Issue 13 - November 2019

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

SCIENCE FOCUS

The Science Focus acknowledges NICD members of staff who have published in peer-reviewed journals. This publication is a compilation of scientific publications where an NICD staff member is either the first or last author.

Editor's Note

The National Institute for Communicable Diseases (NICD) is a health institution with profound local impact and global reach in terms of its research footprint. In this Issue of the Science Focus, we provide a glimpse into the extensive research excellence within the NICD focusing on communicable diseases of public health importance such as HIV, malaria and tuberculosis, among others. This issue has infographics that feature outstanding statistical findings pertaining to research from the two quarters of the financial year 2019/20.

Professor John Frean, Chair of the Research Committee of the Institute, gives a brief commentary on why developing a research culture in the NICD plays an essential role for public health nationally.

The number of peer-reviewed articles produced in the first two-quarters of 2019/20, a breakdown of articles published by NICD centres, the top 5 most published authors within the second quarter, and the top 5 high impact factor score articles can be found in this Issue.

We also feature the newly National Research Foundation (NRF) rated researcher, Dr Halima Said, and, we highlight first and last authors from the NICD who have published articles within the second quarter of 2019/20.

We encourage staff members to continue to send their comments to the Communications Unit and hope this Issue is going to be great reading. Enjoy!

On behalf of the team,

Sinenhlanhla Jimoh
Senior Communications Manager

COMMENTARY

Cultivating research culture – why it's important for the National Institute for Communicable Diseases

Some people may think that research is something done in isolation from the real world. However, according to the National Research Foundation, 'research' is defined as an original investigation that is undertaken to gain knowledge and/or enhance understanding of the world. Generally, it builds on existing knowledge to produce new or substantially improved materials, devices, products, policies, or even processes.

From another point of view, do researchers benefit in a one-sided way by gathering data from communities with the primary objective to publish in journals or earn higher degrees? This was a discussion at a recent meeting with provincial malaria control programme staff in one of the malaria-endemic provinces, where the question was put to researchers from the NICD, universities and other organisations. All the scientific investigations under discussion were directed at various aspects of the public health problem of malaria, but some had been interpreted as optional 'research projects' without wider importance.

Critically, the NICD undertakes many activities in fulfilling its mission to be a world-class public health institution, to address questions and issues that provide new knowledge in order to support the South African National Department of Health, and contribute to the wider African community. Research at the NICD seeks to address broader public health issues, like the distribution and the monitoring of important disease trends over time. More importantly, the formulation of advice on priorities and planning of control programmes, evaluation of public health interventions, and the detection of, and response to, disease outbreaks are focal to the functions of the NICD.

A topical example is the molecular characterisation of clinical *Listeria monocytogenes* isolates, which distinguishes between the expected sporadic infections and those caused by the outbreak strain. In many cases, laboratory findings are directly relevant to the surveillance and epidemiology activities in addressing disease outbreaks, where external support from institutions and organisations play a bigger role in supporting much of the research done at the NICD through grant funding.

In order to continue impacting communities through research, offering grant-funded research projects is a way to attract young scientists and provide opportunities to start research careers – of course, a curious mind being the fundamental requirement!

Prof John Frean
Chairperson: NICD Research Committee

EXCEPTIONAL RESEARCH

NUMBER OF PEER REVIEWED ARTICLES PRODUCED

54

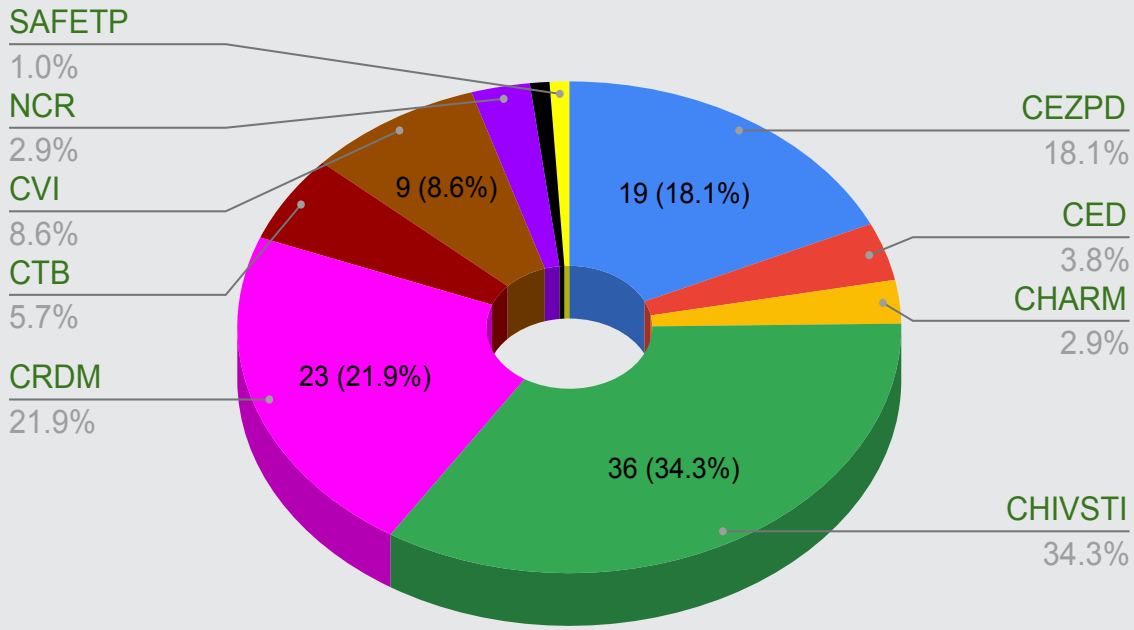
QUARTER ONE
2019/20

50

QUARTER TWO
2019/20

ARTICLES PUBLISHED
PER CENTRE

Data was sourced from Centre Activity Reports consisting of two quarters (quarter one and quarter two) of the financial year 2019/20. The total output of articles for the two quarters were added to make the total contribution per centre.



