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COMMUNICABLE DISEASES SURVEILLANCE BULLETIN

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NICD NHLS

FOREWORD

'The price of liberty is eternal vigilance' – John Philpott Curran (1750-1817)

This issue of the Bulletin highlights some viral diseases of public health importance that are under surveillance. Curran was speaking in a socio-political sense; in our context we can substitute 'freedom from disease' and 'surveillance' for the key words. The measles surveillance programme is a component of the measles elimination strategy in South Africa. Through it, nearly 27 000 specimens from suspected cases were tested during 2010, of which 12 499 (46%) were positive for IgM, evidence of the widespread epidemic affecting all provinces. A mass vaccination campaign was held in the first half of the year and incidence had dropped substantially by year end. Routine immunization against measles must be strengthened to improve coverage rates and reduce risk of future outbreaks. The intrusion of rabies into the greater Johannesburg area is of great concern; of critical importance are the cooperative efforts of veterinary and human health services to mobilize the public to have their pets vaccinated and so reduce the population of susceptible animals, and to ensure people obtain appropriate attention if exposed. Surveillance for acute flaccid paralysis is a vital part of the global campaign to eradicate polio. For the country as a whole the surveillance targets were nearly met in 2010, but there were substantial variations in compliance at provincial level. Polio poses a threat to the region, in the form of cases imported from endemic countries like Nigeria, or from where it has been re-established, such as Angola. As the Spanish-born philosopher, George Santayana (1863-1952) so aptly wrote: *'Those that cannot remember the past are condemned to repeat it'*. Surveillance is the key to not forgetting.

John Frean, Acting Editor

CONTENTS

SUSPECTED MEASLES CASE-BASED SURVEILLANCE, SOUTH AFRICA, 2010

Epidemiology and Surveillance Unit, Specialized Molecular Diagnostics Unit, Viral Diagnostics General Isolation Unit and Serology Unit, National Institute for Communicable Diseases

The case-based measles surveillance system, with laboratory support started in 1998 as part of the National Department of Health's measles elimination strategy. The National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS) is accredited by the World Health Organization (WHO) to perform measles and rubella IgM testing for the national case-based surveillance. The case definition for suspected measles is: any patient who presents with fever ≥38ºC and rash, and at least one of the three Cs (cough, coryza or conjunctivitis). Blood and urine specimens from suspected measles cases nationally are submitted to NICD for laboratory confirmation. The numbers presented here represent specimens received by the NICD and may differ from those presented by the National Department of Health as they may receive information on cases where no specimens were taken.

All blood specimens were tested by Enzygnost (Dade-Behring, Marburg, Germany) diagnostic kits for the presence of anti-measles and anti-rubella immunoglobulin

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M (IgM). Amplification of ribonucleic acid (RNA) for genotyping was attempted in a sample of cases testing positive or equivocal for anti-measles IgM. For molecular analysis RNA was extracted directly from clinical specimens and tested for the presence of measles virus by reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR followed by sequencing.

The current measles outbreak that started in Tshwane district, Gauteng Province in March 2009 continued during the year 2010. In April/May 2010, the Department of Health embarked on a nationwide mass measles vaccination campaign targeting children aged 6 months to <15 years. For the period 1 January to 31 December 2010, the NICD tested about 26 886 specimens that were collected from suspected measles cases nationally. Of these, 12 499 (46%) were positive for measles IgM antibodies and 2 335 (9%) positive for rubella IgM antibodies (Table 1). Of the positive cases, 155 (1%) were positive for both measles and rubella IgM antibodies.

Measles

Laboratory-confirmed measles cases were reported from all nine provinces with KwaZulu-Natal (31%, 3 837/12 499), Mpumalanga (15%, 1 844/ 12 499) and Western Cape (14%, 1 796/12 499) provinces accounting for the highest proportions of the total (Table 1). Timing and distribution of measles cases differed between provinces (Figure 1).

The highest number of measles cases were identified in March and April of 2010 and gradually declined to relatively low numbers towards the end of the year (Figure 2).

Age and sex were known in 11 779 (94%) and 12 031 (96%) laboratory-confirmed measles cases respectively. Age ranged from <1 months to 86 years with a median of three years. Children <5 accounted for 53% (6 195/11 779) of the cases with 27% occurring in those aged 6 to 11 months (Figure 3). Of the 4 220 patients aged < 1 year, 70% (2 933/4 220) were children < 9 months. The gender split was 51% (6 115/12 031) male and 49% (5 916/ 12 031) female.

Molecular characterization was performed on a subset of cases testing positive for measles IgM antibodies. The virus was identified as genotype B3 in all but one case; a similar strain to that was detected throughout the course of the outbreak in 2009. The only other genotype identified was D4. The genotype D4 virus was isolated from a male traveller from France and was unrelated to the ongoing measles outbreak.

Table 1: Number of measles and rubella IgM-positive cases from measles case-based surveillance, South Africa, 2010

Provinces	Measles IgM-positive (N=12 499) (n %)	Rubella IgM-positive (N=2 335) (n %)
Eastern Cape	1 309 (10)	436 (19)
Free State	674 (5)	116 (5)
Gauteng	1 617 (13)	389 (17)
KwaZulu-Natal	3 837 (31)	366 (16)
Limpopo	290 (2)	122 (5)
Mpumalanga	1 844 (15)	188 (8)
Northern Cape	374 (3)	70 (3)
North West	758 (6)	287 (12)
Western Cape	1 796 (14)	361 (15)

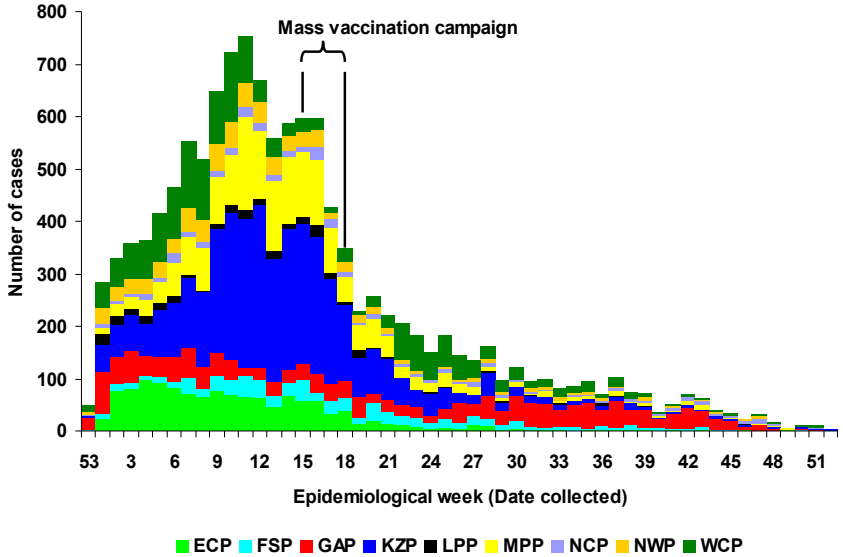

Figure 1: Epidemic curve showing measles IgM positive results per province by week specimens were collected, South Africa, January to December 2010

Province abbreviations: ECP=Eastern Cape; FSP=Free State; GAP=Gauteng; KZP=KwaZulu-Natal; LPP=Limpopo; MPP=Mpumalanga; NCP=Northern Cape; NWP=North West; WCP=Western Cape

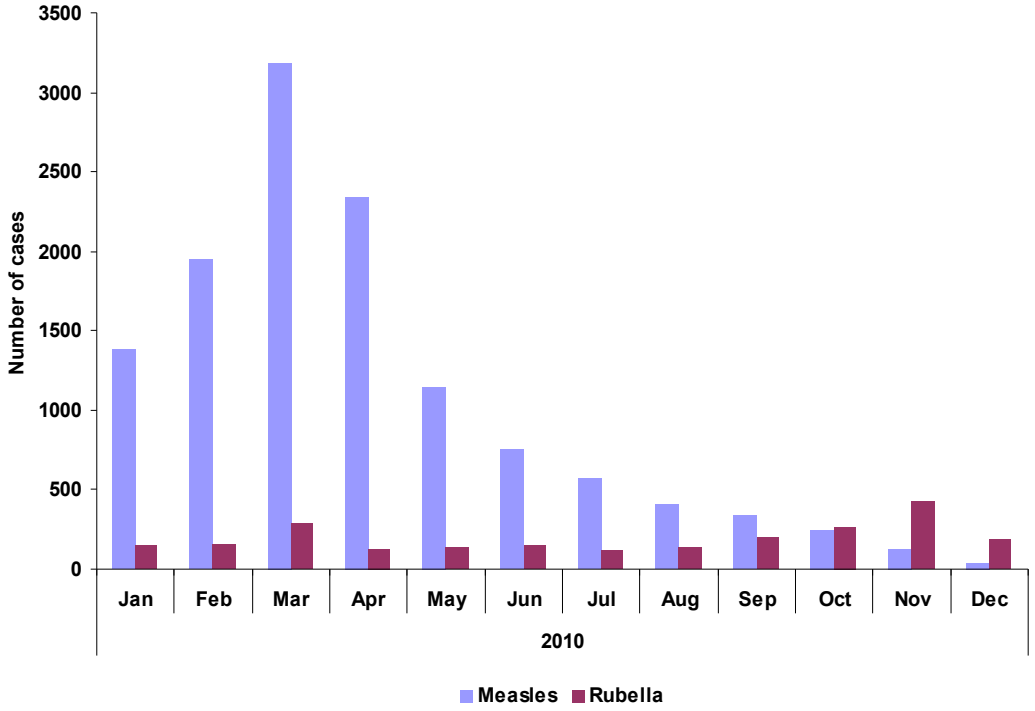

Figure 2: Number of measles and rubella IgM-positive cases by month of specimen collection, South Africa, 2010

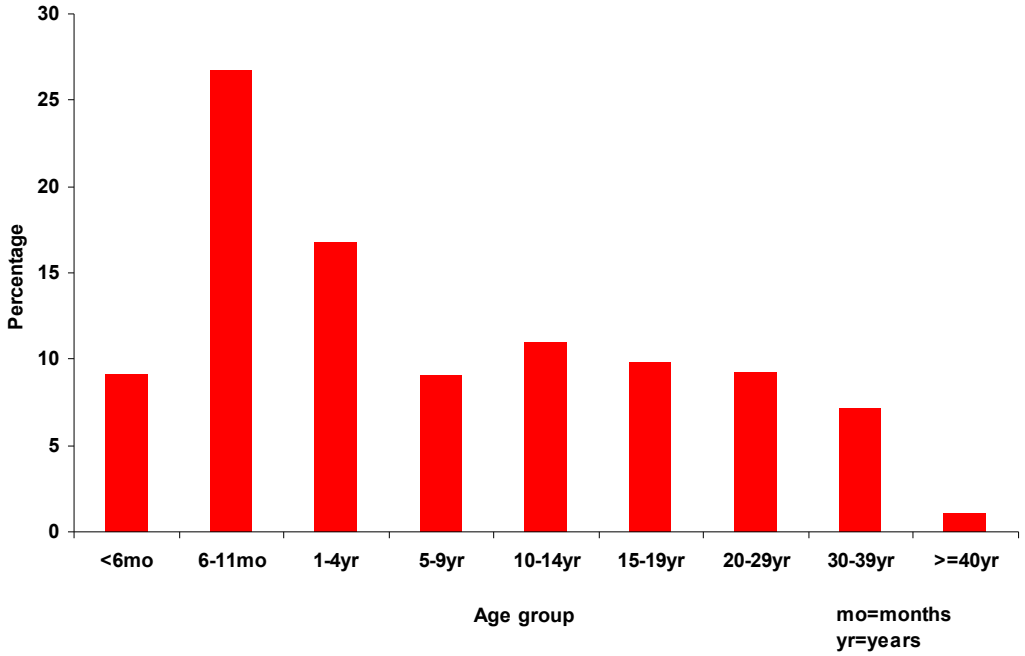

Figure 3: Age distribution of laboratory-confirmed measles cases (N=11 779): South Africa, January to December 2010

Rubella

Of patients with known age (n=2 248), 45% (1 014/2 248) were aged 5-9 years. Age ranged from <1 months to 90 years with a median of six years. A higher proportion of cases was detected during the month of November (Figure 2). Cases were reported from all nine provinces but the Eastern Cape (19%, 436/2 335) and Gauteng (17%, 389/2 335) provinces had proportionally the highest number of cases. Sex was recorded for 2 271 rubella cases and there was equal representation (50%) for both genders. Ten percent of the female cases were in women of reproductive ages (195/1096).

