RESPIRATORY PATHOGENS FROM INFLUENZA-LIKE ILLNESS AND PNEUMONIA SURVEILLANCE PROGRAMMES, SOUTH AFRICA 2014

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Introduction and methods

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. Surveillance was initially conducted in three of South Africa's provinces: Gauteng - Chris Hani-Baragwanath Hospital (CHBH) (this site stopped enrolling patients in December 2013 and was replaced by the Helen Joseph and Rahima Moosa hospital Complex (RMMCH/HJ) in July 2014); KwaZulu-Natal - Edendale Hospital (EDH); and Mpumalanga - Matikwana and Mapulaneng Hospitals (Matikwana/Mapulaneng). This programme has been described previously.[1] Patients were enrolled based on standardised clinical case definitions (table 1). 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. At the same time, the case definition was expanded at KTHC and Edendale Hospital (enhanced surveillance sites) to include cases with severe respiratory illness irrespective of symptom duration, and patients with a clinician admission diagnosis of suspected tuberculosis (TB). This expanded case definition was termed severe respiratory illness (SRI). In 2012, the surveillance was further expanded at the two enhanced surveillance sites (Edendale and KTHC) to include expanded testing of specimens (naso- and oropharyngeal swabs and aspirates) for additional pathogens and collection of additional specimens (induced sputum and oral washes) from patients with SRI (table 2). Also in 2012, the NICD initiated a programme of systematic influenza-like illness (ILI) surveillance at public health clinics. Two primary health care clinics serviced by the two enhanced SRI surveillance sites (Edendale and KTHC) commenced systematically enrolling patients with ILI. Dedicated staff screened and enrolled patients from Monday to Friday each week.

The Viral Watch sentinel surveillance programme, which started in 1984, was specifically designed to monitor influenza activity and has been fully described previously.[2] Participation in the programme is voluntary, and it is mainly composed of general practitioners who are requested to submit specimens from patients with influenza-like illness. During 2014, 171 practitioners registered across South Africa submitted specimens throughout the year. Clinical case definitions for the surveillance programmes are described in table 1 and types of specimens collected and pathogens tested for are described in table 2.

Table 1: Case definitions by age group and surveillance site/programme for the clinical syndromes included in the influenza-like illness and pneumonia surveillance programmes, South Africa, 2014.

Case definition	Criteria	Surveillance site/programme
Influenza-like illness (ILI)	**Patients of all ages** Acute fever of ≥38° Celsius and/or self-reported fever within the last 10 days AND Cough The absence of other diagnoses	Viral watch programme Public health clinic ILI surveillance at Jouberton (Klerksdorp) and Edendale Gateway clinics
Severe acute respiratory illness (SARI) Patient presenting within 10 days of the onset of illness	**2 days-<3 months** Any child hospitalised with diagnosis of suspected sepsis or physician diagnosed lower respiratory tract infection (LRTI) irrespective of signs and symptoms.	EDH, KTHC Complex, Matikwana/ Mapulaneng, RMMCH/HJ
	3 months-<5 years Any child ≥3 months to <5 years hospitalised with physician-diagnosed LRTI including bronchiolitis, pneumonia, bronchitis and pleural effusion.	
	≥5 years Any person hospitalised with an acute respiratory infection with fever (≥38ºC) or history of fever AND cough.	
Severe chronic respiratory illness (SCRI)	Any child or adult meeting the above case definitions presenting with symptom duration >10 days or Any patient with a clinical diagnosis of suspected pulmonary TB AND not meeting any of the above criteria	RMMCH/HJ, EDH, KTHC
Severe Respiratory illness (SRI)	Anyone who meet either SARI or SCRI definitions at RMMHC/HJ, EDH and KTHC hospitals	RMMCH/HJ, KTHC, EDH,

EDH = Edendale Hospital, KTHC = Klerksdorp-Tshepong Hospital Complex, RMMHC/HJ = Helen Joseph and Rahima Moosa Hospital Complex

Table 2: Pathogens tested for by clinical syndrome, surveillance site, type of specimen collected and test conducted - influenza-like illness and pneumonia surveillance, South Africa, 2014.

Pathogen	Programme (syndrome)	Surveillance site	Specimen collected	Test conducted
Influenza and respiratory syncytial virus	Viral watch (ILI)	All Viral watch sites in 9 provinces	Nasopharyngeal (NP) and oropharyngeal (OP) flocked swabs	Multiplex Real-time reverse transcription polymerase chain reaction (RT-PCR)
	Systematic ILI surveillance (ILI)	Edendale Gateway Clinic and Jouberton clinic Klerksdorp	NP and OP flocked swabs > 5 years Nasopharyngeal aspirates (NPA) in children ≤5 years of age	
	Pneumonia surveillance (SARI and SCRI)	EDH, KTHC, Matikwana/Mapulaneng	NP and OP flocked swabs > 5 years. NPA ≤5 years Induced sputum (IS), all ages.	
Human metapneumovirus, Parainfluenza viruses, 1, 2 and 3	Systematic ILI surveillance (ILI)	Edendale Gateway Clinic and Jouberton clinic Klerksdorp	NP and OP flocked swabs > 5 years NPA ≤5 years	Multiplex RT- PCR
	Pneumonia surveillance (SARI and SRI)	EDH, KTHC, Matikwana/Mapulaneng RMMCH/HJ	NP and OP flocked swabs > 5 years NPA ≤5 years	
Bordetella pertussis, Mycoplasma pneumoniae, Legionella spp, Chlamydophila pneumonia	ILI	Edendale Gateway Clinic and Jouberton clinic Klerksdorp	NP and OP flocked swabs > 5 years NPA ≤5 years	Real Time -PCR
	Pneumonia surveillance (SRI)	EDH, KTHC	NP and OP flocked swabs > 5 years NPA ≤5 years Induced sputum	
Streptococcus pneumoniae	SARI, SCRI	EDH, KTHC, Matikwana/Mapulaneng, RMMHC/HJ	Whole blood	PCR for *Lyt A*
Tuberculosis	SRI	EDH, KTHC	Induced or expectorated sputum	GeneXpert and culture
Pneumocystis jirovecii	Pneumonia surveillance (SRI)	EDH, KTHC	Oral washes, Nasopharygeal swabs/ aspirates Induced or expectorated sputum	Real time-PCR

ILI = influenza like illness, SRI = severe respiratory illness, SARI = severe acute respiratory illness, SCRI = severe chronic respiratory illness, EDH = Edendale Hospital, KTHC = Klerksdorp-Tshepong Hospital Complex, RMMHC/HJ = Helen Joseph and Rahima Moosa hospital Complex

The primary objective of the pneumonia and systematic ILI surveillance programmes is to describe the burden and aetiology of outpatient ILI and inpatient severe respiratory illness in HIV-infected and HIV-uninfected children and adults in selected sites in South Africa. This report presents the findings from these surveillance programmes for 2014 for the following pathogens: influenza, respiratory syncytial virus (RSV), human metapneumovirus (hPMV), parainfluenza viruses, 1, 2 and 3 (PIV1-3), *Streptococcus pneumoniae, Bordetella pertussis* and atypical bacterial causes of pneumonia (*Legionella species, Chlamydia pneumoniae* and *Mycoplasma pneumoniae*), tuberculosis and *Pneumocystis jirovecii* (PCP). Data from this

surveillance programme for 2013 were reported in the March 2014 and November 2014 editions of the Communicable Diseases Surveillance Bulletin. [3, 4]

Sample collection and processing

Upper respiratory tract specimens (oropharyngeal - OP, nasopharyngeal - NP and nasopharyngeal aspirates - NPA) were collected in viral transport medium. Whole blood specimens were collected in EDTA containing tubes. Oral washes and sputum were collected in universal containers. Following collection, upper 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. Sputum samples were stored separately at -20^{0}C at the local laboratory before being transported to the NICD on dry ice on a weekly basis. One sputum sample was tested at the surveillance site laboratory for *M. tuberculosis* using GeneXpert and a second sample was tested at the NICD for *M. tuberculosis* by culture, as well as for PCP and bacterial pathogens by PCR (table 2).

Detection of viral pathogens

Respiratory specimens were tested by multiplex real-time reverse-transcription PCR assay for 10 respiratory viruses (influenza A and B viruses, parainfluenza virus 1, 2 and 3; respiratory syncytial virus; enterovirus; human metapneumovirus; adenovirus and rhinovirus). Influenza positive specimens were subtyped using the US Centers for Disease Control and Prevention (CDC) real-time reverse-transcription PCR protocol for characterisation of influenza virus.[5] *Streptococcus pneumoniae* was identified by quantitative real-time PCR detecting the lytA gene from whole blood specimens.

