RESPIRATORY SYNDROMIC SURVEILLANCE REPORT – 2012

Contributors (alphabetical order): Amelia Buys, Cheryl Cohen, Orienka Hellferscee, Jo McAnerney, Jocelyn Moyes, Marthi Pretorius, Florette Treurnicht, Marietjie Venter, Anne von Gottberg, Sibongile Walaza, Nicole Wolter

Centre for Respiratory Diseases and Meningitis, NICD, NHLS

Introduction

The National Institute for Communicable Diseases (NICD) coordinates three main respiratory syndromic surveillance programmes, each focusing on different aspects of respiratory and influenza epidemiology.

These include

1. The Viral Watch and Enhanced Viral Watch surveillance programmes
2. The severe acute respiratory illness (SARI) programme
3. The respiratory morbidity surveillance system

The principal findings of each programme for the year 2012 are summarised below.

Viral Watch and Enhanced Viral Watch surveillance programmes

Viral Watch

The Viral Watch (VW) sentinel surveillance programme was initiated in 1984. It aims to provide information on the geographic spread and timing of influenza virus circulation as well as the type and distribution of circulating influenza viruses each year. Throughout 2012, 183 practitioners registered across South Africa submitted specimens from patients fitting a clinical case definition of influenza like illness (ILI). Of these, 125 submitted specimens to the NICD, 6 to the Department of Virology at Inkosi Albert Luthuli Central Hospital/University of KwaZulu-Natal (KZN), and 52 to the NHLS Tygerberg

Hospital laboratory in the Western Cape (WCP). Positive specimens from the KZN and WCP sites were sent to the NICD for confirmation, serotyping and sequencing, and the databases of all specimens received were sent to the NICD on a weekly basis.

A total of 1945 specimens was submitted during 2012 (KZN: 145; NICD: 1468; WCP: 332). Of these 745 (38%) were positive for influenza. Dual infections were detected in 29 (4%) patients [1 A(H1N1)pdm09 & A (H3N2), 1 A unsubtyped & B, and 27 A(H3N2) & B]. The remaining 716 were further characterized as A (H3N2) (n=411, 57%), B (n=290, 41%), A(H1N1)pdm09 (n=6, 1%) and influenza A unsubtyped (n=9, 1%).

The first influenza detection of the season was made from a specimen collected on 5 June 2012 (week 23), and the last from a specimen collected on 11 October (week 41) (figure 1). The season peaked in week 33, starting 13 August when the detection rate rose to 68%. The season lasted 19 weeks. Sporadic detections were made both before and after the season. The start of the season is defined as the first week in which the influenza detection rate (calculated on specimens tested at the NICD only) rises above 10% and remains above this threshold for two consecutive weeks. The end of the season is defined as the week preceding that in which the detection rate drops below 10% and remains below this threshold for two consecutive weeks.

A further 565 non-influenza respiratory virus detections were made from 485/1200 (40%) patients negative for influenza during 2012. Of these 222 (39%) were rhinovirus, 155 (27%) were adenovirus, 32 (6%) enterovirus, 48 (8%) human metapneumovirus, 24 (4%) parainfluenza virus and 84 (15%) respiratory syncytial virus.

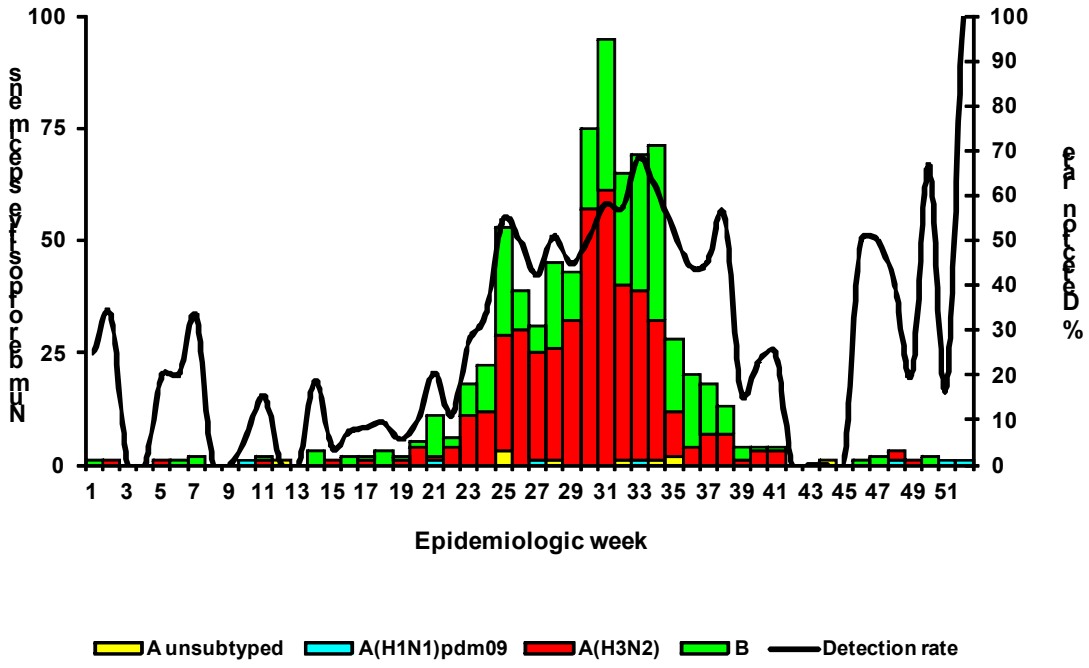


Figure 1. Influenza detection rate and numbers of positive specimens by viral subtype - Viral Watch Surveillance Programme, 2012.

Enhanced Viral Watch

In 2009, in response to the emergence of the influenza pandemic, enhanced Viral Watch centres at 12 public hospitals were enrolled to monitor influenza in hospitalized patients. In 2012, 83 specimens were received from seven of these centres, of which the largest number (n=58, 70%) came from Gauteng. Influenza was detected in the specimens of 8 patients [6 A(H3N2), and 2 B]. Other respiratory viruses were detected in a further 50 patients of which 20 (40%) were respiratory syncytial virus.

