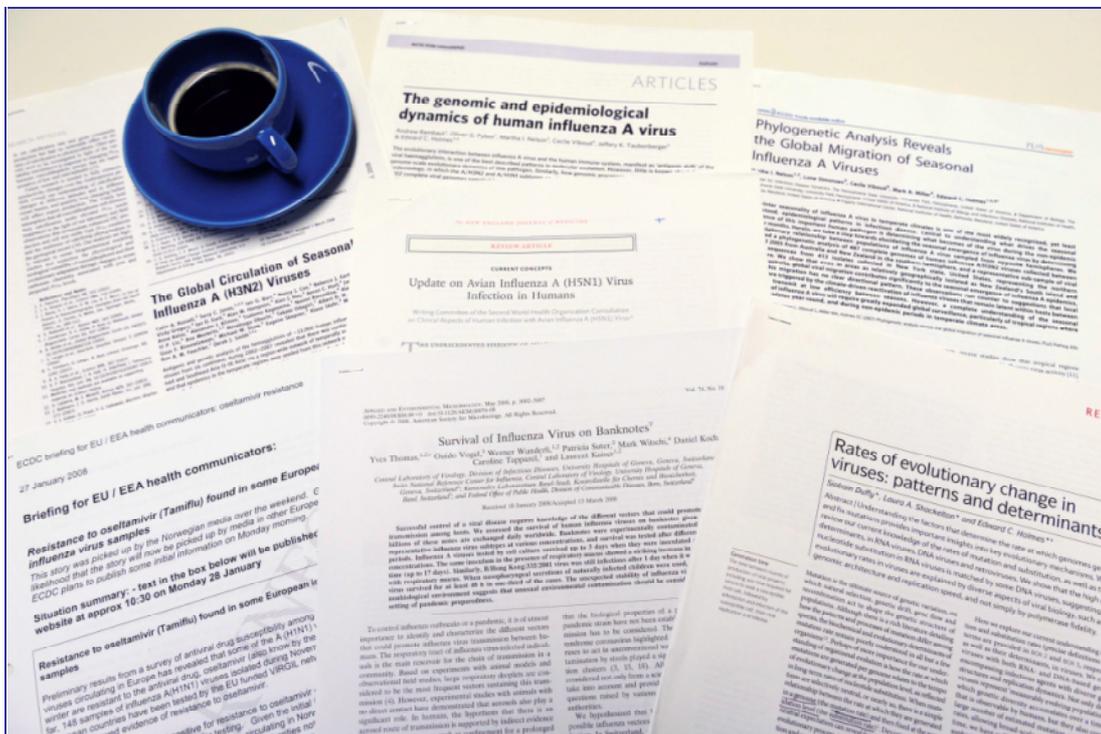


Influenza surveillance in Switzerland Sentinel network report Season 2007 - 2008



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Cover page : influenza headlines in 2007-2008, picture by Pascal Cherpillod

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2. RESUME-SUMMARY- ZUSAMMENFASSUNG

2.1. Résumé

L'épidémie de grippe a été modérée cet hiver. Les virus influenza A (H1N1) ont circulé en majorité la première phase de la saison et les virus influenza B présents dès le début de l'épidémie sont devenus prédominants dans une deuxième phase. Les virus influenza A (H3N2) sont restés sporadiques pendant toute l'épidémie. Les virus influenza A (H1N1) étaient antigéniquement proches de la souche vaccinale influenza A/Solomon Island/03/06. L'analyse génétique a montré que ces virus H1N1 étaient plus proches de la souche plus récente influenza A/Brisbane/59/07. La majorité des virus influenza B appartenait à la lignée Yamagata. Ils étaient antigéniquement et génétiquement proches de la souche influenza B/Jiangsu/10/2003 et des souches plus récentes B/Florida/7/05 et B/Egypt/144/2005. Une minorité de virus influenza B appartenait à la lignée Victoria. Ils étaient proches de la souche vaccinale influenza B/Malaysia/2506/2004. Certains de ces virus B étaient plus proches de la souche plus récente B/Victoria/304/06. Les virus influenza A (H3N2) étaient antigéniquement proches de la souche influenza A/Brisbane/10/2007 apparue récemment. Des virus influenza A (H1N1) résistants à l'Oseltamivir ont été détectés à un taux sans précédent cette année en Europe. Dans le réseau de surveillance Suisse, 18.8 % de virus influenza A (H1N1) présentaient la mutation H274Y dans le gène de la Neuraminidase qui confère la résistance. L'utilisation de l'Oseltamivir ne semble pas être à l'origine de l'apparition de ces virus résistants. En outre, 5/5 virus influenza A (H3N2) détectés dans notre pays présentaient la mutation S31N dans le gène de la Matrice conférant ainsi la résistance à l'Amantadine.

2.2. Summary

The influenza epidemic was of mild intensity in Switzerland during the 2007/2008 season. Influenza A (H1N1) viruses predominated in the first part of the season and influenza B, present since the beginning of the epidemic, became predominant in the second part. Influenza A (H3N2) remained sporadic throughout the entire epidemic. Influenza A (H1N1) viruses were antigenically related to the vaccine strain influenza A/Solomon Island/03/06. Genetic analysis showed that these A (H1N1) viruses were also close to the more recent influenza A/Brisbane/59/07. Most influenza B viruses were of the Yamagata sub-lineage. They were antigenically and genetically related to influenza B/Jiangsu/10/2003 and to the more recent influenza B/Florida/7/05 and B/Egypt/144/2005. A minority of influenza B viruses was of the Victoria sub-lineage and were antigenically related to the vaccine strain influenza B/Malaysia/2506/2004. Some of these B viruses were closer to the more recent B/Victoria/304/06. Influenza A (H3N2) detected were antigenically related to influenza A/Brisbane/10/2007, a variant that appeared recently. Influenza A (H1N1) viruses resistant to oseltamivir were detected at an unprecedented rate this year in Europe. In the Swiss Sentinel network, 18.8% of influenza A (H1N1) viruses harboured the H274Y mutation in the neuraminidase gene conferring resistance to oseltamivir. In addition, of five influenza A (H3N2) viruses detected in our country, all had the S31N mutation conferring resistance to amantadine.

2.3. Zusammenfassung

Die Influenza Epidemie der Saison 2007/2008 war in der Schweiz von einer schwachen Intensität. Influenza A (H1N1) Viren wurden am häufigsten in der ersten Hälfte des Winters nachgewiesen und Influenza B, welche seit Beginn präsent waren, dominierten dann in der zweiten Hälfte. Influenza A (H3N2) Viren traten während der ganzen Epidemie nur sporadisch auf. Influenza A (H1N1) war antigenetisch verwandt mit dem im Impfstoff enthaltenen Influenza A der A/Solomon Islands/03/06. Genetische Untersuchungen zeigten, dass die Influenza A (H1N1) Viren zusätzlich nahe verwandt waren mit dem neu erschienen Influenza A Brisbane/59/07. Die meisten der nachgewiesenen Influenza B Viren gehörten zur Yamagata Linie. Diese waren verwandt mit Influenza B/Jiangsu/10/2003 und mit der neueren Variante Influenza B/Florida/7/2005 und B/Egypt/144/2005. Eine Minderheit der Influenza B Viren gehörte zur Viktoria Linie und war verwandt mit dem Impfstamm Influenza B/Malaysia/2506/2004. Einige der Influenza B waren näher verwandt mit Influenza B/Victoria/304/2006. Die nachgewiesenen Influenza A (H3N2) waren antigenetisch verwandt mit Influenza A/Brisbane/10/2007 einer Variante welche erst kürzlich aufgetaucht war. Gegen Oseltamivir resistente Influenza A (H1N1) traten in Europa in einem noch nie dagewesenen Ausmasse auf. In der Schweizer Bevölkerung wiesen etwa 18,8% der Influenza A (H1N1) die Mutation H274Y im Gen der Neuraminidase auf welche für die Resistenz gegen Oseltamivir verantwortlich ist. Der Gebrauch von Oseltamivir scheint aber nicht die Ursache für das Auftreten dieser Resistenz zu sein. Zusätzlich wurde bei den 5 nachgewiesenen Influenza A (H3N2) die Mutante S31N nachgewiesen welche das Virus resistent gegen Amantadine macht.

3. INTRODUCTION

Influenza epidemic surveillance is conducted in Switzerland by the Federal Office of Public Health (FOPH) in close collaboration with a network of general practitioners representing all regions of the country. Specimens are analyzed at the National Influenza Reference Centre which is part of the Laboratory of Virology, University Hospitals of Geneva, and its associated research centres affiliated to the Medical School of the University of Geneva. For the second consecutive year, the main backbone of our virological surveillance was conducted based on a reverse transcription-polymerase chain reaction (RT-PCR) screening strategy. In addition to a phenotypic analysis based on the inhibition of the hemagglutination (IHA), the hemagglutinin (HA) was sequenced to determine the nature of the influenza virus at the genome level. Neuraminidase (NA) genotyping was also developed and applied to assess the presence of a signature for oseltamivir resistance.

