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FOREWORD

Plague is currently quiescent in South Africa, but recent outbreaks in other countries serve as a warning that activity can resume unexpectedly, making ongoing surveillance of rodent and vector populations in historically plague-endemic areas important. Plague surveillance in South Africa over the last decade is described in this issue, which also includes a report of a new surveillance programme for additional respiratory pathogens. Pathogens under surveillance in this new programme include *Haemophilus influenzae*, *Streptococcus pneumoniae, Bordetella pertussis, Mycoplasma pneumoniae*, *Chlamydophila (Chlamydia) pneumoniae*, *Legionella* species and *Pneumocystis jirovecii*. It is envisaged that this programme will enable descriptions of how co-infections with these pathogens relate to patient outcome.

Also in this issue is the very topical problem of estimating HIV incidence in South Africa. Laboratory methods and post-test algorithms are discussed in the context of South Africa's HIV epidemic. Equally pertinent to the epidemic is the severity of transmitted HIV-1 drug resistance among individuals assumed to be recently infected, such as pregnant women. Data presented in this issue suggest that transmission of drug resistant HIV viruses is occurring in a number of provinces in South Africa.

This is the final issue for 2013 and we wish all our readers and contributors a safe, healthy and joyous holiday season.

Basil Brooke, Editor

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A DECADE OF PLAGUE SURVEILLANCE IN SOUTH AFRICA, 2002-2012

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Introduction

Plague is a potential major public health problem and is subject to the International Health Regulations. Outbreaks involving humans are always notifiable to the World Health Organization. Plague is a zoonotic disease caused by the bacterium *Yersinia pestis.* It is considered to be one of the most pathogenic bacteria to humans due to its rapid progression and high fatality rate (bubonic plague 40%–70%; pneumonic 100%) if not treated timeously.[1,2]

Wild rodents are considered to be the primary natural reservoirs for *Y. pestis* and wild plague exists independent of human populations in natural foci. Transmission of plague from animal to human is usually via the bite of an infected flea (70 - 80% of cases) or handling of an infected animal (20% of cases). Pneumonic plague can be transmitted from human to human at the end-stage of disease by means of aerosols, when an infected person may cough copious amounts of bloody sputum.

Plague is considered a re-emerging disease with 1,000 to 5,000 human cases resulting in 100 to 200 deaths reported to the World Health Organization (WHO) each year. More than 90% of cases occur in Africa.[3]

Plague was first introduced into South Africa during the late 1890s on trade ships visiting its harbours with 2568 cases and 1505 deaths reported between 1899 and 1926.[4] In 1921, plague became notifiable to the National Department of Health (NDoH) and remains a current threat due to the existence of susceptible wild rodent foci in several parts of South Africa.

The last reported outbreak of plague in South Africa occurred in 1982 in the Coega area, Eastern Cape Province (EC), after a dormant period of 10 years.[5] Epizootics should be identified as quickly as possible so that steps can be taken to control disease spread. Plague surveillance is being carried out in this and other natural plague foci by monitoring the susceptible rodent populations in order to alert public health authorities to increased human plague risks.

Materials and methods

Plague surveillance in susceptible rodent populations commenced in July 2002. Surveillance sites were established in the Coega area in the Nelson Mandela Bay Metropolitan (NMBM) (2002 to present), eThekwini Municipality (2003-2010) and the City of Johannesburg (2010 to present). Environmental health officers collected various samples including serum, liver, spleen, heart and lung, and ectoparasites from live-caught rodents. For the period July 2002 to December 2012, 6474 rodent samples were submitted to the Special Bacterial Pathogens Reference Laboratory of the Centre for Emerging and Zoonotic Diseases, National Institute for Communicable Diseases (NICD), for testing (table). Serum samples were tested using a competitive-blocking enzyme-linked immunoassay (EIA) for the detection of *Y. pestis* anti-F1 antibodies.[6] Genotypic species identification of a plague-positive rodent was done by 18S ribosomal DNA sequencing.

Table. Rodent samples received and tested by year for plague surveillance.

Year	Received	Tested
2002	164	160
2003	563	537
2004	414	377
2005	265	204
2006	564	357
2007	827	652
2008	1183	975
2009	761	621
2010	631	560
2011	730	646
2012	327	252
2013	45	31
Total	6474	5372

Results

Over a 10-year period, a total of 5372 rodents with sufficient serum sample volumes was tested, with only one rodent testing positive for plague anti-F1 antibodies. The plague-seropositive rodent was subsequently identified as a southern African vleirat (*Otomys irroratus*) that was trapped in August 2010 in an industrial area close to Motherwell in the Coega area (figure 1). Public health authorities were alerted to increased human plague risks and appropriate steps were taken to prevent disease spread to humans. The NMBM Environmental Health Services conducted flea control that was followed by an intensified rodent control program. An investigation of the area revealed that the rodent trapping site was in relatively close proximity (approximately 1 km) to the 1982 outbreak site (figure 2). In an effort to increase plague awareness in the area, a workshop on plague surveillance and control was conducted by the NICD in collaboration with the NDoH.

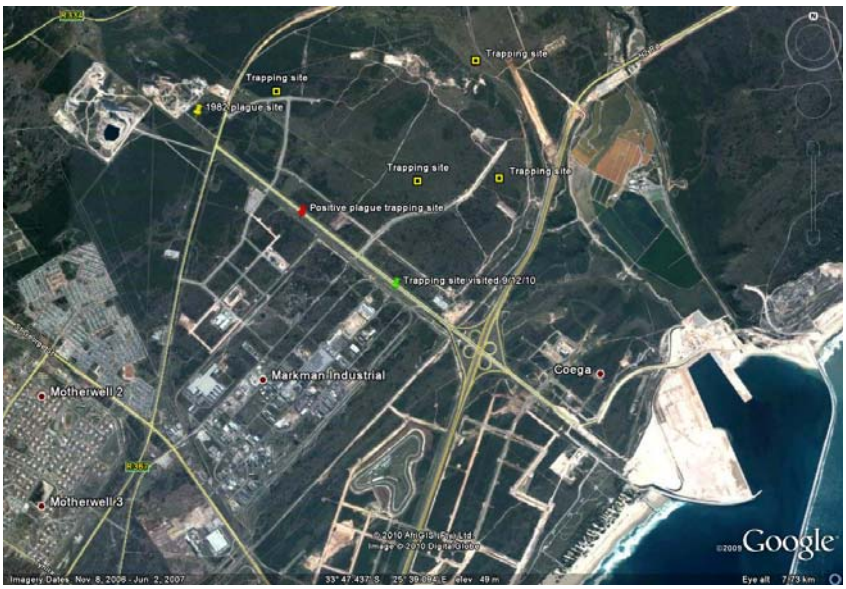

Figure 1: Satellite image of Coega area, Nelson Mandela Bay Metropolitan Municipality, showing rodent trapping sites. Note the site of the 1982 outbreak (upper left quadrant).

Figure 2: Site of 1982 plague outbreak,Coega area, now abandoned. Note environmental disruption from industrial activity in the background, a potential risk factor for plague transmission.

Discussion and conclusion

Even though plague is currently in a quiescent phase in South Africa, experience from outbreaks in other countries (India, Algeria, Libya, Tanzania and Uganda) has shown that plague activity can resume unexpectedly after decades of quiescence. Continued surveillance of rodent and vector populations in historically plague-endemic areas such as Coega is therefore of paramount importance even during periods when no human cases are reported.

Dogs are good sentinel animals for predicting plague outbreaks because they catch rodents and get infected, but generally seroconvert without becoming ill. However, efforts to recruit dog blood samples from the Coega area have been unsuccessful to date. Introducing this strategy may be a future priority if plague activity increases.

Acknowledgements

The authors thank the technical staff of the Centre for Emerging and Zoonotic Diseases, Special Bacterial Pathogens Reference Laboratory and the environmental health officers and officials from participating surveillance sites for their contributions.

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ENHANCED SURVEILLANCE FOR ADDITIONAL RESPIRATORY PATHOGENS, 2012-2013

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Introduction

The National Institute for Communicable Diseases (NICD) has been conducting active, prospective, hospital-based sentinel surveillance for severe acute respiratory illness (SARI) since February 2009 in three of South Africa's provinces (Chris Hani-Baragwanath Hospital (CHBH), Gauteng Province; Edendale Hospital, KwaZulu-Natal Province and Matikwana and Mapulaneng Hospitals, Mpumalanga Province. Patients were enrolled based on a standardised clinical case definition. The programme initially focused on the detection of influenza, but included testing for other respiratory viruses and *Streptococcus pneumoniae*. In June 2010, Klerksdorp-Tshepong Hospital Complex (KTHC), North West Province, was included as a new site and the case definition was expanded to include

cases with a more chronic presentation of severe respiratory illness (SRI), and patients with a clinician admission diagnosis of suspected tuberculosis (TB). In 2012, the surveillance was further enhanced at two sites (Edendale and KTHC) to include expanded testing of specimens (naso- and oropharyngeal swabs and aspirates) and collection of additional specimens (induced sputum and oral washes) from patients with SRI. Respiratory samples were tested for the following pathogens: *Haemophilus influenzae*, *Streptococcus pneumoniae, Bordetella pertussis, Mycoplasma pneumoniae*, *Chlamydophila (Chlamydia) pneumoniae*, *Legionella* species and *Pneumocystis jirovecii*. Induced sputum and oral washes were tested for the following pathogens: *P. jirovecii*, *Mycobacterium tuberculosis*, *H. influenzae*, *S. pneumoniae, B. pertussis, M. pneumoniae*, *C. pneumoniae* and *Legionella* species. In addition, influenza-like illness (ILI) surveillance at two primary health care clinics serviced by the these two enhanced sites (Edendale and KTHC) was started in 2012, and 4 additional clinics were added in 2013. A sample of individuals asymptomatic for respiratory disease was enrolled at ILI sites.

The primary objectives of the surveillance for additional respiratory pathogens were:

- To estimate the prevalence and proportion of patients with *P. jirovecii*, *M. tuberculosis*, *S. pneumoniae, B. pertussis, H. influenzae* and atypical bacterial causes of pneumonia (*Legionella species, C. pneumoniae* and *M. pneumoniae*) in HIV-infected and HIV-uninfected adults and paediatric patients hospitalised with SRI, and to describe the factors associated with positivity for these infections
- To describe the burden and aetiology of outpatient influenza-like illness in children and adults in selected sites in South Africa, in HIV-infected and HIV-uninfected populations

This report details preliminary results from the enhanced surveillance for additional respiratory pathogens from Edendale Hospital and KTHC and the associated ILI sites. From ILI surveillance sites only ILI cases are included in this report.

Methods

Hospitalised patients meeting the clinical case definition for SRI, outpatient cases meeting the case definition for ILI and healthy controls (asymptomatic individuals) were prospectively enrolled from May 2012 to June 2013. Clinical and epidemiological data were collected using standardised questionnaires. Information on in-hospital management and outcome was also collected.

Sample collection and processing

Upper respiratory tract samples (oropharyngeal and nasopharyngeal swabs in patients ≥5 years or nasopharyngeal aspirates in patients <5 years of age) were collected from hospitalised patients (SRI) and outpatients (ILI and healthy controls). Induced sputum, blood and oral washes were collected from hospitalised patients only. In patients ≥5 years, induced sputum and oropharyngeal mouth rinse were collected. In those patients where TB testing was not conducted as part of clinical care, an expectorated sputum or second induced sputum sample (in patients who could not expectorate), was collected and tested at the local laboratory for TB. In patients <5 years, the first induced sputum was tested at the surveillance site laboratory for *M. tuberculosis* using GeneXpert, and a second sample was tested at the NICD for *P. jirovecii*, *M. tuberculosis* and bacterial pathogens. Collections of induced sputum started in June 2012 and November 2012 for adult and paediatric patients respectively.

