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Seasonal and influenza A (H1N1) 2009 virus surveillance in Switzerland

Season 2009 – 2010

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2. RESUME-SUMMARY

2.1. Résumé

La saison 2009-10 de grippe a été exceptionnelle car la première faite pendant une période de pandémie. Un nouveau virus d'origine porcine, le virus influenza A (H1N1) 2009 est apparu au Mexique puis a été détecté et caractérisé à la frontière entre le Mexique et les Etats-Unis au cours du mois d'Avril 2009. Ce virus qui s'est alors répandu chez l'homme sur plusieurs continents en quelques semaines a été détecté en Suisse le 28 Avril 2010 chez une personne de retour du Mexique. Par la suite une première vague concernant surtout des personnes revenant d'Amérique ou du Mexique, puis d'Espagne ou d'Angleterre a eu lieu entre les mois de Juin et d'Août. Après une période d'accalmie pendant le mois d'Août, il a de nouveau circulé largement dans le pays entre les mois de Septembre 2009 et Janvier 2010. Comme dans la grande majorité des pays Européens, l'intensité de cette épidémie a été comparable à celles observées ces dernières années en Suisse.

Le virus influenza A (H1N1) 2009 d'origine porcine a été prédominant en Suisse comme dans tous les pays Européens, aux Etats-Unis et en Amérique du Sud. En plus de la surveillance Sentinelle, un service de diagnostique urgent a été mis en place au laboratoire de Virologie de l'Hôpital Cantonal Universitaire de Genève. Parmi 4893 prélèvements reçus (3560 pour diagnostique et 1333 pour surveillance). 1470 virus influenza ont été détectés, soit 1442 virus A (H1N1) 2009, 22 virus influenza A saisonniers et 3 virus influenza B. Le virus influenza A (H1N1) 2009 a été détecté dès le mois d'Avril 2009, a vraiment circulé entre la fin du mois de mai et le début du mois d'Août avec un pic observé au début du mois de juillet. Puis l'épidémie hivernale a débuté très précocement au début du mois d'octobre pour culminer à la mi-novembre sans réapparition de nouvelle vague grippale durant tout l'hiver.

Les virus influenza A (H1N1) 2009 détectés en Suisse étaient antigéniquement et génétiquement proches de la souche vaccinale pandémique influenza A/California/7/2009. Parmi les virus influenza A saisonniers, 6 virus étaient antigéniquement proches de la souche vaccinale influenza A/Brisbane/10/2007 (H3N2) et 2 étaient proches de la souche influenza A/Brisbane/59/2007 (H1N1).

Parmi les souches influenza B détectées, une était antigéniquement proche de la souche influenza B/Malaysia/2506/2004.

Seules 2/154 souches influenza A (H1N1) 2009 analysées génétiquement par séquençage avaient la mutation H275Y dans le gène de la neuraminidase. Cette mutation confère une résistance à l'oseltamivir. Ces deux cas provenaient de patients immuno-supprimés sous traitement d'oseltamivir à long terme. Les souches A (H1N1) 2009 avaient la mutation S31N dans le gène de la matrice conférant une résistance à l'amantadine. Aucune des 75 souches influenza A (H1N1) 2009 n'avaient la mutation D222G dans le gène de l'hemagglutinine. Cette mutation semble être détectée avec une plus haute fréquence dans les souches influenza A (H1N1) 2009 détectées chez des patients présentant des symptômes graves. En revanche, 16 souches A (H1N1) 2009 présentaient la mutation D222E qui n'a pas de d'influence claire sur sur la pathogénicité du virus.

2.2. Summary

The 2009-2010 influenza season was exceptional as it was the first occurring during a pandemic period. A new swine-origin virus, influenza A (H1N1) 2009 virus, appeared in Mexico and was then dectected and characterized at the frontier between Mexico and the USA during April 2009. The virus circulated in humans in several continents within a few weeks and was detected in Switzerland on 28 April 2010 in an individual returning from Mexico. A first wave concerning particularly persons returning from the USA or Mexico, followed by Spain or the United Kingdom, occurred between June and August. After a brief period of respite during August, the virus circulated widely again throughout the country between September 2009 and January 2010. Similar to most European countries, the intensity of this epidemic was comparable to those observed over the last few years in Switzerland.

Similar to all other European countries, the USA and South America, the influenza A (H1N1) 2009 virus of swine origin was predominant in Switzerland. In addition to Sentinal regular monitoring, an urgent diagnostic centre was set up at the Laboratory of Virology of the University of Geneva Hospitals. Of 4893 samples received (3560 for diagnosis and 1333 for surveillance purposes), 1470 influenza viruses were detected: 1442, influenza A (N1N1) 2009; 22, seasonal influenza A virus; and 3, influenza B virus. Influenza A (H1N1) virus 2009 was detected from April 2009, but only circulated widely between the end of May and the beginning of August with a peak observed at the beginning of July. The winter epidemic began very early at the beginning of October and peaked in mid-November without any reappearance of a new influenza wave during all winter.

The influenza A (H1N1) 2009 viruses detected in Switzerland were antigenically and genetically close to the pandemic vaccine strain influenza A/California/7/2009. Among the seasonal influenza viruses, 6 were antigenically close to the vaccine straine A/Brisbane/10/2007 (H3N2) and 2 were close to the influenza A/Brisbane/59/2007 (H1N1) strain. Of the influenza B strains detected, one was antigenically close to the strain influenza B/Malaysia/2506/2004.

Only 2/154 influenza A (H1N1) 2009 strains analyzed genetically by sequencing had the H275Y mutation, which confers resistance to oseltamivir, in the neuraminidase gene. These two cases concerned immunosuppressed patients receiving long-term oseltamivir treatment. The A (H1N1) 2009 strains carried the S31N mutation in the matrix gene associated with amantadine-resistance. None of the 75 influenza A (H1N1) 2009 strains carried the D222G mutation in the hemagglutinin gene. This mutation appears to have been more frequently observed in influenza A (H1N1) 2009 strains detected in patients with severe symptoms. In contrast, 16 A (H1N1) 2009 strains carried the D222E mutation, which did not appear to have any clear influence on the virus pathogenicity

3. INTRODUCTION

The first influenza A (H1N1) 2009 virus was detected in North America on 28 March 2009 close to the Mexican and USA border. This new swine-origin virus was detected following reports from Mexico of 60 deaths and 600 individuals suffering from respiratory symptoms. The virus circulated also in North America, causing rapidly hundreds of new infections. The epidemic started to circulate in other countries and reached all continents within a few weeks. The new influenza virus reached Europe during the end of April with the first cases identified in the United Kingdom and Spain on 27 and 28 April, respectively. In Switzerland, the first pandemic virus was detected on 28 April 2009 in an individual hospitalised in central Switzerland (Baden, SG) who had returned from travel to Mexico (Figure 1). A new pandemic was declared by WHO on 11 June 2009.

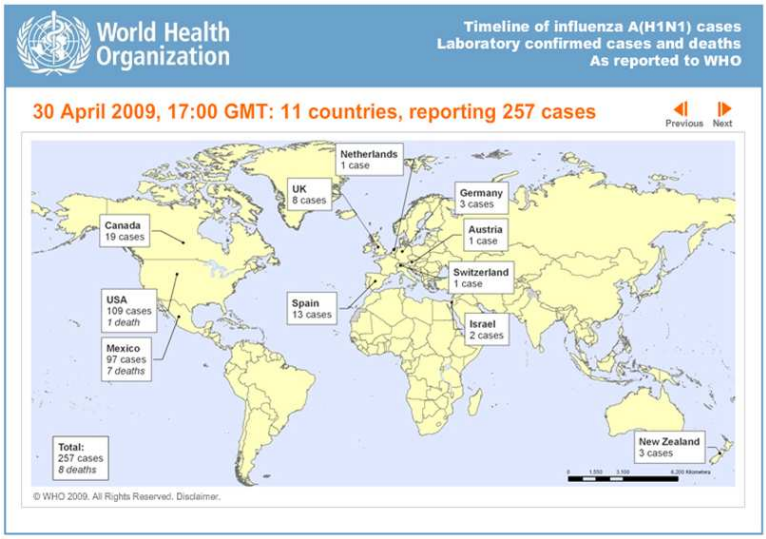


Figure 1: WHO web site update, 30 April 2009 (http://www.who.int)

At this time, detection of influenza A (H1N1) 2009 virus by our Centre was performed on samples sent by Swiss physicians or healthcare authorities independent of their affiliation to the Sentinel network. Similar to many other European influenza surveillance networks, official Swiss Sentinel surveillance started during week 40. Results of mandatory surveillance according to the published official guidelines of the Swiss FOPH (Sentinel surveillance), as well as the results of routine diagnostic activity are presented in this report. From April 2009 to the end of the season,

strategic guidelines for clinical and virological surveillance were adapted and updated on a regular basis (Annex 1).

4. METHOD OF DETECTION FOR INFLUENZA VIRUSES

4.1. Clinical identification of influenza cases

During the 2009-2010 season, a network of 141 practioners participated actively to the clinical surveillance of influenza cases. 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℃ with or with out a feeling of sickness, myalgia, or an alteration of 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 2.

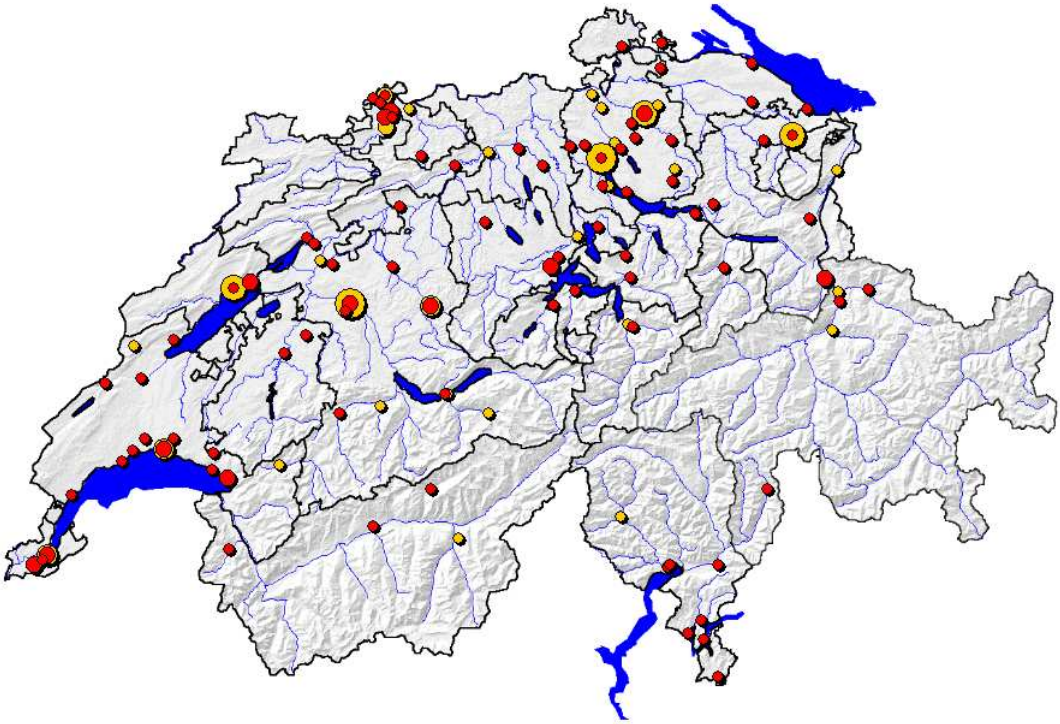


Figure 2: Geographical distribution of the 141 participants of the Sentinel network

Yellow bubble: location of the participants (n=141) conducting clinical surveillance; red bubble: participants conducting both clinical surveillance and specimen collection (n=97). Participants per community (range: 1-6) = bubble size

A subgroup of 91 Sentinel practitioners (50%) provided clinical specimens from selected patients in addition to clinical surveillance. Combined nasopharyngeal and pharyngeal specimens are sent in transport medium by regular mail to the National Reference Centre for Influenza (NRCI) in Geneva for subsequent viral detection and characterization. The sampling selection procedure of specimens is adapted to the epidemic phases as follows.

