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

Annual Report
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Activities.

1) Monitoring of influenza activity in Sweden

2) Reports from SMI on influenza activity in Sweden

3) Characterisation of influenza strains

4) Data from the 2003-2004 season

5) Quality control of laboratory diagnosis of influenza

6) Method Development and Research

1) Monitoring of Influenza activity in Sweden.

1:1) The Sentinel System.

The Swedish sentinel reporting system for influenza consists of 122 sentinel units recruited by the County Medical Officers, including both individual GPs and health care centres of 1-4 GPs. The reports ranged from 3531-15068 weekly out-patient visits during the season. Twenty out of twenty-one counties participated in the system. 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 by either fax or the new web-based reporting system, SentiNet.

1:2) Reports of laboratory verified influenza diagnoses.

Influenza isolation is performed at five virus laboratories, placed at University Hospitals and at SMI. The laboratories are relatively evenly distributed with regard to the population in different areas. The laboratories also perform influenza serology, antigen detection with immunoflourescense (IF) and genome detection by polymerase chain reaction (PCR). Another 19 microbiology laboratories diagnose influenza by IF assays, commercial ELISA KITs or nucleic acid amplification (NAA). During the influenza season, the 24 laboratories send weekly reports on the number of influenza cases, diagnosed by antigen detection, NAA and/or virus isolation. Serology results are not included in these reports.

1:3) Death rates.

As soon as available after the end of the influenza season, information on the weekly death rate in Sweden is purchased from Statistics, Sweden. Mean death rate for influenza-free weeks during the period 1993-98 has been calculated, and is used as reference for the demonstration of weekly excess mortality.

2) Reports from SMI on the influenza activity in Sweden.

2:1) Weekly reports to the Swedish Collaborators.

Each Wednesday, all influenza information collected during the week, including the WHO country reports, is summarised and made available at the SMI home-page (www.smittskyddsinstitutet.se). An electronic newsletter is 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 is distributed towards the end of the summer, when all definitive data are available.

2:2) Other spread of information in Sweden.

The media are constantly interested in influenza, and usually contact SMI to get

information. In most instances those contacts result in correct and informative articles. The institute has a journal ”Smittskydd” and an electronic newspaper “EpiAktuellt”, and during the influenza season reports on the situation are printed there, when appropriate. An information day for the persons who are active in the surveillance system was arranged in Stockholm in September 2003.

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 2000 we have also joined the European Influenza Surveillance Scheme (EISS), and provide weekly information to the EISS home-page.

3) Characterisation of influenza strains.

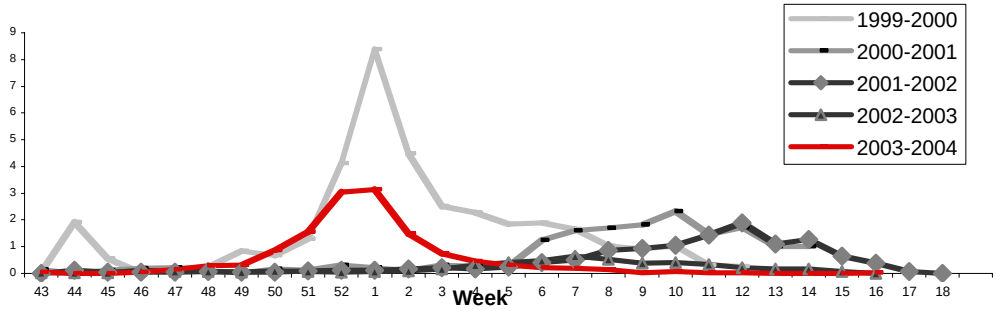
3:1) Genotypic and fenotypic characterisation.
Influenza strains are sent to SMI from the laboratories performing virus isolation. Isolated virus strains are examined for the type and subtype of virus by hemagglutination inhibition (HAI; reagents have been kindly donated from WHO and the Influenza Reference Centre in Rotterdam) and IF with monoclonal antibodies (from Laboratories de Virologie, Lyon). HA and NA-sequencing is also performed. For further characterisation with ferret sera, these strains are also sent to Mill Hill in London.

4) Data from the 2003-2004 season.