TOP 5

MOST PUBLISHED AUTHORS

1

PROF ANNE VON GOTTBERG

2

PROF CHERYL COHEN

3

PROF LYNN MORRIS

4

PROF PENNY MOORE

5

DR SIBONGILE WALAZA

DR MELINDA SUCHARD

TOP 5

HIGH IMPACT FACTOR SCORE ARTICLES

1

Journal Name: Lancet Infectious Diseases
Impact Factor Score: 27.516
Title: Meningitis: A frequently fatal diagnosis in Africa
NICD Author: Anne von Gottberg

2

Journal Name: The Lancet Respiratory Medicine
Impact Factor Score: 22.992
Title: Live attenuated influenza vaccines for African children
NICD Author: Cheryl Cohen

3

Journal Name: Immunity
Impact Factor Score: 21.522
Title: Rapid and focused maturation of a VRC01-class HIV broadly neutralising antibody lineage involves both binding and accommodation of the N276-glycan
NICD Authors: Sharon Madzorera, Dale Kitchin, Bronwen Lambson, Molati Nonyane and Penny Moore

4

Journal Name: Lancet Global Health
Impact Factor Score: 15.873
Title: Global patterns in monthly activity of influenza virus, respiratory syncytial virus, parainfluenza virus, and metapneumovirus: A systematic analysis
NICD Author: Cheryl Cohen

5

Journal Name: Nucleic Acids Research
Impact Factor Score: 11.147
Title: OGRDB: A reference database of inferred immune receptor genes
NICD Author: Cathrine Scheepers

NEWLY NRF RATED RESEARCHER

The National Research Foundation (NRF) rating system is a key driver in the NRF's aim to build a globally competitive science system in South Africa. It is a valuable tool for benchmarking the quality of our researchers against the best in the world. NRF ratings are allocated based on a researcher's recent research outputs and impact as perceived by international peer reviewers.

C – ESTABLISHED RESEARCHER

DR HALIMA SAID

Dr Halima Said is a Medical Scientist who specialises in research on tuberculosis (TB), specifically molecular epidemiology of TB and drug resistance. Her current focus is surveillance and early detection of drug-resistant TB outbreaks using molecular typing methods.

She is responsible for the evaluation of new diagnostic tools, developing research protocols and publishing in peer-reviewed journals.

"My research has grown over the years considering the number of peer-reviewed publications and conference presentations. Up-to-date, I have 17 publications, with 7 first author, all which addressed significant policy directives in South Africa," said Dr Said.

Dr Said believes that her rating will open opportunities for funding and collaborations that will be beneficial to the institution.

ABOUT NRF RATING

The rating process is coordinated by members of academia who are represented in the following committees:

- 27 Specialist Committees coordinated by a Convener
- The Executive Evaluation Committee
- The Appeals Committee

The ratings that are awarded fall within the following categories:

- **A –** Leading international researchers
- **B –** Internationally acclaimed researchers
- **C –** Established researchers
- **P –** Prestigious Awards
- **Y –** Promising young researchers

FEATURED RESEARCH ABSTRACTS FOR THE SECOND QUARTER OF 2019/20

Prof Anne von Gottberg

Meningitis: A frequently fatal diagnosis in Africa

von Gottberg A, *Meintjes G.*

The Lancet Infectious Diseases
Impact Factor: 27.516

Outside of Africa, linkage of multiple national datasets to improve the understanding of meningitis and other diseases happens more often; it is exciting to now see this powerful epidemiological method in use with African datasets, in this report and others.

Collection of large-scale, high-quality, routine data in electronic databases has great potential to enhance epidemiological assessment of common diseases, diagnostic and management practices, and outcomes, and to direct and prioritise interventions and inform policy, practice, and research.

The high mortality reported by Tenforde and colleagues is similar to the mortality in other meningitis cohorts in Africa, although the lack of details regarding presentation and management in this report (e.g. clinical signs and symptoms on presentation, history of length of illness before presenting to hospital, antimicrobial therapy, length of treatment, use of adjuvant therapy) limits the ability to evaluate the major health system contributors. Late presentation, reduced access to optimal diagnostics and antimicrobial therapies, suboptimal acute care, and HIV co-infection have been implicated as major contributors in previous reports.

Of concern is that over the 12-year period covered by Tenforde and colleagues, HIV was still associated with more than two-thirds of meningitis diagnoses and there was no reduction in meningitis mortality over time, despite the scale-up of antiretroviral therapy in Botswana. Reasons for patients continuing to present with complications of advanced HIV (such as meningitis) despite successful antiretroviral therapy programmes include delayed HIV diagnosis and disengagement from antiretroviral care.

Prof Cheryl Cohen

Live attenuated influenza vaccines for African children

Cohen C, *Sullivan SG.*

The Lancet Respiratory Medicine
Impact Factor: 22.992

Africa has the highest burden of influenza-associated mortality globally. Among African children younger than 5 years, the annual influenza-associated rate of hospital admission is estimated to be 174 per 100 000, more than twice the rate reported in children from Europe and North and South America.

In South Africa, 57% of influenza-associated deaths among children younger than 5 years are estimated to occur outside of hospital. Yet, Africa remains the WHO region with the lowest uptake of influenza vaccines.

Contributing factors to this low uptake include limited data for the burden of influenza in most African countries, scant data for costs and cost-effectiveness of influenza vaccination, challenges of administration of an annual vaccination programme, and a paucity of local data for influenza vaccine efficacy and effectiveness.

Dr Erika van Schalkwyk

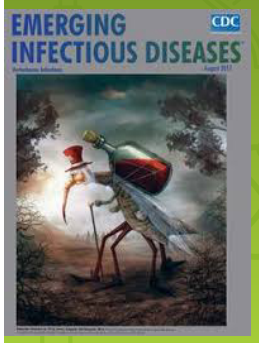
Prof Nelesh Govender

Epidemiologic shift in candidemia driven by *Candida auris*, South Africa, 2016-2017

van Schalkwyk E, *Mpembe RS, Thomas J, Shuping L, Ismail H, Lowman W, Karstaedt AS, Chibabhai V, Wadula J, Avenant T, Messina A, Govind CN, Moodley K, Dawood H, Ramjathan P,* ***Govender NP,*** *GERMS-SA.*

Emerging Infectious Diseases
Impact Factor: 7.785

Candida auris is an invasive healthcare-associated fungal pathogen. Cases of candidemia, defined as illness in patients with *Candida* cultured from blood, were detected through national laboratory-based surveillance in South Africa during 2016-2017. We identified viable isolates by using mass spectrometry and sequencing. Among 6,669 cases (5,876 with species identification) from 269 hospitals, 794 (14%) were caused by *C. auris*. The incidence risk for all candidemia at 133 hospitals was 83.8 (95% CI 81.2-86.4) cases/100,000 admissions. Prior systemic antifungal drug therapy was associated with a 40% increased adjusted odds of *C. auris* fungemia compared with bloodstream infection caused by other *Candida* species (adjusted odds ratio 1.4 [95% CI 0.8-2.3]). The crude in-hospital case-fatality ratio did not differ between *Candida* species and was 45% for *C. auris* candidemia, compared with 43% for non-*C. auris* candidemia. *C. auris* has caused a major epidemiologic shift in candidemia in South Africa.



