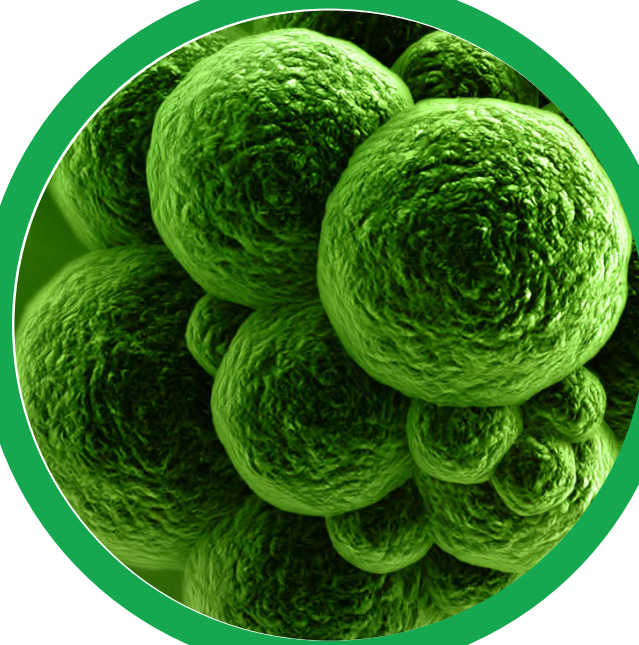
NATIONAL HEALTH LABORATORY SERVICE

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES



NICD ANNUAL REVIEW 2013-14

National Institute for Communicable Diseases (NICD)

The National Institute for Communicable Diseases (NICD) is responsible for surveillance of communicable diseases and is a vital resource of knowledge and expertise in communicable diseases intelligence in South Africa.

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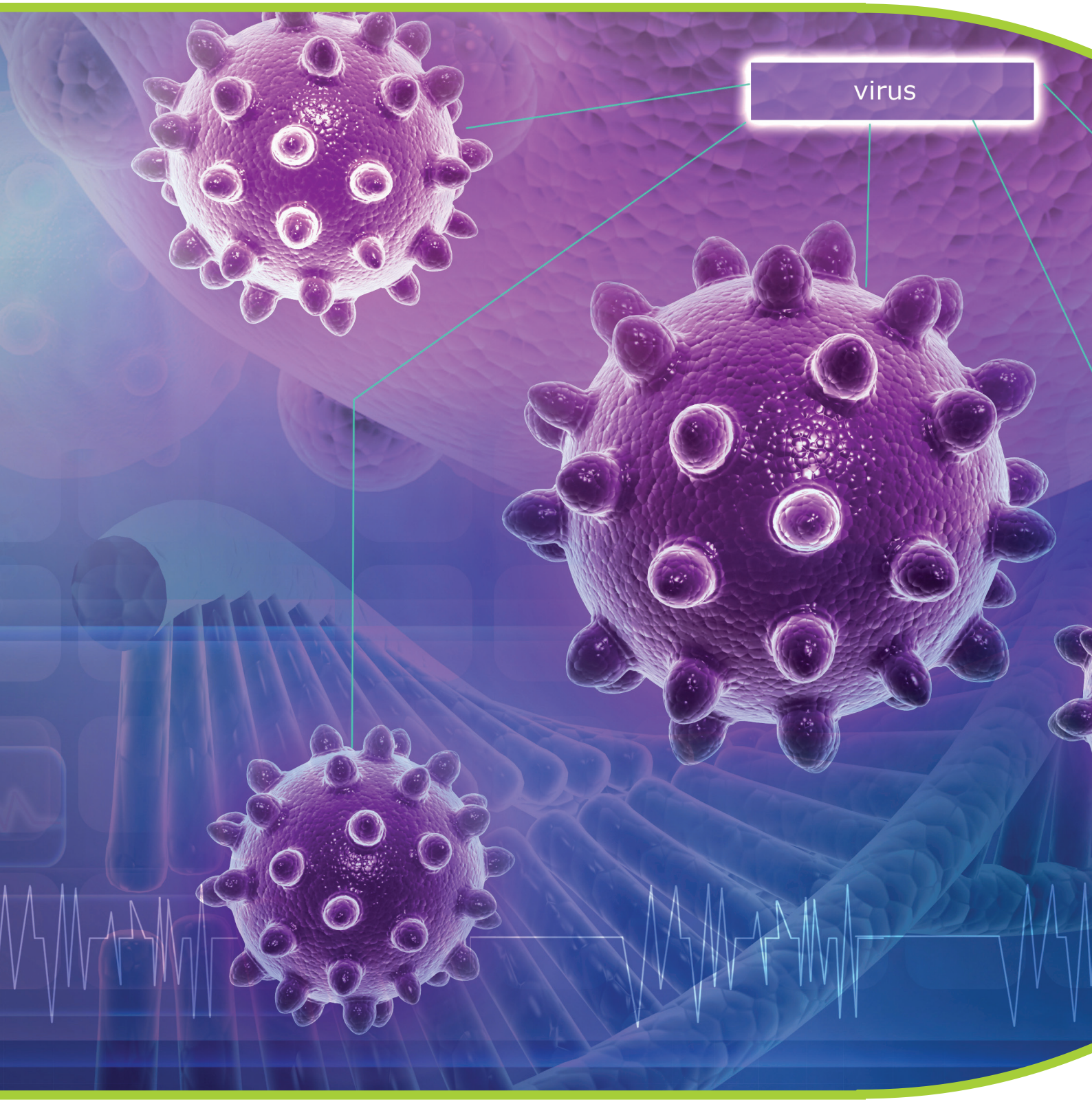
Shutterstock: Digital illustration of viruses in water

DIRECTOR'S OVERVIEW



Shutterstock: Airborne virus

INTRODUCTION

The National Institute for Communicable Diseases (NICD), led by a local scientist and public health specialist, is internationally recognised as an important public-health resource in South Africa and is the only one of its kind in Africa. The past year has seen a number of notable developments at the NICD, including ground-breaking research spanning from providing insight into new pathways for HIV vaccine development to the discovery of new pathogens affecting humans. Furthermore, the sustainability for national public-health surveillance was boosted by a three-year conditional grant from Government, to provide the NICD with transition funding for the GERMS-SA programme. This national laboratory-based and active surveillance programme for communicable diseases was previously grant-funded by the President's Emergency Plan for AIDS Relief (PEPFAR). This provided an opportunity for the re-engineering of the programme to address national health communicable disease priorities, in addition to the focus on HIV. Also, the GERMS-SA programme has set out to expand its surveillance footprint to primary healthcare centres, which will provide a more comprehensive platform for delineating the burden of communicable diseases in South Africa. This expansion will also provide the platform for earlier detection of potential public health threats, such as the surveillance for novel influenza virus strains and outbreaks of water-borne diseases.

The success of public health surveillance in South Africa is currently being challenged by the scarcity of field epidemiologists with a focus on infectious diseases. This is being materially addressed by the NICD in partnership with the Global Diseases Detection Network of the Centers for Disease Control and Prevention (CDC), USA, in the Field and Epidemiology Laboratory Training Program (FELTP). Over the past three years, the FELTP programme has been re-engineered to increase its output of the number of epidemiologists graduating (12 in 2013), including targeted training of individuals who have at least a Masters levels qualification.

These FELTP graduates are expected to be a critical component of the NICD, as it plans on decentralising the support it provides at a provincial level with regard to assisting in infectious disease outbreak responses and providing provincial departments of health with estimates of the burden of communicable disease in their settings. Furthermore, the NICD through the FELTP programme also provided basic Epidemiology skills to 56 Department of Health staff during 2013 and trained a further 46 staff members in health policy and decision making analysis.

Professor Shabir Madhi
Executive Director

Ground breaking research involving new pathway for HIV vaccine development and discovery of new pathogens affecting humans

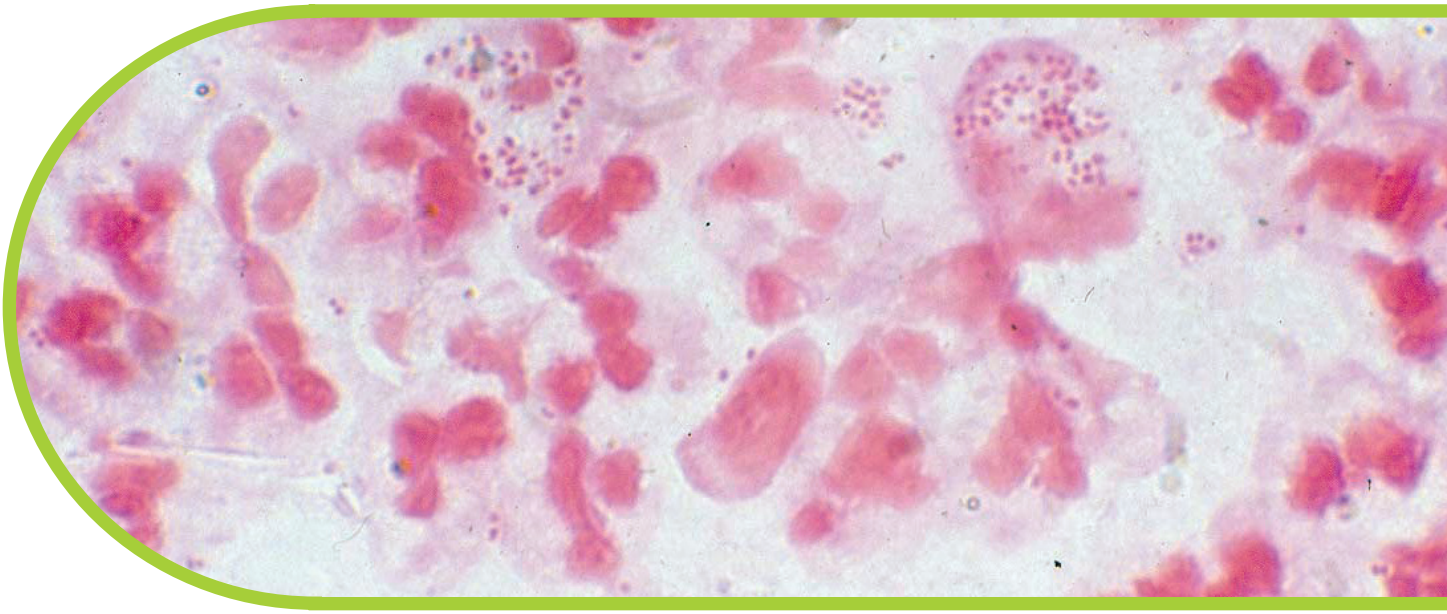
100 000 patients recruited by The Centre for Tuberculosis - the results will inform TB policies for South Africa.

Did You Know?

The CHIVSTI supported the Department of Health through laboratory testing for the 33rd Annual Antenatal HIV-1 Prevalence and HIV Incidence Survey

Some of the key surveillance activities undertaken by the NICD during 2013/14 include collaboration with the National Department of Health (NDoH) and South African Society of Travel Medicine in establishing the South African Travel Health Network in 2013. This network will provide travel-related health guidelines, surveillance for imported priority diseases and mass gatherings support. The Centre for Tuberculosis completed recruitment into the National Tuberculosis Drug Resistance Survey for seven of nine provinces in South Africa with >100 000 patients recruited, the results of which will inform drug-resistant TB policies for the country. The Centre has also completed the first audit of the burden of microbiologically-confirmed TB for South Africa, which covered the period from 2006 to 2013. These data will be instrumental in understanding the interaction between improved HIV management and the burden of TB in South Africa, as well as identify specific hot-spots which need to be targeted for intervention to reduce the burden of TB in South Africa. In addition, the Centre for Respiratory and Meningeal Diseases conducts on-going surveillance on the impact of immunisation of children with pneumococcal conjugate vaccine and rotavirus vaccine on childhood pneumonia and diarrhoea morbidity and mortality. The Centre for HIV and Sexually Transmitted Infections (CHIVSTI) have during 2013 supported the NDoH through laboratory testing for the 33rd Annual Antenatal HIV-1 Prevalence and HIV Incidence survey and the 3rd South African Prevention of Mother-to-Child Transmission (PMTCT) Effectiveness study. The latter study further confirmed the reduction of HIV transmission from HIV-infected mothers to their offspring at the 4-8 weeks time point. Centre staff also provided technical assistance for STI guideline revisions being undertaken by the NDoH's Essential Drugs Programme Primary Care Sub-Committee. The Centre also determined the phenotypic and genetic characteristics of the first two cases of extended-spectrum cephalosporin-resistant *Neisseria gonorrhoeae* in Africa. Both strains belonged to a successful internationally men who have sex with men (MSM) -linked multidrug-resistant gonococcal clone, associated with cefixime treatment failure in Europe and North America, and have implications with regard to future guidelines for STI management.

Although increasingly focused on tailoring the activities of the Institute to being public-health orientated, the NICD nevertheless continues in its pursuit of research excellence and a resource for training South Africans as



CHIVSTI GC Gram

well as scientists from other African countries. The ground-breaking science at the NICD was illustrated by research published in Nature Medicine during 2013, in which as part of an international research consortium involving the Centre for the AIDS Programme of Research in South Africa (CAPRISA), and researchers in the USA and NICD staff in the Centre for HIV and STI thus defined the developmental pathway by which broadly-neutralising antibodies are generated and acquire the requisite molecular characteristics for neutralisation. These data provide important insights relevant to HIV-1 vaccine development. Also, staff in the Centre for Opportunistic, Tropical and Hospital Infections, using specialised DNA analysis, detected a newly-described (and as-yet unnamed) opportunistic fungus in the genus *Emmonsia* that caused disease among HIV-infected patients. This study was done in collaboration with NHLS colleagues in Cape Town and was published in the prestigious New England Journal of Medicine. A discovery by the Centre for Emerging and Zoonotic diseases was a rodent-borne *Bartonella* species not previously described in humans, which was found to be a cause of infective endocarditis. The productivity of NICD staff is indicated by the ongoing year-on-year increase in peer-reviewed publications over the past three years.

On the global front, the NICD continues to support other African countries by being a WHO regional reference centre for diagnostics, training and quality assurance. The Centre for Vaccines and Immunisation is also involved as a WHO reference centre for providing testing and laboratory training for measles and poliomyelitis, which are being targeted for elimination before the end of this decade. Also, the NICD continues to house the only level-4 biosafety laboratory facility in Africa, placing it in a strategic position in dealing with established and emerging highly communicable infectious disease threats in South Africa and the SADC region.

Despite the resource constraints and financially challenging situation under which the NICD operates, the talent of personnel at the NICD enables it to deliver on its mission of being an internationally-acclaimed Institute that monitors communicable diseases in South Africa to help policy development geared toward the protection of South Africans, as well as in the broader African region.

A rodent-borne Bartonella species was discovered as a cause of infective endocarditis by the Centre for Emerging and Zoonotic diseases

CENTRE FOR ENTERIC DISEASES

The Centre for Enteric Diseases is responsible for developing strategies to combat diarrhoeal diseases in South Africa.

Dr Karen H. Keddy;
Centre Head
(Not Photographed)

BACKGROUND

Data from the South African Health Review 2012/13 has revealed that the under-five mortality rate has declined rapidly since 2009 and by 2011 it had exceeded the targets recommended for 2014. The decline could be attributed to the successful implementation of the PMTCT programme and the introduction of the pneumococcal and rotavirus vaccines into the National Immunisation Programme. Currently, there have been few advances in South Africa towards achieving Millenium Development Goal 7, which addresses the issue of safe food and water.

The Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases (NICD) was established in 2012, through the amalgamation of the Enteric Diseases Reference Unit and the Viral Gastroenteritis Unit of the NICD. The Centre is responsible for developing strategies and providing information to combat diarrhoeal diseases in South Africa. The centre currently monitors trends in diarrhoeal pathogen incidence and identifies areas for the introduction of additional interventions.

Dr Nicola A. Page
Centre Head

SURVEILLANCE ACTIVITIES

The Centre focused much of the 2012/13 period on continuing to provide high quality surveillance data for bacteria and viruses associated with diarrhoeal diseases and on amalgamating the two laboratory units into one functional centre. In addition to the regular reports provided to the Department of Health, the Centre was actively engaged with the Department of Agriculture in reviewing research protocols for the Water Research Commission, as well as hosting both local and international training for food and waterborne diseases.

The Centre participated in the investigation of outbreaks in the Northern Cape, Gauteng, Western Cape, and KwaZulu-Natal provinces. The diarrhoeal pathogens implicated in these outbreaks ranged from *Salmonella*, diarrhoeagenic *Escherichia coli*, *Campylobacter, Shigella* spp, typhoid and cholera to rotavirus, norovirus genogroup II, sapovirus and astrovirus.

RESEARCH PROJECTS

Typhoid fever surveillance in sub-Saharan Africa: Burden of Disease Study (TSAP)

Funding: International Vaccine Institute, Seoul, Korea.

This is an international bacteraemia study, to identify which pathogens cause illness in ten sentinel sites in Africa in patients presenting with fever. Data from South Africa on typhoid fever and other febrile illnesses are scanty and driven by outbreak or case reports, with limited information on emerging resistance in the pathogen, or through laboratory-based surveillance systems. Edendale Hospital is the site selected for this study: patients presenting with invasive disease due to various pathogens, including *Salmonella* Typhi are identified and additional information on the patients' history, including antimicrobial exposure, HIV status and outcome data are recorded by a surveillance officer. A Health Care Utilisation Study (HCUS) was undertaken from October – December 2013, to better calculate the burden of disease.

Group for *Enteric Respiratory and Meningeal Pathogens Surveillance* in South Africa (GERMS-SA) – Laboratory-based surveillance for enteric pathogens.

The CED does laboratory-based surveillance and characterisation of bacterial enteric disease in South Africa for: *Salmonella, Shigella, Vibrio cholerae* (O1 and non-O1) and enterohaemorrhagic *E.coli* isolates from all body sites, and diarrhoeagenic *E. coli* isolates from stool. Enhanced surveillance for *Shigella* and *Salmonella enterica* isolates from normally sterile body sites was completed in 2013. EDRU currently receives specimens from over 4000 human cases per annum, according to the definition above. In addition the Centre undertakes to serotype *Salmonella, Shigella* and diarrhoeagenic *E. coli* (DEC) isolates. Regular reports on the isolates received are extracted from the database for information sharing purposes. Molecular methods may be used to establish strain relatedness in outbreaks.

PulseNet Africa

Funding: Global Disease Detection, Centers for Disease Control and Prevention, Atlanta, USA.

PulseNet is an international molecular subtyping network for foodborne/waterborne disease surveillance, using standardised genotyping methods. Information sharing is real-time, which provides global early warning, detection and investigation of foodborne/waterborne disease outbreaks, emerging pathogens and acts of bioterrorism. Surveillance data clarifies the molecular epidemiology of enteric diseases in Africa and assists preventative strategies planning, such as vaccine development and implementation. The PulseNet Africa database has a collection of PFGE patterns for ~3000 enteric pathogen isolates, which includes *Salmonella* species (including *Salmonella* Typhi), *Shigella* species, *Vibrio cholerae* O1 and enterohaemorrhagic *E.coli*. The database mostly includes patterns from South African isolates; however, isolates from eight other African countries are also represented. *Vibrio cholerae* O1 isolates have been received from the AFRICHOL network.

Validation of real-time PCR for detection of *Campylobacter* in stool specimens

Real-time PCR was validated and implemented to detect for *Campylobacter* in stool specimens from children <5 years of age with severe acute diarrhoea, as part of the Rotavirus Surveillance Program. Stool samples were processed to extract microbial DNA and RNA using the QIAGEN QIAamp Viral RNA Mini Kit. An aliquot of the DNA/RNA extraction was used in a multiplex real-time PCR assay, to target genes specific for *Campylobacter jejuni* and *Campylobacter coli*. Results from the analysis of stool samples have found *Campylobacter* PCR detection rates to be 13% and C. *jejuni* is the most commonly identified specimen. These data suggest that rates of *Campylobacter* infection are much higher than previously thought and that many cases of campylobacteriosis have previously been missed in this group of patients.

Validation of multiple-locus variable-number tandem-repeats analysis for molecular subtyping of *Salmonella enterica* serovar Enteritidis

Multiple-locus variable-number tandem-repeats analysis (MLVA), is a technique used to obtain a molecular subtype (DNA fingerprint) for a strain of bacteria. This allows determination of the genetic similarity of strains of bacteria, which can assist with epidemiological investigations of diseases. We validated a 5 locus (SENTR7-SENTR5-SENTR6-SENTR4-SE3) MLVA protocol. The methodology includes a multiplex PCR to target 5 genes, followed by an analysis for the presence and size of PCR products, using an Applied Biosystems 3500 Genetic Analyzer and GeneMapper Software, followed by determination of the MLVA profile. Validation of the methodology was successful and has now been implemented to investigate isolates of *Salmonella Enteritidis.*

Characterisation of *Salmonella enterica* serovar Paratyphi in South Africa, 2003 to 2013

In South Africa, for the 2003 to 2013 period, the CED identified 45 isolates of *Salmonella Paratyphi;* these were further serotyped as follows: *Salmonella Paratyphi* A (24/45; 53%), *Salmonella Paratyphi* B variant Java (13/45; 29%), *Salmonella* Paratyphi B (4/45; 9%) and *Salmonella Paratyphi* C (4/45; 9%). Prevalence of antimicrobial resistance included ampicillin (14%), cotrimaxozole (3%), chloramphenicol (16%), nalidixic acid (50%), ciprofloxacin (49%) and ceftriaxone (0%). For pulsed-field gel electrophoresis (PFGE) pattern analysis: patterns for *Salmonella Paratyphi* A isolates clustered together, with most isolates showing a single pattern; patterns for *Salmonella Paratyphi* B variant Java isolates also clustered together, but isolates showed a diversity of patterns; *Salmonella Paratyphi* B and *Salmonella Paratyphi* C isolates showed a diversity of unrelated patterns.

Azithromycin susceptibility in recent antimicrobial-resistant *Vibrio cholerae* O1 El Tor isolates in South Africa

The aim of this study was to investigate antimicrobial susceptibility (by E-test and agar dilution methods) to azithromycin, as well as to investigate the presence of seven macrolide resistance determinants amongst 100 South African selected antimicrobial-resistant *Vibrio cholerae* O1 El Tor variant isolates. Irrespective of the test method used, all 100 isolates were susceptible to azithromycin and were PCR-negative for the seven macrolide resistance determinants. Statistical comparison of the results showed agreement of MIC values, based on the nearest similar dilution factor at 97% ($p > 0.05$; Mann-Whitney) and MIC correlation at 99% ($p < 0.01$; Spearman correlation coefficient). The agreement between the two azithromycin susceptibility testing methods and significant correlation of MICs, is evidence of the consistency of the test methods.

Did You Know?

The CED does laboratory-based surveillance and characterisation of bacterial enteric disease in South Africa for Salmonella, Shigella, Vibrio cholerae, and diarrhoeagenic E. coli isolates from stool.

Development and evaluation of a multiple-locus variable-number tandem-repeats analysis assay for subtyping *Salmonella* Typhi strains from sub-Saharan Africa

NICD Researchers/Investigators.

Funding: This work was supported in part by a grant from Global Disease Detection, grant 1U19GH000571-02.

The aim of our study was to develop and evaluate a relevant and highly reproducible multiple-locus variable-number tandem-repeats analysis (MLVA) assay, consisting of five variable-number tandem-repeats (VNTR) markers to analyse representative *Salmonella Typhi* strains from Sub-Saharan Africa (SSA). Fifty *Salmonella Typhi* strains from humans were selected from the culture collection at the CED and used to evaluate 13 polymorphic VNTR loci that were previously published. Of these, six VNTR loci showed good allele variation and were found to be suitable for use in MLVA analysis of *Salmonella Typhi* strains. This needs to be narrowed down to a 5-loci MLVA assay, which can be performed in a single multiplex PCR. The assay will then be used to analyse *Salmonella Typhi* strains from Sub-Saharan Africa.

Shutterstock: Salmonella bacteria

The development of real-time detection techniques and increased surveillance of diarrhoeal disease viruses in the under-five South African population

Funding: Poliomyelitis Research Foundation

In order to effectively tackle diarrhoeal diseases in South Africa, it is important to understand which enteric viruses contribute to the disease burden. The aim of the project was to develop real-time detection techniques to identify enteric viruses. Stools samples collected through the rotavirus sentinel surveillance programme between 2009 and 2012 were screened. Of the 4 021 stools screened, *adenovirus* (23%), *norovirus genogroup II* (13%), *sapovirus* (8%), *astrovirus* (7%), *bocavirus* (6%) and *norovirus genogroup* I (3%) were detected. Additional typing was performed in 562 *adenovirus*-positive samples with species A (11.2%), B (19.4%), C (22.8%), D (9.4%), E (0.5%) and F (36.7%) detected. A multiplex real-time detection assay was developed and is being validated.

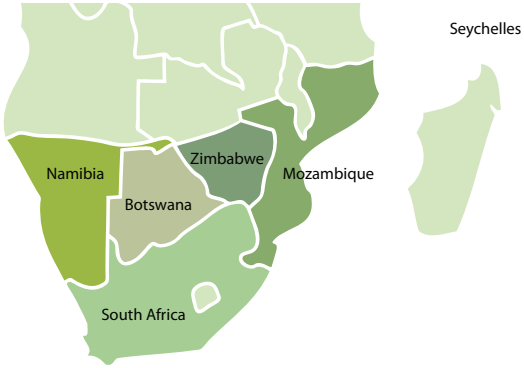
Post-marketing intussusception monitoring after introduction of oral rotavirus vaccine in South Africa

Funding source: The Bill and Melinda Gates Foundation

Intussusception is a rare intestinal blockage which was previously associated with vaccination of infants with a human-simian rotavirus reassortant vaccine formulation. Current rotavirus vaccines did not demonstrate an increased risk of intussusception during large scale vaccine trials. However, post-marketing studies have detected a low-level risk after vaccine administration. No risk data is available for African settings. Active surveillance for intussusception cases has been implemented in seven South African cities. A total of 23 stools were submitted to the laboratory for testing, with rotavirus, norovirus genogroup I and bocavirus detected in one sample each, astrovirus detected in three samples, norovirus genogroup II detected in four samples and adenovirus detected in seven samples. The study will continue for the next three years.

TEACHING AND TRAINING

CED trained 11 epidemiologists and 10 laboratory personnel from other African countries



In addition to postgraduate students enrolling for higher degrees through the Centre, the CED also undertook to train 11 epidemiologists and 10 laboratory personnel from other African countries, namely Zimbabwe, Botswana, South Africa, Namibia, Seychelles and Mozambique as part of the WHO's Global Food Borne Diseases Network (GFN) training. Material covered included investigation of foodborne/waterborne outbreaks, including epidemiological investigation and laboratory characterisation of isolates, water testing and quality management systems. In addition, the CED also ran a PulseNet training course in the genotyping techniques of pulsed-field gel electrophoresis (PFGE) analysis and multiple-locus variable-number tandem-repeats analysis (MLVA) of bacteria. Participants included representatives from the 11 PulseNet Africa member countries, including South Africa, Kenya, The Gambia, Senegal, Cameroon, Malawi, Tanzania, Cote d'Ivoire, Ghana, Uganda and Mozambique.

The CED conducted registrar training by providing a long course in enteric pathogen detection, characterisation and laboratory surveillance from 13-24 May 2013 and an abbreviated short course in the basics of enteric pathogens on 24 July 2013.

The CED provided course content for the training of scientists in the African Rotavirus Network Workshop on 22 July 2013. It also provided training on enteric viruses associated with gastroenteritis to nurses in South Africa through the Allegra Academy Clinical Conference days held on 6, 7, 13 and 14 November 2013.

PROFESSIONAL DEVELOPMENT

Research output

Seheri LM, Page NA, Mawela MPB, Mphahlele MJ, Steele AD. Rotavirus vaccination within the South African expanded program on immunisation. *Vaccine* 2012; **30**:C14-C20.

Msimang VMY, Page NA, Groome MJ, Moyes J, Cortese M, Seheri M, Kahn K, Twine R, Chagan M, Madhi SA, Cohen C. Impact of rotavirus vaccine on diarrhoeal hospitalisation following introduction into the South African public immunisation program. *Pediatr Infect Dis J.* 2013; **32**:1359-64.

Mans J, van Zyl WB, Taylor MB, Page NA, Sobsey MD, Barnard TG, Potgieter N. Applicability of Bio-wipes for the collection of human faecal specimens for detection and characterisation of enteric viruses. *Trop Med Int Health.* 2014; **19**(3):293-300.

Esona MD, Mijatovic-Rustempasic S, Foytich K, Roy S, Banyai K, Armah GE, Steele AD, Volotão EM, Gomez MM, Silva MF, Gautam R, Quaye O, Tam KI, Forbi JC, Seheri M, Page NA, Nyangao J, Ndze VN, Aminu M, Bowen MD, Gentsch JR. Human G9P[8] rotavirus strains circulating in Cameroon, 1999-2000: Genetic relationships with other G9 strains and detection of a new G9 subtype. *Infect Genet Evol.* 2013; **18**:315-24.

Wain J, Keddy KH, Hendriksen RS, Rubino S. Using next generation sequencing to tackle non-typhoidal *Salmonella* infections. J Infect Dev Ctries. 2013; **7**(1):1-5.

Keddy KH,Sooka A; Parsons MB; Njanpop-Lafourcade BM; Fitchet K; Smith AM. Diagnosis of *Vibrio cholerae* O1 Infection in Africa. *J Infect Dis.* 2013 **208**: S23-S31.

Ismail H, Smith AM, Tau NP, Sooka A, Keddy KH for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Cholera outbreak in South Africa, 2008-2009: Laboratory analysis of *Vibrio cholerae* O1 strains. *J Infect Dis.* (2013) **208**: S39-S45.

Smith AM, Mthanti MA, Haumann C, Tyalisi N, Boon GPG, Sooka A, Keddy KH, GERMS-SA Surveillance Network. Nosocomial outbreak of *Salmonella enterica* serovar Typhimurium primarily affecting a paediatric ward in South Africa in 2012. *J Clin Microbiol.* 2014 **52**:627-631.

Nadon CA, Tress E, Ng L-K, Nielsen EM, Reimer A, Maxwell N, Kubota KA, Gerner-Smidt P, MLVA Harmonisation Working Group (Chioy C-S, Izumiya H, Kjeldsen M, Larsson J, Scheutz F, Torpdahl M, Le Hello S, Lindstedt B-A, Malorny B, Pedersen K, Smith AM, Takkinen J, Wain). 2013. Development and application of MLVA methods as a tool for inter-laboratory surveillance. *Eurosurveillance* **18**(35):pii=20565. Available online: **http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20565.**

Larsson JT, Torpdahl M, MLVA Working Group (Aspán A, Bertrand S, Chiou C-S, Goodman C, Heck M, Hiley L, Hopkins K, Hogg G, Eija H-T, Izumiya H, Jernberg C, Le Hello S, Lindstedt B-A, Malorny B, Prendergast D, Ragimbeau C, Sintchenko V, Smith AM, Sorensen G, Tietze E), Nielsen EM. 2013. Proof-of concept study for successful inter-laboratory comparison of MLVA results. *Eurosurveillance* **18**(35):pii=20566. Available online: **http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20566.**

Congresses attended

The CED attended five international and five national conferences during the reporting period.

CENTRE FOR EMERGING AND ZOONOTIC DISEASES

Digital illustration of rabies virus in colour background

Professor Janusz T Paweska
Centre Head

The CEZD provides capacity for investigation of viral haemorrhagic fevers, arboviral diseases, human rabies and rabies-related infections of public health relevance in Africa.

EMERGING AND ZOONOTIC DISEASES

It has been estimated that up to 65% of emerging infectious diseases in the past 60 years have been attributed to zoonotic agents. It is reported that the major zoonotic disease outbreaks (including Rift Valley fever, Nipah and West Nile outbreaks) during 1997 to 2009, cost the global economy a staggering US $ 6.7 billion per year (World Bank, 2012).

The Centre for Emerging and Zoonotic Diseases (CEZD) aims to rapidly detect, identify and thus contribute to the control and prevention of infections, caused by high consequence viral and bacterial pathogens. Emergence or re-emergence of highly dangerous pathogens has special relevance to Africa, due to the extensive human-wildlife interaction, rapidly increasing land-usage and difficult socio-economic conditions. The public health burden of zoonotic agents in South Africa is still poorly understood. The CEZD renders not only diagnostic expertise, but also investigatory capacity on biohazard class three (BSL-3) and four (BSL-4) bacterial and viral pathogens associated with zoonotic diseases. The CEZD employs a "One Health" approach in its investigations, incorporating a better understanding of the ecology and epidemiology of these diseases as a whole.

It is the mission of the CEZD to be a resource for knowledge and expertise to the South African government, the Southern African Development Community (SADC) and elsewhere on the African continent. In this context, the CEZD assists in the planning of relevant policies and programmes and harnesses innovation in science and technology to support surveillance, detection and outbreak response. In observing these goals, the CEZD supports South Africa's commitment to the International Health Regulations.

SURVEILLANCE AND DIAGNOSTIC SERVICES

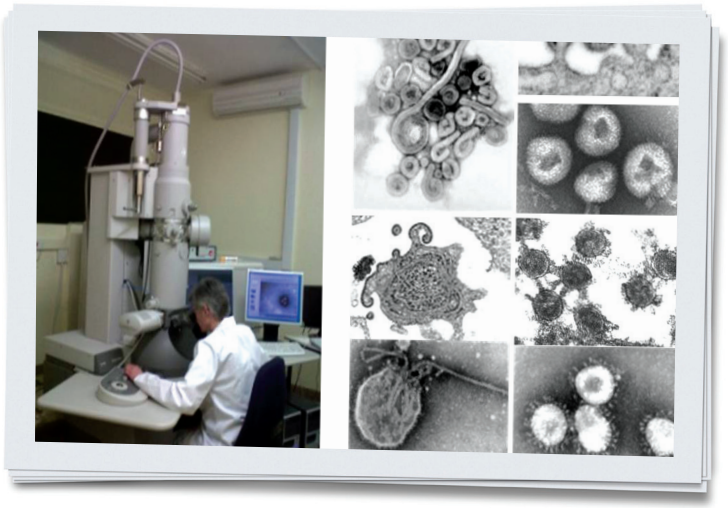
The laboratories of the CEZD provide capacity for the investigation of viral haemorrhagic fevers, arboviral diseases, human rabies and rabies-related infections of public health relevance in Africa. The Centre is accredited for a range of diagnostic tests with the South African National Accreditation System (SANAS). In addition, in 2012, the CEZD obtained approval from the Department of Agriculture, Forestry and Fisheries (DAFF) to perform several tests for the diagnosis of Rift Valley fever, Crimean-Congo haemorrhagic fever and rabies in animals.

Scientists working in the biosafety level four laboratory at the CEZD, NICD.

To provide the diagnosis and perform research on BSL3 and BSL4 viral and bacterial pathogens, the CEZD manages the operation of high and maximum biocontainment facilities. The Centre's BSL4 facility is the only Maximum Biocontainment Suit Laboratory on the African continent and provides an invaluable and strategic resources for the investigation, handling and storage of the most deadly pathogens known to science.

In addition, the CEZD is tasked with the laboratory confirmation and investigation of anthrax, plague, leptospirosis, cat scratch disease (*Bartonella* infection) and botulism. Work on these agents is carried out in a BSL3 facility accredited with the DAFF and the SANAS. The CEZD drives a regional surveillance project for plague (previously the RATZOOMAN project), and is recognised as the Anthrax and Plague Reference Laboratory for Human Diagnostics in South Africa. Recently, the CEZD has expanded the surveillance activities to include other zoonotic pathogens, such as the bacteria that cause brucellosis and Q fever.

The transmission electron microscope is an important tool in the diagnostic arsenal of CEZD for detection of viral pathogens that are not detected by existing PCR protocols, as well as for detecting certain bacteria (particularly obligate intracellular bacteria) and identifying larger pathogens such as microsporidia. The Electron Microscopy capacity provides rapid viral diagnostic screening to both the CEZD and the Division of Public Health and Outbreak Response. For example, conventional resin-embedding and ultramicrotomy techniques were crucial in identifying the microsporidial genus occurring in two paediatric renal-transplant patients, and were also requested for ultrastructural confirmation of *Tropheryma whippelii* infection. Collaborative research support was supplied to the National Institute for Occupational Health for investigations of *Acanthamoeba*-pathogenic biofilms in the water supply systems of certain hospitals in Gauteng.



Using the transmission electron microscope of the CEZD, the NICD detects, identifies and produces imaging of various pathogens associated with zoonotic and other diseases of public health importance to South Africa

Did You Know?