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ACUTE FLACCID PARALYSIS SURVEILLANCE (AFP) IN SOUTH AFRICA AND THE AFRICAN REGION, 2010

Enterovirus Isolation Unit, Epidemiology and Surveillance Unit, Polio Molecular Unit,
National Institute for Communicable Diseases

BACKGROUND

The rationale for acute flaccid paralysis (AFP) surveillance is defined by the WHO as follows: poliomyelitis is targeted for **eradication**. Highly sensitive surveillance for acute flaccid paralysis (AFP), including immediate case investigation and specimen collection, are critical for the detection of wild poliovirus circulation with the ultimate objective of polio eradication. AFP surveillance is also critical for documenting the absence of poliovirus circulation for polio-free certification. Every case of AFP including Guillain-Barré syndrome, in children younger than 15 years of age, or a patient of any age with a clinical diagnosis of polio made by a medical doctor, must be regarded as a possible polio case until proven otherwise. This proof is either by laboratory confirmation or by the consideration of the clinical medical records by the National Polio Expert Committee (NPEC). To meet sample adequacy requirements, all cases require two stool specimens of good condition and sufficient quantity collected at least 24-48 hours apart within 14 days after onset of paralysis, and sent to the NICD for polio identification. During 2010, at a required detection rate of two cases of AFP per 100 000 children under 15 years, 295 cases needed to be identified.Two laboratories at the NICD form part of WHO-supported AFP surveillance network at both a national and regional level. The Enterovirus Isolation Unit of the NICD serves as a national poliovirus isolation laboratory for South Africa as well as for six other southern African countries i.e. Angola, Botswana, Lesotho, Mozambique, Namibia, and Swaziland. The WHO Regional Reference Laboratory for Polio provides the required technical support to characterize wild type and variants using sequence-based technologies and intratypic characterization by real time PCR. There was a significant decrease of reported cases in the one endemic country viz., Nigeria and by contrast a very large outbreak of poliomyelitis was experienced in the Republic of Congo (CNG) affecting mainly males in the younger age groups and with an unusually high case fatality rate. The last wild poliovirus case in the Republic of Congo was reported in 2000. Continued cases of wild type polio were identified in other countries in Central Africa including the Democratic Republic of Congo (RDC), Chad and Angola. In addition, surveillance for circulating vaccine-derived polio virus (cVDPV) was performed and the results of the surveillance are presented.

AFP surveillance in South Africa

There were no wild type poliovirus isolates made from specimens of South African AFP cases. A total of 698 specimens was received from 357 South African cases. Of these, 14 cases had onset of paralysis prior to 2010. In addition 23 cases were classified as not AFP by the NPEC. In the first half of January 2011, specimens were received from a further 14 cases with onset of paralysis in 2010, bringing the total number of suspected cases for 2010 to 334. The case detection rate, calculated only on cases from whom specimens were received, for the country was 2.2 (range per province 0.8 to 2.8, see Figure 1). One specimen only was received from 41 cases, and two or more specimens from 293. The date of onset of paralysis was known for 321 (96%) of cases. Two adequate specimens were received from 260/321 (81%) cases with known date of onset (range per province 50% to 88%- see Figure 1). Non-polio enteroviruses were detected in 71, and non-enteroviruses in 20 of the 698 specimens received in 2010, a non-polio detection rate 13%; poliovirus, identified as Sabin (vaccine) type poliovirus was detected in 11 (2%) specimens of seven patients.

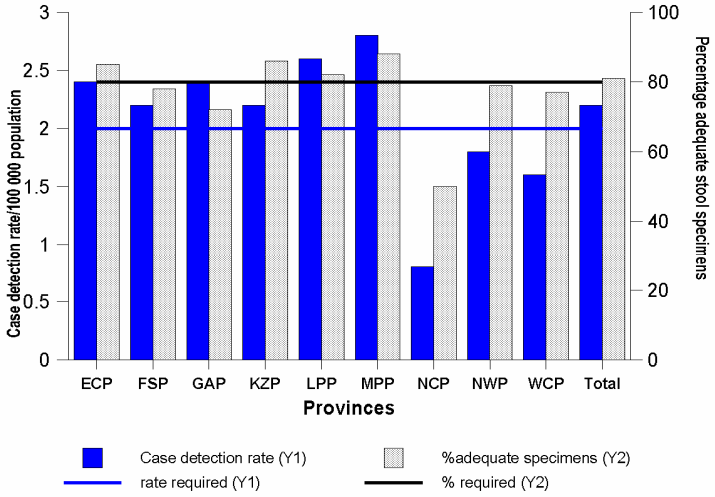

Figure 1: AFP case detection rate and percentage of cases with adequate stool specimens. AFP surveillance, South Africa 2010, per province.

AFP surveillance in southern African block countries

Of the 1480 specimens received from the six southern block countries served by the NICD, 95 were from patients with onset of paralysis prior to 2010. Two adequate stool specimens were received from 597 (86%) of the 695 patients with known onset of paralysis in 2010 (range per country 69% to 100%). Non-polioviruses were detected in 191/1480 (13%) specimens with a non-poliovirus detection rate of 13% (range per country 0% to 18%). Poliovirus was detected in 98 specimens, 56 (57%) of which were identified as wild type poliovirus 1 (PV1), and the remainder as Sabin strains (see details below). The wild type viruses were from 31 patients in Angola with dates of onset ranging from 15 November 2009 to 12 November 2010.

Molecular characterisation of wild-type polioviruses

The laboratory received 1972 specimens in 2010 compared to 2963 specimens in 2009 (Figure 2). These isolates were characterized as vaccine or wild type virus using two real-time methods viz., (i) real-time RT PCR for intratypic differentiation (rRTPCRITD) and (ii) real-time RT PCR for vaccine-derived polioviruses (rRTPCRVDPV).The current real-time PCR methodology contains a considerable advantage over conventional PCR diagnostics in that it allows for the identification of vaccine derived polioviruses as well as viruses which have undergone recombination in either the VP1 or 3D regions of the viral genome without the requirement for the generation of sequence data. The rRTPCRITD positive isolates were tested by rRTPCRVDPV.

The negative rRTPCRVDPV isolates were further analysed by sequencing while negative rRTPCRITD were analysed by sequencing without being tested by rRTPCRVDPV as they were considered to be wild-type viruses.

Two hundred and twenty three (223) cases were identified as wild PV1 in Africa with active outbreaks in Uganda (UGA), the Republic of Congo (CNG), Mali (MAI) and Liberia (LIB). The total number of cases identified from CNG in 2010 was 63. The ages of infected individuals ranged from 1 – 92 years with the 20 – 29 age group being the most affected. Pointe Noire, CNG was the epicentre of the outbreak. Nigeria, classified as an endemic country in Africa had only 7 cases of wild PV1 identified in 2010 compared to 75 cases reported in 2009.

In 2010 PV1 wild type isolates were distributed into two genotypes, SOAS and WEAF-B (Figure 3). The SOAS genotype consists of the viruses from Angola, Democratic Republic of Congo (DRC) and Republic of Congo (CNG). The WEAF-B genotype consists of viruses from Nigeria (data not shown), Niger, Cameroon, Uganda, Senegal, Mali, Serra Leone, Mauritania, Liberia and Guinea.

WEAF-B wild PV3 was identified in Mali, Niger and in Nigeria with only 11 cases reported in Nigeria compared to 313 in 2009. The strains from Mali and Niger were linked to Nigeria strain.

Characterisation of circulating vaccine-derived polioviruses (cVDPV)

Oral polio vaccine (OPV) is an important and effective means of control and eradication of wild polioviruses. The consequence of the use of a live OPV is that there is genetic drift as a result of mutations and recombination events with, for example, non-polio enteroviruses that result in the acquisition of transmissibility and neurovirulence properties similar to the wild polioviruses.

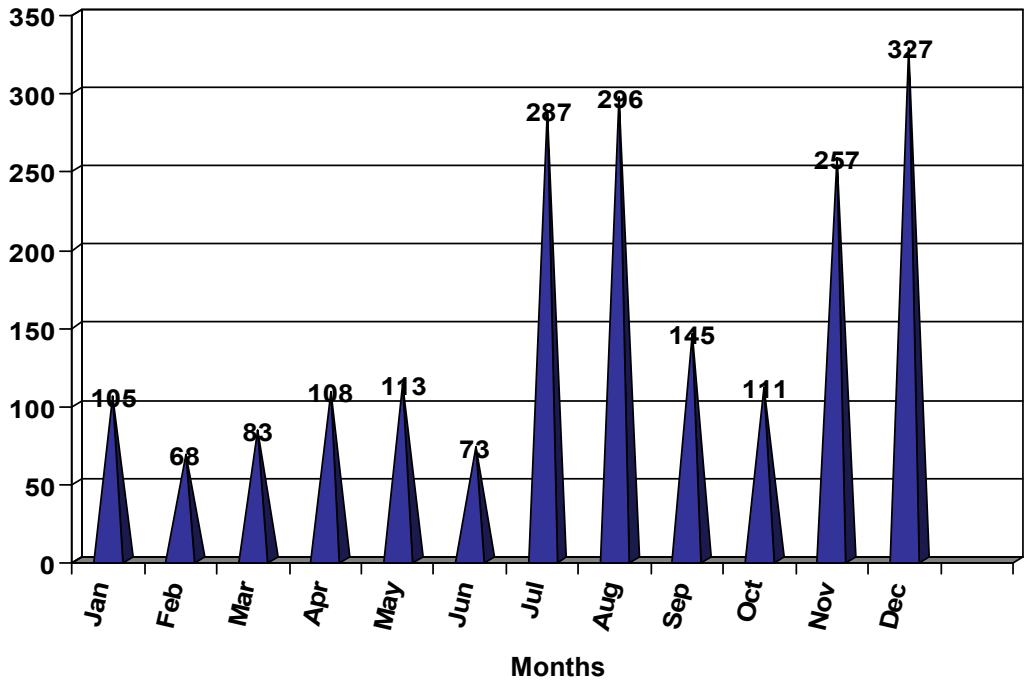

Figure 2: Poliovirus characterization: number of samples received per month, 2010.

One outcome of such events is that of circulating (c) vaccine-derived poliovirus (cVDPV) strains that are transmitted and may cause flaccid paralysis. The VDPVs are Sabin–like viruses that have less than 99% VP1 nucleotide sequence identity to the Sabin oral polio vaccine strains (OPV). cVDPV were identified in the DRC, Niger, Ethiopia and Nigeria. Only 21 cases were in Nigeria compared to 153 in 2009. The Niger case was linked to the Nigerian outbreak. In 2010, Ethiopia experienced an increase in cVDPV type 3 compared to 2009 when only 1 case was reported.

In terms of the targets set to eradicate poliomyelitis the year 2010 was characterized by a decline in the numbers of cases in the one endemic country, namely Nigeria. However, this is in contrast with multiple importations as reflected by the number of countries affected as well as outbreaks.

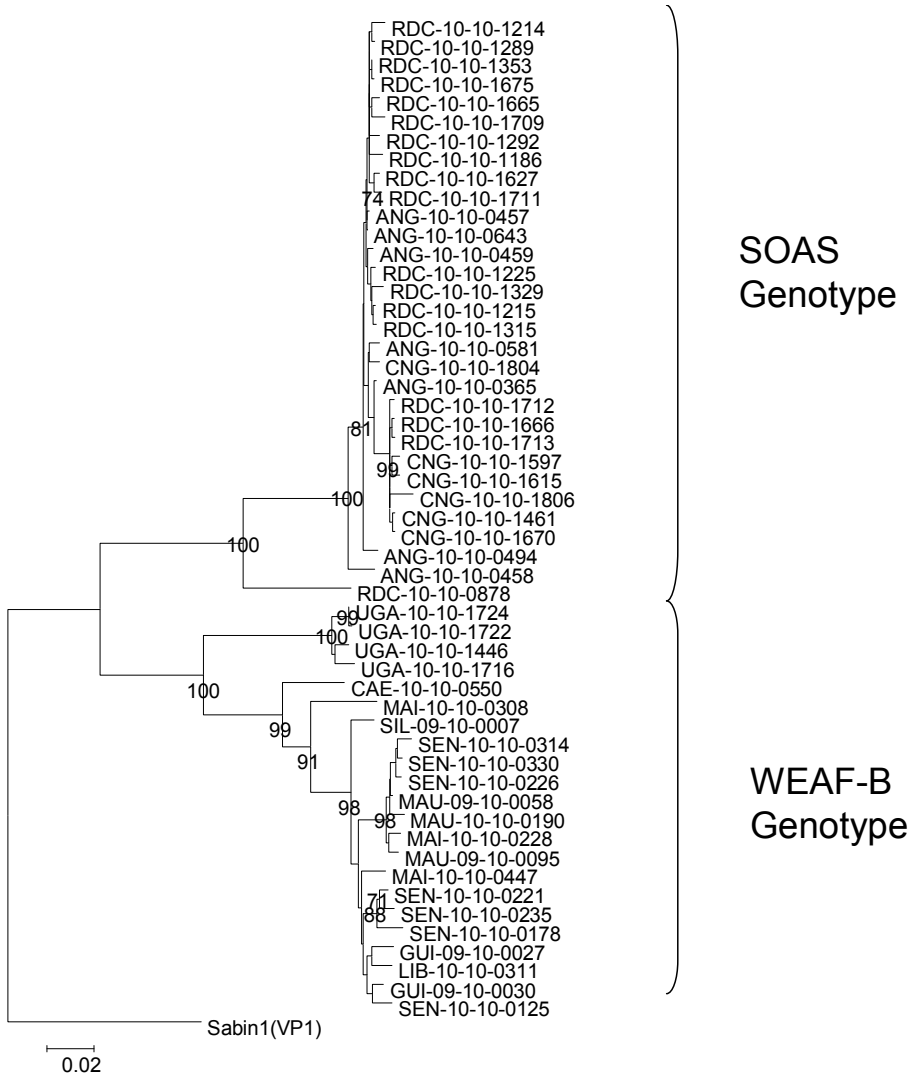

Figure 3: Neighbor-joining tree of the VP1 gene of WEAF-B and SOAS wild PV1 representative of isolates of 2010 from Africa. Bootstrap values of greater than 70% are shown at the branch nodes. Sabin type 1 was used as an out-group.

