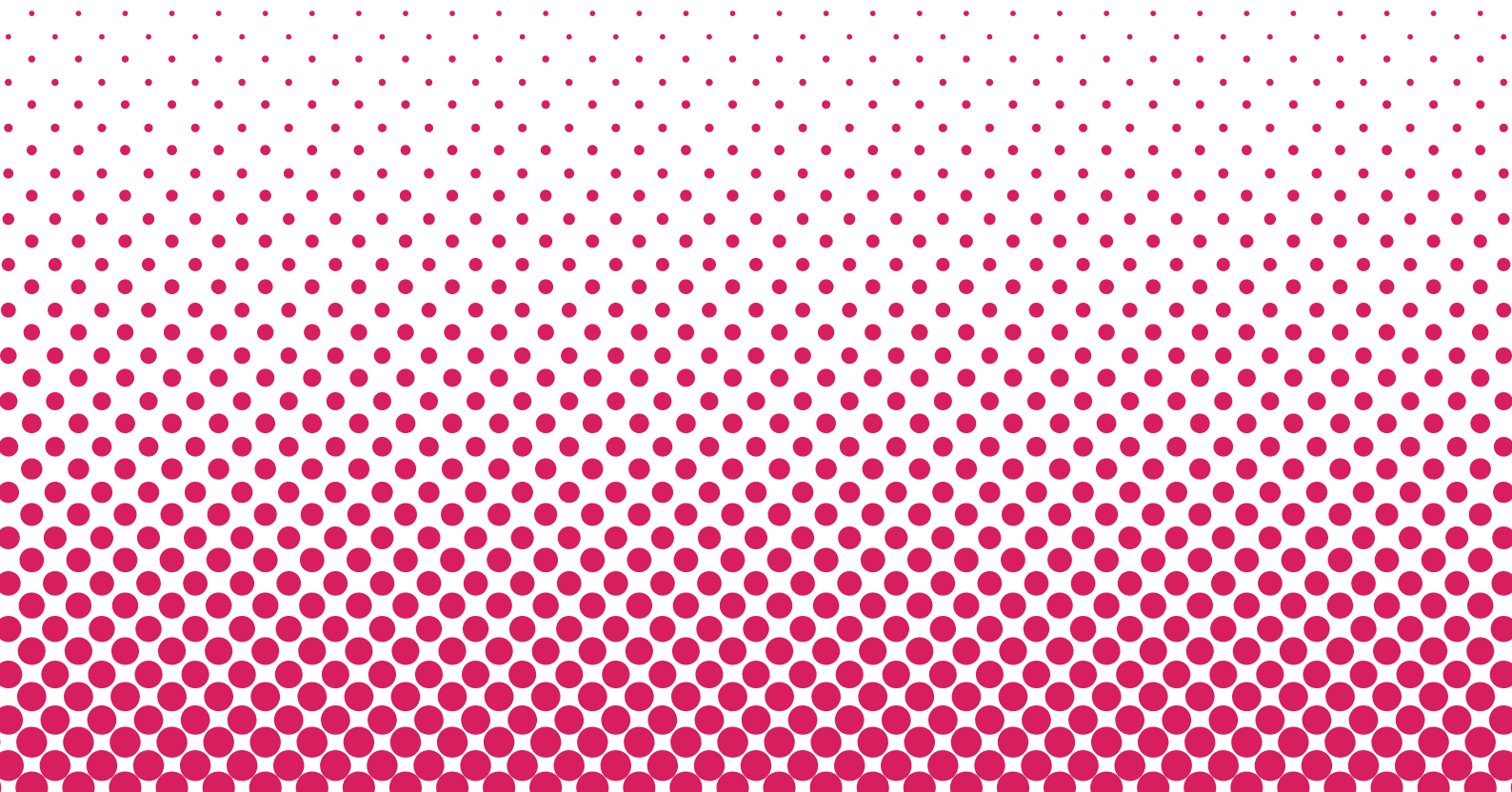


Influenza

Annual Report 2008-2009



The National Influenza Reference Centre
Swedish Institute for Communicable
Disease Control (SMI)

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Contents

1. Monitoring of the influenza activity in Sweden
2. Reports from SMI on the influenza activity in Sweden
3. Characterisation of influenza strains
4. Data from the 2008–2009 season
5. Quality control of laboratory diagnosis of influenza
6. Research and method development

1) MONITORING OF THE INFLUENZA ACTIVITY IN SWEDEN

1:1 Sentinel Surveillance

The Swedish influenza sentinel reporting system 2008–2009 consisted of 102 sentinel units recruited by the County Medical Officers of Communicable Disease Control, including both individual GPs and larger health care centres. All twenty-one counties participated in the surveillance. Date of visit, age and sex of the patients were reported. The reports were sent to the County Medical Officers and to the National Influenza Centre at the Swedish Institute for Infectious Disease Control (SMI), either by fax or by the web-based reporting system “SentiNet”. Each week reports were received from between 28 and 70 of the sentinel units. The total weekly number of out-patient visits at the reporting units ranged from 4112 to 13523. The total number of persons listed by the involved units can’t be given exactly due to the structure of the Swedish health care system, but ranges between 296000 and 53000.

1:2 The Sentinel Sampling System

Sentinel sampling was introduced during season 2006-2007. During the 2008-2009 season, a total of 62 units consisting of sentinel units, infectious disease clinics and paediatric clinics participated. Of these 17 were also reporting to the surveillance system. Thus, not all who samples participated in 1:1. Geographically, the units were evenly distributed throughout Sweden. All Swedish counties were included. During 32 weeks, 1778 samples were received and analysed at SMI. Nine to 112 samples were received per week. The primary diagnostic tool was real-time PCR for influenza type and influenza A subtypes. From week 18 an assay specific for the new pandemic influenza was included, but the pandemic activities will be reported separately. A swabbing protocol for each sample, according to the minimum requirements from the European Influenza Surveillance system (EISS), was completed by the clinicians. Approximately 20 % of the samples analysed were positive for influenza.

1:3 Reports of laboratory verified influenza diagnoses

During the influenza season the 25 laboratories sent weekly reports on the number of influenza cases, diagnosed by antigen detection, nucleic acid amplifications (NAA) and/or virus isolation. Influenza isolation was performed at three virus laboratories, placed at University Hospitals and at SMI. The laboratories that did not perform virus isolation sent representative patient samples to SMI for isolation. Influenza strains were isolated from all regions in Sweden.

1:4 Death rates

Information on the weekly death rate in Sweden was purchased from Statistics Sweden. Mean weekly death rate for each influenza-free set of weeks with the same number between week 40 year 1993 and week 20 year 2009 was calculated, and was used as reference for the evaluation of weekly excess mortality.

2) REPORTS FROM SMI ON THE INFLUENZA ACTIVITY IN SWEDEN

2:1 Weekly reports to the Swedish Collaborators

National and international influenza information, including the WHO and EISS country reports, was collected and summarised in a weekly report. The report was made available at the SMI web-page on Thursday (<http://www.smi.se>). An electronic Newsletter was also sent to all interested parties, including the County Medical Officers of Communicable Disease Control, Departments of Infectious Diseases in Sweden, Microbiological Laboratories and to the National Board of Health and Welfare. A summary of the activity during the entire season was distributed to all parties at the end of the summer, when all definitive data were available.

2:2 Other spread of information in Sweden

Media is constantly interested in influenza, and usually contacts SMI to get information. Generally these contacts resulted in correct and informative articles. The institute has a journal "Smittskydd" and an electronic newspaper "EpiAktuellt", where reports of the season on the influenza situation are published when appropriate. An information day for influenza collaborators was arranged in September 2008.

2:3) Reports to WHO and other National Influenza centres

The Influenza reporting usually starts at week 40 or when the first laboratory verified case occurs. Since May 2009 all European Influenza reporting has been transferred to ECDC:s database TESSy. During the whole season 2008-2009 the Swedish Influenza data were also reported weekly to EuroFlu, WHO.

3) CHARACTERISATION OF INFLUENZA STRAINS

3:1 Genotypic and phenotypic characterisation

Virus strains isolated by SMI or sent to SMI from other laboratories were examined for type and subtype of virus by IF with monoclonal antibodies (WHO, Chemicon) or real-time PCR. HA, NA, NS and M2-sequencing were also performed. For further characterisation with ferret sera, the strains were sent to Mill Hill in London. All the influenza strains were also investigated genotypically and/or phenotypically for antiviral resistance to amantadine and neuraminidase inhibitors.

4) DATA FROM SEASON 2008-2009

4:1 Summary of influenza activity in Sweden

The first laboratory verified influenza cases and the first six ILI cases reported by sentinel physicians were reported in week 40 2008, but were isolated cases. A rather early and intense A3 epidemic hit above all the elderly peaking week 4 2009. It was spread all over the country, and the peak was the highest noted in the laboratory reporting between 2004 and 2009 while the proportion in the sentinel-reporting was less impressive. The many severely ill elderly, not presenting at GPs but at hospitals is the likely main explanation for this discrepancy (see table 4:1 and 4:2). During week 50 to 13, sporadic A(H1N1) strains were detected in the sentinel-system. Sporadic influenza B activity occurred in the end of March. The total number of laboratory diagnoses was 2054 (1926 A and 128 B) compared to 1246 (166 A and 780 B) the previous season. The proportion of influenza A (96%) highly exceeded the number of influenza B (4%).

All samples collected in the sentinel sampling system were analysed with molecular methods. A total of 1778 samples collected during the season were typed and subtyped for influenza. 346/1778 (19,5%) became positive (Fig 4:1:4). During this season, the majority were influenza A (96%, whereof 86% A H3 and 14% A H1) and 4% influenza B..

Fig 4:1:1 Laboratory verified cases of influenza A and B during the 2008-2009 influenza season.

