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FOREWORD

The 2013 winter influenza season in South Africa will once again be carefully monitored by three influenza surveillance programmes coordinated at the NICD. Last year, these programmes showed that the season was biphasic with co-circulation of influenza A(H3N2) and influenza B. The predominant strains identified in 2012 and their genetic sequence characteristics are described in detail in this issue. Interestingly, the influenza B/Brisbane/60-like viruses predominated and low reactors to reference antisera for these isolates were identified for the first time. Also in this issue, the communicable disease surveillance and risk assessment undertaken during the 2013 Africa Cup of Nations soccer tournament is described, the sensitivity of drug resistant HIV-1 isolates to 2nd-generation NNRTI's is assessed, the status of the 'silent' Hepatitis C epidemic in South Africa is analysed using a recently developed surveillance database and a multiplex PCR technique for identification of mammalian blood meals in malaria vector mosquitoes is validated in comparison to the standard ELISA method. Importantly, information concerning currently available data on a recently described coronavirus associated with severe respiratory disease is also provided in this issue. I trust you will find these contributions interesting and useful, and thank all authors for their timely inputs.

Basil Brooke, Editor

CONTENTS

KEEPING WATCH: MONITORING INFECTIOUS DISEASE RISKS TO THE ORANGE AFRICA CUP OF NATIONS, 2013

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Background

The 2013 Africa Cup of Nations (AFCON 2013), a football tournament involving national teams from across Africa, was held in South Africa between 19th January and 10th February, 2013. Sixteen teams qualified for the tournament: Algeria, Angola, Burkina Faso, Cape Verde,

Cote d'Ivoire, Democratic Republic of Congo, Ethiopia, Ghana, Mali, Morocco, Niger, Nigeria, South Africa, Togo, Tunisia and Zambia. Thirty-two games were played in five different stadiums in five provinces: FNB Stadium/Soccer City in Johannesburg, Gauteng Province, Moses Mabhida Stadium in Durban, KwaZulu Natal Province, Nelson Mandela Bay Stadium in Port Elizabeth, Eastern Cape Province, Mbombela Stadium in Nelspruit, Mpumalanga Province and the Royal Bafokeng Stadium in Rustenburg, North West Province. These stadiums have a capacity of 40,000-50,000 people, with 94,000 in Soccer City, which hosted the opening and closing ceremonies.

AFCON 2013 was a series of mass-gathering events defined as gatherings of large numbers of people in the same place and time for a particular purpose. These events included a significant number of international visitors. Planning for mass-gathering events is conducted across government departments, with the health sector typically involved in planning for emergency medicine, disaster management, bioterrorism response, disseminating pre-travel health advice and communicable disease surveillance and response. Communicable disease concerns include the import or export of diseases to susceptible populations, amplification and rapid transmission of outbreaks, impaired outbreak detection and response, and public health infrastructure strained by large numbers of visitors. High profile mass-gathering events can also increase media scrutiny and political pressure on public health activities.

This article briefly describes the communicable disease surveillance and risk assessment undertaken during AFCON and reviews the communicable disease incidents identified.

Methods

The public health surveillance and risk assessment undertaken for AFCON 2013 followed that protocol developed and implemented during the 2010 Federation of Football Associations' (FIFA) World Cup, held in South Africa.

The Role of the Public Health Cluster

Communicable disease surveillance and incident response was overseen by the Public Health Cluster (PHC), which includes senior representatives from the Department of Health Directorates of Communicable Disease Control, Communications, Environment and Port Health, Food Safety, Malaria Control and Surveillance and Epidemiology, the National Institute for Communicable Disease (NICD) and the World Health Organisation (WHO). The PHC met daily at 8am at the Department of Health between 16th January and 13th February 2013. The aims of each meeting were to report any health incidents potentially relevant to AFCON, to conduct rapid risk assessment of each incident, and to report risk assessments to those senior health representatives briefing the Joint Operating Committee 'JOCOM', the pan-departmental committee responsible for ensuring the safety of AFCON.

Figure 1 provides a summary of health reporting arrangements during AFCON. Activities were undertaken in the provinces where games were hosted as well as nationally. The PHC depended on a daily flow of information from each of the departments/ organisations represented at the meeting, as depicted in figure 2.

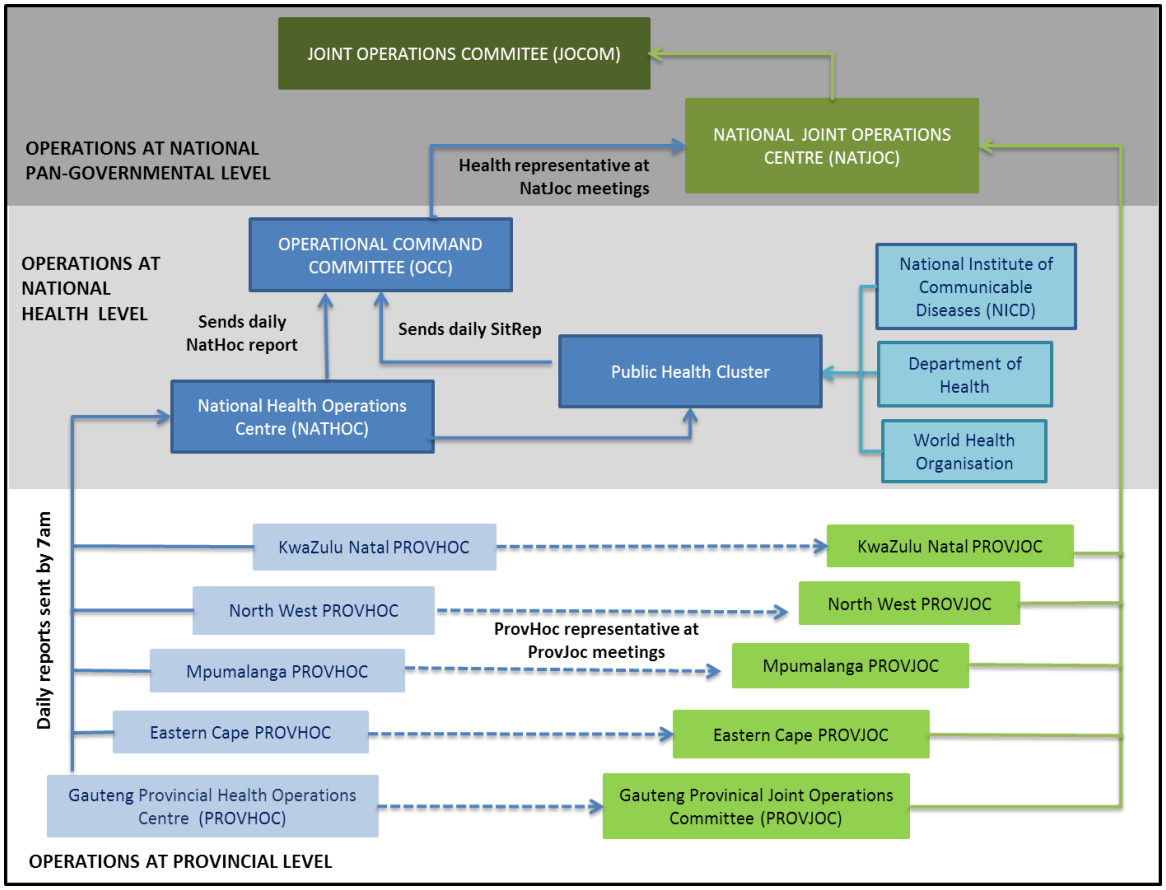

Figure 1. Flow diagram of the reporting structure for public health during AFCON 2013.

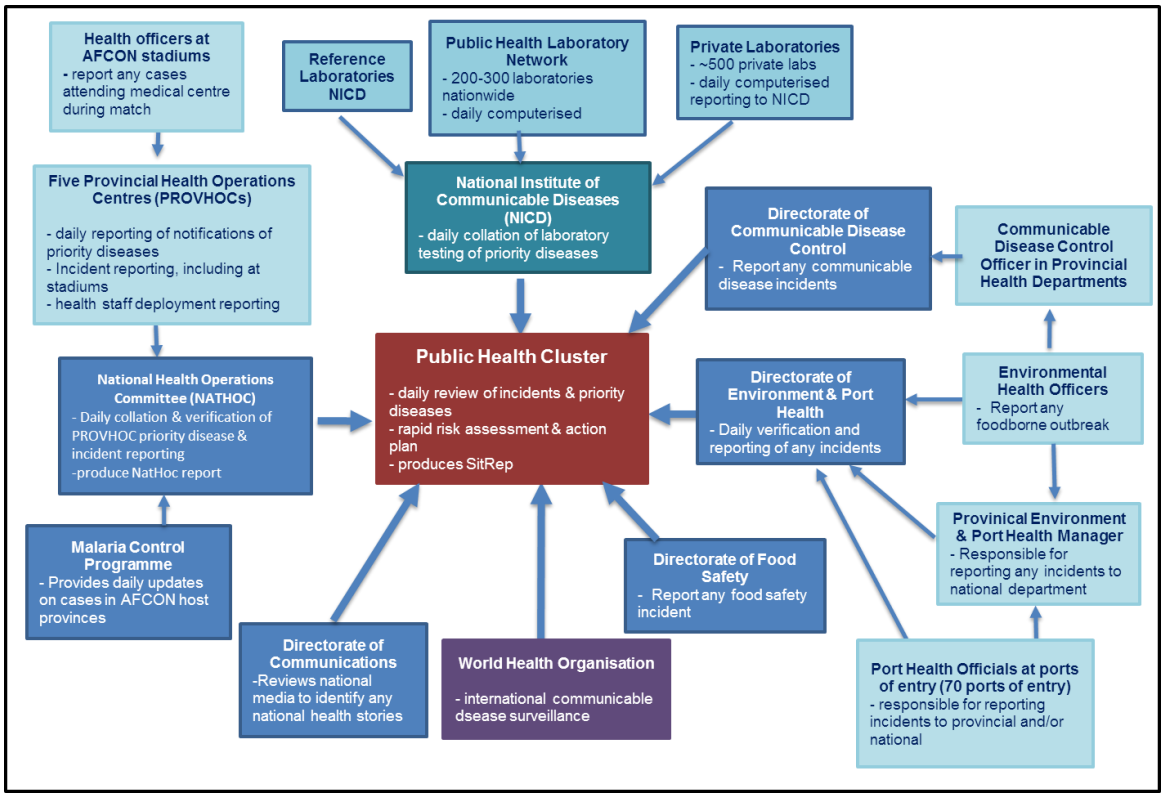

Figure 2. Description of data flows into the Public Health Cluster.

Data Flows into the Public Health Cluster - Enhanced national notification-based communicable disease surveillance and incident reporting

During AFCON 2013 all five provinces hosting matches established both a Provincial Health Operations Centre (PROVHOC), run by staff in the provincial health department, and a cross-departmental Provincial Joint Operations Centre (PROVJOC), which was briefed daily by the PROVHOC. The PROVHOCs were responsible for conducting daily surveillance on 18 priority conditions and collecting information on any public health incident with potential significance for AFCON. The list of priority conditions (described in Table 1) was agreed during preparation for the 2010 World Cup. This was an enhancement of the routine national surveillance of notifiable diseases, requiring daily reporting. The PROVHOCs were required to submit a report to the National Health Operations Centre (NATHOC) in the Department of Health by 5am daily. Each report included data on the priority conditions and associated incidents. The NATHOC was responsible for compiling data on the priority conditions and public health incidents from all five PROVHOCs into one report for the PHC and the Operations Command Committee, to be submitted by 7am daily.

