

RESPIRATORY VIRUS SURVEILLANCE REPORT, SOUTH AFRICA, 2013

Cheryl Cohen, Amelia Buys, Cardia Fourie, Orienka Hellferscee, Jo McAnerney, Jocelyn Moyes, Florette Treurnicht, Marthi Pretorius, Anne von Gottberg, Sibongile Walaza, Nicole Wolter, Marietjie Venter

Centre for Respiratory Diseases and Meningitis, NICD

Introduction

The National Institute for Communicable Diseases (NICD) coordinates four influenza surveillance programmes. These aim to characterise the influenza subtypes circulating in South Africa, as well as to describe the seasonality and epidemiology of the annual influenza season. These programmes collate data on milder influenza-like illness (ILI) and severe acute respiratory (SARI) illness.

The four influenza surveillance programmes include:

1. Viral Watch and Enhanced Viral Watch
2. Severe acute respiratory illness (SARI)
3. Influenza-like illness (ILI) in public health facilities
4. The respiratory morbidity surveillance system

The principal findings of each programme for the year 2013 are given 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. During 2013, 171 practitioners registered across South Africa submitted a total of 2009 specimens throughout the year. Of these, 1803 were submitted to the NICD, 26 to the Department of Virology at Inkosi Albert Luthuli Central Hospital/University of KwaZulu-Natal, and 180 to the National Health Laboratory Service, University of Cape Town laboratory, in the Western Cape Province. Positive specimens from these sites were sent to the NICD for

confirmation, serotyping and sequencing.

Of the 2009 specimens tested, 877 (44%) were positive for influenza. Dual A(H1N1)pdm09 and A(H3N2) infection was detected in five samples (<1%). Other dual infections included one sample positive for A(H1N1)pdm09 and B, and one for A(H3N2) and B. Of the remaining 870 positive specimens, 578 (66%) were influenza A(H1N1)pdm09, 143 (16%) were A(H3N2), 146 (17%) were B, and 3 (<1%) were A unsubtype.

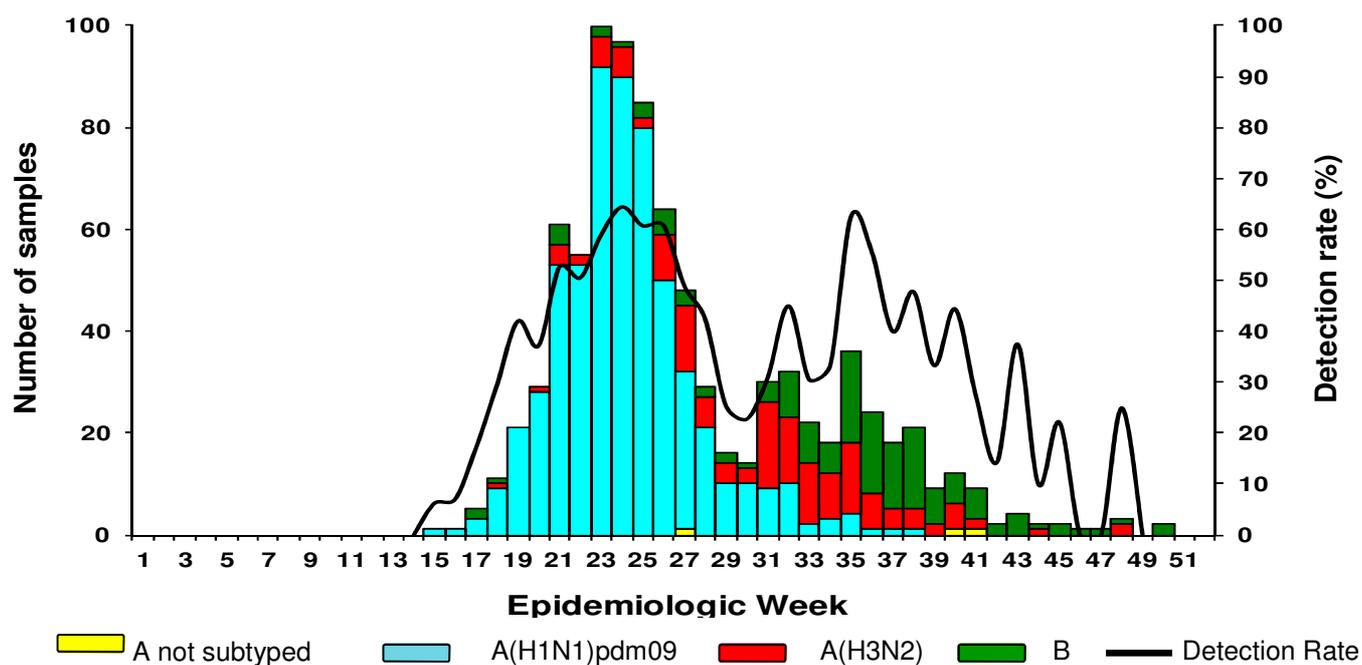
The beginning of the influenza season is defined as the first week the influenza detection rate (calculated on specimens tested at the NICD only) rises above 10% and then consistently remains above this level. The end of the season is defined as the week before the detection rate drops below 10%. The first influenza case of the 2013 season was detected in a specimen collected on 22nd April (week 17), and the last from a specimen collected on 13th October (week 41). The onset of the influenza season in week 17 is one of the earliest recorded since the beginning of the Viral Watch. The season peaked in week 24 when the detection rate rose to 64%. While the average duration of the influenza season over the 9 years prior to 2013 is 17 weeks, the 2013 season lasted 25 weeks (figure 1).