Detection of bacterial pathogens other than tuberculosis

Induced sputum and nasopharyngeal samples were tested for *M. pneumoniae*, *C. pneumoniae, Legionella* spp. and *B. pertussis.* DNA was extracted from the clinical specimens and tested for bacterial pathogens by RT-PCR. 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).[6] A positive result for pertussis was obtained when a specimen was positive for *IS481* and/ or *ptxS*1 genes.[7] Blood specimens were tested using quantitative real-time PCR for the presence of pneumococcal DNA (*lyt*A gene). For *lytA* testing, specimens with a *lytA* Ct-value <40 were considered positive.[8]

Detection of tuberculosis

Tuberculosis testing at the site laboratory was based on the GeneXpert System (Cepheid, Sunnyvale, CA) using the cartridge-based Xpert MTB/RIF (Xpert) assay.[9] Tuberculosis microscopy for acid-fast bacilli was conducted for some patients. All induced sputum specimens were also tested for *M. tuberculosis* by smear for acid-fast bacilli and 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 MTBDRplus v2 assay.[10]

Detection of Pneumocystis jirovecii (PCP)

Pneumocystis jirovecii was tested for on one or more of the following specimens from each patient- oral wash, naso/oropharyngeal sample and induced sputum. DNA was extracted from the clinical specimens using an automated DNA extraction system. Fungal load was determined using a quantitative real-time PCR targeting the region coding for the mitochondrial large subunit rRNA for *P. jirovecii*.[11] All specimens with copy numbers >0 copies/µl were included as positive. These include both cases of infection and colonisation with *P. jirovecii*.

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.

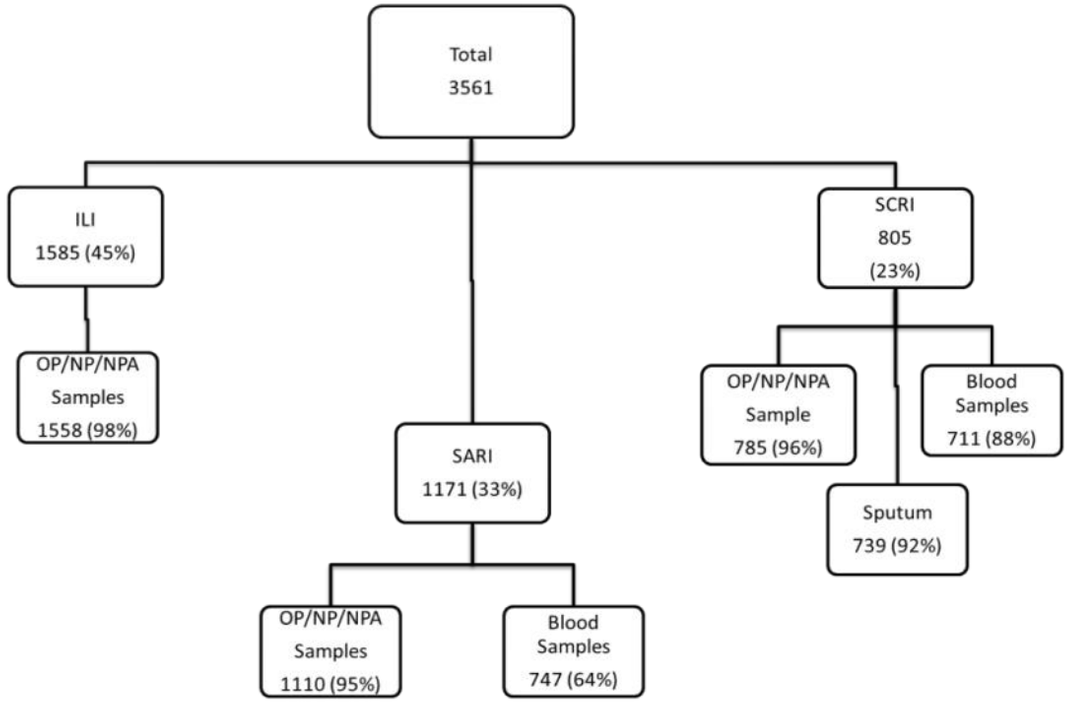
Ethical considerations

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

Results

Of the 3693 patients enrolled into the surveillance programme, 3561 had full data on case definition available, 1585 (45%) fitted the case definition of ILI, 1171 (33%) were diagnosed with SARI and 805 (23%) were diagnosed with SCRI. The demographic characteristics of patients enrolled into the programme are described in table 3. The number of samples tested for each of these groups of patients depended on the samples available and suitable for testing. The number of samples tested for each case definition and pathogen are detailed in figure 1.

Figure 1: Numbers of samples collected by case definition in the influenza like illness and pneumonia surveillance programmes, South Africa, 2014.



ILI = influenza like illness, SARI = severe acute respiratory illness, SCRI = severe chronic respiratory illness, OP=Oropharyngeal, NP= Nasopharyngeal, NPA= nasopharyngeal aspirate

The HIV prevalence varied by case definition and age group; the overall prevalence was lowest in the SARI group (147/638, 23% vs 387/1424, 27% in the ILI group), likely driven by the high numbers of young children in the SARI group. HIV prevalence was highest in the SCRI group (522/723, 72%) and was highest in the 25-44 age group across all case definitions (ILI 236/403, 59%; SARI 51/58, 88%; SCRI 313/337, 93%) (figure 2).

Table 3: Demographic and clinical characteristics of patients with an upper respiratory sample available for testing and enrolled into the systematic influenza-like illness and pneumonia surveillance programmes, South Africa, 2014.

Characteristic	**Influenza-like illness n/N (%) N=1585**	**Severe acute respiratory illness n/N (%) N=1171**	**Severe chronic respiratory illness n/N (%) N=805**
Age group years			
0-4	525/1584 (33)	827/1165 (70)	48/800(6)
5-14	231/1584 (14)	39/1165 (3)	14/800 (2)
15-24	187/1584 (11)	28/1165 (2)	53/800 (7)
25-44	424/1584 (27)	147/1165 (13)	375/800 (47)
45-64	180/1584 (11)	91/1165 (8)	243/800 (30)
≥ 65	37/1584 (2)	33/1165 (30)	67/800 (8)
Female gender	1019/1582 (64)	607/1168 (48)	390/803 (49)
Site			
Edendale Gateway clinic	1025/1585 (67)	N/A	N/A
Jouberton clinic	560/1585 (33)	N/A	N/A
EDH	N/A	324/1171 (28)	204/805 (55)
KTHC	N/A	248/1171 (21)	601/805 (75)
Mapulaneng/Matikwana hospitals	N/A	246/1171 (23)	N/A
RMMCH/HJ	N/A	353/1171 (30)	N/A
In hospital case-fatality ratio	N/A	35/1095 (3)	95/792 (12)

EDH= Edendale Hospital, KTHC= Klerksdorp-Tshepong hospital complex, RMMCH/HJ= Rahima Moosa Mother and Child Hospital/Helen Joseph Hospital

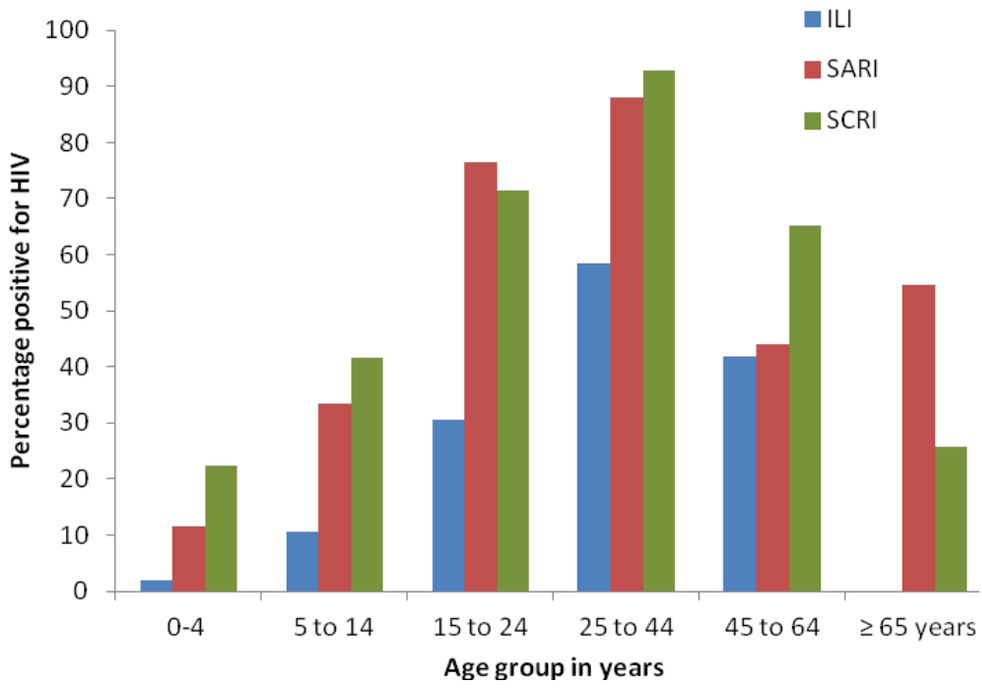


Figure 2: HIV prevalence by age group for case definitions of influenza-like illness (ILI), severe acute respiratory illness (SARI) and severe chronic respiratory illness (SCRI), among patients enrolled in pneumonia and systematic ILI surveillance, South Africa, 2014.