Table 1: List of priority conditions under enhanced surveillance during AFCON 2013.

No.	Name of disease	Reported by NICD	Reported by NATHOC*
1	Anthrax	√	√
2	Botulism	√	√
3	Cholera	√	√
4	Viral Hepatitis	√ (Hepatitis A)	√
5	Influenza	√	√
6	Malaria	√	√ (Malaria programme)
7	Measles	√	√
8	Meningococcal disease	√	√
9	Plague	√	√
10	Polio	√	√
11	SARS	√	√
12	Severe Unexplained Respiratory Illness	X	√
13	Smallpox	√	√
14	Typhoid fever	√	√
15	Rift Valley Fever	√	√
16	Crimean Congo Haemorrhagic Fever	√	√
17	Other Viral Haemorrhagic Fevers	√	√
18	Yellow fever	√	√

*NATHOC = National Health Operations Centre

Enhanced national laboratory based communicable disease surveillance

There is no statutory laboratory notification of diseases in South Africa. However, during the FIFA World Cup and AFCON, laboratory reporting of 17 of the priority conditions was a major component of the daily reporting system. The National Institute for Communicable Diseases (NICD) was responsible for co-ordinating daily reports. These included reports from the Corporate Data Warehouse (CDW), a laboratory data management system run by the National Health Laboratory Service (NHLS) and NICD, as well as reports from the private laboratory networks. The CDW is a database used to collate data from the laboratory information systems of a network of the 250-300 public sector health laboratories across South Africa, allowing real-time alerts on specific organisms diagnosed and the analysis of other positive laboratory test results for specific diseases. During AFCON the NICD/NHLS requested a daily extract of all positive test results of the 17 priority conditions from the CDW. In addition, because private laboratories conduct a major proportion of all diagnostic tests in South Africa, preparation for AFCON by the NICD also involved the inclusion of reports from major private laboratories. Two major laboratory networks, Ampath (300 laboratories) and Lancet, and three major laboratories, vanRensburg and Partners, Pathcare and Vermaak en Vennote, were contacted and agreed to participate by delivering daily reports to the NICD using standard line-lists of the 17 CDW priority conditions.

The NICD provided trend data from a number of well-established surveillance programmes to inform disease risks. These programmes included influenza and severe respiratory diseases, enteric diseases and meningeal pathogens. The NICD was also responsible for providing daily reports on any public health incident reported to the NICD as part of the routine epidemic reporting system and risk assessment.

Reporting to the PHC from other Directorates in the Department of Health

No special arrangements for surveillance or incident reporting were introduced for the other directorates attending the PHC.

The Directorate of Communicable Disease Control (CDC) was responsible for obtaining reports from their local and provincial counterparts on communicable disease incidents reported to the PHC by NATHOC.

The Directorate of Port and Environmental Health was responsible for informing the PHC of any incident reported by their local counterparts, such as port health officials, assessed to be of relevance to AFCON.

The Directorate of Food Safety was represented to provide expertise on food safety issues in the event of a suspected food borne outbreak reported through the communicable disease data flows (CDC, NATHOC and NICD).

The Directorate of Communications was responsible for reviewing local and national South African media daily to identify any health topics covered in relation to AFCON, and for reporting such stories to the PHC.

International communicable disease surveillance

Representatives from the World Health Organization (WHO) were responsible for identifying and reporting international communicable disease outbreaks that had the potential to spread to South Africa during AFCON 2013. Methods for identifying potential outbreaks of concern included an internal daily review of the WHO's 'Event Management System' (EMS) on global public health incidents; contacting WHO counterparts in other national offices for situation updates; and daily monitoring of internet sources. Communicable disease events in Africa were the main focus of surveillance activities.

Internet sources used included ProMED-mail (http://www.promedmail.org/), HealthMap (http://healthmap.org/en/), and Google news.

Results

The Public Health Cluster held 28 meetings between 16th January 2013 and 12th February 2013, and produced 23 situation reports (SitReps). No major public health incidents related to AFCON occurred during the tournament.

Seven health events assessed to be relevant to AFCON were identified. These were reported by the PHC in the SitReps. All of these events were given a risk assessment rating of "Minor or no risk to the ORANGE AFCON to South Africa or internationally".

Three events related to seasonal communicable disease activity with no direct link to AFCON participants or spectators but with potential media interest were reported: two unrelated cases of Crimean Congo Haemorrhagic Fever (CCHF) following tick bites; 462 Malaria cases, including 2 Odyssean clusters; and fourteen suspected measles cases.

Two reports of possible new outbreaks were received during the AFCON surveillance activities and included in the SitRep. Both were found to be false alarms. One was a ProMed report of a diarrhoeal outbreak in Cape Town unsubstantiated by local authorities and the other was a suspected food-borne outbreak at Mbombela Stadium which was ruled out on investigation.

One ongoing outbreak of *Shigella flexneri* in the New Brighton area, Eastern Cape Province, with 67 laboratory confirmed cases was reported in the SitRep. This is a suburb of Port Elizabeth where matches were played. This outbreak was considered low risk to AFCON because visitors were considered unlikely to visit the affected area and because the outbreak was declining.

Five incidents were reported from airport port health officers involving travellers from countries endemic for Yellow Fever who were without proof of vaccination. No incidents were reported by port health officers at overland or sea ports of entry.

A major public health event did occur in South Africa during AFCON as a consequence of extensive flooding in the north of the country, affecting Limpopo, Mpumalanga, KwaZulu-Natal and Gauteng provinces. This event did not significantly impact on the tournament although the flood response could have affected the public health workforce and associated surveillance activities.

Twelve communicable disease events that occurred outside of South Africa but within the African continent were risk assessed by the PHC, including outbreaks of cholera and VHFs. All were assessed to be of low risk for AFCON.

Discussion

No major public health incidents related to AFCON occurred during the tournament. This could be the result of chance, relatively small numbers of international visitors, or preventative public health action. AFCON was always anticipated to be a relatively small-scale mass-gathering event compared to other global events, such as the 2010 FIFA World Cup. Although no figures have yet been provided, anecdotal reports suggest that the number of international visitors attending AFCON was relatively small, and therefore the risk of the importation of communicable diseases was low.

It is also possible that the preparations by public health in advance of and during the event reduced the risk of

health incidents. Heightened health protection controls and greater vigilance, particularly among food safety, environmental health and port health, all of whom implemented systems developed during the World Cup, may have played a role.

The risk assessment and surveillance activities led by the PHC generally worked well. Of the seven incidents reported in the SitReps, all were reported to the PHC meeting within 24 hours. The PHC conducted risk assessment on all seven events, as well as reported international events, and was characterised by a positive and collaborative group dynamic.

A full discussion of the challenges and lessons learned is outside the remit of this article. The key challenge to laboratory surveillance was that as a passive surveillance system it was dependent on clinical recognition and submission of appropriate patient specimens to laboratories.

Strengthening surveillance and response capacity should remain a priority during mass gatherings. Public health systems either developed for or enhanced during mass gatherings should provide a legacy for improving public health surveillance systems within South Africa.

Acknowledgements

All members of the AFCON Public Health Cluster are thanked for their inputs. These include the National Institute for Communicable Diseases, National Health Laboratory Service, Vermaak and Partners Pathologists, Lancet Laboratories and Pathcare Reference Laboratories.

RESPIRATORY SYNDROMIC SURVEILLANCE REPORT – 2012

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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.

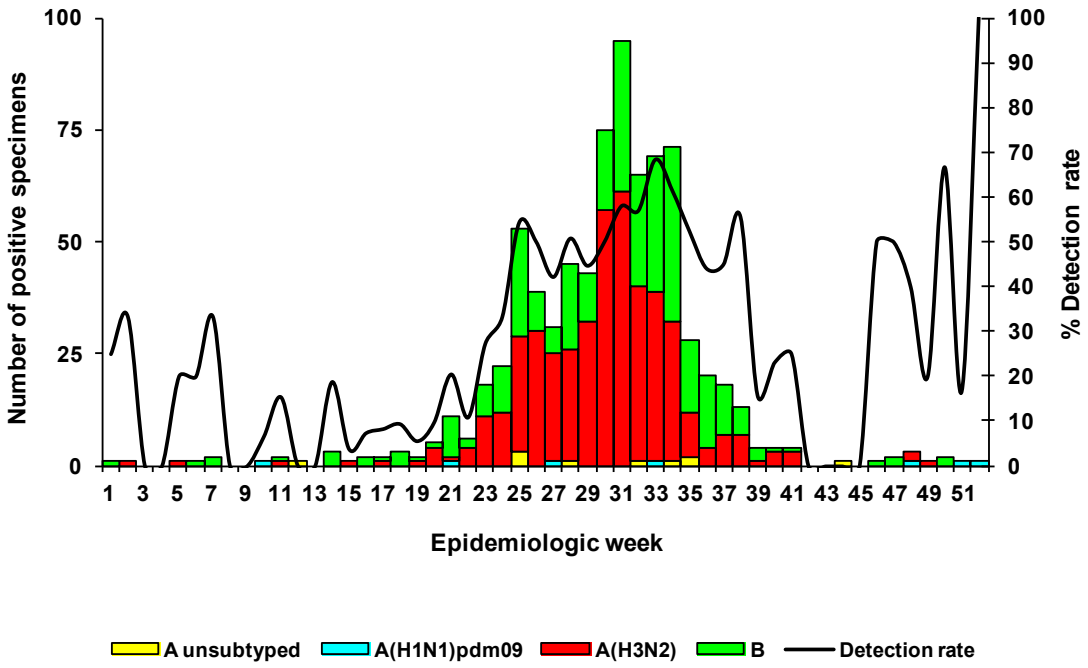


Figure 1. Influenza detection rate and numbers of positive specimens by viral subtype - Viral Watch Surveillance Programme, 2012.

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).