Collected upper respiratory specimens were placed in 4 ml cryovials containing virus transport medium. Oral washes and sputum were collected in universal containers. Whole blood samples were collected in EDTA-containing vacutainer tubes within 24 hours of hospital admission.

Following collection, respiratory and blood samples were kept at 4°C at the local laboratory, and were transported to the NICD on ice within 72 hours post-collection. At the start of the programme, sputum samples were transported together with the oropharyngeal/nasopharyngeal samples. From July 2013 sputum samples were stored separately at -20^{0}C at the local laboratory and transported to the NICD on dry ice on a weekly basis.

Laboratory procedures

DNA was extracted from the clinical specimens and tested for bacterial pathogens and *P. jirovecii* by real-time polymerase chain reaction (PCR).

Detection of bacterial pathogens

Induced sputum and nasopharyngeal samples were tested for *M. pneumoniae*, *C. pneumoniae, Legionella* spp. and *B. pertussis.* A specimen was considered positive for *M. pneumoniae* if the *MP181* target was detected (Ct<45), *C. pneumoniae* if the *CP-Arg* target was detected (Ct<45) and *Legionella* spp. if the Pan-Leg target was detected (Ct<45).[1] This multiplex real-time PCR assay is only able to identify *Legionella* spp., but further assays are required to identify samples to species level. Any specimen that was positive for the *MP181*, *CP-Arg* or Pan-Leg targets was DNA re-extracted and the PCR was repeated in duplicate. If there was an insufficient amount of primary specimen, the initial DNA extract was repeated in duplicate. A specimen was only reported as a positive if the PCR result was positive in at least 2 of the 3 reactions i.e. identified through two extracts. A positive result for pertussis was obtained when a specimen was positive for *IS481* and/or *ptxS*1 genes.[2] A specimen was considered negative if the organism-specific targets (*MP181*, *CP-Arg* and Pan-Leg)[1] were not detected (Ct>45) and the *RNAse P* target was positive (Ct ≤45).

Blood specimens were tested using quantitative real-time PCR for the presence of pneumococcal DNA (*lyt*A gene), and for *H. influenzae* targeting *IgA, bexA* and region II of the *cap* locus of *H. influenzae.* For lytA testing, specimens with a lytA Ct-value <40 were considered positive.[3]

Determination of TB status

TB testing at the local laboratory or at the NICD was based on the GeneXpert System (Cepheid, Sunnyvale, CA) using the cartridge-based Xpert MTB/RIF (Xpert) assay. TB microscopy for acid-fast bacilli was conducted for some patients. All induced sputum specimens were also tested for *M. tuberculosis* by culture in liquid media using BD Bactec MGIT 960 at the NICD. Positive cultures were identified as *M. tuberculosis* complex using Ziehl-Neelsen staining and antigen testing. Genotypic resistance to isoniazid and rifampicin was tested using the Hain MTBDR*plus* v2 assay. A laboratory-confirmed TB case was defined as an individual with a positive result for *M. tuberculosis* on microscopy, culture or PCR from the GeneXpert MTB/RIF test at either the local hospital or the NICD TB Laboratory.

Detection of P. jirovecii

Induced sputum, nasopharyngeal and oral wash samples were tested for the mitochondrial gene coding for the large ribosomal subunit (mtLSU) of *P. jirovecii*. Polymerase chain reaction for *P. jirovecii* detects the organism at much lower levels than do staining techniques and allows for less invasive specimens to be used.[4] However, *P. jirovecii* DNA has been found in patients with no clinical symptoms or signs of pneumonia.[5] Further studies are required to distinguish between colonisation and disease when using PCR. For our study, a specimen was considered positive if *P. jirovecii* DNA was positive on PCR. No distinction was made between true infection and colonisation.

Determination of HIV status

HIV status data was obtained from two data sources. Firstly, for some patients HIV testing was requested by admitting physicians as part of clinical management. This included HIV enzyme-linked immunosorbent assay (ELISA) testing with confirmation by ELISA on a second specimen for patients ≥18 months of age, and qualitative HIV PCR testing for confirmation of HIV-infection status in children <18 months of age. Secondly, for consenting patients, linked anonymous HIV PCR testing for children <18 months of age or ELISA for patients ≥18 months of age was performed using a dried blood spot or whole blood specimen.

Data management

Data management was centralised at the NICD where laboratory, clinical and demographic data from enrolled patients were recorded on a Microsoft Access database.

Ethical considerations

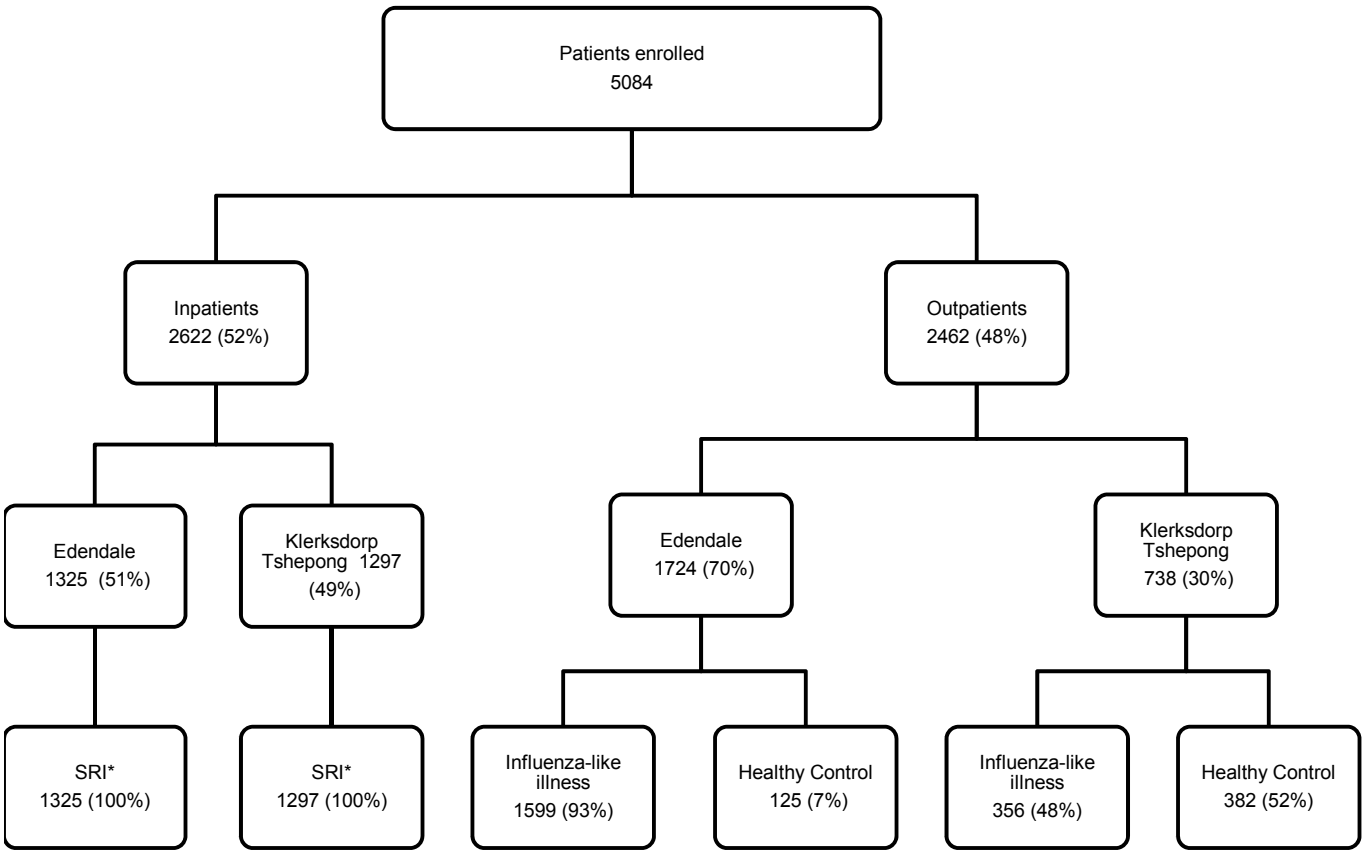
The protocol was approved by the Research Ethics Committees of the University of the Witwatersrand and University of KwaZulu-Natal.

Results

Enhanced surveillance patient enrollment

For the period May 2012 to June 2013 a total of 5084 subjects was enrolled at the two enhanced sites. Of these, 52% (2622/5084) and 48% (2462/5084) were hospitalised patients and outpatients respectively. Similar proportions of patients were enrolled from the two hospital sites, 51% (1325/2622) from Edendale Hospital and 49% (1297/2622) from KTHC. For ILI, the majority of patients (65%; 1599/2462) were enrolled from clinics that refer to Edendale Hospital (figure 1). Of the 5084 enrolled subjects, 10% (507) were healthy controls and these were not included for further analysis.

Figure 1: Numbers and proportions of patients and controls enrolled at enhanced sites for surveillance of additional respiratory pathogens, May 2012 - June 2013.



*SRI= severe respiratory illness.

Characteristics of cases with severe respiratory illness and influenza-like illness enrolled at enhanced surveillance sites

For the period May 2012 through June 2013, 4577 patients meeting the criteria for SRI and ILI were enrolled at the enhanced sites i.e. 2622 (57%) and 1955 (43%) for SRI and ILI respectively (table 1). Patient enrollment varied by age group with the highest proportion (34%, 1538/4577) drawn from the 24-44 year age group. 57% (2577/4521) of enrolled patients were female. HIV status was available for 85% (2220/2622) of patients hospitalised with SRI, of which 56% (1226/2200) were HIV infected. HIV infection varied by age group with the highest proportion that tested HIV positive (89%, 692/770) drawn from the 25-44 year age group. Of the 2425 patients admitted with SRI that had outcome data, 208 (9%) died.

Table 1: Characteristics of cases enrolled with severe respiratory infection and influenza-like illness at enhanced sites, May 2012 - June 2013, South Africa.

Characteristic	Influenza-like illness Number (%)	Severe respiratory illness Number (%)
Age Group (years)		
0-4	406/1955 (21)	878/2622 (33)
5-24	610/1955 (31)	242/2622 (9)
25-44	659/1955 (34)	879/2622 (34)
45-64	233/1955 (12)	480/2622 (18)
≥65	47/1955 (2)	146/2622(6)
Gender		
Female	1225/1905 (64)	1352/2616 (52)
Hospital/Clinic name		
Edendale Hospital		1325/2622 (51)
Klerksdorp-Tshepong Hospital Complex		1297/2622(49)
Imbalenhle/Edendale Gateway clinic	1599/1955 (82)	
Jouberton/Tshepong Gateway clinic	356/1955 (18)	
Underlying illness		
No	1910/1941 (98)	2302/2616 (88)
Outcome		
Died	0/1955(0)	208/2425 (9)

Bacterial pathogens

Bordetella pertussis

Among the 3664 patients with severe respiratory infection and influenza-like illness who were tested for bacterial pathogens, 42 (1%) were positive for *B. pertussis* of which 31 (74%) presented with SRI and 11 (26%) with ILI. The majority of cases occurred in the winter and spring months (figure 2), and cases occurred at all study sites (figure 3). The highest detection rates of *B. pertussis* were in the 25-44 (17/1230, 1.4%) and 45-64 (10/549, 1.8%) year age groups (figure 4). Cases of *B. pertussis* were detected either in nasopharyngeal specimens (30/42, 71%), or induced sputa (8/42, 19%) or in both specimen types (4/42, 10%).