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Specialized Molecular Diagnostics Unit: Elizabeth Both, Mark Goosen

RESPIRATORY VIRUS SURVEILLANCE, SOUTH AFRICA, 2010

Epidemiology and Surveillance Unit, Respiratory Virus Unit, Viral Isolation Unit,
National Institute for Communicable Diseases

The NICD coordinates 4 influenza surveillance programmes, each focusing on different aspects of influenza epidemiology. These are:

1. The viral watch influenza-like illness (ILI) surveillance programme
2. The severe acute respiratory infections (SARI) programme
3. The respiratory morbidity data mining surveillance system
4. Influenza-associated mortality surveillance programme

This bulletin will provide surveillance findings for the year 2010 for the first 3 programmes.

Respiratory virus surveillance

Viral Watch

The Viral Watch sentinel surveillance programme, begun in 1984, was specifically designed to monitor influenza activity in the community. In 2010 It started with 247 practitioners registered across South Africa. However, 67 practitioners did not submit any specimens during the year. Of the 180 who provided specimens, 125 submitted them to the NICD, 8 to the virology laboratory at the University of the Free State (FSP), 9 to the Department of Virology at Inkosi Albert Luthuli Central Hospital/University of KwaZulu-Natal (KZP), and 38 to the NHLS Tygerberg Hospital laboratory in the Western Cape (WCP). Positive specimens from these sites are sent to the NICD for confirmation, serotyping and sequencing, and the databases of all specimens received are sent to the NICD on a weekly basis. A total of 2309 specimens was submitted throughout the year (FSP: 45; KZP: 102; NICD: 1926; WCP 236).

From these 2309 specimens 917 (40%) influenza detections were made which were further characterised as 468 (51%) influenza B, 238 (26%) influenza A/H3N2 and 211 (23%) influenza A/H1N1(2009).

The first influenza detection of the season was made from a specimen collected on 7 June (week 23), and the last from a specimen collected on 8 October (week 40). Sporadic detections were made both before and after the season. The start and end of the season are defined as the weeks where the influenza detection rate (calculated on specimens tested at the NICD only) rises above 10% for 2 consecutive weeks, or falls below 10% for 2 consecutive weeks after a sustained period of high detection rates (Figure 1).

The season peaked in week 33 when the detection rate rose to 65%. The season was longer than any previous season over the past 26 years, lasting 18 weeks. The

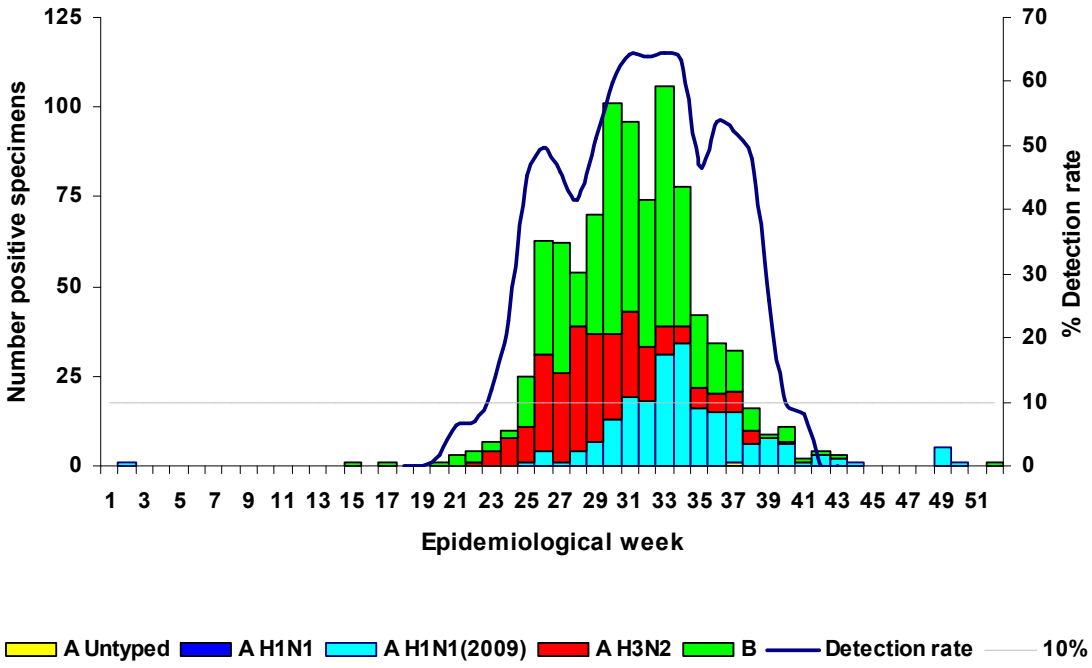


Figure 1: The number of influenza positive samples and detection rate, by viral subtype. Viral Watch Surveillance Programme 2010

average duration of the influenza season over the previous 26 years has been 9 weeks with a range of 7 to 17 weeks. A further 235 respiratory virus detections were made during the year of which 121 (52%) were adenovirus, 27 (12%) human metapneumovirus, 28 (12%) parainfluenza virus, and 59 (25%) respiratory syncytial virus.

Enhanced Viral Watch

In 2009, in response to the emergence of pandemic influenza A/H1N1, enhanced Viral Watch centres at 12 public hospitals in 8 provinces were enrolled to detect influenza strains in hospitalized patients. One hundred and eighty-eight specimens were received from 6/12 hospitals enrolled, the majority 131/188 (70%) of which came from Gauteng, followed by 45/188 (24%) in the Western Cape. Influenza was detected in the specimens of 13 patients (3 A/H1N1 (2009), 5 A/H3N2, 5 B), and other respiratory viruses in a further 56 of which 36 (64%) were respiratory syncytial virus, 13 (23%) adenovius, 4 (7%) parainfluenza virus and 3 (5%) human metapneumovirus.

Severe Acute Respiratory Infections (SARI) Surveillance

The severe acute respiratory infections (SARI) surveillance was initiated in 2009. The aim of the programme is primarily to describe trends in numbers of SARI cases at sentinel surveillance sites and to determine the relative contribution of influenza and other respiratory viruses. Due to the hospital-based, sentinel system that has been established by the SARI surveillance network, the programme has been able to add on testing for SARI-related pathogens such as pneumococcus. In 2010 the SARI programme expanded to include an additional site, namely the Klerksdorp hospital complex (this includes both an adult hospital and a children's hospital). The inclusion of this site brings the number of SARI sites to 6 hospitals at four sites. (Chris Hani Baragwanath hospital, Soweto, Edendale Hospital, Pietermaritzburg and the two Agincourt Hospitals, Matikwana and Mapulaneng in Mpumalanga).

The SARI case definition was also expanded at the 2 enhanced surveillance sites (Edendale Hospital and the Klerksdorp Hospital complex) to include patients admitted with suspected tuberculosis (TB), which will allow us to describe the association between influenza and TB. An additional sub-study undertook to test for cryptococcal antigen in those patients fitting the expanded case definition and who were HIV positive.

In 2010, 4557 patients were enrolled into the SARI surveillance programme. The majority (69%, 3151/4557) of enrolled patients were from Chris Hani Baragwanath Hospital (CHBH). Forty-five percent (2032/4552) of enrolled patients were children under the age 5 and 52% (2332/4531) were female. Of the enrolled patients, influenza results were available for 4508/4557 (99%).Of these, 324/4508 (7%) of samples were positive for influenza on multiplex RT PCR, 207/324(64%) were positive for influenza B, 82/324 (25%) were A/H3N2 and 44/324 (14%) were A/H1N1 (2009). Nine samples were co-infected with Influenza A and B The detection rate for influenza peaked at week 27 and dropped around the June school holidays (week 28 to week 30) and during the period of the national public sector strike (week 33 to week 35) (Figure 2).

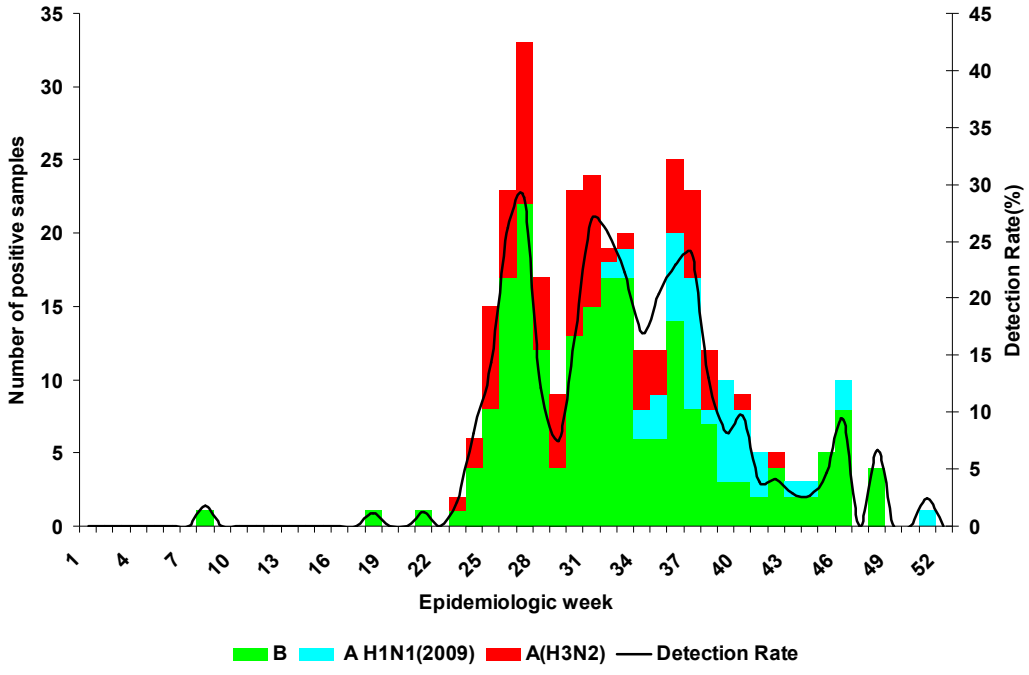
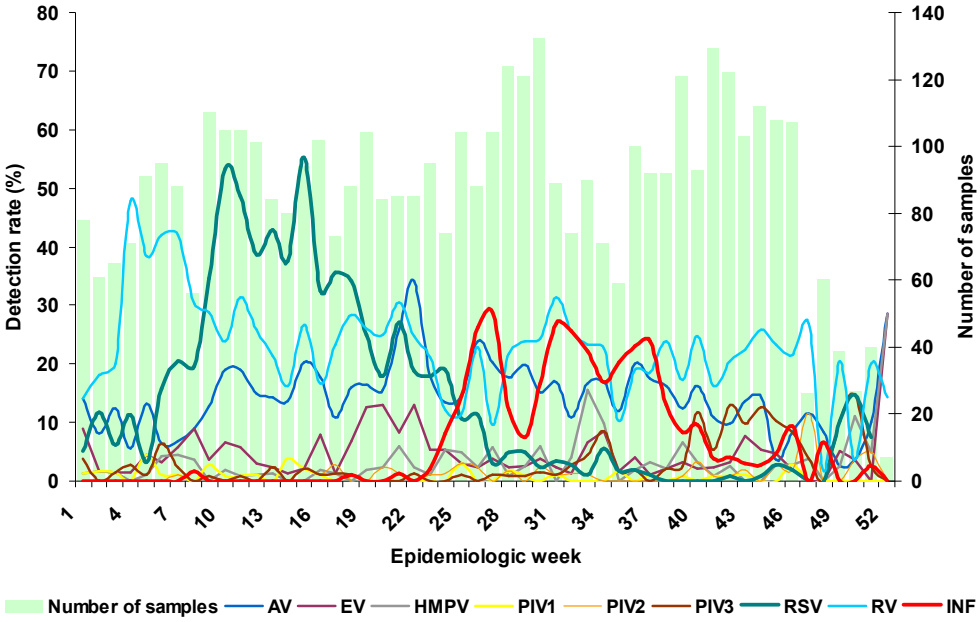


Figure 2: Number of samples positive for influenza by influenza subtype and detection rate, SARI surveillance 2010

Testing for additional respiratory viruses isolated respiratory syncitial virus (RSV) in 14% (648/4508), adenovirus in 15% (662/4508), rhinovirus in 23% (1046/4508), enterovirus in 4% (200/4508), human metapneumovirus in 3% (124/4508), parainfluenza 3 in 3% (140/4508), parainfluenza 2 in 1% (44/4508) and parainfluenza 1 in 1% (39/4508) of patients. As was the case in 2009, the RSV season preceded the influenza season; the detection rate for RSV started increasing from week 6 and reached its peak in week 15 (Figure 3). The detection rate at the peak of the RSV season ranged between 53% and 55% (Figure 4).

