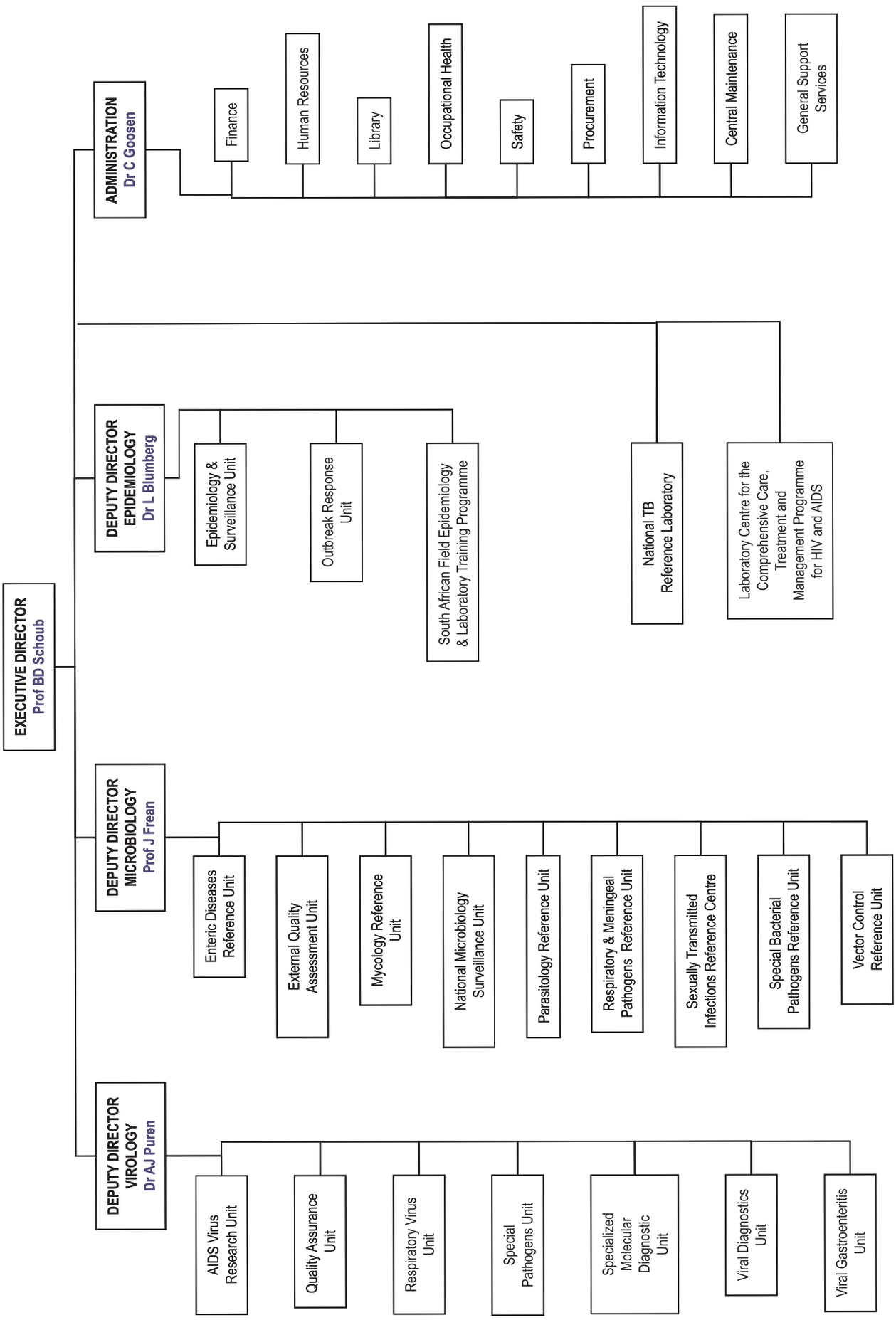
NICD

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

NATIONAL HEALTH LABORATORY SERVICE

ANNUAL REPORT 2006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DIRECTOR'S REPORT

The year 2006 has seen a number of important developments in the NICD which have further enabled this Institute to fulfil its role in contributing to the improvement of the health of the country and the region.

A major component of the NICD's responsibilities is the development of capacity in the field of communicable diseases for this country as well as for neighbouring countries and for other African countries. To this end active steps were taken a few years ago to secure funding to build a training centre. For a number of years short-term training courses in laboratory technologies and data processing were carried out in makeshift lecture theatres and using service laboratories for bench training. In 2006 the Poliomyelitis Research Foundation [PRF], the most important national research funder of medical virology in South Africa, generously donated a large sum of money to erect a training centre at the NICD. The centre consists of a modern 230-seat auditorium, seminar rooms, training laboratories for virology, microbiology and molecular biology and supporting offices. The training centre was officially opened by the Chairman of the Board of Trustees of the PRF, Mr Roy Wiggill, in November 2006. The lecture theatre was named "The James H S Gear Auditorium" to honour the memory of one of the great pioneers of infectious diseases in this country and the Institute's first Director [1953-1976]. One of the flagship training courses which commenced in the academic year 2007 is the South African Field and Epidemiology Laboratory Training Programme [SAFELTP] described in detail in the report. An especially valuable benefit of this course will be the establishment of a network of alumni who will function as field epidemiologists and field laboratory personnel in the future. The training centre will, in addition, provide a facility for many of the national and international training courses which the NICD takes responsibility for.

Official opening of the NICD PRF Training Centre: Tuesday 28th November 2006.
From left to right, Professor Ragnar Norrby (Director-General of the Swedish Institute for Infectious Disease Control), Professor Barry Schoub (Executive Director, NICD), Mr John Robertson (CEO, NHLS), Dr Kamy Chetty (Deputy Director-General, National Department of Health), Mr Roy Wiggill (Chairman, Board of Trustees, Poliomyelitis Research Foundation)

Other major developments which have materialised during 2006/2007 include approval by the CEO of the National Health Laboratory Service [NHLS] and its Board for major funding for the construction and equipping of a National TB Reference Centre. Funding was also received for this programme from the National Department of Health. Construction of the Centre, which is under the directorship of Dr Gerrit Coetzee, is due to commence in the second half of 2007.

The upgrading and modernization of the maximum security BSL-4 laboratory which had to be temporarily closed down, is expected to be completed and the laboratories re-commissioned during 2007. The Board has also approved the purchase of an electron microscope, which is due to be installed in the latter part of 2007. This instrument will be a particularly valuable diagnostic addition to the NICD

The NICD will formally become a member of the International Association of National Public Health Institutes [IANPHI] in May 2007. The Association, which was launched in January 2006 consists of some 50 National Public Health Institutes throughout the world. Its aim is to establish a collaborative network to facilitate scientific exchange and to assist under-resourced Institutes achieve their optimal potential. The NICD has conducted a number of outreach programmes to countries on the African continent by serving as a WHO Regional Reference Laboratory for a number of viral and bacterial infections, a WHO Collaborating Centre for Arboviruses and Haemorrhagic Fevers, and supplying reagents and EQA panels widely to African countries in addition to its extensive training commitments.

The enabling support which NICD enjoys comes from a number of sources and is gratefully acknowledged. In particular, the invaluable support received from our parent body, the National Health Laboratory Service and also the National Department of Health. Significant research funding, in addition to the financing of the PRF training centre, has come from the Poliomyelitis Research Foundation, as well as support received from the South African Medical Research Council, the National Research Foundation and other national bodies. The NICD has received extensive and invaluable international support particularly from the Centers for Disease Control, USA and the World health Organization as well as from a number of research funding bodies such as the National Institutes of Health (USA), Bill & Melinda Gates Foundation (USA), Agence Nationale de Recherches sur le Sida (France), Wellcome Foundation (UK), European Union, Sweden-South Africa Bilateral and Smittskyddinstitutet (Sweden) and a number of other national and international sources. This support has been of immense value to NICD as is detailed in the pages of this report.

Much hard work has gone into making the Institute an important national and global leader in communicable diseases and this preface to the annual report gives me, as Director, an opportunity to say a sincere thank you to all our wonderful staff. Finally, I would like to express my thanks to Liz Millington for again putting together an excellent report.

Barry D Schoub MB BCh, MMed, MD, DSc, FRCPath, FCPath, FRSSAf.
Executive Director.
July 2007.

Visit to NICD by Dr Ruth Bishop, Royal Children's Hospital, Melbourne, Australia - the discoverer of rotavirus: 7th November 2006. From left to right, Professor Barry Schoub, Dr Nicola Page, Dr Ruth Bishop.

PARASITOLOGY REFERENCE UNIT

STAFF

Associate Professor John Frean MB BCh, MMed, MSc, DTM&H, FACTM, Head of Unit
Leigh Dini BSc (Hons), MSc, Laboratory Manager
Rita van Deventer Dip Med Tech (Parasitol), Medical Technologist
Bhavani Poonsamy BSc (Hons), Medical Scientist
Motlabasego Ndou BSc (Hons), Medical Scientist
Pedwell Makutu, Data Clerk
Monde Nkabinde, Professional Nurse

SURVEILLANCE

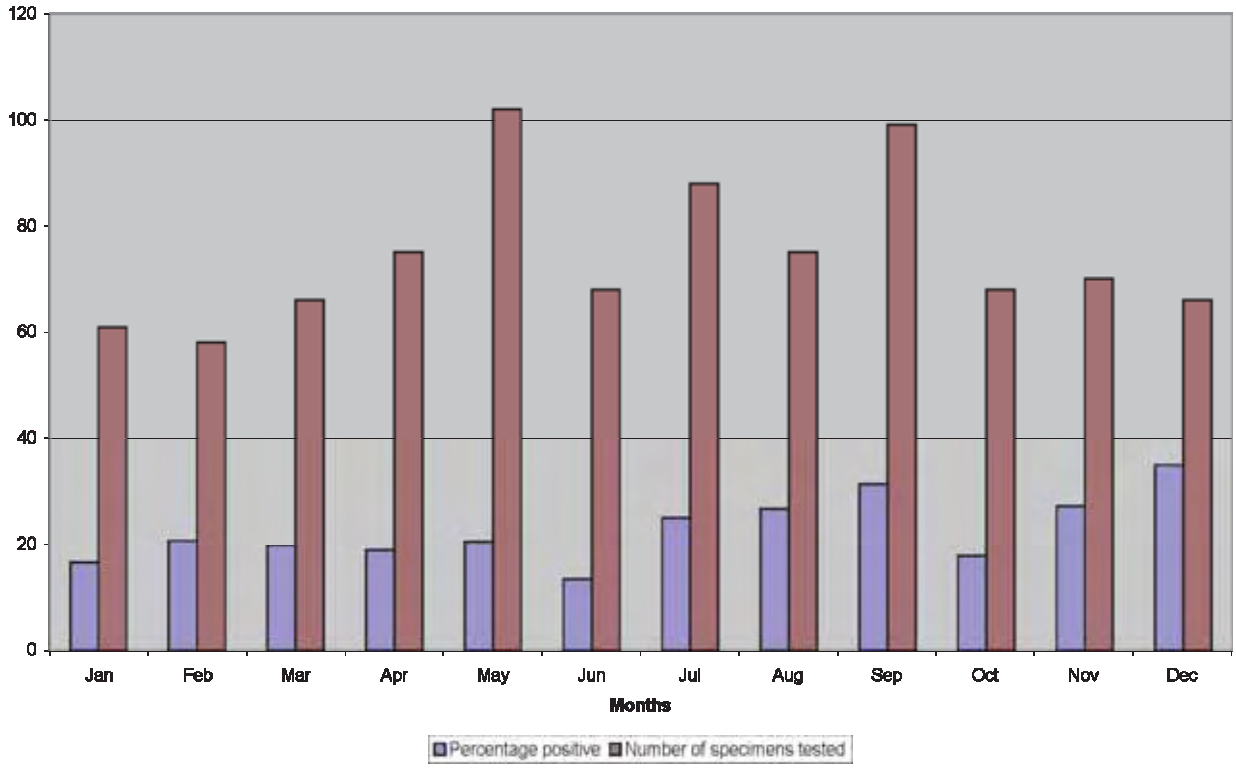
Pneumocystis surveillance was launched in May 2006 at existing GERMS-SA enhanced surveillance sites. The programme aims to gauge the burden of *Pneumocystis* pneumonia (PCP) in South Africa, investigate cotrimoxazole resistance, and strengthen diagnostic capability for this disease in routine laboratories. The shortage of diagnostic expertise contributes substantially to the difficulty in assessing the true impact of this opportunistic pathogen on the HIV-infected population. At least partly reflecting this, the surveillance data to date reveals a wide gap between the expected and submitted numbers of isolates.

CURRENT RESEARCH PROJECTS

Survey and management of drug resistant Pneumocystic pneumonia in South Africa

The project is a collaboration between the NICD and the Swedish Institute for Infectious Disease Control (SMI) in Stockholm, and Chris Hani Barawanath Hospital Respiratory and Infectious Disease units. The objectives of this research programme are to assess the incidence of *Pneumocystis* pneumonia (PCP) in South Africa, to establish genetic markers of drug resistance in *Pneumocystis jirovecii*, and to assess the usefulness of novel procedures for the diagnosis of acute PCP and monitoring of response to treatment. A high rate (50%) of mutations in the *P. jirovecii* dihydropteroate synthase (DHPS) gene, potentially conferring cotrimoxazole resistance, has been found. The value of a serum marker (s-adenosylmethionine) for PCP is under investigation in collaboration with Chris Hani Baragwanath Hospital). Sources of funding for the project are the Swedish-South Africa Health forum and Medical Research Council of South Africa.

2006 *Pneumocystis jirovecii* IFA testing



Assessment of quality of malaria rapid diagnostic tests (RDTs)

RDTs are valuable tools for bedside or clinic diagnosis of malaria where there is no laboratory or microscopist. They form an integral part of the national malaria control programme's efforts to provide an improved diagnostic service to populations at risk. How accurately the tests diagnose malaria depends on a number of factors; storage temperature, correct technique, and operator training can all influence the results and therefore subsequent management of the ill patient. With increasing reliance on malaria RDTs, it is clearly important to assess the quality of the tests' performance in a scientific fashion. PRU, in collaboration with the National Department of Health and WHO (WPRO), is contributing to a programme to effectively monitor the quality of RDTs in use in South Africa.

Epidemiology of toxoplasmosis in South Africa

The protozoan parasite *Toxoplasma gondii* is ubiquitous, yet little is known about the epidemiology of human disease in South Africa, especially in immunocompetent people. Like PCP, toxoplasmosis is an important opportunistic infection in HIV/AIDS patients, but the diagnosis is sometimes difficult to establish with certainty. In the immunologically normal population, pregnant women are at risk for transplacental transmission to the fetus, should they acquire a primary infection during their pregnancy. In collaboration with the Swedish Institute for Infectious Disease Control, the RATZOOMAN project (see SBPRU), and other NICD units, the prevalence of infection in humans and rodents is being investigated.

TRAINING AND EXTERNAL QUALITY ASSESSMENT ACTIVITIES

Unit staff provided the following teaching, training or proficiency testing activities in 2006:

- PCP workshop for GERMS-SA surveillance officers
- PCP laboratory diagnosis training for technologists, Nelspruit NHLS laboratory
- Graduate Entry Medical Programme for 3rd year medical students of the University of the Witwatersrand, and lectures for 3rd year nursing, dental and physiotherapy students in medical parasitology
- Training course for microbiology and clinical pathology registrars, NICD
- BSc student practicals in parasitology
- Laboratory training course in stool and urine parasite identification for technologists
- Two national Parasitology EQA surveys, three times a year: Stool and Urine Parasites (159 participants) and Blood and Tissue Parasites (132 participants)
- Malaria microscopy EQA programme for WHO AFRO, covering 72 sub-Saharan African countries
- Malaria microscopy and quantitation EQA programme for GlaxoSmithKline for their malaria vaccine trial sites in several African countries

Parasitology training

Parasitology stool course

Unit staff received the following training:

- Rita van Deventer was trained in culturing malaria parasites *in vitro*, Imperial College, London, February 2006. She was also instructed in malaria parasite quantitation at the Australian Army Medical Institute in Brisbane, Australia, April 2006.
- Leigh Dini visited the Research Institute for Tropical Medicine in Manila, Philippines, in May for training in malaria identification and quantitation techniques, field collection of malaria parasites, preparation of diluted parasite panels, and quality assessment of malaria RDTs. Later she trained at US NAMRU-2 in Jakarta, Indonesia, in various aspects of malaria laboratory diagnosis and slide bank preparation.
- In November staff were trained at the NICD in real-time PCR for *P. jirovecii* by Dr V Fernandez, of SMI, Karolinska Institute, Stockholm, Sweden.
- Bhavani Poonsamy and Leigh Dini received training in automated sequencing in December.
- During the year staff attended various technical, first aid, and software training courses.

POSTGRADUATE STUDENTS

PhD:
Leigh Dini: A study of *Pneumocystis* pneumonia in South Africa (in progress).

BSc (Hons):
Kesenthri Kistiah: Evaluation of a polymerase chain reaction for the diagnosis of *Pneumocystis jirovecii*.
Natasha Trataris: The burden of non-falciparum malaria in returning travellers in Gauteng Province, South Africa (co-supervised project)

VISITING SCIENTISTS

Dr Antonio Barragan and Dr Victor Fernandez, Swedish Institute for Infectious Disease Control, Stockholm, Sweden
Dr David Bell, WHO WPRO, Manila, Philippines

INTERNATIONAL CONFERENCES AND MEETINGS

Dini L. WHO informal consultation on quality assurance of malaria rapid diagnostic tests and malaria microscopy, 28 Feb - 3 March, Geneva, Switzerland. WHO/NICD malaria microscopy EQA programme (paper).

Dini L, Fernandez V, Wong M, Frean J. Swedish-South Africa Health Forum, April, Stockholm, Sweden. Survey and management of drug resistant *Pneumocystis* pneumonia among HIV/AIDS patients in South Africa (paper).

Dini LA, du Plessis M, Wong ML, Karstaedt A, Fernandez V, Frean J. 9th International Workshop on Opportunistic Protists, 18-24 June, Lisbon, Portugal. High prevalence of DHPS polymorphisms associated with sulfa resistance in South African *Pneumocystis jirovecii* strains (paper).

Frean J. GSK Malaria Slide Reading Meeting, 7-8 September, Brussels, Belgium. Proficiency testing for African malaria laboratories (paper).

Frean J. GERMS-SA Principal Investigator Meeting, 7-8 November, NICD, Johannesburg. Estimating the contribution of PCP to the burden of respiratory disease in HIV-infected persons in South Africa (paper).

PRU staff participated in the WHO Regional Advisory Group Meeting, 12-13 June, NICD, Johannesburg, and presented malaria EQA results for the WHO AFRO Region.

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT

STAFF

Assoc. Professor JA Frean MB BCh, MMed, MSc, DTM&H, FACTM, Head of Unit
L Arntzen Dip Med Tech, MSc, Laboratory Manager (Micro Units)
R Padayachee BSc (Hons), Medical Scientist
M Setshedi Dip Biotech, Biotechnologist
J Mathebula, Laboratory Assistant

The Unit provides a diagnostic and reference service for certain dangerous bacterial diseases, namely, plague, anthrax and botulism, and for this purpose operates a BSL3 (high security) laboratory. Additionally, several other zoonotic infections are also under investigation, including leptospirosis, toxoplasmosis, and bartonellosis. While plague is presently quiescent in South Africa, outbreaks occurred recently in central Africa, and the Unit's expertise in laboratory diagnosis of plague was called upon; in South Africa anthrax outbreaks affecting humans occur periodically, utilising the Unit's capacity to identify *Bacillus anthracis.* The use of molecular diagnostic techniques is becoming increasingly important in the Unit, and has resulted in the realisation that leptospirosis is almost certainly more common than is generally thought. Mrs Arntzen has been recognised by the World Health Organization as a regional expert on plague.

CURRENT RESEARCH PROJECTS

RATZOOMAN is a multicountry, multidisciplinary study of disease risks linked to rodents at the rural/peri-urban interface, primary targeting plague, toxoplasmosis and leptospirosis. The project, which began in 2003, formally terminated in May 2006, with an International Workshop organized by the SBPRU (see conference presentations, below). The workshop was very successful and renewed interest locally in zoonotic diseases and rodent control. Work will continue in the Unit on some of the more than 5 thousand rodent and human specimens that were collected during the study period.

Other research projects that will continue in 2007 are the molecular epidemiology of southern African strains of *B. anthracis* using MLVA (collaborative project with Dr Wolfgang Beyers from the University of Hohenheim, Stuttgart, Germany); optimising PCR diagnosis of leptospirosis in animals and humans; the epidemiology of bartonellosis in humans and animals in South Africa; and the molecular characterisation of plague isolates from the southern African region. Results obtained thus far indicate that ribotype B is the predominant ribotype, with some strains demonstrating a slight difference. These strains will be further characterised. These results suggest a clonal origin of the strains from

Checking a rodent trap: RATZOOMAN project, Mapate Village, Limpopo Province.

Namibia and South Africa and a local divergence in the plasmids. All the strains from the culture collection in SBPRU need to be tested to confirm this observation.

TRAINING AND EXTERNAL QUALITY ASSESSMENT ACTIVITIES

Microbiology and clinical pathology registrars were trained in identification and confirmation of *Yersinia pestis, Bacillus anthracis* and *Clostridium botulinum.* They were also taught laboratory safety procedures that need to be adhered to while working in a BSL3 facility.

The SBPRU, in conjunction with the Quality Assessment Unit, sends plague proficiency testing samples to sixteen African countries and three referee laboratories, CDC in Fort Collins, USA; Institut Pasteur in Madagascar; and Groote Schuur Hospital, Cape Town. Over the past year there has been an encouraging improvement in the results. Still more training is needed in some of the weaker countries.

VISITING SCIENTISTS

Dr Wolfgang Beyers, University of Hohenheim, Stuttgart, Germany
Dr Peter Turnbull, consultant to WHO and Walter Reed Army Medical Centre

INTERNATIONAL CONFERENCES AND MEETINGS

Arntzen L. WHO Interregional meeting on prevention and control of plague, 7-12 April, 2006, Antananarivo, Madagascar. The NICD/WHO plague external quality assessment programme (paper).

Bleeding a vulture to check for anthrax antibodies, REST vulture sanctuary, Gauteng Province.

Arntzen L. RATZOOMAN Workshop, May 2006, Malelane, South Africa. Presentation of zoonoses survey data (paper).

Frean JA. RATZOOMAN Workshop, May 2006, Malelane, South Africa. Plague (paper).

MYCOLOGY REFERENCE UNIT

STAFF

Dr Kerrigan McCarthy, MBBCh, DTM+H, FCPath (Micro), Pathologist, Head of Unit
Dr Jenny Rossouw, PhD (Virology), MSc, Medical Scientist
Muendi Phadagi, BSc (Hons), Medical Scientist
Susan Gould, BSc, Nat Higher Dip Med Tech (Micro), Laboratory Manager
Jaymati Patel, Nat Dip Med Tech (Micro), Medical Technologist
Gloria Zulu, Cert Med Tech, Medical Technician

MYCOLOGY REFERENCE UNIT ACTIVITIES

Laboratory analysis of strains of *Cryptococcus neoformans* obtained from GERMS-SA national surveillance network:

- Characterisation (identification, typing) and storage of over 6000 isolates from across the country was performed by hard-working MRU staff.
- Antifungal susceptibility testing on a limited subset of isolates from the 2005 and 2006 culture collection was performed. Two staff members received training in the Antifungal Unit of the Mycotic Diseases Branch of the Centres for Disease Control and Prevention, Atlanta, USA.
- Details of these and other findings may be found in the GERMS-SA annual report.

Isolation of environmental *Cryptococcus*:
Over 300 environmental samples (organic material, soil and avian droppings) were processed in an attempt to isolate *Cryptococcus* from the environment.

External Quality Assessment Services for Diagnostic Mycology laboratories, NHLS:
EQA for mould and yeast identification was offered to 28 and 111 laboratories respectively. The MRU managed all aspects of the EQA programme, including preparation and distribution of teaching exercises.

Accreditation:
The Molecular and Surveillance laboratory set up procedures and management processes according to ISO15189 in preparation for accreditation in November 2007.

Teaching and training:
Five Microbiology/Clinical Pathology registrars spent two weeks in MRU learning fungal identification, susceptibility testing, and clinical epidemiology of cryptococcosis.

Staff from the MRU taught at the Basic Mycology Training seminar in March 2006 with the Johannesburg Hospital Microbiology Laboratory under the NHLS School of Laboratory Medicine.

Molecular Mycology:

- Molecular epidemiology of South African *Cryptococcus gattii (*serotype B, C) was performed using PCR fingerprinting, sequence analysis, and mating type determination.
- Identification of mould and yeast isolates using D1-D2 sequencing of the fungal ribosomal DNA was established to verify taxonomic position of moulds and fungi with discrepant/discordant biochemical or morphological features.
- Collaborative protocols for molecular analysis of *C. neoformans* var. *grubii* (serotype A) from South Africa and Botswana together with colleagues from Duke University, South Carolina, USA are in progress. This project was initiated with the purchase of a 4 capillary sequencer with software for AFLP analysis and training of staff. An application has been submitted to ISHAM for a travel grant that will facilitate training of a MRU scientist in molecular methods for genotyping and phylogenetic analyses of isolates of *C. neoformans* and *C. Gattii*.

Figure 1. Gloria Zulu and Muendi Phadagi of the MRU reviewing plates for typing of *Cryptococcus* species. Canavanine-glycine bromothymol blue agar plates are turned blue by *Cryptococcus gattii*

CONFERENCES/MEETINGS ATTENDED

- McCarthy K, 16th Congress of the International Society of Human and Animal Mycology, 25-29th June 2006, Paris, France. Emerging epidemiology of Cryptococcosis, 2002-5. Oral and invited presentation.
- Rossouw J, 16th Congress of the International Society of Human and Animal Mycology, 25-29th June 2006, Paris, France. Cryptococcus gattii in HIV-infected and uninfected patients, 2005. Poster presentation.
- Gould S, Patel J, Laboratory Identification of Pathogenic Moulds, 27-28th July 2006, Centre for Disease Control and Prevention, Atlanta, USA. Training course in mould identification and susceptibility testing of yeasts.
- Rossouw J, GERMS-SA Principal Investigator's meeting, 7-8th November 2006, Johannesburg. Molecular epidemiology of cryptococcosis in South Africa.
- Gould S, GERMS-SA Principal Investigator's meeting, 7-8th November 2006, Johannesburg. Antifungal susceptibility testing of cryptococcal isolates from national surveillance.
- McCarthy K, GERMS-SA Principal Investigator's meeting, 7-8th November 2006, Johannesburg, Molecular analysis of cryptococcal isolates from national surveillance.
- McCarthy K., National Institute for Communicable Diseases Academic Day, 27-28th November 2006, Johannesburg. The burden of disease due to AIDS in Gauteng: inferred evidence form results of surveillance for cryptococcosis 2002-2004.
- Rossouw J, University of the Witwatersrand, Health Sciences Research Day, 23rd August 2006, Johannesburg, South Africa. Molecular epidemiology of *Cryptococcus gattii* isolates from HIV-seropositive and negative patients in South Africa.
- Rossouw J, National Institute for Communicable Diseases Academic Day, 27-28th November 2006, Johannesburg, South Africa. Molecular epidemiology of cryptococcosis in South Africa.

EXTERNAL QUALITY ASSESSMENT UNIT

STAFF

Dr Kerrigan McCarthy, MBBCh, DTM&H, FCPath (Micro), Pathologist, Head of Unit
Vivian Fensham, Nat Dip Med Tech (Clin Path, Micro), Laboratory Manager, EQA
Rebecca Landsberg, BTech Biomedical Technology, Medical Technologist, EQA
Helen Haritos, Nat Dip Med Tech (Micro), Medical Technologist, EQA
Marshagne Smith, Nat Dip Med Tech (Micro), Laboratory Manager, National Stock Culture Collection

EQA UNIT ACTIVITIES

External Quality Assessment programmes for the NHLS, private and international sub-Saharan laboratories EQA programmes were provided in the disciplines of bacteriology (115 laboratories), tuberculosis microscopy (253 laboratories), tuberculosis culture (25 laboratories) and syphilis RPR serology (223 laboratories). A new programme for syphilis TPHA serology was introduced in January 2006 to which 34 laboratories subscribed. Results of laboratory performance are available in summary form in a separate EQA annual report.

External Quality Assessment programmes in association with WHO Division for Epidemic Preparedness and Response (Lyons office) EQA programmes in bacteriology, malaria and TB microscopy and were provided for the fifth consecutive year as part of contractual obligations with WHO/EPR/Lyons to 73 national public health laboratories in the African Regional Office (AFRO) of the World Health Organization. These programmes are run with the assistance of NICD consultants and staff in the following units: RMPRU, EDRU, SBPRU, PRU.

The NICD EQA Unit is an advisor and external referee to the EMRO region of the WHO in the WHO/EMRO regional EQA programme.

The NICD hosted the Annual WHO/EPR/Lyons and AFRO EQA review meeting from 12-13 June 2006 attended by the head of WHO/EPR Dr Christian Mathiot, AFRO staff (Laboratory Co-ordinator, Dr JB Ndihokubwayo, TB Programme Director, Dr Daniel Kibuga, and Malaria Programme Director, Dr Noel Chisaka.

NATIONAL STOCK CULTURE COLLECTION (NSCC)

The NSCC continued to provide reference strains from recognised culture collections to NHLS laboratories for quality control purposes.

- 76 NHLS bacteriology laboratories were supplied with a set of 10 reference strains for quality control of antimicrobial susceptibility and other laboratory procedures. A method was developed and perfected by the NSCC in which cultures are lyophilised onto sterile glass beads thus facilitating sequential reconstitution and limiting passage number of reference cultures.
- New strains from recognised culture collections (American Type Culture Collection and National Type Culture Collection, UK) were purchased both for the NSCC and for NICD/NHLS Units. An isolate of each of the 61 new cultures was retained within the NSCC collection.
- Thirty laboratories (NHLS and external) requested 161 cultures from the NSCC for their own purposes. Each culture is supplied with a validation certificate authenticating its identification and susceptibility profile.

TEACHING AND TRAINING ACTIVITIES

A 4-day training course in antimicrobial susceptibility testing was given to Mtatha Tertiary laboratory by EQA and GERMS-SA staff in March 2006.

EQA presentations were given during the course of GERMS-SA training, FELTP training, and in-house NHLS strategic planning:

- Groote Schuur, Tygerberg, PathCare (Cape Town), Green Point (January 2006)
- Mtatha (March 2006)
- All business managers as part of the FELTP QA/Q training course (August 2006)
- NHLS Strategic Planning meeting (October 2006)
- NICD Academic Day (November 2006, Poster presentation)

CONFERENCES/MEETINGS ATTENDED

- V Fensham. Technical Meeting on Rollup of WHO External Quality Assessment Programme in EMRO, 22-23 February 2006, Lyons, France. WHO/NHLS Bacteriology EQA programme to AFRO laboratories.

- All EQA staff and NICD specialist Unit staff. WHO Consultative meeting to review External Quality Assessment Programme in Africa, 12-13 June 2006, Johannesburg, South Africa. General review of the EQA results for the year 2005-2006 and comparison with year 2004-2005 results.
- Vivian Fensham (for K McCarthy, invited speaker). International Symposium on Quality Assurance and Control in Laboratory Medicine, 11-14 September 2006, Geneva, Switzerland. NICD EQA Unit, Role and Functions.
- Sebastien Cognat (WHO/EPR/Lyons) (on behalf of NICD authors). International Congress on Infectious Diseases, June 15 18, 2006. Lisbon, Portugal. Poster presentation: WHO External Quality Assessment Programme in Africa: A 3-Year Review of the Epidemic-prone Diseases Laboratory Diagnostic Capabilities.

Figure 1. Group photo of delegates to WHO Consultative meeting to review External Quality Assessment Programme in Africa, 12-13 June 2006.
From left to right, standing: Dr Anne von Gottberg (RMPRU, NICD), Prof John Frean (SBPRU and PRU, NICD), Dr Daniel Kibuga (AFRO/ATM-TUB), Ms Linda de Gouveia (RMPRU, NICD), Dr Jean-Bosco Ndihokubwayo (AFRO/DSD), Dr Sebastien Cognat (WHO/EPR/Lyon), Dr Noel Chisaka (AFRO/ISCT-MAL), Dr Christian Mathiot (WHO/EPR/Lyon), Ms Lorraine Arntzen (SBPRU, NICD), Dr Bhekithemba Mhlanga (AFRO/HIB)
From left to right, seated: Ms Leigh Dini (PRU, NICD), Dr Kerrigan McCarthy (EQAU, NICD), Ms Vivian Fensham (EQAU, NICD)

ENTERIC DISEASES REFERENCE UNIT

STAFF

Dr KH Keddy MBBCh, BSc (Med), MMed, FCPath (SA), DTM&H, Senior consultant, Head of Unit
Dr AM Smith PhD, Senior Scientist
Mrs A Sooka Dip Med Tech, MSc, Laboratory Manager
Ms S Nadan MSc, Medical Scientist
Mrs F Mnyameni Dip Biomed Tech, Senior technologist
Ms M Ngomane Dip Biomed Tech, Senior technologist
Ms A Sitsula Dip Biomed Tech, Technologist
Mr E Khomane Laboratory technician
Mr M Mtambo Laboratory technician
Mrs P Mogale Data Capturer

Post-graduate students
Ananta Nanoo (MSc Epidemiology)
Masters Dissertation: The impact of cotrimoxazole usage in HIV on cotrimoxazole resistance in non-typhoidal Salmonella

Rugola Mtandu (MSc Epidemiology)
Masters Dissertation: The impact of HIV on clinical-microbiologic features and mortality among patients with Invasive Non Typhoidal Salmonella infection in South Africa.

CURRENT RESEARCH PROJECTS

Molecular epidemiology of South African isolates of Salmonella enterica serotype Typhimurium
Much research has focused on molecular (genotypic) methods for serotyping *Salmonella*, as an alternative to the conventional serotyping method which is reliant on the phenotypic expression of bacterial properties. Expression of genes is mostly controlled by various internal regulatory mechanisms; however expression may also be influenced by external environmental conditions. Therefore, a bacterial strain may fulfil all the generic requirements (structural genes) for a particular serotype, but due to various reasons may not express the gene, resulting in an incomplete serotype determination. EDRU is increasingly encountering this type of problem, particularly with respect to *Salmonella* which are serogrouped to the level of the O:4,5 antigen, but then fail in their H antigen serotyping. An O:4,5 antigen is highly suggestive of the *Salmonella* Typhimurium serotype (O:4,5:H:i;1,2), but without a conclusive H antigen type, we cannot make the diagnosis of serotype *Salmonella* Typhimurium. This is of particular concern for us, as *Salmonella* Typhimurium is the most commonly isolated *Salmonella* serotype in the human population, and nontype-ability of H-antigen could possibly skew our surveillance data for *Salmonella* Typhimurium. This problem can be overcome by using molecular methods (PCR) to diagnose serotype. These methods target two structural genes (*fliC* and *fljB)* coding for the protein component of flagella and detect for specific allelic variants of these genes. In *Salmonella* Typhimurium, the *fliC* gene variant encodes antigenic type (H:i), while the *fljB* gene variant encodes antigenic type (H:1,2).

