FEDERAL OFFICE OF PUBLIC HEALTH
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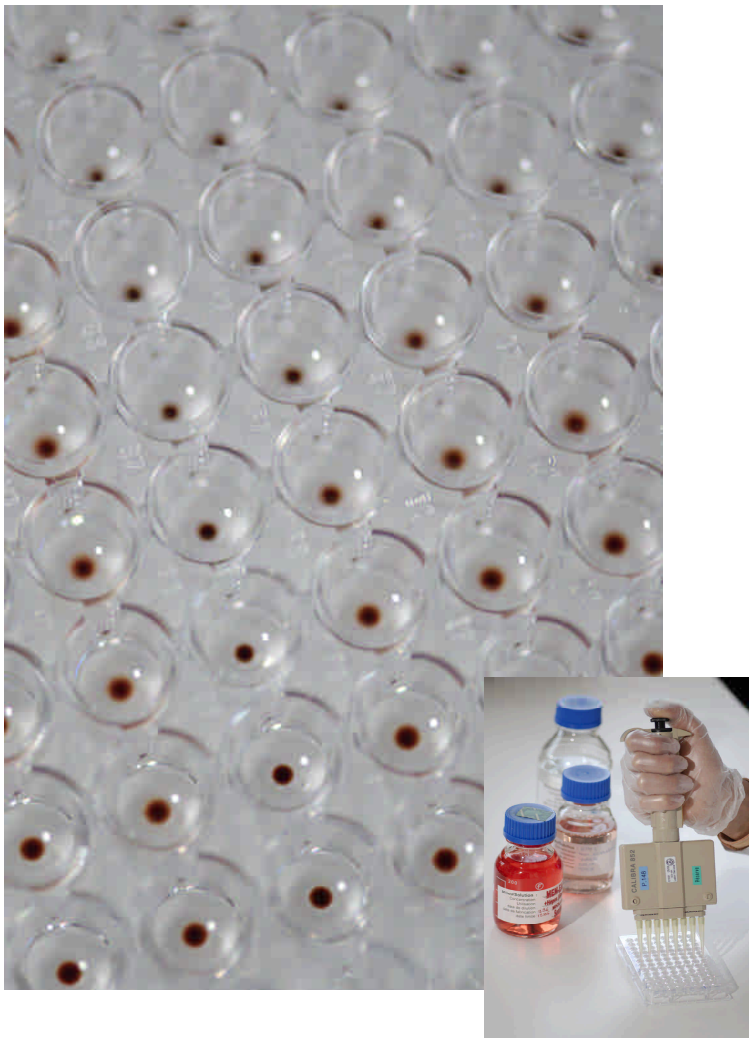
HUG
Hôpitaux Universitaires de Genève

Influenza surveillance in Switzerland
Sentinella study

Winter Season 2005 – 2006



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1. ACKNOWLEDGEMENTS

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2. RESUME-SUMMARY- ZUSAMMENFASSUNG

2.1. Résumé

La surveillance de la grippe par le réseau Sentinelle a permis de mettre en évidence une saison modérée cette année. Une grande diversité de virus a circulé et les personnes de moins de trente ans ont été plus fortement touchées. L'épidémie grippale a débuté tardivement avec une augmentation significative du nombre de virus détectés à partir de la semaine 1 en 2006, pour atteindre un maximum au cours des semaines 8 et 12. Les virus influenza B ont été prédominants cette année: 80 % ont été du type B et 20 % du type influenza A. Parmi ces virus influenza A, 57% étaient des virus influenza A (H3N2) et 30% étaient des virus influenza A (H1N1). Les virus influenza B se distribuaient en 2 populations antigéniquement différentes. 68% étaient proches des souches influenza B/Malaysia/2506/2004 et B/Shandong/7/97, toutes deux appartenant à la lignée influenza B/Victoria/2/87 et 18% étaient proches des souches influenza B/Jiangsu/10/2003 et B/Shanghai/361/2002 de la lignée influenza B/Yamagata/16/88. La majorité des souches influenza A (H3N2) étaient antigéniquement proches de la souche vaccinale influenza A/California/7/04 (H3N2) et de la souche influenza A/Singapore/37/2004 très proche ce la première. Aucune souche voisine de la souche influenza A/Wisconsin/67/2005 (H3N2) n'a été détectée sur le territoire suisse cette année. Enfin, la grande majorité des souches influenza A (H1N1) étaient antigéniquement proches de la souche vaccinale influenza A/New Caledonia/20/99.

Une étude comparative a démontré que la technique de détection du virus influenza par RT-PCR est plus sensible et plus rapide que la culture. La RT-PCR sera adoptée comme technique principale de détection des virus influenza dans la surveillance Sentinelle pour la saison 2006-07.

Enfin, aucun cas d'infection humaine par le virus influenza A (H5N1) n'a été détecté en Suisse. En revanche, ce virus a été trouvé sur 41 oiseaux sur notre territoire. Le séquençage de l'hemagglutinine a permis de montrer que ces virus provenait du lac de Qinghai en Chine, démontrant ainsi le rôle des oiseaux sauvages dans la propagation du virus. Il a également mis en évidence un virus influenza A (H5N1)

variant qui présente des différences génétiques silencieuses sur la séquence de l'hemagglutinine. Ce variant a également été détecté en Allemagne et en France.

2.2. Summary

Influenza surveillance by the Sentinella network this year showed an epidemic of moderate intensity. A wide diversity of viruses circulated with persons under 30 years of age being the most affected population. The influenza epidemic was of late onset with a significant increase in the number of viruses detected from week 1 in 2006 and peaked during weeks 8 and 12. The predominant viruses this year were of type B: 80%, type B; 20%, type A. Among the influenza A viruses, 57% were of type A (H3N2) and 30% of type A (H1N1). The influenza B viruses detected were distributed in two antigenically different groups. 68% were close to the influenza strains B/Malaysia/2506/2004 and B/Shandong/7/95, both of which belong to the lineage influenza B/Victoria/2/87, and 18% were close to the influenza strains B/Jiangsu/10/2003 and B/Shanghai/361/2002 of the lineage influenza B/Yamagata/16/88. The majority of influenza A strains (H3N2) were antigenically close to the vaccine strain influenza A/California/7/04 (H3N2) and to the influenza strain A/Singapore/37/2004, very close to the former. No strain closely related to the influenza strain A/Wisconsin/67/2005 (H3N2) was detected in Switzerland this year. Finally, the overwhelming majority of influenza A (H1N1) strains were antigenically close to the vaccine strain influenza A/New Caledonia/20/99.

A comparative study showed that the use of RT-PCR assay for the detection of the influenza virus is more sensitive and rapid than by culture. For the season 2006-2007, RT-PCR will be used as the principal method for the detection of influenza viruses by the Sentinella surveillance network.

No case of infection in humans by influenza A virus (H5N1) was detected in Switzerland. However, it was found in 41 wild birds across the country. Partial sequencing of the Hemagglutinin allowed to show that these viruses came from Lake Qinghai in China, thus demonstrating the role of wild birds in the spread of the virus. It also highlighted an influenza A virus (H5N1) variant which was characterized by silent genetic differences on the hemagglutinin sequence. This variant has been detected also in France and Germany. As mentioned, Switzerland has not been spared with 41

influenza A/H5 viruses detected in dead wild birds; of these, nine influenza A viruses (H5N1) were confirmed. Three of these influenza A viruses (H5N1), detected by the Zurich veterinary services, have been analyzed by the National Influenza Centre (NIC) and the results are presented in this report. Finally, eight samples from patients with a suspicion of infection by the avian influenza A virus (H5N1) were tested at the NIC : no virus genome was detected in all these human samples.

2.3. Zusammenfassung

In der vergangenen Saison wurde durch die Grippe Überwachung des Sentinella Systems eine mässig intensive Epidemie beobachtet. Eine beträchtliche Anzahl verschiedener Virustypen zirkulierten bei den unter 30 jährigen Patienten. Diese Altersgruppe war von der Epidemie besonders betroffen.
Die Grippe Epidemie begann spät mit einem markanten Anstieg der nachgewiesenen Viren in der 1. Kalenderwoche 2006. Zwei Maxima wurden in den Wochen 8 und 12 registriert. Dieses Jahr dominierten die Influenza B Viren mit einem Anteil von 80%. Der Anteil der Influenza A machte nur 20% aus. Von den Influenza A Viren waren 57% Influenza A (H3N2) und 30% vom Typ (H1N1).
Die Influenza B Viren verteilten sich auf zwei unterschiedliche Gruppen. 68% waren verwandt mit Influenza B Malaysia/2506/2004 und mit B/Shandong/7/97. Diese zwei Vertreter gehören zum Zweig von Influenza B/Victoria/2/87. 18% der B Viren waren verwandt mit Influenza B/Jiangsu/10/2003 und B/Shanghai/361/2002. Diese Vertreter gehören zur Linie der Influenza B/Yamagata/16/88.
Bezüglich der Influenza A (H3N2) Viren war die Mehrzahl mit dem im Impfstoff enthaltenen Influenza A Kalifornien/7/04 (H3N2) und mit Influenza A/Singapore/37/2004 verwandt. Keine Influenza A/Wisconsin/67/2005 (H3N2) Viren konnten gefunden werden. Dieser Stamm wurde vor allem in den USA nachgewiesen. Influenza A (H1N1) war mehrheitlich mit Influenza A/Neu Kaledonien/20/99 verwandt.
In einem Vergleichsexperiment konnte gezeigt werden, dass die RT-PCR empfindlicher und schneller als die Zellkultur ist. Aufgrund der guten Resultate wird diese Technik für die Saison 2006/07 für die Grippeüberwachung als Methode eingesetzt.

Bis jetzt konnte kein einziger humaner Fall in der Schweiz nachgewiesen werden welcher mit Influenza A (H5N1) infiziert worden war. Durch das Überwachungssystem der Veterinäre hingegen konnten 41 Vögel in der Schweiz gefunden werden, welche positiv waren für Influenza A/H5. Die Sequenzierung des Hämagglutinins in unserem Labor von drei Proben ergab, dass das Virus verwandt ist mit denjenigen welche in Tieren im Qinghai See in China nachgewiesen worden waren. Dies ist ein Hinweis, dass unter anderem auch die Zugvögel bei der Ausbreitung eine Rolle spielen können. Durch die Sequenzierung konnte eine Variante identifiziert werden welche auch in Frankreich und Deutschland auftrat.

3. INTRODUCTION

For the 20th consecutive year, influenza surveillance in Switzerland has been carried out by the Sentinella network. This surveillance has allowed to highlight the particularities of any given influenza season. This year, the epidemic began and ended especially late. It was of moderate intensity with a wide diversity of viruses observed throughout the country as reported in other European states. During this season, younger patients (less than 30 years) were more affected than in previous years. This report contains the details of these surveillance results.

Two methods of analysis were used in parallel this year for influenza surveillance: the classic method of culture coupled with immunofluorescence used by the National Influenza Centre (NIC) and real-time PCR. The latter diagnostic technique is now recognized as being one of the most rapid, most sensitive and specific. A comparative study of both techniques is presented in this report.

The season was also punctuated by the spread of the avian influenza epidemic to new continents which had remained untouched until then by the influenza A virus (H5N1). Avian influenza epidemics were reported in Asia, Russia, Africa and Europe and from summer 2005 until June 2006. Very recently, in early July 2006, the virus was detected in a wild bird in Spain. Switzerland has not been spared with 41 influenza A/H5 virus cases detected in dead wild birds; of these, nine were confirmed as influenza A (H5N1). Three of the influenza A (H5N1) viruses detected by the Zurich veterinary services have been analyzed by the NIC and the results are presented in this report. Finally, eight samples from patients with a suspicion of infection by influenza A (H5N1) virus of avian origin have been tested at the NIC.

4. METHOD OF DETECTION FOR RESPIRATORY VIRUSES

4.1. Clinical identification of influenza cases

Clinical identification is based on the network of practitioners in the community who record the total number of flu syndromes observed compared with the total number of consultations carried out during their daily consultations. The case definition used for a flu syndrome is the presence of fever greater than 38° with or without an impression of sickness, myalgia, or a change of general state. In addition to fever, an acute respiratory symptom such as cough or rhinorhea must be present. Among the cases which meet the clinical criteria definitions, a sub-group of 65 practitioners selected each week up to two cases in which a nasopharyngeal or pharyngeal swab was taken for despatch to the reference laboratory. These samples are placed in viral transport medium and shipped to the centre the same day. The geographical distribution of the subgroup of practitioners who took swabs is shown in Figure 1.

Figure 1: Geographical distribution of the 65 participants in the Sentinella network who carried out sampling. Each participant is represented by a white dot. The country is divided into six regions comprising a different number of cantons and each region is distinguished by a number and colour.

4.2. Detection of respiratory viruses

Detection of respiratory viruses was carried out according to two different methods this year (Figure 2). Clinical samples sent by Sentinella practitioners were split into two parts. One was tested by an RT-PCR assay and the other was used to inoculate two different cell lines (MDCK and LLC-MK2) at two different temperatures (37°C and 33°C). The RT-PCR used can detect and differentiate between influenza A and B viruses (Van Elden, 2001). For influenza A viruses, a second RT-PCR must be added to detect the nature of the neuraminidase N1 of influenza A (H1N1) viruses and N2 for influenza A (H3N2) and A (H1N2) viruses (Schweiger et al, 2000).
After seven days of incubation, cultures were screened by the use of monoclonal antibodies for the presence of influenza A and B viruses. In the case of a positive result, further analyses were conducted to define the antigenic properties of the virus strain (see chapter 4.3.).