1) Pre- and post-epidemic phase: the number of MC-ILI by Sentinel practitioners remains below the threshold level of 58 cases per 100,000 inhabitants. During this phase, respiratory screening is performed in all cases.

2) Epidemic phase: the number of persons presenting ILI increases. The MC-ILI is over the threshold of 58 cases of MC-ILI per 100,000 inhabitants. During this phase, respiratory screening is performed in a subgroup of cases according to predefined rules and only 1:5 ILI cases are systematically screened.

3) Special case of influenza A (H1N1) 2009: when activated, guidelines in Annex 1 of general report

4.2. Detection of influenza viruses

4.2.1. Molecular technique

After influenza A (H1N1) 2009 emergence, the strategy for influenza virus detection in Sentinel samples was adapted (Figure 3). The presence of influenza virus in samples was determined by two one-step reverse-transcription polymerase chain reaction (one-step RT-PCR) assays specific for influenza A and B virus detection, respectively.

Influenza A RT-PCR was developed to detect influenza A viruses of animal and human origin: this assay could detect seasonal (H3N2, H1N1) and pandemic viruses (H1N1 2009) of human origin. The primers target the matrix gene of the viruses. The potential advantage of this RT-PCR method is the rapidity to obtain results for all influenza A viruses.

Influenza B RT-PCR was developed in our laboratory and validated in the previous season (Thomas et al, 2009) and did not require new adaptation.

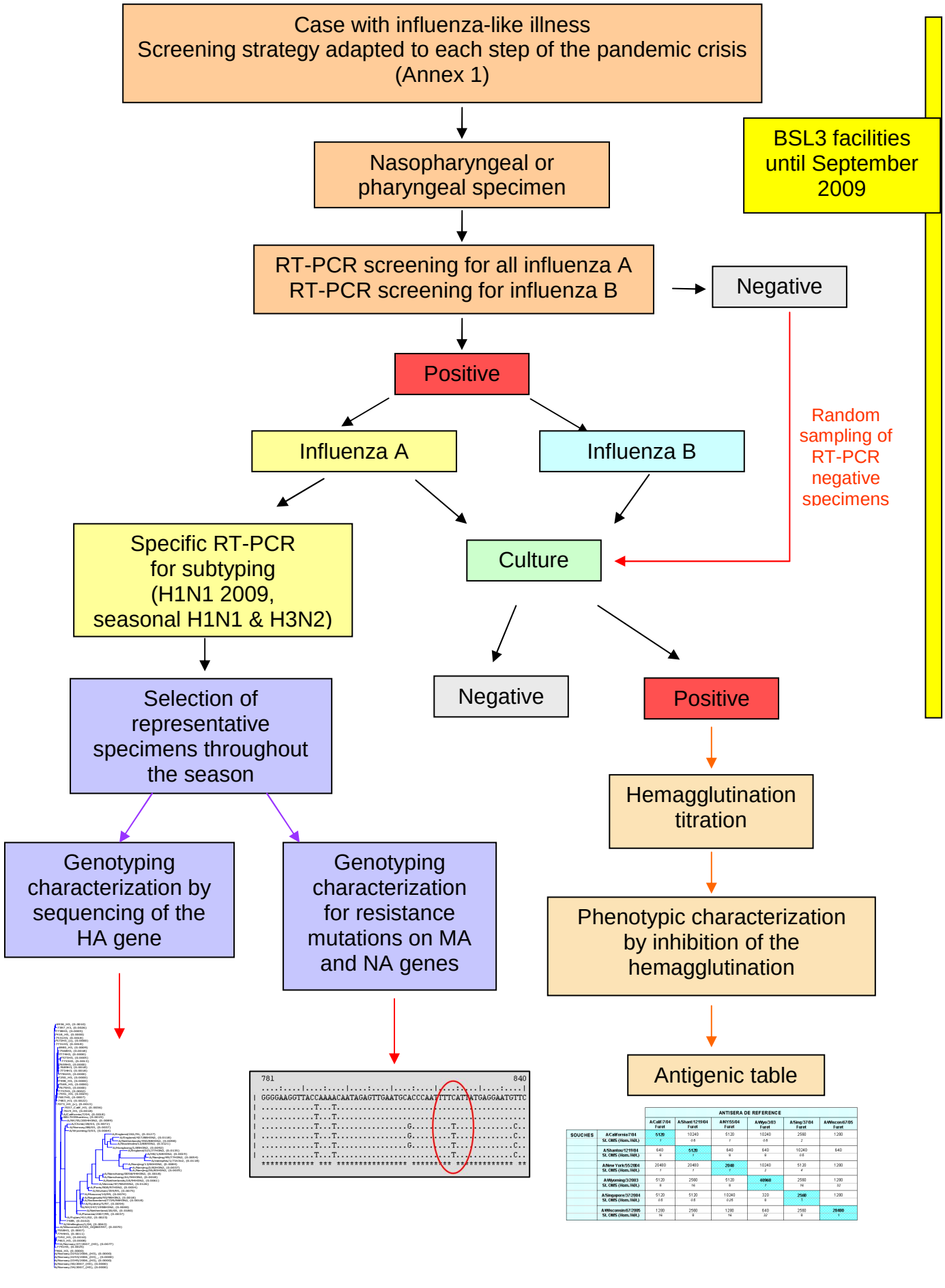


Figure 3 : Procedure used for the detection of influenza viruses by Sentinel surveillance

When influenza A RT-PCR was positive, additional one-step RT-PCR reactions were systematically performed to determine the nature of the hemagglutinin (**HA**) using the following assay: **swiH1-Ge RT-PCR** that recognizes specifically the HA gene of the influenza A (H1N1) 2009 viruses, and the **A/H1-Ge** and influenza **A/H3-Ge** PCR that are specific for seasonal influenza A/H1 and influenza A/H3 viruses, respectively. These latter RT-PCR assays have also been developed in our laboratory.

4.2.2. Cell Culture

PCR-positive samples are cultivated on appropriate cell lines for antigenic characterization by hemagglutination inhibition (**IHA**) to assess the antigenic property of circulating strains and to determine the potential efficiency of recognition by influenza vaccine-induced antibodies (Figure 3). Cell lines used for the surveillance are specific for influenza isolation, 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 assumed to provide a higher efficiency of influenza virus multiplication by cell culture. Cell culture can be

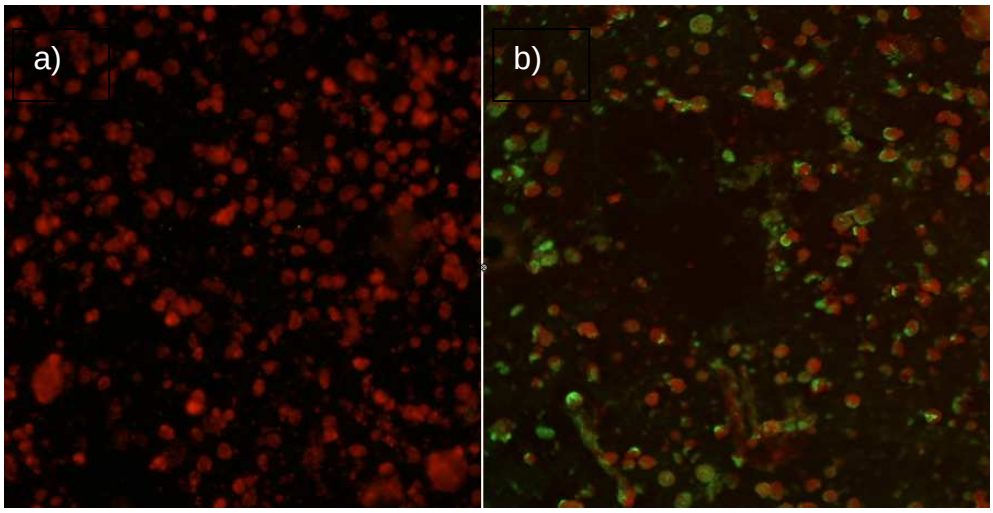


Figure 4: MDCK cells infected by influenza A/Switzerland/01/2009 (H1N1)

Immunofluorescence picture of the first case identified in Switzerland (28 April 2009) that revealed to be not only RT-PCR positive, but also culture-positive with high titer (Genbank no. ACQ57000).

a) Negative control; b) in green, influenza viruses detected with monoclonal anti-influenza A primary antibody and monoclonal FITC conjugate revealing the presence of viral antigen in cells (Chemicon®, USA).

coupled with immunofluorescence detection using viral nucleoprotein-specific monoclonal antibodies. This sensitive method allows the detection of viral antigen, even in low yields of viral culture cases (Figure 4).

During the first and last weeks of surveillance, a random sampling of negative specimens 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 could be the case in 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 RT-PCR detection as the method is intended for human viruses only.

During the first weeks of the H1N1 pandemic, all specimens were inoculated under adapted, upgraded biosecurity containment.

5. CHARACTERIZATION OF INFLUENZA VIRUSES

5.1. Phenotyping and antigenic characterization

In the presence of a positive cell culture, the cell supernatant containing the viral isolate is phenotyped with 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 season, guinea pig red blood cells were used for this reaction. Results are interpreted according to an antigenic table adapted to circulating strains and established at the beginning of the season. The 2009-2010 reference antigenic table comprised 4, 4 and 6 reference ferret antisera/strains for influenza A (H1N1), A (H3N2), and B viruses, respectively (Table 1). Sera specific for influenza A (H1N1) 2009 was produced and used as soon as possible.