Mr David Sacks

Prof Penny Moore

Somatic hypermutation to counter a globally rare viral immunotype drove off-track antibodies in the CAP256-VRC26 HIV-1 V2-directed bNAb lineage

Sacks D, *Bhiman JN, Wiehe K, Gorman J, Kwong PD, Morris L,* ***Moore PL.***

PLOS Pathogens
Impact Factor: 6.463

Previously we have described the V2-directed CAP256-VRC26 lineage that includes broadly neutralising antibodies (bNAbs) that neutralise globally diverse strains of HIV. We also identified highly mutated "off-track" lineage members that share high sequence identity to broad members but lack breadth. Here, we defined the mutations that limit the breadth of these antibodies and the probability of their emergence. Mutants and chimeras between two pairs of closely related antibodies were generated: CAP256.04 and CAP256.25 (30% and 63% breadth, respectively) and CAP256.20 and CAP256.27 (2% and 59% breadth). Antibodies were tested against 14 heterologous HIV-1 viruses and select mutants to assess breadth and epitope specificity. A single R100rA mutation in the third heavy chain complementarity-determining region (CDRH3) introduced breadth into CAP256.04, but all three CAP256.25 heavy chain CDRs were required for potency. In contrast, in the CAP256.20/27 chimeras, replacing only the CDRH3 of CAP256.20 with that of CAP256.27 completely recapitulated breadth and potency, likely through the introduction of three charge-reducing mutations. In this individual, the mutations that limited the breadth of the off-track antibodies were predicted to occur with a higher probability than those in the naturally paired bNAbs, suggesting a low barrier to the evolution of the off-track phenotype. Mapping studies to determine the viral immunotypes (or epitope variants) that selected off-track antibodies indicated that unlike broader lineage members, CAP256.20 preferentially neutralised viruses containing 169Q. This suggests that this globally rare immunotype, which was common in donor CAP256, drove the off-track phenotype. These data show that affinity maturation to counter globally rare viral immunotypes can drive antibodies within a broad lineage along multiple pathways towards strain-specificity. Defining developmental pathways towards and away from breadth may facilitate the selection of immunogens that elicit bNAbs and minimise off-track antibodies.

Dr Nigel Makoah

Prof Lynn Morris

AAV-mediated expression of broadly neutralising and vaccine-like antibodies targeting the HIV-1 envelope V2 region

van den Berg F, ***Makoah N,*** *Ali S, Scott T, Ziki R, Mutsvunguma L, Mkhize NN, Lambson B, Crowther C, Abdool Karim S, Balasz A, Weinberg M, Ely A, Arbuthnot P,* ***Morris L.***

Molecular Therapy: Methods & Clinical Development
Impact Factor: 4.875

HIV-1 infection continues to be a global health challenge and a vaccine is urgently needed. Broadly neutralising antibodies (bNAbs) are considered essential as they inhibit multiple HIV-1 strains, but they are difficult to elicit by conventional immunisation. In contrast, non-neutralising antibodies that correlated with reduced risk of infection in the RV144 HIV vaccine trial are relatively easy to induce, but responses are not durable. To overcome these obstacles, adeno-associated virus (AAV) vectors were used to provide long-term expression of antibodies targeting the V2 region of the HIV-1 envelope protein, including the potent CAP256-VRC26.25 bNAb, as well as non-neutralising CAP228 antibodies that resemble those elicited by vaccination. AAVs mediated effective antibody expression in cell culture and immunocompetent mice. Mean concentrations of human immunoglobulin G (IgG) in mouse sera increased rapidly following a single AAV injection, reaching 8-60 µg/mL for CAP256 antibodies and 44-220 µg/mL for CAP228 antibodies over 24 weeks, but antibody concentrations varied for individual mice. Secreted antibodies collected from serum retained the expected binding and neutralising activity. The vectors generated here are, therefore, suitable for the delivery of V2-targeting HIV antibodies, and they could be used in a vectored immunoprophylaxis (VIP) approach to sustain the level of antibody expression required to prevent HIV infection.

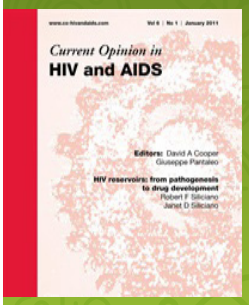
Dr Simone Richardson

Prof Penny Moore

The antibody response in HIV-1-infected donors

Richardson SI, Moore PL.

Current Opinion in HIV and AIDS
Impact Factor: 4.268



Purpose of review: Although the goal of preventive HIV vaccine design is primarily the induction of broadly neutralising antibodies (bNAbs), recent evidence suggests that a protective response will also benefit from Fc effector functions. Here, we provide an update on the antibody response to HIV infection, including both Fab and Fc-mediated antibody responses. We also highlight recent studies showing the interplay between these functions, focusing primarily on studies published in the last year.

Recent findings: Identification and characterisation of bNAb donors continues to provide insights into viral factors that are potentially translatable to vaccine design. Improved and more diverse measures of Fc effector function, and modulators thereof, are enabling a deeper understanding of their role in infection. New data providing mechanistic links between the innate and adaptive humoral immune responses are creating exciting opportunities for vaccine strategies, with the aim of eliciting a polyfunctional protective response.

Summary: New insights into the overall humoral response to HIV infection are defining diverse and synergistic mechanisms required for antibody protection from HIV through vaccination.

Prof Janusz Paweska

Dr Petrus Jansen van Vuren

Evaluation of diagnostic performance of three indirect enzyme-linked immunosorbent assays for the detection of IgG antibodies to Ebola virus in human sera

Paweska JT, *Moolla N, Storm N, Msimang V, Conteh O, Weyer J,* ***Jansen van Vuren P.***

Viruses
Impact Factor: 3.811

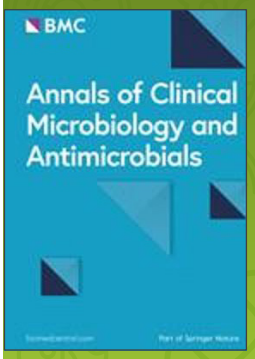
Filovirus serological diagnosis and epidemiological investigations are hampered due to the unavailability of validated immunoassays. Diagnostic performance of three indirect enzyme-linked immunosorbent assays (I-ELISA) was evaluated for the detection of IgG antibody to Ebola virus (EBOV) in human sera. One I-ELISA was based on a whole EBOV antigen (WAg) and two utilised recombinant nucleocapsid (NP) and glycoproteins (GP), respectively. Validation data sets derived from individual sera collected in South Africa (SA), representing an EBOV non-endemic country, and from sera collected during an Ebola disease (EBOD) outbreak in Sierra Leone (SL), were categorised according to the compounded results of the three I-ELISAs and real time reverse-transcription polymerase chain reaction (RT-PCR). At the cut-off values selected at 95% accuracy level by the two-graph receiver operating characteristic analysis, specificity in the SA EBOV negative serum panel (n = 273) ranged from 98.17% (GP ELISA) to 99.27% (WAg ELISA). Diagnostic specificity in the SL EBOV negative panel (n = 676) was 100% by the three ELISAs. The diagnostic sensitivity in 423 RT-PCR confirmed EBOD patients was dependent on the time when the serum was collected after onset of disease. It significantly increased 2 weeks post-onset, reaching 100% sensitivity by WAg and NP and 98.1% by GP I-ELISA.