4.3. Characterization of influenza viruses

If an influenza virus was detected on cell culture, the virus is characterized by a hemagglutination inhibition assay with type specific antisera from immunised ferrets. The results will be interpreted according to an antigenic table adapted and established at the beginning of every season. The aim is to use antisera of strains which have a high probability to be circulating in the human population during the season under study. The choice of these antisera is essential in order to be able to differentiate the sub-types circulating and their antigenic relation with the strains included in the vaccine.
The titers obtained with each strain are identified and compared with standard antisera and allow a precise identification of its antigenic characteristics. The criteria established for the 2005-2006 season are described in Table 1. As mentioned in the legend, the homologous titers allow a comparison with the titers obtained with the circulating influenza strain and to define its antigenic relation to the standard strains. This technique permits the detection of the antigenic variations present on the

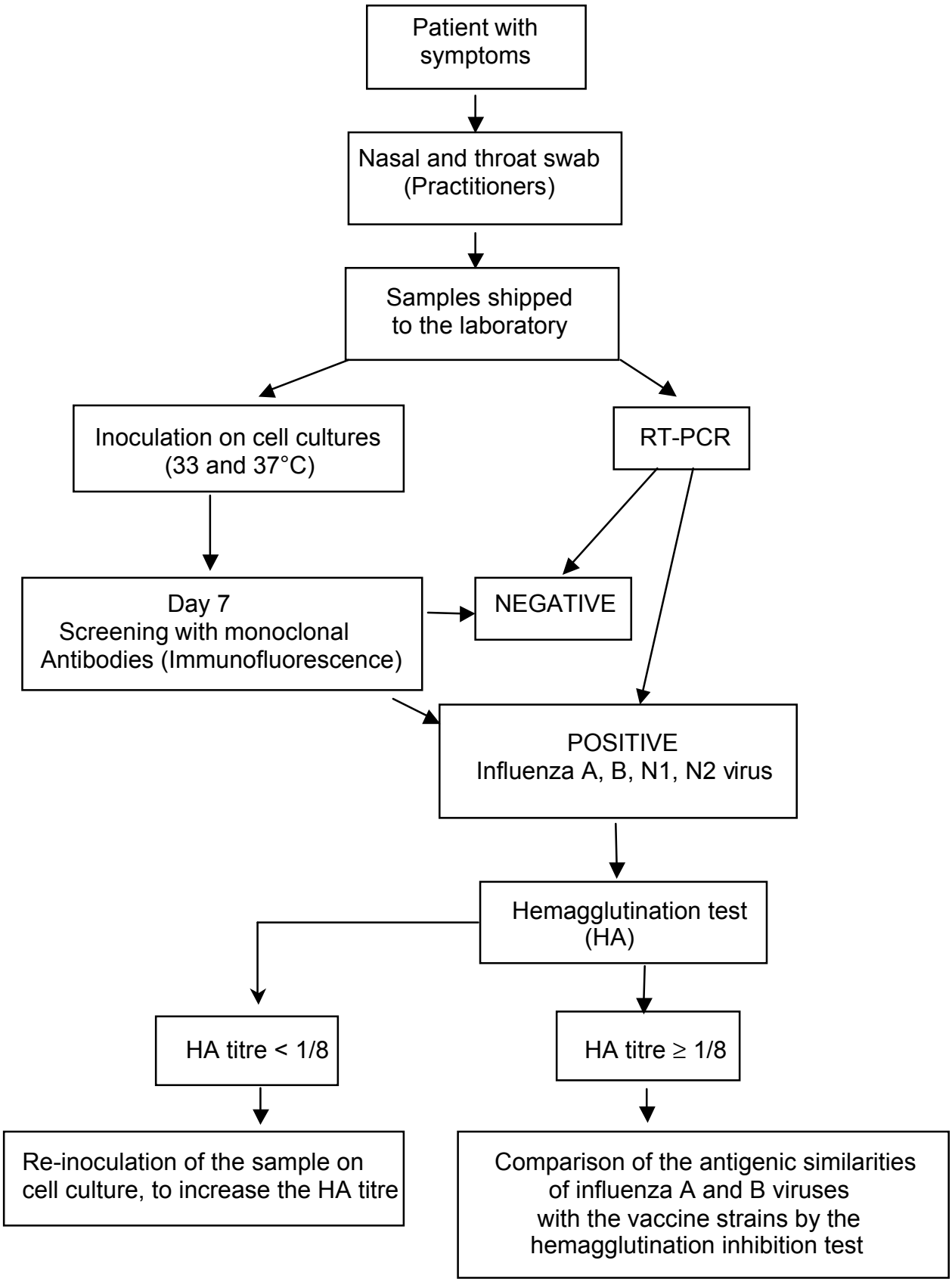


Figure 2: Procedure used for the detection of respiratory viruses by cell culture

Hemagglutinin (HA) which is an essential target of the immune response. However, this technique does not permit to detect antigenic variations related to the neuraminidase (NA). Thus it is not possible to distinguish viruses with the same HA but with a different neuraminidase (e.g., H1N1 and H1N2 viruses). This is important because this type of variant virus circulated in Switzerland during recent years. This demonstrates the importance of RT-PCR for influenza surveillance. In our laboratory we can identify influenza A and B viruses and discriminate between type N1 and N2 (van Elden, 2001, Schweiger et al, 2000) by the RT-PCR technique. A comparative study between RT-PCR and cell culture was made during this season. Results are presented in this report (chapter 5.4).

Table 1: Hemagglutination inhibition (IHA) titers of reference influenza strains incubated with the reference antisera. The IHA titer obtained after incubation of a given strain with its corresponding antiserum is shown in bold type. This value is called the homologous titer (HT). The titer obtained with a strain isolated from a Sentinella sample (Sen) is then compared with the HT titer. If the ratio Sen/HT is low, the strain is considered as antigenically related to the reference strain. If the ratio is high, the strain is considered as antigenically different from the reference strain. The two main lineages of the influenza B viruses are shown in blue: Yam: Yamagata-like ; Vict: Victoria-like.

INFLUENZA B

SAISON 2005/06

		ANTISERA DE REFERENCE					
		B/HK/335/01 Furet	B/Shand/7/97 Furet	B/Brisb/32/02 Furet	B/Jiang./10/03 Furet	B/Shang./361/02 Furet	B/Malaysia/ 2506/04 Furet
SOUCHES	B/Hong Kong/335/2001	**320**	5120	2560	< 80	< 80	640
	St. OMS (Hom./Hét.)	*1*	*0.06*	*0.1*	-	-	*0.5*
	B/Shandong/7/97	320	**5120**	2560	< 80	< 80	640
	St. OMS (Hom./Hét.)	*16*	*1*	*2*	-	-	*8*
	B/Brisbane/32/02	160	5120	**2560**	< 80	< 80	320
	St. OMS (Hom./Hét.)	*16*	*0.5*	*1*	-	-	*8*
	B/Jiangsu/10/2003	< 80	< 80	< 80	**2560**	320	< 80
	St. OMS (Hom./Hét.)	-	-	-	*1*	*8*	-
	B/Shanghai/361/2002	< 80	< 80	< 80	640	**5120**	< 80
	St. OMS (Hom./Hét.)	-	-	-	*8*	*1*	-
	B/Malaysia/2506/2004	160	2560	1280	< 80	< 80	**1280**
	St. OMS (Hom./Hét.)	*8*	*0.5*	*1*	-	-	*1*

INFLUENZA A(H1N1)

SAISON 2005/06

		ANTISERA DE REFERENCE				
		A/N-Caledonia/ 20/99 Furet	A/Beij./262/95 Furet	A/Neth./128/04 Furet	A/HK/2637/04 Furet	A/Egypt/96/02 Furet
SOUCHES	A/N-Caledonia/20/99	**10240**	160	1280	2560	2560
	LCT (Hom./Hét.)	*1*	*64*	8	*4*	*4*
	A/Beijing/262/95	10240	**5120**	2560	1280	640
	St. OMS (Hom./Hét.)	*0.5*	*1*	*2*	*4*	*8*
	A/Netherland/ 128/2004	10240	640	**20480**	10240	1280
	St. OMS (Hom./Hét.)	*2*	*32*	*1*	*2*	*16*
	A/Hong Kong/2637/2004	10240	< 80	1280	**1280**	1280
	St. OMS (Hom./Hét.)	*0.1*	-	*1*	*1*	*1*
	A/Egypt/96/02 (H1N2)	5120	320	1280	640	**1280**
	St. OMS (Hom./Hét.)	*0.25*	*4*	*1*	*2*	*1*

INFLUENZA A(H3N2)

SAISON 2005/06

		ANTISERA DE REFERENCE					
		A/Calif./7/04 Furet	A/Shant/1219/04 Furet	A/NY/55/04 Furet	A/Wyo/3/03 Furet	A/Sing/37/04 Furet	A/Wiscon/67/05 Furet
SOUCHES	A/California/7/04	**5120**	10240	5120	10240	2560	1280
	St. OMS (Hom./Hét.)	*1*	*0.5*	*1*	*0.5*	*2*	
	A/Shantou/1219/04	640	**5120**	640	640	10240	640
	St. OMS (Hom./Hét.)	8	*1*	8	8	*0.5*	
	A/New York/55/2004	20480	20480	**2048**	10240	5120	1280
	St. OMS (Hom./Hét.)	*1*	*1*	*1*	*2*	*4*	
	A/Wyoming/3/2003	5120	2560	5120	**40960**	2560	1280
	St. OMS (Hom./Hét.)	8	*16*	8	*1*	*16*	*32*
	A/Singapore/37/2004	5120	5120	10240	320	**2560**	1280
	St. OMS (Hom./Hét.)	*0.5*	*0.5*	*0.25*	8	*1*	
	A/Wisconsin/67/2005	1280	2560	1280	640	2560	**20480**
	St. OMS (Hom./Hét.)	*16*	8	*16*	*32*	8	*1*

4.4. Identification of influenza A (H5N1) virus

Since 1997, epizootics of avian influenza A (H5N1) virus are regularly observed in humans in South-East Asia (WER 26, 2006). This year, cases of influenza A (H5N1) virus infection in birds have been reported outside Asia. The majority of cases concern wild birds in Russia, some Middle-Eastern countries, but also in Africa and Europe. A summary of this situation will be given in the results section of this report.

It is important to be able to detect the presence of a virus of avian origin in human samples. The selected detection method must be as rapid, specific and sensitive as possible. For this reason, we have developed a Real-Time RT-PCR for the detection of the H5 and N1 genes of the influenza A virus (H5N1).

On the basis of the alignment of the H5 gene sequences of avian and human genomes of the influenza A virus (H5N1), primers and a probe have been selected (Figure 3).

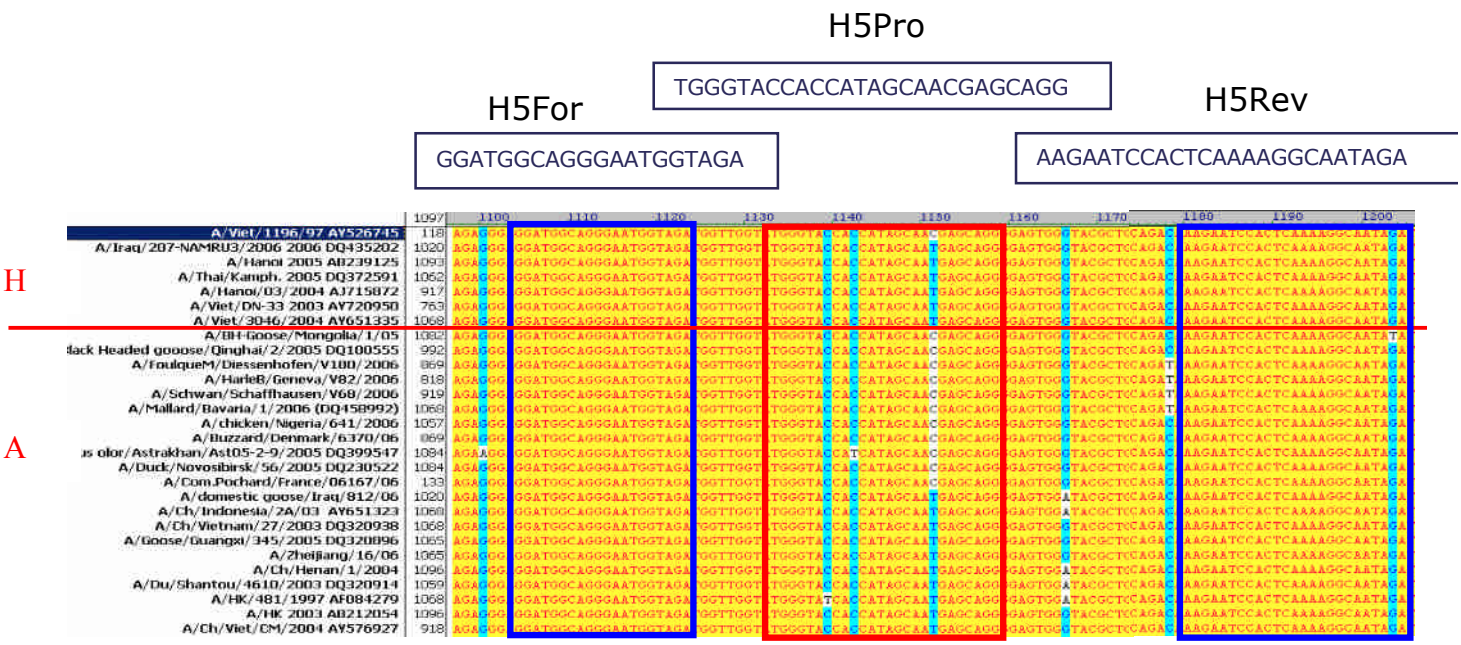


Figure 3: Probe and primers specific for the H5 gene. The regions recognized by the primers are shown in blue. The region recognized by the central, fluorescent probe is shown in red. The consensus sequences are shown in the frames. The sequence of the Reverse H5Rev primer is complementary to the sequence shown in the frame. The virus sequences detected in humans and animals have been identified by "H" and "A", respectively.

To confirm the presence of the H5N1 virus in a sample, we have developed also a PCR capable of detecting the genes coding for the N1 neuraminidase of the influence A virus (H5N1). The primers and the specific probe for N1 have been taken from the article by Payungporn et al (Figure 4).

In a situation such as we are now facing, it is important to develop international contacts to collect information on the epidemics and the virology of the viruses circulating in the infected areas. Given that the influenza virus is evolving constantly, a frequent verification of the validity of the reagents used is necessary. Knowledge of

the sequences as well as the availability of samples containing recently detected viruses is also essential.

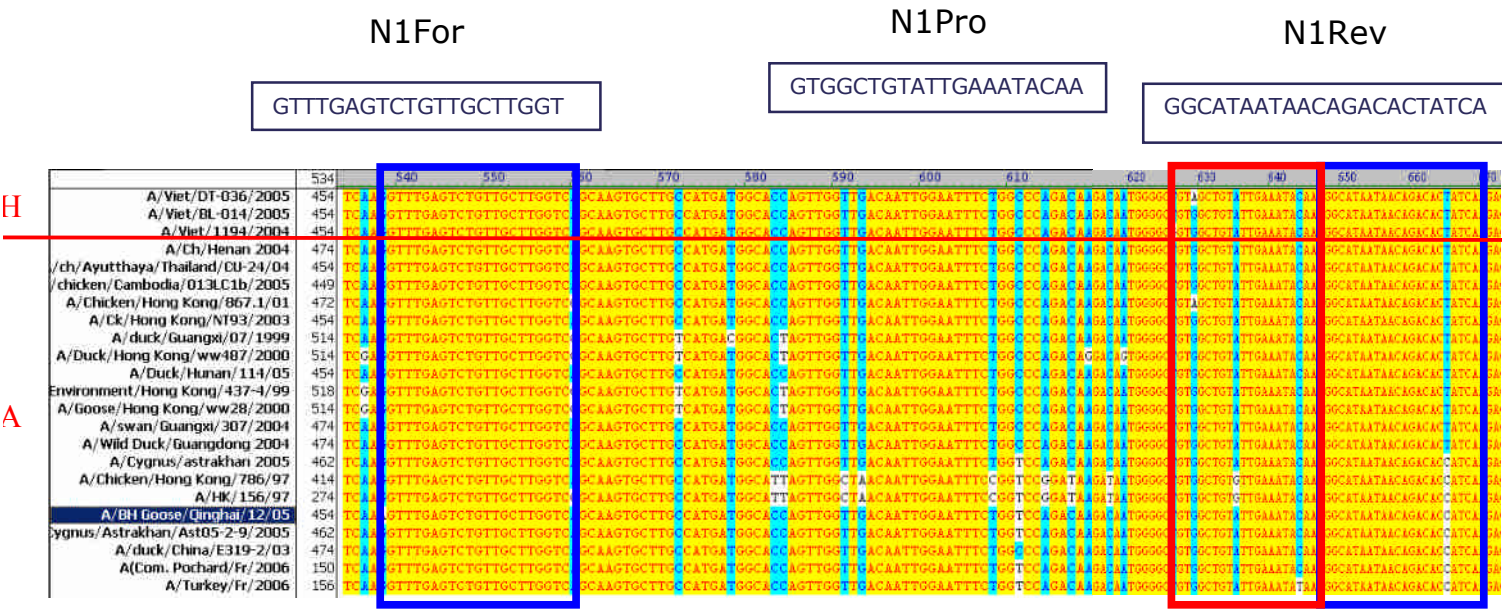


Figure 4: Probe and primers specific for the N1 gene. The regions recognized by the primers are shown in blue. The region recognized by the central, fluorescent probe is shown in red. The consensus sequences are shown in the frames. The sequence of the Reverse N1Rev primer is complementary to the sequence shown in the frame. The virus sequences detected in humans and animals have been identified by “H” and “A”, respectively.

5. RESULTS

5.1. Detection of influenza virus

Surveillance began on 24 September 2005 and end on 21 April 2006 after a period of 21 weeks. 745 samples were obtained from 65 Sentinella practitioners and a few samples were sent in by six hospital laboratories. Of these, 238 influenza viruses were detected, representing a mean positive rate of 31.9% for the whole season (Figure 5a). This percentage varied over the season and two peaks of 70% and 61% occurring during weeks 8 and 13, respectively, were observed in the kinetics of this percentage.

