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NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

# SCIENCE FOCUS

## Special Edition

Science Focus acknowledges NICD staffers who have published in peer-reviewed journals. The Science Focus is a quarterly compilation of the scientific publications included in the quarterly report submitted to the National Department of Health. It includes only publications where an NICD staff member is either the first or last author.

Prof Nazir Ismail

## Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey

***Ismail NA,*** *Mvusi L, Nanoo A, Dreyer A, Omar SV, Babatunde S, Molebatsi T, van der Walt M, Adelekan A, Dyde V, Ihekweazu C, Madhi SA.*

*The Lancet Infectious Diseases*
***Impact Factor: 25.148***


**Background:** Globally, per-capita, South Africa reports a disproportionately high number of cases of multidrug-resistant (MDR) tuberculosis and extensively drug-resistant (XDR) tuberculosis. We sought to estimate the prevalence of resistance to tuberculosis drugs in newly diagnosed and retreated patients with tuberculosis provincially and nationally, and compared these with the 2001–02 estimates.

**Methods:** A cross-sectional survey was done between June 15, 2012–June 14, 2014, using population proportionate randomised cluster sampling in the nine provinces in South Africa. 343 clusters were included, ranging between 31 and 48 per province. A patient was eligible for inclusion in the survey if he or she presented as a presumptive case during the intake period at a drug resistance survey enrolling facility. Consenting participants (≥18 years old) completed a questionnaire and had a sputum sample tested for resistance to first-line and second-line drugs. Analysis was by logistic regression with robust SEs, inverse probability weighted against routine data, and estimates were derived using a random effects model.

**Findings:** 101 422 participants were tested in 2012–14. Nationally, the prevalence of MDR tuberculosis was 2·1% (95% CI 1·5–2·7) among new tuberculosis cases and 4·6% (3·2–6·0) among retreatment cases. The provincial point prevalence of MDR tuberculosis ranged between 1·6% (95% CI 0·9–2·9) and 5·1% (3·7–7·0). Overall, the prevalence of rifampicin-resistant tuberculosis (4·6%, 95% CI 3·5–5·7) was higher than the prevalence of MDR tuberculosis (2·8%, 2·0–3·6; p=0·01). Comparing the current survey with the previous (2001–02) survey, the overall MDR tuberculosis prevalence was 2·8% versus 2·9% and the prevalence of rifampicin-resistant tuberculosis was 3·4% versus 1·8%, respectively. The prevalence of isoniazid mono-resistant tuberculosis was above 5% in all provinces. The prevalence of ethionamide and pyrazinamide resistance among MDR tuberculosis cases was 44·7% (95% CI 25·9–63·6) and 59·1% (49·0–69·1), respectively. The prevalence of XDR tuberculosis was 4·9% (95% CI 1·0–8·8). Nationally, the estimated numbers of cases of rifampicin-resistant tuberculosis, MDR tuberculosis, and isoniazid mono-resistant tuberculosis for 2014 were 13 551, 8249, and 17 970, respectively.

**Interpretation:** The overall prevalence of MDR tuberculosis in South Africa in 2012–14 was similar to that in 2001–02; however, the prevalence of rifampicin-resistant tuberculosis almost doubled among new cases. Furthermore, the high prevalence of isoniazid mono-resistant tuberculosis, not routinely screened for, and resistance to second-line drugs has implications for empirical management.

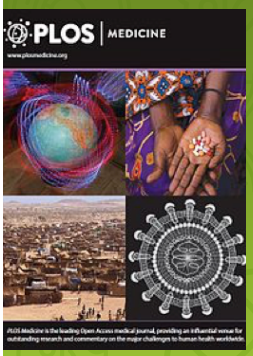
Prof Lynn Morris

Dr Nonhlanhla Mkhize

## Prospects for Passive Immunity to Prevent HIV Infection

***Morris L, Mkhize NN.***

*PLOS Medicine*
***Impact Factor: 11.675***




Despite the widespread global rollout of antiretroviral therapy and its ability to reduce onward HIV transmission, an alarming 1.8 million new HIV infections are estimated to have occurred in 2016. Effective methods to prevent HIV infection include condom use and pre-exposure prophylaxis (PrEP) with antiretroviral drugs; however, owing to barriers such as adherence, an effective vaccine would be the most definitive solution to the ongoing burden of HIV infection. Yet researchers have struggled to design a prophylactic vaccine able to induce protective immunity.

This highlights the urgency of developing new tools to prevent HIV infections and achieve control of the global epidemic. One such approach is passive immunisation with protective antibodies, a strategy that has been used against infectious diseases for over 100 years and has proven useful for post-exposure prophylaxis or against pathogens where no vaccines yet exist. The flourishing field of antibody therapeutics, together with the identification of a growing number of broad and potent HIV monoclonal antibodies, presents an extraordinary opportunity to use this approach for HIV prevention. The protective and therapeutic effects of a number of these broadly neutralising antibodies (bNAbs) have been well demonstrated in animal studies. Furthermore, bNAbs have been shown to have modest antiviral effects in HIV-infected humans, both in reducing viremia and delaying viral rebound after interruption of antiretroviral treatment.

What is not yet known is whether bNAbs are able to protect uninfected humans from acquiring HIV. In this perspective piece, Lynn Morris and Nonhlanhla Mkhize discuss the prospects for using bNAbs to prevent HIV infection.

Prof Gayle Sherman

## Xpert HIV-1 point-of-care test for neonatal diagnosis of HIV in the birth testing programme of a maternity hospital: a field evaluation study

*Technau K, Kuhn L, Coovadia A, Murnane PM,* ***Sherman G.***

*The Lancet HIV*
***Impact Factor: 11.355***


**Background:** Point-of-care testing (POCT) among HIV-exposed infants might improve linkage to care relative to laboratory-based testing (LABT). We evaluated HIV-1 POCT at birth in the context of universal LABT in a maternity hospital and describe our implementation experience.

**Methods:** We did a field evaluation study between October 1, 2014, and April 30, 2016, at the urban Rahima Moosa Mother and Child Hospital (RMMCH), Johannesburg, South Africa. We aimed to sample consecutive neonates at birth with POCT (Cepheid Xpert HIV-1 Qualitative test) and compared the results with those of LABT (Roche COBAS TaqMan HIV-1 Qualitative test) with respect to performance in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Cohen's κ coefficient, result return, antiretroviral treatment (ART) initiation, and coverage.

**Findings:** 18 268 women delivered livebirths at RMMCH and 4267 (23%) were HIV-positive with 4336 HIV-exposed neonates delivered. Mothers of 4141 (96%) HIV-exposed neonates were offered infant birth testing. Mothers of 4112 (99%) neonates consented. In 78 neonates with consent (2%), a test was not done due to early neonatal death (n=13), mother departing before venesection, or staff unavailability. Among 3970 infants who had LABT, 57 (1%) tested positive, 3906 (99%) tested negative, two (<1%) were indeterminate, and five (<1%) had an error result. 2238 (56%) of these infants had concurrent POCT. POCT detected all 30 HIV-infected neonates (sensitivity 100%; 95% CI 88·4–100) with two additional false-positive results (specificity 99·9%; 99·7–100). All positive and 96·2% of negative POCT results were returned, compared with 88·9% of positive and 52·8% of negative LABT results. Although every POCT required 90 min of instrument time, 2·6 h (IQR 2·3–3·1) elapsed between phlebotomy and result return. In days, median time of result return for POCT was 1 day, significantly earlier than 10 days for LABT (p<0·0001). ART was initiated in 30 neonates (100%) with positive POCT compared with 24 (88·9%, p=0·10) of 27 infants who had LABT only, with initiation occurring a median of 5 days earlier in the POCT group (p<0·0001). POCT implementation required additional staff and weekend cover.

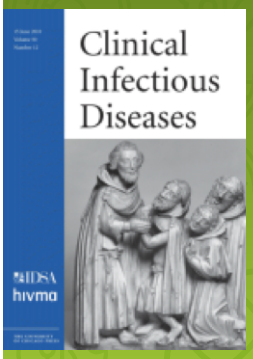
**Interpretation:** Compared with LABT, POCT was associated with good performance, improved rates of result return, and reduced time to ART initiation. Resources needed to integrate POCT into a routine birth testing programme require further evaluation.

Prof Cheryl Cohen

## In- and Out-of-hospital Mortality Associated with Seasonal and Pandemic Influenza and Respiratory Syncytial Virus in South Africa, 2009-2013

***Cohen C,*** *Walaza S, Treurnicht FK, McMorrow M, Madhi SA, McAnerney JM, Tempia S.*

*Clinical Infectious Diseases*
***Impact Factor: 9.117***




Estimates of influenza- and respiratory syncytial virus (RSV)-associated mortality burden are important to guide policy for control. Data are limited on the contribution of out-of-hospital deaths to this mortality.

**Methods:** We modelled excess mortality attributable to influenza and RSV infection by applying regression models to weekly deaths from national vital statistics from 2009 through 2013, using influenza and RSV laboratory surveillance data as covariates. We fitted separate models for in- and out-of-hospital deaths.

**Results:** There were 509 791 average annual deaths in South Africa, of which 44% (95% confidence interval [CI] 43%-45%) occurred out-of-hospital. Seasonal influenza and RSV all-cause mortality rates were 23.0 (95% CI 11.0-30.6) and 13.2 (95% CI 6.4-33.8) per 100 000 population annually (2.3% [95%CI 2.3%-2.4%] and 1.3% [95% CI 1.2%-1.4%] of all deaths respectively). The peak mortality rate was in individuals aged ≥75 years (386.0; 95% CI 176.5-466.3) for influenza and in infants (143.4; 95% CI 0-194.8) for RSV. Overall, 63% (95% CI 62%--65%) of seasonal influenza and 48% (95% CI 47%-49%) of RSV-associated deaths occurred out-of-hospital. Among children aged <5 years, RSV-associated deaths were more likely to occur in-hospital, whereas influenza-associated deaths were more likely to occur out-of-hospital. The mortality rate was 6.7 (95% CI 6.4-33.8) in the first influenza A(H1N1)pdm09 wave in 2009 and 20.9 (95% CI 6.4-33.8) in the second wave in 2011, with 30% (95% CI 29%-32%) of A(H1N1)pdm09-associated deaths in 2009 occurring out-of-hospital.

**Discussion:** More than 45% of seasonal influenza- and RSV-associated deaths occur out-of-hospital in South Africa. These data suggest that hospital-based studies may substantially underestimate mortality burden.

Mr Clement Adu-Gyamfi

Dr Melinda Suchard

## Plasma Indoleamine 2, 3-Dioxygenase, a Biomarker for Tuberculosis in Human Immunodeficiency Virus-Infected Patients

***Adu-Gyamfi C,*** *Snyman T, Hoffmann CJ, Martinson NA, Chaisson RE, George JA,* ***Suchard MS****.*

*Clinical Infectious Diseases*
***Impact Factor: 9.117***


**Background:** There is no biomarker for diagnosing active tuberculosis in patients with human immunodeficiency virus (HIV) infection. Indoleamine 2, 3-dioxygenase (IDO) is an immuno regulatory enzyme that breaks down tryptophan (Trp) to metabolites known as kynurenines (Kyns). We investigated whether IDO activity, as measured by the ratio of Kyn to Trp, could be used to diagnose or predict active tuberculosis disease in HIV-infected adults.