Prof Caroline Tiemessen

Detection and molecular characterisation of urinary tract HIV-1 populations

Mzingwane ML, Hunt G, Lassauniere R, Kalimashe M, Bongwe A, Ledwaba J, Chaisson RE, Martinson N, Richter K, Bowyer SM, ***Tiemessen CT.***

Annals of Clinical Microbiology and Antimicrobials
Impact Factor: 2.924



Background: Identification of all possible HIV reservoirs is an important aspect in HIV eradication efforts. The urinary tract has however not been well studied as a potential HIV reservoir. In this pilot study we molecularly characterised HIV-1 viruses in urine and plasma samples to investigate HIV-1 replication, compartmentalization and persistence in the urinary tract.

Methods: Prospectively collected urine and blood samples collected over 12–36 months from 20 HIV-1 infected individuals were analysed including sampling points from prior to and after ART initiation. HIV-1 pol gene RNA and DNA from urine supernatant and urine pellets respectively were analysed and compared to plasma RNA viruses from the same individual.

Results: HIV-1 nucleic acid was detected in urine samples from at least one time point in 8/20 (40%) treatment-naïve subjects compared to 1/13 (7.7%) individuals on antiretroviral treatment (ART) during periods of plasma viral suppression and 1/7 (14.3%) individuals with virological failure. HIV-1 RNA was undetectable in urine samples after ART initiation but HIV-1 DNA was detectable in one patient more than 6 months after treatment initiation. There was co-clustering of urine-derived *pol* sequences but some urine-derived sequences were interspersed among the plasma-derived sequences.

Conclusions: Suppressive ART reduces HIV-1 replication in the urinary tract but HIV-1 DNA may persist in these cells despite treatment. A larger number of sequences would be required to confirm HIV compartmentalisation in the urinary tract.

Dr Jaishree Raman

Absence of kelch13 artemisinin resistance markers but strong selection for lumefantrine-tolerance molecular markers following 18 years of artemisinin-based combination therapy use in Mpumalanga Province, South Africa (2001–2018)

Raman J, *Kagoro FM, Mabuza A, Malatje G, Reid A, Frean J, Barnes KI.*

Malaria Journal
Impact Factor: 2.798

Background: The ability of *Plasmodium falciparum* parasites to develop resistance to widely used anti-malarials threatens malaria control and elimination efforts. Regular drug efficacy monitoring is essential for ensuring effective treatment policies. In low transmission settings where therapeutic efficacy studies are often not feasible, routine surveillance for molecular markers associated with anti-malarial resistance provides an alternative for the early detection of emerging resistance. Such a longitudinal survey of changes in the prevalence of selected molecular markers of resistance was conducted in the malaria-endemic regions of Mpumalanga Province, South Africa, where malaria elimination at a district-level is being pursued.

Methods: Molecular analyses to determine the prevalence of alleles associated with resistance to lumefantrine (*mdr86N, crt76K* and *mdr1* copy number variation) and sulfadoxine–pyrimethamine (*dhfr* triple, *dhps* double, SP quintuple) were conducted between 2001 and 2018, while artemisinin resistance markers (*kelch13* mutations) were assessed only in 2018.

Results: Parasite DNA was successfully amplified from 1667/2393 (70%) of malaria-positive rapid diagnostic tests routinely collected at primary health care facilities. No artemisinin resistance-associated *kelch13* mutations nor amplification of the *mdr1* gene copy number associated with lumefantrine resistance were observed. However, prevalence of both the *mdr86N* and *crt76K* alleles increased markedly over the study period, with all isolates collected in 2018 carrying these markers. SP quintuple mutation prevalence increased steadily from 14% in 2001 to 96% in 2018. Mixed alleles at any of the codons assessed were rare by 2018.

Conclusion: No *kelch13* mutations confirmed or suspected to be associated with artemisinin resistance were identified in 2018. Although parasites carrying the *mdr86N* and *crt76K* alleles associated with reduced lumefantrine susceptibility were strongly selected for over the study period, nearing fixation by 2018, the marker for lumefantrine resistance, namely increased mdr1 copy number, was not observed in this study. The increase in mdr86N and crt76K allele prevalence together with intense regional artemether–lumefantrine drug pressure, raises concern regarding the sustained artemether–lumefantrine efficacy. Regular, rigorous anti-malarial resistance marker surveillance across all three South African malaria-endemic provinces to inform case management is recommended.

Ms Ashley Burke

Prof Basil Brooke

Anopheles parensis contributes to residual malaria transmission in South Africa

Burke A, *Dahan-Moss Y, Duncan F, Qwabe B, Coetzee M, Koekemoer L,* ***Brooke B.***

Viruses
Impact Factor: 2.798

Background: Understanding the contribution of outdoor-resting *Anopheles* mosquitoes to residual malaria transmission is important in terms of scaling up vector control towards malaria elimination in South Africa. The aim of this project was to assess the potential role of *Anopheles parensis* and other *Anopheles* species in residual malaria transmission, using sentinel surveillance sites in the uMkhanyakude District of northern KwaZulu-Natal Province.

Methods: Monthly vector surveillance was conducted at the sentinel sites from January 2017 to May 2018. Outdoor-placed clay pot resting traps were used to collect male and female adult *Anopheles* mosquitoes. All *Anopheles gambiae* complex and *Anopheles funestus* group specimens collected were identified to species and all females were screened for *Plasmodium falciparum* circumsporozoite protein (CSP) by enzyme-linked immunosorbent assay (ELISA). Samples showing infectivity for *P. falciparum* were further verified by a nested PCR and subsequent DNA sequence analysis.

Results: From a sample of 491 anophelines, *Anopheles arabiensis* (n = 228) and *An. parensis* (n = 194) were the most abundant. Other species collected included *Anopheles merus* (n = 11), *Anopheles quadriannulatus* (n = 10), *Anopheles leesoni* (n = 29), *Anopheles rivulorum* (n = 18), and *Anopheles vaneedeni* (n = 1). Of the 317 female specimens screened for P. falciparum CSP, one *Anopheles arabiensis* and one *An. parensis* showed positive by ELISA and *Plasmodium* nested PCR. For the *An. parensis* specimen, confirmation of its species identity was based on sequence analysis of the ITS2 region, and the presence of *P. falciparum* DNA was further confirmed by sequence analysis.