In this procedure, the titers 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 titers and the observed titers obtained with the circulating influenza strains define the antigenic relationship to the

Table 1: Hemagglutination inhibition (IHA) titers of reference influenza strains tested with the 2009-2010 reference antisera

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

a) Influenza A (H3N2)

		REFERENCE ANTISERA			
		Brisbane/10/07 Ferret	A/Finland/8/08 Ferret	Johannesb./15/08 Ferret	A/Perth/16/ 2009 Ferret
STRAINS	A/Brisbane/10/2007 WHO strain	**1024**	4096	1024	32
	A/Finland/8/2008 WHO strain	128	**512**	256	128
	A/Johannesburg/15/2008 WHO strain	128	256	**512**	256
	A/Perth/16/2009 WHO strain	<8	<8	<8	**2048**

b) Seasonal influenza A (H1N1) and influenza A (H1N1) 2009

		REFERENCE ANTISERA			
		Seychelles/2239/ 08 Ferret	Brisb/59/07 Ferret	St Peter/05/08 Ferret	A/California/7.09* Ferret
STRAINS	A/Seychelles/2239/08 WHO strain	**128**	64	16	<8
	A/Brisbane/59/2007 WHO strain	128	**1024**	256	<8
	A/St Petersburg/5/2008 WHO strain	128	512	**256**	<8
	A/California/7/2009* WHO strain	<8	<8	<8	**64**

** influenza A (H1N1) 2009 virus of swine origin.*

c) Influenza B

		REFERENCE ANTISERA					
		Brisbane 08 Ferret	Malay04 Ferret	Ferret Flo06 Ferret	Brisbane07 Ferret	Barcelona08 Ferret	Bengla07 Ferret
STRAINS	B/Brisbane/60/08 WHO strain	**128**	**128**	**<8**	**<8**	**<8**	**<8**
	B/Malaysia/2506/2004 WHO strain	**<8**	**128**	**<8**	**<8**	**<8**	**<8**
	B/Florida/4/2006 WHO strain	**<8**	**32**	**2048**	**1024**	**64**	**512**
	B/Brisbane/3/2007 WHO strain	**<8**	**32**	**1024**	**1024**	**64**	**256**
	B/Barcelona/143/2008 WHO strain	**<8**	**<8**	**64**	**32**	**128**	**64**
	B/Bengladesh/3333/2007 WHO strain	**<8**	**16**	**256**	**256**	**128**	**512**

reference strains. If the value of this titer is higher than 4, the strain is considered as antigenically different from the reference strain. A value equal to 4 or lower is considered as similar. By this procedure, any significant antigenic drift of the HA of circulating strains can be identified. Based on this information, an adaptation of the arriving vaccine strain can be performed, as well as urgent sanitary measures such as isolation of individuals in the community. Due to the recent emergence of influenza A (H1N1) 2009, the corresponding reference antiserum was added to the other influenza A (H1N1) virus-specific antisera in the antigenic table (Table 1).

5.2. Genetic characterization

Genetic characterization is performed by sequencing analysis target genes. Genes such as the HA vary more than any others and provide important information on the phylogenic origin of the virus. By sequencing the HA gene, influenza strains can be compared with current and older vaccine strains.

A D222G mutation in the receptor domain of HA gene was detected in a minority of influenza A (H1N1) 2009 strains. A high frequency of these variants were detected in severe case samples in Hong Kong,[5] Norway,[3] Scotland,[5] and Spain[1]. A putative relationship with disease severity was raised, but still needs clarification. The HA gene of some Swiss isolates will be analyzed.

Another important task that revealed to be increasingly important over the last few years is the monitoring of antiviral resistance of influenza viruses. Resistance has been observed with the three antiviral drugs, namely, amantadine, oseltamivir and zanamivir. Influenza viruses have developed a resistance against these antivirals with key mutations inducing a modified protein that is no longer recognised by the antiviral. Each antiviral resistance is associated with specific amino acid mutation on a specific gene, the NA gene for neuraminidase inhibitors and matrix gene for amantadanes. The newly-emerged influenza A (H1N1) 2009 virus has also developed resistance to oseltamivir and is uniformly resistant to amantadine. All known mutations of influenza genes and their corresponding antiviral resistance detected in human patients are summarised in Table 2. Hence, sequencing of the

viral NA and MA genes allow to detect precisely mutations known to be associated with resistance. Phenotypic analysis such as microneutralisation could also be used to detect an antiviral resistance of an isolate. This technique is not intended to be used in a routine clinical laboratory.

Table 2: **Key mutations conferring antiviral mutation to influenza viruses**

Antiviral		Oseltamivir	Zanamivir	Amantadine
Gene		NA	NA	M2
Influenza viruses	H1N1 2009	H275Y	/	S31N
	H1N1	H275Y	/	S31N V27A
	H3N2	E119V	E119G (in vitro)	S31N
		R292K N294S (Low R.)	R292K	/
	B	R152K D198N G402S	R152K D198N	/

6. RESULTS FROM THE 2009-2010 SEASON

6.1. Detection by molecular assays of influenza viruses in respiratory specimens

Since the first influenza A (H1N1) 2009 virus detection in Geneva on 28 April 2009, the NRCI was fully supported by the Laboratory of Virology, University of Geneva Hospitals, for the conduct of influenza diagnosis for Switzerland. A high number of nasopharyngeal samples from all the country were sent by practitioners, hospitals, and other laboratories for diagnostic purposes. In May 2009, other Swiss laboratories were rapidly provided with the real-time RT-PCR developed at the NRCI and with controls and positive samples for influenza A (H1N1) 2009 virus detection. Samples arriving at the NRCI were mainly from the canton of Geneva and other parts of Switzerland that did not have the possibility to perform the A (H1N1) 2009 diagnosis at that time. A real-time RT-PCR analysis was performed on these samples within

24h of receipt, 6 days a week. No systemic antigenic analysis was applied on the positive samples with the exception of cases hospitalised in the intensive care unit. The frequency and positive rate are shown in Figure 5. Sentinel surveillance started at week 40, but only samples sent by non-Sentinel practitioners are represented. The rates observed in Figure 5 represent the activity conducted according to official guidelines (Annex 1). The Sentinel network surveillance will be presented in chapter 6.2.

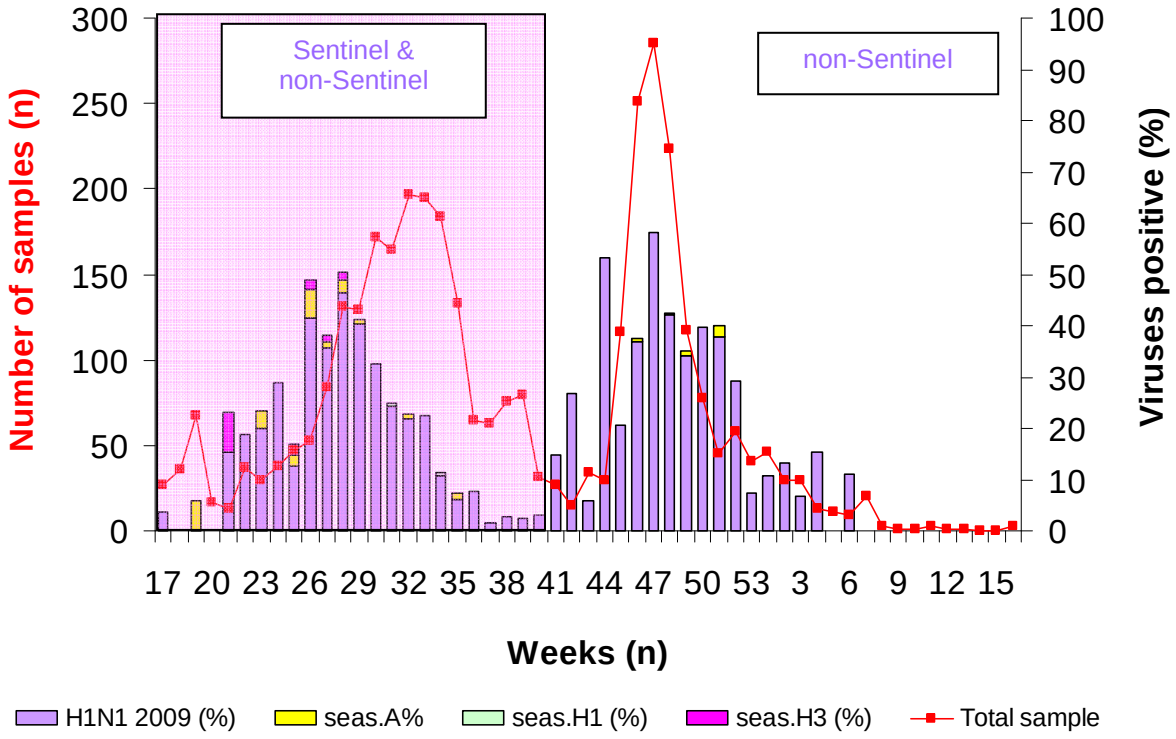


Figure 5: Rate of positive influenza A (H1N1) 2009 virus detected in non-Sentinel patient samples analysed at the University of Geneva Hospitals.

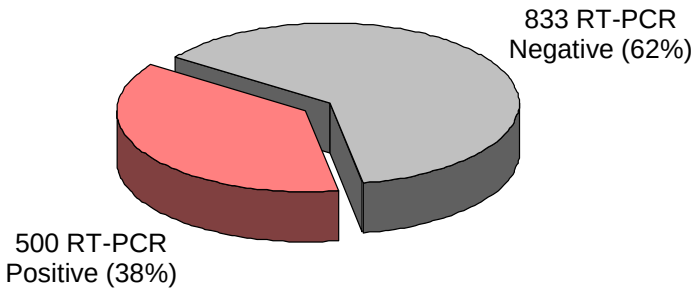
The pink square represents the period when Sentinel and non-Sentinel samples were received. Outside the pink square, data concern the non-Sentinel samples only.

The number of samples received increased regularly from week 17 to week 32 at a rate of 200 samples per week. The number of samples then decreased to week 40. At that time, Sentinel surveillance was officially activated. Influenza A (H1N1) 2009 virus started also to circulate at a higher frequency. The number of samples, together with the positive rate, increased greatly to week 47. Then, the number of samples, and the positive rate decreased with no influenza viruses detected after week 6.