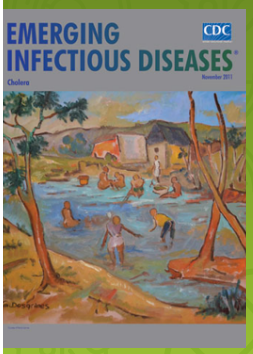
**Methods:** Kyn and Trp concentrations were measured using ultra-performance liquid chromatography mass spectrometry in plasma samples from 32 HIV-infected patients in whom active tuberculosis developed and who were followed up prospectively. We compared to 70 HIV-infected control subjects from the same cohort in whom tuberculosis did not develop, matched by age, sex, and CD4 cell count, and 37 unmatched HIV-infected patients with a diagnosis of pneumonia. Clinical parameters, including body mass index, CD4 cell count, HIV load, and C-reactive protein levels were analysed.

**Results:** At the time of tuberculosis diagnosis, IDO activity was significantly higher in patients with tuberculosis than in controls ($P < 0.001$). Six months before tuberculosis diagnosis, IDO activity was significantly higher in all patients who later developed tuberculosis ($P < 0.001$) than controls. After six months of tuberculosis treatment, IDO activity in patients with tuberculosis declined to levels similar to those in controls. IDO activity was 4-fold higher in patients with tuberculosis than in those with pneumonia, and could be used to distinguish them. With a receiver operating characteristic curve, IDO activity had a sensitivity of 97%, a specificity of 99%, and positive and negative predictive values of 89% and 100% for detecting active tuberculosis disease.

**Conclusion:** Plasma IDO activity is suitable as a biomarker of active tuberculosis in HIV-positive patients.

Dr Erika van Schalkwyk

## Large Outbreaks of Fungal and Bacterial Bloodstream Infections in a Neonatal Unit, South Africa, 2012-2016




***van Schalkwyk E**, Iyaloo S, Naicker SD, Maphanga TG, Mpembe RS, Zulu TG, Mhlanga M, Mahlangu S, Maloba MB, Ntlemo G, Sanyane K, Mawela D, **Govender NP**.*

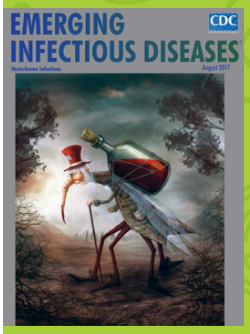
*Emerging Infectious Diseases*
***Impact Factor: 7.422***

Prof Nelesh Govender

Candidemia is a major cause of healthcare-associated infections. We describe a large outbreak of *Candida krusei* bloodstream infections among infants in Gauteng Province, South Africa, during a 4-month period; a series of candidemia and bacteremia outbreaks in the neonatal unit followed. We detected cases by using enhanced laboratory surveillance and audited hospital wards by environmental sampling and epidemiologic studies. During July-October 2014, among 589 patients, 48 unique cases of *C. krusei candidemia* occurred (8.2% incidence). Risk factors for candidemia on multivariable analyses were necrotizing enterocolitis, birthweight <1,500g, receipt of parenteral nutrition, and receipt of blood transfusion. Despite initial interventions, outbreaks of bloodstream infection caused by *C. krusei,* rarer fungal species, and bacterial pathogens continued in the neonatal unit through July 29, 2016. Multiple factors contributed to these outbreaks; the most functional response is to fortify infection prevention and control.

Prof Janusz Paweska

## Marburg Virus Infection in Egyptian Rousette Bats, South Africa, 2013-2014




***Paweska JT,** Jansen van Vuren P, Kemp A, Storm N, Grobbelaar A, Wiley MR, Palacios G, Markotter W.*

*Emerging Infectious Diseases*
***Impact Factor: 7.422***

We detected a high seroprevalence of Marburg virus (MARV) antibodies in fruit bats in South Africa; 19.1% of recaptured bats seroconverted. The MARV RNA isolated closely resembled the 1975 Ozolin strain. These findings indicate endemic MARV circulation in bats in South Africa and should inform policies on MARV disease risk reduction.

Ms Maimuna Carrim

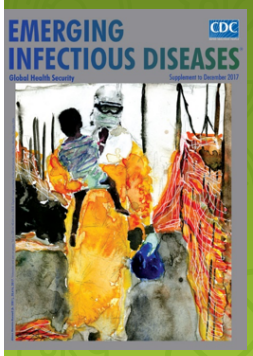
Prof Anne von Gottberg

## Epidemiology and molecular identification and characterisation of *Mycoplasma pneumoniae*, South Africa, 2012-2015

***Carrim M,*** *Wolter N, Benitez AJ, Tempia S, du Plessis M, Walaza S, Moosa F, Diaz MH, Wolff BJ, Treurnicht FK, Hellferscee O, Dawood H, Variava E, Cohen C, Winchell JM,* ***von Gottberg A.***

*Emerging Infectious Diseases*
***Impact Factor: 7.422***

During 2012–2015, we tested respiratory specimens from patients with severe respiratory illness (SRI), patients with influenza-like illness (ILI), and controls in South Africa by real-time PCR for *Mycoplasma pneumoniae,* followed by culture and molecular characterisation of positive samples. *M. pneumoniae* prevalence was 1.6% among SRI patients, 0.7% among ILI patients, and 0.2% among controls (p<0.001). Age <5 years (adjusted odd ratio 7.1; 95% CI 1.7–28.7) and HIV infection (adjusted odds ratio 23.8; 95% CI 4.1–138.2) among *M. pneumoniae*–positive persons were associated with severe disease. The detection rate attributable to illness was 93.9% (95% CI 74.4%–98.5%) in SRI patients and 80.7% (95% CI 16.7%–95.6%) in ILI patients. The hospitalisation rate was 28 cases/100,000 population. We observed the macrolide-susceptible *M. pneumoniae* genotype in all cases and found P1 types 1, 2, and a type 2 variant with multilocus variable number tandem repeat types 3/6/6/2, 3/5/6/2, and 4/5/7/2.




Prof Nazir Ismail

## Defining Bedaquiline Susceptibility, Resistance, Cross-Resistance and Associated Genetic Determinants: A Retrospective Cohort Study

***Ismail NA****, Omar SV, Joseph L, Govender N, Blows L, Ismail F, Koornhof H, Dreyer AW, Kaniga K, Ndjeka N.*

*EBioMedicine*
***Impact Factor: 6.183***

**Background:** Bedaquiline (BDQ) is a novel agent approved for use in combination treatment of multi-drug resistant tuberculosis (MDR-TB). We sought to determine BDQ epidemiological cut-off values (ECVs), define and assess interpretive criteria against putative resistance associated variants (RAVs), microbiological outcomes and cross-resistance with clofazimine (CFZ).

**Methods:** A retrospective cohort study was conducted. Minimal inhibitory concentrations (MIC) to BDQ were determined using 7H9 broth microdilution (BMD) and MGIT960. RAVs were genetically characterised using whole genome sequencing. BDQ ECVs were determined using ECOFFinder and compared with 6-month culture conversion status and CFZ MICs.

**Findings:** A total of 391 isolates were analysed. Susceptible and intermediate categories were determined to have MICs of ≤0.125µg/ml and 0.25µg/ml using BMD and ≤1µg/ml and 2µg/ml using MGIT960, respectively. Microbiological failures occurred among BDQ exposed patients with a non-susceptible BDQ MIC, an Rv0678 mutation and ≤2 active drug classes. The Rv0678 RAVs were not the dominant mechanism of CFZ resistance and cross-resistance was limited to isolates with an Rv0678 mutation.

**Interpretation:** Criteria for BDQ susceptibility are defined and will facilitate improved early detection of resistance. Cross- resistance between BDQ and CFZ is an emerging concern but in this study was primarily among those with an Rv0678 mutation.

Ms Simone Richardson

Prof Lynn Morris

## HIV-specific Fc effector function early in infection predicts the development of broadly neutralising antibodies

***Richardson SI,*** *Chung AW, Natarajan H, Mabvakure B, Mkhize NN, Garrett N, Abdool Karim S, Moore PL, Ackerman ME, Alter G,* ***Morris L.***

*PLOS Pathogens*
***Impact Factor: 6.158***


While the induction of broadly neutralising antibodies (bNAbs) is a major goal of HIV vaccination strategies, there is mounting evidence to suggest that antibodies with Fc effector function also contribute to protection against HIV infection.

Here we investigated Fc effector functionality of HIV-specific IgG plasma antibodies over 3 years of infection in 23 individuals, 13 of whom developed bNAbs. Antibody-dependent cellular phagocytosis (ADCP), complement deposition (ADCD), cellular cytotoxicity (ADCC) and cellular trogocytosis (ADCT) were detected in almost all individuals with levels of activity increasing over time. At six months post-infection, individuals with bNAbs had significantly higher levels of ADCD and ADCT that correlated with antibody binding to C1q and FcγRIIa respectively. In addition, antibodies from individuals with bNAbs showed more IgG subclass diversity to multiple HIV antigens which also correlated with Fc polyfunctionality. Germinal centres activity represented by CXCL13 levels and expression of activation-induced cytidine deaminase (AID) was found to be associated with neutralisation breadth, Fc polyfunctionality and IgG subclass diversity.

Overall, multivariate analysis by random forest classification was able to group bNAb individuals with 85% sensitivity and 80% specificity based on the properties of their antibody Fc early in HIV infection. Thus, the Fc effector function profile predicted the development of neutralisation breadth in this cohort, suggesting that intrinsic immune factors within the germinal centre provide a mechanistic link between the Fc and Fab of HIV-specific antibodies.

Dr Gillian Hunt

## Rates of virological suppression and drug resistance in adult HIV-1 infected patients attending primary healthcare facilities in KwaZulu-Natal, South Africa

***Hunt GM,*** *Dokubo EK, Takuva S, de Oliviera T, Ledwaba J, Dube N, Moodley P, Sabatier J, Deyde V, Morris L and Raizes E.*

*Journal of Antimicrobial Chemotherapy*

***Impact Factor: 5.217***


**Background:** KwaZulu-Natal (KZN) Province in South Africa has the highest HIV disease burden in the country, with an estimated population prevalence of 24.7%. A pilot sentinel surveillance project was undertaken in KZN to classify the proportion of adult patients failing first-line ART and to describe the patterns of drug resistance mutations (DRMs) in patients with virological failure (VF).

**Methods:** Cross-sectional surveillance of acquired HIV drug resistance was conducted in 15 sentinel ART clinics between August and November, 2013. Two population groups were surveyed: on ART for 12–15 months (Cohort A) or 24–36 months (Cohort B). Plasma specimens with viral load ≥1000 copies/mL were defined as VF and genotyped for DRMs.

**Results:** A total of 1299 adults were included in the analysis. The prevalence of VF was 4.0% (95%CI 1.8–8.8) among 540 adults in Cohort A and 7.7% (95% CI 4.4–13.0) of 759 adults in Cohort B. Treatment with efavirenz was more likely to suppress viral load in Cohort A (P"0.005). Independent predictors of VF for Cohort B included male gender, advanced WHO stage at ART initiation and treatment with stavudine or zidovudine compared with tenofovir. DRMs were detected in 89% of 123 specimens with VF, including M184I/V, K103N/S, K65N/R, V106A/M and Y181C.