Conclusions: *Anopheles parensis* is a potential vector of malaria in South Africa although its contribution to transmission is likely to be minimal at best owing to its strong zoophilic tendency. By contrast, *An. arabiensis* is a major vector that is primarily responsible for the bulk of residual malaria transmission in South Africa. As all recently collected sporozoite-positive *Anopheles* mosquitoes were found in outdoor-placed resting traps, it is necessary to introduce interventions that can be used to control outdoor-resting vector populations while maintaining the efficacy of South Africa's indoor house spraying operations.

Ms Hetani Ngobeni

Prof Cheryl Cohen

The performance of different case definitions for severe influenza surveillance among HIV-infected and HIV-uninfected children aged <5 years in South Africa, 2011-2015

Ngobeni H, *Tempia S, Cohen AL, Walaza S, Kuonza L, Musekiwa A, von Gottberg A, Hellferscee O, Wolter N, Treurnicht FK, Moyes J, Naby F, Mekgoe O,* ***Cohen C.***

PLOS One
Impact Factor: 2.776

In 2014, the World Health Organization (WHO) proposed a new severe influenza surveillance case definition, which has not been evaluated in a high human immunodeficiency virus (HIV) prevalence setting. Our study aimed to assess the performance of this proposed case definition in identifying influenza among HIV-uninfected and HIV-infected children aged <5 years in South Africa. We prospectively enrolled children aged <5 years hospitalised with physician-diagnosed lower respiratory tract infection (LRTI) at two surveillance sites from January 2011 to December 2015. Epidemiologic and clinical data were collected. We tested nasopharyngeal aspirates for influenza using reverse transcription polymerase chain reaction. We used logistic regression to assess factors associated with influenza positivity among HIV-infected and HIV-uninfected children. We calculated sensitivity and specificity for different signs and symptoms and combinations of these for laboratory-confirmed influenza. We enrolled 2,582 children <5 years of age with LRTI of whom 87% (2,257) had influenza and HIV results, of these 14% (318) were HIV-infected. The influenza detection rate was 5% (104/1,939) in HIV-uninfected and 5% (16/318) in HIV-infected children. Children with measured fever (≥38°C) were two times more likely to test positive for influenza than those without measured fever among the HIV-uninfected (OR 2.2, 95% Confidence Interval (CI) 1.5-3.4; p<0.001). No significant association was observed between fever and influenza infection among HIV-infected children. Cough alone had sensitivity of 95% (95% CI 89-98%) in HIV-uninfected and of 100% (95% CI 79-100%) in HIV-infected children but low specificity: 7% (95% CI 6-8%) and 6% (95% CI 3-9%) in HIV-uninfected and HIV-infected children, respectively. The WHO post-2014 case definition for severe acute respiratory illness (SARI-an acute respiratory infection with history of fever or measured fever of ≥ 38°C and cough; with onset within the last ten days and requires hospitalisation), had a sensitivity of 66% (95% CI 56-76%) and specificity of 46% (95% CI 44-48%) among HIV-uninfected and a sensitivity of 63% (95% CI 35-84%) and a specificity of 42% (95% CI 36-48%) among HIV-infected children. The sensitivity and specificity of the WHO post-2014 case definition for SARI were similar among HIV-uninfected and HIV-infected children. Our findings support the adoption of the 2014 WHO case definition for children aged <5 years irrespective of HIV infection status.

Dr Selamawit Woldesenbet

Prof Adrian Puren

An overview of the quality assurance programme for HIV rapid testing in South Africa: Outcome of a 2-year phased implementation of quality assurance programme

Woldesenbet SA, *Kalou M, Mhlongo D, Kufa T, Makhanya M, Adelekan A, Diallo K, Maleka M, Singh B, Parekh B, Mohlala A, Manyike PT, Tucker TJ,* ***Puren AJ.***

PLOS One
Impact Factor: 2.776

Objective: This is the first large-scale assessment of the implementation of HIV Rapid Test Quality Improvement Initiative in South Africa.

Methods: We used a quasi-experimental one group post-test only design. The intervention implemented starting April 2014 comprised health-care worker training on quality assurance (QA) of HIV rapid testing and enrolment of the facilities in proficiency testing (PT), targeting 2,077 healthcare facilities in 32 high HIV burden districts. Following the intervention, two consecutive rounds of site assessments were undertaken. The first, conducted after a median of 7.5 months following the training, included 1,915 facilities that participated in the QA training, while the second, conducted after a median of one-year following the first-round assessment included 517 (27.0%) of the 1,915 facilities. In both assessments, the Stepwise-Process-for-Improving-the-quality-of-HIV-Rapid-Testing (SPI-RT) checklist was used to score facilities' performance in 7 domains: training, physical facility, safety, pre-testing, testing, post-testing and external quality assessment. Facilities' level of readiness for national certification was assessed.

Result: Between 2016 and 2017, there were four PT cycles. PT participation increased from 32.4% (620/1,915) in 2016 to 91.5% (1,753/1,915) in 2017. In each PT cycle, PT results were returned by 76%-87% of facilities and a satisfactory result (>80%) was achieved by ≥95% of facilities. In the SPI-RT assessment, in round-one, 22.3% of facilities were close to or eligible for national certification-this significantly increased to 38.8% in round-two (P-value<0.001). The median SPI-RT score for the domains HIV pre-testing (83.3%) and post-testing (72.2%) remained the same between the two rounds. The median score for the testing domain increased by 5.6% (to 77.8%).

Conclusion: Facilities performance on the domains that are critical for accuracy of diagnosis (i.e. pre-testing, testing and post-testing) remained largely unchanged. This study provided several recommendations to improve QA implementation in South Africa, including the need to improve routine use of internal quality control for corrective actions.

Dr Gillian Hunt

Prevalence of HIV-1 drug resistance amongst newly diagnosed HIV-infected infants age 4-8 weeks, enrolled in three nationally representative PMTCT effectiveness surveys, South Africa: 2010, 2011-12 and 2012-13

Hunt GM, *Ledwaba J, Salimo A, Kalimashe M, Dinh TH, Jackson D, Sherman G, Puren A, Ngandu NK, Lombard C, Morris L, Goga A.*

BMC Infectious Diseases
Impact Factor: 2.565

Background: South Africa (SA) has expanded efforts to reduce mother-to-child transmission of HIV (MTCT) to less than 2% at six weeks after birth and to less than 5% at 18 months postpartum by 2016. Despite improved antiretroviral regimens and coverage between 2001 and 2016, there is little data on infant HIV drug resistance. This paper tracks the prevalence of HIV drug resistance patterns amongst HIV infected infants from three nationally representative studies that assessed the effectiveness of national programmes to prevent MTCT (PMTCT). The first study was conducted in 2010 (under the dual therapy PMTCT policy), the second from 2011 to 12 (PMTCT Option A policy) and the third from 2012 to 13 (PMTCT Option A policy). From 2010 to 2013, infant non-nucleoside reverse transcriptase inhibitor (NNRTI) exposure increased from single dose to daily throughout breastfeeding; maternal nucleoside reverse transcriptase inhibitor (NRTI) and NNRTI exposure increased with initiation of NNRTI-and NRTI- containing triple antiretroviral therapy (ART) earlier in gestation and at higher CD4 cell counts.

