pdm2009, 216/225 (96%) were A(H3N2) and 7/225 (0.3%) could not be subtyped due to the low viral load. Of the 25 analysed influenza B viruses, 20/25 (80%) were B/Yamagata lineage and 3 (12%) were B/Victoria lineage. For two samples the lineage could not be determined due to low viral load (Figure 3).

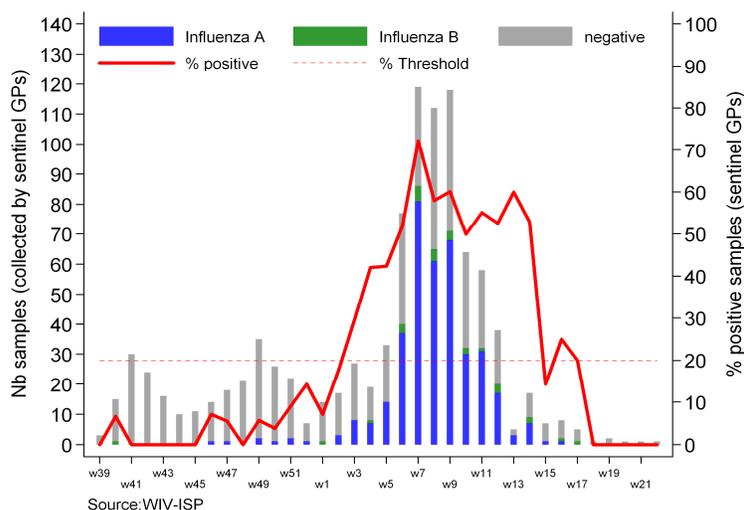


Figure 3. Weekly detection of influenza viruses in Belgium from week 40-2011 to week 20-2012 in the network of sentinel GPs



D.2 Sentinel Surveillance of SARI

D.2.1 Virological surveillance

Surveillance of SARI started week 3/2012, after the first influenza cases were recorded by the SGP sentinel GPs network and ended week 16-2012, about one month after the end of the epidemic.

Origin of samples

A total of 288 samples were collected and sent by the hospital network, among which 281 were accompanied of clinical data and were analysed.

The following hospitals participated to the SARI surveillance season 2011-2012

- CHU UCL (Mont-Godinne) (Yvoir)
- CHU Saint-Pierre (Brussels)
- AZ St Jan (Brugge)
- UZ Brussel
- Jessa Ziekenhuis (Hasselt)
- Cliniques du Sud-Luxembourg (Arlon)

Typing and subtyping results

From week 5-2012 to week 16-2012, 281 respiratory samples (40% children and 60% adults) from de sentinel network of hospitals were sent and analysed by the National Centre for Influenza among which 88 (31.3 %) were positive for influenza with 86/88 (98%) influenza A and 2/88 (2 %) influenza B (Figure 4). Among the analysed influenza A viruses, 81/86 (94 %) were A(H3N2) and 5 (6 %) were non subtypable due to the low viral load. Two influenza B were detected and belonged to the Yamagata lineage.

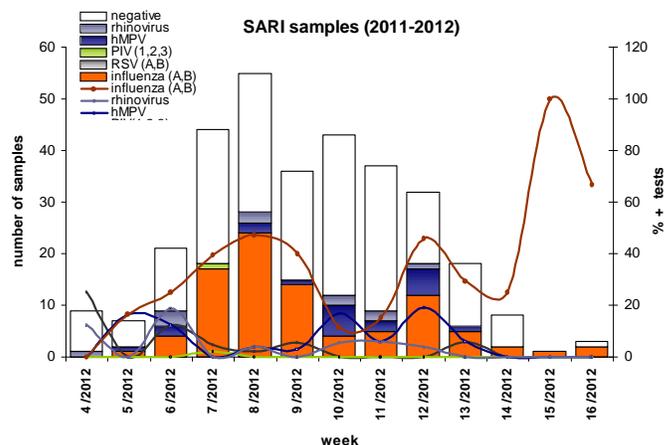


Figure 4. Weekly detection of influenza viruses and other respiratory viruses in Belgium in the SARI network from week 5-2012 to week 16-2012



Age distribution of influenza viruses and other respiratory viruses

The distribution of the different viruses (influenza and other respiratory viruses) were different in the children and adult groups, with a higher percentage of influenza viruses in adults. In children the most predominant viruses were influenza A, RSV, Rhinoviruses and hMPV viruses (Figure 5).