Influenza B viruses were predominant this year; of a total of 238 influenza viruses detected, 191 (80%) influenza B viruses were identified (Figure 5a). Only 47 (20%) influenza A viruses were detected. Among type A viruses, 27 (57%) were influenza A (H3N2) viruses and 14 (30%) were influenza A (H1N1) (Figure 5b). Six (13%) influenza A viruses could not be subtyped and this point will be discussed in more detail in paragraph 5. The details of the influenza virus strains detected are shown in annex 2.

The first case of influenza was detected during week 42 (15-21 October 2005) and identified as an influenza A (H1N1) virus. During weeks 44 and 45, two influenza B viruses were then detected. Between this period and the end of the year, no influenza virus was detected. The first positive case in 2006 was an influenza B virus during week 1 (Figure 6). From then on, the number of viruses continued to increase to a maximum of 35 detected during week 8 (Figure 6). The observed incidence was 70% of positive samples received by the laboratory. A decrease of cases was then observed followed by a second increase to a rate of 31 viruses detected during week 12 (incidence, 53%). The number of samples as well as the number of viruses detected began to decrease with only one virus detected during week 16 (Figure 6).

The influenza B viruses, the majority detected this year, circulated throughout the season. The detection kinetics followed the number of samples received over the weeks. Two peaks for the detection of influenza B can be observed; one during week 8 and the other during week 12 (Figure 6). The influenza A viruses circulated in a much weaker proportion. Two small peaks can be distinguished but much later than those observed for influenza B virus. One detection peak appeared during week 11 with eight viruses, and a second peak can be observed at the end of the season during week 15 with six viruses (Figure 6). Influenza A (H1N1) and influenza A (H3N2) viruses co-circulated throughout the season (Figure 7). A majority of influenza A (H3N2) viruses was observed until week 12. From then on, the influenza A (H1N1) viruses circulated in the majority, thus explaining the second peak observed (Figure 6).

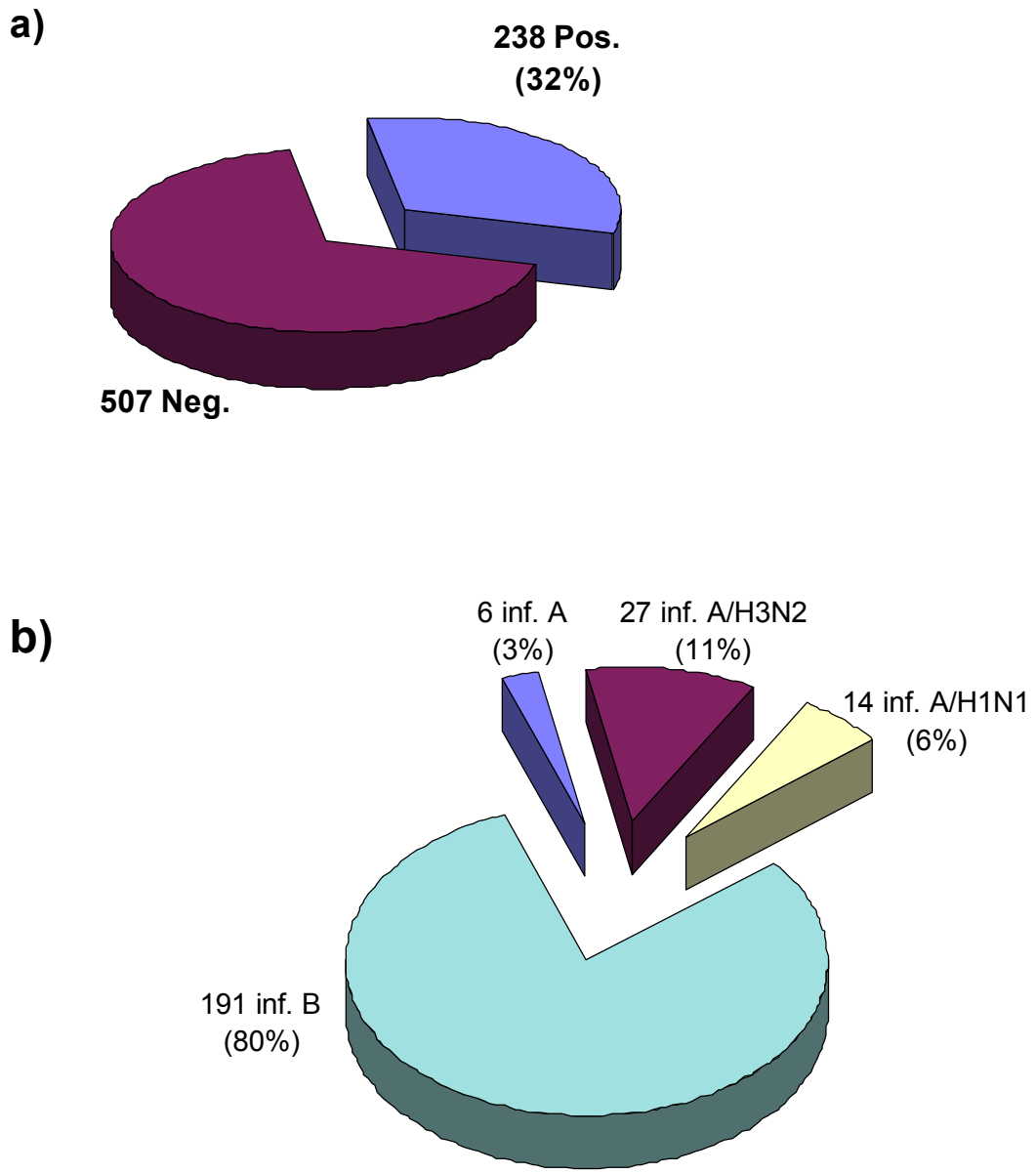


Figure 5: Number and percentage of nasopharyngeal samples positive for influenza virus during during the 2005/06 season (n = 745). **a)** Number of positive and negative samples received during the season. **b)** Number and percentage of different types and subtypes of influenza viruses detected during the season (Inf. = influenza viruses)

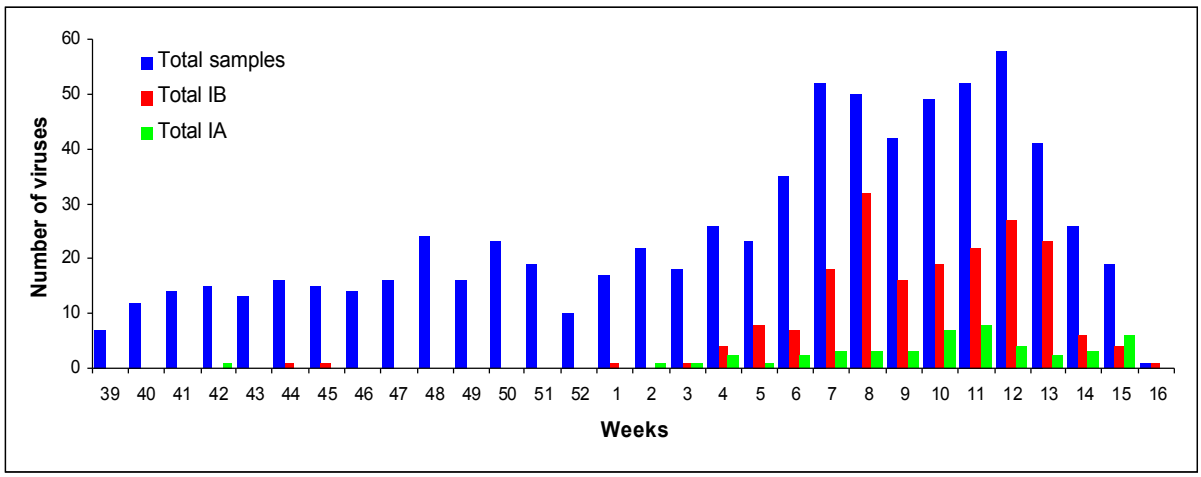


Figure 6: Number of samples received and influenza viruses detected per week.
IA: influenza A virus; IB: influenza B virus.

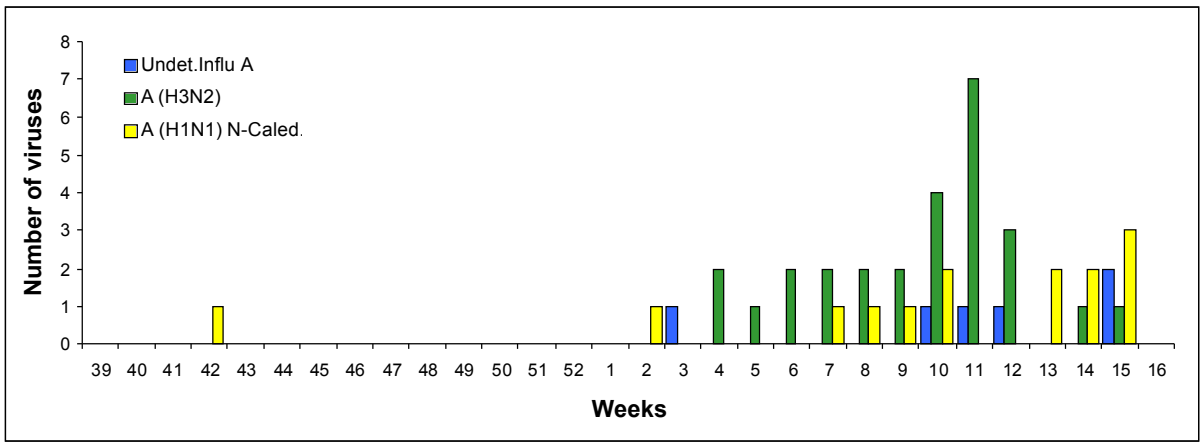


Figure 7: influenza A viruses detected and distribution of subtypes per week. A (H3N2): influenza A (H3N2) virus, A (H1N1): influenza A (H1N1) virus, undetermined influ. A: influenza A viruses unable not be subtyped.

5.2. Antigenic characterisation of influenza viruses detected

5.2.1. Influenza B

Influenza B viruses were mainly detected (191 [80%]). Subtyping analysis was carried out by inhibition of the hemagglutination. The influenza B viruses were distributed into two antigenically different populations: 129 were close to the lineage B/Victoria/2/87 (68%) and 35 were close to the lineage B/Yamagata/16/88 (18%)

(Figure 8 and Annex 2). Twenty-seven (14%) influenza B viruses could not be typed as they either did not grow or insufficiently grew on cell culture (Annex 2).

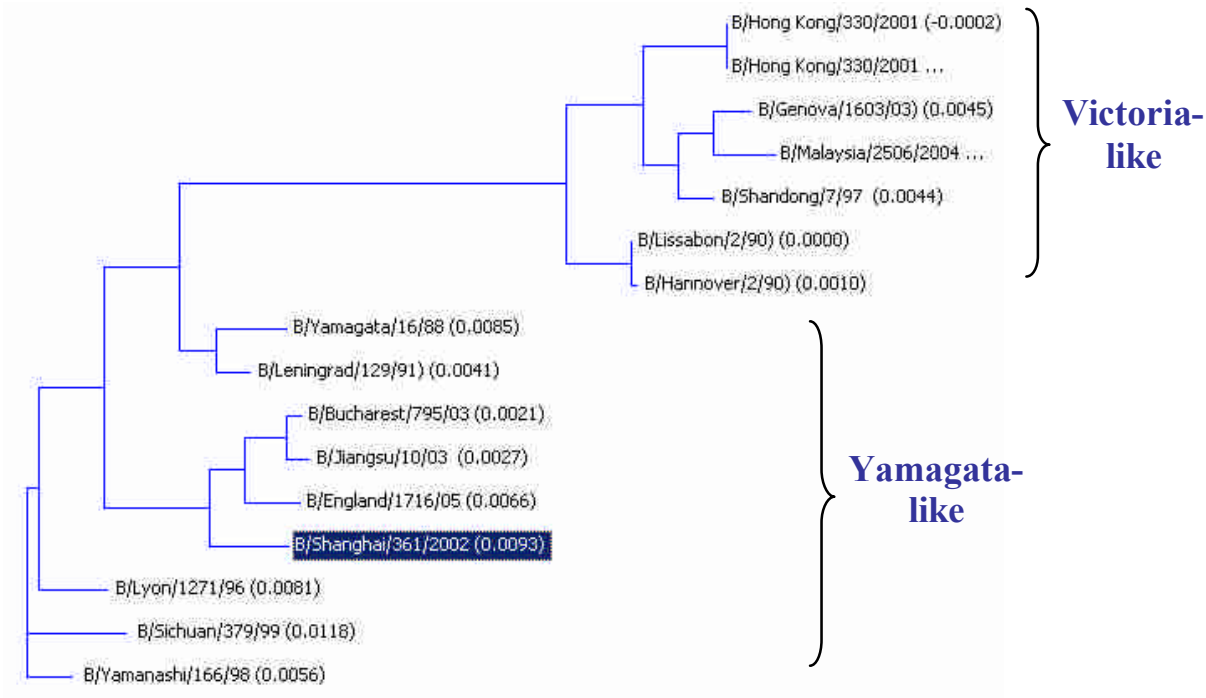


Figure 8: Phylogenetic comparison of nucleotide sequences encoding for the hemagglutinin of influenza B virus. 2005-06 vaccine strain is underlined in blue.

Among the strains of the lineage B/Victoria/2/87, 106 were close to the influenza strain B/Malaysia/2506/2004 and 23 to the influenza strain B/Shandong/7/97, very close to the former (Figure 8, Annex 2).

Among the viruses close to the lineage B/Yamagata/16/88, 11 were close to the influenza strain B/Shanghai/361/2002, a strain included in the 2005-2006 vaccine. Twenty-four were closer to the influenza strain B/Jiangsu/10/2003 which remains close to the former (Figure 8).

5.2.2. Influenza A (H3N2)

Influenza A viruses were detected at a lesser rate: 47 (20%) viruses of 238. Among these, 27 (57%) were influenza A viruses (H3N2) and 14 (30%) were influence A virus (H1N1). Six (13%) influenza viruses were not able to be subtyped due to an insufficient titer after culture. This proportion is similar to that observed for non-subtyped influenza B viruses (14%). The majority of these viruses was detected by RT-PCR alone and did not grow by cell culture, rendering impossible any antigenic study.

Among the influenza A viruses (H3N2), five were antigenically close to the strain influenza A/Singapore/37/2004 and 11 were close to the vaccine strain influenza A/California/7/04. These two variants are very close (Figure 9 and Annex 4). Finally, 11 influenza A (H3N2) strains were influenza A (H3N2) strains which could not be analyzed by inhibition of the Hemagglutination test. Eight of these strains were subtyped by PCR but could not be antigenically analyzed. The three other A viruses (H3N2) showed a decrease of recognition by the standard A (H3N2) antisera. Additional analyses conducted at the National Institute of Medical Research (NIMR) in London showed that these viruses were closer to the more recent influenza A (H3N2) strains, i.e., A/Annecy/1138/05 and A/Hong Kong /4443/05 (not shown on the phylogenetic tree). No strain close to the influenza A/Wisconsin/67/2005 (H3N2) strain was detected in Switzerland this year (Figure 9). In contrast, in the USA, a majority of influenza A (H3N2) strains circulated this winter. Twenty-four percent of these strains were antigenically close to the influenza A/Wisconsin/67/2005 strain. For this reason, this strain was included in the flu vaccine composition for the 2006-2007 season.

