NHLS

NICD Annual Review 2012-13

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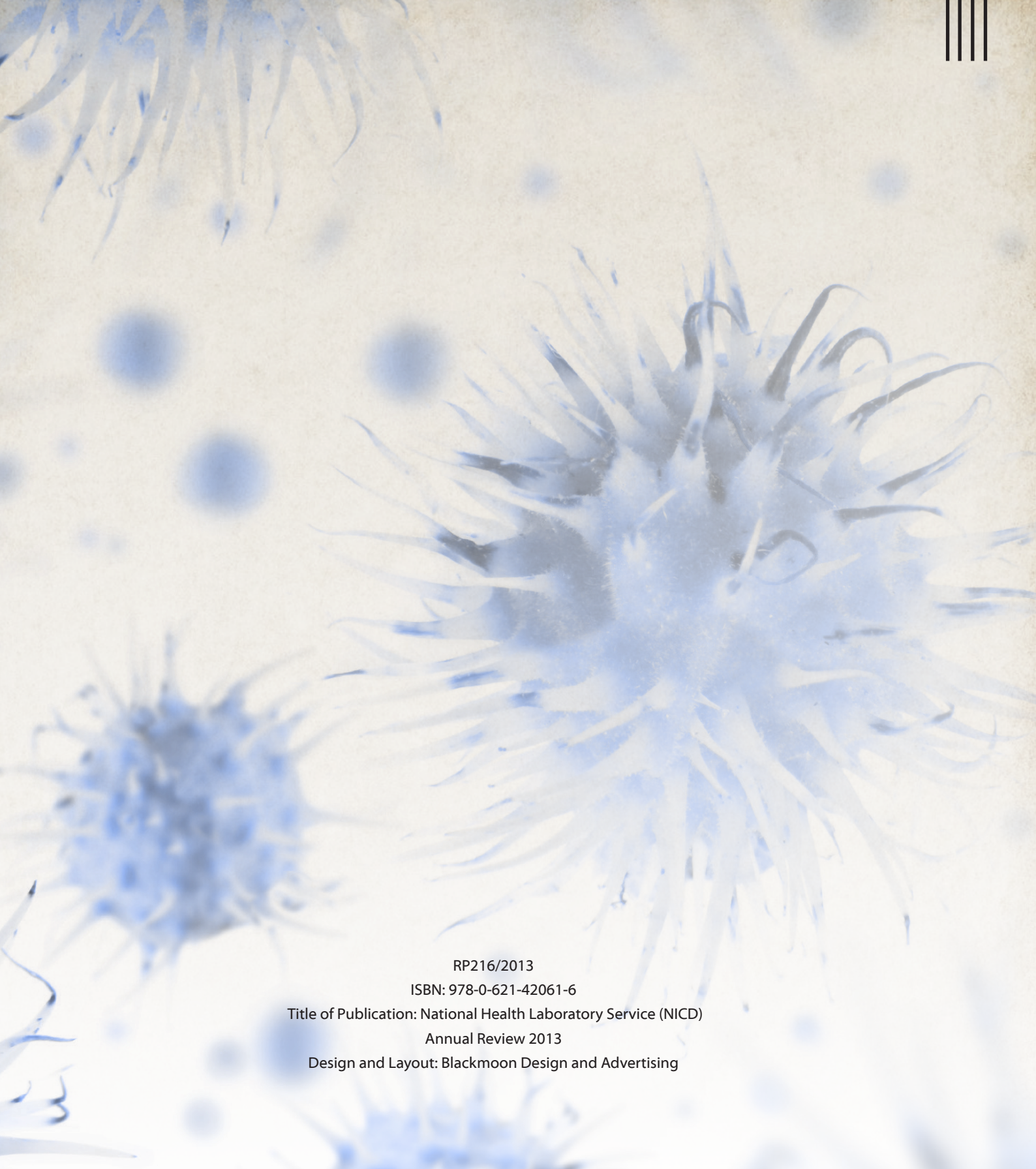
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service



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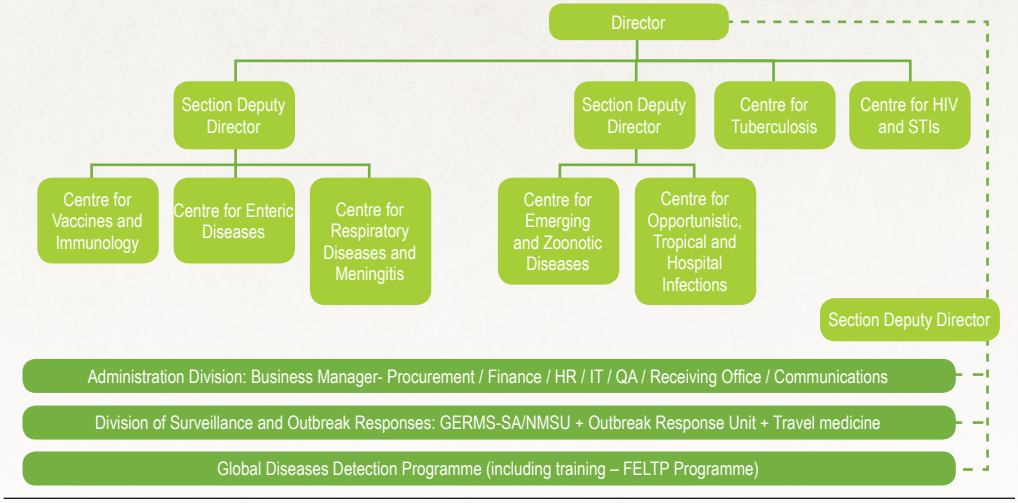
Professor Shabir Madhi
Executive Director

Although increasingly focused on its public health orientation, NICD nevertheless continues its pursuit of research excellence and its quest to be a resource for training not only South Africans but also scientists from other African countries.

The National Institute for Communicable Diseases (NICD) is internationally recognised as an important public health resource in South Africa and, led by local scientists and public health specialists, is unique in Africa. During the past year, it has consolidated the restructuring initiated in 2011 and is now organised around seven thematic centres and a division for surveillance and outbreak response (see Figure 1).

The NICD is also aggressively pursuing the recruitment of epidemiologists to complement the laboratory-based expertise in communicable diseases for which it has become renowned. The expansion of activities aims to entrench NICD in comprehensively servicing the communicable diseases agenda in South Africa's public health arena.

Figure 1: The NICD organogram



As part of the restructuring, it is expected that each centre will develop a portfolio of activities spanning basic science research, laboratory-based surveillance, molecular characterisation of infectious pathogens, and field epidemiology.

Although increasingly focused on its public health orientation, NICD nevertheless continues its pursuit of research excellence and its quest to be a resource for training not only South Africans but also scientists from other African countries. The groundbreaking science at NICD was illustrated by research published in *Nature Medicine* during 2012. The study led by Dr Penny Moore and Professor Lynn Morris provided insights into new potential targets for the development of an HIV vaccine, which has remained elusive to scientists globally.

Centres at NICD have also been involved in measuring the impact on improving child health through the introduction of life-saving vaccines, such as those for rotavirus and pneumococcal conjugate, since they were launched in South Africa's public immunisation programme. These studies will inform not only assessments of the health gains but also the future planning of interventions aimed at reducing mortality among children under five years of age.

In addition, NICD has initiated a national survey of drug-resistant (including multidrug-resistant) tuberculosis (TB). Samples from approximately 160,000 individuals are to be screened to determine the prevalence of TB associated with drug resistance. This will help in tailoring empirical treatment regimens, and will also provide a benchmark against which newer interventions may be evaluated, such as improvement in TB diagnosis using the GeneXpert platform – a cartridge-based, automated diagnostic test. The training offered at NICD includes the two-year Field Epidemiology and Laboratory Training Programme (FELTP), which attracts postgraduate students from across Africa and is expected to produce epidemiologists with specialised skills and a focus on communicable diseases. Such new expertise will further strengthen public health surveillance of communicable diseases in South Africa.

As a World Health Organization (WHO) regional reference centre, NICD continues to service other African countries by providing information on or assistance with diagnostics, training and quality assurance. Furthermore, NICD is home to the only level 4 biosafety laboratory in Africa, placing it in a strategic position to deal with established and emerging highly communicable, infectious disease threats in South Africa and the region.

The 2013/2014 period will witness further expansion of NICD at national level, which in part has been made possible through a grant from government, aimed at enabling the institute to take ownership of laboratory-based and active surveillance for communicable diseases in South Africa.

This expansion will assist NICD in contributing to the agenda of establishing an efficient National Health Insurance (NHI) programme to help improve the lives of all South Africans. Together with the talent of our personnel, we relish the task ahead and look forward to raising our status further as an internationally acclaimed institute.

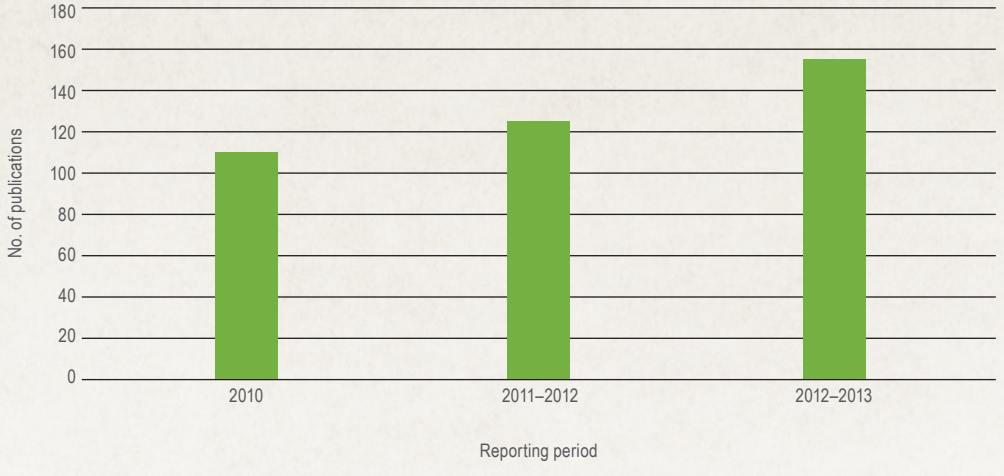
Samples from approximately **160,000** *individuals are to be screened*

The total number of publications generated by public health surveillance and research studies undertaken at the NICD has increased by 37% over the past three years.

There were 155 publications in the current 12-month reporting period (2012-2013) compared with 113 publications in 2010. This is reflected by the year-on-year increase in the average number of publications per 12-month time period as shown in Figure 2.

Note: The 2011-2012 reporting period was from 1 January 2011 to 31 March 2012 (15 months) and the 158 publications from this period were therefore adjusted to 126 in order to reflect a 12-month reporting period, compatible with the duration of the other two reporting periods.

Figure 2: The number of publications generated by NICD staff for three consecutive 12-month reporting periods



Professor Janusz Paweska
Head

CEZD supports South Africa's commitment to International Health Regulations.

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 such as bacteria. This results in a direct cost of US$20 billion to the affected economies (World Bank, 2010). Zoonotic pathogens continue to emerge; the effects of climate change, urbanisation and farming practices, coupled with increasing travel and trade, are shared drivers globally for infectious diseases.

65%
of emerging infectious diseases in the past 60 years have been attributed to zoonotic agents

Some have special relevance to Africa, including extensive human–wildlife interaction, increased land-usage and current socio-economic conditions. The public health burden of zoonotic agents in the South African context is still poorly understood. The Centre for Emerging and Zoonotic Diseases (CEZD) renders diagnostic expertise and investigatory capacity on highly dangerous bacterial and viral pathogens associated with zoonotic disease in South Africa and on the African continent.

The CEZD aims to function as a resource for knowledge and expertise to the South African government, the countries in the Southern African Development Community (SADC) and elsewhere on the African continent, in order to assist in planning relevant policies and programmes and to harness innovation in science and technology to support surveillance, detection and outbreak response systems. In observing this goal, the CEZD supports South Africa's commitment to International Health Regulations.

Surveillance and diagnostic services

The laboratories of CEZD test viral haemorrhagic fevers (VHFs), arboviral disease, human rabies and rabies-related infections. The laboratory is accredited for a range of diagnostic tests with the South African National Accreditation System (SANAS). In addition, in 2012 the laboratory obtained approval from the Department

of Agriculture, Forestry and Fisheries (DAFF) for tests for Rift Valley fever, Crimean-Congo haemorrhagic fever and rabies. To provide the diagnosis and perform research on class 3 and 4 viral pathogens, the laboratory manages high and maximum biocontainment facilities.

laboratory for human diagnostics in South Africa. The transmission electron microscope is an integral tool in the diagnostic arsenal of CEZD, not only in screening for viral pathogens undetected by molecular methods, but in revealing the presence of certain bacteria

Scientists working in the biosafety level 4 laboratory at CEZD, NICD

The biosafety level 4 (BSL4) facility was commissioned and certified after extensive refurbishment and upgrading in May 2011. In June 2012, the facility was decommissioned for routine maintenance, constituting the first successful shut-down period for the laboratory. The facility was recommissioned in December 2012. This facility is the only positive-pressure-suit maximum biocontainment laboratory on the continent.

In addition, CEZD is tasked with the laboratory confirmation and investigation of anthrax, plague, leptospirosis, cat scratch disease (*Bartonella* infection) and botulism. The laboratory operates a BSL3 facility and is accredited with DAFF and SANAS. CEZD drives a regional surveillance project for plague (previously the RATZOOMAN project) and is recognised as the anthrax and plague reference

(particularly obligate intracellular bacteria) and in identifying larger pathogens such as microsporidia. The electron microscope laboratory is also SANAS-accredited.

Current research and surveillance

Genotyping and epidemiology of Bacillus anthracis in South Africa

NICD researcher: Dr J Rossouw

Collaborators: Dr W Beyer (University of Hohenheim, Stuttgart, Germany) and Dr H van Heerden (University of Pretoria)

Subtyping of *Bacillus anthracis,* the etiologic agent of anthrax, is problematic owing to its being one of the most genetically homogeneous pathogens. Modern molecular

strain typing techniques, such as multiple-locus variable number tandem repeat analysis (MLVA), are making it possible to distinguish between outbreak strains, to trace the origin of an outbreak strain back, and to track the routes of transmission of an outbreak strain within and among animal populations. In this study, a 31 loci MLVA was used to study the genotypic diversity of southern African *B. anthracis* isolates (collected 1960 to 2013) in relation to the spatial and temporal dynamics behind the spread of the disease and possible relationships between genotype and host species.

Sindbis fever in South Africa, 2006–2010

NICD researchers: N Storm, Dr J Weyer, PA Leman, A Kemp, V Dermaux-Msimang, Professor JT Paweska

Collaborators: Professors W Markotter and LH Nel (University of Pretoria, Viral Zoonosis Group)

Sindbis virus (SINV) is an avian mosquito-borne virus. Sindbis cases are confirmed in South Africa annually. The study provided basic descriptive epidemiology for Sindbis fever cases that were laboratory confirmed during 2006–2010 based on retrospective data analysis.

Although SINV infection is generally considered to cause mild and self-limiting disease, it is noteworthy that the patients in this study did seek medical consultation and testing. It may be suggested that SINV infection be considered a differential diagnosis for mild flu-like illness, especially during periods of increased mosquito activity.

Human Wesselsbron disease cases, South Africa 2010–2011

NICD researchers: Dr J Weyer, Dr J Thomas, PA Leman, AA Grobbelaar, A Kemp, Prof JT Paweska

Wesselsbron disease is a neglected, mosquito-borne zoonotic infection reported from Africa. The disease primarily affects sheep and other ruminants with incidental spillover to humans. This study reports on the clinical histories of two human cases of Wesselsbron disease that were laboratory confirmed during the 2010/2011 Rift Valley fever outbreak investigation in South Africa. This report describes the first confirmed human cases of Wesselsbron disease since 1996.

Surveillance for zoonotic pathogens in South African bats

NICD researchers: Professor JT Paweska, Dr P Jansen van Vuren, Dr J Weyer, A Grobbelaar, A Kemp

Collaborators: Professor W Markotter (University of Pretoria, Viral Zoonosis Group, Department of Microbiology and Plant Biology)

The CEZD in collaboration with the Microbiology Department of the University of Pretoria is conducting research on bat-borne viral zoonotic pathogens in South African bat populations. Bats are increasingly being implicated as hosts of zoonotic pathogens of potential public health importance, including paramyxoviruses, rabies-related viruses, filoviruses and coronaviruses. Serological results of this study suggest the presence of rabies-related viruses and filoviruses in cave-dwelling fruit and insectivorous bats in

Limpopo province. Intensive monthly sampling of the affected bat roost is ongoing, to understand better the seasonality and ecology of these pathogens in bat populations.

Teaching and training

In addition to extensive in-house training of staff in specialist techniques and the requirements for working in BSL3 and BSL4 biocontainment facilities, the CEZD contributes to human resource capacity development by supporting postgraduate studies in the fields of medical microbiology, medical virology and public health through collaborative projects with South African universities as well as universities elsewhere in the world. In addition, the CEZD is involved in training microbiology and clinical pathology registrars, intern scientists and technologists on an ongoing basis. International scientists frequent the laboratories for specialist diagnostic training, for training related to working in biocontainment facilities and for collaborative research projects. 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) on the dissection and storage of rodent organs for plague surveillance purposes.

A regional workshop on the laboratory diagnosis of emerging and dangerous pathogens was held on 10–14 September 2012. The workshop was sponsored by the World Health Organization as part of the activities of the Emerging and Dangerous Pathogens Laboratory Network (EDPLN). The delegates represented 11 countries from all over Africa and focused on the establishment of diagnostic capacity for the haemorrhagic fever viruses in the countries where these diseases occur.

(left) Donning BSL3 personal protective equipment while preparing to enter the cave containing a large *Rousettus aegyptiacus* roost in Limpopo Province

(right) Bats are captured and screened for the presence of a number of pathogens. The bats are caught while exiting their cave habitat

Professional development

Eight MSc students and one PhD student who were supervised or co-supervised by CEZD staff graduated during the review period.

A CEZD staff member completed the BTech (Quality) qualification at the University of South

Africa (UNISA) in 2012. One CEZD staff member registered for a BTech Biomedical Technology at the Technical University of Pretoria. One CEZD staff member registered for a BSc (Microbiology with Business Management) at UNISA.

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 15 papers in peer-reviewed journals and one book chapter during 2012/2013. The top five publications from this list are as follows.

Bats' susceptibility to Marburg virus

Paweska JT, Jansen van Vuren P, Masumu J, Leman PA, Grobbelaar AA, Birkhead M, Clift S, Swanepoel R, Kemp A. Virological and serological findings in *Rousettus aegyptiacus* experimentally inoculated with Vero cells-adapted Hogan strain of Marburg virus. *PLoS One* 2012; 7(9): e45479. doi:10.1371/journal.pone.0045479.

Synopsis: Filoviruses cause severe to fatal hemorrhagic fever in humans and non-human primates in Africa. The Egyptian fruit bat, *Rousettus aegyptiacus*, is currently regarded as a potential reservoir host for Marburg virus (MARV). This work confirms the susceptibility of *R. aegyptiacus* to infection with MARV and contributes to establishing a bat–filovirus experimental model. Further studies are required to uncover the mode of MARV transmission, and to investigate the putative role of *R. aegyptiacus* as a reservoir host.

Bartonella

Trataris-Rebisz AN, Arntzen L, Rossouw J, Karsteadt A, Frean J. *Bartonella henselae* and *Bartonella quintana* seroprevalence in HIV

Participants in the regional workshop on the Laboratory Diagnosis of Emerging and Dangerous Pathogens receive training in handling specimens in a Class III biosafety cabinet in a mock BSL3 laboratory setting

positive, HIV-negative and clinically healthy volunteers in Gauteng province, South Africa. *Annals of the Australasian College of Tropical Medicine* 2012; **13**: 10–12.

Synopsis: *Bartonella* is a genus of opportunistic, Gram-negative bacilli transmitted from animals to humans.

Bartonellae are emerging pathogens that can cause a variety of clinical manifestations in both immunocompromised and healthy persons. In this study, a cross-sectional survey was carried out in HIV-positive outpatients at the Chris Hani Baragwanath Hospital HIV clinic, sera selected retrospectively from HIV-negative antenatal clinic patients, and clinically healthy volunteers.

A high rate of IgM (antibodies) seropositivity in seemingly healthy subjects in this study could be attributed to asymptomatic infections or suggest a low specificity of the IFA in clinically non-infected persons as previously reported elsewhere. Nevertheless, the high seropositivity in the immunocompromised cohort is comparable to rates reported in other studies.

Further study is needed to elucidate the role of Bartonella as an opportunistic infection that can potentially cause severe illness in immunocompromised patients.

Wesselsbron disease

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.

Synopsis: The paper reports on the clinical histories of two human cases of Wesselsbron disease that were laboratory confirmed during the 2010/2011 Rift Valley fever outbreak investigation in South Africa.