A further 565 respiratory virus detections were made from the 486/1132 (43%) patients who tested negative for influenza during 2013. Of these, 112 (23%) were adenovirus (AV), 47 (8%) were enterovirus (EV), 59 (10%) were human metapneumovirus (HMP), 50 (9%) were parainfluenza viruses (PIV) 1-3, 56 (10%) were respiratory syncytial virus (RSV) and 229 (40%) were

rhinovirus (RV).

Enhanced Viral Watch

In 2009, in response to the prevailing influenza pandemic, enhanced Viral Watch centres at 12 public hospitals were initiated to detect influenza strains in patients hospitalized with severe respiratory illness. In 2013, 327 specimens were received from six of these centres. Of these specimens, the largest number (287, 88%) came from Gauteng. Influenza was detected in the specimens of 30 (10%) patients of which 23 were A(H1N1)pdm09, two were A(H3N2), and five were influenza B. Two hundred and forty-eight other respiratory viruses were detected in a further 196 patients of which 71 (29%) were RV, 63 (25%) were RSV and 55 (22%) were AV.



A(H1N1)pdm09 n= 584, A(H3N2) n=143, influenza B n=146, A not subtyped n=3

Figure 1: Numbers of samples and influenza detection rate, by influenza subtype and week, in patients enrolled into the Viral Watch surveillance programme, 2013.

Severe acute respiratory illness (SARI) surveillance programme

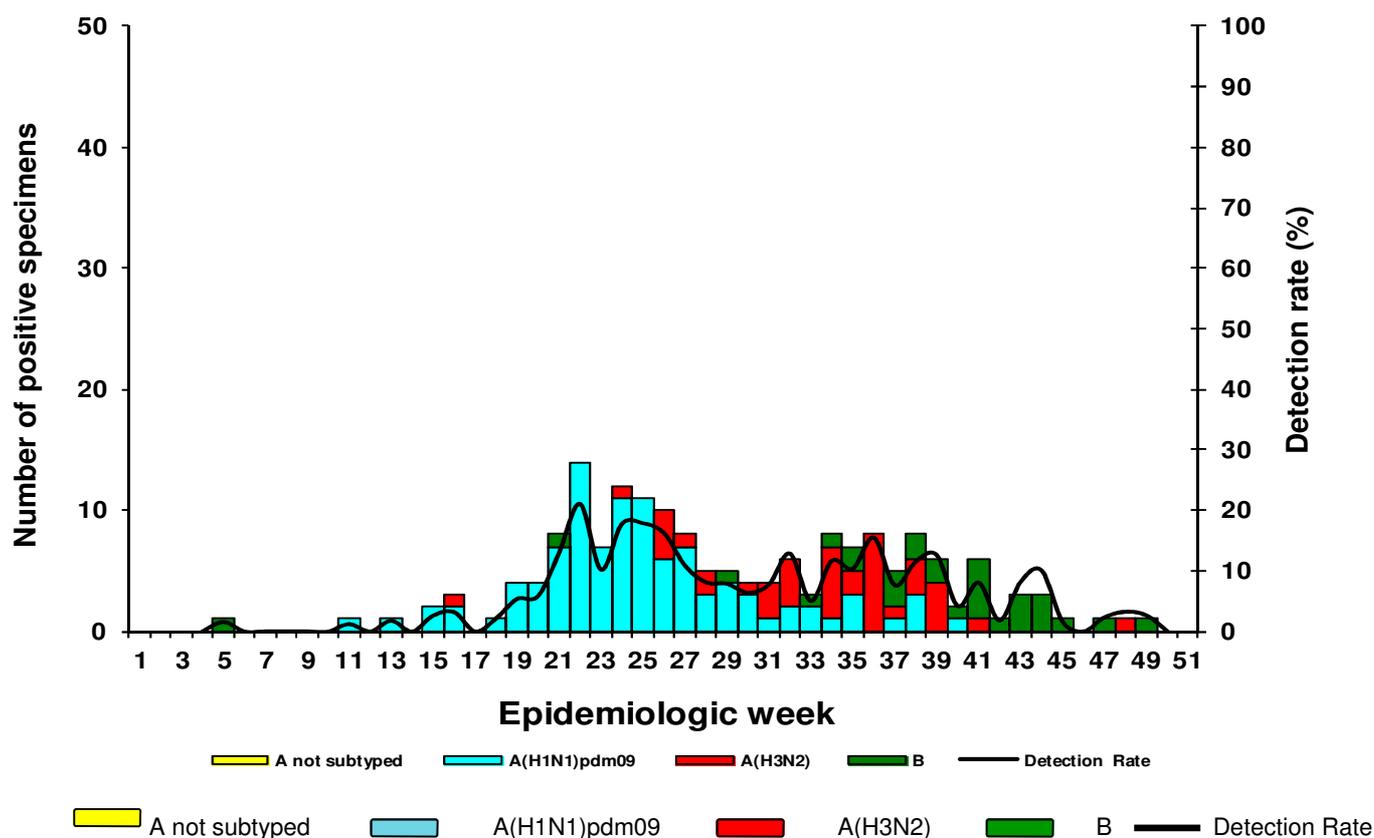
The SARI sentinel surveillance programme was initiated in April 2009 and is presently operational at six public hospitals in four provinces. The primary aims of the programme are to describe trends in the numbers of SARI cases at sentinel sites and to determine the relative contribution of influenza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