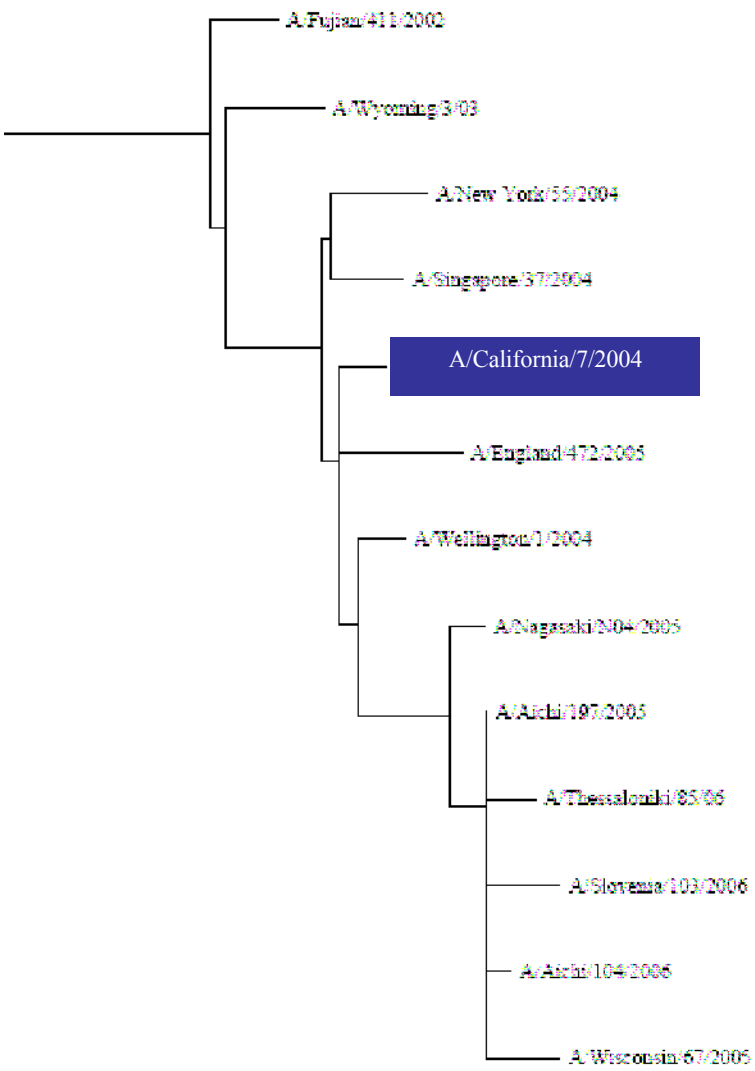


Figure 9: Phylogenetic comparison of nucleotide sequences encoding hemagglutinins of influenza A (H3N2) viruses; the 2005-2006 vaccine strain is highlighted in blue.

5.2.3. Influenza A (H1N1)

Of the 47 influenza A viruses detected during this season, 14 (30%) viruses were of subtype influenza A (H1N1), nine were antigenically close to the vaccine strain influenza A/New Caledonia/20/99 (Annex 3). Two strains were close to the more recent A/Netherland/128/2004 strain. However, this latter strain is very close to the vaccine strain A/New Caledonia/20/99. Finally, three influenza A (H1N1) viruses were detected by RT-PCR alone and could not be antigenically typed.

5.2.4. Co-infection by influenza A and B

Two patients were co-infected by two different influenza viruses (detected by RT-PCR). One was a 34-year-old woman who had received the flu vaccine, and a 17 year-old boy. Both were victims of a virus A and B infection. In the former, it was an influenza A (H3N2) virus close to the vaccine strain and a B virus which could not be typed. For the latter, it was an influenza B/Malaysia/2506/2004 strain distinct from the vaccine strain; the influenza A strain could not be subtyped. In general, subtyping of influenza strains from a co-infection is only carried out on one of the two strains. The reason being that strain subtyping requires a large amount of viral material. To obtain this, the strain must be cultured on cells for several days. After culture, only one strain is detected and is usually the one which grew faster than the second. The latter ends up by no longer growing at all and thus renders impossible any characterization.

Co-infection is rarely observed; in this case, only two of 236 patients positive for influenza. Nevertheless, it could be detected only by RT-PCR which was used this year for all Sentinella samples.

5.3 Patients with influenza infection

5.3.1. Frequency of viruses detected in a particular age group

Each year, the viruses circulating in the population are different from the previous seasons and it is important to observe if certain age categories are more affected than others. To evaluate this aspect, the samples received (Figure 10) and the proportions of viruses detected (Figure 11) during the season have been classified by patient age.

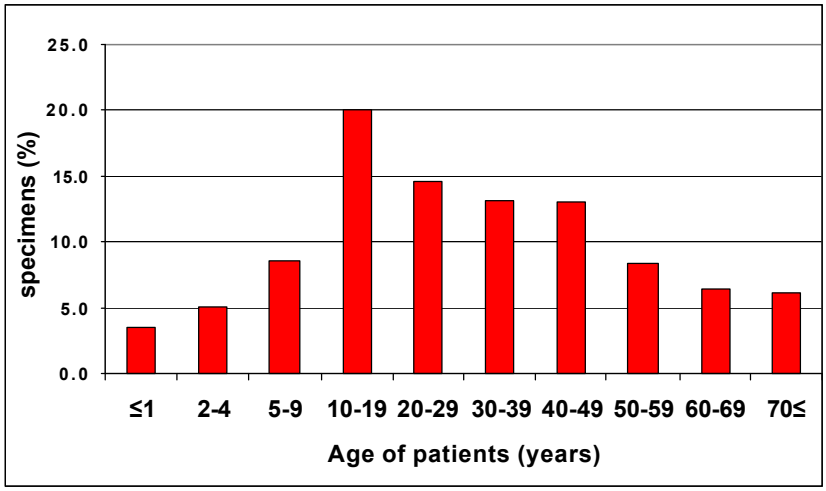


Figure 10: Number of samples received classified according to age group

Most samples were taken from patients aged between 10 and 19 years, i.e., 1 of 5 (Figure 10). These are followed by samples from patients aged between 20 to 49 years. The viruses detected have also been stratified by age group and type of virus (Figure 11). The largest proportion of viruses was detected in patients aged between 5 and 29 years, with the highest rate of detected viruses in persons aged from 10 to 19 years. These viruses represented the majority of influenza B viruses (27%). These viruses evolve slowly. Thus, older persons have probably been exposed more frequently to this type of virus than those who are younger. In contrast, influenza A viruses were more prevalent in patients between 30 and 60 years (13%), in particular, those in the forties age group (6%). The majority of this population represents employed persons. In the case of illness, a medical certificate must be given to the employer. Thus, a medical consultation takes place and the patient is seen by a Sentinella practitioner. The elderly or younger population do not necessarily consult a doctor for a flu condition and this is why this segment of the population is less represented in the graphic.

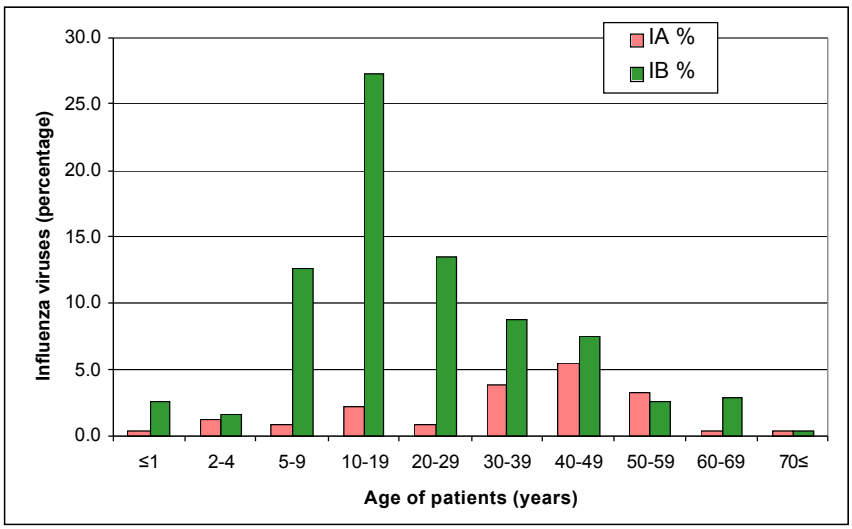


Figure 11: Percentage of viruses detected according to age group

5.3.2. Symptoms of influenza-infected patients

Described symptoms as reported by the practitioners were recorded for all patients. Positive cases have only been considered. Symptoms have been counted and are represented as a percentage of the total of the positive samples (Figure 12).

The most frequent symptom reported by influenza-infected patients is fever (92%), followed by headache and cough. No differences were observed this year in symptoms and these observations are in accordance with the previous season.

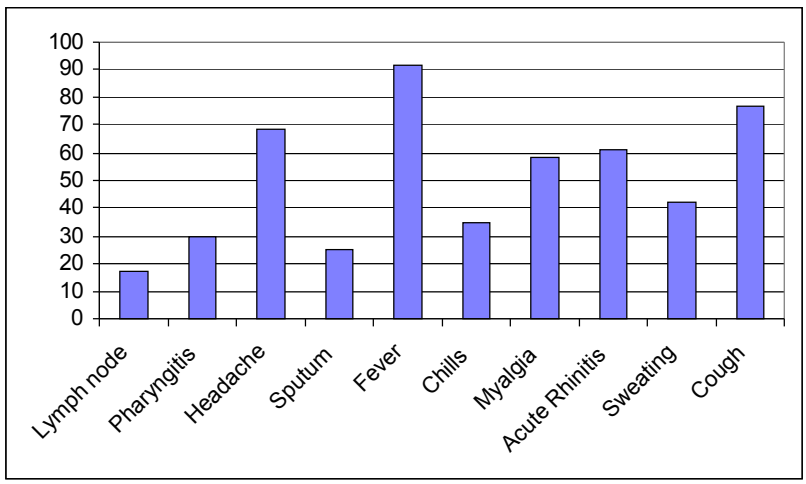


Figure 12: Percentage of symptoms recorded in influenza-infected patients

5.3.3. Influence of the influenza epidemic on the mortality rate in the canton of Geneva

Patients aged over 60 years represent the main population at risk to develop complications following a flu infection. We evaluated the impact of the circulation of the influenza virus on this population residing in the canton Geneva. The choice of Geneva is only due to the fact that we can rapidly obtain data concerning the mortality rate. The weekly death rate of persons over 60 years has been correlated with the rate of medical consultations for flu symptoms and the detection rate of influenza viruses in the canton of Geneva (Figure 13).

In the canton of Geneva, influenza B flu was detected mainly between weeks 7 and 12. Medical consultations confirmed the presence of an epidemic between these same weeks with a rate of medical consultations superior to the normal which is 1.5%. Two distinct peaks appeared in the death rate of persons over 60 years during weeks 7 and 11. These coincide with the observed virus isolates. However, these values are not significantly higher than the mean of the weekly death rate in this population plus two standard deviations. Therefore, the flu epidemic did not cause an identifiable over-mortality in persons over 60 years residing in the canton of Geneva.

5.4. Comparison of influenza surveillance by PCR and cell culture

For several years now, RT-PCR is an increasingly used technique for diagnostic purposes. It is a rapid, sensitive and specific technique and permits the simultaneous analysis of a large number of samples. To evaluate this technique within the framework of flu surveillance by the Sentinella system, we have conducted a comparison of detection by RT-PCR in parallel with the classic detection method of cell culture combined with an immunofluorescence test. The techniques used are described in paragraph 4.2 and the results obtained are shown in Table 2.

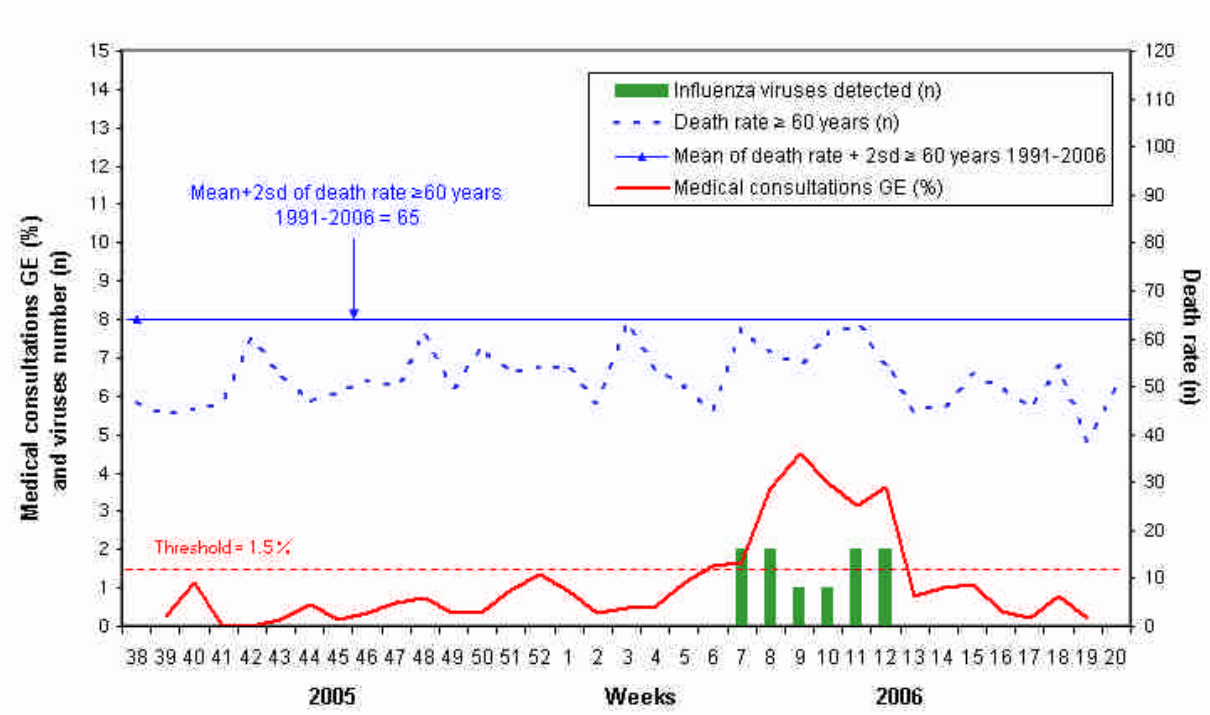


Figure 13: Influence of the influenza epidemic on the mortality of persons over 60 years residing in the canton of Geneva during the 2005-2006 season.

Medical consultations GE (%): medical contacts of influenza-like symptoms in the canton of Geneva; Influenza viruses: influenza A and B viruses detected by the Sentinella network in the canton of Geneva; Death rate: number of weekly death obtained from the records of the Registry Office of the Canton of Geneva published by the public health authorities of Geneva; Mean + two standard deviations: mean number of the weekly deaths of persons older than 60 years + two standard deviations registered between 1991 and 2006.Influence of the influenza epidemic on the mortality in people older than 60 years living in the canton of Geneva during the 2005/06 season.

Of 745 samples received at the NIC, RT-PCR analysis detected 236 positive cases and 509 negative. By culture, 198 samples were positive and 547 were negative (Table 2). The identical results of the analysis obtained by the two methods were as follows: 196 positive and 507 negative. In contrast, an additional 40 influenza viruses were detected only by RT-PCR and remained negative by culture, thus representing 17% of additional viruses. Finally, two influenza viruses grew on culture but were not detected by RT-PCR, representing 0.8% of the total number of viruses. These were an influenza A and an influenza B strain.

These results have allowed us to calculate the sensitivity (Se) of culture and RT-PCR which were 83.2% and 99.2%, respectively (Table 3). RT-PCR is thus more sensitive

Table 2: Results of influenza viruses detected by two techniques (culture and RT-PCR)

		Culture		
		+	-	Total
RT-PCR	+	196	40	236
	-	2	507	509
	Total	198	547	745

than culture. The reliability of each of these techniques can be calculated by the negative (VPN) and positive (VPP) predictive values. Given that the specificity of the two techniques is considered to be equivalent, the VPP is thus 100% also. To summarize, no negative sample is detected positive with either technique. The VPN has been calculated for culture and RT-PCR: 92.6% and 99.5% respectively. The formulae necessary to effects the calculation and the values are mentioned in Table 3.