Bats are increasingly being implicated as hosts of dangerous zoonotic pathogens

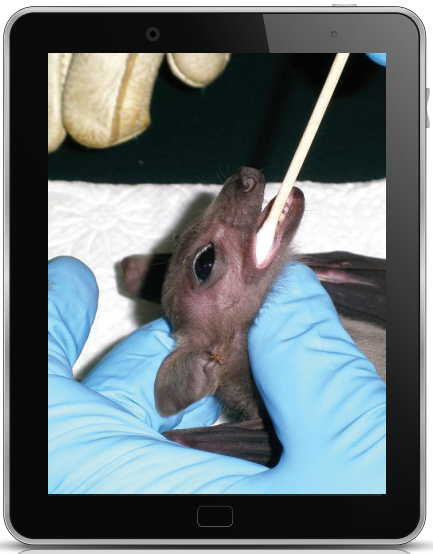
CURRENT RESEARCH AND SURVEILLANCE

A summary of selected projects currently underway at the CEZD:

Surveillance for zoonotic pathogens in South African bats

Bats are increasingly being implicated as hosts of dangerous zoonotic pathogens. Recent serological and molecular results suggest the presence of various known or potential zoonotic pathogens, including filoviruses, rabies-related viruses, paramyxoviruses, and coronaviruses in cave-dwelling fruit and insectivorous bats in Limpopo Province, South Africa. Intensive monthly sampling at the affected bat roost was conducted in 2013 and will continue over the next few years to better understand the seasonality and ecology of these pathogens in bat populations.

Collaborators: Prof Wanda Markotter, University of Pretoria



Oral swab sampling of Rousettus aegyptiacus from the Mahune cave, Limpopo Province

Bartonella – an underdiagnosed, fleaborne disease

The transmission of *Bartonellae* between the reservoir and mammal host is aided by haematophagous arthropods such as fleas, sand flies, mites, lice and ticks. Rodents have been regarded as common reservoirs for zoonoses like *Bartonella*. PCR revealed a 22.8 % (n = 35) *Bartonella* prevalence in the specimens specifically received for *Bartonella* testing, and a 9 % (n=75) *Bartonella* prevalence in the acutely ill patient specimens. What is of great concern however is the *Bartonella* nucleic acid, which was detected in 51 % (n = 105) of a sample of rodents collected in the City of Johannesburg.

Experimental studies with filoviruses in Egyptian fruit bats

The Egyptian fruit bat is postulated to be the reservoir species for Marburg virus, but transmission and shedding mechanisms are yet to be described. Preliminary results indicate that Marburg virus replicates efficiently in various tissues and causes relatively long-lived viraemia, but does not induce disease or overt pathology and is not efficiently transmitted from infected to non-infected bats, despite very close contact between them. Results from this study further contribute to establishing a bat-filovirus experimental model and contribute to a better understanding of the role of bats in the maintenance of filoviruses in nature.

Collaborators: Prof Wanda Markotter, University of Pretoria

Sero-surveys for human exposure to arthropod borne and zoonotic infections in the Kruger National Park

The Kruger National Park has favourable climatic conditions for the breeding of many species of mosquitoes and ticks known to be vectors of pathogens of public health significance. Humans can also acquire infections from the animal hosts of these pathogens, and certain occupational activities increase a person's risk of becoming infected. The project concerns the seroprevalence of selected mosquito-borne, tick-borne and rodent-borne infections amongst the human population that work or reside in the Park. Serological results of surveys conducted in 2013 suggest a high prevalence of tick bite fever and q-fever bacterial infections, but a rather surprisingly low infection rate for arthropod-borne viral and rodent-borne bacterial pathogens.

Collaborators: Dr D Govender, SANParks Scientific Services Skukuza

Prof Frean collecting a blood specimen from a Kruger National Park ranger for serological testing for previous exposures to pathogens such as arboviruses and rickettsia

TEACHING AND TRAINING

In addition to extensive training of staff and national and international research fellows in laboratory techniques ,and introducing them to working in BSL3 and BSL4 biocontainment facilities, the CEZD was actively involved in supporting post-graduate studies in the fields of Medical Microbiology, Medical Virology and Public Health through collaborative projects with South African and international universities. The CEZD is also involved in the training of microbiology and clinical pathology registrars, intern scientists and technologists on an ongoing basis. The CEZD coordinates a number of formal training programmes and is often requested to coordinate specialist diagnostic workshops. This includes training environmental health officers from the City of Johannesburg, Gauteng Province, on the dissection and storage of rodent organs for plague surveillance purposes.

Training of environmental health officers in specimen collection for the national plague surveillance program.

PROFESSIONAL DEVELOPMENT

Two MSc students co-supervised by CEZD staff members graduated during 2013: Marinda Mortlock, MSc:, Detection and characterisation of genetically diverse paramyxoviruses from African bats.

Nadia Storm, MSc: Epidemiology of Sindbis fever in South Africa and development of a real-time pan-alphavirus PCR assay for the detection of Sindbis and other medically important alphaviruses.

HONOURS

Dr Jenny Rossouw has been appointed as a member of the International Health Regulations (IHR) Roster of Experts for Pneumonic plague.

RESEARCH OUTPUT

Updated publications and conference contribution lists are available through the centre webpage located at *www.nicd.ac.za.*

Publications

The CEZD published a total of 17 papers in peer-reviewed journals and two book chapters during 2013/14. A selection of the top five publications are listed here:

Rift Valley fever virus outbreak in South Africa 2008-2011

Archer B, Thomas J, Weyer J, Cengimbo A, Essoya LD, Jacobs C, Ntuli S, Modise M, Mathonsi M, Mashishi MS, Leman PA, le Roux C, Jansen van Vuren P, Kemp A, Paweska JT, Blumberg L. Epidemiological investigations into outbreaks of Rift Valley fever in humans, South Africa, 2008-2011. *Emerging Infectious Diseases* 2013; **19**(2): 1918-1925.

Synopsis: The first sizeable outbreak of Rift Valley Fever in South Africa in 30 years was reported in 2008-2011. A total of 25 deaths were reported from 302 laboratory confirmed human cases during this period. Risk factors, demographics and other epidemiological findings associated with Rift Valley Fever in the South African human population were investigated. The importance of close partnership with animal health and agriculture sectors allowed early recognition of human cases and timely implementation of appropriate preventive health measures.

Improving quality of diagnostic testing for Rift Valley fever

Escadafal C, Paweska JT, Grobbelaar A, le Roux C, Bouloy M, Patel P, Teichmann A, Donoso-Mantke O, Niedrig M. International External Quality Assessment of Molecular Detection of Rift Valley Fever Virus. *PLoS Negl Trop Dis* 2013; 7(5): e2244.

Synopsis: The need to evaluate worldwide laboratories on their performance of Rift Valley Fever (RVF) virus molecular diagnostic methods was prompted by outbreaks of RVF in regions of the world previously regarded free of the virus. This study presents the results of the first international external quality assessment (EQA) for the molecular diagnosis of RVF. Thirty expert laboratories from 16 countries participated. Optimal results were reported by 64% of the analyses, 21% achieved acceptable results and 15% of the results revealed that there is a need for improvement. The Special Viral Pathogens Laboratory (SVPL) of the CEZD validated panels before they were dispatched to participating laboratories. Optimal RVF molecular diagnostic results were achieved by the SVPL.

Rift Valley fever in Tanzania: review of 80 years of data

Sindato C, Karimuribo ED, Pfeiffer DU, Kivaria F, Dautu G, Mboera LEG, Paweska JT. Spatial and temporal pattern of Rift Valley fever outbreaks in Tanzania 1930 to 2007. *PloS One* 2014; **9**(2):e88897.

Synopsis: This study investigated the spatial and temporal pattern of RVF outbreaks in Tanzania over the past 80 years. A retrospective study was carried out, based on disease reporting data at district or village level. The space-time permutation model was applied to identify clusters of cases, and a multivariable logistic regression model was used to identify risk factors associated with the occurrence of outbreaks in the district. RVF outbreaks were found to be distributed heterogeneously and transmission dynamics appeared to vary between areas. The sequence of outbreak waves continuously cover more parts of the country. Whenever infection has been introduced into an area, it is likely to be involved in future outbreaks. The cases were more likely to be reported from the eastern Rift Valley than from the western Rift Valley ecosystem and from areas with clay and loam rather than sandy soil texture.

Lujo virus hemorrhagic fever

Paweska JT, Jansen van Vuren P, Weyer J. Lujo virus Hemorrhagic fever. In book: *Viral hemorrhagic fevers*. Singh SK, Ruzek D, eds. First Edition, CRC Press, 2013: 287-305.

Synopsis: The book is meeting the growing need for a single source of information, focusing on viral hemorrhagic fevers, by editing 30 chapters that represent a major contribution to the virological literature and that are written by many experts worldwide. Lujo virus, a novel and highly pathogenic arenavirus, was identified as the etiological agent of an outbreak of hemorrhagic fever in a South African healthcare facility in 2008. This outbreak heralded the first recognition of this previously unknown arenavirus and was the first report of a highly pathogenic arenavirus from Southern Africa. The chapter summarises laboratory and diagnostic findings resulting from investigations during this outbreak.

Coronaviruses in South African bats

Geldenhuys M, Weyer J, Nel LH, Markotter W. Coronaviruses in South African bats. *Vector borne and Zoonotic Diseases* 2013; **13**(7): 516-9.

Synopsis: The study focused on the testing of a small panel of specimens (n=113) from different species of bats from localities in the North West, Limpopo and Gauteng Provinces of South Africa. RT-PCR testing for a target located in the RNA-dependant RNA polymerase gene of coronaviruses was used to detect three novel viruses from this panel. The sequences produced from these samples clustered with known alphacoronaviruses following phylogenetic analysis. This report provides the first evidence for molecular evidence for the circulation of coronaviruses in selected South African bat species.

Other publications

Journal articles

Bukreyev AA, Chandran K, Dolnik O, Dye JM, Ebihara H, Leroy EM, Mühlberger E, Netesov SV, Patterson JL, Paweska JT, Saphire EO, Smither SJ, Takada A, Towner JS, Volchkov VE, Warren TK, Kuhn JH. Discussions and decisions of the 2012-2014 International Committee on Taxonomy of viruses (ICTV) *Filoviridae Study* Group, January 2012 – June 2013. *Archives of Virology* 2013; DOI 10.1007/s00705-013-1846-9.

Fafetine JM, Domingos A, Antunes S, Esteves A, Paweska JT, Coetzer JAW, Rutten VPMG, Neves L. Generation and characterisation of monoclonal antibodies against Rift Valley fever virus recombinant nucleoprotein. *Transboundary and Emerging Diseases* 2013; **60**(suppl.2): 24-30.

Jäckel S, Eiden M, Balkema-Buschmann A, Ziller M, Jansen van Vuren P, Paweska JT, Groschup MH. A novel indirect ELISA based on glycoprotein Gn for the detection of IgG antibodies against Rift Valley fever virus in small ruminants. *Research in Veterinary Science* 2013; **http://dx.org/10.1016/j.rvsc.2013.04.015.**

Kuhn JH, Bao Y, Bavari S, Becker S, Bradfute S, Brister JR, Bukreyev AA, Caì Y, Chandran K, Davey RA, Dolnik O, Dye JM, Enterlein S, Gonzalez J-P, Formenty P, Hensley LE, Honko AN, Jahrling PB, Johnson KM, Klenk H-D, Kobinger G, Leroy EM, Lever MS, Lofts LL, Mühlberger E, Netesov SV, Olinger GG, Palacios G, Patterson JL, Paweska JT, Pitt L, Radoshitzky SR, Saphire EO, Smither, SJ, Swanepoel R, Takada A, Towner JS, van der Groen G, Volchkov VE, Wahl-Jensen V, Warren TK, Weidmann M, Nichol ST. Virus nomenclature below the species level: a standardised nomenclature for laboratory animal-adapted variants and strains of viruses assigned to the family *Filoviridae.Archives of Virology* 2013; **158**: 1425-1432.

Kuhn JH, Bào Y, Bavari S, Becker S, Bradfute S, Brauburger K, Brister JR, Bukreyev AA, Caì Y, Chandran K, Davey RA, Dolnik O, Dye JM, Enterlein S, Gonzalez J-P, Formenty P, Freiberg AN, Hensley LE, Hoenen T, Honko AN, Ignatyev GM, Jahrling PB, Johnson KM, Klenk H-D, Kobinger G, Lackemeyer MG, Leroy EM, Lever MS, Mühlberger E, Netesov SV, Olinger GG, Palacios G, Patterson JL, Paweska JT, Pitt L, Radoshitzky SR, Ryabchikova EI, Saphire EO, Shestopalov AM, Smither SJ, Sullivan NJ, Swanepoel R, Takada A, Towner JS, van der Groen G, Volchkov VE, Wahl-Jensen V, Warren TK, Warfield KL, Weidmann M, Nichol ST. Virus nomenclature below the species level: a standardised nomenclature for filovirus strains and variants rescued from cDNA, *Archives of Virology* 2013; **158**: 1877-2.

Métras R, Baguelin M, Edmunds WJ, Thompson PN, Kemp A, Pfeiffer DU, Collins LM, White RG. Transmission potential of Rift Valley fever virus over the course of the 2010 epidemic in South Africa. *EID* 2013; **19**(6): 916-924

Pearce MC, Venter M, Schouwstra T, van Eeden C, Jansen van Vuren P, Paweska JT, Liu B, du Plessis A. Serum neutralising antibody response of seronegative horses against lineage 1 and lineage 2 West Nile virus following vaccination with an inactivated lineage 1 West Nile Virus vaccine. *Journal of the South African Veterinary Association* 2013; **84**(1), Art.# 1052, doi:10.4102/jsava.v84i1.1052.

Sindato C, Swai ES, Karimuribo ED, Dautu G, Pfeiffer DU, Mboera LEG, Paweska JT. Spatial distribution of non-clinical Rift Valley Fever viral activity in domestic and wild ruminants in northern Tanzania. *Tanzania Veterinary Journal* 2013; **28**: S21-38.

Storm N, Weyer J, Markotter W, Kemp A, Leman PA, Dermaux-Msimang V, Nel LH, Paweska JT. (2014). Human cases of Sindbis fever in South Africa, 2006-2010. *Epidemiology and Infection* 2014;**142**(2): 234-8.

Storm N, Weyer J, Markotter W, Leman PA, Kemp A, Nel LH, Paweska JT. Phylogeny of Sindbis virus isolates from South Africa. *The South African Journal of Epidemiology and Infection* 2013; **28**(4): 207-214.

Venter M, Jansen van Vuren P, Mentoor J, Paweska JT, Williams J. Inactivated West Nile Virus (WNV) vaccine, Duvaxyn WNV, protects against a highly neuroinvasive lineage 2 WNV strain in mice. *Vaccine* 2013; pii:S0264-410X(13)00851-7. doi:10.1016/j.vaccine.

Weyer J, Thomas J, Leman PA, Grobbelaar AA, Kemp A, Paweska JT. Human cases of Wesselsbron disease, South Africa 2010-2011, *Vector-Borne and Zoonotic Diseases* 2013; **13**(5): 330-336.

Wilson WC, Romito M, Jasperson DC, Weingartl H, Binepal YS, Maluleke MR, Wallace DB, Jansen van Vuren P, Paweska JT. Development of a Rift Valley fever real-time RT-PCR assay that can detect all three genome segments. *Journal of Virological Methods* 2013; **193**(2): 426-431.

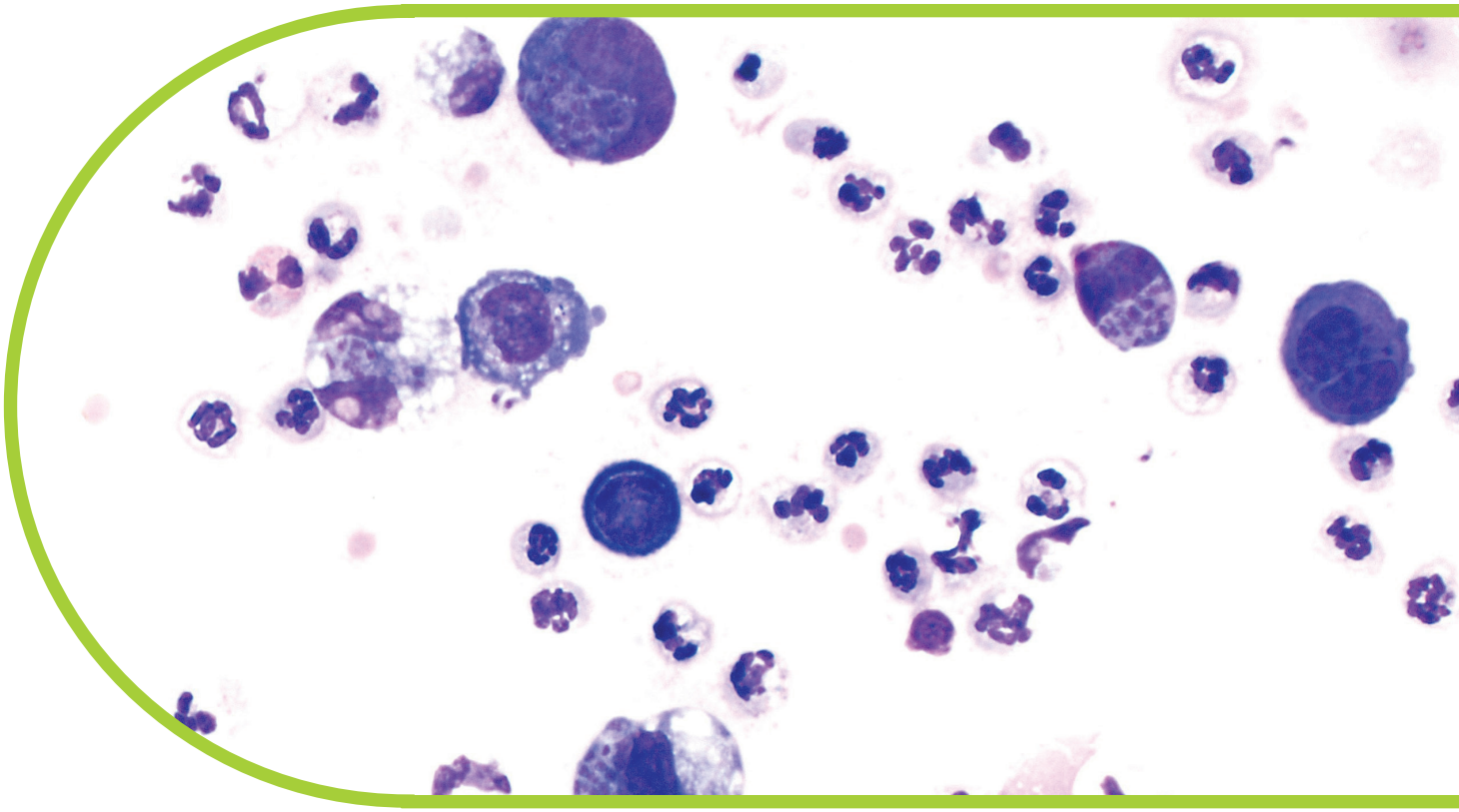
Chapters in books

Paweska JT, Jansen van Vuren P, Weyer J. Lujo hemorhagic fever. In: *Viral haemorrhagic fevers*. Singh SK, Ruzek D, eds. First Edition. CRC Press, 2013; 287-305.

Paweska JT, Jansen van Vuren P. Rift Valley Fever virus: a virus with potential for global emergence. In: *The role of Animals in emerging viral diseases.* Nicholas Johnson, ed. Elsevier Academic Press 2013; 169-200.

Conference presentations

The CEZD presented research findings at five international and two national conferences during the reporting period.



Shutterstock: Toxoplasmosis with Toxoplasmsa gondii within macrophages from a cytology smear. Clusters of parasitic organisms are within macrophages.

CENTRE FOR HIV AND STIs

Professor David Lewis
Centre Head

INTRODUCTION

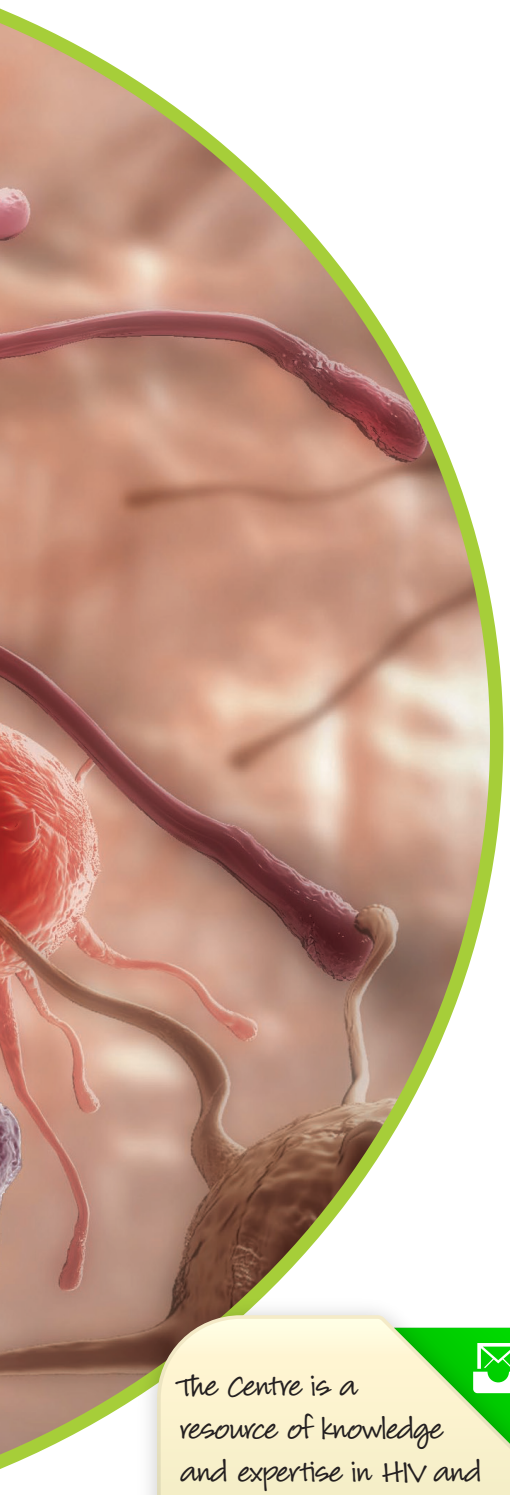
The Centre for HIV and Sexually Transmitted Infections (STI) aims to be a resource of knowledge and expertise in HIV and other regionally relevant STIs to the National South African Department of Health (NDoH), Southern African Development Community (SADC) and the African continent. It aims to assist with the planning of policies and programmes related to the control and effective management of HIV/STIs. The Centre also aims to be a place of academic excellence in terms of both research and teaching/training. It has a strong track record in the research disciplines of HIV virology, HIV immunology, HIV/STI epidemiology, HIV/STI diagnostics and HIV-STI interactions, as well as in successful supervision of PhD and MSc students. The Centre's leadership team consists of Prof David Lewis (Centre Head, STI Section lead), Prof Lynn Morris (HIV Research Section lead), Prof Adrian Puren (HIV Sero-Molecular Diagnostics Section lead), Prof Caroline Tiemessen (Cell Biology Section lead) and Prof Anna-Lise Williamson (HPV Section lead).

SURVEILLANCE/DIAGNOSTIC SERVICES

HIV prevalence and incidence surveillance.

During 2013/14, the Centre supported the NDoH's 33rd Annual Antenatal HIV-1 Prevalence and HIV Incidence survey and the 3rd South African Prevention of Mother to Child Transmission (PMTCT) Effectiveness study. The latter has further confirmed the reduction of HIV transmission from HIV-infected mothers to their offspring at the 4-8 weeks' time point and will be completed in October 2014. The HIV incidence field was advanced by the introduction of a CDC-developed HIV incidence limiting antigen avidity assay (LAg AI). The advance was recognised in a joint WHO/UNAIDS statement, following a review of the evaluation data. In 2013, the Centre performed LAg AI incidence testing on dried blood spot samples (DBS) as part of a national HSRC-led Survey for HIV Prevalence and Incidence. Testing for viral load and antiretroviral drugs levels was included in the recent infection testing algorithm to minimise the false recent rate. Preliminary data from the HSRC study did not show a decline in trend in HIV-1 incidence overall, although there was a decline in younger age groups but higher incidence in the females >25 years of age, which was four times higher than that of males of a similar age group.

The Centre continued its close collaboration with the Public Health England, to further develop HIV surveillance within South Africa. Several objectives were identified and activities undertaken, including the formation of a National HIV Steering Group, the production of a HIV surveillance report based on key quality indicators using the National Institute for Communicable Diseases (NHLS) testing data stored in the NHLS Central Data Warehouse and, finally, the review of the different data sources available. Thesewould be combined to increase the power and accuracy of in-country HIV surveillance, such as the District Health Information System and specifically NDoH databases for treatment, and the vital registration data from Stats SA and modelled data such as Spectrum SA. Initial progress has been made and through the Steering Group, it is hoped to develop work plans to coordinate the sources/ models to better understand the epidemic in the year ahead.



The Centre is a resource of knowledge and expertise in HIV and other regionally relevant STIs to the South African Department of Health and the African continent.

HIV drug resistance surveillance

The Centre's HIV drug resistance laboratory is the designated centre for national surveillance activities and also serves as a World Health Organization regional HIV Drug Resistance Laboratory. Surveillance for transmitted HIV drug resistance (TDR) among individuals assumed to be recently infected was undertaken in 2013/14 using specimens collected as part of the annual antenatal survey conducted by the National Department of Health. A total of 347 HIV-1 positive specimens from primigravid women age <21 years attending public hospitals in five provinces (Eastern Cape, Free State, Gauteng, KwaZulu Natal and the Western Cape) in October 2011, were selected for genotypic analysis. Data were analysed using all available specimens to categorise TDR as low (<5%), moderate (5-15%) or high (>15%). Although 12 samples showed evidence of TDR, the point estimates were <5% in all five provinces for both the nucleoside reverse transcriptase inhibitor (RTIs) and non-nucleoside RTI (NNRTI) drug classes. The data confirm that transmission of resistant viruses, particularly those with NNRTI mutations, is occurring in a number of South African provinces. Plans are in place to sequence specimens collected from across the country from 2012 in order to provide national and provincial TDR prevalence estimates.

STI clinical syndrome, aetiological and gonococcal antimicrobial resistance surveillance

The Gauteng STI surveillance project, run by the Centre in collaboration with the Gauteng Department of Health, continued to collect STI syndrome data from public clinics throughout the review period. In 2013/14, in collaboration with the DoH, Alexandra Health Centre and NHLS laboratories, the Centre undertook aetiological surveillance of three major STI syndromes (male urethritis syndrome, MUS; vaginal discharge syndrome, VDS; genital ulceration syndrome, GUS), as well as surveillance of gonococcal antimicrobial resistance, in Johannesburg, Gauteng and completed testing and data analysis for surveys undertaken in Nelspruit, Mpumalanga and Kimberley, Northern Cape in 2012/13. In addition, a new protocol for national STI aetiological surveillance was revised and approved by the funder and collaborative partner (Centers for Disease Control and Prevention, CDC), as well as in-country. Training of primary healthcare nurses took place in each of South Africa's nine provinces in March 2014.The specimens will be collected and tested in 2014/15.

HPV surveillance

The CDC-supported national STI aetiological survey, mentioned above will also incorporate HPV surveillance through testing for and genotyping of HPV DNA detected in the endocervical specimens collected from 18-20

year-old females attending family planning clinics at primary healthcare clinics across South Africa. In addition, a protocol (currently under review) has been developed to support HPV surveillance, using cervical biopsy material with histologically-confirmed high-grade/cancerous cervical pathology among women attending colposcopy clinics in Cape Town, Johannesburg and Umtata.

HIV-1 rapid testing quality assurance and post-marketing surveillance of HIV rapid test devices

The NDoH re-launched its HIV Counselling and Testing (HCT) Campaign in December 2013. The Centre has revised and introduced a more rigorous HIV rapid test evaluation process, in line with recommendations from the WHO and the CDC. The PEPFAR-funded HIV rapid test quality assurance programme continued its training and M&E of an internal quality assurance control (IQC) programme. Approximately 450 healthcare workers were trained on the IQC programme, and a total of 181 sites visits were performed in 2013/14. Three HIV rapid test kits were awarded to the government tender and post-marketing surveillance of the lots/batches of devices, prior to the release in testing sites had been conducted by the Centre.

HIV external quality assurance (EQA) schemes

Centre staff coordinated the HIV EQA programme for NHLS-participating laboratories. Three NHLS HIV serology surveys were distributed in 2013. The NICD provided the technical support in terms of panel characterisation and selection. Four survey panels for HIV RNA, comprising of two NHLS-specific regulatory and two international panels, were completed and a final report was circulated in March 2014 prior to distribution to 18 participating laboratories. In addition, the Centre provided HIV EQA support to other SADC countries.

Support for HIV vaccine trials

The Centre continued to provide results from validated end-point humoral antibody and molecular HIV assays for the HIV Vaccine Trial Network (HVTN).

Sequencing Core Facility

The Centre currently houses a newly-established Sequencing Core Facility at the NICD, headed by Dr Arshad Ismail. The Core Facility will consolidate all sequencing instruments at the NICD and aims to provide researchers with accurate, cost-effective, high throughput sequencing data. This facility will house both Next-Generation Sequencing (Illumina-MiSeq's and 454 Roche GS junior) and Sanger (3500xl and 3500) platforms. At this early stage, a number of projects have already been completed for the various Centres, justifying the need for centralised capacity and expertise.

Select current research projects

Correlates of protective immunity to HIV-1: a focus on Natural killer (NK) cell responses (2008-2018)

Key collaborator: Dr N Martinson, (PHRU)
Funding: PRF, MRC, NRF, NHLS Trust, NIH

The Centre's earlier studies of maternal-infant HIV-1 transmission identified NK cell responses to HIV-1 peptides in whole blood intracellular cytokine assays that were associated with protection from acquiring HIV-1 in the infants, and with lower viral loads and higher CD4 T cell counts in the mothers. Since this discovery, the centre has utilised similar assays for the study of NK cell responses in long term non-progressors (LTNPs; maintain CD4 counts > 500 cells/µl for >6 years) and elite controllers (ECs; undetectable viral loads < 50 RNA copies/ml). These responses were present in 90% of LTNPs/ECs and in only 10% of HIV-1 progressors. They specifically targeted HIV-1 Env and Vpu peptide pools, and upon further mapping it was evident that response to select Vpu peptides was associated with the elite controller phenotype. Ongoing immunology and host genetic studies will provide important insights into the mechanisms underlying host control of HIV-1 infection in individuals able to spontaneously suppress viral replication in the absence of antiretroviral therapy.

Viral escape from HIV-1 neutralising antibodies drives increased plasma neutralisation breadth through sequential recognition of multiple epitopes and immunotypes (2010-2013)

Key collaborators: Prof SS Abdool Karim (CAPRISA), Prof C Williamson (UCT)
Funding: CAPRISA, CHAVI, DST, NIH, Wellcome Trust

Understanding how broadly cross neutralising (BCN) antibodies develop is an important goal for HIV-1 vaccine development. The Centre previously identified participant CAP257, whose plasma neutralised 84% of global viruses.

The Centre showed that CAP257 breadth was due to the sequential appearance of three distinct BCN antibodies. The first wave targeted a V2 epitope, also recognised by earlier strain-specific antibodies. Strain-specificity was determined by a rare N167 polymorphism in V2, with escape to the more common D167 variant coinciding with breadth. Escape from V2 antibodies by deletion of the N160 glycan exposed the CD4 binding site (CD4bs) that became the target for a second wave of antibodies, with escape mutations in the CD4bs driving increased breadth. The third wave targeted an unknown quaternary epitope. This study showed that the human immune system can generate multiple BCN antibodies, in response to an evolving viral population that exposes new targets through escape from earlier antibodies.

Developmental pathway for potent V1V2-directed HIV-neutralising antibodies (2011-2013)

Key collaborators: Dr PD Kwong, Dr L Shapiro and Dr JR. Mascola (VRC)
Funding: VRC, NIAID, NIH, IAVI, NSF, SCRIPPS, CHAVI-ID, SADST, Wellcome Trust, HERTZ, DDHF, PRF, NRF

Antibodies capable of neutralising HIV-1 often target variable regions 1 and 2 (V1V2) of the HIV-1 envelope, but the mechanism of their elicitation has been unclear. Here the researchers define the developmental pathway by which such antibodies are generated and acquire breadth. Twelve somatically related neutralising antibodies (CAP256-VRC26.01–12) were isolated from CAPRISA donor CAP256. Each antibody contained the protruding tyrosine-sulphated, anionic antigen-binding loop (complementarity-determining region (CDR)-H3) characteristic of this category of antibodies. Their unmutated ancestor emerged between weeks 30–38 post-infection with a 35-residue CDR-H3, and neutralised the virus that superinfected this individual 15 weeks post-infection. Improved neutralisation breadth and potency occurred by week 59 with modest affinity maturation, and was preceded by extensive viral diversification. HIV-1 V1V2-directed neutralising antibodies can thus develop relatively rapidly through initial selection of B cells with a long CDR-H3, and limited subsequent somatic hypermutation. These data provide important insights relevant to HIV-1 vaccine development.

Boosting antibody responses with gp140 protein two years after DNA/MVA priming: Results from the HVTN 073E Phase I vaccine trial conducted in South Africa and the USA (2008-2014)

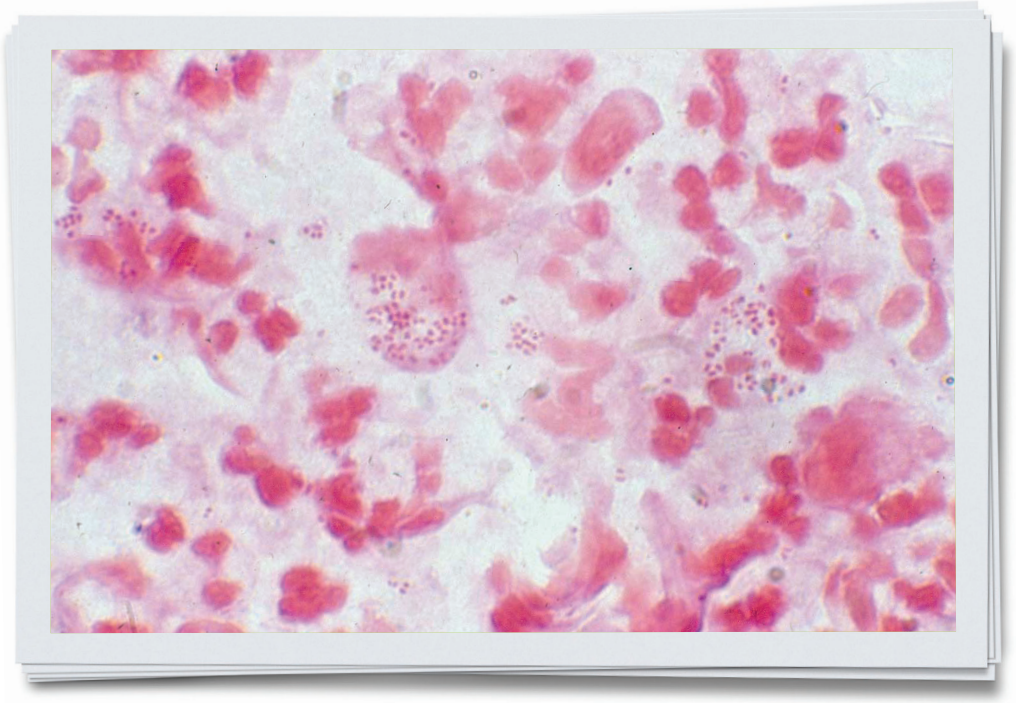
Key collaborators: Georgia Tomaras and David Montefiori (Duke University), Glenda Gray (PHRU)
Funding: NIAID, DAIDS, HVTN

This study was designed to explore whether antibody responses to a DNA/poxvirus vaccine can be improved with the addition of an envelope protein vaccine. 27 HIV-1-uninfected adult participants, who two years prior had been vaccinated with SAAVI DNA-C2 vaccine and SAAVI MVA-C vaccine, were boosted twice, three months apart, with Novartis subtype C oligomeric V2 loop-deleted TV1 gp140 vaccine in MF59 adjuvant. Participants received either DNA/MVA with a protein boost (T1/T2, n=16), DNA/MVA with a placebo boost (T1/C2, n=6), placebo with a protein boost (C1/T2, n=1), or double placebo (C1/C2, n=4). Serum samples from two weeks after the 1st and 2nd protein boosts (visits 19 and 22) and six months later (visit 24) were tested for neutralising antibodies. For all 3 Tier 1 viruses, the area under the magnitude-breadth curve was significantly higher in T1/T2 versus T1/C2 at visits 22 and 24 ($p<0.0001$ and $p=0.0052$). No responses were seen to Tier 2 viruses in the TZM-bl assay or in the C1/T2 and C1/C2 groups. Thus, a protein vaccine was essential for eliciting neutralising responses, and the priming effect of DNA/MVA appears to last for many years with the mobilisation of antigen-specific B cells within weeks of protein boosting.