**Conclusions:** VF in adults in KZN was 8% up to 3 years post-ART initiation but was associated with a high frequency of DRMs. These data identify key groups for intensified adherence counselling and highlight the need to optimise first-line regimens to maintain viral suppression.

Ms Cathrine Scheepers

Prof Lynn Morris

## Serum glycan-binding IgG antibodies in HIV-1 infection and during the development of broadly neutralising responses

***Scheepers C***, *Chowdhury S, Wright WS, Campbell CT, Garrett NJ, Abdool Karim Q, Abdool Karim SS, Moore PL, Gildersleeve JC,* ***Morris L.***

*AIDS*
***Impact Factor: 4.914***


**Background:** The HIV-1 envelope is covered with glycans that provide structural integrity and protect conserved regions from host antibody responses. However, these glycans are often the target of broadly neutralising antibodies (bNAbs) that emerge in some HIV-infected individuals. We aimed to determine whether antiglycan IgG antibodies are a general response to HIV-1 infection or specific to individuals who develop bNAbs.

**Methods:** IgG binding to glycans was assessed using arrays that contained 245 unique components including N-linked carbohydrates, glycolipids, and Tn-peptides. Sera from 20 HIV-negative and 27 HIV-positive women (including 12 individuals who developed bNAbs) were profiled longitudinally. HIV-1 gp120 proteins were used to compete for binding to the array.

**Results:** Antiglycan IgG antibodies fluctuated over a 3-year period, irrespective of HIV infection. However, HIV-positive individuals had elevated binding to 40 components on the array that included Man8, Man9, Tn-peptides, heat shock protein, and glycolipids. Competition experiments confirmed that a proportion of these glycan-binding IgG antibodies were HIV-1-specific, some of which were higher in individuals who developed bNAbs.

**Conclusions:** HIV-1 infection is associated with elevated levels of IgG antibodies to specific glycans. Furthermore, some antiglycan IgG antibodies were more abundant in individuals with bNAbs, suggesting a unique phenotype that may be informative for HIV vaccine design.

Dr Jocelyn Moyes

## Respiratory syncytial virus in adults with severe acute respiratory illness in a high HIV prevalence setting


***Moyes J,*** *Walaza S, Pretorius M, Groome M, von Gottberg A, Wolter N, Haffejee S, Variava E, Cohen AL, Tempia S, Kahn K, Dawood H, Venter M, Cohen C, Madhi SA.*

*Journal of Infection*
***Impact Factor: 4.603***

**Background:** There are limited data on the epidemiology of respiratory syncytial virus (RSV) illness in HIV-infected adults or the elderly in Africa. We studied the epidemiology of RSV-associated severe acute respiratory illness (SARI) hospitalisations in adults in South Africa from 2009 through 2013.

**Methods:** Individuals admitted to sentinel surveillance hospitals were investigated by respiratory tract swabs for RSV, using a multiplex real-time polymerase chain reaction assay. The incidence of RSV-associated SARI was calculated for the one site with population denominators.

**Results:** Of 7796 participants investigated, 329 (4%) tested positive for RSV. On multivariable analysis, HIV-infected individuals with RSV-associated SARI had greater odds of being in the age groups 18-44 and 45-64 years (odd ratios (OR) 26.3; 95% confidence interval (CI) 6.2-112.1 and OR 11.4; 95% CI 2.6-50.0) compared with those ≥65 years and being female (OR 2.7; 95% CI 1.4-5.4). The relative risk of hospitalisation with RSV-associated SARI was 12-18 times higher in HIV-infected individuals compared to that of HIV-uninfected.

**Conclusion:** The incidence of RSV-associated SARI was higher in HIV-infected individuals and those aged 65 years and older. Further studies are warranted to describe the disease association of RSV detected in adults with SARI.

Dr Petrus Jansen van Vuren

Prof Janusz Paweska

## A novel adenovirus isolated from the Egyptian fruit bat in South Africa is closely related to recent isolates from China


***Jansen van Vuren P,*** *Allam M, Wiley MR, Ismail A, Storm N, Birkhead M, Markotter W, Palacios G,* ***Paweska JT.***

*Scientific Reports*
***Impact Factor: 4.122***

Recently a number of novel adenoviruses have been isolated from diverse bat species and from diverse geographical locations. We describe the isolation of a novel adenovirus (Family Adenoviridae, genus Mastadenovirus) from a pool of liver and spleen tissue of an apparently healthy wild-caught Egyptian fruit bat (*Rousettus aegyptiacus*) in South Africa. Genetically the virus is most closely related to four mastadenoviruses recently isolated in China, from *Miniopterus schreibersi* and *Rousettus leschenaultia* bats, which are highly divergent from previously identified bat adenoviruses. The length of the *Rousettus aegyptiacus* adenovirus-3085 (RaegAdV-3085) genome, at 29,342 bp is similar to its closest relatives, and contains 27 open reading frames. The RaegAdV-3085 genome has a low G + C content (36.4%) relative to other viruses in the genus (between 43.6 and 63.9%) but similar to its closest relatives. The inverted terminal repeat (ITR) of RaegAdV-3085 is only 40 bp compared to between 61 and 178 bp of its closest relatives. The discovery of RaegAdV-3085 expands the diversity of known adenoviruses in bats and might represent a member of a new mastadenovirus species in bats.

Dr Ahmad Mazanderani

Prof Gayle Sherman

## Declining Baseline Viremia and Escalating Discordant HIV-1 Confirmatory Results within South Africa's Early Infant Diagnosis Programme, 2010–2016

***Mazanderani AH,*** *Moyo F, Kufa T,* ***Sherman GG.***

*Journal of Acquired Immunodefiency Syndromes*
***Impact Factor: 4.116***

**Objective:** To describe baseline HIV-1 RNA viral load (VL) trends within South Africa's Early Infant Diagnosis programme 2010–2016, with reference to prevention of mother-to-child transmission guidelines.

**Methods:** HIV-1 total nucleic acid polymerase chain reaction (TNA PCR) and RNA VL data from 2010 to 2016 were extracted from the South African National Health Laboratory Service's central data repository. Infants with a positive TNA PCR and subsequent baseline RNA VL taken at age, 7 months were included. Descriptive statistics were performed for quantified and lower than-quantification limit (LQL) results per annum and age in months. Trend analyses were performed using log likelihood ratio tests. Multivariable linear regression was used to model the relationship between RNA VL and predictor variables, whereas logistic regression was used to identify predictors associated with LQL RNA VL results.

**Results:** Among 13 606 infants with a positive HIV-1 TNA PCR linked to a baseline RNA VL, median age of first PCR was 57 days and VL was 98 days. 13195 (97.0%) infants had a quantified VL and 411 (3.0%) had an LQL result. A significant decline in median VL was observed between 2010 and 2016, from 6.3 log10 (interquartile range: 5.6–6.8) to 5.6 log10 (interquartile range: 4.2–6.5) RNA copies per millilitre, after controlling for age (P, 0.001), with younger age associated with lower VL (P, 0.001). The proportion of infants with a baseline VL, 4 Log10 RNA copies per millilitre increased from 5.4% to 21.8%. Subsequent to prevention of mother-to-child transmission Option B implementation in 2013, the proportion of infants with an LQL baseline VL increased from 1.5% to 6.1% (P, 0.001).

**Conclusions:** Between 2010 and 2016, a significant decline in baseline viremia within South Africa's Early Infant Diagnosis programme was observed, with loss of detectability among some HIV-infected infants.

Ms Tanya Murray

## Field Evaluation of Performance of Alere and Cepheid Qualitative HIV Assays for Pediatric Point-of-Care Testing in an Academic Hospital in Soweto, South Africa

***Murray TY,*** *Sherman GG, Nakwa F, MacLeod WB, Sipambo N, Velaphi S, Carmona S.*

*Journal of Clinical Microbiology*
***Impact Factor: 4.054***

Point-of-care (POC) technologies for HIV diagnosis in infants have the potential to overcome logistical challenges that delay treatment initiation and prevent improvements in morbidity and mortality. This study aimed to evaluate the performance of two POC technologies against the current standard-of-care (SOC) laboratory-based assay in South Africa, when operated by nurses in a hospital environment. Children <18 months of age who were treatment naive (excluding prophylaxis) and in whom an HIV PCR test was indicated were eligible for the study. To increase the rate of enrolment of HIV PCR-positive children, HIV-exposed neonates at high risk of mother-to-child transmission and children requiring confirmatory HIV testing were preferentially enrolled. The two POC technologies demonstrated excellent concordance, with 315 (97.8%) results consistent with the SOC result. The POC technologies yielded 102 positive and 220 negative tests each. The SOC assay had 101 positive, 214 negative, 4 indeterminate, 1 invalid, and 2 specimen-rejected results. To include the indeterminate results in sensitivity/specificity calculations, a sensitivity analysis was performed, which yielded a simulated sensitivity of 0.9904 (interquartile range [IQR], 0.9808 to 0.9904) and a specificity of 0.9954 (IQR, 0.9954 to 1.0). This study confirmed that both POC technologies can be successfully used outside the laboratory environment to yield precise sensitivity/specificity values for paediatric, including neonatal HIV testing.


Ms Kedibone Ndlangisa

Prof Anne von Gottberg

## Invasive disease caused simultaneously by dual serotypes of Streptococcus pneumoniae

***Ndlangisa K,*** *du Plessis M, Allam M, Wolter N, de Gouveia L, Klugman KP, Cohen C, Gladstone RA,* ***von Gottberg A.***

*Journal of Clinical Microbiology*
***Impact Factor: 4.054***

There are at least 98 known pneumococcal serotypes. Invasive pneumococcal disease (IPD) is usually caused by a single serotype, and dual-serotype IPD is rare. To assess factors associated with dual-serotype IPD, patient information obtained through laboratory-based surveillance for IPD from 2005 through 2014 in South Africa was reviewed. Genomes of isolate pairs from coinfected individuals were sequenced to determine their molecular characteristics. For 30 (91%) of 33 patients with dual serotypes, one or both isolates were a pneumococcal conjugate vaccine (PCV13) serotype. Dual-serotype IPD was associated with children <5 years of age (adjusted odds ratio [aOR], 4.7; 95% confidence interval [95% CI], 1.8 to 11.7), underlying illness (other than HIV) (aOR, 2.8; 95% CI, 1.1 to 6.6) and death (aOR, 2.5; 95% CI, 1.08 to 6.09). For each coinfecting pair, isolates were genotypically unrelated, and their genotypes were common among isolates of the same serotype in South Africa. Of 701 accessory genes identified among dual-serotype IPD isolates, four were common between isolate pairs. Coinfecting isolate pairs had different genotypic backgrounds. The association of dual serotypes with death warrants increased awareness of IPD coinfection caused by two or more serotypes.