This report describes the first confirmed human cases of Wesselsbron disease since 1996. Molecular sequencing and analysis of the partial NS5 gene of the Wesselsbron genome were used to identify two circulating clades of the virus in southern Africa.

Clade I included isolates collected from South Africa and Zimbabwe, whereas clade II included isolates only from the KwaZulu-Natal province of South Africa. As for other arboviral diseases in Africa, little or no active surveillance is conducted, and the public and veterinary health burden of this disease remains unclear.

Rift Valley fever

Métras R, Porphyre T, Pfeiffer DU, Kemp A, Thomson P, Collins LM, White RG. Exploratory space-time analyses of Rift Valley fever in South Africa in 2008–2011, *PLoS Neglected Tropical Diseases* 6(8): e1808.

Synopsis: The factors explaining the spread of Rift Valley fever (RVF) in domestic livestock during an epidemic are attributed to short- and long-distance mechanisms, including active and passive vector dispersal and movements of infectious animals. However, the scarcity of data makes it difficult to identify and quantify these mechanisms.

2.5%
of the bat colony is actively infected

We have generated hypotheses on the possible mechanisms involved in RVF spread in South Africa between 2008 and 2011. We used descriptive statistics and estimated the space–time interactions as an indicator of the underlying transmission process. Our results confirmed the presence of an intense, short, initial transmission process that could be attributed to active vector dispersal, plus a second transmission event of lower intensity and greater spread that may have resulted from the movements of infectious animals, vector dispersal by wind or artificial vehicle, or emergence of other viral foci. Further data collection and modelling tools are required to confirm these hypotheses

Marburg virus

Amman BR, Carroll SA, Reed ZD, Sealy TK, Balinandi S, Swanepoel R, Kemp A, Erickson BR, Comer JA, Campbell S, Cannon DL, Khristova ML, Atimnedi P, Paddock CD, Kent Crockett RJ, Flietstra TD, Warfield KL, Unfer R, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Rollin PE, Ksiazek TG, Nichol ST, Towner JS. Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. *PLoS Pathogens* 2012; **8**(10): e1002877.

Synopsis: Marburg virus, like its close relative Ebola virus, can cause large outbreaks of haemorrhagic fever with case fatalities nearing 90%. For decades the identity of the natural reservoir was unknown. However, in 2007, Marburg viruses were isolated directly from Egyptian fruit bats *(Rousettus aegyptiacus)* that inhabited a Ugandan gold mine where miners had previously been infected. Soon after, two tourists became ill with Marburg virus after visiting the Python Cave, a popular attraction in Uganda's Queen Elizabeth National Park and less than 40 km from the mine. Python Cave is also home to a large colony of *R. aegyptiacus* bats.

These events prompted a long-term investigation of Python Cave to determine whether *R. aegyptiacus* in the cave carried infectious Marburg virus genetically similar to that found in the tourists, and what ecological factors might lead to transmission of the virus to humans. In the study, we found that approximately 2.5% of the bat colony is actively infected at any one time and that virus isolates from bats are genetically similar to those from the infected tourists. We also found that specific age groups of bats, juveniles of around six months of age, are particularly likely to be infected at specific times of the year. These times coincide approximately with historical dates of emergence of Marburg virus in humans.

Other publications

Journal articles

Fafetine JM, Jansen van Vuren P, Paweska JT. Comparison of a recombinant nucleocapsid IgG indirect ELISA with an IgG sandwich ELISA for the detection of antibodies to Rift Valley fever virus in small ruminants. *Vector-Borne and Zoonotic Diseases* 2012. doi:10.1089/vbz.2012.1006.

Fafetine J, Neves L, Thompson PN, Paweska JT, Rutten VPM, Coetzer JAW. Serologic evidence of Rift Valley fever virus circulation in sheep and

goats in Zambézia Province, Mozambique. *PLoS Neglected Tropical Diseases* 2013; **7**(2): e2065.

Frean J, Perovic O, Fensham V, McCarthy K, Von Gottberg A, De Gouveia L, Poonsamy B, Dini L, Rossouw J, Keddy K, Alemu W, Yahaya A, Pierson A, Dolmazon V, Cognat S, Ndihokubwayo JB. External quality assessment of national public health laboratories in Africa, 2002–2009. *Bulletin of the World Health Organization* 2012; **90**: 191–199A.

Mapaco LP, Coetzer JAW, Paweska J, Venter E. An investigation into an outbreak of Rift Valley fever on a cattle farm in Bela-Bela, South Africa, in 2008. *Journal of the South African Veterinary Association* 2012. doi:10.4102/jsava.v83i1.132.

Kuhn JH, Bao Y, Bavari S, Becker S, Bradfute S, Brister JR, Bukreyev AA, Chandran K, Davey RA, Dolnik O, Dye JM, Enterlein S, Hensley L, Honko AN, Jahrling PB, Johnson KM, Kobinger G, Leroy EM, Lever MS, Mühlberger E, Netesov SV, Olinger GG, Palacios G, Patterson JL, Paweska JT, Pitt L, Radoshitzky SR, Saphire EO, Smither SJ, Swanepoel R, Towner JS, van der Groen G, Volchkov VE, Wahl-Jensen V-W, Warren T, Weidmann M, Nichol ST. Virus nomenclature below the species level: a standardised nomenclature for natural variants of viruses assigned to the family *Filoviridae*. *Archive Virology* 2012. doi: 10.1007/s00705-012-1454-0.

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. doi 10.1007/s00705-012-1594-2.

Lagerqvist N, Moiane B, Bucht G, Neves L, Paweska JT, Lundkvist Å, Falk KI. Stability of a formalin-inactivated Rift Valley Fever vaccine; evaluation of a vaccination campaign in Mozambican cattle. *Vaccine* 2012; **30**(46): 6534–6540.

Rweyemamu M, Kambarage D, Karimuribo E, Wambura P, Matee M, Kayembe JM, Mweene A, Neves L, Masumu J, Kasanga C, Hang'ombe B, Kayunze K, Misinzo G, Simuunza M, Paweska JT. Development of a One Health National Capacity in Africa: the Southern African Centre for Infectious Disease Surveillance (SACIDS) One Health Virtual Centre Model. *Current Topics in Microbiology and Immunology* 2012. doi:10.1007/822012244.

Rweyemamu M, Mmbuji P, Karimuribo E, Paweska JT, Kambarage D, Neves L, Kayembe J-M, Mweene A, Matee M. The Southern African Centre for Infectious Disease Surveillance: A One Health Consortium. *Emerging Health Threats Journal* 2013; **6**: 19958-http://dx.doi.org/10.3402/ehtj.v6i0.19958.

Sabeta C, 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. *Journal of the South African Veterinary Association* 2013; **84**(1): Art.#923, 1-5 http://dx.doi.org/10.4102.v84i1.923.

Samudzi RR, Leman PL, Paweska JT, Swanepoel R, Burt FJ. Bacterial expression of Crimean-Congo haemorrhagic fever virus nucleoprotein, and its evaluation as a diagnostic reagent in an indirect ELISA. *Journal of Virological Methods* 2012; **179**: 70–76.

Scott T, Paweska J, Arbuthnot P, Weinberg MS. Pathogenic effects of Rift Valley fever virus NSs gene are alleviated in cultured cells by expressed antiviral short hairpin RNAs. *Antiviral Therapy* 2012; **17**: 643–656.

Uejio CK, Kemp A, Comrie AC. Climatic controls on West Nile virus and Sindbis virus transmission and outbreaks in South Africa. *Vector Borne Zoonotic Di*seases 2012; **12**(2): 117–125.

Chapters in books

Weidmann M, Abad FX, Paweska JT. Rift Valley fever virus: a promiscuous vector borne virus. In: Elschner M, Cutler S, Weidmann M, Butaye P, eds. *BSL3 and BSL4 Agents: Epidemiology, Microbiology, and Practical Guidelines*, Wiley-VCH Verlag KGaA, 2013: 263–272.

Conference presentations

International: 4.

A total of seven full-length conference papers were presented during 2012.

Dr Karen Keddy

Dr Nicola Page

Centre Heads

Enteric diseases

Data from the South African Health Review 2012/2013 have revealed that the mortality rate of childen under the age of five years has declined rapidly since 2009 and 2011, which is in part attributed to the successful implementation of the HIV-Prevention of Mother to Child Treatment (PMTCT) programme and the introduction of the pneumococcal and rotavirus vaccines into the national immunisation programme.

While these successes should be celebrated, child mortality rates in the poorest quintile are still four times higher than in the wealthiest quintile of the population. The majority of the deaths among the poorest quintile (82.6%) are due to five conditions in children between the ages of one month and one year of age, with half of these children exposed to or infected with HIV. Diarrhoeal diseases are responsible for 20.7% of these deaths, of which 60% are associated with malnutrition.

The Centre for Enteric Diseases (CED) was established in 2012 through the amalgamation of the Enteric Diseases Reference Unit and the Viral Gastroenteritis Unit of NICD. The centre is responsible for developing strategies to combat diarrhoeal diseases in South Africa.

It monitors trends in diarrhoeal pathogen incidence, the impact of rotavirus vaccine on the clinical and molecular epidemiology of rotavirus-associated diarrhoea and also identifies areas for the introduction of additional interventions and evaluation of future diarrhoeal pathogen vaccines.

Surveillance activities

The centre focused much of the 2012/2013 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.

The centre provides regular reports to the Department of Health (DoH), including weekly reports on outbreak-prone enteric pathogens, and monthly reports to the National Outbreak Response Team (NORT).

In addition the centre engaged the Department of Agriculture, Forestry and Fishing (DAFF) on precautionary measures required for humans when administering a new live Salmonella vaccine to chickens and in reviewing current updated research protocols for the Water Research Commission.

The centre expanded the surveillance offerings by including detection assays for bocavirus and Campylobacter surveillance and incorporated the Red Cross Children's Hospital into the rotavirus sentinel surveillance programme. Almost 3,000 specimens, collected as part of the rotavirus sentinel surveillance programme between 2009 and 2012, have been screened for human bocavirus infections, with 6.4% testing positive.

Members of the centre were trained in methods of Campylobacter detection at the Health Protection Agency, London, UK, and the assays are currently being reviewed and validated.

The centre has been actively monitoring the impact of the rotavirus vaccine in the South African expanded programme of immunization (EPI), and current estimates show that rotavirus disease was reduced by 50% in children under five years between 2009 and 2011, with the greatest reductions (up to 64%) seen in children under one year of age.

3,000

specimens, collected as part of the rotavirus sentinel surveillance programme

The centre participated in the investigation of outbreaks in Eastern Cape, Mpumalanga, Gauteng, Western Cape, Free State and Limpopo. The diarrhoeal pathogens implicated in these outbreaks ranged from *Samonella typhimurium, Salmonella hadar, Shigella* spp, typhoid fever and cholera to rotavirus, norovirusgenogroup II, sapovirus and astrovirus.

Current research and surveillance projects

The development of real-time detection techniques and increased surveillance of diarrhoeal disease viruses in the South African population (2009–2014)

NICD researchers: Dr N Page, Ms S Nadan, Mr R Netshikweta.

Funding: Poliomyelitis Research Foundation (PRF).

Elucidating the diarrhoeal disease burden in South Africa can be achieved only if researchers understand the cause of diarrhoeal episodes. The aim of the project was to develop and use real-time detection techniques to identify enteric viruses, other than rotavirus, and to determine the contribution to the overall diarrhoea burden.

Stool samples collected through the sentinel rotavirus surveillance programme between 2009 and 2012 were screened for norovirus, astrovirus, adenovirus, bocavirus and sapovirus. Plans are to genotype adenovirus, bocavirus and other enteric virus strains and to develop detection assays for enteroviruses.

Typhoid fever surveillance in sub-Saharan Africa: burden of disease study

Principal Investigator: (NICD) Karen H Keddy (Centre for Enteric Diseases)
Site coordinators, Edendale: S Haffejee (microbiology), H Dawood (adult medicine), F Naby (paediatric medicine)
Co-investigators: (NICD) A Sooka (Centre for Enteric Diseases)
Investigators: (IVI) F Marks (epidemiologist), J Im (scientist)

The lack of credible data on typhoid fever in many African countries has also limited awareness of the disease among clinical and public health providers. Fever-related diseases are mostly diagnosed based on clinical signs and symptoms. As a result, typhoid fever may be indistinguishable from other serious infections common to Africa such as malaria, tuberculosis and infection due to invasive non-typhoidal Salmonella. This is an international bacteraemia study, to identify which pathogens cause illness in 10 sentinel sites in Africa in patients presenting with fever.

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

Project leader: KH Keddy
(Centre for Enteric Diseases)
Project manager: Ms A Sooka
(Centre for Enteric Diseases)

The centre does laboratory-based surveillance and characterisation of bacterial enteric disease in South Africa; specifically, on all human isolates from diagnostic microbiology laboratories. The case definition for the laboratory-based surveillance includes all *Salmonella, Shigella, Vibrio cholerae* (01 and non-01) and enterohaemorrhagic *Escherichia* coli isolates from all body sites, and diarrhoeagenic *E. coli* isolates from stool only.

The case definition for enhanced surveillance isolates includes only those *Shigella* and *Salmonella enterica* isolates that are from normally sterile body sites in "in-patients" only – that is, the patient should have been admitted to the hospital or enhanced surveillance site. In addition the unit undertakes to serotype *Salmonella, Shigella* and diarrhoeagenic *E. coli* (DEC) isolates for commercial purposes. Regular reports on the isolates received are extracted from the database for the purpose of information sharing. Molecular methods may be used to establish strain relatedness in outbreaks.

PulseNet Africa

NICD researchers: KH Keddy, AM Smith, H Ismail, N Tau, MA Mthanti
Collaborators: P Gerner-Smidt (CDC, USA), PulseNet Africa member countries.

PulseNet is an international molecular sub-typing network for food-borne and waterborne disease surveillance. Pulsed-field gel electrophoresis (PFGE) analysis of bacteria is the primary sub-typing technique used by PulseNet. The network consists of national and regional laboratory networks dedicated to tracking food-borne and waterborne infections worldwide. The Centre for Enteric Diseases,

National Institute for Communicable Diseases is the coordinating laboratory for PulseNet Africa, with member countries including South Africa, Kenya, The Gambia, Senegal, Cameroon, Malawi, Tanzania, Cote d'Ivoire, Ghana, Uganda and Mozambique. The resulting surveillance provides global early warning, detection and investigation of foodborne/waterborne disease outbreaks, emerging pathogens and acts of bioterrorism. Surveillance data help us to understand the molecular epidemiology of enteric diseases in Africa, including diseases such as cholera and typhoid fever. The data are important for preventative strategies planning, such as vaccine development and implementation. Our current PulseNet Africa database has a collection of PFGE patterns for ~2,500 enteric pathogen isolates.

Probable common source for *Salmonella enteritidis* strains isolated from humans with gastroenteritis and from captive wild animals in South Africa

NICD researchers: AM Smith, H Ismail, KH Keddy
Collaborators: MM Henton (Idexx Laboratories, Johannesburg).

Salmonella is well recognised as an aetiological agent of gastrointestinal and diarrhoeal disease. *Salmonella enteritidis* is one of the commonest serotypes associated with food-borne illness. In South Africa, we compared *Salmonella enteritidis* strains isolated from humans with gastroenteritis and strains isolated from captive wild animals, from June 2011 to July 2012. Animal isolates from five different sources were investigated. With the exception of an isolate from a ground hornbill, all animal isolates (jaguar, crocodile, lion and poultry) showed PFGE pattern matches to a human isolate. Animal isolates showed susceptibility to all antimicrobials tested, with the exception of nalidixic acid resistance in isolates from the lion and poultry source. Our data showed similarities between *Salmonella enteritidis* strains isolated from humans and captive wild animals. We suggest that the consumption of contaminated poultry products is the common link and source of related human and animal strains.

Teaching and training

The centre assisted in the training supervision of Field Epidemiology and Laboratory Training Programme (FELTP) students. The centre offers both short and long courses to microbiology registrars from South African universities in specialised techniques that are relevant for the identification of enteric pathogens. The centre assisted in the training of experiential biomedical technology students from the University of Johannesburg.

Research outputs/ publications

The centre's staff authored/co-authored 11 peer-reviewed publications during 2011/2012. A synopsis of the five key manuscripts follows.

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.

Synopsis: The paper gives an overview of rotavirus disease in the South African context, describing clinical features, transmission, seasonality and age distribution. The paper goes on to discuss laboratory detection and the genetic diversity of rotavirus strains at the Dr George Mukhari Hospital since 1983. The paper further gives a summary of the rotavirus vaccine trials conducted in South Africa, the economic burden assessment and a preliminary analysis of the vaccine impact on the burden of rotavirus disease.

Keddy KH, Sooka A, Crowther-Gibson P, Quan V, Meiring S, Cohen C, Nana T, Sriruttan C, Seetharam S, Hoosen A, Naicker P, Elliott E, Haffejee S, Whitelaw A, Klugman KP; for the Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA). Systemic shigellosis in South Africa. *Clinical Infectious Diseases* 2012; **54**(10): 1448–1454. Epub 2012 Apr 3.

Synopsis: The paper gives an overview of invasive shigellosis in South Africa and highlights the importance of HIV infection, particularly in older girls and adults, in disease and the associated mortality in these patients. This emphasises the importance of the role of educating HIV infected individuals who may be involved in child care in South Africa.

Tau NP, Meidany P, Smith AM, Sooka A, Keddy KH for the Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa. *Escherichia coli* O104 associated with human diarrhea, South Africa, 2004–2011. *Emerging Infectious Diseases* 2012; **18**(8): 1314–1317.

Synopsis: *E. coli* O104 is a recognised, though rare, pathogen in South Africa, which may be of either the enteroaggregative (EAggEC) or enteropathogenic pathotypes. The EAggEC strains show some similarity to the *E. coli* O104 responsible for the recent outbreak in Germany.

Tau NP, Smith AM, Sooka A, Keddy KH for GERMS-SA. Molecular characterisation of extended-spectrum {beta}-lactamase-producing *Shigella* isolates from humans in South Africa, 2003–2009. *Journal of Medical Microbiology* 2012; **61**(Pt 1): 162–164.

Synopsis: ESBL producing *Shigella* in South Africa includes a range of serotypes and ESBL production may be due to a number of different mutations. Certain strains carried more than one ESBL resistant determinant. Strains were all multidrug-resistant.

Lunguya O, Lejon V, Phoba MF, Bertrand S, Vanhoof R, Verhaegen J, Smith AM, Keddy KH, Muyembe-Tamfum JJ, Jacobs J. Salmonella typhi in the Democratic Republic of Congo: fluoroquinolone decreased susceptibility on the rise. *PLoS Neglected Tropical Diseases* 2012; **6**(11): e1921. doi: 10.1371/journal.pntd.0001921. Epub 2012.

Synopsis: Widespread multidrug-resistance and decreased fluoroquinolone susceptibility occurring among *Salmonella typhi* isolates from the Democratic Republic of Congo were demonstrated. Strain relatedness occurred in the majority (72%) of isolates but differed from other African patterns. There is a need for increased microbiological diagnosis and surveillance in African countries, as a prerequisite for rational use of antimicrobials and the development of standard treatment guidelines.

Other publications

Smith AM, Keddy KH, Ismail H, Tau N, Sooka A, Archer BN, Thomas J, Crisp N. Possible laboratory contamination leads to incorrect reporting of *Vibrio cholerae* O1 and initiates an outbreak response. *Journal of Clinical Microbiology* 2012; **50**: 480–482.

Frean J, Perovic O, Fensham V, McCarthy K, von Gottberg A, de Gouveia L, Poonsamy B, Dini L, Rossouw J, Keddy K, Alemu W, Yahaya A, Pierson A, Dolmazon V, Cognat S, Ndihokubwayo J-B. External quality assessment of national public health laboratories in Africa, 2002–2009. *Bulletin of the World Health Organization* 2012; **90**: 156–244.

Gonose T, Smith AM, Keddy KH, Sooka A, Howell V, Jacobs CA, Haffejee S, Govender P. Human infections due to Salmonella Blockley, a rare serotype in South Africa: a case report. *BMC Research Notes* 2012; **5**: 562.

Dlamini NR, Bhamjee A, Levick P, Uniacke E, Ismail H, Smith AM. Spontaneous bacterial peritonitis and pneumonia caused by *Bordetellabronchiseptica. Journal of Infection in Developing Countries* 2012; **6**: 588–591.

Ismail H, Smith AM, Archer BN, Tau NP, Sooka A, Thomas J, Prinsloo B, Keddy KH, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Case of imported *Vibrio cholerae* O1 from India to South Africa. *Journal of Infection in Developing Countries* 2012; **6**: 897–900.