Surveillance of acquired HIV drug resistance in adult and paediatric patients in KwaZulu-Natal, South Africa (2013-2014)

Key collaborators: E Raizes and EK Dokubo (CDC)
Funders: CDC-NHLS Cooperative Agreement 5U2GPS001328-05

A pilot cross-sectional sentinel surveillance project was undertaken to classify the proportion of patients failing 1st-line ART and to describe the patterns of drug resistance mutations (DRMs). Three populations were surveyed using consecutive sampling in 15 clinics between August-November 2013: adults on ART for 12-15 months (Surv1) and for 24-36 months (Surv2), and paediatric patients on ART for 12-36 months (Surv3). Specimens with VL ≥1000 cpm were defined as virologic failure (VF) and genotyped for DRMs. Specimens were collected from 1,924 adults and 161 children. VF prevalence was 8.2% (95%CI: 6.3-10.2%) in Surv1, 11.5% (9.7-13.4%) in Surv2, and 33.5% (26.2-40.8%) in Surv3. Of those genotyped, DRMs were detected in 85%, 89% and 97%. The most prevalent mutations were M184V/I, K65R, K103N/S, V106A/M/I and Y181C. Viral suppression rates in the adult population receiving ART were high; however, a third of paediatric participants were viraemic. Among both adult and paediatric failures, DRMs were high. This pilot study highlights the importance of ongoing HIVDR cross-sectional surveys, and the high rate of VF among paediatric populations.



We reported the first internationally verified cases of cefixime resistant gonorrhoea in Africa.

The Orange Farm study part 2: a community study of male circumcision (2007-2014)

Key collaborator: Prof B Auvert (INSERM, University of Versailles)
Funding: ANRS

This project, which commenced in the latter part of 2007, aims to assess the impact of voluntary, safe, medical male circumcision on STI infections in Sub-Saharan Africa. The Centre continues to provide the laboratory testing required for community-based cross-sectional surveys. Male circumcision part D started on 27 January 2014. The approximate sample six is 6 000 specimens,which will be collected from both male and female participants. HIV Serology, HIV Incidence and selected HSV-2 serological and HPV genotyping testing will be done on these samples.

STIs in men-who-have-sex-with-men (MSM) (2011-2014)

Key collaborator: Dr K Rebe (Ivan Toms Centre for Men's Health, ANOVA Health Institute)
Funding: USAID

The Centre, in collaboration with the ANOVA Health Institute, previously determined the prevalence of gonococcal and chlamydial infections at urethral, rectal and pharyngeal sites in symptomatic and asymptomatic MSM attending the Ivan Toms Centre for Men's Health in Cape Town. During 2013/14, the DNA extracts were additionally tested for HPV infection and any HPV positive samples were genotyped. There was a high prevalence of HPV infection, particularly in ano-rectal specimens.

Oral HPV infection in South African men and women recruited for a study on HIV discordant couples (2013-2014)

Key collaborators: Dr T Meiring and Assoc Prof D Coetzee (UCT)
Funding: PRF, SIDA, CANSA, NRF and MRC

Mucosal and cutaneous human papillomavirus (HPV) prevalence from oral samples was investigated in 263 women and 261 men. Oral mucosal HPV prevalence was found to be 14% (73/524) when considering both women and men, when grouped according to gender, 13% (34/263) women and 15% (39/261) men were mucosal HPV positive. Oral cutaneous HPV type prevalence was found to be 17% (91/522), when grouped according to gender, while 18% (48/262) women and 16.5% (43/260) men were cutaneous HPV positive. Fourteen samples that were positive for mucosal HPV types and 14 samples that were positive for cutaneous HPV types, were sent for 454 next generation sequencing. A new HPV type was detected from this sequencing data, the new sequence was confirmed by the WHO Reference Laboratory.

Precious Mahlangu running the Qiagen careHPV™ assay for a collaborative research study.

Detection of cutaneous HPV types in penile samples from South African men (2013-2014)

Key collaborators: Dr T Meiring and Assoc Prof D Coetzee (UCT)
Funding: PRF, SIDA, CANSA, NRF and MRC

Cutaneous human papillomavirus (HPV) prevalence in penile samples was investigated in 139 men, with known mucosal HPV type's status that were previously detected by Roche Linear Array HPV Genotyping assay. A total of 70.5% (98/139) were cutaneous HPV positive. HIV-positive men were more likely to be HPV cutaneous positive than HIV negative (87% 60/69, 54% 38/70, P<0.0001). Those that were positive for mucosal HPV types were more likely to also be positive for cutaneous HPV types (91% 64/70, 49% 34/69, P<0.0001). These findings suggest that in men, genital site cutaneous HPV types are commonly detected. Eighty samples that were positive for cutaneous HPV types were sent for 454 next generation sequencing, to get the HPV genotype detected in these samples and to establish if new types are present.

GRANT FUNDING

Funding to support the Centre's work was obtained from the following organisations:

- Centers for Disease Control and Prevention (CDC, PEPFAR and Global Disease Detection funds)
- Canadian HIV Vaccine Initiative's CANSSA HIV/AIDS network pilot grant
- Department of Science and Technology/National Research Foundation Chair of HIV Vaccine Translational Research
- Medical Research Council
- National Health Laboratory Service Research Trust
- National Research Foundation (NRF) Incentive Funding for Rated Researchers
- National Research Foundation Professional Development Programme
- Poliomyelitis Research Foundation (PRF)
- Technology Innovation Agency (SHARP funding: South African HIV/AIDS Research and Innovation Platform)
- World Health Organization

In addition, research collaborators obtained funding to support Centre activities from the National Institutes for Health, the Agence Nationale de Reserches sur la SIDA et les hépatites virales (collaboration with INSERM at the University of Versailles and Progressus), EPICENTRE, (collaboration with MSF); European Union (via third party agreement with Wits Reproductive Health and HIV Institute), European and Developing Countries Clinical Trials Partnership (EDCTP), and the United States Agency for International Development (collaboration with ANOVA Health Institute). Members of the Centre also participate in major networks such as the Centre for HIV Vaccine Immunology (CHAVI), The Bill and Melinda Gates Centre for AIDS Vaccine Discovery (CAVD) and the HIV Vaccine Trial Network (HVTN).

TEACHING AND TRAINING

During 2013/14, the Centre undertook a variety of teaching and training activities. The Centre offers a thorough and comprehensive training programme for interns and technologists, in line with HPCSA guidelines. The has trained one Intern Medical Scientist as well as Microbiology and Virology Registrars during 2013/14. The Centre is hosting one South African Field Epidemiology and Laboratory Training Programme (FELTP) postgraduate student from Liberia. Several Centre staff have assisted with other aspects of the FELTP teaching programme, as well as facilitated, on behalf of the African Centre for Integrated Laboratory Training, a workshop on the application of the LAg-avidity HIV-1 incidence assay for HIV-1 incidence surveillance. Centre staff trained 1,188 health care workers and professionals on quality management systems for HIV rapid testing using defined curricula. Undergraduate and postgraduate lectures were delivered to medical, nursing, pharmacy, dental and chemistry students at the University of the Witwatersrand and to medical students at the University of Cape Town. In addition, postgraduate lectures on STIs were delivered to practising doctors and to nurses in Cape Town, Johannesburg and Pretoria. In terms of internal, capacity-building, some Centre staff undertook training attachments in the UK at Public Health England, and one staff member spent three months at the Oregon National Primate Research Centre at the Oregon

Health and Science University in Oregon, USA, to learn more about HIV-1 DNA vaccine design. Other staff benefitted from attending a number of training workshops and conferences, within South Africa.

PROFESSIONAL DEVELOPMENT

During 2013/14, two PhD and three MSc students graduated from the Centre:

Anabela Picton received a PhD for her dissertation entitled 'HIV-1 coreceptor CCR5: gene characterisation and expression'.

Hazel Mufhandu received a PhD for her dissertation entitled 'HIV-host interactions: analysis of endemic South African clinical isolates of HIV-1 using anti-gp120 aptamers'.

Xolani Mndende, Simone Richardson and Jinal Bhiman graduated with MSc degrees.

HONOURS

Centre staff received a number of awards and other honours during the reporting period:

- Prof David Lewis commenced his two-year term in July 2013, as elected President of the International Union against STIs and received a NRF B2 rating.
- Prof Caroline Tiemessen was appointed as a member of the Academy of Science of South Africa and an associate member of the Sydney Brenner Institute for Molecular Bioscience, University of the Witwatersrand, as well as Chairperson of the Scientific Advisory Panel) of the Polio Research Foundation.
- Prof Lynn Morris was 2nd runner-up for the 2013 South African Distinguished Women in Science Award and was also elected as a new Board member of the Global HIV Vaccine Enterprise.
- Penny Moore received a research award in recognition of her recent achievements, from the University of the Witwatersrand's Faculty of Health Sciences.
- Dr Anabela Picton was awarded a Claude-Leon postdoctoral fellowship.
- Dr Dshanta Naicker and Jinal Bhiman were invited to join the Golden Key International Honour Society for academic achievers.
- Kurt Wibmer was awarded the 2013 Wits Faculty of Health Science Prize for Research.
- Simone Richardson received the prize for "Best student", during the 4th Infectious Diseases Symposium and 5th ICS workshop held at the University of Cape Town.
- Nancy Tumba won a prize for being one of four young promising postdoctoral students at the CHAVI-ID 2nd Annual Retreat.

RESEARCH OUTPUT

During 2013/14, there were 37 journal articles published in international and national peer-reviewed journals, as well as contributions to four books. In addition, Centre staff delivered 30 presentations at international congresses (two plenary lecture, eight symposium presentations, five oral presentations and 15 posters) and 25 presentations at national congresses (two plenary lectures, 10 symposium presentations, six oral presentations and seven posters).

The following five publications are highlighted as they present work that has advanced public health and/or laboratory science within South Africa:

Auvert B, Taljaard D, Rech D, Lissouba P, Singh B, Bouscaillou J, Peytavin G, Mahiane G, Sitta R, Puren A, Lewis D. Association of the ANRS-12126 male circumcision project with HIV levels among men in a South African township: evaluation of effectiveness using cross-sectional surveys. *PLOS Medicine* 2013; **10**(9): e1001509.

Synopsis: Approximately 2 000 randomly selected men aged 15–49 years were asked about their sexual behaviour and their intention to become circumcised, if uncircumcised, in a baseline survey in 2007/08. A similar follow-up survey was conducted in 2010/11 in which more than 3 000 men were invited to take part. At baseline, 12% of the men surveyed had been circumcised, whereas in the follow-up survey, the overall prevalence of circumcision and the prevalence of circumcision among 15–29 year-olds were 53% and 58%, respectively. The overall HIV prevalence at follow-up was 12% and it was estimated that, without the voluntary medical male circumcisions (VMMCs)

performed during the Bophelo Pele project and the preceding randomised control trial, the prevalence of HIV among men living in Orange Farm would have been 15% in 2011. The study reported a reduction in the rate of HIV incidence rate ranging from 57% to 61% among circumcised men in comparison with uncircumcised men. Importantly, there was no evidence of an association between circumcision status and risky sexual behaviour.

Gentle N, Paximadis M, Puren A and Tiemessen CT. Genetic variability in markers of HLA-C expression shows similarities between two diverse South African populations. *Plos One* 2013; **8**: e67780.

Synopsis: This study examined genetic variation within the HLA-C 3' untranslated region (UTR) of 265 Black and Caucasian South Africans by direct sequencing and identified haplotypes encompassing the 263 insertion-deletion (indel) polymorphism and another indel at position 230 in both populations. Concomitant evaluation of variability at the 235 single nucleotide polymorphism (SNP), identified in previous studies to be in linkage disequilibrium (LD) with the 263 indel and which associated with lower viral setpoint, revealed this polymorphism to be an inappropriate marker for the 263 indel in our populations. These findings provide important insights into genetic variability within the regulatory regions of HLA-C that have potential implications for the understanding of the regulation of HLA-C expression and its impact on HIV-1 disease progression.

Lewis DA, Sriruttan C, Müller EM, Golparian D, Gumede L, Fick D, de Wet J, Maseko V, Coetzee JJ, Unemo M. Phenotypic and genetic characterisation of the first two cases of extended-spectrum cephalosporin resistant Neisseria gonorrhoeae infection in South Africa and association with cefixime treatment failure. *J Antimicrob Chemother* 2013; **68**: 1267-1270

Synopsis: The researchers determined the phenotypic and genetic characteristics of the first two cases of extended-spectrum cephalosporin (ESC) resistant *Neisseria gonorrhoeae* in South Africa, which were in case associated, with a verified cefixime treatment failure. These isolates were cultured from urethral discharge specimens collected by private GPs from men-who-have-sex-with-men. Both strains were resistant to cefixime and possessed a number of identical mutations in key genes contributing to ESC resistance in N. gonorrhoeae. The two strains contained the type XXXIV penA mosaic allele and belonged to a successful international MSM-linked multi-drug-resistant gonococcal clone (MLST ST1901), associated with several cefixime treatment failures in Europe and North America.

Mbulawa ZZA, Johnson LF, Marais DJ, Coetzee D and Williamson A-L. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. *J Infect* 2014; **68**: 185-189.

Synopsis: This study determined cervical, penile and oral HPV types in 221 heterosexual couples, through the use of the Roche Linear Array HPV genotyping assay. Oral HPV prevalence was found to be 6.8% in women and 13.5% in men. The risk of oral infection with a specific HPV type in women was increased when the same type was detected in their genital tract, the genital tract of their male partner or the mouth of their male partner. In men, the risk of oral infection with a specific HPV type was increased when the same type was detected in the genital tract of their female partner or the mouth of their female partner, but not when the same type was present in their own genital tract. These findings suggest that in African settings, oral HPV infection is acquired from sexual partners, and that in women may also be the result of self-inoculation.

Moore PL, Sheward D, Nonyane M, Ranchobe N, Hermanus T, Gray ES, Abdool Karim SS, Williamson C, Morris L. Multiple pathways of escape from 1 HIV broadly cross-neutralising V2-dependent antibodies. *J Virology May* 2013; **87**: 4882-4894.

Synopsis: Broadly cross-neutralising (BCN) antibodies are likely to be critical for an effective HIV vaccine. However, the ontogeny of such antibodies and their relationship with autologous viral evolution is unclear. Here, the study characterised viral evolution in CAP256, a subtype C-infected individual who developed potent BCN antibodies targeting positions R166 and K169 in the V2 region. These data suggest rapid maturation, within 11 weeks, of CAP256 strain-specific antibodies to acquire breadth, with implications for the vaccine elicitation of BCN V2-dependent antibodies. Overall these studies demonstrate that ongoing viral escape is possible, even from BCN antibodies.

LIST OF PUBLICATIONS

Journal articles (37)

Alexandre K, Moore PL, Nonyane M, Gray ES, Ranchobe R, Chakauya E; McMahon J, O'Keefe B, Chikwamba R, Morris L. Mechanisms of HIV-1 subtype C resistance to GRFT, CV-N and SVN. *Virology* 2013; 446: 66-76.

Ameur A, Meiring TL, Bunikis I, Haggqvist S, Lindau C, Hedlund Lindberg J, Gustavsson I, Mbulawa ZZA, Williamson A-L and Gyllensten U. Comprehensive profiling of the vaginal microbiome in HIV positive women using massive parallel semiconductor sequencing. *Sci Reports* 2014**; 4**; 4398.

Andersson E, Shao W, Bontell I, Cham F, Cuong DD, Wondwossen A, Morris L, Hunt G, Sönnerborg A, Bertagnolio S, Maldarelli F, Jordan MR. Evaluation of sequence ambiguities of the HIV-1 pol gene as a method to identify recent HIV-1 infection in transmitted drug resistance surveys. *Infect Genet Evol* 2013; **18**: 125–131.

Auvert B, Taljaard D, Rech D, Lissouba P, Singh B, Bouscaillou J, Peytavin G, Mahiane G, Sitta R, Puren A, Lewis D. Association of the ANRS-12126 male circumcision project with HIV levels among men in a South African township: evaluation of effectiveness using cross-sectional surveys. *PLoS Medicin*e 2013; **10**(9): e1001509.

Cotton MF, Violari A, Otwombe K, Panchia R, Dobbels E, Rabie H, Josipovic D, Liberty A, Lazarus E, Innes S, van Rensburg AJ, Pelser W, Truter H, Madhi SA, Handelsman E, Jean-Philippe P, McIntyre JA, Gibb DM, Babiker AG. Early time-limited antiretroviral therapy versus deferred therapy in South African infants infected with HIV: results from the children with HIV early antiretroviral (CHER) randomised trial. *Lancet* 2013; 382: 1555-1563.

Dinh T-H, Kamb ML, Msimang V, Likibi M, Molebatsi T, Goldman T, Lewis DA. Integration of preventing mother-to-child transmission of HIV and syphilis testing and treatment in antenatal care services in the Northern Cape and Gauteng provinces, South Africa. *Sex Transm Dis* 2013; **40**: 846-851.

Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky, BJ, Ernandes MJ, Georgiev IS, Kim HJ, Pancera M, Staupe RP, Altae-Tran HR, Bailer RT, Crooks ET, Cupo A, Druz A, Garrett NJ, Hoi KH, Kong R, Louder MK, Longo NS, McKee K, Nonyane M, O'Dell S, Roark RS, Rudicell RS, Schmidt SD, Sheward DJ, Soto C, Wibmer CK, Yang Y, Zhang Z, NISC Comparative Sequencing, Mullikin JC, Binley JM, Sanders RW, Wilson IA, Moore JP, Ward AB, Georgiou G, Williamson C, Abdool Karim SS, Morris L, Kwong PD, Shapiro L, Mascola JR. Developmental pathway for potent VIV2-directed HIV-neutralising antibodies. Nature. Published online 2 March 2014 doi:10.1038/nature13036.

Gentle N, Paximadis M, Puren A and Tiemessen CT. Genetic variability in markers of HLA-C expression show similarities between two diverse South African populations. *Plos One* 2013; **8**: e67780.

Georgiev IS, Doria-Rose NA, Zhou T, Do Kwon Y, Staupe RP, Moquin S, Chuang G-Y, Louder MK, Schmidt SD, Altae-Tran HR, Bailer RT, McKee K, Nason M, O'Dell S, Ofek G, Pancera M, Srivatsan S, Shapiro L, Connors M, Migueles SA, Morris L, Nishimura Y, Martin MA, Mascola JR, Kwong PD. Delineating Antibody Recognition in Polyclonal Sera from Patterns of HIV-1 Isolate Neutralisation. *Science* 2013; 340: 751-756.

Gray C, Hong H, Young K, Lewis DA, Fallows D, Manca C, Kaplan G. Plasma interferon-gamma-inducible protein-10 (IP-10) can be used to predict viral load in HIV-1 infected individuals. *JAIDS* 2013; **63**: e115-e116.

Hoffmann CJ, Ledwaba J, Li J-F, Johnston V, Hunt G, Fielding KL , Chaisson RE, Churchyard GJ, Grant AD, Johnson JA, Charalambous S, Morris L. Resistance to tenofovir-based regimens during treatment failure of subtype C HIV-1 in South Africa. *Antivir Ther* 2013; **18**: 915-920.

Hoffmann CJ, Charalambous S, Grant A, Morris L, Churchyard GJ, Chaisson RE. Durable HIV RNA resuppression after virologic failure while remaining on a first-line regimen: a cohort study. *Trop Med Int Health* 2014; **19**: 236-239.

Hong HA, Paximadis M, Gray GE, Kuhn L and Tiemessen CT. KIR2DS4 allelic variants: differential effects on in utero and intrapartum HIV-1 mother-to-child transmission. *Clin Immunol* 2013; **149**: 498-508.

Johnston V, Cohen K, Weisner L, Morris L, Ledwaba J, Fielding K, Charalambous S, Churchyard G, Phillips A, Grant A. Viral suppression following switch to second-line antiretroviral therapy: the role of resistance and 'sub-therapeutic' drug concentrations prior to switch. *J Infect Dis* 2014; **209**: 711-720.

tLacerda M, Moore PL, Ngandu NK, Seaman M,Gray ES, Murrell B, Krishnamoorthy M, Nonyane M, Madiga M, Wibmer CK, Sheward D, Bailer RT, Gao H, Greene KM, Abdool Karim SS, Mascola JR, Korber BTM, Montefiori DC, Morris L, Williamson C, Seoighe Cand the CAVD-NSDP Consortium. Identification of broadly neutralising antibody epitopes in the HIV-1 envelope glycoprotein using evolutionary models. *Virol J* 2013; **10**: 347.

Lassauniere R, Shalekoff S and Tiemessen CT. A novel FCGR3A intragenic haplotype is associated with increased FcγRIIIa/CD16a cell surface density and population differences. *Hum Immunol* 2013; **74**: 627-634.

Lewis DA, Sriruttan C, Müller EM, Golparian D, Gumede L, Fick D, de Wet J, Maseko V, Coetzee JJ, Unemo M. Phenotypic and genetic characterisation of the first two cases of extended-spectrum cephalosporin resistant Neisseria gonorrhoeae infection in South Africa and association with cefixime treatment failure. *J Antimicrob Chemother* 2013; **68**: 1267-1270.

Lewis DA, Gumede LYE, van der Hoven L, de Gita GN, de Kock EJE, de Lange T, Maseko V, Kekana V, Smits F, Perovic O. Antimicrobial susceptibility of organisms causing community-acquired urinary tract infections in Gauteng Province, South Africa. *S Afr Med J* 2013; **103**: 377-381.

Lewis DA. Gonorrhoea resistance among men-who-have-sex-with-men: what's oral sex got to do with it? South Afr. J. Epidemiol. *Infect.* 2013; **28**: 77.

Lewis DA, Marsh K, Radebe F, Maseko V, Hughes G. Trends and associations of Trichomonas vaginalis infection in men and women with genital discharge syndromes in Johannesburg, South Africa. *Sex Transm Infect* 2013; **89**: 523-527.

Lewis DA, Hughes G. Professor Cathy Ison – her contribution to the science and practice of STI control. *Sex Transm Infect* 2013: 610-611.

Lewis DA. The role of core groups in the emergence and dissemination of antimicrobial resistant N. gonorrhoeae. *Sex Transm Infect* 2013; **89**: iv47-iv51.

Lewis DA. Global resistance to Neisseria gonorrhoeae: when theory becomes reality. *Curr Opin Infect Dis* 2014; **27**: 62-67.

Magooa MP, Müller EE, Gumede L, Lewis DA. Determination of Neisseria gonorrhoeae susceptibility to ciprofloxacin in clinical specimens from men using a real-time polymerase chain reaction assay. *Int J Antimicrob Agents* 2013; **42**: 63-67.

Mbulawa ZZA, Johnson LF, Marais DJ, Gustavvson I, Moodley JR, Coetzee D, Gyllensten U and Williamson A-L. Increased alpha 9 human papillomavirus species viral loadin human immunodeficiency virus positive women. *MBMC Infect Dis J* 2014; **14**: 51.

Mbulawa ZZA, Johnson LF, Marais DJ, Coetzee D and Williamson A-L. Risk factors for oral human papillomavirus in heterosexual couples in an African setting. *J Infect 2014*; **68**: 185-189.

Mcleod K, Omar, T, Tiemessen CT, Tshabangu N and Martinson N. Prevalence of premalignant cervical lesions in women with a long term non-progressor or HIV controller phenotype. *JAIDS* 2014; **65**: 29-32.

Moore PL, Sheward D, Nonyane M, Ranchobe N, Hermanus T, Gray ES, Abdool Karim SS, Williamson C, Morris L. Multiple pathways of escape from 1 HIV broadly cross-neutralizing V2-dependent antibodies. *J Virol* 2013; **87**: 4882-4894.

Müller EE, Magooa MP, Lewis DA. Genetic characterisation of Herpes simplex virus type 2 UL23 thymidine kinase in Johannesburg, South Africa. *J Antivir Antiretrovir* 2013; **5**: 080-084.

Ngou J, Magooa MP, Gilham C, Djigma F, Didelot M-N, Kelly H, Yonli A, Sawadogo B, Lewis DA, Delany-Moretlwe S, Mayaud P, Segondy M. Comparison of the CareHPV assay with the Hybrid Capture 2 assays for detection of high-risk HPV DNA in cervical samples from HIV-1-infected African women. J Clin Microbiol 2013; **51**: 4240-4242.

Paximadis M, Schramm DB, Gray GE, Sherman GG, Coovadia A, Kuhn L and Tiemessen CT. Influence of intragenic CCL3 haplotypes and CCL3L copy number in HIV-1 infection in a sub-Saharan African population. *Genes Immun* 2013; **14**: 42-51.

Picton ACP, Paximadis M and Tiemessen CT. Contribution of variable CCL3L copy number to CCL3 protein production in two ethnically divergent South African populations. *Infect Genet Evol* 2013; **14**: 347-356.

Ping L-H, Joseph S, Anderson JA, Abrahams M-R, Salazar-Gonzalez JF, Kincer LP, Treurnicht FK, Arney L, Ojeda S, Zhang M, Keys J, Potter EL, Chu H, Moore PL, Salazar-Gonzalez M, Lyer S, Jabara C, Kirchherrr J, Mapanje C, Ngandu N, Seoighe C, Hoffman I, Gao F, Tang Y, Labranche C, Lee B, Savillle A, Vermeulen M, Fiscus S, Morris L, Abdool Karim SS, Haynes BF, Shaw GM, Korber BT, Hahn BH, Cohen MS, Montefiori D, Williamson C, Swanstrom R and the CHAVI. Comparison of Viral EnvProteins from Acute and ChronicInfections with SubtypeC Human Immunodeficiency Virus Type1 Identifies Differences in Glycosylation and CCR5 Utilization and Suggests a New Strategy for Immunogen Design. *J Virol* 2013; **87**: 7218-7233.

Shalekoff S, Schramm DB, Lassauniere R and Tiemessen CT. Ethnic differences evident within the CXCR4-CXCL12 axis: Implications for cancer and HIV-1 infection. *Cytokine* 2013; **61**: 792-800.

Smeth-Smith, HMB, Harris SR, Skilton RJ, Cutcliffe LT, Radebe FM, Golparian D, Shipitsyna E, Duy PT, O'Neill C, Parmar S, Pitt R, Baker S, Ison CA, Marsh P, Jalal H, Lewis DA, Unemo M, Clarke IN, Parkhill J, Thomson NR. Whole genome sequences of the fastidious bacterium Chlamydia trachomatis directly from clinical samples without culture. *Genome Res* 2013; **23**: 855-866.

Wibmer CK, Bhiman JN, Gray ES, Tumba N, Abdool Karim SS, Williamson C, Morris L, Moore PL. Viral escape from HIV-1 neutralising antibodies drives increased plasma neutralisation breadth through sequential recognition of multiple epitopes and immunotypes. *PLoS Pathogens* 2013; **9**(10); e1003738.

Unemo M, Dillon JR, Lewis DA. Neisseria gonorrhoeaeis evolving into a "superbug" – what measures can be implemented to combat the emergence of multi-drug and extensively-drug resistant gonorrhoea in Latin America and globally? *J Bras Doenças Sex Transm* 2013; **25**: 3-5.

Book chapters (4)

Lewis DA. Chancroid. In: Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. World Health Organization, 2013.

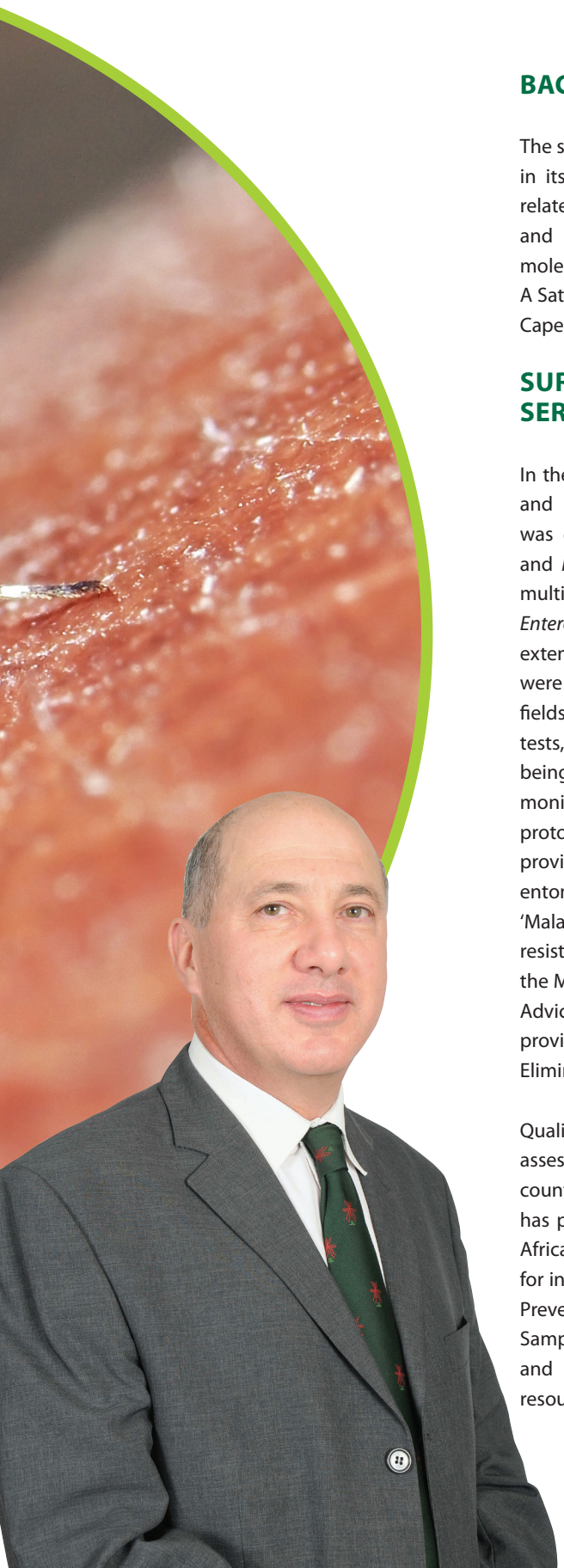
Lewis DA. Donovanosis (granuloma inguinale). In: Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. World Health Organization, 2013.

Lewis DA. Haemophilus ducreyi cultures (section 3.9.4). In: Aerobic bacteriology in the clinical microbiology procedures pandbook. American Society of Microbiology, 2013.

Moore PL, Murphy MK, Derdeyn CA. Role of HIV glycans in transmission and immune escape. Chapter in Book. HIV glycans in infection and immunity. In: HIV glycans in infection and immunity. Springer Science + Business, 2014.

CENTRE FOR OPPORTUNISTIC, TROPICAL AND HOSPITAL INFECTIONS

John Frean
Centre Head



Surveillance functions encompassed national and regional monitoring of cryptococcal meningitis, candidaemia, pneumocystosis, protozoal diarrhoea, and antibiotic-resistant hospital infections

BACKGROUND

The surveillance, reference and research thrusts of the Centre are embodied in its name; namely opportunistic infections, particularly those that are related to HIV/AIDS; tropical infections, especially malaria and its vectors; and nosocomial infections, concentrating on antimicrobial resistance, molecular epidemiology and outbreak investigations in the hospital setting. A Satellite Molecular Epidemiology Unit, based at Groote Schuur Hospital in Cape Town, focuses on nosocomial infections and antimicrobial resistance.

SURVEILLANCE, DIAGNOSTIC AND REFERENCE SERVICES

In the Antimicrobial Resistance Reference Laboratory (AMRRL), phenotypic and genotypic characterisation of mechanisms of bacterial resistance was especially focused on *Staphylococcus aureus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, but a reference service was offered for all multidrug-resistant organisms, such as the emerging carbapenem-resistant *Enterobacteriaceae*. Enhanced surveillance for *S. aureus* infections was extended to the Western Cape Province. Other specialised diagnostic services were offered by the Parasitology and Mycology Reference Laboratories in the fields of opportunistic or unusual parasitic and fungal infections. Molecular tests, particularly PCR and pathogen genomic sequencing, are increasingly being offered. Surveillance functions encompassed national and regional monitoring of cryptococcal meningitis, candidaemia, pneumocystosis, protozoal diarrhoea, and antibiotic-resistant hospital infections. The Centre provides an identification service for medically important arthropods for entomologists, medical practitioners and environmental health officers. 'Malaria vector mosquitoes were routinely identified, and insecticide resistance studies carried out, by the Vector Control Reference Laboratory for the Mpumalanga and KwaZulu-Natal Province Malaria Control Programmes. Advice and expertise, both at the national and provincial levels, was provided to the Department of Health, with participation on the SA Malaria Elimination Committee.

Quality assessment (QA) services provided by the Centre, contributed to assessing diagnostic laboratory proficiency in South Africa and other African countries in malaria, bacteriology, mycology, and tuberculosis. The Centre has played an active role in reporting on laboratory capacity in the WHO African region for the past 12 years, and has supported QA for laboratories for international malaria vaccine trials (GSK Biologicals) and Global Vaccine-Preventable Invasive Bacterial Diseases sentinel sites. The National Biological Sample Collection has resumed its main function and expanded, documented and maintained collections of organisms of national importance, as a resource for scientists and quality controls for routine laboratory tests.

The Centre was also involved in outbreak investigations and responses during 2013-14. These included malaria in communities south of Johannesburg, affecting residents without recent travel history. Entomological investigations revealed no evidence of local breeding of vector anophelines and it was evident that these were odyssean malaria cases, i.e. acquired via infected mosquitoes imported from endemic areas in vehicles, containers or other means. Malaria transmission outside the endemic provinces is of obvious concern, especially if unusual vector breeding patterns are involved. These investigations provide additional support to the Department of Health's malaria elimination activities. An outbreak of carbapenem-resistant *Enterobacteriaceae* at private healthcare facilities in Gauteng was investigated in AMRRL and reported to the Department of Health. The MRL contributed to the investigation of a suspected outbreak of neonatal candidaemia at a Gauteng hospital.

CURRENT RESEARCH AND SURVEILLANCE

Insecticide resistance in malaria vectors

Collaborators: Ms N Coetzer (University of Pretoria)

Anopheles arabiensis is a major malaria vector in much of sub-Saharan Africa, including South Africa. Resistance to insecticides in populations of this species is widespread, necessitating ongoing research into the mechanisms conferring resistance. Recent investigations showed that *An. arabiensis* adults produced from nutrient-deprived larvae are smaller and less tolerant to DDT intoxication. Conversely, well-fed larvae develop comparatively quickly into large, more DDT-resistant adults. In general, larval nutrient deprivation in *An. arabiensis* has important implications for subsequent adults in terms of their size and relative insecticide susceptibility, which may in turn impact on their malaria vector capacity in areas where insecticide-based control measures are in place. It was also demonstrated that permethrin resistance in South African *An. arabiensis* is associated with increased transcription of multiple genes which do not also confer cross-resistance to DDT, unlike a Sudanese strain of *An. arabiensis* in which cross-resistance between pyrethroids and DDT is evident, and which shows strong similarities to the South African strain in terms of gene transcription.

Malaria vector control and transmission dynamics

Collaborators: Dr CL Lyons(University of Stellenbosch) and Dr SL Chown (Monash University,Australia)

Understanding the biology of malaria vector mosquitoes is critical in terms of disease epidemiology and vector control. This is especially important in terms of how climate change is likely to affect malaria transmission. It was shown that three important African malaria vectors, *An. funestus*, *An. arabiensis* and *An. gambiae*, differ significantly in terms of their development rates and survival under different temperature treatments. Increasing temperatures associated with climate change favour survivorship in all three species, but fluctuations in temperatures are detrimental to *An. funestus* and may affect *An. gambiae* in a similar manner. This may have significant implications for disease burden in areas where one or more of these species are primary malaria vectors. Furthermore, in a study designed to compare receptiveness to infection with *Plasmodium berghei* between insecticide-resistant and susceptible laboratory strains of *An. funestus,* it was shown that pyrethroid-resistant females supported the lowest numbers of oocysts and sporozoites, while insecticide-susceptible females produced one of the highest sporozoite indices ever documented in *P. berghei* research. These data suggest that there may be association between insecticide resistance and refractoriness to *Plasmodium* infection, but further elucidation is required.

Laboratory-based antimicrobial resistance surveillance for nosocomial bacteria (LARS)

Laboratory surveillance for antimicrobial resistance (AMR) provides the platform for future coordination with the generation of reliable data on the occurrence of AMR in different geographical regions. A limited number of nosocomial bacterial pathogens such as *S. aureus*, *Klebsiella pneumoniae* (2010-2012), and *P. aeruginosa* (from 2014) were chosen to monitor trends in resistance at sentinel sites at the NHLS over the period.