The first part of this research project will investigate the use of multiplex-PCR for H antigen (flagella) serotyping, to assist with conventional serotyping of *Salmonella*. This will particularly focus on the H antigens (H:i;1,2) of *Salmonella* Typhimurium. This should help to overcome inconclusive H antigen serotyping on strains which can only be serogrouped to the level of the O:4,5 antigen, a group highly suggestive of the *Salmonella* Typhimurium serotype. This will help improve our diagnosis of *Salmonella* Typhimurium serotypes and provide more accurate surveillance data for the South African *Salmonella* population.

The second part of this research project will involve the genotypic fingerprint analysis of *Salmonella* isolates, to investigate the molecular epidemiological status of the South African *Salmonella* Typhimurium population. The following question could then be answered. In South Africa, is the epidemiology *Salmonella* Typhimurium infection dominated by a limited number of clones or is it represented by genetically diverse and unrelated strains? Of most interest will be the data regarding the epidemiology of antimicrobial-resistant *Salmonella* Typhimurium. Antimicrobial selection pressures play a major role in driving the epidemiology of bacterial infections. As antimicrobial usage differs from one country to the next, so the epidemiology of bacterial infection will differ from one country to the next. Our research will enable us to compare the epidemiology of South African *Salmonella* Typhimurium to that of trends observed in the US and European countries. How do major South African clones compare to clones which predominate worldwide? Finally, knowledge of the molecular epidemiology of bacterial infections provides information of major circulating (infecting) clones, so that in times of antimicrobial treatment intervention and vaccine intervention, well-informed and educated decisions can be made to combat the disease.

Methods
Selection of isolates: For the years 2003 to 2005; a total of 610, 823, and 844 *Salmonella* Typhimurium serotypes have been collected by the NICD. This totals a very large pool of 2277 isolates. We would like to

reduce this number by half and so have a more manageable number of isolates. So, for each year, we will randomly select isolates for half the total number collected. This will give us ~1139 isolates.

***PFGE analysis*:** The ~1139 isolates will be analyzed using PFGE following *Xba*I restriction digestion of their genomic DNAs. The resulting PFGE fingerprint patterns will be analyzed and compared using the GelComparII software. The software will draw dendrograms and identify clonal groups and clusters.

***MLST analysis*:** Representative isolates (2-3) of each PFGE clonal group will be analyzed using MLST to obtain sequence types (STs) of isolates. These STs will be compared to those listed in the worldwide MLST database.

***Multiplex-PCR for H antigen serotyping*:** For the years 2003 to 2005; a total of 91 *Salmonella* isolates have serogrouped to the level of the O:4,5 antigen, but then fail in their H antigen serotyping. These isolates will be further investigated using a multiplex-PCR, incorporating primers designed to amplify and detect for the H antigens (H:i;1,2) of ***Salmonella* Typhimurium.**

Outputs / Outcomes

- Improved understanding of the epidemiology and transmission of *Salmonella* Typhimurium
- Insight into the genetic mechanisms for flagellar gene expression in *Salmonella.*

CLINICAL, EPIDEMIOLOGICAL AND LABORATORY STUDY OF INVASIVE *SALMONELLA* TYPHIMURIUM IN HIV INFECTED ADULTS AT A SOUTH AFRICAN HOSPITAL AND RELATEDNESS WITH *SALMONELLA* TYPHIMURIUM ISOLATES FROM NEIGHBOURHOOD FOOD OUTLETS

The incidence of infections caused by non-typhoidal salmonellae has increased considerably in many countries. *Salmonella enterica* serotype Typhimurium is a globally distributed zoonotic serotype and is the most common serotype isolated from animals, particularly cattle and poultry, and humans in the USA and UK. *Salmonella* Typhimurium phage type DT104 is a common infectious agent in cattle and has been contracted by exposed humans. It has become increasingly resistant and many strains are now multidrug-resistant.

There is a known association between infection with invasive *Salmonella* and HIV/AIDS. During 1999, selected *Salmonella* Typhimurium strains were phage typed and indicated the presence of DT 104, a multiresistant type responsible for causing epidemics in several countries. PFGE was performed on a limited number of multiresistant strains and a high degree of clonality was noted.

Methods

Patient isolates of *Salmonella* that are received by EDRU will be serotyped according to current SOPs. Susceptibility testing will be done on all isolates

Macrorestriction analysis of chromosomal DNA is performed using digestion with the enzyme *Xba* I and pulsed-field gel electrophoresis (PFGE).

In-patients identified with multi-drug resistant *Salmonella* Typhimurium will be identified and requested to complete a simple questionnaire on food sources, eating habits, availability of piped water, employment (including self-employment in the informal sector) and other factors that may impact on their acquiring *Salmonella* infection. This data will be collated with information acquired through testing meat specimens obtained from various local sources for ESBL-producing *Salmonella* Typhimurium.

Chicken and beef specimens will be sourced from various informal abattoirs and formal retailers around Soweto, which will be identified with the assistance of local residents. Specimens will be processed and cultured according to recognized methods for food testing. Suspicious colonies will be confirmed as *Salmonella* species on biochemical profiles and submitted for susceptibility testing and serotyping, as per the clinical isolates. Isolates that are confirmed to be ESBL-producing *Salmonella* Typhimurium will be submitted for PFGE, as previously described. ESBL profiles between clinical and food isolates will be compared to assess strain-relatedness between clinical cases and potential food sources of salmonellosis.

Outputs / outcomes

- Better understanding of risk factors, clinical presentation and appropriate therapy for invasive *Salmonella* Typhimurium infections, particularly in HIV/AIDS patients.
- Guidelines on improved hygiene in handing of potentially contaminated food, if HIV-infected.
- Reduction in inappropriate use of antimicrobial agents in agriculture and in medicine.
- Better control of informal slaughterhouses.
- Continued surveillance of patterns and levels of antimicrobial resistance of *Salmonella*, particularly *Salmonella* Typhimurium infections.

EVALUATION OF THE RAPID DIAGNOSTIC TESTS TUBEX TF AND TYPHIDOT IN A TYPHOID ENDEMIC AREA IN SOUTH AFRICA

Typhoid fever is a major cause of morbidity and mortality worldwide, causing an estimated 22 million new infections and 400,000 deaths each year according to WHO. Incidence rates have been estimated as high as 1000 per 100,000 of population per year. In areas of endemicity and in large outbreaks, most cases occur in persons aged between 3 and 29 years1. As in other developing countries, South Africa has a high burden of disease and typhoid fever is endemic in the eastern parts of the country.

Isolation of *Salmonella enterica* serotype Typhi from bone marrow, blood, urine or stool is the gold standard for confirming a case of typhoid fever; however this requires equipment, supplies, and technical training

that are beyond the means of most primary health care facilities. Antibiotic prescribing prior to blood culture may render bacteriological confirmation difficult. As a result, the diagnosis may be delayed or missed entirely while other febrile illnesses are considered, and patients without typhoid fever may receive unnecessary and inappropriate antimicrobial treatment. Emerging drug resistance among circulating *Salmonella* Typhi strains has greatly complicated the treatment of typhoid fever, and heightened the need for rapid accurate diagnosis and the appropriate and selective use of antimicrobial agents to which the organism has so far remained susceptible.

Culture takes at least two days, before the diagnosis can be confirmed. Currently, alternative practices in South Africa include using a modification of the Widal test, specifically a serial dilution and slide agglutination of patients' antisera (http://www.linear.es/), which has not been evaluated (figure 1). This test is dependent on the production of IgM and IgG, but both the O and the H antigen used are non-specific and may cross-react with other *Salmonella* as well as *Brucella*, malaria, miliary tuberculosis etc. This results in both a high false positive as well as a high false negative rate in early disease (figure 1).

TUBEX TF is a rapid diagnostic test that specifically identifies patient IgM against the O antigen of *Salmonella* Typhi (O9). It has been shown to perform well against other rapid tests as well as against culture in the diagnosis of typhoid fever with a sensitivity of 78 to 95% and specificity of 77 to 89% in previous studies, although one disadvantage of the test, is emerging antimicrobial resistance in *Salmonella* Typhi may only then be identified by clinical treatment failures. Nonetheless, the test has excellent potential in typhoid outbreaks and in areas where the antimicrobial resistance patterns of *Salmonella* Typhi are known.

Typhidot is a rapid diagnostic test that detects IgG and IgM against a specific 50kD antigen of *Salmonella* Typhi. It has been extensively evaluated internationally and outperforms the Widal test, offering 72 to 92.3% specificity and 52 to 98.8% sensitivity in some studies. IgM detection suggests acute typhoid and early phase of infections, whereas IgG and IgM detection suggest that the patient is in the midphase of infection.

Both tests use minimal volumes of patient sera, making these appropriate for paediatric use.

Methods

Serum samples: Prospective patient material will be analyzed using TUBEX TF and Typhidot.

Patient population: Consecutive patients with suspected typhoid fever presenting to Rob Ferreira Hospital, Nelspruit, will have blood taken for culture and serology on admission. A maximum amount of 5 ml of blood for serology and culture from adults and 3.5 ml for serology and culture from children under five years of age will be taken, using clotted whole blood (plain-sample additive-free) tubes. Cases will include those who are blood culture-positive for typhoid fever. Controls are those patients in whom blood culture is negative. A total number of 350 patients is anticipated, based on previous serological tests done in this

	Incubation	Active invasion	Established disease	Convalescence	Late complications
Time course	Ingestion ↓ Wk 1	Wk 2	Wk3	Wk4 Wk5	Indefinite
Blood cultures	Negative	← 80-90 % →		Negative unless continued disease or relapse	
Stool cultures	Transiently positive	Negative	← 80% positive →	← 50% → positive	Decreasing incidence of positive cultures with time: 20% at 2 mo 3% at 1yr
Urine cultures	Negative	Negative	←25% positive→	←10%→ positive	Decreasing incidence of positive cultures
Bone marrow culture	Negative	Negative	←80-90% positive→	Decreasing incidence of positive cultures	
Widal test	Negative	← 20% → positive	← 50% → positive	← 80% positive →	

Figure 1. Diagnostic yield of culture and Widal in untreated typhoid fever

laboratory. Patient data will be recorded on the clinical report form, which will be attached to the laboratory report form. The responsibility for recording this data will be that of the student technologist, with assistance of the surveillance officer for Nelspruit-Barberton area.

Tests: The overall sensitivity and specificity of TUBEX TF and Typhidot is to be evaluated using samples as described above. TUBEX TF detects the presence of IgM antibodies against O9, specific for the D-group of Salmonellae to which Salmonella Typhi belongs. Typhidot is a rapid diagnostic test that detects IgG and IgM against a specific 50kD antigen of Salmonella Typhi.

TUBEX TF and Typhidot tests will be run in parallel with blood culture and Widal and modified Widal tests, by independent observes. Blood cultures will be processed according to current standard operating procedures of the National Health Laboratory Service, Nelspruit. Widal tests will be processed on a blinded basis, according to manufacturer's instructions, with the operator unaware of the result from the TUBEX TF and Typhidot tests.

Outputs / Outcomes

- Rapid diagnosis of typhoid fever, particularly in an outbreak situation, will be optimised for South African conditions.

Both isolates of Salmonella Typhi as well as patient sera will be forwarded to Enteric Diseases Reference Unit, NICD, for confirmation of organism identity and parallel evaluation of the TUBEX TF and Typhidot tests

THE MOLECULAR EPIDEMIOLOGY OF *SALMONELLA ENTERICA* SUBSPECIES *ENTERICA* SEROTYPE TYPHI ISOLATES FROM AN OUTBREAK IN DELMAS, SOUTH AFRICA IN 2005

Salmonella Typhi continues to cause severe disease in many parts of the world. The most common clinical complication associated with infection is the perforation of ulcerated Peyer's patches within the small intestine, leading to peritonitis with associated mortality. Variations in disease severity have been associated with differences amongst strains of *Salmonella* Typhi circulating in endemic areas.

Salmonella Typhi has been identified using classical methods such as phage typing and isoenzyme analysis as well as molecular methods such as PFGE, Ribotyping, RAPD and AFLP. Different PFGE patterns between isolates from patients with fatal typhoid fever and isolates from patients with non-fatal typhoid fever have been reported. This implies an association between PFGE patterns within genotypes of *Salmonella* Typhi and the capability to cause fatal disease. PFGE is thus performed to characterize the subtypes of *Salmonella* Typhi associated with or present during a disease outbreak.

Other molecular epidemiological techniques for the discrimination of isolates such as multilocus sequence typing (MLST), which examines the housekeeping genes, may be problematic due to the high identity of gene sequences of closely related *Salmonella* species. The method of VNTR profiling however, has been reported to have the best mix of discriminatory power, reproducibility, typeability, speed, ease of usage and cost for the epidemiological analysis of *Salmonella* Typhi strains when compared to the above mentioned methods.

VNTRs have been increasingly used as molecular markers for strain typing of various bacteria. Slipped-strand misparing that occurs during DNA replication results in various copy numbers of sequence repeats . These variations have been compared between *Salmonella* Typhi strains.

Methods

Pulsed field gel electrophoresis (PFGE): PFGE is used to compare strains of *Salmonella* Typhi from the most recent epidemic with each other as well as with isolates from the previous outbreak. The bacterial cells of the isolates will be immobilized into agarose plugs. Bacteria are lysed to release the chromosomal DNA, followed by *Xba*1 restriction enzyme digestion of DNA. The fragments of genetic material are separated by agarose gel electrophoresis. These restriction patterns may then be compared to identify similarities and differences and determine clones, according to the guidelines of the protocol of the National Molecular Subtyping Network for Foodborne Disease (Pulse-Net) as set out by the Centers for Disease Control and Prevention (CDC), Atlanta, USA.

Multi Locus Variable Number Tandem Repeat Analysis (VNTR): Five identified loci are being investigated: the most discriminatory primer sets will be incorporated in a multiplex PCR. The PCR incorporates primers that have been previously described. The PCR products (fingerprint profiles) will be separated by agarose gel electrophoresis and visualised. Access to a light cycler or real time thermal cycler means the reactions shall be adjusted accordingly to enable quantitative detection of each amplified VNTR product.

Preliminary investigations have shown VNTR to exhibit a high level of discrimination between strains associated with the typhoid outbreak in Delmas and an epidemiologically unlinked strain of *Salmonella* Typhi from Gauteng. This was confirmed by PFGE.

Outputs/outcomes

- PFGE and VNTR will provide fingerprint data that will be analysed and compared using the GelCompare II (Version 4.5) software. This will allow us to determine the relatedness of the strains from 1992 and 2005.
- Acceptable fingerprint profiles will be incorporated in a reference genotypic database for future outbreaks of *Salmonella* Typhi in South Africa and worldwide.

SURVEILLANCE ACTIVITIES

EDRU currently has the responsibility for surveillance and characterisation of bacterial enteric disease in South Africa; specifically, EDRU collects all human isolates from diagnostic microbiology laboratories in South Africa for surveillance, as detailed in table 1 below, from those body sites listed in the same table. These isolates will be characterised at no charge to the laboratory of origin, irrespective of whether the laboratory functions in a private capacity or has a public role. This includes those isolates that may represent carriage of an enteric bacterial pathogen, rather than disease due to that pathogen.

Mpilo Mtambo performing susceptibility testing on isolates received by EDRU

Table 1: Isolates for referral to EDRU, NICD, showing how these isolates are currently characterised.

Culture	Organism	Biochemical identification	Serotyping	Antimicrobial susceptibility testing	Genotyping
All body sites	*Aeromonas*	✓	×	✓	✓
All body sites	*Salmonella* species	✓	✓	✓	✓
All body sites	*Shigella* species	✓	✓	✓	✓
All body sites	*Vibrio cholerae* O1 and non-O1	✓	✓	✓	✓
All body sites	Non cholera vibrios	✓	✓	✓	✓
All body sites	*Yersinia enterocolitica*	✓	×	✓	✓
Stool/ rectal swab	Diarrhoeagenic *Escherichia coli*	✓	✓	✓	✓
All body sites	Suspect EHEC/E coli O157/STEC	✓	✓	✓	✓

- The case definition for these pathogens for all surveillance done by EDRU includes those isolates from body sites as specified below, in both in-patients and out-patients. Specifically this includes those individuals who sought treatment at a hospital or clinic, such as outpatients who have positive stool cultures or rectal swabs, but are not admitted or discharged from casualty. In this instance carriers may be included because they add to the burden of treatment, if not the burden of disease and may represent sub-clinical cases e.g. Cholera.
- The case definition for enhanced surveillance isolates includes only those *Shigella* and *Salmonella enterica* isolates that are from normally sterile body sites in "in-patients" only that is the patient should have been admitted to the hospital or enhanced surveillance site, as currently defined by the Enhanced Surveillance core, or there should have been the intention to admit, to include those patients who may expire in casualty, as established from the bed letter. This also allows for changes in the ES site, either to include new South African sites or to exclude sites which may be viewed as no longer appropriate for the study.
- Although EDRU does not normally do such work for other African countries, the support of the unit may be requested should one of the neighbouring countries require it.
- EDRU currently receives specimens from over 3000 human cases per annum, according to the definition

above. 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.

- Regular reports on the isolates received are extracted from the database for the purposes of information sharing (table 2).
- Where relevant, molecular methods may be used to establish strain relatedness in outbreaks (figure 2).

Table 2: Regular reports submitted to various stakeholders by EDRU

Report	Stakeholder	Frequency
Provincial laboratory report back – *Salmonella*, *Shigella*, DEC	Provincial laboratories and co-ordinators	Quarterly
NICD Bulletin – *Salmonella*, *Shigella dysenteriae* 1, *Vibrio cholerae* O1	Epidemiology unit, NICD	Quarterly
National Outbreak Response Team, National Department of Health – *Salmonella* Typhi, *Vibrio cholerae*	NDOH	Monthly
Enternet *Salmonella*, Enterohaemorrhagic *E. coli*	EU surveillance for food	Quarterly
Global Salm-Surv – *Salmonella*	WHO	Annually

Florah Mnyameni subculturing *Salmonella* for serotyping

Mimmy Ngomane and Asiashu Sitsula serotyping *Shigella* isolates.

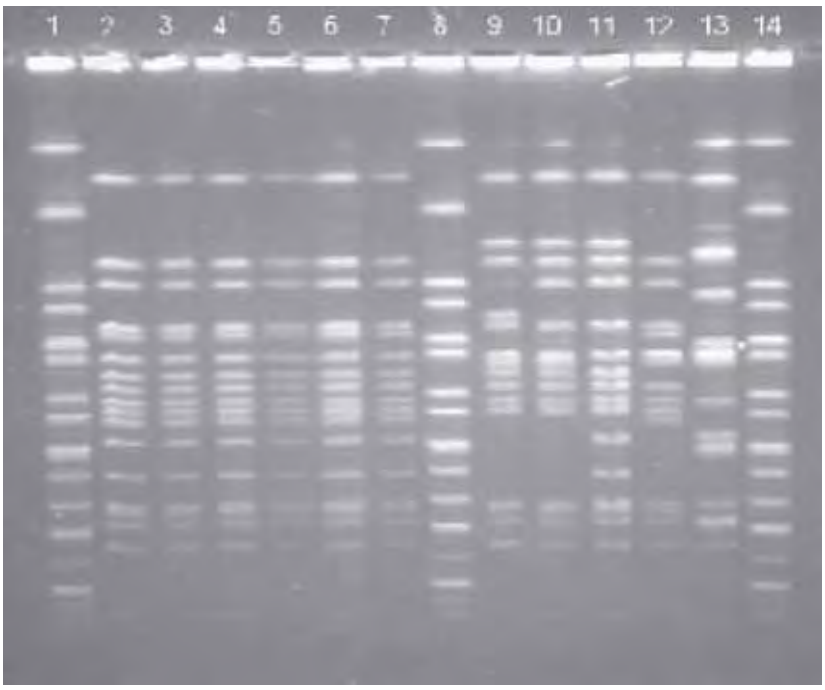


Figure 2. Pulsed-field gel electrophoresis analysis of isolates of *Salmonella* Virchow associated with an outbreak of food-borne disease amongst school teachers in the Mpumalanga province, December 2006. Lanes 2 to 7, selected isolates associated with the Mpumalanga outbreak; lanes 9 to 13, isolates unrelated to the outbreak; lanes 1, 8, 14, molecular size marker.

- EDRU participated in the CAP programme for External Quality Assessment.
- EDRU participated in the Enternet (EU-affiliated) ring trail for identification of verotoxigenic Escherichia coli, 2006.
- EDRU participated in Global Salm-surv Quality Assurance, 2006.

Site training

- Arvinda Sooka and Mimmy Ngomane assisted in site training visits to Northern Cape, from 23-24 November 2006.

INTERNATIONAL MEETINGS ATTENDED

Dr Keddy attended the Annual Enter-net workshop. 2123 September 2006 Prague, Czech Republic (Hosted by the Státní zdravotní ústav).

NATIONAL MEETINGS ATTENDED

Mimmy Ngomane attended the 3rd Public Health Conference, May 15-17, 2006, Midrand, South Africa.

CONFERENCE PROCEEDINGS

- Ngomane M., Sooka A, Mnyameni F, Smith A, Keddy K. Outcome of a diarrhoeal outbreak in a children's home in the Northern Cape. Poster presentation, 3rd Public Health Conference, May 15-17, 2006, Midrand, South Africa.
- Govender N, Quan V, Prentice E, von Gottberg A, Keddy K, and McCarthy K for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). A national South African Surveillance Network for bacterial and fungal diseases. Poster presentation, Programme Book, 3rd Public Health Conference, May 15-17, 2006, Midrand, South Africa.
- Keddy KH. An epidemiological review of recent typhoid outbreaks in South Africa. Annual Enter-net workshop. 21 23 September 2006 Prague, Czech Republic (Hosted by the Státní zdravotní ústav).

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RESPIRATORY & MENINGEAL PATHOGENS RESEARCH UNIT

STAFF

Dr Anne von Gottberg, MBBCh, DTM&H, FCPath (SA) (Micro), Pathologist, Head of Unit
Linda de Gouveia, Nat Dip Med Tech (Microbiology), Laboratory Manager
Ruth Mpembe, Nat Dip Med Tech (Microbiology), Medical Technologist
Olga Hattingh, Nat Dip Biomed Tech (Microbiology & Clinical Pathology), Medical Technologist
Happy Skosana, Nat Dip Biomed Tech (Microbiology), Medical Technologist
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Dr Mignon du Plessis, PhD, Senior Medical Scientist
Nicole Wolter, MSc (Biochemistry), Medical Scientist
Azola Fali, MSc (Biotechnology), Medical Scientist
Kedibone Mothibeli, BSc (Hons), Medical Scientist
Muzi Hlanzi, Matric, Data Clerk
Ethel Maringa, Matric, Diploma in Development Health, Data Clerk
Prof Keith Klugman, MBBCh, PhD, DTM&H, MMEd, FCPath (SA), FRCPath (Lond), FRSSAfr, Professor & Director of Research Unit (RMPRU Bara and NICD)
Phyllis Hyde, BA, Personal Assistant to Research Director

Back row: Linda de Gouveia, Olga Hattingh, Pedwell Makutu (PRU), Muzi Hlanzi

Middle row: Portia Mogale (EDRU), Azola Fali, Kedibone Mothibeli, Lenny Lengwati, Thembi Mthembu (MRU), Ruth Mpembe, Happy Skosana, Ethel Maringa, Phyllis Hyde

Front row: Mignon du Plessis, Nicole Wolter

INTRODUCTION

During 2006, the administration and coordination of the rapidly expanding Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) was successfully handed over to a newly formed unit within the Microbiology Division at the NICD, the National Microbiology Surveillance Unit (NMSU). This unit coordinates bacterial and fungal surveillance involving four units within the NICD (EDRU, MRU, PRU and RMPRU) and Dr Nelesh Govender was hired in March 2006 to run this unit. All medical officer and surveillance officer staff transferred to NMSU. Surveillance database management for all these units is still coordinated by Linda de Gouveia, RMPRU.

Thomas Rafundisani left RMPRU in January 2006 to take up a post as a Sales and Applications Specialist at Dade Behring South Africa, Randjesfontien; and Dr Anthony Smith transferred to EDRU, NICD, to take the lead in their molecular research. Lenny Lengwati and Azola Fali joined the unit in May; and Happy Skosana rejoined us in June 2006.

RMPRU continues to perform active laboratory-based surveillance for invasive disease throughout the country due to *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis*. The unit reports weekly data on these diseases to the Epidemiology Unit, NICD; provides data for presentation at the monthly National Outbreak Response Team (NORT) meeting, National Department of Health, Pretoria and for publication in the quarterly NICD Communicable Diseases Surveillance Bulletin. All of these infections are vaccine-preventable, and our ongoing surveillance data informs pharmaceutical companies, vaccine manufacturers, public health specialists and clinicians working nationally and internationally. In addition we

offer reference diagnostics related to these diseases, as well as outbreak response assistance, in particular for meningococcal cases.

We continue to participate in three external quality assessment programmes relevant to our surveillance and reference functions: SIREVA (Sistema regional de Vacinas) Vigia - Surveillance of *S. pneumoniae, H. influenzae* and *N. meningitidis,* Instituto Adolfo Lutz, Sao Paulo, Brazil; CAP (College of American Pathologists), Bacteriology Survey, Northfield, Illinois, USA; and the EQAS programme CDC/WHO, Collaborating Centre for Antimicrobial Resistance Monitoring, Fred C. Tenover, Director, Public Health Service, Atlanta.

TRAINING ACTIVITIES

RMPRU staff attended various workshops and training courses run by the Continuing Education Unit (CEU), Central Region Branch, NHLS; WITS Health Consortium; Applied Biosystems; and the University of the Witwatersrand. Nicole Wolter was trained in Real Time PCR and gene expression assays, 12-23 June, GR Micro Limited, London, United Kingdom.

RMPRU was involved in training others in the following activities:

- RMPRU hosted and assisted in the facilitation of the laboratory practical sessions for the laboratory track of the South African Field and Laboratory Program, Short Course, 15-24 May 2006, organised by Dr Gill de Jong (Epidemiology Unit, NICD).
- RMPRU provided a 2-week training programme for 5 NHLS clinical pathology and clinical microbiology registrars from 10 to 28 July. Routine phenotypic and genotypic characterisation of bacterial pathogens processed in our laboratory and the epidemiology of these diseases in South Africa were reviewed.
- Linda de Gouveia was recruited as a Laboratory Expert by Dr Jean-Bosco Ndihokubwayo, Regional Adviser - Blood Safety and Clinical Technologies (BCT/DSD/AFRO) within the Division of Health Systems and Services Development (DSD), WHO-AFRO. She was requested to train 10 laboratory technicians from Uíge province, Angola (designated by Dr Bengi Moko Henrique Health Provincial Director, Uíge province) in general diagnostic laboratory principles and techniques. This training took place from 25 August - 8 September, in Luanda, Angola.
- Dr Mignon du Plessis organised a GelCompar software workshop, run by Dr Bruno Pot from Applied Maths, Belgium; 22-23 November, NHLS IT training room, Sandringham, Johannesburg. Eleven NHLS staff attended: 4 from RMPRU (Mignon du Plessis, Azola Fali, Kedibone Mothibeli and Nicole Wolter); 5 from Infection Control, Johannesburg Hospital, NHLS; 1 each from EDRU and MRU, NICD.
- RMPRU staff assisted or took part in training workshops or site visits to laboratories throughout the country coordinated by NMSU and GERMS-SA.
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Staff registered for higher degrees:
Nicole Wolter, PhD, School of Pathology, WITS
Kedibone Mothibeli, MSc, School of Pathology, WITS

External students being co-supervised:
Peter Suwirakwenda Nyasulu, MSc, School of Public Health, WITS
Leigh Dini, PhD, School of Pathology, WITS

SELECTED SURVEILLANCE AND RESEARCH ACTIVITIES IN 2006

Epidemiology of meningococcal disease in South Africa, 1999-2002

This study described the epidemiology of invasive laboratory-confirmed meningococcal disease in South Africa from August 1999 through July 2002, as reported to our laboratory. These data have been accepted for publication (Coulson GB, von Gottberg A, du Plessis M, Smith AM, de Gouveia L, Klugman KP, et al. Meningococcal disease in South Africa, 19992002. Emerg Infect Dis [serial on the Internet]. 2007 Feb [*15/01/2007*]. Available from http://www.cdc.gov/EID/content/13/2/05-1553.htm).

Emergence of endemic serogroup W135 meningococcal disease associated with high mortality in South Africa

In the African meningitis belt serogroup W135 has emerged as an important cause of epidemic disease. The emergence and establishment in other parts of Africa has not been fully elucidated. Cases of invasive meningococcal disease in South Africa from 2000 through 2005 were analysed. The number of cases reported was 2135, of which 1113 (52%) occurred in Gauteng province. In this province, rates of disease increased from 0.81/100,000 in 2000 to 3.98/100,000 in 2005; the proportion of disease due to serogroup W135 increased from 4% (4/54) of cases to 75% (221/295), while the proportion due to serogroup A declined.

Penicillin non-susceptibility in meningococcal isolates causing invasive disease in South Africa

Neisseria meningitidis non-susceptible to penicillin (MIC ≥0.12g/ml) has been reported in several parts of the world, but to our knowledge not from the African continent. Data were analysed from January 2000 through December 2005: 2130 cases of invasive meningococcal disease were reported. Of these cases, 1579 (74%) had viable isolates available for further testing, of which 97 (6%) tested non-susceptible to penicillin. This is the first report of the prevalence of penicillin non-susceptible meningococcal isolates from Africa, and although prevalence rates fluctuated over the years, they were comparable to reports from other countries.

Emergence of fluoroquinolone-resistant invasive pneumococcal infections (IPD) among children, South Africa, 2000-2005

Fluoroquinolone (FQ)-resistant pneumococcal infections are increasing, however remain rare in children. From January 2000 through July 2005, 15,461 IPD cases were reported as part of national surveillance, of which 14,204 (92%) had isolates available for testing. Fourteen isolates were confirmed to be non-susceptible to FQ. The prevalence of non-susceptible isolates increased from 0.05% in 2001 to 0.3% in 2005 (p=0.005, chi-squared test for trend). Non-susceptible isolates were obtained from 4 of 9 provinces, from 9 laboratories. Surprisingly, twelve (86%) cases occurred in children less than 15 years of age.

Molecular epidemiology of South African serotype 3 pneumococci

Pneumococcal serotype 3 is an important cause of invasive pneumococcal disease (IPD) worldwide. This study investigated the genetic clonality of South African pneumococcal serotype 3 isolates. We analyzed 107 (35%) of isolates randomly selected from a total of 303 serotype 3 cases reported from Gauteng Province as part of the surveillance of IPD. The data suggest that the isolates from Gauteng are relatively less clonal compared to isolates from the USA. In addition, the majority of the isolates from Gauteng were classified as ST-458, a clone that is not common in the USA or other parts of the world.

Molecular epidemiology of multidrug-resistant pneumococcal serotype 19A isolates from South Africa

Cases from June 1999 to December 2004 were reviewed to determine if the original multidrug-resistant serotypes 19A clone that was isolated in 1977 is currently circulating among recently isolated multidrug-resistant serotype 19A strains from South Africa,. During the study period 14,177 cases of IPD were reported. Of these, 13,064 (92%) had viable isolates available for further testing and 885 (7%) were identified as serotype 19A. Reduced susceptibility to penicillin was detected in 235 (27%) of these isolates, 25 (11%) of which were multidrug-resistant. PFGE analysis demonstrated diversity among multidrug-resistant isolates. MLST of 15 randomly selected isolates revealed several sequence types (ST), none of which were the same ST as the original 19A clone. Recent South African multidrug-resistant serotype 19A strains appear to be clonally diverse and are not related the original 1977 multidrug-resistant serotype 19A strain.

Staff of the Respiratory and Meningeal Pathogens Research Unit working in the laboratory.

Telithromycin resistance mechanisms in the pneumococcus

Macrolides are commonly used for the treatment of pneumococcal infection. However, due to the global increase in macrolide resistance alternatives are being developed. Telithromycin, a semi-synthetic derivative of erythromycin, is the first ketolide to be approved for clinical use. The mechanism of resistance in a rare highly telithromycin-resistant isolate was investigated and this work has been accepted for publication (Nicole Wolter, Anthony M. Smith, Donald E. Low, and Keith P. Klugman High-level telithromycin resistance in a clinical isolate of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. published ahead of print on 8 January 2007, doi:10.1128/AAC.01153-06).

Clarithromycin alone and in combination with ceftriaxone inhibits the production of pneumolysin by both macrolide-susceptible and resistant strains of *Streptococcus pneumoniae*

The primary objective of this study was to investigate the effects of clarithromycin (0.01-0.5 mg/L) alone or in combination with ceftriaxone (0.1 and 0.25 mg/L) on pneumolysin production by both macrolide-susceptible and resistant (2 *erm*(B) positive and 2 *mef*(A) positive) strains of *Streptococcus pneumoniae. The results have been accepted for publication (*Anderson R, Steel HC, Cockeran R, Smith AM, von Gottberg A, de Gouveia L, et al. Clarithromycin alone and in combination with ceftriaxone inhibits the production of pneumolysin by both macrolide-susceptible and macrolide-resistant strains of *Streptococcus pneumoniae*. J Antimicrob Chemother Advance Access published on January 11, 2007. doi:10.1093/jac/dkl479).

Risk factors for mortality in patients with invasive pneumococcal disease in South Africa

This study describes the risk factors for mortality in the population of laboratory-confirmed invasive pneumococcal disease patients of all age groups in South Africa. We compared risk factors for mortality in this population in relation to antibiotic susceptibility to penicillin, vaccine-serotype disease, HIV-seropositivity, and meningitis compared with other invasive disease. The study period was 1 January 2003 through 31 December 2005, and data were collected from eight of the nine provinces. This work will be submitted as part of an MSc (Med) in Epidemiology & Biostatistics thesis.

