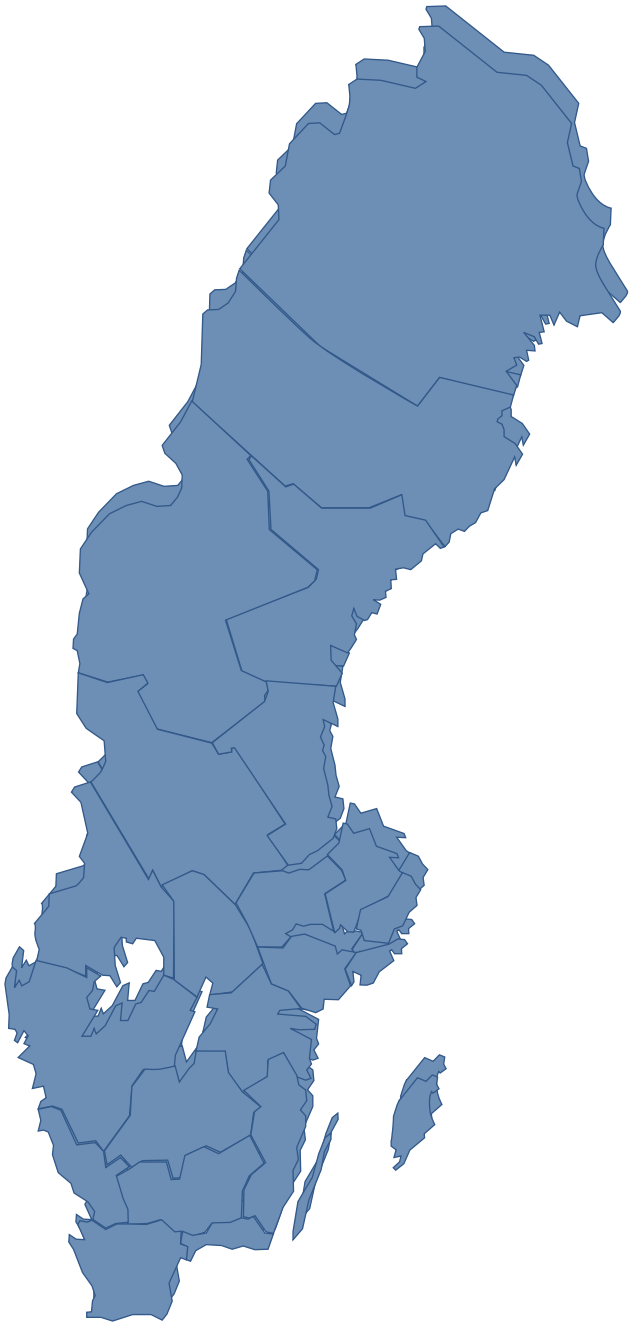
INFLUENZA

Annual Report 2006-2007



SMITTSKYDDSINSTITUTET

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

ANNUAL REPORT

JULY 2006- JUNE 2007

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Contents

1) MONITORING OF THE INFLUENZA ACTIVITY IN SWEDEN

1:1) Sentinel Surveillance.

The Swedish influenza sentinel reporting system 2006–2007 consisted of 149 sentinel units recruited by the County Medical Officers. It included both individual GPs and larger health care centres. Twenty out of twenty-one counties participated in the surveillance (Gotland, the smallest county was not included). Date of visit, age and sex of the patients were reported, and 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 a by web-based reporting system, SentiNet. Each week, reports were received from between 33–98 of the sentinel units. The total weekly number of out-patient visits at the reporting units ranged between 299608–602831.

1:2) The Sentinel Sampling System

Sentinel sampling was introduced during the season 2006-07. A total of 46 units consisting of infectious disease clinics, paediatric clinics and sentinel units have participated. Geographically, the units were evenly distributed throughout Sweden. Nineteen out of 21 Swedish counties were included. During 32 weeks, 1023 samples were received and analysed at SMI. One-57 samples were received per week, more before the first case was identified and less towards the end of the season.
The primary diagnostic tool was real-time PCR for influenza type and influenza A subtypes. A swabbing protocol for each sample, according to the minimum requirements from EISS, was completed by the clinician. Approximately 10 % of the samples analysed were positive for influenza.

1:3) Reports of laboratory verified influenza diagnoses

During the influenza season the 28 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 four 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 the allover 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 2007 has been 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

Each Wednesday, national and international influenza information collected during the week, including the WHO country reports, were summarised and made available at the SMI homepage

(www.smittskyddsinstitutet.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 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. In most instances those 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, and attended by about 100 persons. A short film on influenza vaccination of risk groups was produced and broadcasted repeatedly during the vaccination period in the Swedish State Television.

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

Sweden reports to WHO via Flunet, and starts reporting when the first laboratory verified case occurs. Since year 2000 we participate in the European Influenza Surveillance Scheme (EISS), and provide weekly information to the EISS homepage.

3) CHARACTERISATION OF INFLUENZA STRAINS

3:1) Genotypic and fenotypic characterisation

Virus strains isolated by SMI or sent to SMI from other laboratories were examined for the type and subtype of virus by hemagglutination inhibition (HAI; reagents kindly donated from WHO) and IF with monoclonal antibodies (WHO, Chemicon). HA and NA-sequencing was also performed. For further characterisation with ferret sera, the strains were also sent to Mill Hill in London. All the influenza strains were also investigated genotypically or phenotypically for antiviral resistance to amantane and neuraminidase inhibitors.

4) DATA FROM THE 2006-2007 SEASON

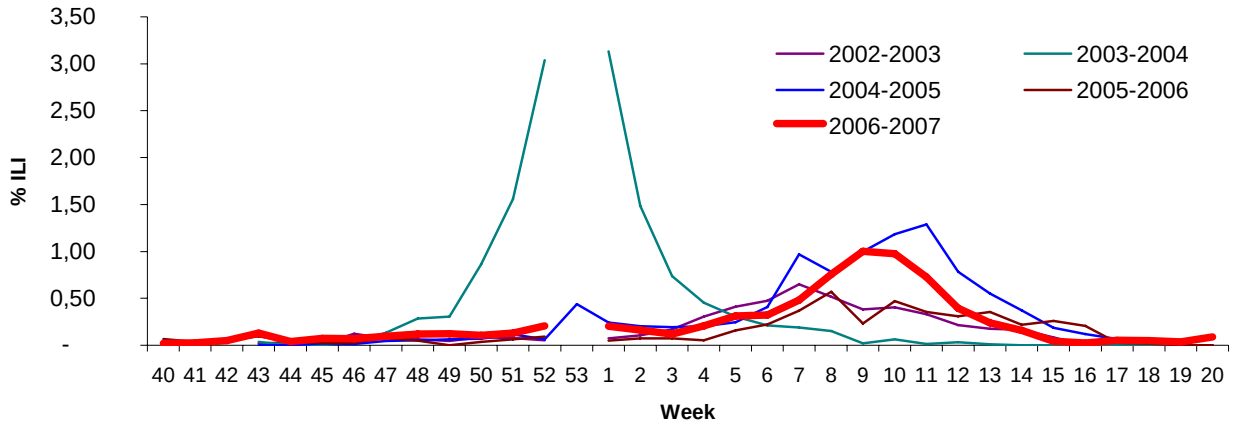
4:1) Summary of the influenza activity in Sweden