Hospitalised patients meeting the clinical case definition of acute respiratory illness were prospectively enrolled. Clinical and epidemiological data were collected using standardized questionnaires. Information on in-hospital management and outcome was also collected. Upper respiratory tract samples (oropharyngeal and nasopharyngeal swabs in patients ≥ 5 years old or nasopharyngeal aspirates in patients < 5 years of age) were 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 were tested for the presence of pneumococcal DNA using quantitative real-time PCR for the *lytA* target. In 2013, due to funding limitations, numbers enrolled at the CHBH site were reduced by systematic sampling of paediatric and adult patients on a 1-2 days per week rotating schedule.

During 2013, 3128 patients were enrolled into the SARI programme, from which 3041 (97%) samples were collected and tested for respiratory viruses. Due to the aforementioned change in enrolment sampling at the

CHBH the number of samples collected at Klerksdorp/Tshepong (KTH) hospitals was higher than that at CHBH for the first time since the introduction of the programme. A third of the samples were collected at KTH (1012/3037, 33%). Children under 5 years accounted for 51% (1594/3128) of patients and 1650/3124 (53%) were male. Of the 3041 patients with influenza results, 174 (6%) were positive for influenza using RT-PCR. Of these, 101 (58%) were positive for influenza A(H1N1) pdm09, 42 (24%) were positive for influenza A(H3N2), 31 (18%) were positive for influenza B and one was a dual infection comprising A(H1N1) pdm09 and A(H3N2).

During week 21 (week starting 20th May), the influenza detection rate rose above 10% and remained above 10% until week 28 (week starting 8th July). The peak detection rate of 18% occurred in week 25 (week starting 17th July). A smaller peak of more than 10% occurred between weeks 34 (week starting 19th August) and week 36 (week starting 2nd September) (figure 2).



A(H1N1)pdm09 n=101, influenza A(H3N2) n=42, influenza B n=31, influenza A(H1N1)pdm09 & A(H3N2) n=1

Figure 2: Numbers of samples positive for influenza and influenza detection rate, by subtype and week, in patients enrolled into the Severe Acute Respiratory Illness (SARI) programme, 2013.

Amongst patients enrolled into the SARI programme, testing for additional respiratory viruses identified RV in 28% (860/3041), AV in 18% (539/3041), RSV in 15% (452/3041), EV in 6% (170/3041), human metapneumovirus (hMPV) in 3% (96/3041), PIV3 in 3% (99/3041), PIV1 in 1% (39/3041) and PIV2 in 1% (29/3041) of samples.