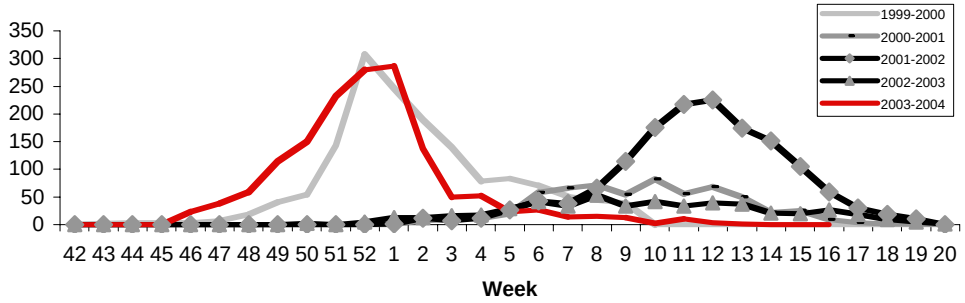
4:1) Summary of the influenza activity in Sweden.
The influenza activity started early this season compared to the previous three seasons. The activity peaked during week 52 and 1, but declined shortly after and remained low during the rest of the season (Fig 4:1). Overall, the activity was widespread, and of medium intensity. A higher proportion of young children were reported this season, compared to previous seasons (Fig 4:3). The total number of laboratory diagnoses was higher in relation to the previous seasons and was 1590, compared to 544 the previous season. 1578 were influenza A and 12 were influenza B (Fig 4:1). Of strains sent to SMI for further subtyping 94% were influenza A H3 (Table 4:5). There was two H1N2 and one B strains. The activity continued until the middle of March. The dominating circulation of H3 probably explains the relatively higher rate of laboratory verified cases in comparison to those reported in the sentinel system, since H3 causes more severe disease, prompting more patients to seek hospital care and thereby increasing the likelihood to have a sample sent to the laboratory. There was excess mortality from week during the peak of the season around Christmas, but the contribution of influenza to the normally high mortality during that period was relatively small (Fig 4:4).

All the influenza strains further characterised were very similar to the strains prevalent in Europe (Fig 4:6, 4:7 and 4:8). No mutations know to induce resistance against neuraminidase inhibitors was identified in the sequenced NA-genes.