The first laboratory verified influenza cases of the season were reported in week 44, and the first positive sentinel case from the sentinel sampling was detected simultaneously. Two weeks earlier sentinel doctors had already reported a few ILI cases. From week 45, 2006 untill week 2, 2007 there was an isolated, and very unusual outbreak of influenza in the northern part of the country. From week 4 there was countrywide activity. It culminated week 10 with more than 200 diagnoses, a figure exceeding the preceding season but very similar to what was noted in 2004. The sentinel reports followed a similar pattern, and the magnitude of the activity was similar to that for 2004-2005 for ILI

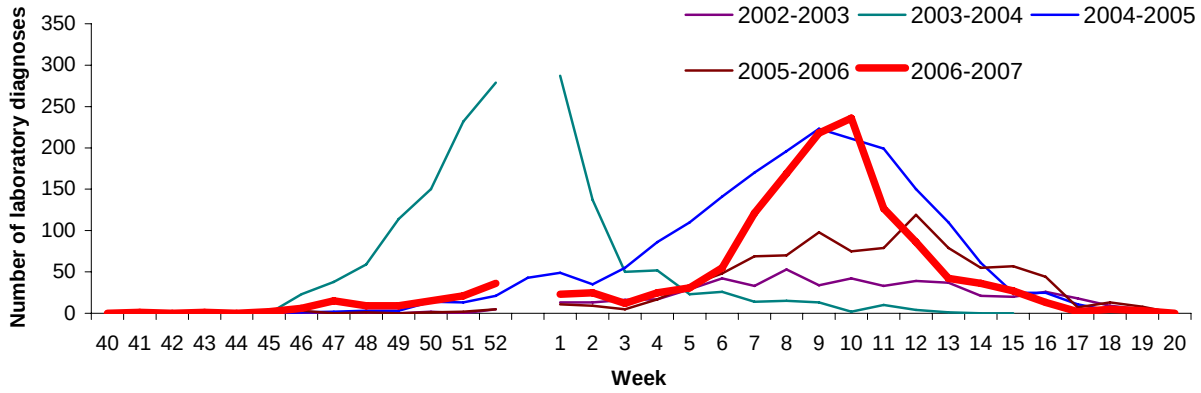
(Fig 4:1:1 and Fig 4:1:2). Overall, the activity was widespread and of medium intensity. The total number of laboratory diagnoses was 1372 (1351 A and 21 B) compared to 908 (446 A and 462 B) the previous season.

All samples collected in the sentinel sampling system were analysed with molecular methods. A total of 1023 samples collected during the season were typed and subtyped for influenza. 131/1023 (12.8%) became positive (fig 4:1:3). During the peak (week 8-10) more than 40% of the samples were influenza A positive.

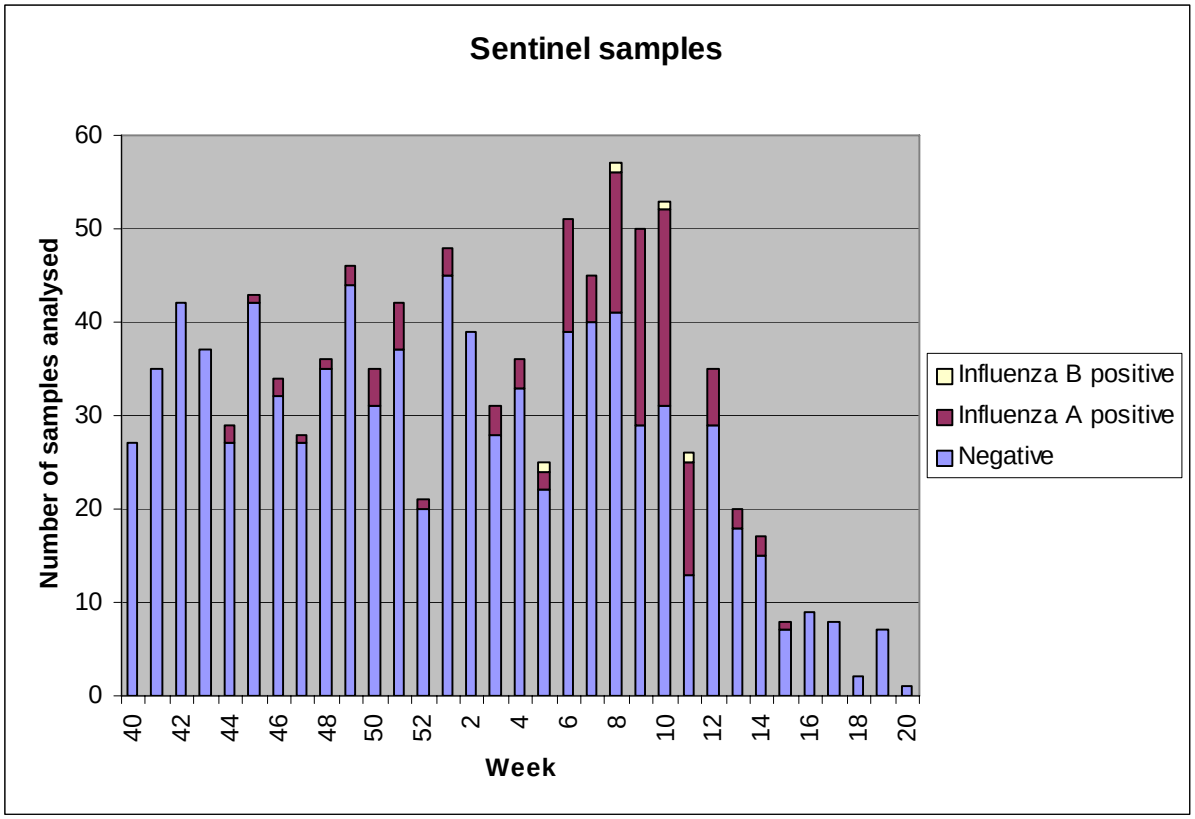
4:1:1) Proportion (%) cases with influenza-like illness (ILI) out of total number of patient visits in the sentinel system



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



4:1:3) Number of laboratory verified influenza cases in the sentinel sampling system.