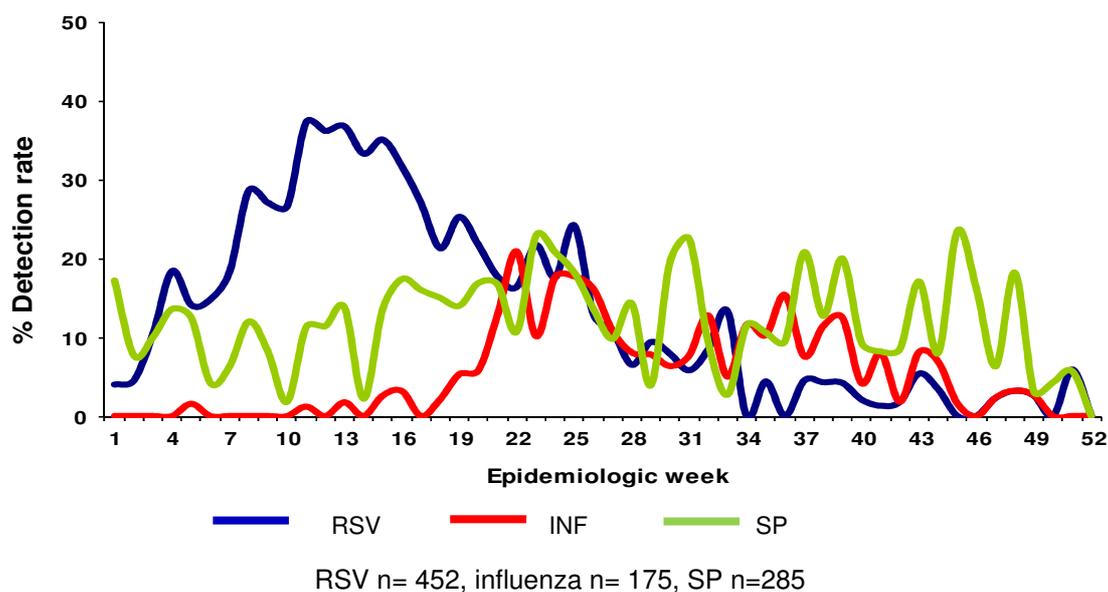


Figure 3: Severe Acute Respiratory Illness (SARI) surveillance programme detection rates for respiratory syncytial virus (RSV), influenza virus (INF) (all subtypes) and *Streptococcus pneumoniae* (SP) by week, 2013.

Of the 3128 patients enrolled into SARI, 2382 (76%) had blood specimens tested for the presence of pneumococcal DNA. Of these, 285 (12%) were positive for *Streptococcus pneumoniae* (SP) (figure 3). Of the patients with influenza, 143/175 (82%) had blood samples taken and 21/143 (15%) were positive for SP. During 2013, the RSV season preceded the influenza season. The

detection rate for RSV remained above 10% from week 3 (week starting 14th January) until week 31 (week starting 22nd July) and reached a peak of 37% in week 11 (week starting 11th March). Figures 4 and 5 show the detection rates for respiratory viruses other than influenza and RSV.

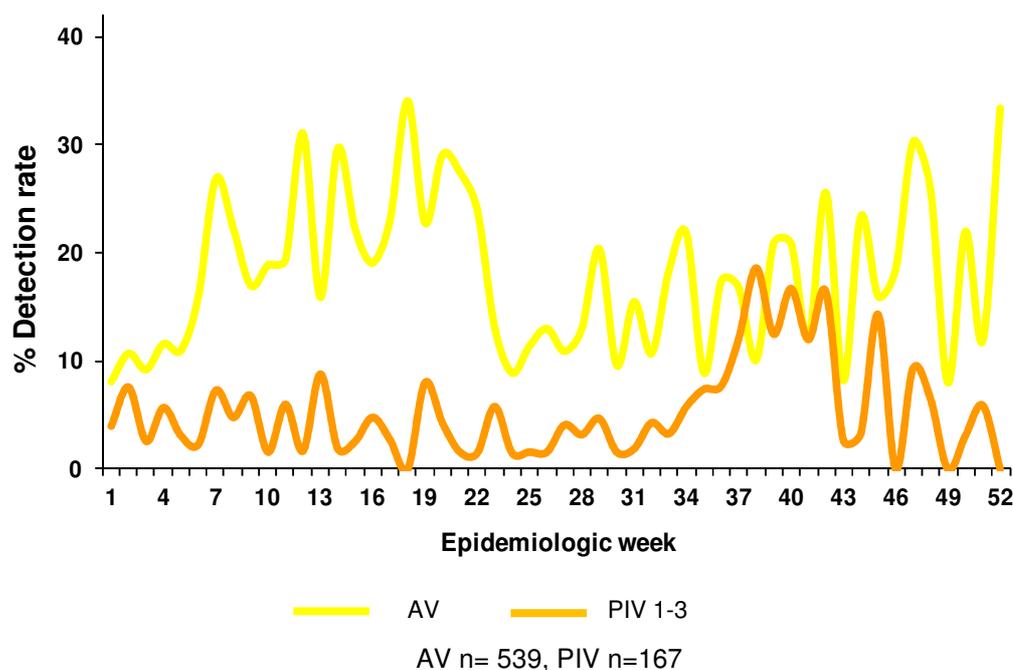


Figure 4: Severe Acute Respiratory Illness (SARI) surveillance programme detection rates for adenovirus (AV) and parainfluenza viruses (PIV) (1-3) by week, 2013.