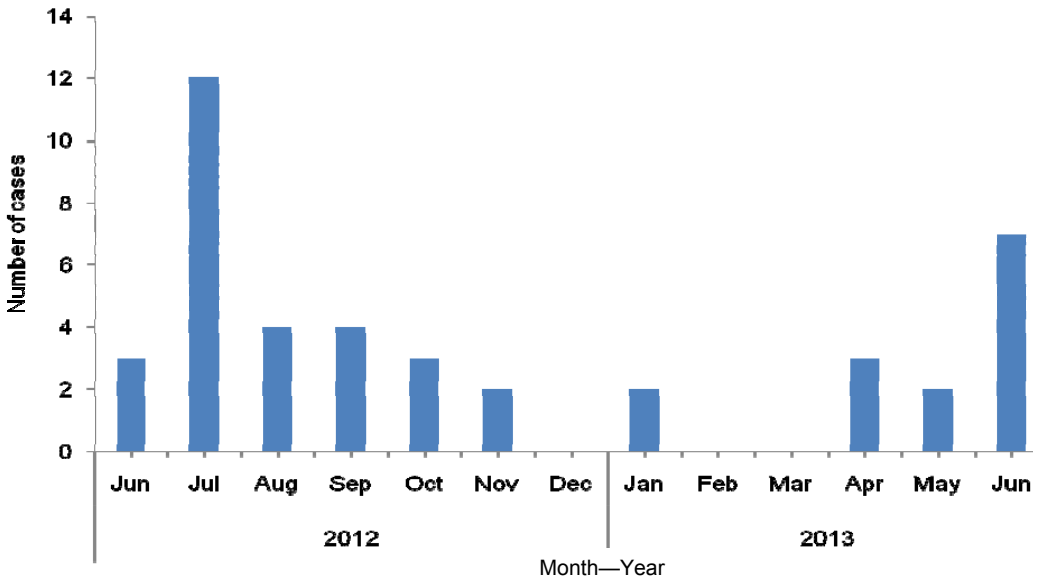


Figure 2: Numbers of cases of *Bordetella pertussis* among patients with severe respiratory infection and influenza-like illness by month and year, May 2012 - June 2013. N=42.

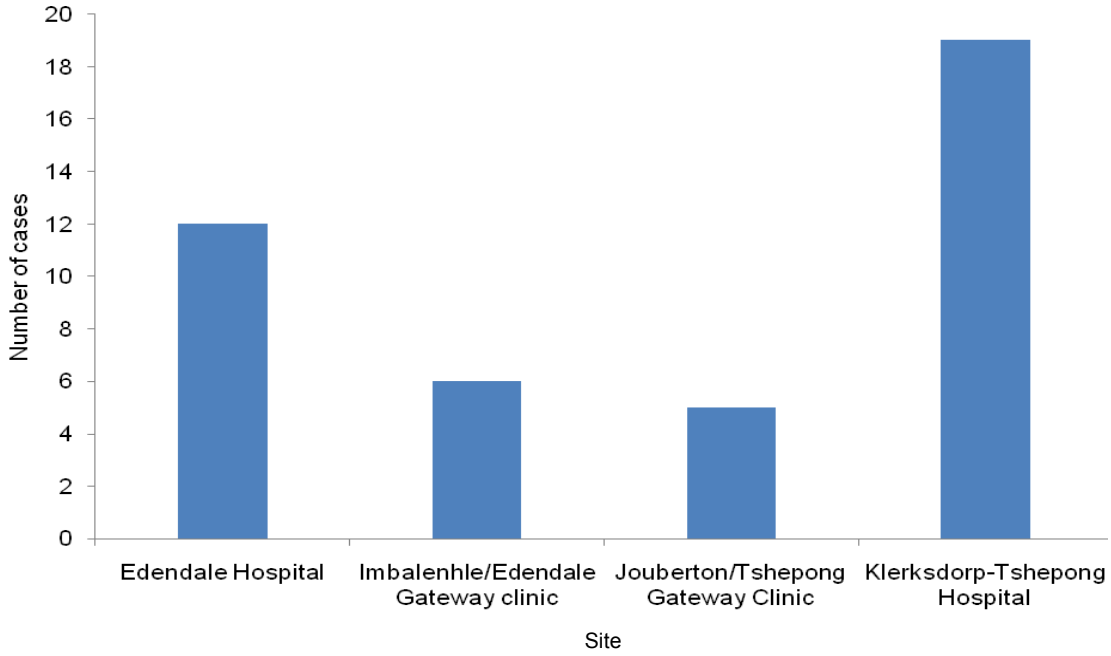


Figure 3: Numbers of cases of *Bordetella pertussis* among patients with severe respiratory infection and influenza-like illness by study site, May 2012 - June 2013. N=42.

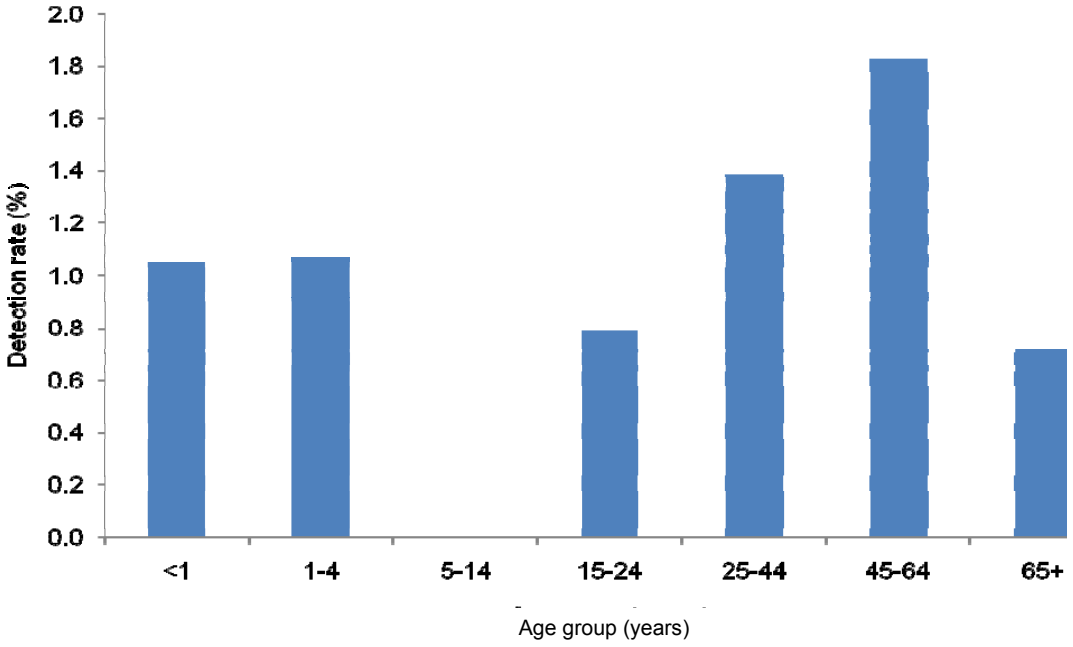


Figure 4: Detection rate of *Bordetella pertussis* among patients with severe respiratory infection and influenza-like illness by age group, May 2012 - June 2013. N=3664.

Atypical bacteria

Among SRI cases, 1964 (75%) were tested for atypical pneumonia causing bacteria. Of these, 33 (2%) were positive for *M. pneumoniae*, 18 (1%) were positive for *Legionella* spp. and 4 (0.2%) were positive for *C. pneumoniae* (figure 5). The majority of *M. pneumoniae cases* were detected at Edendale Hospital (30/33, 91%), whereas the majority of *Legionella* spp. cases were detected at Klerksdorp-Tshepong Hospital (13/18, 72%) (figure 6). The overall detection rate of *M. pneumoniae* was 2% (33/1964), with the highest detection rate in the 1-4 year age group (9/233, 4%) (figure 7).

Cases of *M. pneumoniae* were identified either in naso-pharyngeal specimens (24/33, 73%), or induced sputa (5/33, 15%) or in both specimen types (4/33, 12%). All cases of *Legionella* spp. (18/18) were detected in adult patients, 15-64 years of age, of which 17 (94%) were detected in induced sputa and 1 (6%) was detected in a nasopharyngeal specimen. For patients with induced sputum tested, the detection rate of *Legionella* spp. was 2% (17/718), with the highest detection rates in the 15-24 (2/53, 4%) and 45-64 (7/190, 4%) year age groups (figure 7).

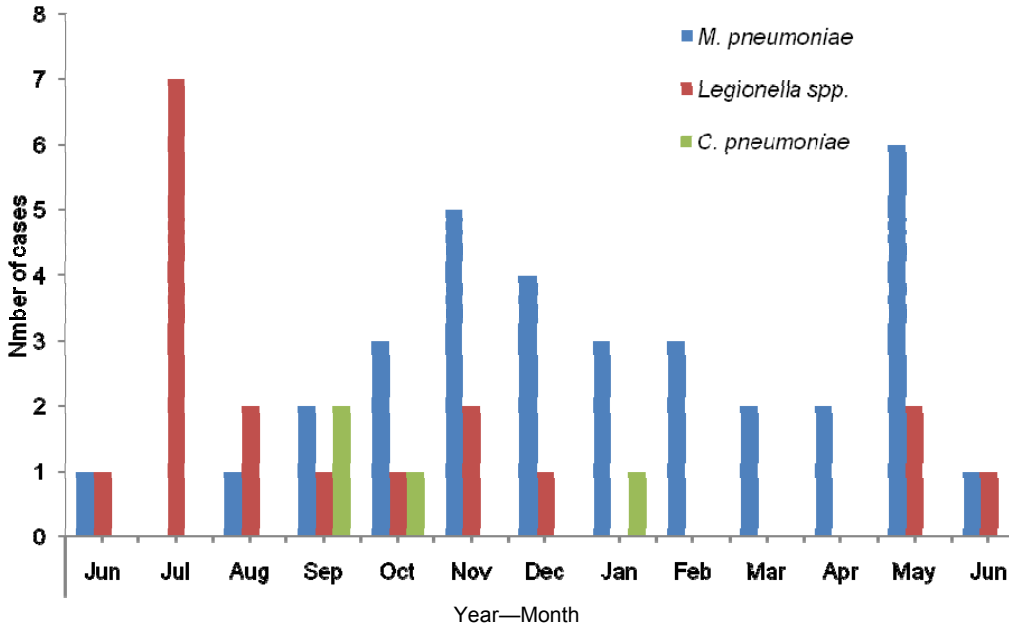


Figure 5: Numbers of cases of *Mycoplasma pneumoniae, Legionella* spp. and *Chlamydophila pneumoniae* among patients with severe respiratory infection by month and year, May 2012 - June 2013.