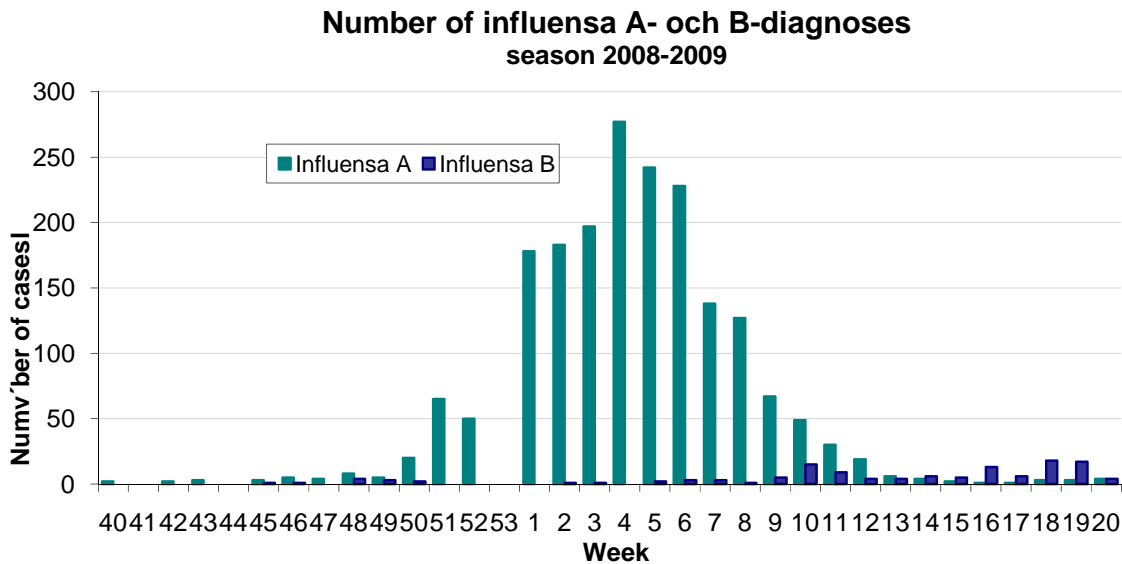


Fig 4:1:2 Number of laboratory verified influenza cases

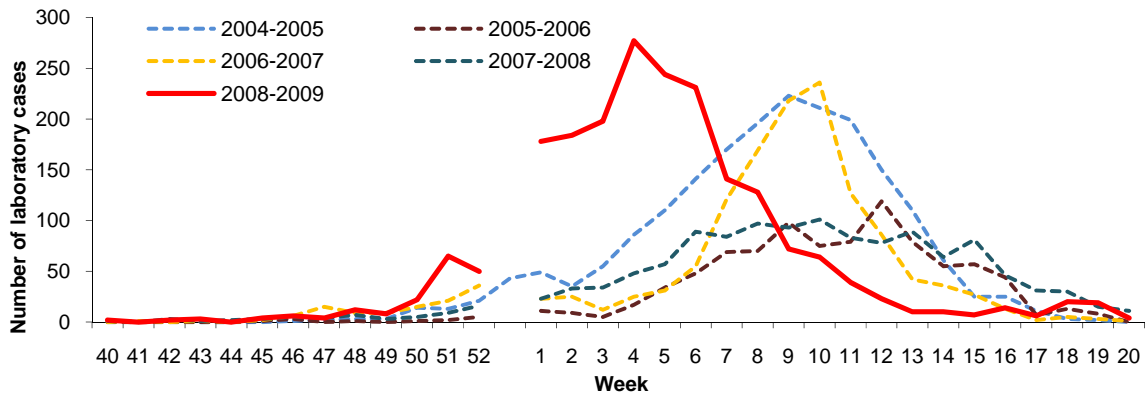


Fig 4:1:3 Proportion (%) cases with influenza-like illness (ILI) out of total number of patient visits in the sentinel system

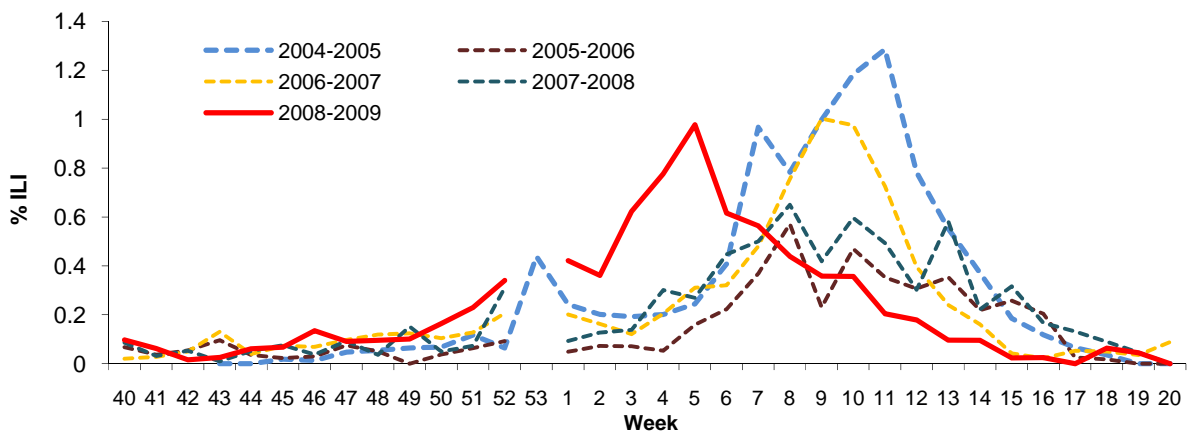
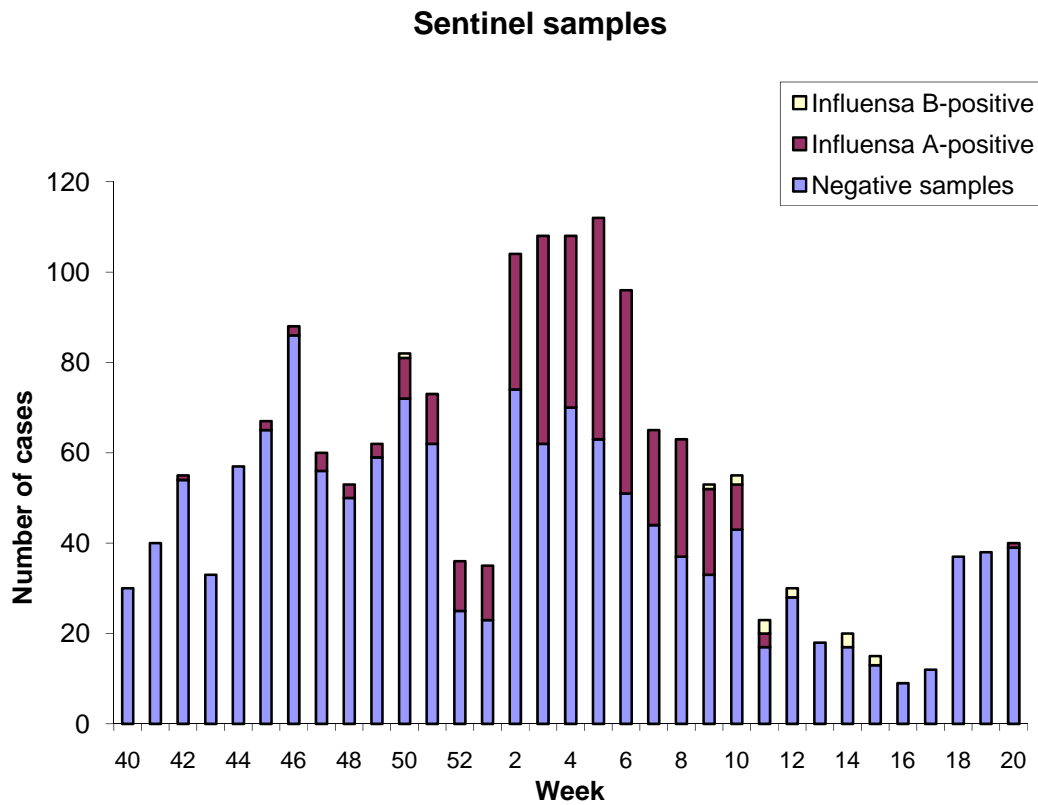


Fig 4:1:4 Number of samples and verified influenza cases in the sentinel sampling system.



4:2 Age distribution in the laboratory and sentinel systems

The age distribution in the two systems reflects that GPs practices, reporting to the sentinel system, mostly cover only otherwise healthy adults. The young children and elderly often get more severely ill and seek hospital care directly if they need medical attention. Usually the specimens are drawn from these more seriously ill patients. The observed distribution of the laboratory cases for the season 2008-09 was considerably higher in the +65 than expected, if the cases had been evenly distributed in relation to the population. 738 persons above 65 were diagnosed with influenza, compared to 356 expected. In the sentinel system, the majority of reported cases were between 15 and 65 years old as usually in Sweden (Table 4:1 and 4:2).

The weekly incidences in the two systems analysed in relation to the expected number of cases with regard to the size of respective populations is shown (Fig 4:2:3 and 4:2:4).

Table 4:1 Laboratory verified cases by age group during season 2008-09.

“Expected cases” is the number of cases that would have occurred if they had been evenly distributed in relation to the population of the respective age groups.