Prof Janusz Paweska

## A Survey on West Nile and Usutu Viruses in Horses and Birds in Poland

*Bazanow B, Jansen van Vuren P, Szymanski P, Stygar D, Fracka A, Twardon J, Kozdrowski R,* ***Paweska JT.***

*Viruses*
***Impact Factor: 3.761***

West Nile virus (WNV) and Usutu virus (USUV) are members of the family Flaviviridae which, natural life cycles involve mosquito–bird–mosquito transmission. Both represent emerging viruses in Europe with potential to cause neuroinvasive disease in humans. This study investigates the seroprevalence of serum neutralising antibodies to WNV and to USUV in birds and in horses in Poland. Antibodies against WNV and USUV were detected in 5 (35.7%) and in 1 (7.14%) of 14 birds and in 62 (15.08%) and in 115 (27.98%) of 411 horses, respectively. Twenty-one WNV serologically positive horses (33.87%) and 67 USUV serologically positive horses (58.26%) did not travel outside Polish borders. Given the high abundance of potentially competent mosquito species in Poland, high populations of horses and different bird species, our findings highlight implementation of active control programmes, including monitoring of geographic spread and dynamics of WNV and USUV transmission in both primary and accidental hosts. It is also important to improve public health awareness about the disease these viruses may cause.


Ms Nadia Storm

Prof Janusz Paweska

## Antibody Responses to Marburg Virus in Egyptian Rousette Bats and their Role in Protection against Infection

***Storm N,*** *Jansen van Vuren P, Markotter W,* ***Paweska JT.***

*Viruses*
***Impact Factor: 3.761***

Egyptian rousette bats (ERBs) are reservoir hosts for the Marburg virus (MARV). The immune dynamics and responses to MARV infection in ERBs are poorly understood, and limited information exists on the role of antibodies in the protection of ERBs against MARV infection. Here, we determine the duration of maternal immunity to MARV in juvenile ERBs, and evaluate the duration of the antibody response to MARV in bats naturally or experimentally infected with the virus. We further explore whether antibodies in previously naturally exposed bats is fully protective against experimental reinfection with MARV. Maternal immunity was lost in juvenile ERBs by 5 months of age. Antibodies to MARV remained detectable in 67% of experimentally infected bats approximately 4 months post inoculation (p.i.), while antibodies to MARV remained present in 84% of naturally exposed bats at least 11 months after capture. Reinfection of seropositive ERBs with MARV produced an anamnestic response from day 5 p.i. Although, PCR-defined viremia was present in 73.3% of reinfected ERBs, replicating virus was recovered from the serum of only one bat on day 3 p.i. The negative PCR results in the salivary glands, intestines, bladders and reproductive tracts of reinfected bats, and the apparent absence of MARV in the majority of swabs collected from these bats suggest that reinfection may only play a minor role in the transmission and maintenance of MARV amongst ERBs in nature.

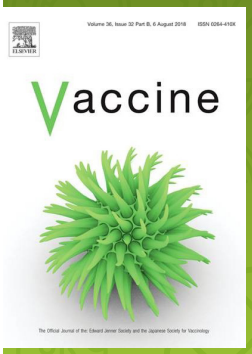
Prof Nicola Page

## Temporal association of rotavirus vaccination and genotype circulation in South Africa: Observations from 2002 to 2014

***Page NA,*** *Seheri LM, Groome MJ, Moyes J, Walaza S, Mphahlele J, Kahn K, Kapongo CN, Zar HJ, Tempia S, Cohen C, Madhi SA.*

*Vaccine*

***Impact Factor: 3.285***




**Background:** Rotavirus vaccination has reduced diarrhoeal morbidity and mortality globally. The monovalent rotavirus vaccine was introduced into the public immunisation programme in South Africa (SA) in 2009 and led to approximately 50% reduction in rotavirus hospitalisation in young children. The aim of this study was to investigate the rotavirus genotype distribution in SA before and after vaccine introduction.

**Materials and Methods:** In addition to pre-vaccine era, surveillance conducted from 2002 to 2008 at Dr George Mukhari Hospital (DGM), rotavirus surveillance among children <5 years hospitalised for acute diarrhoea was established at seven sentinel sites in SA from April 2009 to December 2014. Stool specimens were screened by enzyme immunoassay and rotavirus positive specimens genotyped using standardised methods.

**Results:** At DGM, there was a significant decrease in G1 strains from pre-vaccine introduction (34%; 479/1418; 2002-2009) compared to post-vaccine introduction (22%; 37/170; 2010-2014; p for trend <.001). Similarly, there was a significant increase in non-G1P[8] strains at this site (p for trend <.001). In expanded sentinel surveillance, when adjusted for age and site, the odds of rotavirus detection in hospitalised children with diarrhoea declined significantly from 2009 (46%; 423/917) to 2014 (22%; 205/939; p<.001). The odds of G1 detection declined significantly from 2009 (53%; 224/421) to 2010-2011 (26%; 183/703; aOR=0.5; p<.001) and 2012-2014 (9%; 80/905; aOR=0.1; p<.001). Non-G1P[8] strains showed a significant increase from 2009 (33%; 139/421) to 2012-2014 (52%; 473/905; aOR=2.5; p<.001).

**Conclusions:** Rotavirus vaccination of children was associated with temporal changes in circulating genotypes. Despite these temporal changes in circulating genotypes, the overall reduction in rotavirus disease in South Africa remains significant.

Prof Lucille Blumberg

## The preventable tragedy of diphtheria in the 21st century

***Blumberg LH,*** *Prieto MA, Diaz JV, Blanco MJ, Valle B, Pla C, Durrheim DN.*

*International Journal of Infectious Diseases*
***Impact Factor: 3.202***

The diphtheria outbreak in the Rohingya refugee population in Bangladesh, the outbreak in Yemen due to war, and the more recent outbreaks in Venezuela and Haiti should raise concern that diphtheria remains a public health issue in 2017/2018, almost a century after an effective and safe toxoid vaccine was developed.


Ms Genevie Ntshoe

## Risk factors for pertussis among hospitalised children in a high HIV prevalence setting, South Africa

*du Plessis NM,* ***Ntshoe G,*** *Reubenson G, Kularatne R, Blumberg L, Thomas J, Avenant T. (N.M. du Plessis and G. Ntshoe contributed equally to the article.)*

*International Journal of Infectious Diseases*
***Impact Factor: 3.202***


**Background:** In low- and middle-income countries, including South Africa, the epidemiology of pertussis in relation to immunisation, nutritional, and HIV status is poorly described. This article reports on risk factors in South African children hospitalised with pertussis.

**Methods:** A prospective, hospital-based, sentinel surveillance programme for pertussis was conducted in Gauteng Province, South Africa. Hospitalised children (≤10 years) meeting the surveillance criteria for clinically suspected pertussis were screened and enrolled. Nasopharyngeal specimens were collected for real-time multiplex PCR and culture of *Bordetella* species.

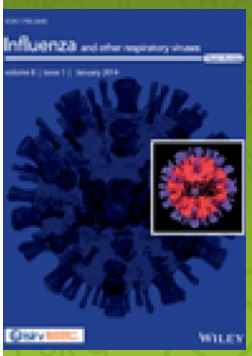
**Results:** *Bordetella pertussis* was detected in 6.2% (61/992) of children. Pertussis was significantly more prevalent in infants younger than 3 months (9.8%; 38/392) and in young children between the ages of 5 and 9 years (12%;4/34) (p=0.0013). Of the 61 confirmed pertussis cases, 17 were too young for vaccination. Of the remaining 44 infants, vaccination DTP1 was administered in 73% (32/44) of pertussis-confirmed patients who were eligible, DTP2 in 50% (16/32), DTP3 in 54% (14/26), and DTP4 in 56% (5/9) of vaccine-eligible cases at 18 months of age. *B. pertussis* infection was less likely in children immunised at least once (5%, 32/692) than in unvaccinated children (10%, 24/230) (p=0.0001). HIV exposure and infection status were determined in 978 (99%) patients: 69% (678/978) were HIV-unexposed and uninfected and 31% (300/978) were HIV-exposed. Of these HIV-exposed patients, 218 (22%) were proven HIV-exposed and uninfected, and 82 patients were HIV-infected (8.4%, 82/978). HIV prevalence was similar in pertussis-positive (6%, 5/82) and pertussis-negative (6%, 55/896) children (p=0.90). *B. pertussis* infection was unrelated to poor nutritional status.

**Conclusions:** In South Africa, *B. pertussis* poses a greater risk to infants who are too young for the first vaccine dose, those who are not vaccinated in a timely manner, and those who do not receive all three primary doses. HIV infection and HIV exposure were not associated with pertussis infection.

Prof Cheryl Cohen

## The effects of the attributable fraction and the duration of symptoms on burden estimates of influenza-associated respiratory illnesses in a high HIV prevalence setting, South Africa, 2013-2015

*Tempia S, Walaza S, Moyes J, Cohen AL, von Mollendorf C, McMorrow ML, Mhlanga S, Treurnicht FK, Venter M, Pretorius M, Hellferscee O, Wolter N, von Gottberg A, Nguweneza A, McAnerney JM, Dawood H, Variava E, Madhi SA,* ***Cohen C.***



*Influenza and Other Respiratory Viruses*

***Impact Factor: 2.954***

**Background:** The attributable fraction of influenza virus detection to illness (INF-AF) and the duration of symptoms as a surveillance inclusion criterion could potentially have substantial effects on influenza disease burden estimates.

**Methods:** We estimated rates of influenza-associated influenza-like illness (ILI) and severe acute (SARI-10) or chronic (SCRI-10) respiratory illness (using a symptom duration cut-off of ≤10 days) among HIV-infected and HIV-uninfected patients attending 3 hospitals and 2 affiliated clinics in South Africa during 2013-2015. We calculated the unadjusted and INF-AF-adjusted rates and relative risk (RR) due to HIV infection. Rates were expressed per 100 000 population.

**Results:** The estimated mean annual unadjusted rates of influenza-associated illness were 1467.7, 50.3, and 27.4 among patients with ILI, SARI-10, and SCRI-10, respectively. After adjusting for the INF-AF, the percent reduction in the estimated rates was 8.9% (rate: 1336.9), 11.0% (rate: 44.8), and 16.3% (rate: 22.9) among patients with ILI, SARI-10, and SCRI-10, respectively. HIV-infected compared to HIV-uninfected individuals experienced a 2.3 (95% CI: 2.2-2.4)-, 9.7 (95% CI: 8.0-11.8)-, and 10.0 (95% CI: 7.9-12.7)-fold increased risk of influenza-associated illness among patients with ILI, SARI-10, and SCRI-10, respectively. Overall 34% of the estimated influenza-associated hospitalisations had symptom duration of >10 days; 8% and 44% among individuals aged <5 and ≥5 years, respectively.

**Conclusion:** The marginal differences between unadjusted and INF-AF-adjusted rates are unlikely to affect policies on prioritisation of interventions. HIV-infected individuals experienced an increased risk of influenza-associated illness and may benefit more from annual influenza immunisation. The use of a symptom duration cut-off of ≤10 days may underestimate influenza-associated disease burden, especially in older individuals.

