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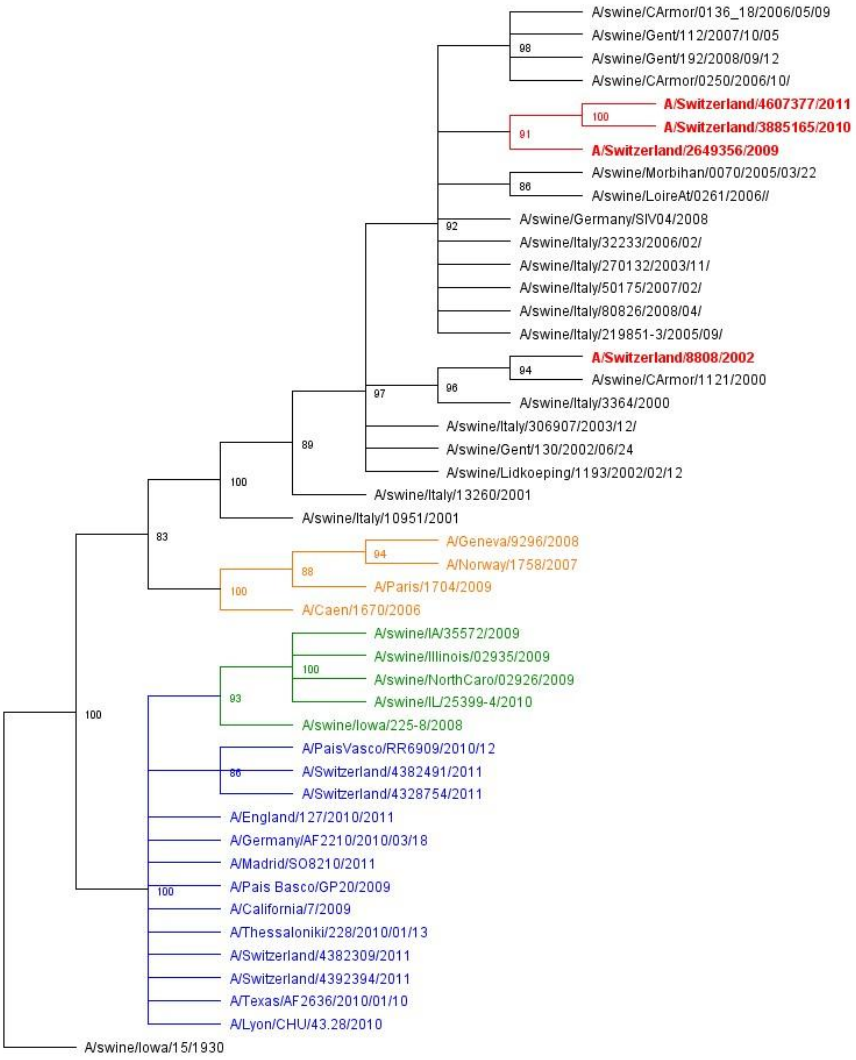
Influenza virus surveillance in Switzerland

Season 2010 – 2011



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Cover : Phylogenetic tree of HA gene sequences of influenza viruses of swine origin detected in swine and humans (see Figure 15 of the present report for further explanations)

Contents

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2. RESUME-SUMMARY

2.1. Résumé

La surveillance de la grippe pendant la saison hivernale 2010-2011 a débuté le 2 octobre 2010 et s'est terminée le 6 mai 2011. Une première vague de virus influenza A (H1N1) 2009 a circulé largement au cours de la saison en Suisse, suivi d'une deuxième vague de virus influenza B qui ont circulé dans des proportions comparables parmi la population. Des cas très sporadiques de virus influenza A (H3N2) ont également été observés au cours de la saison. L'épidémie a débuté au cours de la semaine 52 (fin décembre) puis s'est achevée pendant la semaine 9 (début mars). Cette circulation concomitante de 2 types de virus influenza a induit une activité épidémique d'intensité moyenne comparable à celle de ces dix dernières années. L'épidémie a duré 10 semaines, avec deux pics observés pendant les semaines 1 et 5. Les virus influenza A détectés étaient antigéniquement proches de la souche de référence A/Hong Kong/2212/2010 (H1N1) et restaient bien reconnus par les antisera dirigés contre la souche vaccinale A/California/7/2009 (H1N1). Seuls 7 virus influenza A (H3N2) ont été détectés dont 4 appartenaient au clade génétique Victoria/208 et un au clade Perth/16. La majorité des virus influenza B était antigéniquement proche de la souche influenza B/Hong Kong/514/2009 de la lignée B/Victoria et bien reconnue par l'antiserum vaccinal B/Brisbane/60/2010. Une minorité était antigéniquement proche de la souche B/Wisconsin/1/2010. Aucune souche influenza A des 84 analysées n'était porteuse de mutations reconnues conférant une résistance aux antiviraux oseltamivir et zanamivir. Un nouveau cas d'infection humaine par un virus d'origine porcine a été détecté en avril 2011 en Suisse chez un ouvrier d'une ferme d'élevage de porc. Les séquences partielles de trois gènes HA, NA et MP d'un virus influenza A (H1N1) détecté chez un porc de la même ferme étaient 100% homologues avec celles du virus détecté chez l'homme. C'est le troisième cas humain détecté en Suisse depuis 2009.

2.2. Summary

Influenza surveillance during the 2010-2011 winter season began on October 2, 2010 and finished on May 6, 2011. A first wave of influenza A (H1N1) 2009 virus circulated widely during the season in Switzerland, followed by a second wave of influenza B viruses which circulated in similar proportions among the population. Few sporadic cases of influenza A (H3N2) viruses were also observed during the season. The epidemic began during the week 52 (at the end of December) then completed during week 9 (at the beginning of March). This co-circulation of two types of influenza viruses induced an epidemic activity of medium intensity comparable to one observed the last ten years. The epidemic lasted ten weeks, with two peaks observed during weeks 1 and 5. The influenza viruses detected were antigenically related to the reference strain A/Hong Kong/2212/2010 (H1N1) and remained well recognized by the vaccine antiserum A/California/7/2009 (H1N1). Seven influenza A (H3N2) viruses have been detected, of which four belonged to the Victoria/208 clade and one to the Perth/16 clade. The majority of influenza B viruses was antigenically related to influenza B/Hong Kong/514/2009, of B/Victoria/208 lineage and well recognized by vaccine antiserum B/Brisbane/60/2010. A minority was antigenically closer to the B/Wisconsin/1/2010 strain, of the Yamagata-lineage. None of the 84 influenza A viruses carried known mutations conferring resistance to oseltamivir and zanamivir antivirals. A new case of human infection by a swine influenza virus was detected in April 2011 in Switzerland in a pig farm. The partial sequences of three genes HA, NA and MP of an influenza A (H1N1) virus detected in a pig of the same farm were 100% homolog to the viral gene detected in the man. It is the third human case detected in Switzerland since 2009.

3. Introduction

The emergence of the new pandemic influenza A (H1N1) 2009 virus at the end of March 2009 within an immunologically naïve human population was followed by an atypical early influenza season predominated by the new variant. Since the end of summer, influenza viruses attracted intense scrutiny and raised the following questions: Will the H1N1 2009 continue to predominate over the other human viruses that circulated previously in the population? Will it evolve and come back after antigenic drift? Will the following wave be more intense than the previous one? Will the seasonal influenza A (H1N1) viruses still be detected? We will attempt here to answer some of these questions.

4. Method of detection for influenza viruses

4.1. Clinical identification of influenza cases

During the 2010-2011 season, a network of 157 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°C with or without 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 1.

A subgroup of 85 Sentinel practitioners (54%) 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 72 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 72 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 1:4 ILI cases are systematically screened.

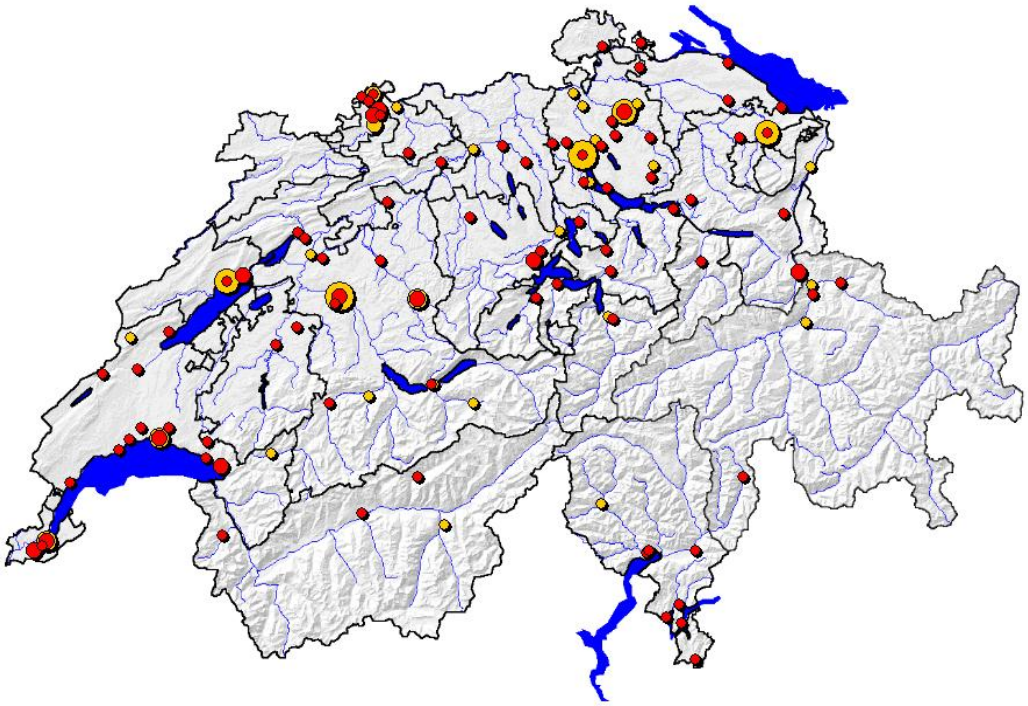


Figure 1: Geographical distribution of the 157 participants of the Sentinel network

Yellow bubble: location of the participants (72) conducting clinical surveillance; red bubble: participants conducting both clinical surveillance and specimen collection (85). Participants per community (range: 1-6) = bubble size

4.2. Detection of influenza viruses

4.2.1. Molecular technique

The first step of influenza virus detection in a nasopharyngeal swab is a real-time reverse transcription polymerase chain reaction. This molecular technique allows a rapid and sensitive detection of any genome of human and animal influenza viruses (Figure 2). Primers specific for influenza A[3], B, and pandemic influenza A (H1N1) viruses were used in a first reaction. Serotypes of seasonal influenza viruses were subsequently determined with hemagglutinin (HA) specific primers (H1 and H3).

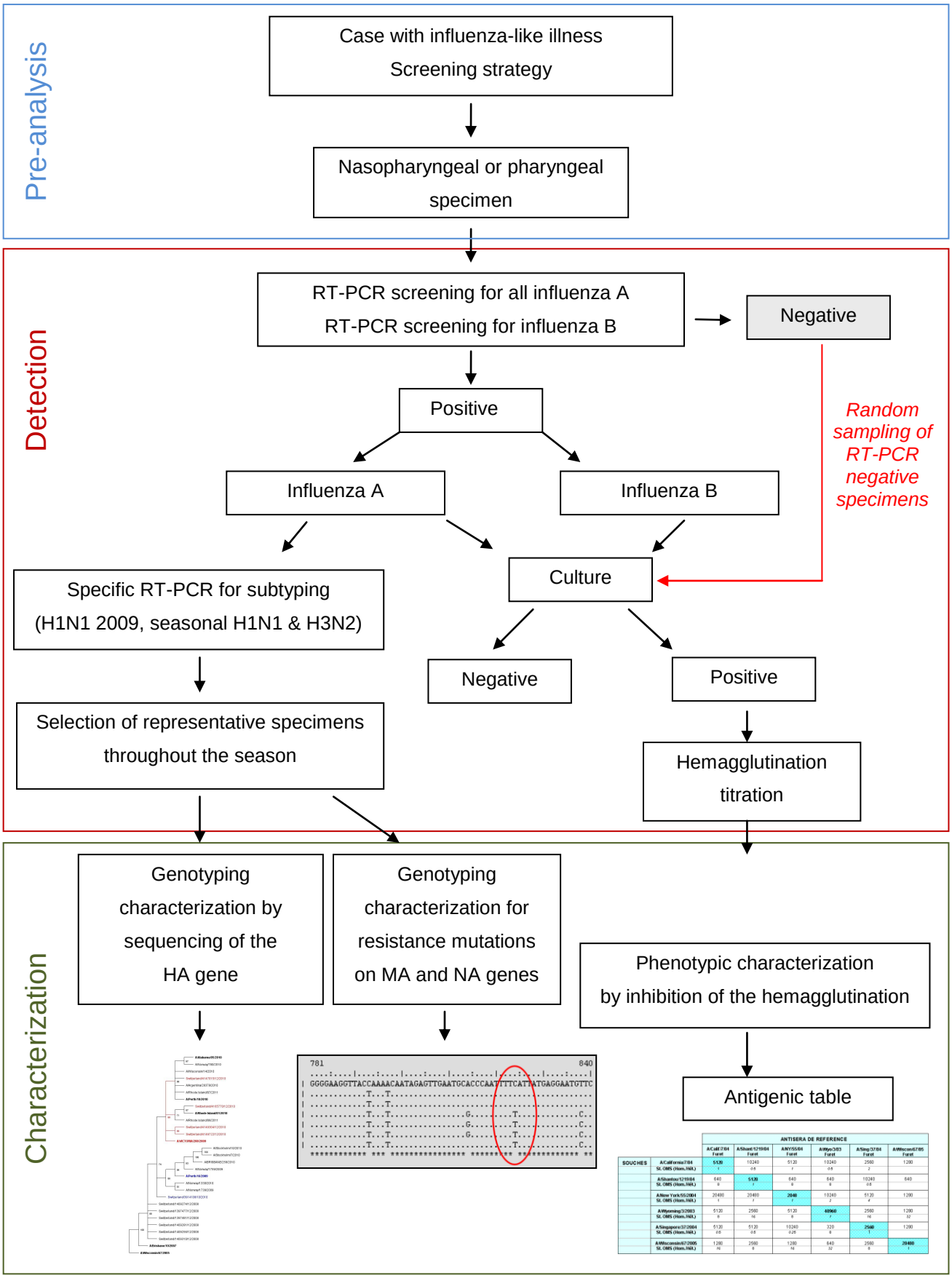


Figure 2 : Procedure used for the detection of influenza viruses by Sentinel surveillance

All positive samples were then cultivated on cells for further studies by inhibition of the hemagglutination (**IHA**). During the pre- and post-epidemic phases, negative samples were also put on cells to determine if influenza viruses of animal origin that remained undetected with first primers could be detected with cell culture.

4.2.2. Cell culture

A major goal of influenza surveillance is to determine the antigenicity of circulating influenza viruses and the potential efficiency of recognition by influenza vaccine-induced antibodies. Cell culture method allows to produce high quantities of influenza virus antigen. Clinical samples detected positive by influenza A or B RT-PCR analysis were incubated for 7 days under 5% CO2 at 33°C on MDCK cells and 37°C on MDCK-SIAT1. The cytopathic effect of the virus on cells is observed with a microscope under visible light (Leica®). If a specific cytopathic effect is observed, the titer of influenza virus is determined by hemagglutination. If no cytopathic effect is observed, an immunofluorescence test is applied to determine the presence of viral antigen (Figure 3). Monoclonal influenza A and B antibodies were used with monoclonal mouse specific FITC-conjugate (Chemicon®, Temecula, CA, USA). In the presence of a positive reaction, a supplementary cell culture cycle could be applied to increase viral titer.

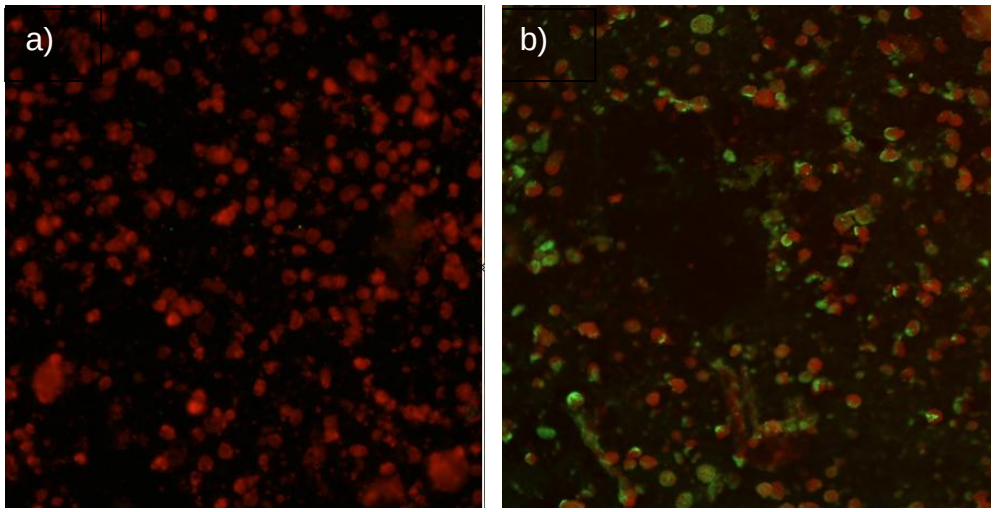


Figure 3: MDCK cells infected by influenza A/Switzerland/01/2009 (H1N1)

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®, Temecula, CA, USA).