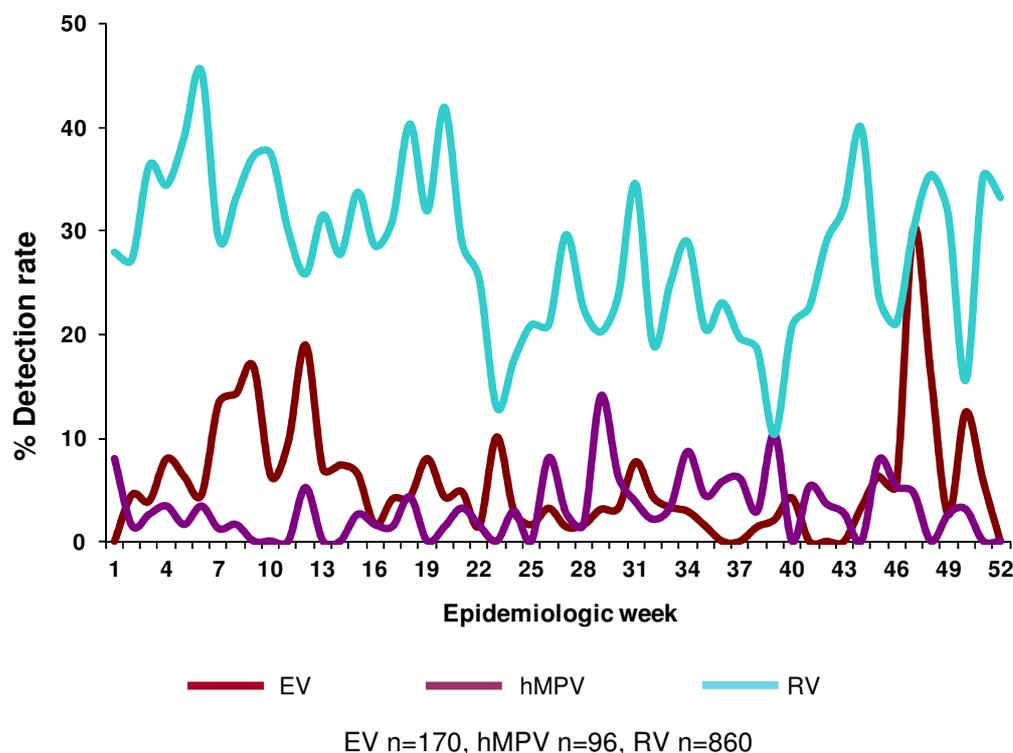


Figure 5: Severe Acute Respiratory Illness (SARI) surveillance programme detection rates for enterovirus (EV), human metapneumovirus (hMPV) and rhino virus (RV) by week, 2013.

Influenza-like illness in primary health care clinics

During 2012, systematic surveillance for ILI was set up at two clinics in two provinces (North West Province and KwaZulu-Natal). An additional four clinics in these provinces were added during 2013. Patients fitting a clinical case definition were prospectively enrolled. Clinical and epidemiological data were collected for each patient. Upper respiratory tract samples (oropharyngeal and nasopharyngeal swabs in patients ≥ 5 years old or nasopharyngeal aspirates in patients < 5 years of age) were collected and tested at the NICD for the presence of influenza and other respiratory viruses using RT-PCR.

During 2013, a total of 1991 specimens was received from all six ILI sites. Of the 243 (12%) positive samples, influenza A(H1N1)pdm09 was detected in 120 (49%), influenza A(H3N2) in 67 (28%), influenza B in 43 (18%)

and influenza A (not subtyped) was detected in three patients ($< 1\%$). There were two dual infections: one A(H1N1)pdm09 and A(H3N2), and one A(H3N2) and influenza B. The first influenza detection of the season was made from a specimen collected on 29th April (week 18), and the last positive specimen was collected in week 46 (week starting 11th November). Sporadic detections were made both before and after the season.

The influenza season started in week 19 and continued to week 26 (6th May through week starting 24th June). A second peak occurred between weeks 37 and 45 (9th September through week starting 4th November). The peak detection rate of 83% was observed in week 44 (week starting 21st October) (figure 6). The two peaks correspond to the influenza season described by the Viral Watch programme.