Severe acute respiratory illness (SARI) surveillance programme

The SARI sentinel surveillance programme was initiated in April 2009 and is presently functioning at six public hospitals in four provinces. The primary aims of the programme are to describe trends in the numbers of SARI cases at sentinel sites and to determine the relative contribution of influenza and other respiratory viruses to the SARI syndrome. The SARI sites are: Chris Hani Baragwanath Hospital (CHBH) in Gauteng, Matikwana and Mapulaneng hospitals which form the Agincourt site in Mpumalanga, Klerksdorp-Tshepong hospital complex in the Northwest Province and Edendale hospital in KwaZulu-Natal.

Hospitalised patients meeting the clinical case definition of acute respiratory illness are prospectively enrolled. Clinical and epidemiological data are collected using standardized questionnaires. Information on in-hospital management and outcome is also collected. Upper respiratory tract samples (oropharyngeal and nasopharyngeal swabs in patients ≥5 years old or nasopharyngeal aspirates in patients <5 years of age) are collected and tested at the NICD for the presence of influenza and other respiratory viruses using real-time reverse transcriptase polymerase chain reaction (RT-PCR). Blood specimens are tested for the presence of pneumococcal DNA using quantitative real-time PCR for the *lyt*A target.

During 2012, 5334 patients were enrolled into the SARI programme. Almost half (2463/5299, 46%) were from CHBH. Children under 5 years accounted for 2342/5334 (44%) of patients and 2761/5299 (52%) were female. Influenza results were available for 4955/5334 (93%) of enrolled patients and 258 (5%) were positive for influenza using RT- PCR. Of these, 134 (52%) were positive for influenza B, 118 (46%) were positive for influenza A(H3N2), 1 (<1%) was positive for influenza A (H1N1) pdm09 and 5 (<1%) were A unsubtyped.

During week 28 (week starting 9 July 2012) the influenza detection rate rose above 10% and remained above 10% until week 38. The peak detection rate of 24% occurred in week 34 (week starting 20 August 2012) (figure 2).

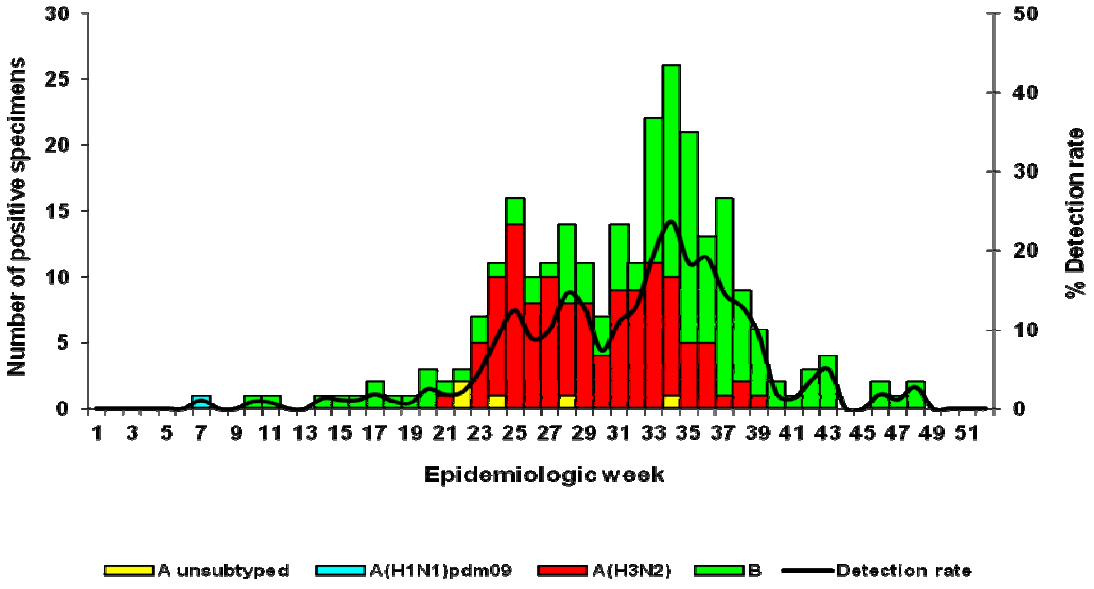
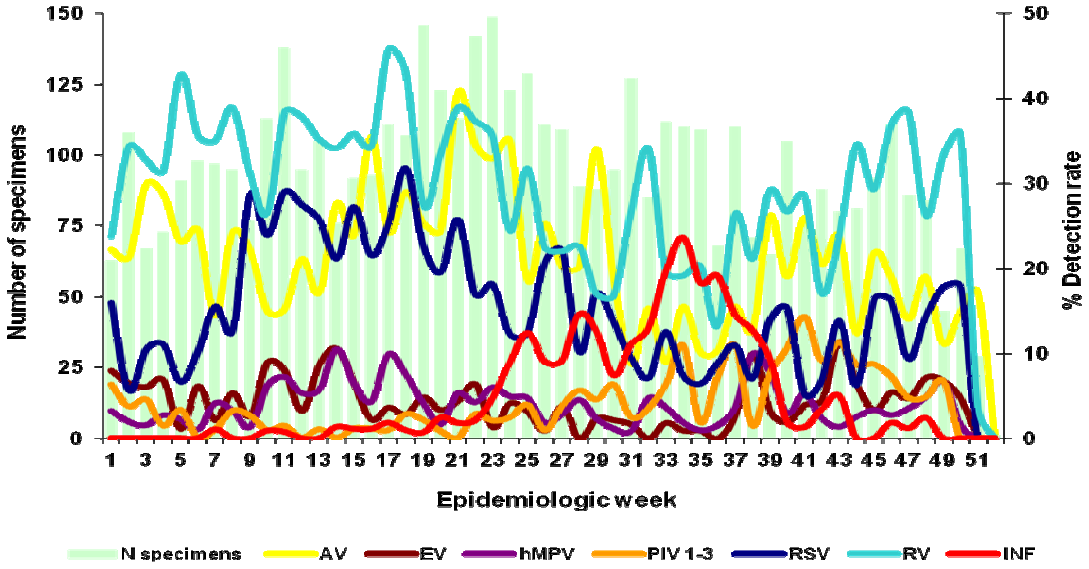


Figure 2. Influenza detection rate and numbers of positive specimens by viral subtype - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.