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.

5. Characterization of influenza viruses

5.1. Phenotyping and antigenic characterization

The immunogenicity of circulating influenza viruses is studied by IHA reaction. The virus obtained by cell culture is submitted to standard antisera. In this latter reaction, the ability of the virus to link to red blood cell receptors 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 at 1.5% in DGV. Results are interpreted according to an antigenic table adapted to circulating strains and established at the beginning of the season. The 2010-2011 reference antigenic table comprised 4, 4 and 6 reference ferret antisera/strains for influenza A (H3N2), A (H1N1) (pandemic and seasonal), and B viruses, respectively (Table 1). The ratio between the homologous titers and the one obtained with the circulating influenza strains define the antigenic relationship to the 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.

Since 2001, two different influenza B virus lineages circulate in Europe. The first is constituted of strains detected in humans for decades worldwide, the B/Yamagata/16/88. A second lineage was detected in 1988 in the southern hemisphere (Asia and Oceania) before arriving in Europe in the early 2000’s, the B/Victoria/2/87. Since then, both lineages circulate alternatively during different

Table 1: Hemagglutination inhibition (IHA) titers of reference influenza strains tested with the 2010-2011 reference antisera

a) Influenza A (H3N2)

Strains \ Ferret antisera	A/Brisbane/10/2007	A/Perth/16/2009	A/Victoria/208/2009	A/Wisconsin/15/2009
A/Brisbane/10/2007	**1024**	16	32	16
A/Perth/16/2009	64	**512**	1024	128
A/Victoria/210/2009	64	512	**2048**	256
A/Wisconsin/15/2009	32	128	64	**32**

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

Strains \ Ferret antisera	A/California/7/2009	A/Bayern/69/2009	A/HKong/2212/2010	A/Brisbane/59/2007 seasonal
A/California/7/2009	***512***	*256*	*128*	*<8*
A/Bayern/69/2009	*128*	***256***	*128*	*<8*
A/HKong/2212/2010	*512*	*256*	***512***	*<8*
A/Brisbane/59/2007 seasonal	*<8*	*<8*	*<8*	***1024***

c) Influenza B

	Victoria-lineage			Yamanashi-lineage		
Strains \ Ferret antisera	B/Brisbane/60/2008	B/HKong/514/2009	B/Paris/1762/2009	B/Florida/4/2006	B/Bangladesh/3333/ 2007	B/Wisconsin/1/2010
B/Brisbane/60/2008	**1024**	256	128	<8	<8	<8
B/HKong/514/2009	64	**512**	256	<8	<8	<8
B/Paris/1762/2009	64	256	**256**	<8	8	<8
B/Florida/4/2006	<8	16	<8	**2048**	512	256
B/Bangladesh/ 3333/2007	<8	16	16	4096	**1024**	512
B/Wisconsin/1/2010	<8	<8	<8	512	128	**128**

seasons in the community. An influenza virus of one lineage is not, or less, recognized by antisera raised against viruses of the other lineage. In the antigenic table (Table 1c), influenza B viruses of the Yamagata lineage (blue section), e.g. B/Florida/4/2006, displayed no, or lower than 16, IHA titers with Victoria-lineage antisera, similar to B/Brisbane/60/2008. After RT-PCR detection of influenza B viruses, the lineage is determined mainly by IHA analysis.

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.

5.2. Genetic characterization

Genetic analysis has been developed and widely used over the last years at the NRCI as sequence determination helps to characterize better the homology between strains. The most variable sequence of influenza virus, the HA1 part of the HA gene, is the target of choice for sequencing analysis. Phylogenetic analysis then consists of comparing these sequences with HA sequences of reference strains. The different levels observed in nucleotide composition help to evaluate the drift importance of the circulating strains. In addition, for influenza A (H1N1) 2009 viruses, the presence of the D222G mutation in the receptor binding region of the HA sequence is associated with more severe cases.[9] Determining the HA sequence of the circulating H1N1 viruses can also help to understand better their pathogenicity.

Another important information that can be revealed by genome sequences is the antiviral resistance. The ability of influenza viruses to replicate in the presence of antiviral drugs can be determined mainly by the neuraminidase (NA) or matrix protein (MP) sequences. Specific mutations in the NA gene induce change in enzymatic activity that remains active in the presence of NA inhibitors (Table 2). Such resistance mutation depends on the type, subtype, and the antiviral. During the 2009-2010 season, H275Y mutation in the NA gene of influenza A (H1N1) 2009 has been observed in several countries, but at a limited rate and mainly in immuno-compromised patients under antiviral treatment.[11,12,14]

Table 2: Key mutations conferring antiviral resistance to influenza viruses

	Mutations conferring antiviral resistance - 06.05.2011 -		
	Oseltamivir (NA)	Zanamivir (NA)	Amantadine (M2)
H3N2	E119V N146K, S219T, A272V D151V,A,N,G I222V (LR) N249S R292K	E119V Q136K D151V,A,N,G R292K (LR)	V27A S31N
H1N1	Y155H G248R+I266V H275Y	Q136K Y155H G248R+I266V	V27A S31N
H1N1p	I117M D199E I223R (LR) I223V H275Y N295S Q313R & I427T	I223R (LR) Q313R & I427T	V27A S31N I43T
B	D197N/E (LR) I221T (LR) H273Y (LR)	D197N/E (LR) I221T (LR)	

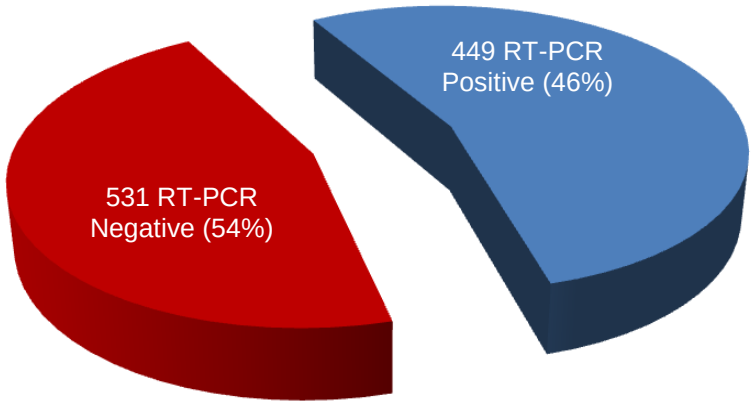
Surveillance of the influenza epidemic during the 2010-2011 season in the United Kingdom revealed a limited number of resistant viruses in patient with no known exposure to oseltamivir.[11] A similar observation was made during the 2008-2009 season with seasonal influenza A (H1N1) viruses that became predominant in the European community in the absence of systematic treatment of influenza infection.[10] Likewise, a S31M mutation in the M gene conferring amantadine resistance has been observed in almost all influenza A (H3N2) viruses[1] and influenza A (H1N1) 2009 viruses[4] circulating in the community over the last years. For some years now, detection of antiviral resistance either in the community or in the patients treated in healthcare facilities has become an important aspect of influenza surveillance.

6. Results for the 2010-2011 season

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

The influenza surveillance period lasted 31 weeks, and started on October 2, 2010 (week 40) and finished on 6 May, 2011 (week 18). 980 nasopharyngeal swabs were sent by 84 Sentinel practitioners. 449 influenza viruses (46%) have been detected by RT-PCR (Figure 4a): 248 influenza A (H1N1) 2009 (55%), 7 influenza A (H3N2) (2%) and 194 (43%) influenza B viruses (Figure 4b).

a)



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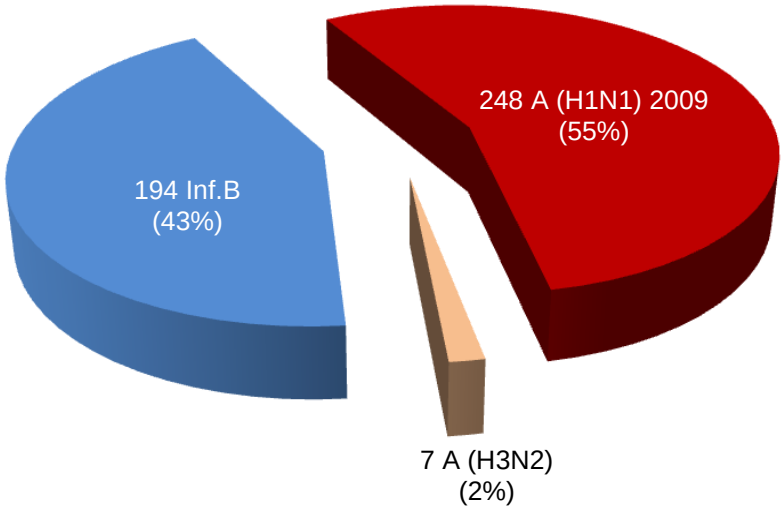


Figure 4: Nasopharyngeal specimens positive for any influenza virus during the 2010-2011 season (n=980)

a) Number of RT-PCR-positive versus negative specimen
b) Distribution of the different types and subtypes

Influenza viruses were to be detected sporadically from September 2011 (Figure 5) to the beginning of December (week 49). The epidemic phase then started with a positive rate of influenza virus detection that increased regularly to culminate during week 52. A slight decrease was then observed before a second increase phase up to week 7. The positive rate then started to decrease regularly up to week 16. Between weeks 52 and 9, the positive rate was above 50% and reached its highest value of 74% during week 7 (mid-February). The epidemic phase lasted for 10 weeks. When compared with the previous season, this duration was quite long. The positive rate returned to the post epidemic level afterwards with no viral detection observed since week 17 (Figure 5).

Influenza A (H1N1) 2009 virus predominated during the season from weeks 49 to 5 and was represented in 10% and 30%, respectively, of samples received. During that period, influenza B virus was detected in only 5 to 22% of samples. This virus then became predominant and was detected in 31% to 61% of the samples. During week 7, the highest positive rate was observed and, influenza B predominated over influenza A (H1N1) 2009 virus with 41% and 33%, respectively, detected. Both viruses continued to co-circulate up to week 14. An influenza A (H3N2) virus was detected early in the season, during week 43 but this virus was detected only sporadically between weeks 43 and 6.

MC-ILI values bypassed the epidemic threshold during week 52 and remained higher for 10 weeks during the season. This observation confirmed the relatively long duration of the 2010-2011 epidemic. A detailed table with influenza virus detection classified according to weeks and viral subtype is appended to this report (Annex 1).

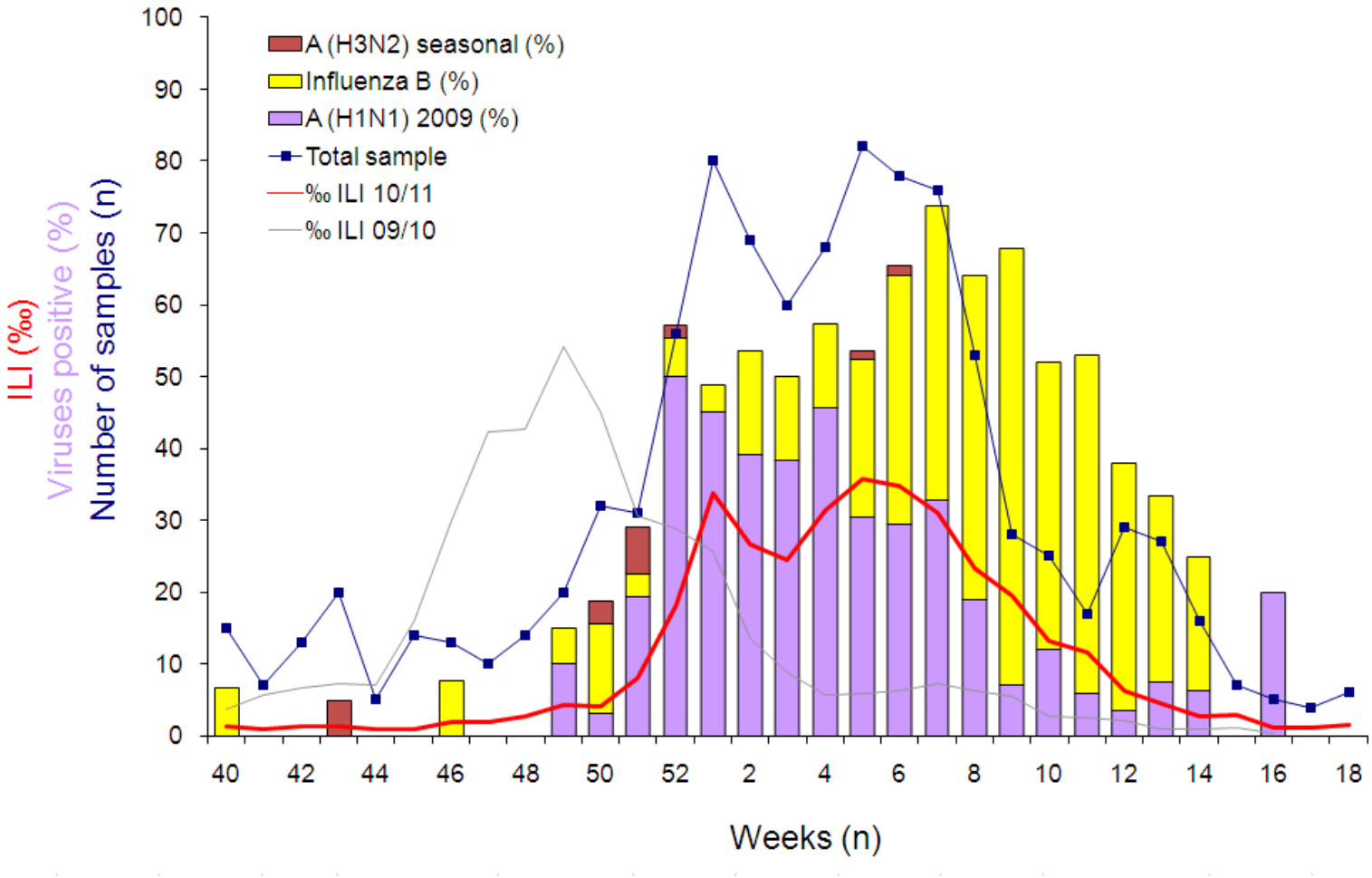


Figure 5: MC-ILI, positivity rate, and distribution of RT-PCR-positive cases according to influenza types.

Proportion of influenza A (H1N1) 2009, A (H3N2) and B viruses (%), total number of samples rested, and MC-ILI (%) 2010-2011 distribution per week. MC-ILI (%) 2009-2010 is also shown for comparative purposes.

6.2. Characteristics of screened Sentinel patients

Many studies showed that influenza A (H1N1) 2009 viruses affected preferentially the patients less than 20 years old.[16] During the 2009-2010 season in Switzerland, 47% of influenza viruses were detected in individuals less than 19 years old.[15] Recent studies conducted in the United Kingdom during the 2010-2011 season showed that the proportion of influenza A (H1N1) 2009 virus was highest among young adults (15-44 years).[5]

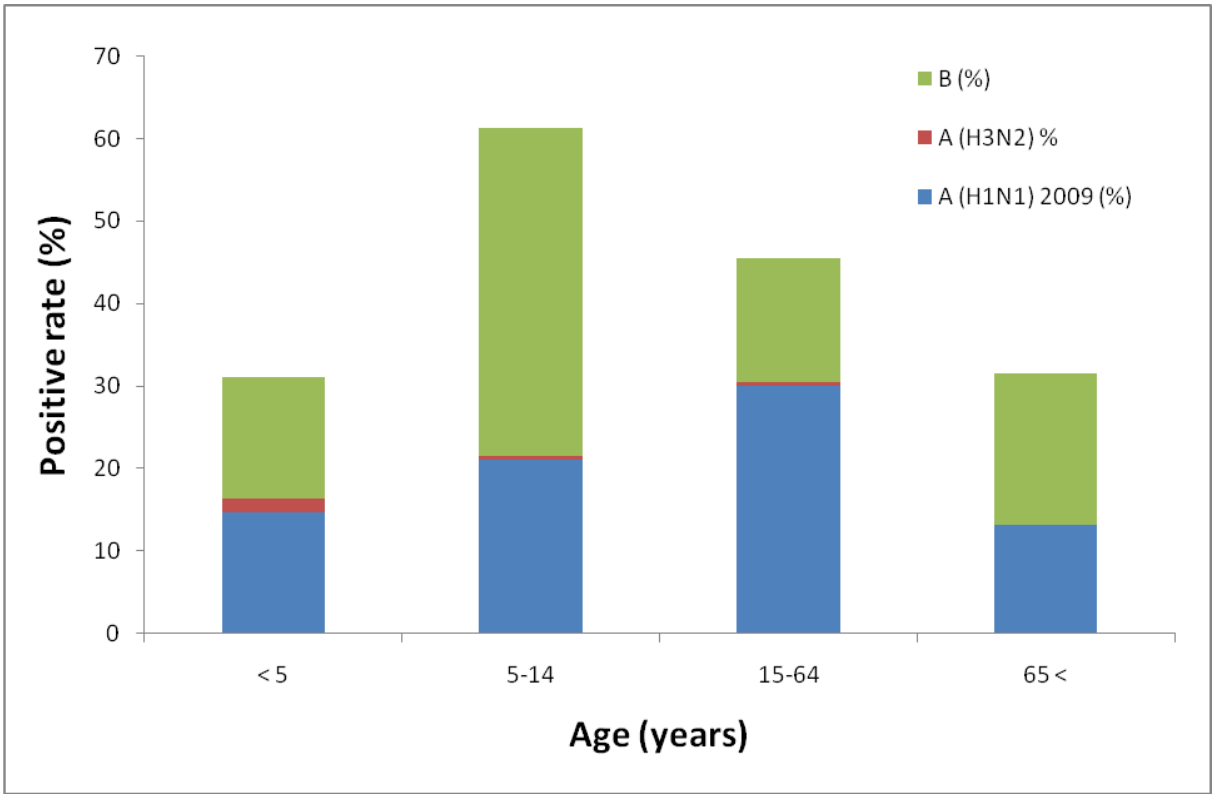


Figure 6: Repartition of viruses (%) detected according to age groups.