4:2) Age distribution in the laboratory and sentinel systems

The age distribution varied was much more in the laboratory than in the sentinel system, most likely reflecting that GPs practices mostly cover otherwise healthy adults. The young children and elderly often get more severely ill and seek hospital care directly if they need medical attention. From these more seriously ill patients specimens are drawn. This season the majority of the reported cases in the laboratory reporting system were young children and elderly people (Table 1, Fig 4:2:1). In the sentinel system the majority of reported cases were between 15 and 65years (Table 2, Fig 4:2:2). The weekly incidences in the two systems have also been analysed in relation to the expected with regard to the size of respective populations (fig 4:2:1 and 4:2:2). The change in the age distribution over time followed a pattern typical for influenza. Early during the season the number of sick children increased, while in the end the elderly patients constituted the majority of cases.

Table. 4:2:2) ILI cases by gender and age group

"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.

Age group	Observed cases	Expected cases
0-4	43	43
5-14	69	87
15-64	593	502
65+	66	133
Total	**771**	

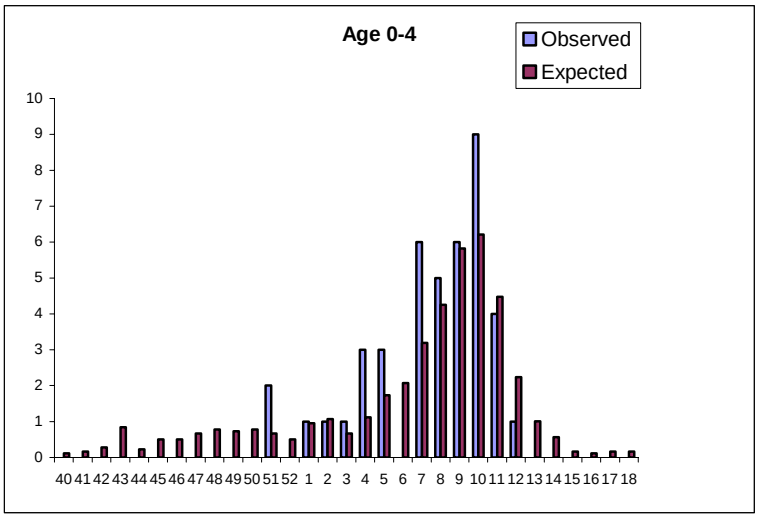
Table. 4:2:1) Laboratory verified cases by gender and age group during the 2006-07 season

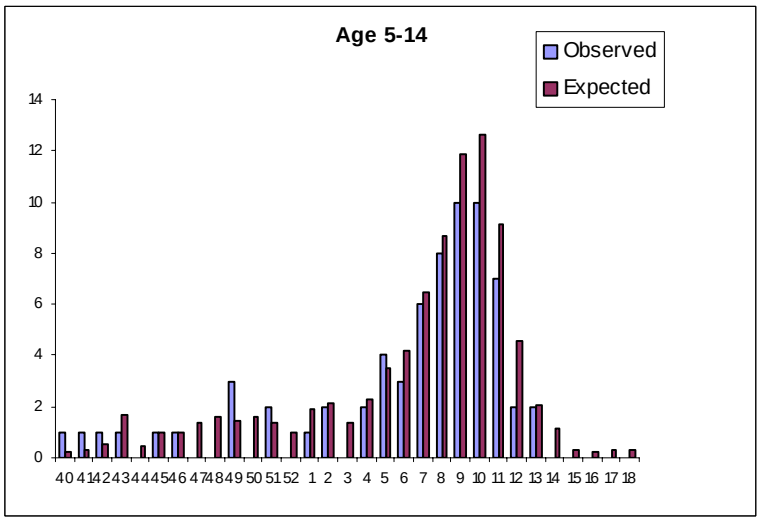
"Expected"is the number of cases that would have occurred if the cases were evenly distributed in relation to the population of the respective age groups.

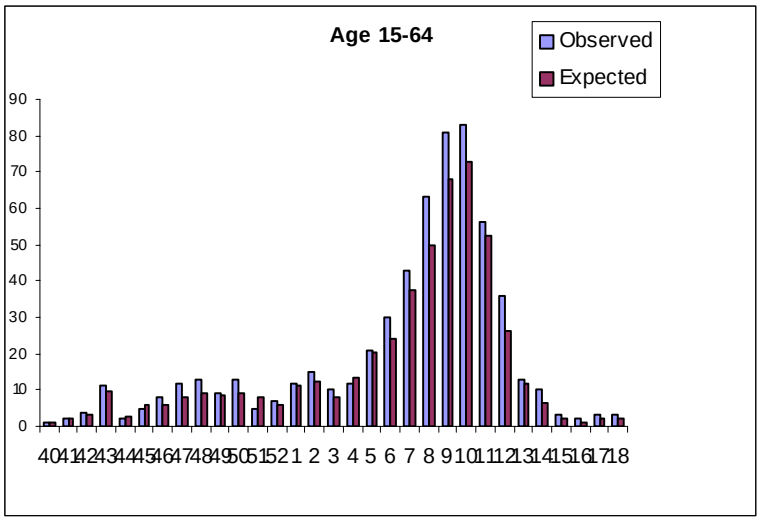
Age group	Observed cases	Expected cases
0-4	103	74
5-14	69	151
15-64	635	873
65+	527	231
Age unknown	38	0
Total	**1372**	

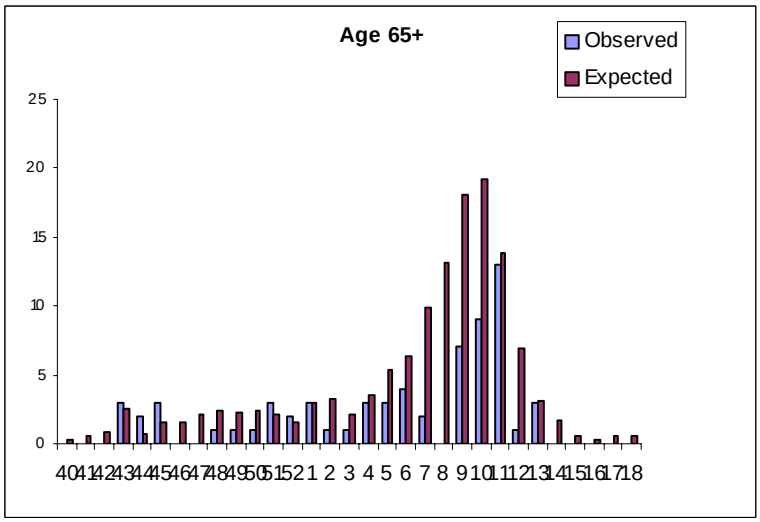
4:2:1) Age distribution of ILI cases during season 2006-07

"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.



