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This 2010/2011 National Institute for Communicable Diseases (NICD) annual review is the first in the new format which will now be incorporated into a composite report of the Institute’s parent organisation, the NHLS. It is also the last report that I will be contributing as Director of this Institute.

It has indeed been a great privilege steering this ship, which started off as a relatively small vessel, as the National Institute for Virology (NIV), to the present ocean liner, the National Institute for Communicable Diseases. Much was achieved in the quarter century of the NIV’s existence, including the construction of one of the first biosafety level 4 laboratories in the world. This period of time saw the final demise of smallpox and also the birth of HIV. The personnel of the NIV were called on to respond to a wide spectrum of viral disease challenges which beset the country as well as globally.

The birth of the NICD, which came about with the establishment of its parent organisation, the National Health Laboratory Service in January 2002, heralded a major new phase and expansion of the Institute’s activities and responsibilities. By amalgamating the microbiology laboratories of the former South African Institute for Medical Research with the NIV, a truly world-class institution was established in South Africa. The NICD expanded greatly and truly flourished under its new parent body, the NHLS. Collaborative programmes and partnerships were established with institutions and international bodies throughout the world. Formal Collaborating and Regional Reference Laboratories for the World Health Organization were created in disciplines ranging from virology to parasitology and entomology.

The NICD has now matured after a fairly lengthy and not always smooth passage from its antecedent NIV to its present NICD. Much has been achieved, and the next phase of the NICD’s development is about to take off. 2011 should see the birth of a new, exciting and challenging phase coinciding with the appointment of a new Director. The new appointee, who will be assuming the post in April, is Professor Shabir Madhi, a young and highly distinguished medical scientist. Professor Madhi comes with an outstanding CV and superb credentials. Amongst others he enjoys an A-rating, the highest rating for scientists bestowed by the National Research Foundation of South Africa. He also holds the position of...
Research Chair in Vaccine Preventable Disease, created by the country's Department of Science and Technology and the National Research Foundation. The NICD is indeed most fortunate to have as its new Director a person of such stature who is now charged with taking the Institute forward to its next phase. Personally, the past three decades as Director of the two Institutes has been an immense privilege. It has been a great honour to have been the founding director of the NICD and to have been given the privilege of establishing the Institute and bringing it to its present status. It is a great pleasure for me to now pass on the baton to my very worthy successor to take the Institute into a new and exciting, albeit challenging, phase of its expansion to serve a greater public health role.

I have enjoyed an extremely rewarding and enriching career and I would like to especially pay tribute to the previous CEO of the NHLS, John Robertson, and the present CEO of the NHLS, Sagie Pillay, for the great encouragement and support they have provided to the NICD. Finally I want to thank all my staff for their loyalty and dedication over the years and to wish them, the new Director and the Institute all the very best for the future.

Microbiology Division
Head: Professor John Frean

Enteric Diseases Reference Unit
Head: Dr K Keddy

The Enteric Diseases Reference Unit (EDRU) was started in 1997, under the guidance of a pathologist and a part-time technologist. Over the next few years, the capacity was increased, and the unit developed full capacity to serotype Salmonella enterica and Shigella species, with training from the Centers for Disease Control (CDC), Atlanta, USA and WHO Reference Centre for Escherichia coli (Statens Serum Institut, Denmark). EDRU took over Vibrio cholerae work from the old Public Health Laboratory and has developed capacity in both phenotyping and genotyping of this group of organisms.

EDRU collects data on patients presenting throughout South Africa with both invasive and non-invasive disease caused by Salmonella species (including Salmonella Typhi), Shigella species, V. cholerae and diarrhoeagenic E. coli. In order to make these data representative and reflective of the disease burden in each province in the country, all diagnostic laboratories throughout the country are motivated to voluntarily submit limited demographic details and isolates to the EDRU. In exchange, serogrouping and serotyping results are offered free of charge, and regular feedback through quarterly reports and aggregated numbers are published in the NICD Bulletin.

In addition to serogrouping and serotyping, Etests are used to determine the minimum inhibitory concentration (MIC) of each isolate to antimicrobial agents, according to CLSI guidelines. Genotypic characterisation of isolates is performed, such as in outbreak situations. The molecular epidemiology of these bacterial pathogens is continually being elucidated, specifically that of outbreak or epidemic-prone pathogens such as Salmonella Typhi, Shigella dysenteriae type 1 and V. cholerae. A multiplex polymerase chain reaction is used to elucidate the presence of toxin genes in diarrhoeagenic E. coli. EDRU is developing its molecular research laboratory involved with characterising the molecular basis for antimicrobial resistance in these pathogens and plans to further characterise the mechanism of disease due to these pathogens at a molecular and cellular level.

Together with collaborators from the CDC, a number of sites in the country are performing ‘enhanced’ surveillance, where additional clinical data on all patients are being collected, by trained surveillance officers, representing almost all the provinces.

EDRU receives funding for its work from the Medical Research Council, NHLS, PEPFAR, WHO and the International Vaccine Institute.

Surveillance

EDRU is responsible for surveillance and characterisation of bacterial enteric disease, specifically collecting all human isolates from diagnostic microbiology laboratories in South Africa. Although EDRU does not normally do such work for other African countries, the support of the unit may be requested should neighbouring countries require it. EDRU receives specimens from over 4,000 human cases per annum. In addition the unit undertakes to serotype Salmonella, Shigella and diarrhoeagenic E. coli (DEC) isolates for commercial purposes and has in the past performed a multiplex polymerase chain reaction (PCR) to diagnose DEC from veterinary specimens.
Multi-country typhoid fever surveillance programme in sub-Saharan Africa: South African study site

More detailed data on typhoid fever epidemiology, particularly in developing countries, are urgently needed. In 2007, the WHO Strategic Advisory Group of Experts on immunisation endorsed the utilisation of typhoid fever vaccines in regions where the disease is highly endemic, and recommended the strengthening of surveillance systems for typhoid fever, including sentinel site surveillance, and the development of reliable and appropriate diagnostics for use in developing countries.

Edendale Hospital in the Umgungundlovu District in KwaZulu-Natal has been identified as the sentinel site as it is already part of the GERMS-SA network and has pre-existing capacity to complete the work. Edendale Hospital attends to 1,600 outpatients a day and usually has 720 in-patients. Umgungundlovu District has a population of approximately 950,000 people with HIV prevalences of up to 60% in some areas; most of these cases are women of child-bearing age. All in-patients admitted and every fifth out-patient with current fever or a subjective history of fever within the previous 72 hours is considered for inclusion in the study. Samples are collected and sent to the laboratory for testing. Positive typhoid fever cultures as well as culture negative cases and dry spot filter paper is sent for PCR confirmation and other tests as required to the reference laboratory at NICD where the typhoid specimen repository is established. Further analyses, such as genotyping (of *Salmonella* Typhi isolates), confirmation of diagnosis and phylogenetics may be conducted at the Bernhard Nocht Institute for Tropical Medicine, Germany.

International collaboration tracks a typhoid fever outbreak over two continents from South Africa to Australia

In May 2010, an increase in *Salmonella* Typhi cases in Pretoria was noted. Further investigation revealed a cluster of eight cases with links to the Pretoria area. The epidemiology of the disease cluster was investigated, including molecular epidemiological analysis (genotyping) of the isolates to determine genetic relatedness of isolates. *Salmonella* Typhi was identified. Genetic relatedness of isolates was investigated using pulsed-field gel electrophoresis (PFGE) analysis of genomic DNA. PFGE patterns were analysed and compared. Patients were interviewed to determine information such as date of onset of disease, symptoms, places of eating and drinking, type of water supply and sanitation at home, travel history, etc. Restaurant X was linked to the outbreak; an audit of the restaurant was conducted including rectal swabs of staff and environmental surface swabs for testing for *Salmonella* Typhi; specimens were all negative for *Salmonella* Typhi.

A further case of the disease was identified from a barman/food handler who recently worked at the restaurant. He became ill on the way to Perth, Australia and was admitted to an Australian hospital where he was diagnosed with typhoid fever; the PFGE pattern of this *Salmonella* Typhi isolate matched the local outbreak pattern. The PFGE pattern of this isolate was obtained from a participating Australian laboratory linked to PulseNet International, a molecular subtyping network for foodborne pathogens. It is speculated that he may have acquired his infection in Lesotho as he had recently travelled to that country and that he may have been the source of this typhoid fever outbreak in Pretoria.

Launch of PulseNet Africa

PulseNet is an international molecular subtyping network for foodborne and waterborne disease surveillance. Each laboratory utilises standardised genotyping methods, sharing information in real time. The resulting surveillance provides early warning of disease outbreaks, emerging pathogens and acts of bioterrorism. Six regional PulseNet networks exist: for the USA, Canada, Latin America, Europe, Middle East and Asia Pacific. On 11-12 August 2010, a consultation was held at the NICD to explore the development of a regional network for Africa, namely PulseNet Africa. Participants included representatives from PulseNet International, CDC, Health Protection Agency, UK, WHO and 11 African countries (South Africa, Kenya, The Gambia, Senegal, Cameroon, Malawi, Tanzania, Cote d’Ivoire, Ghana, Uganda and Mozambique).

The EDRU will be the coordinator for PulseNet Africa. Pulsed-field gel electrophoresis (PFGE) analysis is the primary subtyping technique used by PulseNet, which requires molecular capabilities including PFGE equipment, agarose gel documentation equipment and BioNumerics Software for analysis of PFGE patterns. Of the 11 African countries represented at the meeting, four (Cote d’Ivoire, Ghana, Uganda and Mozambique) lack molecular capabilities and have no PFGE equipment; their priority is to now acquire PFGE capabilities.
Molecular characterisation of cholera outbreak isolates, 2008-2009

Investigators: H Ismail, AM Smith, A Sooka, KH Keddy

In 2008, South Africa experienced two major outbreaks of cholera. From May to July 2008, an outbreak of cholera (outbreak A) was reported in the Ehlanzeni district of Mpumalanga. During mid-November 2008, an outbreak of cholera (outbreak B) was identified in Musina in Limpopo following the importation of cholera cases from Zimbabwe. As part of routine characterisation of *Vibrio cholerae* O1 isolates, isolates undergo serological and biochemical confirmatory identification, antimicrobial susceptibility testing, pulsed-field gel electrophoresis analysis, molecular detection of the cholera toxin (CT) gene ctxA, and the colonisation factor gene tcpA, by PCR analysis.

The molecular epidemiology and mechanism of antimicrobial resistance of 110 antimicrobial-resistant, toxigenic, *V. cholerae* O1 biotype El Tor isolates for the period of 1 January 2008 to 31 May 2009 are currently being investigated, using various molecular techniques. Over the past two decades, a number of new epidemic lineages of *V. cholerae* O1 biotype El Tor variants have emerged or re-emerged. El Tor variants have been described from Bangladesh and Mozambique and produce the CT of the classical biotype. Further analysis will include the molecular screening and nucleotide sequencing of the CT gene ctxB, in order to enhance the understanding of the origin of these antimicrobial-resistant *V. cholerae* O1 biotype El Tor isolates.

Molecular characterisation of extended-spectrum β-lactamase-producing *Shigella* isolates from humans, 2003-2009

Investigators: NP Tau, AM Smith, A Sooka, KH Keddy

Bacillary dysentery caused by *Shigella* species is an important cause of acute diarrhoeal disease in poor communities. Although *Shigella* infection is self-limited, antimicrobial therapy is generally required to manage infection and reduce faecal excretion of the bacterium to prevent further transmission. *Shigella* species have progressively acquired resistance to antimicrobial agents used for treatment of bacillary dysentery due to their ability to acquire mobile genetic elements such as resistance plasmids and transposons. The production of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase (ACBL) is an important mechanism of resistance to β-lactam antimicrobial agents among the *Enterobacteriaceae*. ESBL and ACBL production is often associated with resistance to other classes of antimicrobial agents and as a result, antimicrobial treatment of ESBL-producing isolates becomes limited. This study characterises molecular mechanisms of ESBL-production in human isolates of *Shigella* in South Africa for the years 2003-2009. *Shigella* was serotyped using standard techniques and antimicrobial minimum inhibitory concentrations were determined using Etests and agar dilution methods.

The presence of ESBL-production was established by disc diffusion screening methods. ESBL (*bla*TEM, *bla*SHV and *bla*CTX-M) and ACBL (*bla*CMY) genotypes were determined using PCR and nucleotide sequencing. For the years 2003-2009, the EDRU received 6,833 *Shigella* isolates. Of these, 21 (0.3%) isolates were confirmed ESBL producers; however, only 20 isolates were available for analysis. ESBL-production was not exclusively associated with any particular *Shigella* species/serotype, however *Shigella flexneri* 2a was the most commonly identified. All ESBL-producing *Shigella* strains showed high levels of resistance to ampicillin, co-trimoxazole, ceftriaxone and cefotaxime.

The *bla*CTX-M-15 was the most commonly identified ESBL enzyme. ESBL-producing *Shigella* isolates in the study comprise less than 1% of all *Shigella* isolates received for surveillance; even so, they are clinically relevant as they present a challenge in disease management and patient treatment. It is believed that this is the first report of ESBL-producing *Shigella* isolates from South Africa. The emergence of CTX-M-producing *Shigella* isolates in South Africa is concerning, therefore continued surveillance is needed to monitor the incidence of these isolates.

Characterisation of bacterial causes of diarrhoea in an under-five population

Investigators: Z Makhari, KH Keddy, AM Smith, S Madhi

Diarrhoea is one of the major causes of morbidity and mortality among children under five years of age worldwide. There are various pathogens causing diarrhoeal diseases in both the developed and developing countries which includes bacteria, viruses and parasites. The most commonly isolated diarrhoeal pathogens include: diarrheagenic *Escherichia coli*
(DEC), rotavirus, *Shigella* species, *Salmonella* species, *Vibrio cholerae* and *Campylobacter* species. Rotavirus is known to be the major aetiological agent of gastroenteritis in children under five years of age. For 2007, approximately 527,000 cases of childhood deaths due to diarrhoea were recorded by the WHO, of which 145,000 cases were reported from sub-Saharan Africa.

This study describes the bacterial causes of diarrhoea in South African children under five years of age at four surveillance sites which include: Chris Hani Baragwanath Hospital (Gauteng), Dr George Mukhari Hospital (North West Province), Mapulane and Matikwana Hospitals (Mpumalanga) and Edendale Hospital (KwaZulu-Natal). Stool specimens are collected from children under five years of age, presenting with symptoms of diarrhoea, of which the patient would have presented with three or more episodes of loose stools over a 24-hour period. A combination of standard microbiological methods (culture, biochemical tests, serotyping) and molecular methods (PCR, DNA probing of bacterial colony blots) are used for identification and characterisation of bacterial diarrhoeal pathogens from stool specimens. For April 2009 to December 2010, 1,569 stool specimens were received by the EDRU and 519 were positive for bacterial pathogens.

**Teaching and training**

**Postgraduate**

EDRU also has a team of senior pathologists and scientists who are involved in postgraduate training. A specialised programme for the training of microbiology registrars is offered, and over a two-week period, registrars are exposed to a range of biochemical, serotyping and molecular techniques in the identification of bacterial enteric pathogens. The senior staff members are experienced in postgraduate supervision of scientists and have recently started projects with epidemiology students who are examining the extensive database.

EDRU assists in the training supervision of SA-FELTP students.

**Honours**

Dr A Smith’s National Research Foundation’s research rating was re-evaluated and he was placed in the ‘C’ category at level ‘C2’, which is a category for established researchers with a sustained recent record of productivity in their field of study.
The Unit manages all aspects of a WHO grant-funded bacteriology EQA programme for 81 national public health laboratories in the African Regional Office (AFRO) of the WHO. Seventy participants also subscribe to malaria and 82 to TB microscopy programmes. The Unit provides a bacteriology EQA programme to 11 laboratories in Africa that are involved in a major GlaxoSmithKline (GSK Biologicals) malaria vaccine trial.

The Unit administers proficiency testing programmes for the Parasitology Reference Unit, Mycology Reference Unit, and Viral Serology Unit.

National Stock Culture Collection

There are 478 validated strains in the Collection comprising yeasts, moulds and bacterial strains. A total of 65 new strains were purchased for various units for teaching, training, quality control and research purposes. A total of 83 orders were received for cultures from the collection; 731 strains were prepared by lyophilisation for this purpose.

Surveillance

Laboratory antimicrobial resistance surveillance
This new programme is an integrated, laboratory-based, antimicrobial resistance surveillance system of nosocomial pathogens. The Unit joined the Global Antibiotic Resistance Partnership, South Africa, at its inaugural meeting in Stellenbosch.

The Unit provides surveillance for nosocomial antimicrobial resistance for GERMS-SA.

Research

Microbial aetiology of community-acquired pneumonia in adults in Johannesburg

Principal investigator: Dr O Perovic; investigators: Prof C Felman (Department of Medicine, Wits University); Prof A Duse (Clinical Microbiology and Infectious Diseases, NHLS/Wits University); S Oliver (Division of Medical Microbiology, NHLS/University of Cape Town); Dr A Brink, Ampath

This ongoing study describes the aetiological agents of community-acquired pneumonia (CAP) in one distinct geographical area. The pilot study includes 50 patients from Charlotte Maxeke (Johannesburg) Hospital. This will provide initial information on the aetiological cause of CAP, and potentially lead to a follow-up study with approximately 200 patients from two sites (Cape Town, Johannesburg). Such surveillance will provide appropriate aetiological guidance for empirical antimicrobial treatment for the first time in South Africa.

Training

Postgraduate
Three registrars in clinical microbiology and infectious diseases were trained.

Outreach
Two-day workshops in antimicrobial susceptibility testing and malaria were run in Kampala, Uganda in June 2010.

Mycology Reference Unit

Head: Dr N Govender

The Mycology Reference Unit (MRU) contributes to the control of fungal diseases of public health and clinical importance in South Africa and the southern African region by undertaking laboratory-based surveillance and research, by functioning as a reference laboratory in the field of clinical mycology and providing training to laboratory and clinical personnel. In 2010, the MRU focused on two important fungal diseases – cryptococcosis and candidaemia.

The MRU assisted diagnostic laboratories across the country with identification of unusual or atypical fungal pathogens. In addition, susceptibility testing of clinical isolates was performed upon request.

Surveillance

GERMS-SA cryptococcal surveillance
The cryptococcal surveillance project is nested within GERMS-SA - a national, laboratory-based surveillance programme. The primary objective of this project since 2005 has been to estimate the burden of laboratory-confirmed cryptococcosis in South Africa.
TRAC-South Africa (Tracking Resistance to Antifungal drugs for Candida species in South Africa)

In 2008, a laboratory-based, sentinel surveillance project was designed by MRU, in collaboration with public and private sector laboratory sites across South Africa, and the US Centers for Disease Control and Prevention (CDC), Atlanta. The primary objective was to describe the species distribution of Candida spp. causing bloodstream infection at sentinel sites in South Africa, and to compare the species distribution between the public and private sectors. Another major objective was to describe the prevalence of resistance to nine antifungal drugs (including fluconazole, voriconazole, amphotericin B, caspofungin), among invasive Candida spp. in 2009-2010, and to compare antifungal drug resistance patterns between the public and private health sectors.

Surveillance started on 1 February 2009 and ended on 31 July 2010. During the 18-month surveillance period, 2,164 cases of candidaemia were detected; 1,382 (64%) cases were reported by sentinel laboratories and 778 cases were detected by audit. Sixty-six percent of cases were detected by public sector laboratories. Reference laboratory work is ongoing; by the end of 2010, isolates from close to 800 cases had been characterised. Candida albicans was the most common species causing fungaemia in the public sector. In contrast, Candida parapsilosis was the most common species in the private sector.

Planning enhanced surveillance for candidaemia in 2011

In September 2010, an epidemic intelligence officer from the CDC conducted a pilot study at three hospitals to assess the feasibility of enhanced surveillance for candidaemia. Guided by these findings, prospective enhanced surveillance for candidaemia is planned at nine hospitals in two provinces in early-2011.

Public health advocacy

Along with a group of clinicians and public health workers, the MRU has been involved in efforts to introduce cryptococcal antigen (CrAg) screening directed at all newly-diagnosed South African HIV-infected patients with CD4+ T-cell counts ≤100 cells/µL. At a programmatic level, plasma from EDTA samples sent for CD4+ T-cell count testing could be automatically tested for CrAg at the laboratory, if the CD4+ T-cell count result was ≤100 cells/µL for the first time in that individual. In the future, development of point-of-care CrAg tests could simplify the testing algorithm.

Research

Trends in antifungal drug susceptibility of Cryptococcus neoformans

Widespread use of fluconazole for treatment of cryptococcal meningitis and other HIV-associated
opportunistic fungal infections in South Africa may lead to the emergence of isolates with reduced fluconazole susceptibility. Minimum inhibitory concentration (MIC) testing using a reference broth microdilution method was used to determine if isolates with reduced susceptibility to fluconazole or amphotericin B had emerged among cases of incident disease. Incident isolates were tested from two surveillance periods (2002-3 and 2007-8) when population-based surveillance was conducted in Gauteng. These isolates were also tested for susceptibility to fluconazole, amphotericin B deoxycholate or ≥800 mg fluconazole per day for the duration of hospitalisation, as per guidelines, vs sub-optimal treatment (SOT), which included other regimens. Case data from patients prescribed OT increased significantly over time, even before the guidelines — OT prescription was defined as prescription of at least seven days of amphotericin B deoxycholate or ≥800 mg fluconazole per day for the duration of hospitalisation, as per guidelines, vs. sub-optimal treatment (SOT), which included other regimens. Case data from patients prescribed OT was compared to data from those prescribed SOT. More than half the patients received SOT. However, the proportion of patients prescribed OT increased significantly over time, even before the guidelines. OT prescription was influenced by hospital location, clinical syndrome, and baseline mental status. In contrast, CFR did not change over time, suggesting that other factors, e.g. advanced HIV disease, may be associated with early death, rather than SOT.

Comparison of cerebrospinal fluid parameters between patients with Cryptococcus neoformans and Cryptococcus gattii meningitis

Cryptococcus gattii is a less frequent cause of meningitis than Cryptococcus neoformans. The clinical presentation of C. gattii meningitis may also differ from C. neoformans meningitis. The study aimed to compare cerebrospinal fluid (CSF) parameters between patients with C. gattii vs. C. neoformans meningitis. Laboratory-based surveillance for cryptococcosis was conducted from 1 January to 31 December 2009 at 25 sentinel hospitals in South Africa. Results showed that a significantly higher proportion of cases of C. neoformans meningitis had a low CSF glucose level; however, this finding is of limited diagnostic value.

Multi-locus sequence typing of incident and serially collected isolates of Cryptococcus from HIV/AIDS patients

Up to 23% of South African patients with cryptococcosis will relapse. To determine whether serially collected isolates represented relapse/persistence or new infections, incident and serial isolates from 85 patients were analysed by comparing each isolate's genotype, ploidy and mating type. Surveillance for laboratory-confirmed cryptococcosis was conducted at sentinel sites in South Africa from 2005 to 2008, and isolates were submitted to the reference laboratory. Of 7,807 cases that were detected at sentinel sites during the surveillance period, 634 cases had serially collected isolates, and 185 isolates representing 85 cases were selected. All 85 patients initially received antifungal therapy, and the post-treatment serial isolates from 74 patients had the same genotype. However, the serial isolates from 11 patients possessed genotypes that differed from the original isolate, although in six cases, each pair belonged to the same molecular type. Thus, 13% of the patients with serial isolates were i) co-infected with more than one strain, ii) re-infected with a different strain, iii) and/ or relapsed with a strain that evolved in the patient.

Molecular analyses of paediatric isolates of Cryptococcus neoformans

Compared to adults, cryptococcosis is inexplicably rare among children, even in sub-Saharan Africa, which has the highest prevalence of co-infection with HIV and Cryptococcus neoformans. To explore any mycological basis for this age-related difference in the incidence of cryptococcosis, isolates of C. neoformans recovered from paediatric and adult patients during a two-year period (2005 and 2006) were investigated. One isolate of C. neoformans from each of 82 paediatric patients (<15 years of age) was analysed and the multilocus sequence type (ST), mating type, ploidy and allelic profile determined.
The ratios of each mating type and the proportion of haploids were comparable to isolates that were obtained from 86 adult patients during the same period. Notably, the most prevalent ST was significantly associated with male patients. Overall, these paediatric isolates exhibited high genotypic diversity.