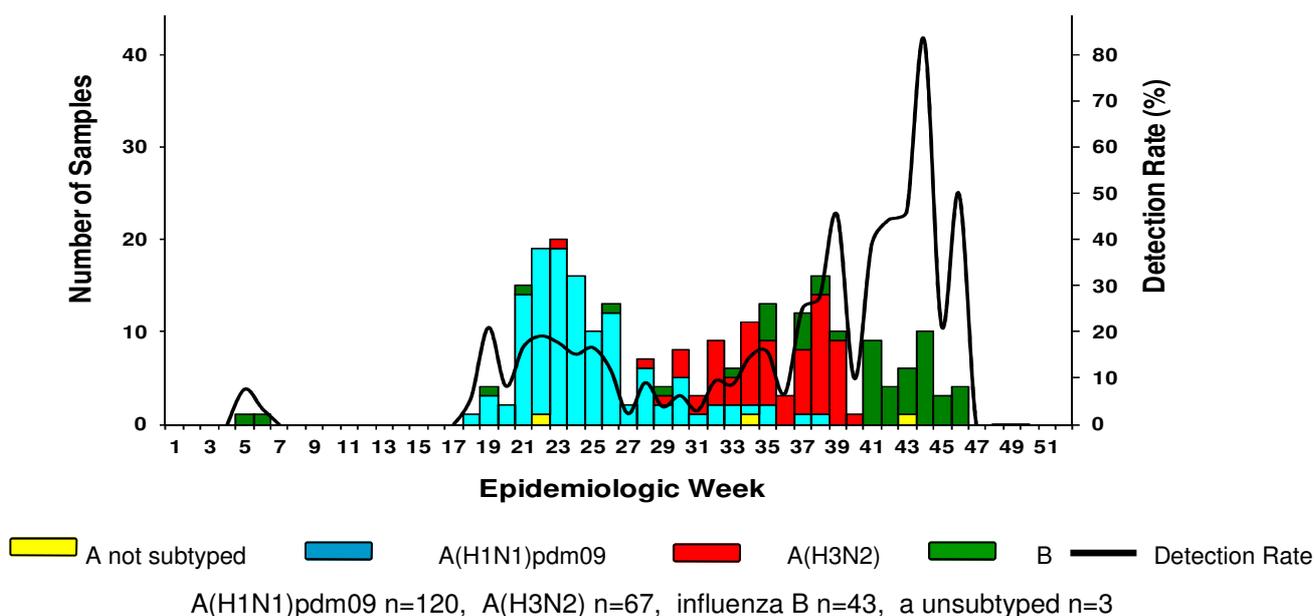


Figure 6: Numbers of samples and influenza detection rate, by influenza subtype and week, in patients enrolled into Influenza-like Illness (ILI) surveillance at public health clinics, 2013.

Respiratory Morbidity Surveillance

In order to describe the influence of the influenza season on the number of pneumonia and influenza (P&I) hospitalizations, the NICD reviews anonymized data from a private hospital group. The numbers of hospitalizations for P&I during the influenza season were compared to those for the periods preceding and following the season. During 2013 there were 1 204 969 consultations reported to the NICD through the respiratory morbidity data mining surveillance system.

Of these, 31637 (3%) were due to P&I.

An increase in P&I consultations and admissions with a second peak following the initial peak was observed during the influenza season as reported in the Viral Watch and SARI programmes (figure 7). The smaller peak preceding the influenza season corresponds to the RSV season observed in the SARI programme. Similarly, the number of hospitalizations for P&I corresponds to the peak RSV and influenza seasons (figure 8).

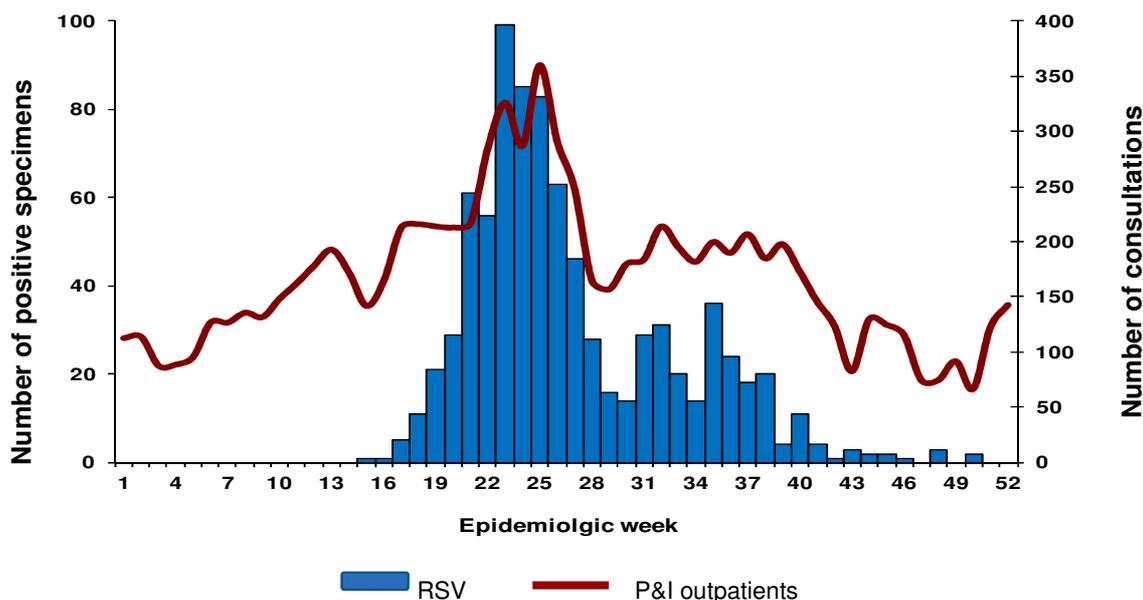


Figure 7: Numbers of private hospital outpatient consultations with a discharge diagnosis of pneumonia and influenza (P&I), and numbers of influenza positive viral isolates (Viral Watch) by week, 2013.