ORAL AND POSTER PRESENTATIONS (NATIONAL)

- **de Gouveia L, Mpembe R, von Gottberg A**, Quan V, Prentice E**, Klugman KP**, **for the** Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). **Surveillance of invasive *Streptococcus pneumoniae*,** *Haemophilus influenzae* and *Neisseria meningitidis* disease from 2003-2005 in South Africa. Poster presentation, Programme Book, 3rd Public Health Conference, May 15-17, 2006, Midrand, South Africa.
- Govender N, Quan V, Prentice E, **von Gottberg A**, Keddy K, and McCarthy K for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). A national South African Surveillance Network for bacterial and fungal diseases. Poster presentation, Programme Book, 3rd Public Health Conference, May 15-17, 2006, Midrand, South Africa.
- **N. Wolter,** AM Smith, DJ Farrell and KP Klugman Telithromycin resistance in a clinical isolate of *Streptococcus pneumoniae* conferred by a deletion in the promoter region of *erm*(B). Oral presentation, WITS Health Sciences Research Day, Aug 23, 2006, Johannesburg, South Africa.
- C. Feldman, R. Anderson, HC Steel, R. Cockeran, **A. von Gottberg, L. de Gouveia,** A. Smith**,** A. Brink, TJ Mitchell and **KP Klugman**. Pneumococcal pneumolysin production is inhibited by clarithromycin, both in the presence and absence of ceftriaxone. Oral presentation, WITS Health Sciences Research Day, August 23, 2006, Johannesburg, South Africa.*
- **K Mothibeli, L. McGee, M. du Plessis, A. von Gottberg,** AM Smith, B. Beall and **KP Klugman**. Molecular epidemiology of invasive pneumococcal serotype 3 isolates from Gauteng province and comparison with serotype 3 strains from United States of America (poster presentation). Poster presentation**,** WITS Health Sciences Research Day, Aug 23, 2006, Johannesburg, South Africa.#
- **M. du Plessis, A. von Gottberg**, C. Cohen, L**. de Gouveia, G. Coulson and KP Klugman** for GERMS-SA. Penicillin non-susceptibility in meningococcal isolates causing invasive disease in South Africa. Poster presentation, WITS Health Sciences Research Day, Aug 23, 2006, Johannesburg, South Africa.
- **Linda de Gouveia, Anne von Gottberg, Ruth Mpembe, Olga Hattingh**, **and Keith Klugman**, for GERMS-SA. Bacterial meningitis in children younger than 5 years in South Africa in 2005. Society of Medical Laboratory Technologists of South Africa (SMLTSA) 1st e'Goli Branch Mini Congress, 5 November 2006, University of Johannesburg, Auckland Park, Johannesburg, South Africa.

  *Prize awarded for the best oral presentation in: HIV and Infectious Diseases Category

  #Prize awarded for the best poster presentation in: HIV and Infectious Diseases Category

ORAL AND POSTER PRESENTATIONS (INTERNATIONAL)

- **Prentice E, von Gottberg A**, Cohen C, **de Gouveia L, Coulson G, Klugman KP**. Emergence of W135 Meningococcal Disease in South Africa. Oral presentation 58, Program and Abstracts Book, International Conference on Emerging Infectious Diseases, March 19-22, 2006, Atlanta, Georgia, USA.
- **Wolter N, Smith AM**, Farrell, DJ, and **Klugman KP**. Telithromycin resistance in a clinical isolates of *Streptococcus pneumoniae* conferred by a deletion in the promoter region of *erm*(B). Oral presentation SY5.02, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.
- **von Gottberg A, Smith AM, de Gouveia L**, Whitelaw A, Hoffman R, **Klugman KP** for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Emergence of fluoroquinolone resistant invasive pneumococcal infections among children, South Africa, 2000-2005. Oral presentation SY13.04, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.
- Quan V, **von Gottberg A, de Gouveia L**, **Klugman KP** for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). History of prior antibiotic use before acute hospital admission for invasive pneumococcal disease in South Africa in 2003 and 2004. Poster presentation PO13.04, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.
- Madhi SA, Cutland CL, Kuwanda L, **de Gouveia L, von Gottberg A, and Klugman KP**. Probing the association between serotype-specific pneumococcal nasopharyngeal colonization and efficacy of the pneumococcal conjugate vaccine against pneumonia. Poster presentation PO7.09, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.
- Adrian PV, van Niekerk N, Jones S, Cutland CL, **von Gottberg A, de Gouveia L, Klugman KP**, Madhi SA. Long-term effects of a 9-valent penumococcal conjugate vaccine (PCV) on nasopharyngeal colonization with pneumococcal serotypes included in the vaccine. Poster presentation PO10.16, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.
- Eastham V, van Niekerk N, Little T, **von Gottberg A, de Gouveia L,** Adrian PV, and Madhi SA. A new method for serotyping pneumococcal strains using

quantitative immunoabsorption as measured by ELISA. PODT.32, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.

- Feldman C, Brink A, **von Gottberg A, de Gouveia L**, Perovic O, and **Klugman KP.** Antimicrobial susceptibility of pneumococcal isolates causing bacteraemic community-acquired pneumonia in the public and private sectors in Gauteng, South Africa. Oral presentation O228, Abstracts and Posters, 16th European Congress of Clinical Microbiology and Infectious Disease, April 1-4, 2006, Nice, France.
- Perovic O, **von Gottberg A**, and Feldman C. Invasive pneumococcal disease in the era of human immunodeficiency virus infection. Oral presentation O221, Abstracts and Posters, 16th European Congress of Clinical Microbiology and Infectious Disease, April 1-4, 2006, Nice, France.
- **du Plessis M, von Gottberg A,** Cohen C, **de Gouveia L, Klugman KP**, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Penicillin non-susceptibility in meningococcal isolates causing invasive disease in South Africa. Poster presentation (13.034), International Journal of Infectious Diseases (volume 10, supp. 1): 12th International Congress on Infectious Diseases (ICID) abstracts; Lisbon, Portugal, June 15-18, 2006.
- Dini LA, **du Plessis M**, Wong ML, Karstaedt A, Fernandez V, Frean JA. High prevalence of DHPS polymorphisms associated with sulfa resistance in South African *Pneumocystis jirovecii* strains. Platform presentation at IX International Workshop on Opportunistic Protists (IWOP-9), Lisbon Portugal, June 2006.
- Charles Feldman, **Anne von Gottberg**, Willem Sturm, Alan Karstaedt, Nelesh Govender, Andrew Whitelaw, **Linda de Gouveia**, **Keith Klugman** for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Nosocomial Invasive Pneumococcal Disease (IPD). Poster presentation, in abstracts of the American College of Chest Physicians (ACCP) 72nd Annual Medical Conference October 21-26, 2006, Salt Lake City, UT, USA.*

*This poster was awarded a "top ten" poster award at the Congress.

INVITED LECTURES, MEETINGS

- Dr Anne von Gottberg attended the EPI/CDC/EPR Quarterly Meeting, 17 February, Roodepoort Civic Centre, Florida Park, and gave a presentation on "Meningococcal Disease in Gauteng, 2005".
- Drs Anne von Gottberg and Elizabeth Prentice attended the National Outbreak Response Team (NORT) meeting, 29 March, Hallmark Building, Pretoria, and gave a presentation on "Meningococcal Disease in Gauteng, 2005".
- Linda de Gouveia was an invited lecturer for the South African Field and Laboratory Program, Short Course, Combined Epidemiology and Laboratory Track, 24 May, NICD, and presented on "Meningococcal Disease in South Africa".
- Dr Anne von Gottberg was a World Health Organization (WHO) temporary adviser at a meeting entitled "Expert Consultative and Planning Meeting to Develop Sub-Regional Reference Laboratory and Surveillance for *Streptococcus pneumoniae* and *Haemophilus influenzae* in the Western African Region", Multi-Disease Surveillance Centre (MDSC), Ouagadougou, Burkina Faso, 23-24 May.
- Dr Anne von Gottberg was an invited lecturer for the Epidemic Preparedness and Response (EPR) Management Workshop, at the Phumelela Conference Facility, Turffontein, Johannesburg, 31 May 2006, and presented on "Meningitis and Meningococcal Disease".
- Linda de Gouveia and Dr Anne von Gottberg participated in a 2-day WHO Consultative meeting to review the External Quality Assessment (EQA) Programme in Africa, NICD, Johannesburg, South Africa, 12-13 June.

NATIONAL MICROBIOLOGY SURVEILLANCE UNIT

STAFF

Nelesh Govender, MBBCh DTM&H Dip HIV Man (SA) FCPath (SA) Micro, Pathologist, Head of Unit
Vanessa Quan, MBBCh DTM&H Dip in Child Health (SA), Medical Officer
Susan Meiring, MBChB, Medical Officer
Elizabeth Prentice, BA MBBCh DTM&H, Medical Officer (until 31 August 2006)
Joy Appolis, Dip General Nursing Intersection of Clinical Care and Research, Surveillance officer
Busisiwe Mbatha, Dip Midwifery Dip Clinical Nursing, Surveillance Officer
Dorothy Hlatshwayo, Dip General Nursing Dip Midwifery Dip Paediatric Nursing Science, Surveillance Officer
Frances Seboya, Dip General Nursing, Surveillance Officer
Molly Morapeli, Dip Comprehensive Nursing, Surveillance Officer
Anna Motsi, Dip General Nursing Dip Midwifery Dip Psychiatric Nursing BA (Hon) Nursing Science, Surveillance Officer
Zodwa Kgaphola, Dip Gen Nursing Dip Midwifery, Surveillance Officer
Pinkie Masuku, Dip Midwifery Dip Psychiatric Nursing, Surveillance Officer
Mumsy Masuku, Dip General Nursing & Midwifery Dip Community Health Dip Psychiatric Nursing, Surveillance Officer
Khasiane Mawasha, Dip Nursing Dip Biomedical Technology, Surveillance Officer
Rebecca Merementsi, Dip General Nursing Dip Midwifery Dip Occupational Health Nursing, Surveillance Officer
Cecilia Miller, Dip General Nursing Dip Midwifery Dip Paediatrics/Child Sciences, Surveillance Officer
Lorato Moapese, Dip General Nursing, Midwifery & Psychiatry Dip Infection Control Nursing, Surveillance Officer
Maria Mokwena, Dip General Nursing & Midwifery, Dip Community Nursing Science, Surveillance Officer
Sylvia Nkomo , Dip in General Nursing & Midwifery, Surveillance Officer
Nazila Shalabi , Dip General Nursing, Surveillance Officer
Khuthaza Mazibuko, Dip General Nursing Dip Professional Nursing, Surveillance Officer
Thandi Mhlanga, Dip General Nursing Dip in Midwifery Dip Psychiatry, Surveillance Officer
Nokuthula Nzuza, Dip General Nursing Dip Midwifery Dip Community Nursing, Surveillance Officer
Weziwe Ngqovu, Dip General Nursing, Surveillance Officer (until 30 April 2006)
Gugu Moyo, Dip Secretarial Studies, Secretary
Muzi Hlanzi, Computer Technician Tertiary Certificate, Data Capture Clerk
Thembi Mthembu, Introductory Business Certificate, Data Capture Clerk
Pedwell Makutu, Dip Electronic Engineering, Data Capture Clerk
Portia Mogale, Senior (Matriculation) Certificate, Data Capture Clerk
Ethel Maringa, Dip Development Studies BA Business Economics BA (Hon) Social Science, Data Capture Clerk
Mashudu Rampilo, BSc, Data Capture Clerk (until 30 April 2006)

Front row (L-R): Vanessa Quan, Gugu Moyo, Busi Mbatha, Nokuthula Nzuza, Rebecca Merementsi, Thandi Mhlanga, Ethel Maringa, Mumsy Msuku, Portia Mogale
Middle row (L-R): Nazila Shalabi, Zodwa Kgaphola, Sylvia Nkomo, Pinkie Masuku, Cecilia Miller, Maria Mokwena, Khuthaza Mazibuko, Anna Motsi, Khasiane Mawasha, Thembi Mthembu
Back row (L-R): Nelesh Govender, Susan Meiring, Molly Morapeli, Joy Apolis, Pedwell Makutu, Lorato Mapese, Frances Seboya, Muzi Hlanzi

INTRODUCTION

The National Microbiology Surveillance Unit (NMSU) was created within the Microbiology Division of the NICD in 2006. Medical officers, surveillance officers and data capture clerks involved in GERMS-SA surveillance activities were transferred to (or hold joint appointment with) this Unit. Nelesh Govender was appointed in March 2006 to manage the Unit.

The NMSU aims to contribute to the control of bacterial and fungal diseases, determined to be of public health importance in South Africa, through the development and coordination of appropriate surveillance systems which provide strategic information for public health action.

Objectives

- To prioritise, develop, coordinate and direct surveillance projects within the Microbiology Division of the National Institute for Communicable Diseases, e.g. GERMS-SA programme.
- To provide laboratory/ technical support for new surveillance projects, where such support does not exist currently.
- To undertake and coordinate collaborative research studies to assist in the understanding of research questions arising from surveillance projects.

SURVEILLANCE (GERMS-SA)

NMSU directs, coordinates and administers the GERMS-SA programme in close collaboration with the Enteric Diseases Reference Unit (EDRU), Mycology Reference Unit (MRU), Parasitology Reference Unit (PRU) and Respiratory and Meningeal Pathogens Reference Unit (RMPRU). A full description of the activities of the GERMS-SA programme in 2006 is contained within the 'GERMS-SA Annual Report 2006'.

Operational surveillance sites

Approximately 120 laboratories actively participated in the surveillance programme in 2006, by submitting isolates along with basic demographic data. Fifteen enhanced surveillance sites were in operation by year end:

- Eastern Cape (1): Nelson Mandela Academic/ Mthatha Provincial hospital complex
- Free State (1): Universitas/ Pelonomi hospital complex
- Gauteng (4): Chris Hani Baragwanath hospital; Johannesburg General hospital; Dr George Mukhari hospital; Pretoria Academic hospital
- KwaZulu Natal (3): King Edward VIII/ Inkosi Albert Luthuli hospital complex; Addington/ RK Khan hospital complex; Prince Mshiyeni Memorial hospital
- Limpopo (1): Polokwane/ Mankweng hospital complex
- Mpumalanga (1): Rob Ferreira/ Themba/ Barberton hospital complex
- Northern Cape (1): Kimberley hospital
- North West (1): Rustenburg Provincial hospital
- Western Cape (2): Groote Schuur/ Red Cross hospital complex; Tygerberg/ Karl Bremer hospital complex

Surveillance site visits and laboratory training activities

- Western Cape (NMSU/ MRU): 19-20 January 2006, Groote Schuur hospital/ NHLS laboratory, Greenpoint NHLS laboratory, Tygerberg hospital/ NHLS laboratory and Cape Town Pathcare laboratory.
- Eastern Cape (NMSU/ MRU/ EQA): 4-6 April 2006, Nelson Mandela Academic/ Mthatha Provincial/ NHLS Mthatha laboratory. This visit included a 3 day laboratory training workshop which focused on antimicrobial susceptibility testing (AST) of fastidious pathogens, identification of bacterial pathogens causing meningitis and quality control procedures for AST in line with CLSI recommended standards.
- Northern Cape (NMSU): 28 March 2006, Kimberley hospital/ NHLS laboratory.
- KwaZulu Natal (NMSU/ RMPRU): 31 May 2 June 2006, King Edward VIII, Addington, Prince Mshiyeni Memorial, RK Khan laboratories and hospitals.
- Gauteng (NMSU): 12 May 2006, Dr George Mukhari hospital/ NHLS laboratory.
- Gauteng (NMSU/ RMPRU): 1 June 2006, Leratong hospital/ NHLS laboratory.
- Gauteng (NMSU): 14 June 2006, Chris Hani Baragwanath hospital/ NHLS laboratory.
- Mpumalanga (NMSU/ PRU): 28-30 June 2006, Rob Ferreira, Barberton and Themba hospitals/ NHLS laboratories. This visit included a 2 day training workshop focused on the introduction of PCP surveillance - provision of start up consumables and training workshop for PCP diagnostics.
- North West (NMSU): 12 July 2006, Rustenberg Provincial hospital/ NHLS laboratory.
- North West (NMSU): 31 July 2006, Leslie Williams Memorial hospital/ laboratory, Carletonville.
- North West (NMSU/ MRU): 7 August 2006, Duff Scott and Westvaal hospitals
- Limpopo (NMSU/ RMPRU): 16-18 August 2006, Tshilidzini, Mankweng and Polokwane hospitals/ NHLS laboratories. This visit included laboratory training lectures dealing with identification/AST of fastidious/ difficult bacterial pathogens.
- Gauteng (NMSU): 4 September 2006, Pretoria Academic hospital/ NHLS laboratory.
- Gauteng (NMSU/ EDRU): 18 October 2006, Johannesburg General hospital/ NHLS laboratory.
- Gauteng (NMSU/ PRU): 19 October 2006, Chris Hani Baragwanath hospital/ NHLS laboratory.
- Free State (NMSU/ EDRU): 23 November 2006, Universitas and Pelonomi hospitals/ NHLS laboratories
- Northern Cape (NMSU/ EDRU): 24 November 2006, Kimberley hospital/ NHLS laboratory. This visit included a laboratory demonstration of diarrhoeal bacterial pathogen serotyping.

Major surveillance programme meetings

Principal Investigators' Meeting, 6-7 November 2006

The 2 day PI meeting, convened at the NICD in Johannesburg, was attended by over 100 local, national and international delegates and representatives from the Department of Health. Representatives of African surveillance networks included Dr Anthony Scott (Wellcome Trust Career Development Fellow in Tropical Medicine, Wellcome Trust/ KEMRI Collaborative Programme, Kilifi, Kenya), Mr Tura Galgalo (Head, Microbiology Reference Laboratory, National Public Health Laboratory Services (NPHLS), Kenya) and Mr Luis Morais (Training Fellow in Microbiology, Manhiça Health Research Centre, Mozambique). Surveillance and research achievements emanating from the GERMS-SA programme were presented by NICD workers and site coordinators. Sessions dedicated to presentation of proposals for new projects as well as novel methods of analysis for existing GERMS-SA data allowed the group to decide on priorities for the upcoming year. Public health advocacy has been identified as a key function of the GERMS-SA programme. An open discussion session enabled the group to identify practical measures to communicate strategic information to those who need to know, e.g. Department of Health.

Surveillance Officer Meetings

7-10 March 2006

The meeting, convened at the NICD in Johannesburg, was attended by 11 surveillance officers from 7 provinces and NICD coordinators.

5-6 November 2006

The meeting, convened at the NICD in Johannesburg, was attended by 18 surveillance officers from 8 provinces, NICD coordinators and Dr Chris Van Beneden and Dr Beth Arthington-Skaggs (CDC, USA).

Surveillance publications

Annual report for 2005 - compiled and distributed in February 2006

Quarterly cumulative statistics by pathogen, laboratory and province

- Q4 (1 January - 31 December 2005) compiled and distributed to all participating laboratories in February 2006
- Q1 (1 January - 31 March 2006) complied and distributed in May 2006
- Q2 (1 January - 30 June 2006) compiled and distributed in August 2006
- Q3 (1 January - 30 September 2006) compiled and distributed in November 2006

Quarterly surveillance network newsletter

- Link volume 13 (December 2005) compiled and distributed to participating laboratories and collaborators in February 2006

GERMS-SA PI meeting November 2006

Back rows L-R: Sr Zodwa Kgaphola, Dr Alan Karstaedt, Sr Molly Morapeli, Ms Jenny Rossouw, Dr Rugola Mtandu, Mr Peter Nyasulu, Dr Victor Fernandes, Dr Anthony Smith, Dr Suzy Budavari, Dr Karen Keddy, Mr Danie Cilliers, Dr Bernice Harris, Ms Carrin Martin, Dr Andre Moller, Sr Nazila Shalabi, Dr Pyu-Pyu Sein, Dr Rena Hoffman, Dr Beth Arthington Skaggs, Sr Cecilia Miller, Dr Andrew Whitelaw, Sr Pinkie Masuku, Ms Boile Mdluli, Mr Tura Galgalo, Sr Lorato Moapese, Dr Linda Meyer, Dr Tom Clark, Ms Kathy Lindeque, Dr Natalie Beylis, Ms Greta Hoyland, Dr Prathna Bhola, Ms Erna du Plessis, Dr Okey Nwanyanwu , Sr Khasi Mawasha, Dr Anne-Marie Pretorius

Front row standing L-R: Sr Thandi Mhlanga, Sr Rebecca Merementsi, Dr Cheryl Cohen, Ms Joy Mnyaluza, Dr Pieter Jooste, Ms Thoko Zulu, Ms Mimmy Ngomane, Sr Kedibone Seboya, Dr Olga Perovic, Mr Luis Morais, Dr Jeannette Wadula, Mr Mani Khoosal, Sr Joy Appolis, Sr Khuthaza Mazibuko, Dr Sharona Seetharam, Sr Sylvia Nkomo

Seated L-R: Ms Jay Patel, Ms Susan Gould, Sr Mumsy Masuku, Dr Vanessa Quan, Sr Nokuthula Nzuza, Dr Mignon du Plessis, Sr Joy McAnerney, Ms Kedibone Mothibeli, Ms Nicole Wolter, Ms Flora Mnyameni, Ms Muendi Phagadi, Ms Asiashu Sitsula, Sr Maria Mokwena, Ms Rita van Deventer, Dr Sandeep Vasaikar, Dr Anthony Scott

Front row seated L-R: Ms Leigh Dini, Dr Susan Meiring, Dr Anne von Gottberg, Dr Maria Botha, Prof John Frean, Dr Chika Somugha, Sr Busi Mbatha, Dr Kerrigan McCarthy, Dr Nelesh Govender, Dr Lucille Blumberg, Dr Claire Heney

- Link volume 14 (April 2006) compiled and distributed in May 2006
- Link volume 15 (July 2006) compiled and distributed in August 2006
- Link volume 16 (December 2006) compiled and distributed in November 2006

Presentation of GERMS-SA surveillance data

- Emergence of W135 Meningococcal Disease in South Africa. **Prentice E**, von Gottberg A, Cohen C, de Gouveia L, Coulson G, Klugman KP. Oral presentation 58, Program and Abstracts Book, International Conference on Emerging Infectious Diseases, March 19-22, 2006, Atlanta, Georgia, USA.
- History of prior antibiotic use before acute hospital admission for invasive pneumococcal disease in South Africa in 2003 and 2004. **Quan V**, von Gottberg A, de Gouveia L, Klugman KP for GERMS-SA. Poster presentation PO13.04, Program and Abstract Book, 5th International Symposium on Pneumococci and Pneumococcal Diseases, April 2-6, 2006, Alice Springs, Central Australia.
- Surveillance of invasive *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* disease from 2003-2005 in South Africa. de Gouveia L, Mpembe R, von Gottberg A, **Quan V, Prentice E,** Klugman KP, for GERMS-SA**.** Poster presentation, Programme and Abstracts Book, Third Public Health Conference, May 15-17, 2006, Midrand, South Africa.
- GERMS-SA: A national South African surveillance network for bacterial and fungal diseases. **Govender N, Quan V, Prentice** E, von Gottberg A, Keddy K, McCarthy K for GERMS-SA. Poster presentation (Poster session P8), Programme and Abstracts Book, Third Public Health Conference, May 15-17, 2006, Midrand, South Africa.
- GERMS-SA: A national South African surveillance network for bacterial and fungal diseases. **Govender N, Quan V, Prentice E**, von Gottberg A, Keddy K and McCarthy K for GERMS-SA. Communicable Diseases Surveillance Bulletin. May 2006. National Institute for Communicable Diseases, of the National Health Laboratory Service. Johannesburg. South Africa.
- GERMS-SA: an update of recent findings. **Govender N**. Oral Presentation, Active Bacterial Core surveillance (ABCs) Surveillance Officer meeting, 12-13 June 2006, Centers for Disease Control and Prevention, Atlanta, USA.
- Nosocomial invasive pneumococcal disease in South Africa. Feldman C, von Gottberg A, Sturm W, Karstaedt AS, **Govender N**, Whitelaw A, de Gouveia L for GERMS-SA. Poster presentation, CHEST 2006, Salt Lake City, Utah, USA, 21-26 October 2006.*
- Extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* in South Africa, 2005. **Govender N,** et al for GERMS-SA. Oral Presentation, National Institute for Communicable Diseases Academic Day, Sandringham, 27-28 November 2006.
- Establishing a GERMS-SA Isolate Bank. **Meiring S, Govender N, Quan V,** von Gottberg A for GERMS-SA. Poster presentation, National Institute for Communicable Diseases Academic Day, Sandringham, 27-28 November 2006.

*This poster was awarded a "top ten" poster award at the Congress.

ONGOING COLLABORATIVE RESEARCH WORK

- Differences in blood culturing practices in rural and urban areas of South Africa (RMPRU)
- Nasopharyngeal carriage study for fluoroquinolone resistant *Streptococcus pneumoniae* in children (RMPRU)
- Validation of genes affecting susceptibility to invasive pneumococcal disease and/or other infectious diseases (RMPRU)

TRAINING ACTIVITIES

Staff registered for higher degrees/ diplomas

- Nelesh Govender, MMed (Micro), Wits
- Elizabeth Prentice, Dip International Research Ethics, UCT

Training courses

- Ethics course (IRENSA), Centre for Bioethics, University of Cape Town (towards a Diploma in International Research Ethics) E. Prentice
- Microsoft Access, 21-22 August 2006 - N. Govender, S. Meiring
- Good Clinical Practice
  - *9-10 March 2006 D. Hlatshwayo, N. Nzuza, K. Mazibuko, N. Shalabi, M. Mokwena, C. Miller, R. Merementsi, M. Masuku, A. Motsi, K. Seboya, B. Mbatha, P. Mogale, E. Maringa and M. Hlanzi
  - *7 June 2006 J. Appolis
  - *31 October 2006 P. Masuku, G. Moyo, T. Mthembu and P. Makutu.

Involvement in SA-FELTP Activities

- Nelesh Govender was invited to lecture at the South African Field Epidemiology and Laboratory Training Program (SA-FELTP) Short Course, Combined Epidemiology and Laboratory Track, 15 May, NICD, on 'Surveillance in South Africa'
- NMSU assisted in the facilitation of the laboratory practical sessions for the laboratory track of the South African Field Epidemiology and Laboratory Training Program Short Course, 15-24 May 2006

SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE

STAFF LIST

A/Professor David Lewis MBBS, FRCP(UK), DTM&H, BA, MSc, PhD, Head of Department
Aulette Goliath, Secretary
Cadwill Pillay BSc (Hons), Research Manager

Laboratory Team:
Dr Inge Zietsman, MBBCh FRCPath (SA) (Micro), Pathologist
Frans Radebe NH Dip Med Tech (Virology) MSc (Med), Laboratory Manager
Etienne Muller BMedSci BSc (Hons) MSc (Med Virology) PhD, Senior Medical Scientist
Lindy Scott Med Tech (Micro), Chief Medical Technologist
Precious Magooa BSc (Hons) (Micro), Medical Scientist
Solomon Mkhonto BSc (Hons) (Micro), Medical Scientist
John Ratabane BSc (Hons) (Micro), Medical Scientist
Marie Slabbert Nat Dip (Micro), Medical Technologist
Sydney Khumalo, Research Assistant
David Mabaso, Research Assistant
Rodgers Chonco, Laboratory Cleaner

Clinical Surveillance and Data Entry Team:
Mireille Cheyip BSc, MSc (Med), Senior Medical Scientist
Stephina Tshelane NP (EH), B Tech (EH), Surveillance Officer
Nthabiseng Khanyile, Data input clerk
Caswell Mavimbela, Data input clerk
Daphne Madondo, Data input clerk
Joseph Masekwameng, Data input clerk
Lena Kwenane, Data Input clerk

Clinical Team:
Obed Mohlamonyane RN, Specialist Nurse
Zanele Jele RN, Nurse Research Coordinator
Duduzile Ntuli RN, Nurse Research Coordinator
David Pitsoane RN, Nurse Research Coordinator
Violet Chiloane RN, Nurse Research Coordinator
Joyce Lethoba RN, Nurse Research Coordinator
Zodwa Mzaidume RN, Nurse Research Coordinator
Thoko Nhlapo RN, Research Nurse
Faith Nyati RN, Research Nurse
Anita van Zijl RN, Research Nurse
Iris Atlee RN, Research Nurse
Thando Mosiro RN, Research Nurse
Gadifele Khasu RN, Research Nurse
Maureen Manuel EN, Research Nurse
Veronica Nosi, HIV VCT Counsellor
Xolani Siyasi, HIV VCT Counsellor
Priscilla Ncameni HIV VCT Counsellor
Lydia Masimola, HIV VCT Counsellor
Alex Vezi, HIV VCT Counsellor
Sipho Mbabela, HIV VCT Counsellor
Sana Mabogwane, HIV VCT Counsellor
Elizabeth Zwane, HIV VCT Counsellor

INTRODUCTION

2006 was a year of substantial progress for the STI Reference Centre, with the successful completion of three major projects and an increase in PEPFAR funding for on-going research projects addressing public health concerns in the field of sexual health. The STI Reference Centre presented papers at international conferences and developed collaborative partnerships with local, national and international colleagues. The Centre also trained a number of personnel throughout the year, including data handlers and programme managers in the national surveillance programme, microbiology registrars, medical and dental students, laboratory and nursing staff both within and outside South Africa. Professor Lewis attended three WHO meetings as a technical advisor during 2006 to discuss rapid STI tests and STI syndromic management incorporation into medical and nursing student curricula in Africa. This annual report contains some of the key achievements and data obtained by the Centre during 2006.

CLINICAL SURVEILLANCE FOR STIs

The STI Reference Centre continued to support the South African National Department of Health (NDoH) with the national STI clinical surveillance programme, funded through the NICD:CDC co-operative agreement. The final draft of the national clinical surveillance report (April 2004 - March 2005) was completed and sent to the NDoH in July 2006 for approval and further dissemination. In the latter half of 2006, staff further developed and fine-tuned the tools for monitoring and evaluation of this programme, following a series of provincial workshops co-ordinated by STI Reference Centre staff.

The Gauteng Clinical STI Surveillance Programme is now in its 12th year and remains highly valued by the Provincial Department of Health in Gauteng. Throughout the year we received data from 21 sentinel sites, produced quarterly reports and an annual report for 2005. This programme remains the only clinical surveillance programme that produces timely and high quality data on STI syndrome caseload in South Africa and prides itself on rapid dissemination of information to the Province. Gauteng Province have re-affirmed their commitment to seeing this surveillance programme continue in the years ahead, certainly until such time as data from the national clinical surveillance programme is reported in the form of timely quarterly and annual reports and demonstrates sustainability akin to the Gauteng surveillance programme.

A pilot SADC STI surveillance programme, funded by the UK Department for International Development through partnership with the Health Systems Trust, covering high transit/cross border sites in Botswana, Namibia, Lesotho and Swaziland was launched in late 2004 and came to an end in September 2006. The final data in relation to establishing enhanced clinical surveillance programmes in the four countries were presented in part by our staff at the SADC conference in Swaziland in September this year. A final report was written at the end of the year and will be submitted to the Health Systems Trust in early 2007.

MICROBIOLOGICAL SURVEILLANCE FOR STIs

The STI Reference Centre continued to co-ordinate the national microbiological surveillance of sexually transmitted infections in South Africa, again supported through PEPFAR funding via the NICD:CDC co-operative agreement. The programme aims to monitor the syndromes of male urethritis, vaginal discharge and genital ulcer disease. The aetiologies of the syndromes, their local or regional epidemiology and antimicrobial susceptibility patterns of *Neisseria gonorrhoeae* are being investigated.

Figure 1. The Salt River Clinic Surveillance team in Cape Town [Right to Left: Ms. Marie Slabbert, Sr. Amila Latif, Sr. Anita van Zijl, Prof. David Lewis]

In the early part of 2006, surveillance was undertaken in the Northern Cape (Kimberley) by the STI Reference Centre. The CDC funds were also used to fund antimicrobial surveillance of gonococci in Kwa-Zulu Natal (Durban) and Mpumalanga with the support of the Microbiology Department, Nelson Mandela School of Medicine, University of KwaZulu Natal who undertook the testing of the specimens. In the last quarter of 2006, the STI Reference Centre employed two staff in the Western Cape and undertook both aetiological and antimicrobial resistance surveillance in Cape Town at Salt River Clinic (see Figure 1). The work was undertaken in collaboration with the Microbiology Department of Tygerburg Hospital, University of Stellenbosch. During 2006, the prevalence of ciprofloxacin resistance gonococci continued to increase (Figure 2) and a key meeting was held at the NDoH in October 2006 to further raise the issue of the failure of current first-line syndromic management protocols to cover gonococcal infections within the country. The STI Reference Centre successfully negotiated space with colleagues at Alexandra Health Centre to commence testing for both aetiological and antimicrobial resistance surveillance in Gauteng starting in January 2007.

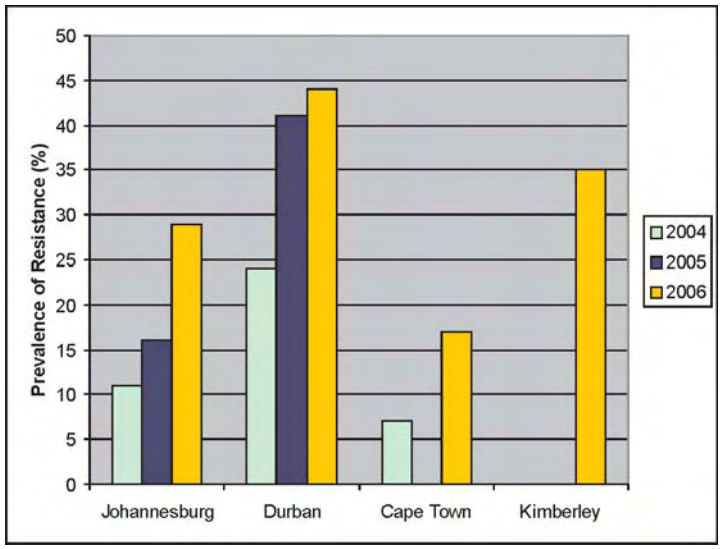

Figure 2. Ciprofloxacin resistance surveillance data for gonococci isolated from Johannesburg, Durban, Cape Town and Kimberley [2004 and 2005 Durban data were presented at the Sun City FIDSSA meeting, July 2005 and the 2006 Durban data were obtained with the financial support of PEPFAR funding administered through the NICD. The STI Reference Centre acknowledge the sharing of these data in NICD communiqués during 2006 by Professors Sturm and Moodley of the Microbiology Department, Nelson Mandela School of Medicine, University of KwaZulu Natal]

During 2006, the STI Reference Centre also assisted the National Departments of Health in Lesotho and Namibia with their national microbiological surveillance initiatives (Figure 3).

Figure 3. Dr Inge Zietsman demonstrating the art of gonococcal culture with laboratory colleagues in Maseru, Lesotho.