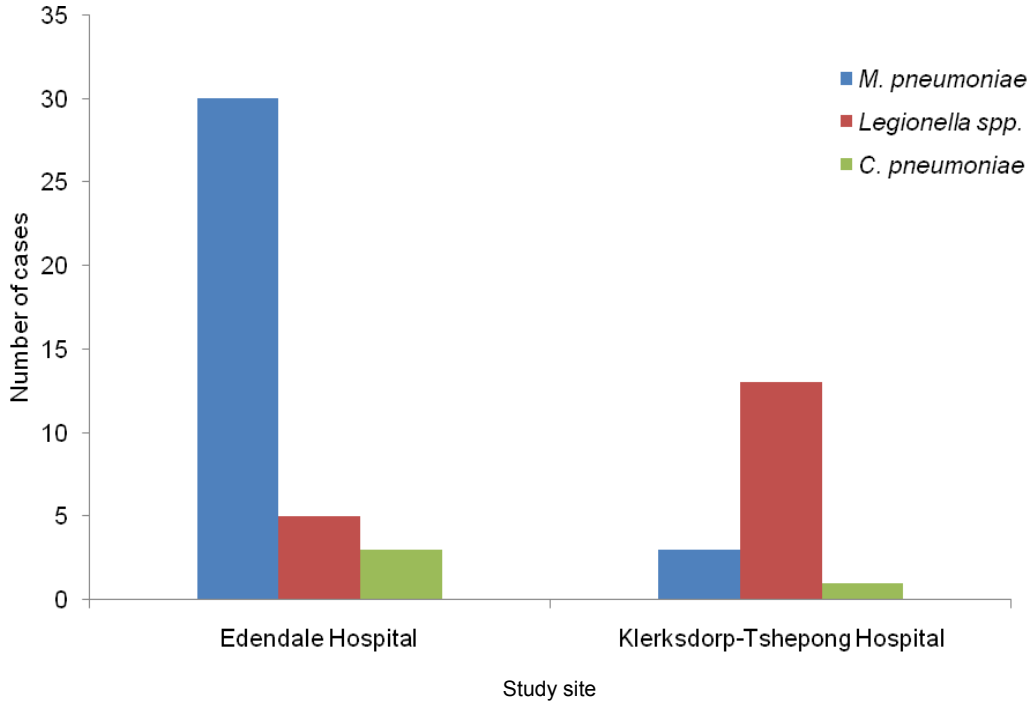
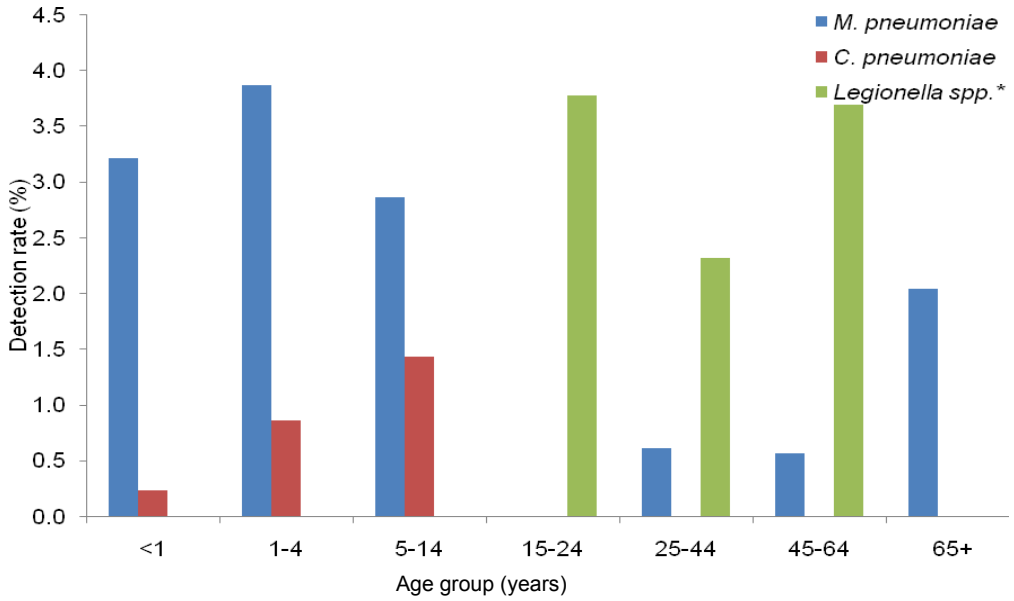


Figure 6: Numbers of cases of *Mycoplasma pneumoniae, Legionella* spp. and *Chlamydophila pneumoniae* among patients with severe respiratory infection by study site, May 2012 - June 2013.

Figure 7: Detection rate of *Mycoplasma pneumonia* (N=1964), *Legionella* spp. (N=718) and *Chlamydophila pneumoniae* (N=1964) among patients with severe respiratory infection by age group, May 2012 - June 2013.



*Detection rate for *Legionella* spp. calculated only for patients with an induced sputum specimen tested.

Streptococcus pneumoniae and *Haemophilus influenzae*

Blood specimens were tested for *S. pneumoniae* for 85% (2141/2522) of SRI patients enrolled from May 2012 to June 2013 and 202 (9%) were positive for *S. pneumoniae*. Pneumococcal infection was detected throughout the year with peaks in the winter and spring months (figure 8), and cases were distributed between the two study sites (figure 9). The overall detection rate of *S. pneumoniae* was 9% (202/2141), which ranged from 16% (13/81) in the 5-14 year age group to 8% (10/130) in the 15-24 year age group (figure 10). Of the 2160 blood specimens tested for *H. influenzae,* 47 (2%) were positive. Of these, 4 (9%) were *H. influenzae* sero-type b with 1 case in the <1 age group,1 case in the 5-14 year age group and 2 cases in the 25-44 year age group. Cases of *H. influenzae* were not seasonal (figure 8), and were detected at both study sites (figure 9). The overall detection rate of *H. influenzae* was 2% (47/2160), with the highest detection rate in the 5-14 year age group (6/85, 7%) (figure 10).

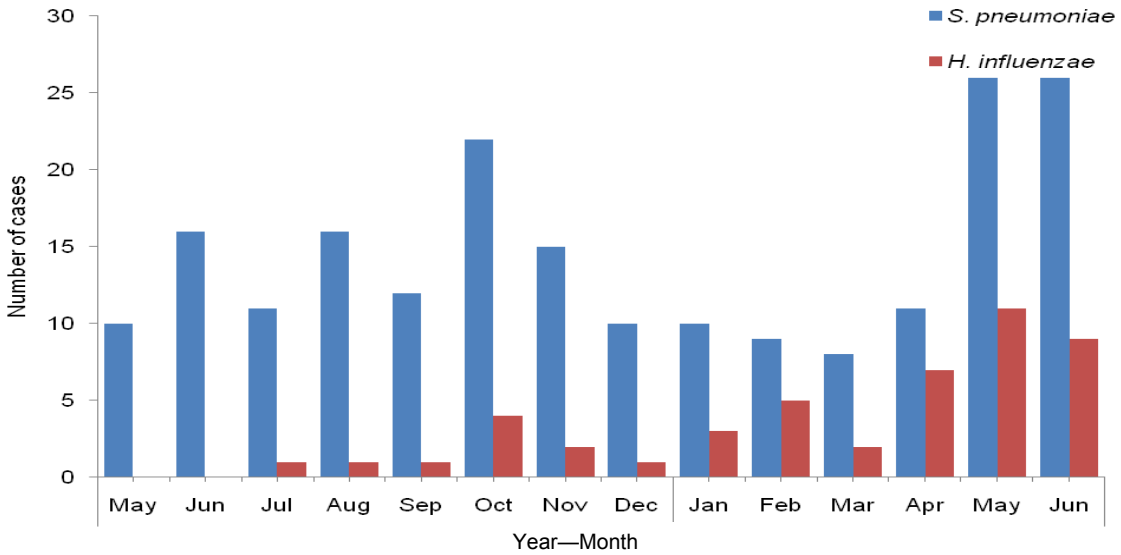


Figure 8: Numbers of cases of *Streptococcus pneumoniae* and *Haemophilus influenzae* from patients with severe respiratory infection by month and year, May 2012 - June 2013.

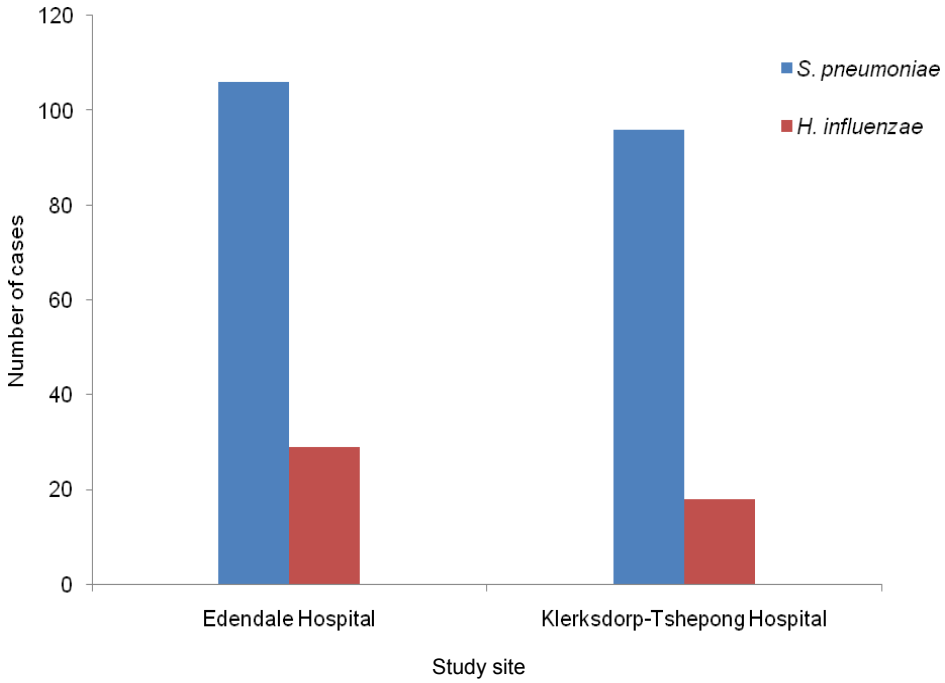


Figure 9: Numbers of cases of *Streptococcus pneumoniae* and *Haemophilus influenzae* among patients with severe respiratory infection by study site, May 2012 - June 2013.

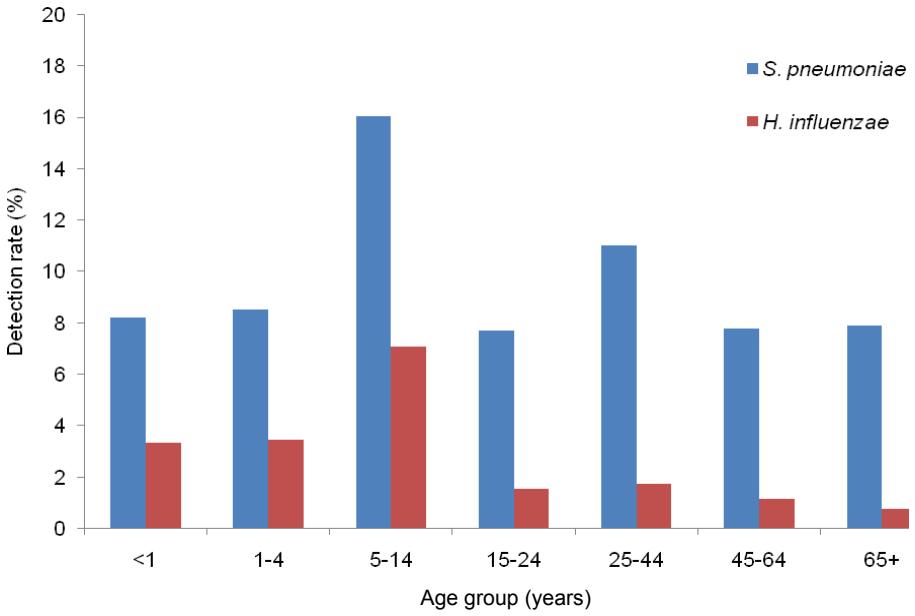


Figure 10: Detection rate of *Streptococcus pneumoniae* (N=2141) and *Haemophilus influenzae* (N=2160) among patients with severe respiratory infection by age group, May 2012 - June 2013.

Tuberculosis

For the period May 2012 through June 2013 59% (1555/2622) of patients admitted with SRI were tested for TB. Testing for TB varied by age group, with the highest proportion tested among the 25-44 years age group (693/879, 79%, p<0.001) (figure 11). Testing for TB also varied by hospital: KTHC (880/1297, 68%) and Edendale (675/1325, 51%). Onsite testing for TB was conducted for 56% (1380/2447) of SRI cases and contributed to 89% (1380/1555) of the total number of cases tested. KTHC tested a higher proportion of cases onsite (783/1200; 65%) than did Edendale Hospital

(597/1247; 48%). Of the total cases tested for TB, 69% (1073) were tested at the NICD. HIV results were available for 88% (1370/1555) of the patients tested for TB of which 66% (908) were HIV infected.

Of the 1555 patients tested for TB 25% (391) were positive. The highest percentage (217/693; 31%) that tested *M. tuberculosis* positive was in the 25-44 years age group. The detection rate for TB was higher in KTHC (254/880; 29%) than in Edendale (137/675; 20%) (figure 12). The HIV prevalence in TB positive cases was 76% (table 2).