Steele AD, Neuzil KM, Cunliffe NA, Madhi SA, Bos P, Ngwira B, Witte D, Todd S, Louw C, Kirsten M, Aspinall S, Jan van Doorn L, Bouckenooghe L, Suryakiran PV, Han HH. Human rotavirus vaccine Rotarix™ provides protection against diverse circulating rotavirus strains in African infants: a randomised controlled trial. *BMC Infectious Diseases* 2012; **12**: 213.

Presentations

Presentations at international conferences, congresses and scientific meetings: 12 papers were presented at international congresses.

Presentations at national/local conferences, congresses and scientific meetings: 5 papers were presented at congresses in South Africa.

Professor David Lewis
Centre Head

To improve surveillance of HIV incidence, methods are being applied to various surveys and, since this methodology is new to the field in South Africa, optimal methods for analysis are being assessed.

HIV and STIs

The Centre for HIV and Sexually Transmitted Infections (STIs) aims to be a resource of knowledge and expertise in HIV and other regionally relevant STIs to the South African government, Southern African Development Community (SADC) and the African continent. It also aims to assist with planning policies and programmes related to the control and effective management of HIV/STIs, and to be a place of academic excellence in terms of 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 Professor David Lewis (Administrative Centre head, STI Section head), Professor Lynn Morris (HIV Research Section head), Professor Adrian Puren (HIV Sero-Molecular Diagnostics Section head), Professor Caroline Tiemessen (Cell Biology Section head) and Professor Anna-Lise Williamson (HPV Section head).

Surveillance and diagnostic services

HIV prevalence and incidence surveillance

During 2012/2013, the centre supported the 32nd Annual Antenatal HIV-1 Prevalence and HIV Incidence survey of the Department of Health (DoH) and the 3rd South African Prevention of Mother to Child Transmission (PMTCT) effectiveness study at 4–8 weeks post-partum. The latter survey has provided supportive evidence of the reduction of HIV transmission from HIV-infected mothers to their offspring as a result of the national PMTCT programme. To improve surveillance of HIV incidence, methods are being applied to various surveys and, since this methodology is new to the field in South Africa, optimal methods for analysis are being assessed.

A major activity to strengthen the role of HIV surveillance in the centre was undertaken

in collaboration with the United Kingdom's Health Protection Agency (HPA). A senior HIV epidemiologist from the HPA, Dr Alison Brown, and staff from our centre explored the existing national HIV surveillance systems and interviewed personnel from key organisations that play a role in HIV surveillance in South Africa. The results were summarised in a report that outlines the key national HIV surveillance activities, the relationships and interactions among the various key organisations, the nature of the surveillance, the key strengths and challenges and proposals for consideration. The centre and HPA are working towards implementing activities, in phases, to support surveillance at different levels ranging from coordination to development of reports that can be used by the DoH. In addition, the centre provides reference laboratory expert support to other SADC countries undertaking HIV prevalence and incidence surveillance.

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 (WHO) regional HIV drug resistance laboratory. The laboratory has recently extended the scope of testing to include genotyping of dried blood spot specimens in addition to plasma, allowing for surveillance of resistance in paediatric patients. Ongoing surveys of transmitted resistance make use of specimens collected from those young women in their first pregnancy who participate in the annual antenatal clinic survey. Surveys completed in three provinces during the reporting period showed that KwaZulu-Natal and Free State have moderate (5–15%) levels of transmitted resistance for the non-nucleoside reverse transcriptase inhibitor (NNRTI) drug class and low (<5%) levels of transmitted resistance for the protease inhibitor (PI) and nucleoside reverse transcriptase inhibitor (NRTI) drug classes. Gauteng had low (<5%) levels of transmitted resistance for all drug classes. This is the fourth consecutive year in which transmitted NNRTI resistance has been seen in KwaZulu-Natal. The data indicate that, while transmitted HIV drug resistance remains fairly low within South Africa, vigilance is required and ongoing genotypic surveillance is essential to inform the national HIV treatment programme on the likely effectiveness of antiretroviral drug regimens.

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 2012/2013, in collaboration with the national and provincial DoHs, Alexandra Health Centre and NHLS laboratories, the centre undertook aetiological surveillance of three major STI syndromes (male urethritis syndrome, MUS; vaginal discharge syndrome, VDS; and genital ulceration syndrome, GUS), as well as surveillance of gonococcal antimicrobial resistance, in Gauteng (Johannesburg),

Mpumalanga (Nelspruit) and the Northern Cape (Kimberley). Molecular, serological and bacteriological methods were employed to test for a variety of STI pathogens. In 2012, the centre reported the first confirmed gonorrhoea treatment failure with an oral cephalosporin (cefixime) in Africa.

Within South Africa, the DoH's 32nd Annual Antenatal HIV-1 Prevalence and HIV Incidence survey was also modified to include HSV-2 testing in the place of syphilis; the HSV-2 testing was applied to at least four of the provinces surveyed as part of an initial pilot to assess the value of including HSV-2. Within the southern African region, the centre also provided technical assistance to the Namibian Ministry of Health and Social Services with their planned 2013 national STI survey.

HPV surveillance

Specific types of human papillomavirus (HPV) are causally associated with cervical cancer and at least 99% of cervical cancers have detectable HPV DNA. In South Africa the annual number of new cervical cancer cases in the year under review was 5,543 and the number of deaths was 3,027, but these figures are likely to underestimate the burden of disease because the cancer registry in South Africa has not been fully functional for some time. South Africa also has some of the world's highest reported prevalence of HPV in women with normal cytology. Women with persistent HPV infection are at a great risk of progressing to cervical cancer. In 2014, South Africa plans to introduce vaccination against human papillomavirus in quintile one, two, three, and four schools, covering around 520,000 girls. A necessary part of the introduction of the vaccine should be a surveillance programme. The centre obtained funding from the President's Emergency Plan for AIDS Relief (PEPFAR) and Global Disease Detection from the Centers for Disease Control and Prevention (CDC) to support HPV surveillance at community level (family planning clinic attendees) and among women with abnormal Pap smear results attending colposcopy clinics. Centre staff provided technical support to DoH on the relative merits of HPV vaccination through an Expert Working Group that reported to the National Advisory Group on Immunization.

99%

of cervical cancers have detectable HPV DNA

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

The DoH has expanded HIV testing in South Africa in the past three years with well over 15 million individuals tested. A critical component is the quality assurance of testing. PEPFAR-funded quality assurance coordinators conducted 235 on-site monitoring and evaluation visits to assess progress with HIV rapid testing and specifically the introduction of the use of internal quality assurance specimens as part of quality assurance monitoring. Three HIV rapid test kits were awarded the government tender in 2011. A key follow-on activity undertaken by the centre was the post-marketing surveillance of the lots/batches of devices prior to release in testing sites.

HIV external quality assurance (EQA) schemes

Centre staff coordinated the HIV EQA programme for NHLS-participating laboratories. Three NHLS serology surveys were assigned to 181 laboratories and four HIV RNA survey panels, composed of two NHLS-specific regulatory and two international panels, were assigned to 18 participating laboratories. Participation in the schemes is mandatory, and in 2012 a review by the NHLS Quality Assurance department included reporting of both the serology and molecular scheme results as part of the quality improvement processes. 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). This includes the HVTN 073e trial, which incorporated a protein boost designed to elicit better antibody responses. While this resulted in higher binding and neutralising antibody titres, these were not broadly neutralising for primary viruses. However, our 2012 publication in *Nature Medicine* detailing the evolution of broadly neutralising antibodies in infected subjects provides encouraging confirmation that the human immune system has the capacity to develop such antibodies, and suggests new pathways to immunogen design.

Selected current research projects

Correlates of protective immunity to HIV-1: a focus on CCR5 (2002–2018)

NICD researchers: Professor C Tiemessen, Dr M Paximadis, Dr D Schramm, Dr S Shalekoff
Collaborators: Dr N Martinson (PHRU), Dr D Spencer (Right to Care), Dr P Ive (CHRU)
Funding: Poliomyelitis Research Foundation (PRF), SHARP

There is little doubt of the importance of CCR5, an important molecule that serves as a co-receptor for HIV-1 and that has many functions in immune response regulation. Striking ethnic or population differences exist in single nucleotide polymorphism frequencies of CCR5. This study particularly focuses on host genetic variances and on how differently CCR5 is expressed on various immune cells in South African black and Caucasian individuals. These populations have divergent evolutionary histories and so provide interesting models for study to elucidate further the factors contributing to differential CCR5 expression and HIV-1 infection/disease outcome.

Comparison of allele-specific PCR and ultra-deep sequencing for the detection of low-frequency HIV-1 NNRTI resistance mutations (2011–2012)

NICD researchers: GM Hunt, L Morris
Collaborators: Dr A Moorthy (John Hopkins University, USA), Professor A Coovadia (Rahima Moosa Mother & Child Hospital), Professor EJ Abrams (Columbia University, USA), Dr R Strehlau (Rahima Moosa Mother & Child Hospital), Dr L Kuhn (Columbia University,

USA) and D Persaud (John Hopkins University, USA)
Funding: NICHD, Secure the Future Foundation

The levels of HIV drug resistance mutations Y181C and K103N in the blood of 105 subtype C HIV-infected infants who failed single-dose nevirapine prophylaxis for HIV transmission were compared using two ultra-sensitive methods. Significant correlations were seen between allele-specific PCR (AS-PCR) and ultra-deep pyrosequencing for the detection of Y181C. Among the five (1%) Y181C and 11 (2%) K103N discordant samples, the majority (3/5 Y181C and 8/11 K103N) were at very low levels (≤5%), most likely due to stochastic variations in the appearance of each mutant in each specimen tested. AS-PCR thus provides a robust, rapid, and cost-effective method for targeted detection of low-frequency HIV-1 drug-resistant mutants and would be suitable for surveillance purposes.

Sensitivity of drug-resistant HIV-1 isolates to second generation NNRTIs (2011–2013)

NICD researchers: Dr AE Basson, Professor L Morris
Collaborators: Dr C Hoffman (Johns Hopkins University, USA), Dr D Katzenstein (Stanford University, USA)
Funding: PRF

Etravirine (ETR) and rilpivirine (RPV) are second generation NNRTIs with a high genetic barrier to the development of resistance and a mutation profile that only partially overlaps that of efavirenz (EFV) and nevirapine (NVP). Samples from patients failing a standard first-line regimen were tested using an in-house single-cycle HIV-1 phenotypic assay to assess their susceptibility to first- and second-generation NNRTIs. Our data suggest that HIV strains from patients failing EFV or NVP would show sensitivity to ETR/RPV and as such these second-generation NNRTIs would be suitable for use in South Africa.

Neutralising antibody response in the HVTN 073E vaccine trial (2012–2013)

NICD researchers: Dr N Mkhize, Ms T Hermanus, Professor L Morris
Collaborators: Dr G Gray (PHRU), Dr A-L Williamson (UCT), Dr C Williamson (UCT), Dr D Montefiori (Duke University, USA), Dr G Tomaras (Duke University, USA)
Funding: HVTN

HVTN 073E is a phase 1 placebo-controlled study extension to HVTN 073/SAAVI 102, to evaluate the safety and immunogenicity of Novartis Sub C gp140 vaccine with MF59 adjuvant, as a boost following SAAVI DNA-C2 vaccine and SAAVI MVA-C vaccine, in HIV uninfected healthy adult participants in South Africa and the USA. Participants received DNA/MVA with a protein boost (T1/T2), DNA/MVA with a placebo boost (T1/C2), placebo with a protein boost (C1/T2), or double placebo (C1/C2). Neutralising antibody responses against Tier 1 viruses and Tier 2 viruses were consistently stronger in T1/T2. Similarly, binding antibody titres were highest in the protein-boosted group that had been primed with DNA/MVA. These data emphasise the importance of including protein in vaccine regimens to stimulate humoral immunity.

Evolution of an HIV glycan-dependent broadly neutralising antibody epitope through immune escape (2011–2012)

NICD researchers: Dr PL Moore, Mr CK Wibmer, Ms JN Bhiman, Mr M Nonyane, Ms NL Tumba, Dr BE Lambson, Ms N Ranchobe Professor L Morris

Collaborators: Dr ES Gray (Edith Cowan University, Australia), Mr D Sheward (UCT), Mr S Bajimaya (Harvard Medical School, USA), Dr M Abrahams (UCT), Dr L Ping (University of North Carolina, USA), Dr N Ngandu (UCT), Professor Q Abdool Karim (CAPRISA), Professor SS Abdool Karim (CAPRISA), Dr RI Swanstrom (University of North Carolina, USA), Dr MS Seaman (Harvard Medical School, USA), Professor C Williamson (UCT)

Funding: CAPRISA, DST, NIH

This study showed, in two HIV-1-infected individuals who developed broadly cross-neutralising (BCN) antibodies targeting the glycan at Asn332 on the gp120 envelope, that this glycan was absent on the initial infecting virus. This BCN epitope evolved within six months through immune escape from earlier strain-specific antibodies and manifested as a shift of a glycan to position 332. Both viruses that lacked the glycan at amino acid 332 were resistant to the Asn332-dependent BCN monoclonal antibody PGT128, whereas escaped variants that acquired this glycan were sensitive. Analysis of large sequence and neutralization data sets showed the 332 glycan to be significantly under-represented in transmitted subtype C viruses compared to chronic viruses, with the absence of this glycan corresponding with resistance to PGT128.

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

NICD researchers: Professor A Puren, Professor D Lewis, Ms E Cutler, Ms B Singh, Ms V Maseko

Collaborators: Professor B Auvert (INSERM, University of Versailles), Dr D Taljaard (Progressus/CHAPS)

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 laboratory testing as part of this research activity. The male circumcision study has also provided an opportunity to assess the use of HIV incidence assays, such as the limiting antigen avidity index (Lag AI) assays, as an alternative or improved assay compared to the BED capture ELISA. A study of the distribution of HPV infections in penile tissues and foreskin surfaces, as well as the effect of soap-washing on HPV detection, formed the basis of an MSc dissertation.

Molecular detection of polymorphism and mutations in the HSV-2 gene encoding UL23 thymidine kinase associated with acyclovir resistance in Johannesburg, South Africa (2010–2013)

NICD researchers: Dr E Müller, Professor D Lewis, Ms P Magooa

Funding: PRF

Acyclovir (ACV) was added in late 2008 as part of the first-line syndromic management treatment algorithm for genital ulceration

in South Africa. To assess the prevalence of mutations in the viral UL23 gene that codes for thymidine kinase, the main mechanism of ACV resistance, among HSV-2 virions detected in genital ulcer specimens, pre- and post-introduction of ACV, the UL23 gene was amplified and fully sequenced using 254 HSV-2 positive specimens obtained from genital ulcer patients recruited in Johannesburg (2007–2011). Although we identified a number of nucleotide UL23 gene mutations, no evidence was found of known ACV resistance mutations in HSV-2 virions following the addition of ACV as first-line therapy for genital ulceration.

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

NICD researchers: Professor D Lewis, Dr E Müller, F Radebe, V Maseko
Collaborators: Dr K Rebe (Ivan Toms Centre for Men's Health and ANOVA Health Institute), Professor J McIntyre (ANOVA Health Institute), Dr Oscar Radebe (ANOVA Health Institute)
Funding: USAID

The centre, in collaboration with the ANOVA Health Institute, 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. Overall, approximately a quarter of the MSM had at least one of these infections at one of the three anatomical sites. MSM with urethral discharges, attending clinics supported by the ANOVA Health Institute in Cape Town and Johannesburg, were tested for the presence of cefixime resistant *Nesseria gonorrhoeae* in an attempt to ensure correct management of patients and to inform on the spread of cefixime-resistant gonorrhoea within these high-risk populations.

Oral HPV infection in South African men and women recruited for a study on HIV discordant couples (2012–2013)

NICD researchers: Professor A-L Williamson, Dr ZZA Mbulawa
Collaborators: Professor M Hoffman (UCT), Professor D Coetzee (UCT), Professor J Moodley (UCT), Dr D Marais (UCT), Dr L Johnson (UCT)
Funding: PRF, SIDA, CANSA, NRF and MRC

Oral human papillomavirus (HPV) prevalence and factors associated with oral HPV infection were investigated in 221 heterosexually active couples. Oral HPV prevalence was found to be 6.8% in the women and 13.5% in the men. In the women, the risk of oral infection with a specific type was significantly increased if the same type was present in the mouth or genital tract of their partner, or in their own genital tract. In men, the risk of oral infection with a specific type was increased only if the same type was present in the mouth or genital tract of their partner.

Typing of HPV from a cohort of young women from KwaZulu-Natal (2012–2013)

NICD researchers: Professor A-L Williamson, Dr ZZA Mbulawa
Collaborators: XK Mndende (UCT), Professor QA Karim (UKZN)
Funding: PRF, NRF, NHLS Research Trust

There is limited information on the prevalence of HPV and the HPV types infecting young

South African women. This study aimed to determine the HPV type-specific prevalence in 223 young women in a longitudinal study. Cervico-vaginal lavage (CVL) specimens were collected at baseline and quarterly visits from sexually active HIV-negative women from KwaZulu-Natal, South Africa, who were ≤ 30 years of age. The Roche Linear Array HPV Genotyping assay was used to determine HPV types from 434 CVL specimens. HPV prevalence was found to be: 67.3% (150/223) at baseline, 65.1% (69/106) at the second visit, 60.9% (42/69) at the third visit. HPV prevalence was high in this population compared to other studies performed on HIV-negative populations of similar age.

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
- Medical Research Council
- National Health Laboratory Service Research Trust
- National Research Foundation Incentive Funding for Rated Researchers
- National Research Foundation Professional Development Programme
- Poliomyelitis Research Foundation
- Technology Innovation Agency (SHARP funding: South African HIV/AIDS Research and Innovation Platform)
- World Health Organization.

In addition, research collaborators obtained funding to support the centre's activities from the National Institutes of Health, the Agence Nationale de Reserches sur la SIDA et les hépatites virales (collaboration with INSERM at the University of Versailles and Progressus), European Union (via third-party agreement with the University of the Witwatersrand Reproductive Health and HIV Institute), 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), Bill and Melinda Gates Centre for AIDS Vaccine Discovery (CAVD) and the HIV Vaccine Trial Network (HVTN).

Teaching and training

During 2012/2013, the centre undertook a variety of teaching and training activities. It offers a thorough and comprehensive training programme for interns and technologists in line with HPCSA guidelines. The centre trained 10 intern medical scientists and two virology registrars during the review period. In addition, it also took part in the NICD training courses for microbiology registrars. Centre staff trained 1,747 healthcare 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 genito-urinary tract infections were delivered

Centre staff trained **1,747** *healthcare workers and professionals on quality management systems for HIV rapid testing*

to practising doctors and nurses in Cape Town, Johannesburg and Nelspruit. Centre staff undertook attachments varying in length from one week to two months at the UK Health Protection Agency. Centre staff also attended (i) a WHO-supported workshop aimed at refining post-marketing HIV rapid test surveillance at the Paul Ehrlich Institute, Frankfurt, Germany; (ii) on-site CDC-hosted training on HIV drug resistance data quality training; and (iii) neutralisation assay-specific training at Duke University, USA.

Professional development

During 2012/2013, there were 13 registered PhD and five MSc students under supervision within the centre. Two PhD and one MSc students graduated.

Honours

Professor Caroline Tiemessen was awarded the DST/NRF Chair of HIV Vaccine Translational Research through the University of the Witwatersrand.

Dr Penny Moore received a CHAVI Young Investigator Award and won the overall prize of the first Norman Letvin Award at the CHAVI Annual Retreat in Durham, USA.

Dr Penny Moore was also the recipient of the 2013 University of the Witwatersrand Faculty of Health Sciences Prize for Research, following her recent publication in *Nature Medicine* on the importance of broadly cross-neutralising anti-HIV antibodies.

Ms Cathrine Mitchell was the winner of the Roche GS Junior Competition.

Ms Simone Richardson received an award for the top Microbiology and Biotechnology Honours student at the University of the Witwatersrand.

Ms Jinal Bhiman and Ms Simone Richardson received University of the Witwatersrand Postgraduate Merit Awards.

Ms Jinal Bhiman, Ms Thandeka Khoza, Ms Cathrine Mitchell and Mr Molati Nonyane were awarded Columbia University-Southern African Fogarty scholarships to undertake training at academic institutions in the USA and South Africa.

Dr Kabamba Alexandre received a James Gear Fellowship to visit the US National Cancer Institute.

Research output

During 2012/2013, 31 journal articles were published in international and national peer-reviewed journals. In addition, centre staff delivered 30 presentations at international congresses (one plenary lecture, five symposium presentations, nine oral presentations and 15 posters) and 36 presentations at national congresses (two plenary lectures, eight symposium presentations, 13 oral presentations and 13 posters). The following five publications are highlighted, as they present work that has advanced public health and/or laboratory science within South Africa.