Methods: Three nationally representative surveys were conducted in 2010, 2011–12 and 2012–13. During the surveys, mothers with known, unknown, or no exposure to antiretrovirals for PMTCT and their infants were included, and MTCT was measured. For this paper, infant dried blood spots (iDBS) from HIV PCR positive infants aged 4 – 8 weeks, with consent for additional iDBS testing, were analysed for HIV drug resistance at the National Institute of Communicable Diseases (NICD), SA, using an in-house assay validated by the Centers for Disease Control and Prevention (CDC). Total viral nucleic acid was extracted from 2 spots and amplified by nested PCR to generate a ~1 kb amplicon that was sequenced using Sanger sequencing technologies. Sequence assembly and editing was performed using RECall v3.

Results: Overall, HIV-1 drug resistance was detected in 51% (95% Confidence interval (CI) [45–58%]) of HIV PCR positive infants, 37% (95% CI [28–47%]) in 2010, 64% (95% CI [53–74%]) in 2011 and 63% (95% CI [47–77%]) in 2012 ($p < 0.0001$), particularly to the NNRTI drug class. Pooled analyses across all three surveys demonstrated that infants whose mothers received ART showed the highest prevalence of resistance (74%); 26% (21/82) of HIV PCR positive infants with no or undocumented antiretroviral drug (ARV) exposure harboured NNRTI resistance.

Conclusions: These data demonstrate increasing NNRTI resistance amongst newly-diagnosed infants in a high HIV prevalence setting where maternal ART coverage increased across the years, starting earlier in gestation and at higher CD4 cell counts. This is worrying as lifelong maternal ART coverage for HIV positive pregnant and lactating women is increasing. Also of concern is that resistant virus was detected in HIV positive infants whose mothers were not exposed to ARVs, raising questions about circulating resistant virus. Numbers in this group were too small to assess trends over the three years.

Mrs Matimba Makhubele

Common mental health disorders among Informal waste pickers in Johannesburg, South Africa 2018 - a cross-sectional study

Makhubele M, *Ravhuhali K, Kuonza L, Mathee A, Kgalamono S, Made F, Tlotleng N, Kootbodien T, Ntlebi V, Wilson K, Naicker N.*

International Journal of Environmental Research and Public Health
Impact Factor: 2.468

Waste-picking is an income-generating opportunity for individuals living in poverty. Waste picking is associated with a range of risk factors for common mental disorders (CMD). This study aimed to determine the prevalence and factors associated with CMD among waste pickers in Johannesburg. A cross-sectional study analysed secondary data for 365 waste pickers. A validated Self-Reporting Questionnaire (SRQ-20) was used to assess CMD. Multivariable logistic regression was fitted to identify factors associated with CMD. The overall prevalence of CMD among waste pickers was 37.3%. The odds of having CMD were 2.5 and 3.2 higher in females and cigarette smokers, respectively ($p = 0.019$ and $p = 0.003$). Life enjoyment (Adjusted odds ratio [aOR] 0.54, $p = 0.02$) and a good quality of life (aOR 0.34, $p \leq 0.001$) were associated with lower odds of CMD. The high prevalence of CMD among waste pickers was significantly associated with cigarette smoking, being female, not enjoying life, and a poor quality of life. Mental health awareness of CMD will assist with the prevention, early detection, and comprehensive management of CMD among waste pickers.

Ms Faith Moyo

Prof Gayle Sherman

Monitoring diagnosis, retention in care and viral load suppression in children testing HIV polymerase chain reaction-positive in two districts in South Africa

Moyo F, *Mazanderani AH, Feucht UD, Ngoma K, Bhardwaj S, Goosen M, Greyling D, Mhlongo OB,* ***Sherman GG.***

South African Medical Journal
Impact Factor: 2.003

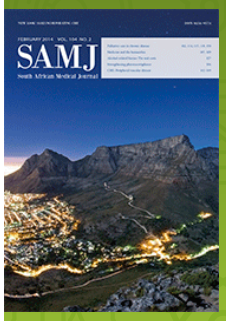
Background: Retention in care is associated with improved virological control and survival among HIV-infected children. However, retention of children in HIV care remains a challenge.

Objectives: To describe, using routine laboratory HIV test data, the retention-in-care and virological outcomes of HIV-infected children aged <18 months in two districts in South Africa.

Methods: HIV polymerase chain reaction (PCR)-positive results of children from uMkhanyakude and Tshwane districts in KwaZulu-Natal and Gauteng provinces, respectively, tested between April 2015 and May 2016, were extracted from the National Health Laboratory Service's Corporate Data Warehouse (CDW). HIV-related tests (PCR, viral load (VL), CD4+) were documented longitudinally for each child for ≥13 months after the first positive PCR result by manually searching demographics within the CDW, supplemented by an automated patient-linking algorithm. Test sets were linked if two or more demographics (surname, name, date of birth, folder number) matched exactly. Programmatic indicators assessed included age at first positive PCR test, presumed confirmatory test rates, retention in care, and VL suppression at 6 and 12 months.

Results: Ninety-four and 304 children tested HIV PCR-positive in uMkhanyakude and Tshwane, respectively. The median age at diagnosis was 3.6 months (interquartile range (IQR) 1.4 - 7.1) for uMkhanyakude and 2.3 months (IQR 0.1 - 6.7) for Tshwane. In uMkhanyakude, confirmed in utero infections accounted for 18.1% of transmissions (n=17), compared with 29.6% (n=90) in Tshwane. Presumed confirmatory test rates following an initial positive PCR result were 77.7% and 71.7% for uMkhanyakude and Tshwane, respectively. Within 6 months of starting antiretroviral therapy, 43 children (58.9%) were lost to follow-up in uMkhanyakude compared with 160 (73.4%) in Tshwane. Of those retained in care at 6 months with a VL measurement, 15 (60.0%) from uMkhanyakude had a VL <1 000 copies/mL, compared with 24 (48.0%) in Tshwane. For both districts, a third of all HIV PCR-positive children were retained in care at the end of follow-up, with 29 (30.9%) in uMkhanyakude and 99 (32.5%) in Tshwane. Of these, 12 (41.4%) had a VL <1 000 copies/mL in uMkhanyakude compared with 28 (28.3%) in Tshwane.