STUDY TO ASSESS THE EFFECT OF ACYCLOVIR TREATMENT ON GENITAL ULCER HEALING IN MEN

This randomised placebo-control trial of acyclovir and its effect on GUD duration and HIV shedding, funded through the NICD:CDC co-operative agreement, came to a successful conclusion in December 2006 (Figure 4). The target of 600 participants was reached with a final recruitment number of 612 men. The trial was conducted at Eloff Street clinic, Alexandra Health Centre, Folang Clinic in Pretoria and in the latter few months in the NHLS Braamfontein Occupational Health Department, where patients were referred from Esselen Street clinic in Hillbrow. Special thanks are due to the Reproductive Health and HIV Research Unit who assisted the STI Reference Centre reach the required target by the end of 2006 through enhanced recruitment of men with genital ulcers at the Esselen Street site. We would also like to thank our colleagues in Regions 7 and 8 within Johannesburg City Health, and within Tswane Metro Health Department, for assisting us with the successful completion of this study. The CDC Principal investigator, Dr Gabriela Paz-Bailey, and the CDC Study Statistician are due to return to the NICD in early 2007 to commence the final analysis of the data. This is a key study regarding genital herpes management and the global STI community await the results with interest.

HIV VCT AND STI SCREENING STUDIES IN CARLETONVILLE

The 3 year expanded periodic presumptive therapy (PPT) study ended in February 2006. Following the preparations undertaken at the end of 2005, two new USAID-funded studies assessing the acceptability of HIV voluntary counselling and testing (VCT) and STI screening in both women at high risk (WAHR) and men living in the Carletonville area were successfully undertaken and completed. The HIV VCT counselling was provided by trained counsellors working in tents placed adjacent to the mobile clinic vans (Figures 5 and 6). The STI Reference Centre employed the first VCT counsellors in the NHLS for this project. The STI Reference Centre teamed up with the Mothusimpilo Project to perform this research and benefited from the close working relationship built up over a number of years between the WAHR and the mobile van nurses (Figure 7).

Figure 4. Episodic Herpes Therapy Study Team [Right to Left: Zanele Jele, Sana Mabogwane, David Lewis (NICD PI), Violet Chiloane, Precious Magooa, Alex Vezi, Lindy Scott, David Pitsoane, Frans Radebe, Sipho Mbabela, Myron Wettrich (CDC-Pretoria), Dudu Ntuli, Aulette Goliath, Tiny Zwane, Gabriela Paz-Bailey (CDC PI)]

Figure 5. Mothusimpilo Project Mobile Clinic for STI screening

Figure 6. Mock demonstration of HIV VCT being performed in tents erected next to the mobile clinics

At the end of 8 months, utilising three mobile vans for four days a week, a total of 1361 WAHR participated in the study; 1285 (94%) underwent screening for STIs and 1137 (84%) took an HIV test. Of the 1137 accepting VCT, 663 (58%) tested HIV antibody positive. In a similar manner, a smaller three month project using all male staff and just one mobile van for 4 days a week managed to enrol 309 men, of whom 303 (98%) agreed to STI screening and 262 (85%) agreed to take an HIV test (28% were HIV antibody positive). Those participants that were HIV seropositive had same day blood tests performed for subsequent CD4 count and HIV viral load and they were referred to local ARV/wellness sites with their results. The STI Reference Centre regrets the decision of USAID to discontinue funding STIRC's planned expansion of such activities to the local community, especially given the success of the project and the hard work of the NICD staff.

Figure 7. Carletonville VCT Study Team [Right to Left: Lydia Masimola, Frans Radebe, Priscilla Ncameni, Thando Mosiro, Sipho Mbabela, Alex Vezi, Veronica Nosi, Inge Zietsman, Xolani Siyasi, Zodwa Mzaidume, Gadifele Khasu, Cadwill Pillay, Iris Attlee]

THE STI REFERENCE CENTRE'S CLINIC FOR MEN IN ALEXANDRA

During 2005, the STI Reference Centre established, in collaboration with Region E, a specialist men's 'drop-in' STI service within 8th Avenue Primary Health Care clinic. This venture would not have been possible without the support and encouragement of Region 7's Regional Health Manager (Ms Ncumisa Mehana) and her team (Gloria Keetse and Zanele Mophosho) and the staff of 8th Avenue clinic, in particular Sr. Wendy Twalo and Sr. Reynolda Kgatuke. This clinic has continued to be popular among men in Alexandra and has enabled the STI Reference Centre and the NICD to make an important contribution to the health of our local community as well as providing a clinical research and surveillance site. A poster describing key findings from the first year's clinic activities was presented at an international meeting by Mr. Obed Mohlamonyane, the STI Reference Centre nurse responsible for service provision.

Figure 8. Mr Obed Mohlamonyane discussing the men's clinic with members of Alexandra's community

In February 2006, the STI Reference Centre hosted a well-attended open day at the NICD for members of the Alexandra community, including students, teachers, social workers, nurses, social workers, press officers and NGO volunteers (Figure 8). STIs and their complications were discussed in the morning along with presentations on the various projects on-going at the STI Reference Centre. The participants were given a tour of the laboratories in the afternoon and particularly enjoyed the opportunity to look at gonococci down the microscope and to see RPR testing in action.

REGIONAL DIRECTORSHIP FOR AFRICA, INTERNATIONAL UNION AGAINST STIs (IUSTI)

Professor Lewis was appointed Regional Director of the African Branch of IUSTI at the start of 2006. The African Region has been relatively inactive for a number of years and this opportunity for developing an STI network across Africa dovetails nicely with the strategic objectives of the NICD. Mrs Aulette Goliath from the Centre supported the work of IUSTI at the October 2006 IUSTI-Europe meeting, where she staffed the first IUSTI membership booth (Figure 9) and attended the IUSTI world EXCO meeting as an observer.

Figure 9. Mrs Aulette Goliath welcomes Dr Pierre Yassa (Zambia) to membership of IUSTI-Africa

CONFERENCES AND PRESENTATIONS

Mpumalanga Provincial Department of Health STI Seminar, Nelspruit, South Africa 13 February 2006
Lewis DA. STI syndromic management**.** (Oral)

3rd Public health Conference of the Pubic Health Association of Southern Africa, Johannesburg, South Africa 15-17 May 2006
Cheyip M. A pilot sentinel STI surveillance system for Botswana, Namibia, Lesotho and Swaziland. (Poster)

British Association of Sexual Health and HIV Spring Meeting, Nottingham, UK 17-19 May 2006 Lewis DA. Lessons from South Africa. (oral)

14th Congress of the International Union against STIs - Asia Pacific Branch, Kuala Lumpur, Malaysia 27-30 July 2006
Lewis DA. The changing tide of STIs in South Africa. (oral)
Lewis DA. Double Trouble: STI-HIV interactions. (oral)

3rd APTIMA Users Meeting, Versailles, France 17-18 October 2006
Magooa P. Comparison of the Aptima Combo 2 and Real Time PCR for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens (oral)

22nd Congress of the International Union against STIs - European Branch, Versailles, France 19-21 October 2006
Lewis DA. Controlling the HIV epidemic in South Africa: future challenges (oral)
Lewis DA. Does periodic presumptive therapy with azithromycin affect the prevalence of STIs and HIV in high risk women? (oral)
Cheyip M. Clinical surveillance of STIs in three population groups in a mining community in South Africa. (poster)
Pillay C. Introduction of voluntary counselling and testing to existing PPT services in the Carletonville mining area of South Africa (poster)
Mohlamonyane O. Unayo i-drop na? Establishment of a specialist men's sexual health clinic in a South African Township. (poster)

National institute for Communicable Diseases' 1st Academic Meeting, Johannesburg, South Africa 27-28 November 2006
Lewis DA. Does periodic presumptive therapy with azithromycin affect the prevalence of STIs and HIV in high risk women? (oral)
Cheyip M. A pilot sentinel STI surveillance system for Botswana, Namibia, Lesotho and Swaziland. (oral)
Pillay C. Introduction of voluntary counselling and testing to existing PPT services in the Carletonville mining area of South Africa (poster)
Mohlamonyane O. Unayo i-drop na? Establishment of a specialist men's sexual health clinic in a South African Township. (poster)
Tshelane S. The Gauteng STI sentinel surveillance programme, South Africa: 2000-2005 (poster)

VECTOR CONTROL REFERENCE UNIT

STAFF

Prof M Coetzee, MSc, PhD, FRES, Head of Unit, Research Professor, School of Pathology of NHLS & Wits University
M Martheze, Secretary
H Saevitzon, Librarian
Dr LL Koekemoer, BSc Hons, PhD, Medical Scientist, Senior Researcher, School of Pathology of NHLS & Wits University
Dr BD Brooke, BSc Hons, PhD, Medical Scientist, Researcher, School of Pathology of NHLS & Wits University
R Naguran, BSc Hons, MSc, Medical Scientist
S Oliver, BSc Hons, Medical Scientist
Z Zulu, Laboratory Assistant
Z Mnisi, Laboratory Assistant
Prof RH Hunt, MSc, PhD, FRES, Honorary Professor, School of Animal, Plant & Environmental Sciences, Wits University

INTRODUCTION

Malaria is the major vector-borne disease in Africa, killing over 1 million people annually, most of them children under five. In South Africa, malaria transmission is confined to the low-lying border areas in the northeast of the country where 7,754 cases were reported in 2005. The Vector Control Reference Unit (VCRU) focuses mainly 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 us with a unique resource for research into insecticide resistance in this important malaria vector. This places the VCRU in a unique position to collaborate 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 collection in the world.

RESEARCH

INSECTICIDE RESISTANCE

Anopheles funestus

Molecular research into pyrethroid resistance in *Anopheles funestus* continues to be a major focus of the VCRU. Metabolic mechanisms involved in the resistance are P450 monooxygenase enzymes and we have demonstrated that a specific CYP6 gene is responsible for pyrethroid resistance in *An. funestus.* Current research using micro-array analysis is being undertaken, in collaboration with colleagues at the Liverpool School of Tropical Medicine, UK, to further investigate this gene and its specific functions.

The DDT and carbamate resistance detected in *An. funestus* from Ghana and Mozambique respectively, are being investigated and strains resistant to each insecticide are being selected.

Anopheles gambiae

Dieldrin resistance in this species has been detected in Ghana populations at unprecedented levels of 100% survival on standard WHO susceptibility tests. The genetic mechanisms responsible for this are being investigated and a laboratory colony is currently under selection for resistance to this insecticide. While dieldrin is no longer in use, either in agriculture or public health, other insecticides in the same broad class of cyclodienes are or may be considered for malaria control. It is important, therefore, that the mechanisms conferring resistance to these insecticides are clearly understood so that informed decisions can be made by malaria vector control programmes.

Anopheles arabiensis

Investigations carried out in collaboration with the Kwazulu/Natal malaria control programme entomologist revealed the presence of DDT resistance in *Anopheles arabiensis*. Research into the resistance mechanisms resulted in the establishment of a DDT-resistant colony. Selection experiments have provided insights into why a major malaria epidemic, similar to that of 2000 caused by pyrethroid resistant *An. funestus,* has not occurred in the area.

The DDT resistance in *An. arabiensis* detected in the Gokwe District of north-central Zimbabwe, is being further investigated under a MIM (Multilateral Initiative for Malaria) grant awarded to Dr Koekemoer of the VCRU in collaboration with Dr Masendu in Zimbabwe.

The "knockdown" mutation (*kdr*) found in the sodium channel gene that confers resistance to pyrethroids in West African *An. gambiae*, has been detected in wild populations of *An. arabiensis* from central Sudan. No clear association between pyrethroid and DDT resistance has been demonstrated but gene frequencies have increased with use of agricultural insecticides. It is

likely that at least one additional metabolic resistance mechanism is involved here.

NOVEL MOSQUITO CONTROL METHODS

Collaboration has been established with colleagues in the Netherlands, UK and Australia to investigate the effect of entomopathogenic fungi on insecticide resistant vector colonies housed at the VCRU.

INTERNATIONAL RESEARCH COLLABORATORS

Prof J Hemingway, Director, Liverpool School of Tropical Medicine, UK
Dr H Ranson, Imperial College, UK
Prof W Takken, University of Wageningen, Netherlands
Dr B Knols, University of Wageningen, Netherlands
Prof D Norris, Johns Hopkins University, USA
Dr T S Awolola, Nigerian Institute of Medical Research, Lagos, Nigeria
Dr H T Masendu, University of Zimbabwe, Harare, Zimbabwe

RESEARCH FUNDING FROM EXTERNAL GRANTING AGENCIES

National Institutes for Health
World Health Organization
UBS Optimus Foundation
SA Medical Research Council
SA National Research Foundation
Wits University Carnegie Foundation

TRAINING

Postgraduate Training

Masters and Doctoral students from all over Africa are trained, many with the support of the World Health Organization and other donor agencies.

DIAGNOSTIC AND OTHER SERVICES

The VCRU provides an identification service 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 Ghana, Guinea Conakry, Mali, Zambia, Botswana and South Africa, for species identification and to detect the presence of *Plasmodium falciparum* sporozoites.

Advice and expertise is provided to the Department of Health both at the national and provincial levels, with participation on the National Malaria Advisory Group.

CONFERENCES

- **Abdalla H, Koekemoer, LL**, Mnzava, AEP, **Hunt RH & Coetzee M** 2006. *Anopheles arabiensis* from Sudan: insecticide resistance and the *kdr* mutation. NICD Academic Day, 27-28 November, Johannesburg

Figure 1: Searching for malaria mosquito larvae in a typical breeding site of *Anopheles gambiae*

- **Amenya DA, Koekemoer LL,** Ranson H, Vaughan A, **Brooke B, Hunt RH, Mafumo H**, Hemingway J & **Coetzee M** 2006. Role of monoxygenases in insecticide resistant *Anopheles funestus* (Diptera: Culicidae). NICD Academic Day, 27-28 November, Johannesburg
- **Brooke BD** 2006. Malaria vector control in South Africa. "I Can Erase Malaria" Wisdom Council Meeting, CIEVRA, Benin. Hosted by Foundation Espace Afrique, Malaria Foundation International & I Can Foundation.
- **Koekemoer LL, Mouatcho JC, Hunt, RH & Coetzee M** 2006. Detection of false positive sporozoites of *Plasmodium falciparum* associated with animal blood in *Anopheles parensis* by ELISA. 27th African Health Sciences Conference, 3-7 December, Durban
- **Koekemoer LL** 2006. CYP6 overexpression in pyrethroid resistant *Anopheles funestus* (Diptera: Culicidae). Health Sciences Research Day, University of the Witwatersrand, 23 August, Johannesburg
- **Oliver SV**, Waite, T.D., Inceoglu, A.B., Cornel, A.J. & **Coetzee,M** 2006. Biochemical analysis of metabolic resistance mechanisms in African malaria vectors. Molecular and Cell Biology Group Symposium, 5th October, Johannesburg
- **Oliver SV** 2006. Biochemical analysis of resistance mechanisms in the African malaria vectors, *Anopheles arabiensis* and *An. funestus*. NICD Academic Day, 27-28 November, Johannesburg

- **Matambo TS, Koekemoer LL & Coetzee M** 2006. Characterization of cytochrome P450 (CYP6P9) from insecticide resistant malaria vector *Anopheles funestus.* Molecular and Cell Biology Group Symposium, 5th October, Johannesburg
- **Mouatcho J** 2006. Resistance in *Anopheles arabiensis* in South Africa. 27th African Health Sciences Conference, 3-7 December, Durban
- **Mouatcho J, Koekemoer LL, Brooke BD,** Mthembu J, Hargreaves K, **Hunt RH & Coetzee M** 2006. Insecticide resistance in the South African malaria vector *Anopheles arabiensis* in Kwazulu/Natal. Health Sciences Research Day, University of the Witwatersrand, 23 August, Johannesburg
- **Mouatcho JC, Koekemoer LL, Hunt RH & Coetzee M** 2006. Detection of false positive sporozoites of *Plasmodium falciparum* associated with animal blood in *Anopheles parensis* by ELISA. Molecular and Cell Biology Group Symposium, 5th October. Johannesburg
- ***Naguran R, Koekemoer LL,** Ranson H & **Coetzee M** 2006. Microarray analysis of gene expression in the major malaria vectors (*Anopheles funestus* and *An. arabiensis*) resistant to insecticides. Molecular and Cell Biology Group Symposium, 5th October. Johannesburg
- **Naguran R** 2006. The use of microarray analysis of gene expression to study insecticide resistance in the major malaria vectors *Anopheles funestus* and *An. arabiensis*. NICD Academic Day, 27-28 November, Johannesburg
- **Okoye PN, Brooke BD, Hunt RH & Coetzee M** 2006. Relative developmental and reproductive fitness associated with pyrethroid resistance in the major southern African malaria vector Anopheles funestus. NICD Academic Day, 27-28 November, Johannesburg

*Awarded the prize for the best poster at this symposium.

Figure 2: Staff and students of the VCRU: From left: S Vezenegho (Research Assistant), T Matambo (PhD), R Naguran (medical scientist & PhD), G Munenga (MSc Zimbabwe University), P Okoye (PhD), M Lo (PhD), J Mouatcho (PhD), H Abdalla (MSc), S Oliver (medical scientist), Dr. L Koekemoer (Head Molecular Lab) and C Kikanke (MSc). Absent: Profs M Coetzee and R Hunt, Dr. E Misiani (post-doctoral fellow), Dr. B Brooke (senior medical scientist), B Spillings (PhD) and M Booman (PhD)..

EPIDEMIOLOGY DIVISION

STAFF

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Dr LH Blumberg MBBCh MMed (Micro) DTM&H DCH DOH, Specialist Microbiologist
Dr GM de Jong MBBCh DTM&H FC Path (Micro), Specialist Microbiologist

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Dr C Cohen MBBCh DTM&H FC Path (SA) Micro MSc (Epidemiology), Specialist Microbiologist/ Epidemiologist
Sr JM McAnerney RN RM Dip Data Dip Method, Nurse Epidemiologist
Dr BN Harris* MBChB Mmed (Community Health), Community Health Specialist
Dr I Weber** MBChB DTM&H, Registrar in Community Health, University of Pretoria
*Left the unit as appointed FELTP Director, March 2006
**On secondment from University of Pretoria, January-July 2006

SA-FELTP
Dr BN Harris MBChB MMed (Community Health), Community Health Specialist, Director of SA-FELTP
Dr E Prentice BA MBBCh DTM&H, Senior Medical Officer
BK Sartorius BSc (Hons) (Micro) MSc (Epidemiology & Biostatistics) EPIET Fellow
B Temane, Secretary

L Millington, Publications Officer/Administration

OUTBREAK UNIT

OVERVIEW

In 2006 the Outbreak Unit continued to provide support for outbreak response, technical expertise for the diagnosis and management of communicable diseases and training of health care workers and laboratory personnel. The unit works in collaboration with the provincial and national Departments of Health to ensure a comprehensive outbreak response and the development of systems for early outbreak detection and improved reporting. In addition, close partnerships with the NHLS diagnostic laboratories and reference units of the NICD aims to deliver appropriate laboratory services during outbreaks and specialized diagnostics services as required.

The Outbreak Unit remains a member of the National Outbreak Response Team (NORT) and continued in 2006 to assist the NORT with development of national guidelines for priority communicable diseases, outbreak response and training.

KEY OUTBREAKS 2006

The unit has worked in partnership with the provincial Communicable Disease Control directorates and NICD reference units in responding to several key outbreaks. The role of the unit varies depending on the nature of the outbreak and may include:

- Outbreak detection and reporting
- Field investigation as required
- Development of clinical and laboratory guidelines
- Interpretation of test results
- Recommendations for control

Rabies
An outbreak in dogs in Limpopo province preceded the recognition of a marked increase in human rabies cases in early 2006. Limpopo had not reported a human rabies case since 1988. From 1 August to 31 December 2006 a total of 21 cases of human rabies were confirmed and an additional 4 probable and 2 possible cases were identified. Drs Blumberg, Cohen and Sartorius conducted field visits to the Vhembe district in partnership with Limpopo Communicable Disease Control to review case files and provide training to health care workers at hospitals and clinics on appropriate post exposure treatment for rabies.

Crimean Congo Haemorrhagic fever (CCHF)
The Outbreak unit continued to work in close collaboration with the Special Pathogens Unit in screening and investigating suspected cases of viral haemorrhagic fever in South Africa.

Investigation of a cluster of CCHF cases in Gauteng

Cases of CCHF in South Africa are largely sporadic with secondary infections an unusual occurrence. However the occurrence of 2 linked cases of CCHF in Gauteng 2006 prompted a field investigation to identify epidemiological links and identify the source of infection.

The index case was a bird breeder which was followed by a secondary case (within 7 days) in a medical technologist who worked in the laboratory in which the index case samples were processed. Although no clear evidence of laboratory transmission could be documented, this was the most likely route of transmission.

Food- borne outbreaks
Several food-borne outbreaks were reported in 2006. Many of these are linked to consumption of carcasses found dead and consumed in poor communities. An outbreak of *Salmonella* spp. food poisoning occurred in December 2006 in Mpumalanga province following consumption of meals prepared at a school kitchen. Twenty five cases were identified with watery diarrhoea, abdominal cramps and low-grade fever. Of these, 92% (n=24) tested positive for *Salmonella* spp. Food samples were obtained but were negative on testing. Improved capacity for investigation of food-borne outbreak will be highlighted in 2007 to ensure adequate epidemiological and microbiological investigations are conducted.

Waterborne disease outbreaks
In 2006 several waterborne disease outbreaks occurred in South Africa.

In the Northern Cape, a large outbreak of diarrhoea was linked to contamination of municipal water. From September 23rd to December 13th a total of 95 cases had been reported with no deaths. Sixteen patients (16.8%) presented with bloody diarrhoea, 6 of whom were confirmed to have *Shigella flexneri* infection on culture of stool samples. The remaining cases reported vomiting and/or watery diarrhoea. Affected patients have a common municipal water supply that has evidence of faecal contamination on laboratory testing.

The Eastern Cape reported an increase in cases of typhoid fever linked to the consumption of faecally contaminated water in a community without piped water systems. Following investigation it was identified that the perceived increase in cases was likely due to improved typhoid diagnostic testing and improved mechanisms of reporting rather than a true outbreak. However the outbreak investigation identified an unacceptably high level of endemic typhoid fever cases in the affected community and prompted mobilization of services to the area.

Common to these waterborne outbreaks is the failure to deliver safe potable water and sanitation particular in rural communities. In addition there remain challenges in ensuring collection and prompt transport of clinical specimens for microbiological analysis.

Institutional outbreaks
The unit provided support for several institutional outbreaks in 2006. These included:

Diarrhoeal disease in a children's home in the Northern Cape

Meningococcal disease in a primary school in Limpopo province: This outbreak was identified in July 2006 with the confirmation of a death in a child due to probable meningococcal meningitis. Further investigation identified an additional 4 cases from the same school (age range: 4-13 years) from June 13th to 29th. All had signs and symptoms consistent with meningococcal disease but only 1 case was laboratory confirmed. Control measures included post exposure prophylaxis for close contacts (if within 10 days) and use of quadrivalent meningococcal vaccine in the exposed school groups.

Three outbreaks of primary varicella zoster: Two of these occurred in hospices for HIV/AIDS patients and one in a long-term psychiatric facility. Control measures included use of varicella immungobulin, isolation and early treatment aimed at reducing morbidity and mortality in these high risk groups.

Anthrax
Two outbreaks of anthrax in animals were reported in 2006. The first was reported in October in Mpumalanga province with several exposures (n=70) in humans through direct contact with carcasses as well as ingestion of infected meat. Exposed individuals received short course prophylaxis with amoxicillin and no illness was reported during the surveillance period.

The second anthrax outbreak occurred in sheep and goats in the Northern Cape in December 2006. One probable human case of cutaneous anthrax was identified and 19 additional individuals who ingested infected meat were given antibiotic prophylaxis.

Hepatitis A
An outbreak of hepatitis A was identified in Mpumalanga province with at least 16 cases, 6 of whom were laboratory confirmed. The cases were linked to 6 families and transmission was likely due to person to person spread in households and at least one instance of food contamination at a household.

Determining burden of hepatitis A and detection of outbreaks is a challenge in South Africa and systems for improved detection and reporting are in development.

International outbreaks
The outbreak unit provides alerts for South Africans, assists with ensuring laboratory preparedness and provides support where required in response to international outbreaks. In 2006 this included information and alerts regarding the following outbreaks on our borders:
- Cholera in Zimbabwe, Angola and Namibia
- Outbreak of polio in Namibia

- Human cases of avian influenza H5N1 and alerts regarding African countries reporting disease in poultry

TRAINING AND CAPACITY BUILDING

Epidemic Preparedness and Response (EPR) training

In 2006 the unit assisted the national and provincial Departments of Health in training provincial public health personnel and doctors in EPR with an emphasis on case management and appropriate laboratory diagnostic tests. These training events took place in several provinces including Northern Cape, Limpopo, Free State and Eastern Cape.

Communicable disease training in Springbok, Northern Cape Province

"Case of the Month" series

This is a laboratory capacity building activity that has been distributed on a quarterly basis to all NHLS laboratories in South Africa 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. Over 300 staff regularly participated in this activity in 2006 for which they earn professional development credits.

Short course on Outbreak Investigation and Response

In collaboration with the SA-FELTP, the unit developed a curriculum and conducted a 9 day initial training program for laboratory staff in Outbreak investigation and response.

FELTP short course 15-22 May 2006

This was followed by a supervised field project and follow up course in October. Key laboratory personnel elected as "OutNet" (see "OutNet" program) representatives were trained together with the provincial communicable disease coordinators (or equivalent) from each province.

External lectures

The unit continues to lecture on communicable diseases as requested to external groups.

Avian influenza (AI) and pandemic influenza preparedness

The unit continues to play a role in national AI and pandemic influenza preparedness. In 2006 activities included:

- collaboration with national DOH in the development of avian influenza case management guidelines and finalization of the national and provincial pandemic preparedness plans
- development of screening criteria for imported AI and distribution of these to key facilities and public health personnel
- distribution of regular AI situation reports to key health personnel
- Screening of suspected imported AI cases and liaison regarding decision making for laboratory testing conducted by the Influenza Reference laboratory at NICD.

"OutNet" program

This program is a laboratory based Outbreak Network for SA which was developed in 2005 and piloted in 2006 with the election and training (in collaboration with the SA-FELTP) of 9 provincial laboratory "OutNet" representatives. These individuals now act as the key points of contact for provincial public health staff and facilitate the role of the laboratory for detection and response to outbreaks in collaboration with the Outbreak unit at NICD.

Laboratory training in outbreak response

OUTBREAK DIVISION PUBLICATIONS

Communiqué
The unit continued to issue a monthly communiqué in 2006 on key communicable disease events in South Africa.

National Guidelines
- Control of meningococcal disease in South Africa - in collaboration with RMPRU and national DoH
- Case Management Guidelines for avian influenza in collaboration with national DoH
- Pandemic influenza preparedness in collaboration with national DoH
- Control of hepatitis A in South Africa

EPIDEMIOLOGY AND SURVEILLANCE UNIT

OVERVIEW

The Epidemiology and Surveillance unit works closely with the National and Provincial Department of Health in the control of measles and polio, liaising between NICD and Department of Health particularly the Expanded Programme on Immunisation (EPI) programme. The unit co-ordinates the "viral watch" system for respiratory virus surveillance. The unit also provides epidemiology support to other units within the NICD through collaborative research projects, analysis and interpretation of epidemiologic data, field visits, teaching and training activities, representation at national EPI meetings and publication of the quarterly Communicable Diseases Surveillance Bulletin.

SURVEILLANCE PROGRAMMES

Suspected measles case-based surveillance
The NICD is accredited by WHO to perform measles and rubella IgM testing for national case-based surveillance. Blood and urine specimens from suspected measles cases nationally are submitted to NICD for confirmation. Approximately 60% of suspected measles cases from Free State Province are tested in that province. The numbers presented here represent specimens received by the NICD and may differ from those presented by the National Department of Health as they may receive information on cases where no specimens were taken.

During 2006 the NICD tested 6620 blood specimens from cases of rash and fever for suspected measles case-based surveillance. The largest number, 1545 (23.3%), were from Gauteng, followed by 1219 (18.4%) from KwaZulu-Natal. Of these specimens 86 (1.3%) were positive for measles IgM antibodies, and 2948 (44.53%) for rubella IgM antibodies.

Measles
The 86 positive measles results were from 82 patients, the majority (29) of whom were from the North West Province (NWP), followed by 24 from Gauteng (Figure 1). Ages of patients with positive measles results ranged from 4 months to 41 years (median 5 years).

In November 2006 Dr Cohen participated in an investigation into a measles outbreak in NWP together

Figure 1. Geographic distribution of measles IgM positive cases confirmed at NICD, South Africa , 2006

section for details.)

RESPIRATORY VIRUS SURVEILLANCE

Viral Watch Surveillance Programme
During 2006 a total of 1449 specimens were received for detection of respiratory virus. Of these 1247 (86.1%) were received from the Viral Watch programme, started in 1984 and expanded substantially in 2005, designed to monitor timing of influenza activity and determine prevalent influenza strains. During 2006 the programme was rolled out in the Eastern Cape, Western Cape, and KwaZulu-Natal, adding a further 42 practitioners. Throat swabs are submitted from these centres throughout the year from patients with respiratory tract infections of recent onset

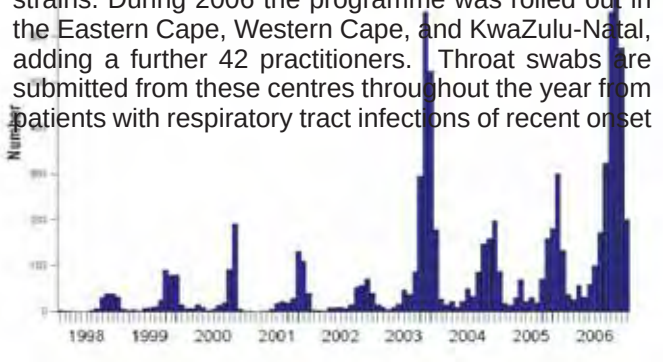

Figure 2: Number of rubella IgM positive cases confirmed at NICD by month, 1998-2006, South Africa

i.e. within 48 - 72 hours, and without obvious bacterial cause, and transported to the laboratory in viral transport medium for isolation of virus. Specimens from Gauteng and the Eastern Cape are submitted directly to NICD, whereas specimens from the Western Cape and KwaZulu-Natal are tested at the respective laboratories and positive specimens sent to NICD for confirmation, serotyping and sequencing.

The first influenza isolate of the season was made from a specimen collected on 27 March, and the last from a specimen collected on 25 October (Figure 5). A total of 554 influenza isolates were made, of which 540 (97.5%) were from the Viral Watch. The isolates were further

identified as 496 influenza A, of which A H3N2 (A/Wisconsin/67/05-like) accounted for the majority, and 58 influenza B, mainly B/Malaysia/2506/04-like.

A further 47 respiratory isolates were made during the year including 23 respiratory syncytial virus, 13 parainfluenza virus (3 type 1, 4 type 2, 5 type 3, 1 untyped), and 2 adenovirus.

Respiratory admissions data mining surveillance system

In 2006 a new surveillance system was established aiming to determine trends in hospital admissions for respiratory illness and examine the association between timing of admissions for respiratory and other

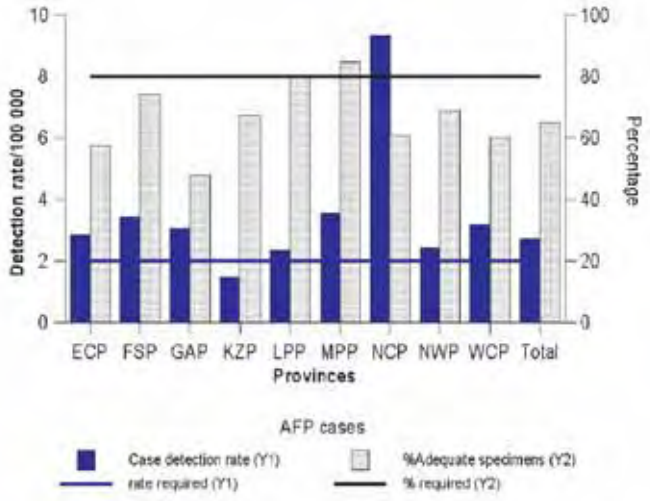

Figure 3. AFP case detection and stool adequacy rate, South Africa, 2006 (only patients from whom specimens were received included)

diagnoses of interest and influenza isolations as determined through the viral watch surveillance system. The system utilizes data on numbers of respiratory and other admissions (classified according to the ICD-10 coding system) extracted from a national private hospital database. The timing of the peaks of admissions for pneumonia, acute upper respiratory infection, influenza and acute lower respiratory infection corresponded with peaks in influenza virus isolations in 2005 and 2006 (Figure 6).

CURRENT RESEARCH PROJECTS

South African age-stratified rubella serosurvey, 2005/2006

A cross sectional age-stratified rubella seroprevalence survey was undertaken to ascertain the susceptibility to rubella and estimate the incidence of congenital rubella syndrome (CRS) in South Africa. 9 545 residual laboratory specimens were collected from January 2005 to March 2006 from all provinces of South Africa. 87.7% (7 916) of the 9 027 specimens with known age

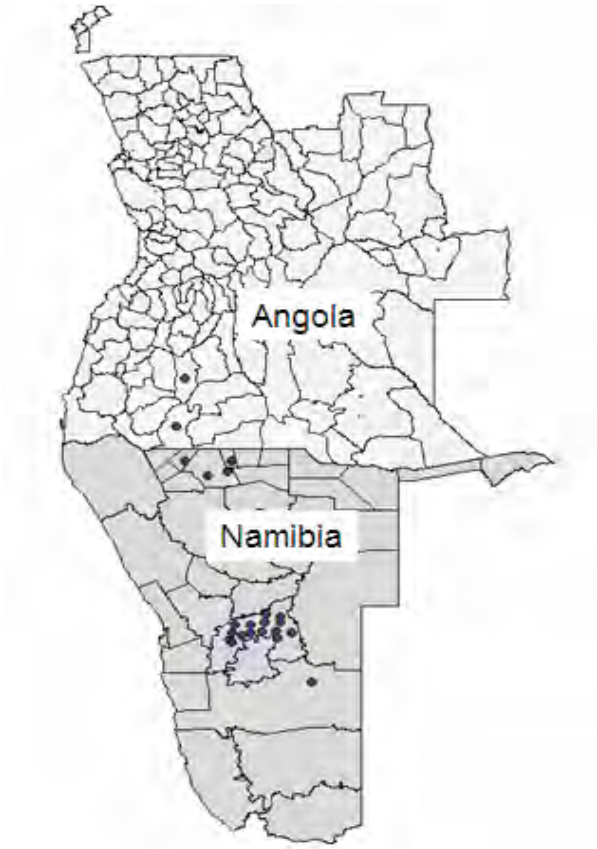

Figure 4. Distribution of laboratory-confirmed wild type polio cases by district, Angola and Namibia 2006

tested rubella IgG positive and prevalence increased with increasing age between the ages of 1 and 15 years (Figure 7). The incidence of CRS in South Africa in 2005 was estimated as 69 per 100 000 live births (95% confidence interval 58-81).