Table 3: Sensitivity (Se), negative (VPN) and positive (VPP) predictive value, and prevalence (p) of culture and RT-PCR

Se = true positive / (true positive + false negative)
Sp = true negative / (true negative + false positive)
p = Prevalence = All positive samples / All samples = 31.9
VPN = Sp x (1-p) / Sp x (1-p) + (1-Se) x p
VPP = Se x p / Se x p + (1-sp) x (1-p)

	Se	**Sp**	**VPN**	**VPP**
culture	83.2%	100%	92,6%	100%
PCR	99.2%	100%	99,5%	100%

However, the VPN is proportional to the prevalence of the influenza and is directly dependent on the virus frequency. We have therefore calculated the evolution of this value over time during the season. The kinetic obtained is shown in Figure 14.

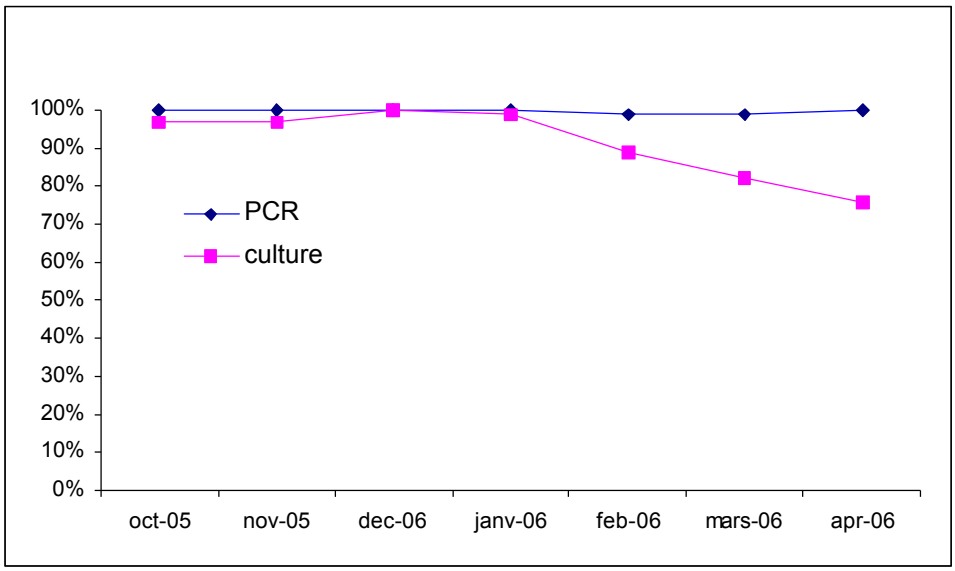


Figure 14: Negative predictive value (VPN) of culture and RT-PCR
VPN by month during the 2005-2006 season

Between October 2005 and January 2006, few influenza viruses were detected. The VPN values were close to a maximum of 100%. From February 2006, the number of positive samples increased considerably. The consequence is that the number of positive samples increased and with these, the number of positive samples by RT-PCR and negative by culture. The VPN observed for culture therefore diminished from February onwards and until March. During April, the VPN difference is even more striking between the two methods, even though the number of positive samples had decreased. This difference comes from the fact that the proportion of influenza A viruses had increased. From April, almost half of all viruses isolated were influenza A viruses (9/20). These grow more badly than influenza B viruses. Thus, certain influenza A viruses were detected by RT-PCR only. For the month of April alone, 20/20 viruses were detected by RT-PCR and only 14/20 by culture. This result explains partially the difference in the VPN values of the two methods.

RT-PCR appears thus as a more rapid and faster methods than culture. We effected a comparison of these two methods from an epidemiological point of view. The weekly rate of detection of influenza viruses for each method is shown in Figure 15. In this figure, the number of positive samples is systematically superior or equal with RT-PCR (blue bars). The kinetic of the detection rate by the two methods appears similar but the differences between the weeks are more marked with RT-PCR. Thus, detection peaks observed during weeks 7, 8, 10 and 15 are higher with

RT-PCR. In addition, of the first three influenza viruses circulating during the season, only an influenza A (H1N1) virus was detected by culture and RT-PCR during week 42. The two influenza B viruses were detected only by RT-PCR during weeks 44 and 45. At the beginning of the 2005-2006 season, the results of the RT-PCR analysis were thus in conformity with the rest of the season, i.e., a large majority of influenza B viruses circulating in the population. This additional accuracy brought by RT-PCR is very valuable in epidemiology.

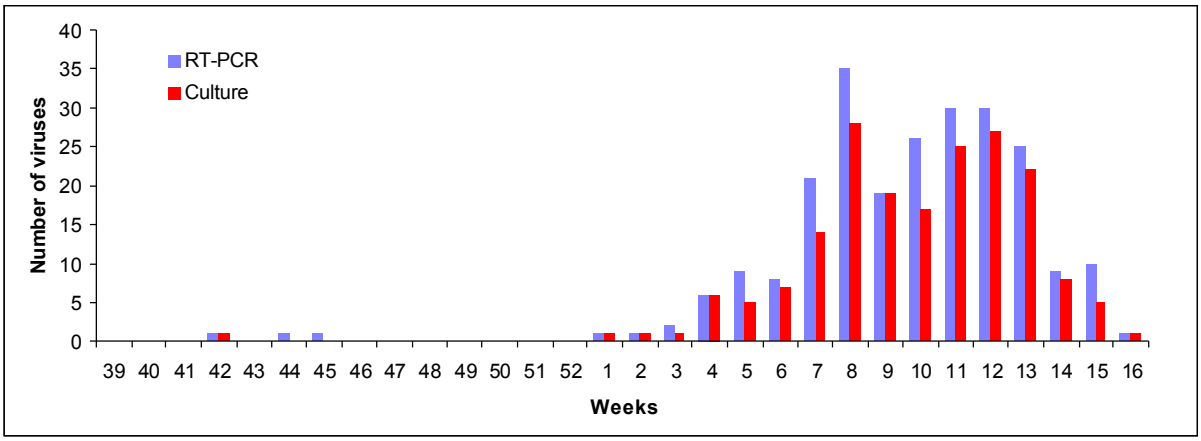


Figure 15: Number of influenza viruses detected by culture and by RT-PCR
Culture: number of viruses detected by culture and immunofluorescence
RT-PCR: number of viruses detected by RT-PCR
The two methods are described in paragraph 4.2

5.5. Influenza in Europe

The epidemic observed in the different European countries was mostly homogenous and a majority of influenza B viruses circulated (58%). Influenza A viruses also circulated (42%). Only 28% of the influenza A strains were subtyped. The influenza A strains circulating are distributed as follows: influenza A (H1N1), 7% and influenza A (H3N2), 11%. Finally, two influenza A (H1N2) viruses were detected in Germany alone.

Sporadic cases of influenza virus were detected from week 40 with influenza A and B viruses detected (Figure 16). The number of influenza viruses began to increase significantly from week 4. Two peaks were then observed during weeks 8 and 12, as was also the case in Switzerland.

In England and Scotland, the epidemic was of earlier onset with a first peak during weeks 2 to 4 of 2006 due to influenza B viruses circulating, and a second, later peak after week 14 due to influenza A (H3N2) and A (H1N1) viruses circulating. In other countries, influenza A and B viruses circulated in parallel during the same periods with only one peak observed (The Netherlands, Belgium, Germany, Poland, Spain and Portugal). The epidemic observed in Italy was marked by the fact that the influenza A (H1N1) viruses were prevailed over the influenza B and A (H3N2) viruses.

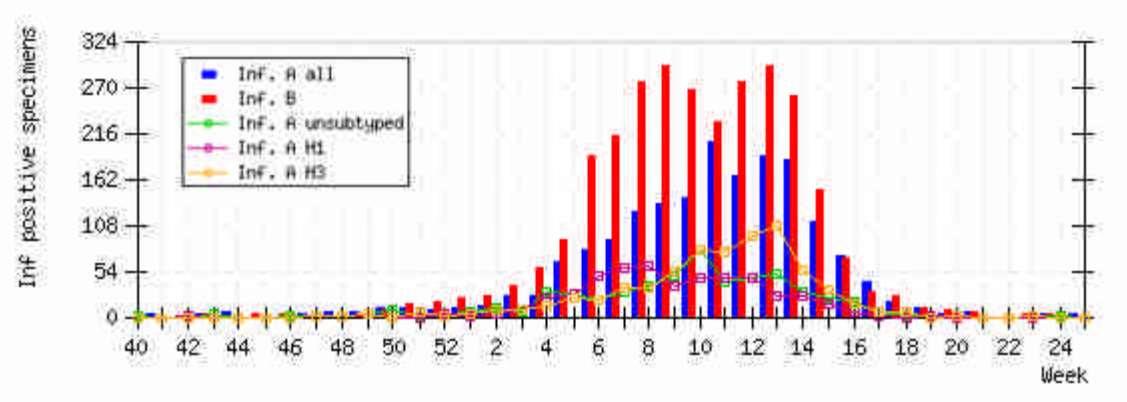


Figure 16: Number of specimens positive for influenza A and B viruses, season 2005-2006. Source: European Influenza Surveillance Scheme

Two thousand one hundred and twenty viruses were analyzed by hemagglutination or by sequencing. 64 percent were influenza B viruses and 36% were influenza A. In Europe, as in Switzerland, influenza B viruses of two different lineages were detected. The majority of the influenza B viruses characterized were antigenically close to the strain B/Malaysia/2506/2004 (95%), belonging to the influenza B/Victoria/2/87 lineage. A minority of influenza B viruses were antigenically close to the vaccine strain influenza B/Jiangsu/10/2003 (5%) which belongs to the second lineage, influenza B/Yamagata/16/88 (Figure 17).

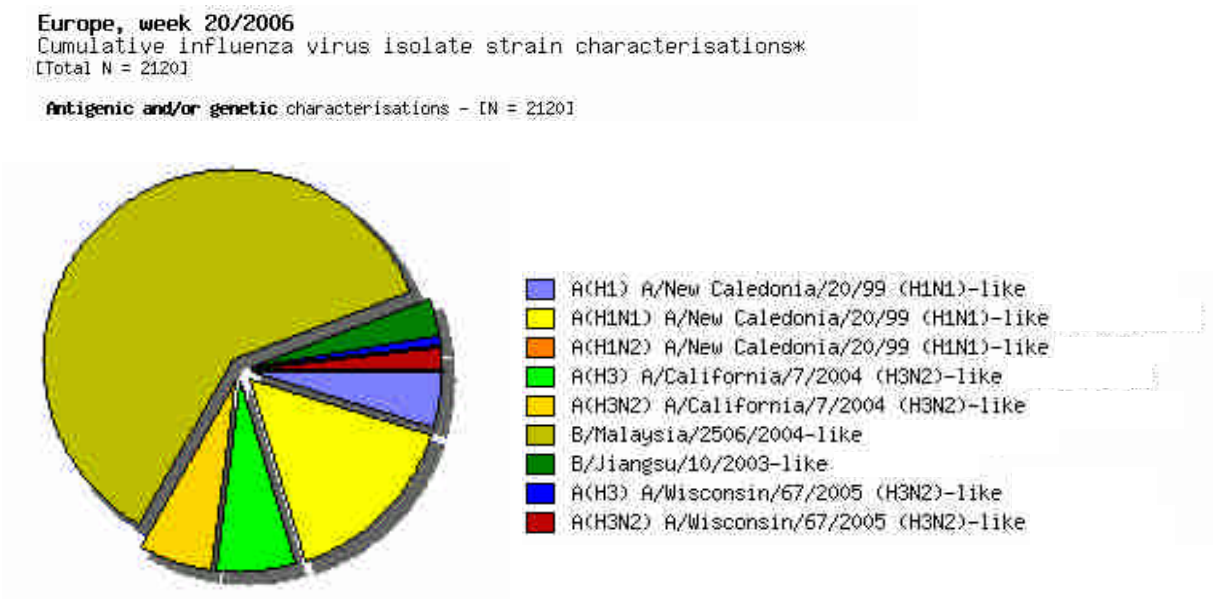


Figure 17: Influenza strains detected in Europe during the season 2005-2006.
Source: European Influenza Surveillance Scheme

84 % of characterized influenza A viruses were of A (H1N1) subtype. All were antigenically related to the vaccine strain influenza A/New Caledonia/20/99 (H1N1). Of the 36% of influenza A (H3N2) viruses, 83% were antigenically related to the vaccine strain influenza A/California/7/2004 (H3N2) and only 17% were more related to influenza A/Wisconsin/67/2005 (H3N2) strain. This last strain was predominant in the United states and caused a mild epidemic.

5.6. Avian influenza

5.6.1 Situation of avian flu worldwide

Since 1997, avian flu epidemics appear regularly in Asia in poultry breeding farms, causing a high rate of animal mortality. These epidemics are due to the appearance and the circulation of the highly pathogenic (HPAI) influenza A (H5N1) virus. If it concerns the low pathogenic (LPAI) influenza A (H5N1) virus, infection in the animal is manifested by a drop in production in domestic batteries (decrease in weight and laying capacity of the sick animals). HPAI influenza A viruses can be transmitted to humans and may result in death in approximately 50% of the cases. This occurs

generally when persons have direct, close contact with breeding animals affected by the virus.

Between June 2003 and February 2004, the influenza A (H5N1) virus caused major epidemics in poultry together with human victims in eight Asian countries (principally Viet Nam and Thailand, then in Cambodia, Laos, Indonesia, China, South Korea, and Japan). Then in April 2005, hundreds of dead, wild birds were discovered in a nature reserve close to Qinghai Lake in China. In all, 6345 birds of different species would die following infection by the influenza A (H5N1) virus. The virus detected was a new variant of the influenza A (H5N1) virus which revealed to be lethal for wild birds. The birds stay usually asymptomatic following infection by the influenza A (H5N1) virus.