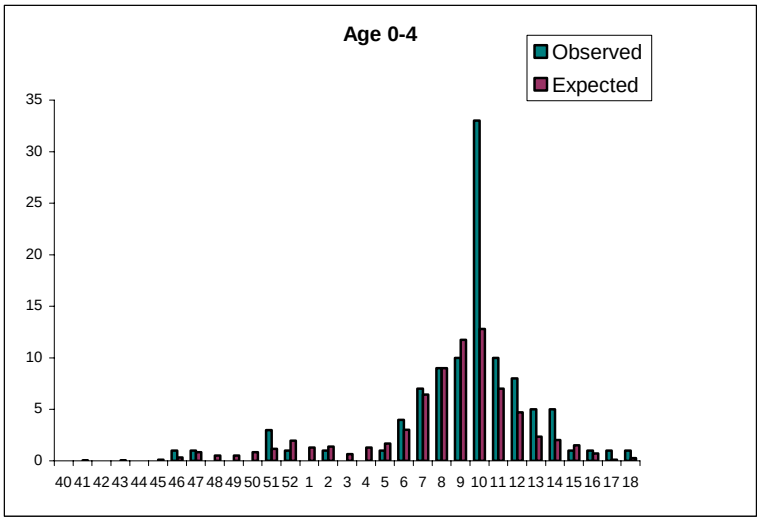


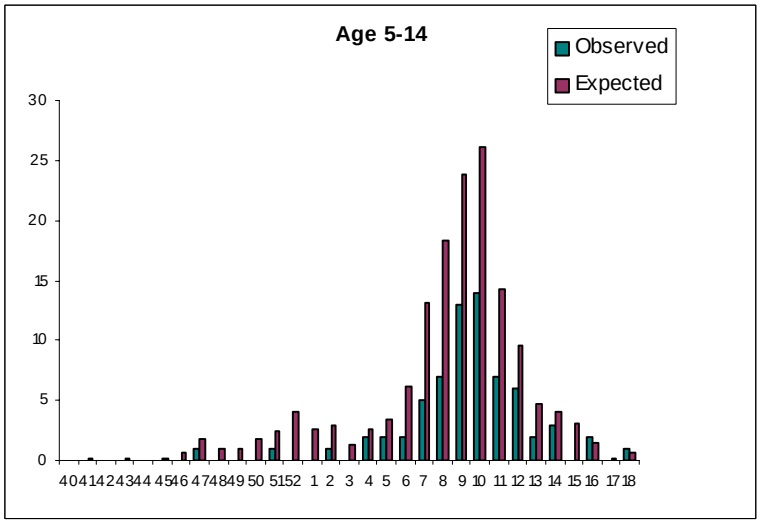

Age 15-64
Observed
Expected
90
80
70
60
50
40
30
20
10
0
40 41 42 43 44 45 46 47 48 49 50 51 52 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

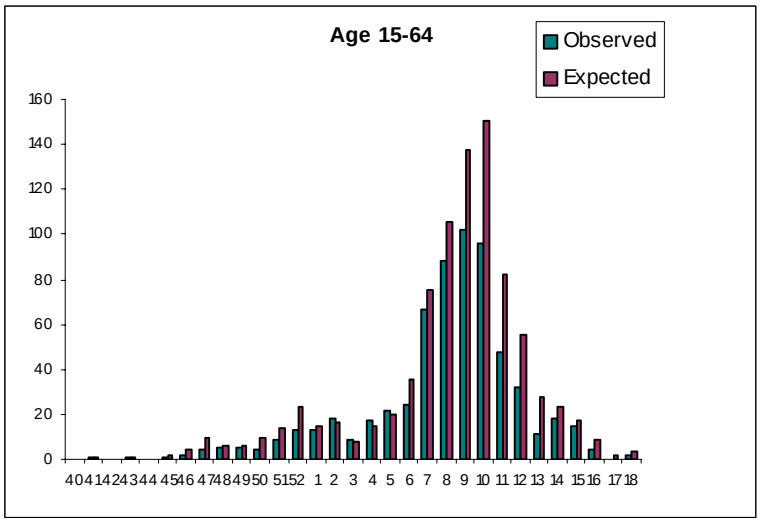

Age 65+
Observed
Expected
25
20
15
10
5
0
40 41 42 43 44 45 46 47 48 49 50 51 52 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

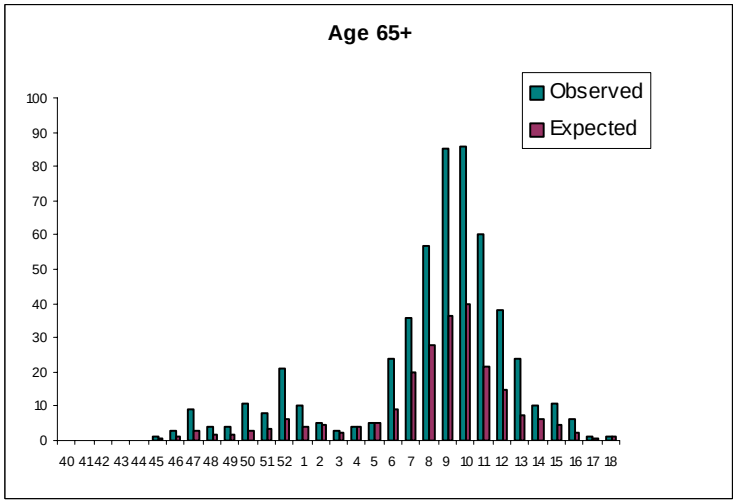
4:2:2) Age distribution of laboratory verified cases during season 2006-07

"Expected" means the number of expected cases in relation to the whole population.