Of the 4557 enrolled SARI patients, 3528 (77%) had blood specimens tested for the presence of pneumoccocal DNA using quantitative real-time PCR (*lytA*). Of these, 218 (6%) were positive for pneumococcal DNA and 10% (21/218) of pnumoccocal positive patients were co-infected with influenza.



AV– adenovirus,EV-enterovirus, HMNPV- humanmetapneumovirus , PIV 1, 2, 3 parainfluenza virus type 1, 2 and 3, RSV –respiratory syncytial virus, INF-influenza virus

Figure 3: Number of samples and detection rate for respiratory viruses by epidemiologic week, SARI

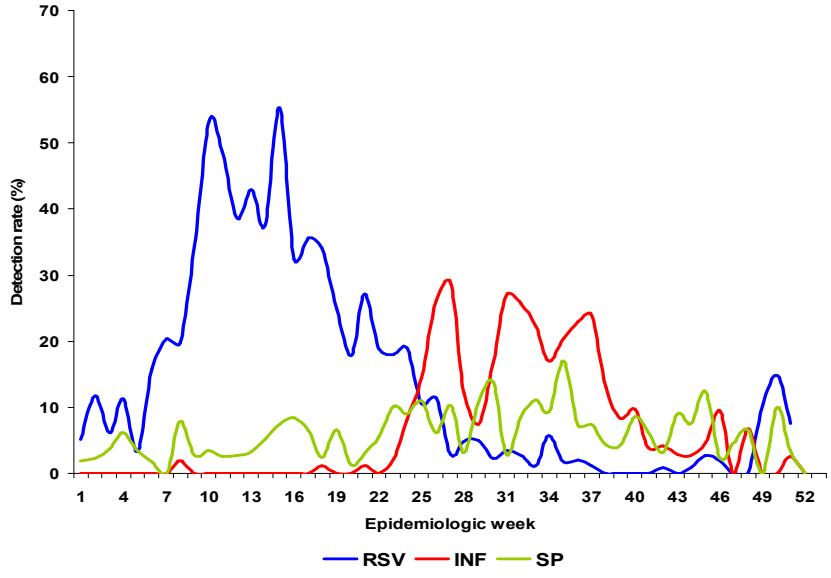


Figure 4: Detection rate for influenza, respiratory syncytial virus (RSV) and pneumococcus (SP) by epidemiologic week, SARI surveillance 2010

Respiratory Morbidity Data Mining Surveillance System

In order to describe the influence of the influenza season on the number of hospitalisations the NICD receives anonymous data from a private hospital group. The number of hospitalisations for pneumonia and influenza are compared during the influenza season. During 2010 there were 1 125 961 consultations reported to the NICD through the respiratory morbidity mining surveillance system. Of these 3% (31 522) were due to pneumonia or influenza (P&I) (ICD codes J10-18). There were 22 208 outpatients and 9 314 inpatients with P&I discharge diagnosis.

An increase in P&I consultations and admissions was observed during the period with a higher number of seasonal influenza virus isolations reported from the VW and SARI surveillance respectively (Figures 5 and 6). A second slightly lower peak is seen preceding the influenza season, this corresponds to the circulation of respiratory syncytial virus.

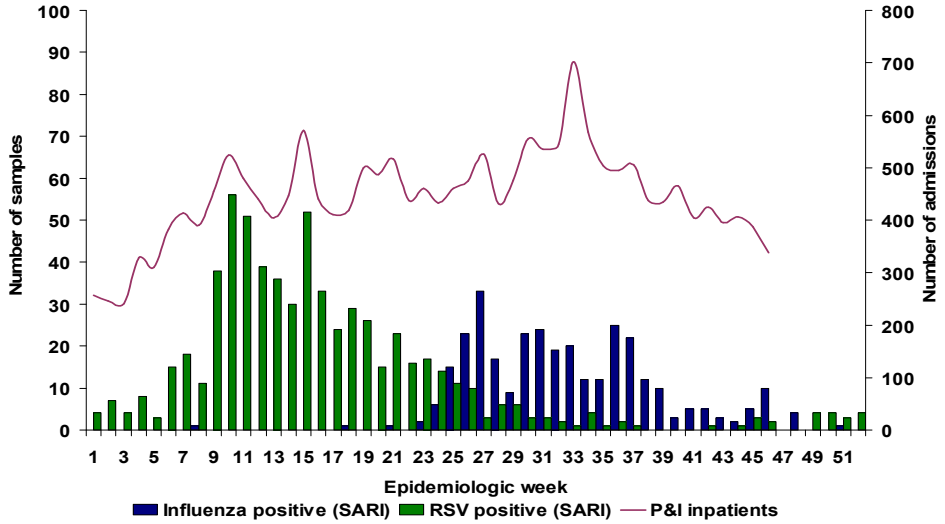


Figure 5: Number of private hospital admissions with a discharge diagnosis of pneumonia and influenza (P&I) and viral isolates (SARI), 2010, South Africa

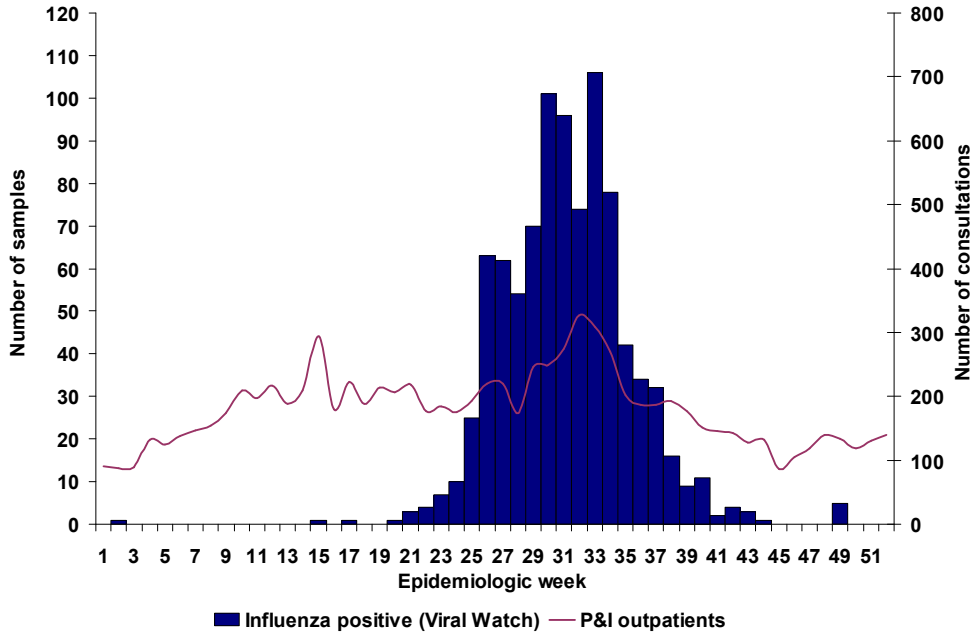


Figure 6: Number of private hospital outpatient consultations with a discharge diagnosis of pneumonia and influenza (P&I) and viral isolates (VW), 2010, South Africa

Influenza and SARI laboratory surveillance in the Respiratory Virus Unit and National Influenza Centre
The Respiratory Virus Unit houses the National Influenza Centre and provides diagnostic testing for respiratory viruses as part of influenza surveillance and is involved in characterizing respiratory viruses for the 3 main active surveillance programmes that are run in collaboration with the Epidemiology Division and Virus Isolation Unit. For the 2010 season the Unit processed, 6915 specimens. A total of 610 influenza A viruses was identified, these were subtyped into 333 A/H3N2 and 277 pandemic H1N1 viruses (Figure 7).

Apart from 2 imported pandemic H1N1 cases in weeks 2 and 14, influenza A cases were only detected from week 23. In contrast to many northern hemisphere countries, pandemic H1N1 cases did not occur during the summer of 2009 or 2010 apart from a few imported cases. Similar to the 2009 influenza season, an apparent bi-phasic curve with H3N2 occurring before pandemic H1N1 was observed in 2010. Sporadic pandemic H1N1 activity was observed until week 52 but was mostly associated with travellers in summer.

Molecular characterization of influenza viruses
Partial sequencing of the HA gene was performed to determine genetic drift over the 2010 influenza season.

A/H3N2
A total of 32 samples was selected for sequencing throughout the season. Specimens grouped with the A/Johannesburg/277/2009 cluster and were more closely related to the A/Perth/16/2009 vaccine strain than to the other 2009 South African cluster which grouped with A/Victoria/502/2009 (Figure 8). SA130506 10G (labelled with a red square) was a vaccine failure in a case from Gauteng Province. P-distance analysis of the amino acid sequence indicated 1.6-2.6% differences between the 2010 strains and the A/Perth/16/2009 vaccine strain. Mutations occurred at positions S45N (new mutation); K62E; K144N; T212A (new mutation); S214I; D53I (new mutation). Only 5 isolates had the ability to agglutinate turkey red blood cells and were antigenically similar to the vaccine strain A/Perth/16/2009 by HAI.

Influenza B
The HA genes of 10 influenza B specimens were sequenced in the 2010 influenza season and included in the phylogenetic analysis. The specimens all grouped with the B/Brisbane/60/2008 vaccine strain (Figure 9). Amino acid p-distance analysis indicated 0-0.6% differences in the South African strains relative to the vaccine strain. Changes were identified in positions S5T, T34P, V87F, I146V, N171S, V225I, N197I, H297Q.

Antigenic analysis of influenza B viruses showed 94 of 116 isolates (81%) were from the B/Victoria-like lineage (B/Brisbane/60/2008-like) and 4 of the 116 (3.5%) were from the B/Yamagata-like lineage (B/Florida/4/2006-like). Eighteen isolates did not yield high enough HA titres to perform the HAI assay. The majority of the B/Brisbane/60/2008-like isolates yielded titres similar to the control antigens, with 2 medium reactors. Of the 4 B/Florida/4/2006-like viruses, 2 low reactors were detected.

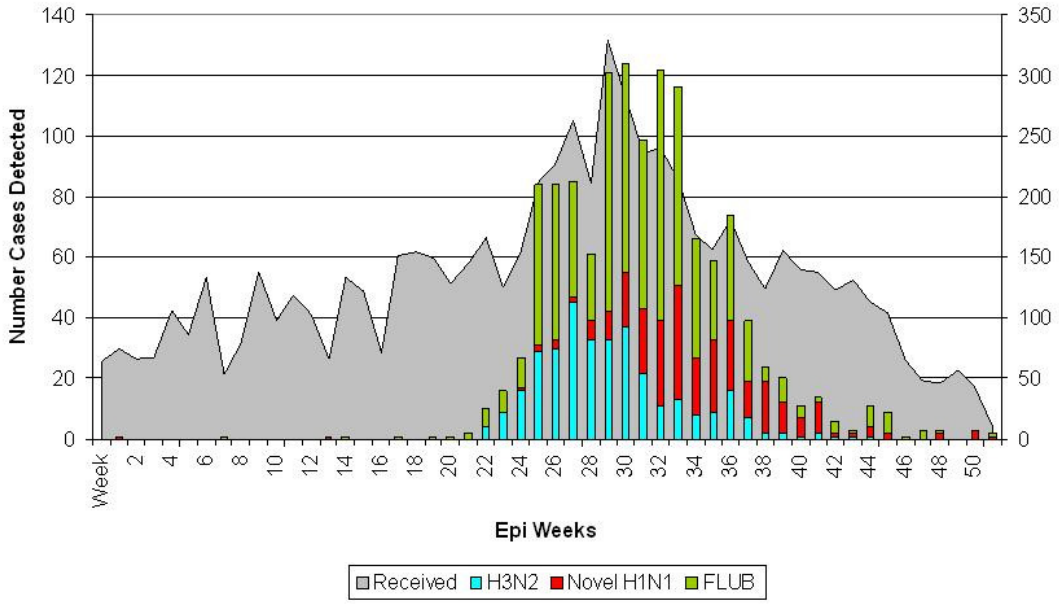


Figure 7: Graph depicting all samples received in the Respiratory Virus Unit and all specimens testing positive for influenza A or B.