**Serum cryptococcal antigen screening in an adult, HIV-infected, antiretroviral-naïve population in Soweto, 2009-2010**

Cryptococcal antigenaemia may precede the onset of symptomatic meningoencephalitis. Screening HIV-infected patients entering into an antiretroviral treatment (ART) programme with a serum cryptococcal antigen test (CrAg) may detect disease before it manifests clinically, and provide an opportunity for early intervention. The study aimed to determine the proportion of adult, HIV-infected patients, entering into an ART programme in Soweto, which was CrAg screen-positive. Whole-blood specimens were collected from patients newly enrolled into the Chris Hani Baragwanath Hospital HIV Clinic, from January 2009 to July 2010. Of 1,581 screened patients, 64 (4%) were screen-positive. Men were significantly more likely to be screen-positive than women. Of 1,012 patients, 992 had no prior diagnosis of cryptococcal disease and 20 (2%) were screen-positive; 332 (32%) had a CD4+ T-cell count <50 cells/ml; 25/332 (8%) were screen-positive. Overall, fewer than one in 20 newly-enrolled patients were CrAg screen-positive.

**Screening for cryptococcosis among patients with suspected or confirmed TB**

A history of pulmonary TB has also been shown to predict development of subsequent cryptococcal disease, independent of factors such as CD4+ T-cell lymphopenia, among HIV-infected adults, newly enrolled into an antiretroviral treatment programme in the Western Cape. Therefore, it may also be appropriate to screen patients with recently diagnosed or suspected TB for sub-clinical cryptococcal disease. A prospective, hospital-based, sentinel surveillance programme for patients with suspected or confirmed TB was initiated in 2010 at Tshepong Hospital Complex (SARI-TB). All eligible patients who were admitted with a new or recent diagnosis of TB were enrolled into the SARI surveillance programme and followed up during hospitalisation, and up to at least four weeks after admission, to establish outcomes. Approximately 200 SARI-TB patients were screened with the serum CrAg test.

**Evaluation of TRAC-SA surveillance**

The purpose of this evaluation, conducted by an epidemic intelligence officer from the CDC in September 2010, was to evaluate the sensitivity and representativeness of the surveillance system, to identify strengths and weaknesses of the system, and to disseminate findings to stakeholders. This is the first surveillance system in South Africa to focus on bloodstream infections caused by *Candida* and include private sector laboratories. Evaluating data from this study can be used to document epidemiological trends in both *Candida* species and antifungal resistance. This information can be used to guide prevention and control measures for candidaemia.

TRAC-SA is a complex system that requires multiple sites and individuals for case finding. It remains separate from other surveillance systems in South Africa which limits its flexibility, stability and acceptability. Auditing by the principal investigator began after the surveillance period ended. Recommendations include the following: increased frequency of auditing, initiating active surveillance and integration into an existing surveillance system. Lessons learned were: i) integration with an established surveillance programme may improve flexibility, acceptability and stability; ii) recognition of the limitations of a surveillance system is important; and iii) providing timely feedback to data collectors is essential to improve the system.

**Molecular and phenotypic identification of clinically important fungi**

Rapid and accurate identification of fungi is required for optimal patient management. Identification based on phenotypic tests is laborious and requires skilled personnel. Phenotypic identification is often restricted to genus level. Absence of typical structures sometimes precludes phenotypic identification. Species-level identification of moulds may be determined by sequencing the Inter Transcription Spacer (ITS) region, which includes the 5.8S rDNA operon. The study aims to validate a sequencing-based assay to identify fungi quickly and accurately. Fungi of uncertain identity were submitted by clinical laboratories. Well-characterised fungi with known identity, obtained from College of American Pathologists Proficiency Testing Scheme (CAP), were identified in parallel. All fungi were identified using standard, phenotype-based algorithms. In addition, DNA was extracted with the Zymo ZR® fungal/bacterial DNA extraction kit.
Thokozile Gloria Zulu prepares mycology samples in the biosafety cabinet while Brian Nemukula and Nthabiseng Matjomane observe.
The ITS region was PCR-amplified using the ITS1 and ITS4 primers; amplified products were sequenced and compared to sequences in a public-domain database. Identification to species-level was determined if >95% similarity was obtained, otherwise the fungus was identified to genus level. Four clinically important fungi with uncertain identity and 14 known fungi (CAP) were tested. All CAP samples were correctly identified. The turnaround time for sequencing was two days whereas phenotypic procedures took up to three weeks. The sequence-based identity was the same as phenotypic identity in all cases. Identification to species-level could be determined for 14/18 (77%) samples; Emmonsia species could only be identified to genus-level because few sequence-based data are available in the public domain. Sequencing of the ITS region allowed identification of clinically important fungi faster than phenotypic methods and was equally accurate. However, identification depends on accuracy of the data in the public-domain database.

A rare, fatal case of invasive spinal aspergillosis
An antiretroviral-naive, HIV-infected man presented with progressive weakness in the lower limbs and urinary and faecal incontinence for two weeks. The patient had been prescribed broad-spectrum antibiotics and prednisone. He had upper motor neuron signs and a sensory level at T1, with accompanying neck stiffness on flexion. Magnetic resonance imaging revealed diffuse abnormal signals of the vertebral bodies in the lower cervical and thoracic areas, with cord compression in the C2 and C3 region and signal distortions of the T2 and T3 vertebral bodies. Chest X-ray and computerised tomography revealed bilateral fungal colonisation of post-tuberculous apical cavities. Histopathology of an extradural spinal lesion at T1/T2 suggested invasive aspergillosis. Post-mortem analysis of the biopsy sample by PCR reaction identified the infectious agent as Aspergillus fumigatus. The patient died within three weeks.

Emmonsia infections among HIV-infected patients in the Western Cape
An unusual cluster of cases of Emmonsia infection was diagnosed retrospectively in HIV-infected patients in the Western Cape by sequencing of fungal DNA. In some cases, the initial diagnosis was disseminated histoplasmosis. The MRU has collaborated with the Western Cape group to identify a potentially new pathogen.

Proficiency testing
In 2010, the MRU distributed three surveys to approximately 100 laboratories participating in the NHLS proficiency testing scheme (PTS) for mycology (yeasts) and approximately 20 laboratories participating in the testing scheme for mycology (moulds). The format of the scheme was updated in line with other schemes coordinated by NHLS.

For the first time, in 2010, the WHO PT programme was expanded to include fungal pathogens. A simulated cerebrospinal fluid specimen was distributed as part of the third survey to 80 laboratories in the WHO-AFRO region; laboratories were expected to perform microscopy (India ink staining) to detect Cryptococcus neoformans.

Teaching and training
Postgraduate
Three mono-speciality registrars were trained for a week and four registrars were trained for a day as part of the short training programme. Refresher mycology courses for registrars close to the specialist exit examinations were held. Training included practical identification of clinically important yeasts and moulds, a review of antifungal susceptibility test methods, an introduction to molecular epidemiology and molecular diagnostic techniques as well as an overview of relevant clinical infectious disease issues.

Staff
A basic mycology workshop was presented to participants from NHLS laboratories in KwaZulu-Natal to improve the capacity for diagnosis of common fungal infections encountered in the province.

A workshop introduced NICD intern scientists to the field of mycology and included an introduction to identification of yeasts and moulds, an overview of antifungal susceptibility testing and a review of molecular techniques used in medical mycology.

National Microbiology Surveillance Unit
Head: Dr N Govender

The National Microbiology Surveillance Unit (NMSU) contributes to the control of bacterial and
fungal diseases, determined to be of public health importance, through laboratory-based surveillance, The NMSU coordinates, directs and provides capacity for the activities of the Group for Enteric Respiratory, and Meningeal disease Surveillance of South Africa (GERMS-SA) for collaborative research studies.

GERMS-SA surveillance programme

GERMS-SA is a national, laboratory-based surveillance programme for bacterial and fungal diseases of public health importance. In 2010, 18,385 laboratory-confirmed cases were reported through this system. A total of 11,359 isolates were submitted to NICD reference laboratories for characterisation. Almost one third of all cases were reported from 25 enhanced surveillance hospital sites. At these sites, clinical case data were obtained by nurse surveillance officers from 3,513 cases (82%); data were obtained from 2,164 (60%) cases through interview. The NMSU directed and coordinated the national surveillance programme in collaboration with the Enteric Diseases Reference Unit, Epidemiology and Surveillance Unit, Mycology Reference Unit, Parasitology Reference Unit and Respiratory and Meningeal Pathogens Reference Unit. In July 2010, Staphylococcus aureus and Klebsiella sp. were included as surveillance pathogens; eight sites are participating and these are nosocomial blood stream infections. This is under the Antimicrobial Resistance Reference Unit. On 31 December 2010, laboratory-based surveillance for Pneumocystis jirovecii came to an end; it may be restarted as syndromic surveillance in the future.

Site visits

In 2010, 58 visits to 41 surveillance sites in nine provinces provided an opportunity for NICD staff to engage with laboratory and hospital staff participating in the surveillance programme. These site visits are core to reporting to participating laboratories, training of staff around GERMS-SA work, as well as in the nested invasive pneumococcal disease case-control study. Four microbiology laboratory training visits were included in the site visit programme.

Communications

The NMSU compiled and distributed regular surveillance publications to the participating laboratory network and other stakeholders. These are available on the GERMS-SA website.

Research projects

The NMSU collaborated with other NICD units on the following projects during 2010:

- A case-control study to estimate the effectiveness of a 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease;
- Cryptococcal meningitis in Gauteng: exploring post-hospital discharge outcomes and uptake of care, 2009;
- Trimethoprim-sulfamethoxazole prophylaxis and antibiotic non-susceptibility in invasive pneumococcal disease;
- Invasive disease due to Haemophilus influenzae serotype b 10 years after routine vaccination introduction, 2003-2009;
- Active, laboratory-based surveillance for invasive and non-invasive shigellosis, 2003-2007: predominant serotypes may guide vaccine development; and
- Nosocomial salmonellosis: analysis of invasive cases occurring in hospitals over an 18-month period: 2007-2008

Parasitology Reference Unit

Head: Prof J Frean

The Parasitology Reference Unit (PRU) provides reference diagnostic services for human parasitic diseases in the NHLS laboratory network, as well as outside laboratories and organisations; administers several national and international external quality assessment programmes for parasitology; undertakes national surveillance for Pneumocystis pneumonia as part of the GERMS-SA; trains pathology registrars, medical scientists, and technologists; and conducts applied research in the field of human medical parasitology.

Diagnostic service

The 2010 diagnostic workload increased compared with the previous year. Apart from Pneumocystis jirovecii, for which the laboratory offers a primary diagnostic service, most specimens are secondarily referred from other laboratories because of their unusual or diagnostically challenging nature, or for surveillance purposes.
Research

Research programme: Pneumocystis jirovecii

Pneumocystis jirovecii is an unconventional opportunistic fungal pathogen which causes the important AIDS-defining infection, Pneumocystis pneumonia (PcP). There are several components to this programme.

Resolution of mixed dihydropteroate synthase (DHPS) genotypes in respiratory specimens from patients with Pneumocystis jirovecii pneumonia from Gauteng

Researcher: B Poonsamy

Point mutations in the fas gene, which codes for the DHPS enzyme, have been associated with resistance to trimethoprim-sulphamethazole (TMP-SMX or cotrimoxazole), used for the treatment and prophylaxis of PcP. In patients with mixed P. jirovecii DHPS genotypes, it is not always possible to resolve individual genotypes by direct sequencing of PCR products. These mixtures can be resolved if their PCR products are cloned into an appropriate vector, amplified by PCR and a number of clones re-sequenced. Of 319 clinical specimens collected to date, 159 (50%) and 213 (67%), respectively, were positive for P. jirovecii by an immunofluorescence antigen (IFA) detection method and real-time qPCR. The relevant DHPS gene segment of all qPCR positive specimens was amplified by nPCR and visualised by gel electrophoresis. All nPCR products were sequenced with the aim of identifying the DHPS genotypes, particularly the mixed DHPS genotypes. Cloning was done on all specimens identified to contain DHPS mixtures, in order to discern their genotypes. After cloning, successful clones were selected and sequenced. Analysis of results is in progress.

Characterisation of the molecular epidemiology of Pneumocystis infections

Researcher: L Dini

P. jirovecii strain characterisation, based on the analysis of ITS haplotypes, was completed on specimens from a subset of 40 patients enrolled in the clinical study. Cloning and sequencing of 10-12 recombinants per specimen was performed. Just over half of the specimens (21/40, 52.5%) contained multiple infections with different P. jirovecii strains. The most prevalent haplotype was the global type Eg (36/40, 90%), followed by Eu (5/40, 13%).

Human cystic echinococcosis

Researcher: B Mogoye

Cystic echinococcosis (hydatid disease) is a zoonotic disease caused by the larval stage of the tapeworm Echinococcus granulosus. It affects various herbivore intermediate hosts like sheep, cattle, goats, camels, etc. Humans can also serve as intermediate hosts of the parasite and acquire the infection by accidentally ingesting parasite eggs. The eggs are produced by adult tapeworms in the small intestines of dogs (definitive hosts) and other canids. The disease is especially prevalent in pastoral communities, where there is contact between humans, dogs and livestock.

In South Africa, hydatid disease epidemiology is poorly understood and studies need to be done to better understand the risk factors for the disease. The study objectives are to understand the molecular epidemiology of E. granulosus, by investigating the strain types prevalent in South Africa, and to investigate risk factors and geographical distribution of the disease, as well as the impact of co-infections like HIV, hepatitis B and tuberculosis on the clinical course, treatment and outcome of hydatid disease.

Quality assessment

The PRU provides several external quality assessment (EQA) or proficiency testing programmes: national parasitology EQA schemes are offered for stool and blood parasites, both being CPD accredited; malaria EQA programmes are designed and specially produced for the WHO (70 African laboratories) as part of a larger NICD EQA contract, and for two international pharmaceutical companies that are trialling antimalarial drugs and vaccines; and PcP EQA which assesses the participating laboratories’ ability to correctly identify Pneumocystis jirovecii.

Surveillance

Pneumocystis pneumonia surveillance

The laboratory-based surveillance that has been running since 2006, was terminated at the end of 2010. The total number of cases acquired by the GERMS-SA surveillance system in 2010 was 298 (371 in 2009). Compared with other opportunistic pathogens under surveillance, this is certainly a substantial underestimate of the burden of disease. There are several reasons for this: the condition is mainly clinically and radiologically...
diagnosed, and laboratory testing is only offered in a few larger centres. Strategies to better estimate the true burden of disease are being pursued with local and international collaborators.

Stool parasite surveillance
Of the 691 rotavirus surveillance specimens received for stool parasite investigation, 114 (16%) were positive for parasites, mainly the protozoan Cryptosporidium parvum. Whereas rotavirus infections peak in winter, stool parasite prevalence shows a summer predominance.

Teaching and training
Teaching and training in various aspects of parasitology and communicable diseases was provided for MSc and Diploma in Tropical Medicine and Hygiene students, medical students, technicians, medical technologists, intern medical scientists, pathology registrars, and SASTM travel medicine course participants. Training was also done in Uganda and Kenya.

Respiratory and Meningeal Pathogens Reference Unit
Head: Dr A von Gottberg

The Respiratory and Meningeal Pathogens Reference Unit (RMPRU) performs laboratory-based surveillance for invasive disease caused by Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis.

The RMPRU reports weekly data on these diseases to the Epidemiology Division, provides data for presentation at the monthly National Outbreak Response Team at the national Department of Health and for publication in the quarterly NICD Communicable Diseases Surveillance Bulletin. Data are also presented and discussed at Department of Health's Expanded Programme on Immunisation (EPI) Task Group and National Advisory Group for Immunisation (NAGI) meetings. The RMPRU also performs national and regional reference laboratory functions for the diagnosis of meningitis and pneumonia caused by the above bacterial pathogens. Laboratory training is offered to local and international students and colleagues.

Surveillance programmes and related research projects

Case-control study to estimate effectiveness of a 7-valent pneumococcal conjugate vaccine (PCV7) against invasive pneumococcal disease

Principal investigators: Dr A von Gottberg, Dr C Cohen

South Africa is one of the first countries in Africa to introduce PCV7 into the Expanded Programme on Immunisation (EPI), and it is the first country with a high prevalence of HIV to introduce the vaccine. Population HIV prevalence in 2008 in persons aged ≥2 years was estimated to be 10%. Clinical trials have suggested a lower vaccine efficacy among HIV-infected children not treated with antiretroviral therapy as compared to HIV-uninfected children. South Africa has elected to utilise a novel vaccination schedule at 6 weeks, 14 weeks and 9 months. This schedule is potentially cheaper than the traditional 3 plus 1 schedules; however, the effectiveness of this schedule administered through a routine immunisation programme among HIV-infected and uninfected children is unknown. Results of the proposed study will help to guide policy decisions about which vaccination schedule should be used. Study findings may be generalised to other countries with a high HIV prevalence, and may thus be of use to policy makers in those countries. A subcontract with Bloomberg School of Public Health, Johns Hopkins University, was finalised to run this study. This grant was awarded by the Accelerated Vaccine Introduction Initiative (AVI), which is funded by the Global Alliance for Vaccine and Immunization Initiative (GAVI). Funds will be routed through the Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA to the NHLS.

Invasive disease due to Haemophilus influenzae serotype b 10 years after routine vaccination, 2003-2009

South Africa started routine infant immunisation against Haemophilus influenzae serotype b (Hib) disease in 1999 with an accelerated three-dose schedule without a booster dose. Following initial declines in Hib disease, national surveillance identified increasing numbers of Hib disease episodes in fully vaccinated children. National laboratory-based surveillance data was reviewed from 2003 to 2009 for invasive Hib disease episodes among children <5 years, including HIV status and vaccination histories.
Hib vaccine failures were defined as invasive Hib disease in children at least four months of age who had received all recommended doses of Hib vaccine. Detection rates in these children increased from 0.7/100,000 population in 2003 to 1.3/100,000 in 2009. Among 263 episodes of invasive Hib disease among children with known vaccination status, 135 (51%) were classified as vaccine failures. Of vaccine failures, 55% occurred among case patients ≥18 months old. HIV status was documented for 90 children with vaccine failure; 53% were not HIV-infected. Vaccine failures, which occurred in both HIV-infected and uninfected children, comprised half of the rise in invasive Hib disease detected in children ten years after national introduction of Hib vaccine. Addition of a booster dose in 2010 should further reduce Hib disease in South African children.

Development of multiplex real-time PCR assays for identification of bacterial meningitis pathogens and Neisseria meningitidis serogroups

Neisseria meningitidis (Nm), Haemophilus influenzae (Hi), and Streptococcus pneumoniae (Sp) are common pathogens for bacterial meningitis. They also cause respiratory infections. Singleplex real-time PCR (rt-PCR) assays have been developed to detect Nm ctrA, Hi hpd and Sp lytA, and specific genes in the cap locus for Nm serogroups (SGs) A, B, C, X, W135 and Y1. To reduce cost and time, it was decided to develop multiplex assays and to improve sensitivities. Collaborators from the Centers for Disease Control and Prevention, Atlanta, Georgia, first re-designed singleplex PCR assays for Nm B, W135 and Y to improve their sensitivity. The new SG assays were tested on a specificity panel of 60 well-characterised isolates belonging to different species and Nm SGs, and a sensitivity panel of 181 Nm isolates of the respective SGs. Three multiplex rt-PCRs were developed for detection of: i) Nm ctrA, Hi hpd and Sp lytA; ii) Nm SGs A, X and W135; and iii) Nm SGs B, C and Y. The assays show high specificity and compare favourably with the singleplex assays.

Molecular serotyping of Streptococcus pneumoniae from isolates and culture-negative clinical specimens

Streptococcus pneumoniae is a leading cause of childhood morbidity worldwide. Ninety-three serotypes have been described, and the phenotypic Quellung reaction is the gold standard for serotyping. PCR-based serotyping assays are less expensive, less time-consuming and enable serotyping to be performed on culture-negative clinical specimens with sufficient DNA. In this study PCR-based serotyping was evaluated. The serotyping PCR (sPCR) assay consists of six sequential multiplex reactions detecting 33 of the most prevalent serotypes. Findings showed that sPCR overcomes many of the disadvantages associated with the Quellung reaction and is a useful alternative for pneumococcal serotyping.

Meningococcal disease surveillance: added value of PCR identification and serogrouping, 2004-2009

Laboratory-confirmed invasive meningococcal disease (IMD) was defined as isolation of Neisseria meningitidis (NM) from normally sterile site specimens. Culture-negative, but antigen-positive for NM or presence of Gram-negative diplococci were excluded due to specificity concerns. Loss of isolate viability during transport resulted in loss of serogrouping data. The aim was to improve the detection and characterisation of NM using molecular methods. PCR for identification (ctrA) and serogrouping (A, B, C, X, Y, W135) was performed on culture-negative clinical specimens (2004-2009); and transport media yielding no growth (2007-2009). Conventional PCR (2004-2007) was replaced by real-time PCR in 2008 (identification) and 2009 (serogrouping). It was found that PCR added significant value in improving data for laboratory-confirmed IMD. Real-time PCR increased the sensitivity of both organism detection and serogroup characterisation.

Global clones and capsule switching identified among invasive Neisseria meningitidis isolates, 2005

Invasive meningococcal clones belong to a few hypervirulent lineages. For selective advantage meningococci may switch their capsule type by replacement of serogroup-specific genes. The aim was to characterise invasive meningococcal isolates from January to December 2005. Isolates were submitted to a national laboratory-based surveillance system. MLST, PorA and FetA typing were used to characterise all serogroup B (seroB) isolates (58), and 20 randomly selected isolates each from seroA, seroC, seroY and seroW135. Capsule switching was identified by isolates of different serogroups sharing a PorA:FetA:ST genotype. Associations between serogroup and clonal complexes (cc) were compared to data on the PubMLST database. Global hyperinvasive lineages associated with specific serogroups were identified in South Africa. cc ST-865 is usually associated with seroB; however, this research documented the expansion of ST-865 among seroC isolates. Capsule switching was identified in the absence of vaccine pressure.
Molecular characterisation of pneumococcal serotypes 1, 3 and 5 causing invasive disease in children under 5 years, 2007

Serotypes 1, 3 and 5 cause invasive pneumococcal disease (IPD) in South Africa, however are not included in the currently used 7-valent conjugate vaccine. A 13-valent conjugate vaccine (PCV13), including these serotypes, is expected to be available in South Africa during 2011. The aim of the study was to genotypically characterise all serotype 1, 3 and 5 isolates causing IPD in children <5 years, prior to vaccine introduction. IPD cases were reported to a national laboratory-based surveillance system for *Streptococcus pneumoniae*.

Multilocus sequence typing was performed. Allele sequences were submitted to the global database to assign allele numbers and sequence types (ST). Serotype 1 comprised two related STs: ST-217 and ST-612. Serotype 3 belonged to three unrelated STs: ST-458, ST-700 and ST-1765. Serotype 5 isolates were ST-289. For serotype 1, ST-217 was prevalent in Africa and Israel but differed from the USA and Europe where ST-306 was predominant. Serotype 3 (ST-458) differed from other countries where ST-180 was common. ST-289, associated with serotype 5, has also been described in the USA and is identical to the Columbia 5.19 international clone.

Molecular characterisation of invasive *Streptococcus pneumoniae* isolates expressing serotype 4, 6A or 6B capsules in children <5 years, 2007

The pneumococcal conjugate vaccine (PCV7) was introduced into the national immunisation programme in 2009. The effect of the vaccine on pneumococcal genotypes is unknown and baseline data are yet to be established. The aim was to genotypically characterise pneumococci expressing serotypes 4, 6A or 6B from children <5 years in 2007, prior to introduction of PCV7. The isolates were obtained through a national laboratory-based surveillance system. All serotype 4, and 50% of serotypes 6A and 6B isolates were randomly selected for multilocus sequence typing. Allele numbers for 6/7 housekeeping genes were used to predict sequence type (ST). ST-2294, ST-1094 and ST-185 were the major STs causing invasive pneumococcal disease caused by serotypes 4, 6A and 6B, respectively. These differed from STs described elsewhere in the world. Serotype 6B ST-185 is identical to the South Africa 6B-8 global clone.

Teaching and training

Staff

Two-day training workshops for medical technologists in basic bacteriology including microscopy, isolation, identification and antimicrobial susceptibility testing of bacteria were held at Kalafong and Mankweng laboratories.

Outreach

A laboratory training workshop was facilitated in The Gambia entitled ‘Fight against pneumococcal disease in West Africa’.

Honours

Dr N Wolter received the Robert Austrian Research Award 2010 in Pneumococcal Vaccinology at the 7th International Symposium on Pneumococci and Pneumococcal Diseases held in Tel Aviv, Israel for a project entitled ‘Impact of the pneumococcal conjugate vaccine on transmission of *Streptococcus pneumoniae* through acquisition of carriage and outcome of that carriage acquisition’. The award includes a $25 000 grant for this project.