4. METHOD OF DETECTION FOR INFLUENZA VIRUSES

4.1. Clinical identification of influenza cases

During the 2007-08 season, a network of 176 practitioners participated actively to the clinical surveillance of influenza cases. This surveillance is based on a weekly count of medical consultations for an influenza-like illness (MC-ILI). The case definition used is the presence of fever of 38°C, with or without a feeling of sickness, myalgia, or an alteration of the general status. In addition to fever, acute respiratory symptoms such as cough and/or rhinorrhea must be present. The geographic distribution of the participating general practitioners is shown in Figure 1.

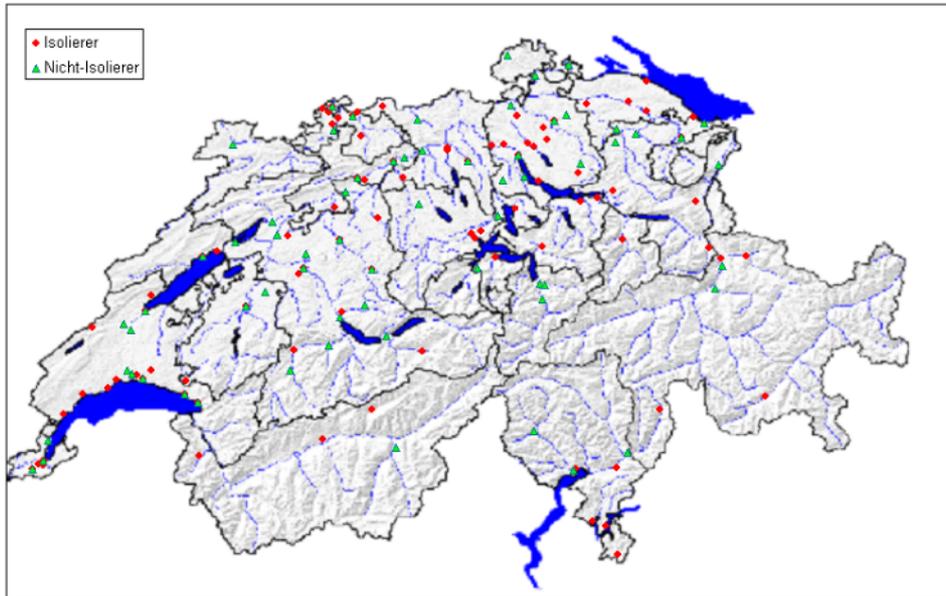


Figure 1: Geographical distribution of the 176 participants in the Sentinel network

Each participant is represented by a coloured dot: green = participants conducting both clinical surveillance and collection of specimens (n=90); red = participants conducting only clinical surveillance (n=86)

A subgroup of 50% of participating practitioners (n=90) provide clinical specimens from a patient selection in addition to clinical surveillance. Nasopharyngeal and pharyngeal specimens are then sent in a transport medium by regular mail to the National Influenza Reference Centre in Geneva for subsequent viral detection and characterization.

A sampling selection procedure of specimens considered for influenza identification was based on the following strategy: 1) before and after the epidemic phase, a sample was taken from all patients consulting a Sentinel practitioner; 2) during the epidemic phase, the sampling strategy was adapted and only one from every eighth patient with a ILI was sampled. The epidemic phase is defined by a threshold of 58 cases of MC-ILI per 100,000 inhabitants.

4.2. Detection of influenza viruses

The presence of influenza virus in samples is determined first by two RT-PCR assays. This allows the rapid detection of the genome of influenza A and B types, even if the virus has been inactivated by the transport. The nature of the NA is also determined by subtype-specific N1 or N2 RT-PCR assays (Schweiger et al, 2000). Positive samples are then cultivated on appropriate cell lines for antigenic characterization by IHA. This allows to specify the antigenic properties of circulating strains and to assess the potential emergence of vaccine escape variants (Figure 2). Cell lines used for the surveillance are specific for influenza culture, i.e. MDCK and SIAT cells. The latter are modified MDCK cells enriched with Sialic acid-coupled protein which is the cellular receptor used by influenza virus to enter the cell. These cells are supposed to provide a higher efficiency of influenza virus multiplication by cell culture.

During the first and last weeks of surveillance, a random sampling of negative specimen are regularly inoculated on cells for virus culture. The goal of this strategy is to detect influenza strains that could escape RT-PCR detection. This event could be explained by the presence of a drifted mutant in the regions of the viral genome targeted by the RT-PCR primers and probes. A virus of animal origin could also escape the RT-PCR detection since the method is intended for human viruses only (Van Elden, 2001). Cell culture is coupled with immunofluorescence detection using viral nucleoprotein-specific monoclonal antibodies. This very sensitive method allows for the detection of viral antigen even in low yields of viral culture cases (see Figure 3).

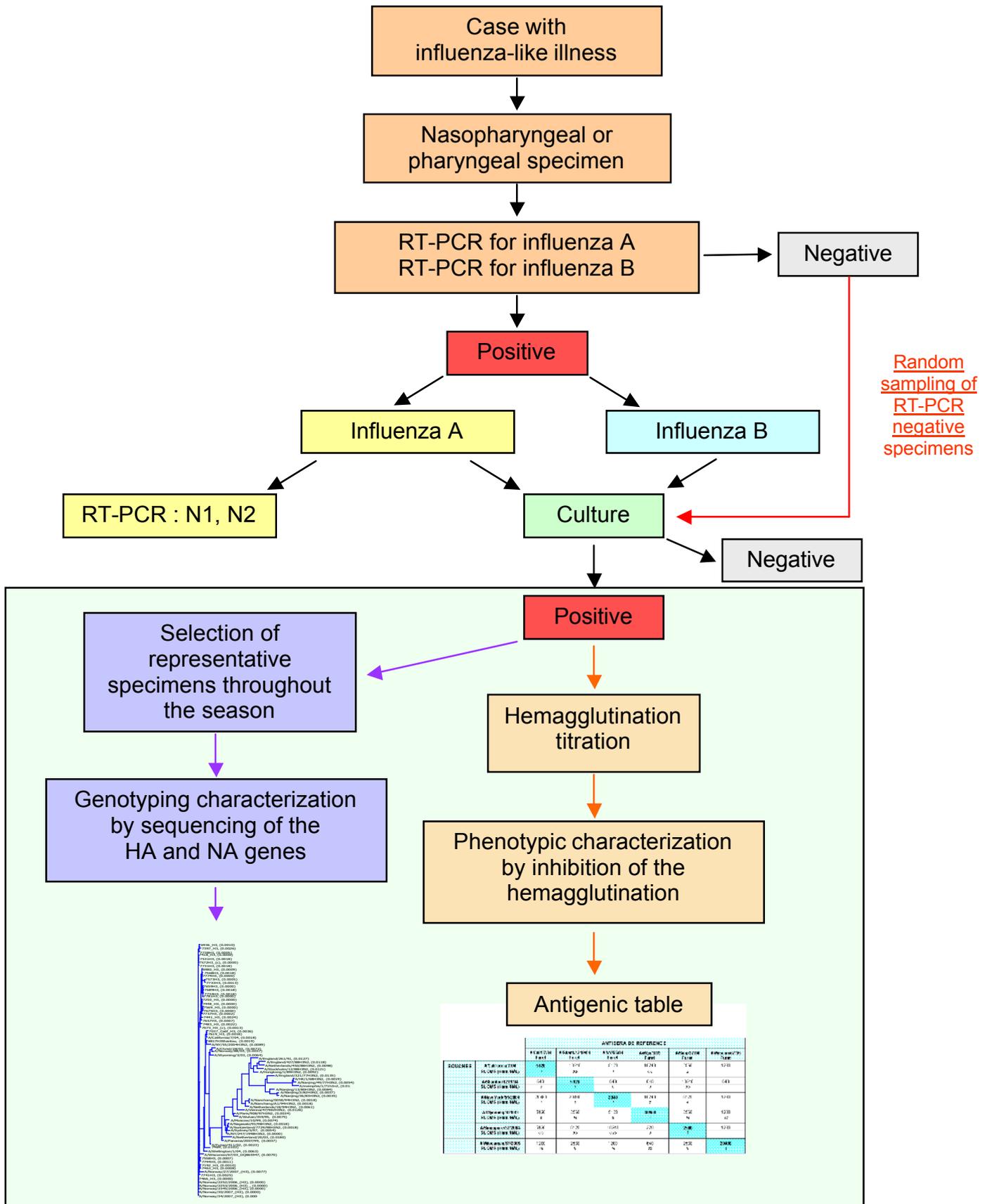


Figure 2 : Procedure used for the detection of influenza viruses in Sentinel surveillance