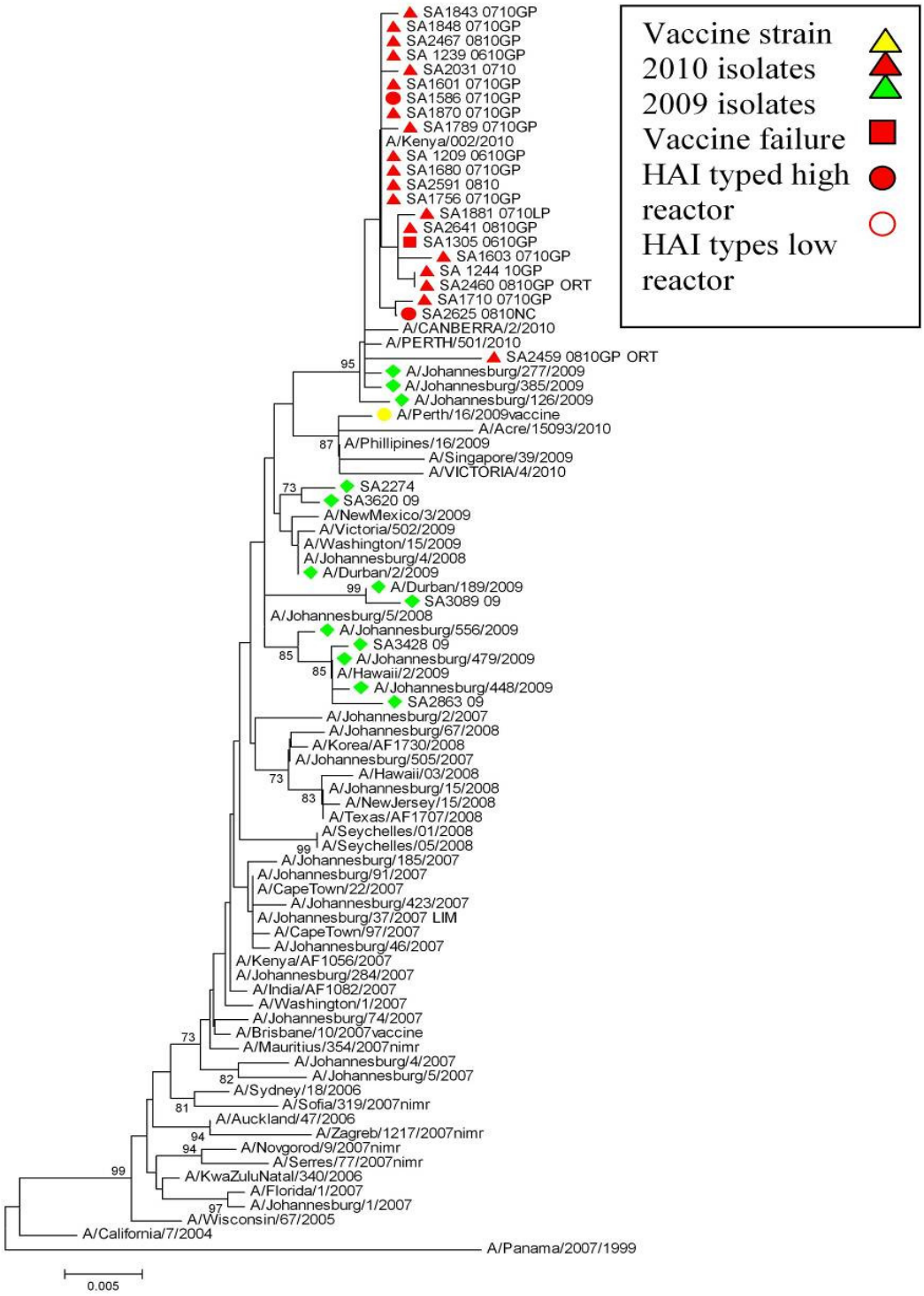


Figure 8: Neighbour-joining tree of the HA gene of influenza A/H3N2 strains. The vaccine strain is indicated in yellow. South African 2009 strains are shown in green while South African 2010 strains are shown in red.

Pandemic H1N1

A total of 42 pandemic H1N1 strains was sequenced throughout the 2010 season and compared to strains from 2009, the vaccine strain and strains identified globally. The 2010 strains grouped separately from the 2009 strains and were further from the root of the tree than the 2009 strains (Figure 10). P-distance analysis indicated 1.2-2.2% differences in the HA protein of 2010 strains relative to the vaccine strain. Three common amino acid changes have been identified in 2009 and 2010 strains; P100S, S220T and !338V. Additional mutations were acquired during the 2010 influenza season, i.e D114N, S202T, E391K and V444I. Antigenic analysis of eighteen pandemic H1N1 isolates showed agglutination with red blood cells ; of these 14 produced HAI results similar to the current vaccine strain (A/California/7/2009). Two medium and 2 low reactors were identified with A/California/7/2009-like antiserum suggesting drift is occurring.

Figure 9: Neighbour-joining tree of the HA gene of influenza B strains. The vaccine strain is indicated in yellow. South African 2009 strains are shown in green while South African 2010 strains are shown in red.

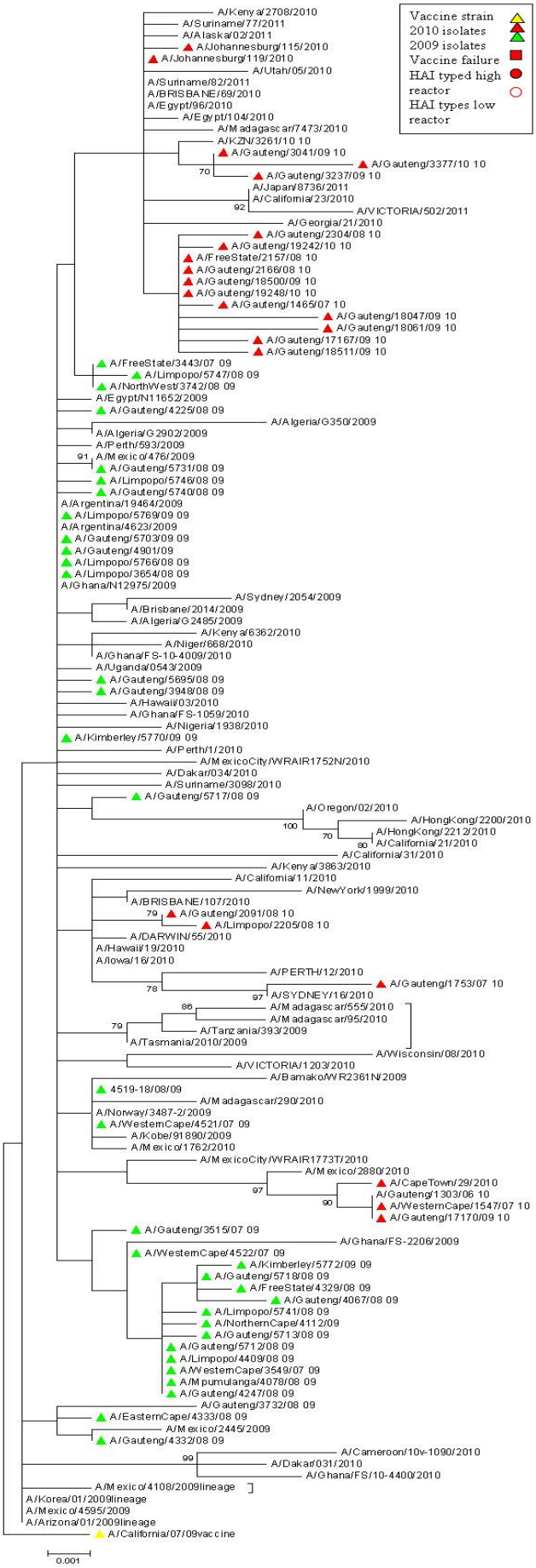


Figure 10: Maximum likelihood tree of the HA genes of the South African 2010 pandemic H1N1 influenza strains (red) relative to 2009 (green) sequences and vaccine strain (yellow).

The Unit has also been actively involved in describing the molecular epidemiology of the influenza A H1N1 pandemic in the country and has presented this data at several international conferences and World Health Organization meetings in 2010. The data were used to help identify the appropriate virus strains to be included in the 2011 southern hemisphere influenza vaccine.

Other causes of SARI

A multiplex assay that detects ten different viruses including influenza A, B, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza virus (PIV) 1, 2, 3, enterovirus (EV), adenovirus (AdV) and rhinovirus (RV) was used to screen 4 526 specimens received through the SARI surveillance from six different government hospitals in Gauteng, Mpumalanga, KwaZulu-Natal and North West Provinces. For 2010 period, RSV preceded the influenza season with most cases of RSV occurring in February to June (Fig 11). Most cases of hMPV occurred in late winter and early spring, while Adv, EV and RV cases were spread thoughout the year (Fig 12). Although RV was detected most frequently, it was also represented in most mixed infections. RV was found together with, in decreasing order, AdV, RSV, EV, PIV-3, hMPV, influenza A/H3N2, influenza B, PIV-1,-2 and pandemic H1N1. For single infections, RV was detected in 36% of cases followed by RSV in 18%, AdV in 11%, EV in 7%, PIV-3 in 6%, influenza A/H3N2 in 6%, pandemic H1N1 in 5 %, and PIV1 and 2 in 1% of cases.

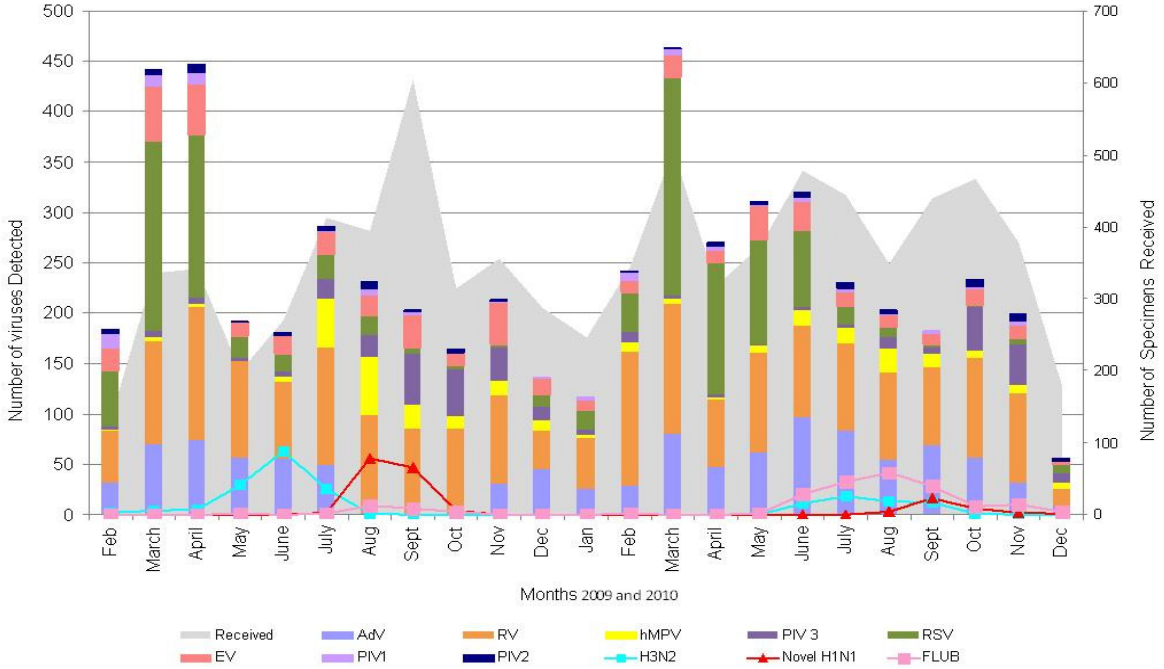


Figure 11: Screening of SARI cases for ten different viruses over 2 years.

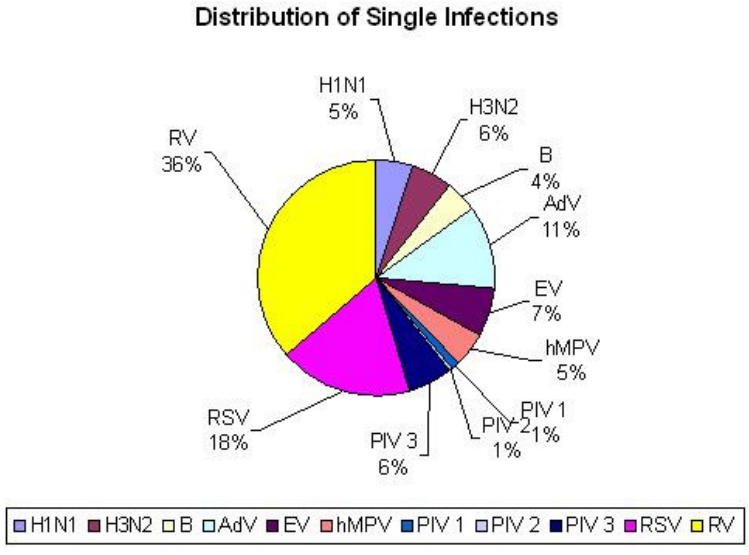


Figure 12: Percentage of specimens where no other virus could be detected

Report compiled by (alphabetical order): Cheryl Cohen, Jo McAnerney, Jocelyn Moyes, Veerle Msimang, Dhamari Naidoo, Marthi Nieuwoudt, Florette Treurnicht, Marietjie Venter, Sibongile Walaza

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- **Patients who kindly agreed to participate in surveillance**

ROTAVIRUS SURVEILLANCE IN SOUTH AFRICA, 2010

Enteric Diseases Reference Unit, Epidemiology and Surveillance Unit, Parasitology Reference Unit and Viral Gastroenteritis Unit, National Institute for Communicable Diseases

Prior to the introduction of the Rotarix® vaccine into the expanded programme of immunisation (EPI) in August of 2009, the NICD started a sentinel surveillance programme for rotavirus in five hospitals. These hospitals include: Chris Hani Baragwanath in Gauteng, Dr. George Mukhari in Gauteng/North West, Mapulaneng and Matikwana in Mpumalanga and Edendale in Kwazulu-Natal Province. The purpose of this surveillance programme is to describe the epidemiology of rotavirus infection and to describe the effect of the introduction of the rotavirus vaccine into the EPI. All children who are admitted to the sentinel hospitals with acute diarrhoea (less than 7 days duration) are enrolled, following informed consent, into the programme. Detailed demographic information, medical histories, clinical presentation data and in-hospital outcomes are recorded for each child. In additional a stool sample is collected for rotavirus (and other diarrhoeal pathogens) testing.