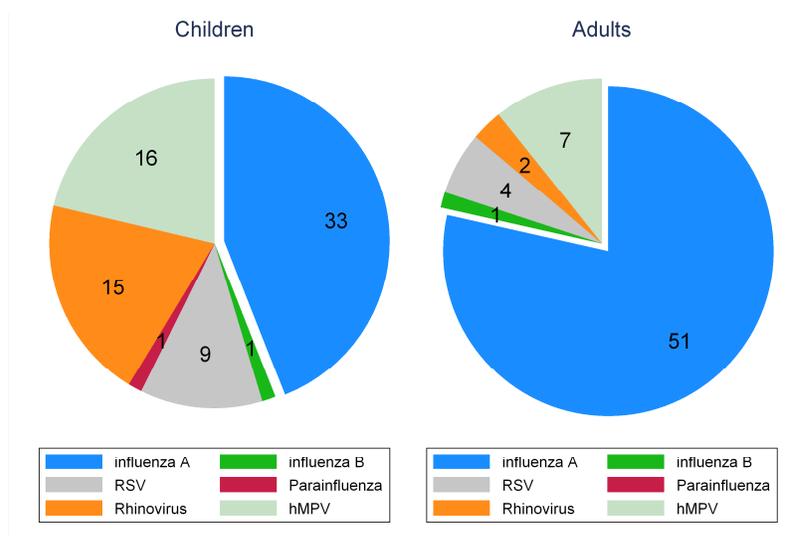


Figure 5. Proportion of the different respiratory viruses in children and adult from week 4/2012 to week 16/2012.

D.3 Non sentinel surveillance

Twenty six respiratory samples from patients with suspected severe influenza were sent from hospitals around the country during the 2011-2012 season and analysed at the NIC for confirmation and subtyping. Nine samples were influenza A positive and all of them were positive for A(H3N2).

D.4. Characterisation of the viruses

D.6.1 A(H1N1)pdm2009

Genetic characterisation

Only two influenza A(H1N1)pdm2009 were detected during this season and these viruses were not sequenced.

Antigenic characterisation

The two samples that were sent to London were unable to grow on cell culture, so no further analysis on the clinical sample was undertaken.



D.6.2 A(H3N2)

Genetic characterisation

A phylogenetic tree showing the vaccine strain, reference strains and 33 A(H3N2) isolates in Belgium during the season 2011-2012 is presented in figure 6. 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.

All sequenced viruses belonged to group3 characterised by V223I substitution. Among them 10 belonged to group 3A represented by **A/Stockholm/18/2011** (characterised by substitution N144D), 13 belonged to group 3B represented by **A/England/259/2011** (characterised by substitution N145S, A198S and N312S) and 5 belonged to group 3C represented by **A/Victoria/361/2011** and characterised by S45N, T48I, A198S and N312S mutations.