6.2. Detection of influenza viruses in nasopharyngeal samples in the Sentinel network

Influenza A (H1N1) 2009 virus was regularly detected during the spring and the beginning of the summer period. During August, the level of positive samples was low and stable. Consequently, epidemic surveillance started on 26 September 2009 (week 40) and ended on 23 April 2010 (week 16) after a period of 29 weeks. 1333 samples from 91 Sentinel participants were analysed by RT-PCR analysis. 500 of these samples were detected influenza-positive, representing an average positive rate of 34% over 30 weeks surveyed (Figure 6a).

a)



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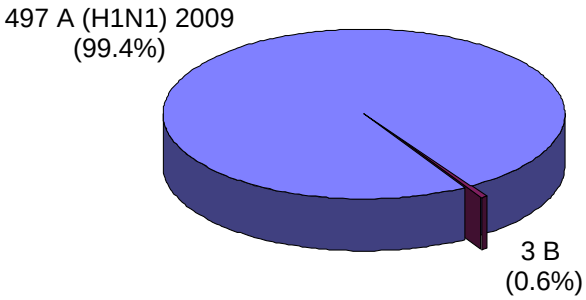


Figure 6: Nasopharyngeal specimens positive for any influenza virus during the 2009-10 season (n=1333)

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

Among the 500 positive cases, 497 (99.4%) were influenza type A viruses and 3 (0.6%) influenza type B (Figure 6b). Among influenza A viruses, all were influenza A (H1N1) 2009 virus which emerged in March 2009 in Mexico. No seasonal influenza A viruses were detected in the Sentinel network during this season.

Influenza A (H1N1) 2009 virus predominated during all the winter period. From a sporadic activity observed at the beginning of the surveillance period, influenza

activity increased and culminated up to week 48. As can be observed in Figure 7, the positive rate remained quite high at around 60% between weeks 48 to 53. Influenza activity then started to decrease and the level returned to sporadic after week 6. The last viruses to be detected in the Sentinel network during the surveillance period was during week 11. Three influenza B viruses only were sporadically detected at the end of the pandemic wave during the weeks 5, 6 and 16. No seasonal influenza A (H3N2) and A (H1N1) viruses were detected in the Sentinel network during the surveillance period.

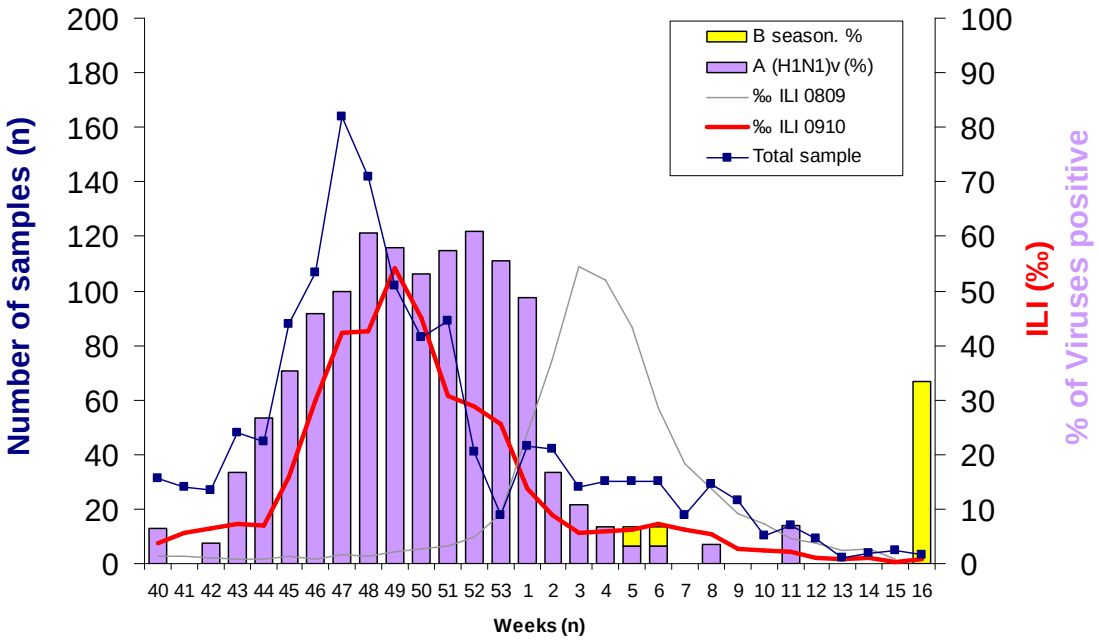


Figure 7: MC-ILI, positivity rate, and distribution type of RT-PCR-positive cases.

Proportion of influenza A (H1N1) 2009 and B viruses (%), samples received and MC-ILI (%) 2009-2010 distribution per week. MC-ILI (%) 2008-2009 is also shown for comparative purposes.

6.3. Characteristics of screened Sentinel patients

The proportion of influenza viruses detected in Sentinel specimens is shown according to the different age groups and virus types (Figure 8). Forty-seven percent of influenza viruses were detected in individuals less than 19 years old and this rose to 65% in those less than 30 years old. This observation confirms reports from other countries[9].This percentage is even higher in some countries, but the Sentinel network has a limited number of paediatricians and children are not the main surveillance target within this network. This population had the highest attack rate than any other

population. Influenza A (H1N1) 2009 constituted the main virus detected during this season and only 3 influenza B viruses were detected.

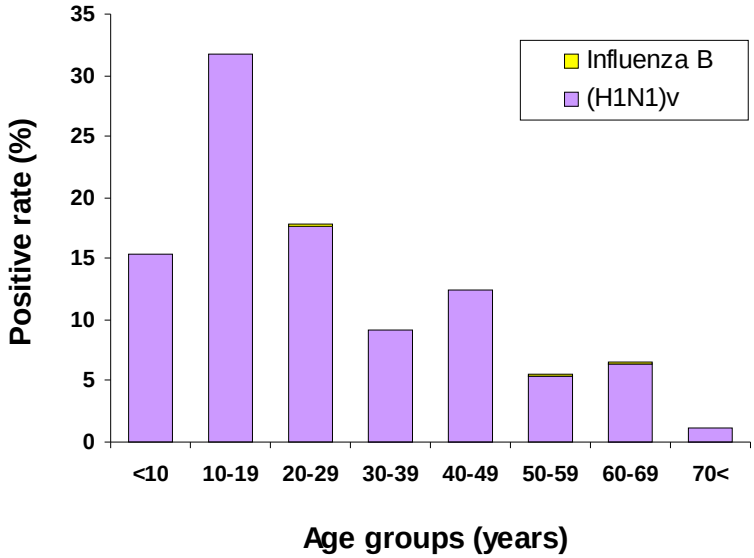


Figure 8: Repartition of viruses (%) detected according to age groups in all positive cases.

6.4. Antigenic and genetic characterization of influenza viruses

Of 1333 samples received, 375 (28%) were sent for urgent analysis, generally according to official guidelines, in order to provide rapid diagnosis for an at-risk population or for individuals requiring antiviral treatment (Figure 9). Of the 958 samples sent for surveillance purposes, 305 (32%) were positive by RT-PCR and were then analysed by cell culture. 207/305 (68%) viruses were able to be grown on cell culture and could be analysed by IHA. Of these 207 viruses, 198 (96%) were influenza A (H1N1) 2009 virus, and one was related to an influenza B/Victoria lineage specimen. Only 8 had an insufficient titer for IHA analysis. A sample of these viruses have also been submitted to sequencing analysis (Figure 9). 76 HA gene (75 A (H1N1) 2009 virus and 1 B virus), 154 NA gene, and 3 MA gene (not shown) were obtained.

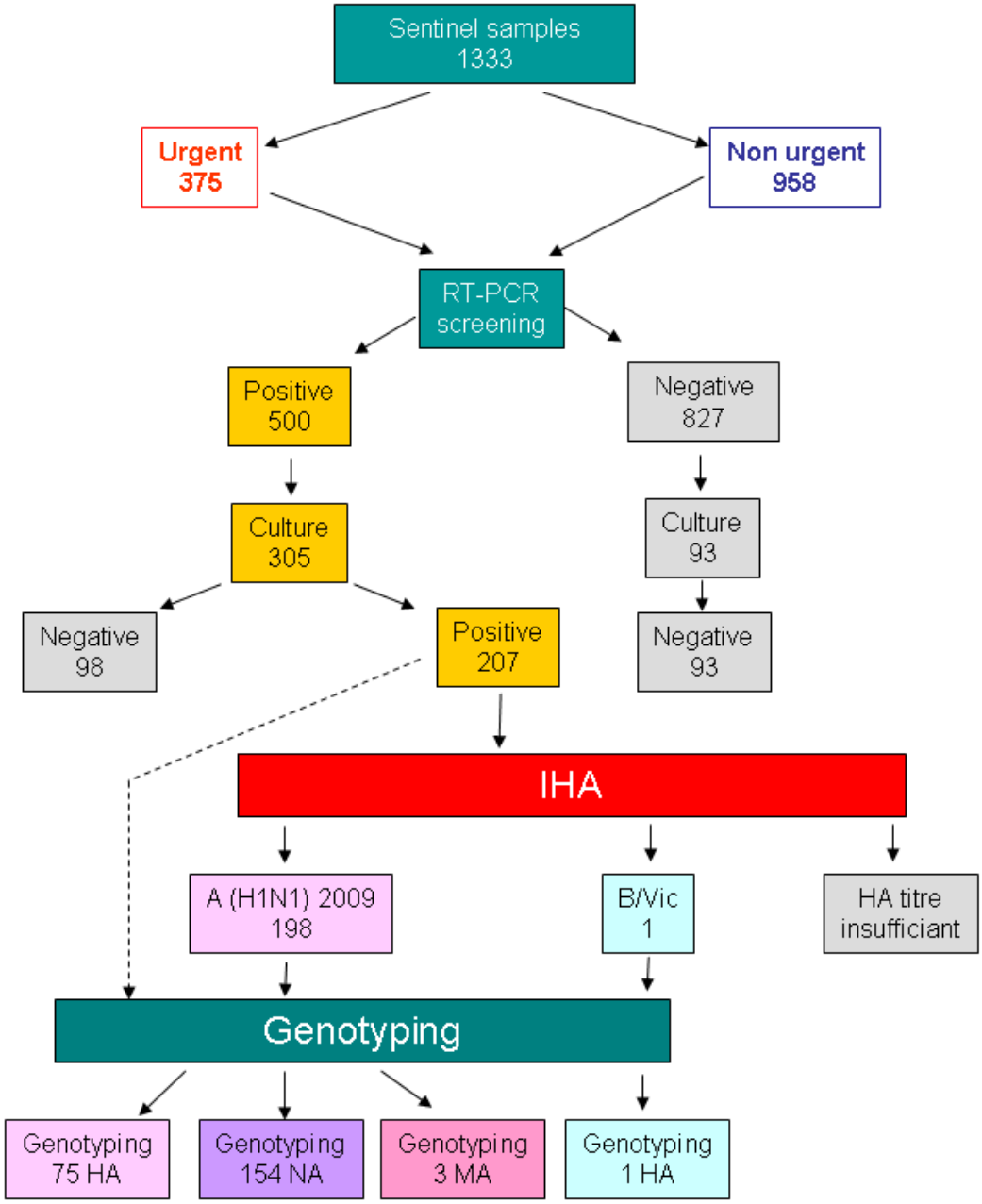
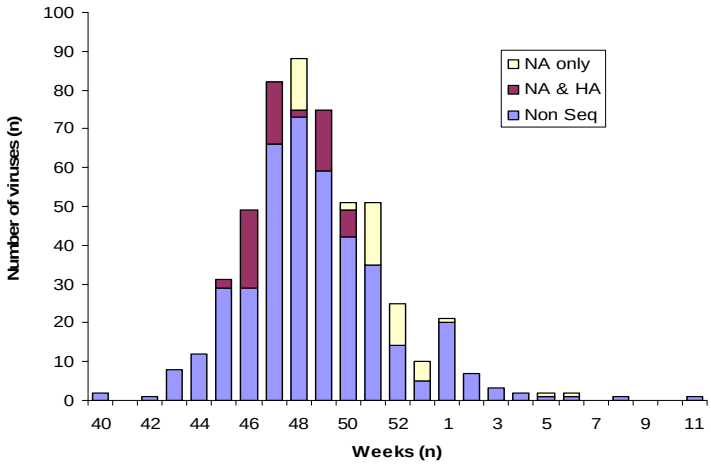


Figure 9 : Summary of the analysis performed on Sentinel samples.