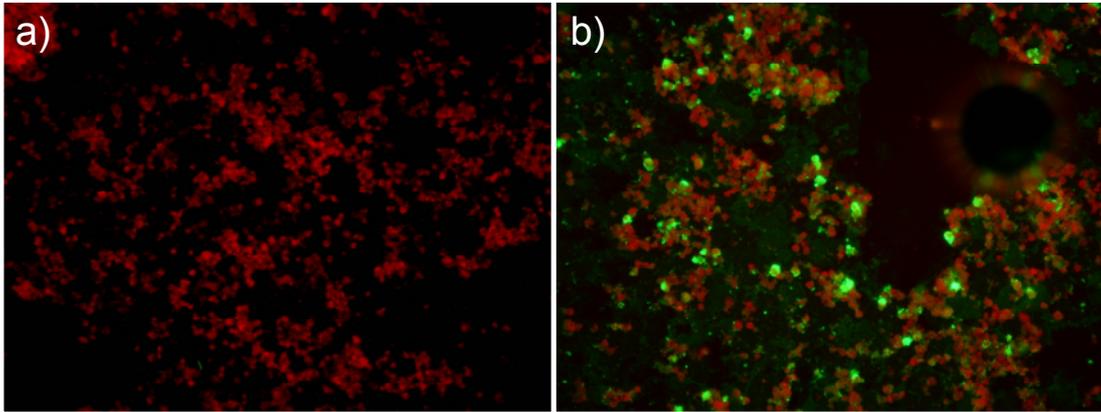


Figure 3: SIAT cells infected by influenza A/Solomon Islands/03/06 (H1N1) virus after 4 days incubation at 37°C with 5% CO₂.

a) Negative control

b) Immunostaining of influenza virus obtained with monoclonal anti-influenza A primary antibody and monoclonal FITC conjugate revealing the presence of viral antigen in cells (Chemicon[®], USA)

4.3. Characterization of influenza viruses

In a presence of a positive cell culture, the cell supernatant containing the viral strain is analysed by hemagglutination and an IHA reaction. In this latter reaction, the ability of the virus to link to the red blood cell receptor is tested in the presence or absence of subtype-specific antisera from immunized ferrets. A specific recognition of the HA by a given antiserum inhibits the interaction between this HA and the red blood cell receptor. In the present analysis, guinea pig red blood cells were used for this reaction. The results are interpreted according to an antigenic table adapted to circulating strains and established at the beginning of the season. The 2007/2008 antigenic features are presented in Table 1.

In this procedure, the titres obtained with each strain are identified and compared with reference antisera adapted to available antisera and circulating strains. This allows a standardized identification of the antigenic characteristics of the HA of a given strain. The ratio between the homologous titres and the observed titres obtained with the circulating influenza strains allows to define the antigenic relationship to the standard strains. In turn, this allows the detection of the antigenic variations present in the HA which is one of the major targets of the immune response.

Table 1 : Hemagglutination inhibition (IHA) titres of reference influenza strains incubated with the 2007/2008 reference antisera

The value obtained in the reaction of the reference strain with the corresponding antiserum represents the homologous titre (HT). The titre obtained with the clinical isolate from a Sentinel sample (Sen) is then compared with the HT titre. If the ratio Sen/HT is ≤ 4 , the strain is considered as antigenically related to the reference strain. If the ratio is > 4 , the strain is considered as antigenically and specifically different from the reference strain.

a) Influenza A (H3N2) :

	Wis67/05 Ferret	Brisban/10/07 Ferret	Nep921/06 Ferret	Hiro52/05 Ferret
A/Wisconsin/57/2005 WHO strain	2048	512	512	1024
Brisban/10/2007 WHO strain	512	256	512	128
A/Nepal/921/06 WHO strain	128	256	1024	128
A/Hiroshima/52/2005 WHO strain	1024	512	512	1024

b) Influenza A (H1N1)

	REFERENCE ANTISERA						
	AN-Cal20/99 Ferret	Thess128/04 Ferret	Egypt39/05 Ferret	Fuku141/06 Ferret	HKong2652/06 Ferret	Solo103/06 Ferret	St Peter Ferret
A/N-Caledonia/20/99 WHO strain	128	128	256	32	8	16	128
A/Thessaloniki/24/2005 WHO strain	128	512	256	32	16	16	128
A/Egypt/39/05 WHO strain	64	64	256	32	16	16	64
A/Fukushima/141/06 WHO strain	128	32	64	128	64	128	< 8
A/Hong-Kong/2652/06 WHO strain	256	32	64	256	256	256	< 8
A/Solomon103/06 WHO strain	32	64	64	128	128	256	< 8
A/St Petersburg/10/07 WHO strain	128	128	128	512	16	16	128

c) Influenza B

	REFERENCE ANTISERA							
	Mal2506/04 Ferret	Bris/32/02 Ferret	Vic/304/06 Ferret	HK/45/05 Ferret	Shang361/02 Ferret	Jiang10/03 Ferret	Egy/144/05 Ferret	Flo/7/05 Ferret
B/Malaysia/2506/2004 WHO strain	256	64	64	64	<8	<8	<8	<8
B/Brisbane/32/2002 WHO strain	256	128	128	32	<8	<8	<8	<8
B/Victoria/304/2006 WHO strain	512	128	256	64	<8	<8	<8	<8
B/Hong Kong/45/2005 WHO strain	256	64	64	32	<8	<8	<8	<8
B/Shanghai/361/2002 WHO strain	64	<8	16	<8	512	64	256	512
B/Jiangsu/10/2003 WHO strain	<8	<8	<8	<8	64	256	128	64
B/Egypt/144/05 WHO strain	64	<8	16	<8	512	64	512	256
B/Florida/7/05 WHO strain	128	16	16	<8	1024	64	512	1024

5. RESULTS FROM THE 2007-2008 SEASON

5.1 Characteristics of patients with influenza infection

5.1.1. Frequency of viruses detected in a particular age group

The proportion of Influenza viruses detected in Sentinel specimens is represented according to age group and virus type (Figure 4). Influenza A and B viruses have been detected in all patient age groups. A mean of 40% of positive samples was observed across all age groups, apart from those over 60 years old. For this latter group, a smaller positive rate was observed. Influenza A (H1N1) virus ratio was low in those over 60 years old (9%) and no influenza virus was detected in persons over 70 years old. In comparison, in the 10-19, 20-29 and under one-year old age groups, the ratio of influenza A (H1N1) detected in samples were higher (20, 20 and 29%, respectively).

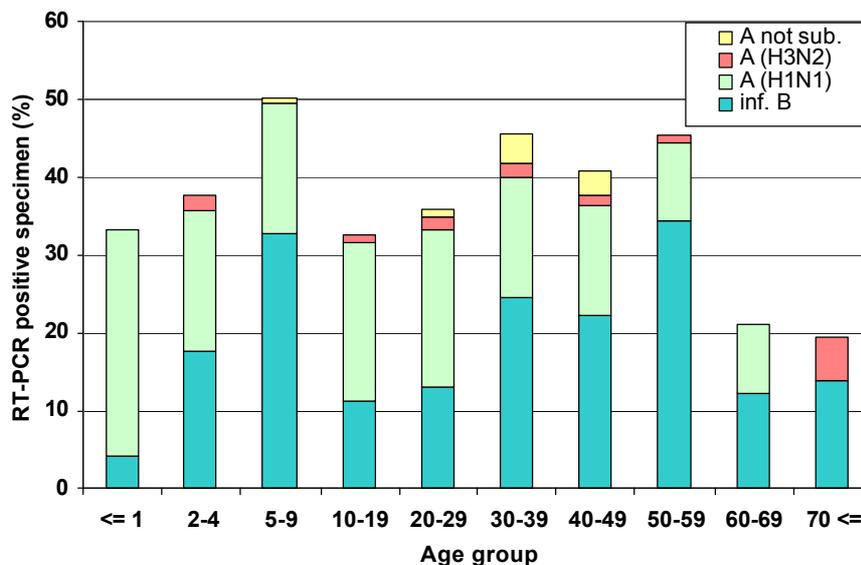


Figure 4: Percentage of viruses detected on number of samples according to age groups

A not sub: influenza A virus not subtyped; A (H3N2): influenza A (H3N2) virus; (H1N1): influenza A (H1N1) virus; inf. B: influenza B virus

5.1.2. Clinical features of participating subjects

45% (419/922) of patients infected with influenza virus were female and 55% (503/922) were male. The age average of patient was of 34 ± 20 years old. The oldest was 90 years old and the youngest was 5 months. Symptoms associated to influenza infection have been recorded. Proportion of the five main symptoms observed in case of a laboratory confirmed influenza infection is shown in Figure 5. The most frequent symptoms observed were the fever and cough, as observed usually during seasons.