Hunt GM, Ledwaba J, Basson AE, Moyes J, Cohen C, Singh B, Bertagnolio S, Jordan MR, Puren A, Morris L. Surveillance of transmitted HIV-1 drug resistance in Gauteng and KwaZulu-Natal provinces, South Africa, 2005–2009. *Clinical Infectious Diseases* 2012; **54**(Suppl 4): S334–S338.

Synopsis: Surveillance of transmitted HIV-1 drug resistance was performed using specimens collected as part of the annual antenatal survey and using primagravid women aged under 21 years as a marker for recent infection. Specimens collected between 2005 and 2009 from Gauteng and KwaZulu-Natal provinces were analysed according to WHO-recommended protocols. The data showed low levels of transmitted resistance in Gauteng but increasing levels of resistance to the NNRTI drug class in KZN in recent years.

Lewis DA, Chirwa T, Msimang V, Radebe F, Kamb M, Firnhaber C. Urethritis/cervicitis pathogen prevalence and associated risk factors among asymptomatic HIV-infected patients in South Africa. *Sexually Transmitted Diseases* 2012; **39**: 531–536.

Synopsis: The prevalence of STIs and associated patient characteristics were determined among individuals asymptomatic for genital discharge who attended a South African HIV treatment centre. Asymptomatic urethritis/cervicitis pathogens were highly prevalent among patients attending this centre. The benefit of introducing such STI screening programmes to improve reproductive health and HIV prevention efforts requires further study.

Mbulawa ZZA, Marais DJ, Johnson LF, Coetzee D, Williamson AL. The impact of human immunodeficiency virus on human papillomavirus transmission in heterosexually active couples. *Journal of Infection*, http:dx.doi.org/10.1016/j.inf.2013.03.009.

Synopsis: This study demonstrates high female-to-male and male-to-female HPV transmission rate among sexually active couples. HIV-positive women were found to be at higher risk of HPV infection transmitted from their male partners than HIV-negative women. HIV infection and low CD4 counts increase the rate of HPV acquisition from sexual partners.

Moore PL, Gray ES, Wibmer CK, Bhiman JN, Nonyane M, Sheward D, Hermanus T, Bajimaya S, Tumba NL, Abrahams M, Lambson BE, Ranchobe N, Ping L, Ngandu N, Abdool Karim Q, Abdool Karim SS, Swanstrom RI, Seaman MS, Williamson C, Morris L and the CAPRISA 002 study team. Evolution of an HIV glycan-dependent broadly neutralising antibody epitope through immune escape. *Nature Medicine* 2012; **18**, 1688–1692.

Synopsis: This study showed how broadly neutralising antibodies, the kind of antibodies that are needed for an HIV vaccine, developed in two unusual HIV-infected women. In both cases, in order to escape from less effective strain-specific antibodies, the viruses in these women added a sugar molecule (glycan) that later became the target of new antibodies. These latter antibodies were broadly neutralising and recognised 88% of global HIV strains. These findings were confirmed

in large sequence data sets, showing that this is a common pattern of evolution, with implications for HIV vaccine design.

Picton ACP, Paximadis M, Shalekoff S, Tiemessen CT. CCR5 promoter haplotypes differentially influence CCR5 expression on natural killer and T cell subsets in ethnically divergent HIV-1 uninfected South African populations. *Immunogenetics* 2012; **64**: 795–806.

Synopsis: CCR5 plays a critical and central role in HV-1 infection. This study evaluated the influence of CCR5 haplotypes on CCR5 expression on various immune cell subsets, and revealed some interesting findings that may help explain why, in earlier studies, possession of the same CCR5 haplotype associates with different disease outcomes in divergent populations. Furthermore, the importance of evaluating cell types other than CD4+ T cells in protection from HIV-1 acquisition and disease progression was highlighted.

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Rivashni Jagaroo Madho performing tests on dried blood spots to detect HIV in infants

Dr Zizi Mbulawa, appointed as an NICD Senior Medical Scientist in 2012, performing HPV testing in Cape Town

Dr Penny Moore's collaborative work on broadly cross-neutralising antibodies was published in *Nature Medicine*

Professor John Frean
Centre Heads

Dr Nelesh Govender

Professor Lizette Koekemoer

Dr Olga Perovic

Opportunistic, tropical and hospital infections

The functional surveillance, reference and research thrusts of the centre are embodied in its name, namely, opportunistic infections, particularly those 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 (GSH) in Cape Town, headed by Professor Mark Nicol, focuses on nosocomial infections and antimicrobial resistance.

Surveillance, diagnostic and reference services

The centre provides a service for the identification of medically important arthropods for entomologists, medical practitioners and environmental health officers. Malaria vector mosquitoes were routinely identified by the Vector Control Reference Laboratory for the Mpumalanga Province Malaria Control Programme.

Laboratory tests were carried out on *Anopheles gambiae* complex mosquitoes from various African countries, including South Africa, for species identification and to detect the presence of sporozoites of *Plasmodium falciparum*, the most deadly agent of malaria.

Advice and expertise was provided to the Department of Health (DoH) at both the national and provincial levels, with participation on the South African Malaria Elimination Committee. Insecticide resistance studies were carried out in collaboration with the Mpumalanga Malaria Control Programme (MMCP), on behalf of the World Health Organization (WHO) Pesticide Evaluation Scheme.

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.

In the Antimicrobial Resistance Reference Laboratory (AMRRL), the phenotypic and genotypic characterisation of mechanisms of resistance was especially focused on *Staphylococcus aureus* and *Klebsiella pneumoniae*, but a reference service was offered for all multidrug-resistant organisms, such as the emerging carbapenem-resistant *Enterobacteriaceae*. Enhanced surveillance for *S. aureus* infections is a new addition to the centre's activities.

Quality assessment (QA) services provided by the centre contributed to assessing diagnostic laboratory proficiency in South Africa and other African countries.

The external QA programmes provided schemes for malaria microscopy, bacteriology, mycology, tuberculosis microscopy and culture, and syphilis serology, and are South African National Accreditation Service (SANAS) accredited for inter-laboratory comparison.

The centre has played an active role in reporting on laboratory capacity in the WHO AFRO region for the past 11 years, and has supported QA for laboratories for international malaria vaccine trials (GSK Biologicals) and Global Vaccine-Preventable Invasive Bacterial Diseases sentinel sites.

The Centre also houses the National Stock Culture Collection, as well as one of the world's largest collections of medically important arthropods.

Outbreak investigations in 2012

Two clusters of malaria cases, including one fatality, were investigated in Gauteng residents without recent travel history.

Entomological investigations revealed no evidence of local breeding of vector anophelines and it is likely that the infections were acquired via infected mosquitoes imported from endemic areas in vehicles, containers or by other means (Odyssean malaria). 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 DoH in its malaria elimination activities.

An outbreak of carbapenem-resistant *Enterobacteriaceae* at private health care facilities in Gauteng was investigated in AMRRL. The Cape Town molecular epidemiology section identified the first patient with carbapenem-resistant OXA-181-producing *Enterobacteriaceae* in a local hospital and investigated the spread of this organism among hospitalised patients, including AIDS patients.

An outbreak of vancomycin-resistant *Enterococcus faecium* in a paediatric oncology ward was also investigated and managed.

A cluster of acute respiratory disease cases following a group visit to the 'Bat Cave' at the Sterkfontein cave complex was investigated. *Histoplasma capsulatum* infection was strongly suspected but laboratory confirmation could not be obtained.

The centre contributed to characterising an outbreak of microsporidial infection in renal transplant cases in Cape Town in collaboration with the local clinicians and with Dr M Birkhead, Electron Microscope Unit (CEZD) at NICD. Molecular studies showed that the three patients were all infected with *Encephalito zooncuniculi,* a parasitic organism. This is primarily an animal pathogen, but is known to be a cause of infections in immunocompromised patients.

Current research and surveillance

Insecticide resistance in malaria vectors

Researchers: L Nardini, Dr RN Christian, Professor M Coetzee, Professor LL Koekemoer
Collaborators: N Coetzer (University of Pretoria), Professor H Ranson (Liverpool School of Tropical Medicine, UK)

In 1999/2000 South Africa suffered the worst malaria epidemic in living memory, with hundreds of people dying from the disease and over 60,000 cases reported. One of the main reasons for the epidemic was the return to South Africa of the *Anopheles funestus* mosquito after an absence of at least 20 years. We showed that this mosquito was resistant to the insecticides that were being used and this resulted in a policy decision to switch insecticides. Subsequently, research into the insecticide resistance mechanisms and behaviour of this important malaria vector, as well as resistance in the other major malaria vector, *Anopheles gambiae*, has been prioritised.

We investigated the detoxification enzymes that are involved in *An. arabiensis* resistance to DDT and pyrethroids in colonies originating from different geographic origins.

These data emphasise the complexity associated with resistance phenotypes and suggest that specific insecticide resistance mechanisms cannot be extrapolated to different vector populations of the same species.

Malaria vector control and transmission dynamics

Researchers: Dr RN Christian, Ms L Nardini, Dr KS Choi, Mr M Osea, Professor LL Koekemoer, Professor M Coetzee
Collaborators: Dr S Blanford, Ms NE Jenkins, Mr BHK Chan, and Professor AF Read (Penn State University, USA); Professor MB Thomas (University of the Witwatersrand); Ms CL Lyons, Dr JS Terblanche, and Dr SL Chown (Stellenbosch University)

Our research on entomopathogenic fungi has continued with respect to optimising longevity of the spores. Spore shelf-life under refrigeration surpassed the standard two-year shelf-life expected of a mosquito control product. We expect optimised formulations could improve spore persistence still further.

In 1999/2000 South Africa suffered the worst malaria epidemic in living memory with hundreds of people dying from the disease

As climates change, concerns have arisen as to how vector-borne diseases will be impacted by changing rainfall patterns and warming temperatures. Despite the importance and controversy surrounding the impact of climate change on the potential spread of malaria, little information exists on the climatic tolerances of the vector mosquitoes.

The effects of age, sex and strain (laboratory vs wild adults) were investigated for critical thermal limit determinations for *An. arabiensis* and *An. funestus* at all life stages. The findings that female mosquitoes have greater tolerance to thermal extremes may have significant implications for future malaria transmission, especially in areas of seasonal transmission and at the extremes of the vector distribution.

The global distribution of malaria vectors was mapped in collaboration with colleagues at Oxford University. This is a follow-on publication to the work published in 2010 by the same authors on the distribution and bionomics of the African malaria vectors.

Population genetic studies on the *An. funestus* group in southern Africa using mtDNA and RFLP markers have been carried out to elucidate further the genetic complexity of this group. Clades I and II (as previously described) have been identified, but additional genetic structuring has been found within *An. funestus, An. funestus*-like and *An. parensis,* indicating further subdivisions.

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

NICD researchers: Dr O Perovic, Dr A Singh-Moodley

Collaborators: GERMS-SA, LARS subgroup

Laboratory surveillance for antimicrobial resistance (AMR) provides a 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 *Staphylococcus aureus* and *Klebsiella pneumoniae*, were identified to monitor trends in resistance at sentinel sites at NHLS.

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

NICD researchers: Dr O Perovic, Dr A Singh-Moodley

Collaborators: Dr V Quan, Dr R Kularatne, Dr T Nana, K Baba, N Govender

Enhanced surveillance at three sentinel sites that participate in the LARS programme describes the prevalence of methicillin-susceptible and resistant *Staphylococcus 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 aid in distinguishing hospital-acquired infections from community-associated infections. Most important, MIC50 and MIC90 on all antimicrobials tested, and

epidemiological analysis, will estimate the 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

NICD researchers: B Poonsamy, Associate Professor J Frean, Dr S Walaza, Dr C Cohen
Collaborators: SARI Study Group

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 in collaboration with the Centre for Respiratory Diseases and Meningitis, NICD.

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

NICD researchers: D du Plessis, Professor J Frean

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, primary hosts (cats) and high-risk humans, and how they compare with strain types in the rest of Africa and the world.

Human cystic echinococcosis in South Africa

NICD researchers: KB Mogoye, Professor J Frean
Collaborators: Dr P Kern, Dr K Wahlers (University Hospitals, Ulm, Germany), Dr T Romig (Hohenheim University, Germany), Professor M Grobusch (Amsterdam University Medical Centre and University of the Witwatersrand), Dr C Menezes, Professor 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 (including sheep, cattle, goats and camels) as well as humans, which serve as accidental intermediate hosts. The disease is especially prevalent in pastoral communities, where there is close contact among humans, dogs and livestock. This study used molecular methods to investigate the genotypes and species affecting humans in South Africa. This is the first time that the genetic structure of the genus has been thus characterised in the country.

Public health programme: cryptococcal screening

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

Laboratory-based screening for cryptococcal disease was implemented at the NHLS CD4 laboratory at Charlotte Maxeke Johannesburg Academic Hospital on 3 September 2012.

Twenty-six healthcare facilities (including three regional hospitals) that refer specimens to this laboratory participated in the programme. Blood samples submitted for a CD4+ T-lymphocyte count from these facilities were tested for cryptococcal antigen (CrAg) using a cryptococcal lateral flow assay (LFA) if the CD4+ T-lymphocyte count was less than 100 cells/µl.

Results for patients who test CrAg-positive were communicated by the laboratory to a pre-selected point of contact at each health facility, as well as added to the CD4 laboratory report to alert the healthcare worker of the CrAg test result. Patients with cryptococcal antigenaemia who provided informed consent were followed up prospectively to collect clinical data indicators. Monthly programme updates were included in the NICD's Surveillance Report. By the end of March 2013, 3,142 patients with a CD4+ T-lymphocyte count < 100 cells/µl had been screened; 144 (4.6%) tested positive for CrAg. Fifty-nine per cent (85/144) of patients were diagnosed at Helen Joseph Hospital and 65% were aged between 30–44 years.

Cryptococcal meningitis: survey of clinical practice for amphotericin B toxicity prevention, monitoring and management at GERMS-SA enhanced surveillance sites

Researchers: Dr ST Miring, Dr NP Govender, L Sabina, Dr M Fortuin-de Smidt and the GERMS-SA team

Amphotericin B is the cornerstone of treatment for cryptococcal meningitis but is associated with several adverse events including serious nephrotoxicity.

This may be a barrier to optimal treatment of this severe opportunistic infection. The World Health Organization issued guidance for amphotericin B toxicity prevention, monitoring and management in 2011.

From October 2012 through February 2013, clinician questionnaires and additional case report forms were completed to determine the current clinical practice at GERMS-SA enhanced surveillance sites.

Detection of cryptococcal antigenaemia from whole blood specimens for rapid diagnosis of cryptococcal disease among HIV-infected adults in South Africa

Researchers: Dr NP Govender, TG Zulu, D Lawrie, Dr N Bosman, Dr T Nana, N Govender, S Lindani, Dr VC Quan, Dr L Coetzee and Professor W Stevens

Accurate detection of cryptococcal antigenaemia (CrAg) from whole blood would facilitate both point-of-care diagnosis of HIV-associated cryptococcal disease and high-volume,

laboratory-based screening to detect cryptococcal disease earlier. Whole blood specimens were tested for CrAg using the lateral flow assay (LFA) in three study groups, namely, Group I: patients with laboratory-confirmed cryptococcal meningitis (CM); Group II: patients who had undergone lumbar puncture but had no laboratory evidence of CM; and Group III: patients with CD4+ T-lymphocyte count < 100 cells/µl who were being screened for cryptococcal disease.

Participants in Groups I and II were prospectively-enrolled in-patients at CMJAH, admitted between March 2011 and September 2012, who provided informed consent for a point-of-care LFA performed by a trained study nurse on fingerprick whole blood. Remnant EDTA-blood samples from participants in Group III were tested with the LFA by the CMJAH CD4 laboratory.

Plasma obtained by centrifugation of EDTA-blood was re-tested at a reference laboratory with the LFA and the latex agglutination test (LA), and whole blood was tested with the LFA.

Clinical epidemiology of candidaemia at sentinel hospitals

Researchers: Dr NP Govender and GERMS-SA team

During the reporting period, cases of candidaemia detected at 11 sentinel hospitals in Gauteng and Western Cape provinces were reported to NICD. Bloodstream isolates and clinical data were collected through the GERMS-SA surveillance programme. The aim of the study is to determine clinical factors associated with disease, antifungal drug resistance and death.

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

Researchers: RE Magobo, S Naicker and Dr NP Govender

Selected isolates of *Candida* spp. submitted through laboratory-based surveillance for candidaemia have been selected for molecular typing. Selected *C. parapsilosis* isolates have been typed to determine the prevalence of cryptic species, *C. orthopsilosis* and *C. metapsilosis,* and to determine the antifungal susceptibility profiles for these cryptic species compared to *C. parapsilosis*. In addition, the reference laboratory has set up PCR assays for amplification of HS1 and HS2 regions of the fks1 subunit of1,3-Greek beta-D glucan synthase, to identify the mutations associated with echinocandin resistance in *Candida* species. Last, the reference laboratory has set up assays for microsatellite typing of *C. parapsilosis* to establish strain relatedness and to uncover previously undetected nosocomial outbreaks.

Research funding

- Centers for Disease Control and Prevention through NHLS/CDC Cooperative Agreement
- Deutscher Akademischer Austauschdienst
- Gates Grand Challenges Explorations
- German Research Foundation
- Global Disease Detection, Centres 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 and 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.

Two graduate research scientists from the Malaria Alert Centre of the Malawi College of Medicine, University of Malawi, were trained in insectary rearing of *An. funestus* and morphological identification of anophelines. Training on molecular entomological techniques was provided to two members of the Okavango Research Institute, University of Botswana.

Professional development

Postgraduate students enrolled: five postdoctoral fellows, six PhD, six MSc, one BSc (Hons), four MMed.

Postgraduate students graduated: three PhD, three MSc, one MSc (Epidemiology).

Dr N Govender and Dr O Perovic successfully completed the UNISA Executive Development Programme.

Honours

Ms L Ndluvo received the prize for the best BSc Honours project in the Faculty of Science, University of the Witwatersrand.

Ms S Oliver received third prize for her presentation at the Molecular Biosciences Research Thrust Annual Research Day at the University of the Witwatersrand in December, and second prize for her presentation at the Wits Cross-Faculty Research Day.

Professor L Koekemoer was nominated as co-chair for the Subcommittee (Vector Control) of the South African Malaria Elimination Committee.

Dr B Brooke was appointed as editor of the NICD Communicable Diseases Surveillance Bulletin and as an academic editor of *PLoS One*.

Professor M Coetzee received the John N Belkin award from the American Mosquito Control Association in recognition of her work on the systematics of African anopheline mosquitoes.

Professor Coetzee, Professor Koekemoer and Dr Brooke received Faculty of Health Sciences certificates at a University of the Witwatersrand awards dinner for achievement in research.

Dr NP Govender received an NHLS top student certificate for the UNISA SBL Executive Development Programme, and was nominated for the ICEID Leaders' Programme at the International Conference on Emerging Infectious Diseases in 2012.
Dr C Bamford received an award for the best poster presentation at the Federation of South African Societies of Pathology (FSASP) Pathpoint 2012 Conference.

Research output

Top six publications from the centre

Choi KS, Koekemoer LL, Coetzee M. Population genetic structure of the major malaria vector *Anopheles funestus* and allied species in southern Africa. *Parasites and Vectors* 2012; **5**: 283.

Synopsis: In this study population genetic data on the An. funestus group in southern Africa were analysed and two genetic clades were identified. This is the first study to describe genetic clades in members of the *An. funestus* group.

Govender NP, Roy M, Oladoyinbo S, Maotoe T, Stevens W, Pinini Z, Spencer D, Venter WDF, Jassat W, Cameron D, Meintjes G, Chiller T, Chetty V, Mbengashe T, Pillay Y, for the South African Cryptococcal Screening Initiative Group. Phased implementation of screening for cryptococcal disease in South Africa. *South African Medical Journal* 2012; **102**: 914–917.

Synopsis: This paper describes operational plans for the first phase of cryptococcal screening in South Africa. South Africa has led the way in programmatic implementation of cryptococcal screening, which is a strategy to reduce deaths associated with this AIDS-defining fungal opportunistic infection.

Jarvis JN, Govender N, Chiller T, Park BJ, Longley N, Meintjes G, Bekker LG, Wood R, Lawn SD, Harrison TS. Cryptococcal antigen screening and pre-emptive therapy in patients initiating antiretroviral therapy in resource-limited settings: a proposed algorithm for clinical implementation. *Journal of the International Association of Physicians in AIDS Care* 2012; **11**: 374–379.

Synopsis: This paper proposes a clinical algorithm that can be used to manage patients identified through a cryptococcal screening programme.

Luengo-Oroz, MA, Arranz A, Frean J. Crowd sourcing malaria parasite quantification: an online game for analysing images of infected

thick blood smears. *Journal of Medical Internet Research* 2012 **14**: e167. doi:10.2196/jmir.2338.

Synopsis: There is increasing interest in computer-aided diagnosis. This research tested the feasibility of a crowd-sourced approach to malaria image analysis by investigating whether anonymous volunteers playing a web-based game, with no prior experience, might be able to count *Plasmodium falciparum* parasites in digitised images of thick blood smears.