Pneumonia surveillance programme results

Viral pathogens (SARI and SCRI)

Of individuals tested for influenza 4% (74/1895) were positive. The season was dominated by influenza A (H3N3) (46/74, 62%) followed by influenza B (25/74, 34%) and influenza A (H1N1)pdm09 (3/74, 4%). The season started in week 30 and continued through week 33. The peak detection rate was 26% in week 33 (figure 3).

The detection rate for RSV was 11% (205/1689). The RSV season preceeded the influenza season, started in week 4 and continued through week 19. The peak detection rate of 52% was in week 10 (figure 4). Parainfluenza viruses 1-3 were detected in 5% (98/1894) of samples and hMPV in 3% (56/1894) of samples. In the same group of patients *S. pneumoniae* was detected in 15% (212/1458) of blood samples (figure 5). Across these pathogens the highest number of cases were in the <5 year age group (influenza 35/73 (48%), RSV 184/205 (90%), PIV1-3 76/97 (78%), hMPV 42/56 (75%) and *S. pneumoniae* 72/211 (34%). The case fatality ratio was highest for patients testing positive for *S. pneumoniae* 18/206 (9%), table 4.

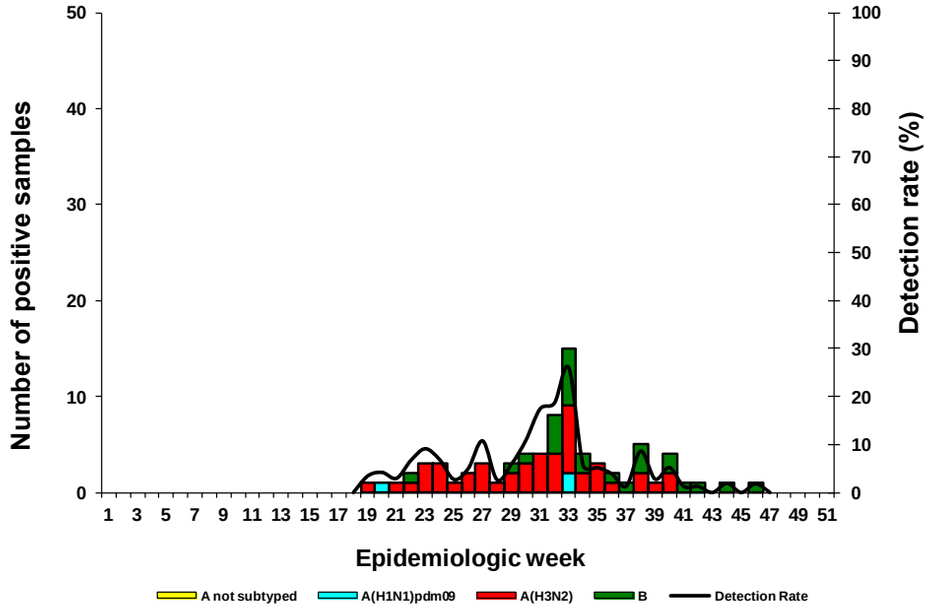


Figure 3: Numbers of samples positive for influenza and influenza detection rate, by subtype and week, in patients enrolled into the pneumonia surveillance fitting the case definition of severe acute respiratory illness (SARI) or severe chronic respiratory illness (SCRI) in South Africa, 2014.

Table 4: Characteristics of patients who tested positive for viral pathogens and *Streptococcus pneumoniae* amongst those patients with severe acute respiratory illness (SARI) or severe chronic respiratory illness (SCRI).

	Influenza n/N(%)	RSV n/N(%)	PIV 1-3 n/N(%)	hMPV n/N(%)	*S. pneumoniae* n/N(%)
Age group, years					
0-4	35/73 (48)	184/205(90)	76/97 (78)	42/56 (75)	72/211 (34)
5-14	2/73 (3)	2/205 (1)	3/97 (3)	0	4/211 (2)
15-24	5/73 (7)	3/205 (1)	4/97 (4)	0	10/211 (5)
25-44	12/73 (16)	7/205 (3)	8/97 (8)	7/56 (13)	71/211 (34)
45-64	13/73 (18)	4/205 (2)	5/97 (5)	5/56 (9)	43/211 (20)
≥ 65	6/73 (8)	5/205 (2)	1/97 (1)	2/56 (4)	11/211 (5)
Female gender	44/73 (59)	88/205 (43)	49/98(5)	31/56 (55)	94/211 (45)
Site					
EDH	14/74(19)	89/205 (43)	33/98 (34)	18/56 (32)	66/212 (31)
KTHC	34/74 (46)	58/205 (28)	25/98 (26)	16/56 (29)	96/212 (45)
Matikwana/ Mapulneng	17/74 (23)	57/205 (28)	10/98(10)	4/56 (7)	38/212 (18)
RMMCH/HJ	9/74 (12)	1/205 (1)	30/98 (31)	18/56 (32)	12/212 (6)
In hospital case fatality ratio	1/73(1)	4/205 (2)	5/98 (5)	0	18/206 (9)

RSV= Respiratory syncytial virus, PIV= parainfluenza virus, hMPV= human metapneumovirus

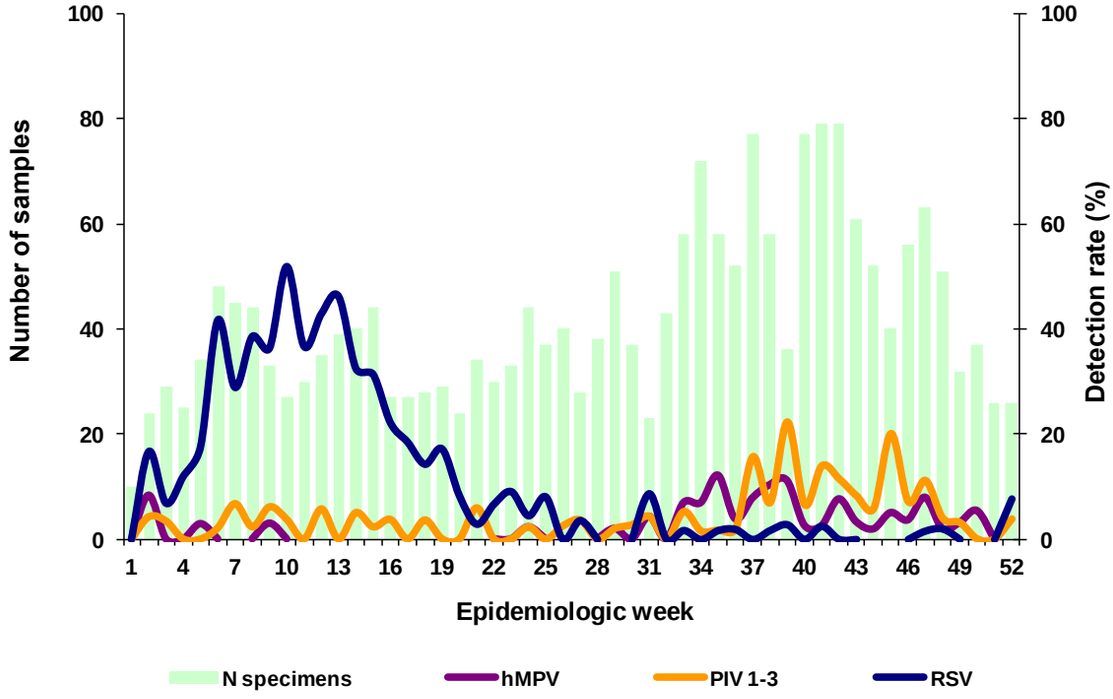


Figure 4: Numbers of samples collected and detection rates for respiratory syncytial virus (RSV), parainfluenza virus 1 -3 (PIV1-3) and human metapneumovirus (hMPV) in patients fitting the case definition for severe acute respiratory illness (SARI) or severe chronic respiratory virus (SCRI), South Africa, 2014.