4:1) Sentinel reports and laboratory verified influenza cases 1999-2004



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*

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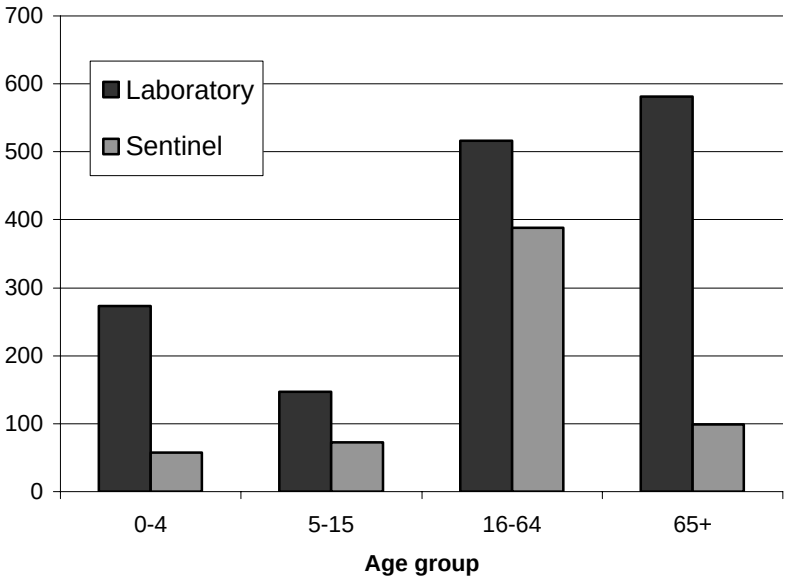
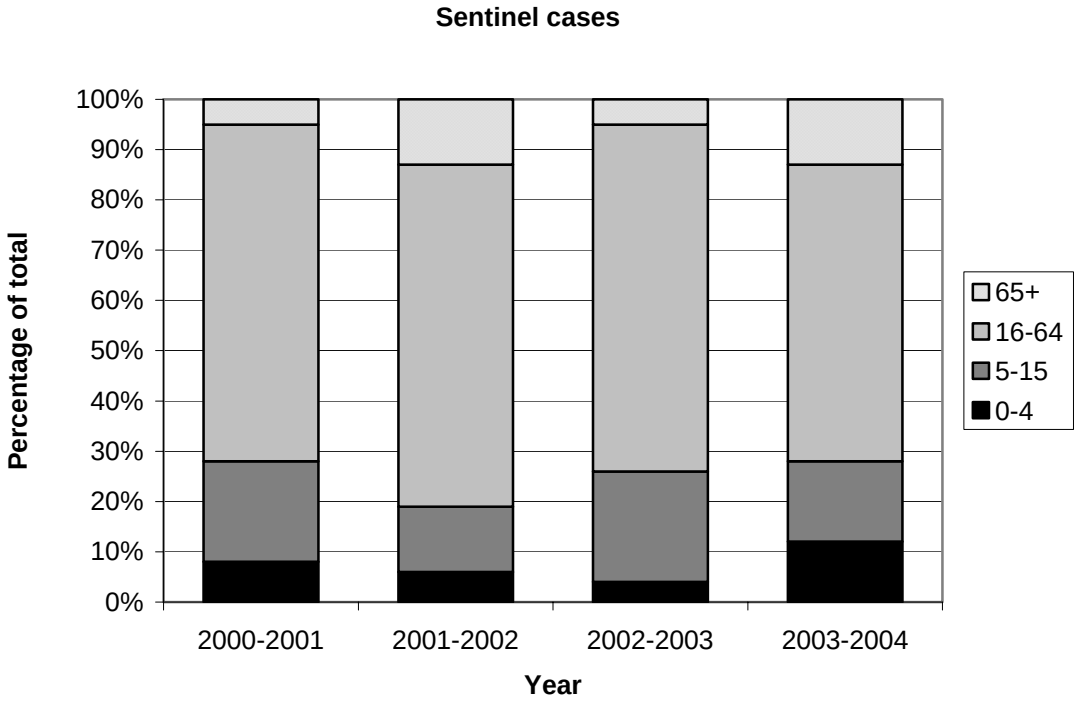
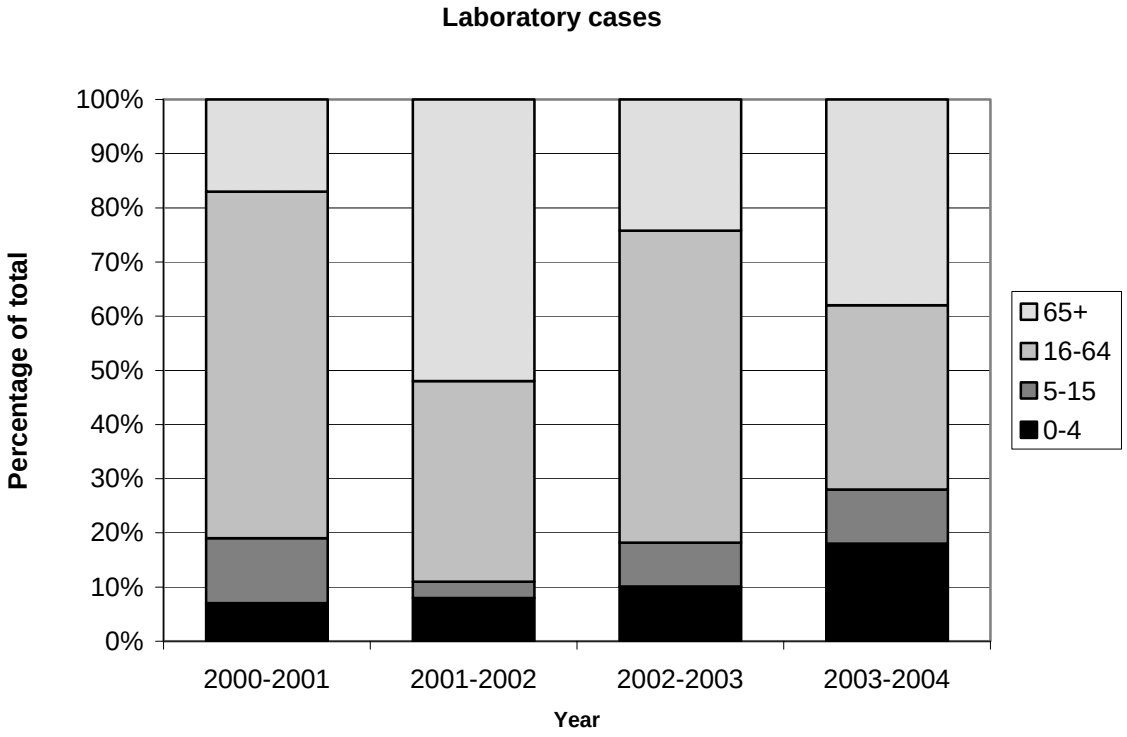
4:2) Age distribution of sentinel cases and laboratory verified cases 2003-2004