To evaluate the impact in the community, influenza viruses detected in Sentinel specimens were classified according to different age groups and virus types (Figure 6). The most frequent influenza associated MC-ILI were observed on the 5-14 years with 61% samples detected positive. If we consider the virus type, influenza B appeared to be the main virus detected in this age group : 40% of specimens were positive for influenza type B and 21.5% only were type A. In contrast, and as observed in the United Kingdom, the proportion of samples positive with influenza A (H1N1) 2009 virus was highest in young adults (15-44 years), with 30% of samples collected in this age group positive for this virus. Adults over 45 years old were less

affected with less than 15% of all screened cases positive for influenza A (H1N1 2009). Children less than 5 years old appeared to be equally affected by influenza A (H1N1) 2009 or influenza B. The small number of positive A (H3N2) samples do not allow to perform an evaluation of differences between age groups.

6.3. Antigenic and genetic characterization of influenza viruses

During the whole season, all positive samples were cultivated, with the exception of weeks 7 to 9. During the latter period (14 February to 4 March 2011), in order to decrease the technical burden only one half of all positive samples was cultivated and 49 randomly selected positive samples were not incubated on cells overall. 402/449 (90%) positive samples identified by RT-PCR analysis were incubated on cells for viral culture.

270 viruses were recovered and analysed by IHA: 141 influenza A (H1N1) 2009 viruses; 1 influenza A (H3N2) virus; and 128 influenza B viruses. By IHA assay 113 influenza B were of the B/Victoria/2/87 lineage, and 15 of the B/Yamagata/16/88 lineage (Figure 6). 177 positive influenza viruses were submitted to a genotypic analysis: 51 HA of influenza A, and 42 of influenza B viruses were sequenced. Of these, 46 HA of A (H1N1) 2009, 5 HA of influenza A (H3N2), 36 HA of B/Victoria lineage, and 6 HA of B/Yamagata lineage. Finally, 84 NA genes were sequenced for antiviral resistance detection in influenza A (H1N1) 2009 viruses (Figure 7).

6.3.1. Influenza A (H1N1) 2009

HA gene:

Influenza A (H1N1) 2009 viruses were relatively well recognized by the vaccine strain influenza A/California/7/2009 (H1N1) and also by the A/Hong Kong/2212/2010 (H1N1) antisera. Of note IHA titers obtained with this last antiserum were regularly equal or higher than the one obtained with the reference strains A/California (Annex 2). This reveals that most strains observed in Switzerland were closer to influenza A/Hong Kong/2212/2010.

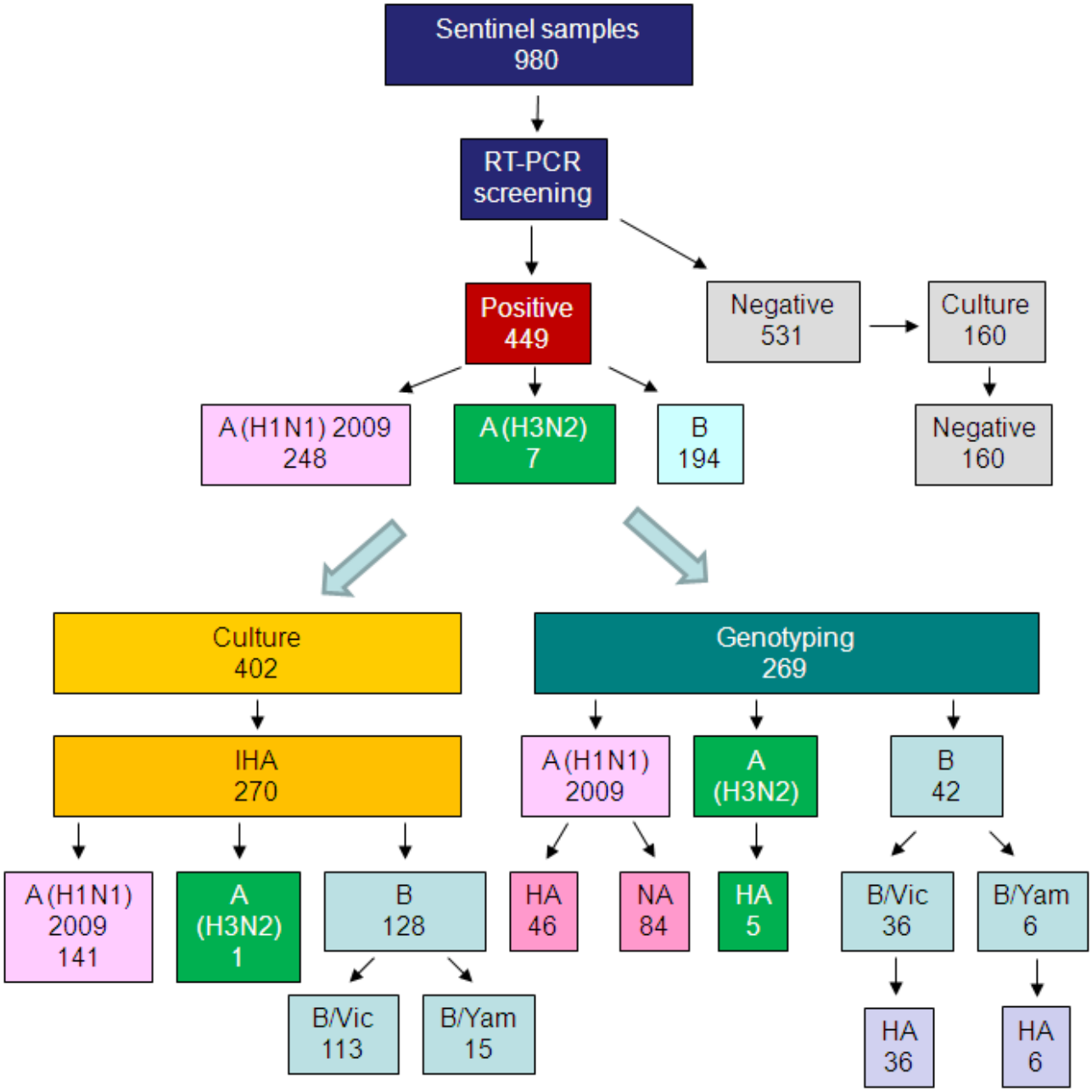


Figure 7 : Summary of the analysis performed on Sentinel samples.

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

The HA gene sequence of a selection of A (H1N1) 2009 viruses detected during the 2010-2011 season in Switzerland has been aligned and a phylogenetic analysis has been conducted. HA sequences of the influenza A (H1N1) 2009 viruses appeared to originate from the same ancestor. However, three different genetic groups could be distinguished by the presence of a specific combination of amino acids' mutation described previously.[13] Group I is constituted of most A (H1N1) 2009 strains detected

this season (red and yellow strains, Figure 8). Seven of these strains had the A134T and S183P mutations and were already observed in United Kingdom (yellow strains, Figure 8).[13] Of note, one influenza strain had the A134T mutation only (influenza A/Switzerland/4149348/2010, yellow star). Nine isolates of this first genetic group displayed the S185T mutation and has been described as the most frequent group of virus (red strains, Figure 8). These strains did not display the S143G mutation as described in other European S185T variant.[13] A second genetic group of 7 strains (green strains, Figure 8) had the R205K, I216V, and V249L and was previously observed in European countries and in Iraq.[13] A D97N mutation was commonly observed in groups II and III in Swiss strains.

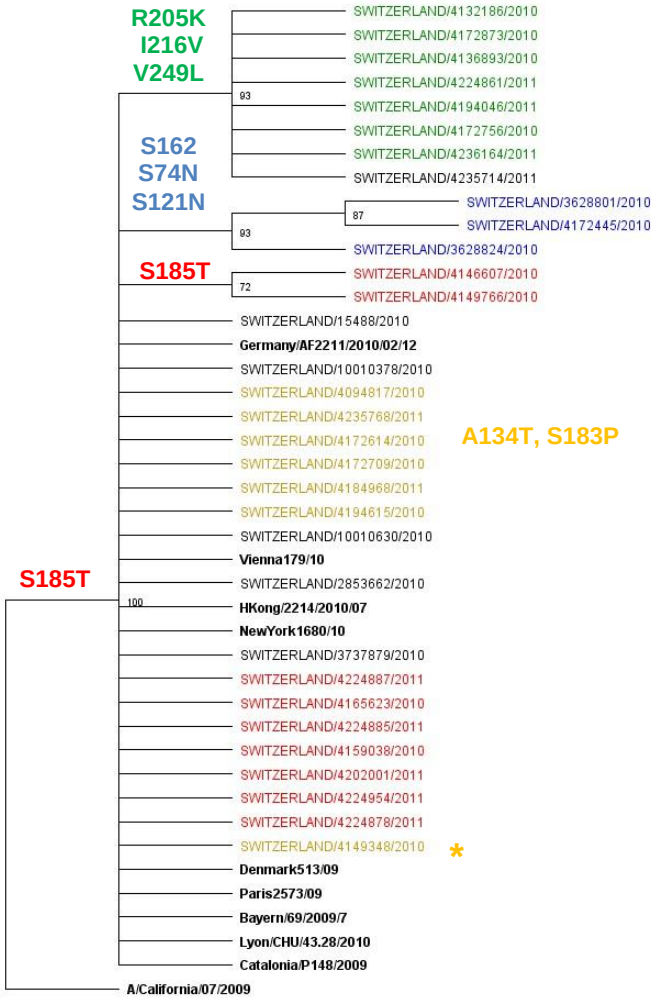


Figure 8 : Phylogenetic analysis of the influenza A (H1N1) 2009 hemagglutinin

Yellow star: Swiss influenza virus with the A134T mutation, but without the S183P

Finally, the third genetic group is constituted by 3 strains that harbored the S162N mutation alone (Figure 8, blue strains), without any association with N31D, A186T and V272I, as observed in Denmark and South Africa.[13] Two of these strains shared two distinct mutations not described before, the S74N and the S121N mutation in the HA1 gene (3628801 and 4172445). The N125D mutation observed in the southern hemisphere was not detected in Swiss strains.

A mutation in the HA was suggested to be associated with more severe cases of A (H1N1) 2009 infection, the D222G mutation.[9] This position was checked in all HA sequences of A (H1N1) 2009 viruses detected in Switzerland. No D222G mutation in HA gene was detected in any viruses (data not shown).

Neuraminidase gene:

The NA sequence of 84 influenza A (H1N1) viruses have been analyzed for the presence of an antiviral resistance marker. No known mutation conferring resistance to oseltamivir was detected. Seven strains detected in Switzerland during this season displayed a Q313R mutation (Figure 9). In association with a I427T mutation, they conferred a 10 to 40x resistance to oseltamivir and zanamivir in an influenza A (H1N1) 2009 strain detected in Georgia in 2009.[8] However, the I427T mutation was not detected in the 7 Q313R Swiss strains, as showed on NA gene (Figure 9).

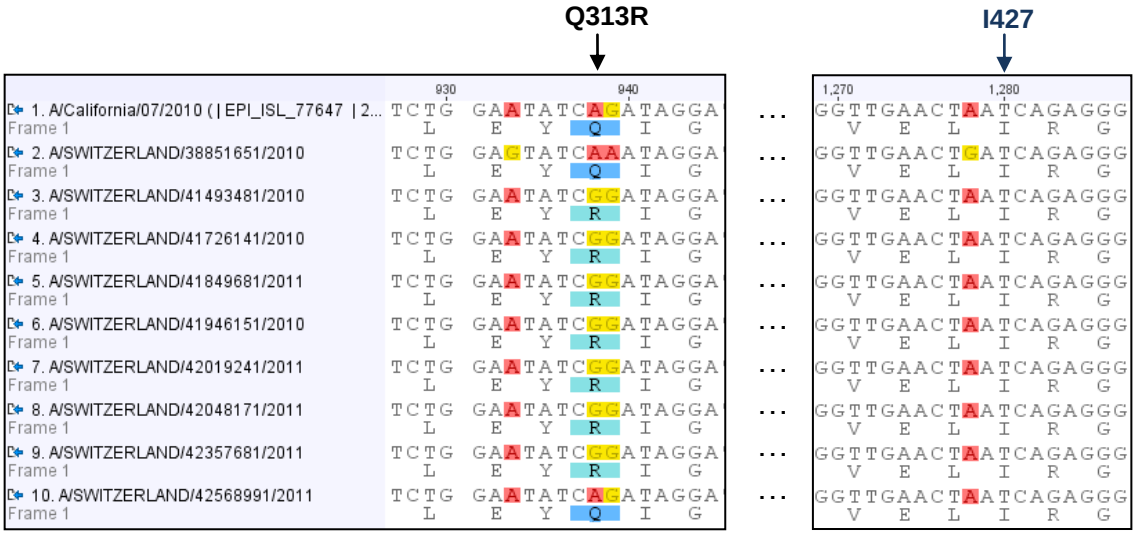


Figure 9: NA sequence alignment of 9 influenza viruses detected in the Sentinel network during the 2010-2011 season

In addition, four Q313R strains have been sent together with Q313 strains, to the French National Centre for influenza viruses in Lyon (France) for the testing of antiviral resistance by phenotypic analysis (MUNANA®, fluorescent-based enzymatic assay of the NA). This test confirmed that in vitro the Q313R strains remained as susceptible as the Q313 strains to oseltamivir (Annex 4).

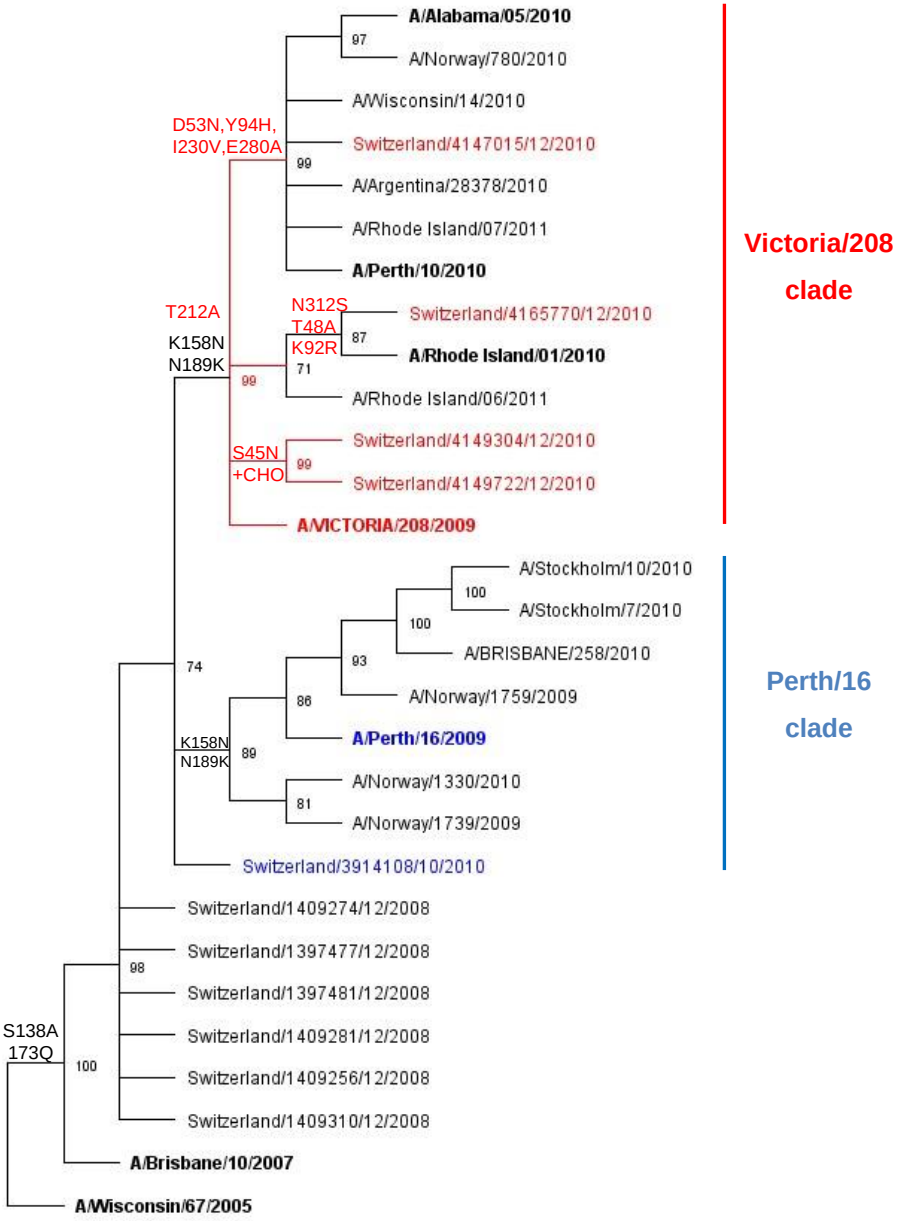


Figure 10: Phylogenetic analysis of HA gene of A (H3N2) viruses.