Meiring ST, Quan VC, Cohen C, Dawood H, Karstaedt AS, McCarthy KM, Whitelaw AC, Govender NP, for the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA). A comparison of paediatric- and adult-onset cryptococcosis detected through population-based surveillance in South Africa, 2005–2007. *AIDS* 2012; **26**: 2307–2314.

Synopsis: Using data from a national surveillance programme, this paper compares the incidence and clinical features of cryptococcal meningitis diagnosed in adults and children.

Nardini L, Christian RN, Coetzer N, Ranson H, Coetzee M, Koekemoer LL. Detoxification enzymes associated with insecticide resistance in laboratory strains of *Anopheles arabiensis* of different geographic origin. *Parasites and Vectors* 2012; **5**: 113.

Synopsis: This paper reports on the first molecular study on a South African malaria vector population to characterise the molecular mechanisms of insecticide resistance.

Other publications

Abu Samra N, Thompson PN, Jori F, Frean J, Poonsamy B, du Plessis D, Mogoye B, Xiao L. Genetic characterization of *Cryptosporidium* spp. in diarrhoeic children from four provinces in South Africa. *Zoonoses and Public Health* 2013; **60**: 154–159.

Blanford S, Jenkins NE, Christian R, Chan BHC, Nardini L, Osae M, Koekemoer LL, Coetzee M, Read AF, Thomas MB. Storage and persistence of a candidate fungal biopesticide for use against adult malaria vectors. *Malaria Journal* 2012; **11**: 354.

Choi KS, Koekemoer LL, Coetzee M. Population genetic structure of the major malaria vector *Anopheles funestus* and allied species in southern Africa. *Parasites and Vectors* 2012; **5**: 283.

Espinel-Ingroff A, Aller AI, Canton E, Castañón-Olivares LR, Chowdhary A, Cordoba S, Cuenca-Estrella M, Fothergill A, Fuller J, Govender N, Hagen F, Illnait-Zaragozi MT, Johnson E, Kidd S, Lass-Flörl C, Lockhart SR, Martins MA, Meis JF, Melhem MS, Ostrosky-Zeichner L, Pelaez T, Pfaller MA, Schell WA, St-Germain G, Trilles L, Turnidge J. *Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole and voriconazole. *Antimicrobial Agents and Chemotherapy* 2012; **56**: 5898–5906.

Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, Fothergill A, Fuller J, Hagen F, Govender N, Guarro J, Johnson E, Lass-Flörl C, Lockhart SR, Martins MA, Meis JF, Melhem MS, Ostrosky-Zeichner L, Pelaez T, Pfaller MA, Schell WA, Trilles L, Kidd S, Turnidge J. *Cryptococcus neoformans-Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for amphotericin B and flucytosine. *Antimicrobial Agents and Chemotherapy* 2012; **56**: 3107–3113.

Klausner J, Govender N, Oladoyinbo S, Roy M, Chiller T. Response to Parkes-Ratanshi R, Wakeham K, Levin J et al. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomized, placebo-controlled trial. *Lancet Infectious Diseases* 2012; **12**: 431–432.

Lyons CL, Coetzee M, Terblanche JS, Chown SL. Thermal limits of wild and laboratory strains of two African malaria vector species, *Anopheles arabiensis* and *Anopheles funestus*. *Malaria Journal* 2012; **11**: 226.

Nardini L, Christian RN, Coetzer N, Ranson H, Coetzee M, Koekemoer LL. Detoxification enzymes associated with insecticide resistance in laboratory strains of *Anopheles arabiensis* of different geographic origin. *Parasites and Vectors* 2012; **5**: 113.

Poonsamy B, Dini L, Frean J. Performance of clinical laboratories in South African parasitology proficiency testing schemes, 2004–2010. *Journal of Clinical Microbiology* 2012; **50**: 3356–3358.

Sinka ME, Bangs MJ, ManguinS, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH, Gething PW, Kabaria CW, Burkot TR, Harbach RE, Hay SI. A global map of dominant malaria vectors. *Parasites and Vectors* 2012; **5**: 69.

Trataris AN, Rossouw J, Arntzen L, Karstaedt A, Frean J. *Bartonella* spp. in human and animal populations in Gauteng, South Africa, from 2007 to 2009. *Onderstepoort Journal of Veterinary Research* 2012; **79**: 18–25.

Trataris-Rebisz AN, Arntzen L, Rossouw J, Karstaedt A, Frean J. *Bartonella hense*lae and *Bartonella quintana* seroprevalence in HIV-positive, HIV-negative and clinically healthy volunteers in Gauteng Province, South Africa. *Annals of the Australasian College of Tropical Medicine* 2012; **13**: 10–12.

Mudau M, Jacobson R, Kuonza L, Morris V, Engelbrecht H, Minenza, N, Nicol M, Bamford C. Outbreak of multidrug-resistant *Pseudomonas aeruginosa* bloodstream infection in the haematology unit of a South African academic hospital. *PloS One* 2013; **8**: e55985.

Conference presentations

Staff and students of the centre presented data at 15 international conferences, nine national conferences, and several local conferences such as university research days and small scientific meetings.

Hunting for mosquito breeding sites. Malaria outbreak, Donkerhoek, near Pretoria

Malaria symposium and exhibition for World Malaria Day, 25 April 2012, held at NICD

Dr Cheryl Cohen

Professor Marietjie Venter

Dr Anne von Gottberg

Centre Heads

The Centre for Respiratory Diseases and Meningitis (CRDM) includes bacteriology and virology laboratories, a team of epidemiologists and surveillance field staff. In the past year, the centre has integrated activities of the different sections and expanded existing syndromic surveillance programmes to include multiple pathogens of public health relevance to South Africa.

The National Influenza Centre (NIC), the World Health Organization (WHO) reference laboratory for influenza surveillance, and the CRDM via its research facility continue to provide data and circulating influenza types to WHO collaborating centres. The information is used to update records annually of the southern hemisphere vaccines as well as any unsubtypeable influenza strains or emerging respiratory viruses.

Through the NIC collaborations CRDM has also been able to respond to new pathogens such as the novel corona virus and avian influenza H7N9. The centre has developed a number of new laboratory diagnostic technologies, one of which has enabled it to introduce syndromic meningitis surveillance.

The CRDM conducts ongoing surveillance and research to monitor the impact and effectiveness of the expanded programme on immunisation vaccination for *Streptococcus pneumoniae* and invasive *Haemophilus influenzae* type b. The centre continues to work with other national surveillance programmes within NICD (GERMS-SA) and supports the Department of Health (DoH) and WHO in monitoring the impact of vaccines, antimicrobial resistance and outbreak response.

A strong research agenda allowed for the development of new laboratory techniques and analysis of epidemiology data that are used to influence policy. The centre is also a source of capacity building and formal training within South Africa and the African region.

Current surveillance programmes and applied research

Respiratory illness syndromic 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 includes collection and testing of specimens for atypical bacterial causes of pneumonia, *Bordetella pertussis* and tuberculosis at selected sites.

Surveillance for influenza-like illness (ILI) in outpatients has been implemented at two primary health clinics adjacent to SARI sites. This programme aims to describe the viruses and bacteria associated with ILI and assess risk factors for SARI in these populations.

The Viral Watch programme, operational for the past 26 years, continues to provide national data on circulating influenza strains. An Enhanced Viral Watch programme is operational in urban and rural hospitals in all nine provinces; it aims to monitor influenza admissions to intensive care units, and the samples collected are also tested for additional viruses.

Surveillance sites have been introduced at the human–animal interface in areas where avian influenza outbreaks occurred in ostriches.

Genetic diversity of human rhinovirus (RV) in patients hospitalised with severe acute respiratory illness (SARI), South Africa, 2009–2010

NICD investigators: M Pretorius, Dr F Treurnicht, Dr C Cohen, Professor SA Madhi, Professor M Venter

Collaborators: SARI surveillance group, Dr S Tempia, CDC (USA) attaché to NICD-NHLS

Human rhinovirus (RV) is a well established cause of upper respiratory illness but is also detected in cases of SARI as a single or co-infection. The disease association and role of different subtypes is not well described. RV subtype C (RV-C) has been postulated to be associated with more severe illness.

The purpose of this study was to characterise the RV species circulating among patients enrolled as part of surveillance for SARI and to describe the clinical presentation.

The VP4/VP2 (420 bp) region was sequenced from a selection of the RV single positive samples with available HIV-status data, and phylogenetic analysis was performed. RV subtype A (RV-A) and RV-C were more commonly identified than RV subtype B (171/381(45%), 161/381(42%) and 49/381(12%), respectively, with no seasonality identified.

On multivariable analysis, RV-C was associated, with having a history of asthma [adjusted relative risk ratio 3.4 (95% CI 1.1-11.1)]. Comparison to mild cases and healthy controls may clarify the disease association.

Excess mortality due to influenza and respiratory syncytial virus (RSV) in children and adults in South Africa, 2000–2012

NICD investigators: Dr S Tempia (USA, CDC attaché to NICD), Dr S Walaza, Professor SA Madhi, Professor M Venter, Sr J McAnerney, Dr C Cohen

Collaborators: SARI surveillance group, Dr A Cohen Influenza Division, National Centre for Immunization and Respiratory Diseases (NCIRD), CDC and Influenza Programme, CDC South Africa

We estimated the influenza- and RSV-associated mortality by applying Poisson regression models to national vital statistics data. In children with respiratory illness <5 years of age, the mean annual number of deaths associated with influenza was 452 (8/100,000 person-years) and that associated with RSV was 546 (10/100 000 person-years).

The mortality rate was higher among infants <1 year of age for both influenza (22/100,000 person-years) and RSV (35/100,000 person years). In children <5 years of age, the influenza-associated mortality rate among all respiratory deaths was greater in HIV-infected (83/100,000 person-years) than in HIV-uninfected (6/100,000 person-years) individuals (age-adjusted relative risk (aRR): 11.5; 95% CI: 9.6–12.6). In persons aged ≥5 years with respiratory illness, the mean annual number of deaths associated with influenza was 3,613 (8.5/100,000 person-years) and that associated with RSV was 429 (1.0/100,000 person-years). Among individuals ≥5 years of age, the influenza-associated mortality rate for all-cause deaths was greater in HIV-infected than in HIV-uninfected individuals (aRR: 7.9; 95% CI: 7.1–8.9).

Estimating the national burden of influenza in South Africa, 2009–2012

NICD investigators: Dr A Cohen (CDC), Dr S Walaza, Professor SA Madhi, Dr J Moyes, Dr S Tempia, A Tshangela, Professor M Venter, Dr C Cohen

Collaborators: SARI surveillance group

Based on data from the published literature and the SARI surveillance programme, we used a multiplier model to estimate the influenza-associated burden of respiratory disease by extrapolating from one hospital with population denominators the annual national number of ILI cases, respiratory hospitalizations, and in-hospital deaths associated with influenza in South Africa from 2009 to2011. We estimated the burden by age and HIV infection status. On average over the 3 years, there were approximately 6.6 million cases of mild influenza-like illness, 31,000 hospitalisations for influenza-associated pneumonia, and 1,200 in-hospital deaths from influenza-associated pneumonia annually in South Africa. The mean incidence rate per 100,000 population was 13,149 cases of influenza-like illness, 63 hospitalisations, and 3 deaths. The highest number of annual hospitalisations and deaths was among children <5 years of age (a mean of 11,992 hospitalisations and 87 deaths annually) and HIV-infected adults 25–64 years of age (a mean of 10,975 hospitalisations and 951 deaths annually). Annual estimates of burden and mortality assist with planning allocation of resources. In addition these data assist with supporting and expanding prevention programmes such as influenza vaccine programmes.

Investigation of avian influenza H5N2 and H7N1 sero-prevalence in humans with an increased risk of exposure during outbreaks in ostriches

NICD investigators: Professor M Venter, Dr F Treurnicht, A Buys, R Samudzi, Professor L Blumberg, Ms C Jacobs, Dr J Thomas, Sr J McAnerney

Following the previous avian influenza H5N2 and H7N1 outbreaks in ostriches, the centre conducted sero-surveys in high-risk humans involved in these outbreaks. Screening of sera from 207 humans who had had exposure to avian influenza H5N2 and 66 veterinarians, ostrich farmers, farm workers and abattoir workers who were involved in the avian influenza H7N1 outbreak during 2011 and 2012 in ostriches identified 4 people with significant HAI antibody titres to avian influenza H5N2 or H7N1. These included a veterinarian who was actively involved in post-mortem investigations of culled ostriches, a farm worker and 2 abattoir workers. This suggested a low risk of infection for humans involved in controlling these outbreaks, with a sero-conversion rate of 1.4% for avian influenza H5N2 and 1.6% for avian influenza H7N1 viruses. Reported symptoms based on a retrospective questionnaire completed at the time of specimen collection included conjunctivitis and ILI.

Replacement and positive evolution of subtypes A and B respiratory syncytial virus (RSV) G-protein genotypes from 1997–2012 in South Africa

NICD investigators: Ms M Pretorius, Dr F Treurnicht, Dr J Moyes, Dr C Cohen, Professor SA. Madhi, Professor M Venter

Collaborators: SARI surveillance group, Ms Stephanie van Niekerk (Zoonosis Research Unit, Department of Medical Virology, University of Pretoria), Stefano Tempia (United States CDC attaché to NICD-NHLS)

Of the RSV genotypes previously described in South Africa from 1997 to 2002, only GA2 and GA5 persisted until 2006, but genotype BA had replaced all previous RSV-B genotypes. This suggests that RSV-A may be more established than RSV-B and that positive selection drives evolution. Randomly selected RSV positive specimens (2009–2012) were subtyped, sequenced, and compared to available RSV sequences (1997–2001; 2006–2009). Phylogenetic analysis indicated that RSV-A genotype GA2 dissolved into 4 new genotypes: SAA2 (unique to South Africa), NA1, NA2 (Japan) and ON1 (Canada) with a 72-base pair (bp) insertion. The GA5 genotype drifted to form three sub-genotypes (GA5 I-III [B1] [B2]). RSV-B genotype BA clustered into newly identified sub-genotypes BA8-10. RSV-A's evolutionary rate was slower than RSV-B and seven new positively selected sites were identified in South African strains; two for RSV-A and five for RSV-B. This demonstrates that positive selection drove both genotypes to evolve, resulting in replacement of all previously identified genotypes in South Africa.

Validation of new assays to detect atypical pneumonia-causing pathogens and pertussis within the syndromic surveillance SARI programme

NICD investigators: F Moosa, M Carrim, Dr N Wolter, Dr M du Plessis, L de Gouveia, Dr A von Gottberg

Collaborators: Dr Jonas Winchell (United States CDC, Atypical Pneumonia Laboratory)

Two projects aimed to describe the atypical bacterial pathogens associated with SRI: "Detection of *Streptococcus pneumoniae, Haemophilus influenzae* and *Bordetella pertussis* in individuals presenting with severe respiratory and influenza-like illness in South Africa, 2012" and "Identification and prevalence of bacteria causing atypical pneumonia in patients with severe respiratory illness and influenza-like illness in South Africa, 2012". For the identification of *B. pertussis* a multiplex real-time polymerase chain reaction (RTPCR) followed by a singleplex RTPCR is used to detect and differentiate *B. pertussis, Bordetella parapertussis, Bordetellaholmesii* and *Bordetella bronchiseptica.* A multiplex real-time PCR is used to identify the atypical pneumonia-causing bacteria. Speciation of *Legionella* is obtained using a multiplex real-time PCR to detect *Legionella pneumophila* serogroup 1.

Preliminary results of 810 induced sputum specimens indicated that five (0.6%) were positive for *B. pertussis,* six (0.7%) for *B. parapertussis*, eight (1%) for M. *pneumoniae*, two (0.2%) for *C. pneumoniae* and 12 (1.5%) for *Legionella* spp. Of 3,382 nasopharyngeal specimens tested for *Bordetella* spp, nine (0.3%) were positive for *B. pertussis* and seven (0.2%) for *B. parapertussis*. Preliminary results of the 1,679 nasopharyngeal specimens tested for atypical pneumonia-causing bacteria are as follows: eight (0.5%) were positive for *M. pneumoniae* and four (0.2%) for *C. pneumoniae.*

Establishing human bocavirus and human coronaviruses (229E, NL63, HKU1 and OC43) in the existing multiplex RTPCR

NICD investigators: Ms O Hellferscee, Professor M Venter

Collaborators: SARI investigators

No epidemiological studies have been conducted to determine the importance and disease association of human bocavirus and human coronaviruses (229E, NL63, HKU1 and OC43) in patients with severe acute respiratory infections, ILI and healthy controls in South Africa over all the age groups. To assess this, RTPCR tests for human bocavirus and human coronaviruses (229E, NL63, HKU1 and OC43) have been optimized and incorporated into the existing multiplex real-time PCR that is being used for routine surveillance. Using this assay will provide the opportunity to investigate patients with SARI, ILI and healthy controls from the same community to explore disease severity and virulence mechanisms of the viral pathogens as single or mixed infections.

Novel corona virus (EMC-2012) assay development

NICD investigators: O Hellferscee, Dr F Treurnicht, Professor M Venter

Collaborators: Viral Watch Programme

The WHO alerted countries to several reports of a new coronavirus (EMC-2012) associated with severe respiratory disease in patients with an epidemiological link to the Arabian Peninsula in June 2012. The novel coronavirus has thus far been identified only in a small number of cases of acute, serious respiratory illness that

presented with fever, cough, shortness of breath and breathing difficulties. The assay for the E protein gene target (UpE) is considered highly sensitive, and has been implemented at the CRDM, since the laboratory is the WHO reference laboratory for testing novel coronavirus in Africa. A second confirmatory PCR on the open reading frame 1b (ORF1b) and a pan-coronavirus PCR will be run on all UpE-positive specimens.

Serological assays and RTPCRs for avian influenza H5, H6 and H7 strains

NICD investigators: Dr F Treurnicht, Ms R Samudzi, O Hellferscee, M Pretorius, A Buys, Professor M Venter

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), and additional antigens can be included as required. Seasonal human influenza virus strains are included as controls. The virology laboratory was accredited by SANAS in May 2011 as well as by the Department of Agriculture, Forestry and Fisheries to support diagnostic testing of avian influenza A/H5 in ostriches during the H5N2 avian influenza outbreak, using the CDC real-time RT-PCR assay. In addition in-house RTPCR assays for avian influenza A/H5, A/H7 and A/H9 have been established, which enhance our capacity to provide reference laboratory support for avian influenza virus diagnosis. We have also established the H7N9 typing RTPCR for detecting cases of the emerging strain from China.

CRDM contribution to the national GERMS-SA programme

The Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) is a national laboratory-based population-based active surveillance programme for invasive bacterial and fungal causes of pneumonia, meningitis and diarrhoeal diseases.

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 surveillance for invasive pneumococcal and Hib disease and case-control and other epidemiologic studies. CDRM also contributes data on numbers and serotypes of *Neisseria meningitidis* and supports diagnostic testing and outbreak response for suspected cases of meningococcal meningitis.

Trends and factors associated with invasive non-typeable and serotype b *Haemophilus influenzae* disease in persons of all ages in South Africa, 2003–2009

NICD investigators: Dr A von Gottberg, Dr C Cohen, Dr A Cohen, L de Gouveia, Dr M du Plessis, Professor S A Madhi
Collaborators: GERMS-SA

The aim of this study was to describe the trends and factors associated with invasive *Haemophilus influenzae* serotype b (Hib) and nontypeable *Haemophilus influenzae* (NTHi) disease in South Africa, 2003–2009, among persons of all ages. Multivariable analysis, restricted to cases presenting to enhanced

surveillance sites, was done to compare characteristics of NTHi cases to Hib cases. A total of 2,563 *H. influenzae* cases in all ages were identified from 2003 through 2009. NTHi accounted for the greatest proportion at 49% (1246/2563), followed by Hib at 33% (860/2563) and non-b encapsulated at 18% (457/2563). Hib among persons <5 years old increased from 10/1,000,000 in 2005 to 21/1,000,000 in 2008 ($p < 0.001$). Hib incidence among persons ≥5 years old increased from 0.5/1,000,000 in 2003 to 1.1/1,000,000 in 2009 ($p = 0.002$). NTHi incidence remained stable among individuals of all ages. Factors associated with NTHi disease compared to Hib disease, after adjusting for other factors, were clinical syndrome (lower respiratory tract infection odds ratio (OR) 8.31, 95% confidence interval (CI) 4.12–16.78; bacteremia: OR 9.24, 95% CI 3.79–22; compared to meningitis), predisposing conditions (OR 3.00, 95% CI 1.68–5.35, compared to no predisposing condition), nosocomial infection (OR 5.67, 95 %CI 1.71–18.82, compared to no nosocomial infection). NTHi cases were less likely to be <5 years old (OR 0.29, 95% CI 0.12–0.76, compared to 25–44-year age group). A booster dose of Hib vaccine at 18 months of age was introduced in 2010 in South Africa, and ongoing surveillance is essential to monitor trends in Hib disease.