Gauteng Malaria Surveillance

Surveillance for malaria cases diagnosed and treated in Gauteng Province between the first of December 2005 and the 30th of November 2006 was conducted in association with the Gauteng Department of Health and the Amayeza Information Centre. Demographic and clinical data on cases was submitted. Between 1 December 2005 and 30 November 2006 the NICD received case reports on 1 704 patients diagnosed with malaria from public (1551 reports) and private sector (153 reports) hospitals. A total of 37 deaths were reported during the study period.

18 months measles vaccination

The cross-sectional survey commenced in Ekurhuleni District in 2006. One hundred and forty caregivers were interviewed and only two of the children presenting for their eighteen-month measles vaccination had no indication of a prior nine-month measles vaccination. Furthermore 14% (n=20) of the children sampled received their nine-month vaccination late and 5% (n=8) were late for their 18-month measles vaccination. Data collection for the rest of Gauteng as well as Limpopo and Mpumalanga provinces will be performed in 2007.

GROUP FOR ENTERIC RESPIRATORY AND MENINGEAL PATHOGENS-SOUTH AFRICA

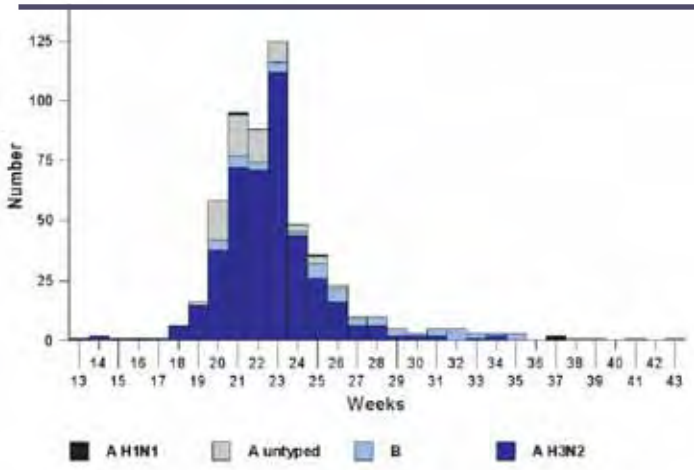

Figure 5. Number of influenza isolates by virus type and epidemiologic week 2006

A further 47 respiratory isolates were made during the year including 23 respiratory syncytial virus, 13 parainfluenza virus (3 type 1, 4 type 2, 5 type 3, 1 untyped), and 2 adenovirus.

Respiratory admissions data mining surveillance system

In 2006 a new surveillance system was established aiming to determine trends in hospital admissions for respiratory illness and examine the association between timing of admissions for respiratory and other diagnoses of interest and influenza isolations as determined through the viral watch surveillance system. The system utilizes data on numbers of respiratory and other admissions (classified according to the ICD-10 coding system) extracted from a national private hospital database. The timing of the peaks of admissions for pneumonia, acute upper respiratory infection, influenza and acute lower respiratory infection corresponded with peaks in influenza virus isolations in 2005 and 2006 (Figure 6).

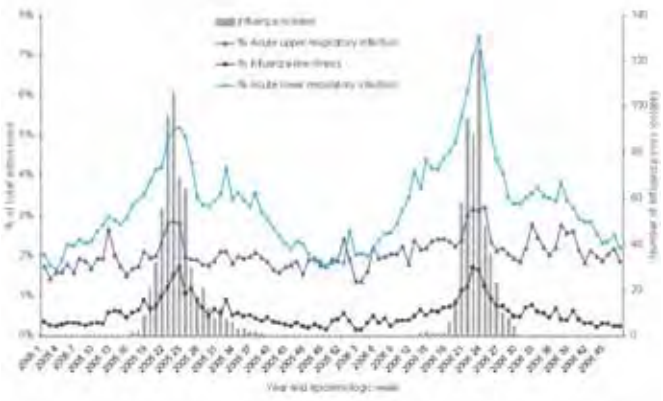

Figure 6. Percentage of admissions due to respiratory diagnoses of interest and influenza isolations by epidemiologic week, 2005-2006

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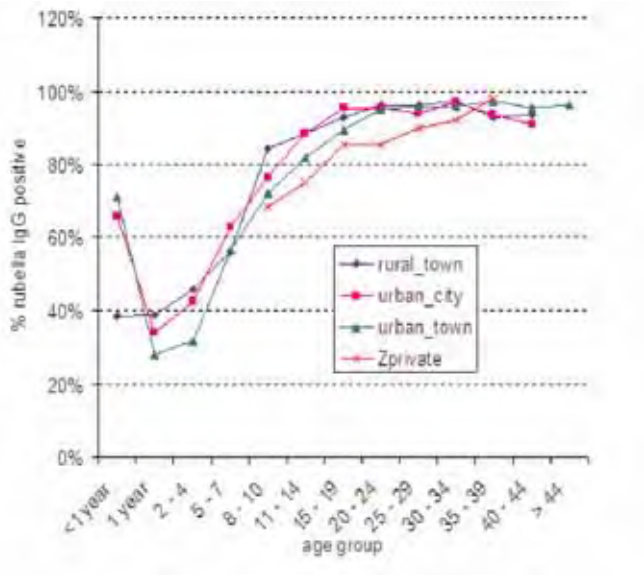

Figure 7. Percentage of specimens seropositive for Rubella IgG by age group and location or type of laboratory from which specimen originates

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services. Additional stand alone short courses were also hosted by the programme, the first of which was held in May 2006 on Outbreak Investigation and Response.

The 2-year MPH accredited programme will commence in January 2007. Fellows will participate in several core modules at the University of Pretoria and NICD and work under a supervisor for the remainder of the two (2) years at a field placement site in their province or in another province should they be willing. This programme is designed to train field epidemiology fellows for positions as national and provincial epidemiologists, public health laboratorians, surveillance officers or other relevant positions in the South African public health system upon successful completion of all the requirements of the training programme.

The 2nd SAFELTP Advisory Committee meeting was held on 4 October to give feedback on progress to the committee members and review the way forward. The committee members also sat in on some of the student presentations and were very impressed with the standard of the projects and recommendations.

On the 4th of September, the SAFELTP short listed applicants for the 2 year residency programme and they were interviewed on the 26th of September by a panel consisting of the SAFELTP staff; Dr Gene MacDonald, the Associate Director for Science, Centers for Disease Control and Prevention, Pretoria, South Africa; Dr Chris Tetteh, Director, FELTP, Kenya and Ms Rika du Plessis, Epidemiology & Surveillance, National Department of Health. The standard of the applicants was generally high and included a number of persons with laboratory backgrounds or who are currently employed by the NHLS.

Dr Harris met with Professor Bah, head of the School of Public Health, Medunsa, Dr Stephen Knight of the School of Public Health, University of KwaZulu Natal and Dr Neil Cameron from the University of Stellenbosch (US) who indicated that they would be very willing to collaborate in presenting an MPH in a SAFELTP sub track, enabling students from their areas easier access to the SAFELTP long course.

SHORT COURSES

From 15-24 May 2006, the SAFELTP ran a short course on Outbreak Investigation and Response. The course comprised 2 tracks (epidemiology and laboratory) with several shared sessions. This short course this proved to be highly successful and well received. The course combined teams consisting of provincial communicable disease coordinators and a representative from the national DOH Communicable Disease Control Directorate as well as an experienced NHLS representative from each province to facilitate the development of effective field laboratory-epidemiology teams during outbreaks.

The general feedback from the participants indicated that the course content was highly applicable and that the skills learned or enhanced would very much compliment their work. Each participant had to complete a project prior to a follow up Disease Surveillance course that was held during 3-5 October 2006. At this course, participants made a formal oral presentation of their project which was rated by an independent panel. 4 participants where selected on the basis of their rating scores to attend the Tephinet Conference in Brazil during 12 -17 November 2006.

A supervision and mentoring short course was held to coincide with the outbreak course to work in partnership with identified supervisors on their role in reaching the expectations and outcomes of the students' projects.

A 3 day course for NHLS Business Managers was held in collaboration with NHLS QA Division from 28-30 August. The course programme consisted of a number of interactive sessions presented by both NHLS staff and external contributors, highlighting certain issues critical for Business Managers. At the end of each session, managers were encouraged to highlight serious problems which may impede QMS implementation and these were recorded for follow up. On the final day, the groups were divided into their relevant Branches and tasked with devising a plan for the implementation of an effective QMS within their respective branches.

The response to the course was positive, as borne out by the surveys conducted, as well as encouraging comments by various individual managers. It was also felt by many that the course was a useful forum for the exchange of ideas, and the discussion of common problems encountered by Business Managers from all the NHLS Branches. It was also the first opportunity for the KZN managers to meet their colleagues from the other Branches. The course should provide a firm basis for the subsequent roll out of QMS training for NHLS laboratory managers by the Branches.

CURRICULUM DEVELOPMENT

An epidemiology track curriculum development work-shop for the 2 year field epidemiology course took place from 3-5 April. The curriculum was accepted by the University of Pretoria as a new sub track of the Masters in Public Health degree. Fellows will receive an MPH from UP upon completion of the programme and a fellowship certificate from the NICD/NHLS.

A laboratory curriculum planning workshop was held from 4 - 6 December to develop the laboratory track for the SAFELTP 2 year course, identify the expected outcome competencies of the candidate in the form of a job description to facilitate placement in the NHLS after completing the program and integrate the coursework to bridge the gap between epidemiologists and laboratorians.

During the meeting, the group discussed the following subject areas:

- Curriculum - Identified the key areas of training for

the laboratory component of the SAFELTP
- Career Path drafted an outcomes-based job description for graduates of the laboratory component of the SAFELTP
- Accreditation - Determined how this track will be incorporated and accredited into the University of Pretoria
- Field Implementation Identified the nature and venue of field placements/implementation
- Recruitment - Determined the qualifications, criteria, and capacity for each cohort of laboratory fellows.

The meeting was attended by the NICD FELTP staff, other members of the Epidemiology Division, members of the NICD reference labs involved with lab based surveillance, the South African CDC office, 2 advisors from CDC Atlanta and a member of SANAS.

This workshop was followed by a TB-QMS Short course planning meeting on December the 7th. South African TB experts from the MRC joined the above group to plan a short course to be run by the SA FELTP in 2007. A one week course covering laboratory, epidemiology, and data management issues was proposed and the course content outlined. This course will be aimed at laboratorians from the NHLS and TB control personnel from the Departments of Health and follow the model of the outbreak course of combined sessions and field work. The course will be presented multiple times to optimize learning in smaller groups.

TEACHING AND TRAINING

Dr Bernice Harris coordinated the Diploma in Tropical Medicine and Health of the University of Pretoria

Dr Bernice Harris was coordinator of the Pretoria University School of Health Systems and Public Health (SHSPH) MPH disease control track

Dr Bernice Harris was external examiner for the Wits MSc in Epidemiology "Topics and Seminars" examinations

Dr Bernice Harris was invited as guest lecturer at an Epidemiology Refresher course for the Tswane Metro Health Department presented by Medunsa and the South African Vaccination and Immunisation Centre (SAVIC).

TRAINING COURSES ATTENDED

Mr Benn Sartorius attended the Outbreak Investigation and Response short course development workshop, CDC Atlanta USA from 22 March April 1, 2006. Activities included finalisation of the timetable and synchronisation with the lab track timetable; development of lectures and exercises to complement the content of each lecture and the structure and content of the large diarrhoea/typhoid case study that will run over 5 days of the course.

Dr Gillian de Jong, Dr Cheryl Cohen and Mr Benn Sartorius were members of the South African team: WHO/CDC Rapid Response Training for avian influenza Workshop, Bangkok Thailand July 16th to 20th 2007.

Dr L Blumberg received a Travel Medicine Fellowship to attend the Gorgas Institute Expert Course in Tropical Diseases, 2006, Lima, Peru 14-25 August.

Dr Elizabeth Prentice completed the IRENSA Ethics course, University of Cape Town and attended the last block from 11 to 22 September.

SPECIAL APPOINTMENTS

Dr Lucille Blumberg was lecturer and examiner to the University of the Witwatersrand Certificate course in Travel Medicine.

Dr Lucille Blumberg was appointed as lecturer for under and postgraduates, Department of Microbiology, University of Stellenbosch.

Dr Lucille Blumberg was appointed as a member of the National Rabies Advisory Group.

Dr Lucille Blumberg continued as a member of the National Malaria Advisory Group.

Dr Lucille Blumberg was inducted as a Fellow of the Faculty of Travel Medicine, Royal College of Surgeons and Physicians, Glasgow.

Dr Bernice Harris was nominated to be an associate member of the College of Public Health Medicine of the Colleges of Medicine of South Africa (CMSA).

Dr Cheryl Cohen was appointed as a member of the National EPI Task Force.

Dr Cheryl Cohen was appointed as a member of the National Certification Committee for the Eradication of Polio.

Dr Cheryl Cohen was appointed as a lecturer at the School of Public Health, University of the Witwatersrand.

CONFERENCES ATTENDED

Local

Malaria prophylaxis, **Blumberg L**. Oral presentation. Infectious Diseases for the General Practitioner. Bakubung, NW Province, 4-5 February 2006.

Influenza Surveillance, **McAnerney J**. Oral Presentation. Influenza Symposium, NICD, 7-8 March 2006.

An update on the recent measles outbreak in South Africa and lessons learned**.** Vaccine-preventable

respiratory and meningeal pathogens, **Harris BN** (invited guest speaker), Limpopo 2006 EPI Symposium organised by the South African Vaccination and Immunisation Centre (SAVIC) and the Limpopo Provincial Department of Health, Aventura Spa Warmbaths, 10-11 May 2006.

Public Health Association of South Africa (PHASA) Congress, Midrand, South Africa, 16-18 May 2006.

- Outbreak of Human Rabies Limpopo Province 2005-2006, **Cohen C, Blumberg L, Sartorius B**, Mogoswane M, Sutton C, Toledo M. Oral presentation.
- The burden of Cryptococcosis in Gauteng by subdistrict in 2002-4; Implications for provision of local health care services for AIDS, McCarthy K, **Cohen C**, Schneider H, Gould S for the Gauteng Cryptococcal Surveillance Initiative. Oral presentation.
- Should rubella immunization be introduced in South Africa? Modelling the impact of rubella vaccination. **Harris BN**, Schoub BD. Oral presentation.

Vaccinology 2006 Congress, Hermanus, South Africa 22-25 October 2006.

- Rabies vaccines, **Blumberg L.** Oral presentation.
- Influenza surveillance: Vaccine Implications, **McAnerney J**. Oral Presentation.
- Surveillance of Vaccine Preventable Diseases, **Cohen C**. Oral Presentation.
- Vaccines and outbreak response - a South African perspective. **de Jong GM**. Oral presentation.
- Ethics of Routine Immunisation **Prentice E**. Oral presentation.
- Rubella seroprevalence: Its influence on vaccine strategies, **Harris BN.** Oral presentation.

Linking Surveillance Programmes. **Cohen C.** Group for Enteric Respiratory and Meningeal Pathogens South Africa (GERMS-SA) Principle Investigators Meeting, 7-8 November 2006, NICD, Johannesburg, South Africa.

International

Failed rabies post exposure prophylaxis in two cases in South Africa, **L Blumberg,** J Paweska. SEARG - South East African Rabies Group, Windhoek, Namibia, 22-25 January 2006.

TB and HIV in South Africa- challenges in diagnosis and management, **Blumberg L** (invited speaker). National TB Conference. St Petersburg, Russia 26-27 January 2006.

Penicillin non-susceptibility in meningococcal isolates causing invasive disease in South Africa. du Plessis M, von Gottberg A, **Cohen C,** de Gouveia L, Klugman KP, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Poster presentation (13.034), International Journal of Infectious Diseases (volume 10, supp. 1): 12th International Congress on Infectious Diseases (ICID) abstracts; Lisbon, Portugal, June 15-18, 2006.

Emergence of W135 Meningococcal Disease in South Africa. **Prentice E,** von Gottberg A, **Cohen C,** de Gouveia L, Coulson G, Klugman KP. Oral presentation 58, Program and Abstracts Book, International Conference on Emerging Infectious Diseases, March 19-22, 2006, Atlanta, Georgia, USA.

Dr Gillian de Jong was the South African representative at: Wilton Park Conference: International Planning for Pandemics March 2nd to 5th 2006. England, United Kingdom.

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NICD ACADEMIC DAY PRESENTATIONS, 27-28 NOVEMBER 2006, JOHANNESBURG, SOUTH AFRICA

- Outbreak of Human Rabies Limpopo Province 2005-2006, **Cohen C, Blumberg L, Sartorius B,** Mogoswane M, Sutton C, Toledo M (Oral presentation).
- Influenza associated admissions and deaths 2005 - estimated using a data-mining surveillance system. **Cohen C, McAnerney J, Blumberg L**. Poster Presentation.
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- Active Influenza Surveillance in South Africa. **McAnerney J**, Besselaar T, Buys A, **Blumberg L**. Poster Presentation.
- The burden of disease due to AIDS in Gauteng: inferred evidence from results of surveillance for cryptococcosis 2002-4**.** McCarthy KM, **Cohen C,** Schneider H, Gould SM, Brandt ME and Hajjeh RA for the Gauteng Cryptococcal Surveillance Initiative Group.
- The current and future activities of the SAFELTP. **Harris BN, Prentice E, Sartorius B**. Poster presentation.
- "OutNet" - pilot activities of a laboratory-based outbreak network for South Africa. **de Jong GM,**

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- "OutNet" - pilot activities of a laboratory-based outbreak network for South Africa. **de Jong GM, Blumberg L.**

VIRAL DIAGNOSTICS & SURVEILLANCE

Specimen Receiving Laboratory:
Ezekiel Maselesele, Higher Dip Med Tech, Laboratory Manager
Lorraine Cranston, Higher Dip Med Tech, Medical Technologist
Reginah Mokoena, Clerk (Laboratory)
Lazarus Ngwenya, Clerk (Laboratory)
Agripa Chauke, Clerk (Laboratory)
Scharlene Lee, Clerk (Laboratory)
Emmanuel Tetse, Driver

Liquid Media Laboratory:
Ernest Mthethwa, BSc, Biotechnologist
Ananias Selepe, Laboratory Assistant
Frank Boshomane, Laboratory Assistant

General Laboratory Support Staff:
Joseph Masekwameng, General worker
Elvis Mathibela, General worker
Martha Mathebula, General worker
Marcus Mpyana, General worker
Frans Mashangoane, General worker

Cell Culture Laboratory
Megan Vandecar, Dip Med Tech, Medical Technologist
Abraham Sehata, Laboratory Assistant
Roselina Simelane, Laboratory Assistant

Respiratory and General Isolation Laboratory
Amelia Buys, Dip Med Tech, Medical Technologist
Nathi Ndlovu, Dip Med Tech, Medical Technologist
Cardia Esterhuyse, Dip Med Tech, Medical Technologist
Lynn Harvey, Laboratory Assistant
Teresa Mashaba, Laboratory Assistant

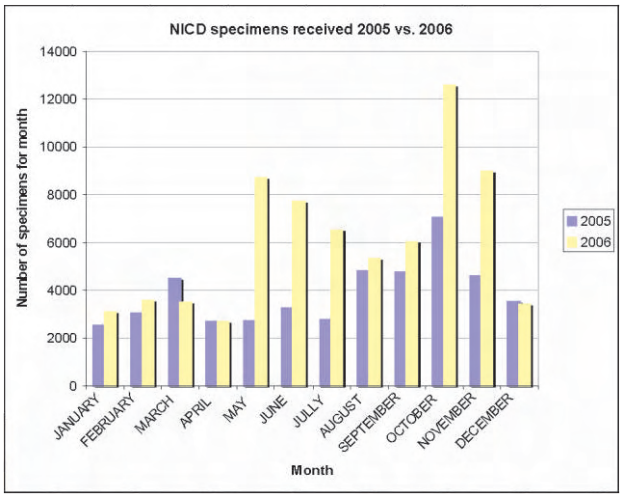
Enterovirus Laboratory
Shelina Moonsamy, Dip Med Tech, Medical Technologist
Areeve Oliver, Dip Med Tech, Medical Technologist
Portia Ngcobondwana Dip Med Tech, Medical Technologist
Doris Lebambo, Laboratory Assistant
Elliot Motaung, Laboratory Assistant

Serology Laboratory
Beverley Singh, Dip Med Tech, Medical Technologist
Beulah Miller, Dip Med Tech, Medical Technologist
Martin Masango, Dip Med Tech, Medical Technologist
Debbie Hlalethoa, Dip Med Tech, Medical Technologist
Joseph Mngoma, Dip Med Tech, Medical Technologist
Mahlatse Maleka, Dip Med Tech, Medical Technologist
Elias Kekana, Dip Med Tech, Medical Technologist
Sweety Zwane, Dip Med Tech, Medical Technologist
Shehaam Majiki, Dip Med Tech, Medical Technologist
Sarah Hloma, Laboratory Assistant

SPECIMEN RECEIVING LABORATORY

Activities

The specimen receiving laboratory experienced two surges in specimen load during the course of 2006 compared to 2005. The increase in specimen load coincided with increased surveillance activities of the NICD linked as well as seasonal variation of various infections e.g., influenza and rubella that require laboratory investigation. Specific examples include an increase in specimen load for the influenza, rubella and HIV-1 antenatal survey.



Courses Attended

- IATA course, October 2006 - Ezekiel Maselesele, Lorraine Cranston and Megan Vandecar (DGM Company, external)
- Management course, November 2006 - Ezekiel Maselesele and Lorraine Cranston (CEU NHLS Central)
- Autoclave course, August 2006 - Joseph Masekwameng and Ananias Selepe (CEU NHLS Central)
- DISA Comms course, July 2006 - Agripa Chauke, Reginah Mokoena and Lazarus Ngwenya (CEU NHLS Central)
- Driver's training, July 2006 - Emmanual Tetse (NHLS Customer Service)
- Course for health and safety representatives, July 2006 - Ezekiel Maselesele and Lorraine Cranston (NHLS Safety Officer)
- Biogical waste disposal, March 2006 - Ezekiel Maselesele (CEU NHLS Central)

CELL CULTURE LABORATORY

Activities

The major function of the cell culture laboratory is the production and banking of various cell lines used by the Enterovirus and General Isolation laboratories for routine testing. With the increased influenza surveillance activity (Viral Watch) there has been an increase in supply of the MDCK cells for virus isolation as well as distribution of viral transport medium to Viral Watch centres. The Vero Slam cell line has been introduced as the preferred cell line for the isolation of measles and rubella viruses. The laboratory kept up with the demand for the LB20 and RD cells lines required by the Enterovirus laboratory during the Namibia polio outbreak. The laboratory also provided LB20 and RD cells line to 15 WHO-supported polio laboratories in Africa as well as the Vero Slam cell lines for measles and rubella isolation.

Training

Four trainee students.

Meeting Presentation

Update on the performance of regional cell banking 2005/2006 at the Regional Polio Heads and Technical Supervisors 24-28 July 2006, Burgers Park Hotel Pretoria.

RESPIRATORY & GENERAL ISOLATION LABORATORIES

The Diagnostic Isolation Laboratories of the NICD have been serving the local academic hospitals and some private laboratories / clinicians as a diagnostic facility to facilitate effective and early detection of disease. The laboratory has been part of the VIRAL WATCH programme for 22 years, providing data on circulating, seasonal Influenza strains. The laboratory serves as a National Influenza centre (NIC) for World Health Organization (WHO). The data is also used by WHO global Influenza programme to help formulate the annual Influenza vaccine. During 2006 the programme was expanded to include sites from the Eastern Cape and also involved referral laboratories from Kwa Zulu-Natal and Western Cape. A total of 1481 respiratory specimens were tested and 554 Influenza isolates were identified (37 % isolation rate). The majority of the isolates were Influenza A (496). The isolates were subtyped using the Heamagglutination Inhibition test (HAI). The reagent for HAI are supplied by WHO Collaborating Centre (WHO CC) for Reference and Research on Influenza in Melbourne, Australia).

The average numbers of specimens received by the General Isolation laboratory for virus isolation were 64 specimens per month. These included specimens such as urines, bloods (for CMpp65 Ag), swabs and vesicle fluids.

The General Isolation Laboratory also received 4935 urines for measles investigations. Of these the serology IgM positive urines were processed and stored for further molecular analysis.

EQA Participation

The laboratory participates in a bi-annual UK NEQAS programme for virus identification.

Meetings / Workshops attended in 2006:

- Cardia Esterhuyse attended the WHO/CDC Inter-Country Training workshop on Surveillance and Laboratory Diagnosis of H5N1 in Nairobi, Kenya from 25-29 September 2006.
- Amelia Buys and Cardia Esterhuyse attended an Essential PCR course held by DNABOITEC, from 31/05/06- 02/06/06.

Table 1: Viral Watch Activities

No of specimens received: 1481												
H1N1	H3N2	Untyped	B/Mal*	B/SH**	Untyped	RSV	ADENO	CMV	PIV 1	PIV2	PIV3	Pool-untyped
6	425	65	48	7	3				2	4	5	
496			58						PIV Untyped: 2			
554						23	2	2	13			11
Total # +ves : 605												

*B/Mal = Influenza B/Malaysia/2506/2004
**B/SH = Influenza B/Shanghai/361/2002

Table 2: General Isolation Laboratory Activities

No of specimens received	703			
	CMV	CMpp65	HSV 1	HSV 2
	33	35	2	0
Total no of isolates	70			

ENTEROVIRUS LABORATORY

The primary activity for 2006 has been the continued support for AFP surveillance at a national and regional level. The laboratory experienced an increased work load during the course of the Namibia polio outbreak. The NICD laboratory serves as the National Polio Lab for SOA (South Africa), SWZ (Swaziland), LES (Lesotho), MOZ (Mozambique), BOT (Botswana), NAM (Namibia) and ANG (Angola). Routinely two tests (RD and L20B) are performed on each sample.

Table 3: Samples received per country with a break-down of results

Country	V1*	V2	V3	W1**	W2	W3	NPENT***	NEGATIVE	TOTAL
ANG	7	4	2	2	0	0	86	297	398
BOT	0	0	0	0	0	0	0	58	58
ERI	2	0	1	0	0	0	3	42	48
ETH	0	0	0	4	0	0	0	0	4
LES	0	0	0	0	0	0	2	38	40
MOZ	0	1	5	0	0	0	21	238	265
NAM	24	0	0	30	0	0	40	574	668
NIE	0	2	1	10	0	9	0	1	23
NIG	0	0	0	2	0	0	1	0	3
RDC	0	0	0	0	0	0	0	2	2
SEY	1	0	1	0	0	0	0	0	2
SOA	3	3	0	0	0	0	134	666	806
SWZ	0	0	2	0	0	0	2	24	28
Total	37	10	12	48	0	9	289	1940	2345

*: V= Vaccine strain; **: W: Wild type virus; ***: NPENT: Non polio enterovirus

General virus isolation from faecal samples

A total of 91 samples were received from non-AFP cases for routine virus culture. On the majority of these three tests per sample were performed, including RD, L20B and Adenovirus SVC.

Coxsackie and polio serology

Serum samples are tested for antibody titres to coxsackie B1-6 and polio 1-3 by means of a neutralization assay using known virus. Therefore, 6 tests are performed on one sample for coxsackie B serology and 3 for polio. Determination of polio titres to all three serotypes is essential to indicate the immune status of an individual. Staff are routinely screened for polio titres and where necessary provided with the polio vaccine.

Table 4: Total number of samples received vs total number of tests performed

Test	# of samples	Total # of tests
Coxsackie	310	1860
Polio	300	900

Confirmatory testing of faecal samples

Confirmatory testing was performed on samples received from ERI (Eritrea), ETH (Ethiopia), NIE (Nigeria), NIG (Niger), RDC (Democratic Republic of Congo) and SEY (Seychelles). The numbers of samples received and breakdown of results are given in Table 3 above.

Confirmatory testing is usually performed when there is a discrepancy in results between the original testing laboratory (stool processing laboratory) and the ITD laboratory, OR when ITD/sequence results indicate the possibility of a laboratory contamination. The stools are then requested and repeat testing is performed to confirm results. However, this does not apply to samples received from ERI. Due to problems encountered by the national laboratory that supports Eritrea all stools from Eritrea were forwarded to NICD for testing

EQA Participation

WHO Proficiency Testing - Enterovirus, specifically poliovirus isolation

UK NEQAS - General enterovirus isolation .

Conferences/Meetings

- GCLP Course attended by Shelina Moonsamy, Johannesburg, February 2006
- Ad5 Assay training for endpoint vaccine testing, Merck, Philadelphia, April 2006 attended by Shelina Moonsamy
- HVTN Conference attended by Shelina Moonsamy, Washington, May 2006
- Global Polio Lab Network Meeting attended by Shelina Moonsamy, Geneva, June 2006
- AFRO Polio Lab Network Meeting, Pretoria, July 2006 Attendees included Shelina Moonsamy, Portia Ngcobondwana, Elliot Motaung and Doris Lebambo. Presentation by Shelina Moonsamy on SOPs - Application and Revision.
- Shelina Moonsamy attended the EPI Task Group Meeting, Kopanong, August 2006, and presented on general poliovirus activities.
- Risk Assessment Presentation, NHLS, September 2006
- HVTN Conference, Seattle, October 2006 attended by Shelina Moonsamy

SEROLOGY LABORATORY

Activities

Several activities were undertaken during the course of 2006 and the distribution of specimens is summarized in the figure. The Serology laboratory continued to provide necessary support for both research and surveillance activities. In the case of research activities the serology laboratory has provided testing for three projects that are investigating the relationship between HIV-1 and co-infections and specifically HSV-2. Thus, for example, the relationship between HIV incident infections and HSV-2 incident infections is currently studied in the Stepping Stones project. Specimens that were collected at specific visits for HIV-1 are currently being re-tested for HSV-2 using different HSV-2 IgG-specific. Approximately 5835 specimens have been tested to date. The role of HSV-2 infection and male circumcision is also ongoing and similar to the

incidence of HIV-1 recent HSV-2 infections that are averted by circumcision. Two different types of HSV-2 kits are currently being used for this purpose. Finally, the laboratory has also supported HSV-2 testing in a research programme that investigates the relationship between HIV-1 shedding in the presence of treatment for HSV-2. The Laboratory has supported various surveillance activities related to HIVI-1 including the annual antenatal survey for the National Department of Health as well as the survey for the Department of Correctional Services. In the case of the antenatal survey a larger sample was collected for testing purposes in order that analysis can be extended to a district level compared to previous surveys where results were applicable at national and provincial levels only. Additional testing included specimens from the national cancer registry survey. A principle activity has been the utilisation of the BED CEIA for the determination of HIV-1 incident infections. As part of the validation of the assay and the use of modified formulae specimens that form part of the antenatal survey from three provinces are being assessed.

In addition to the above projects the serology laboratory serves as the reference laboratory for measles and rubella serology surveillance. The results for 2006 are presented in the Table 5. South Africa is currently at the elimination phase for measles and the number of likely measles cases by serology numbered 86 and 120 measles IgM equivocal results. For effective measles elimination vaccine coverage requires to be above 90%. Under such circumstances laboratory testing becomes a critical component in surveillance in the context of local outbreaks and importation of measles. It is noteworthy that 20 of the cases were positive for both measles IgM and rubella IgM. It is likely in some cases that the measles results are false positives (a significant decrease in PPV value with low incidence). A small number of the measles IgM equivocal results were PCR positive. The test algorithm to include reflex PCR testing and/or rising IgG titres is being explored and this has partly been performed currently (see section on molecular measles characterisation).

With the introduction of a comprehensive treatment and care programme is the requirement for increased access to testing at counselling and testing sites. Testing for HIV-1 at these sites utilises rapid HIV-1 kits. In the main the kits perform equally as well as ELISAs but requires appropriate evaluations to ensure that standard of testing is maintained. To this end the NICD evaluated 42 kits, and subgroup were included in the national tender. A more detailed report following the WHO/UNAIDS guideline was adapted to improve interpretation of the usefulness of the kit tested. In addition the NICD has played a role with various partners including the CDC in the development of an appropriate curriculum to be used at the counselling sites. The training activities related to the curriculum were implemented in a modified format.

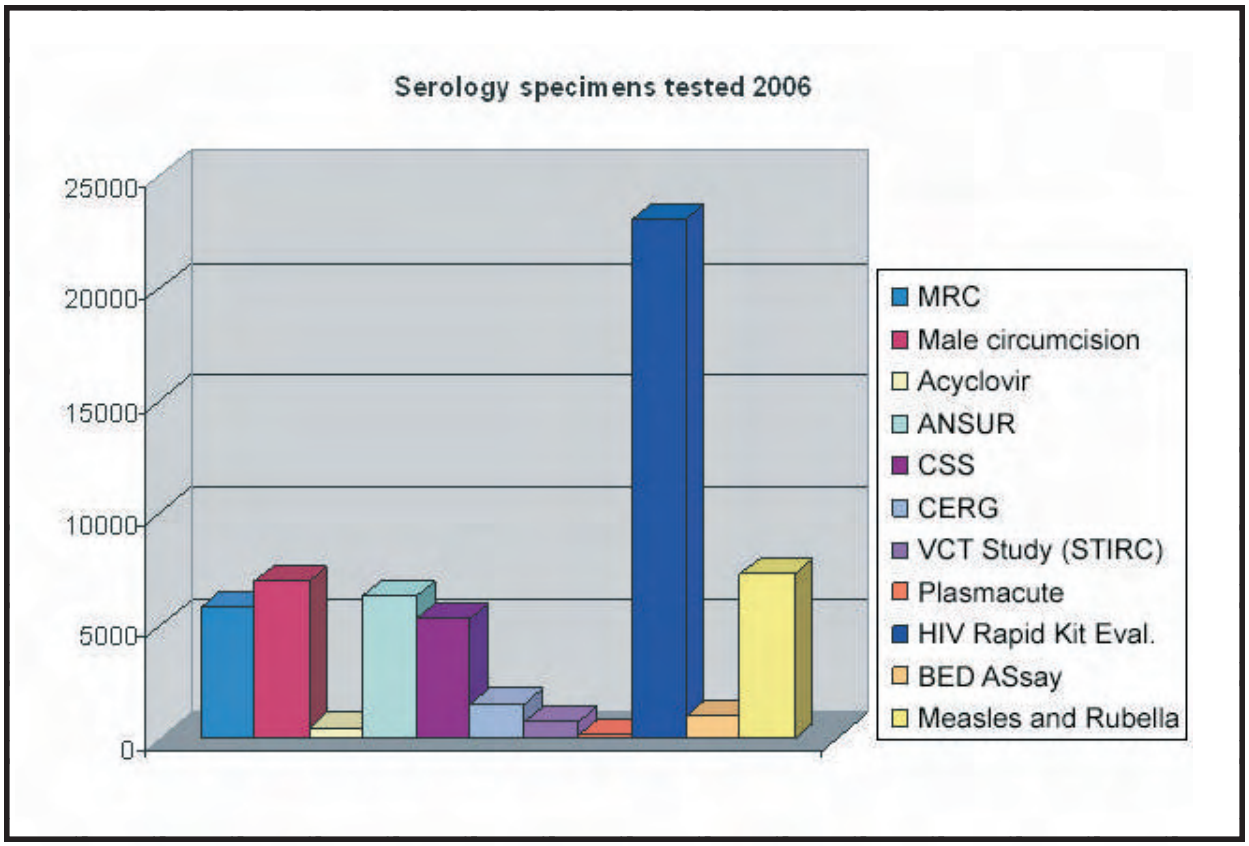


Table 5: Test results of measles case based surveillance

RESULTS										
	Measles Positive			Measles Negative			Measles Equivocal			Total
Month	Rubella Pos	Rubella Neg	Rubella Equiv	Rubella Pos	Rubella Neg	Rubella Equiv	Rubella Pos	Rubella Neg	Rubella Equiv	
1	0	1	0	38	124	10	0	0	0	173
2	0	1	0	26	156	5	0	2	0	190
3	2	6	0	54	179	12	1	1	0	255
4	0	0	0	32	117	11	0	2	0	162
5	1	3	0	57	126	15	1	2	1	206
6	1	5	0	97	133	30	1	9	1	277
7	3	4	0	157	130	12	2	2	1	311
8	1	15	0	311	211	39	7	5	1	590
9	2	9	0	631	474	142	15	10	3	1286
10	8	13	1	727	624	153	14	11	2	1553
11	2	3	0	545	467	129	13	4	1	1164
12	0	5	0	196	196	37	3	4	1	442
Total	20	65	1	2871	2937	595	57	52	11	6609

EQA PARTICIPATION

HIV Serology

- UKNEQAS 3 distributions consisting of 6 panels per distribution.
- CDC NEWBORN SCREENING FOR HIV-1 ANTIBODY ON DBS 4 distributions consisting of 5 panels each.
- Model Performance Evaluation Programme (MPEP) for HIV-1 Antibody - 4 Distributions consisting of 6 panels.
- NHLS HIV EQA - 3 Distributions consisting of 6 panels.
- CDC Proficiency Test Panel for HIV-1 Incidence Testing 2 Distributions, 8 panels. Tested on the Calypte HIV-1 BED Incidence CEIA.