Sexually Transmitted Infections Reference Centre

**Head: Prof D Lewis**

The Sexually Transmitted Infections (STI) Reference Centre is a resource of knowledge and expertise in regionally relevant STIs to the South African Government, to SADC countries and to the African continent at large, in order to assist in the planning of policies and programmes related to the control and effective management of STIs. Intelligence on the aetiology of major STI syndromes, as well as antimicrobial resistance data related to gonococcal infections, are communicated annually to the national and relevant provincial health departments in South Africa as well as to those working in public health and directly with STI patients. The STI Reference Centre also undertakes teaching and training activities, assisting with training of medical technologists, medical scientists, doctors, nurses and other healthcare staff. The STI Reference Centre also aims to be a centre of scientific excellence in the field of STIs; operational research relevant to public health is pursued, and to that end, it has established several international links with STI researchers overseas.
The STI Reference Centre is the operational base of the African Region of the International Union against STIs (IUSTI).

**Surveillance and research projects**

**Microbiological and clinical surveillance of STIs**
In 2010, operational aspects of microbiological surveillance activities for sexually transmitted diseases were completed in Gauteng (Johannesburg), and the Eastern Cape Province (East London and Mthatha). In addition, surveillance activities were commenced in Limpopo and the North West provinces. Negotiations were undertaken to commence similar surveillance in Mpumalanga in 2011.

The microbiological surveillance consists of two components: i) aetiological surveillance of three major STI syndromes: the male urethritis syndrome (MUS), the vaginal discharge syndrome (VDS) and the genital ulcer syndrome (GUS); ii) antimicrobial surveillance of resistance of isolated gonococci from MUS patients to ciprofloxacin and ceftriaxone.

The aetiological surveillance confirmed that gonorrhoea continues to be the main cause of the MUS; that STIs account for less than half of VDS presentations in women, and that genital herpes accounts for the vast majority of GUS cases. Antimicrobial resistance testing demonstrated a continued high prevalence of isolation of ciprofloxacin-resistant gonococci in Alexandra (25%) and similarly high prevalence in East London (30%).

The Gauteng STI surveillance project, run in collaboration with the Gauteng provincial health department, continued to collect data in 2010. The data for 2010 showed no major change to the data of the previous year.

**Implementation of gonococcal resistance surveillance in Africa**
With WHO funding, an aetiological and antimicrobial susceptibility STI survey was set up in Harare, Zimbabwe in November 2010. In addition, the head of the Centre, Professor D Lewis assisted with STI microbiological surveillance protocol revision at the National Microbiological Reference Unit in Antananarivo, Madagascar. He also attended a WHO consultation at the National Microbiological Reference Unit in Mwanza, Tanzania.

**Updating of the SADC Framework for the Management of STIs**
The SADC secretariat had requested Professor Lewis to support the updating of the SADC Framework for the Management of STIs as a technical expert in 2009. The finalised document was approved by the SADC minister in 2010.

**Detection of HIV-specific T- and B-cell immunity in highly exposed HIV-seronegative individuals**
This collaborative study involves a non-governmental organisation working with women at high risk (WAHR) in Carletonville (the Mothusimpilo Project), the STI Reference Centre and the NICD’s Immunology laboratory of the AIDS Virus Research Unit. The aims are to follow up on a group of HIV-seropositive and HIV-seronegative WAHR for one year at three-monthly intervals to study T-cell responses, to identify the receptor profile of natural killer cell subsets that provide protection from HIV infection, and to determine the associations between frequencies of host genes with immune function and clinical data.

**Orange Farm 2 Study: a community study of male circumcision**

Principal investigator: Prof B Auvert (Pubic Health, University of Versailles); co-principal investigators: Prof D Lewis (STIRC) and Dr D Taljaard (Progressus, South Africa)
Funding: French Agence Nationale de Reserches sur la SIDA et les hépatites virales

The current research project, which commenced in the latter part of 2007, aims to establish a male circumcision intervention in Orange Farm in order to evaluate its impact on knowledge, attitudes and practice regarding male circumcision, existing means of prevention (behaviour change, condom use, STI treatment-seeking behaviour and HIV VCT), and the spread of HIV, herpes simplex virus-2, gonorrhoea and chlamydial infection. This project, which was tailored to low income settings, has been a success: community support and participants’ satisfaction with services are high and uptake is steadily increasing. It demonstrated that adult male circumcision could be rolled-out. By the end of 2010, a total of approximately 22,500 circumcisions had been undertaken at the Bophelo Pele Male Circumcision Centre. The project is now considered a model for the roll-out of comprehensive adult male circumcision services, and could be tailored for implementation in other rural and urban low-income communities.
settings in eastern and southern Africa. A paper, entitled ‘A model for the roll-out of comprehensive adult male circumcision services in African low income settings of high HIV incidence: the ARNS 12126 Bophelo Pele Project’, was published in *PLoS Medicine* in 2010. Since adult male circumcision scale-up is expected to take effect within the next two years, this paper is likely to be of high interest to a wide audience, from scientists, medical professionals and public health specialists, to activists, government officials and international organisation officers.

**Alexandra men’s STI clinic**

The STI clinic for men in Alexandra, staffed by the STI Reference Centre, had another successful year in 2010. Numbers of weekly attendances have risen since commencement of the clinic in 2005, from about four to six attendances per clinic to 12-15 attendances per clinic. Over time, there has been a trend to see young men and the proportion of those under 25 has risen substantially. The clinic has been the site of STI surveillance activities as well as a study looking at the prevalence of human papillomavirus infection among men with and without genital warts.

**Teaching and training**

**Postgraduate**

During 2010, three intern medical scientists were trained in various aspects of STI diagnostics. The STI Reference Centre participated in the NICD training rotation for microbiology registrars. Two MSc microbiology students from the London School of Hygiene and Tropical Medicine undertook a two months summer project at the STI Reference Centre.

**Outreach**

STI clinical training was undertaken at a number of external venues, including over 100 African dermatovenerologists on STIs at the 15th Continuing Medical Education and Graduates’ Reunion Conference at the Regional Dermatology Training Centre in Moshi, approximately 50 South African doctors at two separate HIV management courses run by Right to Care, and approximately 150 healthcare workers at the two-day STI update course in Harare, Zimbabwe organised by the Zimbabwe Community Health Intervention Project. In addition, in collaboration with WHO, training of several nurses, doctors and technologists in microbiological surveillance in Madagascar and Zimbabwe was undertaken.

**Special Bacterial Pathogens Reference Unit**

**Head: Prof J Frean**

The Special Bacterial Pathogens Reference Unit (SBPRU) carries out diagnostics and research on zoonotic diseases such as anthrax, plague, leptospirosis, bartonellosis, malaioidosis, and botulism. The unit has a specialised biosafety level 3 (BSL-3) laboratory for handling dangerous bacterial pathogens and serves as the WHO Regional Reference Laboratory for plague in Africa. In addition, the SBPRU carries out plague surveillance by monitoring the susceptible rodent populations in plague-endemic areas in order to alert public health authorities to increased human plague risks. The Unit retained its ISO15189 accreditation status and was certified as a Department of Agriculture Fisheries and Forestry (DAFF) compliant BSL-3 facility.

**Surveillance and research projects**

**Bartonella species in human and animal populations in Gauteng**

**Researcher: Ms AN Trataris**

* Bartonella* is a genus of opportunistic, Gram-negative bacilli transmitted from animals to human hosts. *Bartonella* are highly adaptive, newly emerging pathogens that have the ability to evade the host immune system and cause persistent bacteraemia by occupying the host’s erythrocytes and can cause a variety of clinical manifestations in both immunocompromised and healthy persons. The aims of this study included: determining the IgG and IgM seroprevalences of *B. henselae* and *B. quintana* in immunocompromised and immunocompetent individuals using an immunofluorescence assay (IFA), and investigating the carriage of *Bartonella* spp circulating in human and animal populations in Gauteng using culturing and PCR detection.

The study found that *Bartonella* spp. are important opportunistic pathogens particularly problematic for immunosuppressed individuals. Prevalences suggest that approximately one in four HIV-positive individuals is found to carry the organism which could cause illness. Efforts should be made to better understand the mechanisms of infection and disease progression in this susceptible population.
The detection of *Burkholderia* spp. and pathogenic *Leptospira* spp

**Researcher:** AN Saif

The genus *Burkholderia* consists of more than 45 known species, all of which occupy extremely diverse ecological niches ranging from soils to the respiratory tracts of humans and animals. They are also among the most antibiotic-resistant bacteria encountered. *Burkholderia pseudomallei*, for example, is inherently resistant to many antibiotics, requiring a large combination of antibiotics over many months; it is therefore difficult to eliminate in a clinical setting.

*Leptospirosis* is a common zoonosis worldwide and causes a wide spectrum of disease ranging from subclinical infection to a severe syndrome which includes multi-organ failure and consequent high mortality. This study's objectives, therefore, are to determine the prevalence of pathogenic *Burkholderia* and *Leptospira* spp. in the environment at certain sites in South Africa using molecular and culture techniques; to determine the prevalence of these bacteria in the human population in selected areas using molecular, serological and culture techniques; and to determine whether the human populations in these areas are at risk from these organisms.

**Leptospirosis pilot surveillance study**

Leptospirosis is a well-known cause of febrile illness in many areas of the world, but its importance in South Africa is not known. It has been recognised as a cause of sporadic disease and outbreaks elsewhere in Africa. Recent interest has been stimulated by surveys of humans and rodents in southern Africa, which showed serological evidence of previous exposure, and a small number of human cases have been recently identified locally. There is a lack of awareness of the disease among clinicians, and the absence of sensitive and specific laboratory tests suitable for use in routine diagnostic laboratories; in contrast, in the veterinary field, animal infections have been historically well-catered for by the reference microagglutination test (MAT). Two methods of detection are being applied: a screening test (ELISA) that demonstrates the prevalence of antibodies in the patients’ serum, and PCR, targeting the ribosomal gene sequences specific for pathogenic leptospires. To date, small numbers of samples have been tested, but it is hoped to improve awareness of this infection among clinicians and generate interest in sending clinically relevant specimens.

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**Immunophrophylaxis and molecular epidemiology of anthrax and the fate of *Bacillus anthracis* in living vectors and the environment of Namibia and South Africa**

**Collaborator:** Dr W Beyer (University of Hohenheim, Stuttgart, Germany)

The frequency of outbreaks of anthrax varies from year to year and from season to season within the endemic regions of southern Africa. Although anthrax is an age-old disease, ecological conditions leading to its various epidemiological manifestations (e.g., sporadic cases vs. epidemics and differences in seasonality and species affected in different regions) are still not understood. Until recently the lack of reliable methods to differentiate isolates from different sources was a fundamental hindrance to understanding the epidemiology of anthrax, including the interrelationships between the disease in wild and domestic animals and humans. Programme components are:

**Genotyping and epidemiology of *B. anthracis***

*B. anthracis* appears to be one of the most monomorphic species known; with techniques available in the past, isolates from various sources or geographical locations have been indistinguishable phenotypically and genotypically, with a few rare exceptions. Modern molecular strain typing techniques make it possible to distinguish between outbreak strains, to trace an outbreak strain back to its possible origin, and to track the routes of transmission of an outbreak strain within and between animal populations. It is becoming possible to study genotypic diversity in relation to the spatial and temporal dynamics behind the spread of the disease and possible relationships between genotype and host species. This study investigates the correlation between genotypic diversity and i) spatial and temporal distribution of outbreak strains of *B. anthracis* and ii) the possible host specificity; the correlation between anthrax outbreaks in wildlife and livestock in endemic areas in southern Africa; practices of disposal of the carcasses of animals that have died from anthrax and their role in local epidemics; and the roles of vultures, flies and other insects, and other living vectors, in the epidemiology of anthrax.

**Fate of *B. anthracis* in natural environmental habitats and its role in the epidemiology of anthrax**

Current understanding of the fate of *B. anthracis* in different environments is fragmentary. After the death
of an animal, the vegetative cells of *B. anthracis* are shed into the environment, mainly in the blood oozing from the orifices of the carcass or in body fluids spilled by scavengers. Sporulation of the released bacilli is induced by oxygen. The spores possess a high tenacity and, in some locations, remain viable for many decades. Where proper disposal of the carcasses is not carried out, newly contaminated regions are permanently created and become the potential sources of new infectious cycles for future years.

Contaminated soil is considered the main source of infection causing a new epidemic cycle in nature. One of the important unknowns in this regard is the fate of *B. anthracis* (germination, sporulation, and replication) in natural environmental habitats, such as soil or faecally contaminated sludge. Anthrax spores are notorious for their ability to survive for decades even under harsh environmental conditions. Whether or not free living amoeba can play a role either as a host, protecting *B. anthracis* from environmental influences, or as a vector in oral infections is being investigated in this study. The study followed the fate of *B. anthracis* from environmental influences, or as a vector in oral infections is being investigated in this study. The study followed the fate of *B. anthracis* (germination, replication, re-sporulation) in natural habitats like soil and water holes under the different seasonal conditions and the fate of *B. anthracis* in flies, after feeding on anthrax carcasses.

**Plague surveillance**

Plague is one of three epidemic diseases still subject to the International Health Regulations and notifiable to the WHO. Plague is a zoonotic disease caused by *Yersinia pestis* and is considered as one of the most pathogenic bacteria to humans due to its rapid progression and high fatality rate (bubonic plague 40%-70%; pneumonic 100%) if not treated timeously. The last reported outbreak of plague in South Africa occurred in 1982 in the Coega area, Eastern Cape. Epizootics should be identified as quickly as possible so that steps can be taken to control disease spread. Therefore, plague surveillance has been carried out in this area by monitoring the susceptible rodent populations in order to alert public health authorities to increased human plague risks. In August 2010, a southern African Vlei Rat (*Otomys irroratus*) trapped during routine surveillance in the Coega area tested positive by ELISA for antibodies to the plague bacterium, *Y. pestis*.

**Anthrax repository**

The national anthrax repository is housed within the Unit’s BSL-3 facility. The Unit currently keeps all the historical and new *B. anthracis* isolates from the Kruger National Park as well as other isolates from the rest of South Africa and neighbouring countries.

**Quality assessment**

A plague EQA programme is produced for WHO-AFRO as part of a larger WHO-NICD collaboration and is sent out three times a year to 18 laboratories in plague-endemic countries in Africa, India and Madagascar.

**Teaching and training**

**Postgraduate**

Microbiology and clinical pathology registrars were trained in BSL-3 laboratory principles and activities in the Unit. Staff members were also involved in presenting lectures on the principles of PCR, PCR troubleshooting, and PCR calculations as part of the MSc (Biological and control of African disease vectors) degree presented by the Vector Control Reference Unit.

Intern scientists were trained in the routine diagnostics of special bacterial pathogens e.g. *Bacillus anthracis*, *Yersinia pestis*, *Bartonella* spp., *Leptospira* spp. and *Clostridium botulinum*.

**Outreach**

The SBPRU trained environmental health officers from the City of Johannesburg on the dissection and storage of rodent organs for surveillance purposes; pest control, health and veterinary personnel from the Eastern Cape on plague surveillance and management.

**Vector Control Reference Unit (VCRU)**

*Head: Prof L Koekemoer*

Malaria is the major vector-borne disease in Africa, killing close to one million people annually, most of them children under five years. In South Africa, malaria transmission is confined to the low-lying border areas in the north-east of the country where approximately 5,000 cases were reported in 2009. The Vector Control Reference Unit (VCRU) focuses on the anopheline mosquitoes responsible for malaria transmission. The Unit houses a unique collection of live mosquito colonies of the three most important vector species in Africa, *Anopheles gambiae*, *An. arabiensis* and *An. funestus*, plus the minor vector *An. merus*, and the non-vector species of the *An. gambiae* complex, *An. quadriannulatus*. 
Three colonies of *An. funestus* from Mozambique and Angola continue to provide the VCRU with a unique resource for research into insecticide resistance in this important malaria vector. This places the VCRU in a unique position to offer collaboration with international institutions investigating similar problems and to play a role in influencing policy decisions on vector control strategies in the region. In addition, the VCRU houses the largest museum collection of African arthropods of medical importance in Africa, the third largest such collection in the world. The high level of expertise in the VCRU has been recognised within the University of the Witwatersrand as a research unit called the Malaria Entomology Research Unit (MERU).

Research projects

**Insecticide resistance**

**Anopheles funestus:**

Investigators: BD Brooke, M Coetzee, RH Hunt, ML Kaiser, LL Koekemoer, TS Matambo, SV Oliver, O Wood

Molecular research into pyrethroid resistance in *Anopheles funestus* is a major focus of the VCRU. As part of an initiative aiming to develop alternative insecticides for public health, the toxicity of a range of concentrations of chlorfenapyr against pyrethroid-resistant and -susceptible laboratory reared *An. funestus* was assessed using standard WHO protocols. Chlorfenapyr has been identified as an important addition to insecticides available for malaria vector control, and could be used as a resistance management tool to either circumvent or slow the development of resistance. In addition, the molecular characterisation of mono-oxygenase based pyrethroid resistance was investigated. It has previously been established that increased cytochrome P450 (mono-oxygenase) activity is responsible for the pyrethroid resistance in this species. A mono-oxygenase gene, *CYP6P9*, is highly overexpressed in the pyrethroid-resistant strain compared with a susceptible strain. The full length of the *CYP6P9* sequence was isolated, sequenced and compared between the pyrethroid-resistant and -susceptible strains. Sequence identity between the two strains was 99.3%; the sequence differences were mainly outside of the conserved regions. The functional significance is still unknown, but it is feasible that these variations are associated with differences in insecticide metabolism. A second *CYP6* gene (*CYP6P13*) was also isolated; it shared close similarities with *CYP6P9*. The putative redox partners, cytochrome b5 (cyt b5) and NADPH-cytochrome P450 reductase (CPR), were isolated from *An. funestus* (resistant strain) and showed high levels of sequence identity to other insect cyt b5 and CPRs. Isolation of the coding sequences *CYP6P9* and its cognate redox partners enables the expression of a functional recombinant protein for biochemical and structural analysis. Microarray analysis in the coming year will provide a more detailed understanding of this complex resistance mechanism.

These molecular mechanisms are likely to have occurred in conjunction with other factors that improve production of the resistance phenotype. A strong candidate is cuticle thickening. This is because thicker cuticles lead to slower rates of insecticide absorption, which is likely to increase the efficiency of metabolic detoxification. Measures of mean cuticle thickness in laboratory samples of female *An. funestus* were obtained using scanning electron microscopy. There was a significant and positive correlation between mean cuticle thickness and time to knock down during exposure to permethrin. Mean cuticle thickness was significantly greater in those samples characterised either as more tolerant or resistant to permethrin exposure compared to those characterised as either less tolerant or permethrin susceptible. Further, insecticide susceptible female *An. funestus* have thicker cuticles than their male counterparts.

Pyrethroid resistance in *An. funestus* was also identified in Malawi. Large numbers of *An. funestus* were found resting inside houses in Likomo island, Malawi. WHO insecticide susceptibility tests indicated that wild caught females had developed resistance to deltamethrin, bendiocarb and propoxur. This locality is 1,500 km north of the currently known distribution of pyrethroid-resistant *An. funestus* in southern Africa. The susceptibility results mirror those found in southern Mozambique and South African populations, but are markedly different to *An. funestus* populations in Uganda, indicating that the Malawi resistance has spread from the south.

**Anopheles arabiensis:**

Investigators: BD Brooke, M Coetzee, RH Hunt, C Kikankie, LL Koekemoer

*Anopheles arabiensis* is one of South Africa’s major malaria vectors. Owing to insecticide resistance in this species, alternative interventions to the currently...
available conventional chemical insecticides are urgently needed. The use of fungal pathogens as biopesticides is one such possibility. However, the challenges to this approach are the potential influence of varied environmental conditions and the immunological responses of target species that could affect the efficacy of a biological ‘active ingredient’. An initial investigation into these was carried out to assess the susceptibility of insecticide-susceptible and insecticide-resistant laboratory strains and wild-collected An. arabiensis mosquitoes to infection with the fungus Beauveria bassiana under two different laboratory temperature regimes. Survival data showed no relationship between insecticide susceptibility and susceptibility to B. bassiana infection. All tested colonies showed complete susceptibility to fungal infection despite some showing high resistance levels to chemical insecticides. There was, however, a difference in fungus-induced mortality rates between temperature treatments with virulence significantly higher at 25°C than 21°C. However, because malaria parasite development is also known to slow at lower temperatures, expected reductions in malaria transmission due to fungal infection under the cooler conditions would still be high. These results provide evidence that the entomopathogenic fungus B. bassiana has potential for use as an alternative vector control tool against insecticide-resistant mosquitoes under conditions typical of indoor resting environments. Nonetheless, the observed variation in effective virulence reveals the need for further study to optimise selection of isolates, dose and use strategy in different eco-epidemiological settings.

Insecticide resistance in An. arabiensis from Ethiopia was also investigated. Malaria vector control in Ethiopia is based on selective indoor residual spraying using DDT, distribution of long lasting insecticide-treated nets and environmental management of larval breeding habitats. It was therefore necessary to verify the insecticide susceptibility status of An. arabiensis. Knockdown and mortality results following exposure to insecticide showed resistance to DDT in all villages, resistance to deltamethrin and permethrin in the Ghibe River Valley and permethrin resistance in Gorgora. Bioassay susceptibility tests also indicated the presence of cross-resistance between DDT and permethrin, but not between DDT and deltamethrin. The knockdown resistance (kdr) mutation of leucine to phenylalanine in the sodium ion channel gene was detected in populations from Gorgora and the Ghibe River Valley. Since An. arabiensis shows high levels of resistance to DDT in all villages tested and varying pyrethroid resistance in Gorgora and the Ghibe River valley, precautionary measures should be taken in future vector control operations. Moreover, the status of resistance in other locations in Ethiopia and the spread of resistant gene (s) should be investigated.

Anopheles gambiae:

Investigators: BD Brooke, KS Choi, M Coetzee, RH Hunt, ML Kaiser, C Kikankie, LL Koekemoer, BL Spillings

Insecticide use in public health and agriculture presents a dramatic adaptive challenge to target and non-target insect populations. The rapid development of genetically modulated resistance to insecticides is postulated to develop in two distinct ways: by selection for single major effect genes or by selection for loose confederations in which several factors, not normally associated with each other, inadvertently combine their effects to produce resistance phenotypes. Insecticide resistance is a common occurrence and has been intensively studied in the major malaria vector Anopheles gambiae, providing a useful model for examining how insecticide resistance develops and what pleiotropic effects are likely to emerge as a consequence of resistance. As malaria vector control becomes increasingly reliant on successfully managing insecticide resistance, the characterisation of resistance mechanisms and their pleiotropic effects becomes increasingly important.

An. gambiae is a major vector of malaria in the West African region. Resistance to multiple insecticides has been recorded in An. gambiae S form in the Ahafo region of Ghana. A laboratory population (GAH) established using wild material from this locality has enabled a mechanistic characterisation of each resistance phenotype as well as an analysis of another adaptive characteristic - staggered larval time-to-hatch. An. gambiae GAH showed varying levels of resistance to all insecticide classes. Metabolic detoxification and reduced target-site sensitivity mechanisms were implicated. Most wild-caught families showed staggered larval time-to-hatch. However, some families were either exclusively early hatching or late hatching. Most GAH larvae hatched early but many egg batches contained a proportion of late hatching larvae. Crosses between the time-to-hatch selected sub-colonies yielded ambiguous results that did not fit any hypothetical models based on single-locus Mendelian inheritance.
There was significant variation in the expression of insecticide resistance between the time-to-hatch phenotypes. An adaptive response to the presence of multiple insecticide classes necessarily involves the development of multiple resistance mechanisms whose effectiveness may be enhanced by intra-population variation in the expression of resistance phenotypes. The variation in the expression of insecticide resistance in association with selection for larval time-to-hatch may induce this kind of enhanced adaptive plasticity as a consequence of pleiotropy, whereby mosquitoes are able to complete their aquatic life stages in a variable breeding environment using staggered larval time-to-hatch, giving rise to an adult population with enhanced variation in the expression of insecticide resistance.

Malaria vector control and transmission dynamics

Investigators: KS Choi, M Coetzee, LL Koekemoer

Anopheles longipalpis is morphologically similar to the major African malaria vector Anopheles funestus at the adult stage although it is very different at the larval stage. Despite the development of the species-specific multiplex PCR assay for the An. funestus group, the genomic DNA of An. longipalpis type C specimens can be amplified with the Anopheles vaneedeni and Anopheles parensis primers from this assay. The standard, species-specific An. funestus group PCR, results in the amplification of two fragments when An. longipalpis type C specimens are included in the analysis. This result can easily be misinterpreted as being a hybrid between An. vaneedeni and An. parensis. An. longipalpis type C can be identified using a species-specific PCR assay but this assay is not reliable if other members of the An. funestus group, such as An. funestus, An. funestus-like and An. parensis, are included. This study provides a multiplex assay that will identify An. longipalpis along with other common members of the African An. funestus group, including Anopheles leesoni. An RFLP method for the group was developed that is more accurate and efficient than those used before. Hence, this assay would be useful for field-collected adult specimens to be identified routinely in malaria vector research and control studies.