Amongst patients enrolled into the SARI programme, testing for additional respiratory viruses identified rhinovirus in 30% (1492/4954), adenovirus in 22% (1078/4957), respiratory syncytial virus (RSV) in 16% (791/4956), enterovirus in 4% (204/4957), human metapneumovirus in 4% (197/4957), parainfluenza 3 in 4% (139/4954), parainfluenza 1 in 1% (36/4957) and parainfluenza 2 in 1% (26/4956) of samples (figure 3).

The RSV season preceded the influenza season in 2012. The detection rate for RSV remained above 10% from week 9 until week 33 and reached a peak of 32% in week 18. Of the 5334 patients enrolled into SARI 4083 (77 %) had blood specimens tested for the presence of pneumococcal DNA. Of these, 320 (8%) were positive for pneumococcus and 12 of these patients (11%) were co-infected with influenza (figure 4).

Figure 3. Numbers of specimens received and detection rate of respiratory viruses by epidemiologic week - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.



*N specimens=number of specimens, AV=adenovirus 1078/4957(22%); EV=enterovirus 204/4957(4%); hMPV=human metapneumovirus 197/4957 (4%); PIV1-3=parainfluenza virus type 1, 2, 3 202/4957(4%); RSV=respiratory syncytial virus 791/4956(16%); inf=influenza 258/4955(5%).

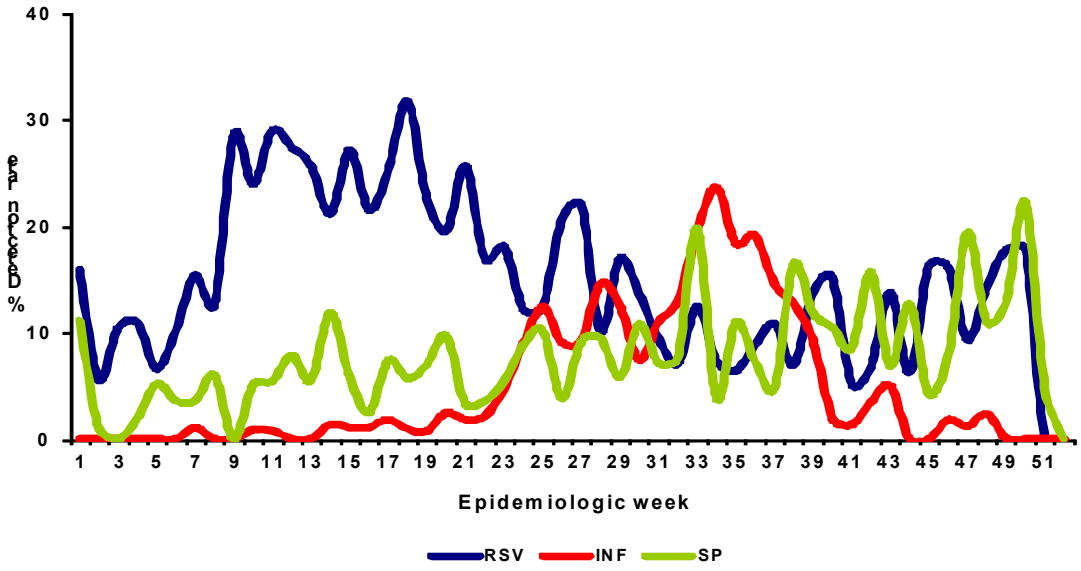


Figure 4. Detection rate for influenza (INF), respiratory syncytial virus (RSV) and pneumococcus (SP) by epidemiologic week - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.

Respiratory Morbidity Surveillance

In order to describe the influence of the influenza season on the number of pneumonia and influenza consultations and hospitalisations, the NICD reviews anonymized data from a private hospital group. The numbers of hospitalizations for pneumonia and influenza during the influenza season are compared to those for the periods preceding and following the influenza season as defined by the Viral Watch programme for influenza consultation and the SARI programme for hospitalisations.

During 2012 there were 994 402 consultations reported to the NICD through the respiratory morbidity data mining surveillance system. Of these, 29 589 (3%) were due to pneumonia or influenza (P&I) (International Classification of Diseases 10 codes J10-18). There were 21 596 (73%) inpatients and 7993 (27%) outpatients with P&I discharge data.

An increase in P&I consultations and admission was observed during the period with a higher number of seasonal influenza virus isolations reported to the Viral Watch and SARI surveillance programmes respectively (figures 5 and 6). A second lower peak was seen proceeding the influenza season, corresponding to the circulation of respiratory syncytial virus.