Enhanced surveillance for hospital versus community associated infections by methicillin-resistant *Staphylococcus aureus*

Collaborators: Dr R Kularatne and Dr T Nana(University of the Witwatersrand), Dr R Lekalala (University of Pretoria), Dr Colleen Bamford (University of Cape Town), Prof Andrew Whitelaw (University of Stellenbosch)

Enhanced surveillance at five sentinel sites that participate in the LARS programme describes prevalence of methicillin-susceptible and resistant *S. aureus*, trends in frequency of resistance, and characterises mechanisms of antimicrobial resistance. Genotypic methods are used to determine the prevalence and pattern of specific antimicrobial resistance genes. Sequence and phylogenetic analysis are used to distinguish hospital-acquired

infections from community-associated infections. Most importantly, MIC50 and MIC90 on all antimicrobials tested, and epidemiological analysis, estimate burden of community- vs. hospital-associated MRSA. Additionally, clinical data risk factors associated with *S. aureus* infections will be analysed. Information will feed into a network of national and global data on the antimicrobial resistance of *S. aureus*.

Pneumocystis jirovecii pneumonia (PCP) in hospitalised patients with severe acute respiratory infections (SARI), using an existing surveillance network in South Africa

Early in the HIV epidemic in Africa, PCP was rarely diagnosed. More recent studies show that PCP is an increasingly important contributor to pneumonia in Africa. This is in contrast with industrialised nations, where the number of PCP cases has fallen since the early days of AIDS. Surveillance is being done for PCP in adults and children at sentinel sites in North West and KwaZulu-Natal provinces. The relative contribution of PCP to the burden of severe acute respiratory infections is being determined together with the Centre for Respiratory Diseases and Meningitis, NICD.

Sentinel surveillance for parasitic causes of diarrhoea in hospitalised children

Five sentinel sites provide stool samples from under-five children with diarrhoea, as part of a rotavirus surveillance programme; residual samples are screened for bacterial and parasitic pathogens. About 13% of samples contain pathogenic parasites; the vast majority (>95%) are *Cryptosporidium* species. Genotyping has previously shown that these are predominantly *C. hominis*. This human-specific species is therefore emerging as an important cause of childhood diarrhoea in South Africa.

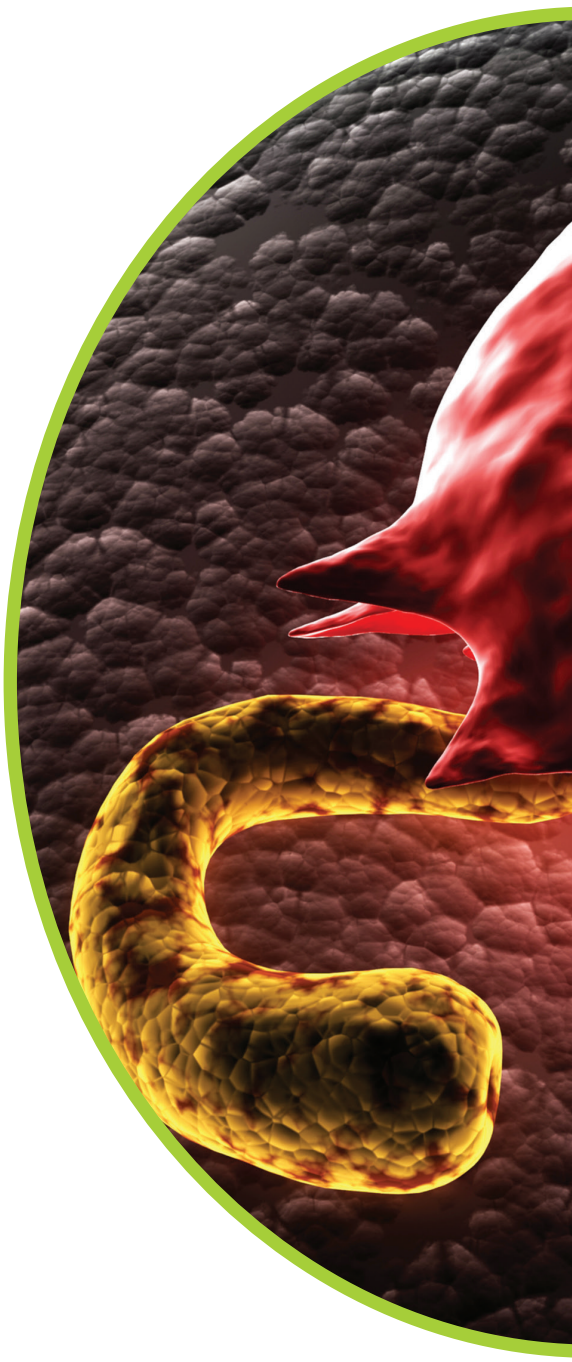
Analysis of the strain types of *Toxoplasma gondii* prevalent in humans and animals in South Africa

The obligate intracellular protozoan parasite *Toxoplasma gondii* is a significant cause of congenital disease and an increasingly important AIDS-defining opportunistic pathogen. This project is investigating the genotypes and virulence markers of *Toxoplasma* prevalent in food animals, rodents, primary hosts (cats) and high-risk humans, and how they compare to strain types elsewhere in Africa and the world.

Human cystic echinococcosis in South Africa

Collaborators: Drs P Kern, K Wahlers (University Hospitals, Ulm, Germany), Dr T Romig (Hohenheim University, Germany), Prof M Grobusch (Amsterdam University Medical Centre and University of the Witwatersrand), Prof C Menezes and Prof M Wong (University of the Witwatersrand)

Cystic *echinococcosis* (hydatid disease) is a zoonosis caused by the tapeworm *Echinococcus granulosus*. It affects various herbivore intermediate hosts (sheep, cattle, goats, camels etc), as well as humans that serve as accidental intermediate hosts. The disease is especially prevalent in pastoral communities, where there is close contact between humans, dogs and livestock. This study used molecular methods to investigate the genotypes and species of affecting humans in South Africa, and showed that most hydatid disease (74% of specimens tested) is caused by the G1 genotype, *E. granulosus*. Less common species/genotypes found locally were *E. canadensis* (G6/7)(19%) and *E. ortleppi* (G5) (7%). This is the first time that the genetic structure of the genus has been characterised in South Africa.



Public health programme: cryptococcal screening

Programme partners: Department of Health, USAID, CDC, PEPFAR partners

Cryptococcal meningitis (CM) has a fatal outcome in more than half of cases in routine care in South Africa. In 2012, 6 817 new cases of laboratory-confirmed CM were detected by the NICD; the incidence was 119 reported cases per 100 000 HIV-infected persons in South Africa. Detectable in blood for weeks to months before onset of CM, cryptococcal antigen (CrAg) is a strong predictor of the development of CM. Following the inclusion of a cryptococcal screen-and-treat intervention in the National Strategic Plan for HIV/AIDS, STIs and TB, South Africa is currently conducting a phased implementation of a national screen-and-treat programme. A reflex laboratory-based testing approach, paired with intensive monitoring and evaluation (M&E), has been used at 100 facilities in Johannesburg and Ekurhuleni Metro districts in Gauteng Province, with planned expansion of this approach to facilities in two rural Free State districts. From September 2012 through December 2013, 10,601 persons were screened for *cryptococcal antigenaemia*; 473 (5%) tested CrAg+.

Multilocus sequence typing of serially-collected isolates of *Cryptococcus* from HIV-infected patients in South Africa

Collaborators: Prof TG Mitchell, Dr AP Litvintseva (Duke University, North Carolina, USA)

Patients with cryptococcal meningitis in Sub-Saharan Africa frequently relapse following treatment. The natural history and aetiology of these recurrent episodes warrant investigation. We used multilocus sequence typing (MLST) to compare the molecular genotypes of strains of *Cryptococcus* isolated from serial episodes of cryptococcal meningitis that were separated by at least 110 days. The most common MLST genotypes among the isolates were the dominant global clinical genotypes of molecular type VNI (M5 and M4), as well as the subpopulation of VNI genotypes apparently restricted to southern Africa. In addition, there was considerable genetic diversity among these South African isolates, as 15% of the patients had unique genotypes of *Cryptococcus neoformans* or *Cryptococcus gattii*. Eleven per cent of the patients were re-infected with a genetically different strain of following their initial diagnosis and treatment. However, most serial episodes (89%), were caused by strains with the same genotype as the original strain. These results indicate that serial episodes of cryptococcosis in South Africa are frequently associated with persistence or relapse of the original infection.

Emmonsia: A newly-discovered fungus causes a potentially deadly disease among HIV-infected South Africans

Collaborators: Prof C Kenyon (University of Cape Town)

Did you know?
We detected a newly-described opportunistic fungus in the genus Emmonsia that caused disease among HIV-infected patients.

Using specialised DNA analysis, the study detected a newly-described (and as-yet unnamed) opportunistic fungus in the genus *Emmonsia* that caused disease among HIV-infected patients. This fungus was genetically related to but entirely separate from known, similar fungi. In 2013, the researchers published information about thirteen cases of disseminated fungal disease that occurred among HIV-infected patients. All thirteen patients had advanced HIV disease, and most were thought to have TB. Some had skin changes suggesting a widespread fungal infection. Once the diagnosis had been made, most patients experienced dramatic and rapid responses to antifungal treatment. In most patients, the skin lesions healed almost completely, they gained weight and their lungs recovered. The infection was fatal in three patients. The infection was probably acquired after inhalation of fungal spores from the environment. No person-to-person transmission occurred with this infection. Since the last publication, at least 30 more cases have been identified through passive surveillance; two patients were HIV-uninfected. Current work on this fungal pathogen is focused on development of an accurate diagnostic assay (antigen and/or molecular assays), whole genome sequencing and exploration of its ecological niche.

Clinical epidemiology of candidaemia at sentinel hospitals

Patients with candidaemia were identified through active laboratory-based surveillance at nine sentinel hospitals in Gauteng and Western Cape provinces in 2012-2013. An incident case of candidaemia was defined as the isolation of *Candida* species from the first submitted blood culture. Detailed clinical information was collected, including underlying diseases and in-hospital mortality. Identification of isolates and antifungal susceptibility testing was performed at the Centre. For cases identified in 2012, a multivariable logistic regression analysis, stratified by age (neonates (≤ 30 days), children (>30 days and <15 years) and adults (≥15 years), was performed to determine factors associated with mortality. Just under half of patients with culture-confirmed candidaemia died in hospital. Predictors of mortality differed by age group and receipt of antifungal treatment was shown to be protective among neonates and adults.

Molecular typing of ***Candida*** isolates causing hospital-associated bloodstream infections

Selected isolates of *Candida* spp. submitted through laboratory-based surveillance for candidaemia have been selected for molecular typing. Selected *Canada parapsilosis* isolates have been typed to determine the prevalence of cryptic species. In addition, the Centre has set up PCR assays for amplification of HS1 and HS2 regions of the fks1/fks2 genes to identify the mutations associated with elevated echinocandin levels in *Candida* species. Lastly, the Centre has set up assays for microsatellite typing of *C. parapsilosis* to establish strain relatedness and to uncover previously-undetected nosocomial outbreaks. Analyses of these data are ongoing.

RESEARCH FUNDING

- Centers for Disease Control and Prevention through NHLS/CDC Cooperative Agreement
- Deutscher Akademischer Austausch Dienst
- Gates Grand Challenges Explorations
- German Research Foundation
- Global Disease Detection, Centers for Disease Control and Prevention
- Hillel Friedland Fellowship
- Innovative Vector Control Consortium
- International Atomic Energy Agency (IAEA)
- Medical Research Council of South Africa
- National Energy Commission of South Africa (NECSA)
- National Health Laboratory Service Research Trust (NHLS Research Trust)
- National Institutes of Health
- National Institutes of Health (ICEMR – Johns Hopkins Malaria Institute)
- National Research Foundation (SARChI,NRF Incentive, DST-NRF Centre of Excellence for Invasion Biology, and DST-NRF Research Chair awards)
- Pennsylvania Department of Health (Tobacco Settlement Funds)
- Research and Policy for Infectious Disease Dynamics (RAPIDD) Programme
- Stellenbosch University Hope Project.

TEACHING AND TRAINING

Teaching and training in various aspects of bacteriology, parasitology, mycology, entomology and communicable diseases was provided to students at postgraduate level (MSc, PhD), medical students, technicians, medical technologists, intern medical scientists, pathology registrars, SASTM travel medicine course participants, as well as doctors enrolled in a Postgraduate Diploma in Tropical Medicine and Hygiene (DTM&H). The Centre assisted the Department of Health with development of laboratory and clinical training materials for the relevant disease programmes.

PROFESSIONAL DEVELOPMENT

Students graduated

Luisa Nardini, PhD. Detoxification enzymes associated with insecticide resistance and exposure to entomopathogenic fungi in *Anopheles arabiensis.*

Manzane Frans Mbokazi, MSc. Ecology of breeding sites and insecticide resistance of potential malaria vectors in Mpumalanga Province, South Africa.

Amoge Eunice Agubuzo, MSc. Malaria vector species composition and insecticide resistance in Zambia.

Benjamin Kgaile Mogoye, MSc. Human cystic echinococcosis in South Africa.

Honours

Prof L Koekemoer received a C1 rating, and Prof M Coetzee a B1 rating, from the National Research Foundation.

Assoc Prof J Frean was appointed Editor-in-Chief of the Annals of the Australasian College of Tropical Medicine.

Dr N Govender was elected Deputy Chair of the South African Society for Clinical Microbiology.

RESEARCH OUTPUT

Top five publications from the Centre

Mogoye BK, Menezes CN, Wong ML, Stacey S, von Delft D, Wahlers K, Wassermann M, Romig T, Kern P, Grobusch MP, Frean J. First insights into species and genotypes of *Echinococcus* in South Africa. *Veterinary Parasitology* 2013; **196**: 427-432.

Synopsis: Hydatid disease is widespread in South Africa but is a particularly common parasitic zoonosis amongst the pastoralists of the Eastern Cape Province. In this paper, for the first time, the genetic structure of *Echinococcus* species causing human hydatid disease in South Africa was revealed, with the G1 genotype predominating amongst the several genotypes present.

Coetzee M, Koekemoer LL. Molecular systematics and insecticide resistance in the major African malaria vector, *Anopheles funestus. Annual Review of Entomology* 2013; **58**: 393-412.

Synopsis: *Anopheles funestus* is one of three major African vectors of malaria. This article examines the progress made on the systematics of the *An. funestus* group and reviews research on insecticide resistance and its mechanisms.

Oliver SV, Brooke BD. The effect of larval nutritional deprivation on the life history and DDT resistance phenotype in laboratory strains of the malaria vector *Anopheles arabiensis. Malaria Journal* 2013; **12**: 44.

Synopsis: *Anopheles arabiensis* is a major malaria vector in Africa. This article shows that larval nutrient deprivation in this species has important implications for subsequent adults in terms of their size and relative insecticide susceptibility, which may in turn impact on their malaria vector capacity in areas where insecticide based control measures are in place.

Govind CN, Moodley K, Peer AK, Pillay N, Maske C, Wallis C, Viana R, Chetty A, Perovic O. NDM-1 imported from India – first reported case in South Africa. *South African Medical Journal* 2013; **103**(7): 476-478.

Synopsis: Carbapenem-resistant Enterobacteriaceae have been increasingly reported throughout the world. Gauteng Province reported the first New Delhi metallo-beta-lactamase-producing organism in South Africa in August 2011. Despite laboratories maintaining a high degree of vigilance, the next case was seen in KwaZulu-Natal almost a year later. This is the first case in South Africa showing this direct epidemiological link with India.

Kenyon C, Bonorchis K, Corcoran C, Meintjes G, Locketz M, Lehloenya R, Vismer HF, Naicker P, Prozesky H, van Wyk M, Bamford C, du Plooy M, Imrie G, Dlamini S, Borman AM, Colebunders R, Yansouni CP, Mendelson M, Govender NP. A dimorphic fungus causing disseminated infection in South Africa. *New England Journal of Medicine* 2013; **369**: 1416-24 .

Synopsis: This study describes thirteen cases of disseminated disease in HIV-infected persons, caused by an entirely novel dimorphic fungal pathogen, *Emmonsia*.

Other publications

Brooke BD, Koekemoer LL, Kruger P, Urbach J, Misiani E, Coetzee M. Malaria vector control in South Africa. *South African Medical Journal* 2013; **103**(10 Suppl 2): 784-788.

Blumberg L, Frean J, Moonasar D and the SA Malaria Elimination Committee. Successfully controlling malaria in South Africa. *South African Medical Journal* 2014; **104** (3 Suppl 1): 224-227 .

Choi KS, Coetzee M, Koekemoer LL. Detection of clade types (clades I and II) within *Anopheles funestus sensu stricto* by the hydrolysis probe analysis (Taqman assay). *Parasites & Vectors* 2013; **6**: 173.

FACT

The New England Journal of Medicine 2013 published **NICD's study of thirteen cases** of **disseminated disease** in HIV-infected persons, caused by an entirely novel dimorphic fungal pathogen, **Emmonsia.**

Coetzee M, Kment P. *Rusingeria* nom. nov, a new substitute name for *Usingeria* Coetzee & Segerman, 1992 (Hemiptera: Heteroptera: Cimicidae). *Zootaxa* 2013; **3664**: 99-100.

Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, Besansky NJ. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa* 2013; **3619**: 246-274.

Coetzee M, Kruger P, Hunt RH, Durrheim DN, Urbach J, Hansford CF. Malaria in South Africa: 110 years of learning to control the disease. *South African Medical Journal* 2013;**103**(10 Suppl 2): 770-778.

Lewis DA, Gumede LYE, van der Hoven LA, de Gita GN, de Kock EJE, de Lange T, Maseko V, Kekana V, Smuts FP, Perovic O. Antimicrobial susceptibility of organisms causing community-acquired urinary tract infections in Gauteng Province, South Africa. *South African Medical Journal* 2013; 103(6): 377-381.

Fettene M, Olana D, Christian RN, Koekemoer LL, Coetzee M. Insecticide resistance in *A. arabiensis* from Ethiopia. *African Entomology* 2013; **21**: 89-94.

Frean J, Poonsamy B, Shandukani B, Moonasar D, Raman J. Case management of malaria: diagnosis. *South African Medical Journal* 2013; 103(10 Suppl. 2): 789-793.

Govender NP, Meintjes G, Bicanic T, Dawood H, Harrison TS, Jarvis JN, Karstaedt AS, Maartens G, McCarthy KM, Rabie H, Variava E and Venter WDF. Guideline for prevention, diagnosis and management of cryptococcal meningitis among HIV-infected persons: 2013 update. *South African Journal of HIV Medicine* 2013;**14**(2): 76-86.

Haji KA, Khatib BO, Smith S, Ali AS, Devine GJ, Coetzee M, Majambere S. Challenges for malaria elimination in Zanzibar: pyrethroid resistance in malaria vectors and poor performance of long-lasting insecticide nets. *Parasites & Vectors* 2013; **6**: 82.

Kanza JPB, Fahime EE, Alaou S, Essassi EM, Brooke B, Malafu AN, Tezzo FW. Pyrethroid, DDT and malathion resistance in the malaria vector *Anopheles gambiae* from the Democratic Republic of Congo. *Transactions of the Royal Society of Tropical Medicine & Hygiene* 2013; **107**: 8-14.

Lo TM, Coetzee M. Marked biological differences between insecticide resistant and susceptible strains of *Anopheles funestus* infected with the murine parasite, *Plasmodium berghei. Parasites & Vectors* 2013; **6**: 184.

Loyse A, Thangaraj H, Easterbrook P, Ford N, Roy M, Chiller T, Govender N, Harrison TS, Bicanic T. Cryptococcal meningitis: improving access to essential antifungal medicines in resource-poor countries. *Lancet Infectious Diseases.* 2013; **13**(7): 629-637.

Lyons CL, Coetzee M, Chown SL. Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors, *Anopheles arabiensis* and *Anopheles funestus. Parasites & Vector*s 2013; **6**: 104.

Morris N, Frean J, Baker L, Ukpe IS, Barnes KI, Kruger P, Mabuza A, Raswiswi E, Maharaj R, **Blumberg L**, Moonasar D. Re-defining the extent of malaria transmission in South Africa: implications for chemoprophylaxis. *South African Medical Journal* 2013; 103(11): 861-864.

Nardini L, Christian RN, Coetzer N, Koekemoer LL. DDT and pyrethroid resistance in *A. arabiensis* from South Africa. *Parasites & Vectors* 2013; **6:** 229.

Strydom KA, Ismail F, Frean J. Plasmo*dium ovale* malaria: a case of not-so-benign tertian malaria. *Malaria Journal* 2014; **13**: 85.

Vezenegho SB, Chiphwanya J, Hunt RH, Coetzee M, Bass C, Koekemoer LL. Characterisation of the *Anopheles funestus* group including *Anopheles funestus*-like from northern Malawi. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2013; **107**: 753-762.

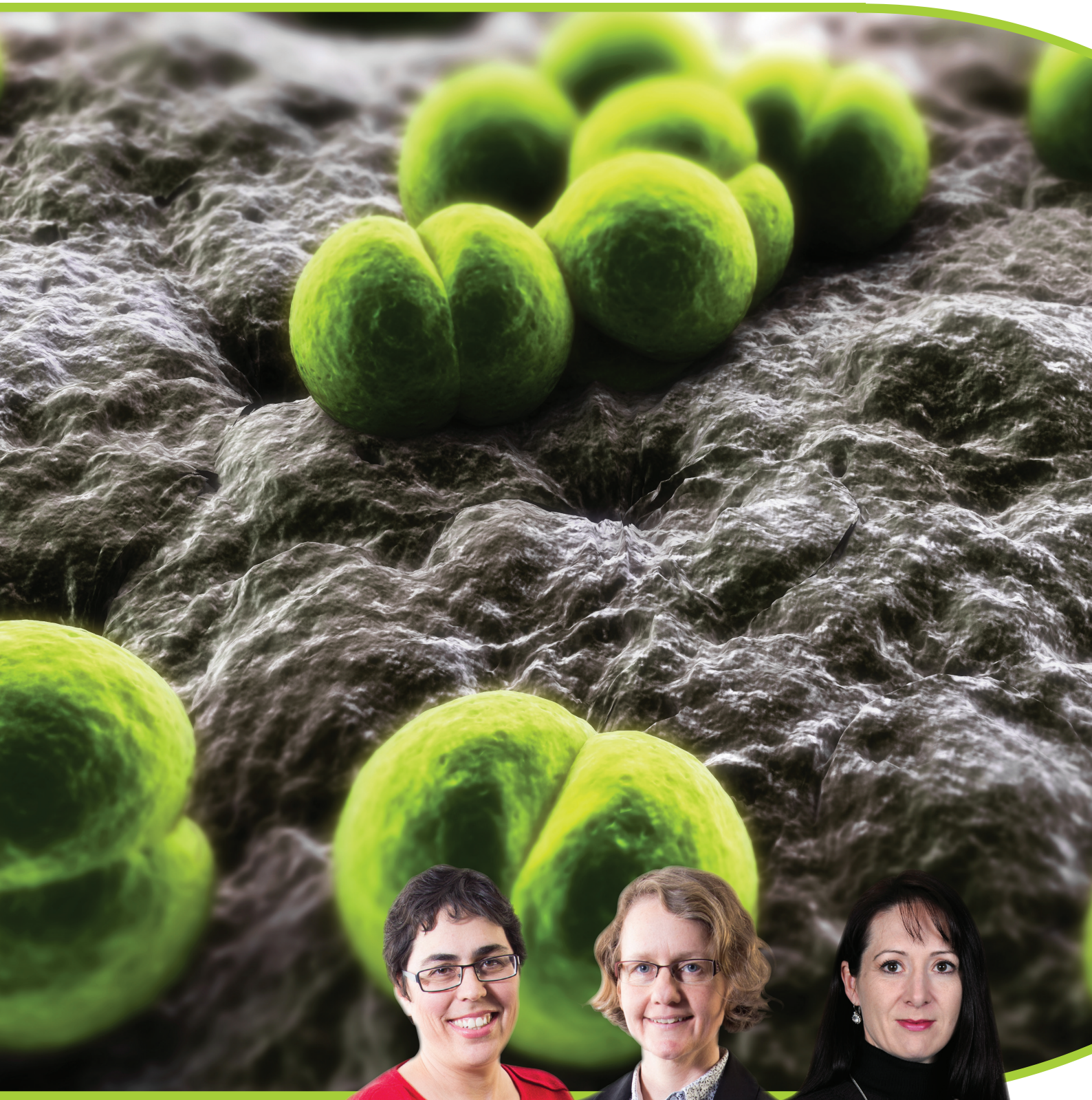
Book Chapter

Stein CM, Parry C, Frean J, Warrell D, Suputtamongkol Y, Griffith K, *et a*l. Multi-system diseases and infections. In: Davidson R, Brent A, Seale A, eds. *Oxford Handbook of Tropical Medicine*, 4th Ed. Oxford: Oxford University Press, 2014.

Conferences

Staff and students of the Centre presented data at 19 international conferences, 13 national conferences and several local conferences.

CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS



Shutterstock: Meningococcus

BACKGROUND

The year under review continued to see the Centre for Respiratory Diseases and Meningitis (CRDM) perform its core functions of syndromic surveillance for pneumonia at sentinel sites within South Africa and laboratory-based surveillance for important bacterial causes of invasive disease and meningitis throughout the country through the GERMS-SA platform. Using these surveillance programmes, the Centre identified key seasonal and epidemiological variations of viral and bacterial pathogens. These data are essential to implement and monitor the impact of ongoing interventions and plan for future interventions to reduce the burden of severe disease and death due to pneumonia and meningitis. The CRDM is expanding its capacity to use new diagnostic technologies such as Taqman array card (TAC, Life Technologies) or Fast-Track Diagnostics (FTD, Junglinster, Luxembourg), that may assist in future surveillance, outbreak investigation or individual patient diagnostics. In addition, the Centre is moving into the era of whole genome sequencing for almost all of its pathogens, hosting the first annual Investigator's Meeting for the Global Pneumococcal Sequencing Project (GPS). It also underwent a formal assessment in 2013 of the capacity and quality management of the laboratory with regard to it's ability to provide polymerase chain reaction (PCR) testing of samples as the WHO/AFRO (World Health Organization Regional Office for Africa) Regional Reference Laboratory for vaccine-preventable invasive bacterial diseases (VP-IBD) for the southern and eastern Africa region. The Centre has been commended for the excellent standard of work and quality measures currently in place. The CRDM continues to function as a WHO National Influenza Centre (NIC) and with a newly established biosafety level three laboratory the CRDM/NIC will continue and expand work to support the diagnosis of known and emerging causes of respiratory diseases and meningitis within South Africa and the region.

From left:
Dr Cheryl Cohen
Dr Anne von Gottberg
Prof Marietjie Venter *(resigned)*
Centre Heads

CURRENT SURVEILLANCE PROGRAMMES

Pneumonia surveillance

The Severe Acute Respiratory Illness (SARI) programme, which initially aimed to describe the contribution of influenza, other respiratory viruses and pneumococcus to the syndrome of SARI, has been expanded to include patients with more chronic respiratory illness and include the collection and testing of specimens for atypical bacterial causes of pneumonia, *Pneumocystis jirovecii, Mycobacterium tuberculosis, Streptococcus pneumoniae, Bordetella pertussis, Haemophilus influenzae* and atypical bacterial causes of pneumonia (*Legionella* species, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) at selected sites. Surveillance for influenza-like illness (ILI) is ongoing in outpatient clinics. As part of this programme, asymptomatic patients are also enrolled, in order to describe the association between organisms identified and disease.

Ongoing influenza surveillance and outbreaks preparedness

In 2013, the majority of influenza A isolates were influenza A(H1N1)pdm09 (41/46) and 50% (18/36) of egg isolations were successful. Haemagglutination inhibition assays characterised 98% (40/41) of A(H1N1)pdm09 isolates with normal reactivity to the A/California/7/2009 reference. Three A(H3N2) isolates reacted with ≥2 fold lower titres to the reference antiserum, A/Perth/16/2009. Influenza B virus isolates showed normal reactivity to B/Wisconsin/1/2010 (Yamagata lineage) antisera. H3N2 haemagglutinin (HA) genes from 2013 are in genetic group 3 and specifically 3C. For 70 A(H1N1)

pdm09 positive samples sequenced 99% (69/70) are in lineage 6. Seven influenza B strains belong to clade two of the B/Yamagata lineage. Influenza A(H1N1)pdm09 positive samples (n=203) tested for the H275Y oseltamivir resistance mutation were wild type. For influenza virus isolates [A(H1N1)pdm09: n=16, A(H3N2): n=1, influenza B: n=7] tested for phenotypic resistance to oseltamivir and zanamivir, 1 A(H1N1)pdm09 was resistant and 1 showed reduced sensitivity.

Assays to test for the MERS-coronavirus by real time reverse transcription PCR (RT-PCR) of the E and orf-1b genes were established. The assay for the E protein gene target (UpE) is considered highly sensitive, and has been implemented at the CRDM. A second confirmatory PCR on the open reading frame 1b (ORF1b) and a pan-coronavirus PCR can run on UpE positive specimens. For A(H7N9) preparedness,the CRDM established the real time RT-PCR assays to test for the H7 and N9 genes as well as other avian H5, H6; H7 and H9 influenza strains. Serum haemagglutination inhibition assays have been established for serological surveillance for human exposure to the following avian influenza virus strains: inactivated A(H5N2), A(H6N2), A(H6N8); A(H7N1), A(H7N7). Establishment of RT-PCR assays for avian influenza A strains enhance our capacity to provide reference laboratory support for avian influenza virus diagnosis and enable the Centre to detect cases of the emerging H7N9 strain from China.

Specific/selected projects

Circulating seasonal and pandemic influenza A and influenza B virus genotypes from 2009-2012: Vaccine strain match and break-through infections in South Africa

NICD investigators: F Treurnicht, J Manamela, D Naidoo, J McAnerney, C Cohen, M Venter

Vaccination against seasonal influenza viruses is important to reduce morbidity and mortality annually in the most vulnerable of the population. The HA1 region of the influenza hemagglutinnin gene was amplified by strain-specific nested RT-PCR followed by direct sequencing of amplicons. Sequence alignment and phylogenetic analysis was performed using BioEdit version 7.1.7 and MEGA 5 programmes. The ATIVS tool was used to predict antigenic distances between A(H3N2)vaccine and seasonal strains where antigenic distances ≥3.25 predict low antigenic reactivity to vaccine strain-derived antisera. The mechanism for vaccine escape needs to be explored further in cases where the antigenic distance between the vaccine and seasonal viruses is <3%. The emergence of the pandemic influenza A/H1N1 strain in 2009 demonstrates vaccine escape as a result of antigenic shift. Antigenic drift over time led to vaccine escape for influenza A(H3N2) strains in 2009, which conincided with introduction of a vaccine strain matched to circulating strains. Vaccine strain mismatch is also predicted for influenza A(H3N2) lineage 7 and 3 A viruses when compared to the 2012 and 2013 Southern Hemisphere vaccines.

Healthcare utilisation surveys in Soweto and Klerksdorp

NICD investigators: C Cohen, C von Mollendorf, S Walaza

Collaborators: CDC: K Wong, AL Cohen, S Tempia; PHRU: N Martinson; University of Witwatersrand: S Norris, E Variava

This project aimed to describe healthcare-seeking behaviours for common infectious syndromes and to identify reasons underlying decisions to seek care. There were 1 442 households in Klerksdorp and 973 households in Soweto interviewed between August and September 2012. Public clinics were most frequently consulted for illnesses (Klerksdorp: 43%; Soweto: 42%), although private-sector facilities also comprised a substantial proportion of consultations. Common barriers to accessing healthcare included perceived deficiencies in medical resources and limited personal resources. In Klerksdorp, seeking care with hospitals, clinics, or doctors was associated with being HIV-infected (OR: 2.8; 95% CI: 1.1–7.2); in Soweto, it was associated with being female (OR: 2.0; 95% CI: 1.2–3.3) and <18 years old (OR: 2.5; 95% CI: 1.4–4.4).

Epidemiology of influenza types and subtypes among patients hospitalised with pneumonia in South Africa, 2009-2012

NICD investigators: C Cohen, M Venter, O Hellferscee, M Pretorius, F Treurnicht, S Walaza, S Madhi, N Wolter, A von Gottberg
Collaborators: SARI surveillance group; CDC: AL Cohen, S Tempia

This analysis compared clinical and epidemiologic characteristics associated with infections due to influenza types and subtypes among children and adults hospitalised with influenza-confirmed severe acute respiratory illness (SARI). Respiratory specimens were tested, typed, and subtyped for influenza virus by polymerase chain reaction assay. 1,239 (8%) of 16,005 SARI patients tested positive for influenza. Case-patients with influenza B were more likely to be HIV-infected (adjusted odds ratio [aOR] 1.4, 95% CI 1.02-1.8) than patients with influenza A. A higher proportion of case-patients infected during the first A(H1N1)pdm09 wave were 5-24 years of age (19%) compared with the second wave (9%). There was no difference in case-fatality or severity between infections with different types and subtypes nor the first and second A(H1N1)pdm09 wave.

Effectiveness and knowledge, attitudes and practises of seasonal influenza vaccine in primary health care settings in South Africa, 2010-2013

NICD investigators: JM McAnerney, S Walaza, A Buys, M Venter, L Blumberg, C Cohen
Collaborators: A L. Cohen, J Duque (United states CDC)

The effectiveness of the influenza vaccine was estimated using a test-negative case-control design amongst individuals with influenza-like illness (ILI) from 2010-2013. Mean annual influenza vaccine coverage among controls was 5% for the four years. Annual VE estimates adjusted for age, underlying medical conditions and seasonality for 2010-2013 were 54% (95% confidence interval (CI): 2% -79%), 57% (95% CI: 16%-78%), 38% (95% CI: -72%-78%), and 87% (95% CI: 67%-95%), respectively. The vaccine was significantly protective in 2010, 2011 and 2013, but not in 2012 when the circulating A(H3N2) strain showed genetic drift. A knowledge, attitudes and practices (KAP) influenza vaccine survey of programme clinicians was conducted in 2013 which showed that >90% of clinicians were familiar with the indications for and the benefits of influenza vaccination. Vaccine coverage was low despite good clinician knowledge of vaccination indications.

Excess mortality associated with influenza among tuberculosis deaths in South Africa, 1999-2009

NICD investigators: S Walaza, A Nanoo, S Madhi, JM McAnerney, C von Mollendorf, J Moyes, C Cohen
Collaborators: S Tempia, AL Cohen

The excess mortality attributable to influenza was modeled by applying Poisson regression models to monthly pulmonary tuberculosis (PTB), extra-pulmonary TB (EPTB) and respiratory non-TB deaths, using national influenza laboratory surveillance data as a covariate. Among individuals of any age the estimated mean annual influenza-associated mortality rates per 100,000 person-years were 164 (n=437) among those with PTB; among those without TB, the rate was 27 (n=1125) for HIV-infected and 5 (n=2367) for HIV-uninfected. No influenza-associated mortality was found among EPTB deaths. Among individuals <65 years of age, those with PTB experienced a greater risk of influenza-associated mortality as compared to HIV-infected individuals with respiratory non-TB disease (relative risk (RR) 5.2; 95% CI: 4.6-5.9) and HIV-uninfected individuals with respiratory non-TB disease (RR: 61.0; CI: 41.4-91.0). Similarly, among individuals ≥65 years of age there was increased influenza-associated mortality in individuals with PTB as compared to HIV-uninfected individuals with respiratory non-TB (RR 13.0; 95% CI: 12.0-14.0). In the ≥65 year age group where the HIV burden is lowest, no influenza-associated mortality was found among HIV-infected respiratory non-TB.

The detection of *Bordetella pertussis* in individuals presenting with severe respiratory illness and influenza-like illness in South Africa, May 2012 to April 2013

NICD investigators: F Moosa, M du Plessis, N Wolter, M Carrim, C Cohen, S Walaza, A von Gottberg
Collaborators: SARI surveillance group

This study determined the prevalence of *Bordetella pertussis* in patients with severe respiratory illness (SRI) and influenza-like illness (ILI) at Edendale and Klerksdorp-Tshepong complexes. Real-time PCR (targeting IS481 and ptxS1) was conducted on induced sputa from SRI patients and nasopharyngeal/oropharyngeal swabs from all patients. The prevalence of pertussis was 0.5% (8/1597), 0.3% (3/1086) and 0.2% (1/452) in SRI, ILI and asymptomatic individuals, respectively. Positive cases were identified in <2 years (0.5%, 2/414), 15-45 years (0.8%, 5/628), >45 years (3%, 2/58) age groups for SRI patients and 2-4 years (0.8%, 1/129) and 15-45 years (3%, 2/58) for ILI patients. Detection rates in induced sputa and nasopharyngeal/oropharyngeal swabs collected from SRI patients were 0.3% (5/1525) and 0.7% (5/685), respectively; with two patients positive for both specimen types. There was no difference in the pertussis positivity rate for SRI cases detected in HIV-positive and -negative patients (4/792, 0.5% vs. 1/570, 0.2%, p=0.4).