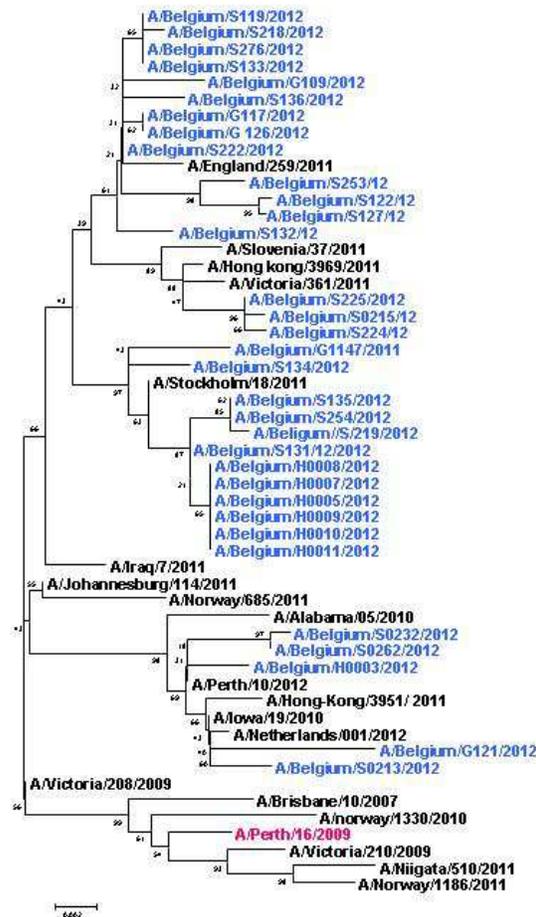


Figure 6. Phylogenetic analysis of circulating influenza A(H3N2) strains during the 2011-2012 season.



Antigenic characterisation

From the 7 A(H3N2) samples sent to CC WHO London, 4 recovered well in cell culture. Test viruses gave low reactivity (≥ 8 -fold reductions) with sera raised against the vaccine virus A/Perth/16/2009 in comparison with the homologous titre of the serum with the vaccine virus. They all showed better reactivity with sera raised against the more recently isolated reference viruses A/Alabama/5/2010, A/Hong Kong/3969/2011 and A/Stockholm/18/2011.

Post infection ferret sera										
Viruses	Collection Date	A/Bris 10/07 F29/09	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Vic 210/09 F11/10	A/Ala 5/10 F27/10	A/Perth 10/10 F03/11	A/HK 3969/11 F27/11	A/Stock 18/11 F28/11	A/Iowa 19/10 F15/11
Genetic group					group 1	group 5	group 5	group 3C	group 3A	group 6
REFERENCE VIRUSES										
A/Brisbane/10/2007	2007-02-06	1280	80	80	160	40	160	320	80	80
A/Perth/16/2009	2009-07-04	<	1280	80	640	320	640	1280	320	320
A/Victoria/208/2009	2009-06-02	640	1280	5120	5120	1280	5120	5120	2560	5120
A/Victoria/210/2009	2009-06-02	640	2560	2560	5120	640	2560	1280	1280	2560
A/Alabama/5/2010	2010-07-13	<	80	40	80	320	320	640	320	320
A/Perth/10/2010	2010-05-25	320	640	1280	2560	1280	2560	2560	1280	2560
A/Hong Kong/3969/2011	2011-05-19	160	320	160	640	320	640	1280	640	640
A/Stockholm/18/2011	2011-03-28	80	160	80	160	160	320	640	640	320
A/Iowa/19/2010	2010-12-30	160	1280	5120	2560	2560	5120	5120	5120	5120
TEST VIRUSES										
A/Belgium/G1063/2011	3B 2011-11-25	40	40	80	80	160	320	640	320	320
A/Belgium/G1109/2011	3C 2011-12-05	40	160	320	160	640	1280	2560	640	1280
A/Belgium/G1125/2011	3B 2011-12-10	40	80	160	160	160	640	1280	320	320
A/Belgium/G1147/2011	3A 2011-12-16	80	160	320	160	320	640	1280	1280	640
Vaccine virus										

Table 3. Antigenic analyses of influenza A H3N2 viruses

D.6.3 B Yamagata

Genetic characterisation

Thirteen samples positive for influenza B Yamagata lineage were sequenced (Figure 7). All belonged to the Yamagata lineage and were close to the reference strains B/Bangladesh/3333/07 and B/Brisbane/3/2007. Viruses within this clade have characteristic amino acid substitutions of S150I, N165Y and G229D.