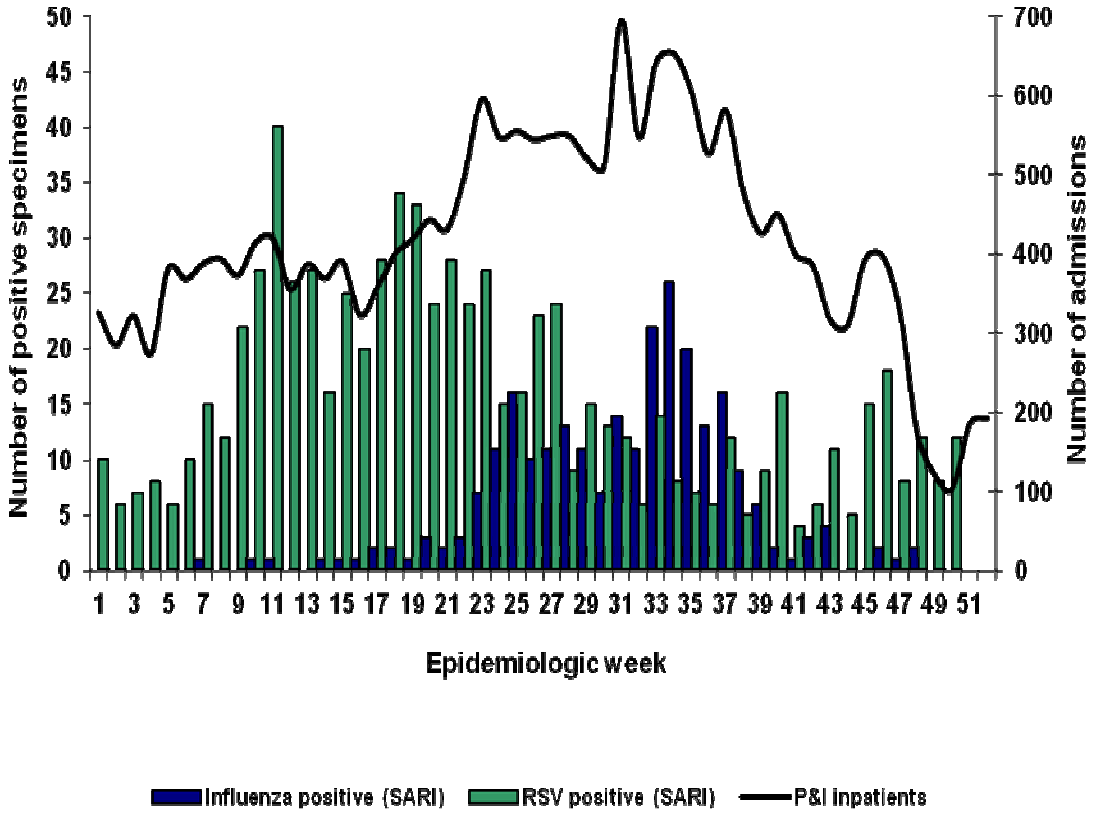


Figure 5. Numbers of private hospital admissions with a discharge diagnosis of pneumonia and influenza (P&I) and viral isolates by epidemiological week - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.

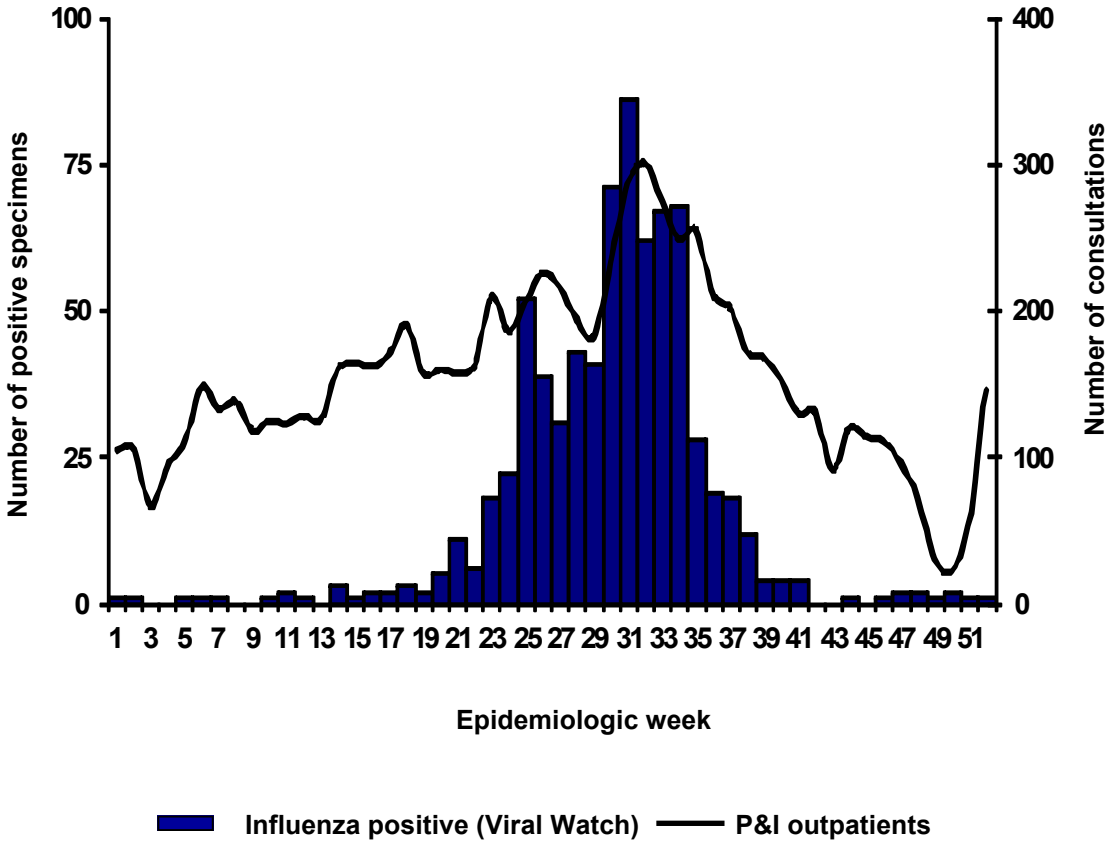


Figure 6. Numbers of private hospital outpatient consultations with a diagnosis of pneumonia and influenza (P&I) and viral isolates by epidemiological week - Severe Acute Respiratory Illness (SARI) Surveillance Programme ,2012.

Molecular characterization of influenza virus strains

Influenza A(H3N2)

Sixty influenza A(H3N2) strains were selected for sequencing throughout the 2012 season from both SARI and Influenza Like illness cases. All 2012 strains clustered within the A/Victoria/208/2009 genetic group with the majority of viruses in lineages 7 and sub-lineage 3A. Viruses that belonged to sub-lineages 3B, 3C and lineage 6 were also circulating (figure 7). The emerging genetic lineage 7 identified in 2011 became the dominant circulating H3N2 strain in 2012 and had acquired an additional D291G mutation.