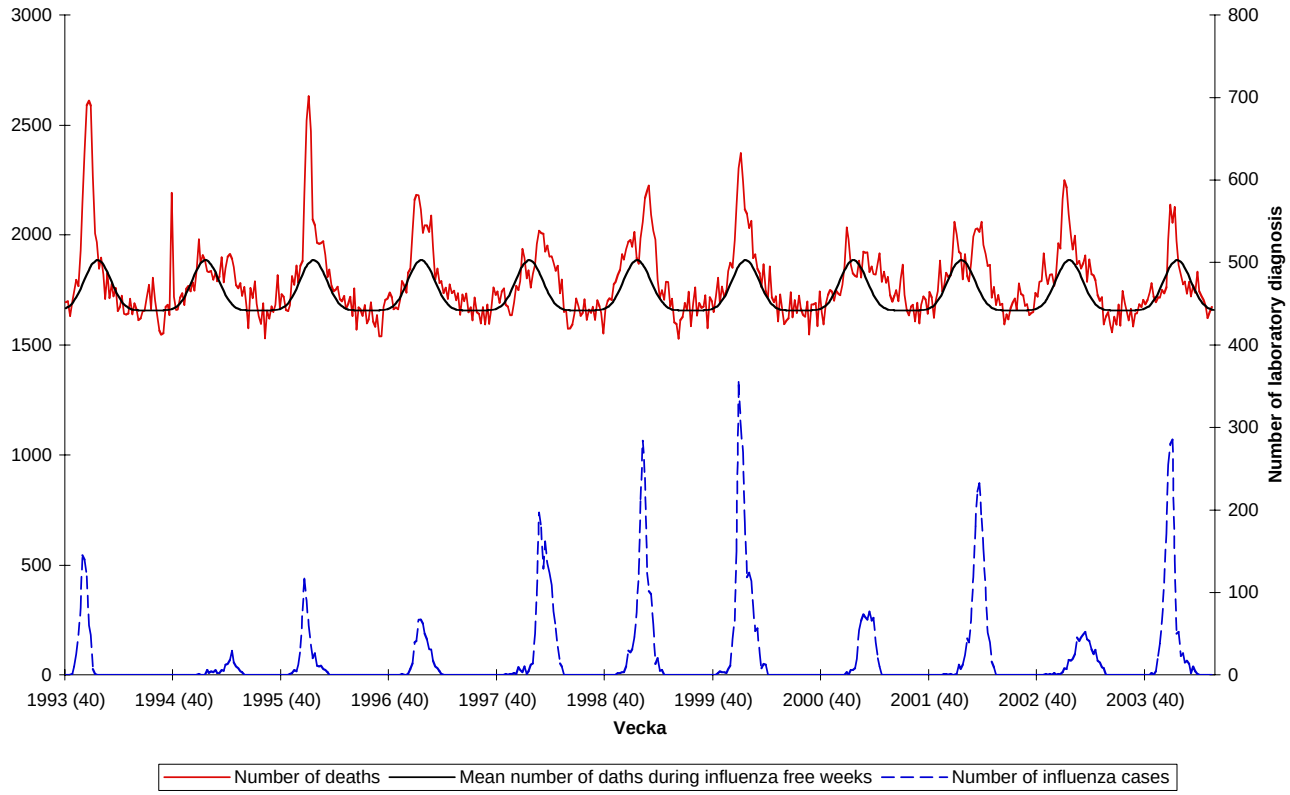


4:3) Age distribution during the last four seasons for sentinel and laboratory cases





4:4) Diagram of the weekly number of deaths in Sweden from week 40 1993 to week 20 2004, and the number of laboratory verified influenza cases during the same period. (The peak of mortality in September 1994 reflects the Estonia ferry catastrophe). Adjusted mean for corresponding influenza-free weeks is also included.