Age group	Observed cases	Expected cases
0-4	167	115
5-14	145	233
15-64	999	1345
65+	738	356
Total	2049	2049

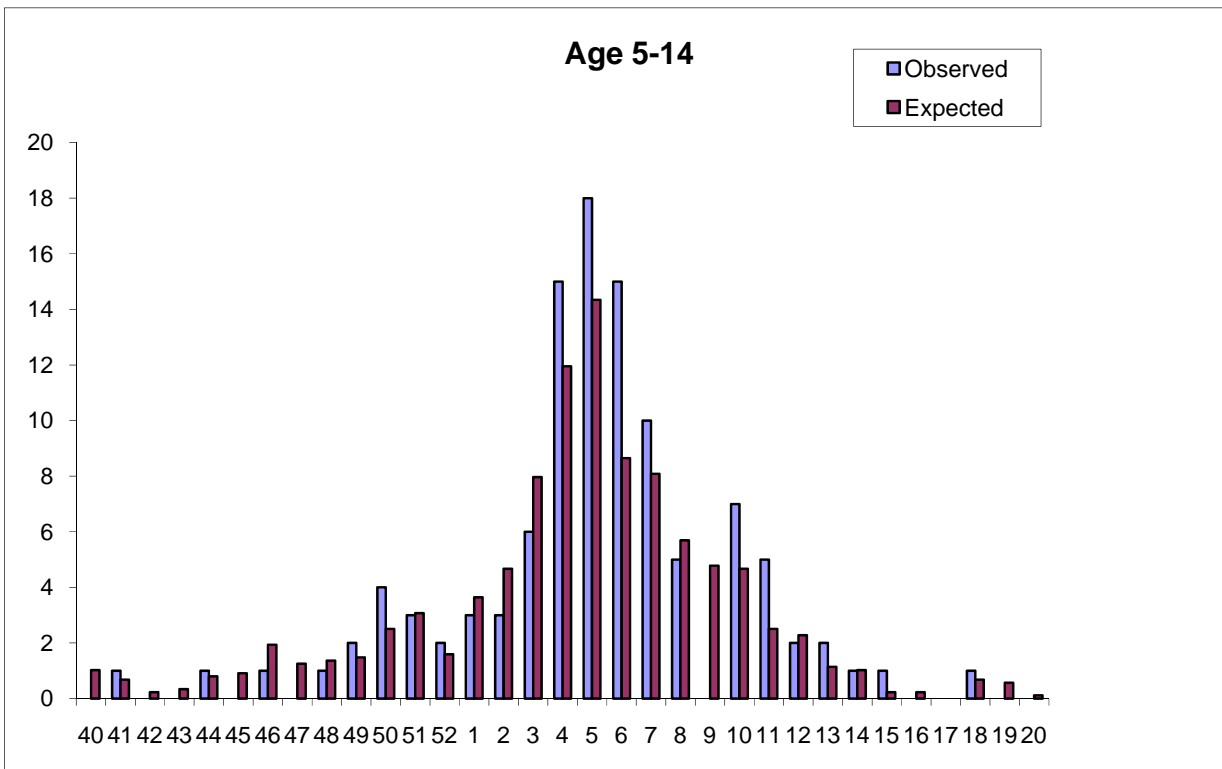
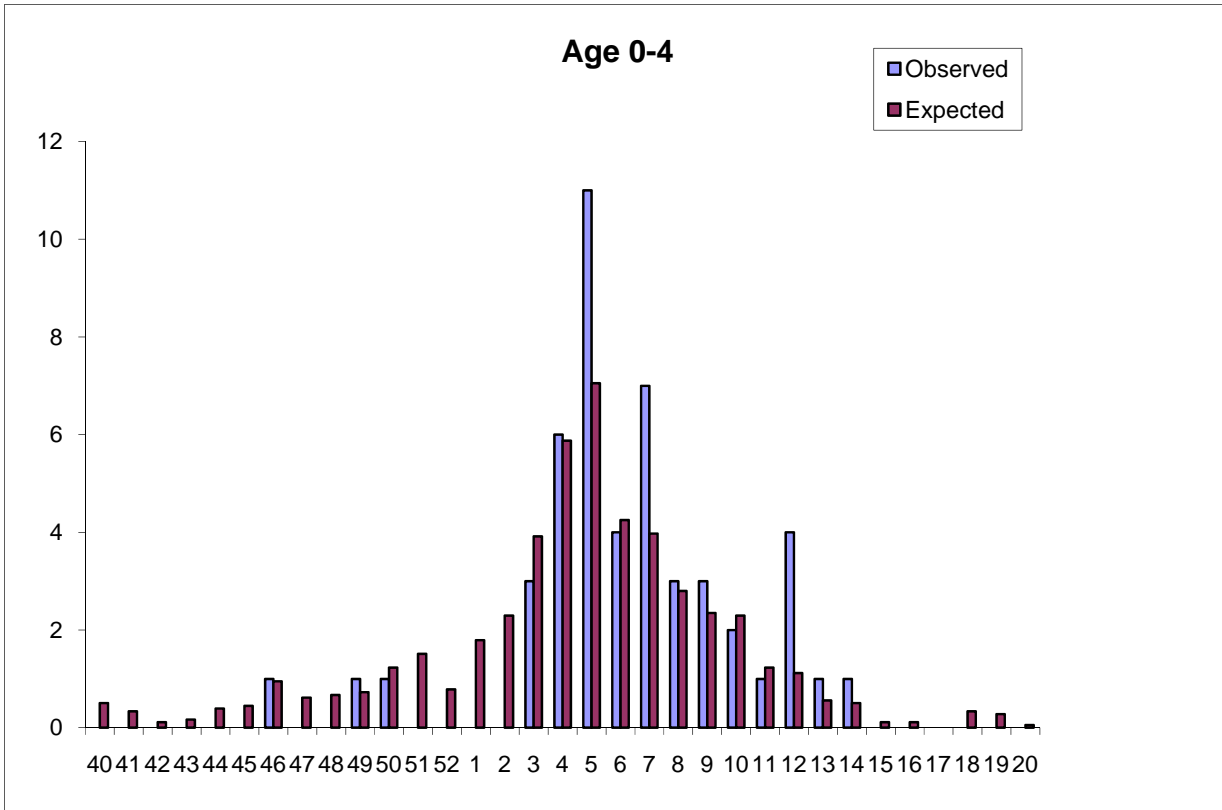
Table 4:2 ILI cases by age group.

“Expected cases” is the number of cases that would have occurred if they had been evenly distributed in relation to the population of the respective age groups.

Age group	Observed cases	Expected cases
0-4	49	49
5-14	109	100
15-64	628	579
65+	96	153
Total	882	882

Fig 4:2:3 Age distribution of ILI cases during season 2008-09

"Expected" is the number of cases that would have occurred if the cases were evenly distributed in relation to the population of the respective age groups.