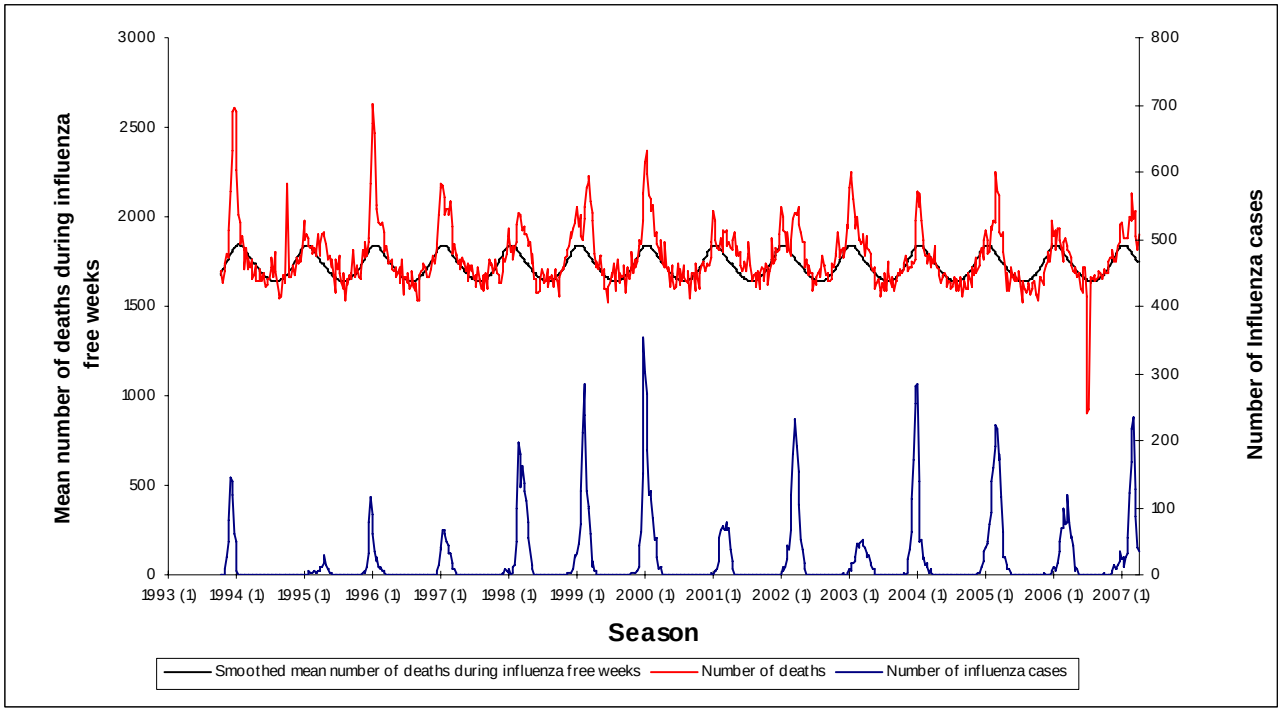




4:3) Estimated excess mortality

There was a peak of mortality related to the increase in influenza activity, and the area above the mortality curve for influenza-free weeks contained just above 2000 persons. Due to methodological difficulties, the number is by no means exact, but calculated with the same method since 1994, the excess mortality has varied from 100 – 4000, with a mean of round the figure for the past season.

4:3:1) Diagram of the weekly number of deaths in Sweden from week 40 1993 to week 20 2007, and the number of laboratory verified influenza cases during the same period. (The peak of mortality in September 1994 reflects the Estonia ferry catastrophe, the decrease in mortality in the summer 2006 has no explanation). Adjusted mean for corresponding influenza-free weeks is also included.



4:4) Characterisation of influenza strains.

All samples collected in the sentinel sampling surveillance were analysed with molecular methods. A total of 1023 samples collected during the season were typed and subtyped for influenza A. 131/1023 (12.8%) samples became positive. Out of these, 127/131 (97%) were influenza A and the remaining 3% influenza B (fig 4:1:3). When the influenza A positive samples were subtyped, influenza A H3 was detected in 115/127 (90%), influenza A H1 in 2/127 (1%). For the remaining 10/127 (9%) of the influenza A positive samples the subtype could not be analysed. Further, from seven of the positive samples virus was isolated and characterised by genotypic and phenotypic methods (table 4:4).

Of strains sent from other laboratories to SMI for further subtyping 91 % were influenza A H3. Only one H1N1 (4%) and one B (4%) strain were received for further characterisation, (table 4:4).
All the influenza strains further characterised were similar to the strains prevalent in Europe (Fig 4:4:1-4:4:4). No mutation known to induce resistance against neuraminidase inhibitors in the genes encoding the neuraminidases for influenza A (N1 and N2) and B was identified. However, 54% of the H3N2 isolates had mutations in the M2-gene, inducing amantadine resistance (fig 4:4:2).

Table 4:4) Table of isolates for which extended feno- and genotyping was performed.

Name of the isolate	Date of sample collection (yyyy-mm-dd)	Subtype	Information	Origin
A/Stockholm/18/06	2006-08-19	H3N2		Lund
A/Stockholm/19/06	2006-08-28	H3N2	Traveller from India	Skövde
A/Umeå/5/06	2006-10-26	H3N2	Probably caught it from a friend who had visit UK	Umeå
A/Umeå/6/06	2006-11-07	H3N2		Umeå
A/Umeå/7/06	2006-11-10	H3N2		Umeå
A/Stockholm/20/06	?	H3N2	Traveller from Thailand	Västerås
A/Stockholm/21/06	2006-12-05	H3N2	Traveller from Syria, came home 1/12	Linköping
A/Stockholm/22/06	2006-12-06	H3N2		Uppsala
A/Stockholm/23/06	2006-12-01	H1N1		Stockholm
A/Stockholm/24/06	2006-12-29	H3N2		Karlstad
A/Stockholm/25/06	2006-12-29	H3N2		Falun
A/Stockholm/1/07	2007-01-12	H3N2		Stockholm
A/Stockholm/2/07	2007-01-15	H3N2	Travelled in Southkorea, home 14 of jan	Stockholm
A/Umeå/1/07	2007-01-01	H3N2		Umeå
A/Stockholm/3/07	?	H3N2	infl vaccinated	Göteborg
A/Stockholm/4/07	?	H3N2	infl vaccinated	Göteborg
A/Stockholm/5/07	?	H3N2	infl vaccinated	Göteborg
B/Stockholm/1/07	2007-03-26	B		Stockholm
A/Stockholm/6/07	2007-03-12	H3N2		Stockholm
A/Stockholm/7/07	2007-03-12	H3N2		Stockholm
A/Stockholm/8/07	2007-03-04	H3N2		Stockholm
A/Stockholm/9/07	2007-03-06	H3N2		Stockholm
A/Stockholm/10/07	2007-03-12	H3N2		Stockholm
A/Sweden/1/06	2006-11-16	H3N2	Sentinel doctor	Göteborg
A/Sweden/2/06	2006-12-18	H3N2	Sentinel doctor	Sorsele
A/Sweden/3/06	2006-12-14	H3N2	Sentinel doctor	Sorsele
A/Sweden/1/07	2007-01-02	H3N2	Sentinel doctor	Sundsvall
A/Sweden/2/07	2007-03-05	H3N2	Sentinel doctor	Aneby
A/Sweden/3/07	2007-03-06	H3N2	Sentinel doctor	Göteborg
A/Sweden/4/07	2007-03-06	H3N2	Sentinel doctor	Halmstad

Figure 4:4:1) The phylogenetic tree of the amino acid sequences of HA of influenza A/H3 strains isolated in Sweden 1996-2007 compared to the vaccine strains.