Mr Jacek Zawada

Prof Lizette Koekemoer

## Molecular and physiological analysis of *Anopheles funestus* swarms in Nchelenge, Zambia

***Zawada JW,*** *Dahan-Moss YL, Muleba M, Dabire RK, Maiga H, Venter N, Davies C, Hunt RH, Coetzee M,* ***Koekemoer LL.***

*Malaria Journal*
***Impact Factor: 2.845***

**Background:** *Anopheles funestus* has been recognised as a major malaria vector in Africa for over 100 years, but knowledge on many aspects of the biology of this species is still lacking. *Anopheles funestus,* as with most other anophelines, mate through swarming. A key event that is crucial for the *An. funestus* male to mate is genitalia rotation. This involves the 135° to 180° rotation of claspers, which are tipped with claws. This physical change then enables the male to grasp the female during copulation. The aim of this investigation was to molecularly characterise wild *An. funestus* swarms from Zambia and examine the degree of genitalia rotation within the swarm.

**Methods:** *Anopheles funestus* swarms were collected from Nchelenge, northern Zambia, during dusk periods in May 2016. All the adults from the swarm were analysed morphologically and identified to species level using a multiplex PCR assay. *Anopheles funestus s.s.* specimens were molecularly characterised by restriction fragment length polymorphism type and Clade type assays. The different stages of genitalia rotation were examined in the adult males.

**Results:** A total of six swarms were observed during the study period and between 6 and 26 mosquitoes were caught from each swarm. Species analysis revealed that 90% of the males from the swarms were *An. funestus s.s.* MW-type, with 84% belonging to clade I compared to 14% clade II and 2% failed to amplify. Very few specimens (3.4%) were identified as *Anopheles gambiae s.s.* Eighty percent of the males from the swarm had complete genitalia rotation.

**Conclusions:** This is the first time that *An. funestus* swarms have been molecularly identified to species level. *Anopheles funestus* swarms appear to be species-specific with no evidence of clade-type differentiation within these swarms. The *An. funestus* swarms consist mainly of males with fully rotated genitalia, which strongly suggests that swarming behaviour is triggered primarily when males have matured.

Ms Erica Erlank

Prof Maureen Coetzee

## The importance of morphological identification of African anopheline mosquitoes (Diptera: Culicidae) for malaria control programme

***Erlank E,*** *Koekemer L,* ***Coetzee M.***

*Malaria Journal*
***Impact Factor: 2.845***

**Background:** The correct identification of disease vectors is the first step towards implementing an effective control programme. Traditionally, for malaria control, this was based on the morphological differences observed in the adults and larvae between different mosquito species. However, the discovery of species complexes meant that genetic tools were needed to separate the sibling species and today there are standard molecular techniques that are used to identify the two major malaria vector groups of mosquitoes. On the assumption that species-diagnostic DNA polymerase chain reaction (PCR) assays are highly species-specific, experiments were conducted to investigate what would happen if non-vector species were randomly included in the molecular assays.

**Methods:** Morphological keys for the Afrotropical Anophelinae were used to provide the a priori identifications. All mosquito specimens were then subjected to the standard PCR assays for members of the *Anopheles gambiae* complex and *Anopheles funestus* group.

**Results:** One hundred and fifty mosquitoes belonging to 11 morphological species were processed. Three species (*Anopheles pretoriensis, Anopheles rufipes* and *Anopheles rhodesiensis*) amplified members of the *An. funestus* group and four species (*An. pretoriensis*, *An. rufipes, Anopheles listeri* and *Anopheles squamosus*) amplified members of the *An. gambiae* complex.

**Conclusions:** Morphological identification of mosquitoes prior to PCR assays not only saves time and money in the laboratory, but also ensures that data received by malaria vector control programmes are useful for targeting the major vectors.

Dr Ziyaad Valley-Omar

Dr Florette Treurnicht

## Intra-host and intra-household diversity of influenza A viruses during household transmissions in the 2013 season in 2 peri-urban communities of South Africa

***Valley-Omar Z,*** *Iyengar P, Mollendorf v, Tempia S, Moerdyk A, Hellferscee O, Martinson N, McMorrow M, Variava E, Masoneke K, Cohen AL, Cohen C,* ***Treurnicht FK.***

*PLOS ONE*

***Impact Factor: 2.766***

Limited information is available on influenza virus sequence drift between transmission events. In countries with high HIV burdens, like South Africa, the direct and indirect effect of HIV on influenza sequence drift between transmission events may be of public health concern. To this end, we measured hemagglutinin sequence diversity between influenza transmission events using data and specimens from a study investigating household transmission dynamics of seasonal influenza viruses in 2 peri-urban communities in South Africa during the 2013 influenza season.

Thirty index cases and 107 of 110 eligible household contacts were enrolled into the study, 47% (14/30) demonstrating intra-household laboratory-confirmed influenza transmission. In this study 35 partial hemagglutinin gene sequences were obtained by Sanger sequencing from 11 index cases (sampled at enrolment only) and 16 secondary cases (8 cases sampled at 1 and 8 cases sampled at 2 time-points). Viral sequence identities confirmed matched influenza transmission pairs within the 11 households with corresponding sequenced index and secondary cases. Phylogenetic analysis revealed 10 different influenza viral lineages in the 14 households. Influenza A(H1N1)pdm09 strains were shown to be genetically distinct between the 2 communities (from distinct geographic regions), which was not observed for the influenza A(H3N2) strains. Intra-host/intra-household influenza A(H3N2) sequence drift was identified in 2 households.

The first was a synonymous mutation between the index case and a household contact, and the second a non-synonymous mutation between 2 serial samples taken at days 0 and 4 post-enrolment from an HIV-infected secondary case. Limited inter-household sequence diversity was observed as highlighted by sharing of the same influenza strain between different households within each community. The limited intra-household sequence drift is in line with previous studies also using Sanger sequencing, corroborating the presence of strict selective bottlenecks that limit sequence variance.

We were not able to directly ascertain the effect of HIV on influenza sequence drift between transmission events.

Ms Pinky Manana

Prof Nazir Ismail

## Feasibility of using postal and web-based surveys to estimate the prevalence of tuberculosis among health care workers in South Africa

***Manana PN,*** *Kuonza L, Musekiwa A, Koornhof H, Nanoo A,* ***Ismail N.***

*PLOS ONE*

***Impact Factor: 2.766***

**Introduction:** Health care Workers (HCWs) are among the highest risk groups for contracting tuberculosis (TB), which is ranked the third most common occupational health disease in South Africa. Little is known about the true extent of the burden of TB among South African HCWs and current surveillance approaches are inadequate. The study aimed to determine the feasibility of using postal and web-based surveys accessed through registries of registered professionals to estimate the prevalence of TB among HCWs in South Africa.

**Materials and methods:** We conducted a cross-sectional survey on a sample of professional nurses and doctors (general practitioners) registered on the Medpages database platform; a subscription based registry for practising health care professionals. The survey included professionals who were actively involved in the clinical management of patients, either in public or private health care facilities. The paper based survey, including pre-paid return envelopes, was distributed via the post office and web-based surveys were distributed via e-mail through a hyperlink. Descriptive statistics were used to summarise the data and the Chi-square test to determine associations between categorical variables. Active TB was defined as any history of TB.

**Results:** Out of a total of 3,400 health care professionals contacted, 596 (18%) responses were received: 401 (67%) web-based and 195 (33%) postal. A significantly higher percentage of complete forms were from postal compared to web-based (97% [189/195] versus 87% [348/401], p<0.001). Younger (<60 years) professionals were more likely to use the web-based compared to postal (87% [236/270] versus 71% [134/189], p<0.001). Overall, the prevalence of active TB infection was 8.7%, (95%CI: 6.3%–11.7%) and there was no difference observed between doctors and nurses (10.8% [18/167] versus 7.5% [22/292], p = 0.236).

**Conclusion:** This novel approach demonstrated the feasibility of using an existing registry of professionals to conduct surveys to estimate the prevalence of TB. Our findings showed a high TB prevalence; however the estimate might have been biased by the low response rate. Further research to optimise our approach could lead to a viable option in improving surveillance among health care professionals.

Dr Shune Oliver

Prof Basil Brooke

## The effect of metal pollution on the life history and insecticide resistance phenotype of the major malaria vector *Anopheles arabiensis* (Diptera: Culcidae)

***Oliver SV, Brooke BD.***

*PLOS ONE*

***Impact Factor: 2.766***

Metal exposure is one of the commonest anthropogenic pollutants mosquito larvae are exposed to, both in agricultural and urban settings. As members of the *Anopheles gambiae* complex, which contains several major malaria vector species including An. *arabiensis*, are increasingly adapting to polluted environments. This study examined the effects of larval metal exposure on various life history traits of epidemiological importance. Two laboratory strains of An. *arabiensis*, SENN (insecticide susceptible) and SENN DDT (insecticide resistant), were reared in maximum acceptable toxicity concentrations, (MATC-the highest legally accepted concentration) of cadmium chloride, lead nitrate and copper nitrate. Following these exposures, time to pupation, adult size and longevity were determined. Larvae reared in double the MATC were assessed for changes in malathion and deltamethrin tolerance, measured by lethal time bottle bioassay, as well as changes in detoxification enzyme activity. As defence against oxidative stress has previously been demonstrated to affect the expression of insecticide resistance, catalase, glutathione peroxidase and superoxide dismutase activity was assessed. The relative metal toxicity to metal naïve larvae was also assessed. SENN DDT larvae were more tolerant of metal pollution than SENN larvae. Pupation in SENN larvae was significantly reduced by metal exposure, while adult longevity was not affected. SENN DDT showed decreased adult size after larval metal exposure. Adult insecticide tolerance was increased after larval metal exposure, and this effect appeared to be mediated by increased β-esterase, cytochrome P450 and superoxide dismutase activity. These data suggest an enzyme-mediated positive link between tolerance to metal pollutants and insecticide resistance in adult mosquitoes. Furthermore, exposure of larvae to metal pollutants may have operational consequences under an insecticide-based vector control scenario by increasing the expression of insecticide resistance in adults.

Dr Ranmini Kularatne

## For Trends in the relative prevalence of genital ulcer disease pathogens and association with HIV infection in Johannesburg, South Africa, 2007–2015 - plos one

***Kularatne RS,*** *Muller EE, Maseko DV, Kufa-Chakezha T, Lewis DA.*

*PLOS ONE*

***Impact Factor: 2.766***

**Background:** In South Africa, treatment of genital ulcer disease (GUD) occurs in the context of syndromic management. GUD aetiological studies have been conducted in Johannesburg since 2007. We report on GUD pathogen prevalence, sero-prevalence of STI co-infections and aetiological trends among GUD patients presenting to a community-based primary healthcare facility in Johannesburg over a 9-year period.

**Methods and Findings:** GUD surveys were conducted from January to April each year. Consecutive genital ulcers were sampled from consenting adults. Swab-extracted DNA was tested by multiplex real-time PCR assays for herpes simplex virus (HSV), Treponema pallidum (TP), Haemophilus ducreyi (HD) and Chlamydia trachomatis (CT). HSV-positive DNA extracts were further subtyped into HSV-1 and HSV-2 using a commercial PCR assay; CT-positive extracts were tested with an in-house PCR assay specific for serovars L1-L3 (lymphogranuloma venereum). Sera were tested for HIV, HSV-2, and syphilis co-infections. Giemsa-stained ulcer smears were screened for Klebsiella granulomatis by microscopy. Data were analysed with STATATM version 14. Of 771 GUD specimens, 503 (65.2%) had a detectable pathogen: HSV 468 (60.7%); TP 30 (3.9%); CT L1-3 7 (0.9%); HD 4 (0.5%). No aetiological agents were detected in 270 (34.8%) ulcer specimens. Seroprevalence rates were as follows: HIV 61.7%; HSV-2 80.2% and syphilis 5.8%. There was a strong association between GUD pathogen detection and HIV seropositivity ($p < 0.001$); 68% of cases caused by HSV were co-infected with HIV. There was a significant decline in the relative prevalence of ulcer-derived HSV over time, predominantly from 2013-2015 (p-value for trend = 0.023); and a trend towards a decrease in the HIV seropositivity rate (p-value for trend = 0.209).