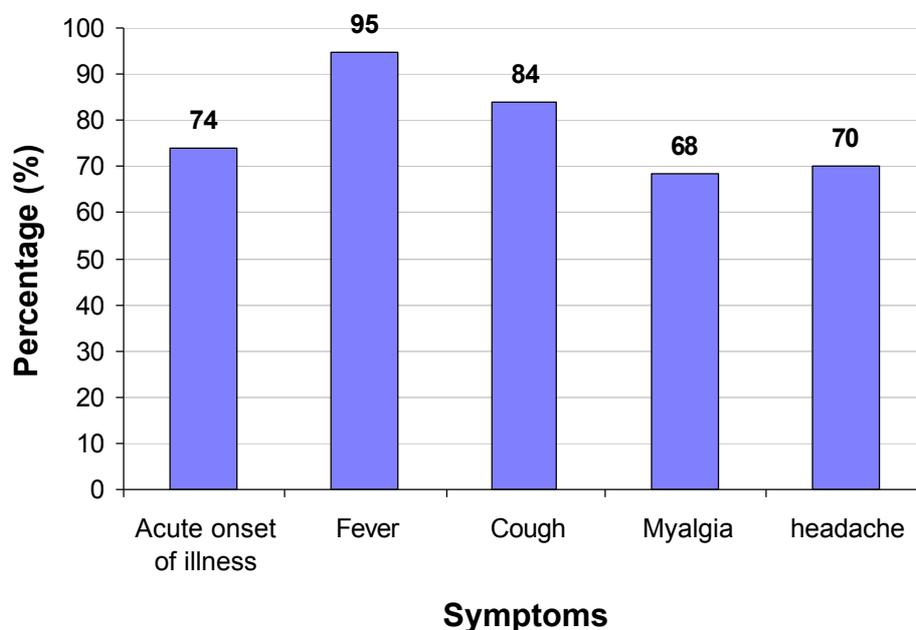


Figure 5: Symptoms recorded according to the presence or absence of influenza virus

5.2. Detection by molecular assays of influenza viruses in nasopharyngeal specimens

The active surveillance phase began on 22 September 2007 and ended on 18 April 2008 after a period of 30 weeks. Nine hundred and twenty-two samples from 99 different Sentinel practitioners were analysed. Of these, 352 influenza viruses were detected positive by RT-PCR, representing an average positive rate of 38% over 33 weeks surveyed (Figure 6a).

Among the 352 positive cases, 164 (47%) were influenza type A viruses and 188 (53%) influenza type B (Figure 6b). Among influenza A viruses, 143 (41%) were from the H1N1 subtype and 12 (3.4%) of the H3N2 subtype (9 (2.6%) influenza A viruses could not be subtyped).

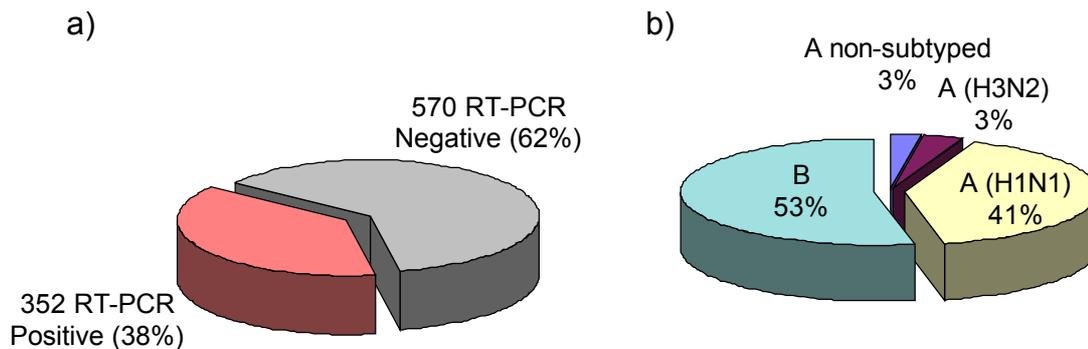


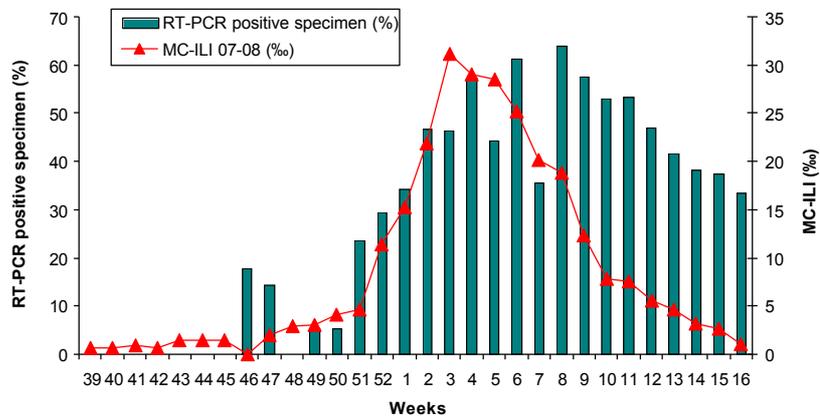
Figure 6: Nasopharyngeal specimens positive for any influenza virus during the 2007/2008 season (n=922)

a) Number of RT-PCR-positive versus -negative specimens; b) distribution of the different types and subtypes of influenza viruses detected.

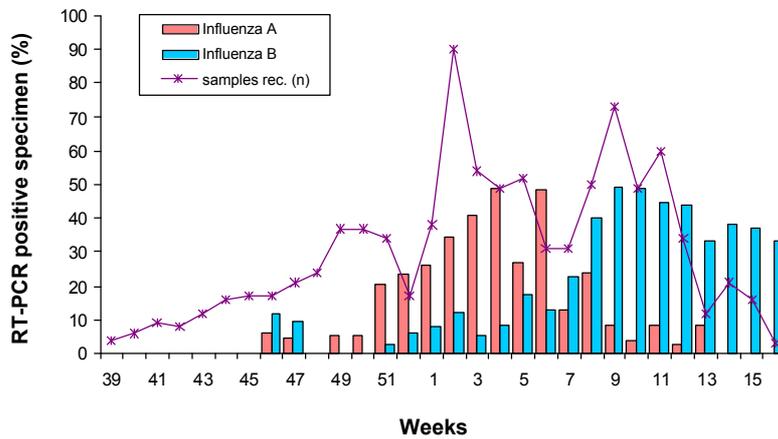
Influenza A and B viruses started to be detected concomitantly in early November 2007 (week 46) as sporadic cases (Figure 7a). Both viruses co-circulated throughout the season until week 13, although differential kinetics have been observed. Influenza A viruses were the dominant strain during the first peak of the season and culminated at week 4, whereas influenza B viruses became dominant four weeks later, peaked at week 9, and dominated the second part of the season when influenza A viruses were less observed (Figure 7b).

The MC-ILI kinetic as reported in our system well paralleled that observed for influenza A virus detection (Figure 7a). On the other side, influenza B detection rate did not parallel the kinetic of MC-ILI cases. In addition, the percentage of positivity was relatively high. Whether this is due to the smallest member of different pattern of disease between influenza A and B cannot be clarified in this epidemiological surveillance.

a)



b)



c)

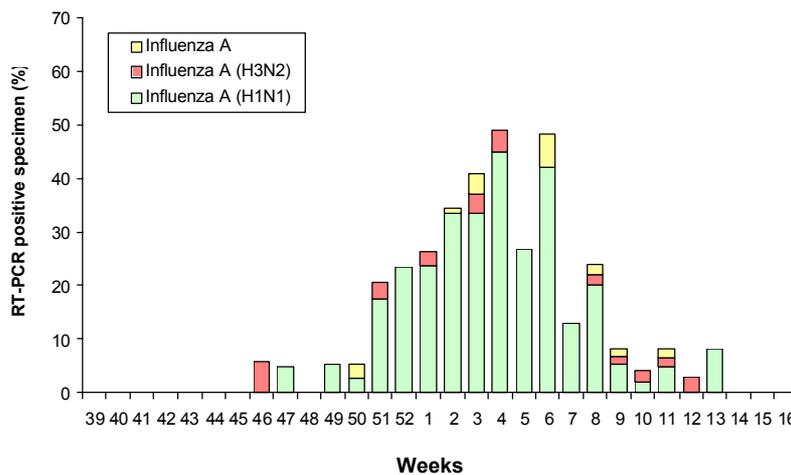


Figure 7: MC-ILI, positivity rate, and type distribution of RT-PCR-positive cases

a) Proportion of influenza viruses and MC-ILI 2007/2008 distributed per week. b) Percentage of influenza A and B viruses detected and number of samples received per week. c) Weekly subtype distribution of influenza A cases, sample rec: number of samples received. MC-ILI: medical consultations for influenza-like illness

Ninety-seven percent of influenza A viruses could be subtyped. Influenza A (H1N1) viruses were predominant among influenza A viruses (Figure 7c) and only a few influenza A (H3N2) viruses were detected sporadically during the whole season.