6.3.2. Influenza A (H3N2)

The 6/7 influenza A (H3N2) viruses grew very poorly and gave very low HA titer that did not allow IHA analysis. Only one influenza A (H3N2) could be subtyped by IHA and was well recognized by the vaccine strain influenza A/Perth/16/2009 (H3N2). The HA1 gene could be sequenced from 5/6 influenza A (H3N2) viruses that gave low HA titers and was compared to both reference and recent strains sequences available at Global Initiative on Sharing All Influenza Data (GISAID) database. Influenza A (H3N2) viruses drifted from the Swiss strains detected during the 2008-2009 season (Figure 10). They share S138A, K158N, K173Q and N189K mutations observed in recent influenza A (H3N2) strains, which distinguish them from the previous influenza A/Brisbane/10/2007 vaccine strain (Rod Daniels, Ljubljana 2011). However, they clustered in two genetically distinct groups. The influenza A/Switzerland/3914108/2010 is related to the A/Perth/16/2009 clade with a T amino acid at the position 212, which is specifically observed in these clade strains[13]. The 4 other Swiss strains were related to the influenza A/Victoria/208/2009 clade and have a T212A mutation. The influenza A/Switzerland/4165770/2010 virus has additional mutations that have been observed in European strains circulating during this season and constituting a subgroup in the Victoria/208 clade: N312S, T48A, and K92R mutations.[13]

6.3.3. Influenza B

128 Influenza B viruses have been typed by IHA. 113/128 (88%) were typed as B/Hong Kong/514/2009 based on their relatively high titers (B/Victoria lineage) (Annex 3). Generally, these strains harbored a lower IHA titer with the vaccine strain B/Brisbane/60/2008 antiserum (Annex 3). 15/128 (12%) were antigenically related to influenza B/Wisconsin/1/2010 virus, a B-Yamagata-like lineage.

42 HA1 genes from influenza B viruses have been sequenced. Of these, 36 were related to the Victoria lineage and 6 to the Yamagata lineage. A phylogenetic analysis showed that all of these viruses were related to the Brisbane/60 clade 1 (Figure 11). The specific N75K and S172P mutations were detected in all B-Victoria lineage viruses. In addition, several of these viruses harbored the L58P mutation (brown star Figure 11) already described.[13]

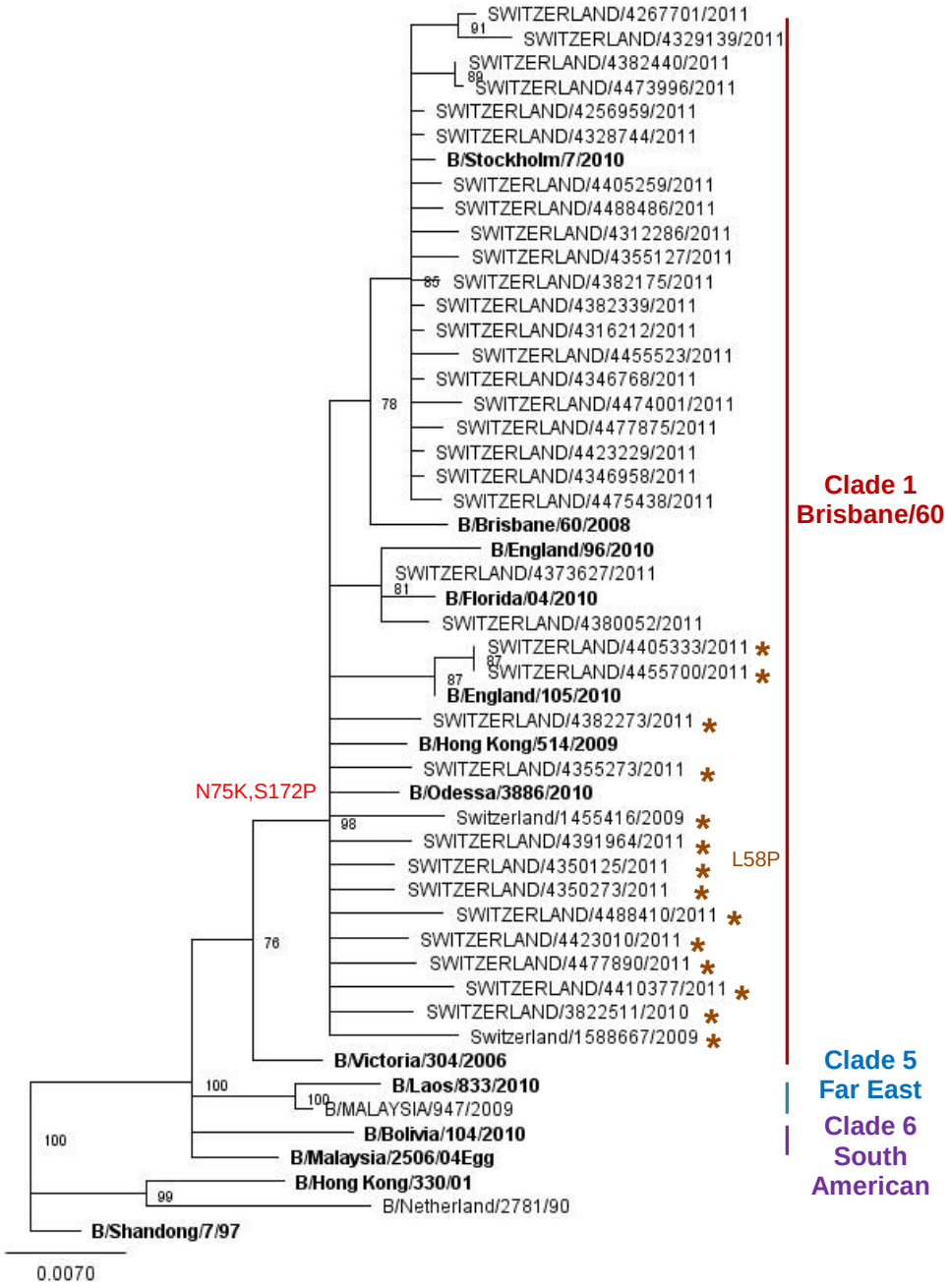


Figure 11: Phylogenetic analysis of the HA gene of influenza B Victoria-like viruses.

Brown stars: influenza B virus of the Brisbane/60 clade displaying the L58P mutation

Influenza B viruses of the Yamagata lineage are related to the Bangladesh/3333 clade which contains influenza B/Wisconsin/1/2010. All these viruses displayed the S150I, N165Y and G229D mutations that have been observed in recent influenza

viruses of many countries (MRC report). These strains are antigenically distinct from the vaccine strain B/Florida/4/2006.

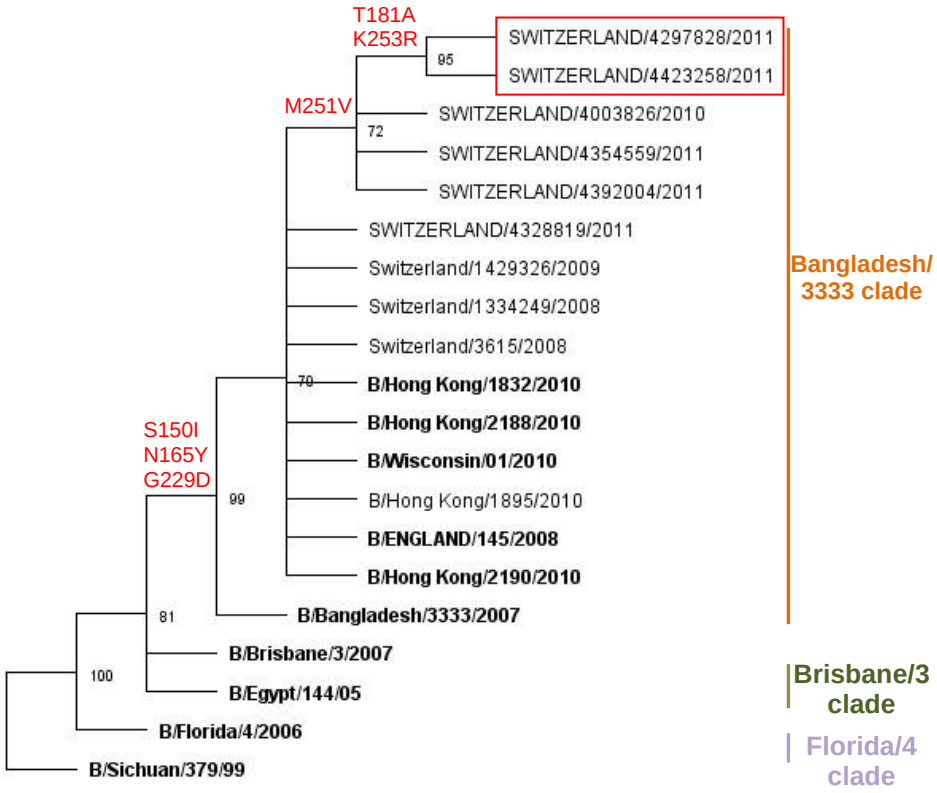


Figure 12: Phylogenetic analysis of the HA gene of influenza B Yamagata-like viruses.

Red square: HA sequences having T181A and K253R mutations and constituting a distinct genetic group.

A M251V is present in 5/6 influenza B viruses of the B-Yamagata lineage detected during the season 2010-2011. The A/Switzerland/4328819/2011 did not display this mutation and keeps a M251, as observed in influenza B viruses detected in Switzerland during 2008 and 2009. However, this strain harbors the N202T mutation in place of the N202S previously observed in some influenza B strains of the Yamagata lineage detected in 2010 in Asia and the USA.[13]

Two strains had the T181A and K253R mutations and constitute a distinct genetic group at the top of the phylogenetic tree, marked with a red square (Figure 12).

7. Influenza activity in European countries

Influenza A and B viruses circulated successively in European countries. Influenza A viruses were predominant over influenza B viruses with 72% and 29% of detection respectively. A first peak in influenza activity was observed during week 3 due to circulating influenza A viruses and a second distinct peak was reached during week 5 due to the circulation of influenza B viruses. 96% of influenza A viruses were subtyped as A (H1N1) 2009 viruses, and 4% were A (H3N2) viruses that remained sporadically detected in Europe. Based on IHA analysis, influenza A (H1N1) 2009 viruses were antigenically related to the vaccine strain influenza A/California/7/2009. Influenza B viruses were related to influenza B/Brisbane/60/2008 (91%) of the B/Victoria/2/87 lineage, to B/Florida/4/2006 (8.9%), and to B/Bangladesh/3333/2007 (0.1%), both of the B/Yamagata/16/88 lineage.[2]

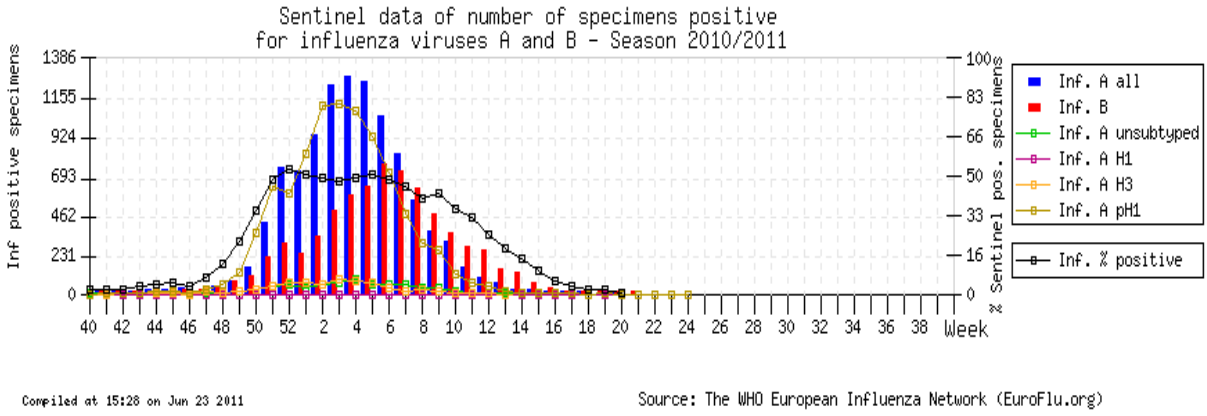


Figure 13: Influenza viruses detection rate in European countries, EuroFlu, WHO-Europe.

585 influenza viruses have been analyzed genetically. 28% belonged to A/California/7/2009 A(H1N1) clade, 2% to the A/Christchurch/16/2010 A(H1N1) clade and 8% to the A/Hong Kong/2213/2010 (H1N1) clade. 23% belonged to the new emerged influenza A/England/142/2010. The influenza A (H3N2) viruses belonged to the A/Perth/16/2009 clade (2%), to the A/Hong Kong/2121/2010 clade (5%) which belong to the A/Victoria/208/2009 clade (1%). The influenza B viruses were related to influenza B/Bangladesh/3333/2007 (3%) of the B/Yamagata lineage and to B/Brisbane/60/2008 of the B/Victoria lineage.

Antiviral susceptibility has been screened on 3519 viruses in 9 European countries (Denmark, Germany, Ireland, Italy, Netherlands, Norway, Spain, Switzerland, and the United Kingdom). 87% of influenza viruses were susceptible to both NA inhibitors. 2.9% had the previously described H275Y mutation known to confer an oseltamivir resistance. These viruses remained sensitive to zanamivir. Nine influenza A (H3N2) viruses have been tested and were found to be sensitive to both inhibitors. 340 influenza B viruses were tested for resistance to both inhibitors and were found sensitive to the antiviral. Finally, amantadine susceptibility has been tested in 197 influenza A (H1N1) 2009 and 10 A (H3N2) viruses , all were found to be resistant.[2]

8. Human infection by an influenza A (H1N1) virus of swine origin

On 6 April, 2011, a 22 year old man working in a pig farm in Switzerland presented respiratory symptoms. A swab was taken on 7 April by a member of the veterinarian surveillance network that performs influenza virus surveillance in pigs (Monika Engels, Vetvir). The sample was sent to the NRCI for further analysis. An influenza A (H1N1) virus of swine origin was detected, named the A/Switzerland/7377/2011 (H1N1) virus (**7377/11**). For details see Annex 6. Since the official report made to the FOPH, further sequencing analysis have been conducted (Table 3).

Table 3: Gene and region of influenza A/Switzerland/7377/2011 virus sequences analyzed.

	Gene fragments sequenced					
A/Switzerland 7377/2011	PB2	PB1	PA	HA	NA	MP
Length (nts)	2229	1797	1657	850	514	728
Region	1-2229	18-1814	495-2151	82-932	616-1129	68-796

Sick animals at the same farm were sampled and analyzed in another specialized laboratory a swine influenza A (H1N1) virus was also detected. The HA-1, NA, and the MP genes were sequenced in this laboratory specialized in animal surveillance (Monika Engels, VetVir, Veterinarian University of Zürich). An alignment analysis of 373 nucleotides of the HA-1 gene showed 100% homology between the 7377/11 sequence and the 59-11 swine sequence (Figure 14). The same analysis was conducted with NA (490 nucleotides) and MP genes (290 nucleotides) and showed also 100% homology (data not shown). These results support an animal-to-human infection.