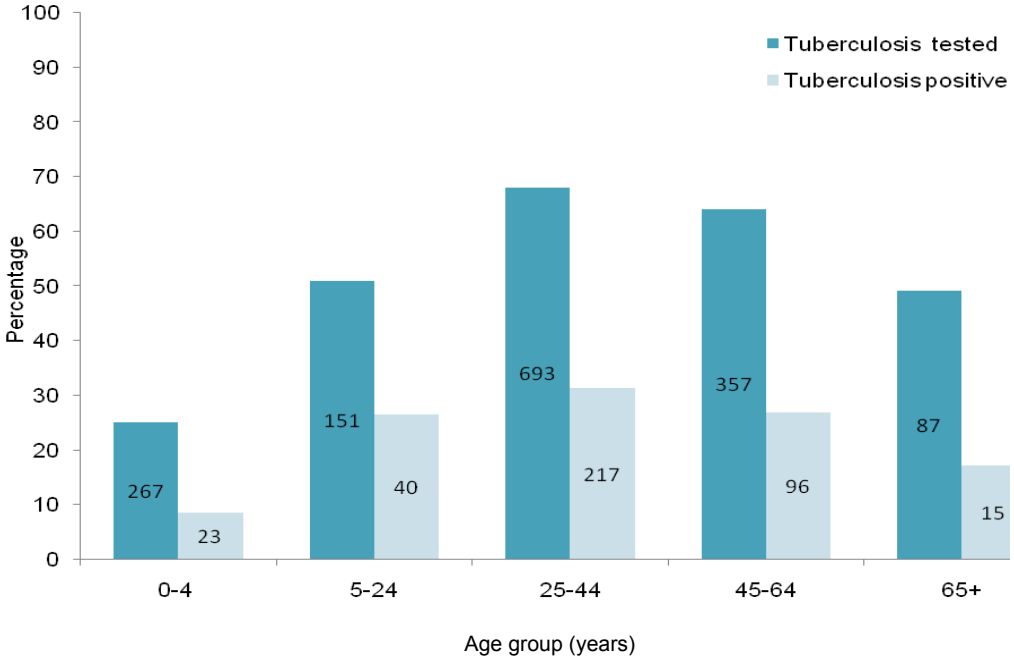


Figure 11: Proportions tested and detection rate for *Mycobacterium tuberculosis* in patients admitted with severe respiratory illness by age group, May 2012 - June 2013.

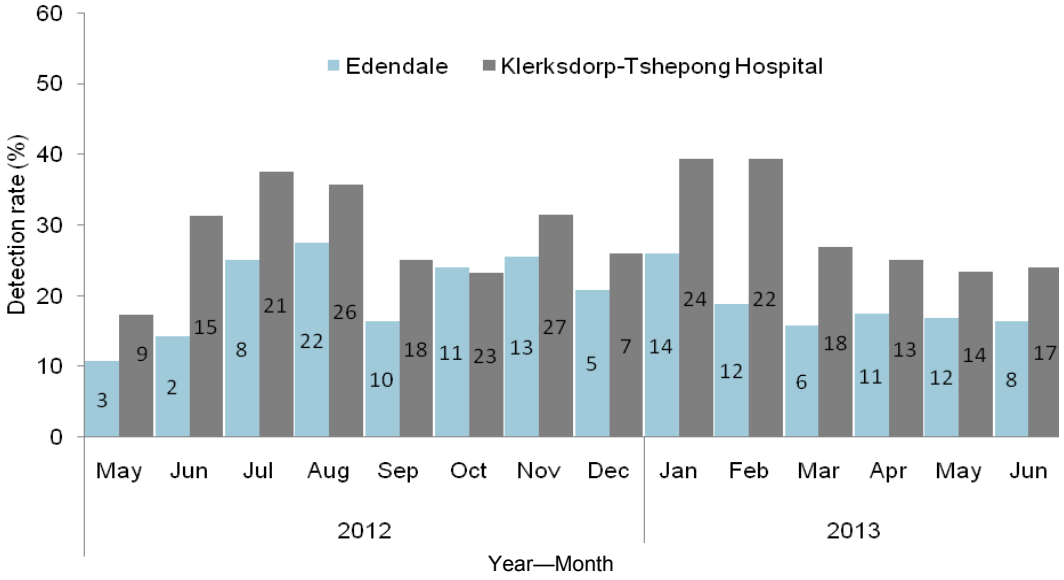


Figure 12: Detection rate of *Mycobacterium tuberculosis* in patients admitted with severe respiratory illness by site, month and year, May 2012 - June 2013.

Table 2: Characteristics of patients with severe respiratory illness who tested positive for *M. tuberculosis*, May 2012 - June 2013.

Characteristics		Laboratory confirmed tuberculosis n/N (%)
Age (years)	<5	23/391 (6)
	5-24	40/391 (10)
	25-44	217/391 (55)
	45-64	96/391 (25)
	≥65	15/391 (4)
Sites	Edendale Hospital	137/391 (35)
	Klerksdorp-Tshepong	254/391 (65)
Gender	Female	192/391 (49)
HIV status	HIV-infected	261/339 (76)
Outcome	Died	40/361 (11)

Pneumocystis jirovecii

For the period June 2012 to June 2013, 4330 specimens (oral rinse, nasopharyngeal and induced sputum) from 2233 SRI patients were tested for *P. jirovecii* (figure 13). Of the patients tested, 329 (15%) were positive for *P. jirovecii* DNA. Nasopharyngeal specimens were the most common specimen type received (2098/4330, 48%). However, induced sputum samples gave the highest detection rate (175/1060, 17%) compared to nasopharyngeal (186/2098, 9%) and oral rinse (63/1172, 5%) specimens (figure 14). The majority (126/329, 38%) of *P. jirovecii*-positive cases was in the 25-44 year age group. Among cases positive for *P. jirovecii*, the HIV prevalence was 68% (199/293) and more than two-thirds (88%, 266/302) survived (table 3).

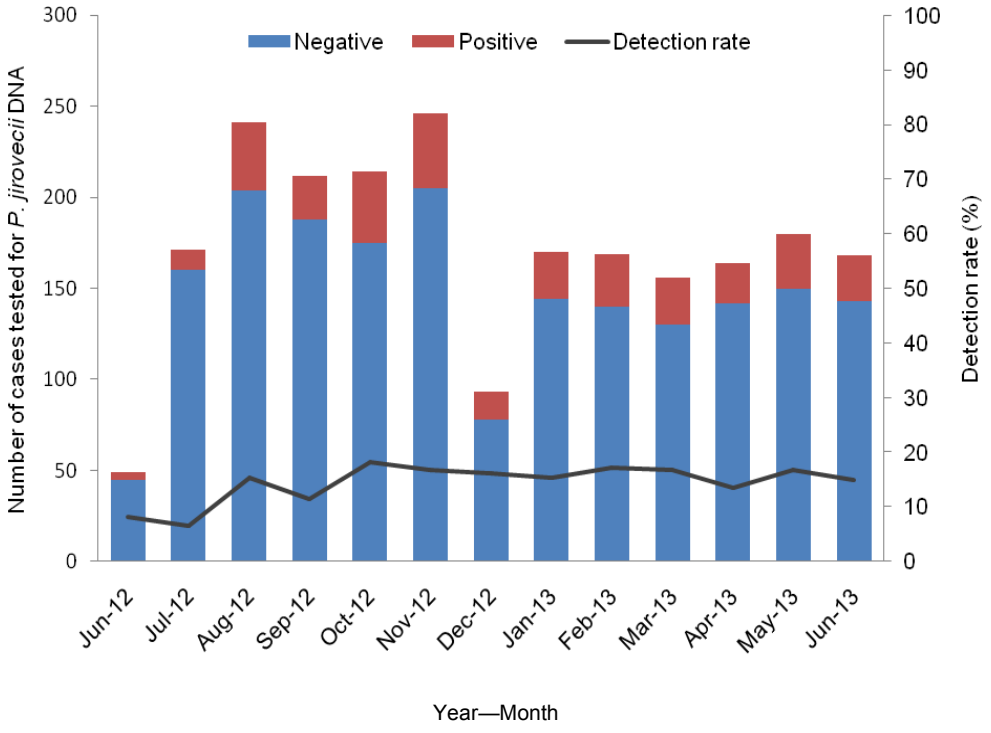


Figure 13: Numbers of cases tested for *Pneumocystis jirovecii* and detection rate by month, June 2012 – June 2013. N=2233.

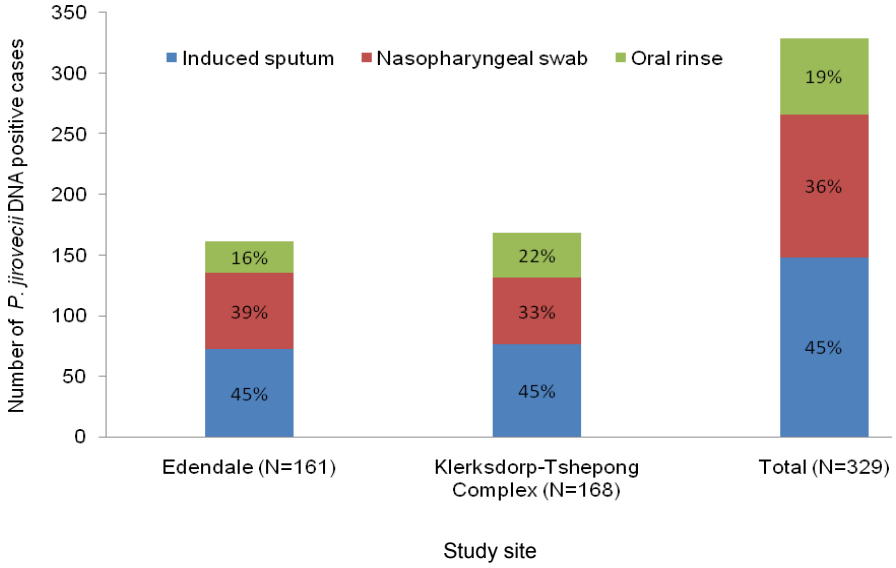


Figure 14: Numbers of *Pneumocystis jirovecii* DNA positive cases by site and specimen type, June 2012 - June 2013. N=329.

Table 3: Characteristics of *Pneumocystis jirovecii* DNA positive cases, June 2012 – June 2013.

Characteristics		n/N (%)
Age (years)	<5	112/329 (34)
	5-24	20/329 (6)
	25-44	126/329 (38)
	45-64	63/329 (19)
	≥65	8/329 (2)
Sites	Edendale	161/329 (49)
	Klerksdorp-Tshepong	168/329 (51)
Gender	Female	181/329 (55)
HIV status	HIV infected	199/329 (68)
Outcome	Died	36/302 (12)

Discussion

By expanding the existing respiratory surveillance to include additional pathogens common in South Africa's high HIV prevalence setting and by including surveillance for milder infections, this surveillance programme has enabled descriptions of the prevalence of additional respiratory pathogens in patients with different clinical presentations at the enhanced sites during its first year. These pathogens are not routinely tested for in the public hospital setting due to cost and the difficulty in obtaining appropriate specimens.

The proportion of community-acquired pneumonia (CAP) cases attributed to atypical pneumonia-causing bacteria differs amongst studies based on the populations studied and the methods used for identification. However, it has been reported by Bartlett et al.[6] that atypical pneumonia-causing bacteria are responsible for approximately 10- 20% of CAP worldwide. A study done in Cape Town on specimens collected from hospitalised adults from July 1987 through July 1988, using serology for identification, found that approximately 36% of pneumonia cases were due to atypical bacteria.[7] *Chlamydophila pneumoniae* was identified in 21% of cases, *Legionella pneumophila* in 9% and

M. pneumoniae in 1% of cases. This differs from the findings presented in this study, using real-time PCR for detection, in which *M. pneumoniae* was found in 2% of cases, *Legionella* spp. in 1% and *C. pneumoniae* in 0.2% of cases. The highest detection rate of *M. pneumoniae* was in children aged 1 to 4 years of age. However, it has previously been reported to cause pneumonia in patients 5-25 years of age.[6] *Legionella pneumophila* was common in adults 40-70 years of age. The data presented here indicate that all positive *Legionella* cases were detected in adult patients in the 15-64 years of age category.