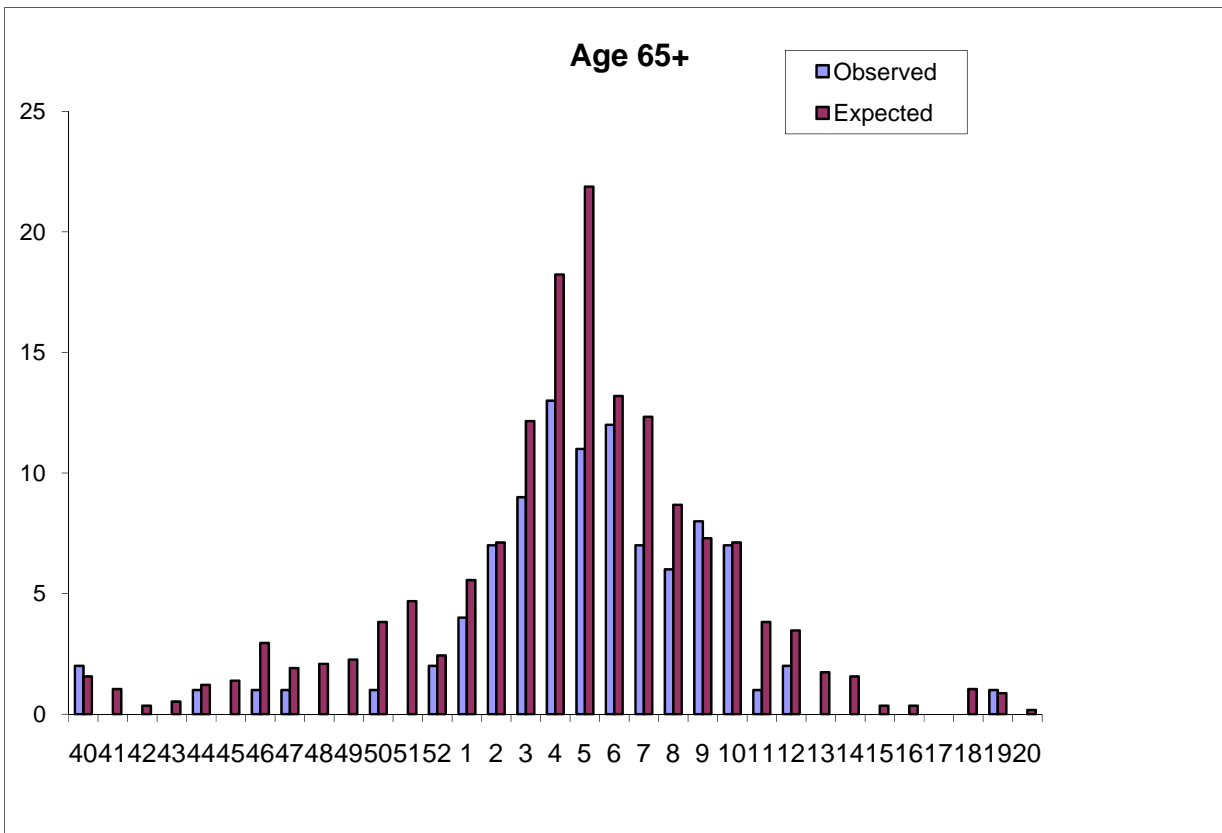
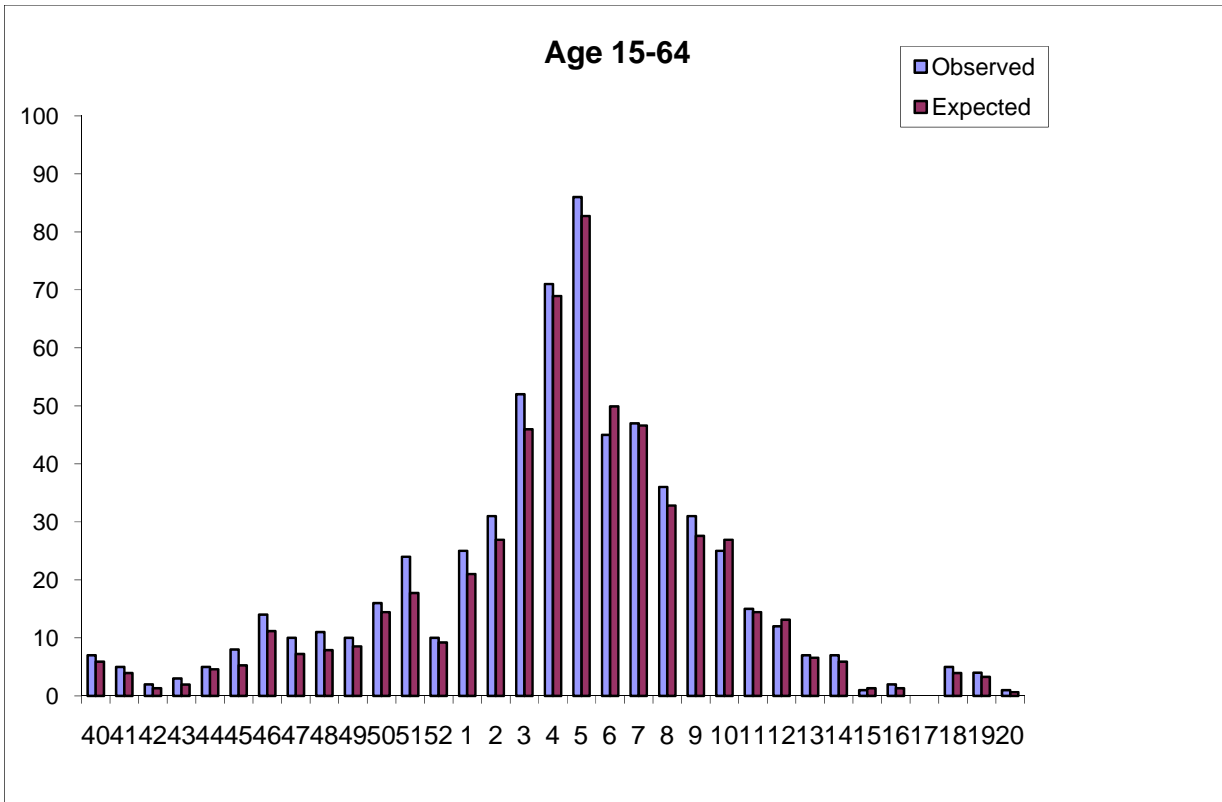
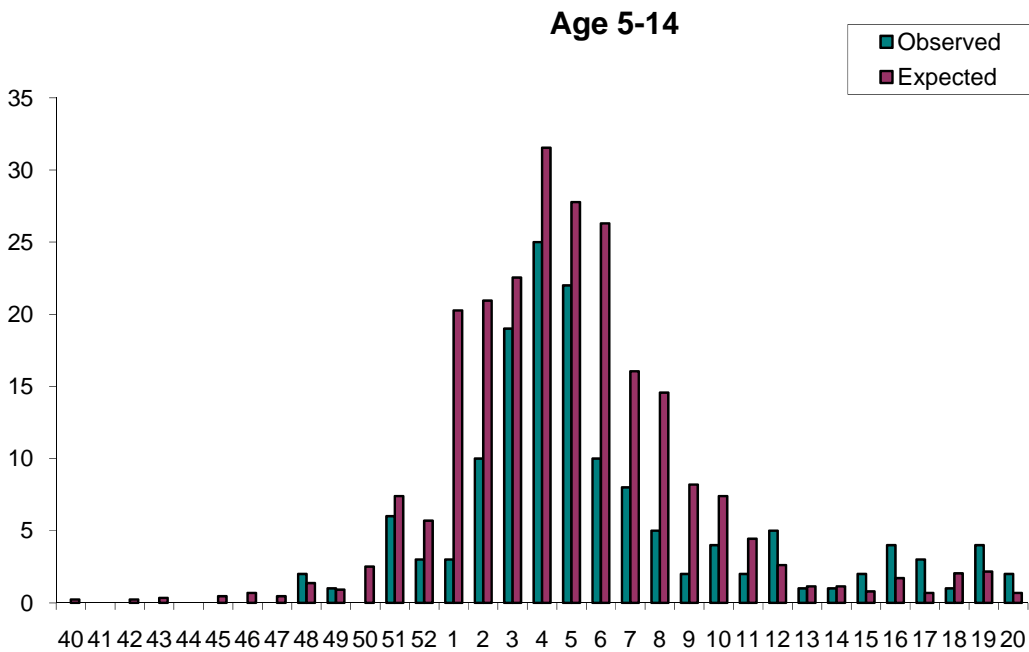
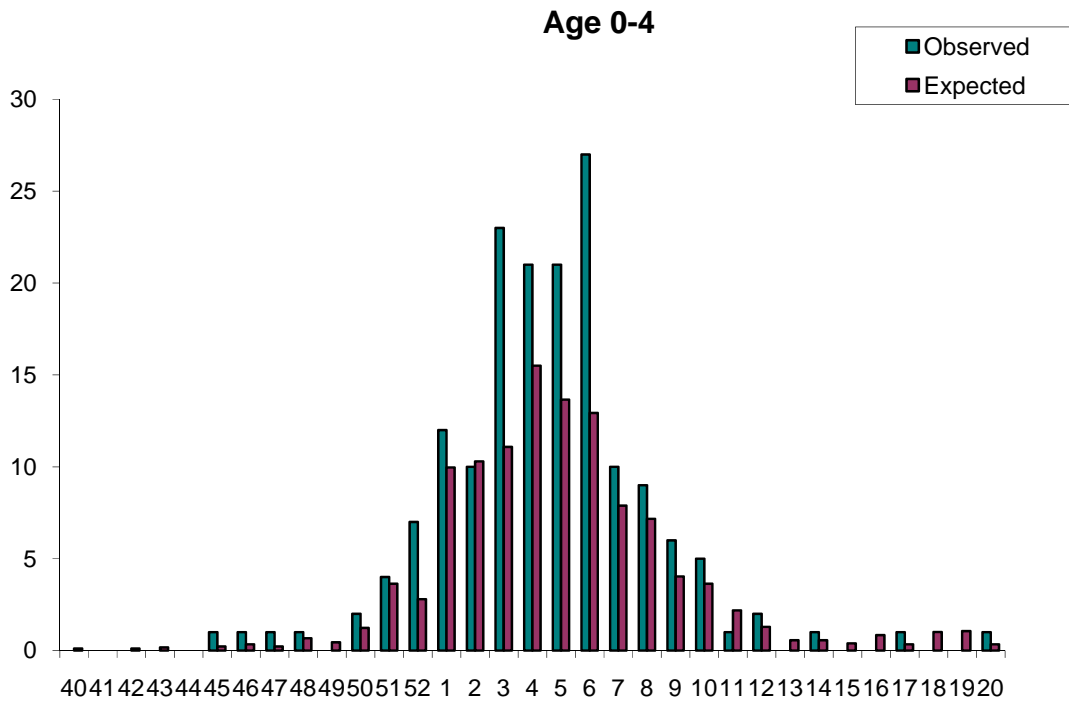
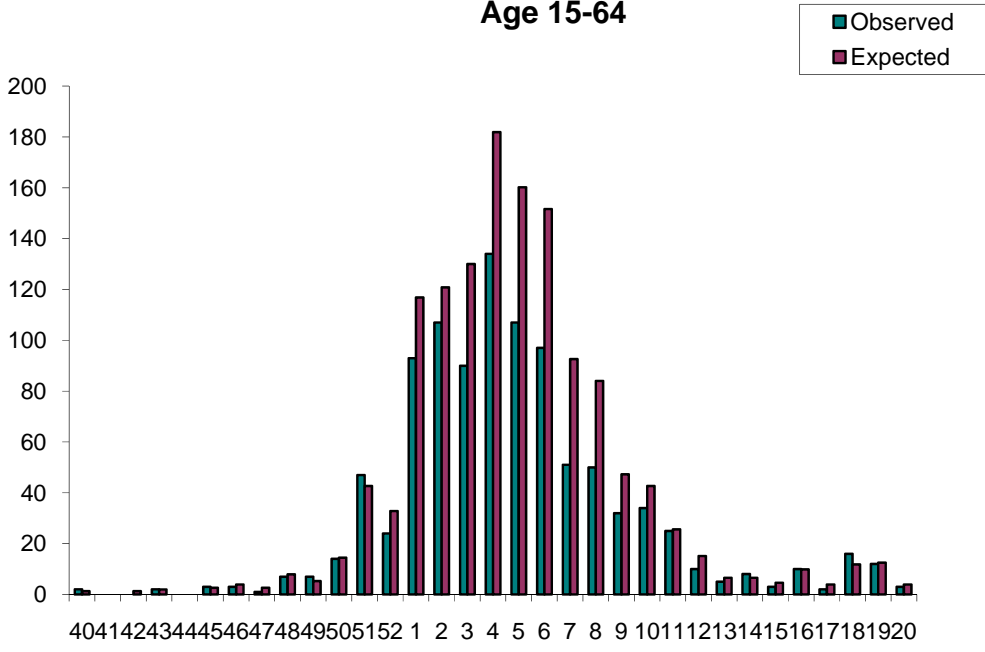


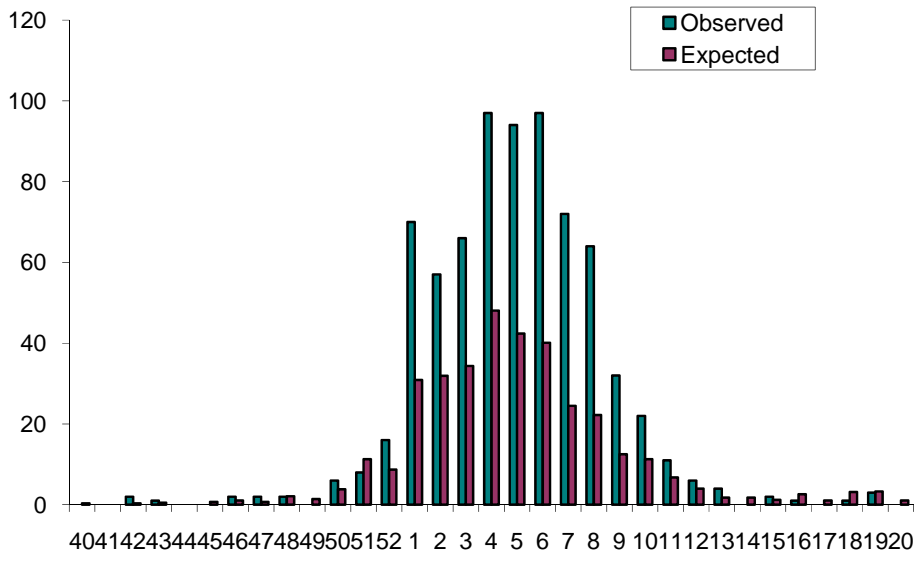
Fig 4:2:4 Age distribution of laboratory verified cases during season 2008-09
"Expected" means the number of expected cases in relation to the whole population.