Risk factors for invasive pneumococcal disease (IPD) in children enrolled in an IPD case-control study in South Africa 2010–2012

NICD investigators: Dr C von Mollendorf, Dr C Cohen, Dr V Quan, S Lindani, N Govender, Dr S Meiring, L De Gouveia, N Naidoo, Dr M Fortuin de Smidt, Dr A Von Gottberg

Collaborators: GERMS-SA, Dr C Whitney, Dr K O'Brien (CDC)

Risk factors for IPD among children eligible to receive PCV-7 were evaluated by a case-control study nested within the GERMS-SA surveillance programme. Controls were matched to cases by age, HIV status and hospital. Among HIV-uninfected children, risk factors for IPD were previous upper respiratory tract infection, daycare attendance, increased numbers of children in household and HIV exposure. Children who had received two or more doses of vaccine were protected. Among HIV-infected children, factors associated with IPD included malnutrition and currently receiving TB treatment. These data provide insight into the groups of children who should be targeted for pneumococcal prevention in the era following vaccine introduction.

Meningitis surveillance and new assays

Macroarray assay for differential diagnosis of meningoencephalitis in southern Africa

NICD investigators: Professor M Venter, Dr J Weyer, Dr JT Paweska (CRDM, Special Viral Pathogens Reference Laboratory, Centre for Emerging and Zoonotic Diseases)

Collaborators: D Zaayman, V Stivaktas, S Goolab, S van Niekerk and Professor R Swanepoel (Zoonosis Research Unit, University of Pretoria)

Many cases of acute febrile illness with central nervous system manifestations go undiagnosed, partly because the potential

pathogens are not routinely investigated. We developed a multiplex PCR-based macroarray procedure for the detection of genomic targets of 29 pathogens associated with febrile disease and meningoencephalitis, including viruses, bacteria, and parasites. Pathogens were identified by hybridization of PCR amplicons with capture probes on a macroarray chip, with colorimetric detection of positive reactions. Positive control specimens for all 29 targets were detected with high sensitivity.

In addition, 25 clinical samples previously found positive for a variety of aetiologies of febrile disease and meningoencephalitis plus 2 quality control samples were all identified. Testing of a blinded panel of 16 specimens in triplicate produced results with high repeatability, sensitivity and specificity. Screening of 138 specimens from patients with febrile and/or neurological signs that tested negative in routine investigations yielded five diagnoses.

TaqMan Array Card

NICD investigators: Dr N Wolter, Dr M du Plessis, Ms M Moleleki, Ms A McIvor, Dr A von Gottberg, Professor M Venter, Ms O Hellferscee, Professor SA Madhi, Dr A Cohen (CDC), Dr C Cohen

Collaborators: Professor S Velaphi (Chris Hani Baragwanath Hospital), Dr J Winchell, Dr S Shrag, Dr A Dimirjian, Dr Danielle Iuliano, Dr Marc-Alain Widdowson (CDC, Atlanta, USA)

The bacteriology and virology laboratories have set up the technology to test clinical samples using the TaqMan Array Card (TAC). This technology will allow for simultaneous diagnosis of multiple viral and bacterial pathogens from neonates presenting with early-onset and community-acquired sepsis and children <5 years with SARI. The technology will be validated in the South African setting in a project called Sepsis Aetiology in Young Infants in South Africa (SAYISA) using specimens collected from infants at Chris Hani Baragwanath Hospital and utilized in a case control study evaluating aetiologies of pneumonia (SARI) among children less than five years of age.

Inactivated West Nile virus (WNV) vaccine, Duvaxyn WNV, protects against highly neuroinvasive lineage 2 WNV strains in mice

NICD investigators: Professor M Venter (CRDM), Mr P Janse van Vuuren, Professor J Paweska (Centre for Emerging diseases and Zoonoses)

Collaborators: Ms J Mentoor; Dr J. Williams (Zoonoses Research Unit, University of Pretoria), M Pearce, (Pfizer international)

Lineage 2 WNV is endemic to southern Africa and Madagascar, and has recently been associated with encephalitis outbreaks in humans and horses in South Africa, central Europe, Italy and Greece. Commercial vaccines have mostly been evaluated against WNV lineage 1 strains. To evaluate protection of Duvaxyn WNV vaccine against lineage 2 strains associated with encephalitis in South Africa, mice were vaccinated twice intramuscularly three weeks apart, and challenged four weeks later with highly neuroinvasive lineage 1 strain NY385/99 or lineage 2 strain SPU93/01. Neutralising antibody titres were measured at the time of challenge and three weeks later.

Immunohistochemistry and RTPCR were conducted on the brains of mice that succumbed during the trial, on controls and on vaccinated mice that survived. Duvaxyn WNV vaccine provided complete protection against challenge with lineage 2 WNV and stimulated significant cross-protective neutralising antibodies in mice against lineage 2.

Meningococcal serogroup Y lpxL1 variants from South Africa are associated with ST-23/ Cluster A3 clonal complex

NICD investigators: Dr M du Plessis, Ms N Wolter, Ms P Crowther,Ms C Moodley, Dr C Cohen, Dr A von Gottberg

Collaborators: Dr HJ Hamstraand Dr P van der Ley (Department of Vaccinology, National Institute of Public Health and the Environment, Bilthoven, The Netherlands), Dr K Schipper, Dr D van de Beek and Dr A van der Ende (Academic Medical Center, Center for Infection and Immunity, Amsterdam, The Netherlands)

Lipopolysaccharide (LPS) is an important initiator of host inflammatory responses. Between 5% and 10% of invasive meningococcal isolates harbour underacylated LPS caused by a mutation in lpxL1. These isolates induce a reduced cytokine response in vitro and patients have less severe disease. Patient data and serogroup Y isolates were collected from 2003 to 2007 through national surveillance for invasive meningococcal disease (n = 218). Isolates were characterised and screened for IL-6 induction. LpxL1 genes from low inducers were sequenced. Clonal complex ST-175 (cc175) and ST-23/Cluster A3 (cc23) accounted for 83% (176/213) and 11% (24/213), respectively. Low cytokine induction was evident in 15% (32/218). Patients with lpxL1 variants were more likely to be 5–14 years old or 15–24 years old compared to patients <5 years of age, and were more likely to have meningitis than sepsis. On multivariable analysis, age remained significant. Among serogroup Y, lpxL1 variants were associated with cc23 and disease in older children and young adults compared to wild-type strains.

Funding sources

- NICD/NHLS Funds
- Co-operative agreements with the Centers for Disease Control and Prevention, Atlanta, USA
- Pfizer
- Accelerated Vaccine Introduction Initiative (AVI)
- National Research Foundation (NRF)
- NHLS Research Trust
- Medical Research Council (MRC)

Honours

At the University of Pretoria, Faculty of Health Sciences Faculty Dinner 2012, the following CRDM staff received awards: Best Overall Publication Non-clinical runners-up 2012: Professor Marietjie Venter; Best Publication from Team Effort runners-up 2012: Professor M Venter; Best Overall Publication Clinical runners-up 2012: Professor M Venter.

Professor Marietjie Venter received a merit certificate at the Exceptional Achievers dinner for academic and NRF-rated scientists of the University of Pretoria, 25 April 2012.

Charmaine van Eeden, PhD student in the Zoonosis Research Unit, received the Norval Young award to participate in the 11th International Congress of the Society of Tropical Veterinary Society and received a student travel award from the Belgian Ambassador to attend the meeting of the International Society for Veterinary Epidemiology and Economics, Maastricht, The Netherlands, 20–24 August. She also received a student travel award from the Arbozoonet of Europe to attend the Joint Conference on Emerging and re-Emerging Epidemics affecting Global Health.

Stephanie van Niekerk, PhD student at Zoonosis Research Unit, was awarded second prize for oral presentation basic science at the University of Pretoria, Faculty Research Day Award, 28 August 2012.

Professor Marietjie Venter was selected as a member of the South Africa Young Academy of Science (SAYAS) on 7 September 2012.

Nicole Wolter was awarded an NRF promising young researcher, Y2 rating.

Teaching and training

CRDM convened the Annual SARI and rotavirus surveillance investigators meeting on 11 December 2012. Its purpose was to present surveillance results and discuss new projects.
CRDM convened the SARI and rotavirus surveillance officer meeting on 3 and 4 December. The purpose was to provide feedback on results, training and updating to all site surveillance officers.

CRDM convened the 9th Influenza Symposium, "Influenza Symposium 2013", on 18–19 March 2013 at the James Gear Auditorium, NICD.

The first NICD Pneumococcal Surveillance Workshop was held at the NICD 17–19 October 2012. Delegates from 14 African countries met to discuss how to work together and overcome challenges facing surveillance activities in their respective countries.

Regional Reference Laboratory activities: CRDM provides laboratory support to southern African countries conducting surveillance for invasive bacterial diseases (IBD) and contributes to overall strengthening of microbiology surveillance in support of new vaccines introduction. During 2012, 796 samples were received from sites in Africa for detection and confirmation of bacterial pathogens. PCR was performed on all cerebro-spinal fluid received and, where positive, molecular serotyping was done. Antimicrobial susceptibility testing was performed on viable isolates. Five site-visits of the sentinel surveillance site laboratories and hospitals were carried out in Swaziland (2 sites), Zimbabwe (1 site), Namibia (1 site) and Madagascar (1 site) during 2012.

The NIC at CRDM was identified as one of three reference laboratories in Africa to assist with requests for testing severe pneumonia cases with travel history or history of exposure for the novel human coronavirus-2012. The CRDM supported response activities to the novel coronavirus identified in two persons with a history of travel to Saudi Arabia.

Professional development

Graduated students: Two completed scientist internships, one BSc Honours, five MSc, one PhD.

Registered students: Five MSc, nine PhD, 1 FELTP, three scientist interns.

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

Research output

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Dr Nazir Ismail

Dr Chikwe Ihekweazu

Centre Heads

The functions of the NICD Centre for Tuberculosis (CTB) are to provide public health surveillance for TB in South Africa, specialist diagnostic services, assistance with policy and guideline development as well as standardisation of diagnostic methods, and the development and evaluation of novel technologies to advise strategic planning and policy.

One of the main activities this year was and continued to be the development and implementation of an integrated surveillance system providing epidemiological data across the country for the public, the government and the scientific community.

The centre also utilises surveillance and microbiological data to design and implement epidemiological research to guide the national response to the TB epidemic. It also supports the national Department of Health (DoH) in the development of new TB guidelines and policy, participates in training programmes and is actively involved in monitoring trends in disease prevalence and TB drug resistance for ongoing evaluation of the impact of TB control measures instituted by the national DoH, as well as early detection of and integrated response to outbreaks.

The CTB provides the DoH on an ongoing basis with data on the frequency of resistance to anti-TB drugs in *Mycobacterium tuberculosis* isolates in South Africa. Based on information stored at the Corporate Data Warehouse (CDW) of the NHLS, the CTB issues weekly notification of new multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB cases identified by laboratories of the NHLS to provincial coordinators of the National Tuberculosis Control Programme (NTBCP).

Surveillance data generated by the centre are available for ongoing assessment of the impact of TB control measures instituted by the national DOH. The centre also plans to assist the TB control activities of other Southern African Development Community countries.

Current surveillance and research programmes

Integrated public health surveillance and reference laboratory services

In support of the NTBCP, the CTB conducts national surveillance of new cases of laboratory-confirmed TB and drug-resistant TB including MDR-TB and XDR-TB. This surveillance is essential for assessing the overall performance of the programme in achieving the Millennium Development Goals and guiding targeted action to areas of need to direct human and financial resources. The value of this surveillance is not only to advance policy changes but also to assess these and allow for regular updating to meet the huge challenges to reduce the burden of TB, including drug-resistant TB, in South Africa.

TB surveillance based on routinely collected data from corporate data and the electronic TB registries

Laboratory-generated data on TB diagnosis and treatment monitoring are available from the CDW for eight provinces from 2004, and, in addition, for KwaZulu-Natal (KZN) since 2011. An automated system with electronic matching and allocation of unique patient identifiers was developed to transform specimen-based data to patient-based data. This automated process has been extended with 'probabilistic matching', to improve data matching further. This has been especially important for TB surveillance as the diagnostic tools used are for both diagnosis and monitoring and therefore specimen-level information needs to be synthesized to a patient-level system. Furthermore, recurrent episodes of infection are not uncommon and being able to monitor these episodes is a special need.

Utilisation of the CDW data for surveillance and feedback to district and municipal level over time has the potential to provide information on the rate, nature of TB, and laboratory requisition patterns (penetration rates). Benefits envisaged for such a CDW-based system are the provision of good, timely and accurate surveillance data to the national programme and provincial health departments on MDR-TB and XDR-TB identified throughout the country.

The data from CDW are also being linked with clinical data collected via the Electronic TB Register, which includes patients' clinical profiles, treatment histories, and treatment outcomes. The initial linking of these two data sets will provide a rich historical resource of TB in South Africa. The data will subsequently be analysed on a quarterly basis. The data generated will be correlated with TB mortality data, HIV prevalence data and ARV uptake data to draw conclusions on the evolution of the TB epidemic in the context of other relevant public health trends. In addition, routine drug resistance monitoring is being enhanced by matching this data to the Electronic Drug-Resistant TB Register (EDR), which was introduced in 2009 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. The impact of integrating these systems will provide a comprehensive

picture of the burden of disease and also monitor the linkages between diagnosis and treatment – an essential process that is required to reduce this burden.

These data will provide the DoH with the information it needs to estimate the impact of its control measures for TB, which is number 8 of the Minister's 10-point plan in the National Strategic Plan 2010–2013. This will also support the assessment of South Africa's performance in achieving the Millennium Development Goals, especially Goal 7, which is "to reduce TB prevalence rates by 50% relative to 1990 levels".

Survey of drug resistance in TB in South Africa

Following the first South African survey in 2001/2002, and as part of the World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) Global Tuberculosis Surveillance Project, the second 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.

With a sample size of 160,000 patients to be recruited in over 400 clinics across the country, it is the largest survey of its kind conducted anywhere in the world and will provide a comprehensive picture of the epidemiology of drug-resistant TB in South Africa as well as robust estimates for the nine provinces. Sputum samples from all survey participants are also being examined for HIV antibodies, as well as cultured for TB, and when TB organisms are found to be present, the isolates are tested for susceptibility to 11 anti-TB drugs.

This information will be invaluable in guiding future policy decisions on the choice of treatment regimens containing drug combinations appropriate for both drug susceptible and resistant TB, including combinations with the new drugs in the pipeline. The survey has been initiated in all 400 survey clinics across the country and is due to be completed in the second quarter of 2014.

Prospective sentinel surveillance of rifampicin-resistant TB in South Africa: GERMS-SA

South Africa continued its phased implementation of the Xpert MTB/RIF rapid TB diagnostic test in TB suspects, started in 2012. With the realisation that rifampicin (RIF) resistance is not an absolute surrogate marker for MDR-TB and may vary among 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

With a sample size of **160,000** *patients to be recruited in over 400 clinics across the country, it is the largest survey of its kind conducted anywhere in the world*

TB is being 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 previous and current antimicrobial use, HIV status and hospital admission data by patient interview or medical record review. Sputum conversion status and early outcome information on patients with RIF-resistant TB at six months after treatment initiation, using clinical data collected on the EDR, will provide evidence-based information for future planning and policy.

Enhanced surveillance of RIF resistance was initiated at Chris Hani Baragwanath Hospital in October 2012 as a pilot study and is being phased in at other sentinel sites in the country.

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

M. tuberculosis is an important aetiological agent and cause of death in patients with severe acute respiratory infections (SARI). Using the NICD enhanced surveillance network, TB as a cause of SARI was surveyed at sentinel sites in hospitalised patients in South Africa. Surveillance officers are operating the SARI programme and are tasked to identify cases that fit the current SARI and TB case definitions. During 2012, TB-incorporated SARI surveillance was initiated at Chris Hani Baragwanath Hospital (Gauteng), Edendale Hospital (KwaZulu-Natal)), Mapulaneng and Matikwana Hospitals (Mpumalanga), and the Klerksdorp/Tshepong Hospital complex (North West province).

Oropharyngeal (throat) and nasopharyngeal swabs were taken for microbiological investigations in ≥5-year-old patients or nasopharyngeal aspirates in cases of patients <5 years of age for detection of respiratory viruses. To determine *M. tuberculosis* as one of the SARI spectrum of aetiological agents, specimens were taken for conventional TB culture and drug susceptibility testing (DST), as well as for PCR-based molecular testing for rapid diagnosis.

In addition, clinical and epidemiological information was collected regarding the onset and progression of symptoms, age group, HIV status and immunization history, as well as socio-demographic information through interview, hospital record review 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 group of importance for policy decisions.

Molecular surveillance of TB

The CTB of NICD is in the process of establishing strain typing for drug-resistant TB and integrating this into its surveillance systems. Expertise in strain typing techniques including spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing has recently been developed by the centre. TB genotyping, when combined with epidemiological data, helps to confirm epidemiological links and detect outbreaks and unsuspected transmissions, and

serves as a valuable tool to monitor progress toward the elimination of TB transmission. The objectives of this project include: to describe the genetic diversity and identify genotypic clusters of MDR-TB in South Africa; assess the occurrence of common patterns of virulence or/and resistance associated with genotypic clusters of MDR-TB in order to refine MDR-TB treatment strategies; assess the epidemiological and demographic factors associated with genotypic clusters of MDR-TB in order to refine MDR-TB control strategies; and, finally, to describe and monitor the molecular epidemiology of TB in South Africa in order to guide the potential implementation of vaccination programmes in future.

The CTB has already introduced molecular-based typing methods for differentiation of *M. tuberculosis* strains circulating in South Africa. These typing techniques have different discriminatory ability and are regular tools in epidemiological studies involving surveillance and outbreak investigations.

Restriction Fragment Length Polymorphism (RFLP) typing, a technique for DNA fingerprinting of *M. tuberculosis* strains, together with spoligo- and MIRU-typing methods, were introduced by the CTB in 2012. RFLP typing is performed by enzymatic restriction of the *M. tuberculosis* genome into fragments at IS6110 insertion sequence sites, resulting in different numbers and lengths of gene fragments obtained for each strain. This technique has excellent discriminatory power, especially when there are sufficient IS6110 insertion sites in strains, and is regarded as the "gold standard" of TB strain typing. Spoligotyping targets polymorphism of various spacer sequences present in the direct repeat (DR) region of the TB genome by a reverse dot procedure.

It is less discriminating than RFLP typing and is useful for the identification of *M. tuberculosis* strains endemic in regions and the study of lineages involved in the evolution of *M. tuberculosis* over time. PCR-based MIRU typing targets mycobacterial interspersed repetitive units (MIRUs) located in minisatellite-like regions (similar to those found in the human chromosomes), and these regions comprise a variable number of tandemly repeated sequences (VNTRs) 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.

Introduction and evaluation of DNA sequencing platforms

The CTB has access to three next-generation sequencing systems: the 454 GS Junior (Roche), the Ion Torrent (Life Technologies) and the MiSeq (Illumina) sequencers, and will evaluate their performance in sequencing resistance genes and phylogenetic markers relevant to the TB control programme. All sequencing platforms rely on sequencing-by-synthesis design but they differ in the details of sequencing chemistry and the approach used to read sequences. Sequencing-by-synthesis relies on template DNA (from

libraries of clonally amplified templates) and genomic DNA, which go through four main processes, starting with DNA fragmentation to generate random overlapping DNA fragments, followed by tagging, amplification and sequence recognition.

Early work published by the centre in the *Journal of Clinical Microbiology* on the Ion Torrent platform has shown that sequencing is a reliable tool for detecting resistance to multiple drugs rapidly and provides additional information on phylogeny. All these are important for patient management and programmatic response planning. Further work has been undertaken to evaluate the other two systems to determine optimal work flows and the most cost-effective approach to utilise these technologies for diagnostics as well as surveillance. This is an exciting and rapidly expanding area and CTB is ensuring that South Africa keeps abreast of the latest technologies and remains at the forefront globally.

Performance of minimum inhibitory concentration (MIC) determinations

Minimum inhibitory concentration (MIC) determination of *M. tuberculosis* strains, a technique not generally available in TB laboratories, was introduced by the CTB early in 2013 in order to differentiate between strains with mutations-associated high and low levels of resistance to anti-TB drugs. This is an important new 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 are optimal for both HIV-positive and HIV-negative patients. MIC determinations will be available for specific projects but will also be performed on strains from the DRS to provide baseline data from which to monitor trends.

As a special project the CTB started the evaluation and comparison of liquid medium-based MIC methods, including the VersaTREK Culture System (TREK Diagnostics), and the MGIT 960 system (Becton Dickinson)-based method with a solid medium-based MIC agar proportion method.