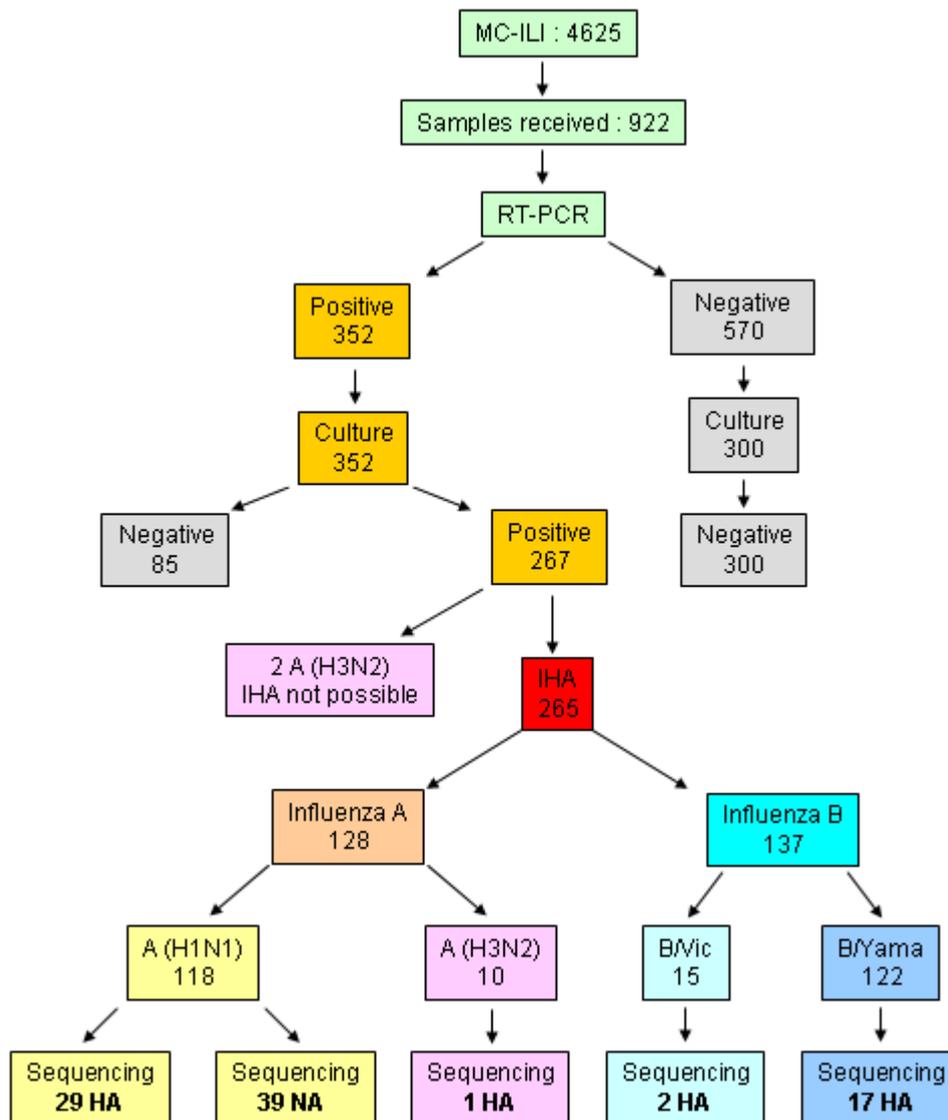


Figure 8 : Summary of the analysis performed on Sentinel samples at the NRCI

IHA: inhibition of the hemagglutination, B/Vic: B/Victoria-like sublineage, B/Yama: B/Yamagata sublineage, HA: hemagglutinin, NA: Neuraminidase.

5.3. Antigenic and genetic characterization of influenza viruses

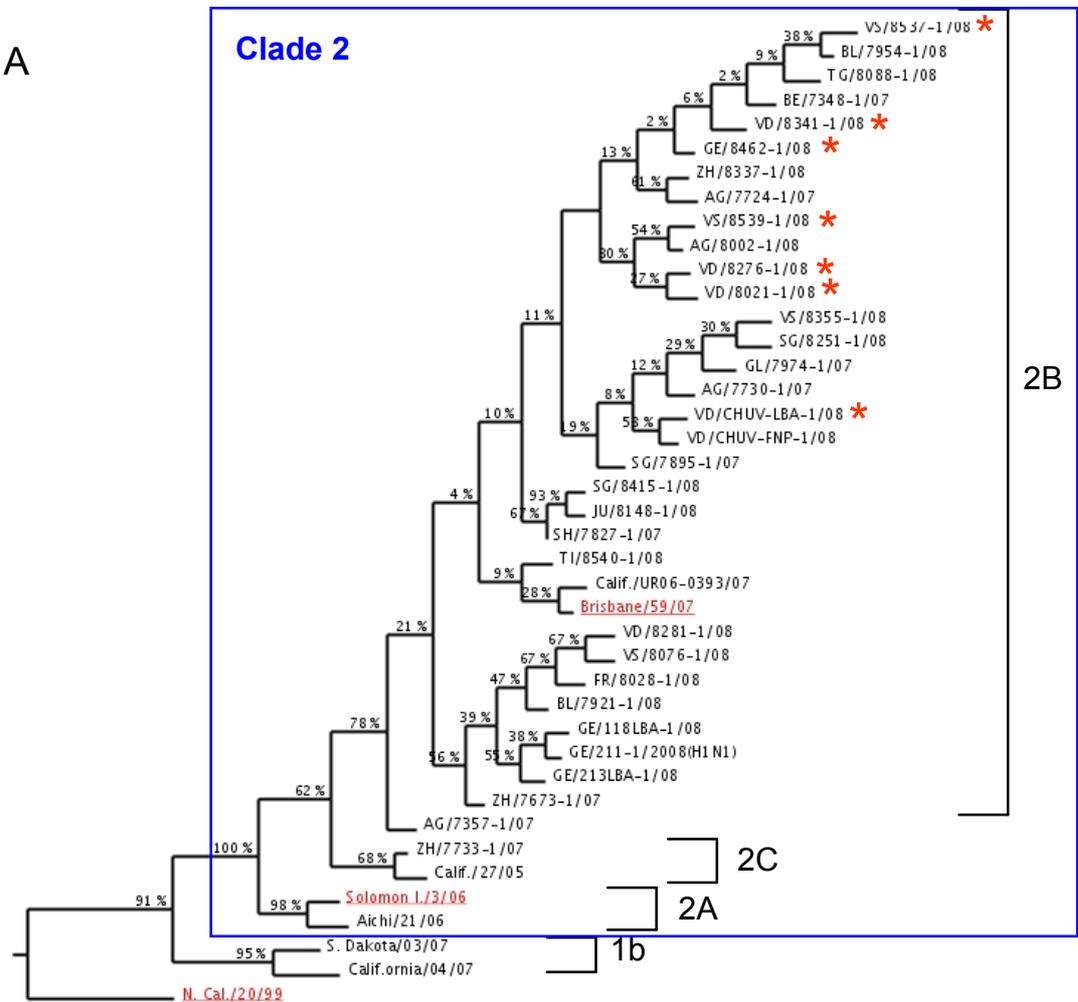
Of all influenza viruses detected by RT-PCR, 267 of 352 (76%) revealed to be culture positive (Figure 8). 137 influenza B viruses and 128 influenza A viruses, representing 118 influenza A (H1N1) and 10 influenza A (H3N2), were phenotyped. Sequencing analysis of a subgroup of these culture positive strains confirmed the nature of HA in 50 cases (29 influenza A/H1, 1 influenza A/H3 and 20 influenza B) (Figure 8). In addition, 39 influenza A (H1N1) viruses were analysed for resistance to oseltamivir by NA sequencing and these results are discussed in chapter 5.3.4.