IHA: inhibition of the hemagglutination; B/Vic: B/Victoria-like sublineage; HA: hemagglutinin gene; NA: neuraminidase gene, MA: Matrix gene

6.3.1. Influenza A (H1N1) 2009

All 198 influenza A viruses that could be analysed by IHA analysis were antigenically related to influenza A (H1N1) 2009 virus. As mentioned in Annex 2, all titers obtained with the A (H1N1) 2009 antiserum were equal or lower to 4 when compared with the homologous rate (Annex 2). No antigenic variant was detected by IHA analysis.

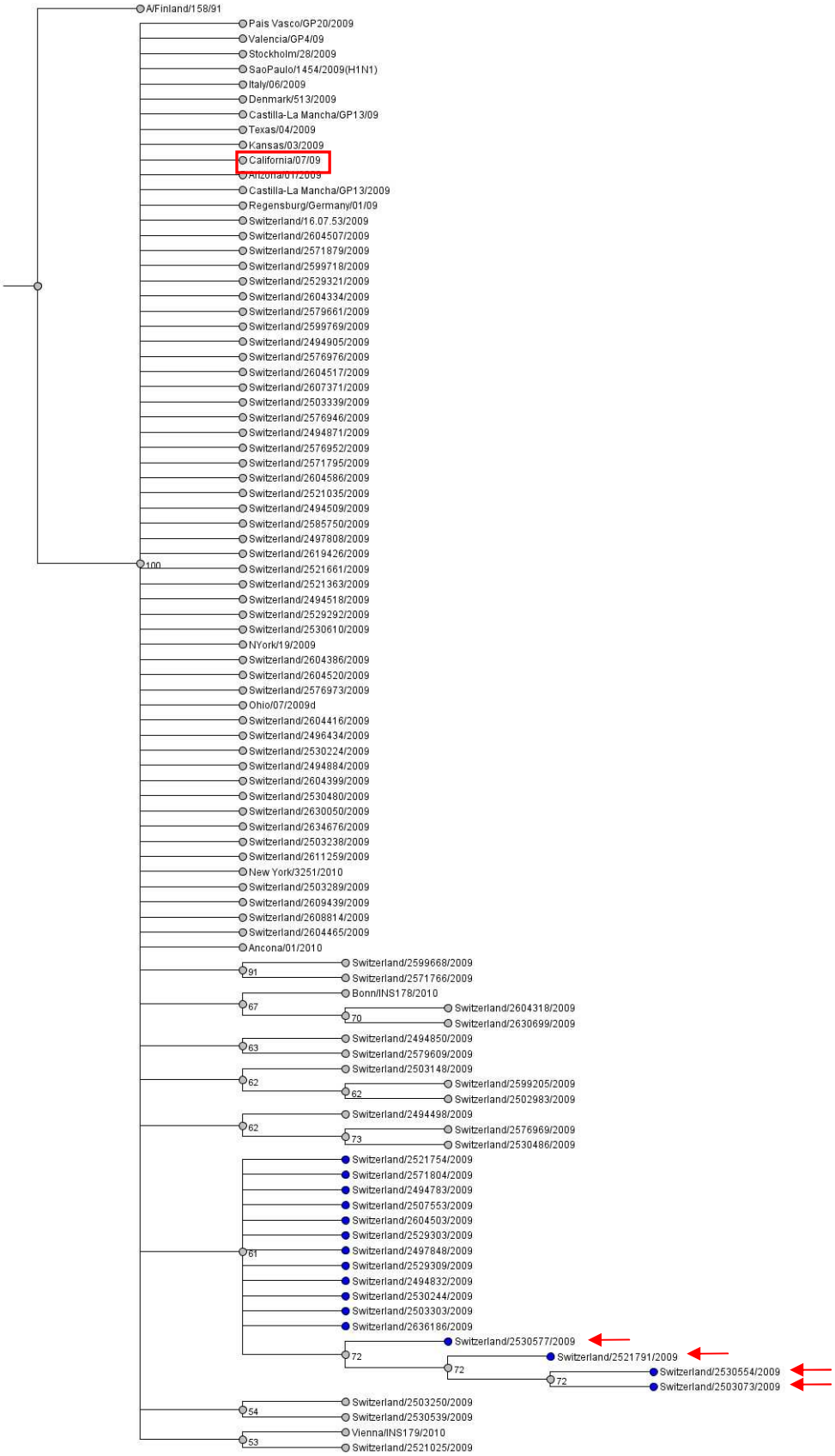


<u>Figure 10</u>: Frequency of viral gene sequencing during the 2009-2010 season

NA : neuraminidase gene sequenced, HA: hemagglutinin gene sequenced, non seq: non-sequenced viruses

Phylogenetic analysis of the HA could be performed on a subgroup of 75 of these culture-positive strains. The HA gene of influenza A (H1N1) 2009 strain detected in Switzerland cannot be distinguished from the one detected in North and South America, and in Asian and European countries during the same period. This result is in accordance with the IHA results.

A mutation in the HA gene was detected recently in an influenza A (H1N1) 2009 virus in Norway first, and in other European countries. This change is located in the receptor recognition site of the HA and is suggested to be associated with an increased pathogenicity activity of the virus. Following a request from the Swiss FOPH, the 75 sequences of influenza A (H1N1) viruses detected by Sentinel



<u>Figure 11</u>: Phylogenetic analysis of the influenza A (H1N1) 2009 hemagglutinin (n=75).

Viruses are named according to country or city name, and sample number together with year of detection. The pandemic vaccine strain is is shown in the red square, blue circles indicate the D222E variants, and red arrows indicate a cluster of genes localised in the same Sentinel participant in the canton of Saint-Gall.

practitioners were analysed to check the amino acid origin at the position 222. No sequence harboured a D222G mutation. However, 16/75 (21%) displayed a D222E mutation (blue circle, Figures 11 and 12). These strains have been collected between the 9 November and 15 December 2009 by 11 different practitioners in 9 different cantons (Table 3). It can be observed that the same Sentinel practitioner from the canton of Saint-Gall sent 4/16 D222E variants (red arrows, Figure 11) with 3 of collected on the same day (Table 3). Patients consulted with ILI symptoms and no complication was reported for any of these Sentinel cases. This D222E mutation was also detected in other European countries[1] and did not appear to be associated with an increase of pathogenicity.

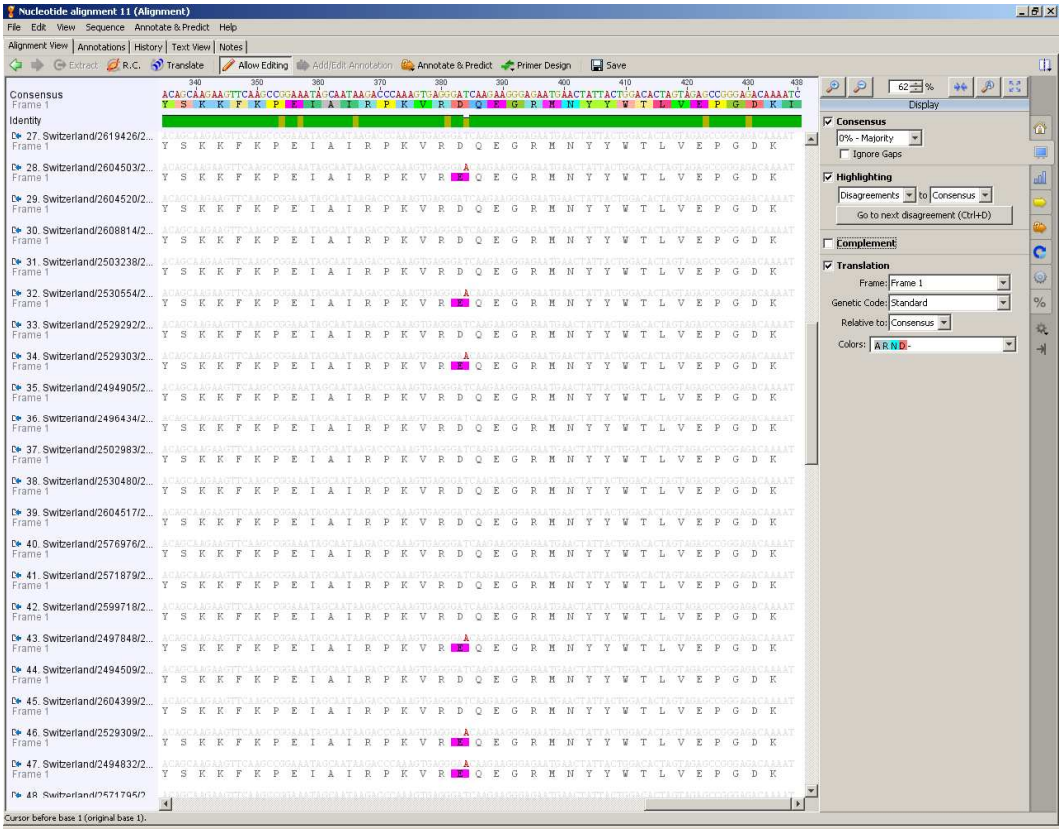


Figure 12: Representation of the hemagglutinin gene alignment of some viruses detected in Sentinel patients.

Table 3: Characteristics of D222E mutated strains.