In addition, the influenza A(H3N2) M gene sequences generated from 70 clinical samples were analysed and all contained the S31N mutation in the M2 protein which confers resistance to adamantanes.

Influenza A(H1N1)pdm09

In the 2012 season only 7 influenza A viruses were sub-typed as A(H1N1)pdm09 and all had cycle threshold (CT) values greater than 30. A single virus isolate was recovered from cell cultures but no hemagglutination of turkey red blood cells could be demonstrated. The HA gene was sequenced from one and the M gene from 3 clinical samples with all 3 carrying the S31N amantadine resistance mutation.

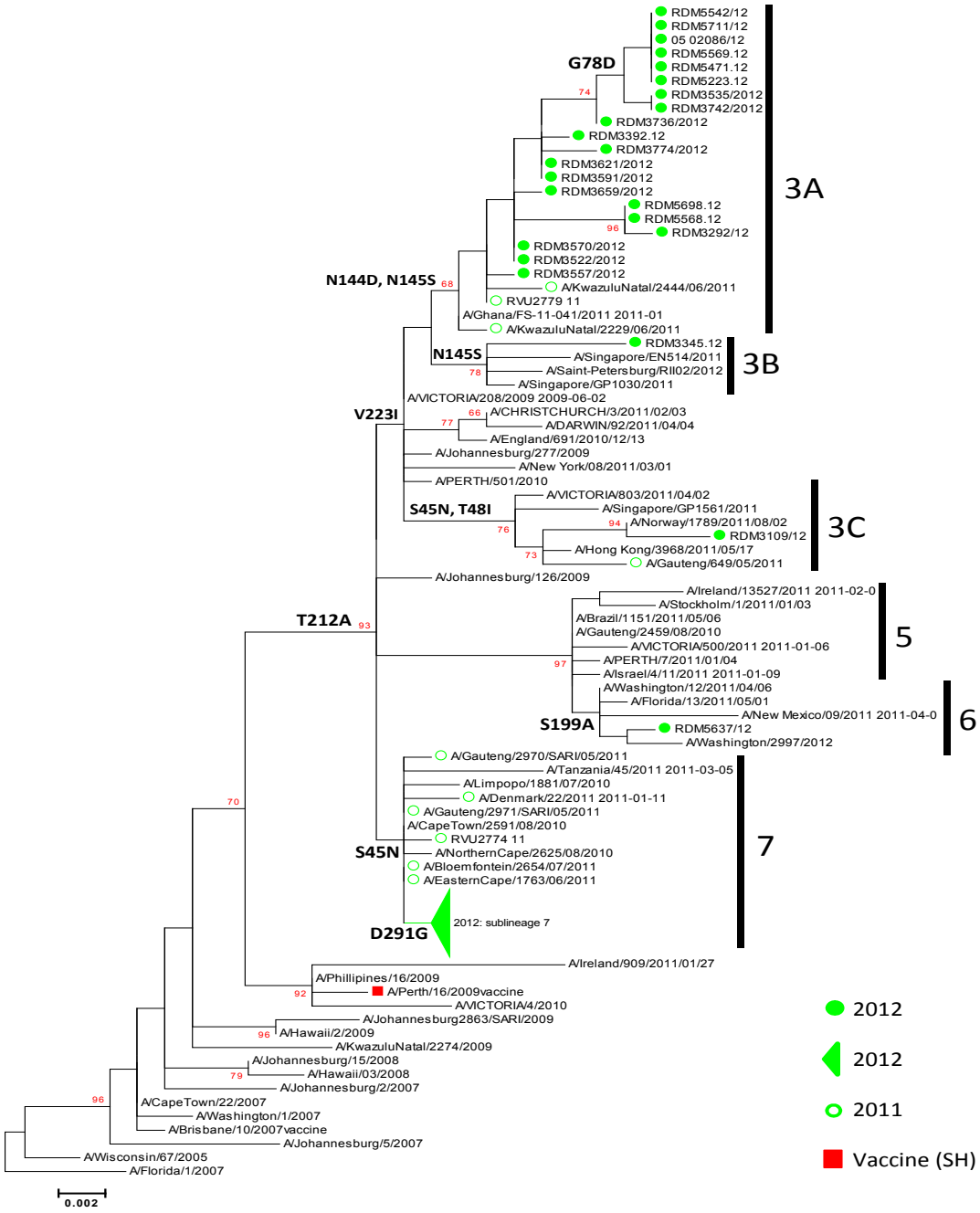


Figure 7. Maximum likelihood tree of the A(H3N2) HA1 region (900bp), South Africa 2012. The 2012 South African strains are indicated in green solid circles (open green circles = 2011). The current southern hemisphere (SH) vaccine strain is indicated by a red square. Amino acid changes corresponding to different groups are indicated. Collapsed 2012 South African strains (solid green triangle) are shown in lineage 7, n=37. Sub-lineage 3C is the lineage representative of viruses similar to the 2012/2013 vaccine strain for the northern hemisphere.

Influenza B

The HA1 region of the HA genes from a total of 66 clinical samples positive for influenza B was sequenced and characterised with 45 grouping in the B/Victoria lineage and 21 in the B/Yamagata lineage.

B/Victoria lineage

Phylogenetic analysis and comparison of the deduced amino acid sequence alignments for the 45 B/Victoria/ lineage-like viruses (figure 8) showed that the majority are B/Brisbane/60/2008-like (or genetic clade 1).

B/Yamagata lineage

Seven viruses belong to clade 2 which is characterised by the mutations R48K, P108A and T181A and the other fourteen are in clade 3 (characterised by the amino acid mutations S150I, N165Y and G229D) as shown by phylogenetic analysis (figure 9).

The NA genes of 31 Influenza B viruses from 2012 were sequenced of which 25 were B/Brisbane-like and 6 were B/Yamagata like (figure 10). Amino acid alignments highlighting mismatches to the vaccine or reference strains were deduced and no mutations known to confer phenotypic drug resistance were detected.