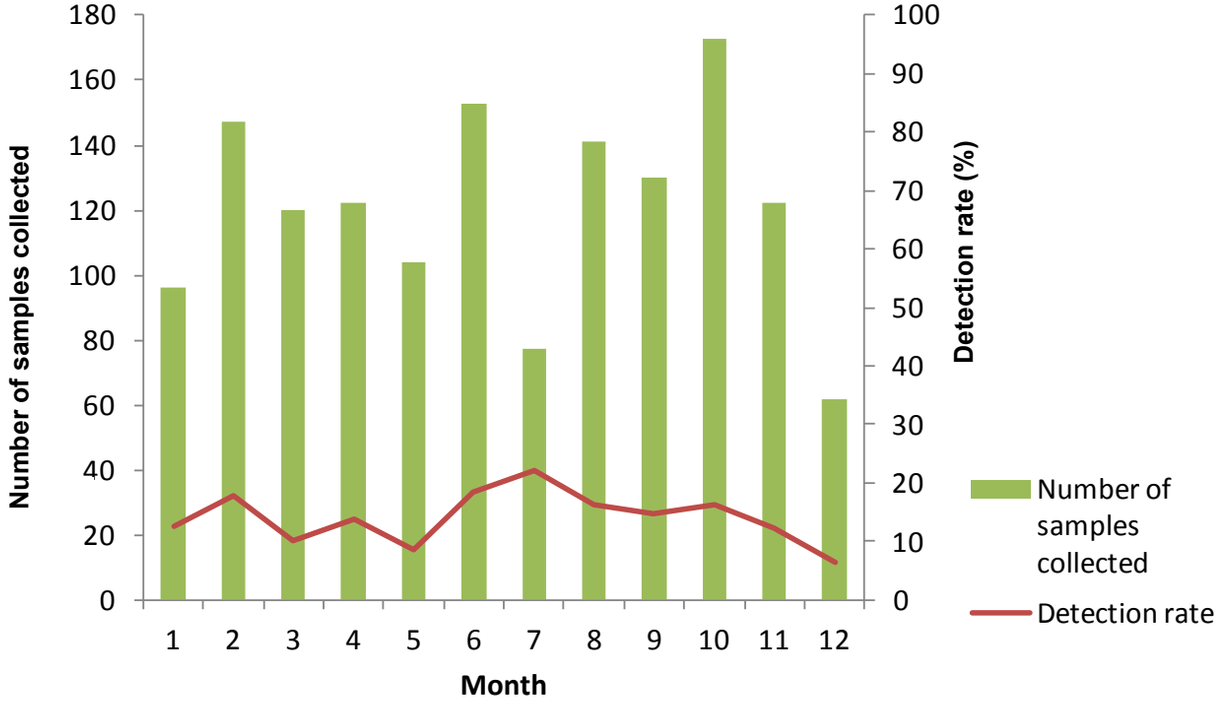


Figure 5: Numbers of blood samples collected and detection rate of *Streptococcus pneumoniae* from patients with severe acute respiratory illness (SARI) and severe chronic respiratory illness (SCRI) by month, South Africa, 2014.

Bacterial pathogens (SARI and SCRI)

Of the 1667 respiratory samples tested for *B. pertussis*, 46 tested positive (3%), 14/1662 (1%) for *M. pneumoniae*, 3/1662 (0.2%) for *C. pneumoniae* and 1/1661 (0.06%) for *Legionella spp*. The highest number of positive samples for *B. pertussis* and *M. pneumoniae* were in the <5 year and 25 to 44 year age groups (table 5). Similarly, the highest number of cases for *C. pneumonia* was in the <5 year age group. There was no clear seasonality for any of the bacterial pathogens (figure 6).

Table 5: Numbers of samples collected, detection rate and characteristics of patients fitting the severe acute respiratory illness (SARI) and the severe chronic respiratory illness (SCRI) case definition who were tested for *Streptococcus pneumoniae, Bordetella pertussis, Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella spp,* pneumonia surveillance, South Africa, 2014.

	B. pertussis **n/N (%)**	***M. pneumoniae*** **n/N (%)**	***C. pneumoniae*** **n/N (%)**	***Legionella spp*** **n/N (%)**
Age group, years				
0-4	13/45 (29)	5/14 (36)	2/3 (67)	0
5-14	2/45 (4)	1/14 (7)	0	0
15-24	2/45 (4)	2/14 (14)	0	1/1 (100)
25-44	13/45 (29)	5.14 (36)	1/3 (33)	0
45-64	11/45 (24)	1/14 (7)	0	0
≥ 65	4/45 (9)	0	0	0
Female gender	23/46 (50)	5/14 (36)	0/3	0/1
Site				
EDH	14/46 (30)	5/14 (36)	1/3 (33)	0/1
KTHC	30/46 (65)	7/14 950)	2/3 (67)	1/1 (100)
RMMCH/HJ	2/46 (4)	2/14 (14)	0/3	0/1

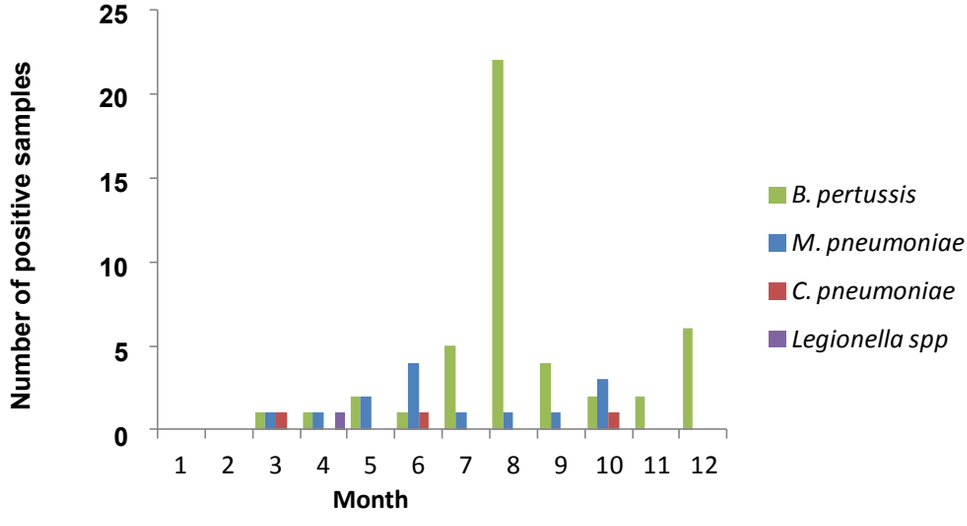


Figure 6: Numbers of positive samples of *Bordetella pertussis*, *Mycoplasma pneumoniae*, *Legionella spp* and *Chlamydia pneumonia* among patients with severe acute respiratory illness (SARI) and severe chronic respiratory illness (SCRI) by month, South Africa, 2014.

Tuberculosis and Pneumocystis jirovecii (PCP)

Of the 945 samples tested for TB, 195 (24%) were positive. Tuberculosis and PCP had no obvious seasonality (figures 7 and 8). The majority of samples that tested positive for tuberculosis were collected at the KTHC site (138/195, 71%) and were in the 25 to 44 year age group (113/223, 51%) (table 6).

Of the 266 samples that tested positive for PCP, 108 (41%) were from nasophyngeal samples, 34 (13%) were oral washes and 124 (48%) were from sputum. Half the patients with positive samples were in the age group 25 to 44 years (133/266, 50%) and the majority was from KTHC 176/266 (66%).

Table 6: Detection rate and characteristics of patients fitting the case definition of severe respiratory illness enrolled into pneumonia surveillance and testing positive for tuberculosis and *Pneumocystis jirovecii*, South Africa, 2014.