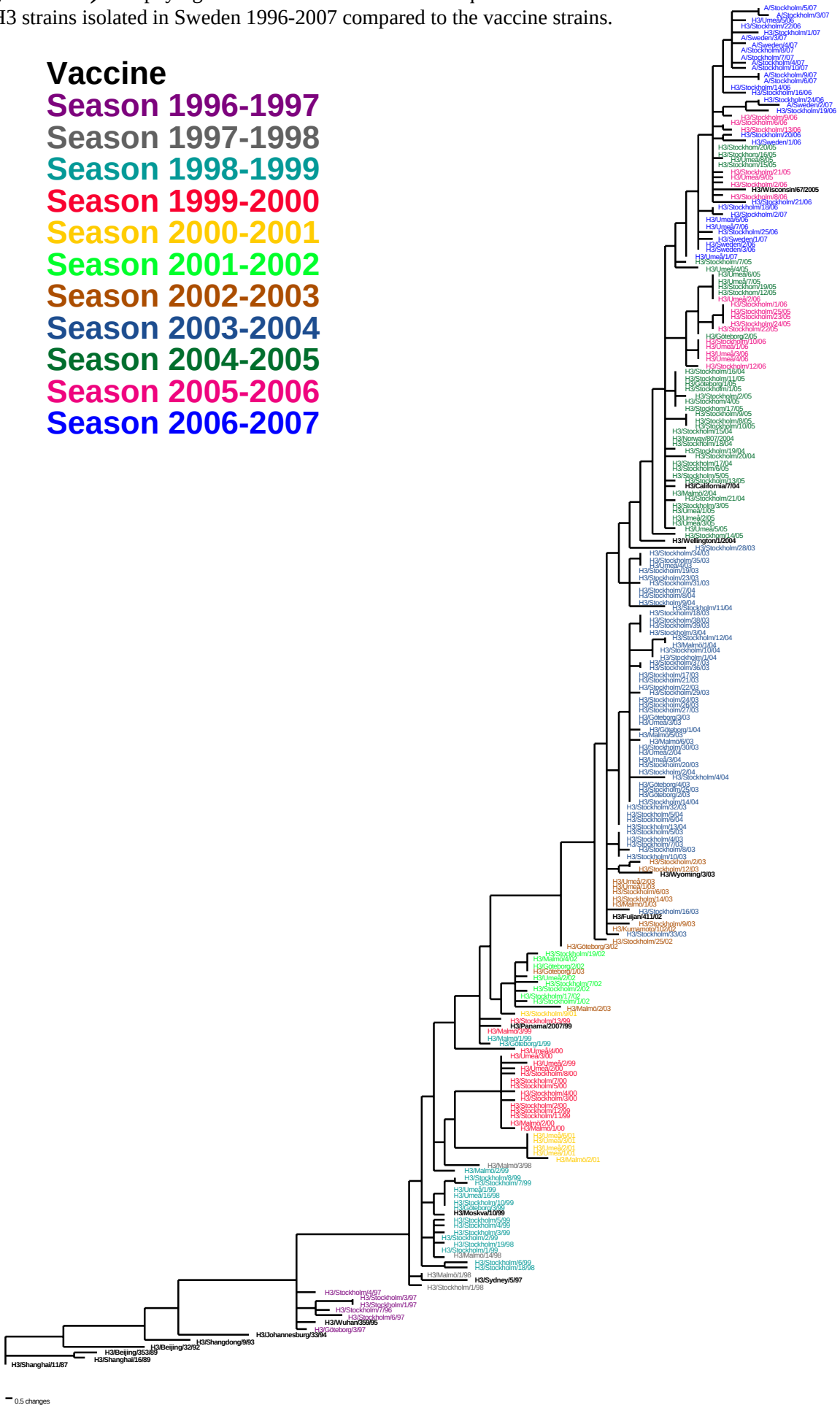


Figure 4:4:2) The phylogenetic tree of the amino acid sequences of HA of influenza A/H3 strains isolated in Sweden 2003-2007 compared to the vaccine strains. Amantadine resistant strains are indicated in bold. All alterations determined in the strains from the last season are shown.