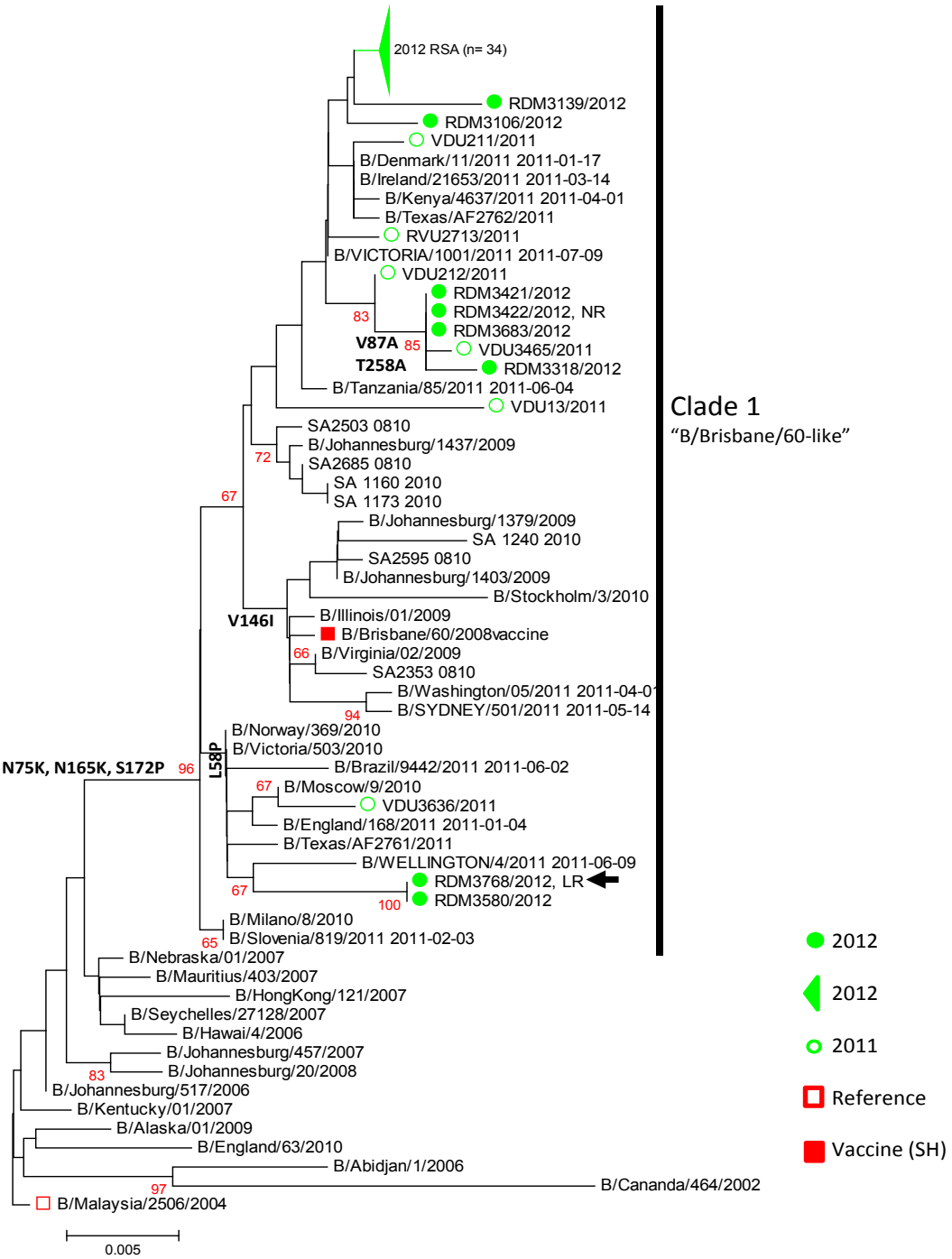


Figure 8. Maximum likelihood tree of the HA1 region of influenza B/Victoria-like viruses (954bp), South Africa 2012. The current southern hemisphere (SH) vaccine strain is indicated in solid red and 2012 strains from South Africa are indicated in solid green. LR = Low Reactor indicated by black arrow, NR= Normal Reactor, B/Malaysia/2506/2004 as root and reference indicated by open red box.