Diagnostic and other services

The VCRU provides a service for the identification of medically important arthropods for entomologists, medical practitioners and health inspectors. Malaria vector mosquitoes were routinely identified by PCR for the Mpumalanga Province Malaria Control Programme. ELISA and PCR tests were carried out on the An. gambiae complex specimens from various African countries, including South Africa, for species identification and to detect the presence of Plasmodium falciparum sporozoites. Advice and expertise are provided to the Department of Health both at the national and provincial levels, with participation on the National Malaria Advisory Group.

Teaching and training

Ad hoc training in morphology, insecticide resistance, PCR techniques, biochemical analysis and ELISA was given to students from Nigeria.

Postgraduate

During 2010, two PhD students graduated while seven PhD, five MSc and one Hons students received training.

Honours

Prof L Koekemoer was promoted to Reader in the School of Pathology.
Prof M Coetzee received the 2009-2010 NSTF award for her outstanding contribution to science through research outputs over the last five to 10 years. The NIH Centre of Excellence in Malaria Research has been awarded to The Johns Hopkins School of Public Health Malaria Institute and Prof Coetzee is a partner in this award. It’s a seven-year grant and the Centre’s role will be to study insecticide resistance in malaria vector mosquitoes in Zambia and Zimbabwe.

Dr K Choi was awarded the prestigious Hillel Friedland post-doctoral fellowship.

Conference presentations

The number of presentations by staff from the Microbiology Division was:
International: 21
National: 4

Virology Division

Head: Prof Adrian Puren

AIDS Virus Research Unit

Head: Prof L Morris

The AIDS Virus Research Unit (AVRU) comprises three laboratories, namely the Virology Laboratory, the Cell Biology Laboratory and the Immunology Laboratory. The Unit is the largest at the NICD and conducts research projects primarily on the virology and immunology of HIV. It also serves important functions for drug resistance surveillance for the national Department of Health as well as validated end-point assays for HIV vaccine trials. Researchers in the unit were involved in important clinical trials including the ground-breaking CAPRISA 004 study which showed that 1% tenofovir gel could prevent HIV infection by 39%. Samples from a phase I clinical trial of the SAAVI HIV vaccines were tested at the unit for immunogenicity. The AVRU raises a large amount of external funding for the various projects with numerous collaborators and serves an important role in training and capacity building, including running workshops.

Virology Laboratory

Head: Prof L Morris

Research projects

HIV-1 neutralisation breadth develops incrementally over four years and is associated with CD4+ T-cell decline and high viral load during acute infection

Understanding how broadly neutralising activity develops in HIV-1-infected individuals is needed to guide vaccine design and immunisation strategies. A large panel of 44 HIV-1 envelope variants (subtypes A, B and C) was used to evaluate the presence of broadly neutralising antibodies in serum samples obtained three years after seroconversion from 40 women enrolled in the CAPRISA 002 Acute Infection Cohort. Seven of 40 participants had serum antibodies that neutralised more than 40% of viruses tested and were considered to have neutralisation breadth. Among the samples with breadth, CAP257 neutralised 82% while CAP256 neutralised 77% of the panel. Analysis of longitudinal samples showed that breadth developed gradually from year 2 with the numbers of viruses neutralised as well as the antibody titres increasing over time. Neutralisation breadth peaked at four year’s post-infection but was not associated with a change in disease progression.

The extent of cross-neutralising activity correlated with CD4+ T-cell decline, viral load and CD4+ T-cell count at six months post-infection, but not at later time points, suggesting that early events set the stage for the development of breadth. However, in a multivariate analysis, CD4 decline was the major driver of this association as viral load was not an independent predictor of breadth. Mapping of the epitopes targeted by cross-neutralising antibodies revealed that in one individual these recognised the membrane proximal external region, while in two other individuals, cross-neutralising activity was adsorbed by monomeric gp120 and targeted epitopes that involved the N-linked glycan at position 332 in the C3 region. Serum antibodies from the other four participants targeted quaternary epitopes, at least two of which were PG9/16-like and depended on N160 and/or L165 residues in the V2 region. These data indicated that less than 20% of HIV-1 subtype C infected individuals developed antibodies with cross-neutralising activity after three years of infection, and that these target different regions of the HIV-1 envelope including as yet uncharacterised epitopes.
Potent and broad neutralisation of HIV-1 subtype C viruses by plasma antibodies targeting a quaternary epitope including residues in the V2 loop

The targets of broadly cross-neutralising (BCN) antibodies are of great interest in the HIV vaccine field. A subtype C HIV-1 superinfected individual, CAP256, with high-level BCN activity was identified; antibody specificity mediating breadth was characterised. CAP256 developed potent BCN activity peaking at three years’ post-infection, neutralising 32 of 42 (76%) heterologous viruses with titres exceeding 1:10,000 against some viruses. CAP256 showed a subtype bias, preferentially neutralising subtype C and A viruses over subtype B viruses. The CAP256 BCN serum targeted a quaternary epitope, which included the V1V2 region. Further mapping identified residues F159, N160, L165, R166, D167, K169 and K171 (forming the FN/LRD-K-K motif) in the V2 region as crucial to the CAP256 epitope. However, the fine specificity of the BCN response varied over time and, while consistently dependent on R166 and K169, became gradually less dependent on D167 and K171, possibly contributing to the incremental increase in breadth over four years. The presence of an intact FN/LRD-K-K motif in heterologous viruses was associated with sensitivity, although the length of the adjacent V1 loop modulated the degree of sensitivity, with a shorter V1 region significantly associated with higher titres. Repairing the FN/LRD-K-K motif in resistant heterologous viruses conferred sensitivity, with titers sometimes exceeding 1:10,000. Comparison of the CAP256 epitope with that of the PG9/PG16 monoclonal antibodies suggested that these epitopes overlapped, adding to the mounting evidence that this may represent a common neutralisation target that should be further investigated as a potential vaccine candidate.

Isolation of a human anti-HIV gp41 membrane proximal region neutralising antibody by antigen-specific single B cell sorting

Broadly neutralising antibodies are not commonly produced in HIV-1-infected individuals or by experimental HIV-1 vaccines. When these antibodies do occur, it is important to be able to isolate and characterise them to provide clues for vaccine design. CAP206 is a South African subtype C HIV-1-infected individual previously shown to have broadly neutralising plasma antibodies targeting the envelope gp41 distal membrane proximal external region (MPER). A fluorescentinated peptide tetramer antigen with specific cell sorting was used to isolate a human neutralising monoclonal antibody (mAb) against the HIV-1 envelope gp41 MPER. The study data indicate that there are multiple immunogenic targets in the C-terminus of the MPER of HIV-1 gp41 envelope and suggest that gp41 neutralising epitopes may interact with a restricted set of naive B cells during HIV-1 infection.

HIV-1 drug resistance at antiretroviral treatment initiation in children previously exposed to single-dose nevirapine

In this study, the prevalence of HIV-1 drug resistance mutations at the time of treatment initiation in HIV-infected children previously exposed to single dose nevirapine (sdNVP) for prevention of transmission was investigated. Drug resistance mutations were measured at time of treatment initiation in 253 infants and young children below 2 years of age who had been exposed to sdNVP a median of 36 weeks previously. Samples were tested using population sequencing and real time allele-specific PCR. The study showed that the prevalence of mutations known to compromise primary non-nucleoside reverse transcriptase inhibitor (NNRTI)-based therapy is high in sdNVP-exposed children, particularly young infants. A sizable proportion continued to have mutations detected through 24 months of age, albeit at low levels. This is of concern since new guidelines recommend treatment initiation on HIV-1 diagnosis. These results support recommendations to use boosted protease-inhibitor-based regimens in all NNRTI-exposed children under 2 years of age.

Cell Biology Laboratory

Head: Prof C Tiemessen

Research projects

Innate immunity and HIV-1: HIV-specific NK cell responses

The unexpected discovery of unusual NK cell (non-T/CD3-negative) responses to HIV-1 peptides whilst studying the role of HIV-specific T-cell responses and maternal-infant HIV-1 transmission was recently reported by this laboratory. Unlike T-cell responses which broadly respond to the various HIV-1 proteins, these responses that mainly targeted the envelope (Env) and regulatory (Reg) peptide pools were associated with reduced maternal-infant HIV-1 transmission. Importantly, these responses were not detected in uninfected control mothers or their
infants. Furthermore, these responses could only be detected in whole blood assays, requiring a non-specific soluble plasma factor for detection in this study and HIV-specific IgG in another study. Next, the role of these responses in control of HIV-1 infection in the mothers was assessed. HIV-specific NK responses among HIV-1 infected women were associated with lower viral loads and higher CD4 T-cell counts and stronger HIV-specific T-cell responses. Broader NK cell responses (i.e. to both Reg and Env) and responses of higher magnitude had the strongest association with better control of HIV-1 infection. Although magnitudes of CD4 and CD8 T-cell responses to Gag correlated significantly with viral load (not CD4 T-cell counts) as shown previously, representation or magnitude of either of these responses were not responsible for why NK responders had better control of infection than NK non-responders. However, when looking at individuals with CD4-supported CD8 T-cell response to Gag (who had lower viral loads) compared to CD8 T-cell responses to Gag alone, these individuals had higher magnitude CD3-negative cell responses to Env (but not Reg). It was previously suggested that these CD4 T-cell responses to Gag may indicate more functionally effective Gag-specific CD8 T-cell responses; these may be comparable to CD8 T-cell responses of greater quality by measure of the presence of multiple functions simultaneously by polychromatic flow cytometry.

These findings can be interpreted in a number of ways: i) in vivo CD4 Gag responses may provide help for HIV-specific NK cells; ii) HIV-specific NK cells provide help to maintain and preserve CD4 T-cell responses; or iii) NK-CD4 T-cell responses help each other in a bidirectional fashion. All these possibilities may in turn also lead to better quality CD8 T-cell responses. Immune response interactions, particularly in the context of existing HIV-1 infection with its accompanied immune dysregulation, are enormously complex and control of HIV-1 infection is unlikely to be explained by just one type of immune response.

Genetic variation within the gene encoding the HIV-1 CCR5 co-receptor
The human co-receptor, CCR5, acts as the principal co-receptor required for macrophage-tropic (R5) HIV-1 virions to gain entry to a cell. Polymorphisms within the open reading frame as well as the promoter and regulatory regions can influence the amount of CCR5 expressed on the cell surface and hence an individual's susceptibility to HIV-1. CCR5 genes were characterised within the South African African (SAA) and Caucasian (SAC). Full length CCR5 sequences were obtained for 70 individuals (35 SAA and 35 SAC) and sequences were analysed for the presence of single-nucleotide polymorphisms (SNPs), indels and intragenic haplotypes. The results showed that the two population groups showed differences in both haplotype arrangement as well as SNP profile. A better understanding of the role played by host genes in response to HIV-1 exposure will contribute towards a better understanding of the protective immunity to HIV-1 and of the disease process in HIV-1-infected individuals. CCR5 is increasingly being shown to play a critical and central role in HIV-1 infection and to date a number of genetic mutations within the gene have been found to positively or negatively influence an individual's susceptibility and rate of disease progression. Thus, studies such as these which provide valuable new information regarding the genetic diversity within this gene, are important to the further understanding of the impact of CCR5 expression on host susceptibility to HIV-1.

Effect of maternal HIV-1 status and antiretroviral drugs on haematological profiles of infants in early life
Maternal HIV-1 status and antiretroviral drug exposure may influence the haematological profiles of infants. The researchers recruited infants from 118 uninfected control women and from 483 HIV-1-infected women who received no antiretroviral drugs (n=28), or received single-dose NVP (sdNVP) (n=424) or triple-drug combination therapy (n=31) to reduce HIV-1 transmission. Blood was drawn from infants within 24 hours of delivery or six to 12 weeks post-delivery and full blood counts performed. Exposed uninfected (EU; no NVP) differed from control infants only in having lower basophil counts and percentages. In all infant groups, leucocyte profiles showed characteristic quantitative changes with age in the first six weeks of life. HIV-1 infected infants displayed by six weeks elevations in white blood cells, lymphocyte, monocyte and basophil counts, and monocyte and basophil percentages, when compared to EU infants. At birth EU NVP-treated infants exhibited elevated monocyte percentages and counts and basophil counts that did not persist at six weeks. Interestingly, EU newborns of mothers with high CD4 counts (> 500 cells/μl) that had taken sdNVP had significantly elevated white blood cell, monocyte and basophil counts when compared to newborn infants of mothers with similar CD4 counts that had not taken
sdNVP; this was not evident in infants of mothers with CD4 counts <200 cells/μl. These previously undescribed features may affect immune response capability in early life and clinical consequences of such changes need to be further investigated.

**Novel methods for the engineering of microbicide-secreting vaginal Lactobacillus species and the identification of predominant vaginal Lactobacillus species**

Vaginal mucosal microflora are typically dominated by Gram-positive Lactobacillus species, and colonisation of vaginal mucosa by exogenous microbicide-secreting Lactobacillus strains have been proposed as a means of enhancing this natural mucosal barrier against HIV infection. The researchers questioned whether an alternative strategy could be utilised whereby anti-HIV molecules are expressed within the cervico-vaginal milieu by endogenous vaginal Lactobacillus populations, which have been ‘engineered’ in situ via transduction. In this study, they therefore investigated the feasibility of utilising transduction for the expression of the HIV co-receptor antagonists, the CC-chemokines CCL5 and CCL3 in a predominant vaginal Lactobacillus species, Lactobacillus gasseri. Their findings illustrated the potential use of transduction of vaginal Lactobacillus species as a novel strategy for the prevention of HIV infection across mucosal membranes. Encouraged by this investigation, they next sought to identify and develop novel transduction models for predominant vaginal Lactobacillus species. They therefore isolated and characterised the predominant culturable vaginal Lactobacillus species and associated bacteriophages from pre-menopausal women and investigated the effect of vaginal discharge syndrome (VDS) and bacterial vaginosis (BV) on the distribution of predominant Lactobacillus species. Lactobacillus species isolated from the vaginal swabs of volunteers, with and without vaginal discharge syndrome (VDS) and/or bacterial vaginosis (BV) were identified by 16S rRNA sequencing. Bacteriophages isolated from predominant strains were visualised by electron microscopy.

They observed that Lactobacillus jensenii, L. crispatus, L. iners, L. gasseri, and L. vaginalis were the predominant culturable vaginal Lactobacillus species (24%, 22%, 10%, 10% and 9%, respectively). L. crispatus isolates were almost equally distributed between individuals with and without VDS, and not significantly reduced in women with BV versus normal microflora. L. jensenii isolates were, however, significantly reduced in women with VDS, and reduced in women with BV versus normal microflora. L. jensenii isolates were also significantly reduced in women with BV-associated VDS versus women without VDS and normal microflora. Lysogeny was commonly observed for L. crispatus (77%). Only 24% of L. jensenii isolates yielded phage particles. Therefore, due to its high frequency of lysogeny and persistence within individuals with VDS and BV, L. crispatus represents the most favourable vaginal Lactobacillus isolate for the development of novel transduction models, specific for the South African population.

**Immunology Laboratory**

*Head: Prof C Gray*

**Research projects**

**CD4 cells in acute and early HIV-1 infection**

The functional integrity of CD4+ T cells is crucial for well-orchestrated immunity and control of HIV-1 infection, but their selective depletion during infection creates a paradox for understanding a protective response. To address the ontogeny of Gag-specific CD4+ T cells during the acute stage of infection, 12 acutely HIV-infected subjects, including two viral controllers, five viral non-controllers and five intermediate controllers, were analysed. The aim was to understand the profile of CD4+ T cell activation and the association with kinetics of HIV-1 specific CD8+ T cells. Autologous and consensus Gag peptide pools were used at various times post Fiebig staging. The data from this study indicate that Gag-specific CD4+ T cells during acute infection mirror the course of viral replication for a short period, but then cease to be functional beyond 90 days after infection. The appearance of antigen-specific CD4+ T cells peaks prior to antigen-specific CD8+ T cells, irrespective of levels of viral replication. Levels of CD4+ T cell activation tend to reflect viral load and these cells appear to have short-lived ability to proliferate.

In a further cohort, activation, memory maturation and multiple functions of total and antigen-specific CD4+ T cells in 14 HIV-1 and cytomegalovirus-co-infected individuals were analysed at three and 12 months post infection, after the initial burst of CD4+ T cells. Both total memory and Gag-specific CD4+ and CD8+ T cells were characterised by elevated levels of CD38, HLA-DR and Ki67. Significant positive correlations between three and 12-month activation and memory events
highlighted that a steady state of CD4+ T cell activation and memory maturation was established during primary infection and that these cells were unlikely to be involved in influencing the course of viraemia in the first 12 months of HIV-1 infection. It would thus appear, taking these findings together that during acute HIV-1 infection, there is rapid HIV-1 specific CD4+ T cell expansion, which is most likely driven by antigen. After three months, the levels of antigen-specific CD4+ T cells remain constant and at a ‘set point’ likely determined by the amount of replicating virus in the host.

**CD8 cells in acute and early HIV-1 infection**

Due to the possible protective effects of CD8+ T cells during early stages of HIV-1 infection, it is critical to understanding the course and kinetics of these cells during acute and chronic infection. The hierarchy of HIV-1 specific T-cell IFN-γ ELISPOT responses during acute subtype C infection in 53 individuals and associated temporal patterns of responses with disease progression in the first 12 months were characterised. There was a diverse pattern of T-cell recognition across the proteome, with recognition of Nef being immunodominant as early as three weeks post-infection. Over the first six months, it was found that there was a 23% chance of an increased response to Nef for every week post-infection, followed by a non-significant increase to Pol (4.6%) and Gag (3.2%). Responses to Env and regulatory proteins appeared to remain stable. Three temporal patterns of HIV-specific T-cell responses could be distinguished: persistent, lost or new. The proportion of persistent T-cell responses was significantly lower in individuals defined as rapid progressors when compared with those progressing slowly, and who controlled viraemia. Almost 90% of lost T-cell responses were coincident with autologous viral epitope escape. Regression analysis between the time to fixed viral escape and lost T-cell responses showed a mean delay of 14 weeks after viral escape. Collectively, T-cell epitope recognition is not a static event and temporal patterns of IFN-γ-based responses exist. This is partly due to viral sequence variation, but also to recognition of invariant viral epitopes that leads to waves of persistent T-cell immunity, which appears to associate with slower disease progression in the first year of infection.

**Honours**

Immunopaedia, an on-line training site in immunology, developed by Prof C Gray, head of the Immunology laboratory, received the prestigious Science Prize for Online Resources in Education (SPORE). The US-based SPORE awards have been established to encourage innovation and excellence in science education, for the provision of high-quality online resources accessible to students, teachers and the public.

Dr E Gray was selected by the Gates Foundation to be honoured in April 2010 by the Collaboration for AIDS Vaccine Discovery (CAVD) Council of Principal Investigators as a young/early career investigator who has made important scientific contributions to the work of CAVD. She received a Fogarty award to attend two months training at Duke University Medical Center, Durham, USA; her training involved isolating the immunoglobulin genes from a single human B-cell for expression of monoclonal antibodies.

Ms A Picton was awarded a South African Fogarty AIDS and TB Training and Research Programme Fellowship to train at the University of Texas.

**Electron Microscope Unit**

**Head: Dr M Birkhead**

The Electron Microscope Unit (EMU) was established primarily to assist in diagnostic screening for unknown viral pathogens or those that are undetected using current molecular methods. The technique used for rapid viral diagnostics is that of negative staining, and if a suitable sample is obtained, a virus can be identified to Ordinal level within an hour of sample receipt. Negative staining is of little value in bacterial and other microbiological diagnostics, due to the large size of the pathogens and the generalised lack of differential surface structures. However, the alternative preparative method for these samples is to cut 70 nm sections of resin-embedded material, to give insight into the internal ultrastructure of the organisms. Although diagnostically relevant to some microbial pathogens such as microsporidia, resin-embedding is of greater value as a research tool and is certainly not particularly rapid (up to two weeks to process, section, stain and view).

**Diagnostics**

Autopsy tissues received for diagnostic screening highlighted the need for a rapid resin-embedding facility; so a microwave tissue processor was ordered during 2010 that should reduce the processing time.
by several days. Another piece of new equipment acquired and operational towards the end of 2010 was an ultracentrifuge designed specifically for pelleting samples for electron microscopy (Beckman Coulter Airfuge®). This should increase the number of viral identifications made by the EMU, as concentration of the virus, if present, improves the sensitivity of electron microscopy diagnostics. An order was placed for a new 11 megapixel camera for the transmission electron microscope (TEM), which will be installed during 2011 and should greatly increase the resolution of the digital images taken with the TEM, enabling the addition of gold-labelling techniques to the menu offered by the EMU. The value of other EM techniques, specifically scanning electron microscopy (SEM), became apparent in the classification of a new species of Emmonsia being described by the Mycology Reference Unit. The EMU is therefore hoping to acquire a SEM. This will be extremely valuable in providing morphological descriptions of many microbiological organisms and pathogens.

Quality assurance

2010 was the first year in which the EMU participated in the external quality assurance programme for rapid viral diagnostics, co-ordinated by the Robert Koch Institute, Berlin. The favourable results of this exercise will facilitate the Unit’s application for SANAS accreditation on the 15189 schedule, the audit set for early 2011.

Training

The EMU assists in intern and registrar training courses conducted by the NICD.

Respiratory Virus Unit

*Head: Prof M Venter*

The Respiratory Virus Unit (RVU) focuses on research, surveillance and training on respiratory viruses associated with influenza-like illness (ILI) and severe acute respiratory infections (SARI). The Unit is affiliated to the respiratory and zoonotic virus programme at the Department of Medical Virology, University of Pretoria; research on these viruses is conducted and training offered to postgraduate science and medical students. RVU houses the National Influenza Centre which is a WHO Regional Reference Laboratory for influenza. The Unit plays a key role in the development of influenza laboratory and surveillance capacity in the Southern African Development Community and is tasked with pandemic preparedness and response in the region. The RVU performs laboratory surveillance, molecular diagnosis and typing of influenza viruses and investigates annual influenza molecular epidemiology, genetic drift and drug resistance. Monitoring of the resistance to influenza antiviral drugs is conducted annually to determine the effectiveness of treatment with these drugs. The information generated is annually shared with the WHO Global Influenza Surveillance Network to assist in making decisions regarding the composition of the annual influenza vaccine for the southern hemisphere.

Diagnostics and surveillance

The RVU provides diagnostic testing for respiratory viruses as part of influenza surveillance and is involved in characterising respiratory viruses for three main active surveillance programmes, namely: the Viral Watch programme – an ILI surveillance programme in which 246 general practitioners and primary healthcare nurses have been recruited from all nine provinces; the SARI surveillance programme - a hospital-based surveillance programme in which detailed epidemiological data and specimens are collected from hospitalised patients with severe respiratory disease; and enhanced Viral Watch surveillance – consisting of 11 hospitals in nine provinces that collect samples from hospitalised patients with SARI.

Influenza and SARI surveillance

For the 2010 season, the RVU processed 6,915 specimens. The influenza season was dominated by the circulation of influenza B viruses, in which 710 viruses were identified. A total of 610 influenza A viruses were identified, these were subtyped into 333 A/H3N2 and 277 pandemic H1N1 viruses. In contrast to many northern hemisphere countries, pandemic H1N1 cases did not occur during the summer of 2009 or 2010 apart from a few imported cases. Similar to the 2009 influenza season, an apparent bi-phasic curve with H3N2 occurring before pandemic H1N1 was observed in 2010.

Molecular epidemiology of influenza viruses

Partial sequencing of the HA gene was performed to determine genetic drift over the 2010 influenza season:
- A/H3N2
  A total of 32 samples were selected for sequencing throughout the season. Specimens grouped with the A/Johannesburg/277/2009 cluster and were more closely related to the A/Perth/16/2009 vaccine strain than to the other 2009 South African cluster which grouped with A/Victoria/502/2009.

- Influenza B
  The HA gene of 10 influenza B specimens were sequenced in the 2010 influenza season and included in the phylogenetic analysis. The specimens all grouped with the B/Brisbane/60/2008 vaccine strain. Amino acid p-distance analysis indicated 0-0.6% differences in the South African strains relative to the vaccine strain. Antigenic analysis of influenza B viruses showed 94 of 116 isolates (81%) were from the B/Victoria-like lineage (B/Brisbane/60/2008-like) and four (3.5%) were from the B/Yamagata-like lineage (B/Florida/4/2006-like).

- Pandemic H1N1
  A total of 42 pandemic H1N1 strains were sequenced throughout the 2010 season and compared to strains from 2009, the vaccine strain and strains identified globally. The 2010 strains grouped separately from the 2009 strains and were further from the root of the tree than the 2009 strains. P-distance analysis indicated 1.2-2.2% differences in the HA protein of 2010 strains relative to the vaccine strain. Three common amino acid changes have been identified in 2009 and 2010 strains. Additional mutations were acquired during the 2010 influenza season, i.e D114N, S202T, E391K and of these 14 produced HAI results similar to the current vaccine strain (A/California/7/2009). Two medium and two low reactors were identified with A/California/7/2009-like antiserum suggesting drift is occurring.