**Conclusions:** HSV remains the leading cause of pathogen-detectable GUD in South Africa. The prevalence of HIV co-infection among GUD patients is high, underlining the importance of linkage to universal HIV testing and treatment in primary healthcare settings.

Prof Caroline Tiemessen

## Age at antiretroviral therapy initiation and cell-associated HIV-1 DNA levels in HIV1—infected children

*Kuhn L, Paximadis M, Da Costa Dias B, Loubser S, Strehlau R, Patel F, Shiau S, Coovadia A,* ***Tiemessen CT.***

*PLOS ONE*

***Impact Factor: 2.766***

**Background:** The latent viral reservoir is the major obstacle to achieving HIV remission and necessitates life-long antiretroviral therapy (ART) for HIV-infected individuals. Studies in adults and children have found that initiating ART soon after infection is associated with a reduction in the size of the HIV-1 reservoir. Here we quantified cell-associated HIV-1 DNA in early-treated but currently older HIV-infected children suppressed on ART.

**Methods:** The study participants comprised of a cohort of 146 early-treated children with HIV-1 RNA <50 copies/ml enrolled as part of a clinical trial in Johannesburg, South Africa. A stored buffy coat sample collected after a median 4.3 years on ART and where HIV-1 RNA was <50 copies/ml was tested for cell-associated HIV-1 DNA levels. An in-house, semi-nested real-time quantitative hydrolysis probe PCR assay to detect total HIV-1 subtype C proviral DNA was used. Children were followed prospectively for up to 3 years after this measurement to investigate subsequent HIV-1 RNA rebound/failure while remaining on ART. Age at ART initiation, HIV-1 RNA decline prior to HIV-1 DNA measurement and other factors were investigated.

**Results:** A gradient between age at ART initiation and later HIV-1 DNA levels was observed. When ART was started <2 months of age, the lowest levels of cell-associated HIV-1 DNA (median 1.4 log10copies/106 cells, interquartile range [IQR] 0.95-1.55) were observed compared to ART started at 2-4 months (median 1.68, IQR 1.26-1.97) or 5-14 months of age (median1.98, IQR 1.69-2.25). A low CD4 T-cell count pre-treatment predicted higher levels of HIV-1 DNA on later testing. The probability of HIV-1 RNA rebound >50 copies/ml whilst on ART within 3 years after the DNA measurement was 2.07 (95% CI: 1.352-3.167) times greater if the HIV-1 DNA level was above the median of 55 copies/106 cells.

**Conclusions:** Cell-associated HIV-1 DNA levels measured after more than 4 years on ART were lower the younger the age of the child when ART was initiated. This marker of the size of the viral reservoir also predicted subsequent viral rebound/treatment failure while ART was sustained. The results provide additional evidence of the benefits of prompt diagnosis and early ART initiation in new-borns and infants.

Dr Anthony Smith

## Laboratory-acquired infections of *Salmonella enterica* serotype Typhi in South Africa: phenotypic and genotypic analysis of isolates

***Smith AM,*** *Smouse SL, Tay NP, Bamford C, Moodley VM, Jacobs C, McCarthy KM, Lourens A, Keddy KH and GERMS-SA Surveillance Network.*

*BMC Infectious Diseases*
***Impact Factor: 2.620***


**Background:** Workers in clinical microbiology laboratories are exposed to a variety of pathogenic microorganisms. *Salmonella* species are among the most commonly reported bacterial causes of laboratory-acquired infections. We report on three cases of laboratory-acquired *Salmonella enterica* serotype Typhi (*Salmonella Typhi*) infection which occurred over the period 2012 to 2016 in South Africa.

**Methods:** Laboratory investigation included phenotypic and genotypic characterisation of isolates. Phenotypic analysis included standard microbiological identification techniques, serotyping and antimicrobial susceptibility testing. Genotypic analysis included the molecular subtyping methodologies of pulsed-field gel electrophoresis analysis, multilocus sequence typing and whole-genome sequencing (WGS); with WGS data analysis including phylogenetic analysis based upon comparison of single nucleotide polymorphism profiles of isolates.

**Results:** All cases of laboratory-acquired infection were most likely the result of lapses in good laboratory practice and laboratory safety. The following critical issues were highlighted: there was misdiagnosis and misreporting of *Salmonella Typhi* as nontyphoidal *Salmonella* by a diagnostic laboratory, with associated public health implications; we highlight issues concerning the importance of accurate fluoroquinolone susceptibility testing and interpretation of results according to updated guidelines; and we described potential shortcomings of a single disk susceptibility screening test for fluoroquinolone susceptibility and suggest that confirmatory minimum inhibitory concentration testing should always be performed in cases of invasive *Salmonella* infections. These antimicrobial susceptibility testing issues resulted in inappropriate ciprofloxacin therapy which may have been responsible for failure in clearance of pathogen from patients. *Salmonella Typhi* capsular polysaccharide vaccine was not protective in one case, possibly secondarily to a faulty vaccine.

**Conclusions**: Molecular subtyping of isolates proved effective to investigate the genetic relatedness of isolates. Molecular subtyping data interpreted together with epidemiological data allowed us to pinpoint the most likely sources for our cases of laboratory-acquired infection.

Prof Janusz Paweska

## Mutation of adjacent cysteine residues in the NSs protein of Rift Valley fever virus results in loss of virulence in mice

*Monteiro GER, Jansen van Vuren P, Schreur PJW, Odendaal L, Clift SJ, Kortekaas J,* ***Paweska JT.***

*Virus Research*
***Impact Factor: 2.484***


The NSs protein encoded by the S segment of Rift Valley fever virus (RVFV) is the major virulence factor, counteracting the host innate antiviral defence. It contains five highly conserved cysteine residues at positions 39, 40, 149, 178 and 194, which are thought to stabilize the tertiary and quaternary structure of the protein. Here, we report significant differences between clinical, virological, histopathological and host gene responses in BALB/c mice infected with wild-type RVFV (wtRVFV) or a genetic mutant having a double cysteine-to-serine substitution at residues 39 and 40 of the NSs protein (RVFV-C39S/C40S). Mice infected with the wtRVFV developed a fatal acute disease; characterised by high levels of viral replication, severe hepatocellular necrosis, and massive up-regulation of transcription of genes encoding type I and –II interferons (IFN) as well as pro-apoptotic and pro-inflammatory cytokines. The RVFV-C39S/C40S mutant did not cause clinical disease and its attenuated virulence was consistent with virological, histopathological and host gene expression findings in BALB/ c mice. Clinical signs in mice infected with viruses containing cysteine-to-serine substitutions at positions 178 or 194 were similar to those occurring in mice infected with the wtRVFV, while a mutant containing a substitution at position 149 caused mild, non-fatal disease in mice. As mutant RVFV-C39S/C40S showed an attenuated phenotype in mice, the molecular mechanisms behind this attenuation were further investigated. The results show that two mechanisms are responsible for the attenuation; (1) loss of the IFN antagonistic propriety characteristic of the wtRVFV NSs and (2) the inability of the attenuated mutant to degrade Protein Kinase R (PKR).

Dr Ahmad Mazanderani

Prof Gayle Sherman

## Differentiating clearly positive from indeterminate results: A review of irreproducible HIV-1 PCR positive samples from South Africa's Early Infant Diagnosis Programme, 2010–2015

***Mazanderani AH****, Moyo F, Kufa T, Maritz J,* ***Sherman GG****.*

*Diagnostic Microbiology and Infectious Disease*
***Impact Factor: 2.341***


We describe the extent of and variables associated with irreproducible HIV-1 PCR positive results within South Africa's Early Infant Diagnosis (EID) programme from 2010 to 2015 and propose criteria for differentiating indeterminate from clearly positive results using the COBAS® AmpliPrep/COBAS® TaqMan HIV-1 Qualitative Test version 2.0 (CAP/CTM Qual v2.0). Fourteen percent of specimens with an instrument-positive result that were repeat-tested yielded a negative result for which cycle threshold (Ct) proved to be the only predictive variable. ACt b33.0 was found to be the most accurate threshold value for differentiating clearly positive from irreproducible cases, correctly predicting 96.8% of results. Among 70 patients with an irreproducible positive result linked to a follow-up HIV-1 PCR test, 67 (95.7%) were negative and 3 (4.3%) were instrument-positive. Criteria differentiating clearly positive from indeterminate results need to be retained within EID services and infants with indeterminate results closely monitored and final HIV status determined.

Ms Faith Moyo

Prof Gayle Sherman

## Introduction of Routine HIV Birth Testing in the South African National Consolidated Guidelines

***Moyo F,*** *Mazanderani AH, Barron P, Bhardwaj S, Goga AE, Pillay Y,* ***Sherman GG.***

*The Pediatric Infectious Disease Journal*
***Impact Factor: 2.305***


**Background:** South Africa represents the first high-burden setting to introduce routine virologic testing at birth within its early infant diagnosis programme, implemented in June 2015. National HIV birth testing coverage, intrauterine transmission rates and case rates for the first year since introduction of universal birth testing are reported.

**Methods:** HIV polymerase chain reaction (PCR) test data from June 2015 to May 2016 were extracted from the National Health Laboratory Service's central data repository by year, month, age, result and geographic location. Birth testing was defined as all HIV PCR tests performed at <7 days of life; coverage as the proportion of all HIV-exposed neonates born who were tested at birth; estimated intrauterine transmission rate as the percentage of HIV PCR positive tests in HIV-exposed neonates tested and case rates as the number of HIV PCR positive tests per 100,000 total live births.

**Results:** Between June 2015 and May 2016, the South African national monthly birth testing coverage increased from 39% (8636 tests) to 93% (20,479 tests). During this period, the number of positive tests at birth increased from 114 to 234 per month, equating to a national intrauterine transmission rate of 1.1% and a birth case rate of 247 per 100,000 live births.

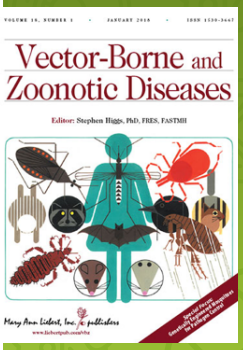
**Conclusions:** Universal birth testing for all HIV-exposed neonates is rapidly being achieved in South Africa, facilitating earlier detection of intrauterine infected neonates. However, the successful linkage into care of HIV infected neonates and their treatment outcomes remain to be assessed.