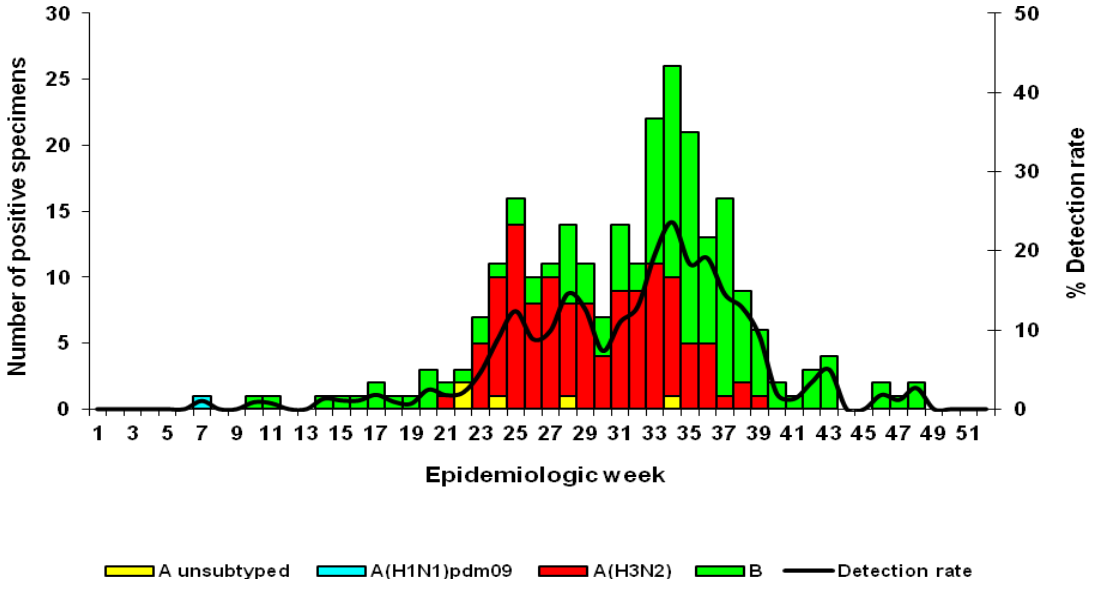
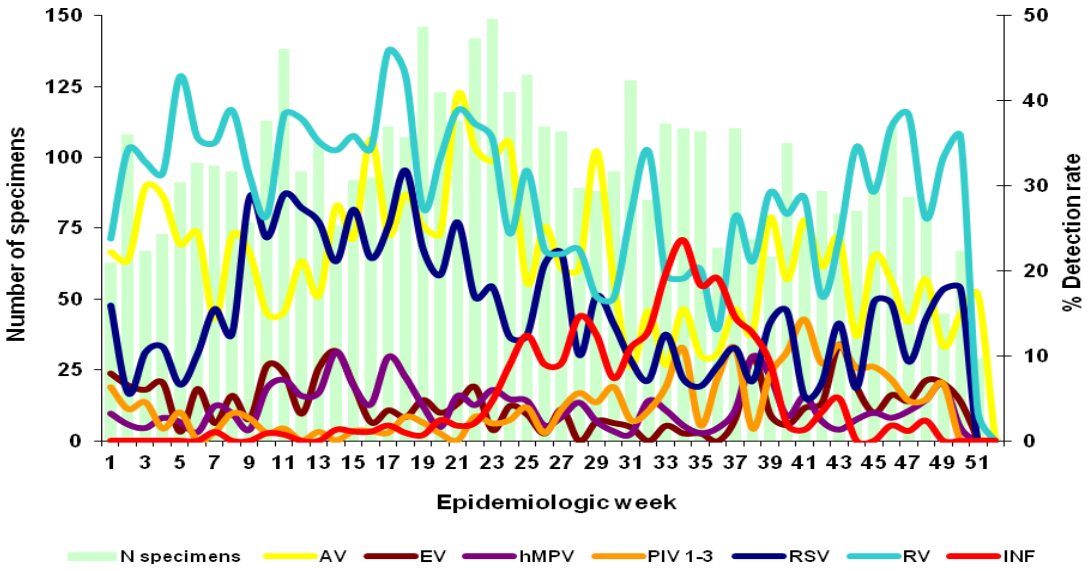


Figure 2. Influenza detection rate and numbers of positive specimens by viral subtype - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.

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%).

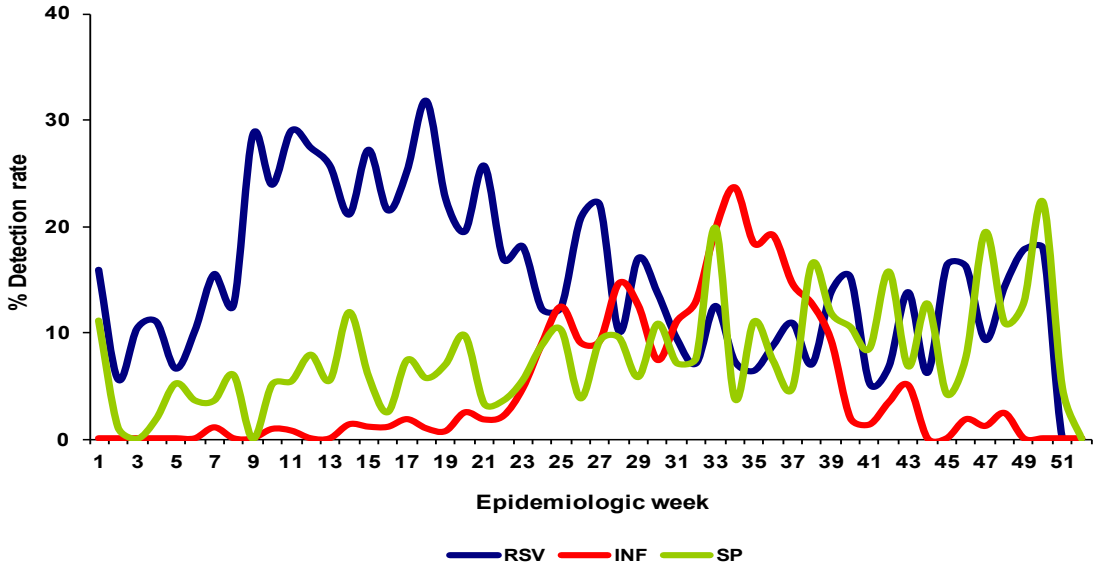


Figure 4. Detection rate for influenza (INF), respiratory syncytial virus (RSV) and pneumococcus (SP) by epidemiologic week - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.

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.

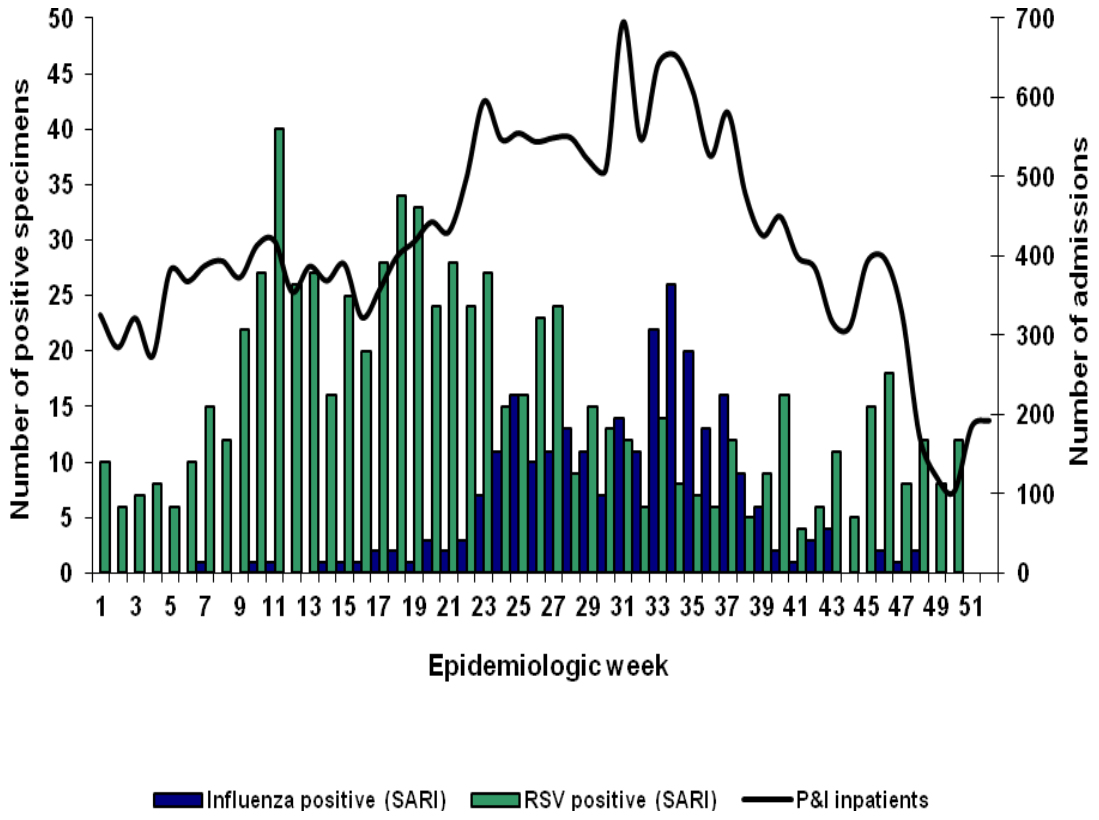


Figure 5. Numbers of private hospital admissions with a discharge diagnosis of pneumonia and influenza (P&I) and viral isolates by epidemiological week - Severe Acute Respiratory Illness (SARI) Surveillance Programme, 2012.

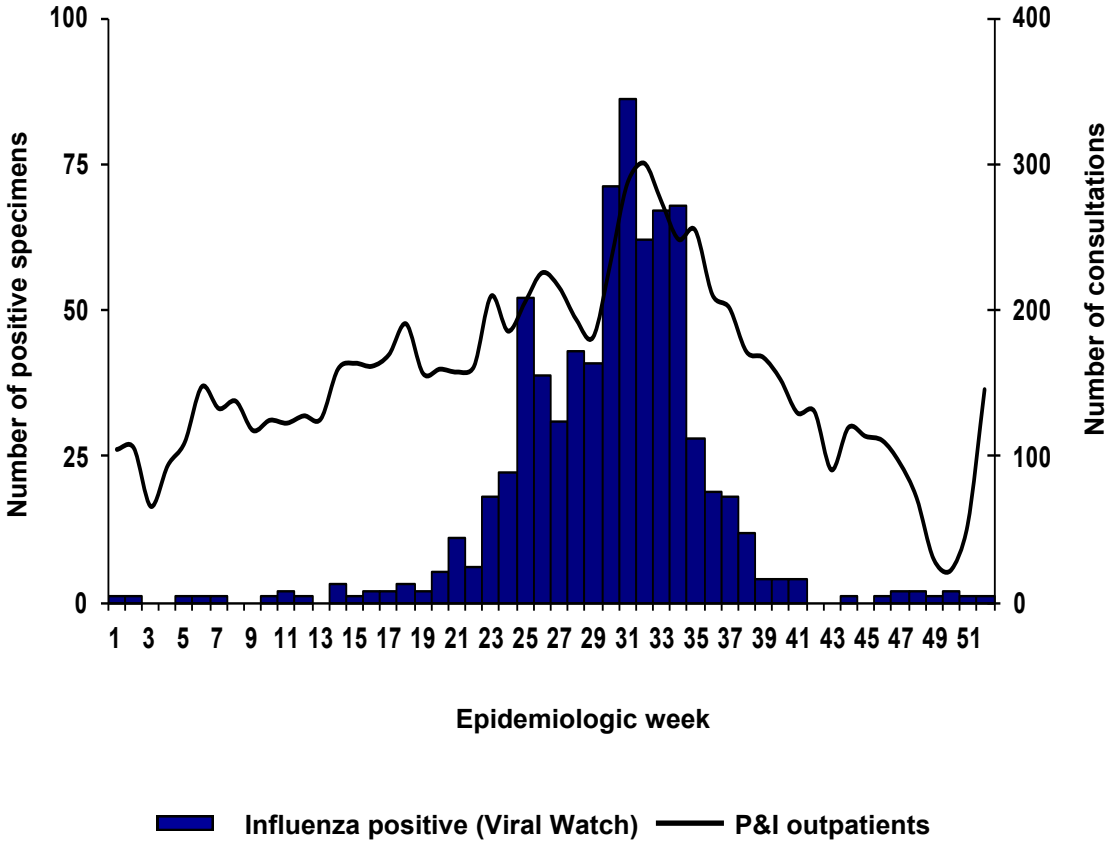


Figure 6. Numbers of private hospital outpatient consultations with a diagnosis of pneumonia and influenza (P&I) and viral isolates by epidemiological week - Severe Acute Respiratory Illness (SARI) Surveillance Programme ,2012.