Syphillis Serology

- NHLS Syphillis Serology for RPR 3 Distributions, 3 panels each.
- NHLS Syphilis Surveillance Proficiency Panel for RPR 1 Distribution, 20 panels.
- UKNEQAS for Syhilis Serology 2 Distributions, 6 panels. Tested

Rubella Serology

- UKNEAS for Rubella IgG Serology 2 Distributions, 6 panels.
- UKNEQAS Diagnostic Exanthem Screen 2 Distributions, 3 panels.

TRAINING

- Seventy four personnel from various organisations including PHRU, NEW START, HSRC trained in quality management of HIV-1 rapid kit testing
- Two laboratory technologist from Mozambique on the testing of Measles and Rubella IgM assays using the Dade Behring kit
- One technologist from Lesotho and 1 from American Society for Clinical Pathology (ASCP) in June on Laboratory Quality Systems.
- Trained a laboratory technologist from Kenya in December to perform the BED Incidence assay.
- Trained 9 senior laboratory technologists in Lesotho on Laboratory Quality Systems in May
- GCLP Course attended by Beverley Singh, Johannesburg, February 2006.
- SANAS Laboratory Systems Course and Internal Auditing Course attended by Martin Masango, Johannesburg, April and May 2006.

SITE VISITS (Technical Assistance): CDC Cooperative agreement activity:

- Lesotho: Training of laboratory staff on TQM and Technical assistance
- Swaziland: Laboratory review

SPECIALIZED MOLECULAR DIAGNOSTICS UNIT

STAFF
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LA Short, Secretary

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E Cutler BSc, Medical Scientist
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M Vos BSc (Hons), Medical Scientist
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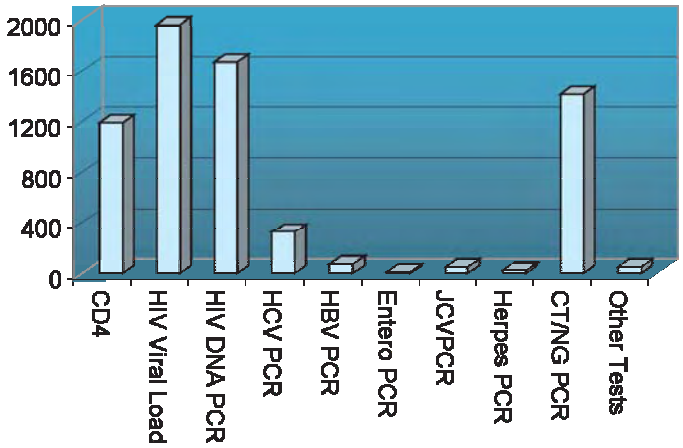
Busisiwe Guliwe Nat Dip Biotech, Biotechnologist
Mashudu Rampilo BSc, Data Manager
Dr Thami Sithebe PhD, Consultant for Polio Containment



SMDU tests performed 2006

THE BLOOD BORNE AND ASSOCIATED VIRUSES
SMDU continued to test a wide range of specimens during 2006. The testing was applied to both diagnostics and operational research activities. The unit worked closely with various other Units at NICD (Serology, STIRC) and indicates the growing integration of programmatic activities.

Renovations creating new research, extraction and post PCR laboratories, offices, were completed to improve work flow and separation of technologies.

Routine HIV-1 DNA PCR DBS specimens included 970 specimens that were processed in support of Lesotho laboratories as part of the Clinton Foundation-supported programme of expanded testing of infants, 150 from Malawi and 550 for the Serethi Study. Validation and implementation of the real time HIV DNA PCR on DBS on the LightCycler 480 is ongoing. The current design of the EQA programme supported by CDC indicates that current extraction technologies such as MagNA Pure may lack analytical sensitivity. This may not be problematic in diagnosis under certain circumstances but could be problematic under conditions such as the timing of the specimen taken, where there is limited material or suppression of viral replication.

Qualitative HBV and HIV DNA PCR testing was used in the 2005-HMCO-BSSA001 study to assess the sterility of reusable biopsy forceps used in gastro-intestinal endoscopy.

In collaboration with STIRC and the Serology Unit, HIV viral load and CD4+ counts (BD Biosciences FacsCount) were performed for the Plasmacute and VCT studies.

A major study that the Unit participated in was to assess the effect of episodic Acyclovir therapy on ulcer duration and HIV shedding from genital ulcers among men in South Africa (HSV-4294).

A randomized placebo controlled trial to assess the efficacy of HSV-2 episodic therapy started in March 2005 and was completed in December 2006. The impact of episodic therapy for herpes as HIV prevention strategy was investigated by measuring the HIV RNA levels in genital fluids, genital swabs and semen. Plasma HIV RNA levels and CD4+ counts were performed on specimens during the 7 visits (28 days) of

surveillance. In total 624 CD4+ counts, 1224 plasma viral loads (Standard Roche Ampliprep/COBAS), 1050 ulcer lavage viral loads (Ultra-sensitive Roche Ampliprep/COBAS), 100 ulcer swab viral loads (Ultra-sensitive Roche Ampliprep/COBAS) and 63 semen (EasyQ) specimens were tested.

The study of associations of STIs with male circumcion continued in 2006 and included analysis of urine specimens using the Roche Amplicor CT/NG test as well as the development of a light cycler method for Trichomonas Vaginalis. In addition, the introduction of a HPV screening test to be followed by genotyping was introduced and will be applied to penile swabs from participants in the trial.

Routine diagnostic tests included Enterovirus Real time PCR, Herpes 1 and 2 Real time PCR, Qualitative HBV PCR and JC PCR. Quantitative HBV PCR was introduced towards the end of the year and testing is performed using Roche Ampliprep/Taqman.

HCV SURVEILLANCE

South African studies published to date indicate a low HCV prevalence rate of 1.6% in this country. However, since HCV genotypes are geographically distributed and may have an impact on response to therapy and an effect on vaccine design in the future, it is necessary to assess the molecular epidemiology of HCV in South Africa with special reference to genotypic frequencies. To this end we have optimized and are validating, methodologies for a cohort of HCV RNA positive specimens from Johannesburg hospitals and validation panels. As part of the broader function of surveillance at the NICD, the Hepatitis Laboratory in the Unit offers diagnostic testing for HCV by qualitative and quantitative PCR (viral load) and genotyping. The latter is determined by sequencing a fragment of the 5'untranslated region (UTR) and/or the non-structural 5B region of the HCV genome. In 2006, 323 specimens were tested for qualitative PCR of which 114 (35%) were found to be positive, 59 of these were tested for quantitative PCR and 24 specimens (of 59) were genotyped by sequencing the 5'UTR amplicon. Forty-six percent (11/24) were classified as genotype 1, 12% (3/24) were genotype 3a and 42% (10/24) were genotype 5a. Our accuracy for qualitative and genotyping tests were shown to be 100% and 80% respectively by our participation in the QCMD programme. Our only failures in the QCMD panel identification involved genotype mixtures which could not be identified by standard PCR and sequencing.

TRAINING

SMDU was involved in molecular technology transfer training sessions. These include Roche PCR Academy applications and HIV DNA training in October and November 2006 and two FACSCount (BD Biosciences) training sessions for CLS and IAVI staff. Registrars and medical technology students were trained on a regular basis and introduced to the full range of molecular tests performed in the unit.

EQA

During the year, the laboratory successfully completed proficiency panels referred by NEQAS, REQAS, QCMD (Quality Control for Molecular Diagnostics), Centers for Disease Control (DBS HIV-1 DNA PCR) and Virology Quality Assessment Program.

DEVELOPMENT OF BIOINFORMATICS AT THE NICD IN 2006

In 2004 the National Bioinformatics Network (NBN) was formed to co-ordinate and promote Bioinformatics in South Africa. Bioinformatics, in its broadest sense, is simply the use of computers to solve biological problems. The NICD was included in this initiative from the outset. The NBN has offered training and support to NICD staff members. In 2006 year the bioinformatics group organized four, very useful, report back sessions to impart knowledge gained at the 5 day Phylogenetics Workshop presented in May 2006 by Dr Martin Coetzee and hosted at the Forestry and Agricultural Biotechnology Institute, FABI, University of Pretoria. Although this workshop covered the basics of Phylogenetics it particularly concentrated on the tests and statistics which can be applied to data before and after analysis. We were also introduced to the relatively new Bayesian inference method. The latter has the accuracy of maximum likelihood (ML) methods but without the inhibitory slowness typical of ML when taxa number of more than 20. Because Bayesian methods calculate the significance of clades at the same time as tree selection their speed and accuracy includes confidence measures.

Staff training has included mastering of the LINUX operating system to enable access to, and use of, the vast software resources available on the internet for machines running this system. A PC with the latest Fedora Core LINUX version and requested programs is maintained and available to all staff and we endeavour to provide users with the necessary support, either internally or externally. Phred Phrap, a sequence calling programme available only in LINUX, was used to produce a “quality” plot of the chromatograms produced from sequencing the 2006 HCV genotyping panels during the last external quality assurance run. For the first time mixtures were coded among the specimens and Phred Phrap enabled us to validate the quality of the single peaks produced during sequencing illustrating that the PCR technique was not capable of picking up more than one signal under our running conditions, even in a 50:50 mixture.

A Bioinformatics group has also been established at the NICD in order to share available Bioinformatic expertise and knowledge. In addition, the forum of the meetings is

designed to encourage staff to ask questions and to share their Bioinformatics needs. If the latter are too complex for our own staff to handle relevant lectures and workshops, overseen by external experts, are organized. In 2006 we staged 15 meetings of which 5 were Workshops where one computer between two was available and each person received hands-on training. More than 60 members of staff have shown an interest in the activities of the group and they have included representatives from every department of the NICD as well as members of the South African Vaccine Producers (Pty) Ltd, a wholly owned subsidiary of the National Health Laboratory Services, which is also situated on the Sandringham site. Highlights of our meetings included the two workshops on PAUP and MODELTEST, respectively. The PC, as opposed to Macintosh, version of PAUP is not user friendly, although the programme is regarded as one of the best phylogenetic packages available since it enables transparent and intelligent parameter selection. MODELTEST determines, from raw sequence data, the optimal model for distance/ likelihood methods taking the guesswork out of model selection. Better insight into programme variables vastly improves the quality of the tree being produced and enables sound reproduction of conditions within and between analyses. This information is now a requirement for acceptance of work by the more prestigious scientific journals.

Although we are slowly building a core of expertise in Bioinformatics at the NICD, we realize that more complex mathematical and statistical applications still require assistance from relevant collaborators and, to this end, it is hoped that in 2007 we will be able to establish joint projects, using data generated at NICD, to solve pertinent surveillance and public health problems.

WHO REGIONAL REFERENCE UNIT FOR POLIO

MOLECULAR EPIDEMIOLOGY OF POLIOVIRUSES IN SUB-SAHARAN AFRICA

The WHO Regional Reference Unit for Polio of the NICD also serves as a reference laboratory for countries which fall under the WHO Polio laboratory Network.

During 2006, the Unit received 1278 poliovirus isolates (Figure 1), which were characterized as vaccine or wild type using two intratypic differentiation (ITD) methods, PCR and ELISA. These isolates were received from National and Regional laboratories throughout Africa namely, Angola, Benin, Burkina Faso, Botswana, Burundi, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Congo, Comoros, Eritrea, Ethiopia, Guinea, Kenya, Lesotho, Liberia, Madagascar, Mali, Malawi, Mozambique, Nigeria, Niger, Democratic Republic of Congo (DRC), Rwanda, Sierra Leone, Senegal, Seychelles, South Africa, Somalia, Sudan, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe . Original specimens from AFP cases were received from several southern African countries and any polio isolates for ITD analysis.

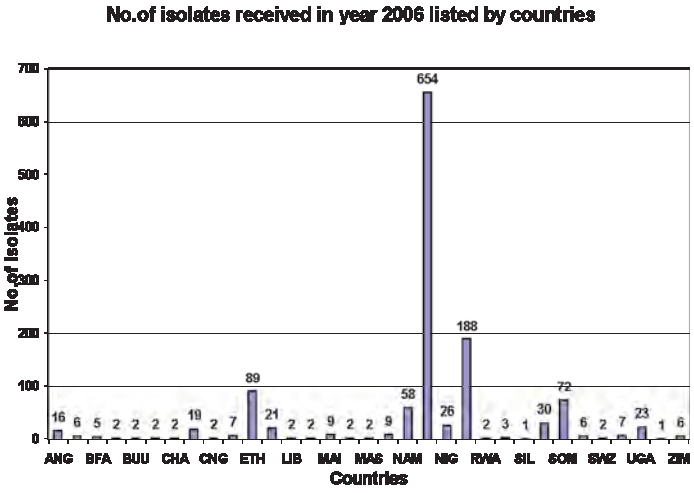
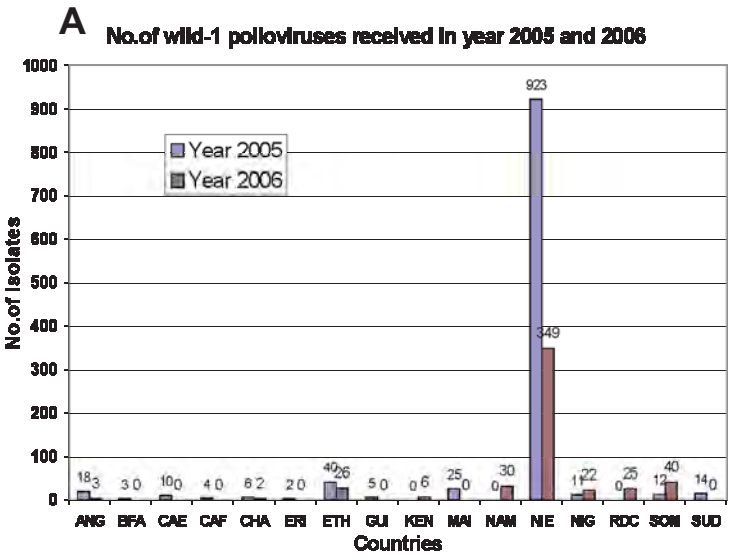


Figure 1: Poliovirus samples received from African countries



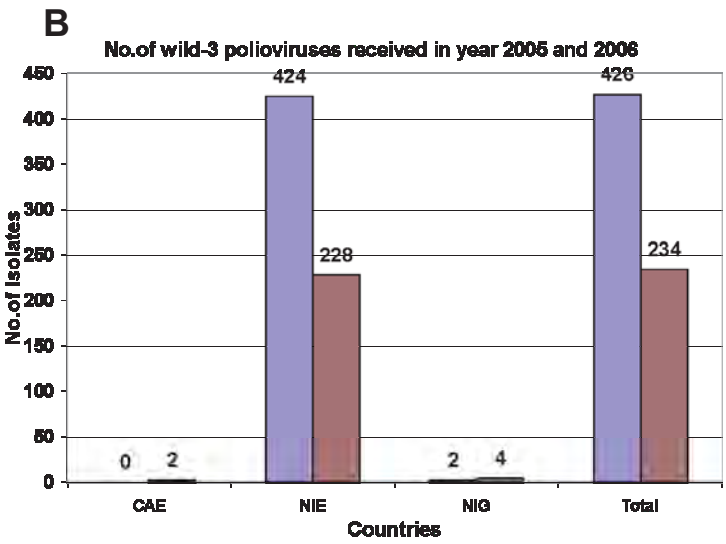


Figure 2. Wild-type isolates of type 1 (A) and type 3 (B) identified in 2006.

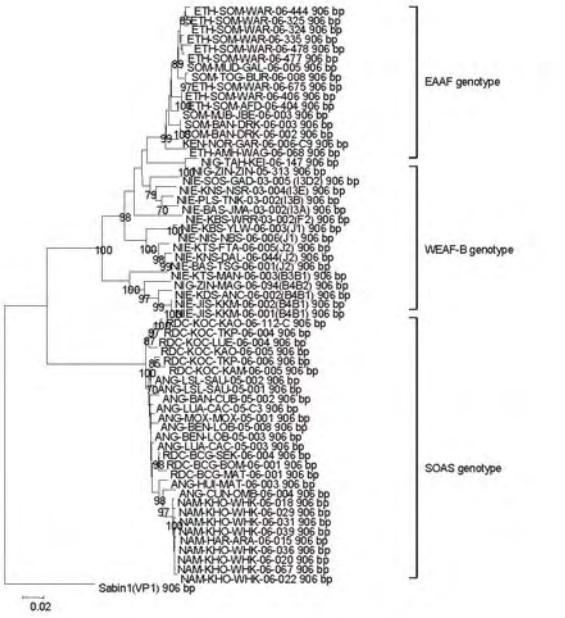


Figure 3. Neighbor-joining tree of the VP1 gene of WEAF-B wild PV1 representative of isolates of 2003 2006 from Africa. Bootstrap values of greater than 70% are shown at the branch nodes. Sabin type 1 was used as an out-group.

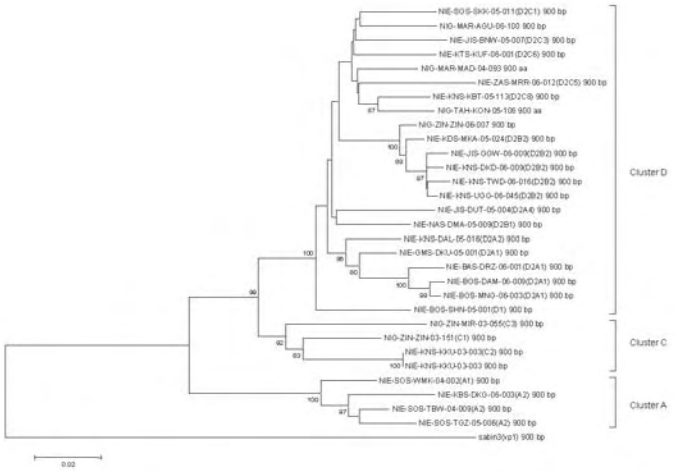


Figure 4. Neighbour-joining tree of the VP1 gene of WEAF-B wild PV3 representative of isolates of 2003 2006 from Africa. Bootstrap values of greater than 70% are shown at the branch nodes. Sabin type 3 was used as an out-group.

Of the identified wild-type polioviruses, 577 were from Nigeria (Figure 2), 349 were wild-type polio type 1 (WPV 1) and 228 were wild-type polio type 3 (WPV 3). Other wild-type polioviruses identified in 2006 were from Angola, Chad, Ethiopia, Kenya, Namibia, Niger, Democratic Republic of Congo and Somalia.

In 2006 PV1 wild-type isolates were distributed into three genotypes, SOAS, WEAF-B and EAAF. The WEAF-B genotype circulated in Nigeria, Niger, and Chad. The EAAF genotype circulated in Ethiopia, Kenya and Somalia. Following the SOAS outbreak in Angola in 2005, two countries were also affected by the same strain in Africa in 2006 namely: Namibia and DRC. Nineteen cases of PV1 wild-type viruses and a contact were identified during the Namibia outbreak which belonged to the SOAS genotype (Figure 3).The index case for Namibia was a 39 year old man from the Hardap region, south-east of the capital Windhoek, who had onset of acute flaccid paralysis (AFP) on the 6th of May 2006. As of October, 2006, 306 AFP cases were reported, 19 cases were confirmed as wild-type 1. Six of the confirmed cases died. In addition to the case from the Hardap region, the wild-type confirmed cases were reported from two main regions, which are the most populated areas in the country: 1) Windhoek in the Khomas region and 2) a northern area bordering Angola with three adjacent regions namely Omusati, Oshana and Ohangwena. The genetic analysis of the VP1 region showed that the virus was imported from Angola The sequence of the index case showed a 97.46% identity to the case from Angola.

WEAF-B wild PV3 is divided into four clusters A-D. Cluster A represents local circulation in Kebbi (KBS) Kano (KNS), Katsina (KTS), Jigawa (JIS), Zamfara (ZAS) and Kaduna (KDS) northern provinces of Nigeria. Cluster B is not represented. Cluster D and cluster C are resolved into two countries, Nigeria and Niger. Two other cases of wild-type 3 were identified in Cameroon and Chad (data not shown).

CONTAINMENT OF POLIOVIRUSES IN SOUTH AFRICA

The South African National Department of Health in cooperation with the World Health Organization's campaign to eradicate polioviruses has appointed the National Certification Committee (NCC), and its sub-committee, the National Task Force (NTF), to oversee the polio certification and laboratory containment activities in South Africa. The NCC and NTF enjoys support from the National Polio Expert Committee (NPEC), which is involved in final classification of all AFP cases, as well as the National Department of Health, which serves as the secretariat of all the committees.

In 2006 the NTF committee sent out laboratory survey and inventory forms requesting all biomedical/ medical/pharmaceuticals laboratories, vaccine manufacturers and other relevant organizations/ stakeholders to search all storage spaces for materials that contain poliovirus or potentially infectious materials. Information gathered from this search will be used to compile a National Inventory of all laboratories with infectious and/or potentially infectious poliovirus materials. Laboratories with such materials will be given appropriate advice on storage and on precautionary measures. A report has been submitted to the African

Regional Commission for Certification (ARCC) and subsequently, the Global Commission for Certification (GCC).

CONFERENCES/MEETINGS/TRAINING

HN Gumede, Alfred Mawela, Busisiwe Guliwe, Olivia Lentsoane, Mbali Nyuswa, Veruschka Singh: On site Data Management workshop conducted by facilitators from CDC, Atlanta, USA, January 2006.

HN Gumede, Alfred Mawela, Mashudu Rampilo, Olivia Lentsoane, and Mbavhalelo Denga: 9th Meeting of Laboratory Directors: Pretoria, South Africa, July 2006.

Mbavhalelo Denga: Inter-regional polio laboratory workshop: Uganda Virus Research Institute (UVRI), Entebbe, 8-16 November 2006.

Presentation of the annual Task Force on Immunisation in Africa award to NICD "for outstanding support to the polio eradication programme in Africa. From left to right, Nicksy Gumedi-Moeletsi, Olivia Lentsoane, Mr John Robertson, Shelina Moonsamy, Prof Barry Schoub.

RESPIRATORY VIRUS UNIT

STAFF

Dr Terry G Besselaar, MSc, PhD, Head of Unit
Sheilagh Smit, BSc (Hons), Senior Medical Scientist
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Background

MOLECULAR INFLUENZA LABORATORY

The influenza laboratories at NICD have been involved since the mid-1980s in monitoring seasonal influenza activity and comprise one of the few active WHO National Influenza Centres (NICs) in Africa. The laboratories play an important role in providing information towards the WHO annual decision on updating the influenza vaccine formulation for the southern hemisphere. In 2006, highly pathogenic avian influenza H5N1 virus was isolated for the first time in several countries in Africa and the NICD laboratories are collaborating with WHO and CDC to strengthen preparedness in Africa for the rapid detection of possible avian H5N1 influenza cases in humans.

MOLECULAR EPIDEMIOLOGY OF THE 2006 INFLUENZA SEASON IN SOUTH AFRICA

Both subtypes of influenza A and B viruses circulated during the season and a total of 554 viruses were isolated during March October in 2006. The isolates were subtyped by the haemagglutination inhibition (HI) test using the kit supplied by the WHO Collaborating Centre (WHO CC) for Reference and Research on Influenza, Melbourne, and a proportion of them were characterised by sequencing the HA1 subunit of the haemagglutinin (HA) gene and performing phylogenetic analysis.

The majority of the isolates (496 or 89.5 %) were influenza A while the remaining 58 (10,5%) were influenza B. Of the influenza A isolates, 6 were identified as subtype H1N1, 425 as subtype H3N2 and 65 were untyped. Most of the influenza B isolates were B/Malaysia/2506/04-like, belonging to the B/Victoria/2/87 lineage. Seven viruses belonging to the B/Yamagata/16/88 lineage (B/Shanghai/361/02-like viruses) were also identified. The largest numbers of virus isolates were made in week 23 (week starting 4 June).

Sequence analysis of the HA1 subunit revealed the H1N1 viruses isolated during the season showed very little genetic drift from the A/New Caledonia/20/99 vaccine strain. Amino acid changes were observed in the isolates sequenced at five residues. These South African isolates were very similar to viruses isolated in countries such as Egypt, Israel and Sweden (Alan Hay, pers. comm).

The molecular characterisation of representative influenza H3N2 isolates revealed that the viruses circulating in South Africa exhibited extensive genetic drift relative to the A/California/7/04 vaccine strain. These changes mapped to antigenic sites A and B. The isolates shared a greater homology with the A/Wisconsin/67/05 strain with the characteristic S193F (antigenic site B) and D225N (receptor binding site) mutations seen in the A/Wisconsin/67/05-like viruses. All the isolates differed from the A/Wisconsin/67/05 strain at residues 122 (antigenic site A), 195 and 223 and sporadic mutations were seen at several other residues in antigenic site B.

Phylogenetic analysis of the HA1 subunit of representative South African 2006 influenza B viruses from both the B/Victoria/2/87 and B/Yamagata/16/88 genetic lineages showed that the 2006 B/Victoria/2/87-like isolates were closely homologous to the B/Malaysia/2506/04 vaccine strain and shared only one common amino acid change at position 109. In the B/Shanghai-like viruses, substitutions were seen at four common residues and sporadic changes were seen at several additional other residues.

Global influenza activity in the southern hemisphere during this period was generally low, with the exception of outbreaks of influenza A H3N2 reported in New Zealand and influenza B outbreaks in Brazil. Based on the data generated mainly from viruses circulating in South Africa, New Zealand and Australia, the influenza vaccine for the southern hemisphere 2006 season has been updated to contain the following strains:
An A/New Caledonia/20/99 like virus (H1N1)
An A/Wisconsin/67/05 like virus (H3N2)
A B/Malaysia/2506/04-like virus

AVIAN H5N1 INFLUENZA IN HUMANS

Several patients with severe respiratory infection with a travel history to countries with ongoing H5N1 virus activity were tested for the presence of H5 by RT-PCR. All tested negative.

HIGHLIGHTS

One of the highlights of 2006 was the inclusion of Dr Terry Besselaar as an expert member of the newly formed WHO Global Influenza Pandemic Task Force for 2006- 2007. The purpose of the task force is to firstly provide advice at the request of the Director General of WHO on key changes to the pandemic alert phase and declaration of a pandemic and on the appropriate response measures to be recommended. The second objective is to provide technical advice on other relevant matters relating to avian and/or pandemic influenza.

Another highlight was participating as a facilitator at the two WHO/CDC Inter-country Training Workshops on Laboratory Diagnosis of H5N1 for strengthening laboratory capacity in Africa which were held in Nairobi. Cardia Esterhuyse also represented NICD in the second workshop where she gave a country presentation of the influenza activities undertaken in South Africa and participated in all the activities (Figure 1). In March 2006 Dr Besselaar was also invited to consult for WHO to assess the laboratory capacity in Nigeria for detecting avian influenza virus. By participating in these activities, NICD has played a key role in helping to strengthen avian influenza preparedness in Africa. NICD was awarded a CDC grant in 2006 to support avian/pandemic influenza preparedness in South Africa and neighboring countries.

ACKNOWLEDGEMENTS

Virus isolation and subtyping was carried out by the Respiratory Virus Isolation section under the expert technical guidance of Amelia Buys. Sequences from other countries were obtained from the WHO Collaborating Centres for Reference and Research on Influenza, London and Melbourne, the LANL influenza database: http://www.flu.lanl.gov and from Dr Olav Hungnes, Norwegian Institute of Public Health, Oslo. We would like to thank WHO, WHO AFRO, Dr Gene MacDonald (US Centres for Diseases Control and Prevention, Pretoria), CDC (Atlanta) and the CDC International Emerging Infections Program (IEIP) in Nairobi, Kenya for their collaboration and support.

CONFERENCES AND MEETINGS

Dr TG Besselaar, L Botha, Amelia Buys, Cardia Esterhuyse and Jo McAnerney. "Antigenetic and genetic characterization of influenza viruses circulating in South Africa in 2006" Oral presentation. NICD Academic days, 27 28 November 2006

Sr J McAnerney, **Dr TG Besselaar** and Dr C Cohen: "Influenza surveillance: vaccine implications" Oral presentation. Vaccinology meeting, Hermanus, 23 -24 October 2006

Dr TG Besselaar: WHO Influenza Pandemic Task Force. First meeting, WHO, Geneva, 25 September 2006

Dr TG Besselaar: Participated in the WHO Consultation on the Composition of Influenza Vaccine for the Southern Hemisphere, 2007. WHO, Geneva, 18 -20 September 2006

Dr TG Besselaar: Facilitator: WHO/CDC Inter-country Training Workshop on Surveillance and Laboratory Diagnosis of H5N1, Nairobi, 25 -29 September 2006

Dr TG Besselaar: Facilitator: WHO/CDC Inter-country Training Workshop on Laboratory Diagnosis of H5N1, Nairobi, 25 -30 June 2006

Figure 1: Workshop participants wearing personal protective equipment for avian influenza diagnosis at the WHO/CDC Inter-country Training Workshop on Laboratory Diagnosis of H5N1 in Nairobi, September 2006. (Photo courtesy of Cardia Esterhuyse).

MOLECULAR MEASLES LABORATORY

BACKGROUND

Extensive measles vaccination campaigns have been held in Africa over the past 5 years to reduce measles-related deaths. It is therefore important to identify which strains of measles virus are being transmitted in order to determine whether endemic transmission has been interrupted, to focus vaccination efforts on those countries where endemic transmission is still occurring, and to determine the origin of imported strains. This information is shared with the World Health Organization (WHO) Global Measles Laboratory Network. The molecular measles laboratory at NICD functions on a national level (identifying strains circulating in South Africa) as well as on a regional level (identifying strains circulating in the southern block countries of Africa). The laboratory also provides a service to other African countries that do not have access to molecular technology.

MOLECULAR EPIDEMIOLOGY OF MEASLES VIRUS IN SOUTH AFRICA IN 2006

The serology laboratory received just over 6600 sera as part of the national measles surveillance program; 81 sera (86 including duplicates; 1.2%) were shown to be measles IgM-positive. Of these specimens, 61 were tested for the presence of measles virus genome. Only 15 (24.6%) were PCR-positive. Fourteen of these were from the Mafikeng area of North West Province and clearly represented a measles outbreak a single strain of virus, genotype D4, was identified. This same strain was circulating in Botswana, Zimbabwe and Zambia in 2005/6 and it is thus likely to have been introduced into South Africa from one of these countries (Botswana being the most likely when considering the geographical location of Mafikeng). The other PCR-positive measles specimen was from an isolated case in the Ekhuruleni district of Gauteng. This virus, identified as genotype B2 and therefore clearly an imported strain, was very closely related to the strain circulating in the DRC and Angola.

62 of the 120 sera that had equivocal measles IgM results were also tested for the presence of measles virus genome. Only 3 were PCR-positive: two of these, from Sedibeng district in Gauteng and Capricorn district in Limpopo, were identified as having genotype B2 and were identical to the strain circulating in the DRC and Angola. The other case, from Metsweding district of Gauteng, was also shown to have an imported measles virus genotype B3. This particular strain of virus is circulating widely in Africa (Nigeria, Burkina Faso, Cote d'Ivoire, Kenya, Uganda, Tanzania, Zambia) and was also introduced into Europe, Canada and the USA in 2006. The 2006 South African strains are shown in blue in Fig. 1.

MOLECULAR EPIDEMIOLOGY OF MEASLES VIRUS IN SOUTHERN AFRICA IN 2006

449 Sera from National Laboratories in 10 African southern block countries were received for confirmatory serological testing as part of the WHO-Quality Assurance program 59 sera were IgM-positive for measles while 8 were IgM-equivocal. To date, 15 (26.8%) were PCR-positive and are highlighted in red in Figure 1.

- 5 sera (Zambia, Zimbabwe, Botswana) were shown to have genotype D4 viruses. These viruses were identical in sequence, and were not the same strain of D4 that had been identified in 2004. There is no data available to indicate the origin of these viruses.
- 2 sera (Lesotho, Malawi) contained genotype D2 viruses. These were the same strain that had caused the extended outbreak in South Africa from 2003-2005.
- 3 genotypes vaccine, B2, B3 were identified in 5 sera from Angola.
- 2 sera (Botswana, Zambia) were shown to have genotype B3 viruses.

MOLECULAR EPIDEMIOLOGY OF MEASLES VIRUS IN AFRICA IN 2006

319 Specimens, including sera, throat swabs, urines and viral isolates, were sent from Uganda, Côte d'Ivoire, Democratic Republic of Congo, Algeria, Angola, Kenya, Lesotho and the Comoros specifically for genotypic analysis. Since Uganda and Côte d'Ivoire are regional reference laboratories for the eastern/central and western blocks of African countries respectively, we therefore also had access to specimens from Rwanda, Benin, Burkina Faso, Mali, Cameroon and Liberia. Sequences were obtained from all PCR-positive specimens (196; 61.4%) and are shown in green in Figure. 1.