Sample n°	Sample date	Date of birth	Canton	Région	Code
2521754	17.11.2009	01.01.2009	TI	6	HUG
2571804	30.11.2009	25.09.1978	BL	3	371
2494783	09.11.2009	14.04.1946	SZ	4	478
2529303	16.11.2009	10.10.1978	ZH	5	533
2604503	08.12.2009	11.05.1963	VS	1	651
2507553	13.11.2009	15.08.2000	ZH	5	666
2497848	11.11.2009	15.09.1966	VD	1	702
2503303	12.11.2009	23.10.1993	VD	1	709
2494832	09.11.2009	20.06.1979	SH	5	751
2530244	13.11.2009	25.05.1991	TI	6	767
2530577	17.11.2009	12.08.1998	SG	5	801
2530554	17.11.2009	19.09.2000	SG	5	801
2521791	17.11.2009	01.01.1977	SG	5	801
2503073	12.11.2009	28.11.1997	SG	5	801
2529309	17.11.2009	22.07.1998	NE	1	1123
2636186	15.12.2009	27.03.1969	FR	2	HUG

6.3.2. Influenza B

Since the 2002-2003 season, two distinct influenza B lineages have circulated in Europe, either both during the same season (2004-2005, 2005-2006), or alternatively in successive seasons. The first lineage is represented by the B/Victoria/02/87 strain and the second by the influenza B/Yamagata/16/88 strain. Antisera directed against the strain of one lineage showed either no or limited cross-reactivity with strains of the other lineage.

Three influenza B viruses only were detected during the 2009-2010 season. One of these could be analysed by IHA analysis and was antigenically related to an influenza B/Malaysia/2506/2004 virus.

6.3.3. Seasonal influenza viruses

Surprisingly, no seasonal influenza A viruses were detected in the Sentinel network. Four seasonal influenza A (H3N2) viruses were detected in non-Sentinel patients hospitalised at the University of Geneva Hospitals and were antigenically related to the seasonal vaccine strain influenza A/Brisbane/10/2008 (H3N2).

6.3.4. Antiviral resistance

Since the arrival of the last generation of antivirals against influenza virus in 2000, resistant variants have emerged and carefully monitored over the past years. Table 2 provides an update on resistant viruses detected in European countries as established by EuroFlu, the European Network of National Influenza Centres. The NA sequence of 154 influenza A (H1N1) 2009 viruses randomly selected have been analysed to detect the presence of mutations known to confer an antiviral resistance. None of the viruses detected in the Sentinel network had such a mutation. However, two H275Y mutations known to confer an oseltamivir resistance were detected in bronchoalveolar or nasopharyngeal samples from patients hospitalised at the University of Geneva Hospitals. Both viruses were detected in samples taken several days after treatment with oseltamivir had been initiated. In both cases, the last samples had a low viral load and in patient 2, the virus detected by RT-PCR lost the mutation conferring the resistance.

Table 4: Description of patients presenting samples with the H275Y mutation in the NA gene conferring resistance to oseltamivir.

Patient	Sample	Sample date	RT-PCR	Sequencing analysis
Patient 1 (N.H.)	NSP[1]	03.12.2009	+++	S
	NSP	09.12.2009	++	S
	BAL[2]	09.12.2009	+++	**H275Y**
	NSP	11.12.2009	++	**H275Y**
Patient 2 (S.S.)	NSP	26.11.2009	+++	S
	NSP	30.11.2009	++	**H275Y**

[1]NSP: nasopharyngeal swab;
[2]BAL: bronchoalveolar lavage

The mutation S31N in the matrix gene of influenza A viruses was detected in almost all influenza A (H3N2) that circulated during the most recent epidemics.[4,7,8] The mutation results from the exchange of a G to A. The matrix gene of the first influenza A (H1N1) 2009 viruses detected for diagnostic purposes in three non-Sentinel patients were analysed by sequencing analysis. The sequences obtained confirmed the presence of the S31N mutation resulting from a G645A nucleotide exchange and confirmed that influenza A (H1N1) 2009 viruses were resistant to amantadine.

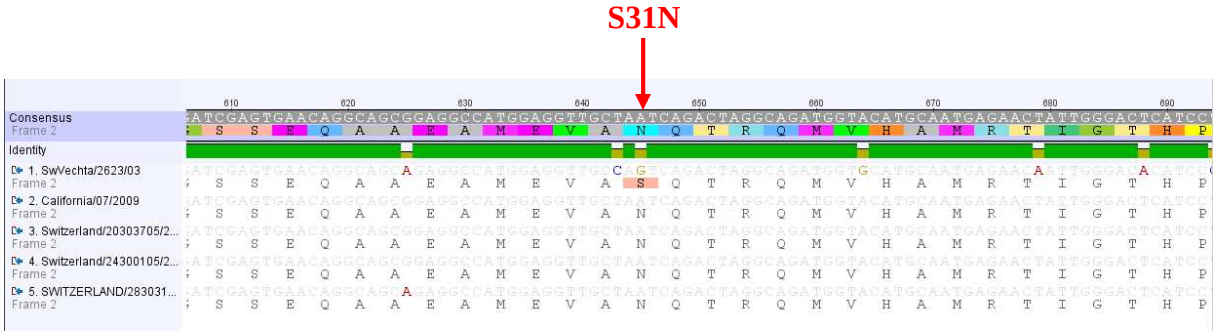


Figure13: Alignment of partial MP gene sequence of influenza A (H1N1) 2009 viruses detected at the University of Geneva Hospitals.

In red, the nucleotide position that confers resistance to amantadine.

6.4. Overview of influenza epidemics around the world

For epidemiological and virological information, see Annex 4.

7. WHO RECOMMENDATION FOR THE COMPOSITION OF INFLUENZA VIRUS VACCINES FOR USE IN THE 2010-2011 NORTHERN HEMISPHERE INFLUENZA SEASON.

The annual meeting for the composition of the influenza vaccine took place on 14-18 February 2010 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 2010-2011 season[10] (Table 5). Since seasonal influenza A (H1N1) viruses circulated at a very low rate in countries, such a strain was replaced by a pandemic influenza virus. A more recent influenza A (H3N2) strain, the influenza A/Perth/16/2009 virus replaced the 2009-2010 vaccine influenza A/Brisbane/10/2007 strain. The influenza B strain of a B-lineage recommended for the 2009-2010 influenza vaccine remained unchanged for the 2010-2011 vaccine strain.

Table 5 : Recommended composition of influenza vaccine for the 2010-2011 and 2009-10 seasons[10]

	Vaccine strain 2010/2011	Vaccine strain 2009/2010
A (H1N1) 2009	A/California/7/2009	A/Brisbane/59/2007
A (H3N2)	A/Perth/16/2009[1]	A/Brisbane/10/2007
B	B/Brisbane/60/2008[2]	B/Brisbane/60/2008[2]

1. A/Wisconsin/15/2009 is an A/Perth/16/2009-like virus and a 2010 southern hemisphere vaccine virus.
2. B/Brisbane/60/2008 is a B/Victoria/2/87-lineage virus.

8. DISCUSSION

In April 2009, 41 years after the 1968 influenza A (H3N2) pandemic virus, the emergence of a new influenza A (H1N1) virus of swine origin caused the first pandemic of the 21st century. Limited immunoprotection in the population was expected as this H1N1 virus has previously circulated only in animals for decades. In this context, influenza surveillance appeared as an essential tool to detect and follow viral circulation and to help healthcare authorities to take adapted decisions.

The Swiss Sentinel network experienced its first outbreak at a national level and the NRCI, together with the Laboratory of Virology of the University of Geneva Hospitals, faced unexpected challenges, similar to other response centres worldwide. The volume of samples received for influenza detection was considerably higher than usual: almost 5000 compared to 1000 during a usual season. Although the duration of surveillance ranged 12 months from May 2009 to April 2010, the peak of activity was concentrated within a few weeks. Surveillance was done also in coordination with other clinical laboratories in Switzerland during several months. We faced the unusual problems that any other laboratory faced around the world: volume increase, new analyses, new developments within a few days, new turn-around time required, and an overflow of requests and demands from the community and journalists, with

all the subsequent consequences in terms of management, financial aspects and organisation. Thanks to the support of the Swiss FOPH and our large institutional laboratory the response was appropriate.

The evolution of MC-ILI and the number of viruses detected over the last decade are presented in Figure 14. Based on these numbers and compared to previous seasonal outbreaks, the A (H1N1) 2009 pandemic had an impact on the population that was theoretically similar to the one of a usual season in terms of MC-ILI. As an example, the peak and the duration of the outbreak was quite similar to 2008-2009 season where influenza A (H3N2) and influenza B viruses co-circulated. However, significant differences could be observed during the 2009-2010 season. First, the influenza A (H1N1) 2009 virus circulated in two waves of different intensity. It started to be detected during the winter period in the Southern hemisphere (March 2009), reached countries from the Northern hemisphere very rapidly, and caused a small epidemic during the summer period, a completely unusual feature for human influenza in our country. The first Swiss case was detected on 28 April 2009 and a small epidemic was then observed between end of June and August, again an unusual period for the Northern hemisphere. During that period (labelled “Diagnostic” in purple in figure 14), samples were sent by any practitioners and laboratories in Switzerland according to pre-established guidelines published by the Swiss FOPH. The surveillance was community-based and according to medical encounters, MC-ILI, and pre-defined risk exposure (travellers). Sporadic cases were observed during the summer with a very limited sustained cluster of cases mostly linked to travel exposure. The second wave was also unusually early in autumn, between mid-October 2009 and ending early in January 2010, although the intensity was similar to previous Swiss influenza epidemics.