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.

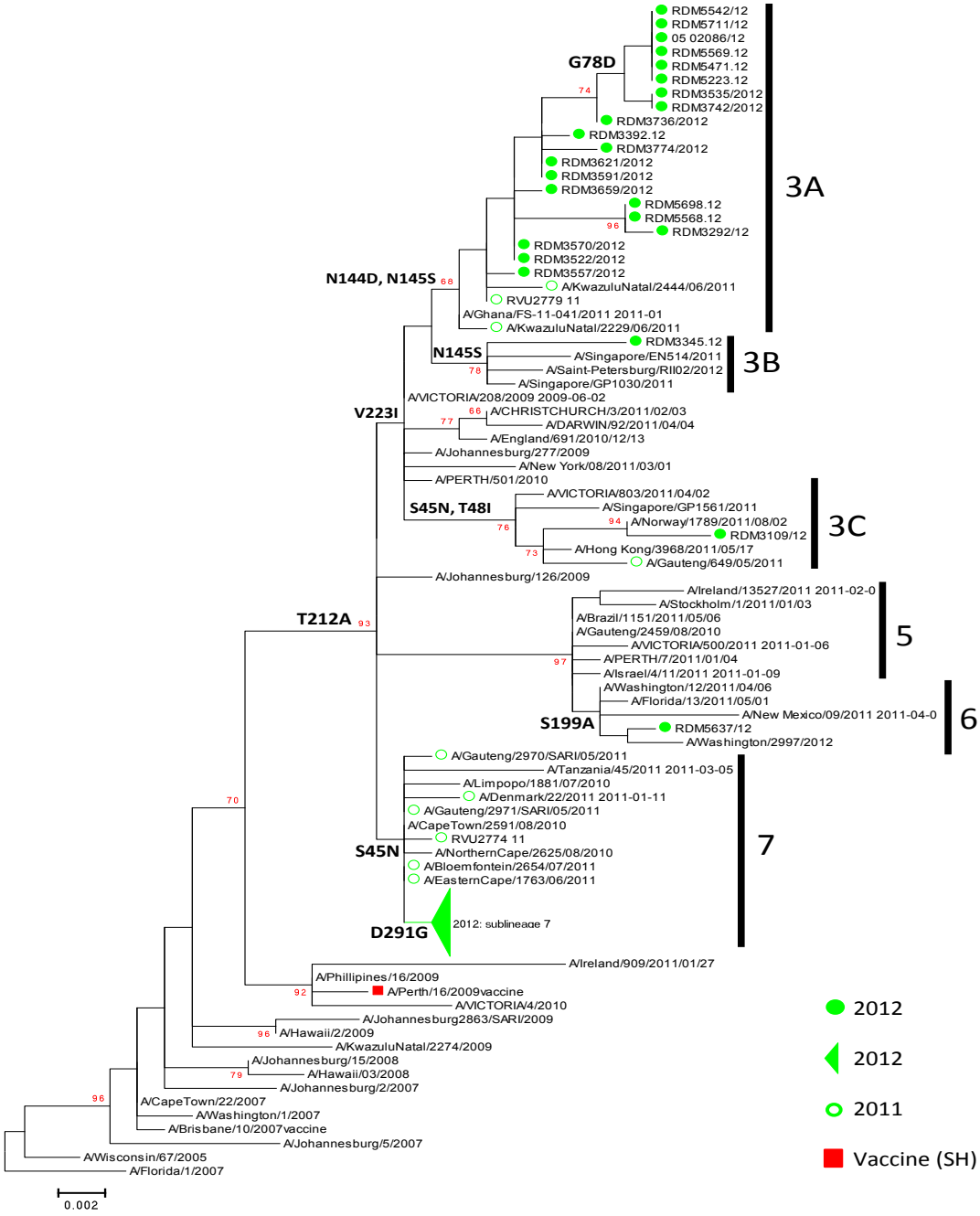


Figure 7. Maximum likelihood tree of the A(H3N2) HA1 region (900bp), South Africa 2012. The 2012 South African strains are indicated in green solid circles (open green circles = 2011). The current southern hemisphere (SH) vaccine strain is indicated by a red square. Amino acid changes corresponding to 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.

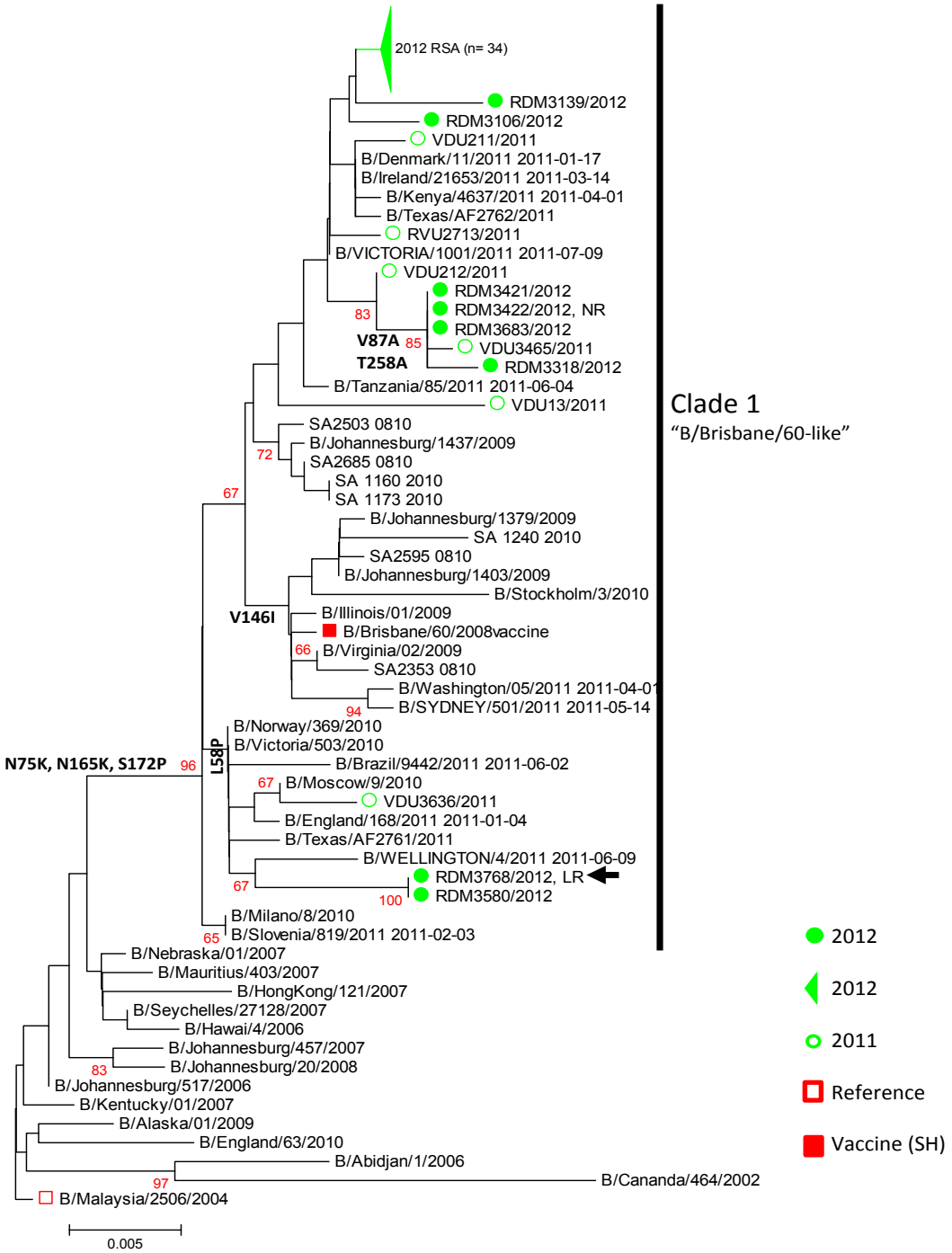


Figure 8. Maximum likelihood tree of the HA1 region of influenza B/Victoria-like viruses (954bp), South Africa 2012. The current southern hemisphere (SH) vaccine strain is indicated in solid red and 2012 strains from South Africa are indicated in solid green. LR = Low Reactor indicated by black arrow,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

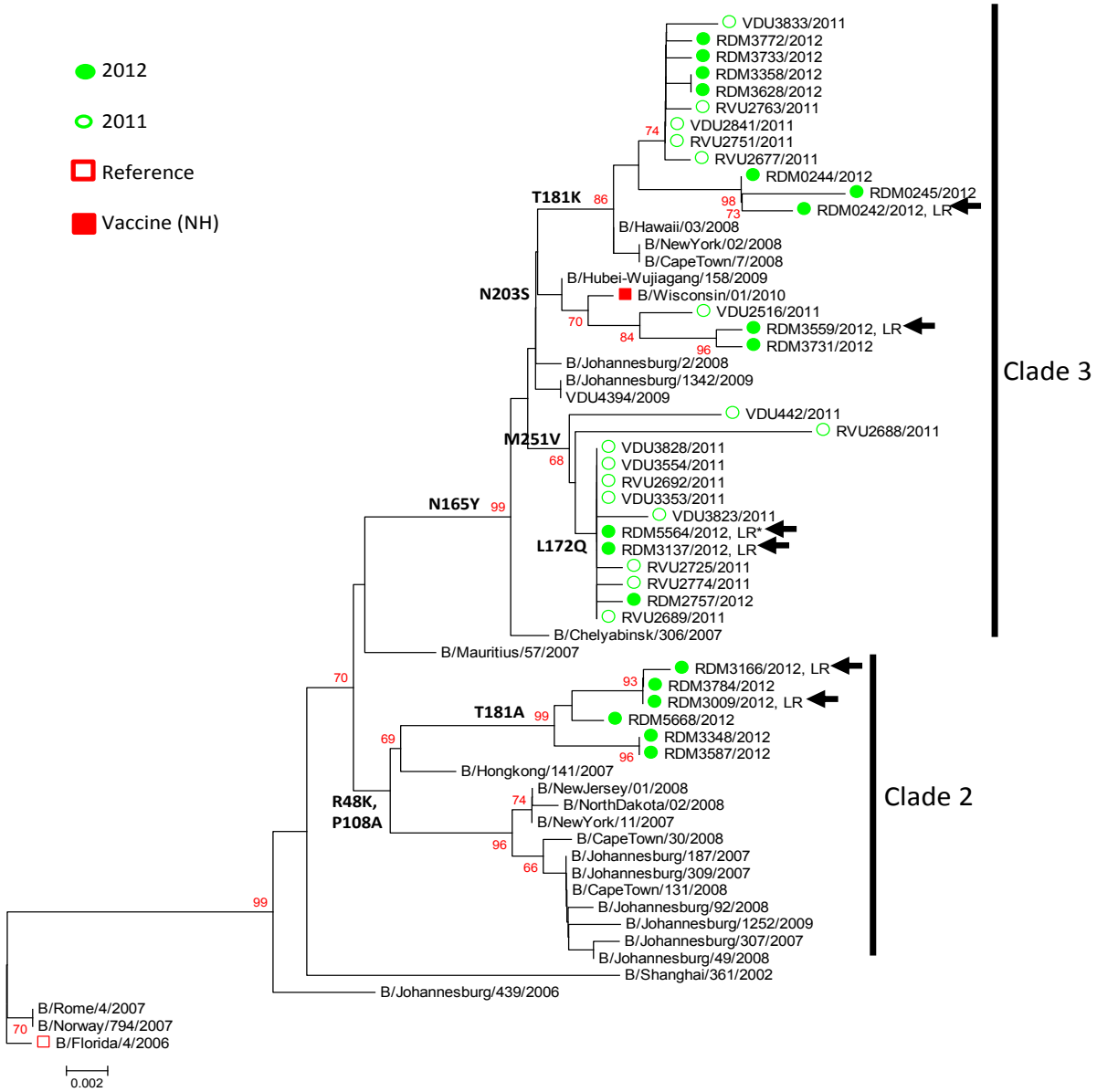


Figure 9. Maximum likelihood tree of the HA1 region of influenza B/Yamagata-like viruses (954bp), South Africa 2012. The 2012/2013 vaccine strain selected for the northern hemisphere (NH) is indicated in solid red and 2012 strains from South Africa are indicated in solid green. LR = Low Reactor indicated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