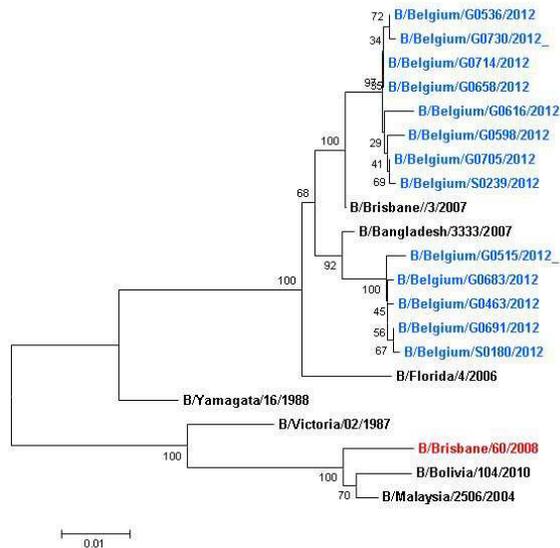


Figure 7. Phylogenetic analysis of circulating influenza B Yamagata strains

Antigenic characterisation

The two samples that were sent to London were unable to grow on cell culture, so no further analysis on the clinical samples was undertaken.

D.6.3 B Victoria

We only detected 3 influenza B of Victoria lineage. These viruses were not characterized.

D.5 Recommended composition of influenza virus vaccines for use in the 2012-2013 northern hemisphere influenza season

The vaccine against influenza is a trivalent vaccine and contains the three viruses currently circulating in the human population: A (H1N1), A (H3N2) and B. Due to the constant evolution of the genome of influenza virus ("antigenic drift"), the composition of the trivalent vaccine against influenza should be regularly adjusted (Figure 8).

Twice a year, in February for the Northern Hemisphere and in September for the southern hemisphere, a WHO consultation held to determine the composition of the vaccine for the following season. Vaccine production is a process that lasts about 6 months. Two influenza strains were changed in the vaccine composition. Influenza A/Perth/16/2009 (H3N2) has been replaced by influenza A/Victoria/361/2011 (H3N2).

Influenza B/Brisbane/60/2008 strain, a Victoria-like lineage, has been replaced by a Yamagata-like lineage, the influenza B/Wisconsin/1/2010 strain.



- A/California/7/2009 (H1N1)pdm09-like virus;
- cell-propagated prototype virus A/Victoria/361/2011;
- B/Wisconsin/1/2010 (YAM).

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/Floria/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)

Figure 8. Evolution of the composition of the trivalent influenza vaccine 2000 – 2013

E. Conclusion

The influenza season 2011-2012 started later than usual. Compared to the previous seasons, the intensity was mild. After two seasons dominated by the pandemic influenza virus A (H1N1)pdm09 virus, influenza A (H3N2) viruses predominated during the 2011-2012 season constituting 86% of all the analysed influenza viruses. Influenza A (H1N1)pdm09 was detected sporadically and influenza B viruses were also detected at a low rate. Influenza viruses started to be detected from mid-December 2011 and then peaked during week 7-2012. During this peak period, the positive detection rate increased to 73%. The epidemic continued until mid-March with a positive detection rate that fell below 20% during the last weeks.

An interesting aspect was the diversity of influenza A (H3N2) variants that circulated during the epidemic. The circulating variants were genetically and antigenically distinct from the vaccine strain A/Perth/16/ 2009. Furthermore, most of the influenza B viruses which circulated in Belgium and Europe during this season were from the Yamagata lineage while the vaccine strain B/Brisbane/60/2008 was from the Victoria lineage. Consequently, most circulating influenza viruses during this epidemic were less covered by the 2011-2012 vaccine. This could have contribute to the low to moderate influenza "vaccine effectiveness" this season, which has been observed in several countries in Europe. For this reason, two influenza strains have been adjusted in the 2012-2013 vaccine.



F. Acknowledgements

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