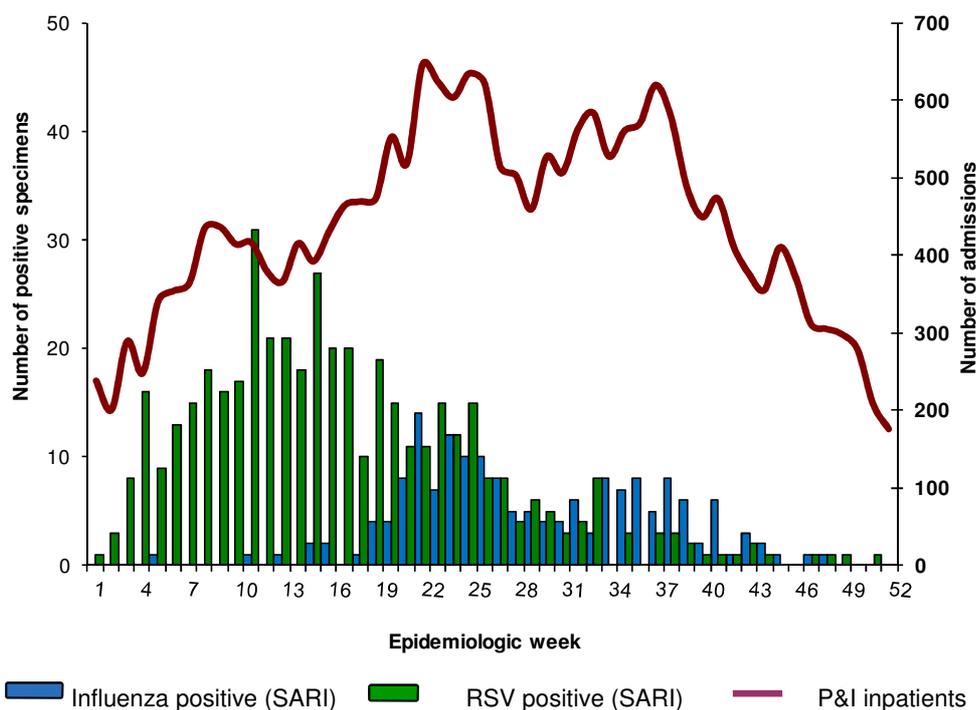


Figure 8: Numbers of admissions for pneumonia and influenza (P&I inpatients), as well as numbers of influenza positive viral isolates (Viral Watch) and respiratory syncytial virus (RSV) positive isolates (SARI) by week, 2013.

Molecular characterizations of influenza virus strains

Genetic characterisations of Influenza A (H3N2), A (H1N1)pdm09 and Influenza B strains are used to monitor genetic drift as well as the emergence of new lineages which are identified by specific amino acid mutations relative to a designated reference strain. The naming of lineages is decided by the WHO Vaccine Consultation Meeting team.

Influenza A(H3N2)

H3N2 HA gene sequences were generated from 9 clinical specimens selected from the 2013 season for both the ILI and SARI surveillance programs. All 2013 strains are within genetic group 3, specifically subgroup 3C, of the seven described lineages. Subgroup 3C is characterised by the following amino acid mutations: Q33R, N145S and N278K relative to A/Perth/16/2009 as reference. Other mutations that can also occur in

subgroup 3C viruses are S45N, T48I, A198S and V223I. The 2013 vaccine strain A/Victoria/361/2011 is also in subgroup 3C.

Influenza A(H1N1)pdm09

In the 2013 season, the HA gene from 70 influenza A (H1N1)pdm09 positive clinical samples was sequenced. Influenza viruses representing 99% of the samples (69/70) were lineage 6 (CDC classification) and one was lineage 7. Influenza A(H1N1)pdm09 strains from five individuals who reported that they received the vaccine are lineage 5 (n=1) and lineage 6 (n=4). The signature amino acid mutation, K283E, characterises the subgroup in lineage 6 containing the 2013 South African viruses.

Influenza B

The HA1 region of the HA genes from a total of seven clinical samples positive for influenza B was sequenced

and characterised. No B/Victoria lineage strains were identified.

B/Yamagata lineage

Seven viruses sequenced belong to clade 2 of B/Yamagata lineage viruses which is characterised by the mutations R48K, P108A and T181A, whereas in 2012 two-thirds of B/Yamagata lineage viruses were in clade 3 which is characterised by the amino acid mutations S150I, N166Y and S230D in reference to the B/Florida/4/2006 strain. In SARI cases positive for influenza B, all samples (n=71/86) subtyped belonged to the B/Yamagata lineage.

Isolation and antigenic characterisations of influenza virus strains

During the 2013 influenza season, a total of 63/91 (69%) influenza virus isolates were successfully obtained from clinical samples that tested positive for influenza on a real-time multiplex PCR assay with a crossing point value ≤ 30 . Of these, 53 were influenza A and 10 were influenza B viruses. The majority of influenza A isolates (89%, n=41/46) were A(H1N1)pdm09 which dominated the season. Of the embryonic egg isolations attempted, 50% (18/36) were successful, of which 14 were influenza A(H1N1)pdm09, three were influenza A(H3N2) and one was influenza B.

A total of 52 virus isolates could be characterised antigenically by hemagglutination inhibition assay (HIA) of which 80% (40/49) were influenza A(H1N1)pdm09 and showed normal reactivity to the A/California/7/2009 reference antiserum. Four influenza A(H3N2) isolates were typed of which three reacted with a 4-fold lower titre and one reacted with a ≤ 2 -fold lower titre compared to the control or reference antiserum, A/Perth/16/2009. Eight influenza B virus isolates reacted with titres similar to the control antiserum for the influenza B/Yamagata lineage, B/Wisconsin/1/2010.