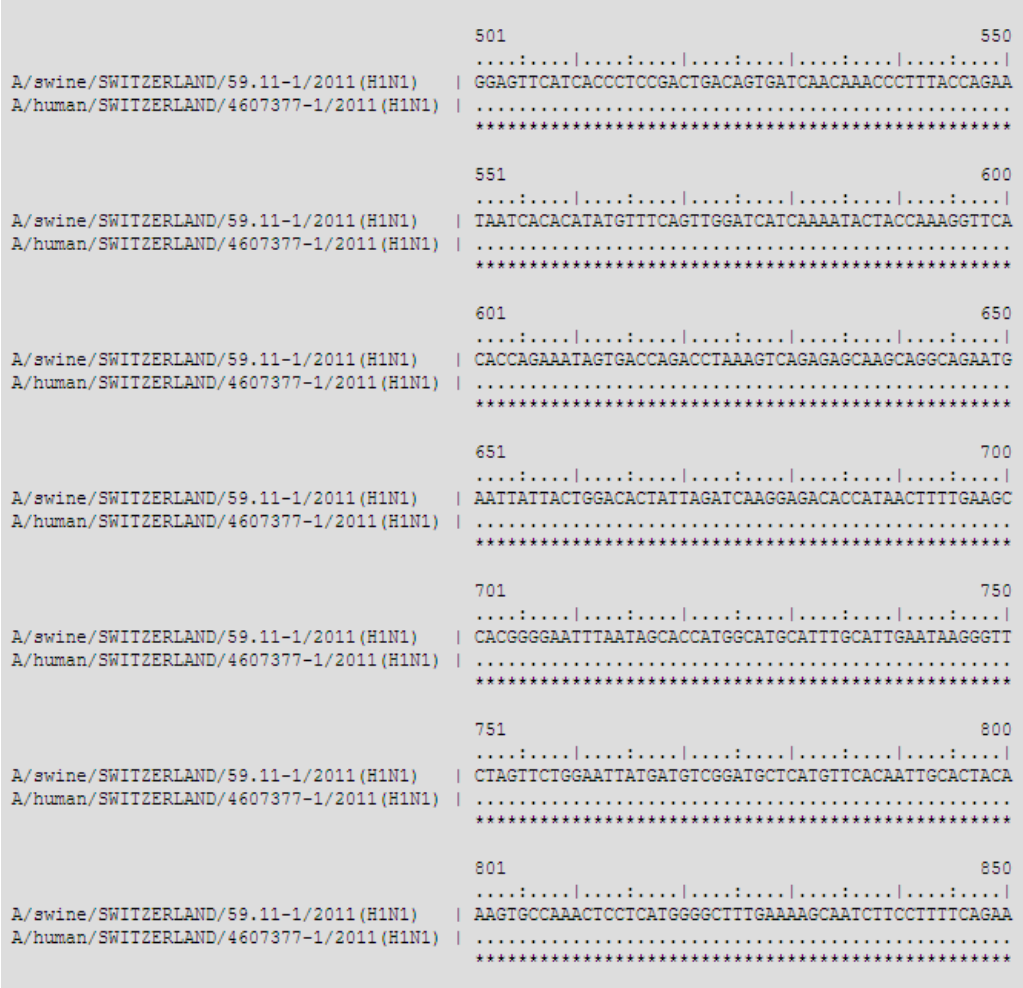


Figure 14: Alignment of the HA gene of influenza A/Switzerland/7377/2011 (H1N1) detected in a human and A/sw/Switzerland/59-11/2011 (H1N1) detected in a pig of the same farm

A blast analysis of the HA1 sequence, the most variable part of the genome, of the 7377/11 virus revealed a close relationship with influenza A/Switzerland/5165/2010

(H1N1) (**5165/10**) and influenza A/Switzerland/9135/2009 (H1N1) (**9135/09**), the two influenza viruses of swine origin detected in humans in Switzerland in 2010. The 7377/11 virus showed 3/850 differences in 5165/10 virus nucleotide composition, and 21/850 differences in 9135/09 virus nucleotide composition (Table 4). These observations suggest a common ancestor for the three viruses.

Table 4: Blast analysis (Smartgene® platform) and sequence homology of the HA sequence of A/Switzerland/4607377/2011 (H1N1) influenza virus.

Query sequence - locus HA									
Select	Action	Dataset	Seq. length	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Country of collection [iso_3166-1]	Internal NCI remark	s4-HA length
☐	more...	HUG Influenza Private Samples	850	A/human/Zurich/4607377-1/2011(H1N1)		human	CH	old number - Human infection with swine origin inf ...	850

Similar sequences found										
Select	Action	Dataset	Official strain name	s4-HA AC	Length	Seq. length	Identities	Mismatches	Match length	Score
☐	more...	IDNS Influenza References (1)	A/Human/Aargau/5165/2010 (H1N1)	CY079544	1723	1723	847 (99.65%)	3	850	1661
☐	more...	IDNS Influenza References (1)	A/Human/Zurich/9356/2009 (H1N1)	CY079537	1715	1715	829 (97.53%)	21	850	1518
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/270132/2003 (H1N1)	GQ175968	984	984	826 (97.18%)	24	850	1495
☐	more...	IDNS Influenza References (1)	A/Swine/Spain/53207/2004 (H1N1)	CY010580	1738	1738	825 (97.06%)	25	850	1487
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/2929-6/2005 (H1N1)	GQ175959	983	983	824 (96.94%)	26	850	1479
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/285983-7/2005 (H1N1)	GQ175969	983	983	822 (96.71%)	28	850	1463
☐	more...	IDNS Influenza References (1)	A/Swine/Gent/132/2005 (H1N1)	FJ791277	1114	1114	821 (96.59%)	29	850	1455
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/295855/2004 (H1N1)	GQ175970	983	983	821 (96.59%)	29	850	1455
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/50175/2007 (H1N1)	FJ770258	983	983	821 (96.59%)	29	850	1455
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/219851-3/2005 (H1N1)	GQ175965	998	998	820 (96.47%)	30	850	1451

A phylogenetic analysis of the HA gene was performed and compared with the HA-1 genes of recent swine influenza A (H1N1) viruses of the classical USA lineage (green, Figure 15) and of avian lineage (black, Figure 15), together with seasonal and pandemic influenza A (H1N1) viruses (yellow and blue, Figure 15). This analysis confirmed the swine origin of the 7377/11 and illustrates the genetic distance between the viruses of different origin: human (seasonal and pandemic) and swine. It also provides an indication of the close relationship between the three influenza strains of swine origin detected in humans in Switzerland in 2009, 2010 and 2011, and the swine strains of the avian lineage recently detected in European countries.

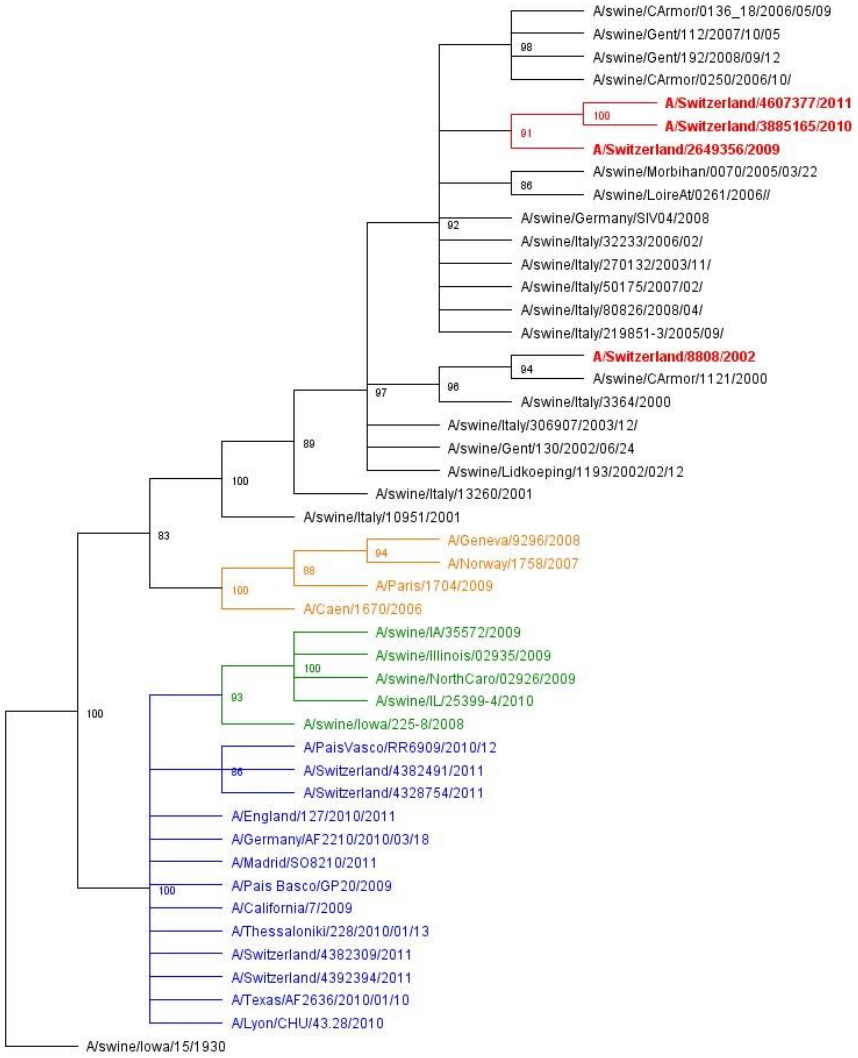


Figure 15: Phylogenetic tree of swine and human HA gene sequences of influenza A (H1N1) viruses.

Red: influenza viruses of swine origin detected in Switzerland. **Blue**: pandemic influenza A (H1N1) 2009 viruses detected in humans since 2009. **Green**: classical swine influenza A (H1N1) viruses of the USA lineage. **Yellow**: seasonal influenza A (H1N1) viruses detected in humans. **Black**: swine influenza A (H1N1) viruses of the avian lineage, which predominate in Europe.

9. WHO recommendation for the composition of influenza virus vaccines for use in the 2011-2012 northern hemisphere influenza season.

The annual meeting for the composition of the influenza vaccine took place on 15-17 February 2011 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 2011-2012 season.[7] Influenza A (H1N1) 2009 viruses

predominated in European countries and circulated at a lower rate in the USA and Asia. Most strains were related to influenza A/California/7/2009 and the vaccine containing antigen related to this strain stimulated anti-HA antibodies sufficient for recent circulating A (H1N1) 2009 viruses. Influenza A/California/7/2009 virus is recommended to be included in the 2011-2012 vaccine (Table 5).

Table 5 : Recommended composition of influenza vaccine for the 2011-12 seasons[17]

	Vaccine strain 2011/2012
A (H1N1) 2009	**A/California/7/2009**
A (H3N2)	**A/Perth/16/2009**
B	**B/Brisbane/60/2008**

Influenza A (H3N2) viruses circulated at a low rate in Europe but was predominant in the USA and Asia. Most were antigenically related to influenza A/Perth/16/2009 (H3N2). This strain is recommended to be included in the 2011-2012 vaccine (Table 5). Influenza B viruses circulated widely thorough the world. B/Victoria/2/87 and B/Yamagata/16/88 lineages were represented but the former predominated in all but one country, China, where the Yamagata lineage was predominant. Most of influenza B viruses of the Victoria lineage were related to influenza B/Brisbane/60/2008, and this strain is recommended for the 2011-2012 vaccine (Table 5).

10. Discussion

After emergence of the new influenza A (H1N1) 2009 virus, several questions were pending: will this virus will continue to circulate? If yes what will the target populations be? Will this virus remain genetically and antigenically stable and as a consequence acquire a higher pathogenic property? Will this virus behave as other subtype influenza A viruses in terms of period of the year and co-circulation with other viruses? Our surveillance provide answers to some of these questions.

This season was classical in Switzerland and in other European countries. First the winter period when influenza viruses circulated was the same as that observed in most previous seasons. Preliminary viruses started to be detected at the beginning of December, and peaks of activity were observed around weeks 1 and 7, in the middle of winter season. Second, even if A (H1N1) 2009 viruses has been circulating worldwide since March 2009 and without any significant antigenic or genetic modification, this virus predominated during the influenza season.

In addition, and as observed with seasonal influenza A viruses, A (H1N1) 2009 co-circulate in a sequential manner with other influenza viruses particularly influenza B viruses. IHA titers obtained with the vaccine antiserum A/California/7/2009 (H1N1) were conserved and did not indicate any dramatic antigenic drift in the influenza A (H1N1) 2009 virus. Genetic analysis showed the existence of a minor genetic drift in the HA sequence with three different groups of mutation constellation, but these changes remained limited and probably unsignificant at the antigenic level. For this reason, the A (H1N1) 2009 vaccine strain for 2011-2012 will remain the same.

By comparison, influenza A (H3N2) strains showed a more complex diversity even if the virus did not circulate widely during the season. A great diversity of well known mutation constellation was observed in the few Swiss influenza A (H3N2) viruses. Such viruses circulated at a low rate in Europe, but were more represented in the influenza epidemic in the USA. Based on USA and European virological results, no change was performed on the vaccine strain that remains the influenza A/Perth/16/2010 virus.

Two influenza B lineages circulated in Switzerland during the 2010-11 season, a majority were related to the B-Victoria lineage and a smaller percentage were related to the B-Yamagata lineage. The 2010-11 vaccine strain, the influenza B/Brisbane/60/2008, was a related to Victoria lineage. Most influenza B-Victoria like strains showed a decreased IHA titer with vaccine antiserum B/Brisbane/60/2008 and were more related to influenza B/Hong Kong/514/2009 that emerged after the B/Brisbane/60/2008. Influenza B virus of the Yamagata lineage were not recognized efficiently by the 2010-11 vaccine induced antibodies, this suggests that vaccine

induced protection against influenza B was not optimal. Additional analysis would be required to confirm this question.

The recently emerged A (H1N1) 2009 virus appears to replace almost completely seasonal influenza A (H1N1) virus worldwide, China excepted. This suggests that similar subtypes of influenza viruses do not co-circulate easily and that the most adapted variant of A (H1N1) viruses competes host infection with other variants of the same subtype. In addition, influenza A (H3N2) did not co-circulate with A (H1N1) 2009 viruses. Such observation was done regularly in previous season. Since 1994, one influenza A virus has predominated over the other subtype during the whole season, with the exception of season 1995-1996 (Table 6).

Table 6: Relative detection rate of influenza A and B subtypes on the total number of viruses detected during the seasons between 1994 and 2011 at the NRCI.

	Seasons	Strains (%)			
		A (H3N2)	A (H1N1) A (H1N2)	A (H1N1)p	B
Culture-based screening	1994/95	79	1	-	20
	1995/96	38	51	-	11
	1996/97	68	1	-	32
	1997/98	98	2	-	-
	1998/99	37	-	-	63
	1999/00	100	-	-	-
	2000/01	1	89	-	10
	2001/02	44	-	-	56
	2002/03	68	3	-	28
	2003/04	99	-	-	1
	2004/05	75	12	-	14
	2005/06	12	6	-	82
RT-PCR-based screening	2006/07	87	13	-	-
	2007/08	3	42	-	55
	2008/09	76	-	-	23
	2009/10	-	-	100	-
	2010/11	2	-	55	43

A comparison of the peak of MS-ILI during the last seasons shows that, the one observed during this season was relatively low (Figure 16). But the number of influenza viruses detected can be compared with the number detected during previous seasons and this is probably related to the fact that the season was relatively prolonged.

Young adults (15-44 years) appeared to be more affected by the A (H1N1) 2009 virus than with usual seasonal influenza viruses. Compared to 2009 the younger age groups (less than 14 years) seem to be less affected by this virus than previously described, but this might be also related to mild diseases or large circulation of this virus in 2009. In contrast, the 5-14 years' age group was probably more affected by influenza B viruses infection than other individuals this year.

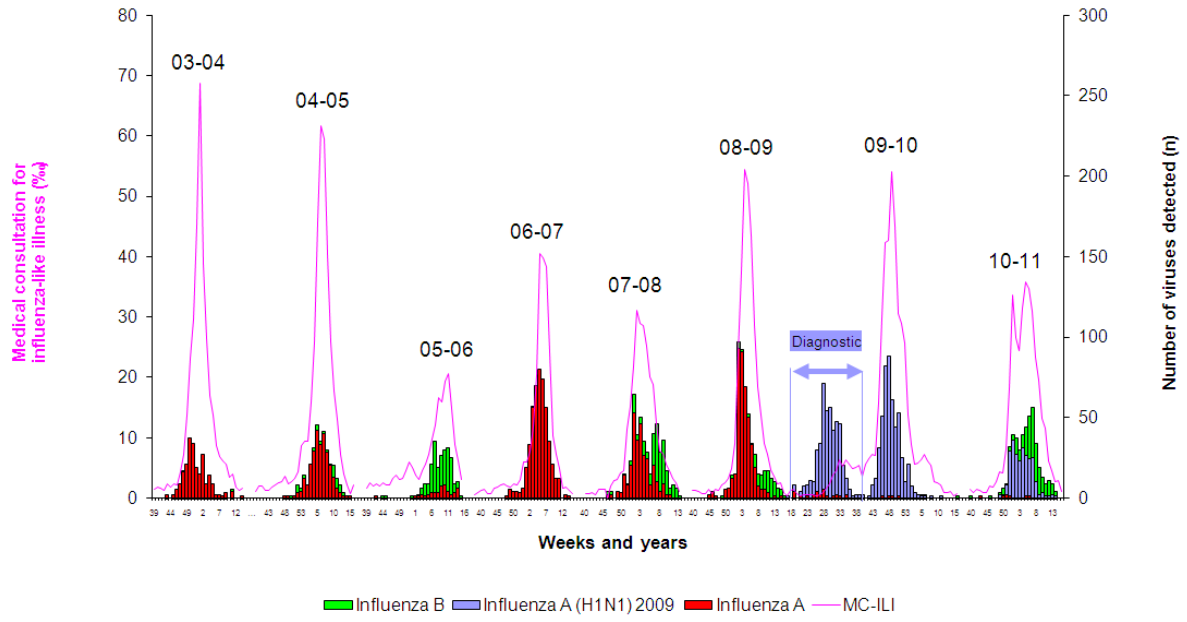


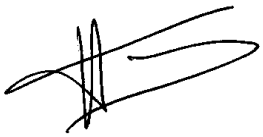
Figure 16: Number of influenza viruses and medical consultations for influenza-like illness registered from 2001 to 2011 in Switzerland.

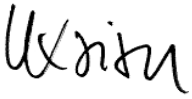
A recent study reported H275Y mutation resistance of 6/159 influenza A (H1N1) 2009 viruses in the United Kingdom with no known oseltamivir exposition in 5/6. We did not observe such spontaneous resistance to oseltamivir in influenza A (H1N1) 2009 viruses detected in the community by Sentinel practitioners. In addition, we did not detect resistance to oseltamivir or zanamivir in samples tested from hospitalized patients from Geneva, Basel, Lausanne or Zürich hospitals (data not shown).