- All isolates from the Democratic Republic of Congo, obtained from specimens collected throughout the country, were genotype B2. Several different strains were identified which implies that endemic circulation is still occurring. One of these strains was also circulating in Rwanda. There were 3 different strains of genotype B2 circulating in Angola. One of these was identical to the predominant strain circulating in the DRC. The same strain was also detected in one of the specimens from Kenya.
- Genotype B3.1 strains were circulating in Cote d'Ivoire, Benin, Mali, Burkina Faso, Algeria, Cameroon, Kenya, Zambia, Botswana and Angola.
- Kenya also had a small proportion of circulating genotype D4 viruses. These were unrelated to the D4 strains that circulated in Kenya in 2002.
- An outbreak of measles in the Comoros islands was initially masked by an ongoing Chikungunya outbreak. It was caused by a genotype D4 virus, but the source of the virus is unclear.

the source of the virus is unclear.

CONFERENCES AND MEETINGS

Sheilagh Smit, Jo McAnerney, Bernice Harris and Caroline Tiemessen. Molecular measles surveillance in Africa. Poster presentation. NICD Academic days, 27 to 28 November 2006.

Sheilagh Smit. Vaccinology meeting, Hermanus, 23 to 24 October 2006.

Sheilagh Smit. Characterization of viruses identified in the African region and challenges in improving surveillance. Oral presentation. The fourth WHO Global

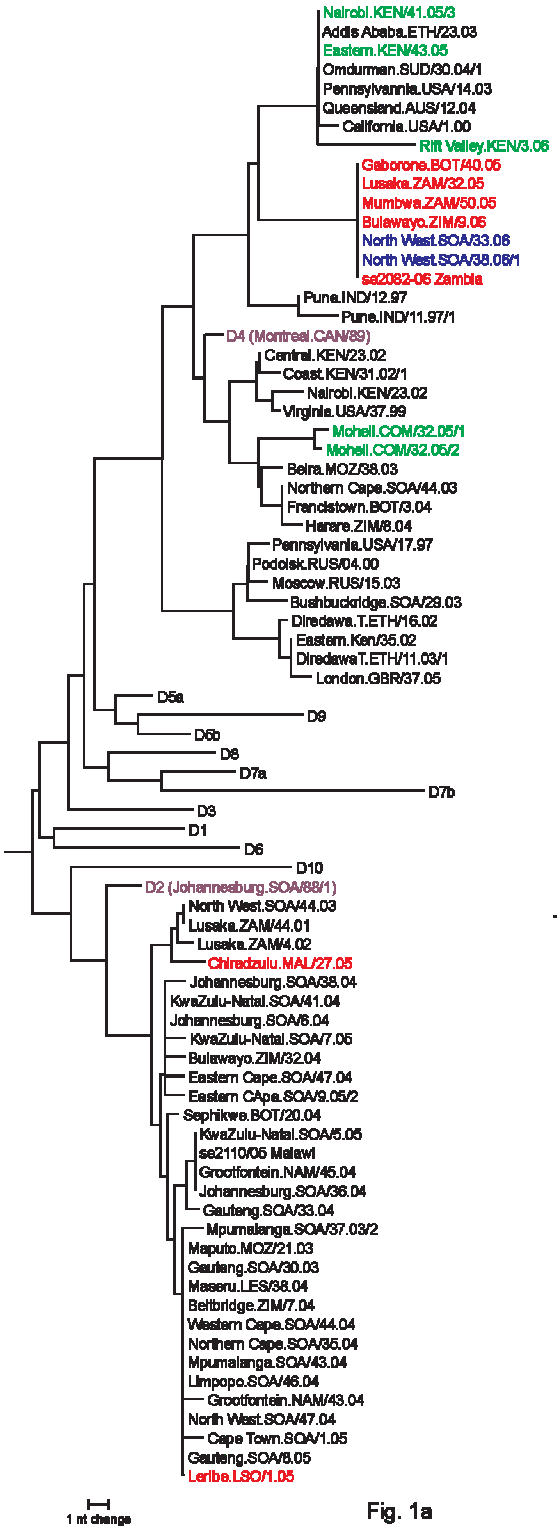

Fig. 1a

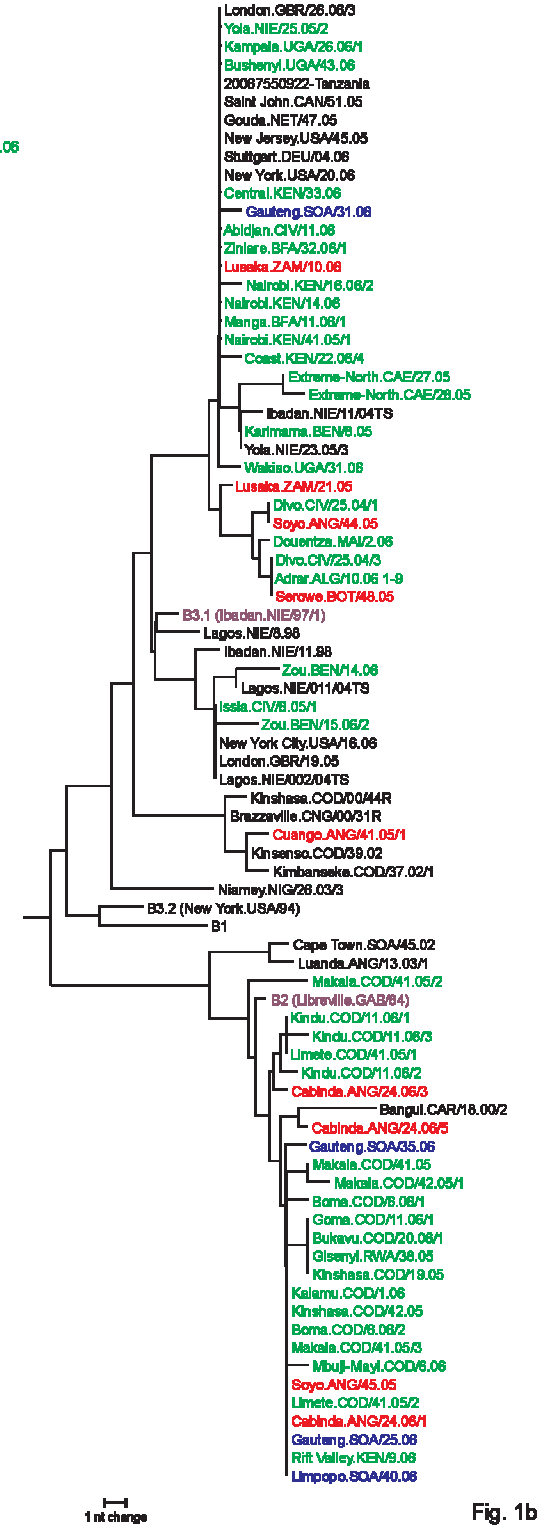

Fig. 1b

Figure 1: Phylogenetic analysis of representative measles virus sequences (450 nucleotides from the 3' end of the nucleoprotein gene) showing (a) Clade D viruses, (b) Clade B viruses. South African 2006 strains are shown in blue, strains from southern African countries are marked in red, other African strains are highlighted in green. Relevant reference strains are shown in purple.

SPECIAL PATHOGENS UNIT

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The Special Pathogens Unit of the National Institute for Communicable Diseases (SPU-NICD) is primarily responsible for the diagnosis and investigation of biohazard class 3 and 4 viruses. The Unit is recognized as a WHO Regional Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses. Class 3 and 4 viruses known to occur in Africa include Rift Valley fever, Crimean-Congo haemorrhagic fever, Marburg, Ebola, Lassa fever-related arenaviruses, and hantaviruses. The Special Pathogens Unit is also responsible for the diagnosis of rabies, rabies-related, and other biohazard class 3 viruses including West Nile, dengue, yellow fever and chikungunya viruses. The SPU-NICD's maximum-security laboratory (BSL-4) was closed in April 2004 for major upgrading, upscaling, and refurbishment. It is planned that it will be fully operational late in 2007. Meanwhile, research projects involving the use of non-infectious nucleic acid preparations of BSL-3 and -4 viral agents and diagnostic service has continued in BSL-2 and BSL-3 laboratories of the Unit.

SPU team and other NICD staff members

COMPARISON OF SPECIMENS RECEIVED IN 2005 AND 2006

The total number of specimens tested in 2006 was higher compared to 2005. However, there was a significant reduction in submissions for diagnosis of suspected viral haemorrhagic fever cases from other countries (Table 1). This is most likely due to the temporary closure of our BSL-4 facility.

Table 1: Comparison of specimens received in the Special Pathogens Unit in 2005 and 2006

Specimens	Received in 2005	Received in 2006
Diagnostic:		
Suspected VHF (South Africa)	52 (47 patients)	82 (45 patients)
Suspected VHF (other countries)	211 (197 patients)*	11 (11 patients)
VHF contacts	55 (55 persons)	1 (1 person)
Undiagnosed fevers	100 (100 patients)	24 (20 patients)
Sub-total	*318*	*118*
Suspected rabies	37 (21 patients)	101 (53 patients)
Rabies immunity	72 (68 persons)	84 (84 persons)
Ticks	5 (4 accessions)	2 (2 accessions)
Miscellaneous	969 (44 accessions)	553 (16 accessions)
Surveys:		
Occupational/residential groups	78 (1 group)	117 (3 groups)
Domestic animals for zoonoses	170 (1 accession)	125 (1 group)
Wild animals for zoonoses	202 (1 accession)	1101 (2 accessions)
Ticks	0	0
Grand total specimens:	1951	2201

INVESTIGATION OF SUSPECTED VIRAL HAEMORRHAGIC FEVERS (VHF)

Eight cases of Crimean-Congo haemorrhagic fever (CCHF) were confirmed in South Africa during 2006 (Table 2), most of them resulted from bite by infected tick and 50% were fatal. Although there is no specific treatment for CCHF infections there is some evidence that Ribavirin can improve the prognosis if administered before day 5 after onset of illness.

A total of 186 cases of CCHF have been diagnosed in southern Africa from the time that the presence of the disease was first recognized in 1981 up until the end of 2006, including seventeen in Namibia, one in DRC, one in Tanzania, and 167 cases in South Africa. The largest group of cases, 85/186 (45,7%), arose from known tick bite or the squashing of ticks; 72/186 (38.7%), arose from known or potential contact with fresh blood or other tissues of livestock and/or ticks; 7/186 (3.8%) nosocomial infections arose from contact with blood or fomites of known CCHF patients, while in 21/186 (11.3%) cases there was no direct evidence of contact with livestock or ticks, but the patients lived in or visited a rural environment where such contact was possible. Most patients were employed in the livestock industry, and males constitute 156/186 (83.9%) of all cases of the disease diagnosed to date. The case fatality rate fluctuated around 30% in the first few years when CCHF was initially recognized in South Africa, but gradually declined to an overall rate of 19.9% (29/146) for the period of 1981-1998, most likely as a result of increased awareness leading to earlier recognition and institution of appropriate supportive therapy. Unfortunately, the case fatality rate drastically increased to 58.9% (23/39) for a period of 1999-2006 which suggests that there is a decline in awareness of the disease among clinicians, resulting in delayed recognition of cases.

Table 2: Confirmed cases of Crimean-Congo haemorrhagic fever virus infection in Southern Africa, January to December 2006.

Location of exposure	Month	Age/Sex	Virus isolation	PCR	Antibody	Died/ Survived	Source of infection
Bloemfontein, Free State	Jan	51 F	Pos.	Pos.	Neg.	Died	Tick bite
Upington, Northern Cape	Feb	61 M	Pos.	Not done	Pos.	Survived	Tick bite
Boshof, Free State	Feb/March	50 M	Neg.	Pos.	Pos.	Survived	Ticks/livestock?
Vereeniging, Gauteng	March	33 M	Pos.	Pos.	Neg.	Died	Tick bite
Vereeniging, Gauteng	April	25 M	Pos.	Pos.	Pos.	Died	Unknown
Hopetown, Northern Cape	April	33 M	Pos.	Pos.	Pos.	Survived	Ticks/livestock?
Prieska, Northern Cape	Dec	35 M	Pos.	Pos.	Pos.	Died	Tick bite
Keimoes, Northern Cape	Dec	25 M	Pos.	Pos.	Pos.	Survived	Tick bite

* Demonstration of IgM and/or IgG antibody responses.

RABIES IN SOUTH AFRICA, 2006

A total of 31 cases of human rabies were confirmed by the SPU during 2006 (Table 3). The number of cases confirmed was higher than in 2005 (8 cases) due to a rabies outbreak in Limpopo Province (LP). The majority of patients contracted rabies from contact with rabid dogs in LPP (21 cases), Kwa-Zulu Natal (KZN 6 cases) or the Eastern Cape Province (ECP 3 cases). In one case, rabies-related Duvenhage virus (DUVV) was isolated from a 77-year old man who was scratched on the face by what appears to have been an insectivorous bat in February 2006 in North West Province (NWP), about 80 km from the location where the first DUVV infection occurred 36 years earlier.

Table 3: Confirmed cases of rabies, 2006

Name	Age/ sex	District of exposure	Exposure: bitten by	Onset	Admitted hospital	Died	Final hospital
RK	12/f	Tshandama, LP	Dog Oct 05	2005/12/29	2006/01/02	2006/01/06	Donald Fraser
NW	10/f	Marindili, LP	Dog 06	2006/01/26	2006/01/30	2006/02/11	Polokwane
IN	9/m	Thavhani, LP	Dog Dec 05	2006/02/07	2006/02/07	2006/02/09	Tshilidzini
SM	5/f	Ha-Budeli, LP	Dog 06	2006/02/06	2006/02/12	2006/02/15	Tshilidzini
AS	7/m	Budeli Mwiini, LP	Dog Oct 05		2006/02/11	2006/02/17	Tshilidzini
NM	3/m	Scottburgh, KZN	Dog Jan 06			2006/03/03	GJ Crookes
NN	4/m	Mufulwi, LP	Dog Jan 06		2006/03/10	2006/03/13	Donald Fraser
NR	5	Vhutalu, LP	Dog Feb 06		2006/03/17	2006/03/18	Donald Fraser
AM	6/m	LP	Dog Feb 06	2006/03/07	2006/03/15	2006/04/01	Tshilidzini
AK	77/m	Sun City, NWP	Bat Feb 06	2006/03/23	2006/03/25	2006/04/05	Durbanville Clinic
TM	11/m	Ha- Mavhunda, LP	Dog Sept 05		2006/03/31	2006/04/02	Tshilidzini
PN	9/m	Dzimauli, LP	Dog Mar 06	2006/04/04	2006/04/04	2006/04/12	Donald Fraser
TT	11/f	Pile Modale, LP	Dog Feb 06		2006/04/14	2006/04/15	Donald Fraser
AN	27/f	Giyani, LPP	Dog Apr 06	2006/04/18	2006/04/21	2006/04/25	Tshilidzini
TB	12/f	Siloam Nzhelele, LP	Dog Dec 05	2006/04/30	2006/05/02	2006/05/09	Polokwane
MM	11/f	Tshino, LP	Dog Feb 06	2006/04/29	2006/05/01	2006/05/06	Tshilidzini
ZR	52/f	Hibberdene, KZN	Dogs Dec-Feb 06	2006/05/11		2006/05/13	Port Shepstone
LS	18/f	Vhurivhuri, LP	Dog 06	2006/05/19	2006/05/19	2006/05/23	Donald Fraser
NRF		Umzimkulu, ECP		2006/05/06		2006/06/06	Port Shepstone
MK	11/f	Tshilidzini, LP	Dog Mar 06	2006/07/02	2006/07/04	2007/07/08	Tshilidzini
CS		Gcilima, KZN	Dog June 06		2006/07/05	2006/07/07	Port Shepstone
LM	Ad/m	Idutywa, ECP		2006/06/24	2006/07/05	2006/07/15	CMH East London
MM	9/f	Mukovhawabale, LP	Dog May 06		2006/07/26	2006/08/01	Donald Fraser
MM	4/m	Maniini, LP	Dog Sept 06	2006/09/21	2006/09/21	2006/09/25	Tshilidzini
LM	8/m	Umtata, ECP	Dog July 06		2007/09/26	2006/09/28	NMAh
MK	11/m	Ha- Mutsha, LP	Dog Sept 06	2006/10/05	2006/10/08	2006/10/13	Tshilidzini
RB	3/f	Mankweng Hosp.,LP	Dog Aug 06	2006/10/09	2006/10/12	2006/10/17	Mankweng
LM	8/m	Paddock, KZN	Dog Sept 06		2006/10/28	2006/11/03	Inkosi Albert luthuli
PM	50/m	Hibiscus, KZN	Dog Oct 06		2006/11/29	2006/11/29	Port Shepstone
BS	70/f	Nweshula, KZN	Dog July 06		2006/12/06	2006/12/23	Port Shepstone
HN	32/m	Duthuni, LP	Dog Feb 06		2006/12/21	2007/01/01	Tshilidzini

ARBOVIRUS SECTION

The number of southern African specimens submitted to the Arbovirus Unit in 2006 (Table 4) was higher than the 281 from 258 patients submitted in 2005. A possible reason for the increase in awareness created by the extensive chikungunya (CHIK) outbreak in the islands of the western Indian Ocean and Madagascar in 2005 and 2006. We did receive a number of submissions from local medical practitioners involving either South African tourists or visitors from the affected regions, as well as sera from 12 patients from the Seychelles. The Seychelles submissions included 8 sera that were positive for CHIK by PCR and virus isolation. Of the 4 virus/viral RNA-negatives, 3 were positive for IgM and all 4 for haemagglutinating antibodies. Interestingly, all 12 sera reacted against the flaviviruses that cause West Nile and yellow fever, 10 reacted against dengue virus/es and 1 against Rift Valley fever virus in the haemagglutination inhibition (HAI) test. Twelve serum specimens originated from suspected CHIK patients who had travelled to or lived in Mauritius. Of these 3 were viraemic and 6 were IgM positive. One 12-year-old patient visiting South Africa on a school tour from Reunion in March became febrile upon arrival whereas a tourist to Reunion and Madagascar towards the end of the outbreak in July became ill but had recovered by the time she returned to South Africa. The first tested positive for CHIK virus by virus isolation and the second had recently seroconverted and was IgM positive. A South African and a Botswana resident both visited Madagascar at the height of the outbreak period in April and tested positive for CHIK by virus isolation and the presence of IgM, respectively. A further 12 South African residents tested positive for CHIK, including two who were viraemic at the time of sampling and seven who were IgM positive; all but the viraemic patients were positive by HAI test.

Table 4: Specimens received for arbovirus testing, 2006

Suspected arbovirus infections	No. of sera (No. of patients)
South Africa, Botswana, Namibia	466 (441)
Other countries	18* (18)
SPU referrals	3 (3)
Total submissions	488 (462)
Undiagnosed fevers	263 (249)
Positive arbovirus results:	
SIN	16 (15)
CHIK	25 (24)
WN	60 (57)
RVF	3 (3**)
DEN	34 (34)
YF	87 (84***)
Total number of positives	225 (217)

* Not including one Seychelles patient who was not tested due to lack of specimen
** 1 vaccination
*** Including at least 4 vaccinees

RIFT VALLEY FEVER VIRUS NUCLEOCAPSID (N) PROTEIN-BASED INDIRECT ELISA

Rift Valley fever virus (RVFV) is an important zoonotic and potential biothreat agent. There is an increased international demand for standardized and safe immunoreagents for diagnosis of RVF. Serodiagnosis of RVF currently relies on the use of live or inactivated whole virus requiring high levels of biological containment for preparation of antigens and performing tests. In collaboration with our colleagues at the North-West University, Potchefstroom and the Onderstepoort Veterinary Institute, Onderstepoort, we evaluated the recombinant nucleocapsid (N) protein of RVF virus for its potential as a diagnostic antigen in an indirect enzyme-linked immunosorbent assay (I-ELISA) for the detection of specific IgM and IgG antibody in humans and ruminants. There was a high correlation (R2 = 0.8789) between virus neutralising titres and IgG I-ELISA readings in human vaccinees (Figure 1). The IgG I-ELISA was more sensitive than virus neutralisation and haemagglutination-inhibition tests in detecting early immune responses in experimentally infected sheep. The I-ELISAs demonstrated that the IgG and IgM responses to the Smithburn vaccine strain were slower and the levels of antibodies induced markedly lower than to wild type RVFV infection.

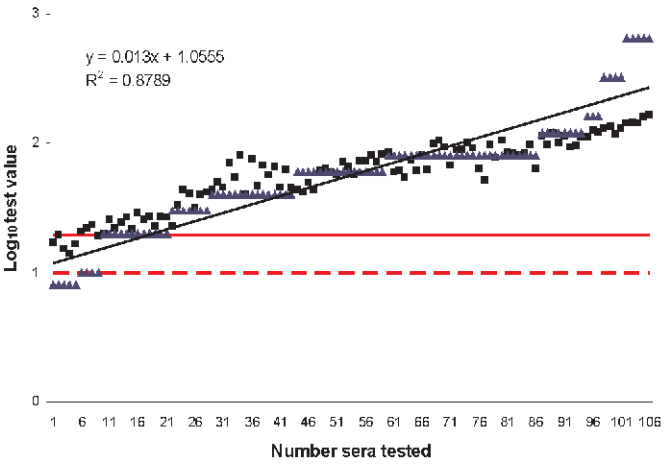


Figure 1. Distribution of the IgG-ELISA PP values (■) and virus neutralising titres (▲) in 106 sera from individuals immunised with the inactivated RVF vaccine. Horizontal lines indicate: (- - -) the cut-off for the virus neutralisation test, (-) the cut-off for the IgG I-ELISA.

OPTICAL FIBER IMMUNOSENSOR FOR SERO-DIAGNOSIS OF RVF

In collaboration with the Department of Biotechnology Engineering, Ben-Gurion University of the Negev, and the National Institute of Biotechnology in the Negev, Beer-Sheva, Israel, we developed and evaluated an optical fiber immunosensor (OFIS) technique for the detection of IgG antibody to RVFV in humans. The OFIS was based on a sandwich enzyme-linked immunosorbent assay (S-ELISA) format, whereby gamma-irradiated RVFV and control antigens were immobilized on the optical fiber surface coated with a

mouse anti-RVFV antibody (Figure 2). Compared to standard colorimetric S-ELISA, the OFIS technique was more sensitive in detecting smaller quantities of RVFV specific IgG. Our results demonstrate that the OFIS assay is a robust and highly accurate system (diagnostic sensitivity = 97.22%; diagnostic specificity = 98.86%) for the detection of specific antibodies to RVFV and has the potential to be used in early diagnosis of infection, disease surveillance and bio-defence monitoring. The OFIS assay lends itself to further development into a portable device for easy deployment in the field.

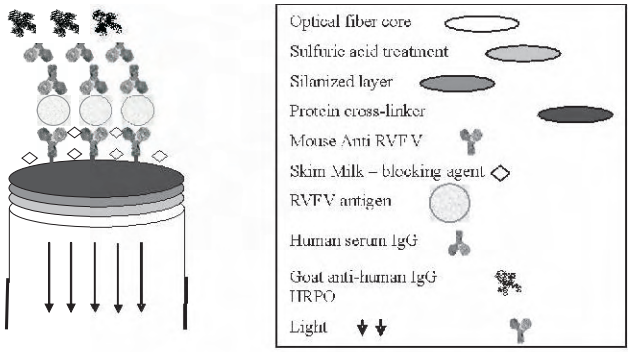


Figure 2. Optical fiber immunosensor based on a sandwich enzyme-linked immunosorbent assay technique for the detection of IgG antibody to RVFV in humans.

VIRUS DETECTION AND MONITORING OF VIRAL LOAD IN CCHF PATIENTS

In collaboration with Bundeswehr Institute of Microbiology, Munich, Germany and Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany we developed a real-time RT-PCR for the detection of a global spectrum of CCHF virus genetic variants. The assay was 100% concordant with nested PCR on 63 samples from 31 confirmed patients. This is the first PCR assay for which validation included representative virus strains from various CCHF endemic regions worldwide (Figure 3). Its high sensitivity enables reliable detection of virus in early stages of CCHFV infection. By eliminating the need for post amplification product processing, real-time RT-PCR allows shortened turnaround times for reporting results. Quantification of viral load can provide an added value in estimating the patient's infectivity. It may also assist in predicting the clinical outcome, and could be used to monitor viral load in patients with ribavirin treatment. Our study provides baseline data on CCHF viral load throughout the acute stage of the illness, and in correlation with the immune response. High viral load seem to correlate with fatal outcome, and detectable antibodies with lower viral loads. Since it is known that detectable humoral antibody response correlates with recovery from infection, monitoring of viral load will be useful for prediction of clinical progress. These preliminary findings are highly encouraging for studies on larger patient cohorts.

VISITORS TO SPU, 2006

MSc students, Department of Biotechnology Engineering, Ben-Gurion University of the Negev Beer-Sheva, Israel: Ariel Sorbazo and Tamy Avida discussing the optical fiber immunosensor technique with SPU staff members.

Dr. Roman Wölfel, a medical microbiologist at the Department for Virology and Rickettsiology of the Bundeswehr Institute of Microbiology in Munich, Germany. Major Wölfel is working in the area of medical defence against biological warfare and terrorism and his interests include viral hemorrhagic fevers as well as rickettsial diseases. He visited SPU to jointly evaluate a real-time RT-PCR for the detection of a global spectrum of CCHF virus genetic variants.

CCHF VIRUS LOW-DENSITY MACROARRAY DETECTION SYSTEM

In collaboration with Bundeswehr Institute of Microbiology and Bernhard Nocht Institute for Tropical Medicine, a low-density macroarray system manufactured by Chipron, Berlin, Germany, was evaluated for rapid detection and identification of CCHF virus in clinical material. The macroarray is a

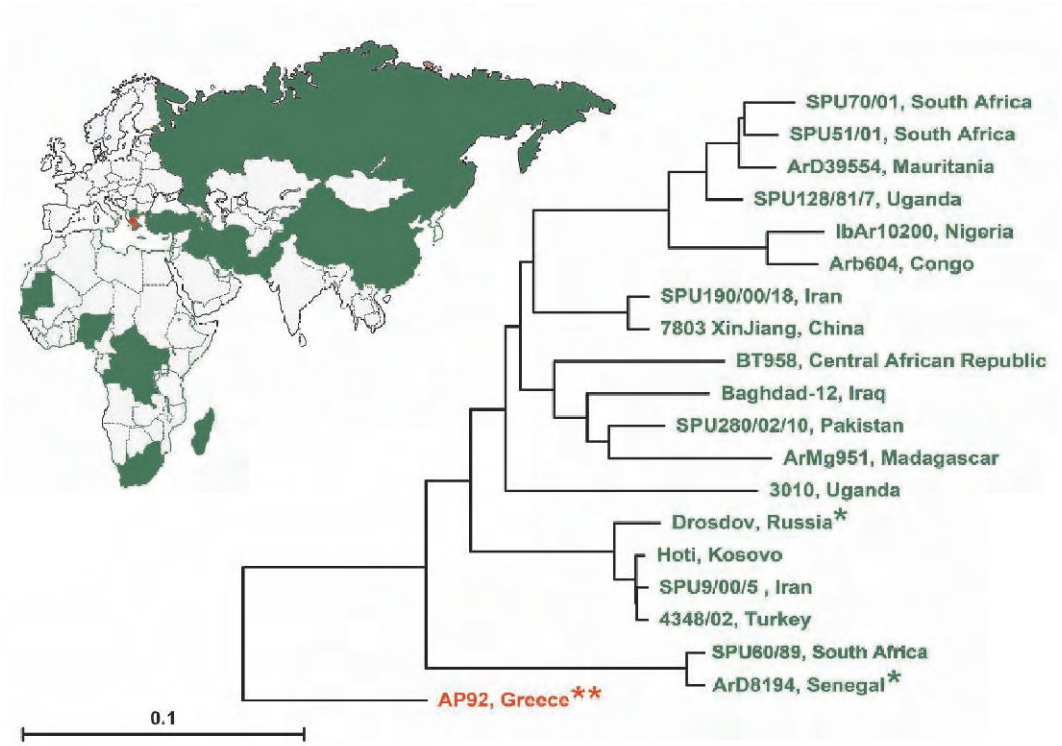


Figure 3. Distribution and phylogenetic relationships of CCHF virus strains selected for the design and validation of the real-time RT-PCR for the detection of a global spectrum of CCHF virus strains. Phylogenetic analysis was based on 450bp sequences of the CCHF virus small (S) segment and the phylogenetic tree was generated by the neighbour-joining method.

transparent, pre-structured polymer support containing eight identical arrays in well-separated, individually addressable hybridization fields (Figures 4 and 5). The non-fluorescent detection principle, based on the formation of a clearly visible substrate precipitate, allows the use of a simple transmission-light film scanner to generate images for the data analysis. The 20 CCHF virus-specific capture probes are between 16 and 22 nt long, and carry an immobilization tag at their 5' end. They are spotted as duplicates in a 9 x 9 pattern, with average spot diameters of 300 nm. Each array is designed to detect CCHF virus specific sequence motifs within the PCR fragments. Four capture probes for the competitive internal control PCR product are included at the bottom of each array (Figures 4 and 5). The assay takes 4 hours including sample preparation and analysis and detects as few as 6.3 genome copies of CCHF virus RNA per reaction. The pattern of hybridization spots is sufficient to differentiate between virus strains. The results of a feasibility study using specimens from CCHF patients showed that this assay provides a highly specific, sensitive, and rapid method for detection of CCHF virus and will be useful for laboratory diagnosis and surveillance of CCHF infections under basic laboratory conditions in developing countries or in field situations.

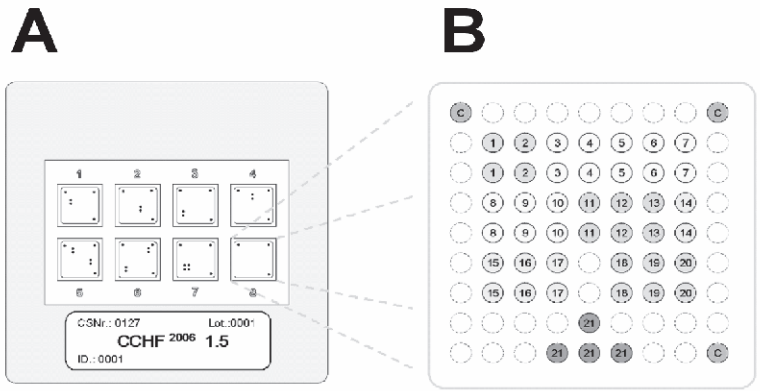


Figure 4. Schematic diagram of the CCHF low-density macroassay. A: Polymer support with eight identical array fields. B: Spotting pattern of one array field; 20 CCHF virus-specific capture probes are immobilized in vertical duplicates. Four capture probes for the internal control of PCR product are included at the bottom of each array. Additional functional control probes to visualize successful hybridization and staining are immobilized in three corners of each field.

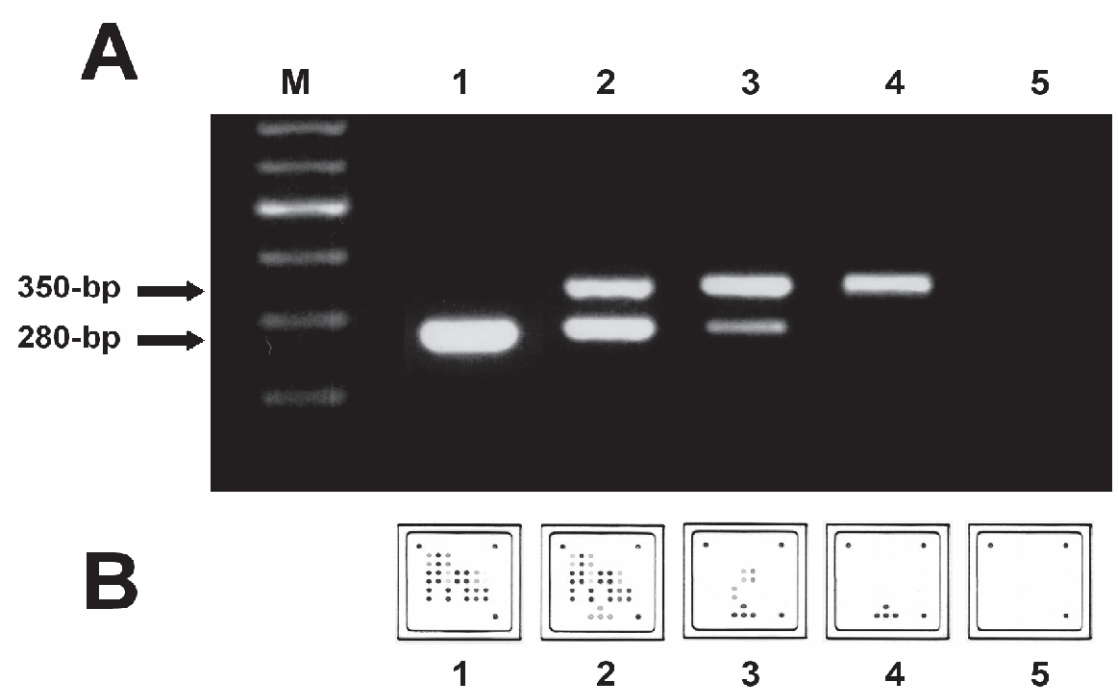


Figure 5: RT-PCR amplifications with the CCHF virus-specific assay (280-bp amplicon) and the internal control (350-bp amplicon). A: Conventional agarose gel analysis of the PCR products. B: Specific hybridization pattern of both CCHF virus-specific amplicons and internal control amplicon on the macroassay. Functional control spots are always visible at three corners.

RAPID MOLECULAR FILOVIRUS DETECTION AND CHARACTERIZATION

In collaboration with our colleagues at the Jerome L. and Dawn Greene Infectious Disease Laboratory, Mailman School of Public Health, New York, a simple consensus reverse transcription-PCR method (cRT-PCR) for filovirus recognition and characterization was developed. To address the diversity of filovirus genomes cRT-PCR utilises a cocktail of specific primers and thus is less sensitive to sequence drift than assays currently in use. This method has been linked with a web-based sequence analysis of amplification products that allows automated speciation. Performance with clinical materials was evaluated using blood collected from human victims of filovirus hemorrhagic fever (HF) infections during recent filovirus epidemics, including cases from the 1995 Ebola HF outbreak in Kikwit, cases from the 2000 Marburg HF outbreak in Durba, DRC, and cases from the 2005 Marburg HF outbreak in Uige, Angola.