Data from the molecular epidemiology investigation of the influenza A H1N1 pandemic were used by the WHO to help identify the appropriate virus strains to be included in the 2011 southern hemisphere Influenza vaccine.

Comparison of influenza in ILI and SARI cases
In both programmes, influenza B predominated followed by influenza A H3N2 and then pandemic H1N1. Far less cases of pandemic H1N1 were detected in patients with SARI relative to ILI surveillance. Cases of pandemic H1N1 were also detected much earlier in ILI surveillance than in SARI surveillance. The main difference between the two programmes is that the ILI surveillance includes outpatients and are mostly carried out in the private sector and includes a higher percentage of older patients and the sites are more widely distributed throughout the country while SARI surveillance includes hospitalised patients and enrolls more paediatric patients from six public sector hospitals in four provinces, of which three are in rural areas. Nevertheless, pandemic H1N1 does appear to be less associated with cases of SARI than the other subtypes in the 2010 season.

Other causes of SARI
A multiplex assay that detects 10 different viruses including influenza A, B, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza virus 1, 2, 3, enterovirus (EV), adenovirus (Adv) and rhinovirus (RV) was used to screen 4,526 specimens received through the SARI surveillance from six different government hospitals in Gauteng, Mpumalanga, KwaZulu-Natal and North West provinces. For 2010 period, RSV preceded the influenza season with most cases of RSV occurring in February to June. Most cases of hMPV occurred in late winter and early spring, while Adv, EV and RV cases were spread though out the year. Although RV was detected most frequently, it was also represented in the most mixed infections.

Research projects

Respiratory viruses
A 14-plex real-time PCR that can detect all major and newly described viral causes of respiratory tract disease, was used to screen 30-50% of all respiratory virus specimens that tested negative for conventional viruses in the Department of Medical Virology, University of Pretoria/NHLS Tshwane routine diagnostic laboratory during 2006 and 2007 and identified viruses in 70% of specimens. Findings of this study suggest that respiratory syncytial virus (RSV) remains the number one cause of severe pneumonia in children in hospitals in Pretoria. Various newly identified respiratory viruses were detected that play an important role in lower respiratory tract disease and have not previously been investigated in South Africa.
This test was subsequently used to screen specimens from patients in Kenya and the results indicated that an intervention that prevents RSV could reduce severe pneumonia admissions by a third in children. The group participated in an international collaboration investigating the impact of the pandemic influenza A(H1N1) 2009 virus on seasonal influenza A viruses in the southern hemisphere. A new BSL-3 laboratory in the Unit is nearing completion. This will enable the group to do research on avian influenza and other respiratory viruses that need to be contained.

A study of the genetics of severe pneumonia in children was completed and polymorphisms in the VDR and JUN genes identified that are associated with enhanced disease in children during RSV infection. An investigation of differences in the G and N5 proteins of RSV strains associated with mild and severe disease is completed. In this study it was shown that subtype A genotypes have remained more constant over the past 10 years with GA2 and GAS still circulating while subtype B genotypes have been replaced completely with a new genotype called BA which have a 60bp insertion in the ectodomain. Strains that have most of the G-protein deleted were also identified in children with pneumonia, suggesting that RSV does not require the G-protein to cause severe disease in immunocompromised children.

A study on differences in cytokine expression patterns in nasal secretions of HIV-positive and negative children with RSV, suggests elevated levels of interferon gamma in HIV-positive children may be associated with severe disease.

**Zoonosis**

For the emerging neurological diseases and zoonosis programme, the pathogenesis of zoonotic viruses such as West Nile virus (WNV) was investigated. In order to determine if WNV is being missed as a cause of neurological disease, a three-year study using horses as sentinel animals for detecting WNV activity, was completed. WNV was identified in 42/260 cases of neurological disease with a fatality rate of 40%, suggesting that at least 16% of unexplained neurological infections in horses may be due to WNV. A surveillance network has been established which consists of veterinarians from across the country who submitted cases of neurological disease in animals to the group for investigations. In the process, the first lineage 1 strain was identified in a pregnant mare that aborted and died a day later of fatal neurological disease. WNV lineage 1 was detected in the brain of both the mare and foetus. Screening of human cases of neurological disease from public sector hospitals in Pretoria identified WNV in seven cases of severe neurological disease that were negative for other causes, suggesting WNV is being missed as a cause of neurological disease in humans in South Africa as well.

Virus discovery projects were also carried out on cases that tested negative for common viruses, and Wesselsbron virus, Sindbis virus, Middelburg virus and an uncharacterised Bunyavirus, named Shuni virus, were identified as causes of neurological disease in horses that have zoonotic potential and should be investigated in human cases of neurological disease. These viruses were also identified in several fatal cases of neurological disease in wildlife, including rhinoceros, buffalo, warthogs and crocodiles with paralysis. Genome sequencing of these isolates are underway.

A new differential diagnostic tool for aseptic meningitis has been developed and validated against clinical specimens. This low density macroarray does not require expensive equipment and is invaluable in the identification of causes on aseptic meningitis outbreaks in Africa. Using this test, the group was able to identify clinical cases of WNV, Rift Valley fever, rabies, as well as 30 other causes of neurological infections in Africa.

**Honours**

Prof M Venter was a finalist in the National Science and Technology Forum awards, for the category ‘Individual with the best output the last 5-10 years or less’. The RVU received the prize for the Best Poster Presentation at the WHO meeting on influenza in Morocco and Prof Venter and her students received the prize for the best poster at the 7th International Respiratory Syncytial Virus Symposium, Rotterdam, Netherlands.

**Training**

**Under and postgraduate**

Lectures are presented to pre and postgraduate medical students in the Department of Medical Virology, University of Pretoria. The RVU participates in training of registrars on influenza diagnostics. Six MSc and three PhD students are registered under Prof Venter’s leadership and joint appointment at the University of Pretoria’s Department of Virology.
Special Pathogens Unit

Head: Prof J Paweska

The Special Pathogens Unit (SPU) is tasked with the laboratory confirmation and investigation of diseases caused by biohazard class 3 and 4 viral agents. These include, among others, the viral haemorrhagic fevers (VHF) caused by Crimean-Congo haemorrhagic, Marburg, Ebola, Lassa and Rift Valley fever and hantaviruses. The SPU is also responsible for the laboratory investigation and confirmation of arboviral disease of public health importance including West Nile, dengue and yellow fever, and Sindbis and Chikungunya virus. The Unit is the only laboratory for human rabies testing in South Africa. The Unit operates high (biosafety level 3) and one of the only maximum (biosafety level 4) biocontainment facilities on the African continent. The Arbovirus Laboratory has been accredited by SANAS under ISO 15189 since 2000.

SPU participates and drives several projects that are aimed at the enhancement of regional capacity for outbreak response and diagnosis of VHF. Research interests include development and improvement of diagnostic tools, molecular epidemiology, pathogenesis and molecular biology of viruses that cause VHF, arboviral disease, rabies and other emerging and re-emerging zoonoses. The Unit is involved in training international scientists on the diagnosis of VHF and arboviral disease and contributes to the training of several postgraduate students in the field.

Diagnostics and surveillance

Comparison of specimens received in 2009 and 2010

Nearly double the number of suspected VHF patients were investigated during 2010 (n=102) compared to 2009 (n=58 patients). This could be attributed to the heightened awareness of VHF disease that accompanied the Rift Valley fever outbreak in the country. Inversely, only seven suspected VHF cases were investigated involving patients from outside South Africa in the absence of notable VHF outbreaks elsewhere on the continent during 2010. This compared to 66 during 2009. The latter mostly related to the outbreak of Ebola haemorrhagic fever in the Democratic Republic of Congo in 2009. The most commonly requested test in the Unit remains the rabies immunity check for vaccinated individuals; a total of 189 specimens were tested in 2010.

Investigation of suspected VHFs

Five cases of Crimean-Congo haemorrhagic fever (CCHF) were laboratory-confirmed in 2010, compared to only three in 2009 and 11 cases in 2008. The cases were all male between the ages of 23 and 67 and were reported from the Free State (n=3) and Northern Cape (n=2) provinces. Two cases were also confirmed from Namibia. Of these seven confirmed cases, one patient passed away. Four of the seven cases had definitive tick exposures before onset of illness.

Human cases of CCHF have been reported annually from South Africa since 1981. Through nearly 30 years of passive surveillance, a total of 182 cases have been laboratory-confirmed from all nine provinces of South Africa. The majority of cases reported tick bites or squashing of ticks, whilst contact with infected blood or tissues was the second most important source of exposure. A strong link with occupational exposure is also noted with the majority of patients employed in the livestock industry (e.g. farmers, farm workers, slaughtermen) and being male.

Outbreak of rabies in Gauteng

Canine rabies has been introduced to South Africa on a number of occasions throughout the colonial period without evidence of sustained cycles. This changed in 1976 when canine rabies was introduced into KwaZulu-Natal from Mozambique. This outbreak spread extensively and canine rabies has been endemic to KwaZulu-Natal and Eastern Cape since. In the past decade, outbreaks of rabies in domestic dogs have also been recognised from the Free State since 2000, Mpumalanga since 2008 and Limpopo since 2005. Although several wildlife species also support cycles of rabies in southern Africa, the domestic dog proves to be the most important source of exposure to humans. Rabies cases have been intermittently reported from Gauteng, particularly on the outlying provincial borders which constitute more farmland and mostly involving mongoose, jackal and other wildlife. In June 2010, three cases of rabies in domestic dogs were confirmed by the Agriculture Research Council-Onderstepoort Veterinary Institute.

These cases were not epidemiologically linked and occurred in kept pets in the greater Johannesburg area. In the following months, several additional cases were reported with the peak of the outbreak in October 2010. The cases were reported from south-western Johannesburg with a hotspot of activity in Soweto.
A total of 37 cases were confirmed over the six-month period with the last laboratory-confirmed case occurring early December 2010. Molecular characterisation of outbreak isolates indicated that these viruses were very closely related and likely associated with a single introduction into Gauteng from KwaZulu-Natal. This is the first reported outbreak of rabies in domestic dogs in Johannesburg. In October 2010, a 3-year-old child from Soweto was also confirmed with rabies. The infant contracted the disease after a scratch exposure to a pet puppy and received no rabies post exposure prophylaxis. This case represents the first report of human rabies contracted in Johannesburg. A total of 11 human rabies cases were confirmed for South Africa during 2010, compared to 15 for the previous year and 17 in 2008.

Investigation of arboviral disease
The Rift Valley fever (RVF) outbreak of 2010 meant a four-fold increase in the number of submitted specimens compared to 2009 and a record number of tests for the arbovirus laboratory of the SPU. RVF outbreaks are episodic occurrences in South Africa, with this year’s being only the third significant outbreak event recorded thus far, interspersed by several smaller, animal-associated outbreaks in the interepidemic periods. It is notable that there has been an upsurge in the number of West Nile and Sindbis cases, two arboviruses which are considered to be more typically endemic than RVF virus based on previous arbovirus laboratory records.

Several patients who had travelled in the Far East and Indian Ocean islands had IgM antibodies to dengue and Chikungunya viruses or active infections. These viruses are epidemiologically rare in southern Africa but are responsible for extensive epidemics in south-east Asian countries and commonly reported in returning travellers from these areas.

Outbreak of RVF
In 2010, 238 human cases of RVF were laboratory-confirmed for South Africa with 26 deaths. The first cases were reported in February 2010 from the Free State. The peak of the outbreak appeared to have occurred in April 2010 with the last case reporting an exposure event during September 2010. The most affected regions included the Free State and Northern Cape, but cases were also reported from the Eastern Cape, North West and Western Cape. A total of 13,902 animal cases were confirmed during the same period with a recorded 8,581 deaths. A strong occupational exposure link could be established for the outbreak with 93% of the confirmed cases reporting direct contact with infected ruminants or their tissues. These included farmers; farmworkers; veterinarians; veterinary technologists; slaughtermen and abattoir workers. Confirmation of all suspected human RVF cases were conducted by the SPU.

Research

Molecular characterisation of RVF outbreak isolates
Phylogenetic studies of RVF viruses collected from 15 African countries, Saudi Arabia and Madagascar over the past 67 years reveal that they belong to one of 15 lineages. Virus diversity was found to be low, with pairwise nucleotide differences ranging from 0-5.4% at the nucleotide level and 0-2.8% at the deduced amino acid level. The low genetic diversity observed among isolates suggests either that the overall tolerance for mutation is low or that this collection of viruses have a relatively recent common ancestor. A total of 47 isolates from five provinces of South Africa (including Western Cape, Free State, Northern Cape, North West, Eastern Cape) were partially sequenced and phylogenetic analysis of 46 isolates indicated a low genetic diversity of less than 1% difference between isolates at the nucleic acid level. They were closely related to a 2009 Northern Cape isolate and ancestral to a 2004 Namibian isolate. The 2010 isolates were distant from 2009 KwaZulu-Natal and 2008 isolates from Mpumalanga, Gauteng, Limpopo and the North West. One 2010 isolate was phylogenetically distant from the other 2010 isolates and additional sequencing confirmed it to be a reassortant RVF virus. This recent outbreak in South Africa was caused by multiple genetic variants of the virus.

Host gene expression in liver of mice immunised with a recombinant RVFV nucleocapsid protein
The RVFV NSs protein has been implicated as the virulence factor of the virus as a result of its ability to counteract the actions of type I interferon, an integral part of the vertebrate host’s innate immune system. Not much else is known about the molecular pathogenesis of RVFV. The expression of an array of genes involved in innate and adaptive immune responses was compared in liver tissue of mice immunised with a recombinant NP and non-immunised mice after severe RVFV challenge. This was the first study exploring the molecular pathogenesis of RVFV in vivo in a target...
organ, and yielded some insights into the action of the virus on the host innate and adaptive immune systems. The results from this study might be useful for future vaccine, antiviral or gene therapy developments.

**Bats as reservoirs for emerging infectious diseases**

In the past decade several human viral pathogens have emerged that can be linked to bat reservoirs. In fact, more than 70 different viruses (not all pathogenic) have been isolated or detected in tissues of different bat species. The list of pathogens includes severe acute respiratory syndrome (SARS) coronavirus, and the paramyxoviruses, Nipah and Hendravirus. Also, it is now widely agreed that the available evidence does substantiate bats as the reservoir of Marburg - and possibly Ebola - virus. Currently, more than 900 bat species are known to exist, which represents almost 20% of the mammalian diversity; it is therefore not surprising that these animals are proving to be reservoirs of several human pathogens. SPU conducts research towards elucidating the ecology, epidemiology and pathology of zoonotic agents associated with bats. During 2010, SPU was involved in a European Union-funded study investigating the pathology and pathogenicity of SARS coronavirus through the use of knockout recombinant viruses in a bat model. SPU participated in an ongoing mission of the CDC and the Ugandan Ministry of Health to investigate the role of Egyptian fruit bats (Roussettus aegyptiacus) as a reservoir of Marburg virus in that country.

**Mission to DRC to investigate feasibility of Ebola fever ecological studies**

Investigators: Prof J-J Muyembe and Dr KS Shamamba (National Institute for Biomedical Research), Dr M Mossoko (Ministry of Health, DRC), Prof JT Paweska, Prof R Swanepoel, Mr A Kemp (SPU)

Luebo district in the Occidental Kasai Province of the Democratic Republic of the Congo (DRC) was the site of Ebola fever outbreaks in 2007-9. The 2007 outbreak was linked to the seasonal catching of fruit bats for human consumption along the Lulua River. Apparently fruit bats were particularly numerous in April and May 2007 on the islands of Ndongo and Koumulele. Therefore, the Luebo area might represent a potentially important ecosystem in which to conduct Ebola virus reservoir host studies. The purpose of the mission was to try to confirm these earlier observations and to determine the feasibility of conducting an international research expedition to the area to sample bats, particularly Hypsignatus monstrosus, but also other fruit bat species implicated as potential reservoirs of Ebola virus.

*The research team being welcomed by the community on the overgrown airstrip near CPC Mission to the north of the Lulua River, DRC.*
The following major observations were made during the mission:

- The village people hunt bats mainly for their own consumption; no indication was found that there is a regular large trade in bats as food animals;
- Differing opinions on seasonal bat activity were obtained. The local chief of Koulimulele Island agreed with village people and bat catchers from Kampungu/Benamonyu/Luebo that large fruit bats were most prevalent on the island in December. Elsewhere in Luebo district estimates of peak fruit bat activity ranged from February to August; and
- The descriptions for the March to May migrations through the islands and palm groves at Boutamila fitted the known behaviour of Eidolon helvum fruit bats. Epomops franqueti bats were caught on Koulimulele Island.

Due to the remoteness, poor infrastructure and road conditions, it was not considered possible to organise a large international ecostudy mission to Luebo district until more information on logistics is obtained, particularly the possibility of sending equipment by air to Kananga and from there by road to Luebo.
Considering the very high costs of deploying a large scale international expedition for Ebola ecology study in Luebo district, and the time needed for detailed planning, budgeting and acquisition of equipment for the research mission, it was proposed that a second exploratory mission to Luebo be undertaken, specifically to seek an indication of the prevalence of fruit bats in the forests along the Lulula River and on the islands.

Molecular diagnosis of human rabies

Human rabies remains an underreported disease in most of the developing world for a variety of reasons, first and foremost because it is not easy to diagnose clinically with two forms of presentation, the encephalitic and paralytic form. There are no routine blood tests that are informative in rabies cases and specific and specialised tests are required to confirm cases. In collaboration with the University of Pretoria, SPU has developed and validated a real time PCR assay for the detection of all rabies-causing lyssaviruses. This assay allows for sensitive and rapid (two hour turnaround time from receipt of specimen in the laboratory and result) detection of lyssavirus RNA in clinical specimens. It improves on previously reported molecular assays for rabies by including the detection of all known lyssaviruses (especially the lyssaviruses circulating in Africa and South Africa) through the use of a single fluorescent-labelled probe.

Specialised Molecular Diagnostic Unit

Head: Prof A Puren

Diagnostic services

The Specialised Molecular Diagnostics Unit (SMDU) has functions consisting of clinical diagnostics, research, surveillance and training. Primary diagnostic tests include CD4 counts, HIV DNA PCR for early infant diagnosis, HIV viral load monitoring, as well as specialised PCR tests for diagnosis of herpes simplex virus, cytomegalovirus, enterovirus and JC virus. The SMDU also assists technically with the hepatitis, polio and measles/rubella units. During 2010, SMDU also provided support to the AIDS Virus Research Unit for HIV drug resistance testing, and to the Outbreak Unit for rapid diagnosis of, for example, enterovirus outbreaks.

Clinical trials and surveillance

SMDU takes part in a number of clinical trials concerning HIV testing for DNA PCR or viral load, such as the HIV Vaccine Trials Network (HVTN), and collaborates closely with the Serology department in this regard. SMDU performs the nucleic acid testing for five HVTN experimental vaccine trials in South Africa. In 2010, the unit was selected as the primary site for HIV DNA PCR testing for a newly introduced surveillance study to evaluate the effectiveness of the national Prevention of Mother-to-Child Transmission (PMTCT) Programme on infant HIV at six weeks postpartum. SMDU also provides HIV DNA PCR testing for a number of respiratory virus and gastroenteroviral surveillance projects, such as those for severe acute respiratory infections and rotavirus. The SMDU assists with hepatitis B and C surveillance for the national Department of Health, as well as for the South African National Blood Service. During 2010, SMDU assisted the Respiratory Virus Unit and Viral Watch surveillance in testing samples with a newly developed multiplex PCR detecting 10 different viruses associated with respiratory infections.

Test statistics

SMDU observed an increase in testing in 2010, most notably for cytomegalovirus, herpes simplex virus and enterovirus PCRs. HIV DNA PCR for early infant diagnosis still accounts for the largest part of the work done by SMDU. This is performed on dried blood spot specimens and is composed mostly of samples sent from Lesotho and Swaziland as part of the NICD’s continuous support and technology transfer to bordering countries.

New tests

A new hepatitis B virus genotyping assay was validated and placed in routine use during 2010. SMDU also initiated testing for a three-part, cross-sectional and prospective, observational, cohort study to estimate HIV incidence among sexually active adult females. For this study, samples are pooled in groups of six and tested with the Roche Ampliscreen assay for HIV.

EQA and accreditation

SMDU maintained its accreditation status in 2010 with SANAS for ISO 15189. The following tests have been selected to be put forward for extension of scope in 2011: i) hepatitis B virus genotyping using line probe assay; and ii) cytomegalovirus real-time PCR. SMDU was also audited due to participation in ongoing trials.
for the HIV Vaccine Trials Network, and has successfully maintained its accreditation status. The Unit annually participates in external quality assessment (EQA) and inter-laboratory comparison for all diagnostic tests performed. In 2010, SMDU took part in 17 different EQA schemes, and submitted 73 datasets in total.

Research projects

Quality assurance initiatives in Lesotho

Researchers: Prof AJ Puren, Dr L Berrie
Funding: PEPFAR/CDC

The NICD aims to assist the Lesotho government in laboratory capacity building. The objectives include laboratory-based training, external auditing of laboratories, evaluation of quality assurance activities, laboratory support for PCR diagnosis and viral load testing, and to provide support to inventory all available equipment. Two full time staff members have been appointed to carry out these objectives, and the SMDU is assisting the Ministry of Health and Social Welfare with the implementation of an expanded programme for HIV early infant diagnostics and HIV viral load testing. The NICD has supported activities to improve laboratory infrastructure and equipment in 21 hospital laboratories, through a laboratory needs assessment. NICD is also supporting minor renovation, furnishing, transport and logistic support to district hospital laboratories and referral networking laboratories.

Comparison of two automated systems, using nucleic acid detection of HIV-1 for infant diagnosis, from dried blood spot specimens

Researchers: M Goosen, Dr L Berrie, Prof AJ Puren

Early infant diagnosis (EID) of HIV-1 has been shown to have significant importance for the survival rates of infants, ensuring earlier initiation of antiretroviral treatment. The aim of this study was to compare the performance of NHLS’ currently used Roche COBAS® AmpliPrep/COBAS TaqMan system for EID of HIV-1 with the new Gen-Probe TIGRIS DTS system. Analytical and clinical sensitivity, specificity, precision, accuracy and robustness of the two systems were assessed. In addition, the throughput performance, ease of use and costing of both the analysers were investigated. The results obtained support the use of the APTIMA HIV-1 screening assay in high throughput laboratories for the early detection of HIV-1 infection.

HIV-1 NAT EQA

Researchers: Ms M Vos, Dr L Berrie
Funding: PEPFAR/CDC

It has been proposed that EQA and internal quality control (IQC) be an integral part of a laboratory’s quality management system (QMS) that will detect weak spots in performance as well as improve on the reliability and confidence when reporting results. Aims of this project are to develop an EQA/proficiency testing molecular programme (HIV DNA PCR and HIV viral load testing) for NHLS laboratories that form part of the National Priority Programme within South Africa; to develop an IQC programme for both HIV DNA and HIV viral load testing; and to create a defined molecular QA network in Africa for both clinical laboratories and blood banks (where applicable). In 2010, HIV EQA panels showed a 100% participation rate by southern African laboratories compared to 82% in 2009, with many errors seen in the past no longer being made.

The Orange Farm part 2 study: a community study of male circumcision

Researchers: Ms E Cutler, Prof AJ Puren
Collaborators: D Taljaard (Progressus, SA), B Auvert (INSERM; University of Versailles, France)

The main aim of this study is to improve knowledge on human papillomavirus (HPV) infections among men by comparing the prevalence of HPV across a variety of sampling sites. Other objectives involve the investigation of the association and prevalence of high risk (hr) HPV, low risk (lr) HPV and HIV infection with male circumcision; and the evaluation of the role of the male foreskin in HPV infection, prevalence and transmission. A randomised, controlled intervention trial was conducted in a general population to ascertain if male circumcision provides protection against HIV-1 infection. A total of 3,274 uncircumcised men, aged 18-24 years, were selected. In an intention-to-treat analysis, the prevalence of hr-HPV among the intervention and control groups was 14.8% and 22.3%, respectively. The prevalence of hr and lr HPV was 14.0% and 17.3%, respectively. When controlling for all covariates, HIV incidence increased significantly with hr-HPV positivity and with the number of hr-HPV genotypes.
Development and comparison of real-time PCR diagnostic assays for currently causative viruses of aseptic paediatric meningitis and important virus infections in immunocompromised patients

Researchers: E Botha, Dr L Berrie, Prof AJ Puren

The aim of this study is to develop a multiplex real-time PCR assay for the diagnosis of the causative agents of aseptic meningitis, targeting enteroviruses, herpes simplex virus, varicella zoster virus, cytomegalovirus, Epstein Barr virus, human herpes virus-6 and mumps. In addition, the GeneXpert enterovirus qualitative assay was evaluated as a potential assay for point-of-care use. A QCMD EQA enterovirus panel was used for the evaluation and was tested in parallel with both the SMDU in-house test and the GeneXpert; 100% comparable results were obtained. Due to the ease of use of the test method and the low level of skills needed to perform the assay, the test will be recommended for use as a point-of-care assay.