	Tuberculosis n/N(%)	***Pneumocystis jirovecii* n/N(%)**
Age group, years		
0-4	11/195 (6)	61/266 (23)
5-14	2/195 (1)	2/266 (1)
15-24	24/195 (12)	10/266 (4)
25-44	113/195 (58)	133/266 (50)
45-64	44/195 (23)	49/266 (18)
≥ 65	4/195 (2)	11/266 (4)
Female gender	111/195 (57)	145/266 (60)
Site		
EDH	57/195 (29)	90 (34)
KTHC	138/195 (71)	176 (66)

EDH= Edendale Hospital, KTHC= Klerksdorp-Tshepong hospital complex

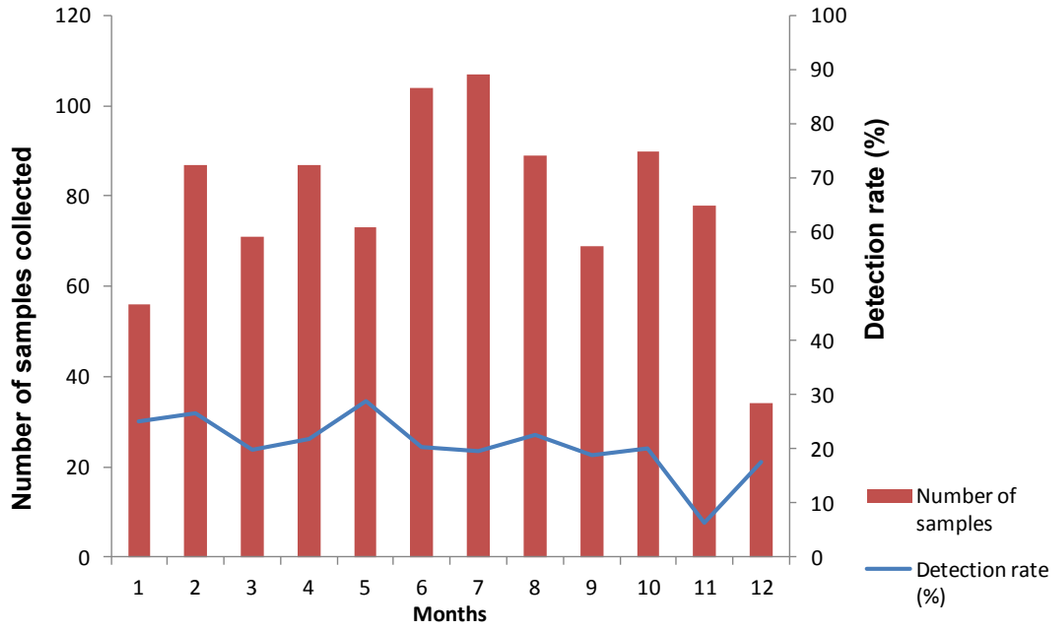


Figure 7: Numbers of samples collected for tuberculosis testing and TB detection rate for patients fitting the severe acute respiratory illness (SRI) case definition, pneumonia surveillance, South Africa, 2014.

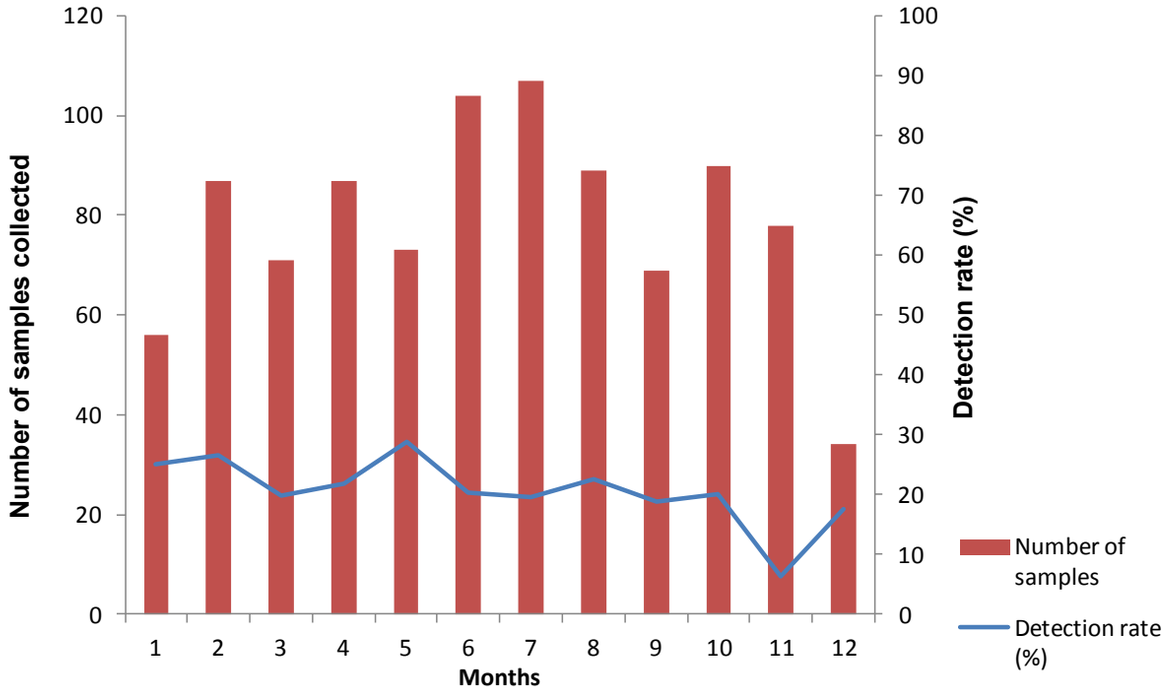


Figure 7: Numbers of samples collected for tuberculosis testing and TB detection rate for patients fitting the severe acute respiratory illness (SRI) case definition, pneumonia surveillance, South Africa, 2014.

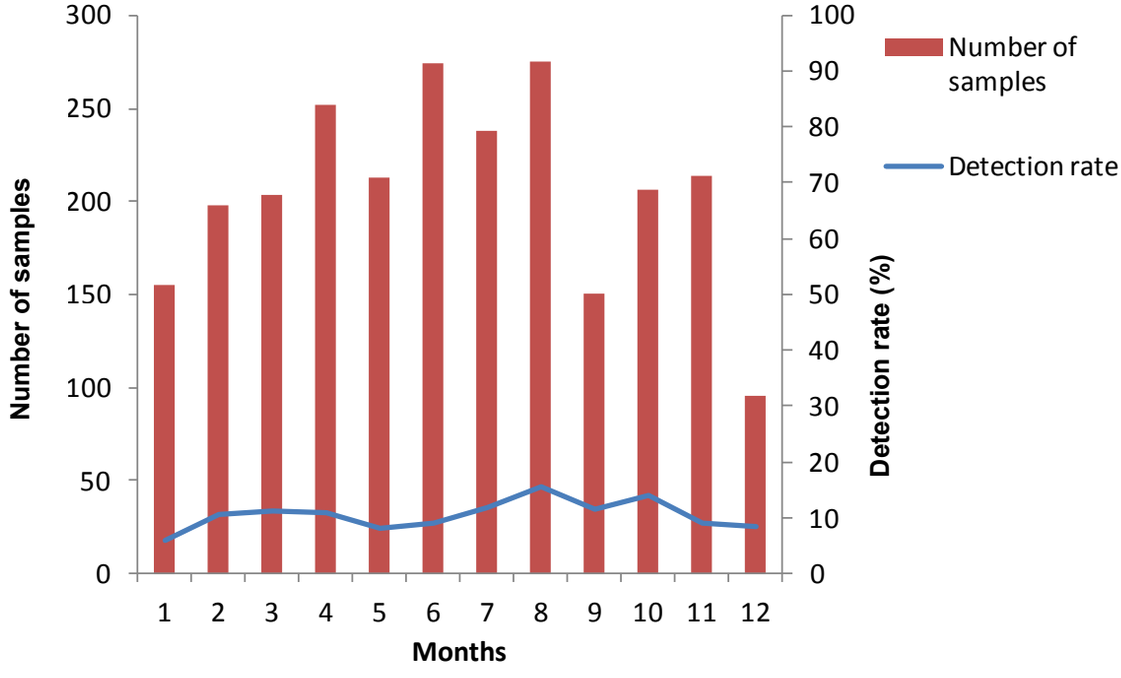


Figure 8: Numbers of samples collected for *Pneumocystis jirovecii* and detection rate for patients fitting the severe respiratory illness (SRI) case definition, pneumonia surveillance, South Africa, 2014.

Systematic ILI surveillance at public health clinics

Influenza and viral pathogens

During 2014, 1585 patients with ILI were enrolled at the two clinics and 1558 (98%) upper respiratory samples were tested. The overall detection rate for influenza was 13% (n=202). Excluding non-subtyped samples, 80% (156/195) were influenza A(H3N2), 15% (30/195) were influenza B and 5% (9/195) were influenza A(H1N1) pdm09. There were no dual infections. Influenza positive samples were detected from week 19. The detection rate reached 10% in week 24 and remained above 10% until week 38 (figure 9).