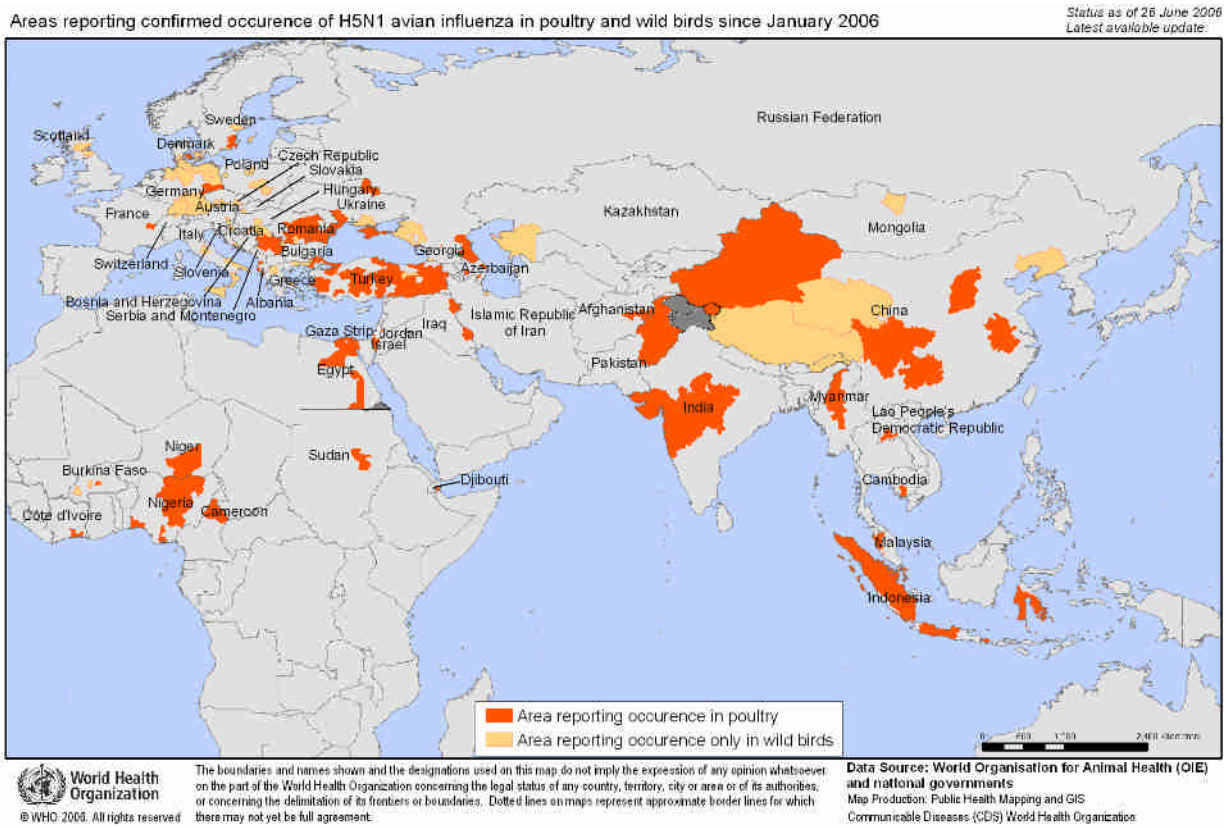


Figure 18: Areas reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds since January 2006, status at 26 June 2006 (latest available update). Source: World Organisation for Animal Health (OIE) and national governments

New epidemics then occurred in wild birds in countries lying north-west of China: Siberia (July 2005), Kazakhstan, Tibet, Mongolia (August 2005). From then on, the epidemics appeared to follow the main migratory routes of Northern Europe (Azerbaijan, Georgia, Bulgaria, Ukraine …), then Central Europe (Romania, Bosnia,

Slovenia, Hungary …) and, finally, Western Europe from February 2006 (Germany, Austria, Italy, France, Switzerland …) (Figure 18). Even more recently, Spain reported a case of a wild bird infected by the influenza (H5N1) virus in the Basque region at the beginning of July 2006 (this case is not shown on the map, Figure 18). In parallel, the Middle Eastern countries (Turkey, Iran, Iraq, Egypt …) and Africa (Nigeria, Niger and Cameroon) also reported epidemic cases in wild birds in the majority, but also in poultry (Figure 18). The origin of the spread to this part of the world remains a subject of controversy, but the poultry trade between Nigeria and Asia appears to play an important role (Ducatez et al, 2006).

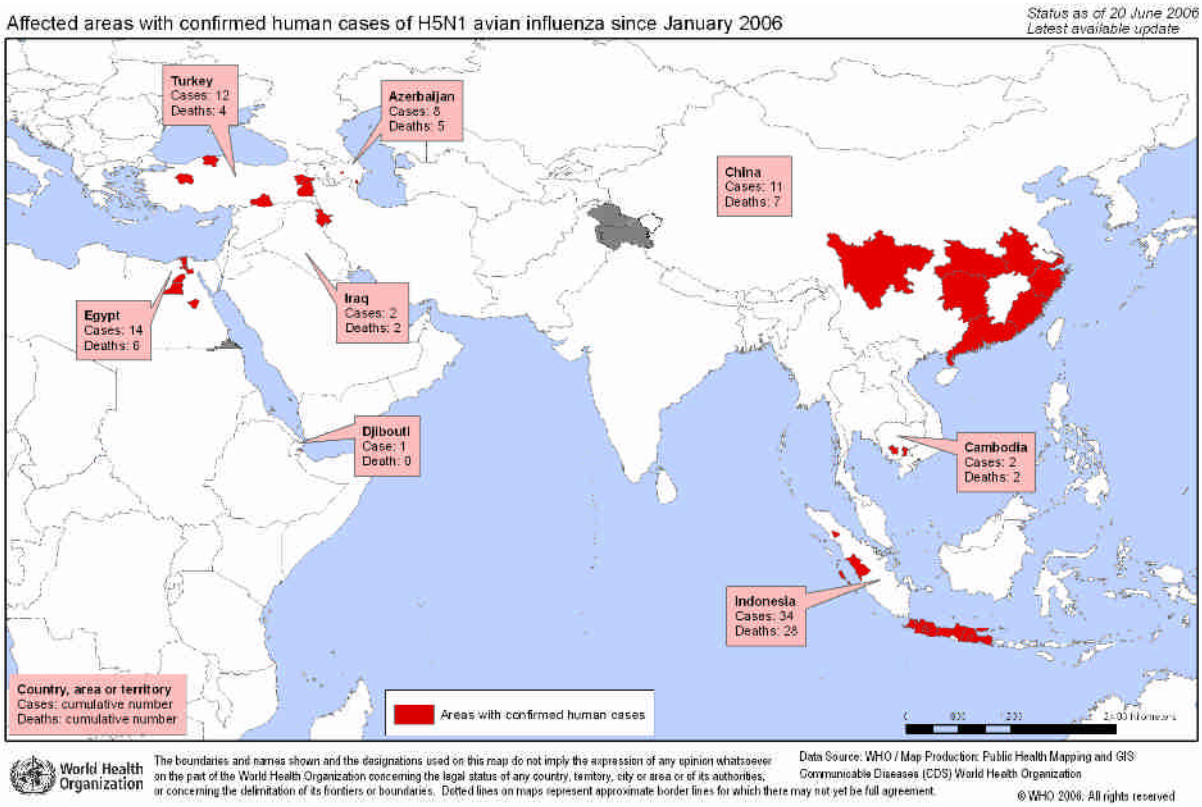


Figure 19: Affected areas with confirmed case of H5N1 avian influenza in human since January 2006, status at 20 June 2006 (latest available update). Source: World Organisation for Animal Health (OIE) and national governments

Certain avian epidemics were accompanied by sporadic case of infection in humans by the influenza A (H5N1) virus, essentially in countries were domestic poultry was affected. This was the case in several Asian countries (Viet Nam, Thailand, Indonesia, Cambodia, China), but also since 2006 in Middle Eastern countries (Turkey, Egypt, Iraq, Azerbaijan) and Africa (Djibouti) (Figure 19 and Table 4). Since

2003, 229 human victims have been identified with 131 deaths following infection by the influenza A (H5N1) virus of avian origin.

Table 4: Total number of cases includes number of deaths
Source: WHO, http://www.who.int/csr/disease/avian_influenza/country/cases_table_2006_07_04/en/index.html
status at 4 July 2006

Country	2003		2004		2005		2006		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	8	5	8	5
Cambodia	0	0	0	0	4	4	2	2	6	6
China	0	0	0	0	8	5	11	7	19	12
Djibouti	0	0	0	0	0	0	1	0	1	0
Egypt	0	0	0	0	0	0	14	6	14	6
Indonesia	0	0	0	0	17	11	35	29	52	40
Iraq	0	0	0	0	0	0	2	2	2	2
Thailand	0	0	17	12	5	2	0	0	22	14
Turkey	0	0	0	0	0	0	12	4	12	4
Viet Nam	3	3	29	20	61	19	0	0	93	42
Total	3	3	46	32	95	41	85	55	229	131

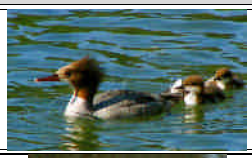
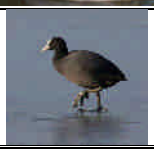
5.6.2. Avian flu in Switzerland

5.6.2.1 Avian flu detected in animals

Switzerland has not been spared with the detection of a first migratory bird on 1 March 2006. Forty-one influenza A/H5 viruses, of which nine were influenza A (H5N1), have been discovered in dead, wild birds. Three inactive samples from birds tested positive for avian flu have been sent to us to test the reliability of our RT-PCR. These concerned three different species of wild, migratory birds detected at three different places in Switzerland (see Table 5). These samples contained only the viral genome. We subjected them to RT-PCR testing developed in our laboratory (see paragraph 4.4). This method allows to detect the hemagglutinin H5 and the neuraminidase N1 of only avian origin. The detection reaction of the viral genome by RT-PCR is shown in Figure 20. The three samples were detected as positive by the RT-PCR H5 and the RT-PCR N1. These results have reassured us of the validity of

our method for the detection of the influenza A (H5N1) viruses circulating at present. However, given that the virus is constantly evolving, it is essential to test regularly the detection method used in the laboratory with positive samples.

Table 5: Samples from birds infected by the influenza A (H5N1) virus in Switzerland. (Material obtained partially from Professor Richard Hoop, University of Zurich)

Sample	English name	Scientific name	Canton	Migration	Picture
V82	Common Merganser	Mergus merganser	GE	Yes	
V68	Mute Swan	Cygnus Olor	SH	Yes/No	
V180	Common coot	Fulica atra	TG	Yes	

The hemagglutinin of the three birds was sequenced in our laboratory. The alignment of these three sequences with the hemagglutinin sequences of other viruses detected in 2005 and 2006 were conducted and can be seen in Annex 5. The name of the aligned influenza A (H5N1) strains is given in Table 6. The results of this analysis demonstrate clearly the common origin of the viruses detected in the Swiss birds as the same as those in Italy, Africa, and the viruses detected at Lake Qinghai, China, in April 2005. This result confirms the role of wild birds in the spread of the influenza A (H5N1) virus from the Asian continent towards Europe. However, five silent mutations can be observed on the three Swiss sequences as well as on the sequence of the influenza A/Mallard/Bavaria/1/2006 (H5N1) virus detected in Germany. Finally, two of these five mutations are also found on the partial sequence of the hemagglutinin of the influenza A/Turkey/France/06222/2006 virus detected in the Ain Department of France, close to the Swiss-French border. These differences show that the virus is still evolving and that influenza A (H5N1) strains with different genetics are now present in Europe.

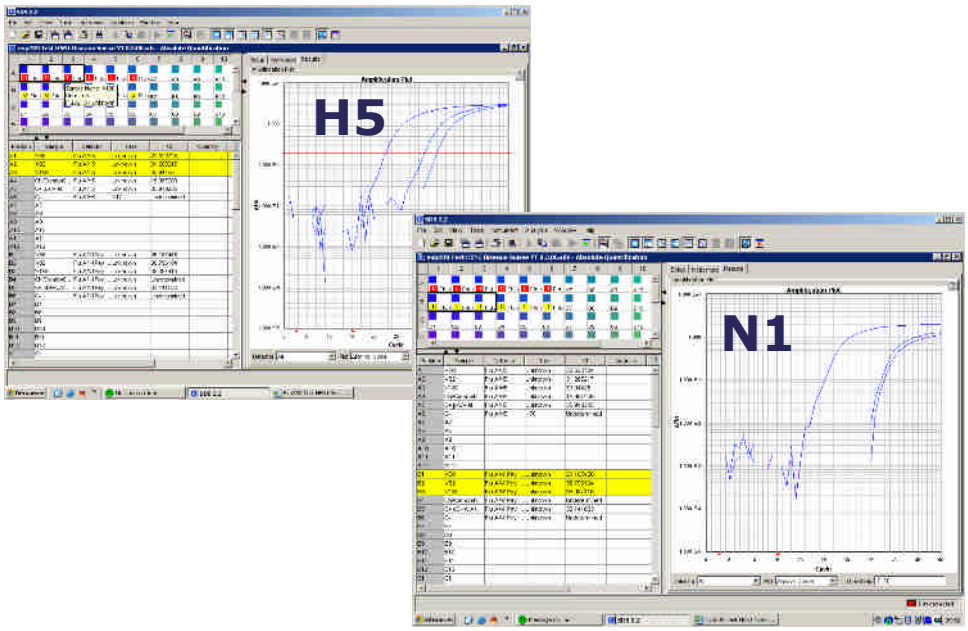


Figure 20: Detection by RT-PCR (Real-Time): H5 (FluH5) and N1 (FluN1) in the V68, V82 and V180 samples

Table 6: Name of the influenza A (H5N1) strains used for the alignment of hemagglutinin sequences, see Annex 5

Strains	Country
A/FoulqueM/Diessenhofen/V180/2006	Switzerland
A/HarleB/Geneva/V82/2006	Switzerland
A/Schwan/Schaffhausen/V68/2006	Switzerland
A/Mallard/Bavaria/1/2006	Germany
A/Turkey/France/06222/2006	France
A/BH Goose/Qinghai/2/2005	China
A/Du/Novosibirsk/56/2005	Russia
A/Ck/Nigeria/641/2006	Nigeria
A/Swan/Italy/179/2006	Italy
A/Com.Pochard/France/06222/2006	France
A/Buzzard/Denmark/6370/2006	Denmark

5.6.2.2 Avian flu detected in humans

Since November 2005, eight suspected cases of influenza A (H5N1) virus infection in humans have been tested by the NIC in Geneva (Table 7). All cases concerned persons returning to Switzerland after travel in risk zones. Risk zones are considered as countries where avian flu epidemics have been reported since 2005. None of

these cases in humans was positive. Of note, an influenza A (H5N1) virus was detected in a patient returning from travel in Africa (case 8, Table 7). This example well illustrates the ease of importation of an influenza virus from one continent to another. Finally, human influenza viruses are circulating in Africa but, unfortunately, very little epidemiological information is available.

Table 7: Case of suspected influenza A (H5N1) infection in humans tested at the NIC, Geneva, Switzerland

Cases	Date of sample	Canton	Countries visited	Animal exposure	Test H5N1	Other result
1	29.10.05	VD	Viet Nam & Cambodia	Non	Neg	Neg
2	03.11.05	GE	China	Domestic poultry	Neg	Neg
3	18.12.05	SG	Thailand	Non	Neg	Neg
4	20.12.2005	NE	Indonesia	Non	Neg	Neg
5	03.03.2006	BS	Turkey	Domestic poultry	Neg	Neg
6	26.03.06	GE	Burkina Faso	Domestic poultry	Neg	A (H1N1)
7	07.06.06	SZ	China	Non	Neg	Neg
8	27.06.06	VD	Thailand	Non	Neg	Neg

As shown, the sources of importation of a flu virus circulating in a country far from Switzerland are many. For this reason, it is important to be prepared to diagnose avian flu in humans.

5.6.3. Evaluation of the "home-made" RT-PCR

The real-time RT-PCR specific for the detection of the hemagglutinin H5 developed by the NIC has already been presented in the previous report (Thomas et al, 2005). The PCR specific for the detection of the N1 of influenza A (H5N1) avian viruses is based on the article of Payungporn et al (*J Virol Methods*, 2006). The specificity of these PCRs has been tested by many positive controls received from European

partners who are members of the EISS network. The origins of the different samples (inactive virus, RNA and cDNA) belonging to the three “clades” (WHO, 2005) have been tested (Table 8). The RT-PCR H5 detected all positive-tested controls belonging to the three clades. The RT-PCR was able to detect positive controls belonging to clades 1, 2 and 3. The influenza A/Duck/Potsdam/619/85 (H5N2) and A/Duck/Singapore/Q-F119-3/97 (H5N3) viruses were well detected by RT-PCR H5. However, they were not detected by RT-PCR N1 as they possessed a neuraminidase of types 2 and 3, respectively.

No tested human viruses (A (H1N1), A (H3N2), and B) was detected by the RT-PCRs H5 and N1, thus demonstrating RT-PCR specificity.

5.6.4. Evaluation of a commercial kit for the detection of avian A/H5 influenza

Some commercial kits for the detection of influenza A (H5N1) virus are now available. We have tested one of these, the “Taqman® influenza A/H5 1.0” manufactured by ABI, Warrington, England. A series of dilutions of the influenza A/Duck/Vietnam/TG24-01/05/2005 (H5N1) virus underwent a parallel detection by the commercial kits and our RT-PCR. A comparable sensitivity of detection was observed for both tests with this strain (Table 9). Both methods are capable of detecting the presence of the virus up to a concentration of 100 genome equivalents/ml. In the light of these results, the sensitivity of these two methods is comparable for the detection of the above-mentioned virus but it remains to be tested on a much larger variety of influenza (H5N1) viruses.