5.3.1. Influenza A (H1N1)

Influenza A (H1N1) viruses were predominant during the first part of the season. One hundred and eighteen influenza A (H1N1) viruses detected by RT-PCR could be cultivated and antigenically characterized by IHA. Of these, 80 (68%) were antigenically closely related to the vaccine reference strain influenza A/Solomon Islands/03/06 (H1N1) and 38 (32%) to the very close variant influenza A/Fukushima/141/06. A subgroup of 29 influenza A (H1N1) viruses obtained by cell culture and collected over the season was sequenced. Alignment and phylogenetic analysis were conducted and are presented in Figure 9. Based on this analysis, it appeared that the influenza A (H1N1) viruses that circulated in Switzerland were related to influenza A/Solomon Islands/3/06 (H1N1), but more closely related to clade 2B represented by the influenza A/Brisbane/59/07 (H1N1). This strain has been selected to be included in the 2008/2009 influenza vaccine.

Oseltamivir-resistant strains were detected among influenza A (H1N1) viruses that circulated in Switzerland and a detailed analysis of this finding is presented in chapter 5.3.4.

Influenza A



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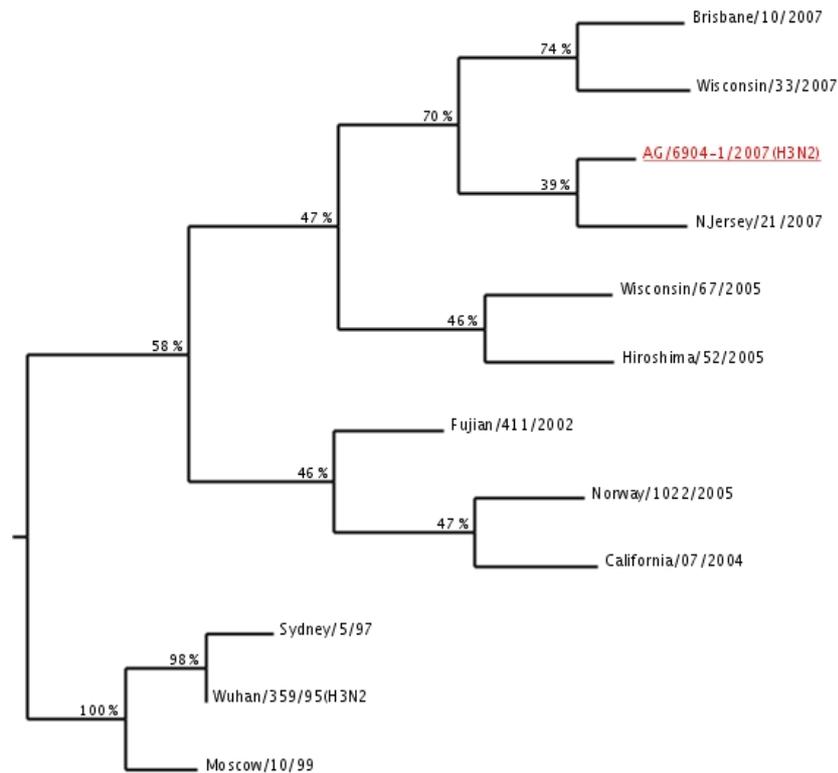
Figure 9: Phylogenetic tree of 29 influenza A (H1N1) viral hemagglutinin recovered in Switzerland.

The virus name is composed of the two letters of the origin county, the four-digit registration number of the patient attributed by the laboratory followed by the viral isolate identification number and the year; LBA: bronchoalveolar lavage, FNP: nasopharyngeal swab; reference strains are in red. Percentage values represent the bootstrap values. The numbers in bracket indicate the different clades. Red stars indicate influenza A (H1N1) viruses resistant to oseltamivir. The two specimen labelled CHUV are non-Sentinel samples.

5.3.2. Influenza A (H3N2)

Only 12 influenza A (H3N2) viruses were detected in Switzerland this season and all but one were antigenically related to influenza A/Brisbane/10/2007 (H3N2). This strain is a new variant closely related to the vaccine strain influenza

A/Wisconsin/67/2005 (H3N2). One influenza A (H3N2) provided reduced titre with our panel of antisera and could not be characterized. The HA of one influenza A (H3N2) strain was sequenced (influenza A/AG6904-1/2007 (H3N2)) and its close relation with the A/Brisbane/10/2007 was confirmed (Figure 10).



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Figure 10: Phylogenetic tree sequences of influenza A (H3N2) viral hemagglutinin including one Swiss isolate labelled in red.

5.3.3. Influenza B

The percentage of influenza B viruses detected (53%) was similar to the number of influenza A virus cases (47%). 137/188 (73%) influenza B positive with RT-PCR were isolated by cell culture and analysed by IHA; 51/188 (27%) influenza B viruses could not be isolated. Two different sublineages, Yamagata-like and Victoria-like, co-circulated. The latter was detected in a minority of cases: 122/188 (65%) influenza B viruses were of the B/Yamagata sublineage; and 15/188 (8%) were of the B/Victoria sublineage. IHA showed that the Yamagata sublineage strains were antigenically related to influenza B/Jiangsu/10/2003 and to the more recent influenza

B/Florida/7/05 (Figure 11). Some of these viruses were also analysed by the WHO Collaborating Centre (MRC) in London. Six of these Jiangsu-like viruses were antigenically closer to influenza B/Egypt/144/2005. This sublineage was not included in the 2007/2008 influenza vaccine. The B/Victoria sublineage strains were predominantly antigenically related to the influenza B//Malaysia/2506/2004 reference strain. This strain was predominant during the 2005/2006 season, but did not circulate last season. This lineage has been included in the 2008/2009 vaccine. Two B/Victoria-like strains were closer to the more recent influenza B/Victoria/304/06 strain.

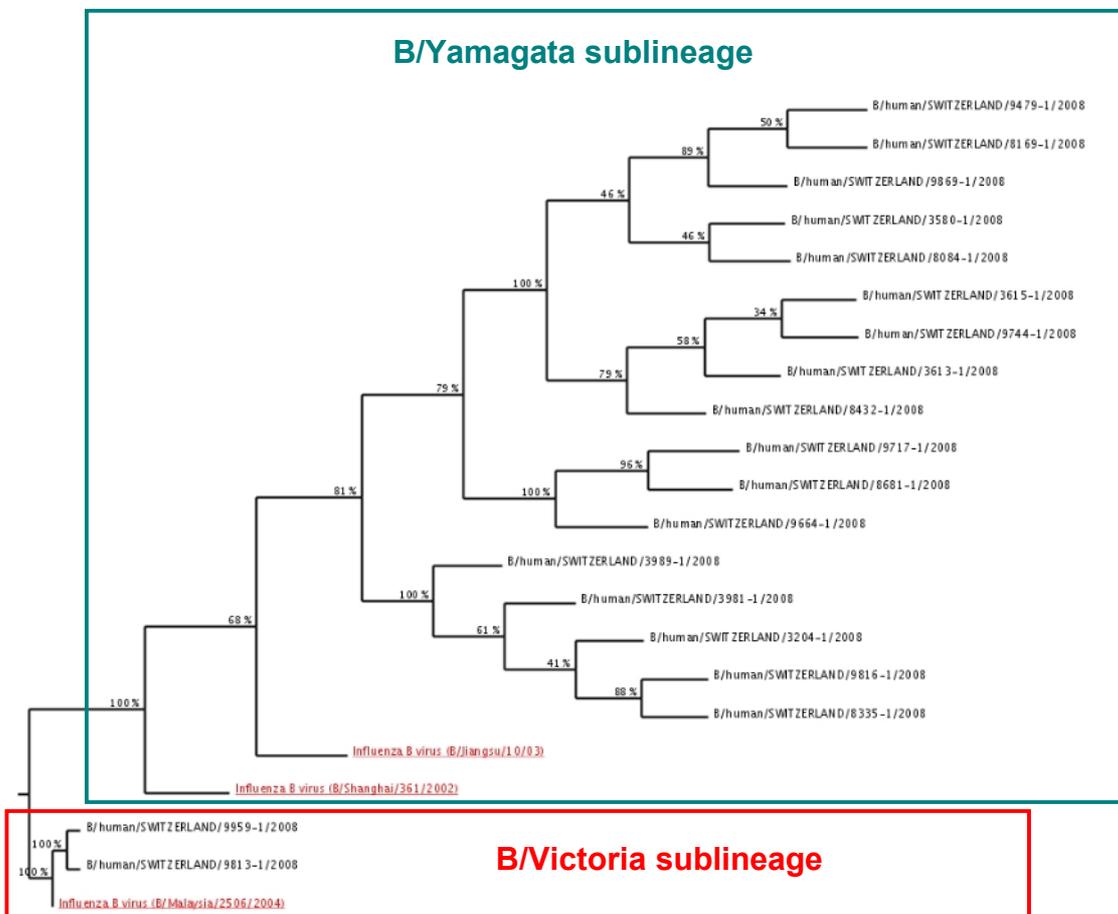


Figure 11: Phylogenetic comparison sequences of influenza B viral hemagglutinin

The vaccine strains are labelled in red. Both sublineages observed in influenza B viruses are labelled with orange and yellow boxes.