Of the 1557 samples tested, 71 (5%) tested positive for parainfluenza 1-3, 63 (4%) for RSV and 49 (3%) for human metapneumovirus. Only RSV demonstrated a defined seasonality which preceded the influenza season. The detection rate for RSV rose above 10% in week 5, peaked in week 12 and remained above 10% until week 17 (figure 10). In the group of patients with ILI: 1% (16/1463) tested positive for *B. pertussis,* 0.5% (7/1460) for *M. pneumoniae* and 0.5% (7/1460) for *C. pneumonia.* The highest numbers of positive samples for *B. pertussis* were in the <5 and 25 to 44 year age groups. Similarly, in *C. pneumonia* the highest number of cases was in the <5 year age group. The highest number of cases of *M. pneumonia* was in the 5 to 14 year age group (table 7). There were no positive tests for *Legionella spp.* There was no seasonality for bacterial pathogens (figure 11).

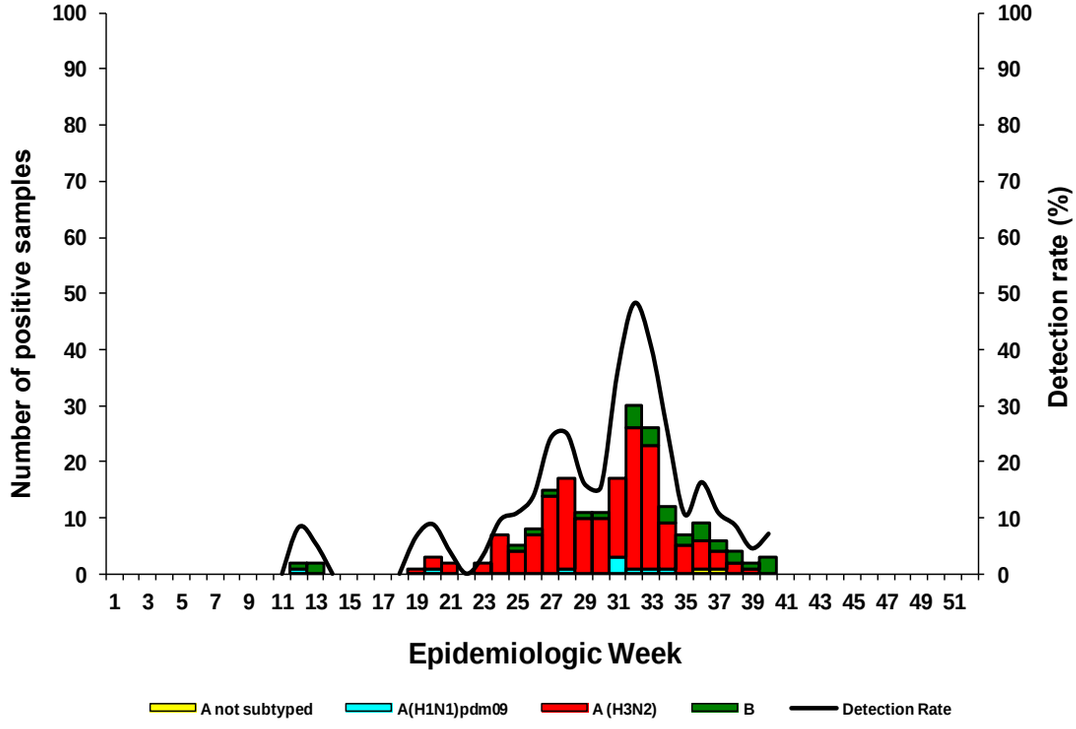


Figure 9: Influenza detection rate by subtype and week in patients with influenza-like illness (ILI) at public health clinics at two sites, South Africa, 2014.

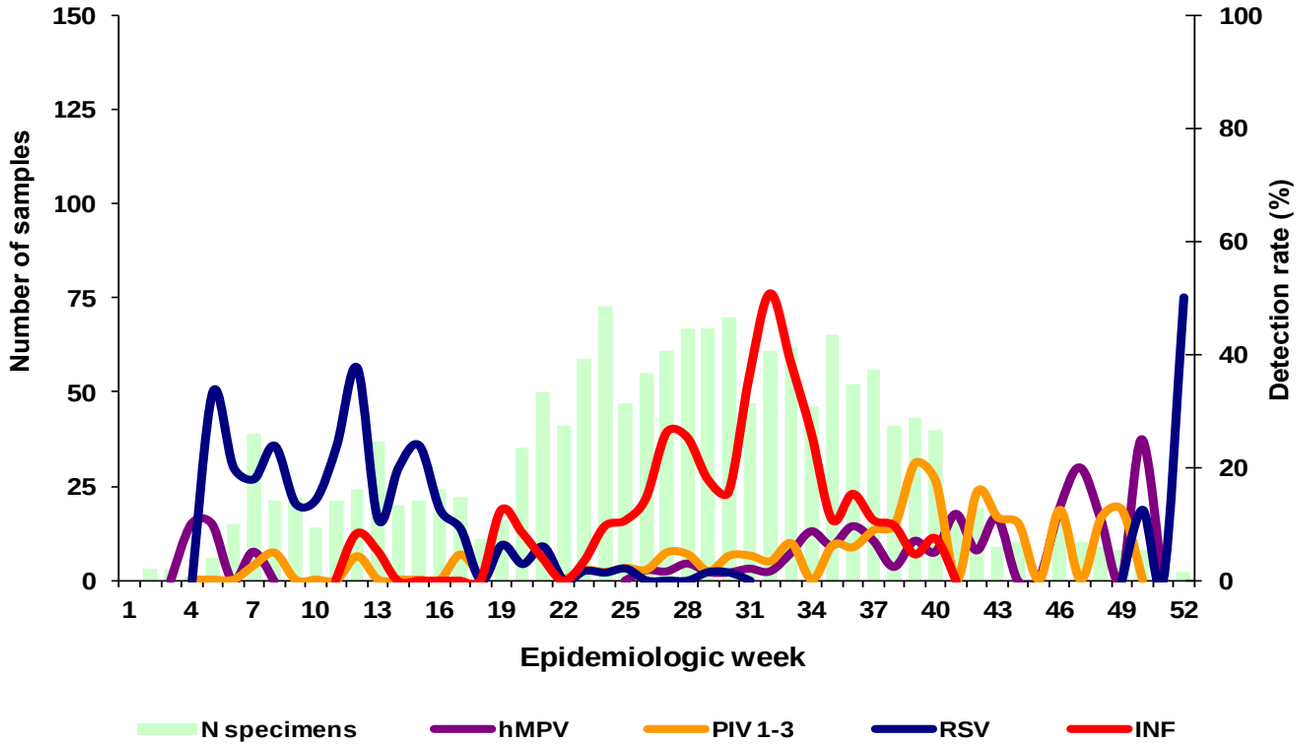


Figure 10: Detection rates of human metapneumonvirus (hMPV), parainfluenza (PIV) 1-3, respiratory syncytial virus (RSV) and influenza by week in patients fitting the influenza-like illness (ILI) case definition at public health clinics, South Africa, 2014.

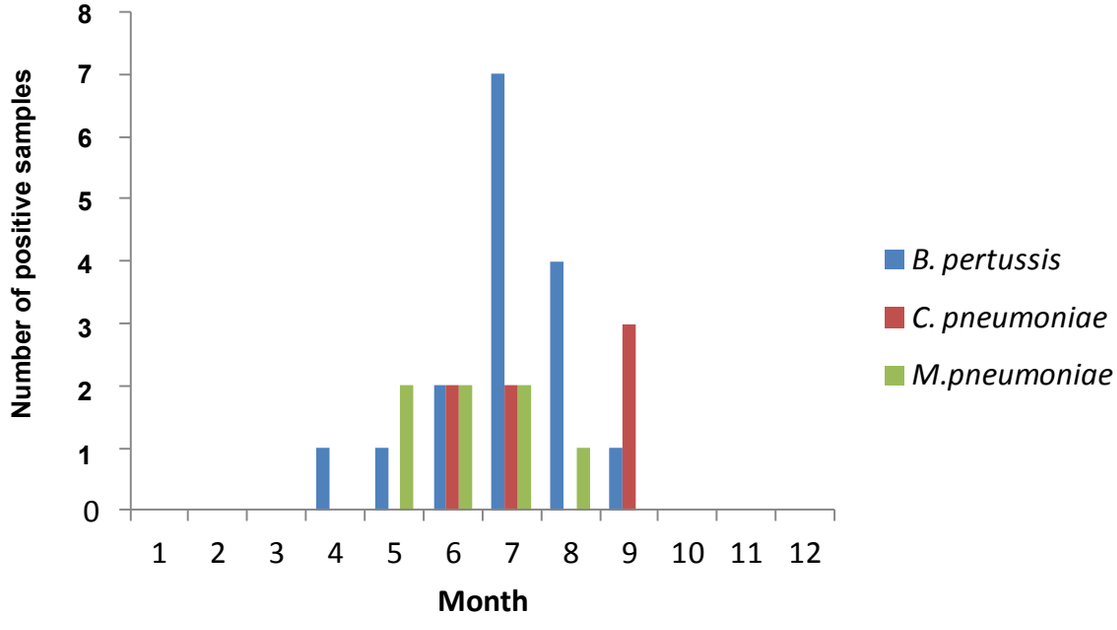


Figure 11: Numbers of positive samples of bacterial pathogens by week and pathogen, in patients fitting the influenza-like illness (ILI) case definition, South Africa, 2014.