However, a controversial point of these types of commercial kits is that the sequence of primers used is not made public. Thus, a comparison of the primer sequence cannot be compared with the sequence of the viral genomes which are changing permanently in the case of avian influenza virus. The reliability of the test can only be verified with the availability of positive controls. This is not always assured in real time. In addition, this test only detects the presence of hemagglutinin H5 and the detection of neuraminidase N1 is not assured. Finally, the method does not allow to preserve the cDNA for later analyses as is the case with our RT-PCR. The Abi kit uses a One-Step PCR from RNA and this particularity makes the necessary

Table 8: Material tested by the RT-PCRs H5 and N1 and results obtained

Subtype	Strain designation	Material	Origin	Clade	H5 PCR	N1 PCR
A (H5N1)	A/Duck/Vietnam/ TG24-01/05	Virus	Schweiger B. Berlin	I	+	+
A (H5N1)	A/Vietnam/1203/04 (H5N1)	cDNA	A. Hay, MRC London	I	+	+
A (H5N1)	A/Vietnam/4207/2005 (H5N1)	cDNA	A. Hay, MRC London	I	+	+
A (H5N1)	A/Ck/Cambodia/7/04	Transc. RNA	JT Aubin Paris	I	+	ND
A (H5N1)	A/Turkey/Turkey/1/2005	cDNA	J.Ellis, HPA London	II	+	+
A (H5N1)	A/Indonesia/6/2005	cDNA	J.Ellis, HPA London	II	+	+
A (H5N1)	A/Parrot/QAV/2005	cDNA	J.Ellis, HPA London	II	+	+
A (H5N3)	A/Duck/Singapore/ Q-F119-3/97 (H5N3)	cDNA	J.Ellis, HPA London	III	+	ND
A (H5N2)	A/Duck/Postdam/ 619/85 (H5N2)	cDNA	Schweiger B. Berlin	?	+	ND
A(H1N1) Hu	A/New Caledonia/ 20/99 (H1N1)	Virus	A. Hay, MRC London		-	-
A(H3N2) Hu	A/California/7/04 (H3N2)	Virus	A. Hay, MRC London		-	-
B (Hu)	B/Shanghai/361/2002	Virus	A. Hay, MRC London		-	-

confirmatory reactions more difficult when a sample is positive. The detection of other respiratory viruses for a differential diagnosis is equally more difficult without available cDNA.

Table 9: Comparison of the RT-PCR of the NIC and the commercial kit

Eq.gen/ml: genome equivalent/ml estimated by B Schweiger, RKI, Germany; ABI One-Step: Taqman® Influenza A/H5 ABI kits, ABI, city. The values mentioned represent the mean of the Ct obtained with the different dilutions of viral solution analyzed in three parallels

Eq.gen/ml	In House RT-PCR	ABI One-Step
10^7	21 ± 1	21.9 ± 0.9
10^6	26 ± 0.3	25.5 ± 0.1
10^5	29 ± 1.6	28.2 ± 0.3
10^4	33 ± 0.9	31.9 ± 0.1
10^3	35 ± 1.4	35.7 ± 0.6
10^2	38 ± 1.3	38.6 ± 0.8
10^1	-	-
C-	-	-

6. INFLUENZA VACCINE COMPOSITION FOR THE 2006-07 SEASON FOR THE NORTHERN HEMISPHERE

The annual meeting on the composition of the influenza vaccine took place on the 15 of February 2006 at WHO in Geneva. The following recommendations were given for the composition of the influenza vaccine for the 2006-07 season:

- An influenza A/New Caledonia/20/99(H1N1)-like virus
- An influenza an A/Wisconsin/67/2005 (H3N2)-like virus[a]
- An influenza a B/Malaysia/2506/2004-like virus[b]

[a] *A/Wisconsin/67/2005 (H3N2) and A/Hiroshima/52/2005 can be used*
[b] *B/Malaysia/2506/2004 virus and B/Ohio/1/2005 can be used*

7. DISCUSSION

The influenza season 2005-2006 was of moderate intensity in Switzerland as in the rest of Europe. The number of samples received as well as the number of influenza viruses detected was inferior to those observed during the preceding seasons, such as those of 1996-1997 and 1997-1998 (Thomas et al, 1997; Thomas et al, 1998). Even the medical consultation rates were moderate. No excess mortality was observed during or after the flu epidemic in persons over 60 years. Moreover, the epidemic was of late onset with no virus detected at all during December 2005. It affected a majority of young persons aged between 5 and 29 years with a maximum of viruses detected in those between 10 to 19 years. This predominance was certainly due to the type of viruses circulating and which represented a majority of influenza B viruses. These viruses are antigenically stable and have already circulated during past seasons. Older persons have therefore been exposed on several occasions and have probably acquired an immunity more developed than the younger population. This resulted in a decreased detection frequency of influenza B viruses in those aged over 30 years.

Several influenza virus variants circulated in parallel this year. Two distinct lineages of influenza B viruses were present. A minority of influenza B viruses were antigenically close to the vaccine strain influenza A/Jiangsu/10/2003 belonging to the Yamanashi lineage. This lineage is the oldest present in Europe (Figure 21). The influenza strain B/Beijing/184/93 circulated between 1995 and 1999 before being replaced by the influenza B/Sichuan/379/99 strain which circulated between 2000 and 2005. The great majority of influenza B viruses detected during the season 2005-2006 were close to the influenza B/Malaysia/2506/2004 strain belonging to the Victoria lineage. These strains were only partially covered by the vaccine. This is the fourth year that this lineage has been detected in Switzerland as in Europe. After having circulated for several years exclusively in Asia, the Victoria lineage arrived in Europe in 2002. In Switzerland, these flu strains circulated exclusively during the 2002-2003 season and in parallel with the Yamagata lineage strains during the 2004-2005 season and the past 2005-2006 season (Figure 21). The disappearance of this strain during 2003-2004 remains an enigma. It suggests the existence of a human reservoir of influenza B strains during several months, or even years, which would explain the re-emergence of this strain. The influenza B/Malaysia/2506/2004 strain

was chosen to replace the influenza B/Jiangsu/10/2003 strain in the flu vaccine for the 2006-2007 season.

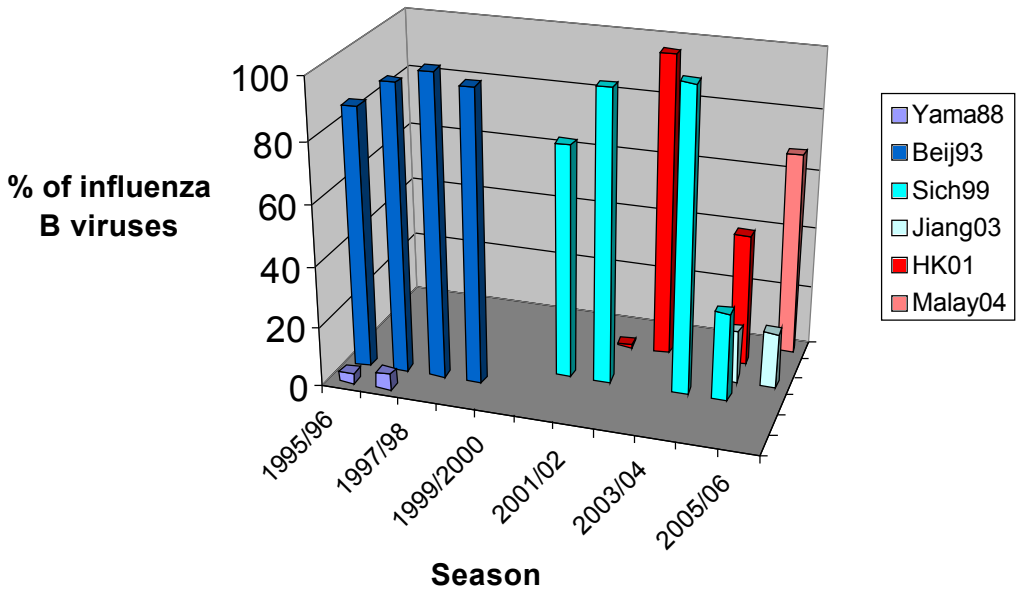


Figure 21: Influenza B strains circulating in Switzerland since 1995. Each colour represents a particular strain. Blue: Yamagata-like strain; red: Victoria-like strain. The values represent the rate of each strain in comparison with all influenza strains detected during the season.

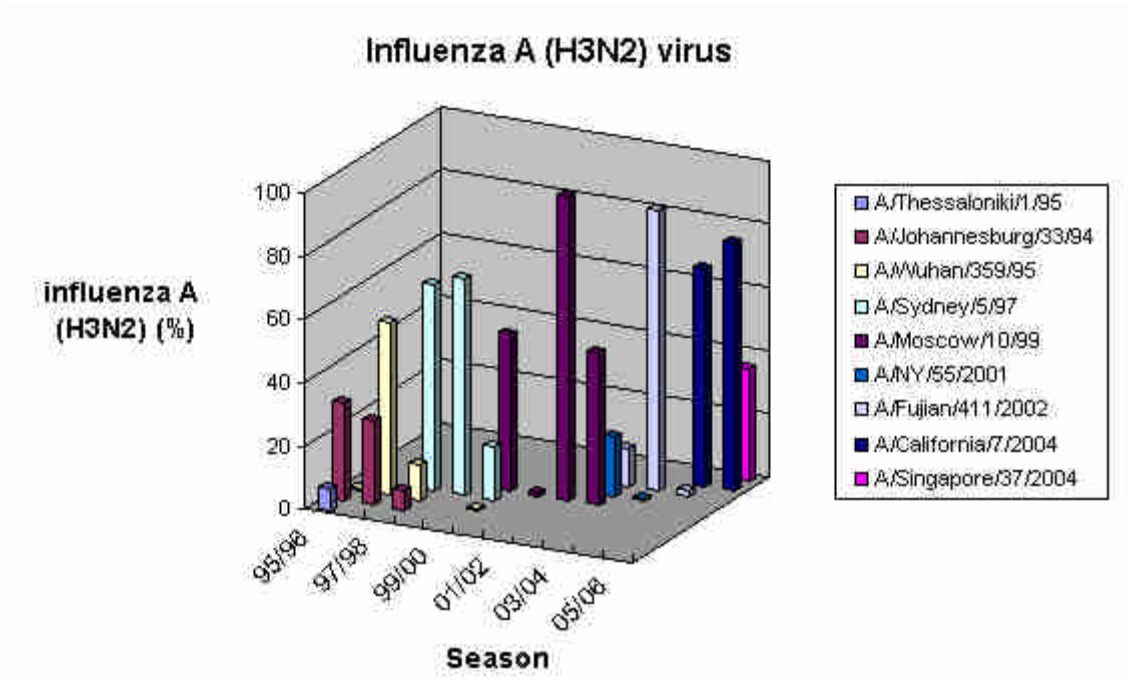


Figure 22: Influenza A (H3N2) strains circulating in Switzerland since 1995. Each colour represents a particular strain. The values represent the rate of each strain in comparison with all influenza A (H3N2) strains detected during the season.

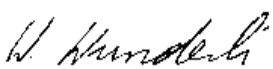
A different situation has been observed for influenza A strains. The influenza A (H3N2) strains in particular appear and then circulate for between two to four years consecutively before definitively disappearing. This phenomenon is shown in Figure 22. The influenza A/Moscow/10/99 strain was present for the longest period, i.e., four years. In contrast, the strains which circulated more recently, influenza A/New York/55/2001, influenza A/Fujian/411/2002 and influenza A/California/7/2004 only circulated over two years. Strains close to the influenza A/Singapore/37/2004 strain began to circulate this season and appeared to supplant the influenza A/Califronia/7/2004 strain. In contrast, the influenza A/Wisconsin/67/2005 strain which was predominant this season in North America was not detected in Switzerland. However, it was detected in Europe: 56 strains were characterized as close to this strain and it has been selected to replace the influenza A/California/7/2004 (H3N2) strain in the 2006-2007 vaccine.

A very significant stability was observed for influenza A (H1N1) strains with the presence of strains since several years close to the influenza A/New Caledonia/20/99 strain.

This year, a comparative study of the two methods for the detection of influenza viruses was conducted on all the Sentinella samples. The results of this study showed that RT-PCR is much more sensitive than cell culture. This represented an advantage which allowed this year for example to acquire information on the first viruses which were detected uniquely by RT-PCR at the beginning of the surveillance period (paragraph 5.4). Other advantages of this diagnostic method are its rapidity and capacity to deal with a large number of samples. Taken together, all these advantages show that the RT-PCR assay is the best quality technique to study the epidemiology of influenza. In consequence, it will be used for the next session by the NIC for Sentinella influenza surveillance. It allows an initial, wide, rapid and more sensitive screening of samples to detect influenza viruses. Negative samples by RT-PCR will be put aside and positive ones will be cultured for strain characterization.

Finally, during this season, the influenza A (H5N1) virus responsible for large epidemics of avian influenza in poultry, but also of epizootics in human, circulated once again. This year, the new event was that the virus left the Asian continent and spread across several continents and countries within only a few months: in Russia, the Middle Orient, Africa and Europe. In Switzerland, 41 birds were detected positive for influenza A/H5, of which nine were influenza A (H5N1) viruses. We obtained

material from three avian influenza A/H5 viruses and were able to detect the type of neuraminidase and to sequence the hemagglutinin. These analyses allowed to show that the origin of these viruses was probably Lake Qinghai in China. This result confirms the role of wild birds in the dissemination of the influenza A (H5N1) virus across different continents. The evolution in the hemagglutinin sequence of these viruses was also detected in viruses detected in neighbouring countries of Switzerland, namely, Germany and France. This result shows that this virus is in constant evolution. It is absolutely necessary to have regular access to the sequences of the most recently detected viruses to verify the validity of primers used for the detection of influenza A (H5N1). This is a critical point for the use of commercial kits for the detection of influenza A (H5N1) viruses which are in constant evolution. We have shown that one of these commercial kits is as sensitive as the method developed in our laboratory for the detection of the inactivated influenza A/Vietnam/TG24-01/2005 (H5N1) virus. However, the sequence of primers used is unknown. For this reason, the verification of the efficacy of these primers for the detection of emerging influenza A/H5 viruses is impossible. This kit could be used eventually for a confirmation of analyses, but in parallel with a test developed and adapted under laboratory conditions.