Conclusions: We demonstrate the value of routine laboratory data in monitoring diagnosis, retention and VL suppression in HIV-infected children. This approach is scalable, can be reported near real-time, is relatively inexpensive to implement, and provides a tool for improving paediatric HIV services until clinical databases can assume this role.

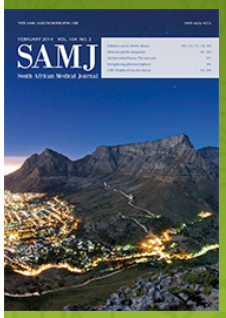


Dr Claire von Mollendorf

Factors associated with missed and delayed DTP3 vaccination in children aged 12 - 59 months in two communities in South Africa, 2012 – 2013

Mthiyane TN, Cohen C, Norris SA, Walaza S, Tempia S, Cohen AL, von Gottberg A, ***von Mollendorf C.***

South African Medical Journal
Impact Factor: 2.003



Background: Although immunisation services are available to all children in South Africa (SA), many children miss or have delays in receiving vaccines. There are limited data on factors associated with missed or delayed vaccination in children in this setting.

Objectives: To assess vaccination coverage and factors associated with missed and delayed diphtheria-tetanus-pertussis vaccine third dose (DTP3) vaccination in children aged 12 - 59 months in two SA communities.

Methods: We used data from household-level healthcare utilisation surveys conducted in Soweto in 2012 and in Pietermaritzburg in 2013. Information on vaccination status was recorded from the Road to Health cards or vaccination history from clinics for children aged <5 years. Factors associated with missed or delayed DTP3 vaccination were assessed using unconditional logistic regression.

Results: Of a total of 847 eligible children aged 12 - 59 months, 716 had available vaccination information. Overall DTP3 vaccination coverage was high for both sites: 90.6% in Pietermaritzburg and 93.9% in Soweto. However, 32.6% and 25.2% of DTP3 vaccinations were delayed (received after 18 weeks of age) in Pietermaritzburg and Soweto, respectively. The median delay for DTP3 vaccinations was 4.7 weeks (interquartile range 1.7 - 23.0). Factors associated with delayed DTP3 vaccination included being born in 2010 (adjusted odds ratio (aOR) 3.0, 95% confidence interval (CI) 1.4 - 6.3) or 2011 (aOR 2.7, 95% CI 1.3 - 5.7) compared with being born in 2008, probably due to vaccine shortages; a low level of education of the primary caregiver, with children whose caregivers had completed secondary education having lower odds of delayed vaccination (aOR 0.5, 95% CI 0.3 - 0.9) than children whose caregivers only had primary education; and maternal HIV status, with unknown status (aOR 3.5, 95% CI 1.6 - 7.6) associated with higher odds of delay than positive status. Factors associated with missed DTP3 vaccination (not vaccinated by 12 months of age) included two or more children aged <5 years in a household (aOR 2.4, 95% CI 1.2 - 4.9) compared with one child, and household monthly income <ZAR500 (aOR 3.4, 95% CI 1.1 - 11.4) compared with ≥ZAR2 000.

Conclusions: Despite high overall DTP3 coverage observed in two communities, many vaccinations were delayed. Vulnerable groups identified in this study should be targeted with improved vaccination services to enhance uptake and timeliness of vaccination.

Dr Anthony Smith

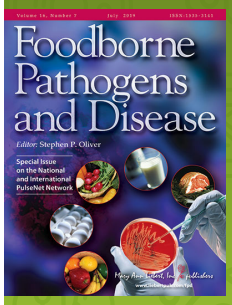
Dr Juno Thomas

Outbreak of *Listeria monocytogenes* in South Africa, 2017-2018: Laboratory activities and experiences associated with whole-genome sequencing analysis of isolates

Smith AM, *Tau NP, Smouse SL, Allam M, Ismail A, Ramalwa NR, Disenyeng B, Ngomane M,* ***Thomas J.***

Foodborne Pathogens and Disease
Impact Factor: 1.981

In South Africa, a progressive increase in listeriosis cases was noted from mid-June 2017, heralding what was to become the world's largest listeriosis outbreak. A total of 1060 cases were reported for the period January 1, 2017 to July 17, 2018. We describe laboratory activities, experiences, and results of whole-genome sequencing (WGS) analysis of *Listeria monocytogenes* isolates associated with this outbreak. Bacteria were identified using the VITEK-2 COMPACT 15 microbial identification system. WGS was performed using Illumina MiSeq technology. WGS data were analysed using CLC Genomics Workbench Software and free-to-use on-line analysis tools/pipelines. Multilocus sequence typing (MLST) showed that 91% of clinical isolates were sequence type 6 (ST6), determining that the outbreak was largely associated with *L. monocytogenes* ST6. Epidemiological and laboratory findings led to investigation of a large ready-to-eat processed meat production facility in South Africa, named Enterprise Foods. *L. monocytogenes* ST6 was found in environmental sampling swabs of the production facility and in ready-to-eat processed meat products (including polony, a product similar to bologna sausage) manufactured at the facility. ST6 isolates, sourced at the Enterprise Foods production facility and from Enterprise food products, were shown by single nucleotide polymorphism (SNP) analysis to be highly related to clinical isolates; these nonclinical ST6 isolates showed <10 SNP differences when compared to clinical ST6 isolates. Core-genome MLST showed that clinical ST6 isolates and Enterprise-related ST6 isolates had no more than 4 allele differences between each other, suggestive of a high probability of epidemiological relatedness. WGS data interpreted together with epidemiological data concluded that the source of the listeriosis outbreak was ready-to-eat processed meat products manufactured by Enterprise Foods. Listeriosis has now been added to the South African list of mandatory notifiable medical conditions. Surveillance systems have been strengthened to facilitate prevention and early detection of listeriosis outbreaks.



Dr Jacqueline Weyer

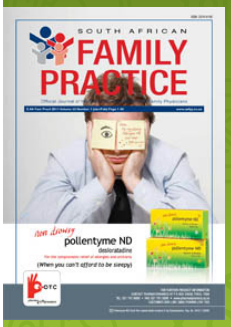
Prof Lucille Blumberg

Management of rabies

Weyer J, Blumberg L.