Prof Lucille Blumberg

## Prevalence of Selected Zoonotic Diseases and Risk Factors at a Human-Wildlife-Livestock Interface in Mpumalanga Province, South Africa

*Simpson G, Quan V, Frean J, Knobel D, Rossouw J, Weyer J, Tanguy M, Godfroid J,* ***Blumberg LH.***

*Vector-Borne and Zoonotic Diseases*
***Impact Factor: 2.171***




A lack of surveillance and diagnostics for zoonotic diseases in rural human clinics limits clinical awareness of these diseases. We assessed the prevalence of nine zoonotic pathogens in a pastoral, low-income, HIV-endemic community bordering wildlife reserves in South Africa. Two groups of participants were included: malaria-negative acute febrile illness (AFI) patients, called febrilers, at three clinics (n = 74) and second, farmers, herders, and veterinary staff found at five government cattle dip-tanks, called dip-tanksters (n = 64). Blood samples were tested using one PCR (*Bartonella* spp.) and eight antibody-ELISAs, and questionnaires were conducted to assess risk factors. Seventy-seven percent of febrilers and 98% of dip-tanksters had at least one positive test. *Bartonella* spp. (PCR 9.5%), spotted fever group (SFG) *Rickettsia* spp. (IgM 24.1%), *Coxiella burnetii*. (IgM 2.3%), and *Leptospira* spp. (IgM 6.8%) were present in febrilers and could have been the cause of their fever. Dip-tanksters and febrilers had evidence of past infection to Rickettsia spp. (IgG 92.2% and 63.4%, respectively) and *C. burnetii* (IgG 60.9% and 37.8%, respectively). No Brucella infection or current *Bartonella* infection was found in the dip-tanksters, although they had higher levels of recent exposure to *Leptospira* spp. (IgM 21.9%) compared to the febrilers. Low levels of West Nile and Sindbis, and no Rift Valley fever virus exposure were found in either groups. The only risk factor found to be significant was attending dip-tanks in febrilers for Q fever (p = 0.007). Amoxicillin is the local standard treatment for AFI, but would not be effective for *Bartonella* spp. infections, SFG rickettsiosis, Q fever infections, or the viral infections. There is a need to revise AFI treatment algorithms, educate medical and veterinary staff about these pathogens, especially SFG rickettsiosis and Q fever, support disease surveillance systems, and inform the population about reducing tick and surface water contact.

Prof Gayle Sherman

## Closing the gaps to eliminate mother-to-child transmission of HIV (MTCT) in South Africa: Understanding MTCT case rates, factors that hinder the monitoring and attainment of targets, and potential game changers

*Goga A, Chirinda W, Mgandu NK, Ngoma K, Bhardwaj S, Feucht U, Davies N, Ntloana M, Mhlongo O, Silere-Maqetseba T, Moyo F,* ***Sherman G****.*

*South African Medical Journal*
***Impact Factor: 2.163***


**Background:** Ninety percent of the world's HIV-positive pregnant women live in 22 countries. These 22 countries, including South Africa (SA) have prioritised the elimination of mother-to-child transmission of HIV (EMTCT). Since 2016 all 22 countries recommend lifelong antiretroviral treatment for all HIV-positive pregnant and lactating women. To measure South African national, provincial and district-level progress towards attaining EMTCT, we analysed the number of in utero (IU) paediatric HIV infections per 100 000 live births (IU case rate), and synthesised factors hindering the monitoring of EMTCT progress and attainment from the viewpoint of provincial and district-level healthcare managers and implementers. We highlight potential innovations to strengthen health systems and improve EMTCT programme delivery.

**Methods:** We reviewed national, provincial and district-level birth HIV testing data from routine National Health Laboratory Service's (NHLS) records between April 2016 and March 2017. To obtain a qualitative perspective from healthcare managers and implementers, we synthesised information from the nine 2016 provincial-level EMTCT stock-taking workshops. These workshops involve key provincial and district-level staff, mentors and supporting partners. Lastly, we highlight potential innovations presented at these workshops to overcome operational challenges.

**Results:** The national IU mother-to-child transmission (MTCT) rate was 0.9%, which translated to an IU case rate of 245 HIV-positive neonates per 100 000 live births. Provincial IU percent MTCT risk ranged from 0.6% to 1.3%, with IU case rates ranging between 168 and 325 cases per 100 000 live births. District-level IU percent MTCT risk ranged from 0.4% to 1.9%. Potential game changers include: pre-conception counselling to optimise maternal-partner health, weekly dissemination of HIV polymerase chain reaction (PCR) and viral load reports from the NHLS to specific individuals who trace mothers and infants needing care, use of ward-based outreach teams and community caregivers to trace HIV-infected mothers and their infants to link them into care, inclusion of a unique identifier in patient-held infant Road to Health booklets to facilitate infant tracing and continuous quality improvement (CQI) processes within facilities and districts and implementation of an HIV-positive baby tool to understand the characteristics and risks of HIV-positive infants. On an ecological level, provinces and districts using community-based approaches and CQI methodology seemed to have lower MTCT and IU case rates.

**Conclusions:** More quantitative analyses are needed to understand what proportion of the success can be attributed to community-based and CQI approaches, and the impact of the potential game changers on progress towards EMTCT.

Ms Faith Moyo

Prof Gayle Sherman

## Near-real-time tracking of gaps in prevention of mother-to-child transmission of HIV in three districts of KwaZulu-Natal Province, South Africa

***Moyo F,*** *Mazanderani AH, Bhardwaj S, Mhlongo OB, Kufa T, Ng'oma K, Smith BA,* ***Sherman GG.***

*South African Medical Journal*
***Impact Factor: 2.163***


**Background:** Identifying and addressing gaps in the prevention of mother-to-child transmission of HIV (PMTCT) is required if South Africa (SA) is to achieve targets for eliminating MTCT (eMTCT). Potential PMTCT gaps that increase MTCT risk include late maternal HIV diagnosis, lack of or delayed antiretroviral therapy (ART) during pregnancy and breastfeeding, and lack of effective prophylaxis for HIV-exposed infants.

**Objectives:** To investigate, in near real time, PMTCT gaps among HIV-infected infants in three districts of KwaZulu-Natal Province, SA.

**Methods:** Between May and September 2016, PMTCT co-ordinators from eThekwini, uMgungundlovu and uMkhanyakude districts received daily email notification of all HIV polymerase chain reaction (PCR)-positive results. Co-ordinators reviewed facility records for each infant to identify gaps in PMTCT care, including maternal age, timing of maternal HIV diagnosis, maternal treatment history and maternal viral load (VL) monitoring. Data were submitted via the mobile phone SMS (text message) service using Rapid Pro technology and analysed in Stata 14.

**Results:** Data on PMTCT gaps were received for 367 (91.8%) of 400 infants with HIV PCR-positive results, within a median time of 12.5 days (interquartile range (IQR) 6 - 23). The median maternal age was 25 years (IQR 22 - 30), with 48 teenage mothers (15 - 19 years). The sample size was too small to determine whether there were significant differences in PMTCT gaps between the 48 teenage mothers and 293 older (20 - 34 years) mothers. Of the mothers, 220 (60.0%) were first diagnosed prior to conception or at their first antenatal care (ANC) visit, and 127 (34.6%) at or after delivery; 137 (37.3%) transmitted HIV to their infants despite receiving >12 weeks of ART. VL results were unavailable for 70.0% of women. Only 41 (17.5%) of women known to be HIV-positive during ANC had confirmed virological suppression. No statistically significant differences in PMTCT gaps were observed between districts, owing to small sample sizes in uMgungundlovu and uMkhanyakude.

**Conclusions:** The findings highlight the need to improve services during ANC, in particular prioritising maternal VL monitoring. We intend to use improved technology to streamline data collection and reporting towards eMTCT.

Dr Ashika Singh-Moodley

Prof Olga Perovic

## Phenotypic and genotypic correlation of carbapenememase-producing Enterobacteriaceae and problems experienced in routine screening

***Singh-Moodley A, Perovic O.***

*South African Medical Journal*
***Impact Factor: 2.163***


**Background:** The emergence and transmission of carbapenem-resistant Enterobacteriaceae (CRE) is a concern in both the clinical and public health arenas. Reliable and accurate detection of these organisms is required for patient management and infection prevention and control purposes. In the routine laboratory, phenotypic methods are utilised for identification of CRE.

**Objectives:** To investigate the phenotypic profiles of suspected carbapenemase-producing Enterobacteriaceae (CPE) isolates generated by the automated MicroScan Walkaway system making use of the Clinical and Laboratory Standards Institute (CLSI) guidelines, and correlate these with carbapenemase production by molecular methods.

**Methods:** Antimicrobial susceptibility testing was performed using the MicroScan Walkaway system, and the presence of six carbapenemase genes (*bla*NDM, *bla*VIM, *bla*IMP, *bla*OXA-48 and variants, *bla*GES and *bla*KPC) was screened for using a multiplex real-time polymerase chain reaction.

**Results:** A total of 2 678 isolates were evaluated. *Klebsiella pneumoniae* accounted for 62.9% of the isolates (n=1 685), followed by *Enterobacter cloacae* (n=361, 13.5%). Carbapenemases accounted for 75.2% of isolates; *bla*OXA-48 and its variants predominated (n=978, 36.5%), followed by *bla*NDM (n=904, 33.8%), *bla*VIM (n=108, 4.0%), *bla*IMP (n=35, 1.3%), *bla*GES (n=24, 0.9%) and *bla*KPC (n=18, 0.7 %).

**Conclusions:** A considerable number of isolates expressing a carbapenemase or carbapenemases (the majority of which were *bla*OXA-48 producing) were susceptible to third-and fourth-generation cephalosporins and carbapenems, demonstrating that confirmed carbapenemase-producing isolates are not presenting as possible carriers of carbapenemases using routine diagnostic methods. Similar results were obtained when CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints were applied and are suitable for the purpose of patient management. However, since genotyping assays are costly, it is suggested that routine laboratories first perform comprehensive phenotypic screening for CPE.

Mr Phuti Sekwadi

## Waterborne outbreak of gastroenteritis on the KwaZulu-Natal Coast, South Africa, December 2016/ January 2017

***Sekwadi PG**, Ravhuhali KG, Mosam A, Essel V, Ntshoe GM, Shonhiwa AM, McCarthy K, Mans J, Taylor MB, Page NA, **Govender N.***

*Epidemiology and Infection*
***Impact Factor: 2.044***


An unexpected increase in gastroenteritis cases was reported by healthcare workers on the KwaZulu-Natal Coast, South Africa, January 2017 with >600 cases seen over a 3-week period. A case–control study was conducted to identify the source and risk factors associated with the outbreak so as to recommend control and prevention measures. Record review identified cases and controls and structured-telephonic interviews were conducted to obtain exposure history. Stool specimens were collected from 20 cases along with environmental samples and both screened for enteric pathogens. A total of 126 cases and 62 controls were included in the analysis. The odds of developing gastroenteritis were 6.0 times greater among holiday makers than residents (95% confidence i nterval ( CI) 2 .0–17.7). Swimming in the lagoon increased the odds of developing gastroenteritis by 3.3 times (95% CI 1.06–10.38). Lagoon water samples tested positive for norovirus (NoV) GI.6, GII.3 and GII.6, astrovirus and rotavirus. Eleven (55%) stool specimens were positive for NoV with eight genotyped as GI.1 (n = 2), GI.5 (n = 3), GI.6 (n = 2), and GI.7 (n = 1). A reported sewage contamination event impacting the lagoon was the likely source with person-to-person spread perpetuating the outbreak. Restriction to swimming in the lagoon was apparently ineffective a t p reventing t he o utbreak, p ossibly d ue t o inadequate enforcement, communication and signage strategies.