Testing for rotavirus is performed at the Viral Gastroenteritis Unit (VGU), NICD and at the Diarrhoeal Pathogens Research Unit (DPRU), University of Limpopo Medunsa campus using the ProSpecT Rotavirus ELISA kit (Oxoid, UK) to assign stools as rotavirus positive or negative.

A total of 1237 children was enrolled into the surveillance program in 2010. Stool samples were available for 1174 children and 1140 (97% of the samples and 92% of the children enrolled) samples were sufficient for testing.

The total number of rotavirus positive samples for 2010 was 241 (21%). A consistent increase in the detection rate marking the start of the rotavirus season was seen in late May 2010 (week 20) The peak detection rate was 68% (17/25) in the middle of June (week 24). The exact end of the season was hard to define due to the decrease in hospitalisations (all cause) resulting from the national health care workers strike in August/September of 2010. However, by mid-September (week 38) the detection rate for rotavirus had dropped to below 10%.

Comparing the period April to September of 2009 to the same period in 2010 the number of diarrhoea cases and the detection rate of rotavirus was lower for 2010 than in 2009. A total of 705 diarrhoea cases was enrolled in 2009 and 564 for the same period in 2010. This represented a 20% decrease in number of diarrhoea cases. The number of rotavirus positive cases decreased from 395 to 201, a 49% decrease. The rotavirus season started a month later in 2010 compared with 2009. Although this is only one year of data post-introduction of the vaccine; the later start of the season, the decrease in total number of diarrhoea cases and the lower detection rate of rotavirus is consistent with findings in the United States of America and Central America following the introduction of a similar vaccine into these regions.[1-3]

Molecular characterization of rotavirus positive specimens collected from sentinel surveillance sites in South Africa in 2009 and 2010

The naked rotavirus particle has an icosahedral structure composed of three protein layers. These layers consist of the core, inner capsid and outer capsid. The viral genome of 11 distinct segments of double stranded (ds) RNA is enclosed within these protein layers. Rotaviruses are differentiated into groups based on epitopes on the inner capsid protein and seven genogroups (A-G) have been described. Group A rotaviruses are the leading cause of mild to severe gastroenteritis in infants and young children.[1]

Group A rotaviruses are classified according to the smooth outer capsid protein (encoded by the VP7 gene) and short spike protein (encoded by the VP4 gene),

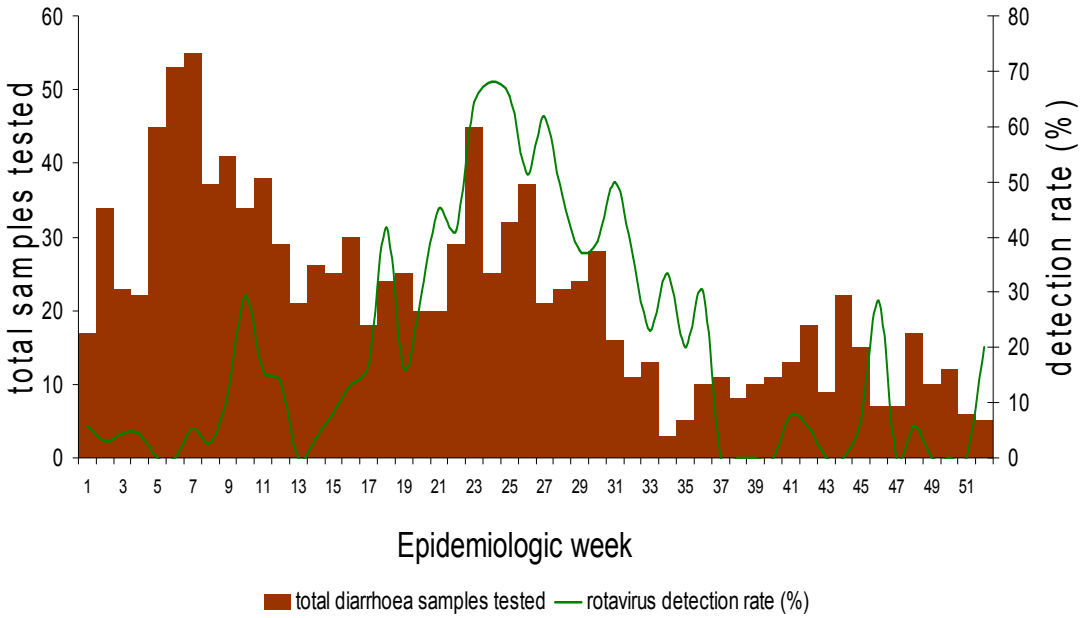

Figure 1: Number of diarrhoea samples tested and rotavirus detection rate (%) by week in children < 5 years of age hospitalised for diarrhoea, South Africa 2010.

specifying G and P types, respectively. These proteins are the major antigens inducing neutralizing immune responses during rotavirus infections. Although 12 different G genotypes and 15 P genotypes[3] have been detected in humans, serotypes G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are thought to be the predominant circulating strains causing diarrhoea in infants and young children worldwide.[3]

Between 20 April 2009 and 31 December 2010, stool specimens were received in the Viral Gastroenteritis Unit (VGU) from sentinel surveillance and case-control study sites including Chris Hani Baragwanath Hospital (CHBH), Mapulaneng Hospital (MP), Matikwana Hospital (MK), Edendale Hospital (EdH), Ngwelezane Hospital (NgH) and Red Cross Children's Hospital (RCCH). Screening for rotavirus antigens was done utilizing the GastroVir Strip (Coris Bioconcept, Belgium) in 2009 and the ProSpecT™ Rotavirus Microplate Assay (Oxoid, UK) in 2010. RT-PCR genotyping was performed on rotavirus positive specimens utilizing standardised methods described in the WHO Manual of Rotavirus Detection and Characterization Methods.[2] The VP7-specific primers used included G1, G2, G4, G3, G8, G9, G10 and G12. The VP4-specific primers utilized included P[4], P[6], P [8], P[9], P[10], P[11] and P[14].

A total of 650 stool specimens was received from CHBH (66%), MP (16%) and MK (17%) sites in 2009. Twelve specimens did not have sufficient clinical material to conduct further analysis. A total of 261 rotavirus-positive specimens were detected using the GastroVir strip and a further 43 were detected by RT-PCR in specimens that gave equivocal results using the dipstick.

A total of 1218 stool specimens was received from all the sites in 2010 and in the following proportions: CHBH (47%), MP (6%), MK (18%), EdH (7%), NgH (8%) and RCCH (13%). Seventy-two specimens did not have sufficient clinical material to conduct further analysis. A total of 262 rotavirus-positive specimens was detected using the ProSpecT assay.

The number of diarrhoeal specimens received in VGU in 2010 almost doubled and this was mainly due to the inclusion of three additional sentinel sites. However, while the number of sites and specimens increased, the number of rotavirus positive specimens decreased from 304 in 2009 to 262 in 2010; a 25% decrease. This may be due to the introduction of the rotavirus vaccine into the national expanded program of immunization (EPI) in August 2009.

The genotyping results show a shift in genotype from G1P[8] and G2P[4] in 2009 to G1P[8], G8P[4] and G12P [8] in 2010 (Tables 1 and 2). At CHBH, G1P[8] strains were predominant in 2009 and continued to circulate in 2010 (Table 1). However, G1P[8] strains have continued to decrease from 57% in between 2005 and 2007[4] to 35% in 2009 and 26% in 2010. The second most common strain in 2009 (G2P[6]) decreased and was replaced by G8P[4] strains in 2010. Conversely to G1P[8] strains, G8P[4] strains have increase in number from low levels between 2005 and 2007[4] to 5% in 2009 and 23% in 2010. While this trend should be monitored, a decrease was noted in both rotavirus strains represented in the vaccine formulation (55% to 38%) and strains not found in the vaccine formulation (40% to 33%) at CHBH.

At the Mpumalanga sites, G1P[8] strains were predominant in 2009 (Table 1), consistent with previous genotyping results from this area in 1998/1999.[5] Serotype G1P[8] strains were replaced by strains not

represented in the vaccine formulation (G8P[4] and G2P [4]) in 2010 (Table 1). At EdH, G2P[4] and G2P[6] strains detected in 2009 were replaced by G12P[8] strains in 2010 (Table 2). A similar trend was noted in the other costal sites (NgH and RCCH) where G12P[8] strains were frequently detected. In addition, very few G1P[8] strains were detected at the coastal sites, which may be partly due to the natural fluctuations of rotavirus strain circulation and partly due to vaccine introduction.

Continuous monitoring of the circulating rotavirus strains will be required to assess the impact of vaccination with a monovalent vaccine on rotavirus serotype epidemiology and the emergence of any strains in response to the vaccine pressure.

Table 1: Summary of the rotavirus genotyping results for Chris Hani Baragwanath (CHBH), Mapulaneng and Matikwana Hospitals in 2009 and 2010.

	CHBH				Mapulaneng				Matikwana			
	2009		2010		2009		2010		2009		2010	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Rotavirus strains with VP7 or VP4 genotypes in the monovalent vaccine												
G1P[8]	69	35	31	26	20	39	1	11	39	70	6	17
G1P[6]	12	6	0	0	3	6	0	0	3	5	0	0
G1P[4]	6	3	1	1	1	2	1	11	3	5	0	0
G2P[8]	3	2	2	2	0	0	0	0	0	0	0	0
G8P[8]	0	0	4	3	3	6	0	0	1	2	1	3
G12P[8]	15	8	8	7	4	8	0	0	2	4	0	0
G9P[8]	5	3	0	0	6	12	0	0	1	2	4	6
Total	**110**	**55**	**46**	**38**	**37**	**73**	**2**	**22**	**49**	**88**	**11**	**31**
Rotavirus strains without VP7 or VP4 genotypes in the monovalent vaccine												
G2P[4]	24	12	6	5	6	12	1	11	4	7	8	22
G2P[6]	40	20	3	3	4	8	1	11	1	2	0	0
G8P[4]	10	5	27	23	3	6	3	33	0	0	6	17
G8P[6]	2	1	0	0	0	0	0	0	0	0	0	0
G9P[4]	0	0	1	1	0	0	0	0	0	0	0	0
G9P[6]	0	0	0	0	1	2	0	0	0	0	0	0
G12P[4]	2	1	2	2	0	0	0	0	1	2	0	0
G12P[6]	1	<1	0	0	0	0	0	0	0	0	0	0
Total	**79**	**40**	**39**	**33**	**14**	**27**	**5**	**56**	**6**	**11**	**14**	**39**
Mixed or not typed rotavirus strains												
Mixed	6	3	7	6	0	0	0	0	0	0	1	3
G1P?	1	<1	0	0	0	0	0	0	0	0	0	0
G2P?	0	0	3	3	0	0	1	11	0	0	2	6
G8P?	0	0	10	8	0	0	0	0	0	0	0	0
G9P?	0	0	0	0	0	0	0	0	0	0	4	11
G12P?	1	<1	1	1	0	0	0	0	0	0	0	0
G?P[4]	0	0	0	0	0	0	0	0	1	2	2	6
G?P[8]	0	0	1	1	0	0	0	0	0	0	0	0
ND	3	2	13	11	0	0	1	11	0	0	2	6
Total	**11**	**6**	**35**	**29**	**0**	**0**	**2**	**22**	**1**	**2**	**11**	**31**
Grand total	**200**		**120**		**51**		**9**		**56**		**36**	

Table 2: Summary of the rotavirus genotyping results for Edendale Hospital in 2009 and 2010 and for Ngwelezane and Red Cross Children's Hospitals in 2010.