Molecular detection of *Mycoplasma pneumoniae* among patients with severe respiratory and influenza-like illness in South Africa, May 2012 – August 2013

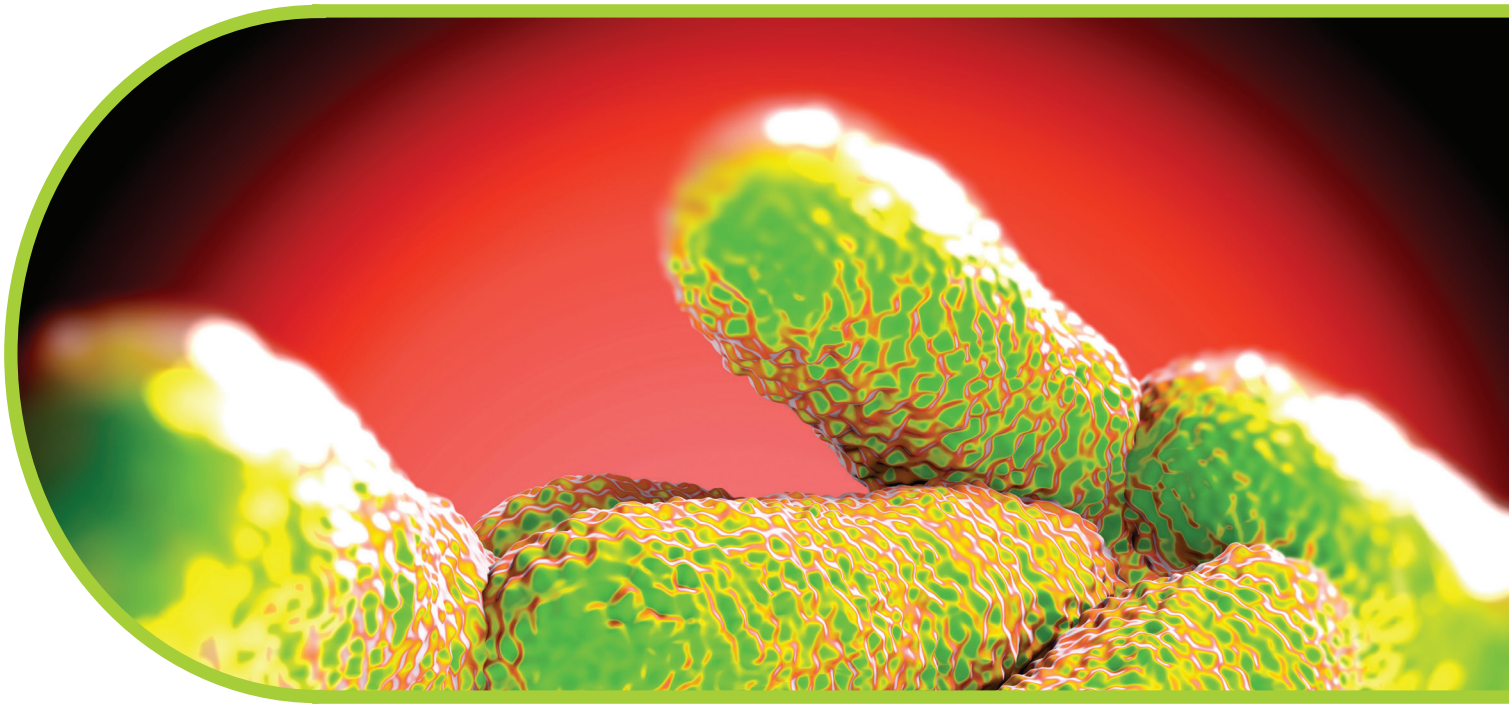
NICD investigators: M Carrim, N Wolter, M du Plessis, L de Gouveia, S Walaza, F Moosa, C Cohen and A von Gottberg
Collaborators: SARI surveillance group

This study describes the prevalence of *M. pneumoniae*, using real-time PCR, in patients with severe respiratory illness (SRI), influenza-like illness (ILI) and asymptomatic individuals. Nasopharyngeal/oropharyngeal specimens were collected from all patients; induced sputum was collected from SRI patients. Macrolide susceptibility testing using high-resolution melt curve analysis (HRM), and MLVA analysis was performed on 30/43 *M. pneumoniae*-positive nasopharyngeal/oropharyngeal specimens. The PCR detection rate for *M. pneumoniae* was 2% (46/2280), 1% (15/1657) and 0.2% (1/525) for SRI, ILI and asymptomatic individuals, respectively. Among the 46 *M. pneumoniae*-positive SRI cases, 24, 16 and 6 were from nasopharyngeal/oropharyngeal specimens, induced sputum and both specimen types, respectively. *M. pneumoniae* was detected in all age groups with 61% (28/46) occurring in children <5 years. Of those tested, MLVA identified three distinct types, and macrolide resistance was not detected.

Bacterial aetiology of severe lower respiratory tract infection (LRTI) in South Africa, 2012-2013

NICD investigators: N. Wolter, M. du Plessis, C. Cohen, S. Walaza, M. Venter, M. Carrim, F. Moosa, A. von Gottberg
Collaborators: H. Dawood, E. Variava

The bacterial aetiology of severe LTRI was determined in patients enrolled at two sentinel sites, using real-time PCR. Whole blood, induced sputum and nasopharyngeal/oropharyngeal swabs/aspirates were tested for *S. pneumoniae/Haemophilus influenzae,* Legionella spp. and *Mycoplasma pneumoniae/Chlamydophila pneumoniae,* respectively. 2291 cases were enrolled: 1102 (48%) were male and 739 (32%) were <5 years old. For cases with known HIV status, 57% (1067/1861) were HIV-positive. 137/1704 (8%) bloods were positive for *S. pneumoniae* with the highest detection rate in the 5-14 year age group (15%; 9/62). 1% (19/1720) of bloods were positive for *H. influenzae,* of which 11% (2/19) were serotype b. Legionella spp. were detected in 2% (13/708) of induced sputa, with 10 cases (77%) occurring in the 15-45 year age group. *M. pneumoniae* and *C. pneumoniae* were detected in 1% (17/1505) and 0.3% (5/1505) of nasopharyngeal/oropharyngeal specimens, respectively. Although *S. pneumoniae* is the most commonly detected bacterial cause of severe LRTI, other bacterial pathogens should be considered in the differential diagnosis.



Shutterstock: Legionella Pneumophila Bacteria

High nasopharyngeal pneumococcal density, increased by viral co-infection, is associated with invasive pneumococcal pneumonia in South Africa

NICD investigators: N Wolter, C Cohen, SA Madhi, M Venter, J Moyes, S Walaza, B Malope Kgokong, M du Plessis, V Magomani, M Pretorius, O Hellferscee, A von Gottberg
Collaborators: S Tempia, M Groome, H Dawood, K Kahn, E Variava, KP Klugman

Factors associated with pneumococcal colonisation (NPC), high colonisation density and invasive pneumococcal pneumonia (IPP) were determined among patients hospitalised with acute lower respiratory-tract infection (ALRI). In 2010, 4025 cases were enrolled in ALRI surveillance. 969 (24%) had nasopharyngeal specimens tested for respiratory viruses and *S. pneumoniae* by real-time PCR. Of these, 749 (77%) had blood tested for *S. pneumoniae.* NPC was detected in 55% (534/969). On multivariable analysis, controlling for age and tuberculosis treatment, influenza infection, adenovirus, rhinovirus and HIV were associated with NPC. High NPC density was associated with respiratory virus co-infection and IPP, controlling for age and gender. 7% (52/749) of cases had pneumococci detected in blood. On multivariable analysis, among NPC cases, IPP was associated with HIV, influenza, high NPC density and ≥5 days hospitalisation. Respiratory virus infection was associated with elevated colonisation density and, in turn, IPP.

Real-time *lytA* PCR cycle threshold values required for successful real-time PCR-based deduction of pneumococcal serotypes

NICD investigators: N Wolter, V Magomani, C Cohen, S Walaza, J Moyes, A von Gottberg
Collaborators: S Tempia, CDC (USA) attaché to NICD-NHLS

Serotyping is key for pneumococcal disease surveillance. The study assessed the *lytA* cycle threshold values (CtV) that could predict real-time PCR-based serotype deduction (targeting 42 serotypes/groups). *LytA* PCR was conducted on blood collected from patients hospitalised for severe acute respiratory illness. Positive samples (CtV ≤40) were PCR serotyped. The proportion of "PCR-serotypable" samples with CtV≤30 was compared to the proportion of serotypable samples with individual CtV from 31 to 40 using multinomial regression. Of 14,311 samples tested, 953 (7%) were *lytA* positive of which 820 (86%) were available for PCR serotyping, and 398 (49%) tested positive for serotype/serogroup . For serotyped samples CtV ranged from 25-40 (median CtV 36). A progressive reduction in the proportion of PCR-serotypable samples was observed for CtV≥34: 61% (37/61; CtV 34) to 9% (4/45; CtV 39). Compared to samples with CtV≤30, the decrease in PCR-serotypable specimens was statistically significant for samples with individual CtV≥35. Low pneumococcal load (*lytA* CtV ≥35) predicts a significant loss in sensitivity of real-time PCR-based serotype deduction.

LABORATORY-BASED SURVEILLANCE FOR BACTERIAL CAUSES OF MENINGITIS AND PNEUMONIA

The CRDM continues to contribute to the evaluation of the impact of both the pneumococcal conjugate vaccines (PCV) and the *Haemophilus influenzae* serotype b conjugate vaccine (Hib CV) through national, laboratory-based, population-based, active surveillance for invasive pneumococcal and Hib disease and case-control and other epidemiologic studies. The CDRM also contributes data on numbers and serogroups of *Neisseria meningitidis* and supports diagnostic testing and outbreak response for suspected cases of *meningococcal meningitis.* During 2013, the CRDM developed more specific plans to integrate surveillance with the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa, (GERMS-SA) programme, specifically syndromic surveillance for pneumonia.

Update on ongoing monitoring of the impact of the polysaccharide-protein conjugate vaccine (PCV) on invasive pneumococcal disease in South Africa

NICD investigators: A von Gottberg, L de Gouveia, V Quan, S Meiring, C von Mollendorf, SA Madhi, C Cohen
Collaborators: S Tempia, ER Zell, J Verani, KL O'Brien, CG Whitney, KP Klugman and The Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) investigators

South Africa introduced the seven-valent pneumococcal protein-polysaccharide conjugate vaccine (PCV-7) in 2009. PCV-7 impact was assessed using active, national disease surveillance data for invasive pneumococcal disease (IPD) (n=35,192 cases). From 2005-2012, the percentage change in IPD incidence, focusing on individuals aged <2 and 25-44 years, between post-vaccine (2011-2012) and pre-vaccine years (average of 2005-2008) was calculated. Rates of PCV-7 IPD among children <2 years declined by 89% in 2012, from 32.1 to 3.4 cases per 100,000 person-years (95% confidence interval [CI], -92% to -86%). Among adults aged 25 to 44 years, vaccine serotype IPD declined by 57% (95% CI, -63% to-50% [3.7/100,000 to 1.6/100,000]). The large reduction in disease caused by vaccine serotypes among both children and adults likely reflects substantial direct and indirect benefits from PCV-7 introduction. The smaller reduction in nonvaccine-serotype IPD suggests HIV prevention efforts are also playing a role in prevention of serious pneumococcal disease.

Epidemiology of invasive pneumococcal disease (IPD) in HIV exposed-uninfected children <1 year of age in South Africa, 2009 to 2013

NICD collaborators: C von Mollendorf , A von Gottberg , S Meiring , L de Gouveia , V Quan , S Lengana, C Cohen
Collaborators: GERMS- SA investigators, S Tempia, KL O'Brien , KP Klugman , C Whitney

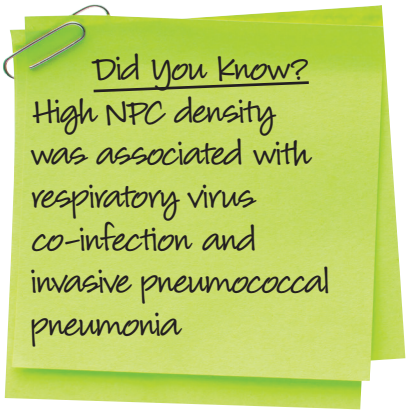
A cohort study in children (<1 year) was conducted using pneumococcal surveillance data from the GERMS-SA programme for 2009 through 2013. There were 2 099 IPD cases in children <1 year of age from 2009 through 2013. Across all five years, the highest incidence of IPD was in HIV-infected children (323/100,000-473/100,000 population) compared with HIV-unexposed uninfected (HUU) children (17/100,000-23/100,000; relative risk 14.3 [95% CI 10.3-19.8]) and HIV-exposed uninfected (HEU) children (42/100,000-60/100,000; relative risk 7.9 [95% CI 5.5-11.3]). HEU children also had a higher incidence of IPD than HUU children (relative risk 1.8 [95% CI 1.4-2.4]). In children <6 months of age, HUU children were at significantly lower risk of dying then HEU children (ARRR=0.54 [95% CI 0.33-0.90]).

Whole genome resolution of selected *Neisseria meningitidis* serogroup W strains, South Africa, 2003-2012

NICD investigators: M du Plessis, S Govindpershad, C Cohen , L de Gouveia, N Wolter and A von Gottberg
Collaborators: MCJ Maiden, KA Jolley, SD Bentley, J Parkhill, KP Klugman

Expansion of the hypervirulennt ST-11 clone caused serogroup W (MenW) to increase in South Africa, replacing serogroup A as the predominant serogroup. Selected MenW isolates, collected through national surveillance, were analysed at the genome level. One MenW isolate per year (N=10) was randomly chosen for analysis. The BIGS database was used to analyse ST-11 isolates using MLST, rMLST and 1,975 defined loci (whole genome MLST, wgMLST). South African ST-11 isolates were compared to endemic and outbreak ST-11 genomes from the UK. 8/10 SA MenW isolates were ST-11, had identical finetyping antigens, and were closely related using genome analysis. Using rMLST, ST-11 isolates responsible for the Southampton outbreak were distinct. SA isolates clustered more closely with the endemic UK isolates. wgMLST analysis further resolved the UK endemic and SA strains into two groups.

HONOURS



1. Prof M Venter was a finalist in the NSTF-BHP Billiton Awards: T W Kambule NRF-NSTF Awards, category: To an Individual for an Outstanding Contribution to SETI through Research and its Outputs over the last 5 to 10 years – sponsored by the National Research Foundation (NRF).
2. Ms M Pretorius won first prize for her oral presentation, category Basic Sciences and second prize in Clinical Sciences at the University of Pretoria Faculty of Health Science Faculty day, University of Pretoria gala dinner and Awards Ceremony 16 November
3. University of Pretoria, Faculty of Health Science Faculty day:
   a. Best Publication by a Young Researcher (<35 years) Clinical: D Zaayman and M Venter, West Nile Virus Neurologic Disease in Humans, South Africa, September 2008-May 2009. Emerging Infectious Diseases.
   b. Best Publication representing the Best Team Effort: C van Eeden, JH Wiliams, TGH Gerdes, E van Wilpe, A Viljoen, R Swanepoel and M Venter. Shuni virus as a cause of Neurologic disease in horses. Emerging Infectious Diseases.

TEACHING AND TRAINING

1. CRDM staff travelled to the Seychelles Public Health Laboratory to conduct an assessment and laboratory training for WHO hospital-based sentinel surveillance for invasive vaccine preventable diseases, 13-16 May 2013.
2. CRDM staff conducted a WHO Site Assessment and lab training visit, Lusaka, Zambia from 9-11 July 2013 (Pediatric Bacterial Meningitis Surveillance).
3. CRDM hosted Mr John Mwaba from Lusaka University Teaching Hospital, Zambia which is a PERCH (Pneumonia Etiology Research for Child Health) study site. Mr Mwaba spent a week (22-26 July 2013) at CRDM learning the Quellung method for the serotyping of pneumococci.
4. CRDM hosted Dr Maaike Alaerts from the Malawi Liverpool Welcome Trust lab in Malawi, from 25-26 July 2013, to train her set up a real-time PCR for pneumococcal detection and serotyping.
5. CRDM staff travelled to Luanda, Angola for a WHO Invasive Bacterial Disease assessment and conducted hands-on lab training from 5-8 August 2013.
6. CRDM travelled to Zambia from 18 – 22 November 2013 on invitation from the WHO to train the virology laboratory at the University Teaching Hospital in Lusaka on Influenza virus isolation techniques.
7. Dr S Lengana attended the Health Economic (Health Policy and Decision Making Analysis) workshop from18-28 June. The workshop was hosted by Centers for Disease Prevention and Control (USA) and the NICD.
8. CRDM hosted the African Influenza and Emerging Respiratory Virus Preparedness Meeting, at the Westin Hotel, Cape Town, on the 4 September 2013.
9. Dr F Treurnicht attended the theoretical session of the Global Initiative on Sharing Influenza Data (GISAID)/ International society for Influenza and other Respiratory viruses (ISIRV)-Anti-Virus Group (AVG) Training Workshop on Sequence analysis and Detection of Antiviral Resistance on 7 September 2013 in Cape Town.
10. Ms L de Gouveia attended a WHO Global NUVI (New and Underutilized Vaccine Implementation) Strategic Review meeting in Geneva, Switzerland from 16 – 19 September 2013.
11. Dr J Moyes participated in the Global Estimates Network (RSV) meeting in Edinburgh from 4-6 November 2013.

Professional development

Number of postgraduate students who graduated: 1 MSc, 2 PhD
Number of postgraduate students currently registered: 1 B Tech, 4 MSc, 1 MMed and 6 PhD
Number of scientist interns currently registered: 3

CRDM staff lecture at the Universities of Witwatersrand and Pretoria and are involved in registrar training, and ongoing postgraduate supervision of students.

RESEARCH OUTPUT

Top five papers

Wolter N, Cohen C, Tempia S, Madhi SA, Venter M, Moyes J, Walaza S, Malope KB, Groome M, du Plessis M, Pretorius M, Dawood H, Kahn K, Variava E, Klugman KP, von Gottberg A. HIV and influenza virus infections are associated with increased blood pneumococcal load: a prospective, hospital-based observational study in South Africa, 2009-2011. *J Infect Dis* 2014;**209**:56-65.

On multivariable analysis, HIV infection (adjusted odds ratio [aOR], 2.4; 95% confidence interval [CI], 1.6-3.6), influenza virus coinfection (aOR, 1.4; 95% CI, 1.2-2.1), oxygen therapy during admission (aOR, 1.6; 95% CI, 1.1-2.3) and in-hospital death (aOR, 2.1; 95% CI, 1.1-4.0) were significantly associated with increased pneumococcal load. Among lytA-positive patients, after adjustment for length of hospitalization, duration of symptoms, and oxygen therapy during admission, pneumococcal loads ≥10,000 DNA copies/mL (aOR, 3.6; 95% CI, 1.8-7.2) were associated with increased risk of death. HIV and influenza virus infections were associated with elevated pneumococcal loads, which, in turn, were associated with increased risk of death.

von Gottberg A, Cohen C, de Gouveia L, Meiring S, Quan V, Whitelaw A, Crowther-Gibson P, Madhi SA, Whitney CG, Klugman KP. Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era: South Africa, 2003-2008. *Vaccine* 2013;**31**:4200-4208.

In 2008, reported incidence of IPD was 6-fold higher in children <1 compared to children 1-4 years of age: 87 per 100,000 population and 14/100,000, respectively. The relative risk of IPD was 21-fold (95% CI, 19-24) and 34-fold (29-41) greater in HIV-infected compared to HIV-uninfected children in the <1 year and 1-4-year-old age groups respectively. On multivariable analysis serotypes 6B (relative risk ratio (RRR) 0.7; confidence interval (CI) 0.5-0.9), 18C (RRR 0.3; CI 0.1-0.5), 1 (RRR 0.2; CI 0.1-0.4) and 8 (RRR 0.2; CI 0.1-0.4) were significantly less common in HIV-infected individuals than serotype 14. All vaccine formulations have the potential to prevent most cases and deaths from IPD in children in South Africa. Vaccines with protection against 19A would be advantageous in South Africa.

Cohen C, Moyes J, Tempia S, Groom M, Walaza S, Pretorius M, Dawood H, Chhagan M, Haffejee S, Variava E, Kahn K, Tshangela A, von Gottberg A, Wolter N, Cohen AL, Kgokong B, Venter M, Madhi SA. Severe Influenza-associated Respiratory Infection in High HIV Prevalence Setting, South Africa, 2009-2011. *Emerg Infect Dis.* 2013 Nov;**19**(11):1766-74.

This paper describes severe influenza in HIV-infected individuals. Influenza-associated acute lower respiratory tract infection incidence was 4-8 times greater for HIV-infected (186-228/100,000) than for HIV-uninfected persons (26-54/100,000). HIV-infected patients were more likely to have pneumococcal co-infection; to be infected with influenza type B compared with type A; to be hospitalized for 2-7 days or >7 days; and to die from their illness. These findings indicate that HIV-infected persons are at greater risk for severe illnesses related to influenza and thus should be prioritized for influenza vaccination.

Moyes J, Cohen C, Pretorius M, Groome M, von Gottberg A, Wolter N, Walaza S, Haffejee S, Chhagan M, Naby F, Cohen AL, Tempia S, Kahn K, Dawood H, Venter M, Madhi SA; South African Severe Acute Respiratory Illness Surveillance Group. Epidemiology of respiratory syncytial virus-associated acute lower respiratory tract infection hospitalizations among HIV-infected and HIV-uninfected South African children, 2010-2011. *J Infect Dis.* 2013 Dec 15;208 Suppl 3:S217-26.

This paper describes the increase risk of hospitalisation with RSV-associated acute lower respiratory tract infection (ALRTI) in HIV infected children, after adjusting for age, HIV-infected children had 3 to 5-fold increased risk of hospitalization with RSV-associated ALRTI (2010, relative risk [RR] 5.6, 95% confidence interval [CI] 4.5-6.4, 2011, RR 3.1 95% CI 2.6 -3.6). In addition HIV infected children admitted with RSV-associated ALRTI had more severe illness, with a higher odds of death (odds ratio [OR] 31.1, 95% CI 5.4-179.8) and hospitalization for >5 days (OR 4.0, 95% CI 1.5-10.6) than HIV-uninfected children.

Venter M, van Vuuren PJ, Mentor J, Paweska J, Williams J: Inactivated West Nile Virus vaccine, Duvaxyn WNV, protects against neuroinvasive lineage 2 WNV strain in mice. *Vaccine* 2013 Aug; **31**(37): 3856-62.

Serum neutralising antibodies in vaccinated mice were detected but low three weeks after primovaccination. Three weeks post-challenge, vaccinated mice had significantly higher serum neutralising antibody titres against both lineages than unvaccinated controls. After challenge, all vaccinated mice remained healthy but all unvaccinated mice demonstrated severe neurological signs with 75% mortality rate. WNV was not detected in brains of vaccinated mice whereas virus replicated in most unvaccinated mice challenged with either lineage. Gross and microscopic lesions were found only in unvaccinated mice challenged with both lineages. Duvaxyn WNV vaccine provided complete protection against challenge with lineage 2 WNV and stimulated significant cross protective neutralising antibodies in mice against lineage 2.

List of publications

Adler D, Laher F, Wallace M, Grzesik K, Jaspan H, Bekker L-G, Gray G, Valley-Omar Z, Allan B and Williamson A-L. High Rate of Multiple Concurrent Human Papillomavirus Infections among HIV-Uninfected South African Adolescents *J Immunol Tech Infect Dis* 2013, **2**:1

Ampofo WK, Al BS, Cox NJ, Giovanni M, Hay A, Huang S, Inglis S, Katz J, Mokhtari-Azad T, Peiris M, Savy V, Sawanpanyalert P, Venter M, Waddell AL, Wickramasinghe G, Zhang W, Ziegler T. Strengthening the influenza vaccine virus selection and development process: outcome of the 2nd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at the Centre International de Conferences (CICG) Geneva, Switzerland, 7 to 9 December 2011. *Vaccine* 2013;**31**: 3209-21.

Cohen C, Moyes J, Tempia S, Grooem M, Walaza S, Pretorius M, Dawood H, Chhagan M, Haffejee S, Variava E, Kahn K, Tshangela A, von Gottberg A, Wolter N, Cohen AL, Kgokong B, Venter M, Madhi SA. Severe influenza-associated respiratory infection in high HIV prevalence setting, South Africa, 2009-2011. *Emerg Infect Dis* 2013;**19**:1766-74.

du Plessis M, Wolter N, Crowther-Gibson P, Hamstra HJ, Schipper K, Moodley C, Cohen C, van de Beek D, van der Ley P, von Gottberg A, van der Ende A. Meningococcal serogroup Y lpxL1 variants from South Africa are associated with clonal complex 23 among young adults. *J Infect* 2014;**68**: 455-61.

Feikin DR, Kagucia EW, Loo JD, Link-Gelles R, Puhan MA, Cherian T, Levine OS, Whitney CG, O'Brien KL, Moore MR. Serotype-specific changes in invasive pneumococcal disease after pneumococcal conjugate vaccine introduction: a pooled analysis of multiple surveillance sites. *PLoS Med* 2013;**10**: e1001517. Anne von Gottberg, Serotype Replacement Group.

Gumede N, Lentsoane O, Burns CC, Pallansch M, de Gourville E., Yogolelo R, Muyembe-Tamfum JJ, Puren A, Schoub BD, Venter M. Emergence of vaccine-derived polioviruses, Democratic Republic of Congo, 2004-2011. *Emerg Infect Dis* 2013;**19**: 1583-89.

Haynes AK, Manangan AP, Iwane MK, Sturm-Ramirez K, Homaira N, Brooks WA, Luby S, Rahman M, Klena JD, Zhang Y, Yu H, Zhan F, Dueger E, Mansour AM, Azazzy N, McCracken JP, Bryan JP, Lopez MR, Burton DC, Bigogo G, Breiman RF, Feikin DR, Njenga K, Montgomery J, Cohen AL, Moyes J, Pretorius M, Cohen C, Venter M, Chittaganpitch M, Thamthitiwat S, Sawatwong P, Baggett HC, Luber G, Gerber SI. Respiratory syncytial virus circulation in seven countries with Global Disease Detection Regional Centers. *J Infect Dis* 2013;208 Suppl 3:S246-S254.

Lwande OW, Lutomiah J, Obanda V, Gakuya F, Mutisya J, Mulwa F, Michuki G, Chepkorir E, Fischer A, Venter M, Sang R. Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. *Vector Borne Zoonotic Dis* 2013;**13**:637-42.

Madhi SA, Kuwanda L, Venter M, Violari A. Prospective cohort study comparing seasonal and H1N1(2009) pandemic influenza virus illnesses in HIV-infected children during 2009. *Pediatr Infect Dis J* 2014;**33**:174-76.

Moyes J, Cohen C, Pretorius M, Groome M, von Gottberg A, Wolter N, Walaza S, Haffejee S, Chhagan M, Naby F, Cohen AL, Tempia S, Kahn K, Dawood H, Venter M, Madhi SA. Epidemiology of respiratory syncytial virus-associated acute lower respiratory tract infection hospitalizations among HIV-infected and HIV-uninfected South African children, 2010-2011. *J Infect Dis* 2013;208 Suppl 3:S217-S226.

Nair H, Simoes EA, Rudan I, Gessner BD, Azziz-Baumgartner E, Zhang JS, Feikin DR, Mackenzie GA, Moisi JC, Roca A, Baggett HC, Zaman SM, Singleton RJ, Lucero MG, Chandran A, Gentile A, Cohen C, Krishnan A, Bhutta ZA, Arguedas A, Clara AW, Andrade AL, Ope M, Ruvinsky RO, Hortal M, McCracken JP, Madhi SA, Bruce N, Qazi SA, Morris SS, El AS, Weber MW, Scott JA, Brooks WA, Breiman RF, Campbell H. Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. *Lancet* 2013; **381**:1380-1390.

Ntshoe GM, McAnerney JM, Archer BN, Smit SB, Harris BN, Tempia S, Mashele M, Singh B, Thomas J, Cengimbo A, Blumberg LH, Puren A, Moyes J, van den Heever J, Schoub BD, Cohen C. Measles outbreak in South Africa: epidemiology of laboratory-confirmed measles cases and assessment of intervention, 2009-2011. *PLoS One* 2013;**8**: e55682.

Nunes MC, Shiri T, Van Niekerk N, Cutland CL, Groome MJ, Koen A, von Gottberg A, de Gouveia L, Klugman KP, Adrian PV, Madhi SA. Acquisition of Streptococcus pneumoniae in pneumococcal conjugate vaccine-naive South African children and their mothers. *Pediatr Infect Dis J* 2013;**32**: e192-e205.

Nzenze SA, Shiri T, Nunes MC, Klugman KP, Kahn K, Twine R, de Gouveia L, von Gottberg A, Madhi SA. Temporal changes in pneumococcal colonization in a rural African community with high HIV prevalence following routine infant pneumococcal immunization. *Pediatr Infect Dis J* 2013;**32**: 1270-1278.

Ping LH, Joseph SB, Anderson JA, Abrahams MR, Salazar-Gonzalez JF, Kincer LP, Treurnicht FK, Arney L, Ojeda S, Zhang M, Keys J, Potter EL, Chu H, Moore P, Salazar MG, Iyer S, Jabara C, Kirchherr J, Mapanje C, Ngandu N, Seoighe C, Hoffman I, Gao F, Tang Y, LaBranche C, Lee B, Saville A, Vermeulen M, Fiscus S, Morris L, Karim SA, Haynes BF, Shaw GM, Korber BT, Hahn BH, Cohen MS, Montefiori D, Williamson C, Swanstrom R. Comparison of viral Env proteins from acute and chronic infections with subtype C human immunodeficiency virus type 1 identifies differences in glycosylation and CCR5 utilization and suggests a new strategy for immunogen design. *J Virol* 2013;**87**: 7218-33.

Pretorius MA, van Niekerk S, Tempia S, Moyes J, Cohen C, Madhi SA, Venter M. Replacement and positive evolution of subtype A and B respiratory syncytial virus G-protein genotypes from 1997-2012 in South Africa. *J Infect Dis* 2013;208 Suppl 3: S227-S237.

Sayer J, Sunderland T, Ghazoul J, Pfund JL, Sheil D, Meijaard E, Venter M, Boedhihartono AK, Day M, Garcia C, van Oosten C., Buck LE. Ten principles for a landscape approach to reconciling agriculture, conservation, and other competing land uses. *Proc Natl Acad Sci U S A* 2013;**110**: 8349-56.

Shiri T, Auranen K, Nunes MC, Adrian PV, Van Niekerk N, de Gouveia L, von Gottberg A, Klugman KP, Madhi SA. Dynamics of pneumococcal transmission in vaccine-naive children and their HIV-infected or HIV-uninfected mothers during the first 2 years of life. *Am J Epidemiol* 2013;**178**: 1629-37.

Valley-Omar Z, Muloiwa R, Hu NC, Eley B, Hsiao NY. Novel respiratory syncytial virus subtype ON1 among children, Cape Town, South Africa, 2012. *Emerg Infect Dis* 2013;**19**: 668-70.

Van Eeden C, Zaayman D, Venter M. A sensitive nested real-time RT-PCR for the detection of Shuni virus. *J Virol Methods* 2014;**195**: 100-105.

Venter M, van Vuren PJ, Mentoor J, Paweska J, Williams J. Inactivated West Nile Virus (WNV) vaccine, Duvaxyn WNV, protects against a highly neuroinvasive lineage 2 WNV strain in mice. *Vaccine* 2013**;31**: 3856-62.

von Gottberg A, Cohen C, de Gouveia L, Meiring S, Quan V, Whitelaw A, Crowther-Gibson P, Madhi SA, Whitney CG, Klugman KP. Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era: South Africa, 2003-2008. *Vaccine* 2013;**31**: 4200-4208.

Wolter N, Cohen C, Tempia S, Madhi SA, Venter M, Moyes J, Walaza S, Malope KB, Groome M, du Plessis M, Pretorius M, Dawood H, Kahn K, Variava E, Klugman KP, von Gottberg A. HIV and influenza virus infections are associated with increased blood pneumococcal load: a prospective, hospital-based observational study in South Africa, 2009-2011. *J Infect Dis* 2014;**209**: 56-65.

Madhi SA, Wals PD, Grijalva CG, Grimwood K, Grossman R, Ishiwada N, Lee PI, Nascimento-Carvalho C, Nohynek H, O'Brien KL, Vergison A, Wolter J. The Burden of Childhood Pneumonia in the Developed World: A Review of the Literature. *Pediatr Infect Dis J;* 2013; 32 (3); e119-e127.

Madzivhandila M, Adrian PV, Cutland CL, Kuwanda L, Madhi SA. Distribution of pilus islands of group B streptococcus associated with maternal colonization and invasive disease in South Africa. *J Med Microbiol.* 2013; **62** (2): 249-53.

Madhi SA, Izu A, Violari A, Cotton MF, Panchia R, Dobbels E, Sewraj P, van Niekerk N, Jean-Philippe P, Adrian PV. Immunogenicity following the first and second doses of 7-valent pneumococcal conjugate vaccine in HIV-infected and -uninfected infants. *Vaccine.* 2013; **31**(5): 777-83.

Kwatra G, Madhi SA, Cutland C, Buchmann E, Adrian PV. Evaluation of Trans-Vag broth, CNA agar and CHROMagar StrepB for the detection of Group B Streptococcus in vaginal and rectal swabs from pregnant women in South Africa. *J Clin Microb.* 2013. **51**(8): 2515-9.

Ditse Z, Adrian PV, Kuwanda L, Madhi SA. Association of Streptococcus pneumoniae common protein antibodies and pneumococcal nasopharyngeal colonization in HIV-infected and HIV-uninfected African children. *Vaccine.* 2013. **31**(40): 4421-7.

Jones SA, Groome M, Koen A, van Niekerk N, Sewraj P, Mabunda X, Kuwanda L, Izu A, Adrian PV, Madhi SA. Immunogenicity of seven-valent pneumococcal conjugate vaccine administered at 6, 14 and 40 weeks of age in South African infants. *PlosOne 2013*. **8**(8): e72794.

Zar HJ, Madhi SA, Aston SJ, Gordon SB. Pneumonia in low and middle income countries: progress and challenges. Thorax. 2013; **68**(11): 1052-6.

Nascimento-Carvalho CM, Madhi SA, O´Brien KL. Review of Guidelines for Evidence-based Management for Childhood Community-acquired Pneumonia from between Developed and Developing Countries. *Pediatr Infect Dis J. 2013*. **32**(11): 1281-2.

Shiri T, Nunes MC, Adrian PV, Van Niekerk N, Klugman KP, Madhi SA. Interrelationship of Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus colonization within and between pneumococcal-vaccine naive mother-child dyads. *BMC Infect Dis*. 2013; **13**(1): 483.

Kayhty H, Auranen K, Dagan R, Goldblatt D, O'Brien K, Scott A, Antonio M, Lucero M, Madhi SA, Klugman KP, Mulholland K. Case for carriage. *Vaccine* 2013; **32**(1): 180-203.