Table 7: Detection rate and characteristics of patients with influenza-like illness who were tested for bacterial pathogens at public health clinics at two sites, South Africa, 2014.

	Bordetella pertussis **n/N (%)**	***Mycoplasma pneumoniae*** **n/N (%)**	***Chlamydia pneumoniae*** **n/N (%)**
Age group, years			
0-4	4/16 (25)	2/7 (29)	4/7 (57)
5-14	5/16 (31)	3/7 (43)	3/7 (43)
15-24	3/16 (19)	0/7	0
25-44	4/16 (25)	1 (14)	0
45-64	0/16	0 /7	0
≥ 65	0/16	1/7 (14)	0
Female gender	9/16 (56)	5/7(71)	6/7(86)
Site			
Edendale Gateway clinic	6/16 (38)	5/7(71)	5/7(71)
Jouberton clinic	10/16 (62)	2/7(29)	2/7 (29)

Additional surveillance activities

Viral watch (VW)

In 2014, 117 general practitioners across South Africa's 9 provinces participated in the VW programme. A total of 1054 samples was tested for influenza; of these 515 (49%) tested positive for influenza. The season was dominated by influenza A(H3N2) in which 351/515 (68%) of samples tested positive for influenza A(H3N2), 19% tested positive for influenza B and 12% (64/515) tested positive for influenza A(H1N1)pdm09. The season started in week 22 and the detection rate remained above 10% until week 41 (figure 12).

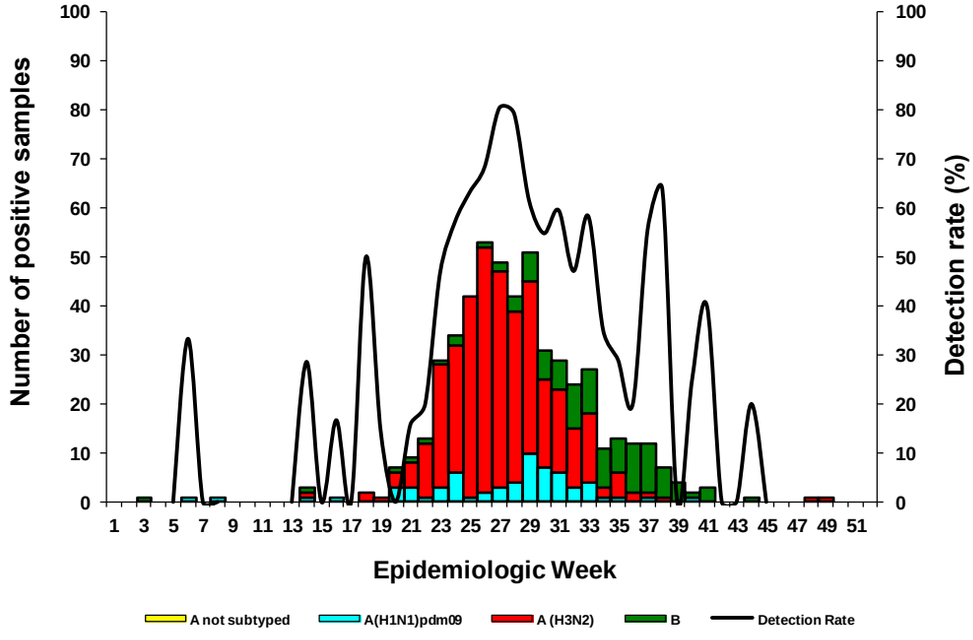


Figure 12: Numbers of samples and influenza detection rates by viral subtype and week in patients fitting the case definition of ILI enrolled into the Viral Watch programme, South Africa, 2014.

Respiratory Morbidity Surveillance

In order to describe the influence of the influenza season on the number of pneumonia and influenza hospitalizations, the NICD reviewed anonymized data from a private hospital group. The numbers of hospitalizations for pneumonia and influenza during the influenza season were compared to those for the periods preceding and following the season. During 2014 there were 672 598 consultations reported to the NICD through the respiratory morbidity data mining surveillance system. Of these, 29 558 (4%) were due to pneumonia or influenza (P&I) (International Classification of Diseases 10 codes J10-18). There were 22 059 (75%) inpatients and 7499 (25%) outpatients with P&I discharge data.

An increase in P&I consultations and admission was observed during the period with a higher number of seasonal influenza virus isolations reported to the Viral Watch, ILI and SARI surveillance programmes respectively (figures 13, 14, and cross reference figure 3; ILI influenza figure 9; ILI Viral Watch influenza figure 12). A second lower peak preceded the influenza season, corresponding to the circulation of respiratory syncytial virus (figures 13, 14 and cross reference figure 4; SARI viruses, figure 10; ILI viruses).

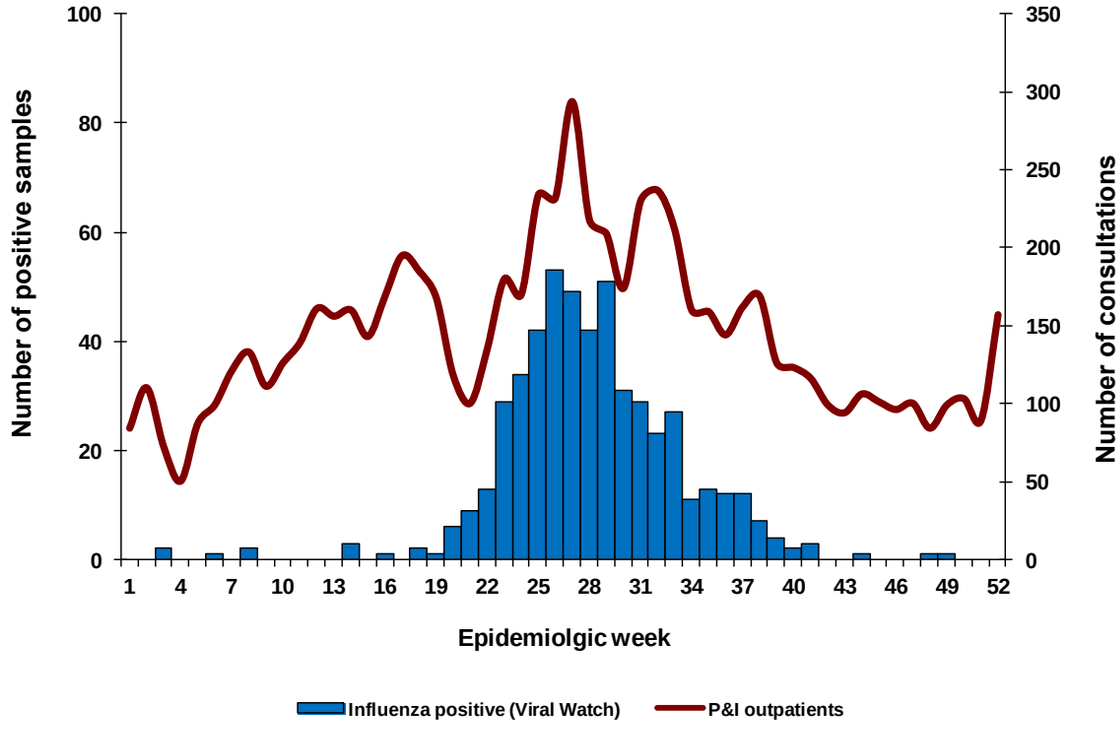


Figure 13: Numbers of private hospital outpatient consultations with a discharge diagnosis of pneumonia and influenza (P&I), and numbers of influenza positive viral isolates (Viral Watch) by week, South Africa, 2014.