However, these samples did not come from a well established surveillance network and are addressed in a care by care decision between practitioners working in hospitals. Thus, we cannot conclude the absence of such resistance treated patients.

The additional detection of an influenza A (H1N1) virus of swine origin in a pig farm employee in Switzerland demonstrates that collaboration between veterinary and reference center is fruitful to provide opportunities to screen humans exposed to animal viruses. This surveillance provides information on the viral exchange that apparently occurs regularly between swine and humans. The proximity and exposure of humans to swine have always been the source of such contamination. However, to better understand and evaluate human infection by swine virus, systematic surveillance samples could be helpful in farm workers and family members of the exposed individuals. The recent emergence of the influenza A (H1N1) 2009 pandemic virus highlights that human infections by swine influenza viruses could lead to a new pandemic.

Geneva, 4 August 2011





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11. References

1. Bright, R. A., D. K. Shay, B. Shu, N. J. Cox, and A. I. Klimov. 2006. Adamantane resistance among influenza A viruses isolated early during the 2005-2006 influenza season in the United States. JAMA 295:891-894.

2. Brown, C., Mott, J., Jorgensen, P., and Pereyaslov, D. Euroflu weekly electronic bulletin - Week 18. 13-5-2011.

3. CDC. CDC protocol of realtime RT-PCR for influenza A (H1N1). 1-7. 28-4-2009. 28-4-2009.

4. Deyde, V. M., T. G. Sheu, A. A. Trujillo, M. Okomo-Adhiambo, R. Garten, A. I. Klimov, and L. V. Gubareva. 2010. Detection of molecular markers of drug resistance in 2009 pandemic influenza A (H1N1) viruses by pyrosequencing. Antimicrob. Agents Chemother. 54:1102-1110.

5. Ellis, E., Galiano M., Pebody R., Lackenby A., Thompson C.I., Bermingham A., McLean E, Zhao H., Bolotin S., Dar O., Watson J.M., and Zambon M. 2011. Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in Winter 2010/11. Eurosurveillance 16.

6. Ferraris, O., N. Kessler, and B. Lina. 2005. Sensitivity of influenza viruses to zanamivir and oseltamivir: a study performed on viruses circulating in France prior to the introduction of neuraminidase inhibitors in clinical practice. Antiviral Res. 68:43-48.

7. GISN-WHO. WHO recommendation for the vaccine composition 2011-12 - Northern hemisphere influenza season. 17-2-2011.

8. Gubareva, L., Nguyen, H., Sleeman, K., Ballenger, K., Mishin, V. P., Sheu, T. G., Trujillo, A. A., Deyde, V., Levine, M., Craine, P., Okomo-Adhiambo, M., and Klimov, A. Comprehensive Assessment of the Drug Susceptibility of 2009 H1N1 Influenza Viruses. Options for the Control of Influenza VII. Options for the Control of Influenza VII , 18-19. 3-9-2010.

9. Kilander, A., R. Rykkvin, S. G. Dudman, and O. Hungnes. 2010. Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010. Euro. Surveill 15.

10. Lackenby, A., O. Hungnes, S. G. Dudman, A. Meijer, W. J. Paget, A. J. Hay, and M. C. Zambon. 2008. Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe. Euro. Surveill 13.

11. Lackenby, A., G. J. Moran, R. Pebody, S. Miah, L. Calatayud, S. Bolotin, I. Vipond, P. Muir, M. Guiver, J. McMenamin, A. Reynolds, C. Moore, R. Gunson, C. Thompson, M. Galiano, A. Bermingham, J. Ellis, and M. Zambon. 2011. Continued emergence and changing epidemiology of oseltamivir-resistant influenza A(H1N1)2009 virus, United Kingdom, winter 2010/11. Euro. Surveill 16.

12. Longtin, J., S. Patel, A. Eshaghi, E. Lombos, R. Higgins, D. Alexander, R. Olsha, J. Doyle, D. Tran, A. Sarabia, C. Lee, N. Bastien, Y. Li, D. Low, G. Boivin, and J. Gubbay. 2011. Neuraminidase-inhibitor resistance testing for pandemic influenza A (H1N1) 2009 in Ontario, Canada. J Clin. Virol. 50:257-261.

13. McCauley, J., daniels, R., Yi, P. L., Zheng, X., Hou, T., Gregory, V., Whittaker, L., Cattle, N., Kloess, J., and Halai, C. Report of MRC for WHO annual consultation on the composition of influenza vaccine for the northern hemisphere. Interim report Feb 2011, 1-58. 14-2-2010.

14. Robert N. and on behalf of NISN. An overview of antiviral drug-Resistance data presented at Options for the control of Influenza VII-September 2010 Hong Kong. 1-18. 1-12-2011.

15. Thomas Y. and Kaiser L. Influenza surveillance in Switzerland - Sentinel network report - Season 2009-2010. 1-55. 1-7-2010.

16. WHO. 2010. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med 362:1708-1719.

17. WHO. 2010. Recommended viruses for influenza vaccine for use in the 2010-2011 Northern Hemisphere influenza season. Weekly Epidemiological Record 85:81-92.

Annex 1: Influenza virus detection according to weeks and nature of the virus.

Sentinel Surveillance Winter 2010-11																
Weeks	Dates		‰ ILI	Total sample	A/H1N1 2009			A (H3N2) seasonal			Influenza B				Total virus (n)	% pos
					undet.	HK10	Total	undet	Perth09	Total	undet	BHK09	Bwiscon	Total		
40	02-oct-10	08-oct-10	1.4	15	**0**		**0**			**0**		**1**		**1**	**1**	**7**
41	09-oct-10	15-oct-10	1	7	**0**		**0**			**0**				**0**	**0**	**0**
42	16-oct-10	22-oct-10	1.4	13	**0**		**0**			**0**				**0**	**0**	**0**
43	23-oct-10	29-oct-10	1.3	20	**0**		**0**		**1**	**1**				**0**	**1**	**0**
44	30-oct-10	05-nov-10	1	5	**0**		**0**			**0**				**0**	**0**	**0**
45	06-nov-10	12-nov-10	1	14	**0**		**0**			**0**				**0**	**0**	**0**
46	13-nov-10	19-nov-10	2	13	**0**		**0**			**0**			**1**	**1**	**1**	**8**
47	20-nov-10	26-nov-10	1.9	10	**0**		**0**			**0**				**0**	**0**	**0**
48	27-nov-10	03-déc-10	2.7	14	**0**		**0**			**0**				**0**	**0**	**0**
49	04-déc-10	10-déc-10	4.3	20	**2**		**2**			**0**	**1**			**1**	**3**	**15**
50	11-déc-10	17-déc-10	4.2	32	**1**		**1**		**1**	**1**	**1**	**2**	**1**	**4**	**6**	**19**
51	18-déc-10	24-déc-10	8.1	31	**0**	**6**	**6**		**2**	**2**	**0**	**1**		**1**	**9**	**29**
52	25-déc-10	31-déc-10	18.2	56	**7**	**21**	**28**		**1**	**1**	**1**	**2**		**3**	**32**	**57**
1	01-janv-11	07-janv-11	33.7	80	**12**	**24**	**36**			**0**	**0**	**3**		**3**	**39**	**49**
2	08-janv-11	14-janv-11	26.6	69	**13**	**14**	**27**			**0**	**2**	**8**		**10**	**37**	**54**
3	15-janv-11	21-janv-11	24.5	60	**13**	**10**	**23**			**0**	**2**	**5**		**7**	**30**	**50**
4	22-janv-11	28-janv-11	31.4	68	**16**	**15**	**31**			**0**	**1**	**6**	**1**	**8**	**39**	**57**
5	29-janv-11	04-févr-11	35.8	82	**10**	**15**	**25**		**1**	**1**	**4**	**12**	**2**	**18**	**44**	**54**
6	05-févr-11	11-févr-11	34.7	78	**8**	**15**	**23**	**1**		**1**	**12**	**12**	**3**	**27**	**51**	**65**
7	12-févr-11	18-févr-11	31	76	**15**	**10**	**25**			**0**	**18**	**12**	**1**	**31**	**56**	**74**
8	19-févr-11	25-févr-11	23.3	53	**4**	**6**	**10**			**0**	**7**	**14**	**3**	**24**	**34**	**64**
9	26-févr-11	04-mars-11	19.5	28	**2**		**2**			**0**	**6**	**11**		**17**	**19**	**68**
10	05-mars-11	11-mars-11	13.2	25	**1**	**2**	**3**			**0**	**2**	**7**	**1**	**10**	**13**	**52**
11	12-mars-11	18-mars-11	11.6	17	**1**		**1**			**0**	**1**	**6**	**1**	**8**	**9**	**53**
12	19-mars-11	25-mars-11	6.2	29	**0**	**1**	**1**			**0**	**2**	**7**	**1**	**10**	**11**	**38**
13	26-mars-11	01-avr-11	4.5	27	**1**	**1**	**2**			**0**	**3**	**4**		**7**	**9**	**33**
14	02-avr-11	08-avr-11	2.8	16	**1**		**1**			**0**	**3**			**3**	**4**	25
15	09-avr-11	15-avr-11	2.9	7			**0**			**0**				**0**	**0**	0
16	16-avr-11	22-avr-11	1.1	5	**1**		**1**			**0**				**0**	**1**	20
17	23-avr-11	29-avr-11	1.1	4			**0**			**0**				**0**	**0**	0
18	30-avr-11	06-mai-11	1.5	6			**0**			**0**				**0**	**0**	0
				980	**108**	**140**		**1**	**6**		**66**	**113**	**15**		**449**	
					248			**7**			**194**					

undet*: subtype and lineage undetermined;* ***HK10:*** *A/Hong Kong/2212/20010 (H1N1),* ***BrisH1:****Influenza A/Brisbane/59/2007 (H1N1) ;* ***Perth09****: Influenza A/Perth/16/2009 (H3N2);*
BHK09: *Influenza B/Hong Kong/514/2009, Victroria-like;* ***Bwiscon*** *: Influenza B/Wisconsin/01/2010, Yamagata-like;*

Annex 2: Inhibition of the hemagglutination titers of pandemic influenza A (H1N1) 2009 viruses

	A/California/7/2009	A/Bayern/69/2009	A/HKong/2212/2010
A/California/7/2009	**512**	256	128
A/Bayern/69/2009	128	**256**	128
A/HKong/2212/2010	512	256	**512**
N° prel			
4172614	>16000	4096	>16000
4194683	>16000		>16000
4201988	>16000		>16000
4204781	>16000		>16000
4297945	>16000		>16000
4146607	> 16000	2048	>16000
4172774	> 16000		>16000
4195436	> 16000		>16000
4124921	8192	2048	>16000
4165667	8192	8192	>16000
4380097	8192		>16000
4194046	>16000		> 16000
4194089	>16000		> 16000
4172446	8192	2048	> 16000
4149348	>8192	2048	>8192
4149766	4096	2048	>8192
4201924	>16000		8192
4224874	8192		8192
4226434	8192		8192
4267082	8192		8192
4288860	8192		8192
4172709	4096	1024	8192
4194656	4096		8192
4373360	4096		8192
4475480	4096		8192
4194152	2048		8192
4328754	8092		8092
4194633	8192		4096
4532372	8192		4096
4204758	4096		4096
4235768	4096		4096
4236164	4096		4096
4267342	4096		4096
4288765	4096		4096
4297928	4096		4096

	A/California/7/2009	A/Bayern/69/2009	A/HKong/2212/2010
A/California/7/2009	**512**	256	128
A/Bayern/69/2009	128	**256**	128
A/HKong/2212/2010	512	256	**512**
N° prel			
4317931	4096		4096
4329162	4096		4096
4404483	4096		4096
4579436	4096		4096
4579455	4096		4096
4616050	4096		4096
4194073	2048		4096
4203654	2048		4096
4204801	2048		4096
4224887	2048		4096
4373593	2048		4096
4380121	2048		4096
4380132	2048		4096
4328800	4036		4036
2548644	2048		2048
4165623	2048	512	2048
4193892	2048		2048
4194141	2048		2048
4194615	2048		2048
4195266	2048		2048
4204876	2048		2048
4225019	2048		2048
4289964	2048		2048
4297862	2048		2048
4328760	2048		2048
4347087	2048		2048
4355259	2048		2048
4477845	2048		2048
4172639	1024	1024	2048
4195363	1024		2048
4201826	1024		2048
4202001	1024		2048
4295988	1024		2048
4346511	1024		2048
4350000	1024		2048
4236312			2048
4149641	1024	128	1024
4172440	1024	512	1024
4172445	1024	512	1024
4172630	1024	256	1024
4258439	1024		1024

	A/California/7/2009	A/Bayern/69/2009	A/HKong/2212/2010
A/California/7/2009	**512**	256	128
A/Bayern/69/2009	128	**256**	128
A/HKong/2212/2010	512	256	**512**
N° prel			
4289803	1024		1024
4194037	1024		1024
4201801	1024		1024
4201939	1024		1024
4224954	1024		1024
4256937	1024		1024
4288688	1024		1024
4296000	1024		1024
4346894	1024		1024
4350057	1024		1024
4350206	1024		1024
4350222	1024		1024
4354554	1024		1024
4355317	1024		1024
4410443	1024		1024
3628824	512		1024
4073222	512	256	1024
4235714	512		1024
4256899	512		1024
4410390	512		1024
4422980	512		1024
4382491	2048		512
4194677	1024		512
4142095	512	256	512
4147066	512	256	512
4194183	512		512
4201894	512		
4201955	512		512
4224894	512		512
4224987	512		512
4256916	512		512
4256939	512		512
4267096	512		512
4267304	512		512
4268085	512		512
4288795	512		512
4289977	512		512
4316089	512		512
4318532	512		512
4318716	512		512
4318779	512		512

	A/California/7/2009	A/Bayern/69/2009	A/HKong/2212/2010
A/California/7/2009	**512**	256	128
A/Bayern/69/2009	128	**256**	128
A/HKong/2212/2010	512	256	**512**
N° prel			
4328742	512		512
4329095	512		512
4382309	512		512
4094817	256	256	512
4147094	256	128	512
4172852	256		512
4256922	256		512
4288995	256		512
4317930	256		512
4328751	256		512
4329364	256		512
4423338	256		512
4579455	256		512
4165739	128	128	512
4204817	64		512
4296045	512		256
4195415	256		256
4201917	256		256
4224878	256		256
4236209	256		256
4296076	256		256
4172873	1024	128	128
3628801	1024		
4288925	128		128

ND: Not done

Annex 3: Inhibition of the hemagglutination titers of influenza B viruses

	Victoria-lineage			Yamagata-lineage		
	B/Brisbane/60/2008	**B/HKong/514/2009**	**B/Paris/1762/2009**	**B/Florida/4/2006**	**B/Bengladesh/3333/2007**	**B/Wisconsin/1/2010**
B/Brisbane/60/2008	**1024**	256	128	<8	<8	<8
B/Hong Kong/514/2009	64	**512**	256	<8	<8	<8
B/Paris/1762/2009	64	256	**256**	<8	8	<8
B/Florida/4/2006	<8	16	<8	**2048**	512	256
B/Bengladesh/3333/2007	<8	16	16	4096	**1024**	512
B/Wisconsin/1/2010	<8	<8	<8	512	128	**128**
N° Sample						
4003826	<16	<16	<16	256	64	32
4297828	<16	<16	<16	256	64	64
4329311	<16	<16	ND	128	ND	64
4354559	<16	<16	ND	ND	128	128
4373554	<16	<16	ND	ND	512	256
4423028	<16	<16	ND	ND	64	64
4423258	<16	<16	ND	ND	64	64
4512312	<16	<16	ND	ND	128	64
4514614	<16	<16	ND	ND	128	128
4537490	<16	<16	ND	ND	32	64
4607252	<16	<16	ND	ND	128	64
4350025	<16	32	ND	ND	128	64
4410377	<16	32	ND	ND	512	256
4112157	<16	16	<16	128	64	32
4328819	<16	16	<16	512	64	64
4312294	256	2048	ND	ND	<16	<16
4226486	128	1024	512	<16	<16	<16
4346753	128	1024	ND	ND	<16	<16
4346958	128	1024	ND	ND	<16	<16
4391972	128	1024	ND	ND	<16	<16
4404530	128	1024	ND	ND	<16	<16
4149710	256	1024	256	< 8	<8	< 8
4195335	256	1024	542	ND	ND	ND
4346794	256	1024	ND	ND	<16	<16
4350125	512	1024	ND	ND	<16	<16
4267326	16	512	128	<16	<16	<16
4312286	64	512	ND	ND	<16	<16
4312330	64	512	256	<16	<16	<16