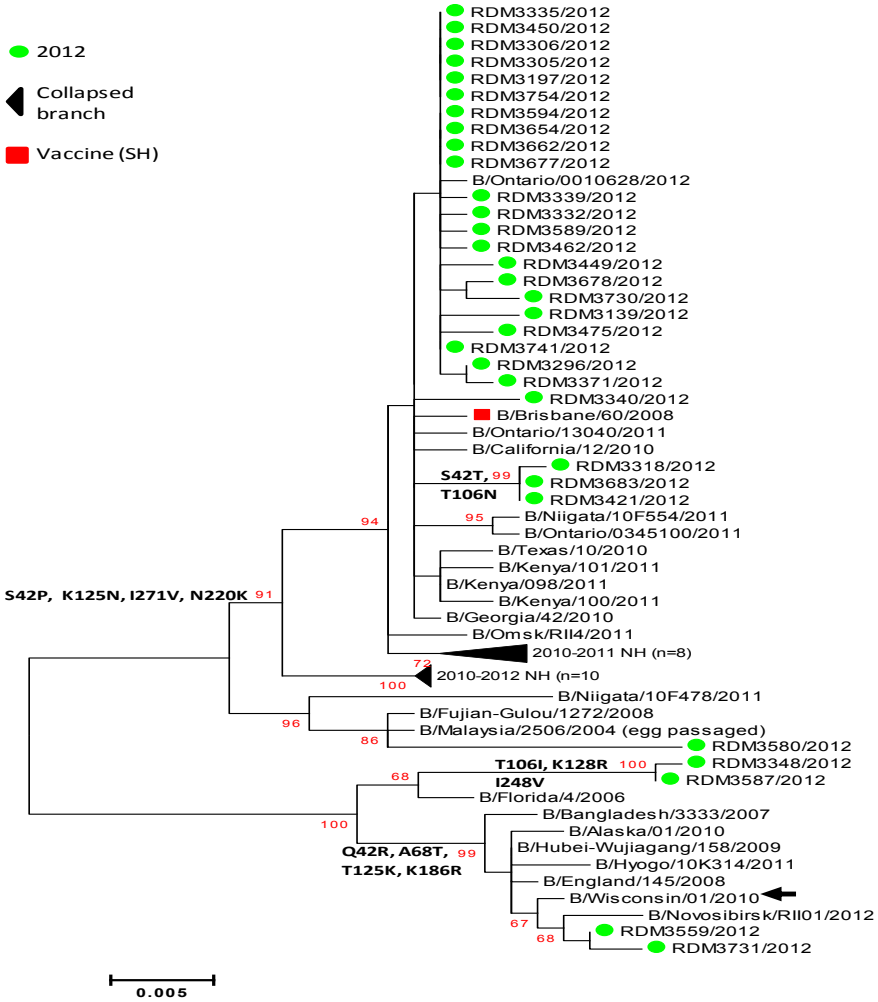


Figure 10. Unrooted maximum likelihood tree of the 5'NA gene region of influenza/Victoria-like and B/Yamagata-like viruses (807 bp), South Africa 2012. The 2012/2013 vaccine strain selected for northern hemisphere is indicated by a black arrow and the current southern hemisphere (SH) vaccine strain is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

Acknowledgements:

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SENSITIVITY OF DRUG RESISTANT HIV-1 ISOLATES TO 2ND-GENERATION NNRTI'S

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Introduction

South Africa has an estimated 5.7 million people infected with HIV-1 of whom ~1.8 million were receiving antiretroviral treatment by 2011.[1] The first-line regimen for adults is non-nucleoside reverse transcriptase inhibitor (NNRTI)-based, comprising of either efavirenz (EFV) or nevirapine (NVP), in combination with two nucleos(t)ide reverse transcriptase inhibitors. Both these first-generation NNRTIs have a low genetic barrier to the development of resistance and share substantial cross-resistance. Recent studies have shown that approximately 80% of first-line failures contain NNRTI resistance mutations.[2-4] As a consequence, failure on either drug necessitates a switch to a protease-based regimen. Although protease inhibitors are effective in controlling HIV-1 viremia, they are less tolerable and more expensive than the NNRTI-based regimen. Due to the extensive use of first-generation NNRTIs in South Africa and other developing countries, accessibility to alternative NNRTIs with an unrelated resistance profile is needed in order to delay regimen switch to a protease -based regimen.

Etravirine (ETR) and rilpivirine (RPV) are second generation NNRTIs with a high genetic barrier to the development of resistance and a resistance profile that only partially overlaps that of EFV or NVP.[5] In the DUET trials, ETR was demonstrated to be safe, tolerable and active in treatment-experienced patients with NNRTI resistant strains.[6] In the ECHO and THRIVE trials, rilpivirine had a non-inferior efficacy in treatment-naive patients compared to EFV, with a favorable safety profile.[7,8] Both ETR and RPV retain activity against viruses containing the K103N mutation in HIV-1 reverse transcriptase, the most prevalent NNRTI resistance mutation in current first-line failures. Although an accumulation of NNRTI mutations are required for resistance to ETR or RPV, a single mutation at codon Y181, frequently selected for by NVP, could be sufficient to cause resistance to both.[9] Indeed, an increased risk in ETR failure has been associated with the prior use of NVP.[10]

The aim of this study was to assess whether South African patients failing on an EFV/NVP-based first-line regimen would harbor viruses sensitive to second generation NNRTIs.

Methods

Phenotypic susceptibility testing was performed on 33 patients using an in-house single-cycle HIV-1 phenotypic assay to assess their susceptibility to both first- and second-generation NNRTIs. Standard population-based Sanger-sequencing was performed to identify NNRTI resistance mutations present in the reverse transcriptase portion of the *pol* gene of HIV-1 in the samples.

Results

As expected, most samples (n=31/33, 94%) were resistant to both EFV and NVP while only 36% (n=12/33) and 21% (7/33) were resistant to ETR and RPV respectively (table 1). Apart from sample DR64, with a single Y181C mutation, all samples resistant to ETR and/or RPV contained ≥2 NNRTI resistance mutations. In contrast, a single NNRTI resistance mutation was sufficient to cause resistance to EFV and/or NVP. The majority of samples with high-level resistance to ETR contained the Y181C mutation (58%, n=7/12). This mutation had a lower impact on RPV resistance as only 3 highly resistant samples contained Y181C.

Table 1. Genotype and phenotypic susceptibility of patient samples to first- (EFV and NVP) and second-generation (ETR and RPV) NNRTIs.

Patient ID	NNRTI exposure	Genotype NNRTI resistance mutations	Phenotype (Fold-change) ETR	RPV	EFV	NVP
SAVE 1186	EFV	V106M,Y188C	0.5	0.6	42.7	59.9
SAVE 1302	EFV	K101H,K103N,G190A	0.7	1.1	42.7	59.9
SAVE 2229	EFV	V106M	0.7	0.2	4.0	5.4
SAVE 2041	EFV	K103N	0.8	0.6	41.6	59.9
TOGA 134184	NVP	A98G,K103N	1.1	0.9	42.7	59.9
SAVE 1412	*NR	K103N	1.3	1.3	42.7	59.9
SAVE 1383	EFV	K103N,V106A,G190A	1.3	1.9	42.7	59.9
TOGA 311368	EFV	M230L	1.4	0.9	1.3	1.9
TOGA 605248	NVP	K101H,G190A	1.4	0.8	42.7	59.9
SAVE 1379	EFV	V106M,G190A	1.5	0.8	42.7	59.9
TOGA 357702	EFV	K103N,V108I	1.6	1.1	42.7	59.9
TOGA 064124	NVP	K103N	1.7	1.3	40.2	59.9
TOGA 56522	NVP	K103N	1.8	1.3	42.7	59.9
SAVE 1434	EFV	V90I,K103N,P225H	2.3	1.7	42.7	59.9
TOGA 063884	NVP	K103N	2.7	1.7	42.7	59.9
TOGA 480117	NVP	V106M,Y188C	3.0	1.7	42.7	59.9
TOGA 301226	EFV	K103N,H221Y,P225H	3.2	1.6	42.7	59.9
SAVE 1500	NVP	K101E,V106M,E138A,F227L	3.2	1.8	42.7	59.9
TOGA 437809	EFV	A98G,K103N,V108I,P225H	4.8	5.0	42.7	59.9
SAVE 2452	EFV	K103N,E138A,P225H	5.3	7.4	42.7	59.9
TOGA 102710	EFV	K103N,Y181C	8.6	5.2	28.1	59.9
DR150	NVP	V106M,Y181C	10.9	1.2	42.7	59.9
SAVE 1400	EFV	K101E,Y188L	11.2	75.8	42.7	59.9
TOGA 62695	EFV	V106M,V179D,Y188C	12.6	2.4	42.7	59.9
DR146	EFV	K103N,V108I, Y181C,H221Y	17.8	4.9	42.7	59.9
SAVE 1252	EFV	K101E,G190A,H221Y,M230L	19.0	18.8	42.7	59.9
TOGA 33184	EFV	L100I,K103N	24.8	12.4	42.7	59.9
DR49	NVP	V108I,Y181C	25.1	3.3	40.6	59.9
TOGA 135372	NVP	Y181S,Y188H,H221Y	42.5	8.2	42.7	59.9
SAVE 1154	EFV	V106M,M184V,Y188L,H221Y	47.7	75.8	42.7	59.9
DR64	NVP	Y181C	47.7	17.0	27.9	59.9
DR41	NVP	A98G,Y181C,M230L	47.7	16.9	28.9	59.9
DR122	NVP	Y181C,H221Y	47.7	23.3	30.7	59.9
Total number susceptible			18	20	1	1
Total number with low-level resistance			1	2	1	0
Total number with intermediate resistance			2	4	0	1
Total number with high-level resistance			12	7	31	31

Susceptible Low-level resistance Intermediate resistance High-level resistance

*NR: Not reported

NOTE: This table indicates the genotypic and phenotypic resistance profiles of patients (n=33) failing on an EFV- or NVP-based first-line regimen. The genotype indicates the various NNRTI resistance-associated mutations present in the reverse transcriptase (RT) gene of the HIV-1 strain obtained from the patient. The phenotype indicates the level of phenotypic resistance to ETR, RPV, EFV and NVP. Values represent the ratio, or fold-change (FC), of the inhibitory concentration-50 (IC_{50}) of the sample virus compared to that of a wild-type reference virus for a particular drug. A value of FC>1 indicates a decrease in drug susceptibility that infers some level of drug resistance. The level of phenotypic resistance is classified as “susceptible” (FC=1), “Low-level resistance” and “Intermediate resistance” (FC>1 but <10), and “High-level resistance” (FC≥10). The samples are ordered according to ETR FC.