Nineteen HA of influenza B viruses were submitted to sequencing analysis. The phylogenetic tree of this analysis confirmed that both lineages co-circulated in

Switzerland this season. 17/19 HA were of the Yamagata lineage and close to the B/Jiangsu/10/2003 reference strain (Figure 11). 2/19 HA were of the B/Victoria lineage and close to the B/Malaysia/2506/2004 reference strain.

5.3.4. Antiviral resistance

In January 2008, the Norwegian Institute of Public Health and the UK Health Protection Agency detected an unusually high percentage of oseltamivir-resistant viruses, one of the two antivirals used for the treatment of influenza illness (Lackenby et al., 2008). All resistant viruses were influenza A (H1N1) subtype which was one of the predominant circulating strains in Europe at that time. An update performed on 23 June 2008 showed that 709 of 2850 (25%) influenza A (H1N1) viruses recovered in different European countries were oseltamivir resistant (Figure 12).

A(H1N1) viruses resistant to oseltamivir, aggregated according to date of specimen collection			
Country	A(H1N1) viruses tested for resistance	A(H1N1) viruses resistant to oseltamivir[†]	Proportion A(H1N1) viruses resistant to oseltamivir
Austria	164	12	7.3
Belgium	32	17	53.1
Bulgaria	9	0	
Croatia	6	0	
Czech Republic	24	0	
Denmark	46	2	4.3
Finland	11	3	27.3
France	495	231	46.7
Germany	524	72	13.7
Greece	48	5	10.4
Hungary	11	0	
Ireland	61	6	9.8
Italy	106	1	0.9
Latvia	15	0	
Luxembourg	226	59	26.1
Netherlands	141	42	29.8
Norway	273	184	67.4
Poland	8	1	12.5
Portugal	29	6	20.7
Romania	55	4	7.3
Serbia	13	0	
Slovakia	5	0	
Slovenia	28	1	3.6
Spain	79	2	2.5
Sweden	20	3	15.0
Switzerland	53	10	18.9
Turkey	3	0	
Ukraine	25	9	36.0
United Kingdom	345	38	11.0
Europe [#]	2855	708	24.8

Figure 12: Resistance to oseltamivir detected in European countries

The basis of the resistance arose from a C to T substitution that produces a H274Y mutation in the NA gene. This substitution has been associated with an alteration of the enzymatic properties of the NA gene. In Europe, data on the rate of resistance are available thanks to the surveillance network for vigilance against viral resistance (VIRGIL) which actively screens circulating influenza viruses (Meier et al., 2007).

For the first year, a sequencing method of NA was implemented in a systematic fashion at the National Influenza Reference Centre (NIRC). A total of 76 Swiss samples were analysed including some through the VIRGIL network (Table 2a). The samples were composed of 53 influenza A (H1N1), 5 influenza A (H3N2), and 18 influenza B viruses. The H274Y mutation conferring oseltamivir resistance was detected in 10/53 (18.8%) NA of influenza A (H1N1) (Table 2b). None of the 18 influenza B nor five of the influenza A (H3N2) viruses had a NA gene with this mutation (Table 2b). At this time, no other mutation known to be associated with antiviral resistance has been documented. Phylogenetic study of the HA sequence of the oseltamivir resistant viruses did not reveal a cluster significantly different from the other A (H1N1) viruses : these strains are not genetically different than the influenza A (H1N1) viruses sensitive to oseltamivir (Figure 9, resistant strains are noticed with a red star). Phenotypic analysis of these strains did not reveal a difference of IHA titres neither. The resistant strains are not antigenically different from sensitive strains.

Of note, amantadine resistance has also been tested for influenza A (H1N1) and A (H3N2) viruses by genotyping analysis. 5/5 (100%) influenza A (H3N2) viruses from Switzerland harboured the S31N mutation conferring amantadine resistance (Table 2b). This phenomenon has also been observed in other European countries and in the USA (Bright et al, 2006). None of the 20 influenza A (H1N1) viruses tested harboured the mutation.

Table 2 : Influenza virus resistance assessed by genotyping and/or enzymatic assay

a) Antiviral resistance tested according to the networks (VIRGIL: European Surveillance Network for Vigilance against Viral Resistance, genotypic and enzymatic test; NIRC : National Influenza Reference Centre, genotypic analysis. b) Antiviral resistance tested according to drug and influenza virus type.

a)

	Tested	Oseltamivir-resistant
VIRGIL	28	3
NIRC	33	2
NIRC & VIRGIL	15	5
Total	76	10

b)

Viruses	Oseltamivir-resistant	Zanamivir	Amantadine-resistant
A (H1N1)	10/53 (18.8%)	0/53	0/20
A (H3N2)	0/5	0/5	5/5 (100%)
B	0/18	0/18	-

5.4. Overview of influenza epidemics around the world

5.4.1. Influenza in Europe

As observed in Switzerland, both types of influenza A and B viruses circulated at the same frequency in Europe during the influenza epidemic 2007/2008. Initially, influenza A viruses started to circulate and culminating during weeks 5 and 6. Influenza B viruses then began to circulate concomitantly with A viruses and became predominant at the end of the season (Figure 13a).

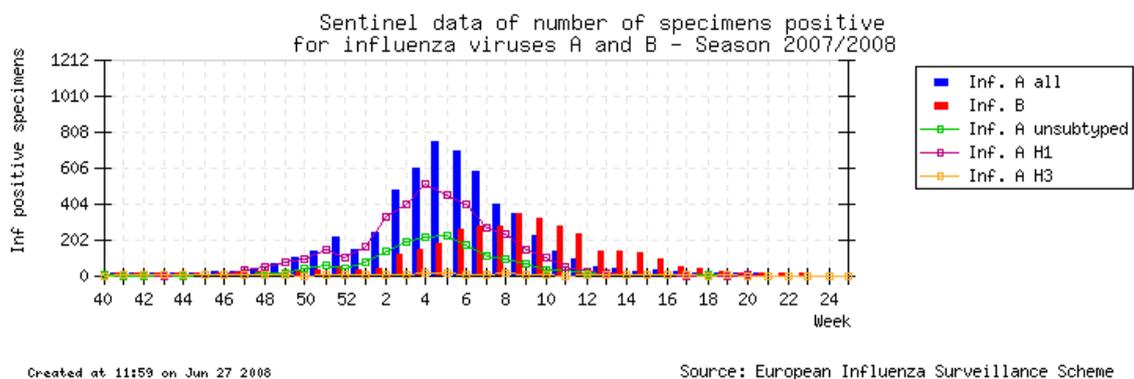
Influenza A (H1N1) viruses were related to the vaccine strain A/Solomon Islands/3/2006 (55%) (Figure 13b). Very few influenza A/New Caledonia/20/99 (0.1%) were detected. Influenza A (H3N2) viruses were also detected at a very low percentage (1.7%). Twenty percent of these were related to influenza A/Wisconsin/67/2005 and 80% to the more recent strain influenza

A/Brisbane/10/2007. Influenza B viruses were related to two distinct lineages, influenza B/Yamagata/16/88 and B/Victoria/02/87. The predominant strains were related to influenza B/Florida/4/2006 (42%). A very low ratio of influenza B viruses was related to the Victoria lineage and were related to influenza B/Malaysia/2506/2004 (0.4%).

5.4.2. Influenza epidemic in North America

In the USA, the influenza epidemic seems to have had quite different characteristics than in Europe. Influenza A viruses were predominant (71%) compared to influenza B viruses (29%). Most viruses were of the A/H3N2 subtype (74%) and a minority (26%) of the A/H1N1 subtype.

a)



b)

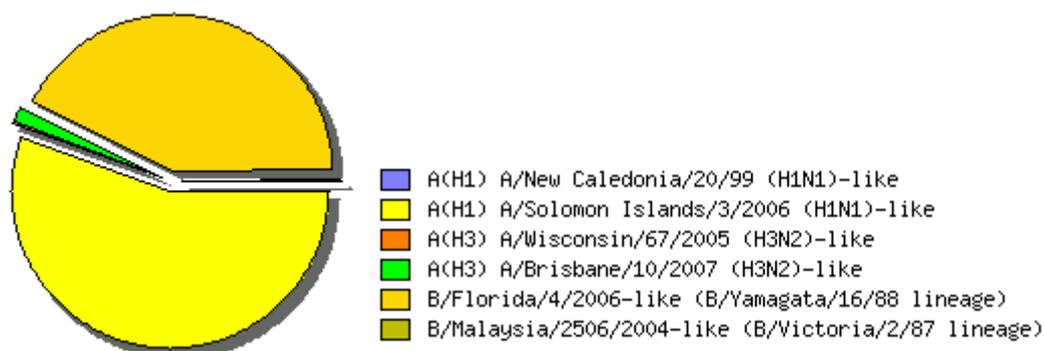


Figure 13: Influenza viruses detected in the Sentinel networks in European countries

a) Type and subtype of influenza viruses by week. b) Influenza viruses antigenic and genetic characterization. Data provided by EISS (<http://www.eiss.org>)

The US circulating influenza A (H3N2) viruses were related to influenza A/Brisbane/10/2007 (60%) and in a smaller proportion to the influenza A/Wisconsin/67/2005 vaccine strain (23%). Influenza A (H1N1) viruses were related to the vaccine strain influenza A/Solomon Islands/03/06 (66%) and to a new variant A/Brisbane/59/2007 (29%). Influenza B viruses were related to the two distinct lineages influenza B/Yamagata/16/88 and B/Victoria/02/87. They were antigenically related to influenza B/Florida/4/2006 of the former lineage (98%), and of the latter to influenza B/Malaysia/2506/2004 and the more recent influenza B/Ohio/01/2005 (MMWR, 2008).