National TB repository

The CTB initiated the establishment of a national mycobacterial culture collection of isolates stored in our repository, which will provide an important source of material for TB surveillance, encourage research and development, and assist in providing a reference material for monitoring evolution of unusual cases of TB detected through the network of laboratories in South Africa. Isolates are stored at –70 °C and will be archived using specialised software systems and following quality assurance processes to ensure the integrity of this national asset.

As part of the surveillance work on these isolates, research-related information generated on these cultures over time will be archived and increase the usefulness and value of this collection.

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 revolutionize management of drug-susceptible and especially drug-resistant TB. Examples of this are the evaluation and roll-out of the GenoType MTBDR plus assay (Hain Lifescience) and more recently the GeneXpert (Cepheid) technologies in South Africa, in which the NTBRL and subsequently the NPP played a prominent role. Future evaluation and roll-out of other novel technologies will keep South Africa on the cutting edge of TB management. Evaluation of several other systems has been initiated and includes real-time PCR-based systems, comprising high throughput and point-of-care, as well as hybrid models. In addition, evaluation of alternative screening algorithms and diagnostic approaches are also being considered. The diagnostic gap for additional resistance testing using new PCR modalities is being investigated, as well as sequencing-based approaches.

National implementation of Xpert MTB/RIF

The NHLS' National Priority Programme (NPP) was tasked with the roll-out of the Xpert MTB/RIF, and from the outset the CTB supported this implementation and Dr Linda Erasmus was seconded for this major task. The South African DoH and NHLS have been recognised internationally for their role as global leaders in the implementation of Xpert MTB/RIF as part of the NTBCP. In March 2011 they initiated the introduction of this test for the rapid diagnosis of pulmonary TB and RIF resistance for use throughout the country. The phased roll-out has progressed well with the Xpert MTB/RIF replacing smear microscopy as the primary diagnostic test while also screening for drug-resistant TB.

By March 2013, 203 Xpert instruments of varying capacity had been placed in 142 testing centres nationally. The NHLS assisted the DoH with clinical training of healthcare workers on the new diagnostic algorithm. More than 1,180,669 assays were performed, and *M. tuberculosis* was detected in 16–18% of specimens during the first year and 14% in the second year. On average, 7.19% of *M. tuberculosis*-positive samples were rifampicin resistant with some geographical variation noted. Errors rates range from 2–3%.

More than 1,180,669 assays were performed

Current research projects

Epidemiology of drug-resistant TB among children and adolescents in KwaZulu-Natal, Eastern Cape, Limpopo and Gauteng, South Africa

NICD researchers: C Ihekweazu, A Nanoo, N Ismail, L Erasmus

Collaborators: H Menzies, S Bamrah, M van der Walt, D Mametja, N Ndjeka

The CTB collaborated with the Centers for Disease Control and Prevention (CDC), Atlanta, USA, the South African Medical Research Council and national DoH in a study to describe

the epidemiology of and access to treatment for drug-resistant TB among children and adolescents in the Eastern Cape, Gauteng, KwaZulu-Natal and Limpopo. In addition, the study evaluated current recording and reporting systems by comparing MDR-TB case data recorded at the facility, and provincial and national levels with data recorded on the NHLS CDW. This information was fed back to all levels of the health system, including the participating MDR testing facilities. The study has demonstrated major challenges with the recording and reporting systems and highlighted the need for integration of these systems. These findings support the integration project being conducted jointly by the NICD NHLS and national DoH and highlight the large numbers of patients who are not documented as being on treatment. This primary defaulter group has serious implications for control programmes utilising information from the integrated surveillance system and can have a significant impact on case finding.

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

NICD researchers: N Ismail, S Omar
Collaborators: N Beylis, 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 NTBRL/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 system has an automated slide loader and image capturing hardware, which capture fields from stained slides. Images that have been captured are then interpreted using TBDx software. A fully functional system has been developed, and assessment of the performance of the system has reached the stage of clinical evaluation under routine laboratory testing conditions. Further cascade testing is being incorporated to determine the cost differences when the system is used as a pre-screening tool.

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

NICD researchers: N Ismail, H Koornhof
Collaborators: G Kaplan, D Fallows, B Kreiswirth, C Gray, K Klipstein-Grobusch

As part of a cross-sectional investigation on pathogen-related factors in the MDR/XDR-TB epidemic in Gauteng, more than 300 *M. tuberculosis* drug-resistant isolates from Gauteng province are undergoing molecular typing and drug resistance profiling. This study will provide information on drug-resistance-related mutations in *M. tuberculosis* isolates in Gauteng and changes in these mutations over a three-year period. The second cross-sectional study collecting TB isolates from Gauteng commenced in 2013.

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), rifampicin (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 whole genome sequencing on a selective basis. By April 2013, 78 HIV-negative and 55 HIV-positive MDR/XDR-TB patients had been enrolled into the study. Additional components of this study include immunological profiling of cytokines and other biomarkers as well as measurements related to changing nutritional status before and during treatment.

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.

Laboratory support for Médecins Sans Frontieres TB research in Swaziland

NICD researchers: N Ismail, B Magazi, H Koornhof, Z Bhyat
Collaborators: G Mpalala, S Zwane

The NTBRL/CTB continued to provide laboratory support for a project on TB management in Swaziland conducted by Médecins Sans Frontières (MSF). Part of this project is the evaluation of the performance of the GenoType MTBDR plusline probe assay (LPA) at Mbabane and Manzini sites in Swaziland compared with smear microscopy, culture and drug susceptibility testing (DST). The LPA together with conventional smear microscopy, culture and DST are performed at the CTB on specimens as they arrive and results are issued within acceptable turnaround times. Additional drug resistance testing is performed on MDR cases.

Transmission of HIV-associated XDR-TB in South Africa (TRAX Study)

NICD researchers: N Ismail, BT Magazi, SV Omar
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 the transmission of XDR-TB in that province. The study aimed 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 refers 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.

Teaching and training

Eligible microbiology technologists and medical scientists performing routine or research-based testing for *M. tuberculosis* by culture or molecular techniques were offered special training and structured courses by the CTB.

The centre also assisted the African Centre for Integrated Laboratory Training (ACILT) with training courses for technicians, technologists and medical scientists in TB smear microscopy (two courses), DST of *M. tuberculosis* cultures (one course), and *M. tuberculosis* culture, molecular detection and identification (one course).

Additional training is provided to rotating registrars and intern scientists covering both reference mycobacteriology testing and public health aspects.

Professional development

Postgraduate candidates

Number of candidates enrolled: 2 PhD, 0 MMed, 3 MSc, 1 BSc (Hons).
Number of candidates graduated: 1 PhD, 0 MMed, 1MSc (Microbiology), 1 FELTP.

Honours

None

Research output

Publications

Said HM, Kock MM, Ismail NA, Baba K, Omar SV, Osman AG, Hoosen AA, Ehlers MM. Evaluation of the Genotype® MTBDRsl Assay for susceptibility testing of second-line anti-tuberculosis drugs. *International Journal of Tuberculosis and Lung Disease* 2012; **16**:104–110.

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Conference presentations

A total of 26 presentations were made at scientific meetings for the year. Nine of these presentations (three oral presentations and six posters) took place at international meetings; 17 (15 oral presentations and two posters) at national meetings; and one oral presentation at a local meeting.

Acknowledgements

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

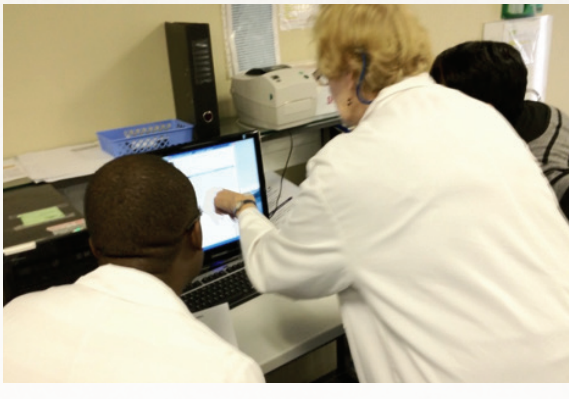
Winter 2012: A snowy day at the Centre for Tuberculosis

(left)
Centralised DR survey training

(right)
DR survey training at the primary healthcare clinics

(left)
Pre-packed DR survey material ready for distribution across South Africa

(right)
DR survey: The CTB laboratory processing samples and reporting results



Professor Barry Schoub
(1 April 2012–28 February 2013)
Head

The Centre for Vaccines and Immunology conducts poliovirus isolation, identification and molecular analysis for South Africa, Angola, Botswana, Lesotho, Mozambique, Namibia, Swaziland, Angola and the Democratic Republic of Congo.

The Centre for Vaccines and Immunology provides laboratory support to South African and southern African departments of health for surveillance of vaccine-preventable diseases including acute flaccid paralysis (polio) and measles.

The unit houses two World Health Organization (WHO) regional referral laboratories. Specialised molecular diagnostic services are offered to South African stakeholders for hepatitis B, hepatitis C, enteroviruses, JC and BK viruses.

Surveillance and diagnostic programmes

Polio surveillance

In support of the Global Poliomyelitis Eradication Initiative (GPEI) initiated in 1988 by the World Health Assembly, any new onset of hypotonic weakness (acute flaccid paralysis) in a child aged younger than 15 years is investigated for poliovirus. Acute flaccid paralysis surveillance is a GPEI strategy to detect poliovirus circulation, re-importation of wild poliovirus into polio-free areas or regions and emerging vaccine-derived polio viruses (VDPVs).

The Centre for Vaccines and Immunology conducts poliovirus isolation, identification and molecular analysis for South Africa, Angola, Botswana, Lesotho, Mozambique, Namibia, Swaziland, Angola and the Democratic Republic of Congo. The majority of the samples were received from South Africa (34.5%), Mozambique (29.45%) and Angola (27.7%). Polioviruses isolated include 20 type 1 Sabin-like, eight type 2 Sabin-like, 30 type 3 Sabin-like and six vaccine-derived polio virus type 2.

A total of 319 non-polio enteroviruses was isolated with an isolation rate of 13.5% (target = 10%). Sequencing analysis identified 21 cases as circulating VDPV type 2 in three

countries; Somalia (1), Kenya (3) and the Democratic Republic of Congo (17). Only six wild poliovirus type 1 (WPV 1) cases were identified from Chad and Niger and no wild poliovirus type 3 cases were identified.

The GPEI has been highly successful in reducing cases of polio. Globally, annual cases have been reduced from 350,000 in 1988 to fewer than 1,000 by the end of 2011, and Pakistan, Afghanistan and Nigeria are the only remaining endemic countries.

Only three African countries reported wild polioviruses (WPV) in 2012 (Nigeria, Niger and Chad) compared to 13 countries in 2011. In South Africa, the last wild poliovirus case reported was in 1989. Adequate surveillance remains the keystone of global polio eradication.

Measles surveillance

As one of the most contagious infectious diseases, measles causes significant morbidity and mortality in children, and especially those who are malnourished and/or immune-compromised.

Since the aim of Millennium Development Goal 4 is to reduce the overall number of deaths among children under five years of age by two-thirds from 1990 to 2015, routine measles vaccination coverage was selected as an indicator of progress towards this goal. Aggressive efforts to improve such coverage resulted in an estimated 86% reduction in measles-related mortality globally between 1990 and 2008, representing a 23% reduction in all-cause mortality in the under-five age group in this period.

The Centre for Vaccines and Immunology is the national and WHO regional referral laboratory for measles surveillance. Serology, specifically the detection of measles-specific IgM antibodies, is the most commonly used method of laboratory diagnosis of acute measles infection.

Since rubella presents with similar clinical symptoms, laboratories often test for IgM against both viruses in suspected measles cases. Measles and rubella IgM testing was performed on 6,815 serum specimens. These comprised 6,273 South African specimens (92%), 26 proficiency specimens (20 WHO and six UKNEQAS) and 516 specimens (7.6%) from southern African countries.

Of the South African specimens, 27 tested positive for measles IgM (0.4%), 20 tested indeterminate for measles IgM (0.3%), 2,132 (34%) were rubella IgM-positive and 537 (8.6%) were rubella IgM-indeterminate. Routine rubella IgM testing of rash surveillance samples was discontinued in March 2013.

Hepatitis B testing

Hepatitis B vaccination was introduced into the South African Expanded Programme of Immunisation in 1995. The HIV epidemic has increased the burden of disease in South Africa from hepatitis B. There is currently no national laboratory-based surveillance for hepatitis B,

but multiple sites perform diagnostic testing. The Centre for Vaccines and Immunology has analysed the epidemiological data of the cases it receives for diagnostic testing and genotyped the strains.

The number of laboratory-confirmed cases of hepatitis B for the period was 1,152. The age group 26–48 years was most prevalent (52%) and males accounted for 55% of cases. Genotype A was the predominant genotype (39%) with genotype D (1.73%) and genotype E (1.21%) also detected.

Specialised viral diagnostics

The National Institute for Communicable Diseases and the National Health Laboratory Service Tygerberg were the only public laboratories performing accredited specialised molecular tests for enteroviruses, Epstein Barr virus, herpes simplex virus (HSV)1 and HSV2, *Varicella zoster* virus and polyoma viruses BK and JC. In addition, NICD offers diagnostic testing for hepatitis C. Hepatitis C isolates from 2012 were characterised further and genotyped. In the reporting period, there were 1,002 positive specimens for hepatitis C.

A total of 16 HCV subtypes and mixed intergenotypic infections (6.7%) were identified. Genotype 5a is predominant in South Africa and accounted for 35% of the laboratory-confirmed cases, followed by 1b (22%), 3a (11%) and 4 (9%). Clinical studies in collaboration with gastrointestinal clinics demonstrate that patients with genotype 5a respond better on combination therapy than those with genotype 1 and 4, as noted globally. Hepatitis C epidemiological data will support decision-making regarding treatment programmes.

Research projects

Evolution of poliovirus type 3 using known immunodeficient vaccine-derived polioviruses (iVDPV)

NICD researchers: Dr N Gumede-Moeletsi, H du Plessis, Professor B Schoub

Synopsis: Immunodeficiency vaccine-derived polioviruses [iVDPVs] have more amino acid changes than cVDPV with the inclusion of mixed bases. Immune pressure varies and immune escape leads to different lineages. A plaque assay will be performed on previously identified iVDPVs and different colonies will be sequenced to characterise different populations.

Epitope analysis of genotype 5a hepatitis C virus against South African HLA backgrounds

NICD researchers: Dr N Prabdial-Sing, Professor A Puren
Collaborators: Dr S Bowyer
Funding: Poliomyelitis Research Foundation

Synopsis: The study investigates HCV variability within well studied epitopes identified in genotype 1 and uses algorithms to predict the immunogenicity of their variants from other less studied genotypes. This will inform the most promising vaccine candidates locally. Six class I- and seven class II-restricted epitope sequences within the HCV genome were

compared across the six HCV genotypes using local genotype 5a sequence data together with global data.

Despite the homogeneity of genotype 1 and genotype 5 over the epitopes, there was limited promiscuity to local HLA-alleles.

The prevalence of hepatitis C viral mutations and IL28B single nucleotide polymorphisms and response to pegylated and ribavirin therapy in a Johannesburg study group

NICD researchers: Professor A Puren and Dr N Prabdial-Sing
Collaborators: R Williams
Funding: Poliomyelitis Research Foundation

Synopsis: Preliminary data indicated four to six mutations in the core region and five mutations in the interferon sensitivity-determining region for genotype 5a samples.

All genotype 5a samples analysed had a R70Q mutation, which has been associated with poor viral response. Preliminary data on the prevalence of SNP rs8099917 in various ethnic groups were obtained.

Characterisation of non-polio enteroviruses which have the potential to cause acute flaccid paralysis

NICD researchers: W Horward, A Puren
Collaborators: L Berrie
Funding: Poliomyelitis Research Foundation

Synopsis: As the global eradication of polioviruses approaches, there is consideration of the need to identify other viruses that cause the syndrome of acute flaccid paralysis. There are no vaccines currently available, and it is necessary to assess the prevalence and epidemiology so as to ascertain the burden and implement measures to reduce transmission.

The goals of the project are to screen for non-polio enteroviruses and determine genotypes by molecular tests in order to understand the prevalence of enteroviruses and enterovirus genotypes in the country.

Teaching and training

Expanded Programme of Immunisation (EPI) surveillance workshop

The Department of Health (DoH) in partnership with WHO and NICD conducted a National EPI Disease Surveillance workshop to roll out the revised surveillance tools, manuals and standard operating procedures on 19–28 November 2012.

The workshop cascaded information to clinicians in hospitals and healthcare facilities and shared revised EPI tools.

Postgraduate training

Ten registrars received training and four intern medical scientists successfully completed their internships registered with HPCSA as medical scientists.

Professional development

Postgraduate candidates: registered six (five MSc, one B.Tech).
Graduated: one PhD (April 2012; one MSc (November 2012).

Honours

Dr N Gumede-Moeletsi: 2012 Best PhD Achiever (University of Pretoria).

Professor BD Schoub: Order of Mapungubwe (Silver), 27 April 2012; Lifetime Achievement Award, African Society of Laboratory Medicine [ASLM], December 2012; Research Award from the Faculty of Health Sciences, University of the Witwatersrand, 10 July 2012.

Research output

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Omer SB, Bednarczyk R, Madhi SA, Klugman KP. Benefits to mother and child of influenza vaccination during pregnancy. *Human Vaccines & Immunotherapeutics* 2012; **8**(1): 130–137.

Professor Lucille Blumberg
Head

In this reporting period, an additional pathogen, rifampicin-resistant TB, was added to the organisms under surveillance from September 2012.

Public Health, Surveillance and Response

The Public Health, Surveillance and Response Division incorporates the National Outbreak Response unit, surveillance under the platform of the GERMS-SA surveillance programme and travel medicine within the newly formed South African National Travel Health Network (SANTHNeT).

The division facilitates communication and data sharing between the national and provincial health departments and the National Institute for Communicable Diseases and provides epidemiological input to other NICD units through collaborative projects and support of surveillance and epidemiological activities and outbreak responses.

GERMS-SA

Section co-ordinator: Dr Vanessa Quan

Surveillance

GERMS-SA is a laboratory-based surveillance programme for invasive bacterial and fungal causes of pneumonia, meningitis and diarrhoeal diseases. It is coordinated by the National Microbiology Surveillance Unit (NMSU) and spans many of the centres at the NICD, including the centres for Enteric Diseases; Respiratory Diseases and Meningitis; Opportunistic, Tropical and Hospital Infections; and Tuberculosis. GERMS-SA is an active surveillance programme and relies not only on participating laboratories to submit isolates but also makes use of the Corporate Data Warehouse (CDW) to ensure that all cases meeting the case definition are included in the database. In this reporting period, an additional pathogen, rifampicin-resistant TB, was added to the organisms under surveillance from September 2012.

Surveillance for *Klebsiella* spp. was part of the GERMS-SA pathogen list only until 31 July 2012. The pathogens include: *Salmonella enterica, Shigella* spp., *Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Staphylococcus aureus, Klebsiella* sp., *Cryptococcus* spp, *Candida* 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 South Africa on HIV-associated opportunistic infections, evaluating the impact of vaccines included in the Expanded Programme on Immunisation (EPI) and monitoring rifampicin-resistant TB. Approximately 180 laboratories (public and private), from all nine provinces, reported 15,400 cases according to specific GERMS case definitions.

One third of the cases (5,458 or 35%) came from 25 sentinel sites across the country where 30 surveillance officers collected clinical information on 3,570 (66%) of patients relating to these specific pathogens; this is an underestimation since not all forms have been captured.

The work carried out by the GERMS-SA team has significantly contributed to the development of clinical guidelines for pneumonia, meningococcal disease, cholera, cryptococcosis and typhoid fever and the introduction of pneumococcal conjugate vaccine (PCV) and the *Haemophilus influenzae* type b vaccine booster dose into the EPI. Data emanating from the GERMS-SA activities have contributed to the rolling out by the Department of Health (DoH) of a cryptococcal antigen screen and treat programme, which will facilitate the early diagnosis and treatment of cryptococcal meningitis. In the reporting period, GERMS-SA data have contributed to the new *Cryptococcus* management guidelines (*South African Medical Journal* 2013); the burden of cryptococcosis, the degree of

Initiating rifampicin-resistant TB surveillance in Kimberley, March 2013. From left to right: Lindani, Sr van Niekerk, Sr Barnes, Dr Linda Erasmus and Sr Siyaka

immune-suppression among cryptococcosis patients on presentation and the spectrum of anti-fungals prescribed in local hospitals. GERMS-SA data along with preliminary data from the PCV-7 invasive pneumococcal disease case-control study were used to support recommendations for including a third primary dose of PCV for HIV-infected children on the EPI. In addition, GERMS-SA has conducted numerous site visits to NHLS laboratories, providing training and development of laboratory staff. Such activities continue to contribute to the expansion of knowledge of public health and ensure quality contributions that assist GERMS-SA in determining the burden of disease.