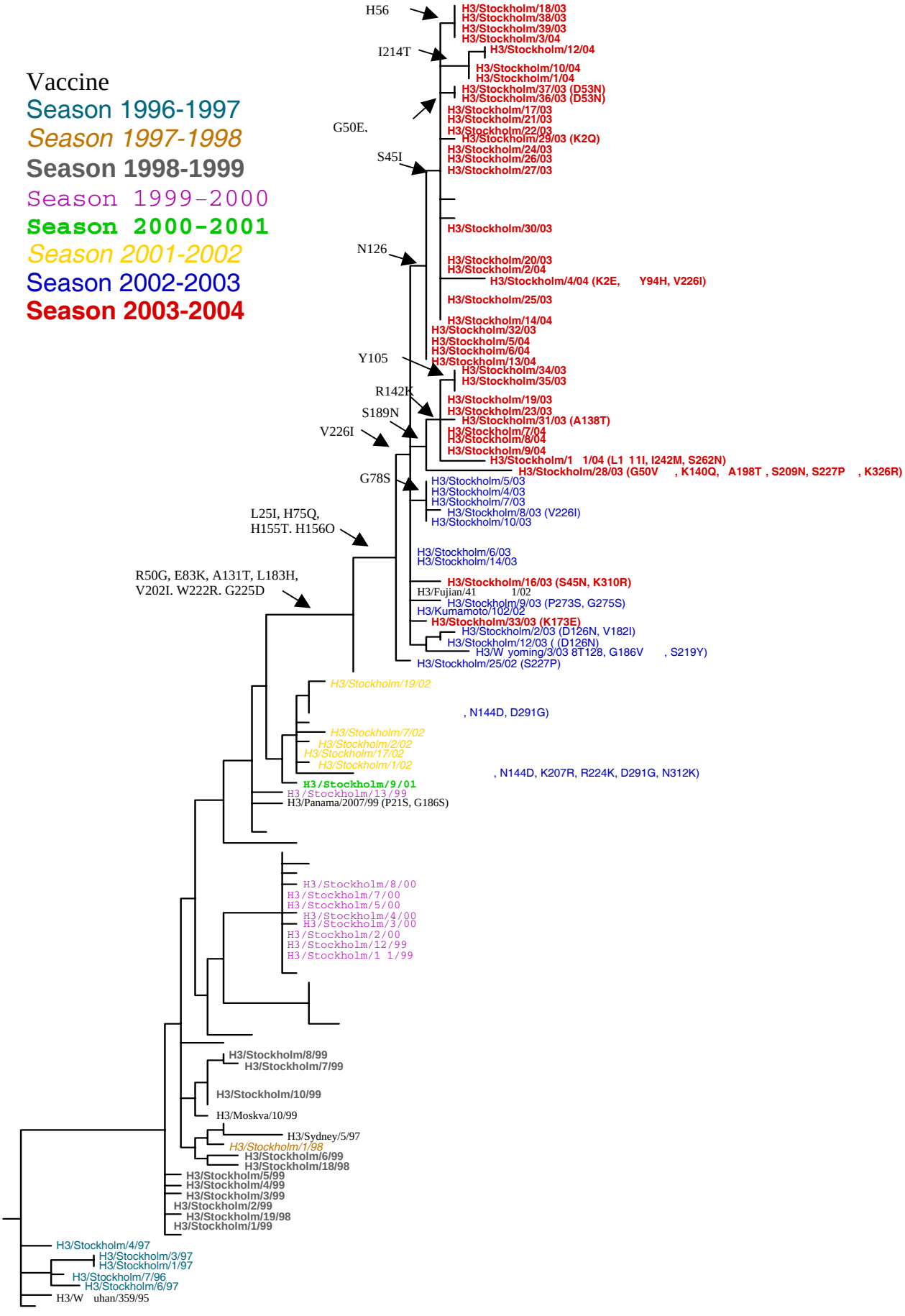


4:5) Table of isolates for which extended feno- and genotypings were performed.