During the one year period of this survey, 42 (1%) pertussis cases among patients with SRI and ILI were detected as compared to 60 pertussis cases that were previously reported nationally to the Department of Health over a period of four years (2000-2004).[8] Most clinicians do not consider pertussis in adults and although it is a notifiable condition it is seldom reported. The highest number of pertussis cases was detected in patients with SRI and the majority of cases were detected in samples from the Klerksdorp-Tshepong hospital complex. While disease was identified in all age groups, disease burden was greatest in the 25-64 year age group. Previously, disease was most prevalent in the infant and adolescent age groups, but more recent studies have demonstrated disease burden in the adolescent and adult age groups,[9,10] as indicated in the data presented here. *Bordetella pertussis* was detected in nasopharyngeal and induced sputum specimens although nasopharyngeal specimens gave the highest positive yield (71% vs. 19%).

In this survey the prevalence of TB was 25%. This is lower than that reported in a study conducted in KwaZulu-Natal which was based on the aetiology of CAP. The Kwazulu-Natal study reported *M. tuberculosis* as the commonest isolated pathogen in cases admitted with CAP. *Mycobacterium tuberculosis* was the commonest agent among CAP patients in HIV and non-HIV-infected subjects (40% and 35%, respectively).[11] From the data presented here, nine percent of children less than five years admitted with severe respiratory illness had laboratory confirmed TB. This was similar to the 8% prevalence of culture-confirmed TB reported in other studies in HIV-infected and HIV–uninfected children hospitalised for acute pneumonia.[12,13] The high prevalence of TB in patients admitted with SRI highlights the importance of early detection leading to a reduction of missed opportunities when active testing for TB, particularly in high risk groups, is conducted. Although this survey aimed to screen all SRI patients enrolled in the study for TB, only 59% of patients were tested, and testing varied by age group and site, with a higher proportion of cases tested in the older age groups. Challenges in sample collection have been reported previously, particularly in children.

In this survey the prevalence of *P. jirovecii* in HIV-infected and HIV-uninfected patients of all ages was 15%. The pathogenesis of *P. jirovecii* is not completely understood, which makes the interpretation of whether a positive PCR test is significant or not difficult.[14] Those studies based solely on colonization showed that in immunocompetent children prevalence varied between 16% to 100%, was 20% in immunocompetent adults, and varied from 20% to 43% in HIV-infected adults.[15] Differences in prevalence rates are likely due to differences in study populations, specimen type and the diagnostic tests used. In a previous South African study, the prevalence of *P. jirovecii* pneumonia in children <10 years was 10%[16] but can be as high as 49% in HIV-infected children with severe pneumonia.[17] The use of different specimen types in different subsets of the population as well as clinical factors need to be taken into consideration in order to distinguish between colonisation and true disease when PCR is used for diagnostic purposes.

Further analysis of data gathered from this surveillance programme will allow for the identification of risk groups to be targeted for interventions and to describe how co-infections with these pathogens relate to patient outcome.

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HIV INCIDENCE ESTIMATES FOR SOUTH AFRICA: UPDATE ON LABORATORY METHODS AND POST-TEST ALGORITHMS

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Introduction

One of the key indicators of the South African National Strategic Plan on HIV, STIs and TB (2012–2016) is a reduction by 50% of new HIV infections.[1] The South African strategic plan is in line with the Millennium Development Goal 6. Thus, the May 2013 WHO/ UNAIDS Technical Update on HIV Incidence Assays for Surveillance and Epidemic Monitoring has provided a new lease of life for laboratory-based measures of incidence.[2] The Technical Update followed the presentation of results from the evaluations of HIV incidence assays conducted by the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) at the Conference for Retroviruses and Opportunistic Infections (CROI) held in March 2013. The assays evaluated by CEPHIA were the BED capture enzyme immunoassay (BED-CEIA), limiting-antigen avidity EIA (LAg AI), Vitros-Less Sensitive and Bio-Rad Avidity Index EIA. Based on the preliminary analysis by CEPHIA, none of the assays evaluated completely met the recommended target product profile for an incidence assay. Nevertheless, the assays can be used in an algorithm (see discussion below on the Recent Infection Testing Algorithm [RITA]) that would provide credible estimates of HIV incidence. This is in contrast to the UNAIDS statement of 2005 which cautioned against the use of the BED-CEIA for purposes of estimating HIV incidence at a population level.[3] Subsequent to the 2005 statement, there have been several developments at a laboratory level (i.e. assay development with at least 10 assays described) and at the post-test laboratory level (i.e. mathematical and statistical tools for the establishment of, for example, the mean duration of recent infection) that culminated in the 2013 Technical Update. The critical importance of HIV incidence estimates is explored in detail below. The National Institute for Communicable Diseases (NICD) currently applies incidence assays to various surveys, including the national annual HIV antenatal prevalence surveys and the general household surveys conducted by the Human Sciences Research Council (HSRC), described below. In addition, incidence testing has been applied to interventions studies such as the roll-out of male circumcision.

National HIV prevalence surveillance systems

National HIV surveillance comprises three major programmes in South Africa. Firstly, one of the long-standing and major contributors to our understanding of the HIV epidemic in South Africa is the annual antenatal survey. Antenatal-based HIV surveillance was initiated in 1990 and is a national, anonymous, unlinked surveillance system that focuses on first-time antenatal attendees. The surveys are conducted in 52 districts and currently aim to recruit 36,000 pregnant women. The participants are recruited from 1445 public sector antenatal clinics annually in the month of October. There have been several refinements to the surveys, with the following objectives: to determine (1) the national point prevalence, (2) the prevalence in each province and district, (3) age-related prevalence, and (4) trends. The justification of the use of such surveys is that approximately 89% of pregnant mothers in South Africa utilise the public service, and of these 90% are black women. The most recent report showed a prevalence of 29.5% for 2011 which was similar to the prevalence of 30.2% for 2010.[4] In addition to the antenatal surveys, there was the national household survey for HIV prevalence in 2002 and the HIV prevalence and incidence surveys in 2005, 2008 and 2012, all led by the Human Sciences Research Council (HSRC). The reported national HIV prevalence for these surveys was 11.4% (2002), 10.8% (2005), 10.6% (2008) and 12.3 % (2012).[5] In general, one can conclude from both types of surveys that South Africa has a hyperendemic, generalized and mature HIV epidemic with approximately 6.4 million people infected. Within this epidemic, one can identify subsets that are at higher risk based on higher prevalence compared with the general population. The most-at-risk populations (MARPS) include African females aged 20–34 years,

African males aged 25–49 years, men who have sex with men (MSM), high-risk drinkers, people who use drugs for recreational purposes, and people with disabilities.

The epidemic has been addressed in several ways; one programme was the introduction of antiretroviral treatment in 2004 and, in 2007, the National Strategic Plan (NSP) established universal access targets (defined as the annual enrolment of 80% of those newly eligible onto antiretroviral therapy) over five years. An important estimate in the HSRC study that may explain the increase in prevalence is that approximately 2.1 million individuals are currently on antiretroviral therapy. The effect of expanded antiretroviral therapy coverage on life expectancy with a concomitant increase in prevalence has been noted in South Africa.[6] In the case of prevention of mother-to-child transmission, antiretroviral therapy was introduced in 2002 and the programme has undergone various iterations to keep pace with policy changes. The third national surveillance system is the South African Prevention of Mother-To-Child Transmission Effectiveness (SAPMTCTE) study, which examines the effectiveness of the prevention of mother-to-child transmission of HIV. Two surveys have been conducted to date, and a third is in the process of completion. The initial surveys show a declining trend, with transmission rates of 3.5% for 2010 and 2.7% for 2011.[7] A limitation is that the data apply to 4–8 weeks post birth period and do not take into account HIV transmission in the post-weaning phase. Nevertheless, a target for elimination of mother-to-child transmission has been set for 2015.

The leading edge of the epidemic: HIV incidence

The HIV prevalence surveillance studies have contributed to understanding the nature and magnitude of the epidemic, the identification of geotypes that are affected (e.g. rural versus urban), as well as the age groups and genders affected. The three general household surveys in particular have certainly provided critical information that has complemented the ongoing annual antenatal surveys. It is nevertheless recognised that prevalence studies have limitations. HIV prevalence, based on the data from the antenatal surveys and general population surveys, has reached a plateau in South Africa. There is thus a need to understand the underlying dynamics of the epidemic, especially in the context of various interventions, such as the expanded antiretroviral programme. A critical approach for understanding the dynamics of the epidemic is to measure incidence; i.e. the number of infections/person-years of observation or as an annual percentage of the population that acquire infections.[8] The information that can be derived from incidence measurements includes an understanding of transmission patterns, enabling a rational basis for the introduction of interventions (e.g. prevention and treatment programmes), the subsequent evaluation of these interventions, and the projection of the burden of HIV infections.

Estimated and observed HIV incidence in South Africa

The UNAIDS Report on the Global AIDS Epidemic 2013 reports a 52% reduction in new HIV infections among children, and a combined 33% reduction among adults and children since 2001. A summary of the estimates for South Africa is presented in table 1. The estimates are based on the Global AIDS Response Progress Reporting (GARPR), an online tool to which countries submit their most recent data on global indicators, and are also based on modelled HIV estimates created in standard modelling software by national epidemiological teams. The reported estimates of new infections for all ages in 2012 are 350,000, compared with 640,000 in 2001.

Other approaches in modelling incidence include, for example, a modelled cohort approach. The cohort approach was applied to the HSRC national household surveys conducted in South Africa in 2002, 2005 and 2008. In the case of the 2008 study, the extent of antiretroviral use was taken into account in the calculations.[9] In the period 2002–2005, the HIV incidence rate in men and women aged 15–49 years was estimated to be 2.0 infections per 100 person-years. The highest incidence rate was in women in the age group 15–24, with an estimate of 5.5 per 100 person-years. The incidence for the 2005–2008 period for the 15–49 age group was 1.3/100 person-years, but this decline was not significant. Nevertheless, when the estimate was calculated in women in the 15–24 age group, there was a 60% decline in incidence (2.2/100

person-years) which was statistically significant. By contrast, data obtained from longitudinal studies have shown a high and sustained HIV incidence in rural KwaZulu-Natal, whether at a general population level with an incidence of 3.4 per 100 person-years over a 5-year period, or in a cohort study of women where the incidence of 6.5 per 100 person-years and 6.4 per 100 person-years in rural and urban settings, respectively, was observed.[10,11] The household survey incidence estimates have generated lively debate as to the validity of the different approaches; e.g., statistical modelling versus laboratory-based prevalence and its use in HIV incidence estimates.[12,13] An additional and critical limitation is that the incidence estimates are retrospective, which highlights the need for more real-time estimates.