Age 15-64



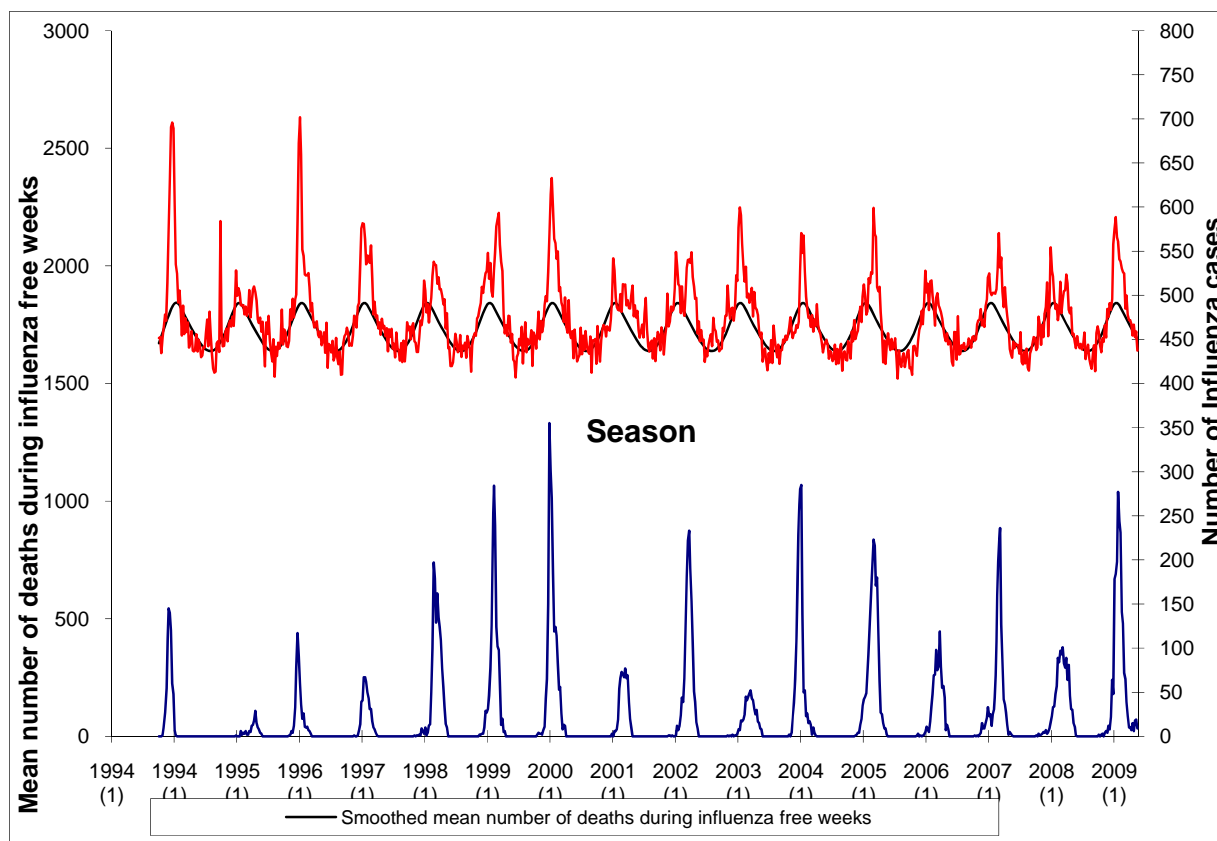
Age 65+



4:3 Estimated excess mortality

There was excess mortality related to the influenza activity, and the area above the mortality curve that has been normalised for absence of influenza activity (red – black in fig 4:3:1) was around 1000 persons. According to a scientific modelling, including both the effect of other infections and temperature, the excess mortality related to influenza was 1640 (Andersson M. Et al; manuscript2011).

Fig 4:3:1 The weekly number of deaths in Sweden from week 40, 1993 to week 20, 2009 (red), and the number of laboratory verified influenza cases during the same period (blue). (The peak of mortality in September 1994 reflects the Estonia ferry catastrophe). Adjusted means for corresponding influenza-free weeks are also included (black).



4:4 Characterisation of influenza strains

Of strains sent from other laboratories to SMI for further characterising and sentinelsamples, 14/64 (22 %) were influenza A/H1N1, 4/64 (3%) pandemic A/H1N1,42/64 (66%) A/H3N2, and 4/64 (6%) influenza B (table 4:4). During the period (week 50-13) when seasonal H1N1 was circulating SMI requested samples from the Swedish diagnostic laboratories to be able to monitor the oseltamivir

resistant H1N1. Thirty samples were sent from one laboratory. The samples were isolated and analysed in real-time PCR. All of them were A/H3 and no further analyses were made.

All the influenza strains characterised were similar to the strains prevalent in Europe. The vaccine used in A/Brisbane/10/2007 (H3N2) and B/Florida/4/2006 (B/Yamagata/16/1988-like). Twelve of the A/H1N1 strains were similar to A/Brisbane/59/2007 (clade 2B) and two strains were similar to A/Hong Kong/2652/2006 (clade 2C) (Fig 4:4:1). All 45 A/H3N2 strains were similar to A/Brisbane/10/2007 (Fig 4:4:2 and 4:4:3). The four influenza B strains characterised belonged to the B/Victoria/2/1987-like lineage (all were similar to clade repr. B/England/393/2008) and no one was similar to the vaccine B/Florida/4/2006 (Fig 4:4:4). The vaccine strains for season 2009-10 are A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2) and B/Brisbane/60/2008 (B/Victoria/2/1987-like) The two pandemic A/H1N1 strains were similar to the A/California/7/2009 (Fig 4:4:5).

An increased frequency of A/H3N2 strains resistant to amantadine due to mutations in the M2-gene was noted during previous seasons, and characterisation for amantadine resistance was therefore performed. All 45 characterised A/H3N2 and the two pandemic A/H1N1 strains from the 2008-09 season were resistant. One of the 14 A/H1N1 (seasonal) strains was also amantadine resistant.

The increased incidence of oseltamivir resistant A/H1N1 (seasonal) due to H274Y alteration in the N1 gene continues in 2008-09. We found this alteration in 13 out of 14 (93%) characterised strains. During the 2007-08 season the alteration was seen in 4 out of 36 (11%) characterised strains. For A/H3N2 strains a mixture of D or N in position D151 in the N2 gene was seen in 6 of the 45 characterised strains, and mutation D151E were seen in two. These mutations have been reported to induce reduced sensitivity for oseltamivir. However, when three of these strains (two with mixture of D/N and one E in position 151) were analysed with phenotypic assay (MUNANA) none of them become an outlier. No mutation known to induce resistance against neuraminidase inhibitors in the genes encoding the neuraminidases for pandemic influenza A/N1 or B were identified. The neuraminidase inhibition assay was performed for oseltamivir and zanamivir and showed same results as the genotypic results.

Table 4:4) Table of isolates for which extended pheno- and genotyping were performed.

Strain	Sample collection day (dd/mm/YYYY)	Subtype	Information	Origin
A/Stockholm/23/2008	12/10/2008	H3		Gothenburg
A/Stockholm/24/2008	03/10/2008	H3		Stockholm
A/Stockholm/25/2008	24/10/2008	H3		Karlstad
A/Stockholm/22/2008	04/11/2008	H1		Malmoe
A/Stockholm/26/2008	12/11/2008	H3		Stockholm
A/Stockholm/27/2008	08/12/2008	H3		Uddevalla
A/Stockholm/1/2009	07/01/2009	H1		Halmstad
A/Stockholm/2/2009	14/01/2009	H3		Sundsvall
A/Stockholm/9/2009	02/01/2009	H3		Stockholm
A/Stockholm/6/2009	05/01/2009	H1		Stockholm
A/Stockholm/10/2009	05/01/2009	H3		Stockholm
A/Stockholm/11/2009	07/01/2009	H3		Stockholm
A/Stockholm/12/2009	07/01/2009	H3		Stockholm
A/Stockholm/13/2009	07/01/2009	H3		Stockholm
A/Stockholm/22/2009	08/01/2009	H3		Stockholm
A/Stockholm/7/2009	08/01/2009	H1		Stockholm
A/Stockholm/14/2009	08/01/2009	H3		Stockholm
A/Stockholm/23/2009	12/01/2009	H3		Stockholm
A/Stockholm/4/2009	12/01/2009	H1		Stockholm
A/Stockholm/15/2009	12/01/2009	H3		Stockholm
A/Stockholm/3/2009	13/01/2009	H3		Stockholm
A/Stockholm/24/2009	13/01/2009	H3		Stockholm
A/Stockholm/16/2009	13/01/2009	H3		Stockholm
A/Stockholm/17/2009	14/01/2009	H3		Stockholm
A/Stockholm/18/2009	22/01/2009	H3	Vaccinated	Karlstad
A/Stockholm/19/2009	22/01/2009	H3	Vaccinated	Karlstad
A/Stockholm/20/2009	22/01/2009	H3		Vaxjo
A/Stockholm/21/2009	22/01/2009	H3	Vaccinated	Vaxjo
A/Stockholm/8/2009	05/01/2009	H1		Gothenburg
A/Stockholm/5/2009	09/01/2009	H1		Gothenburg
A/Stockholm/29/2008	08/12/2008	H1		Gothenburg
A/Stockholm/28/2008	17/12/2008	H1		Gothenburg
A/Stockholm/30/2008	18/12/2008	H1		Gothenburg
A/Stockholm/31/2008	29/12/2008	H3	Vaccinated	Gothenburg
A/Stockholm/25/2009	10/02/2009	H3		Gothenburg
A/Stockholm/26/2009	10/02/2009	H3	Vaccinated	Gothenburg
A/Stockholm/27/2009	11/02/2009	H3	Vaccinated	Gothenburg
B/Stockholm/1/2009	07/02/2009	B (Victoria/2/87 lineage)		Stockholm
B/Stockholm/2/2009	27/02/2009	B (Victoria/2/87 lineage)		Stockholm
B/Stockholm/3/2009	06/03/2009	B (Victoria/2/87 lineage)		Stockholm
A/Umeå/1/2009	23/01/2009	H3		Umea
A/Umeå/2/2009	03/02/2009	H3		Umea
A/Umeå/3/2009	01/03/2009	H3		Umea