Discussion

These data suggest that HIV strains from patients failing on NVP or EFV would mostly show sensitivity to ETR and RPV. Currently there are no clear guidelines in South Africa for the use of 2nd generation NNRTIs and several factors could impact on their use. The prevalence of ETR/RPV-related NNRTI mutations in sub -Saharan Africa is low and the predicted resistance to ETR and RPV uncommon.[3] The use of these 2nd-generation NNRTI will mostly be influenced by high frequencies of Y181 mutants, as selected for by NVP, and the accumulation of NNRTI mutations. By prioritizing the use of EFV over NVP, and frequent viral load monitoring to prevent the accumulation of resistance associate mutations, 2nd-generation NNRTI might be a viable option for first-line failures.

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THE STATUS OF HEPATITIS C – THE SILENT "VOLCANO" – IN SOUTH AFRICA

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Introduction

Hepatitis C is a "silent" epidemic as very little of its epidemiology is known to the public, health care workers, populations at risk or policy makers. Most carriers do not know that they are infected with the virus as they are asymptomatic for many years of their life. In 20% of cases the source of Hepatitis C Virus (HCV) infection is unknown although intravenous drug use remains the most common risk factor in the UK and US.[1] In South Africa and other African countries, unhygienic injection practices and traditional scarifications are possible routes of HCV transmission.[2]

Testing for hepatitis C is a sensitive issue. Many patients still suffer with the stigma associated with infection. This stigma contributes to the paucity of information concerning the extent of the growing epidemic in which transmission continues. Worldwide, 170-180 million people are infected with HCV.[3] Of these, about 80% will become chronically infected[4], with 25-30% developing liver cirrhosis and/or hepatocellular carcinoma.[5] The seroprevalence of HCV in South Africa ranges from low (1.4-1.8%) in blood donors and health care workers to high (13-33%) in HIV positive individuals and patients with chronic active hepatitis.[6-9] However, even with a seroprevalence as low as 1-1.5%, the USA is currently facing a huge burden of disease as a result of HCV-related hospitalizations and liver transplants, with an estimated cost of $10 billion for the period 2010-2019.[10]

Standard drug therapy for hepatitis C infection is injectable pegylated interferon and oral ribavirin. Direct-acting antivirals are used in South Africa, mostly in the private sector and in combination with pegylated interferon and ribavirin.[11] Response to standard therapy is affected by the virus genotype[12] and/or host genetic factors, such as HLA-type, interleukin-28B, and ethnic group.[13-15] Co-infections (HIV, HBV), co-morbidities (diabetes mellitus, obesity) and social factors (alcohol and substance use) can worsen HCV liver disease.[16-17] The contribution of HCV to liver disease, as well as HCV/HIV co-infection, has not been well characterized in South Africa as studies to date have been small and limited.[8,18]

A comprehensive, national surveillance database is needed to identify demographic trends in infection, changing viral genotypic frequencies, follow-up acute infections/serology-positives for molecular test confirmations (according to the national algorithm, draft National Guidelines for the prevention and control of Hepatitis C virus in South Africa, 2011) as well as for further treatment and management. The database needs to be interlinked with other databases/registries, such as the cancer registry and/or hospital records to model burden of disease and costs related to HCV infection in SA.

Methods

A national HCV surveillance database has been developed at the National Institute for Communicable Diseases (NICD-2012), in collaboration with the NHLS Corporate Data Warehouse (CDW), to enable serology and molecular tests as well as demographic information to be captured on one database. Enhanced data such as transmission risks need to be included. Also, the laboratory at the NICD has a database on laboratory-confirmed cases only.

Results

In a recent preliminary analysis of a total of 2360 viral hepatitis C requests received at the NICD from January 2010 - December 2012, 1002 patient specimens tested positive on viral load (>15 international units/ml). The median age was 41-48 years. Like other studies[19], HCV is mostly detected in persons aged 41-74 years (figure 1). A higher number of males (53%) were infected compared to females (40%).

Genotyping was performed on 886 samples with sufficient volume of specimen and viral load of >200 IU/ml, using either a line-probe assay (LiPA) and/or sequencing of the 5'untranslated region. A total of 16 HCV subtypes and mixed intergenotypic infections (6.6%) was identified. Genotype 5a was dominant confirming previously published data[20,21] and accounted for 36% of the laboratory-confirmed cases (figure 2), followed by 1b (22%), 3a (11.7%) and 4 (8.91%) (table 1). Clinical studies in collaboration with the South African Gastro-Intestinal Tract (GIT) clinics, demonstrate that patients with genotype 5a respond better to combination therapy than those with genotype 1 and 4, as noted in other studies.[22,23]

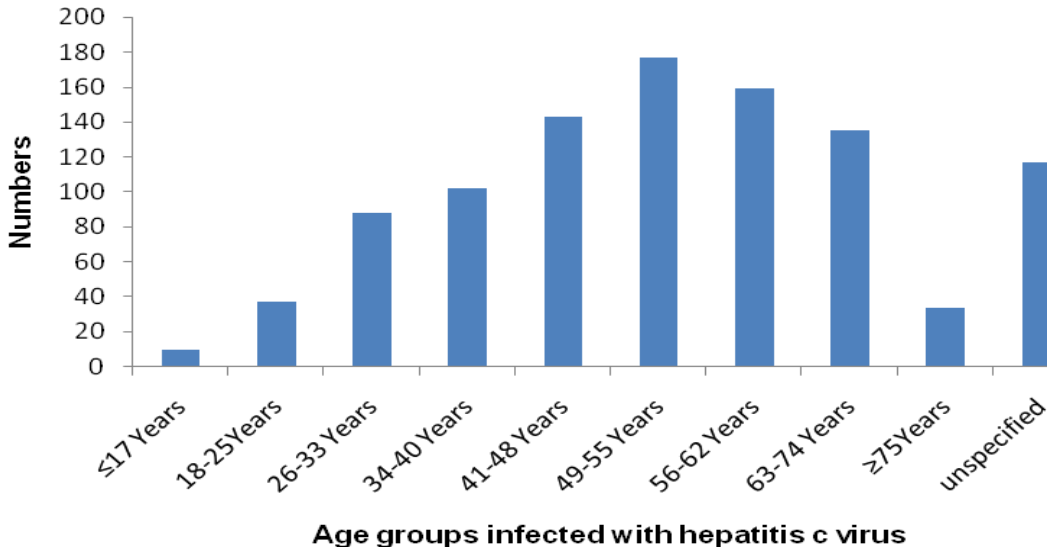

Figure 1. Number of hepatitis C laboratory confirmed cases per age group received by the NICD during the period January 2010 - December 2012.

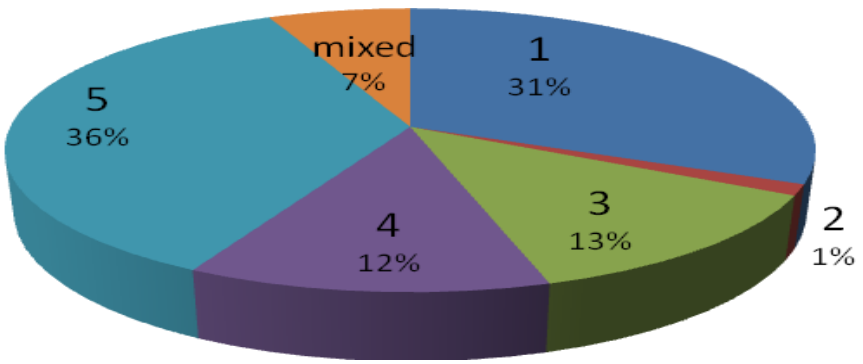

Figure 2. Major HCV genotypes identified in 886 laboratory confirmed hepatitis C positive samples received by the NICD during the period January 2010 - December 2012.

Table 1. A breakdown of all genotypes/subtypes found in hepatitis C positive samples received by the NICD during the period January 2010 - December 2012.

Genotypes/Subtypes	No.	%
1	63	7.11
1a	20	2.25
1b	195	22
2	5	0.56
2a	2	0.23
2a/2c	2	0.23
2b	2	0.23
3	8	0.9
3a	104	11.7
4	79	8.91
4a	2	0.23
4a/4c/4d	11	1.24
4e	7	0.79
4f	3	0.34
4h	8	0.9
5a	316	35.7
mixed	59	6.66
Total	886	100

Discussion

These databases can only be truly comprehensive and functional if the information is supplied on request forms and captured onto the respective reporting systems: DISA or TrackCare. Completeness of data is a challenge. For example, request forms that are used to populate the database are not always complete (11.67% gave no age/birth date). Hepatitis C awareness and training programs, infrastructure and staff are needed to aid in the collection of data for the database. To date, several NICD staff have been trained in appropriate data capture, collaborators at academic hospitals and private laboratories have been informed about the national surveillance database for hepatitis B and C, and HCV advocacy group meetings (Western Cape) and World hepatitis Day initiatives have been facilitated. Public awareness about HCV, diagnosis and treatment accessibility and referrals to academic hospitals need to be strengthened. The development of appropriate surveillance systems and tools can play an important role in informing on policy in terms of the numbers of infected individuals, projections on cost of therapy, potential interventions to prevent new infections and effectiveness of treatment programmes.

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SEVERE ACUTE RESPIRATORY INFECTIONS ASSOCIATED WITH A NOVEL CORONAVIRUS, EMC 2012

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Introduction

In the last few months the World Health Organisation has alerted countries to several reports of a new coronavirus associated with severe respiratory disease in patients with an epidemiological link to the Arabian Peninsula.[1] Coronaviruses are a large family of viruses, some of which may cause respiratory infections in humans and animals. Such respiratory infections may

range from mild respiratory illness (such as the common cold) but can also include more serious disease. The new coronavirus, human coronavirus-Erasmus Medical Centre 2012 (EMC-2012), was first identified in September 2012 from a patient in Saudi Arabia who died from a severe respiratory infection in June 2012.[1] The novel coronavirus has thus far only been identified in a small number of cases of acute, serious respiratory illness who presented with fever, cough, shortness of breath and breathing difficulties. To date, WHO has been informed of a total of 17 confirmed cases of human infection with the novel coronavirus, including eleven deaths globally.[2] These cases are summarized in table 1.

Table 1. Confirmed cases by country of human infection with novel coronavirus, EMC-2012.

Country	# of cases	# cases with travel history	Place Diagnosed	# of deaths	Risk factors
Saudi Arabia	9	Not applicable	Saudi Arabia	6	
Qatar	2	Not applicable	1 United Kingdom 1Germany	1	
Jordan	2	Not applicable	Jordan	2	
United Kingdom	3	1 (Saudi Arabia and Pakistan)	United Kingdom	2	Underlying malignant condition
United Arab Emirates	1	Not applicable	Germany	1	

The aim of this article is to provide information concerning currently available data on this virus to healthcare providers in South Africa.