Table 1: UNAIDS Modelled HIV estimates for South Africa: Comparison of 2001 and 2012 estimates*

Category	2001			2012		
	Estimate	Lower Estimate	Upper Estimate	Estimate	Lower Estimate	Upper Estimate
Prevalence estimates in adults (15-49)	15.3	14.7	15.8	17.9	17.3	18.4
People living with HIV (all ages)	4 300 000	4 100 000	4 700 000	6 100 000	5 800 000	6 400 000
Adults (15+ living with HIV)	4 100 000	3 900 000	4 400 000	5 700 000	5 500 000	6 000 000
New infections (all ages)	640 000	600 000	700 000	370 000	340 000	420 000
New infections (15+)	560 000	530 000	620 000	350 000	320 000	390 000
Percentage of 15-24 living with HIV females/males for 2012	13.9	12.9	16.8	3.9	2.5	5.7
AIDS deaths	200 000	190 000	230 000	240 000	220 000	270 000

*http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf

Laboratory-based incidence measurements

Prospective cohorts that include HIV negative participants that are followed up at regular intervals can be used to directly measure incidence based on seroconversion or detection of virus components e.g. p24 or RNA. There are several disadvantages to such an approach, for example: cohorts may not necessarily reflect trends in the general population; there is difficulty in sustaining large cohort studies; it is costly to sustain cohorts; the introduction of bias caused by behaviour change can result in a lower incidence relative to the population under study. An important consideration is that the window period for these viral markers is relatively brief, 2–4 weeks prior to antibody detection, and would thus require substantial numbers to be tested.[14] In the case of p24, the protein is relatively variable in its detection making it an unreliable marker for detecting primary HIV infections (PHI). Nevertheless, the identification of primary HIV infection (PHI) is important because it is during this phase of HIV infection that individuals are highly infectious and contribute significantly to transmission dynamics.

In order to improve the efficiency of detection of PHI, pooling strategies have been devised to reduce costs. Estimates of PHI in South Africa have varied in different settings; for example, estimates of 2.9% were observed in a high-risk setting.[15] The ideal would be a laboratory-based incidence method(s) that can distinguish between recent HIV infection and established or long-term infection with a sufficiently long window period in order to measure incidence.[16] The requirements of an incidence assay are different from the standard diagnostic assay because the incidence estimates are applied at a population level and thus a high degree of sensitivity and specificity, as is the case of a diagnostic assay, is not required. Standard serological testing for HIV infection cannot distinguish between recent

(incident) HIV infections and long-term infections. Following exposure to HIV, individuals enter a transitory period in which there are specific immune responses such as antibody maturation that can distinguish between recent HIV infection and long-term infection. The duration of these responses is known as the mean duration of recent HIV infection (MDRI) and is highly heterogeneous in a population. The MDRI is an important parameter in incidence calculations (see below). The MDRI varies and depends on the method(s) used for detection. Factors that affect the MDRI include the population under study and the virus subtype. Nevertheless, an MDRI of 4–12 months duration is regarded as ideal in terms of providing information in real time.

Several serological assays have been described and used for the estimation of HIV incidence at both an individual and population level.[17] The underlying principles of the assays used for incidence estimates include for example, quantity/titre of antibody, antibody avidity, IgG subtype and epitope-specific antibodies. Although several assays have been described several are either in-house or commercially available assays used off-label. The BED capture EIA (CEIA) and LAg AI assays have been specifically developed for HIV incidence testing and are commercially available. The principal underlying the BED CEIA assay is to determine the proportion of IgG present.[18] By using a specific normalised cut-off value and a defined window period, incidence can be calculated. To overcome the issue of subtype the assay includes three peptides of the transmembrane gp41 glycoprotein of HIV subtype B, CRF_01AE and subtype D (hence the name for assay, “BED”). Nevertheless, there are differences in window period for the different subtypes but these are not as marked as for the detuned assay, which was amongst the first of the quantitative assays to be used in incidence measurements.[19] The LAg AI assay includes antibody avidity in the presence of limiting antigen on coated wells. The antigen is the same as for the BED assay but is linearized to improve antibody binding.[20] Compared to a conventional two-well avidity assay the LAg AI assay is performed in a single well in the presence of a chaotropic agent. The LAg AI assay has undergone initial validation using a defined protocol by the CDC and recently by CEPHIA (see above). These two assays cannot be used in isolation to calculate incidence because there are variations in the MDRI and misclassification can occur when using these assays. These complications have led to post-test methods/ algorithms (termed RITA) to calculate incidence.

Recent infection testing algorithm (RITA)

An important observation in the use of the BED CEIA was the overestimation of HIV incidence, the result of misclassification.[21] Thus, a second key post-test parameter that is included in the HIV incidence calculation is the misclassification rate or false recent rates (FRR). Four conditions contribute to the false recent rate. Firstly, there is heterogeneous immune response in a population. Thus, when considering the evolution of maturation of antibodies, the majority of individuals will fall within a defined MDRI but there is a small proportion of individuals (2%-5%, depending on various states of the epidemic), such as elite controllers, where immune responses do not evolve. Elite controllers will be falsely classified by a laboratory test as recently infected despite a duration of infection greater than the MDRI. The second condition is the use of antiretroviral drugs that suppress viral replication and antigen production, leading to a reduction of anybody responses. Thirdly, advanced HIV disease (AIDS) with a loss of CD4 cells and antibody responses and, fourthly, unknown factors such as HIV subtype, populations and geographic region, appear to influence responses although the underlying mechanisms are not known.

The current iteration for calculating HIV incidence is known as RITA (Recent Infection Testing Algorithm) and takes into account not only the MDRI but also the FRR (figure 1).[22] An example of RITA would be the use of two different assays, the use of multiple epitope-based assays and would include additional information such as CD4 count, HIV viral load and data on antiretroviral drugs.[23] Some of the incidence formulae are similar but it is recommended that specific FRR need to be determined. However, this may be an onerous requirement to fulfil and would entail testing individuals from the same population who have been infected for periods greater than the MDRI e.g. >12 months. The calculation of accurate incidence would require large

sample sizes compared to that of prevalence given that incident HIV infections are 10-fold less than prevalent infections.[24] For an HIV incidence assay to be suitable for use it is recommended that the FRR should be less than 2%. The algorithm the NICD intends to use will comprise a single incidence assay and the use of an HIV viral load cut-off to exclude elite controllers/viral suppression (figure 2).

$$I_r = \frac{R - \varepsilon P}{(1-\varepsilon)\omega N}$$

Survey counts:

N = no. of HIV-negative persons in survey
P = no. of HIV-positive persons in survey
R = no. of specimens classified as recent

Calibration parameters:

ω = mean duration of recent infection (MDRI, in years)
ε = False recent ratio (FRR)

Figure 1: Formula for estimating assay-based HIV incidence rates.

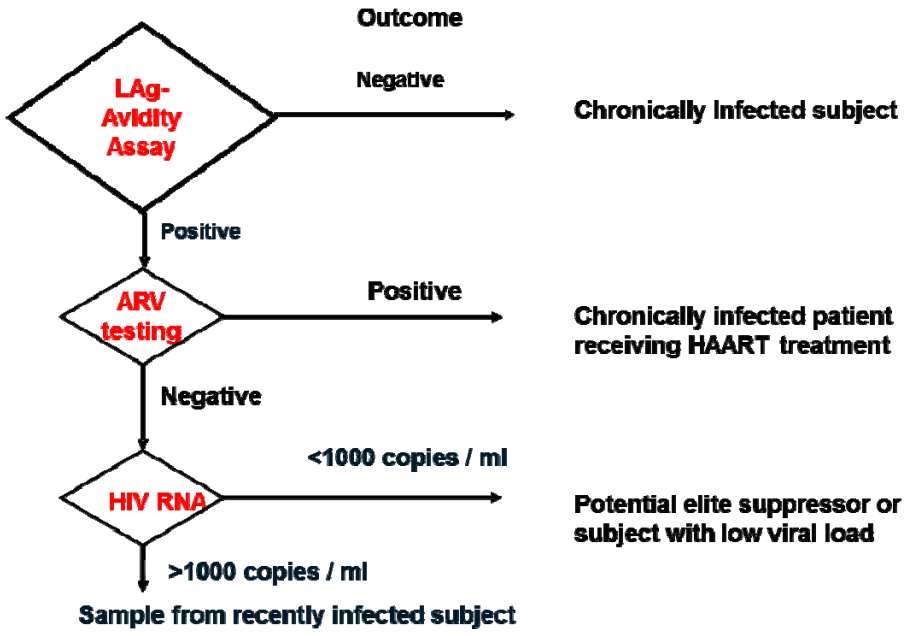

Figure 2: Recent Infection Testing Algorithm (RITA) for calculating HIV incidence.

Conclusion

The HIV epidemic in South Africa has now matured into a diffuse and hyperendemic phase. In addition, there have been multiple interventions and more recently an expanded antiretroviral programme. In this context the usefulness of prevalence surveys to understand the epidemic becomes more difficult with increasing prevalence as reported in the recent HSRC General Population Survey. Obtaining incidence estimates becomes more relevant in understanding the dynamics of the epidemic, patterns of transmission, identifying risks and assessing interventions. This is especially so in the era of rapid evolution of policy, for example the recent WHO recommendation to initiate treatment at CD4 counts of 500 cells/mm^3 or less.[25]

Establishing estimates of incidence has not been straightforward. Initial mathematical models for incidence will become more complicated as various factors such as the effect of antiretroviral therapy are taken into

account. An alternative and appealing approach to link to HIV incidence estimates is the concept of community viral load i.e. population-level aggregate measures of viral load. There is a plausible relationship between suppression of viral load, transmission and HIV incidence but the straightforward relationship belies the complexity underlying this concept.[26] Shortcomings in deriving community viral load estimates include the fact they may not represent actual viral loads, possibly because of dropouts in the treatment cascade and variability of the viral loads in individuals over time. Alternative approaches to incidence estimates include possible biomarkers (e.g. cytokines) or viral diversity and sequence ambiguity. [27,28] In the case of viral diversity, the development of suitable indices that would sufficiently discriminate between early and late infections have been described but are likely to be complicated in that not all settings are there. For example, single founder viruses and super-infections may complicate the interpretation of data. The ideal for HIV incidence estimation remains a cost-effective laboratory-based approach that produces near real time estimates that can inform policy decisions concerning identification of hot spots, transmission patterns and evaluation of interventions. Recent advances such as the LAg AI assay in combination with post-test factors do provide optimism because challenges that have beset incidence measurements to date are being addressed.

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SURVEILLANCE OF TRANSMITTED HIV-1 DRUG RESISTANCE IN FIVE PROVINCES IN SOUTH AFRICA IN 2011

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Introduction

As part of its strategy for surveillance of HIV-1 drug resistance in resource-limited settings, the World Health Organization (WHO) recommends surveillance for transmitted drug resistance (TDR) among individuals assumed to be recently infected, such as pregnant women.[1] The proposed method analyses sequences of ≤47 remnant specimens from individuals consecutively identified as HIV-infected in order to categorize TDR as low (<5%), moderate (5-15%) or high (>15%).[2] In 2012, the WHO published a global HIV drug resistance report detailing increasing levels of transmitted resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) particularly in Africa. This increase was attributed to expanded antiretroviral therapy coverage.[3]

The Centre for HIV and STI, National Institute for Communicable Diseases (NICD), has been performing TDR surveys in pregnant women since 2002, using specimens collected as part of the annual antenatal survey (ANSUR) conducted by the National Department of Health. Previous reports in the November 2012 NICD Bulletin show that while TDR remained low (<5%) to all drug classes in Gauteng, moderate (5-15%) rates of transmitted NNRTI drug resistance were detected in KwaZulu-Natal in 2009 and 2010.[4,5]

In this report an updated dataset with results from the analysis of specimens collected in the 2011 ANSUR survey from Gauteng (GP) and KwaZulu-Natal (KZN) is given. In addition, the first analyses of data from the Orange Free State (OFS) and Eastern Cape (EC) provinces are presented. Previously, specimens collected in the Western Cape (WC) province in 2006 were successfully analyzed whilst specimens from a 2007 survey were also tested but with poor polymerase chain reaction (PCR) amplification rates leading to non-classifiable results. The DNA samples of specimens from a WC 2011 survey were successfully amplified and are also included in this analysis (table 1).