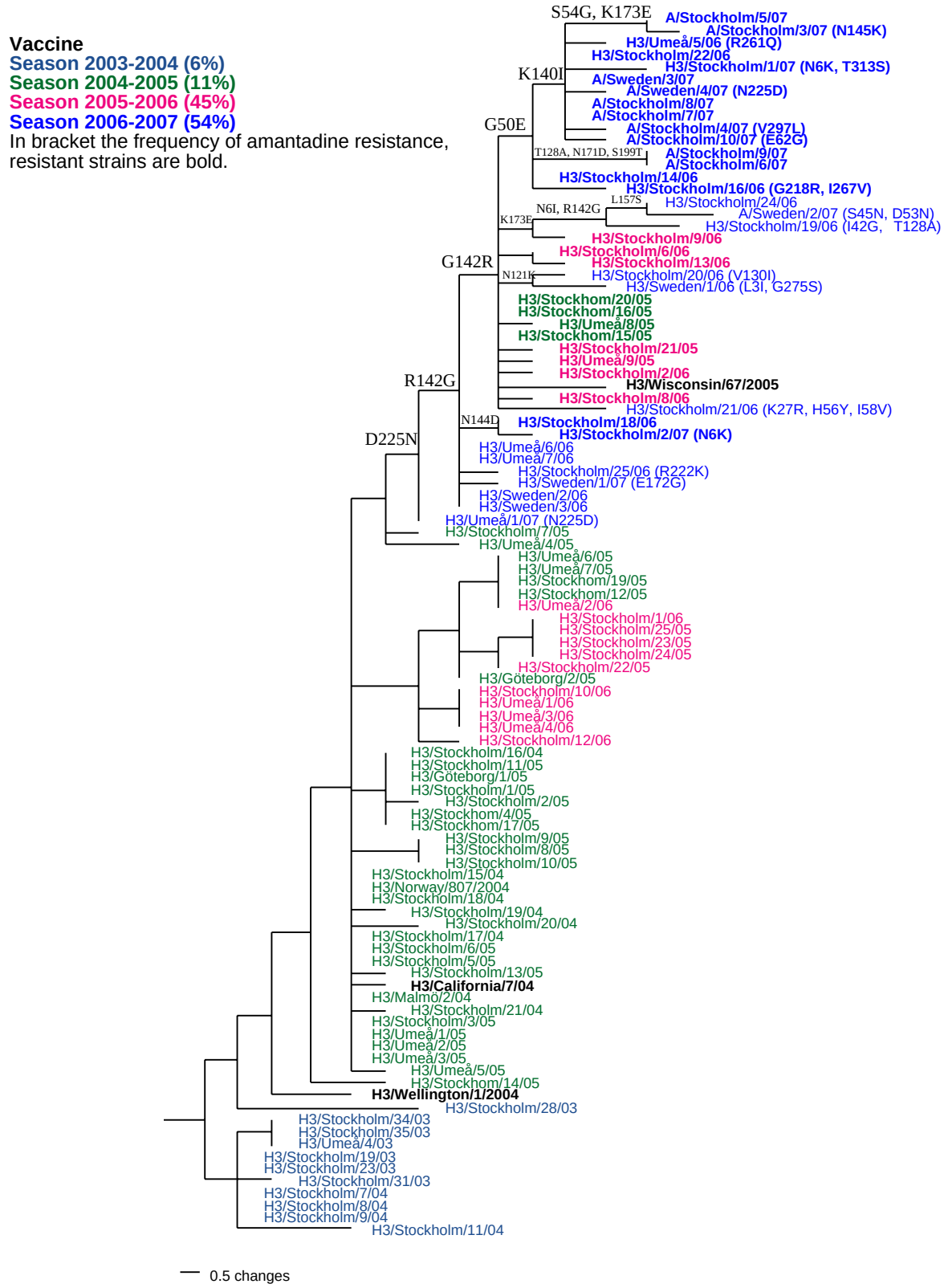


Figure 4:4:3) The phylogenetic tree of the amino acid sequences of HA of influenza A/H1 strains isolated in Sweden compared to the vaccine strains. All alterations determined in the strains from the last two seasons are shown.

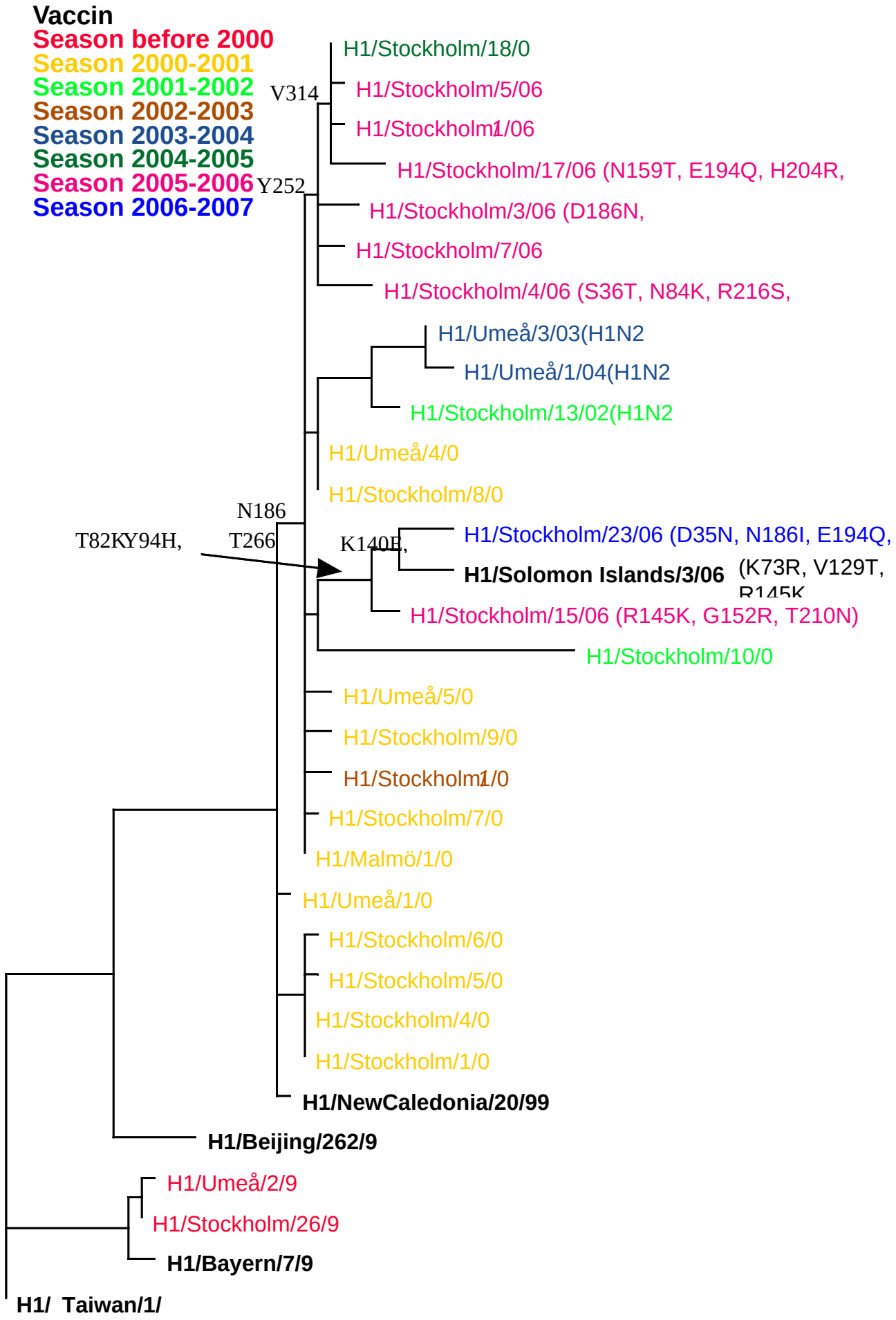
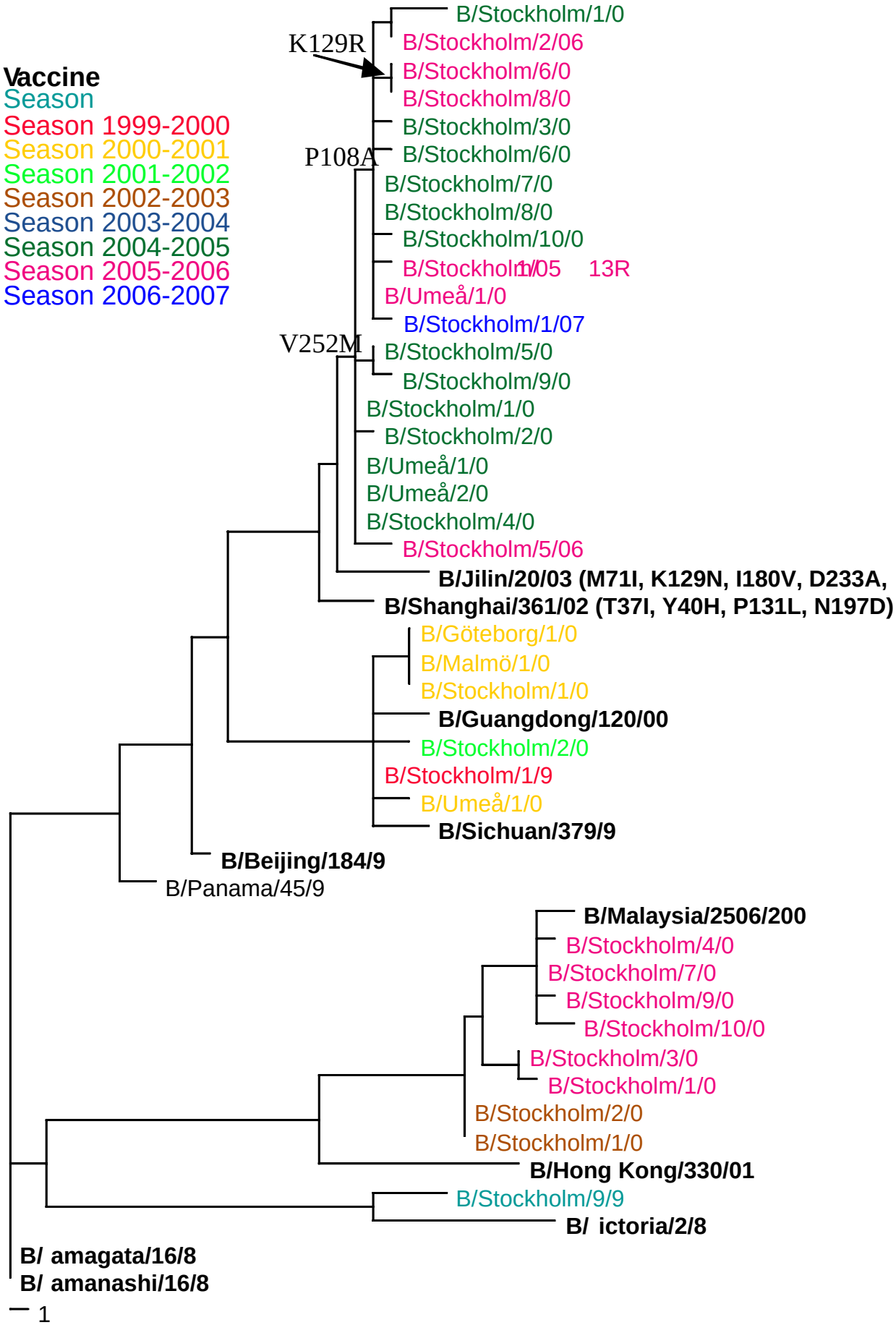