	Victoria-lineage			Yamagata-lineage		
	B/Brisbane/60/2008	**B/HKong/514/2009**	**B/Paris/1762/2009**	**B/Florida/4/2006**	**B/Bengladesh/3333/2007**	**B/Wisconsin/1/2010**
B/Brisbane/60/2008	**1024**	256	128	<8	<8	<8
B/Hong Kong/514/2009	64	**512**	256	<8	<8	<8
B/Paris/1762/2009	64	256	**256**	<8	8	<8
B/Florida/4/2006	<8	16	<8	**2048**	512	256
B/Bengladesh/3333/2007	<8	16	16	4096	**1024**	512
B/Wisconsin/1/2010	<8	<8	<8	512	128	**128**
N° Sample						
4347017	64	512	ND	ND	<16	<16
4347043	64	512	256	<16	<16	<16
4355127	64	512	ND	ND	<16	<16
4382142	64	512	ND	ND	<16	<16
4404566	64	512	ND	ND	<16	<16
4445083	64	512	ND	ND	<16	<16
4455612	64	512	ND	ND	<16	<16
4571778	64	512	ND	ND	<16	ND
4328761	128	512	256	<16	<16	<16
4350158	128	512	ND	ND	<16	<16
4382175	128	512	ND	ND	<16	<16
4382273	128	512	ND	ND	<16	<16
4391964	128	512	ND	ND	<16	<16
4607267	128	512	ND	ND	<16	<16
4607286	128	512	ND	ND	<16	<16
4201976	256	512	256	< 16	<16	<16
4256959	256	512	256	<16	<16	<16
4532255	512	512	ND	ND	<16	ND
4172846	16	256	ND	< 16	<16	< 16
4235813	32	256	128	ND	ND	ND
4236238	32	256	128	<16	<16	<16
4329353	32	256	ND	<16	ND	<16
4379953	32	256	ND	ND	<16	<16
4410333	32	256	ND	ND	<16	<16
4477890	32	256	ND	ND	<16	<16
4512231	32	256	ND	ND	<16	<16
4146934	64	256	128	<8	< 8	< 8
4172747	64	256	128	< 8	<8	<8
4201882	64	256	128	< 8	< 8	< 8

	Victoria-lineage			Yamagata-lineage		
	B/Brisbane/60/2008	**B/HKong/514/2009**	**B/Paris/1762/2009**	**B/Florida/4/2006**	**B/Bengladesh/3333/2007**	**B/Wisconsin/1/2010**
B/Brisbane/60/2008	**1024**	256	128	<8	<8	<8
B/Hong Kong/514/2009	64	**512**	256	<8	<8	<8
B/Paris/1762/2009	64	256	**256**	<8	8	<8
B/Florida/4/2006	<8	16	<8	**2048**	512	256
B/Bengladesh/3333/2007	<8	16	16	4096	**1024**	512
B/Wisconsin/1/2010	<8	<8	<8	512	128	**128**
N° Sample						
4256903	64	256	128	<16	ND	ND
4296058	64	256	128	<16	<16	<16
4297886	64	256	256	<16	<16	<16
4318663	64	256	128	<16	<16	<16
4318680	64	256	128	<16	<16	<16
4346458	64	256	128	<16	<16	<16
4349919	64	256	128	<16	<16	<16
4373678	64	256	ND	ND	<16	<16
4379977	64	256	ND	ND	<16	<16
4392447	64	256	ND	ND	<16	<16
4404512	64	256	ND	ND	<16	<16
4405309	64	256	ND	ND	<16	<16
4410366	64	256	ND	ND	<16	<16
4410458	64	256	ND	ND	<16	<16
4444318	64	256	ND	ND	<16	<16
4445208	64	256	ND	ND	<16	<16
4455523	64	256	ND	ND	<16	<16
4455596	64	256	ND	ND	<16	<16
4455700	64	256	ND	ND	<16	<16
4473996	64	256	ND	ND	<16	<16
4475455	64	256	ND	ND	<16	<16
4488410	64	256	ND	ND	<16	<16
4488431	64	256	ND	ND	<16	<16
4514217	64	256	ND	ND	<16	<16
4532349	64	256	ND	ND	<16	<16
4537416	64	256	ND	ND	<16	<16
4571823	64	256	ND	ND	<16	ND
4571900	64	256	ND	ND	<16	ND
4571930	64	256	ND	ND	<16	<16

	Victoria-lineage			Yamagata-lineage		
	B/Brisbane/60/2008	**B/HKong/514/2009**	**B/Paris/1762/2009**	**B/Florida/4/2006**	**B/Bengladesh/3333/2007**	**B/Wisconsin/1/2010**
B/Brisbane/60/2008	**1024**	256	128	<8	<8	<8
B/Hong Kong/514/2009	64	**512**	256	<8	<8	<8
B/Paris/1762/2009	64	256	**256**	<8	8	<8
B/Florida/4/2006	<8	16	<8	**2048**	512	256
B/Bengladesh/3333/2007	<8	16	16	4096	**1024**	512
B/Wisconsin/1/2010	<8	<8	<8	512	128	**128**
N° Sample						
4607153	64	256	ND	ND	<16	<16
4267701	128	256	64	<16	<16	<16
4346561	128	256	ND	ND	<16	<16
4391961	128	256	ND	ND	<16	<16
4405259	128	256	ND	ND	<16	<16
4423229	128	256	ND	ND	<16	<16
4442437	128	256	ND	ND	<16	<16
4477875	128	256	ND	ND	<16	<16
4289953	256	256	128	<16	<16	<16
4488486	256	256	ND	ND	<16	<16
4226388	16	128	ND	ND	<16	ND
4288179	16	128	64	<16	<16	<16
4316102	16	128	ND	ND	ND	ND
4444336	16	128	ND	ND	<16	<16
4475405	16	128	ND	ND	<16	<16
4514225	16	128	ND	ND	<16	<16
4532312	16	128	ND	ND	<16	<16
4542927	16	128	ND	ND	<16	<16
4579372	16	128	ND	ND	ND	ND
4236256	32	128	128	<16	<16	<16
4268053	32	128	64	<16	<16	<16
4328782	32	128	64	<16	<16	<16
4355273	32	128	ND	ND	<16	<16
4404328	32	128	ND	ND	<16	<16
4445315	32	128	ND	ND	<16	<16
4226368	64	128	128	<16	<16	<16
4329221	64	128	ND	<16	ND	<16
4329242	64	128	128	<16	<16	<16
4329254	64	128	64	<16	<16	<16

	Victoria-lineage			Yamagata-lineage		
	B/Brisbane/60/2008	**B/HKong/514/2009**	**B/Paris/1762/2009**	**B/Florida/4/2006**	**B/Bengladesh/3333/2007**	**B/Wisconsin/1/2010**
B/Brisbane/60/2008	**1024**	256	128	<8	<8	<8
B/Hong Kong/514/2009	64	**512**	256	<8	<8	<8
B/Paris/1762/2009	64	256	**256**	<8	8	<8
B/Florida/4/2006	<8	16	<8	**2048**	512	256
B/Bengladesh/3333/2007	<8	16	16	4096	**1024**	512
B/Wisconsin/1/2010	<8	<8	<8	512	128	**128**
N° Sample						
4354541	64	128	128	<16	<16	<16
4422917	64	128	ND	ND	<16	<16
4423017	64	128	ND	ND	<16	<16
4512218	64	128	ND	ND	<16	<16
4542852	64	128	ND	ND	<16	<16
4475438	128	128	ND	ND	<16	<16
3822511	<16	128	<16	<16	<16	<16
4550263	64	128	ND	ND	<16	<16
4571836	32	64	ND	ND	<16	ND
4571930	<16	32	ND	ND	<16	ND

ND: Not done

Annex 4: Antiviral test analysis report, National Reference Centre of Influenza Viruses South Region, Virology Laboratory, Lyon, France, 22 March 2011.

Résultats Sensibilibité vis-à-vis de l'oseltamivir et du zanamivir, Test MUNANA[6]

	Nom du virus	Type	Passage	Date isolement	Activité Na	IC50 Oselta	IC50 Zana	mutation
			MDCK-Siat		RFU	Moyenne en nM	Moyenne en nM	
1	**4350000**	H1N1p	P2	07/02/2011	181	0,29	0,41	
2	**4350206**	H1N1p	P2	08/02/2011	12	0,25	0,49	
3	**4328742**	H1N1p	P2	31/01/2011	172	0,34	0,25	**Q313R**
4	**4328800**	H1N1p	P2	02/02/2011	60	0,95	0,61	
5	**4267342**	H1N1p	P3	14/01/2011	78	0,47	0,67	
6	**4235768**	H1N1p	P2	14/01/2011	74	0,54	0,30	**Q313R**
7	**4224954**	H1N1p	P4	12/01/2011	7	0,10	0,56	
8	**4256899**	H1N1p	P3	14/01/2011	154	0,45	0,45	
9	**4201924**	H1N1p	P4	06/01/2011	91	0,29	0,36	**Q313R**
10	**4194615**	H1N1p	P3	29/12/2010	141	0,28	0,41	**Q313R**

Les virus porteur de la mutation Q313R apparaissent tous sensibles aussi bien à l'oseltamivir qu'au zanamivir.

Pour Gubareva et al. un virus porteur des mutations Q313R et I427T possède une IC50 de 6,2 vis-à-vis du zanamivir et de 8,5 vis-à-vis de l'oseltamivir.

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UNIVERSITÉ DE GENÈVE

Geneva, 6 June 2011

Laboratory of Virology

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Department of Genetic and Laboratory Medicine

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Laurent Kaiser, MD

Human infection by a swine influenza virus

On 8 April 2011, a nasopharyngeal specimen was sent by the "SchweineGesundheitsDienst" (SGD), a veterinary institute at Sempach (ZH) to the National Centre of Influenza (NCI). The specimen revealed to be positive for an influenza virus of swine origin. Once confirmed by sequence analysis, the finding and confirmation was transmitted immediately on 14 April 2011 to the Swiss Federal Office of Public Health with a preliminary report.

A. Case description

A 22-year-old male employee working on a farm in the county of Zurich presented acute respiratory symptoms (cold, cough, no other identified clinical information available) 24h before a nasopharyngeal swab was sampled on site on 6 April 2011 by a veterinarian in charge of animal surveillance (SGD-Sempach). Animals from the same farm were reported to be sick and three swine specimens had been detected positive for influenza A virus (subtype and sequence non specified; information transmitted by Monika Engels, VETVIR, email of 12 April 2011). The clinical sample was shipped to the NCI on 8 April 2011 and labeled 4607377.

B. Virologic analysis

1. RT-PCR analyses

The nasopharyngeal specimen was screened for influenza using a panel of specific RT-PCR assays (Table 1). A generic influenza A combination[1] specific to animal and human matrix gene sequences of influenza A viruses was positive, but all other combinations targeting human-specific viruses (influenza A, seasonal H1, H1 2009, and H3)[2] remained negative.

Method	Real-Time PCR					culture
Target	Influenza A MP[1]	Influenza A MP[2]	Pandemic influenza A/H1 2009[2]	Seasonal influenza A/H1[2]	Seasonal influenza A/H3[2]	Virus multiplication in cells
Specificity	Animal/ human	Human	Human	Human	Human	Animal/ human
4607377	**++**	**-**	**-**	**-**	**-**	**+++**

Table 1: RT-PCR assays used to screen nasopharyngeal specimens.

2. Viral culture

Influenza A virus was cultivated on MDCK-SIAT-1 cells (37°C, 5% CO2) and a strong and specific cytopathogen effect could be observed after 96 h (Figure 1a). Immunofluorescence analysis using monoclonal antibodies directed against influenza virus nucleoprotein confirmed the presence of viral antigens in cells (Figure 1b).

a) b)

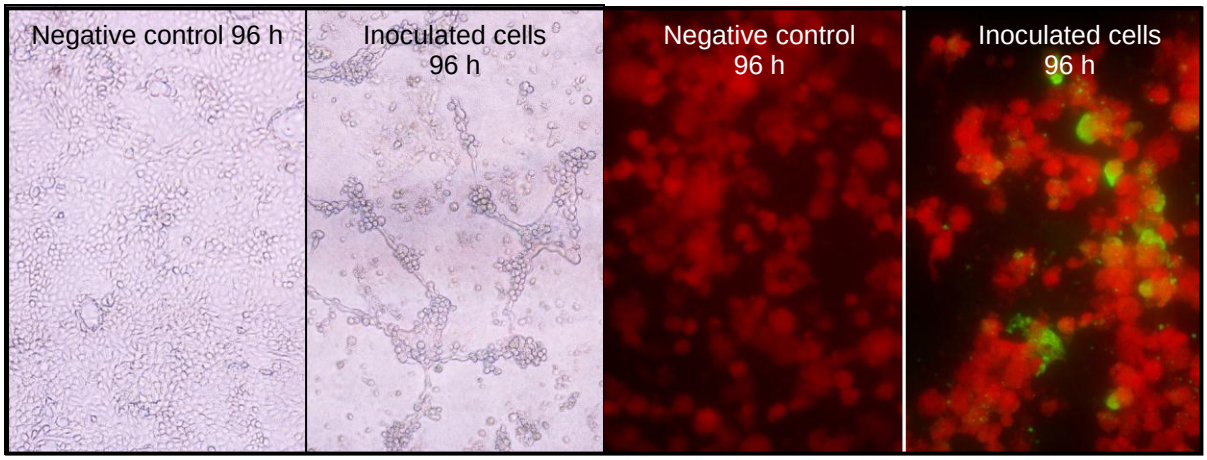

Figure 1: Viral culture of 4607377 sample.

a) Cells inoculated with the index case specimen revealed rapidly a significant cytopathic effect compared to negative controls. b) Indirect, fluorescent, monoclonal antibodies directed against the influenza nucleoprotein confirmed the presence of viral antigen (green colored cells, right panel) compared to non- infected cells (left panel).

3. Sequence analysis

Six of eight genes of the isolated virus were totally or partially sequenced (Table 2). Sequences are available in Annex 1. A method adapted for the complete genome amplification was used as described in a previous report on human infection with swine influenza virus in Switzerland dated 24 November

2010.[3] Due to a lack of sensitivity of this method or for other technical reasons, only partial genome sequences were obtained. Two genome segments (nucleoprotein and NS1/2 sequences) and two internal regions of the PB2 (678 nucleotides) and the PB1 (478 nucleotides) remained unsequenced. The complete HA1 sequence was targeted and completed as it is the most divergent region of influenza genome.

Table 2: Summary of gene sequences obtained for influenza A/Switzerland/4607377/2011 (H1N1) virus.

	Gene fragments sequenced					
A/Switzerland 4607377/2011	PB2	PB1	PA	HA	NA	MP
Length (nts)	1550 (Δ678nt)	1318 (Δ478nt)	1657	850	513	728
Region	11-2239 (Δ760-1438)	18-1814 (Δ738-1216)	495-2151	82-932	616-1129	68-796

Δ: gap in the sequence; nt: nucleotides

3.1. Blast analysis

A blast analysis with publically-available influenza sequences obtained from the NCBI database website were downloaded on the Smartgene® platform and allowed to confirm that the six sequences were of swine origin. We could show also that all six sequences were related to the corresponding genes of the two previous Swiss swine influenza viruses detected in humans in December 2009 and October 2010[4] and reported previously. To illustrate this homology, the HA1 sequence (the most variable genome part of influenza virus) was compared with the recent Swiss strains. The homology rate of 2011 swine strain is of 99.7% with the 2010 strain detected in Aargau county (A/Switzerland/3885165/2010), and 97.5% with the 2009 strain detected in Zürich county (A/Switzerland/2649356/2009, Table 3). The highest homology was observed between the two most recent strains, influenza A/Switzerland/3885165/2010 detected in October 2010, and influenza A/Zürich/4607377/2011 detected in April 2011. Similar to our 2010 report.[4] the present swine virus is closely related to classical European swine avian-like influenza A (H1N1) viruses, which predominate in European swine.

Table 3: Blast analysis (Smartgene® platform) and sequence homology of the HA sequence of A/Switzerland/4607377/2011 (H1N1) influenza virus.

Query sequence - locus HA									
Select	Action	Dataset	Seq. length	Strain name / Strain ID (auto fill)	Antigenic IHA typisation	Host category	Country of collection [iso_3166-1]	Internal NCI remark	s4-HA length
☐	more...	HUG Influenza Private Samples	850	A/human/Zurich/4607377-1/2011(H1N1)		human	CH	old number - Human infection with swine origin inf ...	850

Similar sequences found										
Select	Action	Dataset	Official strain name	s4-HA AC	Length	Seq. length	Identities	Mismatches	Match length	Score
☐	more...	IDNS Influenza References (1)	A/human/SWITZERLAND/3885165-1/2010(H1N1)	CY079544	1723	1723	847 (99.65%)	3	850	1661
☐	more...	IDNS Influenza References (1)	A/human/SWITZERLAND/2649356-1/2009(H1N1)	CY079537	1715	1715	829 (97.53%)	21	850	1518
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/270132/2003 (H1N1)	GQ175968	984	984	826 (97.18%)	24	850	1495
☐	more...	IDNS Influenza References (1)	A/Swine/Spain/53207/2004 (H1N1)	CY010580	1738	1738	825 (97.06%)	25	850	1487
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/2929-6/2005 (H1N1)	GQ175959	983	983	824 (96.94%)	26	850	1479
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/285983-7/2005 (H1N1)	GQ175969	983	983	822 (96.71%)	28	850	1463
☐	more...	IDNS Influenza References (1)	A/Swine/Gent/132/2005 (H1N1)	FJ791277	1114	1114	821 (96.59%)	29	850	1455
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/295855/2004 (H1N1)	GQ175970	983	983	821 (96.59%)	29	850	1455
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/50175/2007 (H1N1)	FJ770258	983	983	821 (96.59%)	29	850	1455
☐	more...	IDNS Influenza References (1)	A/Swine/Italy/219851-3/2005 (H1N1)	GQ175965	998	998	820 (96.47%)	30	850	1451

Identities between nucleotides' composition are given in % and highlighted in the red box

3.2. Phylogenetic analysis of the HA sequence

The HA sequence of the 4607377 sample was compared with other HA sequences publically available in the NCBI database. The HA sequences of previous swine influenza viruses detected in Switzerland have been considered also in the phylogenetic analysis (Figure 3, red strains). A swine influenza A (H1N1) virus detected in humans in Switzerland in 2002 was also introduced in the analysis.[5] The HA sequence of the three influenza strains of swine origin detected between 2009 and 2011 in Switzerland in humans formed a cluster, thus strongly suggesting a common ancestor for these viruses. The 2011 strain appeared to be closer to the 2010 strain than to the 2009 one. These three strains are also closely related to the HA sequence of avian-like swine influenza viruses, which predominate in European pigs (Figure 3, black strains). By contrast, these sequences are distinct from the classical swine virus that predominates in the USA and circulates at a lower rate in Europe (Figure 3, green strains). This phylogenetic analysis confirmed also that HA sequences of swine strains detected in humans are distinct from the human influenza A (H1N1) 2009 strain (blue strains) and also from the seasonal influenza A (H1N1) strains (yellow strains).