A/Stockholm/32/2008	17/12/2008	H3		Stockholm
A/Stockholm/33/2008	22/12/2008	H3		Stockholm
A/Stockholm/46/2009	16/02/2009	H1		Stockholm
A/Stockholm/47/2009	17/02/2009	H3		Stockholm
B/Stockholm/6/2009	08/05/2009	B (Victoria/2/87 lineage)		Stockholm
A/Umeå/5/2009	04/03/2008	H3		Umea
A/Sweden/3/2008	15/10/2008	H3	Sentinel doctor	Falun
A/Sweden/4/2008	03/11/2008	H3	Sentinel doctor	Eskilstuna
A/Sweden/5/2008	02/12/2008	H3	Sentinel doctor	Horndal
A/Sweden/6/2008	08/12/2008	H3	Sentinel doctor	Uppsala
A/Sweden/7/2008	22/12/2008	H3	Sentinel doctor	Umea
A/Sweden/8/2008	09/12/2008	H1	Sentinel doctor	Anderslov
A/Sweden/9/2008	17/12/2008	H3	Sentinel doctor	Lund
A/Sweden/10/2008	18/12/2008	H1	Sentinel doctor	Lerum
A/Sweden/11/2008	19/12/2008	H3	Sentinel doctor	Skelleftea
A/Sweden/12/2008	14/12/2008	H3	Sentinel doctor	Gavle
A/Sweden/1/2009	12/01/2009	H1	Sentinel doctor	Lindome
A/Stockholm/28/2009	30/04/2009	H1 pandemic		Stockholm
A/Stockholm/29/2009	08/05/2009	H1 pandemic		Stockholm

Figure 4:4:1) The phylogenetic tree of the amino acid sequences of the HA of influenza A/H1 strains isolated in Sweden 2001-2009 compared to the vaccine strains.

Figure 4:4:2) The phylogenetic tree of the amino acid sequences of the HA of influenza A/H3 strains isolated in Sweden 1996-2009 compared to the vaccine

Figure 4:4:3) The phylogenetic tree of the amino acid sequences of the HA of influenza A/H3 strains isolated in Sweden 2004-2009 compared to the vaccine strains.

Figure 4:4:4) The phylogenetic tree of the amino acid sequences of the HA of influenza B strains isolated in Sweden 1999-2009 compared to the vaccine strains.

Figure 4:4:5) The phylogenetic tree of the amino acid sequences of the HA of pandemic influenza A/H1v strains isolated in Sweden 2009.

Figure 4:4:1)

Vaccine

Season <2000

Season 2000-2001

Season 2001-2002

Season 2002-2003

Season 2003-2004

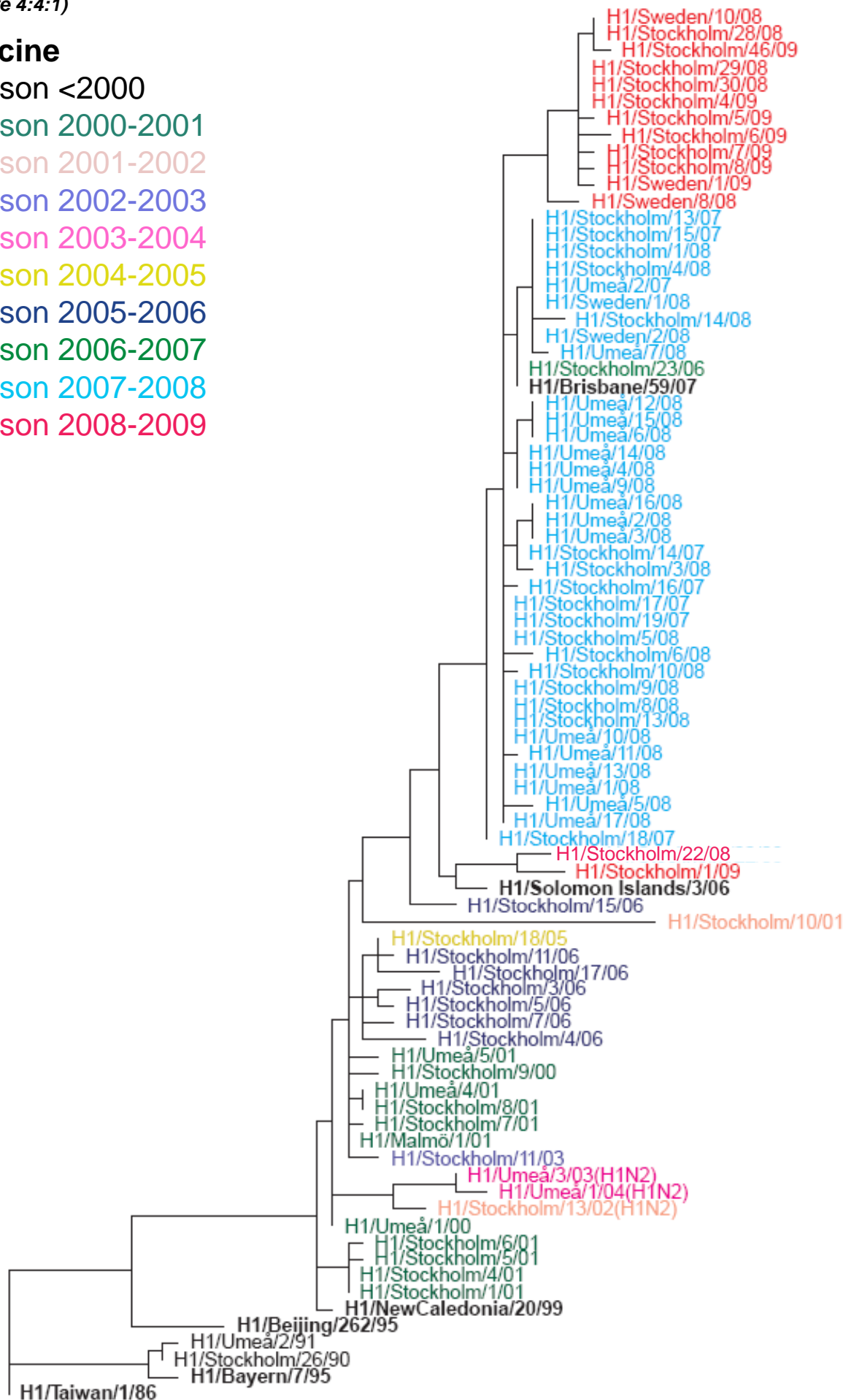
Season 2004-2005

Season 2005-2006

Season 2006-2007

Season 2007-2008

Season 2008-2009



— 1 change

Figure 4:4:2)