Evaluation of the COBAS AmpliPrep/COBAS TaqMan HIV-1 test, the NucliSens EasyMag/EasyQ HIV-1 test, the VERSANT HIV-1 RNA 1.0 assay and the Abbott RealTime HIV-1 test

Researchers: D Greyling, Dr L Berrie, Prof AJ Puren

HIV-1 viral load monitoring was previously performed using the NucliSens EasyMag/EasyQ HIV-1 test system. In 2010, the use of both the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 assay and the Abbott m2000 HIV-1 assay were implemented by the NHLS for this purpose. Additional automated systems are available on the South African market for HIV viral load monitoring, such as the Siemens kPCR system. The aim is to evaluate and compare the analytical and clinical performance as well as workflow and ease of use of these four systems. Reproducibility, limits of detection, contamination risks and throughput of each system are being evaluated.

Teaching and training

SMDU teaches and trains intern medical scientists, technologists and registrars, and has a thorough and comprehensive training programme in line with HPCSA guidelines. The training is conducted within SMDU on currently used molecular methodologies and other relevant laboratory techniques. During 2010, six intern medical scientists, three student medical technologists and three student registrars were trained on this programme. In addition, SMDU also takes part in the long training course and short training course curricula for student registrars. During 2010, nine such students were trained in SMDU.

Two SMDU staff members are also involved in the QA department CEU training course on laboratory quality management provided to NHLS staff.

Viral Gastroenteritis Unit

Head: Dr N Page

The Viral Gastroenteritis Unit (VGU) is responsible for the establishment of a national surveillance system for the detection and characterisation of viruses associated with gastroenteritis. This includes rotavirus, adenovirus type 40 and type 41, astrovirus, norovirus and sapovirus. In addition, the incidence of newly emerging viruses including picobirnavirus, aichivirus, torovirus and picotrinavirus has to be assessed in the South African population. The Unit also aids the Epidemiology Department in identifying any viral aetiology involved in diarrhoeal outbreaks and characterising the viruses isolated.

Surveillance and research

The rotavirus vaccine was launched in South Africa in September 2008 and became available for all children within a specified age range (first dose <14 weeks and the second dose >24 weeks) from August 2009. Rotavirus surveillance was initiated in April 2009 to monitor the impact of the rotavirus vaccine in the national expanded programme of immunisation (EPI). The Unit continued rotavirus surveillance at the five sentinel surveillance sites (Chris Hani Baragwanath, Dr George Mukhari, Mapulaneng, Matikwana and Edendale hospitals).

A case-control study investigating rotavirus vaccine effectiveness in HIV-infected and uninfected children started in April 2010. Two additional sites (Ngwelezane and the Red Cross Children’s hospitals) submit specimens to the Unit for rotavirus screening and genotyping of rotavirus strains. Between January and December 2010, 1,218 diarrhoeal specimens were referred to the unit for rotavirus testing from six of the seven sentinel sites. A total of 1,146 of the specimens had sufficient clinical material for testing and rotavirus was detected in 23% of diarrhoeal cases.
Rotavirus detection rates varied between sites, ranging from 15% at Mapulaneng to 40% at Red Cross Children’s Hospital. There was a reduction in the number of rotavirus cases in children <2 years in 2010, compared to 2009.

Genotyping data revealed that serotype G8P[4], G1P[8] and G12P[8] strains were predominant. However, rotavirus strain prevalence varied between sites with coastal sites (Western Cape and KwaZulu-Natal) showing more G12P[8] strains while inland sites (Gauteng and Mpumalanga) showed more G1P[8] and G8P[4] strains. While the globally important G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] strains were responsible for 26% of infections, unusual G and P combinations were noted in 36% of strains. Genotyping data from Europe show unusual G and P combinations and potential zoonotic infections in only 2% of cases, highlighting the need to continue monitoring circulating rotavirus strains.

In addition to the sentinel surveillance, 666 rotavirus positive stools were received from private laboratories in the Western Cape. The age distribution of the rotavirus-positive cases was similar to previous seasons in the Western Cape (2007-2009), with the bulk of the burden in children <2 years. A positive trend is the decrease in the numbers of rotavirus-positive cases in children of all age groups, except 13-18 months which is only slightly higher than last year (136 in 2009 versus 138 in 2010).

The 2010 rotavirus season in the Western Cape was delayed, with peak detection in June rather than April. This seasonal shift has been previously noted in countries during the first season after rotavirus vaccine introduction and a sharp reduction in the numbers of rotavirus cases is expected in 2011. Of the 666 specimens received, 460 had sufficient clinical material for further analysis. There has been a shift in the predominant genotype circulating in the Western Cape from G1P[8] and G2P[4] in 2007, 2008 and 2009 to G2P[4] and G12P[8] in 2010. This may be partly due to the introduction of the monovalent G1P[8] vaccine and partly due to the natural fluctuation of rotavirus strains from one season to another. Continuous monitoring of the rotavirus strains circulating within the community is required to assess vaccine impact and identify strains that may escape protective immunity. The VGU was involved in the World Cup preparedness and implemented a real-time assay for the detection of norovirus genogroup I and II in diarrhoeal outbreaks.

While the assay was not utilised for the World Cup, the Unit was involved in the investigation of the diarrhoeal outbreak in the neonatal unit of the Charlotte Maxeke Hospital. In addition, the Unit identified a rotavirus outbreak in the neonatal ward of the Rahima Moosa Hospital in western Johannesburg.

Conference presentations

The number of presentations made by staff from the Virology Division during 2010 was:
International: 26
National: 3
Local: 13

Viral Diagnostics Serology Unit

The serology section serves various functions, including the reference laboratory for measles and rubella serology testing for South Africa and as the regional reference laboratory for the WHO-supported programme for measles and rubella control. The NICD’s other major role includes coordinating the laboratory testing for the Annual Antenatal HIV-1 Prevalence Survey, as well as HIV incidence testing for the survey. The laboratory provides end-point diagnostic testing for the HIV Vaccines Trial network (HVTN). Moreover, the lab provides quality assurance testing for major collaborators including the provision of HIV EQA and IQC panels. A major activity for 2010 was the management of laboratory testing for the measles outbreak. The other major large survey that the laboratory participated in was prevention of mother-to-child transmission (PMTCT) study to determine the effectiveness of the current antiretroviral prevention strategies in infants.

Surveillance

Measles and rubella
The measles outbreak continued in 2010 and there was a decline in the number of specimens tested towards the end of 2010. The current strategy is to test for measles and rubella IgM simultaneously. The total number of samples tested in 2010 was 24,560 for measles IgM, 24,548 for rubella IgM, 44 for measles IgG and five for rubella IgG; 12,758 samples were reported positive for measles IgM and 2,271 samples were reported positive for rubella IgM. The extensive laboratory testing was continued during the 2010 period in order to define the nature and extent of the outbreak in support of
the national Department of Health. The usefulness of the laboratory testing was reflected in, for example, defining immunity gaps in the less than 9 months age group following the outbreak vaccine campaign. The vaccine campaign was nevertheless successful as reflected in the decline in the number of newly identified cases.

**Antenatal HIV-1 prevalence survey (ANSUR)**

This survey has been conducted every year since 2000 during the month of October. The survey provides important information about the HIV epidemic in South Africa and evidence over the past three years suggests that prevalence at least in pregnant women has reached a plateau. Testing is conducted for HIV serology and RPR on first time attending pregnant women at the antenatal clinics. The number of pregnant women recruited for testing has expanded over the past three years in order to provide information at a district level rather than at a national and provincial level only. The expected number to be tested is 36,000 pregnant women. The NICD coordinates the testing with nine other NHLS laboratories. The testing in all nine provinces was completed and the results of the survey are expected in 2011.

**HIV-1 incidence testing**

HIV incidence, i.e. the number of new infections in a given period, is a critical measure of where the epidemic is and is also a reflection of the success or otherwise of interventions to prevent new HIV infections. Nevertheless, incidence measurements have been complicated by various factors including the effect of antiretroviral therapy. Incidence testing is conducted each year on samples from the ANSUR survey for three provinces and data for the previous three years will be analysed by various formulae that have been derived based on different correction factors. Published data with longitudinal follow-up have shown incidence estimates as high as 6% in women in, for example, microbicide trials. Additional studies that are being conducted to obtain estimates at a general population level include the International Programme for Microbicides (IPM) preparedness studies and also in preparation for the HSRC-supported 2011 survey for HIV prevalence and incidence.

**PMTCT survey**

The study was conducted with the Medical Research Council as the coordinating partner for the activity that included the NICD as the key supporting laboratory for the study. The impact of national prevention of mother-to-child transmission (PMTCT) programmes on MTCT rates at the population level is unknown in most countries including South Africa where PMTCT was initiated in 2001. A national cross-sectional facility-based survey of infant-caregiver pairs was conducted using a stratified multi-stage sampling design. Dried blood spot (DBS) specimens from 4-8-week-old infants were screened for HIV antibodies. Of 9,610 enrolled infant-caregiver pairs, 2,888 (30.1%) HIV-exposed infants were identified. Factors associated with MTCT were unplanned pregnancy, exclusive breast-feeding, and maternal highly active antiretroviral therapy. After nine years of the national PMTCT programme, the MTCT rate at 4-8 weeks postpartum was around 4%. Additional research is needed to explore the effect of PMTCT on late postnatal transmission.

**HIV Vaccines Trial Network (HVTN)**

The HVTN 503 study involves the use of the Merck Study Vaccine ‘Merck Adenovirus serotype 5 HIV-1 gag/pol/neo’ in participants enrolled in the study. The sites involved are Soweto, Cape Town, CAPRISA, KOSH and Medunsa. The serology section performs the in-study HIV testing and recent exposure/acute infection testing, using the env-based Biorad Multispot Rapid HIV1/2 test kit and the Biorad Genetic Systems HIV-1 Western Blot assay as per algorithm.

**HIV rapid kit post marketing surveillance**

Post marketing surveillance (PMS) is performed on HIV rapid test kits from kits selected from the national Department of Health (NDoH) RT-41 tender. Reports are generated and issued to the supplier and the NDoH. In 2010, PMS testing was conducted for SD Bioline (Pantech), Advanced Quality (Titima Consortium), G-Ocean, Determine (Zenelinde Trading) and First Response (Callcom). The PMS panel consists of 47 samples and the panel is tested on each new batch sent to the lab. In 2010, the usefulness of the PMS activity was demonstrated by confirming good performance of the kits when problems from the testing sites were reported.

**External quality assurance (EQA)**

HIV EQA/proficiency testing distribution

The lab is involved in the characterisation, preparation and distribution of EQA panels to NHLS labs and WHO labs. In 2010, three EQA distributions were issued to 206 NHLS labs and 129 private labs, two WHO distributions were issued to 49 WHO labs and 25 private labs.
An alternative strategy to reduce costs of EQA was piloted using dried tube serum (DTS). The DTS was piloted with an NGO called Newstart and the panels were sent to 17 sites. Results will be available in 2011.

Training

The laboratory trained four medical intern scientists during the year. Three sets of registrars rotated through the lab. Two intern medical technologists were trained.

Hepatitis Unit

The Hepatitis Unit provides services for the detection, surveillance and diagnosis of hepatitis B virus (HBV) and hepatitis C virus (HCV) disease. Laboratory-based surveillance networks for PCR and genotyping of HBV and HCV have been established. The aim of the laboratory is to provide baseline data on HBV and HCV to the national Department of Health (NDoH). The laboratory continually assesses new technologies for research and diagnostics so as to provide cost-efficient and rapid testing to the public. Genotyping of HBV and HCV is of clinical, epidemiological and surveillance importance. From a public health perspective, knowledge of the genotype frequencies and changes in these provide important epidemiological information which informs screening and preventative public programmes.

Surveillance

HCV national surveillance

The national surveillance database was established for the storage and collation of HCV serology and PCR data from NHLS laboratories countrywide by the Corporate Data Warehouse (CDW) and thus reflects HCV-positive laboratory results in the public sector. These initiatives, together with the imminent circulation of the NDoH guidelines for the control and treatment of HCV in SA should increase the number of requests for PCR to confirm seropositive results and increase the care and management of HCV-infected individuals. The establishment of the databases will facilitate better notification of the disease and allow for improved follow-up.

There was a higher recorded HCV seropositivity in 2008 (9.6%) as compared to 2007 (5.28%). HCV PCR prevalence does not appear to have increased significantly over the years but clinicians are becoming more aware of the availability of tests for HCV diagnosis at NHLS laboratories. The mean age for the serology positive group and PCR positive group ranged from 37 to 47 years. Many of the individuals may have had first signs of chronic hepatitis, hence were referred for HCV testing as most cases of persistent disease are asymptomatic in the early stages. The seropositive figures for 2007 and 2008 were higher (1371 and 1,745, respectively), than the PCR positive figures for the same years (195 and 191, respectively), re-iterating the recommendation that antibody results be confirmed by nucleic acid amplification tests (NAAT), such as PCR (as per National Guidelines for the Prevention and Control of Hepatitis C Virus in South Africa, final draft 2010). Nationally, the total number of HCV PCR positives in the public sector, was 621 over the five-year period from 2004-2008.

HCV is a curable disease and patients with genotypes 2 and 3 have a better chance of clearing the infection on combination therapy. It is recommended that genotyping and hence, early treatment be provided for all HCV PCR-positive patients to reduce transmission and the burden of hospital costs. Ongoing genotype and regular viral load surveillance will clarify the level of response to therapy in patients with less-studied genotypes in SA, for example, genotypes 4 and 5.

HBV and HCV genotypes in a clinical setting and volunteer blood donor groups in SA

HBV and HCV genotyping on all patient samples from hospitals around Gauteng began at the NICD in 2009 and 2007, respectively. The Hepatitis Unit is collaborating with the South African National Blood Services (SANBS) that had implemented individual NAAT for HIV, HBV and HCV to ensure the provision of safe blood to the community. Annual HBV and HCV viral load and genotyping tests are conducted on the NAAT positives. In this way, PCR positives and genotypes of HBV and HCV are surveyed in the more general population, as compared to sentinel clinical groups.

In the case of HBV genotype A, it is still seen to be the predominant genotype in both the patient and blood donor groups. Nevertheless, other HBV genotypes are being detected, for example, genotype D, which is usually prevalent in southern Europe, Middle East and India. Mixed genotype infections have been detected in both the study groups, especially genotype A and others (D, E or F). The major HCV genotype in SA is genotype 5a. An increase in the prevalence of genotypes 3 and 4
may be due to travel and migration. Mixed genotype infections were also detected for HCV-positive samples and this comprised mostly of genotypes 1 and 5a. This detection of mixed genotypes has only been possible by the latest technology of the Inno-LiPA (HBV) and LiPA (HCV) assays as sequencing would usually detect a major quasispecies in a mixture. The clinical impact or response to therapy of individuals with mixed genotype infections has not been established but needs to be monitored, especially if there is a shift from a predominant genotype to a more virulent or replication-competent strain.

Polio Unit

Surveillance

Acute flaccid paralysis
The rationale for acute flaccid paralysis (AFP) surveillance is defined by the WHO as follows: Poliomyelitis is targeted for eradication. Highly sensitive surveillance for AFP, including immediate case investigation, and specimen collection are critical for the detection of wild poliovirus circulation with the ultimate objective of polio eradication. AFP surveillance is also critical for documenting the absence of poliovirus circulation for polio-free certification. All cases of AFP including Guillain-Barré syndrome, in children younger than 15 years of age, or a patient of any age with a clinical diagnosis of polio made by a medical doctor, must be regarded as a possible polio case until proven otherwise. This proof is either by laboratory confirmation or by the consideration of the clinical medical records by the National Polio Expert Committee (NPEC). To meet sample adequacy requirements, all cases require two stool specimens of good condition and sufficient quantity collected at least 24-48 hours apart within 14 days after onset of paralysis, and sent to the NICD for polio identification. During 2010, at a required detection rate of two cases of AFP per 100,000 children under 15 years, 295 cases needed to be identified. Two laboratories at the NICD form part of WHO-supported AFP surveillance network at both a national and regional level. The Enterovirus Isolation Unit serves as a national poliovirus isolation laboratory for South Africa as well as six other southern African countries, i.e. Angola, Botswana, Lesotho, Mozambique, Namibia, and Swaziland. The WHO Regional Reference Laboratory for Polio provides the required technical support to characterise wild type and variants using sequence-based technologies and intratypic characterisation by real-time PCR. There was a significant decrease of reported cases in the one endemic country, viz. Nigeria, and by contrast a very large outbreak of poliomyelitis was experienced in the Republic of Congo affecting mainly males in the younger aged groups and with an unusually high case fatality rate. The last wild poliovirus case in the Republic of Congo was reported in 2000. Continued cases of wild type polio were identified in other countries in Central Africa including the Democratic Republic of Congo, Chad and Angola. In addition, the surveillance for circulating vaccine-derived polio virus was also performed.

AFP surveillance in South Africa
There were no wild type polio isolates detected from specimens of South African AFP cases. A total of 698 specimens were received from 357 South African cases. Of these, 13 cases had onset of paralysis prior to 2010. In addition ten cases were classified as not AFP by the NPEC. In the first half of January 2011, specimens were received from a further 16 cases with onset of paralysis in 2010, bringing the total number of suspected cases for 2010 to 350. The case detection rate, calculated only on cases from whom specimens were received, was 2.4 (range per province 0.8 to 3.2). One specimen only was received from 49 cases, and two or more specimens from 301 cases. The date of onset of paralysis was known for 323 (92%) of cases. Two adequate specimens were received from 256/323 (79%) cases with known date of onset (range per province 50% to 88%). Non-polio enteroviruses were detected in 71 cases, and non-enteroviruses in 20 of the 698 specimens received in 2010 (non-polio detection rate 13%), and poliovirus, identified as Sabin (vaccine) type poliovirus in 11 (2%) specimens of seven patients.

AFP surveillance in southern block countries
Of the 1,480 specimens received from the six southern block countries served by the NICD, 95 were from patients with onset of paralysis prior to 2010. Non-polio viruses were detected in 191/1,480 (13%) specimens. Poliovirus was detected in 98 specimens, 56 (57%) of which were identified as wild type poliovirus 1, and the remainder as Sabin strains. The wild type viruses were from 31 patients in Angola.
Research

Characterisation of wild-type molecular polio viruses

The laboratory received 1,972 specimens in 2010 compared to 2,963 specimens in 2009. These isolates were characterised as vaccine or wild type using two real-time PCR methods, viz. i) real-time RT PCR for intratypic differentiation (rRTPCRITD) and ii) real-time RT PCR for vaccine-derived polioviruses (rRTPCRVDPV). The current real-time PCR methodology contains a considerable advantage over conventional PCR diagnostics in that it allows for the identification of vaccine-derived polio viruses as well as viruses which have undergone recombination in either the VP1 or 3D regions of the viral genome without the requirement for the generation of sequence data. The rRTPCRITD positive isolates were tested by rRTPCRVDPV. The negative rRTPCRVDPV isolates were further analysed by sequencing while negative rRTPCRITD were analysed by sequencing without being tested by rRTPCRVDPV as they were considered to be wild type viruses. Two hundred and twenty-three cases were identified as wild PV1 in Africa with active outbreaks in Uganda, the Republic of Congo (CNG), Mali and Liberia. The total number of cases identified from CNG in 2010 was 63. The ages of infected individuals ranged from 1-92 years with the 20-29 age group being the most affected. Pointe Noire, CNG was the epicentre of the outbreak. Nigeria, classified as an the endemic country in Africa, had only seven cases of wild PV1 identified in 2010 compared to 75 cases reported in CNG. In 2010, PV1 wild type isolates were distributed into two genotypes, SOAS and WEAF-B. The SOAS genotype consists of the viruses from Angola, Democratic Republic of Congo and CNG. The WEAF-B genotype consists of viruses from Nigeria, Niger, Cameroon, Uganda, Senegal, Mali, Serra Leone, Mauritania, Liberia and Guinea. WEAF-B wild PV3 was identified in Mali, Niger and in Nigeria with only 11 cases reported in Nigeria compared to 313 in 2009. The strains from Mali and Niger were linked to Nigeria strain.

Characterisation of circulating vaccine-derived polio viruses (cVDPV)

Oral polio vaccine (OPV) is an important and effective means of control and eradication of wild polio viruses. The consequence of the use of a live OPV is that there is genetic drift as a result of mutations and recombination events with, for example, non-polio enteroviruses that result in the acquisition of transmissibility and neurovirulence properties similar to the wild polio viruses. One outcome of such events is that of circulating (c) vaccine-derived poliovirus (cVDPV) strains are transmitted and may cause flaccid paralysis. The VDPVs are Sabin-like viruses that have less than 99% VP1 nucleotide sequence identity to the Sabin oral polio vaccine strains. cVDPV were identified in the DRC, Niger, Ethiopia and Nigeria. Only 21 cases were in Nigeria compared to 153 in 2009. The Niger case was linked to the Nigeria outbreak. In 2010, Ethiopia experienced an increase in cVDPV type 3 compared to 2009 where only one case was reported. cVDPVs tend to occur where the wild type serotype has been eradicated, hence it is common compared to other serotypes. In terms of the targets set to eradicate poliomyelitis, 2010 was characterised by a decline in the numbers of cases in the one endemic country, namely Nigeria. However, this is in contrast with multiple importations as reflected by the number of countries affected as well as by outbreaks.

Measles/Rubella Unit

Molecular characterisation of measles virus in laboratory-confirmed cases of measles, allows genotype determination and thus inference of origin. This information can be used to focus the implementation of control measures. The genotype and sequence data are shared with the Global Measles Laboratory Network of the WHO. The NICD measles laboratories (serology, virus isolation, molecular) have dual functions – as a national laboratory to perform case-based surveillance and identify strains circulating in South Africa, and as a WHO regional reference laboratory (RRL) function to assess quality of the serology results from national labs in southern Africa and to identify strains of measles and rubella viruses circulating in these countries. The molecular laboratory also provides a service to African countries outside our RRL responsibilities that do not have access to nucleic acid sequencing technology, in order to improve molecular surveillance in the continent.

Diagnostics

The measles outbreak that started in 2009 in South Africa continued throughout 2010 although the number of cases decreased after the national mass campaign was conducted in weeks 15-18. A single strain of measles virus (genotype B3) was identified throughout the course of the outbreak. This viral strain
was identical to one of the strains detected in Benin in 2008/9 and thus the South African outbreak was the result of an importation of a single strain of virus into South Africa with subsequent widespread transmission. The presence of a single strain throughout the outbreak reflects the decline in vaccine coverage and an increase in a sufficiently large pool of susceptible individuals for a single genotype to spread rapidly. Several countries reported measles cases with the identical South African strain following the FIFA Soccer World Cup tournament in South Africa.

A sporadic case with a genotype D4 virus was identified during the mass event; however, no further chains of transmissions were identified. This strain has been circulating in Europe and particularly in France, where there has been a prolonged measles outbreak.

With regard to the WHO-RRL function of the molecular laboratory, specimens including clinical material, viral isolates and PCR products were received from the national labs in eight countries for molecular analysis (Botswana, Lesotho, Malawi, Mozambique, Namibia, Senegal, Zambia and Zimbabwe) and from the two other RRL-based in Uganda and Côte d’Ivoire (who forwarded specimens from Burundi, Rwanda, Benin, Côte d’Ivoire, Liberia, Mauritania, Niger, Nigeria, Sierra Leone and Togo). Several different strains of genotype B3 were identified. The genotype B3 strain that was circulating in South Africa was found to be circulating across southern Africa (Malawi, Zambia, Zimbabwe, Botswana, Angola, Namibia, Lesotho, Swaziland, Mozambique). It is very likely that the outbreaks in these countries were linked to the South African outbreak because of the timing of the outbreak reports and the frequency of cross-border travel. Analysis of the sequence data also demonstrated that there was concurrent widespread circulation of genotype B2 strains in Angola and Namibia.

Phylogenetic analysis of the sequence data revealed that a small number of strains of genotype B3 viruses were circulating across the African continent. This apparent reduction in virus diversity may be attributable to the effect of the many rounds of mass measles vaccination campaigns conducted in the region and may even imply interruption of endemic transmission in many of these countries. However, one cannot exclude possible bias in the results due to the relatively small sample numbers analysed from each country.

Quality Assurance Unit

The aim of the Quality Assurance Unit is to improve the effectiveness of the quality management system (QMS) of laboratories in accordance with the principles of Good Laboratory Practice to maintain ISO 15189 and other applicable ISO standards.