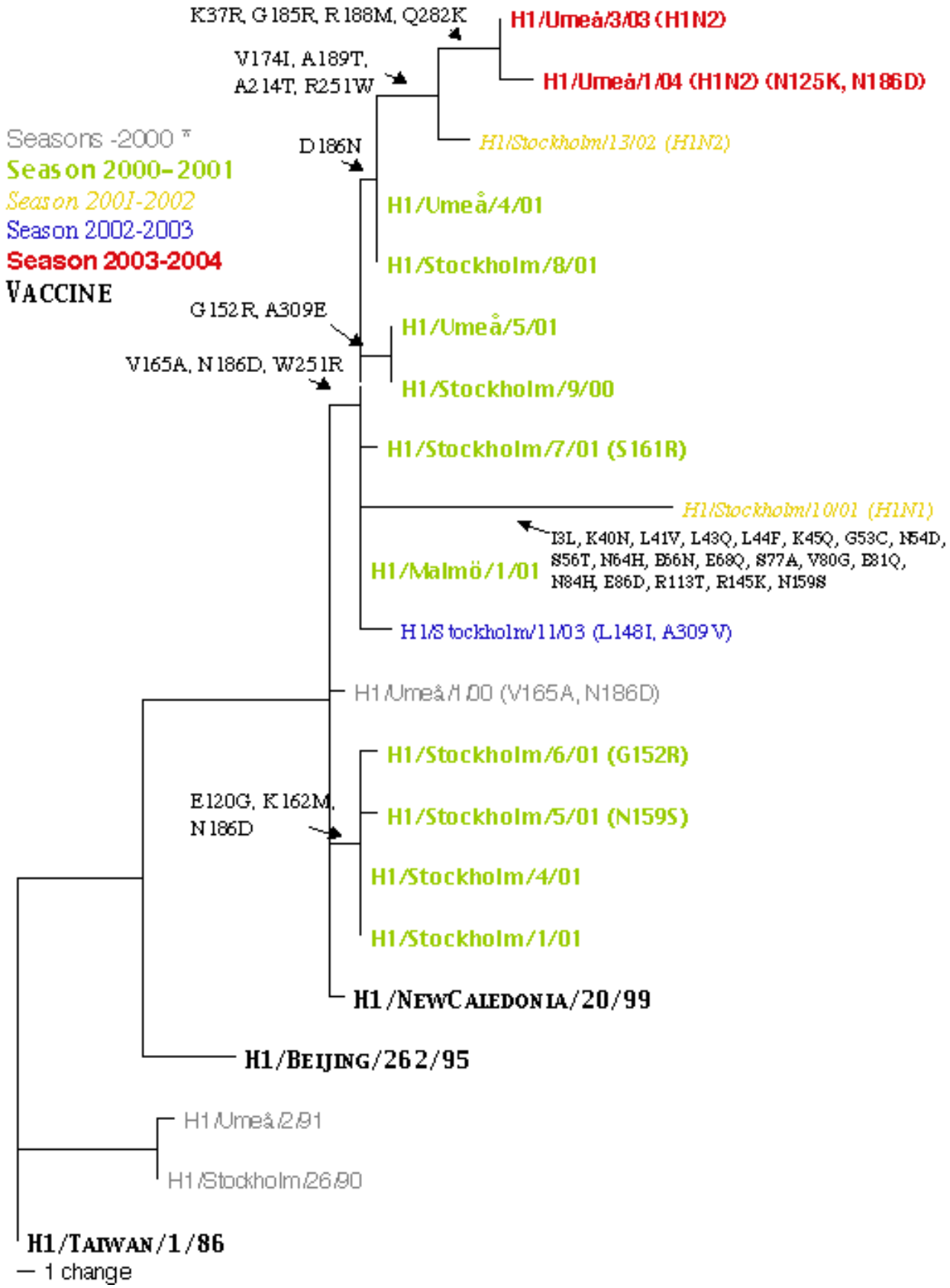
Name	Antigenically similar to:	Comments
Influenza B		
B/Stockholm/1/04	B/Shanghai/261/03	
Influenza H1N2		
A/Umeå/3/03 (H1)	A/New Caledonia/20/99	
A/Umeå/1/04 (H1)	A/New Caledonia/20/99	
Influenza H3N2		
A/Stockholm/16/03 (H3)	A/Fujian/411/02	Traveller from Viet Nam
A/Stockholm/17/03 (H3)	A/Fujian/411/02	Traveller from Scotland
A/Stockholm/18/03 (H3)	A/Fujian/411/02	Traveller from Canary Islands
A/Stockholm/19/03 (H3)	A/Fujian/411/02	
A/Stockholm/20/03 (H3)	A/Fujian/411/02	
A/Stockholm/21/03 (H3)	A/Fujian/411/02	
A/Stockholm/22/03 (H3)	A/Fujian/411/02	
A/Stockholm/23/03 (H3)	A/Fujian/411/02	Traveller from London