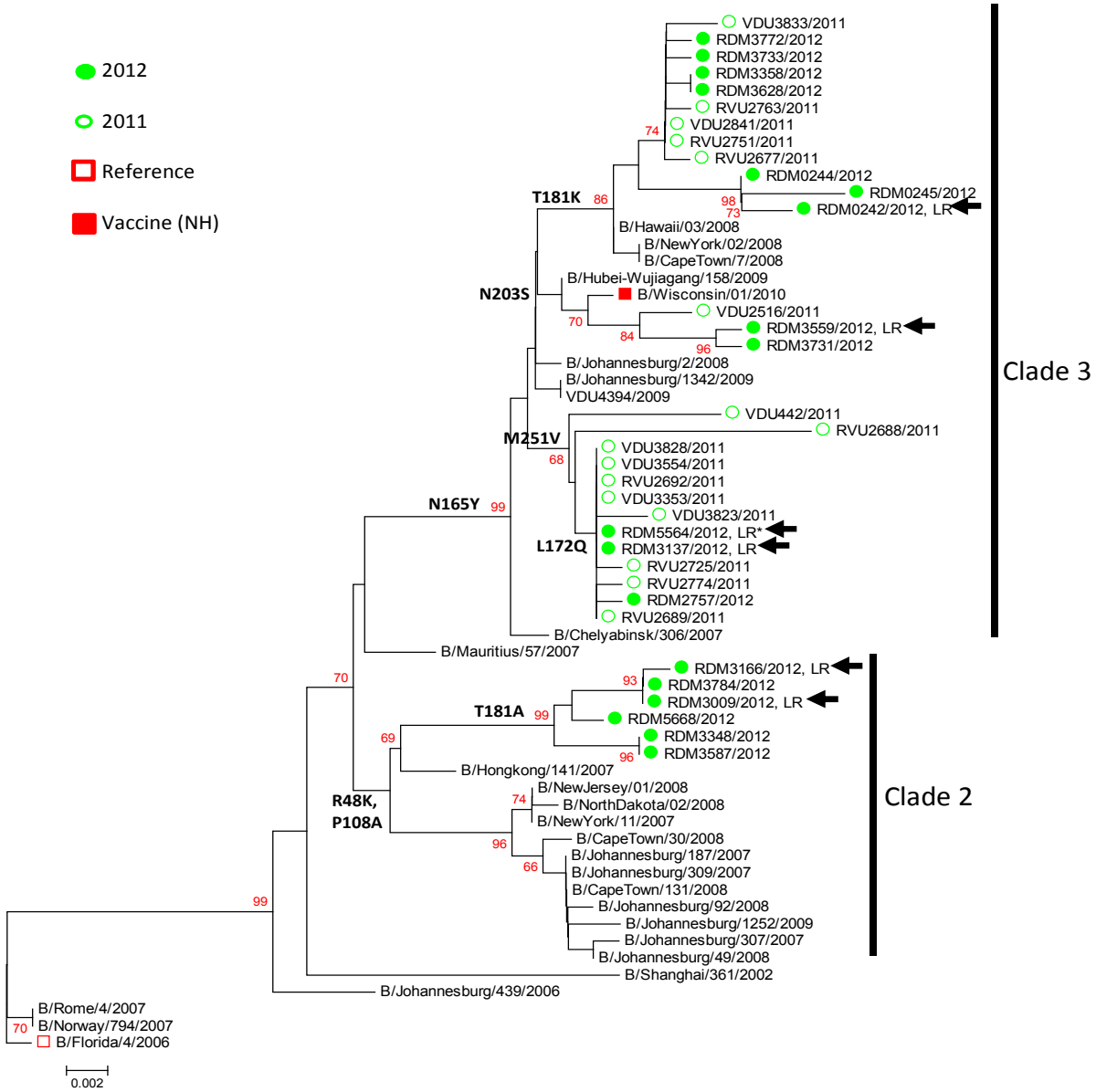


Figure 9. Maximum likelihood tree of the HA1 region of influenza B/Yamagata-like viruses (954bp), South Africa 2012. The 2012/2013 vaccine strain selected for the northern hemisphere (NH) is indicated in solid red and 2012 strains from South Africa are indicated in solid green. LR = Low Reactor indicated by black arrows, B/Florida/4/2006 as root and reference indicated by open red box.

Antigenic characterisation of influenza virus strains

During the influenza season of 2012 influenza virus isolation was attempted on clinical samples that tested positive for influenza on a real-time (RT) multiplex polymerase chain reaction (PCR) assay with a CT value of 30 or less. Both the conventional MDCK cells and the MDCK-SIAT1 cells were used in parallel for virus isolations. A total of 114 influenza virus isolations was obtained of which 83 were from influenza A(H3N2) viruses and 30 from influenza B viruses and a single influenza A(H1N1)pdm09 isolate. Using turkey and guinea pig red blood cells, 48% (55/114) of the cell culture isolates could be tested by hemagglutination inhibition assays and no difference was observed in the success rate for influenza virus isolates generated by either MDCK or MDCK-SIAT1 cell cultures.

Embryonic egg isolations were attempted for 59 clinical samples of which 22 positive cultures were generated for influenza A(H3N2) and 11 for influenza B as measured by immunofluorescence which detects infected cells and measures influenza virus

neuraminidase (NA) activity. All A(H3N2) egg isolates were negative for hemagglutination of either turkey or guinea pig red blood cells, but 19 showed NA activity. The influenza B egg isolations were more successful with 7/11 (64%) giving hemagglutination titres and NA activity whilst the rest showed only NA activity.

The hemagglutination inhibition assay results for antigenic characterization of influenza A(H3N2) and influenza B viruses are summarized in table 1. A total of 26 A(H3N2) virus isolates could be characterised antigenically by hemagglutination inhibition assay (HIA) and all showed normal reactivity to the A/Perth/16/2009 reference antiserum. As mentioned before, the majority of circulating H3N2 strains belong to lineages 7 and 3 and based on global data a new H3N2 vaccine strain for the 2013 Southern Hemisphere vaccine was selected from sub-lineage 3C (A/Victoria/361/2011).

Thirty three influenza B viruses were characterized for reactivity to reference antisera raised against vaccine or other reference antigens using the hemagglutination inhibition assay (table 1). Twenty five isolates reacted to the B/Brisbane/60/2008-like reference antisera of which 6 had low reactivity to the vaccine strain. For the B/ Yamagata-like isolates 7 showed low reactivity to the B/ Wisconsin/1/2010 antisera and 1 had low reactivity to the B/Florida/4/2006 antisera. The B/Wisconsin/1/2010-like virus strain was selected for inclusion in the 2013 vaccine for the southern hemisphere. Representative cell culture and egg isolates as well as clinical samples were sent to the WHO collaborating centres in London and Melbourne for further characterization.