Figure 4:4:4) The phylogenetic tree of the amino acid sequences of HA of influenza B strains isolated in Sweden compared to the vaccine strains. All alterations determined in the Yamagata-like strains from the last two seasons are shown.



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-four laboratories reported altogether 34 data sheets participated. Most of the participating laboratories answered the panel correctly (252/272 analyses). The results of the External Quality control from 1994-2006 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 1994-2006. 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
Imagen	231/273 (85%)	216/249 (87%)	190/258 (74%)
Chemicon indirekt IF	100/102 (98%)	90/95 (95%)	93/97 (96%)
WHO	37/39 (95%)	36/37 (97%)	34/36 (94%)
Biosoft/Argene (1994-1999)	5/6 (83%)	6/6 (100%)	5/7 (71%)
Biotrin (1994-2006,not 1999)	17/18 (94%)	18/19 (95%)	15/15 (100%)
DPC-PathoDx (2000-2006)	124/145 (86%)	135/143 (94%)	108/119 (91%)
Chemicon direkt IF (2000-2006, not 2004)	21/26 (81%)	20/21 (95%)	14/17 (82%)
Becton-Dickinson (2001-2003)	8/10 (80%)	8/8 (100%)	2/6 (33%)
Real-time PCR (2003-2006)	20/20 (100%)	24/24 (100%)	19/19 (100%)
Binax NOW (2004-2006)	17/18 (94%)	23/24 (96%)	10/21 (48%)

6) METHOD DEVELOPMENT AND RESEARCH

6:1) During the last season the frequency of admantane resistant influenza A, H3 and H1 strains, circulating in Sweden was investigated. Since a high frequency of admantane resistant H3N2 strains was found, the investigation continued during this season. No resistance among subtype H1 isolates was found. The mutation serine-to-asparagine at position 31 (S31N) in M2 was determined in 54% of the H3N2 strains analysed during this season (fig 4.4.2).
During the last three seasons, resistant strains were collected during the whole seasons however, all the resistant strains clustered together in the phylogenic analyse of the hemagglutinins (fig 4:4:2).

6:2) A neuraminidase inhibitory susceptibility assay developed by M. Zambon was established. In total 11 isolates were analysed. No resistant strain was found. The influenza B strains were less sensitive to oseltamivir and zanamivir compared to H1N1 and H3N2 strains. In general Victoria-like influenza B strains were less sensitive than Yamagata-like strains. This work will continue.

6.3) During the previous five seasons some outbreaks with extra high mortality or attack rate have been notified. When the hemagglutinin and neuraminidase genes were sequenced no specific pattern found explaining the outbreaks. To be able to study the cause of different outbreaks sequence systems for NS, M and PA were established. We studied 10 strains from each of the five previous seasons. We found that
1) 16% of the strains were reassortants when the M1 and PA gene segments were studied.
2) Reassortants were found in four out of five seasons studied.
3) No co-circulating lineages were found during 2003-2004.
4) The drift pattern was more pronounced for the surface proteins.

6.4) In a project funded by Eurocine SMI is investigating cross protection to influenza A subtypes after nasal immunisation. Studies have been performed in mice and during this autumn a study in humans will start.

6:3) Modelling and prediction

In a study founded 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 have been studied.

6:4) Death rates

SMI participates in an application to EU DG Sanco for European analyses of death rates (coordinator: Ann Mazick, Statens Seruminstitut, Copenhagen).

6:5) Population-based surveillance

Since sentinel reporting by GPs only reveal a part of the true spread of influenza in the society, a study of self-reporting of disease was performed in 2006. The prerequisites for recruiting at least 1% of the population for self-reporting of influenza via SMS, automated telephone requests and the webb were studied. Self-reporting was compared to personal interviews. The results were rather discouraging concerning the automated techniques, and the reasons for the failure were analysed. It turned out that both technical aspects and form for invitation to the study had failed. In October 2007 a new study

encompassing the Stockholm area, using improved techniques, will start. 14 000 persons, geographically and age-wise distributed according to what is found in different regions, will be asked to report influenza, either by the web or by telephone. A scientific application for grants for establishment of true reference numbers by other methods has been submitted.

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Swedish Institute for Infectious Disease Control
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Sweden
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e-mail: Annika.Linde@smi.ki.se

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