Clinical presentation

Patients have generally presented with pneumonia, although a significant proportion have also experienced renal failure. With the exception of one case from the UK cluster, all confirmed cases presented with severe respiratory illness.[2]

Transmission

Based on the small number of cases reported so far, there is limited information on mode of transmission, source of the virus, its geographic extent and the spectrum of illness. Genetic sequencing to date suggests that the virus is closely related to coronaviruses detected in bats.[3]

Infections occurred in clusters in three instances. The first cluster of two fatal cases from Jordan occurred in April 2012. Stored samples from these two cases tested positive retrospectively for the novel coronavirus. These were part of a hospital cluster of 11 cases (2 confirmed and 9 probable cases), 8 of whom were health workers. The second cluster occurred in October 2012 in a family from Saudi Arabia with three confirmed cases and one probable case. Two of them died. The most recent cluster occurred in the UK in February 2013. In this cluster three family members presented with laboratory confirmed novel coronavirus infection. Two of them reported no recent travel history outside of the UK suggesting that transmission had occurred in the UK. One family member had travelled to the Middle East and

Pakistan and was ill on his return. One case with an underlying pre-existing medical condition that might have increased susceptibility to infection died.[4]

Recent information from the UK family cluster suggests that human-to-human transmission does occur and it may have occurred in two instances in the Middle East. The mode of human-to human transmission is unknown but may involve different routes of transmission such as droplet and contact transmission.[2]

Who should be tested for novel coronavirus?

WHO recommends that testing for the new coronavirus should be considered in patients with unexplained pneumonias, or in patients with unexplained, severe, progressive or complicated respiratory illness who are not responding to treatment. Any clusters of severe acute respiratory illness (SARI) or SARI in health care workers should be thoroughly investigated, regardless of where in the world they occur.[2]

A prioritisation process should ensure that testing for the novel coronavirus is undertaken only when there is clinical or epidemiological link to a patient or region with laboratory confirmed case/s. This serves to avoid the inappropriate use of scarce resources, the generation of false positives and the risk of overwhelming the health system. If clinicians are not sure whether a patient meets the criteria for testing, the Centre for Respiratory Diseases and Meningitis (CRDM) of the National Institute for Communicable Diseases (NICD), National Health Laboratory Service, can be contacted through the NICD Hotline: 0828839920. Additional information on laboratory testing and contact details can be accessed from the NICD website (www.nicd.ac.za: About us – Our Centres – Respiratory Disease and Meningitis).[4]

WHO Case definition as of 3 December 2012 [5]

Patients under investigation

A person with acute respiratory infection, which may include history of fever or measured fever (≥ 38°C) and cough,

AND

Suspicion of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome), based on clinical or radiological evidence of consolidation,

AND

Residence in or history of travel to the Arabian Peninsula or neighbouring countries within 10 days prior to onset of illness,

AND

Not already explained by any other infection or aetiology, including all clinically indicated tests for community-acquired pneumonia according to local management guidelines. It is not necessary to wait for all test results for other pathogens before testing for novel coronavirus.

Probable Case

A person with an acute respiratory infection* with clinical, radiological, or histopathological evidence of pulmonary parenchymal disease (e.g. pneumonia or Acute Respiratory Distress Syndrome, (ARDS)),

AND

no possibility of laboratory confirmation for novel coronavirus either because the patient or samples are not available for testing,

AND

close contact** with a laboratory-confirmed case.

* This may include but is not limited to cases with a history of fever or measured fever.

** Close contact includes:

anyone who provided care for the patient, including a health care worker or family member, or who had other

similarly close physical contact;
anyone who stayed at the same place (e.g. lived with, visited) as a probable or confirmed case while the case was symptomatic.

Confirmed Case
A person with a laboratory confirmation of infection with the novel coronavirus (http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html).

Contacts
Individuals with acute respiratory illness of any degree of severity who, within 10 days before onset of illness, were in close physical contact with a confirmed or probable case of novel coronavirus infection, while the case was ill.

Any person who has had close contact with a probable or confirmed case while the probable or confirmed case was ill should be carefully monitored for the appearance of respiratory symptoms. If symptoms develop within the first 10 days following contact, the individual should be considered a "patient under investigation", regardless of the severity of illness, and investigated accordingly.

Clusters
Any cluster of severe acute respiratory infection, particularly clusters of patients requiring intensive care, without regard to place of residence or a history of travel,
AND
Not already explained by any other infection or aetiology, including all clinically indicated tests for community-acquired pneumonia according to local management guidelines.

Health care workers
Health care workers with pneumonia, who have been caring for patients with severe acute respiratory infections, particularly patients requiring intensive care, without regard to place of residence or history of travel,
AND
Not already explained by any other infection or aetiology, including all clinically indicated tests for community-acquired pneumonia according to local management guidelines.

Specimen collection and transport
Based on current but limited information, lower respiratory specimens (naturally produced sputum, broncho-alveolar lavage, tracheal aspirates, and tissue from biopsy/autopsy from lung) appear to have the highest titre. Upper respiratory specimens (nasopharyngeal aspirate, combined nose/throat swab, nasopharyngeal swab) are also recommended (table 2).[5] Paired serum samples should also be collected and stored. Respiratory virus diagnosis depends on the collection of high-quality specimens, their rapid transport to a laboratory and appropriate storage before laboratory testing. Virus is best detected in specimens containing infected cells and secretions. Specimens should be collected as soon as possible, preferably during the first 72 hours after onset of disease. However, specimens will still be processed if collected up to 7 days after the onset of symptoms.[5]

Table 2. Coronavirus specimen collection, packaging and transport.

Specimen type	Transport medium	Transport to laboratory	Dangerous goods shipping category	Comment
Naturally produced sputum*	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	Biological substance, Category B	The preferred sample but need to ensure the material is from the lower respiratory tract.
Bronchoalveolar lavage	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	There may be some dilution of virus but still a worthwhile specimen.
Tracheal aspirate	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Nasopharyngeal aspirate	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Combined nose/throat swab	Virus transport medium	On ice.	As above	Virus has been detected in this type of specimen
Nasopharyngeal swab	Virus transport medium	On ice.	As above	
Tissue from biopsy or autopsy including from lung	Virus transport medium or saline	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Serum for serology or virus detection: always collect paired samples if possible. Acute – first week of illness Convalescent -3 to 4 weeks later	no	On ice **or** frozen	As above	
Whole blood	EDTA anticoagulant	On ice	As above	For virus detection, particularly in the first week of illness

* The collection of induced sputum samples may pose an additional infection risk for health care workers.

Source: Interim surveillance recommendations for human infection with novel coronavirus, 3 December 2012 [5]

Laboratory test methods and algorithm

All suspected cases should be referred to the CRDM which is a WHO reference laboratory for the testing of novel coronavirus in Africa. A number of reverse transcription polymerase chain reaction RT-PCR assays that are specific for the novel coronavirus have been developed and published.[5] The assay for the E protein gene target (UpE) is considered highly sensitive, and has been implemented at the NICD. A second confirmation PCR on the open reading frame 1b (ORF1b) will be performed on all UpE positive specimens and a pan-Coronavirus PCR will be run on all specimens.

The following criteria are used to laboratory confirm a case:

- Positive PCR assay for at least two different specific targets in the novel coronavirus genome; or
- One positive PCR assay for a specific target in the novel coronavirus genome and an additional different PCR product sequenced.[5]

Patients meeting the case definition should also undergo routinely available laboratory investigations for common aetiologies of community acquired pneumonia. The CRDM is able to test for: Para-Influenza viruses 1-3, influenza A virus, influenza B virus, respiratory syncytial virus, enterovirus, rhinovirus, human metapneumovirus, adenovirus, human bocavirus, human coronaviruses 229E, OC43, NL63 and HKU1, by real-time PCR. These tests will be run in parallel with the novel coronavirus specific PCR on all suspected cases.

Infection control

It is advised by WHO that standard and droplet precautions should be applied to all patients with confirmed and suspected coronavirus infection. Airborne precautions should be added when performing aerosol generating procedures.[6] Additional information on infection control can be accessed from the WHO web-site.[7]

Surveillance for novel coronavirus implemented at NICD

Private and public health care professionals are invited to submit cases of severe acute respiratory infections, meeting the case definition of unexplained pneumonia or a travel history to the Arabian Peninsula, for investigation of the novel coronavirus.

Advice on travel

The WHO does not recommend that any travel or trade restrictions be applied. In addition, no screening at points of entry should be enforced.[5]

Updated information

The situation with regards to this virus is rapidly evolving as new cases are detected and reported. For updated information please consult the NICD webpage at www.nicd.ac.za or World Health Organisation at www.who.int/csr/disease/coronavirus_infections.

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OPTIMIZATION AND VALIDATION OF A MULTIPLEX PCR FOR IDENTIFICATION OF MAMMALIAN BLOOD MEALS IN MALARIA VECTOR MOSQUITOES AND TIME-COST COMPARISON BETWEEN THE PCR AND ELISA METHODS

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Background

Blood meal identification for haematophagous insects, including malaria vector mosquitoes, is critical in understanding the vectorial capacity of species and populations. This is because the feeding behaviour of mosquitoes varies greatly, with some species feeding either exclusively on humans (anthropophily) or animals (zoophily), or both. As only those *Anopheles* species that take human blood are potential vectors of human malaria, identification of the blood meal source/s of species-identified female mosquitoes provides important information concerning malaria vector incrimination.

Methods for determining the blood meal source of haematophagous insects have evolved greatly over the years. Precipitin tests have been used to detect the blood meal source of insects for many decades.[1] In the 1980s, an enzyme-linked immunosorbent assay (ELISA) was introduced as a more sensitive alternative.[2] Both methods have their advantages and disadvantages and were often chosen based on the situation and level of accuracy, specificity and sensitivity required.[3] Other methods such as haemoglobin crystallization[4], agglutination reactions[5] and immunoflourescence[6,7] have either been proposed or used. Although each method has proved useful, they are either inadequately sensitive, inadequately specific, unreliable or too elaborate - requiring sophisticated equipment.[2]

The need for a blood meal identification method that is both specific and sensitive led to many laboratories adopting the ELISA method, which is currently the most widely used. However, the advancement of molecular techniques and the requirement to carry out multiple molecular diagnostics on single specimens led to the development of polymerase chain reaction (PCR) techniques for blood meal identification.[8] These methods are mainly based on cytochrome b, a well characterized protein from complex III of the mitochondrial oxidative phosphorylation system.[9,10] In order to reduce the cost and time involved, Kent & Norris[8] developed a multiplex PCR based on cytochrome b for mammalian blood meal identification in malaria mosquitoes. This method directly identifies mammalian blood in mosquitoes by the amplification of size-specific DNA fragments.

The aim of this study was to assess the reliability, cost effectiveness and efficiency of the multiplex PCR technique of Kent & Norris[8] in comparison to the routine ELISA method.[11]

Materials and Methods

Mosquito samples

Indoor resting mosquitoes were collected from six villages spanning two ecological zones (forest and coastal savannah) in Ghana during September to December, 2010. Immediately after collection, mosquitoes were dry preserved using silica gel. Each specimen was identified to species using morphological keys[12,13] and PCR[14,15] as appropriate.