Mr Nevashan Govender

Ms Orienka Hellferscee

## Enterovirus genotypes among patients with severe acute respiratory illness, influenza-like illness, and asymptomatic individuals in South Africa, 2012-2014

***Hellferscee O**, Tempia S, Walaza S, Variava E, Dawood H, Wolter N, Madhi SA, du Plessis M, Cohen C, **Treurnicht FK**.*

*Journal of Medical Virology*
***Impact Factor: 1.988***


Enteroviruses can cause outbreaks of severe acute respiratory illness (SARI) and EV-A, -B, -C, and -D species have different pathogenic profiles and circulation patterns. We aimed to characterise and determine the prevalence of enterovirus genotypes among South African patients with respiratory illness and controls during June 2012 to July 2014. Syndromic SARI and influenza-like illness (ILI) surveillance was performed at two sentinel sites. At each site nasopharyngeal/oropharyngeal specimens were collected from SARI and ILI patients as well as controls. Specimens were tested for enterovirus by real-time PCR. Positive specimens were further genotyped by sequencing a region of the VP1 gene. The prevalence of enterovirus was 5.8% (87/1494), 3.4% (103/3079), and 3.4% (46/1367) among SARI, ILI, and controls, respectively (SARI/controls, $P=0.002$ and ILI/control, $P=0.973$). Among the 101/236 (42.8%) enterovirus-positive specimens that could be genotyped, we observed a high diversity of circulating enterovirus genotypes (a total of 33 genotypes) from all four human enterovirus species with high prevalence of Enterovirus-B (60.4%; 61/101) and Enterovirus-A (21.8%; 22/101) compared to Enterovirus-C (10.9%; 11/101) and Enterovirus-D (6.9%; 7/101) ($P=0.477$). Of the enterovirus genotypes identified, Echovirus 30 (9.9%, 10/101), Coxsackie virus B5 (7.9%, 8/101) and Enterovirus-D68 (6.9%, 7/101) were most prevalent. There was no difference in disease severity (SARI or ILI compared to controls) between the different enterovirus species ($P=0.167$). We observed a high number of enterovirus genotypes in patients with respiratory illness and in controls from South Africa with no disease association of EV species with disease severity

Dr Florette Treunicht

Dr Leonard Dandalo

Prof Lizette Koekemoer

## Population Dynamics and *Plasmodium falciparum* (Haemosporida: Plasmodiidae) Infectivity Rates for the Malaria Vector *Anopheles arabiensis* (Diptera: Culicidae) at Mamgene, KwaZulu-Natal, South Africa

***Dandalo LC**, Brooke BD, Munhenga G, Lobb LN, Zikhali J, Ngxongo SP, Zikhali PM, Msimang S, Wood OR, Mofokeng M, Misiani E, Chirwa T, **Koekemoer LL**.*

*Journal of Medical Entomology*
***Impact Factor: 1.968***


*Anopheles arabiensis* (Patton; Diptera: Culicidae) is a major malaria vector in the Southern African region. In South Africa, effective control of this species using indoor-based interventions is reduced owing to its tendency to rest outdoors. As South Africa moves towards malaria elimination there is a need for complementary vector control strategies. One of the methods under consideration is the use of the sterile insect technique (SIT). Key to the successful implementation of an SIT programme is prior knowledge of the size and spatial distribution of the target population. Understanding mosquito population dynamics for both males and females is critical for efficient programme implementation. It is thus necessary to use outdoor-based population monitoring tools capable of sampling both sexes of the target population. In this project, mosquito surveillance and evaluation of tools capable of collecting both genders were carried out at Mamfene in northern KwaZulu-Natal Province, South Africa, during the period January 2014 to December 2015. Outdoor- and indoor-resting *Anopheles* mosquitoes were sampled in three sections of Mamfene over the 2-year sampling period using modified plastic buckets, clay pots and window exit traps. Morphological and molecular techniques were used for species identifications of all samples. Wild-caught adult females were tested for *Plasmodium falciparum* (Welch; Haemosporida: Plasmodiidae) infectivity. Out of 1,705 mosquitoes collected, 1,259 (73.8%) and 255 (15%) were identified as members of either the *Anopheles gambiae* complex or Anopheles *funestus* group respectively. An. *arabiensis* was the most abundant species contributing 78.8% of identified specimens. Mosquito density was highest in summer and lowest during winter. Clay pots yielded 16.3 mosquitoes per trap compared to 10.5 for modified plastic buckets over the 2-yr sampling period. *P. falciparum* infection rates for An. *arabiensis* were 0.7% and 0.5% for 2014 and 2015, respectively. Logistic regression analysis showed an association between An. arabiensis catches with section and season of collection but not with sex and collection methods. These data confirmed the presence of a perennial An. *arabiensis* population at Mamfene and constitute the first records of *P. falciparum* infective An. *arabiensis* from South Africa, confirming this species as a major vector in the malaria endemic provinces of the country.

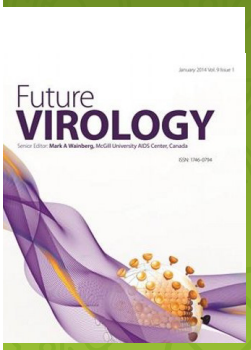
Dr Sabelle Jallow

## Possible implications of maternal HIV infection for increasing measles susceptibility in young infants

***Jallow S**, Madhi SA.*

*Future Virology*
***Impact Factor: 1.121***

Recent outbreaks in LMICs, such as South Africa, observed a demographic shift in measles cases; in addition to high percentage of cases occurring in very young infants (24% of 18,431 laboratory confirmed cases in infants less than 9 months' age); adults in the age group of high HIV prevalence were also affected (20% in adults aged 15–39 years).




Prof Penny Moore

## The Neutralising Antibody Response to the HIV-1 Env Protein

***Moore PL**.*

*Current HIV Research*
***Impact Factor: 1.562***

**Background:** A vaccine able to elicit broadly neutralising antibodies capable of blocking infection by global viruses has not been achieved, and remains a key public health challenge.

**Objectives:** During infection, a robust strain-specific neutralising response develops in most people, but only a subset of infected people develop broadly neutralising antibodies. Understanding how and why these broadly neutralising antibodies develop has been a focus of the HIV-1 vaccine field for many years, and has generated extraordinary insights into the neutralising response to HIV-1 infection.

**Results:** This review describes the features, targets and developmental pathways of early strain-specific antibodies and later broadly neutralising antibodies, and explores the reasons such broad antibodies are not more commonly elicited during infection.

**Conclusion:** The insights from these studies have been harnessed for the development of pioneering new vaccine approaches that seek to drive B cell maturation towards breadth. Overall, this review describes how findings from infected donors have impacted on active and passive immunisation approaches that seek to prevent HIV-1 infection.

Ms Lizanne Basson

## Blowflies as vectors of *Bacillus anthracis* in the Kruger National Park

***Basson L**, Hassim A, Dekker A, Gilbert A, Beyer W, Rossouw J, van Heerden H.*

*Koedoe*
***Impact Factor: 0.919***

Anthrax, caused by *Bacillus anthracis*, is endemic in the Kruger National Park (KNP). The epidemiology of *B. anthracis* is dependent on various factors including vectors. The aims of this study were to examine non-biting blowflies for the presence of *B. anthracis* externally and internally after feeding on an anthrax-infected carcass and to determine the role of flies in disseminating *B. anthracis* onto the surrounding vegetation. During an anthrax outbreak in 2014 in the endemic Pafuri region, blowflies associated with two 2–3-day-old anthrax-positive carcasses (kudu and impala) as well as surrounding vegetation were collected and investigated for the presence of *B. anthracis* spores. The non-biting blowflies ($n$ = 57) caught included *Chrysomya albiceps*, Ch. *marginalis* and *Lucilia* spp. *Bacillus anthracis* spores were isolated from 65.5% and 25.0% of blowflies collected from the kudu and impala carcasses, respectively. *Chrysomya albiceps* and *Ch. marginalis* have the potential to disseminate *B. anthracis* to vegetation from infected carcasses and may play a role in the epidemiology of anthrax in the KNP. No *B. anthracis* spores were initially isolated from leaves of the surrounding vegetation using selective media. However, 170 and 500 spores were subsequently isolated from *Abutilon angulatum* and *Acacia sp.* leaves, respectively, when using sheep blood agar.

**Conservation implications:** The results obtained in this study have no direct conservation implications and only assist in the understanding of the spread of the disease.

Dr Anthony Smith

## Genome Sequence for Shiga Toxin-Producing *Escherichia coli* 026:H11, Associated with a Cluster of Hemolytic-Uremic Syndrome Cases in South Africa, 2017

***Smith AM**, Bosco KJ, Nicol MP, Kleynhans J, McCulloch M, Duze ST, Ismail A, Allam M, Tau NP, Keddy KR.*

*Genome Announcements*
***Impact Factor: 1.18***

Shiga toxin-producing *Escherichia coli* (STEC) strains are primarily foodborne pathogens that may cause diarrheal outbreaks and are associated with severe complications, specifically hemolytic-uremic syndrome (HUS). We report here genome sequence data for STEC O26:H11, which is associated with a cluster of cases of HUS; a rarely described syndrome in South Africa.

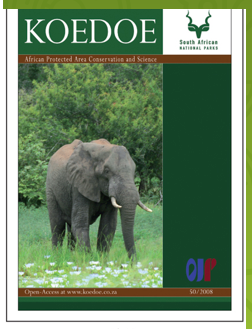



FIGURE 2
A new front-cover style for *Koedoe*, launched in conjunction with *Koedoe*'s 50th volume anniversary.

Dr Mushal Allam

Dr Anthony Smith

## Whole-Genome Sequences of *Listeria monocytogenes Sequence* Type 6 Isolates Associated with a Large Foodborne Outbreak in South Africa, 2017 to 2018

***Allam M***, *Tau N, Smouse SL, Mtshali PS, Mnyameni F, Khumalo ZTH, Ismail A, Govender N, Thomas J,* ***Smith AM***.

*Genome Announcements*
***Impact Factor: 1.18***

We report whole-genome sequences for 10 *Listeria monocytogenes* sequence type 6 isolates associated with a large listeriosis outbreak in South Africa, which occurred over the period of 2017 to 2018. The possibility of listeriosis spreading beyond South Africa's borders as a result of exported contaminated food products prompted us to make the genome sequences publicly available.

Dr Mushal Allam

Dr Shaheed Valley Omar

## Whole-Genome Sequence of a *Mycobacterium goodii* Isolate from a pediatric Patient in South Africa

***Allam M***, *Joseph L, Ismail F, Said H, Ismail NA, Ismail A, Mtshali S, Mnyameni F, Goussard P, Pekeur JC, Lourens A,* ***Omar SV***.

*Genome Announcements*
*Impact Factor: 1.18*

We describe here the draft genome sequence of a *Mycobacterium goodii* isolate from a pediatric patient in Western Cape, South Africa. To our knowledge, this is the second reported genome of this rapidly growing nontuberculous mycobacterial species.

**All Impact Factors were sourced from Web of Science**

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