SANAS accreditation was maintained for all units in the NICD in 2010 with the addition of one extension of scope. A SADC delegation inspected the Viral Diagnostics Serology Unit, Quality Assurance Unit and Training Centre to assess requirements for recommending laboratories to become supranational reference laboratories and/or regional centres of excellence. An additional activity entailed assisting units that provide proficiency testing schemes with the implementation of ISO17043 within the respective schemes. A total of 35 internal audits where conducted to assess maintenance of the QMS within the laboratories/departments.

The QA Unit conducted training on the Introduction to QMS including accreditation and assisted with the internal auditors course and mentoring trainee auditors. Staff working on the CDC-funded project ‘Quality assurance initiatives for Lesotho laboratories’ were mentored.

Epidemiology Division

Head: Professor Lucille Blumberg

Epidemiology and Surveillance Unit

Head: Dr C Cohen

The Epidemiology and Surveillance Unit facilitates communication and data sharing between the national and provincial health departments and the NICD, and provides epidemiological input to other NICD units through collaborative projects and support of surveillance and epidemiology activities. Unit staff is involved in numerous teaching and training activities and represent the NICD at meetings with the Department of Health. In 2010, the Unit coordinated several surveillance programmes including the Severe Acute Respiratory Tract Infections (SARI) surveillance programme, the rotavirus surveillance programme, the Viral Watch influenza-like illness surveillance system, the respiratory hospitalisations
surveillance programme and the influenza-associated mortality surveillance programme and collaborated on several other programmes including the rotavirus vaccine effectiveness case control study and the PCV 7 vaccine effectiveness case control study. The unit also collaborated with the AIDS Unit in setting up a new project to establish a surveillance system for antiretroviral treatment drug resistance. The Unit is also responsible for publication of the quarterly Communicable Diseases Surveillance Bulletin.

**Surveillance programmes**

**Suspected measles case-based surveillance**

The case-based measles surveillance system, with laboratory support, started in 1998. The NICD is accredited by the WHO to perform measles and rubella IgM testing for the national case-based surveillance. Blood and urine specimens from suspected measles cases nationally are submitted to NICD for laboratory confirmation.

The measles outbreak that started in Tshwane district, Gauteng province in March 2009 was ongoing during 2010. In April/May 2010, the Department of Health embarked on a nationwide mass measles vaccination campaign targeting children aged 6 months to <15 years. During 2010, the NICD tested 26,886 specimens that were collected from suspected measles cases nationally. Of these, 12,499 (46%) were positive for measles IgM antibodies and 2,335 (9%) positive for rubella IgM antibodies. Of the positive cases, 155 (1%) were positive for both measles and rubella IgM antibodies.

Laboratory-confirmed measles cases were reported from all nine provinces with KwaZulu-Natal (31%), Mpumalanga (15%) and Western Cape (14%) provinces accounting for the highest proportions of the total. The highest number of measles cases was identified in March and April of 2010 and gradually declined to relatively low numbers towards the end of the year. Children <5 accounted for 53% (6,195/11,779) of the cases with 27% occurring in those aged 6 to 11 months.

Of rubella patients with known age (n=2,248), 45% were aged 5-9 years; age ranged from <1 month to 90 years with a median of six years. Cases were reported from all nine provinces but the Eastern Cape (19%) and Gauteng (17%) provinces had proportionally the highest number of cases. There was equal representation (50%) for both genders, and 10% of the female cases were in women of reproductive ages.

**Respiratory virus surveillance**

The Viral Watch sentinel surveillance programme, started in 1984, is specifically designed to monitor influenza activity in the community. During 2010, 247 practitioners registered across South Africa; however, 67 practitioners did not submit any specimens during the year. Of the 180 who submitted specimens, 125 submitted specimens to the NICD, eight to the virology laboratory at the University of the Free State, nine to the Department of Virology at Inkosi Albert Luthuli Central Hospital in KwaZulu-Natal, and 38 to the Tygerberg Hospital laboratory in the Western Cape. Positive specimens from these sites were sent to the NICD for confirmation, serotyping and sequencing. A total of 2,309 specimens were submitted throughout the year; of these, 917 (40%) influenza detections were made which were further characterised as 468 (51%) influenza B, 238 (26%) influenza A H3N2 and 211 (23%) influenza A H1N1. The first influenza detection of the season was made from a specimen collected on 7 June (week 23), and the last from a specimen collected on 8 October (week 40). The season was longer than any previous season over the past 26 years, lasting 18 weeks - the average duration over the previous 26 years was nine weeks.

A further 235 respiratory virus detections were made during the year of which 52% were adenovirus, 12% human metapneumovirus, 12% parainfluenza virus, and 25% respiratory syncytial virus.

In order to describe the influence of the influenza season on the number of hospitalisations, the NICD receives anonymous data from a private hospital group. The number of hospitalisations for pneumonia and influenza are compared to the influenza season. During 2010 there were 1,125,961 consultations reported to the NICD through the respiratory morbidity mining surveillance system. Of these, 3% were due to pneumonia or influenza.

**Severe acute respiratory tract infection (SARI) surveillance** was initiated in 2009. The aim of the programme is primarily to describe trends in numbers of SARI cases at sentinel surveillance sites and to determine the relative contribution of influenza and other respiratory viruses. In addition, the programme has been able to add on testing for SARI-related
pathogens such as pneumococcus. In 2010, the SARI programme expanded to include an additional site, namely the Klerksdorp Hospital complex (adult and children’s hospitals), bringing the number of SARI sites to six hospitals at five sites (Chris Hani Baragwanath in Soweto, Edendale Hospital in Pietermaritzburg and the two Agincourt hospitals, Matikwana and Mapulaneng in Mpumalanga). The SARI case definition was also expanded at the two enhanced surveillance sites (Edendale Hospital and the Klerksdorp Hospital complex) to include patients admitted with suspected tuberculosis (TB) which will look at the association between influenza and TB. An additional sub-study was undertaken to test for cryptococcal antigen in those patients fitting the expanded case definition and who were HIV-positive.

In 2010, 4,557 patients were enrolled into the SARI surveillance programme, the majority 69% (3,151/4,557) from Chris Hani Baragwanath Hospital. Overall, 45% (2,032/4,552) were children under the age of 5. Influenza results were available for 99% of enrolled patients. Seven percent (324) of samples were positive for influenza on multiplex real-time (RT) PCR; of these, 64% (207/324) were positive for influenza B, 25% (82/324) for A H3N2 and 14% (44/324) were A H1N1. Nine samples were co-infected with Influenza A and B.

Specimens were also tested for additional respiratory viruses and these were identified as follows: respiratory syncitial virus (RSV) in 14%(648/4508), adenovirus in 15% (662/4508), rhinovirus in 23%(1046/4508), enterovirus in 4%(200/4508), human metapneumovirus in 3% (124/4508), parainfluenza 3 in 3%(140/4508), parainfluenza 2 in 1% and parainfluenza 1 in 1% (44/4508) of patients. As was the case in 2009, the RSV season preceded the influenza season.

Of the 4,557 enrolled SARI patients, (77%) 3528 had blood specimens tested for the presence of pneumococcal DNA using quantitative RT PCR (lytA). Of these, (6%) 218 were positive for pneumococcal DNA and 10% (21/218) of pneumococcal-positive patients were co-infected with influenza.

Rotavirus surveillance
Prior to the introduction of the Rotarix® vaccine into the expanded programme of immunisation (EPI) for rotavirus in August of 2009, the NICD started a sentinel surveillance programme in five hospitals, namely Chris Hani Baragwanath in Gauteng, Dr George Mukhari in Gauteng/North West, Mapulane and Matikwana in Mpumalanga and Edendale in KwaZulu-Natal. The purpose of this surveillance programme is to describe the epidemiology of rotavirus infection and to describe the effect of the introduction of the rotavirus vaccine into the EPI. All children who are admitted to the sentinel hospitals with acute diarrhoea (less than seven days duration) are enrolled into the programme. Detailed demographic information, medical histories, clinical presentation data and in-hospital outcomes are recorded for each child. In addition, a stool sample is collected for rotavirus (and other diarrhoeal pathogens) testing at the Viral Gastroenteritis Unit at the NICD and at the Diarrhoeal Pathogens Research Unit at the University of Limpopo Medunsa campus.

A total of 1,237 children were enrolled into the surveillance programme in 2010. The total number of rotavirus-positive samples was 241 (21%). A consistent increase in the detection rate marking the start of the rotavirus season was seen in late May 2010. The peak detection rate was 68% (17/25) in the middle of June. The exact end of the season was hard to define because of the decrease in hospitalisations due to the national healthcare workers strike in August/September of 2010. However, by mid September the detection rate for rotavirus had dropped to below 10%.

Comparing the number of diarrhoea cases and the detection rate of rotavirus for 2010 and in 2009, data show a 20% (705 to 564) decrease in diarrhoea cases and 49% (395 to 201) in rotavirus-positive cases. The rotavirus season started a month later in 2010 compared with 2009. Although this is only one year of data post introduction of the vaccine, the later start of the season, the decrease in total number of diarrhoea cases and the lower detection rate of rotavirus are consistent with findings in the USA and Central America following the introduction of a similar vaccine.

Group for Enteric Respiratory and Meningeal Pathogens – South Africa (GERMS-SA)
The Group participated in a number of collaborative projects with the National Microbiology Surveillance Unit, Respiratory and Meningeal Pathogens Unit, Enteric Diseases Reference Unit and Mycology Reference Unit as part of GERMS-SA.
The invasive pneumococcal disease (IPD) case-control study is a nested matched case-control study to estimate the effectiveness of the 7-valent pneumococcal conjugate vaccine (PCV-7) which was incorporated as part of the South African EPI programme in April 2009. The study is nested in the GERMS-SA surveillance programme and uses surveillance officers at the 25 enhanced surveillance sites to enrol eligible individuals with invasive disease due to *Streptococcus pneumoniae*. The 25 enhanced GERMS-SA hospitals have been combined into 21 IPD sites. The study population includes all children who are eligible to receive PCV-7 through the EPI programme, i.e. born after the 15 February 2009. In the Western Cape and Free State the date of birth criteria differed as the roll-out of PCV-7 was delayed in these provinces.

Data collection started at the Gauteng sites from 1st March 2010 and the other sites started as soon as ethics committee approvals were received. The last site (Dr George Mukhari Hospital) started on 5th October 2010. A total of 270 cases were screened for the study in 2010. Of the screened cases, 164 (60%) children were eligible for the study and only eight (5%) of the eligible children were not enrolled. In terms of controls, 372 were accepted as eligible for the study. Project challenges in 2010, included enrolment of HIV-infected controls, verification of vaccination histories in the absence of Road-to-Health cards and HIV testing in some provinces. Successes included the following:

- Introduction of new strategies to improve HIV-infected control enrolment;
- Review of the Road-to-Health cards and vaccination histories of all children enrolled into the study and the compilation of a report to summarise common errors in documentation and interpretation of vaccine histories;
- Providing training on improving the quality of HIV status data and the uptake of HIV testing;
- Conducting multiple site visits during the year. Approximately 23 formal site visits were done, including two site visits that included CDC visitors. In addition, monthly visits were done to Rahima Moosa Maternal and Child Hospital as this is a non-enhanced site which only works on this project. The other non-enhanced site (Kalafong) was visited four times. At all these visits training was conducted and site audits were also done for sites with challenges;
- Creation of an ACCESS database for the study; and
- Employing a dedicated medical officer and data analyst for the study.

**Communications**

**Web-based surveillance reports**

Web-based reports from influenza surveillance programmes were published weekly in the season and monthly out of the influenza season on the NICD website. Weekly web-based reporting from the measles surveillance programme continued throughout 2010. Rotavirus web-based weekly reports were introduced in April 2010.

**Surveillance publication**

The Epidemiology and Surveillance Unit publishes the quarterly *Communicable Diseases Surveillance Bulletin* which aims to be a scientific publication for the regular dissemination of surveillance and outbreak data as well as relevant recent research from the NICD.

**Teaching and training**

**Staff**

The Unit coordinates the Epidemiology Journal Club and Epidemiology Discussion Group which aim to bring NICD staff involved in epidemiology together in a forum which allows for ongoing education and discussion related to strengthening NICD epidemiology and surveillance activities.

**Postgraduate**

The Unit coordinated the Infectious Diseases Epidemiology module for the MSc (Epidemiology and Biostatistics) and presented lectures for the short course in clinical trials for the School of Public Health, at the University of the Witwatersrand School. Microbiology registrars rotated through the Epidemiology Division. Lectures were given to students on the Field Epidemiology and Laboratory Training Programme.

**Outreach training**

The Unit assisted the City of Johannesburg with training for the expanded programme on immunisation as well as with epidemic preparedness response training, particularly related to the measles outbreak in South Africa.
Outbreak Response Unit

Head: Dr J Thomas

The Outbreak Response Unit provides technical support for all aspects of communicable disease outbreaks and control in the nine provinces, with special emphasis on optimising the role of laboratory services during these events. The Outbreak Response Unit is a source of intelligence during outbreaks, and through working in close collaboration with the provincial and national health departments and other stakeholders, ensures a comprehensive outbreak response and development of systems for early detection and improved reporting of epidemic-prone communicable diseases. In addition, close partnerships with the NHLS diagnostic laboratories and reference units of the NICD deliver appropriate laboratory diagnostic services during outbreaks and specialised diagnostic tests as required. The Unit also participates in training public health and laboratory personnel.

2010 FIFA Soccer World Cup activities

The Division of Epidemiology represented the NICD-NHLS as a member of the Public Health Cluster Workgroup of the National Health Operations Committee of the 2010 FIFA Soccer World Cup. The Outbreak Response Unit assisted the Department of Health with monitoring and response to communicable diseases during the 2010 FIFA Soccer World Cup period, both directly related and unrelated to the football activities. Laboratories were supported by facilitating the collection and testing of clinical and environmental specimens during foodborne disease outbreaks and other communicable disease incidents. Daily situation reports were provided.

The Unit prepared a Synopsis Guide for 2010 FIFA Soccer World Cup visitors to South Africa, which was posted on the NICD website. From February to August 2010, the monthly communiqués included pre- and post-2010 FIFA Soccer World Cup communicable disease alerts for healthcare workers, visitors and the general public.

Key outbreaks in 2010

The Unit is a member of the Multisectoral National Outbreak Response Team (MNORT) and assists with the development of provincial and national guidelines for priority communicable diseases. The Unit’s role in outbreak responses may include, but is not limited to, outbreak detection and reporting, field investigation, development of clinical and laboratory guidelines, management of laboratory surveillance data and interpretation of results, and recommendations for prevention and control.

Rift Valley fever (RFV) outbreak

RVF virus re-emerged in South Africa during 2008 following an extended inter-epizootic period. During 2008 and 2009, sporadic, localised, outbreaks were detected and investigated across the country, with human infection limited to a relatively small number of laboratory-confirmed cases. During 2010, an extensive and geographically widespread outbreak was observed. A total of 238 laboratory-confirmed human RVF cases and 26 deaths were identified. The majority (86%) were male. Of the 238 confirmed cases, data on occupation were available for 226 cases (95%); the majority (82%) worked within occupations where direct contact with animals frequently occurs. Further history regarding exposure was obtained for 89% (212/238); amongst these cases, 93% had a history of direct contact with RVF-infected ruminants, 4% reported exposure to mosquitoes in the absence of direct animal/animal tissue or unpasteurised milk exposure, and 3% report drinking unpasteurised milk but no direct contact with infected animals.

Brucellosis

In December 2010, the cause of several abortions in a goat herd on a farm in Gauteng was confirmed as a Brucella melitensis outbreak by the Department of Agriculture, Forestry and Fisheries. The farmer and one of the farm workers were subsequently diagnosed with brucellosis. Both cases were treated with the recommended triple-drug regimen, and recovered well.

Rabies

Every year the Special Pathogens Unit confirms between five and 31 human cases of rabies throughout South Africa. In 2010, dog rabies was reported from Johannesburg for the first time, although cases of rabies in several wildlife species (mostly jackal and mongoose) have been reported from Gauteng previously. Subsequently, rabies was confirmed as the cause of death of a 3-year-old child from Soweto one month after being scratched by an unvaccinated domestic puppy. A large animal vaccination campaign was initiated by the Department of Agriculture, Forestry and Fisheries to bring the
outbreak under control. Simultaneously, the Unit’s efforts shifted towards improving community and healthcare worker practices of prevention, through appropriate use of post-exposure treatment and prophylaxis.

**Foodborne disease outbreaks**

Due to enhanced disease surveillance systems which were put in place during the 2010 FIFA Soccer World Cup period, a number of foodborne disease outbreaks were detected and reported to the Department of Health and the NICD. Ten outbreaks of foodborne illness were reported from 4th June-9th July 2010: two from Mbombela district in Mpumalanga, two from Umlazi and KwaMashu townships in KwaZulu-Natal, two from Vhembe district in Limpopo, two from Tshwane and City of Johannesburg districts in Gauteng, one from Blauwberg in Western Cape Province, and one from Bloemfontein in the Free State. The successful reporting and investigation of foodborne outbreaks during the 2010 FIFA Soccer World Cup period was thanks to increased awareness of foodborne illness, prompt recognition and timely reporting of suspected outbreaks, and greatly improved communication and co-operation between the role-players involved. Numerous Department of Health directorates (including communicable diseases, food control, environmental health), SA-FELTP residents, NHLS laboratories including the Infection Control Services laboratory and the Outbreak Response Unit worked together efficiently to ensure optimal outbreak response and investigation.

The Unit assisted in the response to and investigation of several other foodborne disease outbreaks/illnesses. These included:

- **Kuruman, Northern Cape:** Foodborne illness affected 72/115 people who had eaten cooked donkey meat; a 4-year-old child died as a result of severe dehydration secondary to diarrhoea and vomiting.
- **Mbombela, Mpumalanga:** 100 learners attending a conference at a lodge fell ill after consumption of a buffet lunch. *Bacillus cereus* diarrhoeal-toxin was detected in food specimens tested.
- **Vhembe district, Limpopo:** Five family members who had eaten elephant biltong fell ill; *Salmonella baiboukoum* (an uncommon nontyphoidal *Salmonella* species) was isolated from the biltong.
- **Tjakastad, Mpumalanga:** Following consumption of a meal served at a funeral, a total of 12 patients became ill and were admitted to hospital, one of whom (a 71-year-old female) died shortly after admission. *Salmonella* Heidelberg was isolated from both clinical and food specimens; additionally, toxin-producing *Bacillus cereus* and enterotoxin C-producing *Staphylococcus aureus* were identified in food samples.
- **Bushbuckridge, Mpumalanga:** 188 learners aged between 5 and 12 years became ill after sharing a meal of porridge and milk at school; 50 cases required admission to Tintswalo Hospital for rehydration and observation (including one admission to ICU). *Salmonella* Virchow was isolated from 12 of 15 stool specimens available for testing.

**Enteroviral meningitis outbreak**

The NICD was requested to support the investigation of a suspected outbreak of meningitis in Prieska, Northern Cape. Over 50 cases of presumptive viral meningitis cases were identified, and enterovirus was detected in numerous cerebrospinal fluid and throat swab specimens. The outbreak subsided rapidly following health promotion interventions.

**Odyssean malaria**

Five confirmed cases and one probable case of *Plasmodium falciparum* malaria were reported from Slovo Park in Soweto, Gauteng. Five of six patients had no history of travel outside of the province in the months preceding infection. The Outbreak Response Unit and Vector Control Research Unit assisted the Department of Health with field investigations. The Unit also assisted the Environmental Health Practitioners from the Department of Health with health promotion activities, including an evaluation of the malaria awareness campaign conducted in Slovo Park.

**Other epidemic-prone diseases**

Pertussis (whooping cough): A 2-month-old child was admitted to a hospital in Gauteng with a diagnosis of pneumonia; among the investigations done, a nasopharyngeal aspirate specimen was submitted for *Bordetella pertussis* PCR, and tested positive. Advice was given regarding treatment and post-exposure prophylaxis for contacts (including healthcare workers).
Diphtheria: A case in an adult patient was reported from Cape Town; the diagnosis was confirmed by isolation of *Corynebacterium diphtheriae*, subsequently shown to be toxin-producing. Public health authorities were notified and contact and droplet precautions were put in place. It was found that a total of 16 healthcare workers were significantly exposed. They were vaccinated, had throat and nose swabs taken and were given a macrolide as prophylaxis.

Viral haemorrhagic fever: Five laboratory-confirmed cases of Crimean-Congo haemorrhagic fever were diagnosed (two from the Free State and three from the Northern Cape).

Travel-associated infections
Legionnaires’ disease: Early in 2010, the European Working Group for Legionella Infections (EWGLINET) reported a cluster of two travel-associated cases of Legionnaires’ disease, possibly associated with visiting a golf resort in the Western Cape, occurring in November 2008 and December 2009, respectively. In both cases, the patients became ill on return to Europe, presenting with pneumonia subsequently diagnosed as Legionnaires’ disease. A collaborative risk assessment and environmental investigation was carried out by teams from the NHLS Infection Control Services laboratory, the Outbreak Response Unit, and Environmental Health Practitioners from Eden District Municipality. Control measures were instituted, and the hotel remains open.

Cholera: A 37-year-old female returning from a business trip to London and India, presented to a private casualty facility in Johannesburg with abdominal cramps and diarrhoea. A stool specimen was sent to the laboratory. *Vibrio cholerae* was isolated, and confirmed at the NICD as toxin-producing *V. cholerae* O1.

Automated laboratory alert systems
During 2010, the Unit strengthened collaborations with the NHLS Corporate Data Warehouse (CDW) in the development and validation of automated systems for alerting response personnel to the diagnosis of priority communicable diseases. The system provides the Outbreak Response Unit with timely notifications and patient information following the confirmation of the following infections by NHLS laboratories throughout South Africa: *Salmonella* Typhi, *Vibrio cholerae*, and *Neisseria meningitidis*. 
A similar system designed to send automated SMS alerts was also piloted during 2010. Furthermore, the CDW served to provide compressive line-list datasets of disease conditions as required during outbreaks to support investigations.

The OutNet programme

OutNet is a laboratory-based outbreak network, which has been running since 2005 with the nomination and training (in collaboration with SA-FELTP) of nine provincial laboratory OutNet representatives who act as the key points of contact for provincial public health staff and facilitate the role of the laboratory for detection and response to outbreak in collaboration with the Outbreak Response Unit. Updates and contact with these representatives are maintained via monthly teleconferences and direct contact during outbreaks. The OutNet representatives are involved in training activities at provincial level. The Unit hosted a two-day OutNet workshop, focusing on laboratory preparedness and response to communicable disease events during the 2010 Football World Cup.

Communications

The Unit publishes a monthly Communicable Diseases Communiqué, which reports recent outbreak and communicable disease cases/issues of relevance. This is distributed to a wide audience including: general practitioners, specialists, infectious diseases and travel medicine societies, and national and provincial public health personnel.

Special urgent advisories and communiqués in response to acute events requiring immediate dissemination of information were published.

National guidelines were published for the 2010 Healthcare Workers’ Handbook on Influenza in South Africa and the 2010 Health Workers’ Guidelines on Rift Valley fever. Numerous fact sheets and clinical management guidelines in response to outbreaks or potential outbreaks during 2010 were prepared including clinical management and prevention of human cases of anthrax and Legionnaires disease.

Teaching and training

Staff
The Case of the Month series has been distributed on a quarterly basis to all NHLS laboratories since 2005. The series aims to train staff in diagnostic laboratories in basic principles of epidemiology as applied to the role of the laboratory in communicable disease control. Approximately 200 laboratories participated in this activity in 2010, for which they earned professional development credits.

Postgraduate
The Unit supports the training of future epidemiologists and public health experts through the SA-FELTP, and provides supervision to residents during outbreak investigations, and gives lectures during both short and long courses offered by the programme. In 2010, the Unit served as a six-month field post for a second year SA-FELTP resident, who assisted in the Rift Valley fever outbreak investigation in Free State Province.

The Unit provided the training to public health specialists, by hosting six-month placements for registrars to gain experience in both outbreak response activities and communicable diseases-related public health. Clinical microbiology and infectious diseases registrars from the University of the Witwatersrand are hosted for a one week training placement, and clinical pathology and microbiology registrars from the University of Pretoria are hosted for a one-day training placement.

The Unit hosted a four-month placement for a Public Health registrar seconded from the Health Protection Agency (UK) to the NICD for the duration of the 2010 FIFA Soccer World Cup activities, to gain experience in communicable disease surveillance and response in mass gathering event situations.

Outreach
The Unit assisted the national and provincial health departments in training public health personnel and doctors in epidemic preparedness and response with an emphasis on case management and appropriate laboratory diagnostic tests; provided training in the investigation and response to foodborne disease outbreaks; and participated in the mass measles and polio vaccination campaign training workshops in several provinces.