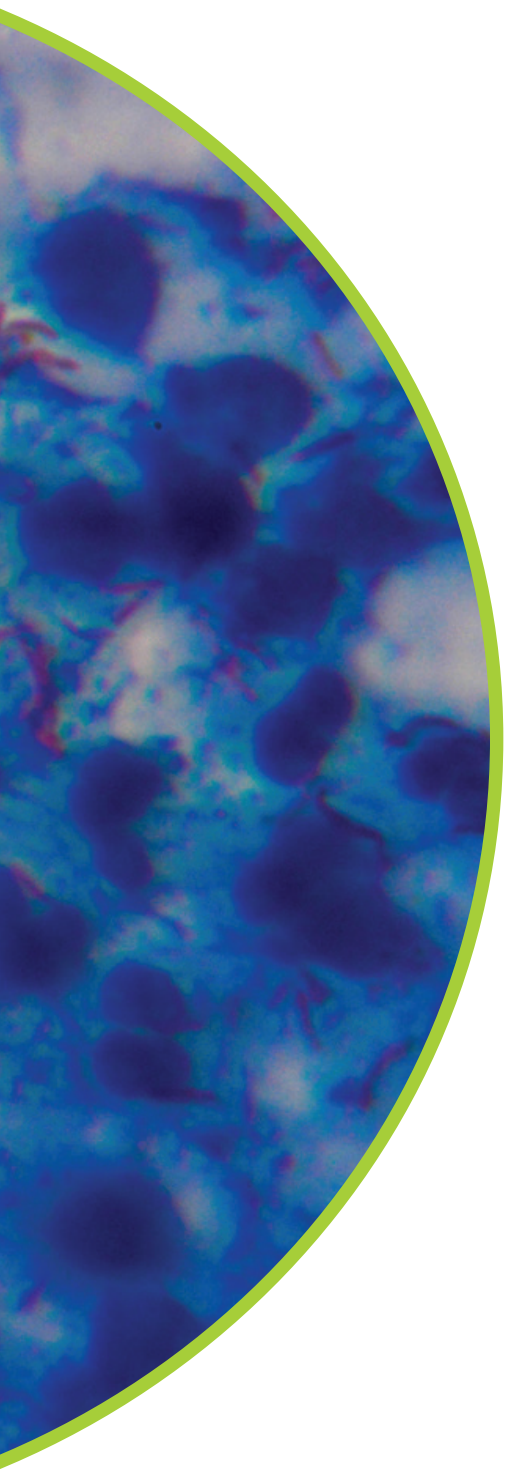
Number of conference presentations:

51 international conference presentations
4 national conference presentations
6 local conference/congress presentations

CENTRE FOR TUBERCULOSIS

Shutterstock: AFP stain show Microbacterium tuberculosis (TB)

Dr Nazir Ismail
Centre Head



BACKGROUND

In line with the national mandate of the National Institute for Communicable Diseases (NICD), the Centre for Tuberculosis (CTB) serves as a National TB Reference Laboratory (NTBRL), participates in epidemiology-oriented training programmes and conducts ongoing laboratory-based public health surveillance of TB in South Africa, utilising modern molecular technology for TB strain characterisation. The CTB also initiates applied research related to the National TB Control Programme (NTBCP). It furthermore advises and works closely with the Department of Health (DoH) on strategic planning and assists with policy and guideline formulation concerning the diagnosis and treatment of TB. The CTB is actively involved as a core function in monitoring trends in disease prevalence and TB drug resistance for ongoing evaluation of the impact of TB control measures instituted by the DoH, as well as early detection of, and integrated response to outbreaks. The Centre continued to utilise surveillance and microbiological data for the design and implementation of epidemiological research geared to advise and guide the national response to the TB epidemic. In support of these activities, the CTB plays a leading role in the evaluation and standardisation of new diagnostic methods for the country and assists with the development and implementation of an integrated surveillance system to serve as a platform for epidemiological planning and response.

An important function of the CTB is to provide the DoH prospective data on the frequency of resistance to anti-TB drugs in Mycobacterium tuberculosis isolates in South Africa. For this purpose, the Centre provides information extracted from the Corporate Data Warehouse (CDW) of the NHLS for weekly notification of new rifampicin-resistant (RR), multidrug-resistant (MDR), and extensively drug-resistant (XDR) TB cases identified by laboratories of the National Health Laboratory Service (NHLS) to provincial and district coordinators of the NTBCP. Such surveillance data are available for ongoing assessment of the impact of TB control activities of the DoH, as well as to inform TB control agencies of other Southern African Development Community (SADC) countries.

SURVEILLANCE /DIAGNOSTIC SERVICES

Integrated public health surveillance and reference laboratory services

The CTB supports the NTBCP by conducting national surveillance of new cases of laboratory-confirmed TB, as well as new drug-resistant, including RR, MDR and XDR TB cases identified by NHLS laboratories. Information on drug resistance prevalence will advise on the performance of the NTBCP in pursuit of the Millennium Development Goals and provide guidance on directing human and financial resources to areas of need targeted for action.

A complementary function of the CTB is to serve as a NTBRL where new molecular techniques are applied for *M. tuberculosis* strain characterisation in terms of drug resistance profiles, restriction fragment length polymorphism (RFLP) types, spoligotypes and mycobacterial interspersed repetitive unit (MIRU) types. The CTB also houses a repository where *M. tuberculosis* strains, including molecularly characterised genotypes and phenotypes, are catalogued and stored.

TB surveillance based on data from corporate data and electronic TB registries

The CDW has been storing TB diagnostic and treatment monitoring data collected routinely from eight provinces from 2004, and in addition from KwaZulu-Natal since 2011. In order to transform laboratory-based data to patient-based information, an automated system with electronic matching and allocation of unique patient identifiers was developed. Applying a "probabilistic matching" approach, the process of data matching was further improved. As the same laboratory tests are used for both diagnostic and treatment monitoring purposes, a specimen-level information system was transformed into a patient-level system while provision was also made to identify recurrent episodes over time in the same patient, reflecting re-infection or reactivation episodes. (Differentiation between the latter could be facilitated by DNA fingerprinting/genotyping, using whole genome sequencing and other molecular typing approaches - See molecular surveillance of TB) on page 57.

The historical analysis of TB trends in South Africa between 2004 and 2012, using laboratory-based information, has been completed. This analysis has assessed changes at national and provincial levels and has been compared with the HIV prevalence and anti-retroviral (ARV) roll-out over the same period. The incidence rates for several provinces are above a 1000 per 100 000 population, which makes these regions among the highest incidence areas globally. Fifty-five percent of cases were males with an average age of 38 years, while among females the average age was 33 years. Thus the pattern is similar to that of the HIV epidemic, with the economically active age group most affected. A positive development is a decline nationally in TB incidence since 2008, a trend closely associated with the expansion of the ARV roll-out.

CDW-based surveillance, linked to district and municipal access points, provides feedback information on the occurrence and nature of TB episodes, as well as laboratory requisition patterns (penetration rates). Such CDW-derived surveillance also provides good, timely and accurate information for the NTBCP, as well as to provincial health departments on RR-TB, MDR-TB and XDR-TB (all three defined on laboratory criteria) and facilitates planning and management of drug-resistant TB in the country. Clinical data, including clinical profiles, treatment histories and treatment outcomes, collected via a TB clinic-based electronic register are being linked to the CDW data set. Such linking of clinical and laboratory data bases affords users a rich historical resource of TB in the country, and, analysed on a quarterly basis, can be correlated with data related to mortality, HIV prevalence and ARV treatment uptake. This will enable conclusions to be drawn on the evolution of the TB epidemic in the context of other relevant public health trends, including those demonstrated by monitoring data related to TB drug resistance. Drug resistance monitoring is being enhanced by matching laboratory generated drug resistance data with those of the Electronic Drug-Resistant TB Register (EDR), introduced to develop an interface that could, on a daily basis, transfer data relating to MDR- TB and XDR-TB patients into the EDRWeb of the national DoH. Integrating these data sets facilitates the identification of patients with laboratory confirmed drug-resistant isolates who fail to present for treatment. Thus, integrating these systems will present a comprehensive picture of the burden of disease, while the monitoring of linkage between diagnosis and treatment which is an essential element for reducing this burden, will further enhance patient management.

Survey of drug resistance in TB in South Africa

As part of the World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUTLD) Global Tuberculosis Surveillance Project, a country-wide drug resistance survey (DRS) was started by the national DoH and CTB in June 2012. This major survey is designed to be nationally representative and is geared to determine the prevalence and trend of drug-resistant TB in the nine provinces, as well as describe on a population basis the types of MDR *M. tuberculosis*-complex strains circulating in the country and HIV prevalence in this group. A population-proportionate cluster sampling strategy was used to determine the sample size and the survey sites and to ensure that the survey population is representative of TB in each of the nine provinces of the country.

This is survey is one of the largest of its kind globally, and patients are recruited from over 400 facilities over a year period. The unique design of this survey is the recruitment of suspects rather than cases allowing assessment of the drug resistance burden in both smear-positive and smear-negative patients – an important issue for high HIV/AIDS settings like South Africa. An additional difference is the testing of all culture confirmed cases for a full range of drugs including second-line agents. This is important as the data will be essential to inform the applicability of new regimens that combine the new agents with existing agents and the levels of resistance to the latter is a critical factor that will influence the success or failure of these new interventions.

The survey is nearing completion with five of the nine provinces having ended their recruitment phase by early April 2014 and the last enrolments likely to be completed at the end of May 2014. The final report of the survey is expected to be completed at the end of 2014. The survey will record rates of drug resistance for each province with additional information on risk factors associated with drug-resistant TB. It has been over 10 years since the last survey was conducted and important changes have occurred since then, including several outbreaks of TB, greater awareness of XDR TB and the emergence of even more resistant types. The data will certainly be important for future policy making in the control of drug-resistant TB for South Africa.

Prospective sentinel surveillance of rifampicin-resistant TB and TB/HIV integration in South Africa: GERMS-SA

The implementation of the Xpert MTB/RIF rapid TB diagnostic test as the initial diagnostic test for people with presumed TB is now complete. With the realisation that rifampicin (RIF) resistance is not an absolute surrogate marker for MDR-TB and may vary between provinces, it was decided to study the clinical and epidemiological features of RIF monoresistance as an entity, as distinct from RIF resistance as part of MDR-TB in South Africa.

Enhanced surveillance of RIF-resistant TB has been introduced at sentinel sites of the Group for Enteric Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA) in order to deliver accurate, timely and detailed information on risk factors such as previous treatment, close contacts, socio-economic and occupational factors, as well as HIV status and hospital admission data. Changes in the frequency, clonality and geographic distribution of RIF-resistant *M. tuberculosis* strains over time are of key interest to this surveillance activity. Enhanced surveillance of RIF resistance was initiated at Chris Hani Baragwanath Hospital, Gauteng, in October 2012 and has now been introduced into five other provinces with the remainder to be completed by the end of 2014.

A new sentinel surveillance project using the GERMS platform is being established in 2014 which will address an important gap that exists between the TB and HIV programmes that often run in parallel rather than together. This surveillance system will enable the country to monitor trends of patients with TB only, HIV only and TB with HIV and also assess changes in risk factors. Additionally, an important area that will be addressed is the prevalence of primary drug resistance for both HIV and TB. This is very important as empiric regimens for both diseases are currently used and the need for surveillance to monitor and feedback information on primary drug resistance is an essential requirement for policy formulation in the country. This project is expected to begin in the second half of 2014.

TB as a cause of severe acute respiratory infections: SARI project

Prospective, hospital-based sentinel surveillance for severe acute respiratory illness (SARI) was initiated in South Africa in 2009 and TB, as a cause of SARI, was included in this programme in 2012. The incorporation of TB into the SARI programme was phased in, starting at Chris Hani Baragwanath Hospital (Gauteng), Edendale Hospital (KwaZulu-Natal), Mapulaneng and Matikwana Hospitals (Mpumalanga), and the Klerksdorp/Tsepong Hospital complex (North West Province). Together with oropharyngeal and nasopharyngeal specimens for general microbiological (virological, bacteriological and yeast) investigations, sputum samples were collected for TB culture and drug susceptibility testing, as well as for PCR-based molecular testing for rapid diagnosis. In addition, clinical and epidemiological information was collected relating to the onset and progression of symptoms, age group, HIV status and immunisation history as well as socio-demographic information through interview, hospital record, and the administration of a questionnaire. This surveillance system adds important information on late presentation TB disease and profiles the severe cases of TB with increased mortality, constituting a target of importance for policy decisions,

Molecular surveillance of TB and Geospatial Scanning

Strain typing for drug-resistant TB is now established at the Centre and is being integrated into its surveillance systems. Expertise in strain typing techniques, including spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) as well as IS6110 restriction fragment length polymorphism (RFLP) typing, is now available at the centre. Additionally, an automated system for IS 6110 RFLP typing has been acquired and recent validations have shown excellent concordance with the existing methods. This is an important improvement on the laborious conventional MIRU-VNTR gold standard typing method which is laborious and time consuming, and South Africa is now one among only a handful of countries with this sophisticated automated at its disposal.

The typing techniques have different discriminatory ability and are used in epidemiological studies involving surveillance and outbreak investigations. However, due to the high burden of disease and the endemicity of strain types in South Africa, the most appropriate tool for a specific setting is being evaluated. RFLP typing is performed by enzymatic restriction of the *M. tuberculosis* genome into fragments of different lengths, resulting in a distinctive pattern for each strain. RFLP typing has excellent discriminatory power, especially if a sufficient number insertion sites are present in the typed strains, and is regarded as the "gold standard" of *M. tuberculosis* strain typing. Spoligotyping is a PCR-based method to type *M. tuberculosis* complex bacteria and the underlying principle is DNA polymorphism present at the Direct Repeat (DR) chromosomal locus, uniquely present in *M. tuberculosis* complex bacteria. The level of differentiation by spoligotyping is less compared to IS6110 fingerprinting for strains having five or more IS6110 copies but higher for strains with less than five copies. It is useful for the identification of *M. tuberculosis* strains endemic in regions and the study of lineages involved in the evolution of the organism. MIRU typing is also PCR-based and targets mycobacterial interspersed repetitive units located in minisatellite-like regions, similar to those found in the human chromosome, and these regions comprise a variable number of tandemly repeated sequences (VNTR) in the M. tuberculosis genome. Primers specific for the flanking regions of these genetic elements are used for PCR amplification. MIRU typing has good discriminatory power and is often used in conjunction with spoligotyping.

TB genotyping, when combined with epidemiologic data, helps to confirm epidemiologic links and detect outbreaks and unsuspected transmissions, and serves as a valuable tool for monitoring progress toward the elimination of TB transmission. The objectives of this project include the description of the genetic diversity and identification of genotypic clusters of MDR-TB in South Africa; assessment of the occurrence of common patterns of virulence and/or drug resistance associated with genotypic clusters of MDR-TB in order to refine MDR-TB treatment strategies; assessment of epidemiological and demographical factors associated with genotypic clusters of MDR-TB in order to refine MDR-TB control strategies and, finally, the description of the molecular epidemiology of TB in South Africa and its monitoring. Such information could guide the implementation of vaccination programmes in future.

It is clear that the drug-resistant TB epidemic in South Africa is not evenly distributed and the identification of hot spots is an important requirement for the effective implementation of control strategies in the country. In the past year the capacity to utilise geographical information systems (GIS) for surveillance has been expanded at the centre and this combined with the available molecular epidemiology data will allow early detection of hot spots of ongoing transmission, as well as determine the emergence of new clones of drug resistance and their temporal and geospatial evolution. Such information will allow for interventions to be planned and effectively applied. The surveillance project will initially focus on 11 high burden districts in South Africa.

Introduction and evaluation of DNA sequencing platforms

Sequencing to detect mutations is rapidly emerging as the method of choice for resistance determination and the introduction of several next-generation sequencing platforms is rapidly changing the sequencing landscape. The CTB has identified the value and importance of this technology as an essential tool required by TB reference laboratories for strain characterisation as well as its utility in surveillance and outbreak investigations. The overall cost of this technology has been decreasing steadily and the performance of whole genome sequencing has become more practical and cost effective compared with targeted sequencing. Furthermore, several recent publications have highlighted the role of compensatory mutations and mutations outside the known regions of resistance encoding as being important for the expression of drug resistance. The UK has already embarked on ambitious plans for whole genome sequencing (WGS) of *M. tuberculosis* strains and CTB is a major partner working with colleagues at the Oxford University and Cambridge University as part of ongoing collaboration with Public Health England. The knowledge and experiences shared will be mutually beneficial and will allow CTB to develop the sequencing capacity in South Africa

The CTB has acquired a next- generation sequencer for WGS as a part of a surveillance project aimed at understanding resistance to pyrazanimide and fluoroquinolones as two key drugs planned for use with the new drug regimens. The MiSeq instrument (Illumina) has a high throughput per run with a low error rate. It is user friendly and has a low hands-on time because template amplification is carried out directly on the instrument without manual intervention. It is able to sequence fragments from both ends (paired-end mode) without changes to the library preparation stage or additional intervention during sequencing.

A workflow for performing WGS for TB at NICD is now established and the system optimised. Most of the processing is performed on the instrument and is automated. Additionally, the bioinformatics for extracting the resistance information from the WGS is being automated through a dashboard. These developments are important steps in establishing South Africa as a forerunner in the use of this first-world technology in the control of TB..

The instrument was placed at the Centre in 2013 and thus by early April 2014 over 600 whole genomes have been sequenced as part of a WHO project. This project involves sequencing of strains from two provinces (Gauteng and KwaZulu-Natal) as part of the current drug resistance survey. The information is being analysed and will inform future screening technologies for drug resistance in South Africa and globally. The South African WGS data will also be compared with sequence data from four other countries: Pakistan, Azerbaijan, Bangladesh and Belarus. Different sequencing approaches are being used with some using Sanger sequencing, others performing WGS (South Africa is presently the only country performing WGS). The project is expected to be completed by the end of the fourth quarter of 2014.

The project has been valuable in developing WGS capacity at the CTB. It is likely that WGS will replace earlier sequencing technology in the medium term.

Determination of minimum inhibitory concentrations (MICs) of anti-TB drugs

The CTB introduced in 2013 minimum inhibitory concentration (MIC) determination of *M. tuberculosis* strains, a technique not generally available in TB laboratories. MIC testing will allow the laboratory to differentiate between strains with mutations associated respectively with high or low levels of resistance to anti-TB drugs. This is an important development as new data are emerging on the need to re-evaluate standard drug concentrations based on the PK/PD modelling. Thus the ability to determine MICs and also to have data on MICs of circulating strains will be important to determine if current treatment regimens for TB are optimal for both HIV-infected and HIV-negative patients.

As another project, the CTB started the evaluation and comparison of liquid medium-based MIC methods, including the VersaTREK Culture System (TREK Diagnostics)and an adapted MGIT 960 system (Becton Dickinson)-based method, with a solid medium-based MIC agar proportion method. Early findings have shown good overall performance: the methods are easy to perform, rendering them suitable for operational settings. The new VersaTrek-based MIC testing method is also being applied to drug-resistant strains identified in the ongoing DRS and will provide an important baseline for future trend analysis. Additionally, drug susceptibility testing for rifabutin susceptibility is also available and initial findings indicate that a small proportion of RIF-resistant strains exhibit low rifabutin MIC values. This finding will be further investigated.

National TB Repository

A national TB repository is being established at the CTB as a national resource for the surveillance of TB in South Africa. This will be a unique resource that will house a variety of data from different sources of information on epidemiology, microbiology and geospatial distribution of TB in South Africa. It will, in addition, incorporate a national mycobacterial culture collection of isolates stored in the repository, which will serve as an important source of culture material and information related to TB surveillance. It will also facilitate and encourage research and development, and provide reference material for monitoring the evolution of unusual cases of TB and TB strains detected through the network of laboratories in South Africa.

A schematic illustration of the structure of the data repository is shown in the figure below. The surveillance data generated by the CTB will available to be accessed through the portal. Progress is being made with the establishment of this system. In addition, isolates are stored and archived using specialised software systems after following quality assurance processes to ensure the integrity of this national asset.

An important element of the repository is a dashboard which will allow registered users to access different levels of the data dependent on the permissions given on the tier access structure. The dashboard will allow users to access information on the epidemiology of TB down to district level, assess trends over time and have the information displayed in chart or map format.

The implementation of the system and access will done in phases with general TB epidemiology data being made available during the first phase.

New and Emerging diagnostics

The CTB continues to evaluate new technologies for diagnosis and treatment monitoring of TB, as well as for surveillance studies. Some of these exciting molecular-based methods evaluated by the CTB produce rapid results and promise to revolutionise management of drug-susceptible and especially drug-resistant TB.

Several evaluation studies are underway, assessing the performance of competitor line probe assays for the diagnosis of RIF- and isoniazid-resistant TB. Additionally, evaluation of an assay for the molecular diagnosis of pyrazanimide resistance is scheduled to start in May 2014. A "collection to detection" system has been evaluated for the diagnosis of TB compared with Xpert MTB/RIF at laboratories and points of care and this data should be available soon. In addition the use of specialised "kits" for next- generation sequencing, using a targeted approach, are being assessed to detect several drug resistance genetic targets. A similar approach is being investigated using a "digital" PCR format.

A major need for the future of TB control is diagnostics that can detect resistance to agents other than RIF and this is a key focus of the CTB. In addition, evaluation of alternative screening algorithms and diagnostic/sampling approaches are also being considered.

Evaluation of the TBDx automated computer-aided smear microscopy system of for diagnosis and use as a cost-effective screening tool

NICD Researchers: N Ismail, S Omar, A Dreyer
Collaborators: J Lewis, D Dowdy, H van der Meulen, D Clark, G Churchyard

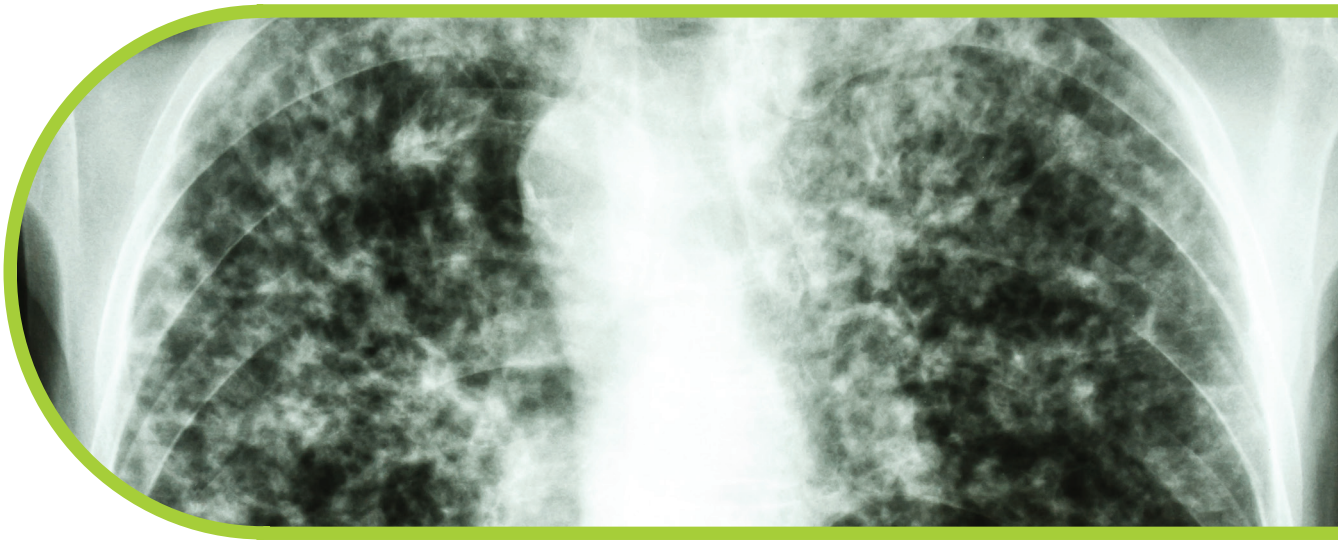
This study is a joint venture of the Aurum Institute for Health Research, Guardian Technologies International, USA and the National Tuberculosis Reference Laboratory/CTB to develop and evaluate the performance of an automated computer-aided smear microscopy system involving digital image scanning of *M. tuberculosis* cells in sputum smears stained with the fluorescent Auramine O stain. The evaluation of the new improved system showed excellent performance as a stand-alone system for TB microscopy which matched a microscopy gold standard of a combined 80 years of experience. A second important finding was the utility of the system to be used as a screening tool to Xpert MTB/RIF. The algorithm incorporating both the TBDx and Xpert MTB/RIF shows that this approach achieved a sensitivity of 80% and a specificity of 99%, whilst reducing the overall use of Xpert MTB/RIF by 75%.

Host and pathogen contributions in the emergence of extensively drug-resistant tuberculosis: Cross-sectional and prospective observational studies

NICD Researchers: N Ismail, L Erasmus
Collaborators: G Kaplan, D Fallows, B Kreiswirth, C Gray, K Klipstein-Grobusch
As part of the first of a two-phase cross-sectional investigation on pathogen-related factors playing a role in the MDR/XDR-TB epidemic in Gauteng, more than 300 *M. tuberculosis* drug-resistant isolates from Gauteng province have undergone molecular typing and drug resistance profiling. The initial results showed important clonal differences of the MDR epidemic in Gauteng, compared with published data from other provinces, and indicated clonality in a sizable proportion of strains exhibiting drug resistance. The second phase of the cross-sectional study, three years after the first, is underway. This study will provide information on drug-resistance-related mutations in *M. tuberculosis* isolates in Gauteng and indicate changes in mutation profiles of isolates over a three-year period.

In a related longitudinal study, cultures from sputum specimens from 200 newly admitted MDR/XDR-TB patients at Sizwe Hospital (100 HIV-positive and 100 HIV-negative) were collected at monthly intervals for the investigation of the acquisition of drug-resistance-determining mutations over time. Genes encoding resistance to isoniazid (katG and inhA), rifampicicn (rpoB, core region), quinolones (gyrA and gyrB), amikacin, kanamycin and capreomycin (rrs and tlyA), streptomycin (rpsL), pyrazinamide (pncA) and ethambutol (embB) are being studied and sequenced when relevant, including WGS on a selective basis. Clonality similar to that observed in the cross sectional study was encountered but evidence of amplification of drug resistance during therapy was also observed.

Additional components of this study conducted by collaborating researchers include immunological profiling of cytokines and other biomarkers, as well as, measurements relating to changing nutritional status before and during treatment. Early findings have identified a putative immunological marker predictive of culture conversion to resistance. These are early but important findings that are still under investigation.



Shutterstock: film chest x-ray show interstitial infiltrate both lung due to Mycobacterium tuberculosis infection (Pulmonary Tuberculosis)

Novel methods for diagnosis of paediatric tuberculosis

NICD Researchers: N Ismail, S Omar
Collaborators: A van Rie, C Hanarahan

Confirmation of the diagnosis of paediatric TB remains a challenge, due to paucibacillary specimens collected for the diagnosis of pulmonary TB in children, and the difficulty for young children to produce sputum on demand. It is widely acknowledged that there is an urgent need for novel approaches to confirm TB diagnosis in children, especially young children with intrathoracic disease, the most frequent type of childhood TB. While it is important for studies of novel diagnostics to be conducted in a wide range of settings, none have investigated the performance of novel diagnostics for paediatric TB at lower level hospitals or community clinics, settings where the vast majority of children first present for care. This cohort study evaluates innovative approaches for the diagnosis of TB in young children presenting at a primary care facility, as well as the utility of using alternative samples for TB diagnosis in these children. It furthermore examines the feasibility of contact tracing in this setting.

HIV Incidence Provincial Surveillance System (HIPSS): A longitudinal sub -study to monitor TB prevalence and incidence trends in the uMgungundlovu District, KwaZulu-Natal, South Africa

NICD Researchers: N Ismail, A Dreyer
Collaborators: Ayesha Kharsany, Cherie Cawood, Carlos Toledo, Gavin George, Kaymarlin Govender, Alex Welte, et al.

Despite improvements in HIV related morbidity and mortality, the rate of new HIV infections remain unacceptably high. In response to the provincial and national priorities to better monitor HIV incidence in high prevalence areas, the HIV Incidence Provincial Surveillance System (HIPSS) is being established in two sub-districts in the province of KwaZulu-Natal. The cohort is a household-based representative sample of men and women in sub-districts of Vulindlela and the Greater Edendale in the uMgungundlovu municipality of KwaZulu-Natal, South Africa. For the sub-study, the prevalence and incidence of TB, sexually transmitted infections (STIs) and hepatitis B and hepatitis C infection will be measured at baseline and at follow-up. This data will be important in determining the rate of new infections and will inform planning of programmatic interventions.

Xpert for people attending HIV/AIDS care: Test or review?

NICD Researchers: N Ismail, L Erasmus
Collaborators: V Chilota, Y Hanifa, H van der Meulen, G Churchyard

This study aims to evaluate strategies for prioritisation of cases with increased likelihood of mortality or transmission, and to assess the impact of this stratified algorithm on the NTBCP. Patients stratified as a high priority based on risk factor criteria are assigned for rapid Xpert MTB/RIF assay testing, as opposed to those assigned a lower priority. All patients undergo follow-up and monitoring to determine the optimal pathway to diagnosis in the context of Xpert MTB/RIF testing. The study is progressing well and is nearing the final stages of enrolment.

Transmission of HIV-associated XDR-TB in South Africa (TRAX Study)
NICD Researchers: N Ismail, BT Magazi, SV Omar
NICD Researchers: N Ismail, L. Erasmus
Collaborators: S Shah, R Rustomjee, B Kreiswirth

Following the disastrous Tugela Ferry outbreak of XDR-TB in KwaZulu-Natal in 2006, the present study was designed to investigate prospectively transmission of XDR-TB in that province. The study aims to determine the proportion of new XDR-TB cases with primary drug resistance, identify risk factors associated with such transmission through epidemiological and social network analysis and, using molecular genotyping, to demonstrate transmission patterns involving persons and locations associated with XDR-TB transmission.

For this study, the CTB collaborates with Inkosi Albert Luthuli Hospital Laboratory, which is responsible for all DST testing in KwaZulu-Natal and refer all laboratory-based XDR-TB isolates from KwaZulu-Natal for confirmatory testing and molecular characterisation. Additional information on risk factors is collected and transmission to close contacts is investigated. Strong emphasis is placed on understanding the transmission dynamics among XDR cases in this high-burden TB and HIV province.

The study is progressing well and an additional component has been added using WGS to elucidate transmission dynamics under higher resolution conditions, as well as the micro-evolutionary pathway of MDR-/XDR-TB.

Teaching and training

Training accommodating experiential as well as didactic learning has been offered to technical laboratory staff from two countries. This has been important in developing capacity in the respective countries. In addition, CTB has participated in developing a train-the-trainer course for SADC countries which will be implemented through the SADC secretariat. Additional training covering both reference mycobacteriology testing and public health aspects of TB is provided to rotating registrars from university-based medical microbiology departments in South Africa, as well as to intern scientists in the country. In addition CTB mentored a FELTP student, further expanding capacity in epidemiology in South Africa.

Professional development

Postgraduate candidates

Numbers of candidates enrolled: 2 PhD, 2 MMed, 3 MSc, 1 BSc [Hons].
Numbers of graduates graduated: 0 PhD, 1 MMed, # MSc, # BSc [Hons].

Honours

Maanda Mudau – Novartis Vaccine Award for Epidemiology of Infectious Diseases
Shingai Machingaizaide – PhD Scholarship from the Centre for International Health, Ludwig Maximillians University, Germany

Research output

Scientific Publications

Rodwell TC, Valafar F, Douglas J, Qian L, Garfein RS, Chawla A, Torres J, Zadorozhny V, Soo Kim M, Hoshide M, Catanzaro D, Jackson L, Lin G, Desmond E, Rodrigues C, Eisenach K, Victor TC, Ismail N, Crudu V, Gle MT, Catanzaro A. Predicting extensively drug-resistant tuberculosis (XDR-TB) phenotypes with genetic mutations. *J Clin Microbiol.* 2014; **52**(3): 781-9.

Ismail N, Koornhof H. Tuberculosis continues to be a public health problem in South Africa from diagnosis to treatment. *Southern Afr J Epidemiol Infect 2013*; **28**(4): 191

Daum LT, Fourie PB, Bhattacharyya S, Ismail NA, Gradus S, Nontuthuko E. Maningi NE, Omar SV, Fischer GW. Next-generation sequencing for identifying pyrazinamide resistance in Mycobacterium tuberculosis. Clin Infect Dis 2014; 58 (6): 903-904; first published online December 12, 2013 doi:10.1093/cid/cit811

Carrim M, Dawood H, Frean J. Fortuin-de Smidt M, du Plessis D. du Plessis M, Moosa F, Moyes J, Naby F, Ismail N, Poonsamy B, Walaza S, Wolter N, Variava E, von Göttberg A. Enhanced surveillance for additional respiratory pathogens, 2012-2013. *NICD Communicable Diseases Surveillance Bulletin* 2013; **11**(4): 101-114

Conference presentations

International Union against Tuberculosis and Lung Disease (IUTLD), Africa Region: 19th Conference, Kigali, Rwanda, 20th June 2013: 1 poster, 1 oral presentation.

IUTLD, 44th Union World Conference on Lung Disease, Paris, 30/10/2013 – 03/11/2013: 6 posters, one oral presentation, two invited speakers

6THSA AIDS Conference, Durban, 18-21 June 2013: one invited speaker, one poster

FIDSSA, Drakensberg : one invited speaker, four posters

20th Conference on Retroviruses and Opportunistic Infections (CROI), Atlanta, Georgia, USA, 3rd March 2013: 1 poster

17th ICASA Conference, Cape Town, 7-11 December 2013: 1 poster

International Conference of Infectious Diseases, Cape Town, 2-5 April 2014: three oral presentations, two posters

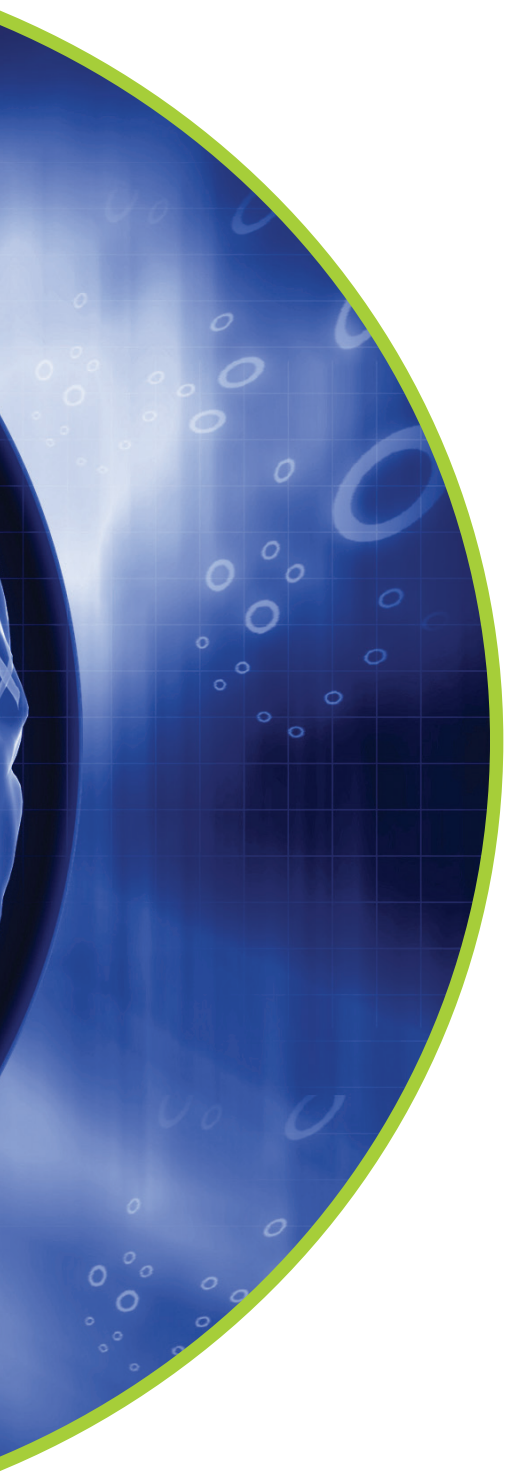
Acknowledgements

The CTB thanks the NICD/NHLS for funding and operational support; the President's Emergency Plan for AIDS Relief (PEPFAR) through the CDC, under terms of agreement 1U19GH000571; the Global Disease Detection (U2GPS001328); and the National Institute of Allergy and Infectious Diseases (1R01 AI089349 and AI080737) for funding support.

CENTRE FOR VACCINES AND IMMUNOLOGY

AFP stain show Microbacterium tuberculosis (TB)

Dr Melinda Suchard
Centre Head



BACKGROUND

The Centre for Vaccines and Immunology has been established to provide laboratory and epidemiological support to three vaccination programmes within the Expanded Programme on Immunisation – polio, measles and hepatitis – as well as to carry out appropriate research to answer public health questions related to vaccines and vaccine-preventable diseases. In addition to the national and provincial Departments of Health, which are the major stakeholders, the Centre functions as regional reference laboratories for polio and measles for the World Health Organization (WHO).