Teaching and training

NMSU assists in the training of registrars and SA-FELTP residents on surveillance. The broader GERMS-SA team performed 31 site visits to laboratories and hospitals participating in the programme and provided feedback on surveillance data as well as and training in clinical and microbiological diagnostics.

Professional development

Postgraduate candidates graduated: one MSc.

Outbreak Response Unit

Section co-ordinator: Dr Juno Thomas

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 National Health Laboratory Service (NHLS) diagnostic laboratories and NICD centres provide appropriate laboratory diagnostic services during outbreaks and specialised diagnostic tests 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.

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 2012, the ORU assisted with a wide spectrum of outbreaks, including shigellosis in the Eastern Cape; cholera in Limpopo; clusters/focal outbreaks (typhoid fever, rabies, Odyssean malaria, enteroviral meningitis); institutional outbreaks (shigellosis, hepatitis A, hepatitis B); and healthcare-associated infection outbreaks (non-typhoidal salmonellosis, varicella

The team from the Outbreak Response Unit, entomologists from the Centre for Opportunistic, Tropical and Hospital Infections and members of the Tshwane Communicable Diseases Directorate on site during the Odyssean malaria outbreak investigation in Tshwane in January 2013

Members of the Outbreak Response Unit and FELTP interact with local community members during the cholera outbreak investigation in Musina in March 2013

zoster virus, and carbapenem-resistant Gram-negative bacteria (NDM-1, VIM)). During 2012, the ORU continued to strengthen networks for the reporting and investigation of foodborne illness, with 92 outbreaks followed up by the unit. The ORU supported communicable disease monitoring during the 2013 African Cup of Nations tournament by providing daily surveillance data and communicable disease intelligence updates. The CDW Alert system, managed by the ORU, facilitates timely notification to healthcare and public health workers of laboratory-confirmed cases of priority communicable diseases detected by NHLS laboratories throughout the country (*Salmonella typhi, Vibrio cholerae, Neisseria meningitidis* and *Bordetella pertussis*). 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, ORU assisted with the development of provincial and national guidelines for priority communicable diseases.

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 disease 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.

Teaching and training

The unit 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. ORU supported the training of future epidemiologists and public health experts through the South African Field Epidemiology and Laboratory Training Programme (SAFELTP).

The unit provides supervision to residents during outbreak investigations, and also gives lectures during both short and long courses offered by the programme.

The ORU supported the training of public health specialists from the University of the Witwatersrand, University of Pretoria and University of Limpopo 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 the Health Protection Agency (UK) were hosted for three-month placements as part of the NICD-HPA exchange programme.

Travel Health

Section co-ordinator: Professor Lucille Blumberg

The South African National Travel Health Network (SANTHNet) was established in 2012 as a partnership between the NICD, the DoH (Communicable Disease Cluster) and the

Inaugural meeting of the WHO Collaborating Centres in Novo Sad, Serbia

South African Society of Travel Medicine. The network focuses primarily on communicable diseases to ensure compliance with the International Health Regulations; and provides an authoritative platform for the development of guidelines for the prevention and treatment of travel-related diseases, guidance for health professionals advising the public travelling locally and abroad, a consultative serve for returning travellers with suspected infectious diseases, and a platform for surveillance for selected travel-related diseases and imported infections such as Dengue fever.

The network functions as a point of contact and liaison internationally for infectious diseases acquired in southern Africa, and assists with training 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 interactions between animal and human health and the environment. A proposed WHO Collaborating Centre for Mass Gatherings Health will be accommodated and will work together with five other centres in Australia, the United Kingdom, Serbia, the United States of America and Saudi Arabia, respectively.

Mass gatherings (MG) are highly visible events that present both challenges and opportunities to host countries and the wider international health community. The health implications of these events range from threats of acute communicable and non-communicable disease (and injury) to the potential for positive and sustainable health legacies.

Drawing on previous experience and programmes established for the 2010 FIFA World Cup, the MG centre was responsible for providing pre-travel advice for the 2013

African Cup of Nations (AFCON) football tournament, which was held from 19 January to 10 February 2013 in South Africa, with 16 countries competing for the coveted title. For the duration of the tournament, the centre assisted the provincial and national DoH with an enhanced surveillance programme focused on communicable diseases considered as potential threats for the tournament.

This enhanced surveillance was facilitated using data gathered from public and private healthcare facilities, as well as laboratory data from NHLS and participating private pathology laboratories. Numbers of laboratory-confirmed cases of selected communicable diseases (including hepatitis A, influenza, malaria, measles, meningococcal disease, and typhoid fever) were collated and reported to the Public Health Cluster each day, and risk assessments for incidents reported during the event. Overall the AFCON was very successful, with a small number of food-borne illnesses reported.

Teaching and training

Undergraduate and postgraduate teaching on travel and tropical diseases was provided for undergraduates and postgraduates at the universities of Stellenbosch, the Witwatersrand and North-West as well as participants attending the travel medicine course and the Diploma in Tropical Diseases (University of the Witwatersrand).

A specialist master class on tropical, travel-related and neglected diseases was held in 2012 at the NICD for infectious diseases sub-specialty fellows from all South African universities.

Honours

Professor Blumberg was appointed as a Bio-weapons Inspector on the United Nations Secretary General's roster. Professor Blumberg received the 2012 Epidemiology award from the South African Society for Veterinary Epidemiologists for contributions in the field of One Health and Zoonoses. Professor Blumberg was also elected chairperson of the SA Malaria Elimination Committee (SAMEC) for a five-year period.

Research output

Abubakar I, Gautret P, Brunette GW, Blumberg L, Johnson D, Poumerol G, Memish ZA, Barbeschi M, Khan AS. Global perspectives on preventing infectious diseases associated with mass gatherings. *Lancet Infectious Diseases* 2012; **12**(1): 66–74.

Archer BN, Timothy GA, Cohen C, Tempia S, Huma M, Blumberg L, Naidoo D, Cengimbo A, Schoub BD. Introduction of 2009 pandemic influenza Avirus subtype H1N1 into South Africa: clinical presentation, epidemiology, and transmissibility of the first 100 cases. *Journal of Infectious Diseases* 2012; **206** (Suppl 1): S148–S153. doi: 10.1093/infdis/jis583

Crowther-Gibson P, Cohen C, Klugman KP, de Gouveia L, von Gottberg A, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Risk factors for multidrug-resistant invasive pneumococcal disease in South Africa, asetting with high HIV prevalence, in the prevaccineera from 2003 to 2008. *Antimicrobial Agents and Chemotherapy* 2012; **56**(10): 5088–5095.

McAnerney JM, Cohen C, Moyes J, Besselaar TG, Buys A, Schoub BD, Blumberg L. Twenty-five years of outpatient influenza surveillance in South Africa, 1984–2008. *Journal of Infectious Diseases* 2012; **206** (Suppl 1): S153–S158. doi:10.1093/infdis/jis575.

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Maanda M, Jacobson R, Kuonza L, Morris V, Engelbrecht H, Nicol M, Bamford C. Outbreak of multidrug-resistant *Pseudomonas aeruginosa* bloodstream infection in the haematology unit of a South African academic hospital. *PLoS One* 2013; **8**(3): e55985. doi: 10.1371.

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* 2013; **8**(2): e55682. doi:10.1371/journal.pone.0055682.

Soeters HM, von Gottberg A, Cohen C, Quan V, Klugman KP. Trimethoprim-sulfamethoxazole prophylaxis and antibiotic non-susceptibility in invasive pneumococcal disease. *Antimicrobial Agents and Chemotherapy* 2012; **56**:1602–1605.

Whitehorn J, Roche RR, Guzman MG, Martinez E, Villamil Gomez W, Nainggolan L, Laksono IS, Mishra A, Lum L, Faiz A, Sall A, Dawurung J, Borges A, Leo YS, Blumberg L, Bausch DG, Kroeger A, Horstick O, Thwaites G, Wertheim H, Larsson M, HienTT, Peeling R, Wills B, Simmons C, Farrar J. Prophylactic platelets in Dengue: survey responses highlight lack of an evidence base. *PLoS Neglected Tropical Diseases* 2012; **6**(6): e1716.

World Health Organization: Clinical management of influenza and other acute respiratory illness in resource-limited settings: learning from the influenza pandemic (H1N1)2009. http://www.who.int/influenza/patient_care/clinical/858-WHOGIPReport_A4_WEB_FA.pdf. Dr Thomas participated in the technical meeting, provided input and reviewed the report.

Chapters in books

Blumberg L, Cohen C, Barnes KA. In: Kibel M, Westwood T, Saloojee H, eds. *Child health for all,* 5th ed. Oxford University Press, 2013.

Conference presentations

International congresses: 9
National congresses: 16
Local congresses: 10

Dr Natalie Mayet
Head

The programmes mission is aimed at strengthening health systems by building capacity to rapidly identify and contain emerging health threats in partnership with all stakeholders.

The South African Global Disease Detection Centre (SARGDDC) was established in July 2010 through a tripartite partnership with the US Centers for Disease Control and Prevention (CDC), the South African Department of Health (DoH) and the National Institute for Communicable Diseases (NICD).

The programme was established as the eighth Global Disease Detection Regional Centre; its mission is aimed at strengthening health systems by building capacity to rapidly identify and contain emerging health threats in partnership with all stakeholders.

The SARGDDC is integrated with and collaborates with the DoH, the centres at the NICD and other stakeholders in the core programmes of:

- International emerging infectious disease detection and response (IEIP)
- The South Africa Field Epidemiology and Laboratory Training Programme (SAFELTP)
- Flu/pandemic preparedness and response
- Emergency preparedness and risk communication
- One Health – a concept for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment
- Laboratory system strengthening.

Many of the specific activities of SARGDDC are integrated in the reports of the centres of the NICD, and SARGDDC continues to collaborate and support 21 projects through a non-research co-operative agreement and 7 projects through a research co-operative agreement.

This year has seen the appointment of Patrick Chong as the Deputy Director of SARGDDC. He is a US Centers for Disease Control and Prevention (CDC) employee and has worked at the Division of Global HIV and AIDS in Vietnam and Thailand as the Country Deputy Director for Thailand and recently he has been assisting CDC programmes in Zimbabwe, Tanzania, Kazakhstan and Papua New Guinea.

NICD and SARGDDC collaborate and support
21
projects

Key highlights

The centre hosted the International Health Regulations Risk Communications workshop on 4–6 September 2012 together with the Division Global HIV/AIDS, CDC-Atlanta and the NDOH. Some 75 participants from sectors including health promotion, communications, communicable diseases control, environmental health, NICD and the private sector attended the training.

Dr Michael Washington from the Heath Economics Technical Support Corporation in CDC-Atlanta, presented a workshop on the Prevention Effectiveness of Heath Economics/Modelling on 20 September 2012 at NICD. This training will be followed with a Health Policy and Decision Analysis workshop planned for 18–21 June 2013. Hands-on expertise for specific projects will be conducted in the week of 24–28 June 2013.

The centre facilitated Africa's first Joint Law Enforcement and Public Health Workshop on 23–24 October 2012. It was attended by 31 participants, 26 from public health and 5 from law enforcement. The CDC and Federal Bureau of Investigation teamed up to give an overview of biological agents and to share their experience of jointly addressing issues of biosafety and biosecurity.

The SARGDDC facilitated the DoH Director General's visit to the CDC in Atlanta on 5 October; it is supporting follow-up activities with the aim of establishing the National Public Health Institute for South Africa and is assisting DoH with the development of a National Surveillance Strategy.

A number of key consultations in the last year have strengthened collaborative efforts.

- Dr Steve Lindstrom and LaShondra Berman, CDC-Atlanta Influenza Division, visited NICD on 12–14 September 2012 to work with the virology laboratory at the National Influenza Centre on quality control and quality assessment of a new multi-pathogen diagnostic assay developed by CDC, the Taqman Array Card (TAC). This assay permits rapid testing of more than 20 pathogens on a single specimen at one time.
- Dr Tony Ao, Epidemic Intelligence Service Officer from Global Disease Detection at CDC-Atlanta, visited South Africa in January and February. Dr Ao visited the country to conduct a case-control study of risk factors for hospitalisation with pneumonia and influenza in rural South Africa and presented his work at the CDC-South Africa Journal Club on 20 February.
- Influenza programme staff also travelled to Madagascar from 4 to 8 March to conduct influenza surveillance and pandemic preparedness reviews. The staff took the opportunity to work with colleagues at the Institut Pasteur-Madagascar on analysing their influenza surveillance and vital statistics data.
- There were a number of visits by senior CDC staff including Dr Scott Dowell, the Director of the Global Disease Detection and Emergency Response Division; Dr Barry Fields, the Laboratory Director for the Global Disease Detection programme in Kenya; and other colleagues from the US Department of Defense and the Defence Threat Reduction Agency.

South Africa Field Epidemiology and Laboratory Training (SAFELTP)

South Africa launched its Field Epidemiology and Laboratory Training programme in May 2006. The SAFELTP was developed in partnership with DoH, NICD, CDC and the University of Pretoria. It is modelled on the CDC Epidemic Intelligence Service (EIS) programme in the USA and is unique in that it is the only one in South Africa designed to train field epidemiology residents for public health leadership positions in the national and provincial departments of health.

The programme provides practical hands-on training with the objectives of providing a platform for building field epidemiology capacity to improve surveillance, detect and respond to public health events, use evidence-based approaches to improve programmes and policy and provide the framework to encourage epidemiological research aligned with national research priorities.

Dr Carl Reddy was appointed as the Programme Director of SAFELTP on 1 March 2013.

The 2013 cohort of nine SAFELTP residents hase been assigned to support national priority programmes and residents have been assigned to assist in Gauteng, Eastern Cape and KwaZulu-Natal. Two residents have been allocated to work with the DoH Communicable Disease Cluster and the national TB programme. Another resident has been assigned to the Cancer Registry at the National Institute for Occupational Health, and the Sexually Transmitted Infections Reference Centre at the NICD also gained a resident. A resident is working with the Epidemiology and Strategic Information unit at the Human Sciences Research Council and will also support the Tshwane NHI District. Another resident is working at the University of South Africa's Institute for Social and Health Sciences and is involved in research in the field of injury and violence prevention. In December 2012, Dr Patience Kweza assisted in the investigation of the typhoid outbreak in Zimbabwe. The SAFELTP team provided outbreak response training to 95 DoH staff in Free State from 29 October–1 November, and the acting programme director served as the external examiner for the Ghana FELTP.

Honours

Riyadh Manesen, a resident on the 2011 cohort, won an award for the best poster at the PHASA Conference for "The Outbreak of diarrhoeal diseases in Verkeerdevlei, Free State Province", and Nomathemba Dube, a resident from the 2010 cohort, achieved her Master of Public Health degree with distinction. Two oral and four poster presentations were made at the TEPHINET Conference in Jordan on 10–15 November 2012. Eight SAFELTP residents completed their training and were awarded Master of Public Health Degrees at the April 2013 graduation ceremony held at the University of Pretoria. The MPH attainment rate has risen from 51% in 2011 to 76% (35/46) for all residents who have completed the programme and it is anticipated that this rate will increase to more than 80% after the September 2013 graduation.

Research output

Journal articles

Dube NM, Summers R, Khin-San T, Mayayise G. A pharmacovigilance study of adults on highly active antiretroviral therapy, South Africa: 2007–2011. *Pan African Medical Journal* 2012; **11**: 39.

Essoya D, Landoh, Tchamdja P, Bayaki Saka, Khin ST, Gitta SN, Wasswa P, de Jager C. Morbidity and mortality due to malaria in Est Mono district, Togo, from 2005 to 2010: a time series analysis. *Malaria Journal* 2012; **11**: 389.

Motladiile TW, Tumbo J, Zvinavhashe HO, Sebekedi C, Rakau ME. Multidrug-resistant nosocomial infections among private hospital patients in the North West province. *Southern African Journal of Epidemiology and Infection* 2012; **27**(3): 111–115.

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Ntshoe GM, McAnerney JM, Archer BN, Smit SB, Harris BN, et al. Measles outbreak in South Africa: epidemiology of laboratory-confirmed measles cases and assessment of intervention, 2009–2011. *PLoS One* 2013; **8**(2): e55682.

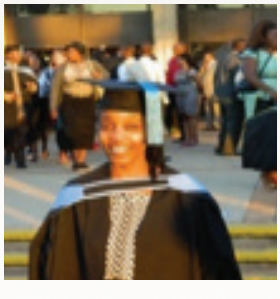
Mamahlodi T, Kuonza L, Candy S. Cervical cancer screening programme in Limpopo province: January 2007 to December 2010. *Southern African Journal of Gynaecological Oncology* 2013; **5**(1): 410.

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 African Medical Journal* 2013; **14**:86.

Conference presentations

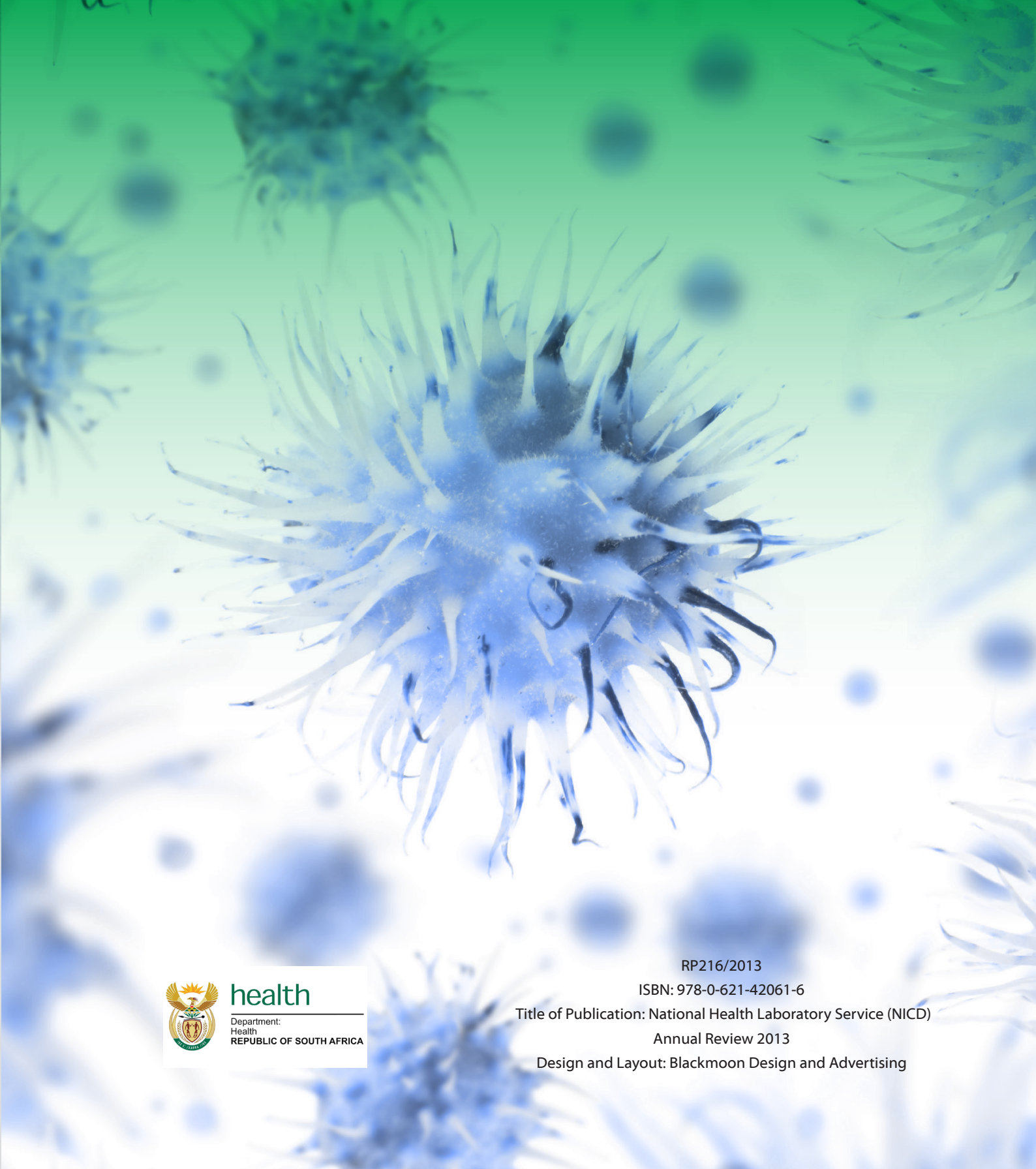
Local: 8
International: 7

Left to right: Queen Ranoto, Joy Ebonwu, Patience Kweza, Lindiwe Cele, Riyadh Manesen, Nkhiphitheni Munyai, Pretty Wongoma, and Tebogo Mamahlodi

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	Fanconi'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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