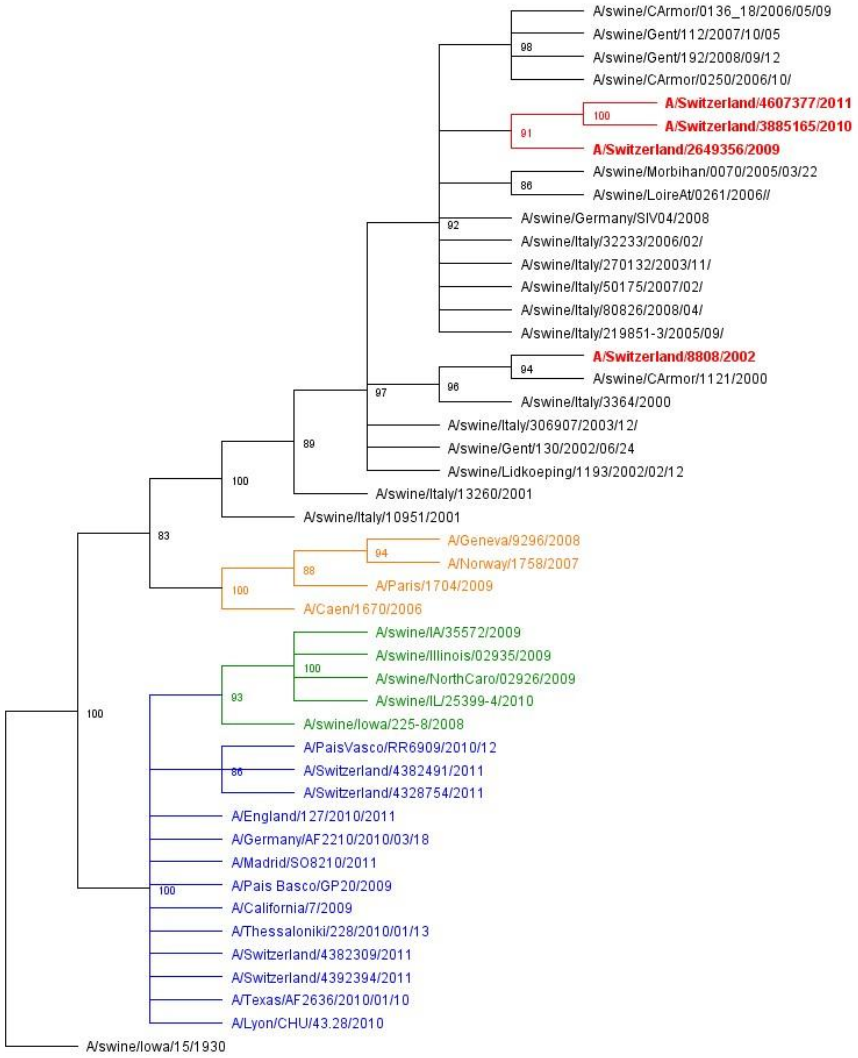

Figure 3: Phylogenetic tree obtained with alignment of swine and human HA gene sequences. Red: influenza viruses of swine origin detected in Switzerland. **Blue**: pandemic influenza A (H1N1) 2009 viruses detected in humans since 2009. **Green**: classical swine influenza A (H1N1) viruses of the USA lineage. **Yellow**: seasonal influenza A (H1N1) viruses detected in humans. **Black**: swine influenza A (H1N1) viruses of the avian lineage, which predominate in Europe.

3.3. Antiviral resistance

The sequence of the three MP genes showed that the mutations V27A and S31N known to confer amantadine resistance[6] were detected in the 4607377 strain, as well as in the two previous swine influenza viruses detected in 2009 and 2010 (2649356 and 3885165, respectively; Figure 4). All these strains are thus amantadine-resistant.

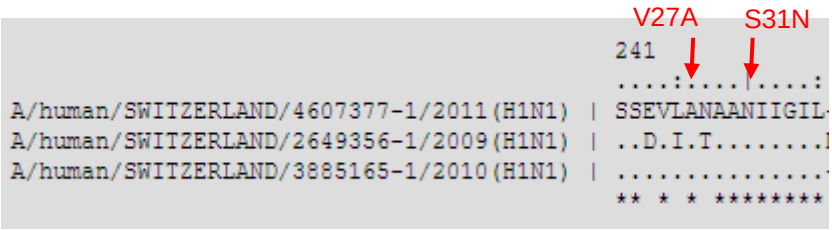

Figure 4: MP sequence alignment of the three recent Swiss swine influenza viruses

Similarly, the NA sequences of the three recent Swiss strains were aligned (Figure 5). Mutations known to confer a resistance to oseltamivir, zanamivir, or peramivir were tested. Specifically, the H275Y mutation was not detected. This mutation is detected in 1% to 3% of pandemic A (H1N1) strains[7] and sporadically in patients on oseltamivir treatment.[8]

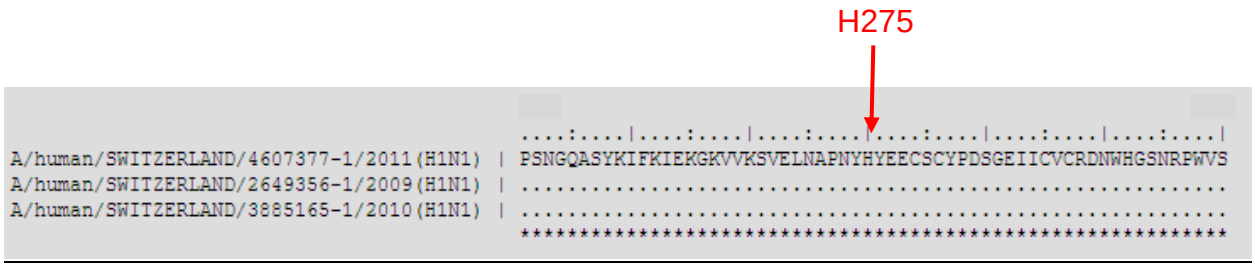

Figure 5**:** NA sequence alignment of swine influenza viruses detected in humans between 2009 and 2011.

Sequences of the six genes of this case are attached to the present report (Annex 1) and will be published in the NCBI database in order to be publically available.

3.4. Polymerase (PB2) sequence

The polymerase PB2 sequence is critical for influenza virus replication and transcription and is usually implicated in the host range restriction in mammalian species.[13,14] Avian-like swine influenza viruses have usually a glutamate (E) at position 627, whereas human influenza viruses have a lysine (K) at the same position. The influenza A/Switzerland/4607377/2011 and the A/Switzerland/3885165/2010 PB2 sequences have a 627E, similar to most European avian-like swine influenza viruses (Figure 4). Another human adaptation could also be provided by a 591K mutation.[15] The two PB2 sequences harbour a 591Q. None of these two mutations conferring a human adaptation has been detected in the 4607377 and 3885165 viruses. The influenza A/Switzerland/2649356/2009 PB2 sequence was not sequenced to determine the presence of the mutation.

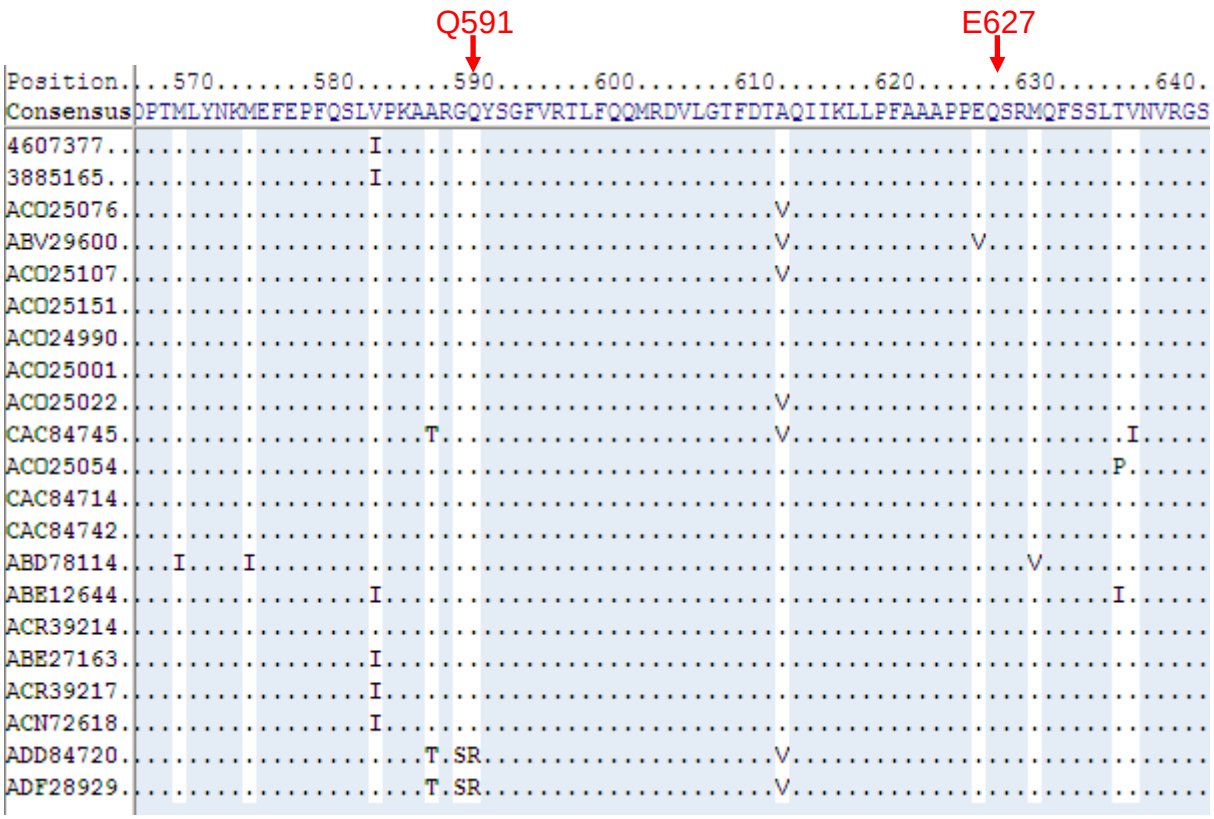

Figure 4: PB2 sequence alignment of 4607377 and 3885165 Swiss strains and European swine influenza A (H1N1) viruses.
The red arrow indicates the Q591 and the E627 position.

C. Conclusion

The influenza strain detected in a Swiss pig farm employee from the county of Zurich was confirmed to be of swine origin. The virus was detected by virus culture, thus confirming that a productive infection occurred. Comparison of the HA1 sequence, the most variable genome part of influenza virus, showed that the strain is an avian-like swine influenza strain, which predominates in European pigs. The strain is also closely related to the swine strains detected in two humans in 2009 and 2010 in Switzerland. Limited evolution of the three strains suggests a common origin and a local circulation of these viruses since 2009. However, due to a lack of swine influenza virus sequences from Switzerland available for our analysis, we could not confirm that these viruses were similar to those circulating in animals. The three Swiss influenza strains are related also to influenza strains detected in Italy, France and Germany, and confirm a common ancestor between animal viruses from Switzerland and those from other European countries. The question as to whether these swine strains circulated in Swiss swine or in humans during these last years remains to be clarified. All three cases had close exposure to pigs and suggest a sporadic animal-to-human transmission. Human-to-human transmission has not been identified at the epidemiological level and no additional testing of potential human contact has been

conducted. None of the two swine influenza viruses 4607377 and 3885165 harbored the previously described mutations in PB2 627K and 591K that confer human adaptation of a swine virus.

From 1958 to 2009, at least 62 human infections by a swine influenza virus were reported from seven countries, of which 13 occurred in one epidemic at Fort Dix military base in the USA.[9,10] In Switzerland, three human infections with swine influenza A (H1N1) viruses were reported in 1986 and 2002.[11,12] In 2010, we confirmed two additional human infections with influenza of swine origin.[4] Together with the present report, three swine influenza viruses have been detected between December 2009 and April 2011. This detection rate confirms sporadic human infections in farmers or farm employees. Of note, compared to the previous years, the number of human samples sent by veterinarians participating in the swine influenza surveillance network has increased over the two last seasons. Since 2008, 25 samples from individuals presenting respiratory symptoms and in close contact with pigs have been sent for influenza analysis. Of these, 17/25 (68%) samples were performed between 2010 and 2011. This surveillance allowed us to detect three cases since December 2009. Human infection by swine influenza virus is expected and certainly underestimated in specific at-risk individuals. Additional information on swine virus sequences would help to clarify potential relevant differences between the A (H1N1) swine viral sequences and the one detected in humans.

Geneva, 6 June 2011

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References

1. CDC protocol of realtime RT-PCR for swine influenza A (H1N1). Geneva: World Health Organization, 2009. http://wwwhoint/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428pdf; accessed 5 April 2011.

2. Thomas Y, Kaiser L. Seasonal and influenza A (H1N1) 2009 virus surveillance in Switzerland Season 2009 – 2010. Berne: Swiss Federal Office of Public Health, 2010:1-41.

3. Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A viruses. Arch Virol. 2001; 146:2275-2289.

4. Thomas Y and Kaiser L. Human infection by swine influenza virus. Berne: Swiss Federal Office of Public Health, 24 November 2010.

5. Gregory V, Bennett M, Thomas Y, Kaiser L, Wunderli W, Matter H, Hay A, Lin YP. Human infection by a swine influenza A (H1N1) virus in Switzerland. Arch Virol. 2003;148:793-802.

6. Klimov AI, Rocha E, Hayden FG, Shult PA, Roumillat LF, Cox NJ. Prolonged shedding of amantadine-resistant influenzae A viruses by immunodeficient patients: detection by polymerase chain reaction-restriction analysis. J.Infect Dis. 1995;172:1352-1355.

7. WHO Update on oseltamivir resistance in influenza A (H1N1) 2009 viruses. Geneva: World Health Organization. 2011. http://www.who.int/csr/disease/influenza/2011_04_08_weekly_web_update_oseltamivir_resistance.pdf; accessed 8 April 2011.

8. Christian R, Boudreault AA, Kuypers J, Lofy KH, Corey L, Boeckh MJ, Englund JA. H275Y mutant pandemic (H1N1) 2009 virus in immunocompromised patients. Emerg Inf Dis. 2011;17:4.

9. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. Clin Inf Dis. 2007;44:1084-1088.

10. Shinde V, Bridges CB, Uyeki TM, Shu B, Balish A, Xu X, Lindstrom S, Gubareva LV, Deyde V, Garten RJ, Harris M, Gerber S, Vagasky S, Smith F, Pascoe N, Martin K, Dufficy D, Ritger K, Conover C, Quinlisk P, Klimov A, Bresee JS, Finelli L. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. N Engl J Med. 2009;360:2616-2625.

11. de Jong JC, Paccaud MF, de Ronde-Verloop FM, Huffels NH, Verwei C, Weijers TF, Bangma PJ, van Kregten E, Kerckhaert JA, Wicki F, et al. Isolation of swine-like influenza A(H1N1) viruses from man in Switzerland and The Netherlands. Ann Inst Pasteur Virol. 1988;139:429-437.

12. Gregory V, Bennett M, Thomas Y, Kaiser L, Wunderli W, Matter H, Hay A, Lin YP. Human infection by a swine influenza A (H1N1) virus in Switzerland. Arch Virol. 2003;148:793-802.

13. Hatta M, Gao P, Halfmann P, Kawaoka Y.Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science. 2001;293:1840-1842.

14. Subbarao EK, London W, Murphy BR. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. J Virol. 1993;67:1761-1764.

15. Mehle A, Doudna JA. Adaptive strategies of the influenza virus polymerase for replication in humans. Proc Natl Acad Sci U S A. 2009;106:21312-21316.

Annex 1: Gene sequences of 4607377 sample.

>A/Switzerland/4607377/2011 (H1N1), PB2

ATGTCGCAGTCTCGCACTCGCGAGATACTGACAAAAACCACTGTGGACCATATGGCAATAATAAA
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>A/Switzerland/4607377/2011 (H1N1), PB1

TCTACTTTTCTTAAAAGTGCCAGCACAAAATGCTATAAGCACCACATTCCCCTACACTGGAGATCC
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GGATGnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
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>A/Switzerland/4607377/2011 (H1N1), PA

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