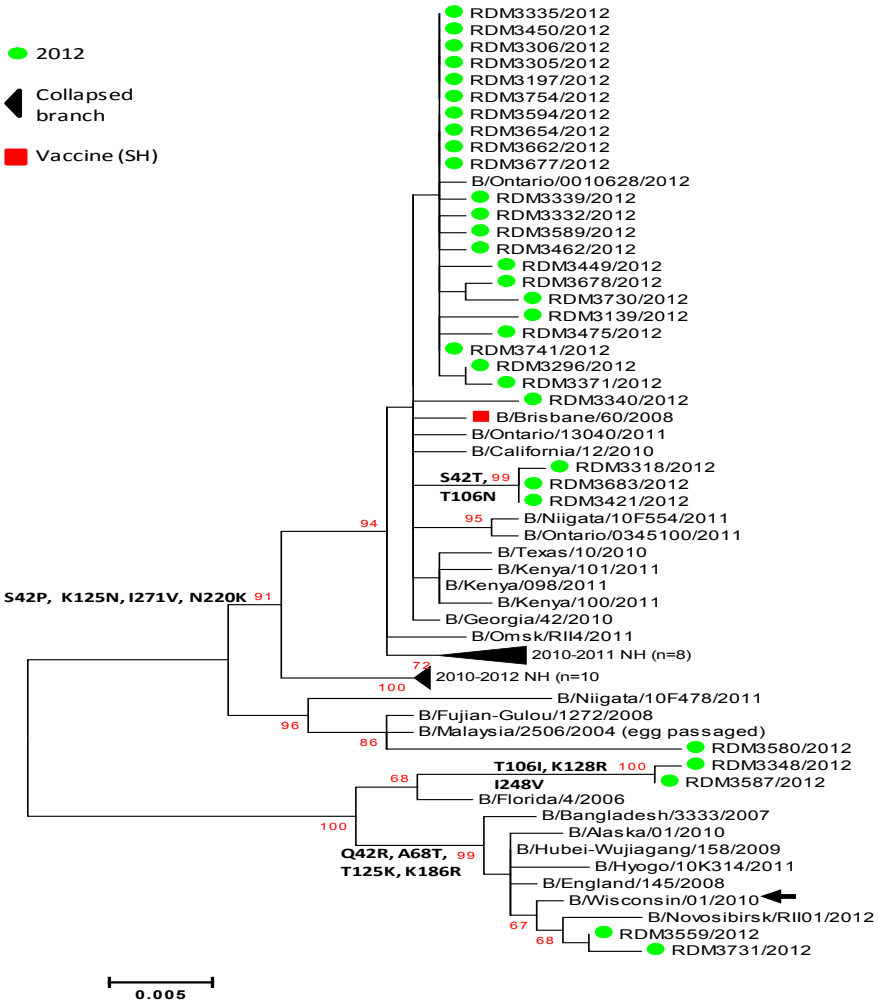


Figure 10. Unrooted maximum likelihood tree of the 5'NA gene region of influenza/Victoria-like and B/Yamagata-like viruses (807 bp), South Africa 2012. The 2012/2013 vaccine strain selected for northern hemisphere is indicated by a black arrow and the current southern hemisphere (SH) vaccine strain is indicated by a solid red box. The 2012 strains from South Africa are indicated by solid green circles.

Table 1: Summary of influenza virus isolation by hemagglutination and results of the hemagglutination inhibition assay, South Africa 2012.

Number of isolates attempted in cell lines and (Eggs): n = 173 114 in cell lines and 59 in eggs (egg isolates)			
Total number of positive cultures: n= 147 **as indicated by immunofluorescence and neuraminidase activity** **(egg isolates)**			
Flu A(H1N1)2009 n = 1	Flu A(H3N2) n = 83 (22)	Flu B n =30 (11)	
Hemagglutination assay results			
HA positive n =59		HA negative n = 88	
Hemagglutination inhibition results **(egg isolates)**			
A/California/7/2009 (H1N1)pdm09-like n = 0	A/Perth/16/2009 (H3N2)-like n = 26	B/Brisbane/60/2008-like n = 21 + (4) 6 low reactors	B/Wisconson/01/2010-like n = 8 8 low reactors

Resistance testing of influenza virus strains

A total of 142 H3N2 positive clinical samples (Viral Watch =115 , SARI =27) were tested for the presence of the E119V mutation associated with oseltamivir resistance by real-time PCR. The 119V oseltamivir resistance variant was not detected with 127/142 samples having the wildtype E119 mutation. There was no amplification in the other 15 samples.

No evidence of phenotypic drug resistance to oseltamivir or zanamivir was detected in influenza A (H3N2) [n= 6] and B [n= 14] virus isolates with relative luciferase units of ~ 40000 and greater.

Discussion

The influenza season of 2012 was biphasic with co-circulation of influenza A(H3N2) and influenza B. Although the detection rate of influenza in the Viral Watch programme was similar to 2011, the detection rate of influenza in the SARI progamme was lower than in previous years (2010:7%, 2011: 9%, 2012 5% $p<0.001$). This trend was evident even when excluding enrolled cases meeting the expanded case definition only and when stratifying by age group (data not shown). This change in detection rate could reflect true differences in influenza virus circulation between seasons or an unmeasured bias in our surveillance programme.

Genetic drift has occurred in influenza A/H3N2 and B strains from the vaccine strains. In contrast to 2011 the Influenza B/Brisbane/60-like viruses predominated and

for the first time we identified low reactors to reference antisera for these isolates. All the B/Yamagata-like virus isolates showed low reactivity with antisera raised against the B/Wisconsin/1/2010 strain or B/Florida/4/2006. Circulating influenza A(H3N2) viruses mainly belonged to lineage 7 - identified in 2011 as an emerging lineage - as well as lineage 3. No neuraminidase inhibitor resistant influenza viruses were detected.

No seasonal A(H1N1) strains were detected in 2012 and only sporadic cases of A(H1N1)pdm09 were detected. The combination of conventional MDCK, MDCK-SIAT1 cell cultures and use of embryonated eggs for influenza virus isolation contributed to the success with which influenza virus strains were isolated. However, influenza A(H3N2) isolates from embryonated egg inoculations did not agglutinate turkey or guinea pig red blood cells.

Vaccine recommendations for the 2013 influenza season in the southern hemisphere include a new influenza A(H3N2) strain from the sub-lineage 3C (A/Victoria/361/2011) and change of the influenza B vaccine strain to a B/Yamagata/ lineage strain namely, B/Wisconsin/1/2010-like. For the first time the WHO has recommended the inclusion of both the B/Brisbane and B/Yamagata-like strains in a quadrivalent vaccine for the 2012/2013 northern hemisphere and 2013 southern hemisphere influenza season.

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