DNA isolation

DNA was extracted[16] from the abdomens of all female mosquitoes. Abdominal DNA from a female mosquito fed on human blood served as a positive control. Other positive controls consisted of DNA extracted from whole cow, pig, goat and dog blood collected from the Department of Agriculture, University of Pretoria. The DNA pellets were re-suspended in 200 µl of 1 x TE Buffer and stored at -20°C until ready for use.

Optimization and validation of the blood meal identification PCR protocol

The blood meal PCR assay was initially implemented according to the protocol of Fornadel *et al.*[17] A hot start *taq* Polymerase is required for this protocol. The PCR reaction was adapted slightly with an annealing temperature of 58°C.

In an effort to reduce the overall cost per specimen, the PCR reaction mixture was halved to 12.5 µl reaction volume and a standard *taq* Polymerase was used. The optimised 12.5 µl reaction mixture consisted of: 1.25 µl 10x PCR buffer, 0.5 mM dNTP mix, 0.75 mM $MgCl_2$, 50 pmol of each primer (UnvRev 1025, Pig573F, Human894F, Goat368F, Dog368F, Cow121F), 0.5U Taq DNA polymerase and 0.5 µl template DNA. The following cycling conditions were used: 95°C for 5 minutes followed by 95°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute for 35 cycles with a final extension at 72°C for 7 minutes.

This optimized Kent & Norris[8] protocol was validated on 350 mosquito samples comprising 243 *Anopheles gambiae* and 107 *An. funestus* specimens. A single PCR amplicon for each blood type identified was sequenced to confirm the accurate identification of the blood meal source. This was accomplished by aligning the sequences obtained to known sequences for each blood source which are available on the NCBI database.

Cost and time comparison between PCR and ELISA

The cost of blood meal identifications using PCR and ELISA was generated in South African Rands (ZAR) and then converted to United States Dollars (USD) using the South African Reserve Bank exchange rate of 8.1897 ZAR : 1.0 USD for 11th July, 2012. These costs only included consumables, with the assumption that equipment is a basic prerequisite for running these assays. The costs were compiled from the suppliers quotes for bulk purchases (including VAT), and refined to cost per analysis of a 96 well plate for ELISA and a 36 well agarose gel for PCR. From these, the costs of supplies needed to analyse one sample for either ELISA or the multiplex PCR were determined.

The time required to conduct blood meal identification using either PCR or ELISA was derived based on a 36 well gel and a 96 well plate respectively. The following was taken into consideration when calculating the time required for PCR identifications: time required to extract DNA, prepare the PCR reaction mixtures, cycling time of the PCR reaction in the PCR machine, preparation of the agarose gel, electrophoresis and gel analysis. The following was taken into consideration when calculating the time required for ELISA identifications: time required to prepare the microtitre plates, process the mosquito homogenates, complete the incubation steps of the ELISA protocol and analyse the optical densities obtained for the ELISA protocol. Hands on time (active work on the process minus waiting times) and whole process time were estimated for both PCR and ELISA.

Results

Optimization and validation of the PCR protocol

Analysis for bloodmeal identification was based on the protocol of Fornadel *et al.*[17] with a change of annealing temperature from the recommended 56°C to 58°C Sequence analysis of selected blood meal PCR amplicons confirmed that the correct host, (human, cow, pig or dog) was amplified. PCR validation of 350 blood fed indoor resting mosquito specimens resulted in 82.3% and 94.3% successful blood meal identifications for *An. gambiae* and *An. funestus* respectively (table 1) based on amplicon sizes visualised by electrophoresis.

Table 1: Identification of blood meal sources of *Anopheles gambiae* and *An. funestus* females from Ghana using the optimized blood meal PCR protocol of Kent &Norris.[8]

Anopheles Spp.	n (%)	No. blood meals successfully identified (%)	Blood meal Source (%)		
			Human	Animal	Mixed
gambiae	243 (69.4)	200 (82.3)	188 (77.4)	1 (0.4)	11 (4.5)
funestus	107 (30.6)	102 (95.3)	102 (82.9)	0 (0)	0 (0)
Total	350	302 (86.3)	290 (96.0)	1 (0.003)	11 (3.6)

Time Cost Analysis for PCR and ELISA

Table 2 shows the time and cost analysis for PCR vs ELISA. The multiplex PCR method requires less time (8.78 hours) to identify five blood meal sources simultaneously, compared to ELISA which requires 23.75 hours for five blood meals assuming that these are performed one at a time. This time can be significantly reduced if all five assays are performed concurrently. PCR is more cost effective and currently costs approximately US $0.93 per five blood meal sources per sample as compared to US $3.19 for the equivalent identifications using ELISA.

Table 2. Comparative cost and time required for the PCR and ELISA methods for mosquito blood meal source identification. Hands on time refers to processing time without incubation periods included.

	Time to Complete (hrs)			
	Whole Process	Hands on	Cost per Sample	
Multiplex PCR for 5 Blood Meals	8.78	4.28	R 7.61	($ 0.93)
ELISA for 1 Blood Meal	4.75	0.25	R 5.22	($ 0.64)
ELISA for 5 Blood meals	23.75	1.25	R 26.10	($ 3.19)

Discussion

Laboratories that process large numbers of mosquito specimens for multiple diagnostic features require methods and processes that are high throughput, efficient and cost effective. Although the ELISA method is sensitive in terms of identifying the source of blood meals[2], it is time consuming, comparatively expensive and is limited in terms of throughput. The optimized multiplex PCR protocol[8] for blood meal identification is equally sensitive. The 13.7% of specimens from which blood meal source could not be identified, even after two repeat attempts, suggests that they may have taken blood meals from other domestic animals such as cats, sheep, chicken, duck, turkey or even peridomestic pests such as rats, mice and bats. Other factors such as DNA degradation, enzyme inhibitors and human error cannot be ruled out.

The multiplex PCR assay, compared to the ELISA method, appears to be quicker and less costly when assaying for several blood meal sources. However, if the aim of identifying blood meal source is only to determine the proportion feeding on humans, such as determination of the human blood index (HBI), then ELISA is a better option in terms of cost and time.

A major advantage of the multiplex PCR over ELISA is that the PCR can be integrated with other molecular diagnostic methods, especially in laboratories that conduct multiple diagnostics on single specimens using the extracted DNA of each specimen.[8] Under these circumstances, apart from tailored primers, all the

reagents used for blood meal identification by PCR are universal for to any diagnostic PCR assay. Purchasing these reagents in large quantities can further reduce the costs for all of the PCR diagnostic processes employed. Furthermore, using single DNA extracts for multiple diagnostics should reduce the time required to prepare specimens and thus enhance throughput.

It is concluded that the multiplex PCR method of Kent & Norris[8] can significantly reduce the time required for and cost of identification of blood meal sources of mosquitoes and other haematophagous insects. This protocol can be adopted for routine blood meal source identification with ELISA retained as an alternative method in situations where only the human blood index is of interest.

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Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD - South Africa, corresponding periods 1 January - 31 December 2011/2012*

Disease/Organism	1 Jan to 31 Dec, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Botulism	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2011	1180	343	1887	1042	404	616	58	445	478	6453
	2012	1067	314	1985	1889	174	365	59	303	566	6722
Haemophilus influenzae, invasive disease, all serotypes	2011	32	25	155	73	7	21	12	8	95	428
	2012	36	18	108	47	3	12	8	6	94	332
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2011	8	3	19	12	2	4	7	2	17	74
	2012	2	5	17	3	1	4	2	2	12	48
Serotypes a,c,d,e,f	2011	1	1	12	3	0	0	0	0	5	22
	2012	2	0	5	0	0	1	0	0	6	14
Non-typeable (unencapsulated)	2011	2	2	30	6	0	1	0	0	14	55
	2012	0	1	18	5	0	0	0	0	14	38
No isolate available for serotyping	2011	5	3	21	15	2	5	1	3	2	57
	2012	6	3	12	8	0	3	2	1	9	44
Measles	2011	4	2	36	23	1	2	8	8	2	86
	2012	0	2	8	6	1	0	0	2	1	20
Neisseria meningitidis, invasive disease	2011	50	26	134	39	8	19	6	5	52	339
	2012	48	13	78	26	3	7	2	8	47	232
Novel Influenza A virus infections	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Plague	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Rabies	2011	2	0	0	1	3	0	0	0	0	6
	2012	1	1	4	0	3	1	0	0	0	10
**Rubella	2011	518	58	750	407	474	429	83	358	191	3268
	2012	369	48	285	784	73	181	212	90	254	2296
Salmonella spp. (not typhi), invasive disease	2011	25	22	259	105	4	35	6	9	58	523
	2012	46	22	311	136	7	43	12	10	108	695
Salmonella spp. (not typhi), isolate from non-sterile site	2011	140	21	503	234	11	62	18	18	240	1247
	2012	144	23	492	211	9	53	7	8	334	1281
Salmonella typhi	2011	10	2	20	12	1	10	0	1	16	72
	2012	4	0	23	12	1	10	0	1	13	64
Shigella dysenteriae 1	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non Sd1)	2011	215	40	598	164	8	30	35	12	451	1553
	2012	275	65	589	231	5	35	31	8	389	1628
Streptococcus pneumoniae, invasive disease, all ages	2011	343	229	1613	553	56	195	68	190	562	3809
	2012	315	219	1294	577	64	163	49	125	422	3228
Streptococcus pneumoniae, invasive disease, < 5 years	2011	48	45	316	86	9	41	20	30	106	701
	2012	53	35	243	80	6	20	7	17	51	512
Vibrio cholerae O1	2011	0	0	0	0	1	0	0	0	0	1
	2012	0	0	0	0	0	0	0	0	0	0
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2011	0	0	0	0	0	0	0	0	0	0
	2012	0	0	0	0	0	0	0	0	0	0
***Other VHF (not CCHF)	2011	17	3	0	0	0	0	3	0	14	37
	2012	0	0	0	0	0	0	0	0	0	0

Footnotes

*Numbers are for cases of all ages unless otherwise specified. Data presented are provisional cases reported to date and are updated from figures reported in previous bulletins.

**Rubella cases are diagnosed from specimens submitted for suspected measles cases

***All cases for 2011 were confirmed as Rift Valley Fever

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 31 December 2011/2012*

Programme and Indicator	1 Jan to 31 Dec, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Acute Flaccid Paralysis Surveillance											
Cases < 15 years of age from whom specimens received	2011	69	29	75	88	73	38	6	17	23	418
	2012	59	29	68	72	45	46	3	18	27	367

Footnotes

*Numbers are for all ages unless otherwise specified. Data presented are provisional numbers reported to date and are updated from figures reported in previous bulletins.

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Monitoring for the presence of polio in a country is based on AFP (acute flaccid paralysis) surveillance – the hallmark clinical expression of paralytic poliomyelitis. The clinical case definition of AFP is an acute onset of flaccid paralysis or paresis in any child under 15 years of age. AFP is a statutory notifiable disease and requires that 2 adequate stool specimens are taken as soon as possible, 24 to 48 hours apart, but within 14 days after onset of paralysis, for isolation and characterisation of polio virus. The differential diagnosis of AFP is wide, the most common cause of which is Guillain-Barre Syndrome. The incidence of AFP in a population has been studied in a number of developing countries and WHO have determined, as a result of these studies, that the criterion for adequate surveillance of AFP is 2 cases per 100 000 population of children less than 15 years of age (it was formerly 1 per 100,000 but this was thought to be inadequately sensitive).

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