A/Stockholm/24/03 (H3)	A/Fujian/411/02	Traveller from Kenya
A/Stockholm/25/03 (H3)	A/Fujian/411/02	
A/Stockholm/26/03 (H3)	A/Fujian/411/02	
A/Stockholm/27/03 (H3)	A/Fujian/411/02	
A/Göteborg/2/03 (H3)	A/Fujian/411/02	
A/Göteborg/3/03 (H3)	A/Fujian/411/02	
A/Stockholm/28/03 (H3)	A/Fujian/411/02	
A/Stockholm/29/03 (H3)	A/Fujian/411/02	
A/Stockholm/30/03 (H3)	A/Fujian/411/02	
A/Stockholm/31/03 (H3)	A/Fujian/411/02	
A/Stockholm/32/03 (H3)	A/Fujian/411/02	
A/Stockholm/33/03 (H3)	A/Fujian/411/02	
A/Umeå/4/03 (H3)	A/Fujian/411/02	
A/Umeå/5/03 (H3)	A/Fujian/411/02	
A/Stockholm/34/03 (H3)	A/Fujian/411/02	
A/Stockholm/35/03 (H3)	A/Fujian/411/02	
A/Malmö/5/03 (H3)	A/Fujian/411/02	
A/Malmö/6/03 (H3)	A/Fujian/411/02	
A/Göteborg/4/03 (H3)	A/Fujian/411/02	
A/Stockholm/36/03 (H3)	A/Fujian/411/02	
A/Stockholm/37/03 (H3)	A/Fujian/411/02	
A/Stockholm/38/03 (H3)	A/Fujian/411/02	
A/Stockholm/39/03 (H3)	A/Fujian/411/02	
A/Stockholm/1/04 (H3)	A/Fujian/411/02	
A/Stockholm/2/04 (H3)	A/Fujian/411/02	
A/Stockholm/3/04 (H3)	A/Fujian/411/02	
A/Stockholm/4/04 (H3)	A/Fujian/411/02	
A/Stockholm/5/04 (H3)	A/Fujian/411/02	
A/Stockholm/6/04 (H3)	A/Fujian/411/02	
A/Stockholm/7/04 (H3)	A/Fujian/411/02	
A/Stockholm/8/04 (H3)	A/Fujian/411/02	
A/Stockholm/9/04 (H3)	A/Fujian/411/02	
A/Stockholm/10/04 (H3)	A/Fujian/411/02	
A/Umeå/2/04 (H3)	A/Fujian/411/02	
A/Umeå/3/04 (H3)	A/Fujian/411/02	
A/Göteborg/1/04 (H3)	A/Fujian/411/02	
A/Malmö/1/04 (H3)	A/Fujian/411/02	
A/Stockholm/11/04 (H3)	A/Fujian/411/02	
A/Stockholm/12/04 (H3)	A/Fujian/411/02	
A/Stockholm/13/04 (H3)	A/Fujian/411/02	
A/Stockholm/14/04 (H3)	A/Fujian/411/02	

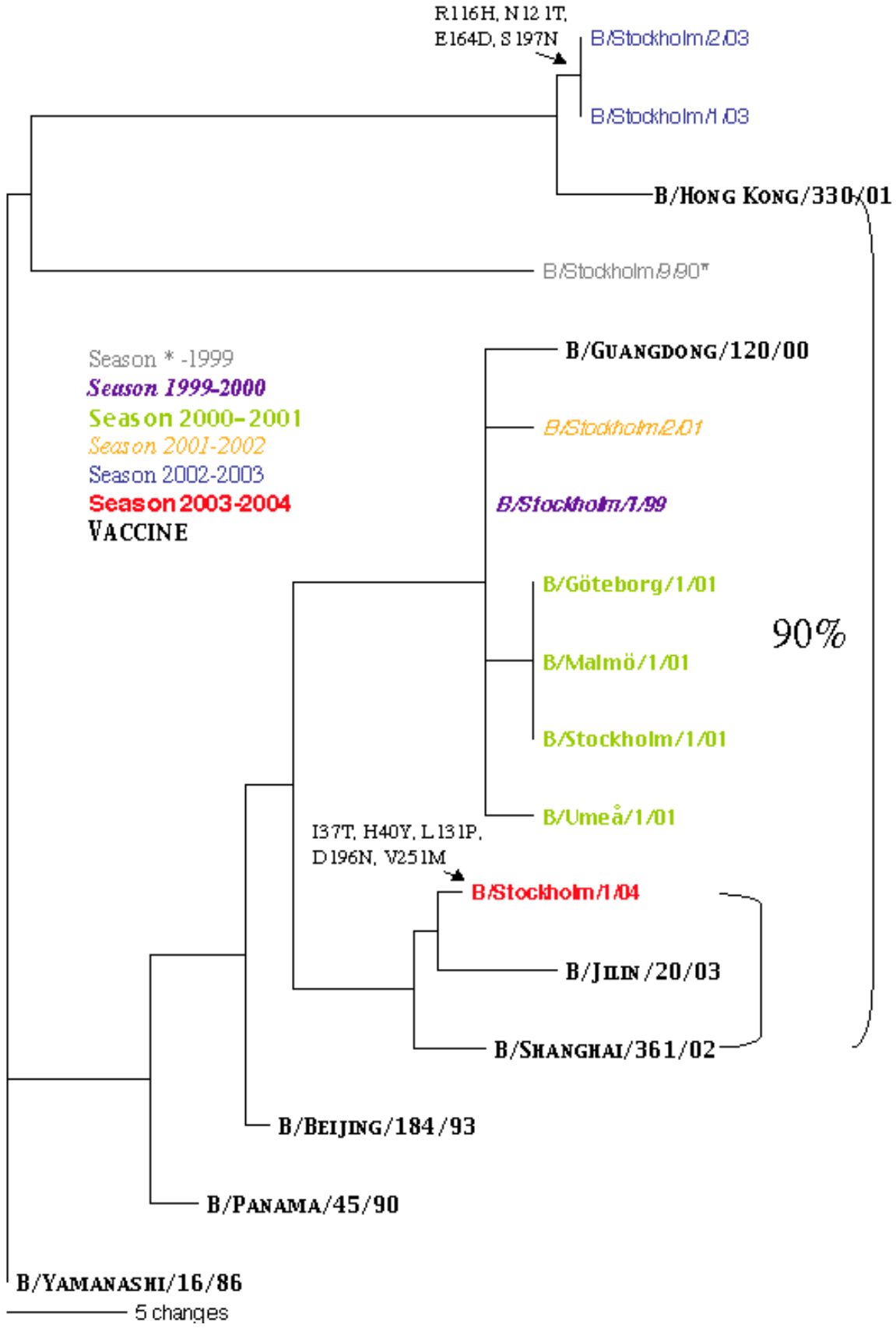
4:6) The phylogenetic tree of the amino acid sequences of HA of influenza A/H3 strains isolated in Sweden 1996-2004 compared to the vaccine strains.