Resistance testing of influenza virus strains

No drug resistant genotypes were detected from a total of 103 influenza A(H1N1)pdm09 positive clinical samples [ILI (Viral Watch)=70 hospitalized cases, (Enhanced Viral Watch)=6; SARI=27] tested for the presence of the H275Y mutation associated with oseltamivir resistance. In addition, 24 influenza virus isolates were tested for phenotypic evidence of reduced susceptibility to the neuraminidase inhibitors oseltamivir and zanamivir. Of these, two showed reduced sensitivity even though both contained the drug-sensitive genotype at position 275.

Discussion

The 2013 influenza season was initially dominated by circulation of influenza A(H1N1)pdm09, mainly in weeks 20-30 (13th May to 22nd July), followed by circulation of predominantly A(H3N2) in weeks 31-40 (29th July to 30th September). The season ended with circulation of mainly influenza B in weeks 41-47 (7th October to 11th November). Although the overall detection rate was higher in the Viral Watch programme (44%) than in the ILI surveillance programme (12%), the season and circulating viral subtypes were similar. The protracted 2013 season is reflected in all surveillance programmes.

The detection rate of 12% for *S. pneumoniae* was somewhat higher than has been recorded in preceding years. This was likely a result of changes to the surveillance programme including improved quality of DNA extraction from blood specimens and changes in the enrolment algorithm at CHBH in 2013.

The A(H1N1)pdm09 strains dominated the season and the majority of viruses were in genetic lineage 6. All viruses showed good antigenic reactivity to antisera raised against the A/California/7/2009 vaccine strain. Genetic drift from the vaccine strains has occurred in the influenza A and B strains. In contrast to 2012 when both

Influenza B lineages co-circulated, the Influenza B/Yamagata-like viruses circulated in 2013. All the B/Yamagata-like virus isolates showed normal reactivity with antisera raised against the B/Wisconsin/1/2010 vaccine strain. Circulating influenza A(H3N2) viruses mainly belonged to lineage 3C. Three A(H3N2) isolates typed showed low reactivity to antisera raised against the A/Victoria/361/2011 vaccine strain. Two isolates from participants with influenza-like illness were identified that showed reduced sensitivity to the neuraminidase inhibitors in the phenotypic resistance assay, yet contained the sensitive genotype for the signature mutation at position 275.

Vaccine recommendations for the 2014 influenza season in the southern hemisphere include a new influenza A(H3N2) strain, A/Texas/50/2012-like, which has antigenic properties similar to the A/Victoria/361/2011 vaccine strain, and a change from the influenza B vaccine strain to a new B/Yamagata/lineage strain namely B/Massachusetts/2/2012-like.

Acknowledgements

We wish to thank all doctors who participated in the Viral Watch and Enhanced Viral Watch programmes in 2013. Contributors to the SARI and Viral Watch Surveillance programmes are thanked for their inputs. These include: Shabir Madhi, Director, NICD; Amelia Buys, Maimuna Carrim, Cheryl Cohen, Mignon Du Plessis, Orienka Hellferscee, Victoria Magomani, Jo McAnerney, Fahima Moosa, Jocelyn Moyes, Marthi Pretorius, Makatisane Papo, Adrian Puren, Florette Treurnicht, Akhona Tshangela, Marietjie Venter, Anne von Gottberg, Sibongile Walaza and Nicole Wolter of the Centre for Respiratory Diseases and Meningitis, NICD; Mark Goosen and Deidre Greyling of the Centre for HIV and STIs, NICD; Andrew Black of Chris Hani Baragwanath Hospital; Michelle Groome, David Moore and Suzan Nzenze of the Department of Science and Technology /

National Research Foundation: Vaccine Preventable Diseases Unit; Meera Chhagan, Halima Dawood, Sumayya Haffejee and Fathima Naby of Edendale Hospital; Keith Klugman of Emory University, Atlanta USA; Erna du Plessis, Omphile Mekgoe and Ebrahim Variava of the Klerksdorp/Tshepong Hospital Complex; Kathleen Kahn, Stephen Tollman and Rhian Twine of the MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt); Frew Benson of the South African National Department of Health - Communicable Diseases Directorate; Adam Cohen and Stefano Tempia of the United States Centers for Disease Control and Prevention (CDC); Keitumetsi Baloyi, Ulentia Chetty, Margaret Hlobo, Sandra Kashe, Agnes Koena, Tselane Makgoba, Julia Malapane, Wisdom Malinga, Seipati Matshogo, Annalet Moodley, Myra Moremi, Thulisile Mthembu, Nomathemba Mofokeng, Bekiwe Ncwana, Wendy Ngubane, Maureen Nkosi, Andrina Sambo, Gabisile Senne, Nelly Sigasa and Khadija Shangase of the Surveillance Officers & Research Assistants group; Nireshni Naidoo, Boitumelo Letlape, Debra Mathebula, Venson Ndhlovu, Kelebogile Motsepe, Robert Musetha, Mpho Ntoyi, Shirley Mhlari, Thembinkosi Matiwane and Dimakatso Maraka of the Data management team.