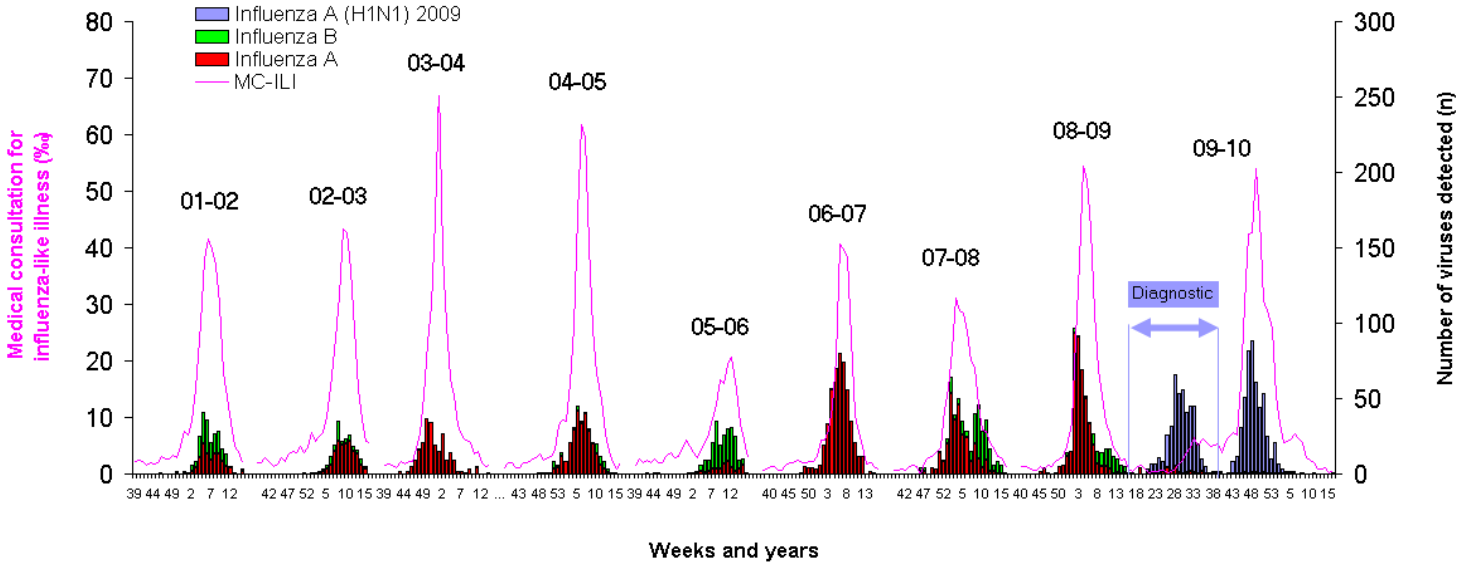


Figure 14: Weekly detection of influenza viruses and medical consultations for influenza-like illness between December 2001 and April 2010.

The period labelled "Diagnostic" comprises samples from Sentinel and non-Sentinel practitioners from all Switzerland.

This H1N1 virus has obviously circulated since 1918 in different animals for decades before reassorting and evolving in its current form. This event has already occurred in the past, but with quite different impacts observed.

- An influenza A (H1N1) virus of avian origin was at the origin of the "Spanish flu" in 1918-19 and caused more than 40 millions deaths.
- Another influenza A (H1N1) virus, but of swine origin, caused also cases in American soldiers in Fort Dix in 1976[6]
- In 2003, an influenza A(H7N7) of avian origin caused almost 300 human cases and one fatal case[2]
- Influenza A (H5N1) viruses of avian origin were detected in humans in 1997 and have been regularly detected each year since 2003. This virus has a death rate of 59% (295/499 cases as of 28 June 2010).

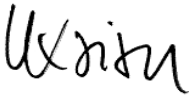
When they emerge in the population, influenza viruses of animal origin seem to be based on these observations associated with a higher death rate. Fortunately, this was not the case for the viruses of swine origin (H1N1 of 1977 and H1N1 2009). Given the diversity and ability of this virus to evolve, we should probably refrain from drawing any strong conclusions here.

Finally, as observed worlwide, this virus affected preferentially individuals less than 20 years old. However, the risk group for severe complications remained the same as the one for seasonal influenza: children less than 5 years old, immunocompromised patients, pregnant women, and those over 65 years old[9] In Switzerland, every second virus was detected in a patient less than 20 years old.

When influenza A (H1N1) 2009 virus started to circulate in Switzerland, it rapidly became predominant and other viruses did not circulate or at a very low non-significant rate. Few seasonal influenza A viruses were detected in the non-Sentinel samples, essentially during spring 2009, and sporadic influenza B viruses were occasionally detected during spring 2010. Similar patterns were observed in most European countries. Influenza B viruses were sporadically detected in the late part of the season, but did not cause any outbreak or epidemic wave. Antigenic and genomic analysis of influenza A (H1N1) 2009 viruses did not reveal different variants from the reference vaccine strain influenza A/California/7/2009 (H1N1) that was originally detected in Switzerland. No D222G mutant was detected in either Sentinel samples or in hospitalised patients with severe disease. D222E mutants in the HA gene were detected in 9 different Swiss cantons, with a putative cluster in Saint-Gall. No additional potential pathogenic feature of these strains was observed.

We did not observe spontaneous resistance to oseltamivir in influenza A (H1N1) 2009 viruses detected. However, resistant strains were detected in immunocompromised patients who were under long-term treatment with oseltamivir. Of note, no systematic resistance surveillance was conducted for hospitalised patients throughout the country. In addition, the matrix gene of influenza A (H1N1) 2009 virus sequences confirmed the S31N mutation responsible for the expected amantadine resistance.

Geneva, 14 July 2010



Yves Thomas, Ph.D **Laurent Kaiser, Pr**

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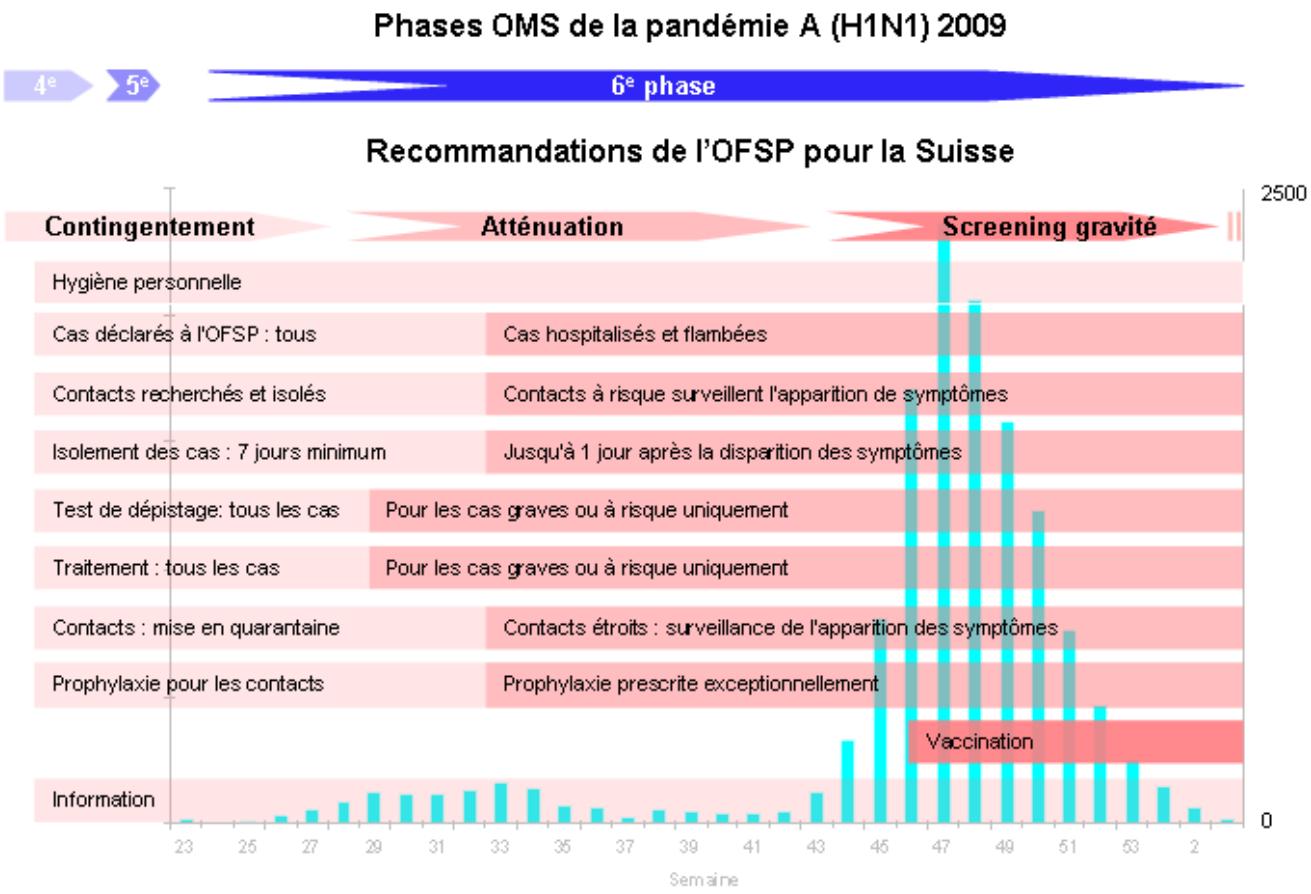
Annex 1: Official guideline adopted by the Swiss Federal Office of Public Health during influenza A (H1N1) 2009 pandemic



Annex 2: Inhibition of the hemagglutination of Influenza A (H1N1) 2009 viruses

	Influenza A (H1N1)			
	A/Seych/08	A/Brib/07	A/S.Peter/08	A/Calif/09
A/Seychelles/2239/08	**128**	64	16	<8
A/Brisbane/59/2007	128	**1024**	256	<8
A/St Petersburg/5/2008	128	512	**256**	<8
A/California/7/2009	<8	<8	nf	**64**

N°	Cells	IHA Result	HA	IHA Calif09 titre
2243133	ZH 33℃	A/California/7/09	16	1024
2243133	ZH 33℃	A/California/7/09	16	256
2253814	ZH 33℃	A/California/7/09	64	2048
2263814	ZH 33℃	A/California/7/09	64	512
2266201	SIAT 37℃	A/California/7/09	8	1024
2296477	ZH 33℃	A/California/7/09	32	256
2296477	SIAT 33℃	A/California/7/09	32	4096
2349079	ZH 33℃	A/California/7/09	32	256
2391565	SIAT 33℃	A/California/7/09	8	256
2391565	SIAT 33℃	A/California/7/09	16	2048
2418073	SIAT 33℃	A/California/7/09	16	256
2418073	SIAT 33℃	A/California/7/09	16	1024
2418104	SIAT 33℃	A/California/7/09	16	256
2428432	ZH 33℃	A/California/7/09	32	2048
2450472	SIAT 33℃	A/California/7/09	16	1024
2450547	SIAT 33℃	A/California/7/09	8	1024
2463780	SIAT 37℃	A/California/7/09	16	2048
2463826	SIAT 37℃	A/California/7/09	8	4096
2463880	SIAT 37℃	A/California/7/09	16	512
2464142	SIAT 37℃	A/California/7/09	32	512
2464318	SIAT 37℃	A/California/7/09	8	256
2467143	SIAT 37℃	A/California/7/09	8	256
2468600	SIAT 37℃	A/California/7/09	8	256
2468656	SIAT 37℃	A/California/7/09	16	512
2468656	SIAT 37℃	A/California/7/09	16	2048
2468686	ZH 33℃	A/California/7/09	32	2048
2468697	SIAT 37℃	A/California/7/09	8	256
2468709	SIAT 37℃	A/California/7/09	16	4096
2468833	SIAT 37℃	A/California/7/09	8	256
2471716	SIAT 37℃	A/California/7/09	8	256
2471729	SIAT 37℃	A/California/7/09	16	256
2471744	SIAT 37℃	A/California/7/09	16	512
2471755	SIAT 37℃	A/California/7/09	8	1024
2475589	SIAT 37℃	A/California/7/09	16	512
2475608	SIAT 37℃	A/California/7/09	16	1024
2479896	SIAT 33℃	A/California/7/09	32	2048
2480535	SIAT 33℃	A/California/7/09	32	1024
2480890	SIAT 33℃	A/California/7/09	32	1024
2494304	SIAT 33℃	A/California/7/09	16	512
2494471	SIAT 33℃	A/California/7/09	8	512
2494498	SIAT 33℃	A/California/7/09	16	1024
2494509	SIAT 33℃	A/California/7/09	16	1024