4:7) The phylogenetic tree of the amino acid sequences of HA of influenza A/H1 strains isolated in Sweden compared to the vaccine strains



4:8) The phylogenetic tree of the amino acid sequences of HA of influenza B strains isolated in Sweden compared to the vaccine strains.



5) Quality control of laboratory diagnosis of influensa.

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 this 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 with different proportions of uninfected cells from a lymphoblastoid cell line. Twenty-four laboratories reporting altogether 34 data sheets participated. Most of the participating laboratories answered the panel correctly (190/200 analyses). The results of the External Quality control from 1994-2003 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 1994-2003. The number (%) of reported correct results related to total number of examinations performed with the methods.

	Influensa A/H1	**Influensa A/H3**	**Influensa B**
Imagen	176/208 (85%)	143/165 (87%)	149/194 (77%)
Chemicon indirekt IF	89/90 (99%)	75/80 (94%)	79/83 (95%)
WHO	24/25 (96%)	18/19 (95%)	21/22 (95%)
Biosoft/Argene (ej 2002, 2003)	5/6 (83%)	6/6 (100%)	5/7 (71%)
Biotrin	10/11 (91%)	9/10 (90%)	8/8 (100%)
DPC-PathoDx	59/73 (81%)	45/50 (90%)	40/48 (83%)
Chemicon direkt IF (2002, 2003)	15/18 (83%)	11/12 (92%)	9/10 (90%)
Becton-Dickinson (2002, 2003)	8/10 (80%)	8/8 (100%)	2/6 (33%)
Realtids PCR (2003)	3/3 (100%)	3/3 (100%)	2/2 (100%)

6) Method development and research.

6:1) Improved diagnostics and typing.

The arsenal of diagnostic methods using molecular techniques has been expanded. Real-time PCR for H1, H3 and the matrix protein have been refined, to enable a rapid and sensitive identification of A viruses and H1 and H3. Common sequencing methods be used for all HA and almost all NA have been established and evaluated. Faecal samples from 1500 ducks passing Öland (an island) during the autumn 2003 is being examined, and around 10% of these have been shown to contain influenza. The H5 has

been expressed in mammalian cells, and the preparations are under evaluation for serological use.

6:2) Evaluation of dry nasal swabs for laboratory diagnosis.
Nasopahryngeal aspirates, and dry nasal swabs were compared for sensitivity of influenza diagnosis with PCR. The materials worked equally well, even after 3 weeks of storage at +4°C. Manuscript in preparation.

6:3) Nasal vaccination with adjuvants.
In an EU-granted study, conventional influenza vaccine has been given intranasally to mice together with different kinds of atoxic, lipid adjuvants (Eurocine). IgG and IgA antibody formation, cellular responses and protection measured as viral growth by quantitative PCR. In all aspects, the vaccine given intranasally with one of the adjuvants is at least equally effective as subcutaneous vaccination in all parameters. A manuscript has been submitted to Vaccine.

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