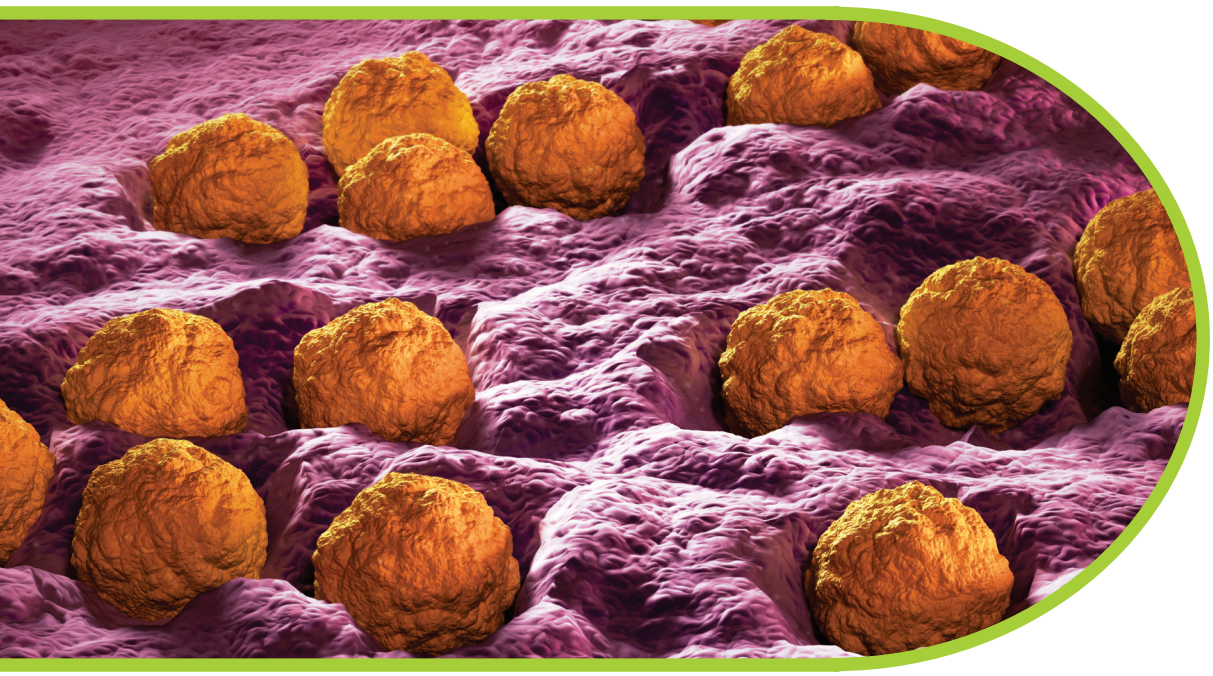
National Polio surveillance

As part of the Global Polio Eradication Initiative, the Centre for Vaccines and Immunology serves as the national reference laboratory for poliovirus isolation. South Africa is monitored by international bodies, such as the WHO, in terms of meeting adequacy indicators for surveillance of poliovirus, including a minimum case detection rate of two suspected acute flaccid paralysis cases per 100 000 population. Stool samples from cases of acute flaccid paralysis are inoculated into cell culture, and any samples with suggestive cytopathic effects undergo molecular characterisation, including sequencing. South Africa has not had a case of endemic polio since 1989, but constant vigilance is required for imported cases. Molecular typing is required to differentiate circulating strains of Sabin vaccine virus from wild polio virus. The Centre processed more than 2 500 samples during the year. Data is shared and cases classified by the National Polio Expert Committee based on history, clinical notes and laboratory findings. Additionally, the Centre provides expertise to the National Task Force and National Certification Committees for polio containment in all laboratories nationally.

Measles surveillance

In 2011, the WHO Regional Committee for Africa established the 2020 measles elimination goal. Routine measles vaccination coverage was selected as an indicator of progress towards achieving the Millenium Development Goal 4 (to reduce the overall number of deaths among children under five years of age by two-thirds from 1990 to 2015). It is estimated that measles deaths have declined by 71% globally from 2000-2012. The last large outbreak of measles in South Africa occurred in 2009/2010, with over 18 000 reported cases.

The Centre for Vaccines and Immunology is the national referral laboratory for measles surveillance. Serology, specifically the detection of measles-specific IgM antibodies, is the mainstay of diagnosis of acute measles infection. The Centre tested more than 8 000 specimens this year in support of the national measles surveillance programme. There were 11 sporadic South African measles cases detected with no clustering. The Centre reports case-based data to the National Department of Health and the WHO, and aggregated data to the Multisectoral National Outbreak Response Team (MNORT). The Centre works closely with the National Expanded Programme on Immunisation Task Group to classify cases and monitor susceptibility.



Shutterstock: Rubella - German measles

RESEARCH AND SPECIAL PROJECTS

Hepatitis B surveillance

Collaborators: Ms S Woodhall, Dr F Ncube (Public Health England),
Dr E Prentice (national Department of Health)

Hepatitis B is a highly infectious virus transmitted by contact with blood or body fluids which causes chronic sequelae such as cirrhosis and hepatocellular carcinoma. The virus poses an occupational health risk for medical staff. Vaccination against Hepatitis B has been part of the routine expanded programme of immunisation in South Africa since 1995. The NICD, together with collaborators, initiated a steering committee comprising representatives from the National Cancer Registry, South African Blood Services (SANBS), clinicians (state and private), academic institutes, Public Health England and the National Department of Health. The aim is hereof is to form a national Hepatitis B surveillance network to focus on strengthening data on hepatitis B infection and disease, and to analyse epidemiological trends to guide national policy and guidelines on prevention. The network will map out existing reportable passive data sources and decide on optimal plans for active surveillance.

Regional Polio surveillance

Collaborators: WHO

Funding source: WHO

The Centre serves as a Regional Reference Laboratory for polio detection in the WHO AFR region, as well as in other African countries. Detection of poliovirus requires labour intensive cell culture isolation methods followed by molecular typing and sequencing. Strict laboratory containment measures are essential for working with a pathogen that has been eliminated in South Africa. There remain three countries worldwide with endemic polio virus (Pakistan, Afgahanistan and Nigeria), but 2013/2014 saw outbreaks of polio virus in previously polio free areas. The Centre confirmed 75 samples from four countries (Ethiopia, Kenya, Somalia and Cameroon) as wild poliovirus type 1 and two as vaccine-derived poliovirus (VDPV) type 2 (Angola and Niger). These results helped inform the regional outbreak response to the identification of wild type virus in previously polio free countries. The early identification of wild poliovirus and VDPVs is vital for immediate action in the endgame strategy for global polio eradication.

Additionally, the Centre provided onsite support to the Inter-Country Polio Laboratory at the Kenya Medical Research Institute as a WHO consultant. The objectives of this were successfully completed, namely the provision of onsite support for testing of acute flaccid paralysis samples to clear the rapidly building backlog, assessment and sensitisation of biosafety in handling specimens, revision of standard operating procedures and training of extra Technicians.

Regional Measles and Rubella surveillance

Collaborators: WHO
Funding source: WHO

Despite intensive efforts to improve measles vaccination coverage globally in order to meet regional elimination and development of goals, global coverage appears to have leveled off. In Africa, very few countries currently offer a second measles vaccine dose in their routine immunisation schedules and rely on periodic supplementary campaigns to provide the second dose. This inadequate coverage is reflected in the global measles outbreak data since January 2012, where seven large measles outbreaks occurred in Africa.

The Centre tests specimens from nine African countries (Botswana, Kenya, Lesotho, Madagascar, Malawi, Mozambique, Namibia, Swaziland, Zambia and Zimbabwe) for measles and rubella as part of the WHO regional quality assurance programme. Additionally, support was given to Namibia during several measles outbreaks. Regionally, intensified efforts are required to increase coverage with two doses of measles vaccine through routine immunisation services, sustaining the implementation of the 'reaching every district' approach, use of supplementary immunisation activities and introduction of a second dose in the routine immunisation schedule where it is not yet offered.

Hepatitis C surveillance

Hepatitis C is a blood-borne virus that causes acute and chronic liver disease. Groups at high risk include those with sexually transmitted infections, men who have sex with men and injecting drug users. There is currently no effective vaccine against hepatitis C. There is a paucity of data on Hepatitis C incidence and prevalence in South Africa. The centre is collating Hepatitis C prevalence and genotyping data. Passive surveillance data from healthy blood donors is collected in collaboration with the South African National Blood Service. Genotyping information can be used to predict response to treatment, with genotype 1 being less responsive and requiring longer treatment than genotype 2 or 3. Genotype 1 (46%) was found predominantly in the anonymous blood donations, followed by 5a (29%) and 3 (17%). Laboratory-based data from diagnosed patient populations is accessed via the National Health Laboratory Service. Genotype 5a accounted for 30% of the laboratory-confirmed cases followed by 1b (22%), 3a (12%), 4 (8%) and mixed intergenotypic infections (5%).

Improvement in laboratory vaccinology techniques

While immunity to most vaccines is measured via serology, cellular immunity and neutralising antibodies are more difficult to monitor. The Centre has have established a laboratory with capacity for viral neutralisation assays for polio, measles and Respiratory Syncitial Virus. Additionally, the laboratory is equipped with a Luminex bead based flow cytometer for multiplexed serological assays of small sample volumes. Such techniques will be optimised for the detection of immunity and evaluated as alternatives to current assays. The assays will be used to assess durability and levels of immunity to measles, pneumococcus, RSV and other pathogens.

The assessment of the combined oral/inactivated polio immunisation schedule in an African setting.

South Africa is the first African country to have implemented an inactivated polio vaccine (IPV) into the expanded programme of immunisation schedule. The WHO endgame includes plans that every country should implement an IPV within the next few years. Effects of the changed schedule on immunity in a Southern African setting have not been assessed. Sera archived at Chris Hani Baragwanath Hospital, prior to the introduction of IPV, will be compared to sera collected after the introduction of IPV for polio neutralising antibodies. Samples from infants at six weeks, 18 weeks, 18 months and three years will be tested for polio neutralising antibodies using the modified polio neutralisation assay against all three polio serotypes. Additionally, the influence of HIV exposure will be determined. These results will inform policy regarding the switch from oral polio virus (OPV) to IPV containing regimens.

Professional development

The Centre holds weekly CPD accredited journal clubs and monthly presentations by staff and students on the theme of vaccines and immunology. The Centre also provides input to the National Health Laboratory Service Immunology Expert Committee with the aim of upskilling the next generation of scientists and clinicians with expertise in immunology and vaccinology.

TEACHING AND TRAINING

Graduated: 1 MSc, 1 B-Tech

Courses:

Short Course in Basic and Advanced Immunology

The Centre for Vaccines and Immunology, together with the University of the Witwatersrand, ran the eighth annual Short Course in Basic and Advanced Immunology. This course gives in-depth understanding of immunological concepts and terminology and brings clinicians and scientists up to date with cutting edge advances in their fields. The course covers both theoretical and practical aspects of humoral, cellular, innate and adaptive immunity.

Measles and Rubella Serology, Laboratory Management & Quality Assurance Training

The NICD, in partnership with the WHO, conducted a measles laboratory workshop for the Eastern & Southern block countries of the WHO Measles/Rubella Laboratory Network. Participants were from 12 countries (Botswana, Ethiopia, Lesotho, Malawi, Mozambique, Namibia, South Sudan, South Africa, Swaziland, Tanzania and Uganda) with facilitators from South Africa, Zimbabwe, Cote d'Ivoire, New Zealand and WHO-AFR.

Research Output

Gumede N, Lentsoane O, Burns CC, Pallansch M, de Gourville E, Yogolelo R, Muyembe-Tamfum JJ, Puren A, Schoub BD, Venter M.Emergence of vaccine-derived polioviruses, Democratic Republic of Congo, 2004-2011. *Emerging Infectious Diseases* 2013; **19**(10): 1583-1589

Gouandjika-Vasilache I, Mazitchi A, Gumede N, Manirakiza A, Manenegu C, Koyazegbe TD, Burns C. Wild poliovirus importation, Central African Republic. *Emerging Infectious Diseases* 2013; **19**(6): 1012-1013

Madhi SA, Dittmer S, Kuwanda L, Venter M, Cassim H, Lazarus E, Thomas T, Liberty A, Treurnich F, Cutland CL, Weinberg A, Violari A. Efficacy and immunogenicity of influenza vaccine in HIV-infected children: a randomised, double-blind, placebo controlled trial. *AIDS*. 2013; **27**(3): 369-79.

Nunes MC, Madhi SA. Review of a new fully liquid, hexavalent vaccine: Hexaxim. *Exp Opinion Bio Therapy*. 2013; **13** (4): 575-93.

Madhi SA, Koen A, Cutland C, Groome M, Santos-Lima E. Antibody Persistence and Booster Vaccination of a Fully Liquid Hexavalent Vaccine Co-Administered with Measles/Mumps/Rubella and Varicella Vaccines at 15-18 Months of Age in Healthy South African Infants. *Pediatr Infect Dis J*; 2013; **32**(8): 889-97.

Madhi SA, Izu A, Violari A, Cotton MF, Panchia R, Dobbels E, Sewraj P, van Niekerk N, Jean-Philippe P, Adrian PV. Immunogenicity following the first and second doses of 7-valent pneumococcal conjugate vaccine in HIV-infected and -uninfected infants. *Vaccine*. 2013; **31**(5): 777-83.

Simani OE, Adrian PV, Violari A, Kuwanda L, Otwombe K, Nunes MC, Cotton MF, Madhi SA.Effect of in-utero HIV exposure and antiretroviral treatment strategies on measles susceptibility and immunogenicity of measles vaccine. *AIDS*. 2013; **27**(10):1583-91.

Madhi SA, Dangor B, Heath PT, Schrag S, Izu A, Sobanjo-terMeulen A, Dull PM. Considerations for a Phase-III trial to evaluate a Group B Streptococcus polysaccharide-protein conjugate vaccine in pregnant women for the prevention of early- and late-onset invasive disease in young-infants. 2013. *Vaccine*. **31** (Suppl 4): D52-7.

Ditse Z, Adrian PV, Kuwanda L, Madhi SA. Association of Streptococcus pneumoniaecommon protein antibodies and pneumococcal nasopharyngeal colonization in HIV-infected and HIV-uninfected African children. *Vaccine.* 2013. **31**(40): 4421-7.

Jones SA, Groome M, Koen A, van Niekerk N, Sewraj P, Mabunda X, Kuwanda L, Izu A, Adrian PV, Madhi SA. Immunogenicity of seven-valent pneumococcal conjugate vaccine administered at 6, 14 and 40 weeks of age in

South African infants. *PlosOne2013*. **8**(8): e72794.
Cheuvart B, Neuzil KM, Steele D.A, Cunliffe N, Madhi SA, Karkada N, Han H.H., Vinals C. Association of serum anti-rotavirus immunoglobulin A antibody seropositivity and protection against severe rotavirus gastroenteritis: analysis of clinical trials of human rotavirus vaccine. *Hum VaccImmuno*. 2014. **10**(2): (In press)

Simani OE, Izu A, Violari A, Cotton MF, van Niekerk N, Adrian PV, Madhi SA. Effect of HIV-1 exposure and antiretroviral treatment strategies in HIV-infected children on immunogenicity of vaccines during infancy. *AIDS*. 2014; **28**(4): 531-41.

Kim SY, Russell LB, Park J, Verani JR, Madhi SA, Cutland CL, Schrag SJ, Sinha A.Cost-effectiveness of a potential group B streptococcal vaccine programme for pregnant women in South Africa. *Vaccine* 2014.1954-1963.

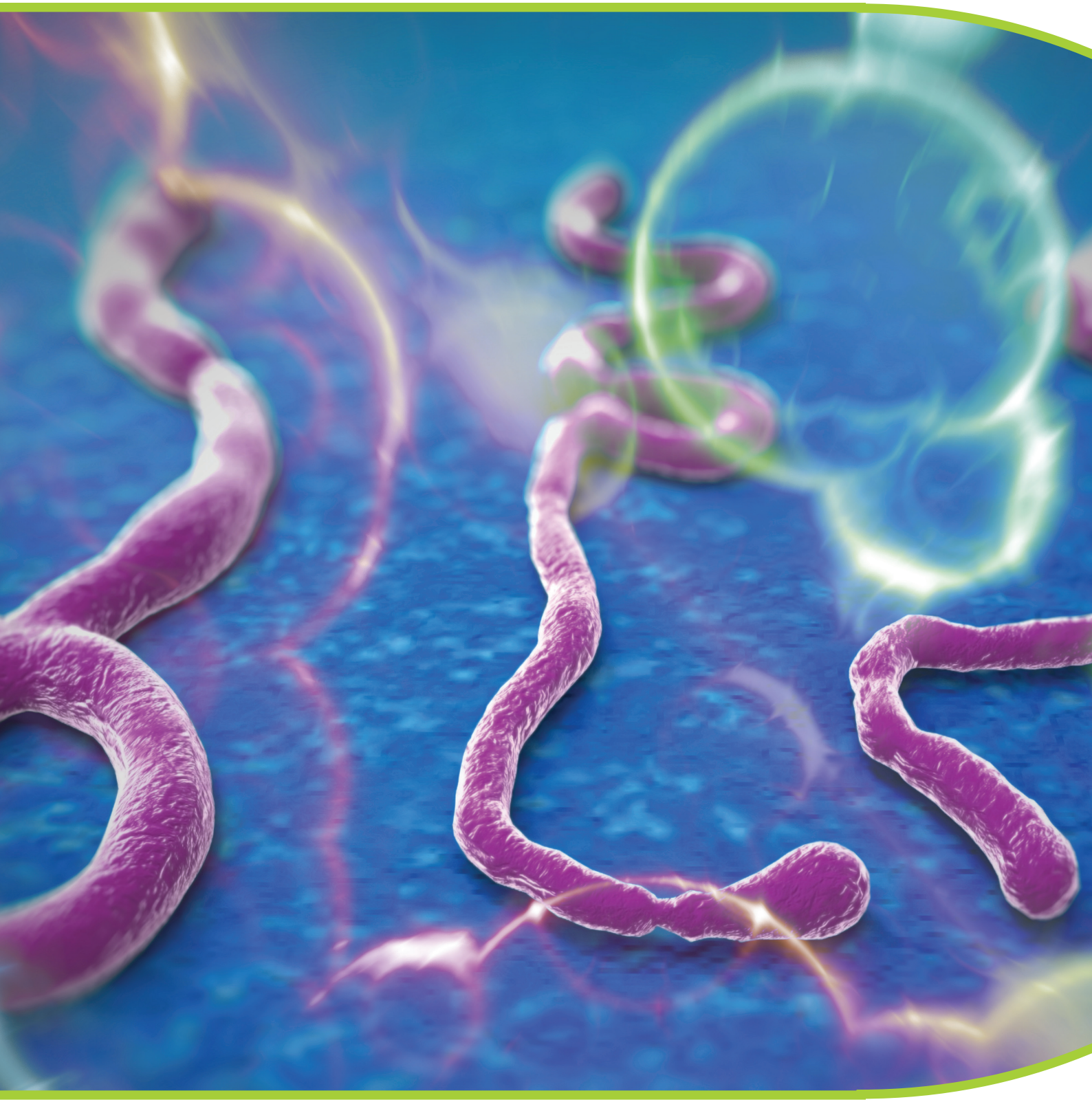
Conference presentations

- World Hepatitis Day event convened at NICD – 147 attendees
- Two invited speakers at international meetings
- Three invited speakers at regional meetings
- One invited speaker at national congress

The Centre for Vaccines and Immunology facilitating a WHO Measles and Rubella Serology, Laboratory Management & Quality Assurance Training at the NICD in November 2013 with participants from 12 countries.

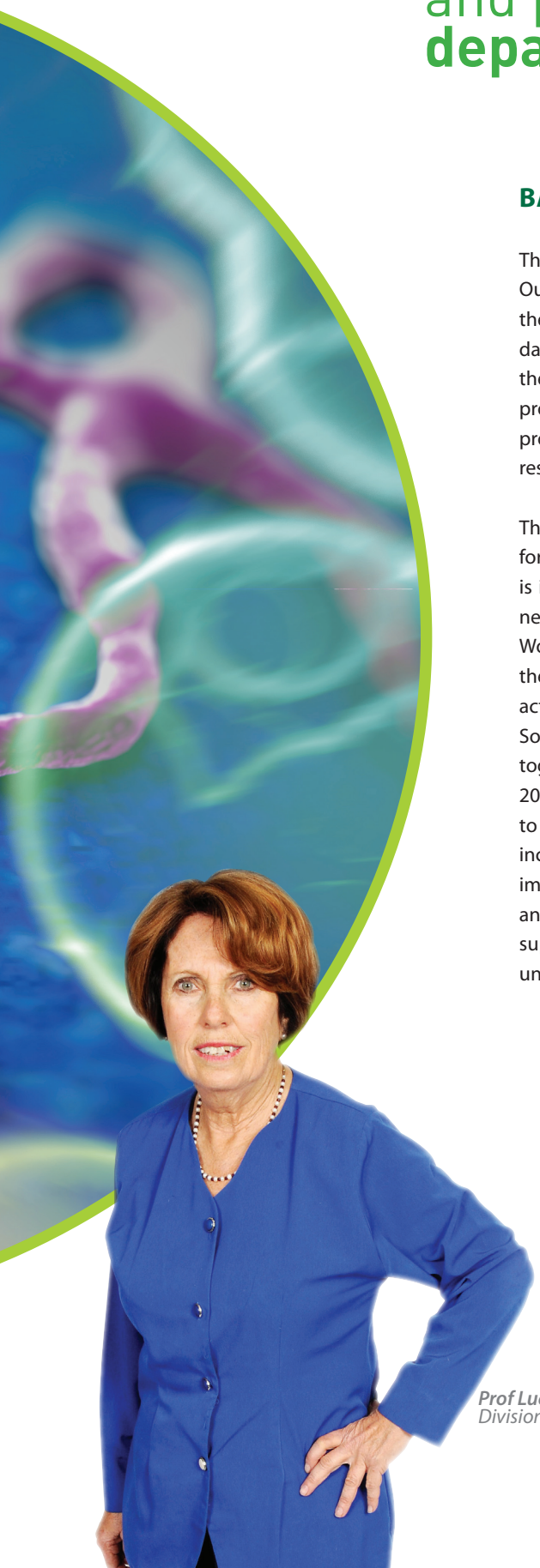
S Moonsamy, R Williams and W Howard presented on Biocontainment in Laboratories and Biorisk Management at the Polio Regional Laboratories Meeting for the AFRO region in Harare, Zimbabwe May 2013.

DIVISION OF PUBLIC HEALTH SURVEILLANCE AND RESPONSE



Shutterstock: Ebola virus

The division **facilitates communication and data sharing** between the **national** and **provincial health departments** and the **NICD**



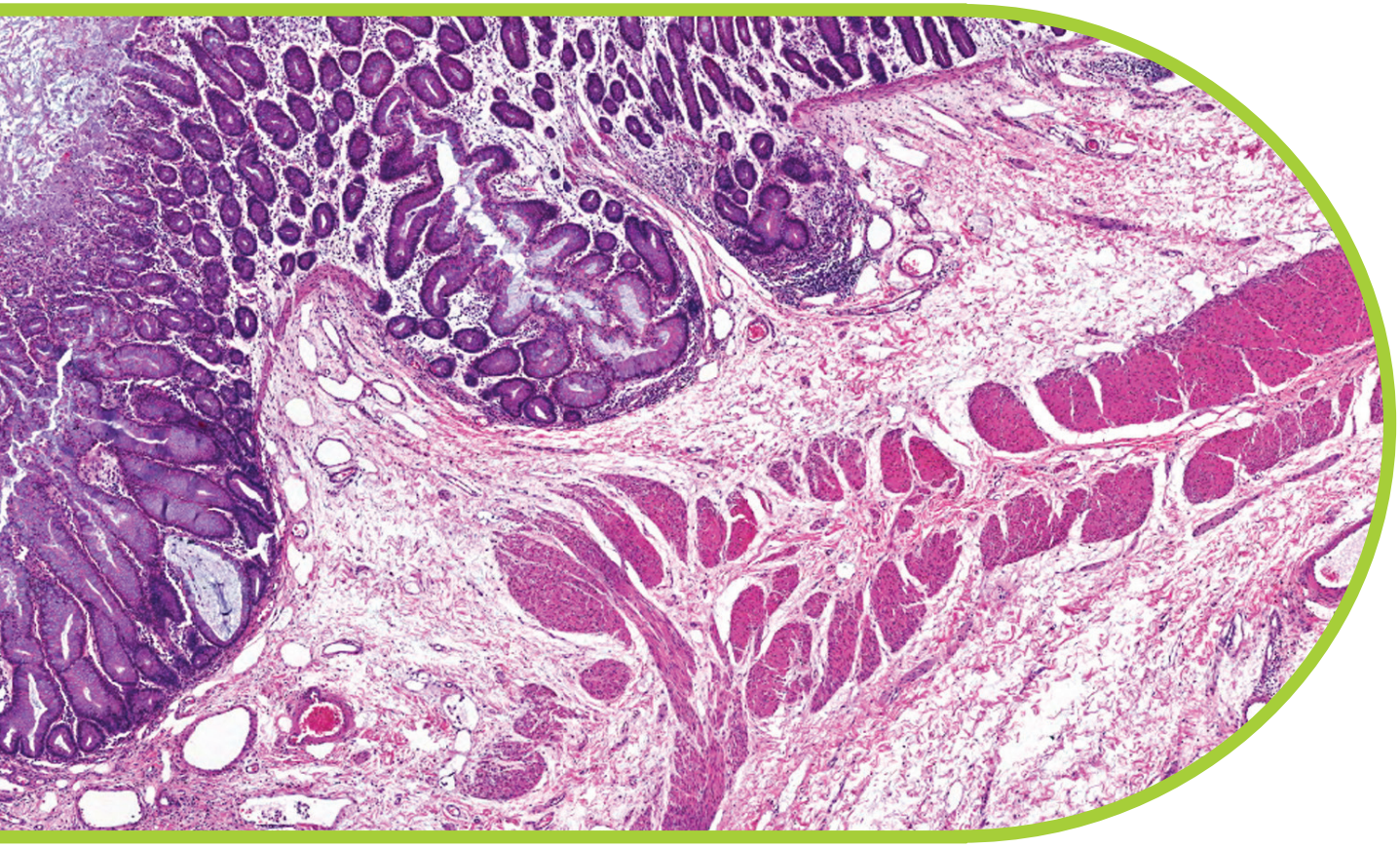
Prof Lucille Blumberg
Division Head

BACKGROUND

The Public Health Surveillance and Response Division includes the Outbreak Unit, the GERMS-SA surveillance programme, Travel Health and the Communications Unit. The division facilitates communication and data sharing between the national and provincial health departments, the National Institute for Communicable Diseases (NICD) and the public. It provides epidemiological input to other NICD units through collaborative projects and support of surveillance and epidemiological activities, outbreak responses.

The Division has expanded significantly in 2013/14. A GERMS programme for surveillance for a number of priority conditions in rural and urban clinics is in the process of being established in all nine provinces supported by a network of NICD appointed epidemiologists. As a legacy of the 2010 FIFA World Cup event, a Mass Gatherings Centre has been established as part of the WHO Mass Gathering Global Network to support research and operational activities on communicable disease monitoring and risk assessments. The South African National Travel Health Network (SaNTHNeT) was established together with the NDoH and South African Travel Medicine Society in August 2013 to provide reliable and current information and guidelines for travellers to the Southern African region. The NICD Communications Unit has been incorporated into this division to strengthen and expand a role in conveying important public health messages and outbreak alerts to both the medical and allied professionals. The National Outbreak Unit has continued in its support of all outbreak-related activities, as part of the National Outbreak unit.

The Mass Gatherings Centre was established to support research and operational activities on communicable disease monitoring and risk assessments



Shutterstock: Severe bacterial (shigella, salmonella, cholera) infection of the intestines resulting in enteritis with ulcers of the intestinal mucosal lining and bacterial colonies adherent to the dead tissue.

GERMS-SA

Surveillance/diagnostic services

GERMS-SA is a laboratory-based surveillance programme for diseases of public health importance. It is coordinated by the National Microbiology Surveillance Unit (NMSU) and spans many of the centres at the NICD. The laboratory surveillance pathogens include: *Candida spp, Salmonella enterica, Shigella spp, Vibrio cholerae, Campylobacter spp, Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Staphylococcus aureus, Pseudomonas aeruginosa, and Cryptococcus spp.* GERMS-SA is an active surveillance programme and relies not only on participating laboratories to submit isolates, but also makes use of the NHLS Corporate Data Warehouse to ensure that all cases meeting the case definition are included in the database. Annually approximately 200 microbiology laboratories, nationally, report roughly 13 000 cases meeting the GERMS-SA case definitions. The enhanced surveillance arm is operational at 25 sentinel sites across the country where 30 surveillance officers collect clinical information on patients relating to specific pathogens: *invasive S. pneumoniae, H. influenzae, N. meningitidis, S. enterica and Shigella spp.; S. aureus and Candida spp bacteraemia; Cryptococococcus spp and rifampicin-resistant TB.*

The aim of GERMS-SA is to use the data to inform and guide public health policy makers in their decisions. The objectives include estimating the burden of both community- and hospital-acquired infectious diseases under surveillance, monitoring antimicrobial susceptibility trends, monitoring the impact of the HIV/AIDS Comprehensive Care, Management and Treatment Programme in SA on HIV-associated opportunistic infections, and evaluating the impact of vaccines included in the Expanded Programme of Immunisation (EPI). The work carried out by the GERMS-SA team has significantly contributed to the development of clinical guidelines for pneumonia, meningococcal disease, cholera, cryptococcosis, typhoid fever, contributed to the situational analysis of antibiotic resistance in South Africa., and the introduction of pneumococcal conjugate vaccine as well as a booster dose for *Haemophilus influenzae* type b into the EPI. Data emanating from the GERMS-SA activities have also contributed to the DoH roll out of the cryptococcal antigen screening programme to facilitate the early diagnosis, and hence treatment, of cryptococcal meningitis.

GERMS-SA work is funded through the NHLS and a CDC cooperative agreement, and more recently by the DoH.

200 microbiology laboratories, nationally, report roughly **13 000 cases** meeting the GERMS-SA case definition

Expansion of the GERMs platform: Xpert MTB/RIF

Xpert MTB/RIF, a rapid diagnostic test that detects both *Mycobacterium tuberculosis* and resistance to rifampicin, has been implemented in all NHLS laboratories nationally and will be now the initial diagnostic test for all TB suspects in South Africa. In response to this implementation, enhanced surveillance for Xpert rifampicin resistant TB was initiated at Chris Hani Baragwaneth Hospital and selected surrounding clinics late in October 2012, and has subsequently been introduced into five other provinces. This surveillance will monitor trends over time, estimate the proportion of Multi-Drug Resistant TB among rifampicin resistant TB cases and the burden of resistance to other TB drugs, and provide information on risk factors including close contact, occupational history and HIV status. Surveillance will be initiated in the remaining three provinces during 2014.

Research projects

Zoonosis Diseases Study

Investigators: V Quan1, J Frean1, G Simpson2, D Knobel2, S Meiring1, J Weyer, 1 J Rossouw1, A Hulth3, L Blumberg1
1. National Institute for Communicable Diseases, a division of the National Health Laboratory Service 2. Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria 3. Division of Global Health, Karolinska Institutet, Sweden

Research funding: This study was partly funded by the Swedish Civil Contingencies Agency (MSB) and Swedish International Development Cooperation Agency (SIDA) and by the Global Disease Detection Program

Project summary: The Mnisi area of Bushbuckridge, Mpumalanga, South Africa has a population of mainly agro-pastoralists. The area is bordered by the Kruger National Park and contact between wildlife, livestock and humans is frequent. This is a One-Health study and done in collaboration with veterinary practitioners and researchers from the University of Pretoria Veterinary Faculty.

The aims of the project:

- Describe the prevalence of zoonotic infections in adult patients presenting with acute febrile illness, who tested negative for malaria.
- Describe the prevalence of previous zoonotic infections in healthy herders and veterinary staff at the dip tanks.

Between October 2012 through June 2013, consenting malaria-negative adult patients with acute febrile illness (AFI) were enrolled if they matched the study criteria of current temperature over 37.5°C or a history of fever in the last 72 hours. Epidemiological information was gathered around exposures and practices that would pose a potential risk for zoonosis. Lab testing was done by PCR and serology for brucellosis, bartonella infections, leptospirosis, Q-fever, tick bite fever (TBF), West Nile virus, Sindbis, Rift Valley fever and chikungunya virus infections. Additionally, healthy herders and veterinary staff at five dip tanks were tested for past zoonoses. Seventy four patients were enrolled and 64 healthy adult herders/veterinary staff were recruited.

Conclusion: TBF and bartonella were shown to be causes of acute febrile illness, suggesting a need to review standard antibiotic regimens for acute febrile illness. Rodent control, as a source of bartonella infections, needs to be addressed. There was a high background exposure to TBF, Q fever and leptospirosis in the Mnisi community in both patients presenting with AFI and herders and veterinary staff.

Early morning at the dip tank: veterinary staff and herders with their cattle showing close contact

Smart phones used for improving the timeliness of infectious disease reporting in rural South Africa

Investigators: Vanessa Quan 1, 2, Lucille Blumberg 1, 2, Gerdalize Kok 3, Anette Hulth 4

1. Division of Public Health Surveillance and Response, National Institute for Communicable Diseases (NICD), South Africa; 2. School of Pathology, University of the Witwatersrand, South Africa; 3. Malaria Control Programme, Mpumalanga, South Africa; 4. Division of Global Health, Karolinska Institute, Sweden

Research funding: This study was partly funded by the Swedish Civil Contingencies Agency (MSB) and Swedish International Development Cooperation Agency (SIDA)

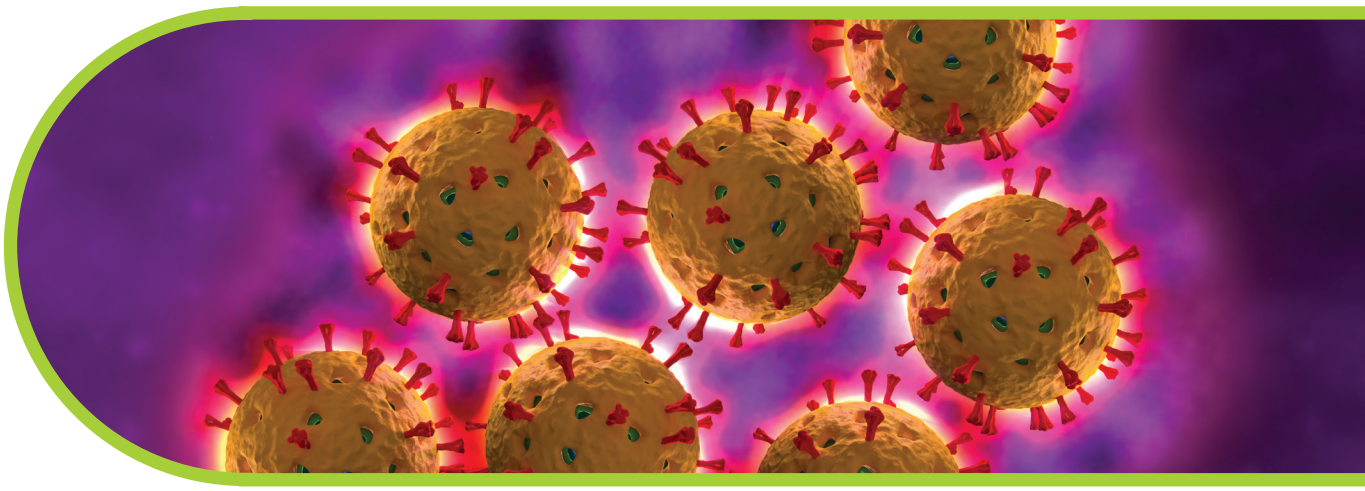
Project summary: The use of a smart phone for data submission to a central server was piloted in a rural area to improve timeliness for reporting of malaria cases, diarrhoeal cases and dog bites. A designated nurse reported patient-level data on positive malaria cases from three clinics in Mpumalanga Province, South Africa from October 2012 to May 2013. A key component of the malaria elimination strategy is a 24-hour reporting time for confirmed malaria cases to allow rapid follow-up and management of additional cases by malaria field staff.

The use of a notification sent via SMS from a mobile phone for each newly diagnosed malaria case was acceptable to users and was technically feasible in this rural area and significantly improved the timeliness of data transmission

Consideration should be given to large-scale use, possibly using a toll-free phone service, within the provincial malaria control programmes.

SR Ennica interviewing and taking blood from herders at the dip tank, to assess risk factors and baseline seroprevalence for zoonoses in healthy herders, Mpumalanga

Teaching SR Ennica, a 70-year-old nurse, how to use the cell phone to capture data on malaria cases, zoonotic diseases and reporting dog bites and diarrhoeal diseases for the project.



Shutterstock: Rotavirus Cells - in fluid

OUTBREAK RESPONSE UNIT

The Outbreak Response Unit (ORU) provides technical support for all aspects of communicable disease outbreaks and control in South Africa. Through close collaboration with provincial and national health departments and other stakeholders, together with systems for early detection and improved reporting of epidemic-prone communicable diseases, the ORU functions as a source of intelligence for outbreak detection and facilitates comprehensive outbreak response activities. In addition, close partnerships with NHLS diagnostic laboratories and NICD centres provide appropriate laboratory diagnostic services during outbreaks and specialised diagnostic testing as required. In April 2012, the National Department of Health Communicable Diseases Directorate and the ORU became functionally integrated as the National Outbreak Unit, a platform for synergistic outbreak detection and response activities throughout the country.

Catching mosquitoes at the home of a patient with odyssean malaria

Public health services

The ORU's role in outbreaks may include, but is not limited to, the following: outbreak detection and reporting, field investigation, development of clinical and laboratory guidelines, management of laboratory data and interpretation of results, and recommendations for prevention and control.