	Edendale				Ngwelazane		Red Cross	
	2009		2010		2010		2010	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Rotavirus strains with VP7 or VP4 genotypes in the monovalent vaccine								
G1P[8]	1	3	1	6	0	0	0	0
G9P[8]	0	0	0	0	0	0	1	2
G12P[8]	0	0	6	38	6	29	19	32
Total	**1**	**3**	**7**	**44**	**6**	**29**	**20**	**33**
Rotavirus strains without VP7 or VP4 genotypes in the monovalent vaccine								
G2P[4]	4	11	2	13	1	5	8	13
G2P[6]	6	16	0	0	0	0	0	0
G8P[4]	0	0	2	13	1	5	1	2
G12P[4]	0	0	0	0	0	0	1	2
Total	**10**	**26**	**4**	**25**	**2**	**10**	**10**	**17**
Mixed or not typed rotavirus strains								
Mixed	0	0	1	6	0	0	1	2
G1P?	1	3	0	0	1	5	0	0
G8P?	0	0	3	19	0	0	1	2
G12P?	1	3	0	0	4	19	3	5
G?P[4]	1	3	0	0	0	0	0	0
G?P[6]	4	11	0	0	0	0	0	0
G?P[8]	1	3	0	0	1	5	0	0
ND	17	45	1	6	7	33	25	42
Total	**27**	**71**	**5**	**31**	**13**	**62**	**30**	**50**
Grand total	**38**		**16**		**21**		**60**	

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OUTBREAK OF RABIES IN GAUTENG PROVINCE, SOUTH AFRICA, 2010

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Introduction

Introduction events of canine rabies in South Africa date back to the 1800s. Nevertheless during this time there was no reported evidence of the establishment of sustained transmission cycles. This situation changed in 1976 when canine rabies was introduced into the eastern coastal province of KwaZulu-Natal from Mozambique, and subsequently spread extensively and became endemic to the coastal provinces of KwaZulu-Natal and Eastern Cape Province. In the past decade, outbreaks of rabies in domestic dogs have also been recognized from the Free State Province since 2000, Mpumalanga Province since 2008 and in Limpopo Province since 2005. Transmission cycles of canid rabies biotype virus are also reported in black-backed jackal from the northern regions of the country and bat-eared fox from the south-west. In addition, a second variant of rabies virus, commonly referred to as mongoose rabies biotype, is regularly reported from the yellow mongoose (*Cynictis penicillata)* and other herpestids from the central plateau of the RSA, but primarily the Free State Province.

Molecular clock analysis of mongoose rabies virus isolates indicated that it was probably introduced to southern Africa more than 200 years ago.[1] Although several wildlife species also support cycles of rabies virus in Southern Africa, the domestic dog proves to be the most important source of exposure to humans.[2] Annually between 6 and 31 cases have been confirmed in South Africa since 1983, but the public health burden of rabies is believed to be much greater. A total of 11 human rabies cases was confirmed in South Africa during 2010, compared to 15 in the previous year and 17 cases in 2008. These cases were reported from the Northern Cape (n=1); Mpumalanga (n=1); Gauteng (n=1); KwaZulu-Natal (n=3), Eastern Cape (n=2) and Limpopo Provinces (n=3).

The first report of locally-transmitted rabies in Johannesburg

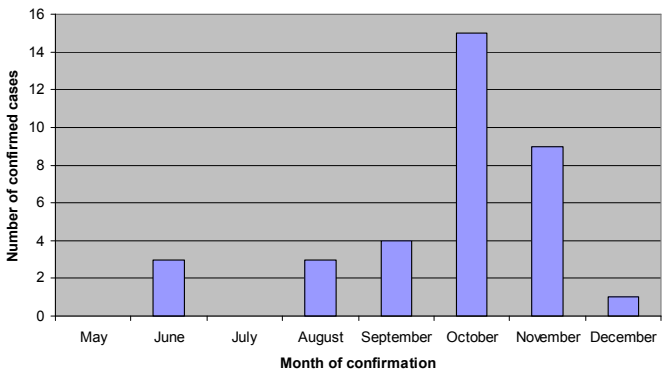
Rabies cases have been intermittently reported from the Gauteng Province, particularly on the outlying provincial borders which constitute mainly farmland and rural areas. These cases have been mostly involving mongoose, jackal and other wildlife animal species. Cases of rabid dogs have also been reported occasionally, mostly linked to the translocation of pets.

In June 2010, three cases of rabies in domestic dogs were confirmed by the Agriculture Research Council-Onderstepoort Veterinary Institute (ARC-OVI). These cases were not epidemiologically linked and occurred in kept pets in the greater Johannesburg area. In the following months several additional cases were reported with the peak of the outbreak in October 2010 (Figure 1). The cases were reported from south-western Johannesburg with a hotspot of activity in Soweto. A total of 37 cases was confirmed over the six-month period with the last laboratory-confirmed case occurring in December 2010. Outbreak response included ring vaccination around the location of confirmed cases, but also centralized campaigns where pet owners could bring their dogs and cats to be vaccinated. In addition rabies vaccination was offered free of charge at private veterinary clinics (Directorate of Veterinary Services, Department of Agriculture, Forestry and Fisheries). A large-scale health education campaign was also executed.

Molecular characterization of outbreak isolates indicated their very close genetic relationships, which suggest that the outbreak resulted from a single introduction of the virus into Gauteng Province from KwaZulu-Natal (Figure 2).[3] This is the first reported outbreak of rabies in domestic dogs in Johannesburg with evidence of local transmission.

In October 2010, rabies was confirmed in a 3-year-old child from Soweto. The child contracted the disease after being scratched by a pet puppy and unfortunately, most likely due to the seemingly benign nature of the exposure, did not receive rabies post-exposure prophylaxis. This case represents the first report of human rabies contracted in Johannesburg.

a)



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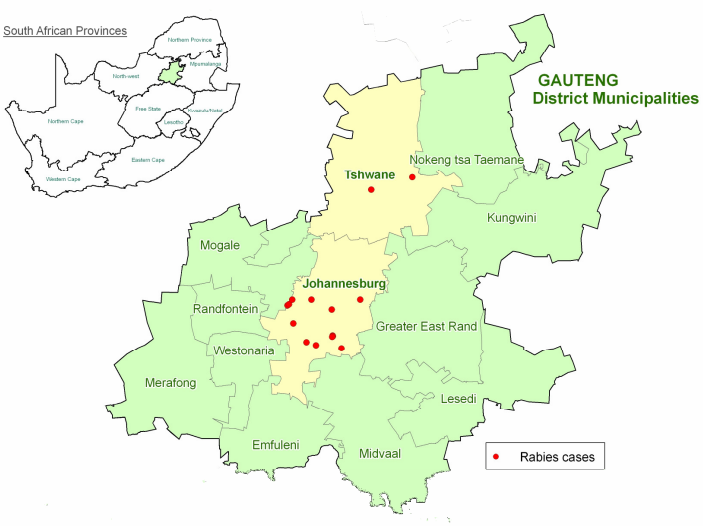

Figure 1: (a) Rabies cases confirmed in animals in Gauteng from May to December 2010 (Data source: Rabies Unit, ARC-OVI), (b) map of Gauteng depicting the distribution of cases confirmed during the outbreak period (map courtesy of Rabies Unit, ARC-OVI).

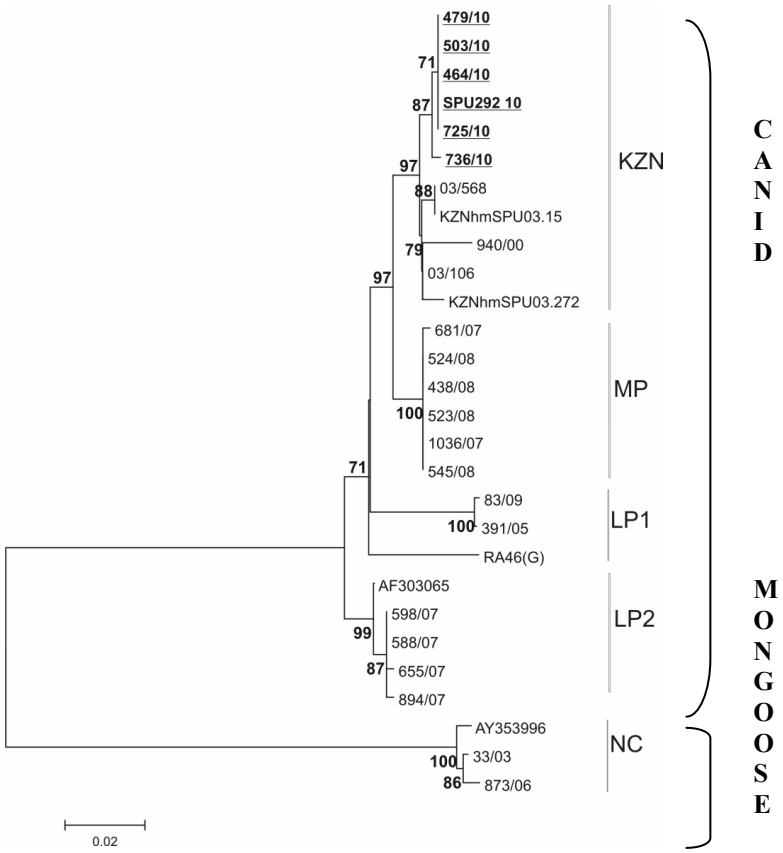

Figure 2: Neighbour-joining tree showing genetic relationships amongst rabies virus isolates recovered from dogs in Gauteng Province in 2000-2010, from a 3-year-old child (SPU 272/10) and previously characterised isolates from South Africa. Selected 2010 outbreak isolates are underlined and in bold. The other outbreak isolates were removed from the analysis as they had 100% sequence identity. Only bootstrap support values ≥70% are shown on the nodes.[3] The scale bar depicts nucleotide substitutions per site. LP = Limpopo Province, NC = Northern Cape Province and MP = Mpumalanga Province.

Report compiled by (alphabetical order): Lucille Blumberg, Pat Leman, Janusz Paweska, Jacqueline Weyer

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VIRAL HAEMORRHAGIC FEVER OUTBREAKS, SOUTH AFRICA, 2010

Special Pathogens Unit and Outbreak Response Unit, National Institute for Communicable Diseases

Introduction

The viral haemorrhagic fevers are characterized by high case-mortality rates of up to 90% and although considered rare they have the potential to cause explosive outbreaks, especially in nosocomial settings. The African viral haemorrhagic fevers include Ebola, Marburg, Crimean-Congo haemorrhagic fever (CCHF), Rift Valley fever (RVF), hantavirus infection with renal syndrome, Lassa fever, and related arenaviral infections. Of these, CCHF and RVF are endemic to South Africa. Here we report on the occurrence of laboratory-confirmed cases of VHF in South Africa during 2010.

Crimean-Congo hemorrhagic fever

A total of five CCHF cases was laboratory confirmed in South Africa during 2010, compared to only three in 2009 and 11 cases in 2008. The cases were all males aged between 23 and 67 years, three from the Free State and two from Northern Cape Province. In addition two cases were confirmed from Namibia. Of the seven confirmed cases one patient died. Four of the seven cases had definitive tick exposures before onset of illness.

Human cases of CCHF have been reported annually from South Africa since 1981. Through nearly thirty years of passive surveillance a total of 182 cases have been laboratory confirmed from all nine provinces of South Africa. The majority of cases reported tick bites or squashing of ticks (n=84, 45%), whilst contact with infected blood or tissues was the second most important source of exposure (n=66, 36%). A strong link with occupational exposure is clearly noted; the majority of patients were employed in the livestock industry (e.g. farmers, farm workers, slaughtermen) and were male (n=151, 83%).

Rift Valley fever

Small, focal outbreaks of RVF were reported in South Africa in 2008 and 2009. In 2008 these outbreaks were reported from Mpumalanga, Limpopo, Gauteng and North West Provinces with a total of 18 confirmed human cases. In 2009 the outbreaks extended to the KwaZulu-Natal and Northern Cape Provinces with a total of 7 confirmed cases. The last reported cases of human RVF in South Africa prior to the 2008 outbreak were more than 30 years ago in the 1970s.[1] In 2010 a total of 242 human cases of RVF was laboratory confirmed in South Africa with 26 deaths.[2] The first cases were reported in mid-February 2010 from the Free State Province. The peak of the outbreak appeared to have occurred in April 2010 with the last case diagnosed in September 2010. The most afflicted regions included the Free State and Northern Cape Provinces, but cases were also reported from the Eastern Cape, North West and Western Cape. A total of 13 902 animal cases was confirmed during the same period with 8 581 deaths (Data source: Directorate of Veterinary Services, Department of Agriculture, Forestry and Fisheries). A strong occupational exposure link could be established during the outbreak with 93% of the confirmed cases reporting direct contact with infected ruminants or their tissues. Cases included farmers, farmworkers, veterinarians, veterinary technologists, slaughtermen and abbatoir workers.