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Annex 1: Detection of respiratory viruses during the 2005/06 season

MC-ILI: proportion of medical consultations for influenza-like illness; influ A: influenza A; not type: influenza A which could not be sub-typed. Influ. B: influenza B

Weeks			Total samples	Flu A			Flu B				Total	Incidence %	Total IA	RT-PCR	Culture	% IA	% IB
				Undet.	A (H3N2)	A (H1N1)	Undet.	Malay.	Jiangsu	Total							
39	24.sept.05	30.sept.05	7								**0**	0	0	0	0	0	0
40	01.oct.05	07.oct.05	12								**0**	0	0	0	0	0	0
41	08.oct.05	14.oct.05	14								**0**	0	0	0	0	0	0
42	15.oct.05	21.oct.05	15			**1**					**1**	7	1	1	1	100	0
43	22.oct.05	28.oct.05	13								**0**	0	0	0	0	0	0
44	29.oct.05	04.nov.05	16				**1**			**1**	**1**	6	0	1	0	0	100
45	05.nov.05	11.nov.05	15				**1**			**1**	**1**	7	0	1	0	0	100
46	12.nov.05	18.nov.05	14								**0**	0	0	0	0	0	0
47	19.nov.05	25.nov.05	16								**0**	0	0	0	0	0	0
48	26.nov.05	02.déc.05	24								**0**	0	0	0	0	0	0
49	03.dec.05	09.dec.05	16								**0**	0	0	0	0	0	0
50	10.dec.05	16.dec.05	23								**0**	0	0	0	0	0	0
51	17.dec.05	23.dec.05	19								**0**	0	0	0	0	0	0
52	24.dec.05	30.dec.05	10								**0**	0	0	0	0	0	0
1	31.dec.05	06.janv.06	17					**1**		**1**	**1**	6	0	1	1	0	100
2	07.janv.06	13.janv.06	22			**1**					**1**	5	1	1	1	100	0
3	14.janv.06	20.janv.06	18	**1**				**1**		**1**	**2**	11	1	2	1	50	50
4	21.janv.06	27.janv.06	26		**2**			**2**	**2**	**4**	**6**	23	2	6	6	33	67
5	28.janv.06	03.febr.06	23		**1**		**3**	**3**	**2**	**8**	**9**	39	1	9	5	11	89
6	04.febr.06	10.febr.06	35		**2**		**2**	**3**	**2**	**7**	**9**	26	2	8	7	22	78
7	11.febr.06	17.febr.06	52		**2**	**1**	**1**	**14**	**3**	**18**	**21**	40	3	21	14	14	86
8	18.febr.06	24.febr.06	50		**2**	**1**	**3**	**23**	**6**	**32**	**35**	70	3	35	28	9	91
9	25.febr.06	03.mars.06	42		**2**	**1**		**14**	**2**	**16**	**19**	45	3	19	19	16	84
10	04.mars.06	10.mars.06	49	**1**	**4**	**2**	**4**	**12**	**3**	**19**	**26**	53	7	26	17	27	73
11	11.mars.06	17.mars.06	52	**1**	**7**		**1**	**15**	**6**	**22**	**30**	58	8	30	25	27	73
12	18.mars.06	24.mars.06	58	**1**	**3**		**4**	**17**	**6**	**27**	**31**	53	4	30	27	13	87
13	25.mars.06	31.mars.06	41			**2**	**5**	**16**	**2**	**23**	**25**	61	2	25	22	8	92
14	01.avr.06	07.avr.06	26		**1**	**2**	**1**	**5**		**6**	**9**	35	3	9	8	33	67
15	08.avr.06	14.avr.06	19	**2**	**1**	**3**	**1**	**3**		**4**	**10**	53	6	10	5	60	40
16	15.avr.06	21.avr.06	1						**1**	**1**	**1**	100	0	1	1	0	100
			745	**6**	**27**	**14**	**27**	**129**	**35**		**238**			**236**	**188**		
				47			**191**										

Annex 2: IHA titre obtained for the influenza B viruses

In red: strains sent to MRC London for further characterisation.

Seq N°	Dte sample	Virus	Typisation	B/Shan	B/Brisb	B/Jiang	B/Shang	B/Malay
4745	25-janv-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
4746	25-janv-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	256	<8
5129	03-fév-06	Influenza B	InfB Jiangsu/10/03	<8	<8	256	256	<8
5174	06-fév-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6063	27-fév-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6065	27-fév-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6131	28-fév-06	Influenza B	InfB Jiangsu/10/03	<8	<8	256	128	<8
6406	08-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	256	256	<8
6630	14-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	256	256	<8
6632	14-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6637	14-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6643	14-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6687	15-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6689	15-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6795	17-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6895	21-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6897	21-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
6907	21-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
7015	23-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
7050	24-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
7058	24-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	256	256	<8
7166	28-mars-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
7525	06-avr-06	Influenza B	InfB Jiangsu/10/03	<8	<8	256	128	<8
7864	20-avr-06	Influenza B	InfB Jiangsu/10/03	<8	<8	128	128	<8
4081	06-janv-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
4469	17-janv-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
4718	24-janv-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
5069	02-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5128	03-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	64
5167	06-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5272	**08-fév-06**	**Influenza A&B**	**InfB Malaysia/2506/04**	256	16	<8	<8	64
5340	09-fév-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
5425	10-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5660	16-fév-06	Influenza B	InfB Malaysia/2506/04	512	64	<8	<8	128
5662	16-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5665	16-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5666	16-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5667	16-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5675	16-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5676	16-fév-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
5730	17-fév-06	Influenza B	InfB Malaysia/2506/04	256	<8	<8	<8	64
5777	20-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5834	21-fév-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
5838	21-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5841	21-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5842	21-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128

Seq N°	Dte sample	Virus	Typisation	B/Shan	B/Brisb	B/Jiang	B/Shang	B/Malay
5843	21-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5844	21-fév-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	64
5845	21-fév-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
5846	21-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5856	21-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5897	22-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5904	22-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5974	23-fév-06	Influenza B	InfB Malaysia/2506/04	256	64	<8	<8	64
5979	23-fév-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
5981	23-fév-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
5982	23-fév-06	Influenza B	InfB Malaysia/2506/04	256	64	<8	<8	64
6006	24-fév-06	Influenza B	InfB Malaysia/2506/04	128	32	<8	<8	32
6007	24-fév-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
6060	27-févr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	64
6061	27-févr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6122	28-févr-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
6123	28-févr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6126	28-févr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6129	28-févr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	64
6130	28-févr-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	64
6183	01-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
6186	01-mars-06	Influenza B	InfB Malaysia/2506/04	512	64	<8	<8	256
6187	01-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6216	02-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6218	02-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6220	02-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6225	02-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6229	02-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6252	03-mars-06	Influenza B	InfB Malaysia/2506/04	256	512	<8	<8	512
6299	06-mars-06	Influenza B	InfB Malaysia/2506/04	256	256	<8	<8	512
6305	06-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
6361	07-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6364	07-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
6369	07-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6370	07-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6404	08-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	128
6415	08-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6417	08-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6467	09-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6474	09-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6475	09-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	128
6509	10-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	64
6581	13-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6584	13-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
6627	14-mars-06	Influenza B	InfB Malaysia/2506/04	256	<8	<8	<8	64
6691	15-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
6754	16-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
6756	16-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6757	16-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128

Seq N°	Dte sample	Virus	Typisation	B/Shan	B/Brisb	B/Jiang	B/Shang	B/Malay
6759	16-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
6793	17-mars-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
6797	17-mars-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
6851	20-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
6857	20-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6896	21-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6900	21-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
6909	21-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6910	21-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
6911	21-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7013	23-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7020	23-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
7051	24-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
7057	24-mars-06	Influenza B	InfB Malaysia/2506/04	512	16	<8	<8	128
7104	27-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7107	27-mars-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
7160	28-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7165	28-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7172	28-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	128
7173	28-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7195	29-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7201	29-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7204	29-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7274	30-mars-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
7280	30-mars-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7281	30-mars-06	Influenza B	InfB Malaysia/2506/04	256	32	<8	<8	128
7282	30-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7328	31-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7331	31-mars-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7352	03-avr-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7354	03-avr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7429	04-avr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
7592	10-avr-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7756	13-avr-06	Influenza B	InfB Malaysia/2506/04	256	16	<8	<8	64
7759	13-avr-06	Influenza B	InfB Malaysia/2506/04	512	32	<8	<8	128
4970	31-janv-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
5775	20-févr-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
5778	20-févr-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	32
5835	21-févr-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
6009	24-févr-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
6221	02-mars-06	Influenza B	InfB Shandong/7/97	512	<8	<8	<8	64
6264	03-mars-06	Influenza B	InfB Shandong/7/97	512	<8	<8	<8	64
6635	14-mars-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
6645	14-mars-06	Influenza B	InfB Shandong/7/97	256	16	<8	<8	32
6755	16-mars-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64

Seq N°	Dte sample	Virus	Typisation	B/Shan	B/Brisb	B/Jiang	B/Shang	B/Malay
6937	22-mars-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	32
6938	22-mars-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	16
7018	23-mars-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
7022	23-mars-06	Influenza B	InfB Shandong/7/97	512	16	<8	<8	64
7024	23-mars-06	Influenza B	InfB Shandong/7/97	512	16	<8	<8	64
7105	27-mars-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	32
7279	30-mars-06	Influenza B	InfB Shandong/7/97	256	16	<8	<8	32
7420	04-avr-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
7423	04-avr-06	Influenza B	InfB Shandong/7/97	512	16	<8	<8	64
7430	04-avr-06	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
7526	06-avr-06	Influenza B	InfB Shandong/7/97	512	16	<8	<8	64
7800	18-avr-06	Influenza B	InfB Shandong/7/97	256	32	<8	<8	32
7926	24-avr-2006	Influenza B	InfB Shandong/7/97	512	32	<8	<8	64
5236	07-févr-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
5270	08-févr-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
5663	16-févr-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
5707	16-févr-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
5858	21-févr-06	Influenza B	InfB Shanghai/361/02	**<8**	**<8**	**128**	**256**	**<8**
5859	21-févr-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
6010	24-févr-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
6306	06-mars-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
6513	10-mars-06	Influenza B	InfB Shanghai/361/02	<8	<8	256	256	<8
6903	21-mars-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
7196	29-mars-06	Influenza B	InfB Shanghai/361/02	<8	<8	128	256	<8
9208	07-nov-05	Influenza B	IB en PCR					
5067	02-févr-06	Influenza B	IB en PCR					
5131	03-févr-06	Influenza B	IB en PCR					
5234	07-févr-06	Influenza B	IB en PCR					
5235	07-févr-06	Influenza B	IB en PCR					
5458	13-févr-06	Influenza B	IB en PCR					
5585	15-févr-06	Influenza B	IB en PCR					
5976	23-févr-06	Influenza B	IB en PCR					
6008	24-févr-06	Influenza B	IB en PCR					
6058	27-févr-06	Influenza B	IB en PCR					
6365	07-mars-06	Influenza B	IB en PCR					
6416	08-mars-06	Influenza B	IB en PCR					
6465	09-mars-06	Influenza B	IB en PCR					
6516	10-mars-06	Influenza B	IB en PCR					
6854	20-mars-06	Influenza B	IB en PCR					
6761	**16-mars-06**	**Influenza A&B**	**IA + IB**	**Co-infection**				
6908	21-mars-06	Influenza B	IB en PCR					
6935	22-mars-06	Influenza B	IB en PCR					
6945	22-mars-06	Influenza B	IB en PCR					
7054	24-mars-06	Influenza B	IB en PCR					
7198	29-mars-06	Influenza B	pas de typisation					
7277	30-mars-06	Influenza B	IB en PCR					

Seq N°	Dte sample	Virus	Typisation	B/Shan	B/Brisb	B/Jiang	B/Shang	B/Malay
7329	31-mars-06	Influenza B	IB en PCR					
7428	04-avr-06	Influenza B	IB en PCR					
7514	06-avr-06	Influenza B	IB en PCR					
7524	06-avr-06	Influenza B	IB en PCR					
7794	18-avr-06	Influenza B	IB en PCR					
9208	07-nov-05	Influenza B	IB en PCR					
9355	10-nov-05	Influenza B	IB en PCR					

Annex 3: IHA titre obtained for the influenza A (H1N1) viruses

In red: strains sent to MRC London for further characterisation.

In yellow: strains that have been detected only by RT-PCR. Influenza A type and influenza A/N1 origin have been detected by RT-PCR only and not by cell culture.

Seq N°	Ddem	DefVirus	Typisation	A/Ncal	A/Beij.	A/Neth.
8761	24-oct-05	Influenza A	InfA H1N1 N-Caled./20/99	512	32	256
4243	11-janv-06	Influenza A	InfA H1N1 N-Caled./20/99	1024	16	512
5977	23-févr-06	Influenza A	InfA H1N1 N-Caled./20/99	1024	32	512
7202	29-mars-06	Influenza A	InfA H1N1 N-Caled./20/99	1024	32	512
7327	31-mars-06	Influenza A	InfA H1N1 N-Caled./20/99	512	16	256
7425	04-avr-06	Influenza A	InfA H1N1 N-Caled./20/99	512	32	512
7426	04-avr-06	Influenza A	InfA H1N1 N-Caled./20/99	1024	64	512
7588	10-avr-06	Influenza A	InfA H1N1 N-Caled./20/99	512	32	512
7757	13-avr-06	Influenza A	InfA H1N1 N-Caled./20/99	512	32	512
6472	09-mars-06	Influenza A	InfA H1N1 Netherland/128/04	2048	128	2048
6215	02-mars-06	Influenza A	InfA H1N1 Netherland/128/04	512	64	1024
6414	08-mars-06	Influenza A	Influenza A H1N1 in PCR			
5733	17-févr-06	Influenza A	Influenza A H1N1 in PCR			
7644	11-avr-06	Influenza A	Influenza A H1N1 in PCR			

Annex 4: IHA titre obtained for the influenza A (H3N2) viruses

In red: strains sent to MRC London for further characterisation; **In green** are mentioned the strains which showed a decreased titre with influenza A (H3N2) of the 2005-06 season. These strains have been tested with influenza A/Wisconsin/67/2005 antiserum received at the end of the season; **In yellow**: strains that have been detected only by RT-PCR. Influenza A type and influenza A/N1 origin have been detected by RT-PCR only and not by cell culture.

Seq N°	Ddem	DefVirus	Typisation	A/Calif	A/Shan	A/Nyork	A/Wyo	A/Sing	A/Wis
5782	20-févr-06	Influenza A	InfA H3N2 Californ./7/04	128	128	128	64	64	
6473	09-mars-06	Influenza A	InfA H3N2 Californ./7/04	64	32	64	16	32	
6511	10-mars-06	Influenza A	InfA H3N2 Californ./7/04	128	32	64	32	64	
5415	10-févr-06	Influenza A	InfA H3N2 Californ./7/04	128	16	32		32	64
6185	01-mars-06	Influenza A	InfA H3N2 Californ./7/04	128	32	64	32	32	128
7016	23-mars-06	Influenza A	InfA H3N2 Californ./7/04	128	32	64	32	64	64
8015	26-avr-06	Influenza A	InfA H3N2 Californ./7/04	128	128	128	128	64	128
4922	30-janv-06	Influenza A	InfA H3N2 Californ./7/04	64	256	64	<8	256	
5417	10-févr-06	Influenza A	InfA H3N2 Californ./7/04	128	256	64	64	128	
6580	13-mars-06	Influenza A	InfA H3N2 Californ./7/04	64	128	32	16	64	
6850	20-mars-06	Influenza A	InfA H3N2 Californ./7/04	64	64	32	16	32	
6761	16-mars-06	Influenza A	InfA H3N2 Singapore/37/04 Co-inf. Flu B	128	64	128	64	64	
6762	16-mars-06	Influenza A	InfA H3N2 Singapore/37/04	256	64	128	64	128	
6942	22-mars-06	Influenza A	InfA H3N2 Singapore/37/04	64	32	16	64	64	
6944	22-mars-06	Influenza A	InfA H3N2 Singapore/37/04	256	256	64	128	128	
7023	23-mars-06	Influenza A	InfA H3N2 Singapore/37/04	128	64	16	64	64	
4923	30-janv-06	Influenza A	Influenza A H3N2	64	64	64	32	32	256
5456	13-févr-06	Influenza A	N2 en PCR						
6062	27-févr-06	Influenza A	N2 en PCR						
6217	02-mars-06	Influenza A	N2 en PCR						
6412	08-mars-06	Influenza A	N2 en PCR						
6899	21-mars-06	Influenza A	Influenza A H3N2	32	16	32	<8	16	64
7161	28-mars-06	Influenza A	**A/Annecy/1138/05**	64	32	32	16	32	64
6128	28-févr-06	Influenza A	N2 en PCR						
6639	14-mars-06	Influenza A	N2 en PCR						
6912	21-mars-06	Influenza A	N2 en PCR						
7840	20-avr-06	Influenza A	N2 en PCR						
5272	08-févr-06	Influenza A	Coinf. InfB Malaysia/2506/04			-			

Annex 5: HA alignment of influenza A (H5N1) viruses detected in 2005 and 2006

Mismatches observed in the hemagglutinin of the influenza A (H5N1) virus detected in birds in Switzerland, France and Germany are coloured **in red**