South African Family Practice
Impact Factor: Score unavailable

Rabies is endemic in South Africa and human rabies cases continue to be reported annually. Most human cases in South Africa are dog-transmitted. Whilst efforts are underway to control and eventually eliminate dog rabies in the country, prevention of the disease through appropriate use of rabies postexposure prophylaxis is critical to save lives. This article provides a summary of rabies in South Africa and key aspects of the prevention of the disease in exposed humans.



Dr Jacqueline Weyer

Paramyxo- and coronaviruses in Rwandan bats

Markotter W, Geldenhuys M, Jansen van Vuren P, Kemp A, Mortlock M, Mudakikwa A, Nel L, Nziza J, Paweska J, ***Weyer J.***

Tropical Medicine and Infectious Disease
Impact Factor: Score unavailable

A high diversity of corona- and paramyxoviruses have been detected in different bat species at study sites worldwide, including Africa, however no biosurveillance studies from Rwanda have been reported. In this study, samples from bats collected from caves in Ruhengeri, Rwanda, were tested for the presence of corona- and paramyxoviral RNA using reverse transcription PCR assays. Positive results were further characterised by DNA sequencing and phylogenetic analysis. In addition to morphological identification of bat species, we also did molecular confirmation of species identities, contributing to the known genetic database available for African bat species. We detected a novel *Betacoronavirus* in two Geoffroy's horseshoe bats (*Rhinolophus clivosus*) bats. We also detected several different paramyxoviral species from various insectivorous bats. One of these viral species was found to be homologous to the genomes of viruses belonging to the *Jeilongvirus* genus. Additionally, a *Henipavirus*-related sequence was detected in an Egyptian rousette fruit bat (*Rousettus aegyptiacus*). These results expand on the known diversity of corona- and paramyxoviruses and their geographical distribution in Africa.

Ms Francinah Mapuroma

Dr Claire von Mollendorf

Healthcare seeking behaviour for common infectious syndromes among people in three administrative regions of Johannesburg, South Africa, 2015: A cross-sectional study

Mapuroma R, *Cohen C, Kuonza L, Musekiwa A, Tempia S, Tshangela A,* ***von Mollendorf C.***

Pan African Medical Journal
Impact Factor: Score unavailable

Introduction: Hospital-based surveillance programmes only capture people presenting to facilities and may underestimate disease burden. We conducted a healthcare utilisation survey to characterise healthcare-seeking behaviour among people with common infectious syndromes in the catchment areas of two sentinel surveillance hospitals in Johannesburg, South Africa.

Methods: A cross-sectional survey was conducted within three regions of Johannesburg from August to November 2015. Premises were randomly selected from an enumerated list with data collected on household demographics and selected syndromes using a structured questionnaire. Fisher's exact or chi-square tests were used to determine association of characteristics among different regions.

Results: Of 3,650 selected coordinates, 3,358 were eligible dwellings and 2,930 (87%) households with 9,850 individuals participated. Four percent of participants (431/9,850) reported influenza-like illness (ILI) in the last 30 days; equal numbers of participants (0.2%, 20/9,850) reported pneumonia or tuberculosis symptoms in the last year and <1% reported diarrhoea or meningitis symptoms. Sixty eight percent (295/431) of participants who reported ILI, 75% (6/8) of children with diarrhoea and all participants who reported pneumonia (20), tuberculosis (20) or meningitis (6) sought healthcare. For all syndromes most sought care at registered healthcare providers. Of these only 10% (24/237) attended sentinel hospitals, predominantly those that lived closer to the hospitals. In contrast, of patients with meningitis, 50% (3/6) sought care at sentinel hospitals.

Conclusion: Patterns of seeking healthcare differed by syndrome and distance from facilities. Surveillance programmes are still relevant in collecting information on infectious syndromes and reflect a proportion of the hospital's catchment area.

Dr Ahmad Mazanderani

Prof Gayle Sherman

Evolving complexities of infant HIV diagnosis within prevention of mother-to-child transmission programmes

Mazanderani AH, Sherman GG.

F1000Research
Impact Factor: Score unavailable

Early diagnosis of HIV infection among infants and children is critical as prompt initiation of antiretroviral therapy prevents morbidity and death. Yet despite advances in the accuracy and availability of infant HIV diagnostic testing, there are increasing challenges with making an early definitive diagnosis. These challenges relate primarily to advances in prevention of mother-to-child transmission (PMTCT) of HIV. Although PMTCT programmes have proven to be highly effective in reducing infant HIV infection, infants who are HIV-infected may achieve virological suppression and loss of detectability of HIV nucleic acid prior to diagnosis because of antiretroviral drug exposure. Hence, false-negative and indeterminate HIV polymerase chain reaction (PCR) results can occur, especially among high-risk infants given multi-drug prophylactic regimens. However, the infant HIV diagnostic landscape is also complicated by the inevitable decline in the positive predictive value of early infant diagnosis (EID) assays. As PMTCT programmes successfully reduce the mother-to-child transmission rate, the proportion of false-positive EID results will increase. Consequently, false-negative and false-positive HIV PCR results are increasingly likely despite highly accurate diagnostic assays. The problem is compounded by the seemingly intractable prevalence of maternal HIV within some settings, resulting in a considerable absolute burden of HIV-infected infants despite a low mother-to-child transmission rate.

Dr Anthony Smith

Mrs Jackie Kleynhans

Shiga toxin-producing *Escherichia coli* O26:H11 associated with a cluster of haemolytic uraemic syndrome cases in South Africa, 2017

Smith AM, *Tau NP, Kalule BJ, Nicol MP, McCulloch M, Jacobs CA, McCarthy KM, Ismail A, Allam M,* ***Kleynhans J.***

Access Microbiology
Impact Factor: Score unavailable

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that may cause diarrhoeal outbreaks and occasionally are associated with haemolytic-uraemic syndrome (HUS). We report on STEC O26:H11 associated with a cluster of four HUS cases in South Africa in 2017.

Methodology: All case-patients were female and aged 5 years and under. Standard microbiological tests were performed for culture and identification of STEC from specimens (human stool and food samples). Further analysis of genomic DNA extracted from bacterial cultures and specimens included PCR for specific virulence genes, whole-genome sequencing and shotgun metagenomic sequencing.

Results: For 2/4 cases, stool specimens revealed STEC O26:H11 containing *eae, stx2a* and *stx2b* virulence genes. All food samples were found to be negative for STEC. No epidemiological links could be established between the HUS cases. Dried meat products were the leading food item suspected to be the vehicle of transmission for these cases, as 3/4 case-patients reported they had eaten this. However, testing of dried meat products could not confirm this.

Conclusion: Since STEC infection does not always lead to severe symptoms, it is possible that many more cases were associated with this cluster and largely went unrecognised.

Thank you for your contributions! Kindly continue to send your contributions to:

Muzikayisem@nicd.ac.za

Editorial and production team:

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