	Influenza A (H1N1)			
	A/Seych/08	A/Brib/07	A/S.Peter/08	A/Calif/09
A/Seychelles/2239/08	**128**	64	16	<8
A/Brisbane/59/2007	128	**1024**	256	<8
A/St Petersburg/5/2008	128	512	**256**	<8
A/California/7/2009	<8	<8	nf	**64**
2494518	SIAT 33℃	A/California/7/09	32	1024

N°	Cells	IHA Result	HA	IHA Calif09 titre
2494783	SIAT 37℃	A/California/7/09	32	2048
2494832	SIAT 33℃	A/California/7/09	32	2048
2494871	SIAT 37℃	A/California/7/09	32	2048
2494884	SIAT 33℃	A/California/7/09	32	2048
2494905	SIAT 37℃	A/California/7/09	16	1024
2496434	ZH 33℃	A/California/7/09	8	128
2503073	ZH 33℃	A/California/7/09	8	128
2503115	ZH 33℃	A/California/7/09	8	512
2503205	SIAT 33℃	A/California/7/09	8	512
2503225	SIAT 33℃	A/California/7/09	8	2048
2503250	ZH 33℃	A/California/7/09	8	256
2503303	ZH 33℃	A/California/7/09	8	256
2503339	ZH 33℃	A/California/7/09	32	1024
2520610	SIAT 37℃	A/California/7/09	32	1024
2521035	SIAT 37℃	A/California/7/09	8	256
2521633	SIAT 37℃	A/California/7/09	4	1024
2521661	SIAT 37℃	A/California/7/09	16	512
2521754	SIAT 37℃	A/California/7/09	8	512
2521769	ZH 33℃	A/California/7/09	8	512
2521791	SIAT 37℃	A/California/7/09	4	1024
2523626	SIAT 37℃	A/California/7/09	4	2048
2530244	SIAT 37℃	A/California/7/09	8	4096
2530437	SIAT 37℃	A/California/7/09	8	2048
2530573	SIAT 37℃	A/California/7/09	32	512
2530577	SIAT 37℃	A/California/7/09	32	2048
2535012	SIAT 37℃	A/California/7/09	16	2048
2535038	SIAT 37℃	A/California/7/09	32	1024
2535118	SIAT 37℃	A/California/7/09	32	2048
2548488	SIAT 37℃	A/California/7/09	32	2048
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2548607	SIAT 37℃	A/California/7/09	8	2048
2548618	SIAT 37℃	A/California/7/09	8	4096
2548642	SIAT 37℃	A/California/7/09	16	1024
2548644	SIAT 37℃	A/California/7/09	32	2048
2548736	SIAT 37℃	A/California/7/09	8	1024
2548824	SIAT 37℃	A/California/7/09	16	1024
2548947	SIAT 37℃	A/California/7/09	32	2048
2548961	SIAT 37℃	A/California/7/09	16	2048
2548980	SIAT 37℃	A/California/7/09	64	2048
2548990	SIAT 37℃	A/California/7/09	32	512
2552028	SIAT 37℃	A/California/7/09	8	1024
2552041	SIAT 37℃	A/California/7/09	8	1024
2552078	SIAT 37℃	A/California/7/09	8	4096

	Influenza A (H1N1)			
	A/Seych/08	A/Brib/07	A/S.Peter/08	A/Calif/09
A/Seychelles/2239/08	**128**	64	16	<8
A/Brisbane/59/2007	128	**1024**	256	<8
A/St Petersburg/5/2008	128	512	**256**	<8
A/California/7/2009	<8	<8	nf	**64**

N°	Cells	IHA Result	HA	IHA Calif09 titre
2552084	SIAT 37℃	A/California/7/09	8	4096
2552101	SIAT 37℃	A/California/7/09	4	4096
2557795	SIAT 37℃	A/California/7/09	8	2048
2557807	SIAT 37℃	A/California/7/09	32	1024
2557807	SIAT 37℃	A/California/7/09	32	1024
2557919	SIAT 37℃	A/California/7/09	16	1024
2557934	SIAT 37℃	A/California/7/09	16	512
2560917	SIAT 37℃	A/California/7/09	64	1024
2571794	SIAT 37℃	A/California/7/09	32	2048
2576969	SIAT 37℃	A/California/7/09	32	2048
2579665	SIAT 37℃	A/California/7/09	32	4096
2585983	SIAT 37℃	A/California/7/09	32	4096
2585998	SIAT 37℃	A/California/7/09	32	2048
2586156	SIAT 37℃	A/California/7/09	32	4096
2599205	SIAT 37℃	A/California/7/09	8	2048
2599668	SIAT 37℃	A/California/7/09	32	2048
2599764	SIAT 37℃	A/California/7/09	32	1024
2599769	SIAT 37℃	A/California/7/09	8	1024
2604318	SIAT 37℃	A/California/7/09	32	4096
2604386	SIAT 37℃	A/California/7/09	32	4096
2604399	ZH 33℃	A/California/7/09	16	4096
2604517	SIAT 37℃	A/California/7/09	16	4096
2607997	SIAT 37℃	A/California/7/09	4	4096
2608031	SIAT 37℃	A/California/7/09	16	512
2608068	SIAT 37℃	A/California/7/09	16	1024
2612852	SIAT 37℃	A/California/7/09	16	2048
2612874	SIAT 37℃	A/California/7/09	16	512
2612922	SIAT 37℃	A/California/7/09	16	2048
2616798	SIAT 37℃	A/California/7/09	4	1024
2616834	SIAT 37℃	A/California/7/09	16	2048
2616851	SIAT 37℃	A/California/7/09	16	1024
2616857	SIAT 37℃	A/California/7/09	32	4096
2616870	SIAT 37℃	A/California/7/09	8	1024
2627556	ZH 33℃	A/California/7/09	32	4096
2627568	ZH 33℃	A/California/7/09	32	4096
2627627	ZH 33℃	A/California/7/09	32	4096
2627996	ZH 33℃	A/California/7/09	32	4096
2631477	ZH 33℃	A/California/7/09	32	4096
2635541	ZH 33℃	A/California/7/09	32	4096
2635804	SIAT 37℃	A/California/7/09	8	2048
2635855	ZH 33℃	A/California/7/09	8	512
2635864	ZH 33℃	A/California/7/09	32	2048
2635877	SIAT 37℃	A/California/7/09	8	1024
2635899	SIAT 37℃	A/California/7/09	8	512
2636037	SIAT 37℃	A/California/7/09	16	2048

	Influenza A (H1N1)			
	A/Seych/08	A/Brib/07	A/S.Peter/08	A/Calif/09
A/Seychelles/2239/08	**128**	64	16	<8
A/Brisbane/59/2007	128	**1024**	256	<8
A/St Petersburg/5/2008	128	512	**256**	<8
A/California/7/2009	<8	<8	nf	**64**

N°	Cells	IHA Result	HA	IHA Calif09 titre
2636061	SIAT 37℃	A/California/7/09	16	2048
2666475	SIAT 37℃	A/California/7/09	16	4096
2666502	SIAT 37℃	A/California/7/09	16	1024
2666526	SIAT 37℃	A/California/7/09	8	1024
2682020	SIAT 37℃	A/California/7/09	16	2048
2682149	SIAT 37℃	A/California/7/09	16	2048
2682158	SIAT 37℃	A/California/7/09	8	1024
2702109	SIAT 37℃	A/California/7/09	2	1024
2705007	SIAT 37℃	A/California/7/09	8	2048
2705041	SIAT 37℃	A/California/7/09	16	4096
2705059	SIAT 37℃	A/California/7/09	32	2048
2714142	SIAT 37℃	A/California/7/09	8	2048
2714145	SIAT 37℃	A/California/7/09	8	4096
2714206	SIAT 37℃	A/California/7/09	4	4096
2714220	SIAT 37℃	A/California/7/09	8	4096
2723927	SIAT 37℃	A/California/7/09	4	2048
2731901	SIAT 37℃	A/California/7/09	8	2048
2731907	SIAT 37℃	A/California/7/09	32	2048
2741313	SIAT 37℃	A/California/7/09	4	2048
2743986	SIAT 37℃	A/California/7/09	2	4096
2768246	SIAT 37℃	A/California/7/09	8	2048
2768257	SIAT 37℃	A/California/7/09	8	1024
2788920	SIAT 37℃	A/California/7/09	16	512
2803034	SIAT 37℃	A/California/7/09	8	1024
2843287	SIAT 37℃	A/California/7/09	16	1024
2853662	SIAT 37℃	A/California/7/09	16	2048
2644674	SIAT 37℃	A/California/7/09	16	512
2644698	SIAT 37℃	A/California/7/09	16	1024
2644707	SIAT 37℃	A/California/7/09	16	1024
2644720	SIAT 37℃	A/California/7/09	16	2048
2644809	SIAT 37℃	A/California/7/09	128	2048
2644892	SIAT 37℃	A/California/7/09	8	2048
2658990	SIAT 37℃	A/California/7/09	32	1024
2659019	SIAT 37℃	A/California/7/09	4	2048
2662451	SIAT 37℃	A/California/7/09	32	2048

Annex 3: Inhibition of the hemagglutination of Influenza B viruses

	ANTISERA DE REFERENCE					
	B/Brisb08	**B/Mal04**	**B/Flo06**	**B/Brisb07**	**B/Barcel08**	**B/Bengl07**
B/Brisbane/60/08	**128**	128	<8	<8	<8	<8
B/Malaysia/2506/2004	<8	**128**	<8	<8	<8	<8
B/Florida/4/2006	<8	32	**2048**	1024	64	512
B/Brisbane/3/2007	<8	32	1024	**1024**	64	256
B/Barcelona/143/2008	<8	<8	64	32	**128**	64
B/Bengladesh/3333/2007	<8	16	256	256	128	**512**

N°	Cells	IHA result	HA	IHA Mal04 titre
2843262	ZH 33℃	B/Malaysia/2506/04	1024	128

Annex 4: World Health Organization. 2010. Recommended viruses for influenza vaccine for use in the 2010-2011 Northern Hemisphere influenza season. Weekly Epidemiological Record 85:81-92. (http://www.who.int/wer/2009/wer8425.pdf)