Methods

Specimen collection and testing

All participants were from the GP, KZN, OFS, EC and WC ANSUR surveys conducted in 2011. HIV-1 positive specimens from primigravid women age <21 years or <25 years (if required) were selected for genotypic analysis.[2] Genotypic resistance was defined as the presence of resistance mutations using the Stanford Calibrated Population Resistance (CPR) algorithm Version 6.0.[6,7] Sequences were ordered according to date of collection and prevalence classification assigned as per the recommended WHO method.[2] If no surveillance drug resistance mutations (SDRM) were found within the first 34 specimens, prevalence was classified as <5%. If resistance was detected, then 47 sequences were evaluated and if the number of sequences with relevant resistance mutations was between 2 or 8, the prevalence of TDR was classified as 5-15%. Ethical approval for drug resistance testing was obtained from the University of the Witwatersrand Human Research Ethics Committee.

Results

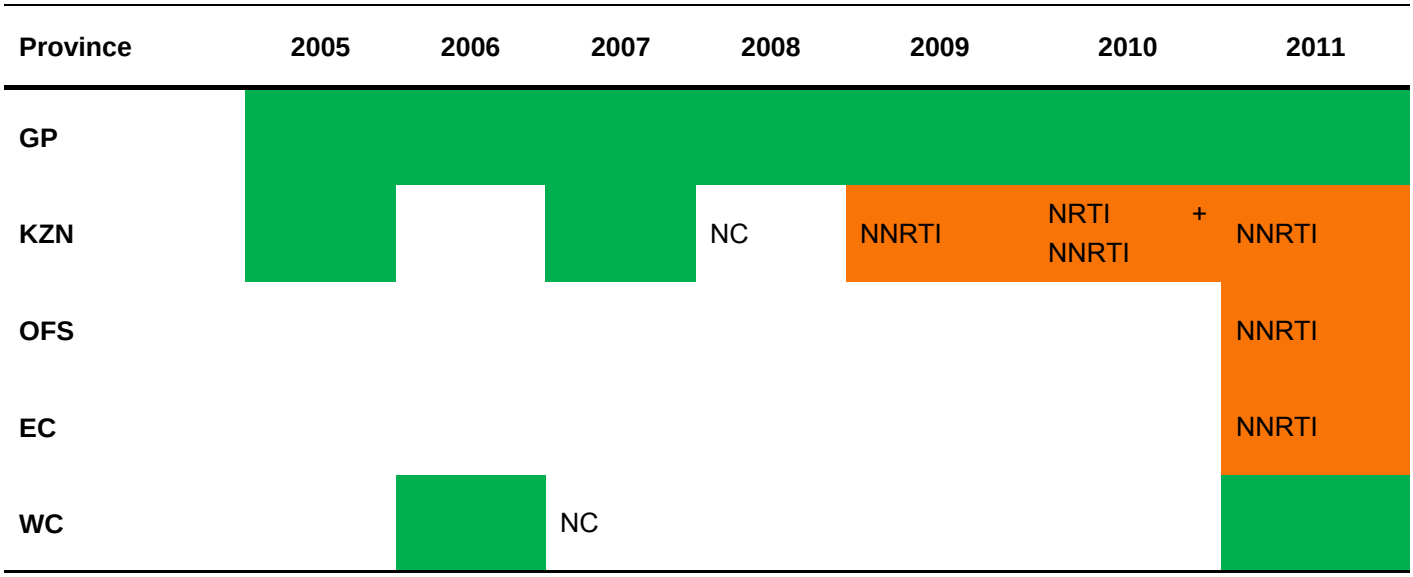
Classification of Threshold Survey (TS) sequence data

Around 100 specimens were collected each from the GP, WC, EC and OFS surveys, while 245 specimens were collected from the KZN 2011 survey due to previously poor PCR amplification rates. The TDR prevalence rates for 2011 by province are:

- GP2011: 2 sequences contained SDRM. One specimen had the M46I protease mutation, and a second contained the nucleoside reverse transcriptase inhibitor (NRTI) mutations D67N and K219N in addition to the NNRTI mutation Y181C. As the number of sequences with relevant resistance mutations to each drug class was one, the prevalence of TDR was low (<5%) for all drug classes.

- KZN2011: 2 specimens contained the NNRTI mutation K103N. The TDR prevalence rate for this province was low for the protease inhibitor (PI) and NRTI drug classes and 5-15% (moderate) for the NNRTI drug class.
- OFS2011: 2 specimens contained NNRTI mutations (Y188L and P225H in 1 sequence, and K103N and G190A in the second). One sequence contained the PI mutation I47A. The TDR rates for this province are <5% for PI and NRTI and 5-15% (moderate) for NNRTI.
- EC2011: 2 specimens contained NNRTI mutations (K103N and G190A). The TDR prevalence rate for this province was low for PI and NRTI, and moderate for NNRTI.
- WC2011: 1 specimen contained K103N, consequently the TDR rate was <5% (low) for all drug classes.

Table 1: Prevalence classification of transmitted drug resistance (TDR) in selected provinces of South Africa as per the WHO recommended method of using annual antenatal survey (ANSUR) specimens, 2005 – 2011.



Green: Low (<5%) prevalence classification of HIV TDR; Orange: moderate (5-15%) prevalence classification of TDR. NC: not classifiable. GP = Gauteng, KZN = KwaZulu-Natal, OFS = Orange Free State, EC = Eastern Cape, WC = Western Cape. NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor.

Modifications to survey sampling strategy and data analysis

Following concerns in the survey design regarding the small number of specimens analyzed and the possibility that the analysis was over-estimating TDR levels, the WHO recently modified the TDR survey analysis sampling plan and strategy to collect and sequence all available remnant specimens from primagravid patients age <25; preferably <22 years, and to determine a point prevalence with 95% confidence intervals to estimate levels of TDR in a country. The data from 2011 was analyzed according to the new modifications (table 2).

In all five provinces the point prevalence TDR estimates were <5% for both the NRTI and NNRTI drug classes. However, due to the relatively low number of specimens analyzed, the confidence intervals were wide and reached the upper limit of ~10%. The point prevalence estimates for NNRTI TDR in KZN, OFS, EC and WC were higher than for GP and for the NRTI drug class, consistent with previous analysis using the previous method.

Discussion

In Gauteng province the levels of transmitted resistance continued to remain low (<5%) for all drug classes. However, levels of transmitted resistance were shown to be moderate (5-15%) for the NNRTI drug class in KZN, OFS and EC. Whilst calculating point prevalence by province has produced figures that are lower than

previously estimated, the confidence intervals indicate that the prevalence of TDR in all five provinces should be classified as low to moderate.

These surveys are limited by sub-optimal PCR amplification rates of specimens tested, probably due to inadequate storage of the serum specimens leading to sub-optimal preservation of viral RNA which is used for resistance testing. Although rates of PCR amplification have improved in recent years, particular care and attention to the preservation of specimens for resistance testing is encouraged.

The data presented here suggest that transmission of NNRTI resistant viruses is occurring in a number of provinces in South Africa. However, these data must be treated with caution and ongoing vigilance is required. Surveys such as these need to be interpreted in conjunction with systematic assessment of the antiretroviral therapy (ART) delivery program in order to minimize the selection of drug resistant viruses and subsequent transmission to newly infected individuals.

Moving forward, this analysis needs to be expanded to all provinces in South Africa. Plans are in place to sequence specimens collected from across the country in order to provide national and provincial TDR prevalence estimates.

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Table 2: Transmitted drug resistance (TDR) sequence data by province, South Africa, from the 2011 ANSUR surveys analyzed using previous and new WHO recommended methods.

Province	# sequences with NRTI mutations	# sequences with NNRTI mutations	NRTI threshold level	NNRTI threshold level	# sequences with NRTI mutations	# sequences with NNRTI mutations	Mutations detected	NRTI Point prevalence (95%CI)	NNRTI Point Prevalence (95%CI)
GP	1/47	1/47	<5%	<5%	1/54	1/54	D67N+K219N+ Y181C	1.9% (0.3 – 10.1%)	1.9% (0.3 - 10.1%)
KZN	0/47	2/47	<5%	5-15%	0/62	2/62	K103N; K103N	0	3.2% (0.9 – 11.0%)
OFS	0/47	2/47	<5%	5-15%	0/67	2/67	Y188L+P225H; K103N+G190A	0	3.0% (0.8 – 10.3%)
EC	0/47	2/47	<5%	5-15%	1/85	3/85	K103N; G190A; K65R+K103N	1.5% (0.3 – 8.0%)	3.5% (1.2 – 9.9%)
WC	0/47	1/47	<5%	<5%	2/79	3/79	K103N; M41L; K103N; K65R+Y181C	2.6% (0.7 – 9.0%)	3.9% (1.3 – 10.9%)
Prevalence classification using the previous denominator of 47 specimens: if the number of sequences with relevant resistance mutations is between 2 or 8, the prevalence of TDR is classified as 5-15%.					**Prevalence classification using the new, modified approach to sequence as many specimens as possible and estimate the point prevalence with 95% confidence intervals.**				

GP = Gauteng, KZN = KwaZulu-Natal, OFS = Orange Free State, EC = Eastern Cape, WC = Western Cape. = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor.

Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD, South Africa, for the corresponding periods 1 January - 30 September 2012/2013*

Disease/Organism	1 Jan to 30 Sep, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Botulism	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2012	892	254	1579	1531	152	305	56	248	506	5523
	2013	563	194	1571	1363	111	266	40	202	483	4793
Haemophilus influenzae, invasive disease, all serotypes	2012	29	15	83	36	1	9	4	5	63	245
	2013	19	13	92	38	1	10	4	3	82	262
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2012	2	5	14	3	1	3	2	2	9	41
	2013	3	1	9	4	0	0	0	0	4	21
Serotypes a,c,d,e,f	2012	1	0	3	0	0	1	0	0	5	10
	2013	0	1	5	0	0	0	1	0	6	13
Non-typeable (unencapsulated)	2012	0	1	18	3	0	0	0	0	7	29
	2013	0	1	11	2	0	0	1	1	14	30
No isolate available for serotyping	2012	6	2	9	5	0	3	1	1	4	31
	2013	1	4	24	9	0	6	1	0	11	56
Measles	2012	0	1	3	5	1	0	0	1	1	12
	2013	1	0	0	1	0	0	0	1	0	3
Neisseria meningitidis, invasive disease	2012	31	9	66	20	2	3	0	7	37	175
	2013	30	11	48	32	2	3	2	4	37	169
Novel Influenza A virus infections	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Plague	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Rabies	2012	1	1	0	4	3	1	0	0	0	10
	2013	0	2	0	1	3	1	0	0	0	7
Salmonella spp. (not typhi), invasive disease	2012	29	13	250	96	4	23	10	5	82	512
	2013	32	15	215	98	5	28	5	4	117	519
Salmonella spp. (not typhi), isolate from non-sterile site	2012	153	24	461	192	8	49	13	11	269	1180
	2013	154	58	734	232	9	103	13	42	398	1743
Salmonella typhi	2012	2	0	16	9	0	3	0	0	12	42
	2013	1	1	21	10	0	10	0	1	12	56
Shigella dysenteriae 1	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non Sd1)	2012	193	43	447	171	3	19	18	5	309	1208
	2013	196	64	525	221	12	55	12	23	222	1330
Streptococcus pneumoniae, invasive disease, all ages	2012	236	164	1040	449	53	123	33	104	320	2522
	2013	223	132	742	349	42	86	62	90	352	2078
Streptococcus pneumoniae, invasive disease, < 5 years	2012	43	25	185	71	4	13	4	15	38	398
	2013	33	28	173	52	5	9	3	27	51	381
Vibrio cholerae O1	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	1	0	0	0	0	1
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	2	0	0	0	2	0	1	0	5
Other VHF (not CCHF)	2012	0	0	0	0	0	0	0	0	0	0
	2013	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.
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, for the corresponding periods 1 January - 30 September 2012/2013*

Programme and Indicator	1 Jan to 30 Sep, 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	2012	49	17	46	53	32	36	3	16	22	274
	2013	44	13	46	58	36	26	5	20	30	278

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

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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