Laboratory investigation included the use of nucleic acid detection (Taqman based, real time PCR and loop-mediated isothermal amplification, or LAMP-based assays),[3] virus isolation in suckling mice and/or cell culture or serological screening (haemagglutination and IgM ELISA).[4] A total of 1588 serum specimens from suspected human cases was tested during the period of

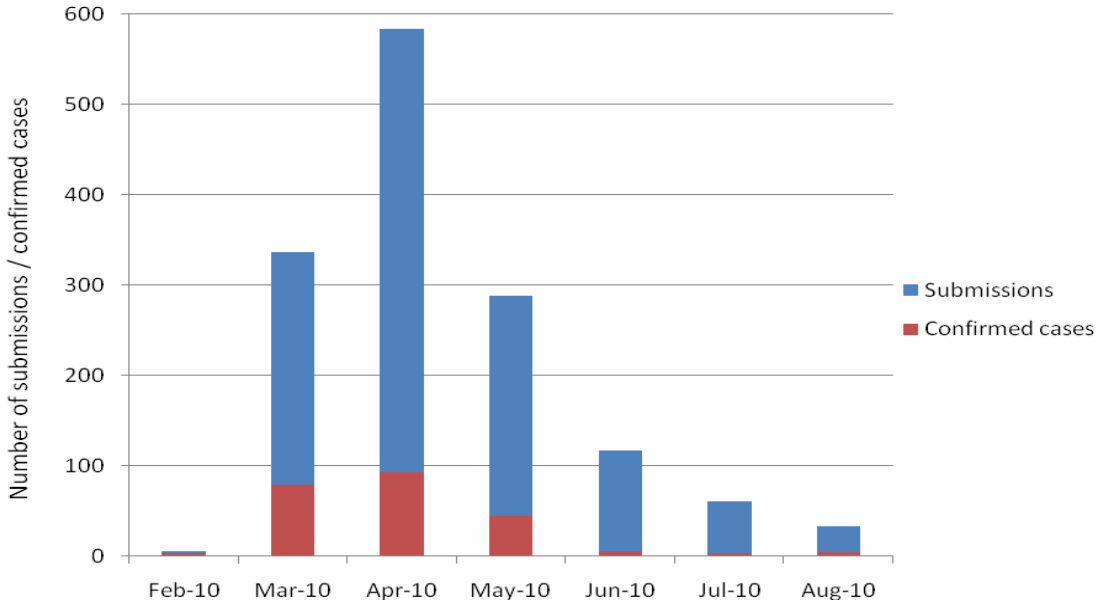

Figure 1: Number of submissions and laboratory-confirmed human cases during the RVF outbreak in South Africa up to September.

February to September 2010, with most specimens submitted and cases diagnosed in April (Figures 1, 2). Of the total number of cases confirmed, 90 were diagnosed by detection of IgM alone, 14 by PCR alone, 117 by PCR and virus isolation, and the remainder by a combination of these three methods (Table 1). One has to emphasize, however, that because of very short duration of viraemia in RVFV-infected individuals and the fact that most infected patients undergo subclinical or mild infections, definitive diagnosis of RVF should not only rely on a single PCR result. Follow-up specimens from suspected RVF cases should be tested if possible and nucleic acid detection assays run in parallel with additional tests. Data given in Table 2 clearly demonstrate that the laboratory results in RVF patients by various assays are influenced by the time when specimens are collected after the onset of illness. However, not only the time at which a specimen is collected after infection might hamper diagnosis of RVF but also the presence in blood or inhibitory factors, which might obscure detection of one or more of the targeted analytes (e.g. antibody, antigen, nucleic acid). This again highlights the need for the use of multiple assays for accurate diagnosis of RVF.

In some cases there was evidence of recent infection with other arboviruses endemic to the country. Whereas the majority of the anti-WN virus antibody-positive submissions in 2010 were clearly historical, based on the absence of specific IgM antibodies, 193 (28.4% of the total number of serology positive specimens) were IgM-positive. Four WN virus isolates were identified, all belonging to the so-called WN lineage II clade. Sindbis virus was not isolated this year but 207 cases (29.8% of the total number of serology- positive specimens) were IgM-positive.

A total of 47 isolates of RVFV from 5 provinces affected by the 2010 outbreak (including Western Cape, Free State, Northern Cape, North West, Eastern Cape) was partially sequenced. Phylogenetic analysis of these isolates indicated less than 1% difference between isolates at the nucleic acid level. They are closely related to a 2009 Northern Cape isolate and ancestral to a 2004 Namibian isolate. The 2010 isolates are distant from 2009 KwaZulu-Natal and 2008 isolates from Mpumalanga, Gauteng, Limpopo and the North West Provinces. One of the 2010 isolate is phylogenetically distant from the remaining isolates and additional sequencing confirmed it to be a reassortant RVF virus.

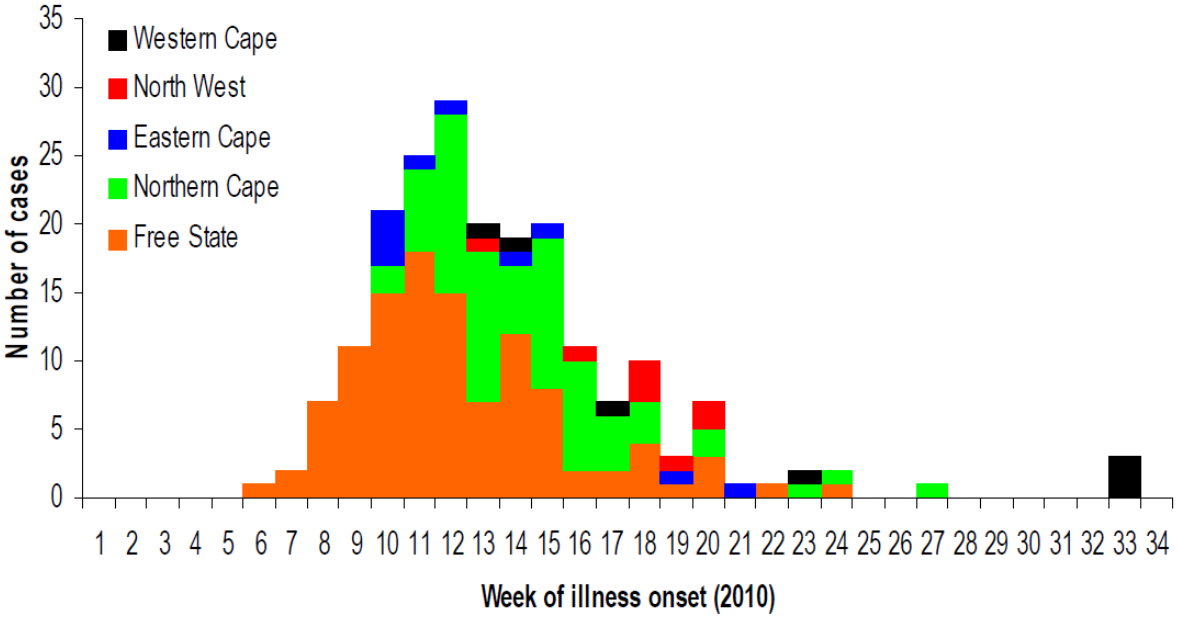

Figure 2: Number of laboratory-confirmed human RVF cases by week of illness onset and province, 2010, South Africa. Data based on the subset of patients for whom date of onset could be determined (n = 237).

Table 1: Laboratory confirmation of RVF human cases by different methods, South Africa, 2010.

Assay	PCR only	PCR + Isolation	PCR + IgM	PCR + Isolation + IgM	Isolation + IgM	IgM only
Number of confirmed cases	14	117	3	15	3	90

Table 2: Comparison of results in RVF patients subjected for follow up laboratory testing.

Case	Date specimen taken	PCR	Virus isolation	ELISA IgM
Case 1	24.03.2010	Positive	Yes	Positive
	13.04.2010	Negative	No	Positive
Case 2	25.03.2010	Positive	Yes	Negative
	10.04.2010	Negative	No	Positive
Case 3	29.03.2010	Positive	Yes	Negative
	07.05.2010	Negative	No	Positive
Case 4	03.04.2010	Positive	Yes	Negative
	06.04.2010	Negative	No	Positive
Case 5	07.04.2010	Positive	Yes	Negative
	19.04.2010	Negative	No	Positive
Case 6	15.04.2010	Positive	Yes	Negative
	20.04.2010	Negative	Yes	Positive
Case 7	06.10.2010	Negative	No	Positive
	22.10.2010	Negative	No	Positive

This report compiled by (alphabetical order): Brett Archer, Lucille Blumberg, Ayanda Cengimbo, Antoinette Grobbelaar, Petrus Jansen van Vuren, Alan Kemp, Pat Leman, Chantel le Roux, Janusz Paweska, Jacqueline Weyer

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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 2009/2010*

Disease/Organism	Cumulative to 31 December, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Botulism	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2009	1271	482	2125	1455	678	823	79	730	528	8171
	2010	1213	451	2097	1059	522	752	65	614	491	7264
Haemophilus influenzae, invasive disease, all serotypes	2009	39	22	159	42	4	27	9	11	72	385
	2010	45	25	167	21	10	16	11	8	92	395
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2009	8	8	15	16	1	2	4	2	18	74
	2010	6	8	27	8	3	8	5	2	15	82
Serotypes a,c,d,e,f	2009	0	1	18	2	0	3	0	1	6	31
	2010	0	2	9	0	1	2	0	0	10	24
Non-typeable (unencapsulated)	2009	1	1	31	12	1	2	1	0	11	60
	2010	1	1	46	4	0	0	1	1	11	65
No isolate available for serotyping	2009	8	4	25	3	1	6	2	4	1	54
	2010	13	4	17	0	2	4	0	1	6	47
Measles	2009	80	165	4114	423	220	131	65	453	209	5860
	2010	1309	674	1617	3837	290	1844	374	758	1796	12499
Neisseria meningitidis, invasive disease	2009	36	18	203	32	3	67	9	19	75	462
	2010	31	26	187	22	13	28	20	11	67	405
Novel influenza A virus infections***	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Plague	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Rabies	2009	8	0	0	5	2	2	0	0	0	17
	2010	2	0	1	3	3	1	1	0	0	11
**Rubella	2009	276	74	681	551	402	401	168	298	130	2981
	2010	436	116	389	366	122	188	70	287	361	2335
Salmonella spp. (not Typhi), invasive disease	2009	39	27	312	92	5	32	12	24	71	614
	2010	35	19	312	69	12	16	13	9	64	549
Salmonella spp. (not Typhi), isolate from non-sterile site	2009	156	38	726	156	5	118	18	52	215	1484
	2010	163	42	634	175	14	90	8	30	146	1302
Salmonella Typhi	2009	12	3	26	4	0	8	1	1	11	66
	2010	11	2	29	9	1	10	0	0	13	75
Shigella dysenteriae 1	2009	0	0	1	0	0	1	0	0	0	2
	2010	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non Sd1)	2009	250	73	606	155	8	71	16	19	414	1612
	2010	282	57	720	144	18	56	36	45	445	1803
Streptococcus pneumoniae, invasive disease, all ages	2009	362	308	2259	529	111	302	88	175	639	4773
	2010	388	318	1847	426	110	240	106	183	587	4205
Streptococcus pneumoniae, invasive disease, < 5 years	2009	97	67	629	166	18	88	46	27	203	1341
	2010	73	51	408	111	16	42	38	33	135	907
Vibrio cholerae O1	2009	0	0	37	0	449	61	0	19	4	570
	2010	0	0	1	0	0	0	0	0	0	1
Viral haemorrhagic fever (VHF)											
Crimean-Congo haemorrhagic fever (CCHF)	2009	0	1	0	0	0	0	1	0	1	3
	2010	0	3	0	0	0	0	2	0	0	5
Other VHF (not CCHF)****	2009	0	0	0	5	0	0	2	0	0	7
	2010	17	124	0	0	0	0	81	9	11	242

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.

*** Confirmed cases. Excludes pandemic influenza H1N1. See weekly influenza reports on www.nicd.ac.za.

**** All Rift Valley fever . For 2010 the total includes 1 case from an unknown province.
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

U =unavailable, 0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 31 December 2009/2010*

Programme and Indicator	Cumulative to 31 December, 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	2009	50	13	66	104	68	35	8	24	20	388
	2010	51	19	67	76	45	33	2	19	23	334
Laboratory Programme for the Comprehensive Care, Treatment and Management Programme for HIV and AIDS											
CD4 count tests											
Total CD4 count tests submitted	2009	388,048	216,936	764,365	955,872	257,526	302,602	62,182	251,613	237,172	3,436,316
	2010	369,377	133,818	643,620	837,362	217,570	241,241	52,587	230,147	216,261	2,941,983
Tests with CD4 count < 200/µl	2009	120,663	64,251	249,617	219,541	82,576	90,599	17,652	72,231	58,570	975,700
	2010	116,665	40,588	214,244	248,709	67,841	73,633	16,970	69,788	57,017	905,455
Viral load tests											
Total viral load tests submitted	2009	137,512	75,297	336,132	334,584	82,725	92,194	25,578	100,567	106,189	1,290,778
	2010	138,683	44,374	288,093	344,257	86,597	83,422	20,866	90,854	83,798	1,180,944
Tests with undetectable viral load	2009	84,451	51,867	230,137	246,440	57,170	69,479	15,749	64,675	79,537	899,505
	2010	84,752	33,114	198,115	245,034	55,269	56,287	13,211	63,587	68,551	817,920
Diagnostic HIV-1 PCR tests											
Total diagnostic HIV-1 PCR tests submitted	2009	29,960	14,654	63,348	78,095	21,385	23,599	4,752	18,770	17,638	272,201
	2010	28,649	10,564	60,965	74,438	16,468	17,508	3,480	16,748	16,834	245,654
Diagnostic HIV-1 PCR tests positive for HIV	2009	2,303	1,249	5,589	5,867	2,001	2,072	389	1,649	1,034	22,153
	2010	2,885	1,260	6,533	7,190	2,095	2,164	401	1,970	1,350	25,848

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.

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

U = unavailable, 0 = no cases reported

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