Vaccin

Säsong <2000

Säsong 2000-2001

Säsong 2001-2002

Säsong 2002-2003

Säsong 2003-2004

Säsong 2004-2005

Säsong 2005-2006

Säsong 2006-2007

Säsong 2007-2008

Säsong 2008-2009

Säsong 2009-2010

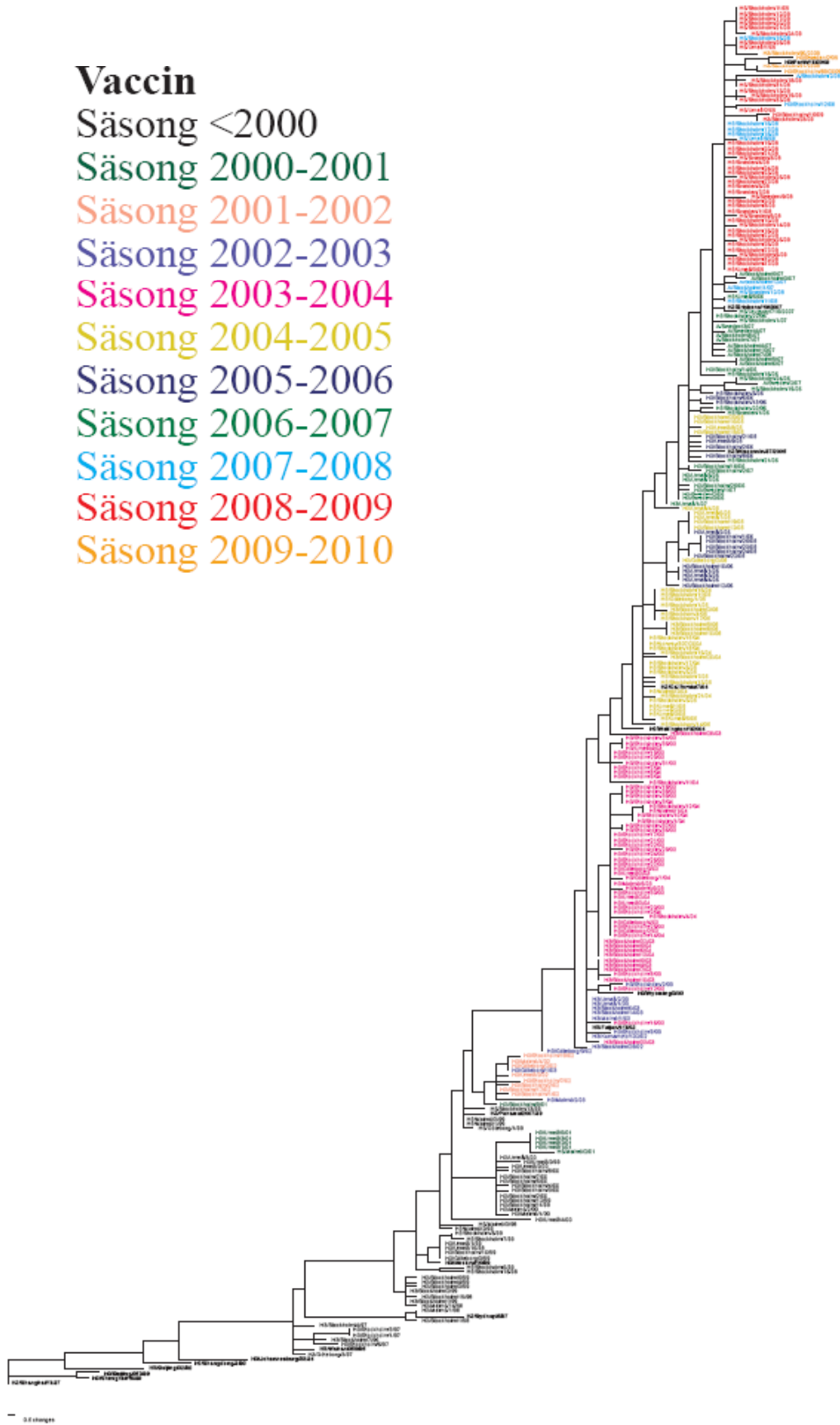


Figure 4:4:3)

Vaccine

Season 2004-2005

Season 2005-2006

Season 2006-2007

Season 2007-2008

Season 2008-2009

Inter-seasonal 2009-2010

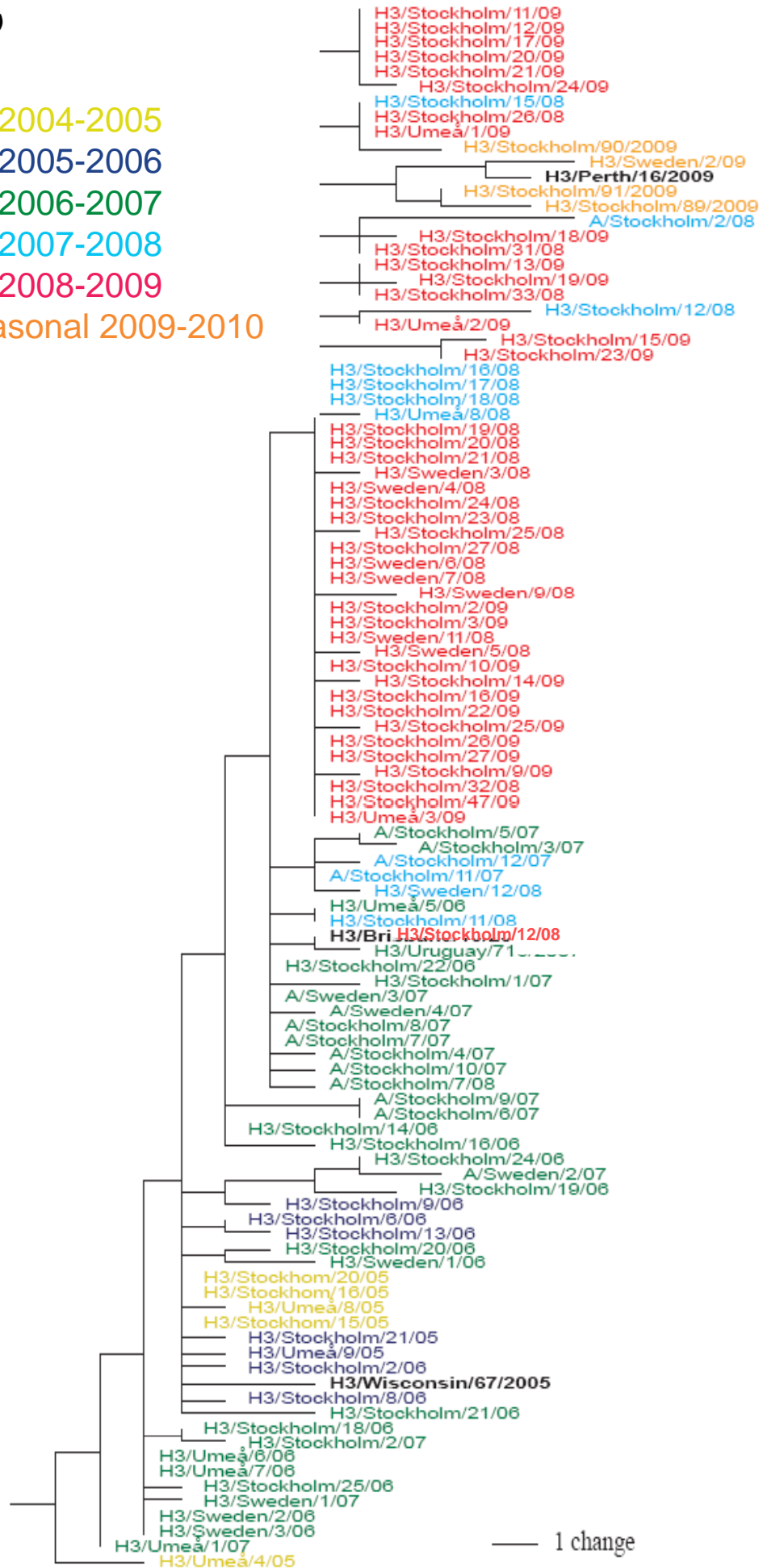
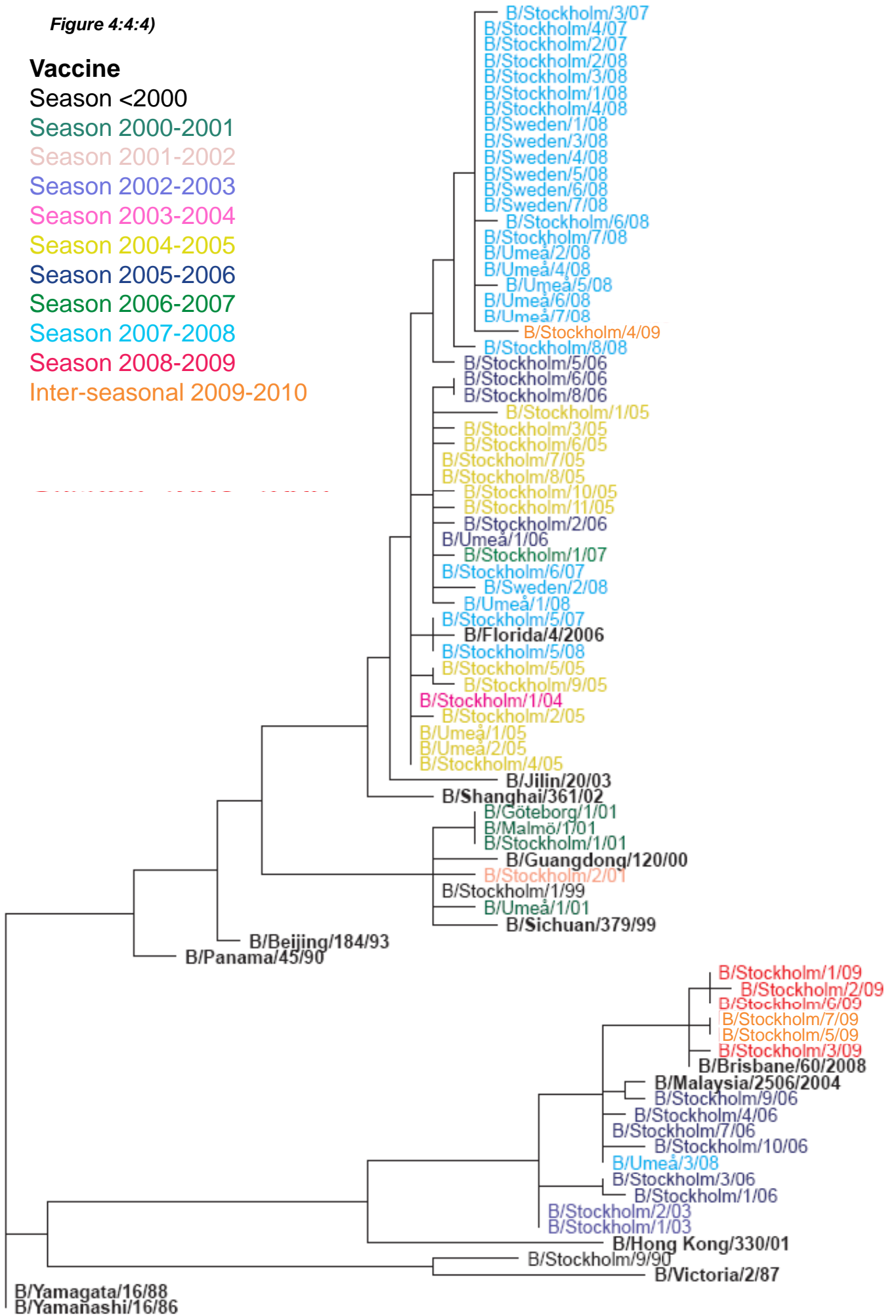


Figure 4:4:4)

Vaccine

- Season <2000
- Season 2000-2001
- Season 2001-2002
- Season 2002-2003
- Season 2003-2004
- Season 2004-2005
- Season 2005-2006
- Season 2006-2007
- Season 2007-2008
- Season 2008-2009
- Inter-seasonal 2009-2010