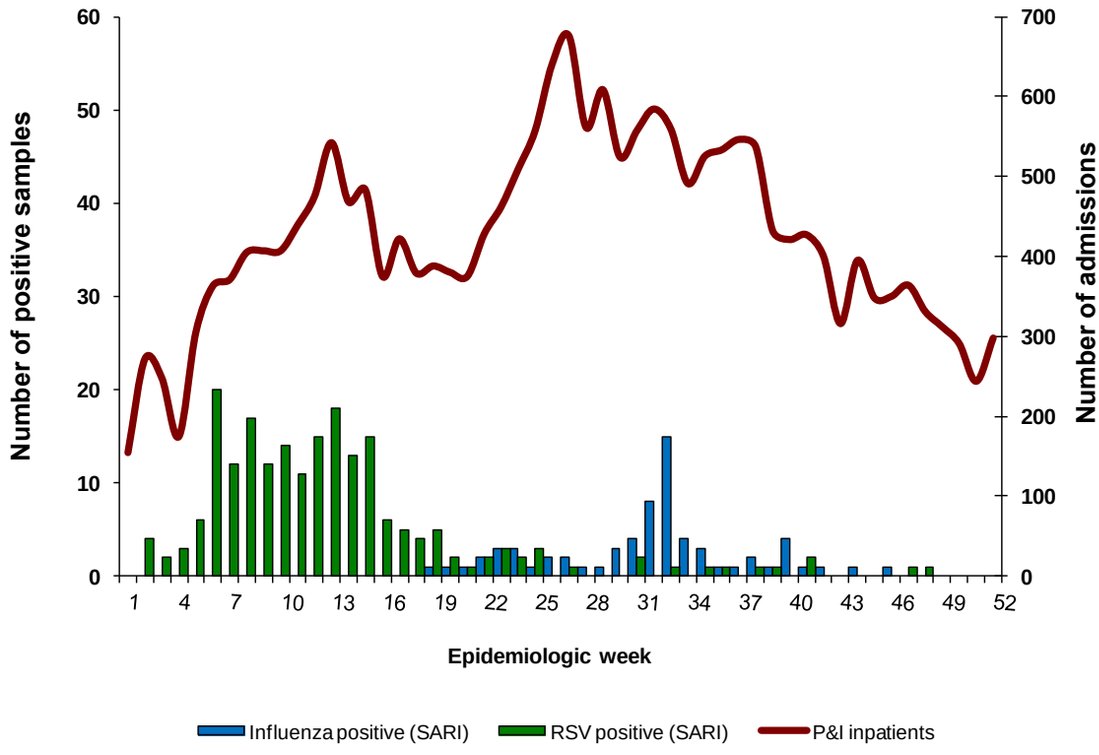


Figure 14: Numbers of admissions for pneumonia and influenza, as well as numbers of influenza positive viral isolates (Viral watch) and respiratory syncytial virus (RSV) positive isolates (SARI) by week, South Africa, 2014.

Molecular characterizations of influenza

During the influenza season of 2014, influenza virus isolation was attempted on clinical samples that tested positive for influenza on a real time multiplex PCR assay with a crossing point value of 30 or less. Madin-Darby Canine Kidney (MDCK) cells were used for virus isolations and about 67% (50/75) were successful. Of the 50 influenza virus isolations obtained, 43 were from influenza A viruses and 7 from influenza B viruses. The majority of influenza A virus isolates (n= 35) were influenza A/H3N2 which dominated the season. Compared to the 3 previous seasons this represents a great success in the isolation of H3N2 viruses in cell cultures. Changes in the phenotypic characteristics of H3N2 viruses were reported by the WHO Collaborating Centres. The embryonic egg isolations attempted were not successful. On investigating the failure of isolates, it was established that the collaborating centres recently used slightly older chicken embryos than normally used.

Antigenic characterization of influenza virus isolates

Turkey red blood cells were used in the hemagglutination and hemagglutination inhibition assays to determine the antigenic reactivity of the influenza virus isolates. A total of 46 virus isolates could be characterised antigenically by hemagglutination inhibition assay (HAI) of which 70 % (32/46) were influenza A(H3N2). Of the influenza A(H3N2) viruses serotyped 69% (22/32) showed normal reactivity to the A/Texas/50/2012 vaccine strain reference antiserum. Five influenza A(H3N2) isolates reacted with a ≥2-fold lower titre than the control or reference antiserum, 3 reacted with a 4-fold lower titre and 2 with 8-fold lower titres. All seven A(H1N1)pdm09 isolates showed normal reactivity to the corresponding reference antiserum. Seven influenza B virus isolates were characterized for reactivity to reference antisera raised against vaccine or reference antigens. Five influenza B isolates reacted to the B/Yamagata lineage reference antisera of which 1

reacted with a 2-fold lower titre. Two reacted with similar titres as the reference B/Victoria lineage strain. However, these 2 samples were collected outside of the influenza season from individuals with travel history.

Genetic characterisation of influenza A (H3N2), A (H1N1)pdm09 and influenza B strains was carried out by sequencing and phylogenetic analysis of the hemagglutinin (HA) genes. Lineages were identified by specific amino acid mutations relative to a designated reference strain as described by the WHO Vaccine Consultation Meeting team.

Influenza A(H3N2)

H3N2 HA gene sequences were generated from 35 clinical specimens selected from the 2014 season for both the ILI and SARI surveillance programs. All 2014 strains are within the genetic group 3 of the seven lineages (WHO CC, London) identified, specifically subgroup 3C.3. Subgroup 3C is characterised by the following amino acid mutations: Q33R, N145S and N278K relative to A/Perth/16/2009 as reference. One strain is in the sub-subgroup 3C.2a identified in February 2014 as one of two emerging lineages with low reactivity to the current vaccine The 2014 vaccine strain A/Texas/50/2012 is in subgroup 3C.1 (CDC nomenclature). The deduced amino acid alignment showing amino acid mutations compared to the A/ Perth/16/2009 reference strain.

The neuraminidase (NA) gene was sequenced for 30 influenza A(H3N2) positive samples and 3/30 had the mutations Y155F and D251V which divide the subgroup 3C. Two sequences showed an additional mutation at position 315 (S>G). All viruses had the N402D mutation that results in the loss of a potential N-linked glycosylation site which, together with the S367N and K369T mutations, results in a shift in potential of the N-linked glycosylation site.

In the 2014 season the HA gene from only 5 influenza A (H1N1)pdm09 positive clinical samples was sequenced. Three of these samples were in subgroup 6B as compared to 2013 when it was in 6C. Subgroups 6B and 6C are characterised by the following mutations: D97N, S185T, S203T and K283E, E3474K, S451N and E499K in HA1 and 2. The amino acid mutations K163Q and A256T characterise subgroup 6B.

Influenza B

The HA1 region of the HA genes from a total of 7 clinical samples positive for influenza B was sequenced and characterised. No B/Victoria lineage strains were sequenced as those identified were from outside of South Africa's influenza season.

B/Yamagata lineage

Seven viruses sequenced belong to clade 2 of B/ Yamagata lineage viruses in reference to the B/ Florida/4/2006 strain. This is on the deduced amino acid alignments showing the identified amino acid changes and the following are characteristic of clade 2 to which the current vaccine strain belongs: R48K, P108A and T181A.

Discussion

The influenza season in South Africa in 2014 was predominated by influenza A(H3N2), followed by influenza B and influenza A(H1N1)pdm09. The season started in week 22 at the ILI sites but the detection rate in the SARI programme only remained constantly above 10% from week 31. In 2014 some changes to the SARI surveillance programme were made - in particular the change of surveillance site in Gauteng Province. As a result of this the total numbers of samples tested for 2014 were slightly lower than previous years.

Other commonly detected pathogens in patients with pneumonia were pneumococcus, tuberculosis, RSV and PCP. Atypical bacterial pathogens and *B. pertussis* were detected in <5% of individuals. However, an increase in

B. pertussis cases was observed in July and August of 2014, followed by a reduction in case numbers without any specific intervention. The increase was investigated and contamination was excluded. The increase likely reflects winter seasonality and disease periodicity which has been reported in some countries. [12,13] A full assessment of this observation is difficult as systematic surveillance for pertusiss has only been implemented since 2012 and baseline data on pertussis epidemiology from South Africa are limited.[14,15] Ongoing systematic pertussis surveillance is needed to provide robust baseline data on disease burden and epidemiology and to monitor for future increases in disease.

The most commonly identified pathogens in patients with ILI were influenza and RSV. A total of 115 A(H3N2) positive clinical samples, mainly from patients with ILI, were tested for the presence of the E119V mutation associated with reduced susceptibility to oseltamivir by real-time RT-PCR. Only the wild-type E119 variant was detected in all samples. Representative cell culture and egg isolates as well as clinical samples were sent to the WHO Collaborating Centres in London and Australia for further characterisation.

The CRDM is working towards comprehensive surveillance for the clinical syndromes of ILI and pneumonia. This is the first report to combine the viral pathogens with the additional testing for bacterial pathogens and some of the atypical causes of pneumonia in our setting. Additional work is being done on the interaction between these pathogens and the risk factors for severe disease which will assist clinicians and policy makers to improve health care and implement prevention strategies such as vaccines.

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