6. WHO RECOMMENDATION FOR THE COMPOSITION OF INFLUENZA VIRUS VACCINES FOR USE IN THE 2008-2009 NORTHERN HEMISPHERE INFLUENZA SEASON

The annual meeting for the composition of the influenza vaccine took place on 13-14 February 2008 at WHO headquarters in Geneva. Based on the epidemiological data available at that time, recommendations were issued for the composition of the influenza vaccine for the 2008/2009 season (WHO, 2008) (Table 3).

Table 3 : Recommended composition of influenza vaccine for the 2008/2009 and 2007/2008 seasons

	Vaccine strain 2008/2009	Vaccine strain 2007/2008
A (H1N1)	A/Brisbane/59/2007	A/Solomon Islands/03/2006
A (H3N2)	A/Brisbane/10/2007^a	A/Wisconsin/67/2005
B	B/Florida/4/2006^b	B/Malaysia/2506/2004

a: A/Brisbane/10/2007 is a current southern hemisphere vaccine virus

b: B/Florida/4/2006 and B/Brisbane/3/2007 are current southern hemisphere vaccine viruses

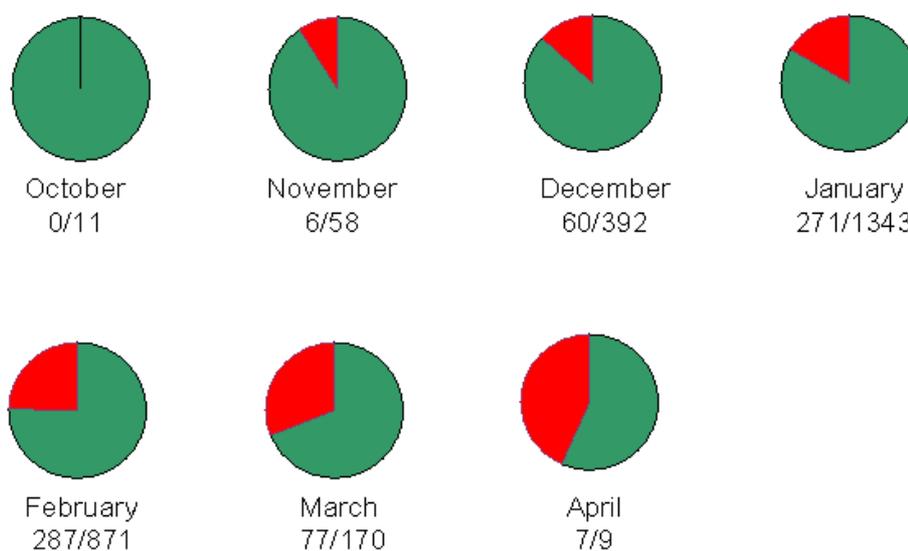
7. DISCUSSION

The main characteristic of the 2007-2008 influenza season in Switzerland was the co-circulation of influenza A viruses followed by influenza B viruses. The influenza B lineage was mainly of the B/Yamagata one and was probably only partially covered by the influenza vaccine. Another important feature of the season was (as in many other European countries) the emergence of resistant strains that have circulated without drug pressure. This illustrates once again the ability of this virus to drift and adapt.

Overall, the 2007/2008 influenza seasonal epidemic was of relatively moderate intensity. Influenza A (H1N1) viruses predominated during the first part of the season. After the peak of ILI diseases observed in the surveyed population, influenza B became the predominant circulating virus. Influenza A (H3N2) viruses did not circulate this year and only few sporadic cases were observed. The influenza A (H1N1) viruses were antigenically related to the 2007/2008 influenza vaccine, an observation that could in part explain the short duration of the epidemic. In contrast, most influenza B viruses detected were distantly related to the vaccine strain and could circulate more easily. Of note, the rate of influenza B detection did not correlate with the kinetic of ILI. Whether this reflects a selection bias or a different disease pattern associated with influenza B could not be decided based on available data.

Influenza A (H1N1) viruses that circulated were antigenically close to the 2007/2008 vaccine strain, the influenza A/Solomon Islands/03/06. However, phylogenetic analysis showed that most of these strains were more related to the recent strain influenza A/Brisbane/59/2007. This antigenic drift was also observed in other parts of the world and, for this reason, influenza A/Brisbane/59/2007 strain has been selected for the influenza vaccine for the 2008/2009 season. Although both influenza B sub-lineages, B/Victoria and B/Yamagata, were co-circulating, only a small percentage of influenza B/Victoria-like viruses was observed (11% of influenza B typed). These viruses were antigenically related to influenza B/Malaysia/2506/2004. The highest percentage of influenza B viruses subtypes observed was of the influenza B/Yamagata sub-lineage (89% of influenza B typed). These viruses were antigenically related to two members of this sublineage, respectively influenza B/Jiangsu/10/03 and the more recent influenza B/Florida/4/06 virus. This latter strain

has been selected to be included in the 2008/2009 vaccine. Therefore, it can be assumed that the protection conferred by the 2007/2008 vaccine against these strains was reduced since a limited cross protection is expected between these different lineages. Influenza A (H3N2) viruses were detected only sporadically throughout the season. Based on both phylogenetic analysis of the HA gene and on the phenotype determined by the IHA of the test, these viruses were related to a new variant, the influenza A/Brisbane/10/2007. This strain has also been selected for the 2008/2009 vaccine despite a low circulation since it has the potential to emerge as a new dominant variant.



EISS unpublished results

Figure 14: Rate of oseltamivir-resistant influenza viruses in Europe over the season

Red: Oseltamivir-resistant influenza A (H1N1) viruses; green: non-resistant influenza A (H1N1) viruses.

A highlight of the present season is the emergence of spontaneously-resistant oseltamivir strains, circulating in a relatively high rate without antiviral pressure. Antiviral-resistant viruses appeared in many different European countries, including Switzerland, and was also observed worldwide. The resistance rate was particularly high in European countries (average 25%, ranging from 0 to 67%) and Canada (25%). This rate was lower in the USA (11%), in Asia or Oceania (4%) (WHO^a, 2008).

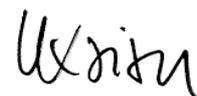
The resistance to oseltamivir was detected in influenza A (H1N1) viruses exclusively and was conferred by a unique H274Y mutation in the NA gene. The number of resistant strains circulating seems to have increased progressively during the season (Figure 14). The highest rate of resistance has been observed in Norway (67%), France (46%) and in Belgium (53%) where the use of antivirals in the community is non significant. In comparison, in Japan, where the use of oseltamivir is one of the highest in the world, the resistant strains was only 2% during the 2007/2008 season (WHO^b 2008). This observation illustrates the ability of this virus to select mutant variants without altering its transmissibility and to survive in a population, despite antiviral pressure. The 18% observed in our survey should not be considered as an absolute reference number but illustrates that these resistant viruses circulate in Switzerland in a significant and unexpected rate.

A phylogenetic analysis of the HA of influenza A (H1N1) viruses did not show any clustering of resistant viruses (compared with sensitive viruses) and did not point out to a common origin of the resistant strains. Interestingly, this resistance pattern was limited to influenza A (H1N1) viruses. Influenza B as well as influenza A (H3N2) viruses that circulated this season remained sensitive to oseltamivir. However, all influenza A (H3N2) strains analyzed harboured amantadine resistance, as already observed since the 2005/2006 season (MMWR^a, 2006). The co-circulation of different influenza subtypes resistant to different drug classes is the confirmation that antiviral resistance screening, either by genotypic or other phenotypic assay, need to be added systematically to any surveillance activity.

Geneva, Friday, 13 August 2008



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