— 1 change

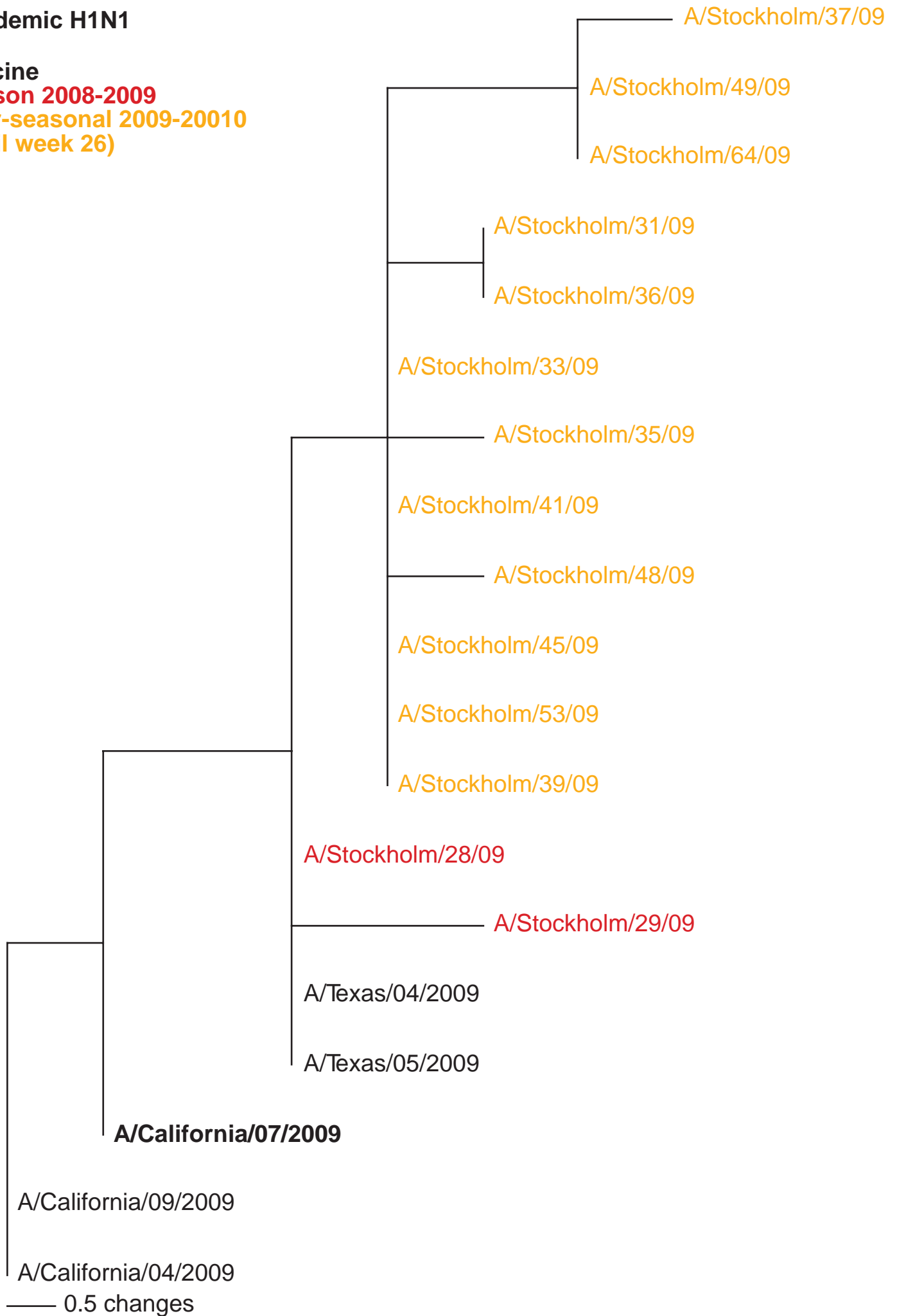
Figure 4:4:5) The phylogenetic tree of the amino acid sequences of the HA of pandemic influenza A/H1v strains isolated in Sweden 2009.

Pandemic H1N1

Vaccine

Season 2008-2009

**Inter-seasonal 2009-2010
(until week 26)**



5) QUALITY CONTROL OF LABORATORY DIAGNOSIS OF INFLUENZA

In collaboration with the organisation for External Quality Assessment in Sweden (Equalis), panels for quality control of antigen detection with IFA or ELISA and PCR, and for virus isolation, were sent to laboratories performing these types of diagnostic assays in Sweden. The influenza panel for IF consisted of 8 different acetone fixed preparations of the influenza strains expected for the season, grown in MDCK cells, and mixed in different proportions with uninfected cells from a lymphoblastoid cell line. Twenty-eight laboratories reported altogether 38 data sheets. Nineteen of the participating laboratories reported correct results (265/300 analyses). The results of the External Quality control from 1994-2008 related to methods is presented (Table 5:1).

Table 5:1 External Quality Control Assessment in Sweden (Equalis).

Results of panels for influenza antigen detection from 1994-2008. The number (%) of reported correct results related to total number of examinations performed with the methods is presented.

	Influenza A/H1	Influenza A/H3	Influenza B
Direct IF			
DPC-PathoDx (2000-2008)	187/211 (89 %)	189/198 (95 %)	160/176 (91 %)
Imagen	275/318 (86 %)	254/287 (89 %)	213/293 (73 %)
Chemicon (2000-2008, except 2004 and 2007)	22/27 (81 %)	20/21 (95 %)	14/20 (70 %)
Indirect IF			
Chemicon	111/114 (97 %)	100/105 (95 %)	102/107 (95 %)
WHO	49/51 (96 %)	46/47 (98 %)	44/46 (96 %)
Biotrin (1994-2008, except 1999)	23/24 (96 %)	23/24 (96 %)	20/20 (100 %)
Immunocromatography			
Binax NOW (2004-2008)	41/42 (98 %)	43/45 (96 %)	23/40 (58 %)
QuikVue Influenza (2006-2008)	12/14 (86 %)	10/13 (77 %)	8/13 (62 %)
Becton-Dickinson (2001-2003)	8/10 (80 %)	8/8 (100 %)	2/6 (33 %)
EIA membran test/BD Directigen (2008)	3/3 (100 %)	2/2 (100 %)	0/3 (0 %)
PCR			
Real time PCR (2003-2008)	60/62 (97 %)	58/58 (100 %)	55/55 (100 %)

6) METHOD DEVELOPMENT AND RESEARCH

6.1. In a project funded by Eurocine, a research company focusing on the development of nasal vaccines, SMI is investigating cross protection to influenza A subtypes after nasal immunisation. Studies have been performed in mice and during this winter a study in humans will start.

6:3. Modelling and prediction

In a study funded by the Swedish Emergency Management Agency, detailed modelling for prediction of spread of influenza in the society is performed by PhD Lisa Brouwers and collaborators. The effect of various measures for social distancing and different strategies for vaccination in a pandemic situation are studied.

6:4. Death rates

SMI participates in European monitoring of excess mortality for public health action, Euro-MOMO. (coordinator: Ann Mazick, Statens Seruminstitut, Copenhagen). A new modeling method for estimation of excess mortality is being developed.

6:5. Population-based surveillance

Before the 2007-08 season, around 14 000 persons in the Stockholm area were sent a letter, asking if they were willing to report upper respiratory tract infections and answer some question concerning their symptoms via the web or by interactive voice response. They were also informed that their data in various population registers would be analysed in an anonymous manner. Around 3500 agreed to report, and the results of the reporting was continuously shown as numbers with ARI and ILI, respectively, at the SMI homepage. The reporting was validated by repeated short questionnaires to part of the 3500 reporters during the season, and by a final questionnaire to all 14000 invited by the end of the season. Evaluation is ongoing, and the main results of the study will be presented late during 2008.

6:6. Webb queries for evaluation of timing and intensity of seasonal influenza activity

A model to estimate the timing and the peak of the influenza season, based on queries related to influenza submitted to a medical web site during two influenza seasons (2005-2007) was developed. The model – which is based on partial least squares regression – consists of two parts: one estimating the number of positive laboratory cases and one estimating the proportion of cases with influenza-like illness as reported by the sentinel GPs. The model was evaluated on previously unseen data during the 2007/2008 season, from which the number of influenza-related web queries (twenty types) for this new season was calculated. The timing of the estimated sentinel peak as well as the estimated laboratory peak coincided with the peaks for the traditional sources. The model over-estimated the intensity, especially so the curve for laboratory verified cases. The dominance of mild influenzas during the season – compared to the two years for which the model was developed – resulting in fewer visits to health care units, could explain the higher intensity found in model. In 2008-09, when H2N3 again dominated, there was a good fit between the web-pattern and sentinel- and laboratory reporting.

During the season, a system was generated for automatic transfer of data, and the curves were automatically generated each Monday.

6:7. Scientific articles and reports:

The Russian influenza in Sweden in 1889-90: an example of Geographic Information System analysis. Skog, L. Hauska, H. Linde, A. Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin. 13(49), 2008 Dec 4.

[Oseltamivir-resistant influenza virus A \(H1N1\), Europe, 2007-08 season.](#)

Meijer A, Lackenby A, Hungnes O, Lina B, van-der-Werf S, Schweiger B, Opp M, Paget J, van-de-Kasstele J, Hay A, Zambon M; European Influenza Surveillance Scheme. Emerg Infect Dis. 2009 Apr;15(4):552-60.

[Encephalitis after influenza in Sweden 1987-1998: a rare complication of a common infection.](#)

Hjalmarsson A, Blomqvist P, Brytting M, Linde A, Sköldenberg B. Eur Neurol. 2009;61(5):289-94.

[Molecular characterization of highly pathogenic H5N1 avian influenza viruses isolated in Sweden in 2006.](#)

Kiss I, Gyarmati P, Zohari S, Ramsay KW, Metreveli G, Weiss E, Brytting M, Stivers M, Lindström S, Lundkvist A, Nemirov K, Thorén P, Berg M, Czifra G, Belák S. Virol J. 2008 Oct 6;5:113.

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