During the year, the ORU assisted with the investigation and response to a wide spectrum of outbreaks, (106 in total), including:

- Rotavirus outbreaks in KwaZulu-Natal and Northern Cape provinces -see below*
- Foodborne illness outbreaks, countrywide
- Institutional outbreaks: TB, hepatitis A, hepatitis B, scabies
- Healthcare-associated infection outbreaks: ESBL-producing Klebsiella, pneumoniae (Gauteng and Limpopo provinces), carbapenemase producing Enterobacteriaceae (Gauteng Province)
- Rabies in KwaZulu-Natal province
- Odyssean malaria, Gauteng Province.

In January 2014 severe malaria was confirmed in two persons residents in the south of Johannesburg, close to a highway. Neither had a history of travel to a malaria transmission area and they were not epidemiologically linked. There was no evidence of breeding of anopheles mosquitoes in their households or surrounds and this was likely Odyssean malaria due to imported mosquitoes. Both patients recovered.

Did you know?

The ORU provides technical support for all aspects of communicable disease outbreaks and control in South Africa

The ORU assisted with the suspected diarrhoeal disease outbreak investigations in Siyanda district (Northern Cape Province) and eThekwini Metropolitan (KwaZulu-Natal Province), together with numerous governmental (District, Provincial and National Departments of Health), non-governmental (including WHO, CDC-SARGDD and US-CDC), diagnostic NHLS laboratories, and NICD entities (Virology Laboratory at Centre for Enteric Diseases (CEDv), Bacteriology Laboratory at Centre for Enteric Diseases, Parasitology Laboratory (Centre of Opportunistic, Tropical and Hospital Infections) and SA-FELTP). Rotavirus was the most common pathogen identified. However, a range of other enteric viruses was also detected, mostly occurring as mixed infections with rotavirus. The increase in diarrhoeal illness cases reported in both Siyanda District and eThekwini Metropolitan coincided with the occurrence of the annual rotavirus season which typically occurs in South Africa between April and August every year. The magnitude of the increase in diarrhoea-related cases was, however, perceived as being higher than previous post-rotavirus vaccine introduction years. Possible reasons for the increase in diarrhoea cases include, among others , suboptimal rotavirus vaccine coverage as well as possibly a 'biennial peak' phenomenon – although the latter was not supported by the Rotavirus Surveillance Programme data from non-KZN sites for 2013. Data from the NICD Rotavirus Surveillance Programme (implemented in April 2009, with seven sites in five provinces [including KZN but not NCP] at present) did not show an overall increase in either the number of cases eligible for enrolment in the study (children <5 years of age admitted to hospital for acute diarrhoea) or the rotavirus detection rate during 2013 as compared to previous post-vaccine introduction rotavirus seasons. However, data from the rotavirus surveillance programme for the KwaZulu-Natal sites showed that the number of stool samples tested during the 2013 rotavirus season was higher than in previous post-rotavirus vaccine introduction years, but the proportion of rotavirus-positive cases was comparable to that reported in previous post-rotavirus vaccine introduction years.

The ORU continued to strengthen networks for the reporting and investigation of foodborne illness, with close to 60 foodborne illness outbreaks reported and followed up. The ORU supported communicable disease monitoring during key mass gathering events, including the Nelson Mandela Memorial week and funeral activities, and the 2014 African Championship (CHAN) by providing daily surveillance data and communicable disease intelligence updates to relevant stakeholders. The CDW alert system, managed by the ORU, facilitates timely notification of laboratory-confirmed cases of priority communicable diseases detected by NHLS laboratories throughout the country (*Salmonella* Typhi, *Vibrio cholerae, Neisseria meningitidis* and *Bordetella pertussis*) to healthcare and public health workers. The OutNet programme is an NHLS laboratory-based outbreak network with nine provincial laboratory OutNet representatives who act as the key points of contact for provincial public health staff and facilitate laboratory functions in outbreak detection and response. As NOU, the ORU assisted with the development of provincial and national guidelines for priority communicable diseases.

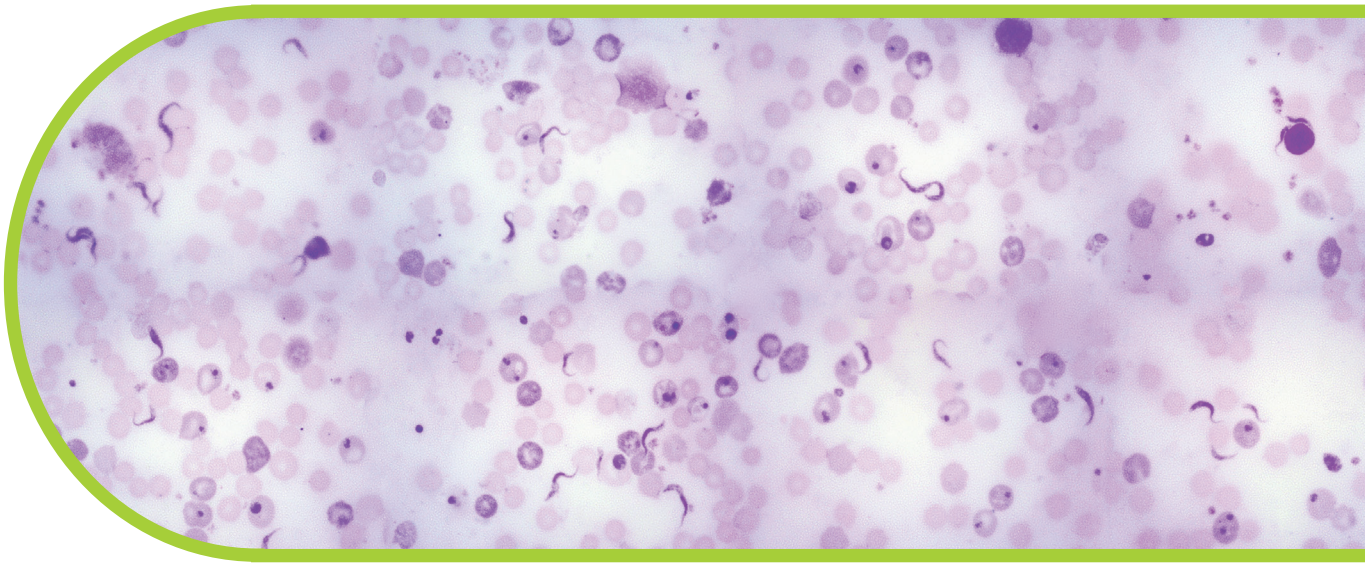
Residents of the Field Epidemiology Programme are seconded to the ORU for 8 weeks to gain field experience of outbreaks under supervision.

The ORU publishes a monthly Communicable Diseases Communiqué, which reports recent outbreak and communicable disease cases/issues of relevance. This is distributed to a wide audience including: general practitioners, specialists, infectious diseases and travel medicine societies, and national and provincial public health personnel. In addition, the unit publishes special urgent advisories and communiqués in response to acute events requiring immediate dissemination of information.

Research Projects

Pertussis surveillance project

Investigators: Dr Juno Thomas and Genevie Ntshoe (ORU, NICD); Dr Gary Reubenson (Rahima Moosa Mother and Child Hospital and University of the Witwatersrand); Dr Ranmini Kularatne (National Health Laboratory Service).
Research funding: Sanofi Pasteur
Project summary: This hospital-based project based at a single site in Gauteng Province aims to describe the prevalence and characteristics of pertussis disease amongst children <10 years of age who are hospitalised with suggestive respiratory illness, or in the case of infants and young children, present with apnoea for investigation. With informed consent from caregivers, key clinical data is recorded and nasopharyngeal swabs are collected for B. pertussis and B. parapertussis PCR testing as well as culture for Bordetella spp. Case enrolment in ongoing and will continue until September 2014.



Shutterstock: real Trypanosoma photomicrograph panorama. The serpentine flagellates can be seen among blood cells and can impair the nervous system. The shadowy appearance is due to photographing a blood smear.

TRAVEL HEALTH

This unit provides a consultative service for health practitioners regarding pre-travel advice for travellers and clinical consultations for returning travellers with suspected infectious diseases; develops guidelines for a number of travel-related diseases and neglected diseases; serves as a point of contact and liaison internationally for infectious diseases acquired in southern Africa, and assists with training of travel health practitioners and those studying tropical diseases. There is a focus on zoonotic diseases and emerging pathogens through the One Health approach brought about by the interactions between animal and human health and the environment.

South African National Travel Health Network (SANTHNet)

http://www.santhnet.co.za

The SANTHNET is a travel health network run by the National Department of Health (NDoH), the National Institute for Communicable Diseases (NICD) and the South African Society of Travel Medicine (SASTM), and was launched in August 2013.

The SANTHNET provides up-to-date information on health risks for travel in the southern African region, with a primary South African focus through developing and providing guidelines on communicable diseases and up-to-date information on disease outbreaks. An informative website has been developed and has attracted a significant number of nationally and internationally visits each month. The network will focus on developing guidelines around travel related health matters. The network will serve as a surveillance platform to gather information around imported communicable diseases e.g. dengue, trypanosomiasis and leishmaniasis as well as expert advice on diagnosis and management of tropical and travel related diseases. The Unit also manages a supply of essential drugs for a selection of tropical and neglected diseases e.g.leishmaniasis, trypanosomiasis and severe malaria.

WHO collaborating centres on health at mass gatherings

http://www.who.int/ihr/publications/mass_gatherings/en/

The Mass Gatherings Centre was established for communicable disease surveillance and risk assessment for the 2010 FIFA World Cup and has now become a part of the WHO 6-centre Mass Gatherings Collaborating Centre network (Disaster Research Centre, Flinders University, Australia; Public Health England, United Kingdom; National Institute of Communicable Diseases (NICD), South Africa; Institute of Public Health of Vojvodina, Serbia; School of Public Health, University of Washington, United States of America; Ministry of Health, Saudi Arabia).

Mass gathering events that were covered by the division and which included daily reporting on risks and potential risks included:-

- 2014 African Nations Championship – 11 January to 1 February 2014 in South Africa
- World Transplant Games – 28 July to 4 August 2013 in Durban, South Africa
- Nelson Mandela Memorial – 5 to 15 December 2013 in South Africa

Shutterstock: The Malaria Virus Cell - 3D illustration

NICD COMMUNICATIONS UNIT

In the past year, the National Institute for Communicable Diseases (NICD) Communication Unit has actively expanded its role in conveying important public health messages and outbreak alerts to both the medical and allied professionals through guidelines and information sheets, as well as to the general public through media releases, interviews on TV, radio and the print media and through the introduction of social media. An active website has continued to grow in attracting the general public, the media and health professionals. To meet the increasing demands for this, media training for senior staff was undertaken.

A general review of the NICD website is planned for the coming year with an interim updating of all Centre information.

A new electronic format has been successfully developed for disseminating the NICD publications with stakeholders in the public and private sector and national and provincial Departments of Health. These include the monthly communiqué which highlights current communicable disease events and outbreaks, the monthly NICD surveillance bulleting which collates data from the NICD surveillance programmes, and the quarterly bulletin which documents more formally outbreaks and surveillance programmers.

New internal publications have been developed: NICD Science Focus, a quarterly compilation of abstracts of scientific publications by NICD staff members published in peer- reviewed journals; NICD newsletter which has a more informal focus on events and happening at the NICD, and staff awards and profiles individual staff members and their work.

Active communication via the NICD website - www.nicd.ac.za continues to grow in attracting the general public, media and professionals

Conference presentations

National: 55

International: 12

Teaching and training

- The Division provides training and mentoring of the SA-FELTP residents. The Unit provides supervision to residents during outbreak investigations, and also gives lectures during both short and long courses offered by the programme.
- Training for GERMS-SA staff on research and surveillance.
- The broader GERMS-SA team performed >30 site visits nationally to laboratories and hospitals participating in the programme and provided feedback on surveillance data, and training in clinical and microbiological diagnostics.
- The ORU assisted national and provincial health departments in training healthcare workers and public health personnel in epidemic preparedness and response, with an emphasis on case management and appropriate laboratory diagnostic tests for a number of epidemic-prone diseases.
- The ORU supported the training of public health specialists from the University of the Witwatersrand, University of Pretoria by hosting six-month placements for registrars to gain experience in both outbreak response activities and communicable diseases-related public health.
- Public health registrars from Public Health England (UK) were hosted for three-month placements as part of the NICD-HPA exchange programme.
- Under-and postgraduate teaching on travel and tropical diseases was provided for undergraduates and postgraduates at the Universities of Stellenbosch, Witwatersrand and North-West.
- Training for the certification in Travel Medicine.
- Training for the Diploma in Tropical Diseases.

Professional development

Postgraduate candidates graduated: One MPH

Postgraduate candidates enrolled: One PhD; One MSc

Awards / prizes

- Prof Lucille Blumberg received the Rotary Foundation Paul Harris Fellowship award (The presentation of Paul Harris Fellow recognition is The Rotary Foundation's way of expressing its appreciation for a substantial contribution to its humanitarian and educational programmes.)
- Mr Sonwabo Lindani won best poster presentation at the 5th FIDSSA conference, 10-12 October 2013.
- Prof Lucille Blumberg received the 2014 WSAVA (World Small Animal Veterinary Association Global One Health Award) which is awarded to an individual or organisation in recognition of their leadership in furthering One Health in southern Africa by working with both the human medical and veterinary communities on diseases of zoonotic importance.
- Dr Pieter de Jager's abstract submitted for the ICID conference in April 2014 was accepted for an oral presentation. It was also selected as one of two abstracts for the Novartis Vaccine Award for Epidemiology of Infectious Diseases.

Committees

- South African Malaria Elimination Committee (SAMEC, Chairman)
- RaVaGES Advisory Group Rabies
- ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium) Advisory Board
- Biological Weapons Working Committee (BWWC) of the South African Council for the Non-Proliferation of Weapons of Mass Destruction
- AHEAD-GLTFCA Advisory Committee as from 2014
- Multisectoral National Outbreak Response Team (MNORT)
- National Advisory Group on Immunisation
- Rabies Advisory Group
- Infectious Diseases Society of Southern Africa (FIDDSA).

Honorary appointments

- Prof Lucille Blumberg was appointed as an Extraordinary Professor in the subject group Clinical Pharmacy by the North-West University as from 3 June 2013.

Research output

Publications

Staff authored/co-authored the following publications:

Archer BN, Thomas J, Weyer J, Cengimbo A, LanDoH DE, Jacobs C, Ntuli S, Modise M, Mathonsi M, Mashishi MS, Leman PA, le Roux C, Jansen van Vuren P, Kemp A, Paweska JT, Blumberg L. Epidemiologic investigations into outbreaks of rift valley Fever in humans, South Africa, 2008-2011. *Emerg Infect Dis*. 2013 Dec;**19**(12). doi: 10.3201/eid1912.121527.

Sartorius B, Cohen C, Chirwa T, Ntshoe G, Puren A, Hofman K. Identifying high-risk areas for sporadic measles outbreaks: lessons from South Africa. *Bull World Health Organ* 2013; **91**: 174-183.

Blumberg L, Frean J, Moonasar D, South African Malaria Elimination Committee. Successfully controlling malaria in South Africa. *SAMJ, Suppl1*, Mar 2014; **104**(3): 224-227.

Metcalf CJ, Cohen C, Lessler J, McAnerney JM, Ntshoe GM, Puren A, Klepac P, Tatem A, Grenfell BT, Bjørnstad ON. Implications of spatially heterogeneous vaccination coverage for the risk of congenital rubella syndrome in South Africa. *J R Soc Interface*. 2013 Jan 6;**10**(78):20120756. doi: 10.1098/rsif.2012.0756.

Morris N, Frean J, Baker L, Ukpe IS, Barnes KI, Kruger P, Mabuza A, Raswiswi E, Maharaj R, Blumberg L, Moonasar D. Re-defining the extent of malaria transmission in South Africa: Implications for chemoprophylaxis. *S Afr Med J.* 2013 Oct 2;**103**(11):861-4. doi: 10.7196/samj.7533.

Ntshoe GM, McAnerney JM, Archer BN, Smit SB, Harris BN, Tempia S, Mashele M, Singh B, Thomas J, Cengimbo A, Blumberg LH, Puren A, Moyes J, van der Heever J, Schoub BD, Cohen C. Measles Outbreak in South Africa: Epidemiology of Laboratory-Confirmed Measles Cases and Assessment of Intervention, 2009-2011. *PLoS ONE* **8**(2): e55682. doi:10.1371/journal.pone.0055682.

Sabeta CT, Weyer J, Geertsma P, Mohale D, Miyen J, Blumberg LH, Leman PA, Phahladira B, Shumba W, Walters J, Paweska JT. Emergence of rabies in the Gauteng Province, South Africa: 2010-2011. *J S Afr Vet Assoc*. 2013 Apr 26;**84**(1):E1-5. doi: 10.4102/jsava.v84i1.923.

von Gottberg A, Cohen C, de Gouveia L, Meiring S, Quan V, Whitelaw A, Crowther-Gibson P, Madhi SA, Whitney CG, Klugman KP. Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era: South Africa, 2003-2008. *Vaccine.* 2013 Aug 28;**31**(38):4200-8. doi: 10.1016/j.vaccine.2013.04.077. Epub 2013 May 16.

Weyer J, Thomas J, Leman PA, Grobbelaar AA, Kemp A, and Paweska JT. Human Cases of Wesselsbron Disease, South Africa 2010–2011. *Vector-Borne and Zoonotic Diseases.* May 2013, Vol. **13**, No. 5: 330-336.

SAMJ, Vol 103, No10 (2013) – Malaria Supplement – Pg 769-806

Blumberg L, Moonasar D. Foreword. S Afr Med J. 2013 Aug 29;103(10):7401. doi: 10.7196/samj.7401. Upke IS, Moonasar D, Raman J, Barnes KI, Baker L, Blumberg L. Case management of malaria: Treatment and chemoprophylaxis. *S Afr Med J.* 2013 Aug 29;**103**(10):793-8. doi: 10.7196/samj.7443.

Moonasar D, Morris N, Kleinschmidt I, Maharaj R, Raman J, Mayet NT, Benson FG, Durrheim DN, Blumberg L. What will move malaria control to elimination in South Africa? *S Afr Med J.* 2013 Aug 29;**103**(10):801-6. doi: 10.7196/samj.7445.

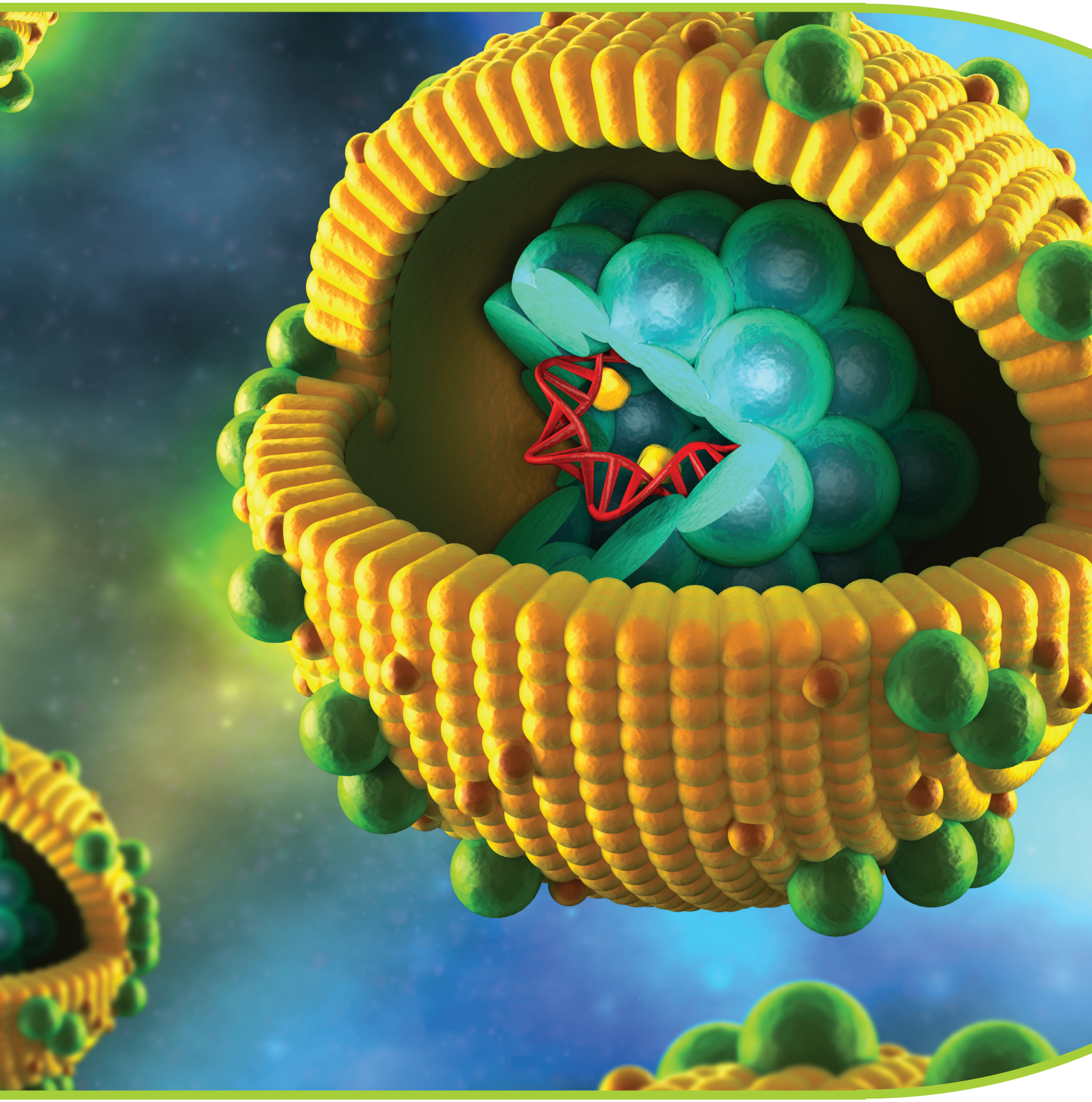
Boyles TH, Bamford C, Bateman K, Blumberg L, Dramowski A, Karstaedt A, Korsman S, Le Roux DM, Maartens G, Madhi S, Naidoo R, Nuttall J, Reubenson G, Taljaard J, Thomas J, van Zyl G, Von Gottberg A, Whitelaw A, Mendelson M. Guidelines for the management of acute meningitis in children and adults in South Africa. *South Afr J Epidemiol Infect,* 2013; **28**(1): 5-15.

Frean J, Brooke B, Thomas J, Blumberg L. Odyssean malaria outbreaks in Gauteng Province, South Africa, 2007 – 2013. SAMJ (In press).

Book chapters

Blumberg L, Enria D, Bausch D. Manson's Tropical Diseases 23rd Edition. Section 5; Viral Infections; Viral Haemorrhagic Fevers. (pg 171-194). (eds. Jeremy Farrar, Peter Hotez, Thomas Junghanss, Gagandeep Kang, David Lalloo, Nicholas White)

THE SOUTH AFRICAN REGIONAL GLOBAL DISEASE DETECTION CENTRE



Shutterstock: Hepatitis Virus Cell - in detailed view

Dr Natalie Mayet
Head

The SARGDDC was established in 2010 in collaboration with the CDC, as the eighth of ten global programmes.

OVERVIEW

The South African Regional Global Disease Detection Centre (SARGDDC) is a collaborative partnership between the US Centres for Disease Control and Prevention (CDC) the National South Africa Department of Health (NDoH) and the National Institute for Communicable Disease (NICD).

The SARGDDC was established in 2010 by the CDC as the eighth of ten global programmes. The core function of the GDD is to integrate with activities and programmes of partners in order to prevent; detect and effectively respond to infectious disease threats. The SARGDDC provides the platform for building capacity through four different focused programmes: the South African Field Epidemiology and Laboratory Training Programme; the Influenza Programme; the International Emerging Infections Programme; and the recently established One Health Programme. The SARGDDC collaborates on specific activities with the DoH, NICD and other partners and supplements some activities through research and non-research co-operative agreements.

HIGHLIGHTS

The appointment of Ms Dorothy Southern, MPH-International Health, as a Scientific Writer and Mr Alfred Musekiwa MSc as a biostatistician provided the opportunity to build capacity in scientific writing and biostatistics. Transfer of these scarce and critical skills is intended to advance the quality of research outputs for emerging researchers.

Professor Marietjie Venter has been appointed as the One Health Programme Director in March 2014 and will drive the interdisciplinary collaboration in aspects of health care for humans, animals and the environment.

SURVEILLANCE

The SARGDDC Co-Directors assisted with the formulation of the Terms of Reference for the National Surveillance Coordinating Forum established by the Deputy Minister of Health, and supported the drafting of the National Surveillance Strategy Document.

Supporting the South African Malaria Elimination agenda by chairing the Surveillance Technical Subcommittee of the South African Malaria Elimination Committee

SPECIAL PROJECTS

The SARGDDC has 18 collaborative projects with the NDoH and NICD, and employs 40 staff through these co-operative agreements. Some of the projects include: Public Health Emergency Preparedness and Response Capacity Building; Strengthening Malaria Surveillance; Supporting PulseNet Africa; Investigation of Vector-borne Viruses as the cause of Neurological Disease of Humans; Harbouring of Viral Zoonotic Agents by Southern African Bat population; Improving Biosafety and Biorisk Management Program in South Africa; Use of New Molecular Technology in Diagnosing Neonatal Sepsis; The prevalence of HPV infections in Men who have Sex with Men and the support for the development of a National Public Health Institute for South Africa.

There have been a number of delegations that have visited South Africa and these include the CDC Cooperative Biological Engagement Program (CBEP) from 27-30 January 2014 and Dr J. Michael Underwood, PhD. Epidemiologist. Division of Cancer Prevention and Control, CDC, Atlanta who provided technical support to the Cancer Registry at the NIOH in March and presented a Public Lecture on the 13 March 2014 titled "HPV Infection and Cancer- the past, present and future", the talk was attended by 30 staff from the NICD, NHLS, NCR and NIOH.

The SAFELTP hosted a pre-conference workshop at PHASA on 24 September 2013 on Building Epidemiology Capacity in South Africa; the workshop was attended by 41 participants including Ms Jeanette Hunter, the DDG Primary Health Care and Dr Frew Benson. The outcome of the workshop was the formation of an Epidemiology Task Team of subject matter experts who will recommend an action plan for building epidemiology capacity in South Africa.

Dr Lazarus Kuonza attended the regional South African Development Community (SADC) meeting in Gaborone, Botswana, 14-16 August 2013 to discuss a regional training on the Domestication of Harmonised Communicable Disease Surveillance Frameworks and Databases across SADC Member States.

Dr's Carl Reddy, Kuonza, and Mayet presented a poster on Building Capacity and Competency for Applied Public Health Practice at the PHASA Conference in September 2013 and mentored 3 residents who had abstracts submitted.

The SARGDDC is supporting the South African Malaria Elimination agenda by chairing the Surveillance Technical Subcommittee of the South African Malaria Elimination Committee and is assisting with the transfer of the Malaria Information System from the MRC to the NICD.

Maanda Mdau, 2010 cohort, facilitated the establishment of a Geographic Information Systems (GIS) working group at the NICD and presented on GIS at the Epidemiology Forum meeting in May 2013.

Dr Patience Kweza, 2011 cohort, was invited by the NDoH to present her findings on the knowledge, attitude and practices on human rabies among primary school children in Vhembe District Limpopo Province, 2012 at a meeting in Upington, Northern Cape on 25 June.

The US government launched the Global Health Security Agenda with more than 25 countries and international organisations on 13 February 2014. South Africa was one of the represented countries and the aim is to strengthen the world's ability to prevent, detect and respond to infectious disease threats, an agenda aligned with the broader GDD mission.

26 districts at NDoH are trained and supported by SARGDDC

Teaching and training

The Communicable Disease Cluster at the NDoH has trained 26 districts, and continues to train, in Epidemic Preparedness and Response with the support of the SARGDDC.

The SARGDDC has supported the training of Ms Shelina Moonsamy in Poliovirus serology at the Polio and Picornavirus Lab Branch/CDC Atlanta from the 12 -24 May 2013 and has funded the training visit of Mr Zibusiso Masuku to the Phillips University BSL4 facility in Marburg Germany from 24-30 November 2013.

The Global Foodborne Diseases Network Training course conducted on the 13-17 May, on behalf of the WHO, was jointly run by staff from the Enteric Disease Technical Support Corps in Atlanta and the Centre for Enteric Disease 2013.

The SARGDDC hosted a workshop on the Introduction to Health Policy and Decision Analysis from 18-21 June 2013. The course was an introduction to applied policy and decision analyses and included the rationale for health economic evaluation and the different methodologies that could be used for the analysis. A diverse group of 46 participants attended the course. The course facilitators, Dr Martin Meltzer, Head of the Health Economics and Modelling unit in the CDC Atlanta Division of Preparedness and Emerging Infections and Dr Michael Washington, an Industrial Systems Engineer, provided intense practical training. This was followed by hands-on consultation with five participants who are currently working on select projects.

Biosafety and Biosecurity Training was conducted with SARGDDC funding for NHLS and 54 staff were trained in both Umtata and KZN.

Two Basic Epidemiology short courses were conducted in KZN, one in April and the other in August. Fourty-one health professionals from the NDoH and the Health Systems Trust were trained to conduct data analysis and participants analysed 12 field projects the different health districts.

SAFELTP staff conducted a Data Management course for Gauteng Department of Health employees from 3 -7 of June.

The SAFELTP Supervisors/Mentorship Training was conducted by Dale Carnegie on 18-19 July 2013, the 24 participants learnt skills and techniques required to effectively mentor and coach.

Professional development

Eight SAFELTP residents were awarded the Master of Public Health Degrees at the April 2013 graduation ceremony and four residents were awarded Master of Public Health Degrees at the September 2013 graduation ceremony at the University of Pretoria. The MPH attainment rate has increased from 51% in 2011 to the current rate of 83%.

Honours

Dr Mazvita Naome Muropa of the 2012 cohort, was selected to present at the prestigious International Night of the EIS conference on "Antiretroviral Initiating Regimens and Discontinuation Patterns, MEDUNSA Pharmacovigilance Surveillance Cohort, South Africa, 2004 -2011" in Atlanta, April 22-26. She was selected to be one of five speakers from more than 200 applicants.

Ms Andronica Rakgantso, of the 2012 Cohort, won the 1st prize at the Faculty Research Day held at the University of Pretoria in August, on her Poster "Gastroenteritis Outbreak Investigation in Siyanda District, Northern Cape Province, South Africa, May-June 2013"

Mr Maanda Mudau was selected for the Novartis Vaccine Award for Epidemiology of Infectious Diseases for his abstract entitled: "Geospatial analysis and identification of space-time clusters of MDR-TB in South Africa, 2006-2012".

Ms Joy Ebonwu, of the 2011 cohort, had her abstract accepted on "Low treatment initiation among newly diagnosed multi-drug resistant tuberculosis patients in Gauteng, South Africa", for an oral presentation at the 44th Union World Conference on Lung Health in Paris, France from 30 October -3 November 2013. With over 1380 abstracts submitted, only the top 180 were selected for oral presentation.

Research output

The research outputs supported by the SARGDDC are listed in the respective Centres of the NICD. The publications listed below are those specific to the SAFELTP Programme.

Mamahlodi T, Kuonza L, Candy S. Cervical cancer screening programme in Limpopo province: January 2007 to December 2010, *South Afr J Gynaecol Oncol* 2013; **5**(1): 4-10.

Khosa E, Kuonza L, Kruger P, Maimela E. Towards the elimination of malaria in South Africa: a review of surveillance data in Mutale Municipality, Limpopo Province, 2005-2010. *Malaria Journal* 2013, **12**: 7.

Khuzwayo L, Kuonza L, Ngcobo J. Evaluating the acute flaccid paralysis surveillance system in South Africa, 2005-2009 - an analysis of secondary data. *Pan Afr Med J* **14**: 1

Kubheka V, Govender P, Margot B, Kuonza L. Dog-bites and human rabies in Uthungulu district of KwaZulu-Natal Province, South Africa, 2008-2010 – a review of surveillance data. *South Afr J Epidemiol Infect* 2013; **29** (2)

Ebonwu JI, Tint KS, Ihekweazu C. Low treatment initiation rates among multidrug-resistant tuberculosis patients in Gauteng, South Africa, 2011. *IJTLD* 2013: 17 pp. 1043-1048(6)

Dube NM, Girdler-Brown BV, Tint KS, Kellett P. Repeatability of manual coding of cancer reports in the South African National Cancer Registry, 2010. *South Afr J Epidemiol Infect* 2013; **28**(3): 157-165

Imanishi M, Kweza P, Slayton RB, Urayai T, Ziro O, Mushayi W, Chizororo M, Kuonza L, Ayers T, Freeman M, Govore E, Duri C, Chonzi P, Zinyowera S, Manangazira P, Kilmarx PH, Mintz E, Lantagne D. the Zimbabwe Typhoid Fever Outbreak Working Group 2011–2012. Household Water Treatment Uptake during a Public Health Response to a Large Typhoid Fever Outbreak in Harare, Zimbabwe. *Am J Trop Med Hyg* 2014 13-0497

Presentations

International conferences: 9
National conferences: 9
Academic research days: 3

Glossary

ACTG AIDS	clinical trials group
ARMS-PCR	amplification refractory mutation system PCR
ART	antiretroviral therapy
ARV	antiretroviral
CANSA	Cancer Association of South Africa
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CCHF	Crimean-Congo haemorrhagic fever
CCMT	Comprehensive Care Management and Treatment
CDC	Centers for Disease Control and Prevention
CDW	Corporate Data Warehouse
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CMV	cytomegalovirus
CNS	central nervous system
CPD	continuing professional development
CPUT	Cape Peninsula University of Technology
CRC	colorectal cancer
CSF	cerebrospinal fluid
CSIR	Council for Scientific and Industrial Research
CVD	cardiovascular disease
DST	drug susceptibility testing
EQA	external quality assurance/assessment
EU	European Union
DGGE	denaturing gradient gel electrophoresis
DGM	Dr George Mukhari Hospital
DST	Department of Science and Technology
EID	early infant diagnosis
ESBL	extended-spectrum beta-lactamase
FA F	anconi's anaemia
FBC	full blood count
FISH	fluorescence in situ hybridisation
FNA	fine needle aspiration
GC-MS	gas chromatography-mass spectrometry
GEMP	graduate entry medical programme
GERMS-SA	Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa
GSH	Groote Schuur Hospital
HA	haemophilia A
HAART	highly active antiretroviral therapy
HBV	hepatitis B virus
HBC	hepatitis C virus
HEU HIV	exposed uninfected
HHV	human herpesvirus
HLA	human leucocyte antigen
hMPV	human metapneumovirus
HPV	human papillomavirus
HVTN	HIV Vaccine Trials Network
IALCH	Inkosi Albert Luthuli Central Hospital
ICU	intensive care unit
IMD	inherited metabolic disease
IPC	infection prevention and control

IRMA	immunoradiometric assay
KEH	King Edward VIII Hospital
KIDCRU	Children's Infectious Diseases Clinical Research Unit
LTI	Laboratory for Tissue Immunology
MDR-TB	multidrug-resistant tuberculosis
MIC	minimum inhibitory concentration
MGIT	mycobacterium growth indicator tube
MLPA	multiplex ligation-dependent probe amplification
MRC	Medical Research Council
MRSA	methicillin-resistant Staphylococcus aureus
MSSA	methicillin-susceptible Staphylococcus aureus
NAAT	nucleic acid amplification test
NIAID	National Institute of Allergy and Infectious Disease
NICD	National Institute for Communicable Diseases
NIH	National Institutes of Health
NRF	National Research Foundation
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PRF	Poliomyelitis Research Foundation
QF-PCR	quantitative fluorescent polymerase chain reaction
RA	rheumatoid arthritis
RCCH	Red Cross Children's (Memorial) Hospital
RFLP	restriction fragment length polymorphism
RIA	radioimmunoassay
RSV	respiratory syncytial virus
RT-PCR	real-time polymerase chain reaction
SAAVI	South African AIDS Vaccine Initiative
SABMR	South African Bone Marrow Registry
SADC	Southern African Development Community
SANAS	South African National Accreditation System
SARI	severe acute respiratory infection
SCC	staphylococcal cassette chromosome
SLE	systemic lupus erythematosus
SME	sub-acute measles encephalitis
SNP	single nucleotide polymorphism
STI	sexually transmitted infection
TB	tuberculosis
TMS	tissue microarray analysis
T-RFLP	terminal restriction fragment length polymorphism
UCT	University of Cape Town
UFS	University of the Free State
UKZN	University of KwaZulu-Natal
US	Stellenbosch University
WHO	World Health Organization
Wits	University of the Witwatersrand

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REPUBLIC OF SOUTH AFRICA

RP204/2014
ISBN: 978-0-621-42903-9
Title of Publication: National Institute for Communicable Diseases (NICD)
Annual Review 2013 - 2014