The Unit presented lectures for various groups, including: general practitioners, hospital-based healthcare staff, medical specialists, private healthcare workers and allied medical workers, veterinarians and animal-health workers.
The South African Field Epidemiology and Laboratory Training Programme (SA-FELTP)

Head: Dr B Harris

Over the past 25 years, field epidemiology training programmes have been established in over 40 countries worldwide. There are now at least 10 such programmes in Africa. The SA-FELTP was launched in 2006, the second programme to have a laboratory component, Kenya being the first. The Department of Health, NICD, NHLS, the US Centers for Disease Control and Prevention (CDC) and the University of Pretoria established this programme to build epidemiological capacity and strengthen public health laboratory practice in South Africa. The programme’s main output is graduates with a Masters degree in Public Health and two years supervised work experience and training aimed at strengthening practical skills and knowledge. The students participate in several core modules at the University of Pretoria and NICD and work under a supervisor for the remainder of the two years at a field placement site at national, provincial or district level within the Department of Health and the NHLS. Two applied field epidemiology short courses are presented annually aimed at public health professionals from national and provincial health departments, local and municipality metro city councils who are involved in communicable diseases control, disease surveillance, outbreak investigations and data management.

The programme is funded by the President’s Emergency Plan for AIDS Relief (PEPFAR), national and provincial health departments, NHLS, NICD, CDC and the African Field Epidemiology Network (AFENET).

During 2010, SA-FELTP assisted in 11 outbreak investigations ranging from a cluster of suspected viral haemorrhagic fever cases to a country-wide Rift Valley fever outbreak. A number of foodborne outbreaks and a suspected Legionnaires’ disease outbreak were also investigated during the 2010 FIFA Soccer World Cup. Eleven surveillance system evaluations including AFP surveillance in Mpumalanga and the laboratory component of the suspected measles case-based surveillance system were undertaken by residents. Eleven analyses of large databases, including the annual antenatal survey data for Mpumalanga, were conducted.

Residents and graduates assisted in preparations and provided support during and participated in intra and post-campaign evaluations of the mass measles and polio, and the influenza vaccination campaigns.

Teaching and training

SA-FELTP
Eleven residents enrolled in the MPH accredited programme and 12 were supported in their second year of this programme.

Postgraduate
The Wits School of Public Health integration of quantitative and qualitative research methodology module was co-ordinated and taught.

The Diploma in Tropical Medicine and Health was co-ordinated and the communicable disease control module co-ordinated and presented for the University of Pretoria. Staff taught the module on public health surveillance data management at this university to 18 postgraduate students, including SA-FELTP residents. Infectious disease control was taught to students studying MSc in the field of Epidemiology and Biostatistics. An introductory epidemiology lecture and a three-day EpiInfo practical was given to the entomology MSc students of the Vector Control Research Unit of the NICD and Wits University and lectures presented on the control of communicable diseases for the Wits MSc in Child Health students.

Outreach
SA-FELTP staff presented a two-week applied epidemiology short course, attended by disease control, port health, epidemiology and surveillance staff from provincial, district and sub district levels of the health departments.

Travel Health Unit

This Unit was established in 2008 and provides a consultative service for health practitioners regarding pre-travel advice for travellers, clinical consultations for returning travellers with suspected infectious diseases; develops guidelines for a number of travel-related diseases and neglected diseases; serves as a point of contact and liaison internationally for infectious diseases acquired in southern Africa, and assists with training of travel health practitioners and those studying tropical diseases. The focus is on zoonotic diseases.
diseases and emerging pathogens through the One Health approach brought about by the interactions between animal and human health and the environment.

**2010 FIFA Soccer World Cup: communicable disease risks and surveillance**

An estimated three million spectators, including in excess of 350,000 foreign visitors, attended the 2010 FIFA Soccer World Cup event. The event posed specific challenges given its size, diversity of attendees and the potential for transmission of communicable diseases. A number of opportunities arose to reduce the risk of communicable diseases which included pre-travel advice, enhanced epidemic intelligence to timeously detect incidents and the provision of standard operating procedures for epidemic response.

Pre-travel advice was developed by the Unit, in consultation with the national Department of Health and in accordance with the international health regulations and published widely. This included recommendations for yellow fever vaccination, influenza vaccination as the event coincided with the southern hemisphere influenza season, hepatitis A vaccine because of the endemicity in South Africa, and measles vaccination for those considered non-immune, because of the measles outbreak in South Africa. A pre-event meeting was organised at the NICD in February 2010 and brought together a number of international and national experts and private and public laboratory representatives to present and formalise the surveillance programme for the event.

There were five foodborne outbreaks reported and investigated that were related to the World Cup. There was very little pandemic H1N1 activity; influenza A H3N2 and B predominated during the influenza season which was later and milder than usual, possibly because of school closure and the large number of attendees who were likely immune to influenza A H1N1 through previous exposure or vaccination. A number of measles cases involving World Cup attendees from other countries were confirmed, and some were further characterised and identified as genotype B3, the genotype currently circulating in South Africa.

The use of well-established laboratory-based surveillance programmes for influenza, meningococcal disease and measles were particularly useful in identifying trends and disease activity. Other achievements included the establishment of the Public Health Cluster, the overall improvement in notifications, especially from the private health sector and an improved response to managing foodborne outbreaks.

**Tropical and travel-related diseases**

The Unit was involved in formulating the national guidelines for the treatment of malaria and the prevention of malaria and these were published in 2010. A number of consultations took place both locally and internationally to provide support for the diagnosis and management of travellers with travel-related diseases, including East and West African trypanosomiasis and leishmaniasis.

**Teaching and training**

**Under- and postgraduate**

Teaching on travel and tropical diseases was provided for undergraduates and postgraduates at the universities of Stellenbosch and the Witwatersrand as well as participants at the travel medicine course and the Diploma in Tropical Diseases. The first infectious diseases Masters class was held at the NICD in November 2010. This course was specifically for residents training in the paediatric or adult infectious diseases subspecialty as well as recently-qualified specialists and covered infectious diseases that may not be routinely encountered during their training, but which they certainly would be called to consult on as infectious diseases specialists. The emphasis was on parasitology, tropical and travel-related diseases including the so-called neglected diseases, as well as those of public health importance, surveillance programmes for influenza, measles and polio, outbreak response, notifiable diseases and post-exposure prophylaxis. Nine infectious diseases residents’ in academic training programmes from universities around the country attended.

**Honours**

The following staff in the Epidemiology Division was honoured for their work:

Dr M Landoh received first prize for best poster presentation at the 6th Tephinet Global Conference in Cape Town.
Mr B Archer, SA-FELTP graduate of the 2007 intake, received a merit certificate at the 2010 University of Pretoria Health Sciences Faculty gala function for completing his master’s degree with distinction. He also received an award as first runner up for best publication by a young researcher for an article on the epidemiology and factors associated with fatal cases of pandemic H1N1 Influenza virus infections in South Africa, 2009.

Conference presentations

Epidemiology Division staff made oral and poster presentations at:
International conferences: 33
National conferences: 6

National Tuberculosis Reference Laboratory
Head: Dr Gerrit Coetzee

The National Tuberculosis Reference Laboratory (NTBRL) was established in 2006, primarily to strengthen and support the National Tuberculosis Control Programme (NTBCP) of the national Department of Health (NDOH) by improving and expanding capacity of tuberculosis (TB) services within the NHLS, and to maintain high diagnostic standards to meet the needs of the country.

The NTBRL serves as a resource and monitoring facility for NHLS smear microscopy laboratories and newly established laboratories for PCR-based line probe assays (LPAs) for the rapid diagnosis of TB and drug-resistant TB, respectively, as well as for laboratories performing conventional TB culture and drug susceptibility testing (DST).

High priorities of the NTBRL are the maintenance of good quality assurance practices in public sector TB laboratories, validation and introduction of state-of-the-art technology, and organisation of training programmes for staff. An important role of the NTBRL is to keep abreast of developments in TB diagnosis, notably molecular technology for the rapid diagnosis of TB and early detection of drug resistance.

Surveillance of newly laboratory-diagnosed TB cases and drug resistance is a prime function of the NTBRL. The Corporate Data Warehouse (CDW) information systems generate useful laboratory-based data on the prevalence of new TB cases and multidrug-resistant (MDR) and extremely drug-resistant (XDR) TB in South Africa. These data are analysed and interpreted by the NTBRL and laboratory-based trends reported to the NDOH and NTBCP on a regular basis.

Strategies in support of the NTBCP

The laboratory plays a pivotal role in the management of the NTBCP and aggressive strategies are required to ensure that the information from TB laboratories in the country is utilised to its full potential. Until recently the laboratory diagnosis of TB relied heavily on the detection of acid-fast bacilli (AFB) in smears prepared for fluorescence microscopy from sputum samples and the more sensitive method of culture for Mycobacterium tuberculosis in the liquid-medium-based MGIT 960 system, especially in HIV/AIDS patients with TB whose smear microscopy tests for AFB are often negative. The MGIT culture system is also used to perform DST in order to assist with the choice of anti-TB drugs and treatment monitoring of patients with drug-resistant TB. Major advances in molecular microbiology led to the introduction of LPA for the rapid diagnosis of MDR-TB into the NTBCP and, following validation and a decision by the NDOH to implement, the NTBRL has the responsibility of introducing this new technology at NHLS laboratories throughout South Africa.

Evaluation of new technologies

New molecular-based technologies have the advantage over conventional culture based methods in that they provide results much more rapidly. The NTBRL has been involved in the evaluation of these techniques, notably the GenoType MTBDRplus assay manufactured by Hain Lifescience, Nehren, Germany, and abbreviated Hain LPA in this report, whose performance was assessed by the NTBRL at different stages of implementation into the NTBCP.

During 2010, the NTBRL evaluated an ancillary study of the Thibela project (see under Aurum Institute for Health Research in Collaborations section), the performance of the GeneXpert in a gold mining setting. This study is ongoing.

The GenoType MTBDRsl assay (Hain Lifescience, Nehren, Germany) has been evaluated on several samples from various studies. It is an extension of the GenoType MTBDRplus assay (Hain LPA) and detects mutations...
associated with resistance to the XDR-TB-defining drugs fluoroquinolones and aminoglycoside/cyclic peptide agents, and ethambutol.

Role of GeneXpert (Xpert MTB/RIF) in NTBCP

The GeneXpert (Xpert MTB/RIF) assay, manufactured by Cepheid, Sunnyvale CA (US), has recently become available internationally for the rapid diagnosis of TB and MDR-TB. It simultaneously detects in sputum samples gene sequences specific for M. tuberculosis and its resistance to rifampicin (RIF) which serves as a marker for MDR-TB. It is a sample processing system based on an automated heminested real-time PCR assay integrated into a single disposable cartridge. This technologically advanced test is more sensitive than smear microscopy for TB detection and like the Hain LPA can detect RIF-resistant TB rapidly. It was cleared for roll-out in the NTBCP after extensive validation in a multicentre trial which included institutions in Cape Town and Durban and following recommendation for use in high TB incidence countries by FIND and WHO. Together with the DOH and other institutions, the NTBRL was involved in determining the role of Xpert MTB/RIF in the NTBCP and planning for its roll-out early in 2011.

Workshop to assess role of Xpert MTB/RIF in NTBCP

A national TB workshop was arranged by the NTBRL and the NDOH to discuss the role of the new Xpert MTB/RIF technology in the NTBCP. Organisations represented included the NDOH, NHLS, medical universities, provincial departments of health, CDC and non-governmental organisations. The workshop concluded that the Xpert MTB/RIF has tremendous potential for improved TB management and control through its ability to rapidly detect with a high degree of sensitivity the presence of TB organisms by means of TB-specific DNA in sputum, as well as MDR-TB through detection of mutations related to rifampicin resistance. The rapid detection of TB and MDR-TB cases will result in improved management of TB patients who will be diagnosed and treated earlier and, of great public health importance, curtail transmission of both TB and MDR-TB.

Computer-aided smear microscopy study

In a collaborative study with Aurum Institute and Guardian Technologies International, Hendon, VA, USA, the performance of an automatic computer-aided smear microscopy system involving digital image scanning of TB organisms in sputum smears stained with the fluorescence-based Auramine O stain, designated digital microscopy (DM), is being evaluated at the NTBRL. The system includes a decision support image assessment tool to assign TB acid-fast status to image indeterminate structures resembling AFBs by a smear microscopy expert (E-DM). The study involved 90 sputum samples from TB suspect patients tested independently by conventional fluorescence microscopy (FM) and the automated DM system. The DM involved 1,733 microscopic fields containing 0-10 AFB per field. Compared with FM, DM was shown to be highly sensitive, approaching 100% sensitivity but had reduced specificity which was improved with E-DM. Further evaluation of the computer-aided microscopy system is ongoing and will include comparison with mycobacterial culture findings.

Quality assurance

External quality assessment (EQA) programmes for TB laboratories in South Africa in smear microscopy, TB culture and DST are provided by agencies outside the NTBRL and are the Tuberculosis Epidemiology & Intervention Research Unit of the Medical Research Council, South Africa for DST performance and the Microbiology EQA Assessment Reference Unit of the NHLS for smear microscopy for AFB, and M. tuberculosis culture and DST. The inter-laboratory proficiency testing of the performance of the Hain LPA in NHLS laboratories was continued during 2010 but will be replaced in 2011 by a new EQA system.

Smear microscopy rechecking programme

Sample sizes of smears for rechecking are based on smear positivity rates and number of AFB-negative smears per business unit and smears are collected according to a randomised system administered by the CDW. Rechecking of smears is performed at quarterly evaluation periods in a blinded fashion by the controller technologist at the NTBRL and smears with discrepant results are re-examined by a second controller whose assessment is accepted as final.

Laboratory audit

During 2010, an audit of the NTBRL facilities and activities was conducted by the Health Professions Council of South Africa, resulting in the recognition of the NTBRL as a training centre for the country. Further accreditation will be sought from SANAS and WHO during 2011.
Roll-out of line probe assay country-wide
As a result of the enormous implications of the rapid detection of MDR-TB for the NTBCP, the introduction of the Hain LPA in NHLS laboratories country-wide became a major priority. The test has the ability to detect MDR-TB within a short turnaround time of less than one week. It detects resistance-generating mutations in genes associated with the mode of action of the two most active anti-TB drugs, isoniazid and rifampicin, and with its introduction, the need for conventional DST with a long turnaround time of four to six weeks, has been drastically curtailed.

Progress with LPA roll-out
Hain LPA technology was introduced in NHLS laboratories in 2007 and the national roll-out of the LPA project commenced in January 2009. Initially, 20 LPA roll out sites to cover eight of the nine provinces were chosen and subsequently additional sites in KwaZulu-Natal (KZN) were added to the list. Standardisation of methodology, including reading, interpretation and reporting of results was instituted. The African Centre for Integrated Laboratory Training (ACILT) trained 60 technicians, 15 in each of four NHLS regions; the Hain Lifescience suppliers of the LPA equipment provided training in instrument use. The introduction of LPA in urban areas and densely populated regions with established laboratories generally went smoothly with large numbers of LPA tests being performed in the Western Cape and Gauteng provinces. Good progress was made during 2010 in the Eastern Cape and Northern Cape and to a lesser extent in the Free State. In rural areas in Limpopo, Mpumalanga and North-West Province progress was slow. It is anticipated that the introduction of automated readers, which has reached an advanced stage of development, will greatly facilitate the smooth integration of LPA into the NTBCP.

Surveillance of drug-resistant TB
CDW-based surveillance
MDR-/XDR-TB data extracted from the CDW are used to maintain registers for provinces to identify new MDR-TB cases for referral to MDR-TB centres. Data from CDW enabled NTBRL to provide detailed figures of MDR-TB and XDR-TB cases which are useful for monitoring the effectiveness of the NTBCP, as well as establishing strategies for TB management. Due to the introduction of a laboratory information management system (LIMS) only concluding late in 2010, reliable MDR-/XDR-TB data were not available on an annual basis for KZN.
A steady but modest increase in new laboratory-confirmed MDR-TB cases continued during the period 2007-2010, approaching 6,000 new cases a year. This modest increase in recent years followed a much steeper increase during the preceding three years. A similar trend was observed in the case of XDR-TB patients but there was a 58% increase from 272 in 2007 to 431 in 2010 in these cases.

**Electronic Drug-Resistant TB Register**

The Electronic Drug-Resistant TB Register (EDRWeb) was introduced into the NTBCP following collaboration between the NTBRL, NDOH, Centers for Disease Control and Prevention (CDC) and Warmtechnology CC 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, developed by Warmtechnology CC. The interface with the NHLS CDW has been running successfully since 2009 in eight of the nine provinces. Completion of the LIMS roll-out in KZN in late 2010 and mapping to the CDW have allowed the extension to KZN.

**National Drug Surveillance Survey**

Protocol development for the National Drug Surveillance Survey was completed and funding for the project, which is managed by the CDC, Atlanta, USA, finalised. Population-proportionate cluster sampling will ensure that specimens are representative of TB patients in South Africa. Prevalence of MDR-/XDR-TB in new and retreated patients will be established and used in rational management planning. The survey commenced in 2010.

**Research and development**

Research performed at the NTBRL is directed mainly at issues related to the NTBCP. The first major FIND-supported project introduced into the NTBCP was the GenoType MTBDRplus assay (Hain LPA) and was first evaluated in three pilot studies in Cape Town, Johannesburg and Kimberley, followed by a major demonstration project involving patients linked to larger centres in South Africa before it was eventually introduced into the NTBCP. Due to the potential of the novel Xpert MTB/RIF test to revolutionise management of TB patients and to have a major impact on the control of TB in this country, the same process as that followed for the Hain LPA will be fast-tracked for rapid implementation of this method.

The computer-aided smear microscopy study involving image scanning of TB organisms in sputum smears from TB suspect patients is another example of the NTBRL’s role in the development of new technologies relevant to TB control in South Africa.

**Mycobactericidal activity of peracetic acid in sputum and effect on Hain LPA performance**

Peracetic acid has previously been shown to have excellent mycobactericidal activity in the presence of organic matter, including in MGIT liquid culture medium but affects the band density of LPA strips when the Hain LPA test is applied to smear microscopy-positive sputum samples treated with 1% or 2% peracetic acid. In collaboration with scientists from Prisman Progressive Chemistry GmbH, Viernheim, Germany, these studies have been extended to investigate the effect of pulsed peracetic acid exposures on mycobactericidal activity and Hain LPA performance, using a neutralising agent to stop peracetic acid action after defined exposure times. In preliminary experiments it was shown that the neutralising agent used in this study on its own affects the band density of Hain LPA strips. Further studies are planned to investigate the effect of modified neutraliser and different concentrations of peracetic acid on Hain LPA performance, while retaining acceptable mycobactericidal activity.

**Collaborative research**

The NTBRL is involved in four major collaborative studies with prestigious international institutions aimed at describing mutations in genes associated with resistance to anti-TB drugs. The studies involve TB patients from Gauteng province in two projects initiated by Professor G Kaplan of the Public Health Research Institute of the University of Medicine and Dentistry of New Jersey (PHRI-UMDNJ); TB patients from the Eastern Cape in a multicentre project of the Division of Microbiology and Infectious Diseases (DMID) of the National Institute of Allergy and Infectious Diseases (NIAID) with Professor A Catanzaro of the University of California as principal investigator; and TB patients from KZN linked to the Nkosi Albert Luthuli Central Hospital TB laboratory under the direction of Professor S Shah and in collaboration with Professor B Kreiswirth of the PHRI-UMDNJ for genetic studies.
The studies in collaboration with PHRI-UMDNJ are:

Cross-sectional observational studies in Gauteng
Two cross-sectional studies over six months, four years apart, will be conducted to evaluate progression of the MDR/XDR-TB epidemic in Gauteng. Each study will characterise sputum cultures from 350 newly diagnosed MDR-TB patients by molecular typing and drug resistance profiling through DNA sequencing of target genes. In the process, strain diversity and identification of clusters will illustrate possible transmission patterns. By the end of 2010, collection of approximately 300 cultures for the first cross-sectional study had been completed and strains shipped to the USA.

Prospective observational study in Gauteng
This study comprises 100 HIV-negative and 100 HIV-positive newly diagnosed MDR-TB patients enrolled over a four-year period. These patients are examined at monthly intervals with sputum culture and assessment of nutritional and immunological status. Clinical progress of patients is being monitored and, based on sputum conversion, will be categorised into fast and slow responders and risk factors for poor outcome relating to host and microbial factors determined accordingly. Time to record positive growth in the MGIT culture system on culture of sputum samples will also be assessed as a predictor of poor outcome. At least 10 colonies from Middlebrook 7H11 agar plates will be collected from each base-line sputum to determine culture diversity and assess genetic heterogeneity and mixed infections in sputum samples of participating MDR-TB patients.

Genetic profiling and molecular typing of drug-resistant strains in KZN
Drug-resistant isolates of *M. tuberculosis* from KZN are being collected for molecular characterisation at the PHRI TB Center UMDNJ. The study relates to clinical and epidemiological aspects of drug-resistant TB in KZN.

Guardian Technologies International
The computer-aided smear microscopy study involves image scanning of TB organisms in sputum smears conducted in collaboration with Guardian Technologies, USA, Aurum Institute for Health Research, Johannesburg, Medicine in Need Inc., MA, USA and Medicine in Need (Pty) Ltd, Pretoria.

Aurum Institute for Health Research
The NTBRL provides laboratory support for the Thibela TB study, which evaluates the effect of community-wide preventive therapy with isoniazid among gold mine employees, on the incidence of TB in a cluster randomised study. Nested in the main project are ancillary studies including TB case ascertainment and culture-confirmed prevalence studies, as well as FIND-supported studies evaluating the performance of the new molecular-based Hain LPAs, together with the Xpert MTB/RIF. These new technologies for the rapid detection of MDR-TB are being compared with smear microscopy, and culture using the MGIT 960 system at the NTBRL. Cultures from these studies are shipped to the PHRI TB Center in Newark, New Jersey, USA for molecular characterisation, including sequencing of target genes related to drug-resistance.

Médecines Sans Frontières (MSF): Khayelitsha project
The NHLS TB Laboratory in Green Point participated earlier in a MSF project which focused on a patient-centered approach to drug-resistant TB treatment in the community. The laboratory at the time performed culture and DST against first- and second-line anti-TB drugs. As an extension of this project the NTBRL performed DST on isolates from the Khayelitsha project on the first- and second-line drugs isoniazid, rifampicin,
streptomycin, ethambutol, ethionamide, amikacin, kanamycin, capreomycin, para-aminosalicylic acid, pyrazinamide, ofloxacin and moxifloxacin on highly drug-resistant isolates from Khayelitsha. These strains are being stored at the NTBRL repository as local reference strains for future studies.

**MSF Swaziland study**
The MSF is conducting a TB management project in Swaziland and requested the NTBRL to assist with the laboratory component of the study. The NTBRL performs routine and new diagnostic tests for the management of Swaziland patients with acceptable turnaround times to optimise patient management. DST against first- and second-line anti-TB drugs is performed on all culture-positive specimens that show resistance to either rifampicin or isoniazid on Hain LPA testing.

**Training**

**African Centre for Integrated Laboratory Training**
The NTBRL has close links with the African Centre for Integrated Laboratory Training (ACILT) which was established to help create a new generation of laboratory experts, particularly in the fields of HIV, TB and malaria throughout Africa. National and international organisations contributed to the establishment of ACILT including the NDOH, NHLS, NICD, CDC, WHO AFRO, United States Agency for International Development, American Society for Microbiology, and Foundation for Innovative New Diagnostics.

In the field of TB, ACILT courses focus on TB culture/DST, microscopy and molecular diagnostics, laboratory management and accreditation, quality management systems (QMS) and commodity management.

ACILT assisted with the training of 60 student technicians, 15 for each of four NHLS regions (Coastal, Central, KZN and Northern regions) recruited to manage the roll-out of the Hain LPA project country-wide. These students received comprehensive training, including measured outcomes in TB laboratory diagnosis, as well as in-house training in their own laboratory.

Since the establishment of ACILT three years ago it has offered courses on TB culture and TB/DST, microscopy and molecular diagnostics, HIV early infant diagnosis PCR, BED incidence, quality assurance in HIV rapid testing, bio-safety and bio-security, national laboratory strategic planning, strengthening laboratory management, QMS and commodity management. All these courses have a strong practical orientation and are subjected to a stringent evaluation component. Courses are offered by ACILT staff, NHLS and NICD experts and are supported by CDC, APHL, Becton Dickenson (US) and others. Participants have included laboratory staff from Nigeria, Uganda, Ethiopia, Mozambique, Namibia, Angola, Rwanda, Botswana, Malawi, Ivory Coast, Tanzania, Zambia, Zimbabwe and South Africa. During 2010, 20 courses were presented. ACILT also participated in WHO accreditation audits in 14 laboratories in Lesotho.
Research output

Publications


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