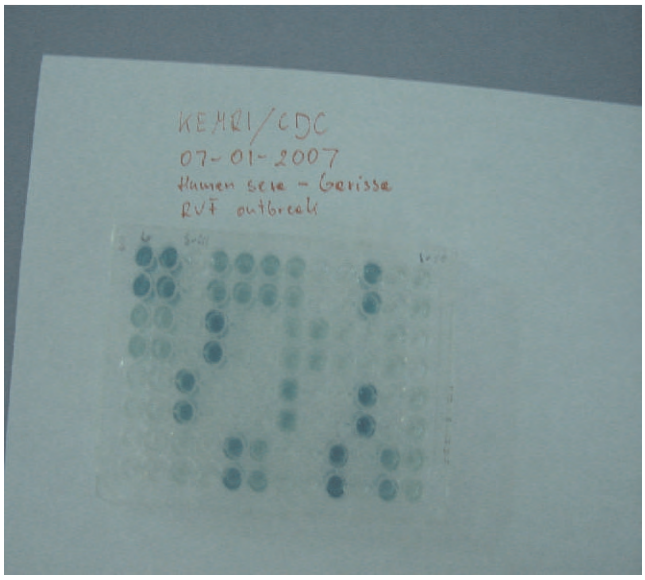
RIFT VALLEY FEVER OUTBREAK IN KENYA, 2006-2007

The re-emergence of RVF in Kenya was laboratory confirmed in mid December 2006 by the KEMRI/CDC in Nairobi. By the end of January 2007, more than 400 cases and 120 deaths of RVF had been reported in the flood-affected areas, mostly in the North Eastern, Central and Coast Provinces of Kenya. This outbreak caused not only severe infections in humans and animals but also resulted in food crises in East Africa due to restrictions in livestock movement and closing of slaughterhouses and livestock markets in affected areas. During RVF outbreaks, at most risk are: 1/ people involved in the livestock industry, e.g. animal herdsmen, slaughterhouse workers, veterinarians, and others when handling diseased animals or having contact with blood, body fluids or tissues of infected animals; 2/ people who are bitten by mosquitoes during an outbreak; 3/ health care workers and other people caring for infected individuals. Humans infected with RVFV typically develop a mild self-limited febrile illness, but retinal degeneration, severe encephalitis, fatal hepatitis and hemorrhagic fever may also occur. Dr Janusz T. Paweska (SPU-NICD) was invited by the Kenyan Ministry of Health and KEMRI/CDC to assist in setting up a local RVF diagnostic capacity and visited two laboratories for diagnosis of RVF in Kenya, one in Garissa and one in Nairobi.

Flooded areas in Garissa district, north-eastern province of Kenya.

Settlement of nomadic pastoralists in Kenya: RVF virus infections were often diagnosed in pastoralists who had handled infected ruminants or their products.

24 hour accident and emergency hospital in Garissa: Dr JT Paweska visiting RVF patient.



ELISA plate: indirect ELISA based on the RVF virus recombinant nucleocapsid protein developed in-house at the Special Pathogens Unit, NICD.

The two pictures above are of the KEMRI/CDC BSL-3 laboratory where the 2006 RVF outbreak was first diagnosed: Dr JT Paweska demonstrating to the KEMRI/CDC and KABETE medical and veterinary diagnosticians a recombinant RVF ELISA.

FATAL HUMAN INFECTION WITH DUVENHAGE VIRUS IN SOUTH AFRICA

Two rabies-related viruses, Lagos bat virus (LBV) and Duvenhage virus (DUVV) are associated with bats in Africa. Although 15 isolates of LBV have been field-recovered so far, including 8 from fruit bats and a cat in KwaZulu-Natal Province, the virus has never been associated with human disease. DUVV was first isolated in 1970 from a fatal human case as a result of a bite by an insectivorous bat about 150 km northwest of Johannesburg. The virus was subsequently isolated in 1981 from a bat (possibly *Miniopterus schreibersi*) in Limpopo Province, and in 1986 from *Nycteris thebaica* in Zimbabwe. DUVV infection was confirmed in a 77-year old man who was scratched on the face by what was most likely an insectivorous bat in February 2006 in North West Province, about 80 km from the location where the first DUVV infection occurred 36 years earlier. The patient did not seek medical care, and thus no postexposure treatment was given. He become ill 27 days later and died on day 14 of his illness. RT-PCR on saliva taken on day 10 of his rabies-like illness and on brain tissue collected post-mortem revealed the presence of lyssavirus nucleic acid. Nucleotide

sequencing of the PCR products and phylogenetic analysis confirmed the identity of the virus as DUVV (Figure 6). The virus was also isolated from saliva and brain tissue by mouse inoculation. It appears that rabies-related viruses are widely endemic in South Africa; this warrants active investigation of the bat-associated lyssaviruses in the country.

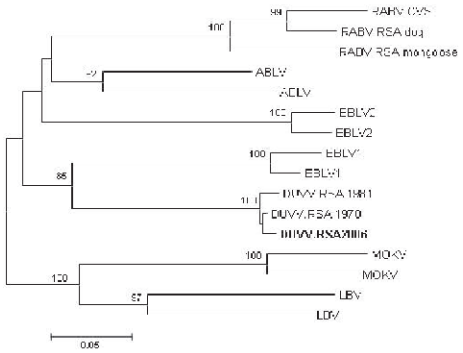


Figure 6. Neighbor-joining tree relating a 372-bp nucleotide sequence of the nucleoprotein gene of the 2006 Duvenhage virus isolate (boldface) to representative sequences of known lyssavirus genotypes, including South African dog and mongoose isolates and the reference challenge virus strain (CVS) of rabies (RABV). ABLV: Australian bat lyssavirus, EBLV: European bat lyssavirus, MOKV: Mokola virus, LBV: Lagos bat virus.

The following two pictures are of a site visit to locations associated with Duvenhage virus infection in humans. Tooysdraal, Limpopo, where Duvenhage virus was first discovered in 1970 when it caused fatal rabies-like disease in a person bitten by an unidentified insectivorous bat.

Alan Kemp (Special Pathogens Unit, NICD)

Dr Charles Ruprecht (CDC, Atlanta), Pat Leman (Special Pathogens Unit, NICD), Prof Louis Nel (University of Pretoria) investigating bat roosting sites in derelict mine tunnels.

ANTIBODY AGAINST SARS-RELATED CORONA-VIRUSES IN AFRICAN BATS

Asian bats have recently been identified as potential reservoir host of coronaviruses associated with the severe acute respiratory syndrome (SARS-CoV). With our colleagues at the Robert Koch Institute, Berlin, Germany, the Bernhard Noch Institute for Tropical Medicine, Hamburg, Germany and Conservation International, South Africa, we tested 705 archived bat sera collected from 1986-99 in Southern Africa for unrelated purposes. Antibody activity to SARS-CoV antigen was detected by an ELISA in 6.7% bat sera, with highest prevalence in the fruit bat *Rousettus aegyptiacus* (16.4%) and the insectivorous bat *Mops condylurus* (12.2%).

DO RABIES VACCINES PROTECT AGAINST THE MONGOOSE RABIES STRAIN▲

Rabies virus is endemic in southern Africa with two biotypes of rabies circulating, the canine biotype, associated with dogs, black-backed jackals and bat-eared foxes and the mongoose biotype associated mainly with viverrids. The disease is universally fatal in all species, however efficacious vaccines are available for pre- and post-exposure prophylaxis and the vaccine is complemented with a rabies immunoglobulin for post-exposure protection. Rabies vaccines are known to protect against classical rabies and antigenically similar genotypes. In southern Africa most patients contract rabies from contact with rabid dogs. There have been recorded instances in which patients were bitten by rabid mongooses and, despite receiving adequate PEP, they contracted the disease and died. Documentation of possible vaccine failures associated with viverrid rabies constitutes an important public health issue. The NIH mouse potency test was used to determine the degree of protection provided by rabies human diploid cell vaccine (HDCV) against challenge with a lethal dose of rabies virus isolated from viverrids and canids from

southern Africa. The relative potency of the vaccine was determined by comparison of the number of survivors after challenge with viverrid and canid biotypes. Preliminary studies showed that the HDCV gave 100% protection against both viverrid and canid biotypes when immunized mice received lethal doses of virus.

ALPHAVIRUS-DERIVED REPLICON VACCINE AGAIST RIFT VALLEY FEVER VIRUS

Vaccination of livestock provides the most practical and effective means of controlling transmission of RVFV to humans and preventing spread of the virus by livestock trade to non-endemic areas. The development of safe and effective vaccines would therefore impact on human and livestock health as well as economic development. Antibody responses against the viral glycoproteins are believed to mediate protective immunity against RVFV. Previous studies using recombinant RVFV G1 and G2 proteins have demonstrated that protective immunity can be achieved by immunization with both G1 and G2 proteins or G2 proteins only. In collaboration with our colleagues at the University of North Carolina, Sindbis virus replicon vectors expressing the RVFV glycoproteins and the non-structural nsM protein, were constructed and evaluated for their ability to induce protective immune responses against RVFV. These replicon vectors produced the RVFV glycoproteins to high levels in vitro and induced systemic anti-RVFV antibody responses in immunized mice, as determined by RVFV-specific ELISA, fluorescent antibody tests, and demonstration of neutralizing antibody. Vaccination with replicon particles expressing the RVFV M segment provided 100% protection against lethal systemic or nasal RVFV challenge. Furthermore, preliminary results indicate that the replicon vectors elicited high levels of RVFV-specific neutralizing antibody in vaccinated sheep. These results suggest that alphavirus-based replicon vectors induce protective immunity against RVFV, and that this approach merits further investigation into its potential utility as a RVFV vaccine.

CONFERENCES ATTENDED, STUDY VISITS

Dr L. Blumberg, Dr JT Paweska, Prof R Swanepoel: 8th Meeting of the Southern and Eastern African Rabies Group (SEARG), Heja Game Lodge, Windhoek, Namibia, 22-26 January 2006.

Dr JT Paweska: The United States Department of Agriculture National Veterinary Stockpile Rift Valley Fever Countermeasures Working Group, Paris, 11-12 April 2006.

Dr JT Paweska: Visited European BSL-4 facilities at Bernard-Nocht Institute, Hamburg, Germany; Health Protection Agency, Porton Down, UK; Swedish Institute for Infectious Disease Control, Stockholm, Sweden; National Institute for Health and Medical Research in Lyon, France, 02 March -13 April 2006.

Prof R Swanepoel: Conservation International Global Symposium, Antananarivo, Madagascar, 20th - 24th June 2006.

Prof R Swanepoel: 56th Afro Regional Committee Meeting, Addis Ababa, Ethiopia 28 Aug - 1 Sep 2006.

Prof R Swanepoel: Royal Society International Workshop on Science and Technology Developments Relevant to the Biological and Toxins Weapons Convention, London, England, 1-4 September 2006.

Dr JT Paweska, Prof R Swanepoel: 3rd International Filovirus Meeting Winnipeg, Canada, 17 - 20 September 2006.

Prof R Swanepoel: WHO Eastern Mediterranean Regional Office Intercountry Workshop on Crimean-Congo Haemorrhagic Fever Prevention and Control, Istanbul, 6 - 8 November 2006.

Prof R Swanepoel: WHO Variola Research Committee, WHO, Geneva, Switzerland 16 - 17 November 2006.

HIV/AIDS RESEARCH UNIT

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Virology Laboratory
A/Prof L Morris DPhil, Chief Specialist Scientist, Head
S Doig, Personal Assistant
S Herrmann, Admin Assistant
Dr P Moore PhD, Senior Medical Scientist
Dr V Pillay PhD, Senior Medical Scientist
Dr A Basson PhD, Senior Medical Scientist
Dr G Hunt PhD, Senior Medical Scientist
S Cohen BTech, Laboratory Manager
K Alexandré BSc (Hons), Research Assistant
E Cave MSc, Research Assistant
I Choge MSc, Research Assistant
Z El-Khatib, Msc PhD, Visiting student
E Gray MSc, Research Assistant, PhD Student
M Langan BSc (Hons), Laboratory Assistant**
J Ledwaba MSc, Research Assistant
N Leseka MSc, Research Assistant
S Loubser, Research Assistant*
S Nkosi MSc, Research Assistant (Deceased 11/7/2006)
M Phoswa, Lab Technician
M Rakgotho MSc, Research Assistant
N Ranchobe BTech, Laboratory Assistant**
N Taylor-Meyer MSc, Project Coordinator
P Walker, Visiting PhD Student*

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Dr S Meddows-Taylor PhD, Senior Medical Scientist
Dr M Paximadis PhD, Senior Medical Scientist
Dr D Schramm PhD, Senior Medical Scientist
A Oliver Dip Med Tech, Laboratory & Admin Manager
J Mans, Postgraduate Student (PhD)
N Mohanlal, Postgraduate Student (PhD)
H Mathe, Postgraduate Student (PhD)
S Donninger, Postgraduate Student (MSc)
F Anthony, Postgraduate Student (MSc)
S Smit, Postgraduate Student (MSc)
M Suchard, Postgraduate Student (MMed) (shared with Prof C Gray)

Immunology Laboratory
Prof CM Gray MSc PhD, Chief Specialist Scientist, Head
Dr V Morafo MSc PhD, Senior Medical Scientist
Dr D de Assis Rosa BSc (Hons) PhD, HLA & QA Manager
Dr C Riou PhD, Visiting Scientist**
Dr M Suchard BSc MBBCh FC Path SA (Clin) DTM&H, Visiting MMed Student
GR Khoury Btech Med Tech, Operations Manager
NS Nyoka NHDIP Med Tech (Virology) Chief Medical Technologist
PS Xaba BSc Nat Dip Med Tech, Medical Technologist, Laboratory Manager
PP Mokgotho BSc (Hons), Repository Manager
PS Kay, M Tech Med Tech, Project & Laboratory Manager
MD Mlotshwa M Tech (Biomed Tech), Medical Scientist
NO Malatsi M Tech (Biomed Tech), Medical Scientist
H Maila MSc, Research Assistant
TY Mathebula BSc MSc, Research Assistant
P Maenetje M Tech (Biotech), Research Assistant
P Mohube HDip (Psy), Research Assistant
M Phatsoane B Med Sci (Hons), Research Assistant
CS Ndlovu M Tech (Biotech), Research Assistant
MK Mufhandu Nat Dip Med Tech, Project Coordinator*
VK Dyer BSc, Laboratory Assistant
KJ Ihlenfeldt, Administrative Manager

* Left during 2006
**Joined during 2006

The AIDS Unit continues to play a leading role in HIV research both within South Africa and internationally. The Unit is primarily focussed on research into HIV-1 subtype C with a major focus on understanding the correlates of protective immunity. Major projects within the Unit during the year have contributed towards the immunological evaluation of candidate HIV vaccines, addressing issues of HIV drug resistance and understanding mechanisms of mother-to-child transmission. The Unit trains junior scientists and has a number of post-graduate students and also contributes to training local scientists and those from other African countries through laboratory-based training and workshops. Members of the Unit are involved in a number of networks including the HIV Vaccine Trials Network (HVTN), the Center for HIV and AIDS Vaccine Immunology (CHAVI) and the WHO Global HIV Drug Resistance Network. Funding of research is largely through grants from the South African AIDS Vaccine Initiative (SAAVI), National Department of Health, Center for the AIDS Programme of Research in South Africa (CAPRISA, an NIH funded study), CHAVI, HVTN, Centers for Disease Control, USAID, The Wellcome Trust, The Fogarty International Centre, Bristol Myers Squibb Secure-the-Future Program, Doris Duke Human Pathogenesis Program and the Center for HIV Vaccine

Discovery (CAVD) funded by the Global HIV vaccine Enterprise and the Bill and Melinda Gates Foundation.

In 2006, the HLA lab was relocated from the Specialised Molecular Diagnostics Unit, under the leadership of Dr Adrian Puren, to the Immunology Lab, under the leadership of Prof Clive Gray. This lab is now being run by Dr Debra de Assis Rosa.

In November 2006 Professor Clive Gray convened a workshop as part of the Academy of Science of South Africa (ASSAf) Nutrition Panel, which was held at the Department of Science and Technology, Pretoria. The title of the workshop was "Diet and nutrition in relation to the functioning of the gastrointestinal tract in HIV-infected individuals".
Prof Clive Gray organized and hosted a Becton Dickinson Biosciences LSR II Multicolor Flow Cytometry Workshop from 27 Nov - 1 Dec 2006. The workshop was run by Dr Holden Maecker from BD Biosciences in California. Both NICD staff and international visitors attended the workshop.

A planning meeting for the Southern Africa Treatment and Resistance Network (SATuRN) was held at the NICD on the 5th and 6th July, attended by 70 delegates. The main workshop was preceded, on the 4th July, by a focused one day informatics workshop. SATuRN is a collaborative research project between institutions from South Africa, Zimbabwe, USA and Sweden to monitor HIV Drug Resistance.

Dr Penny Moore, Elin Gray, Eleanor Cave, Pholo Maenetje and Isaac Choge were all awarded Colombia University - Southern Africa Fogarty AIDS Training and Research Program (AITRP) Fellowships to allow them to train in various laboratories in the USA for 3-6 months.

Jabulani Nhlapo, Mia Coetzer and Dianna Schramm were all awarded their PhD in 2006. Polly Walker submitted her PhD in 2006.

Dr Mia Coetzer was awarded a James Gear Fellowship from the Poliomyetis Research Foundation to support her post-doctoral studies at The Scripps Research Institute in San Diego, USA.

Kabamba Alexandre won a prize for the best poster and the best young researcher at the Research Day, Health Sciences Faculty, University of the Witwatersrand on 23 August 2006.

Sadly, Sibusiso Nkosi, a research assistant and PhD student in the AIDS Unit died unexpectedly on 11th July. He was 29 years old and was the recipient of a prestigious Mellon Award. A memorial service was held on 14th July at NHLS. He is survived by his wife Netty Malatsi, also an employee in the AIDS Unit and his 3 year old daughter. Numerous messages of condolences were received from local and international scientists many of which were read at the memorial service.

New staff to the Unit in 2006 included Martina Langan and Nthabeleng Ranchobe.

Visitors to the Unit included Dr Julie McElrath, Dr Pat D'Souza, Dr Cecilia Cheng-Meyer, Dr Guido Ferrari, Dr Ron Veazey, Dr David Katzenstein, Dr Deenan Pillay and Dr Sylvia Bertagnoli.

LONGITUDINAL ANALYSIS OF HIV-1 SUBTYPE C ENVELOPE SEQUENCES FROM SOUTH AFRICA

The envelope genes of 23 subtype C viral isolates from five individuals with early HIV-1 infection, followed for 2-4 years, were sequenced, analyzed and correlated to coreceptor usage. Isolates from three participants used the CCR5 coreceptor at all time points, with no significant adaptations in the variable loop lengths, predicted N-linked glycosylation sites or predicted change in sensitivity to monoclonal antibodies with disease progression. However, two individuals Du151 and Du179, who had previously shown to be dually infected with two phylogenetically distinct subtype C strains, were able to use CXCR4 with disease progression. The intra-person genetic diversity were 9% for Du151 and 3% for Du179 compared to <2% for participants who did not undergo a coreceptor switch. In both cases this coreceptor switch was associated with specific amino acid changes in the crown, an increased net amino acid charge in the V3 loop and an increase in the length of the V1 region. *This work is in press at AIDS Research and Human Retroviruses.*

PHENOTYPIC CHARACTERIZATION OF HIV-1 ISOLATES FROM GHANA

Viral isolates from 27 HIV-1 infected patients in Ghana, most of who were symptomatic, were characterised for co-receptor usage using MT-2 and U87.CD4 cells. Irrespective of clinical status, most infections were caused by CCR5-tropic viruses although 3 CXCR4-tropic viruses were also found. Genotyping was performed by sequencing the gp41 region. Seven viruses clustered with subtype G reference strains, while the remaining 20 viruses clustered within the subtype A reference viruses. Most subtype A isolates clustered loosely with the CRF02_AG viruses and are described as CRF02_AG-like. The V3 loop was sequenced in selected isolates including all isolates capable of using CXCR4. The V3 region of CXCR4-using viruses contained genetic traits characteristic of CXCR4-using subtype B and C viruses, such as increased charge, presence of positively charged residues at positions 11 and 25 and loss of a predicted glycosylation site. This study supports previous work showing that CRF02_AG is responsible for most HIV-1 infections in Ghana at this time. The predominance of CCR5-using viruses, even in symptomatic patients, suggests that CCR5-blocking strategies may be useful for prevention and treatment of HIV-1 infections in Ghana. *This work is in press at AIDS Research and Human Retroviruses.*

POLYMORPHISMS IN NEF ASSOCIATED WITH DIFFERENT CLINICAL OUTCOMES IN HIV-1 SUBTYPE C INFECTED CHILDREN

The human immunodeficiency virus type 1 (HIV-1) negative factor, or Nef, has a variety of functions which are important in viral pathogenesis. Sequence analysis has identified *nef* mutations that are linked to the rate of disease progression in adults and children infected with HIV-1 subtype B. Here we have sequenced and analysed HIV-1 subtype C *nef* sequences from 34 children with rapid (RP) or slow progressing (SP) disease and identified polymorphisms associated with disease stage including motifs involved in specific pathogenic functions. Unlike subtype B, insertions and deletions in the N-terminal variable region were observed exclusively in SP children (8 out of 25). Strong positive selection pressures were found in sites of known functional importance amongst SP sequences, whereas RP had strong negative selection across the gene. A lineage analysis of selection pressures indicated weaker pressure across the *nef* gene in SP sequences bearing a deletion in region 8-12, suggesting this deletion has functional importance *in vivo*. Together these results suggest a differential adaptation of certain Nef functions related to disease progression, some of which may be attributable to immune-imposed pressures. These data broadly reflect previous studies on subtype B, corroborate the decreased cytopathicity of SP viruses, but also highlight potential subtype differences that require further investigation. *This work is in press at AIDS Research and Human Retroviruses.*

HIGH SPECIFICITY OF V3 SEROTYPING AMONGST HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 SUBTYPE C INFECTED PATIENTS WITH VARYING DISEASE STATUS AND VIRAL PHENOTYPE

V3 serotyping is a technique for determining HIV-1 genetic subtype based on the binding of antibodies from patient sera or plasma to synthetic V3 peptides derived from subtype consensus sequences. Variation in the performance of this assay has been attributed to V3 sequence heterogeneity, the degree of which varies with patient disease progression, virus coreceptor usage and genetic subtype. In this study we assessed the performance of a competitive peptide enzyme immunoassay in samples from HIV-1 subtype C infected patients with varying disease profiles, including those with syncitium (SI) and non-syncitium inducing (NSI) viruses. Out of 90 sera tested, 94.4% reacted strongly against the subtype C peptide. There was no significant difference in assay sensitivity amongst samples from advanced AIDS patients in which humoral immune response is lower, nor amongst SI viruses which carry changes in the V3 sequence. We found 4 samples which were cross-reactive with other subtypes and 1 acutely infected patient was found to be non-reactive due to low anti-gp120 antibody titers. A significantly higher number of samples showed secondary reactivity to subtype A, compared to other subtypes ($p<0.005$). We conclude that the assay is able to identify HIV-1 subtype C infection with a high level of sensitivity (94%) irrespective of the stage of disease and therefore provides a valuable resource for the large-scale epidemiological monitoring of the spread of HIV-1 subtypes in South Africa. *This work has been published in the Journal of Medical Virology.*

INTRODUCTION OF GLYCAN 295 IN SUBTYPE C VIRUSES RENDERS PARTIAL SENSITIVITY TO 2G12 ANTIBODY NEUTRALIZATION

HIV-1 subtype C viruses are generally resistant to the neutralizing MAb 2G12. This has been attributed mainly to the absence of a glycan at position 295 in gp120. We introduced an N-linked glycan by site-directed mutagenesis at position 295 into three subtype C envelope functional clones: Du151.2, COT9.6 and COT6.15. Binding of 2G12 to the wild-type or mutant envelopes was assessed by ELISA and flow cytometric analysis and neutralization sensitivity was evaluated in a pseudovirion-based assay. The V295N mutation increased 2G12 binding to all 3 viruses although the levels varied considerably. COT9-V295N showed the highest levels of 2G12 binding and was the only mutant to become sensitive to neutralization by this MAb (Figure 1). The introduction of another N-linked glycan at position 448 in COT6-V295N resulted in a virus that was marginally more sensitive to 2G12. We also determined that an N-linked glycan at position 442, common amongst subtype C viruses, influenced the sensitivity to 2G12 since COT9-V295/S442N showed the highest sensitivity to 2G12. Conversely deletion of this glycan in COT6-V295N/S448N/N442Q resulted in a loss of sensitivity to 2G12. Collectively our results indicate that the epitope recognized by the MAb 2G12 is not readily reconstituted on a subtype C envelope and the requirements for the binding and neutralization of this antibody may differ from what has been described for subtype B viruses.

NEUTRALIZING ANTIBODY RESPONSES IN ACUTE HIV-1 SUBTYPE C INFECTION

The study of the evolution and specificities of neutralizing antibodies during the course of HIV-1 infection may be important in the discovery of possible targets for vaccine design. In this study we assessed the autologous and heterologous neutralization response in 14 HIV-1 subtype C infected individuals using envelope clones obtained within the first 2 months post-infection. Our data shows that potent, but relatively strain-specific, neutralizing antibodies develop within 3-12 months of HIV-1 infection and these were not related to the clinical status (Figure 2). The magnitude of this response was associated with shorter V1-V5 envelope lengths and fewer glycosylation sites particularly in the V1-V2 region. Anti-MPER antibodies were detected in 4 of 14 individuals within a year of infection, while antibodies to CD4-induced epitopes developed to high titer in 12 participants and in most cases before autologous neutralizing antibodies. However, neither anti-MPER nor anti-CD4i antibody specificities conferred

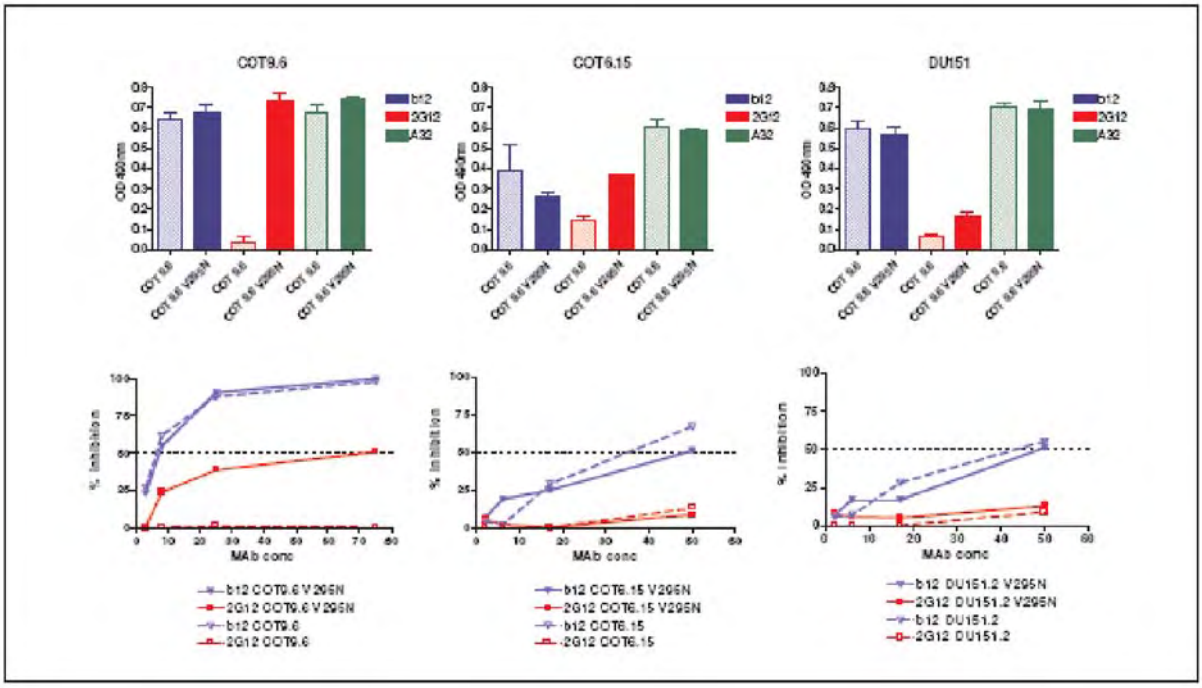


Figure 1. 2G12 binding (top) and neutralization (bottom) of wild-type and V295N mutant envelopes from 3 subtype C viruses. IgG1b12 and A32 are used as control antibodies.

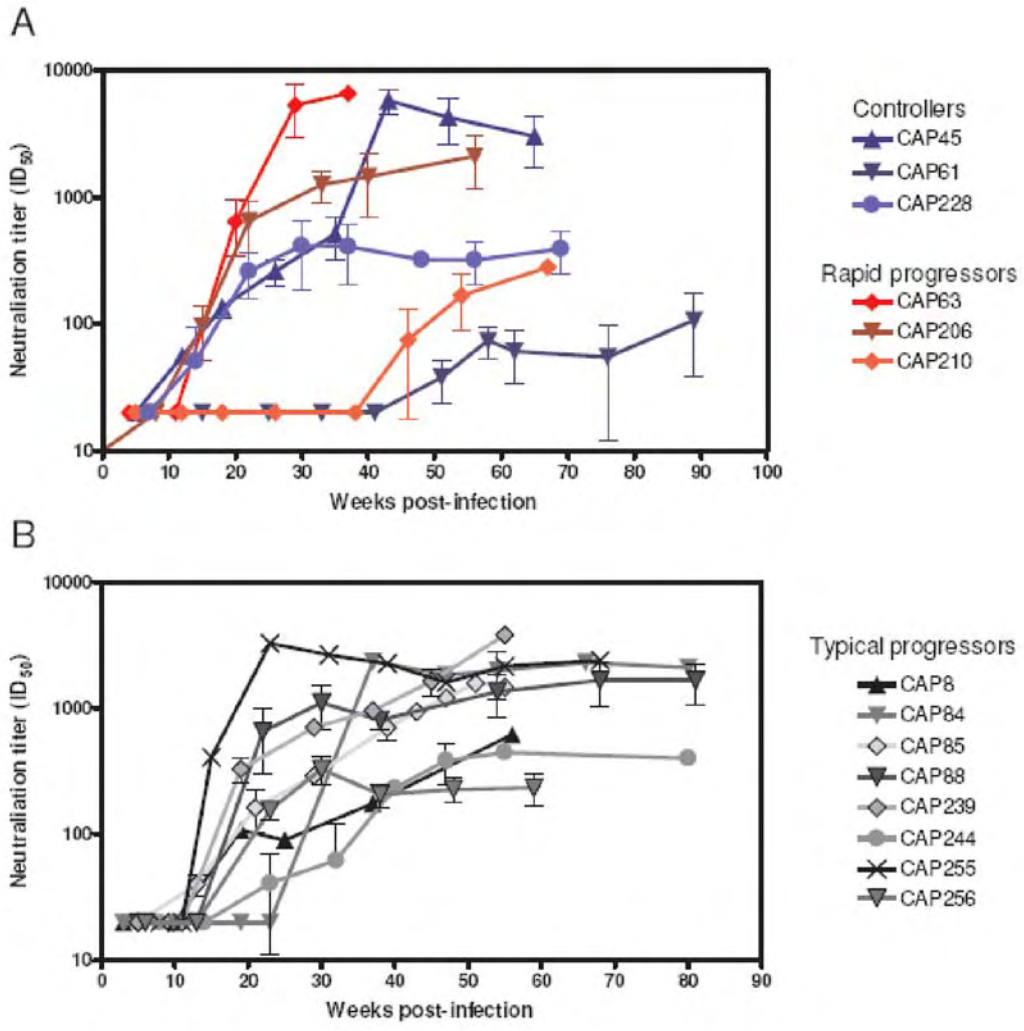


Figure 2: Autologous neutralizing antibody titers among 14 HIV-1 subtype C acutely infected individuals defined as controllers or rapid progressors (A) or typical progressors (B)

neutralization breadth. These data provide insights into the kinetics, potency, breadth, and epitope specificity of neutralizing antibody responses in acute HIV-1 subtype C infection. *This paper is in press at Journal of Virology*

ANTIRETROVIRAL DRUG RESISTANCE SURVEILLANCE AMONG DRUG-NAIVE HIV-1 INFECTED INDIVIDUALS IN SOUTH AFRICA

Surveillance for transmitted HIV-1 drug resistance was conducted among drug-naïve HIV-1 infected pregnant women in South Africa, where single dose nevirapine (NVP) has been in use since 2001 and a national antiretroviral treatment program started in 2004. All subjects were from the Gauteng Province and were part of the 2002 and 2004 annual ante-natal HIV seroprevalence survey conducted by the South African National Department of Health. All subjects met the inclusion criteria as set out by the WHO guidelines for HIV-1 transmitted drug resistance surveillance (women <21 years of age and in first pregnancy). Genotyping was performed on viral RNA by sequencing the protease and reverse transcriptase genes. Samples were also tested for the K103N mutation using a highly sensitive allele-specific real time PCR assay (ASC-PCR). Of 128 eligible participants from 2002, 65 (51%) were successfully amplified. None of the samples from 2002 had evidence of resistance mutations on genotyping or by ASC-PCR. Of 117 eligible participants from 2004, 48 (41%) samples were successfully amplified. Of these, one had T69D and one had the K70R resistance mutation, to give a total of 2/48 (4.2%) participants with evidence of resistance mutations by genotyping. One sample was also positive for K103N by ASC-PCR. All samples clustered phylogenetically with HIV-1 subtype C, the predominant subtype circulating in South Africa. Using the threshold survey, resistance prevalence overall and for each drug class in 2002 and 2004 was <5% for the Gauteng province of South Africa. The detection of a low frequency of resistance mutations in the 2004 survey suggests that surveillance should be conducted annually among untreated populations to determine if this increases with time. *This work is in press at Antiviral Therapy.*

THE HUMAN EXPERIMENTAL MODEL OF MATERNAL-INFANT HIV TRANSMISSION EXTENDS OUR UNDERSTANDING OF PROTECTIVE IMMUNITY TO HIV

HIV vaccine researchers have hedged their bets widely concluding that the likely immune responses capable of preventing HIV are a combination of virus-specific neutralizing antibodies and cell-mediated immune responses ($CD4^+$ and $CD8^+$ T cell responses). It stands to reason that innate immune responses should be essential too, given that they are the first to act upon initial encounter with HIV and on subsequent recall. Furthermore, it is the innate immune system that instructs the development of adaptive immunity. It is therefore somewhat surprising that components of the innate immune system have received so little attention in relation to their role in HIV protective immunity. To unravel the complexities of innate immunity, the maternal-infant model is particularly useful; in part because of the central place of innate immunity among newborns, and primarily because HIV-exposed and unexposed babies can so easily be compared to examine the effects of past exposure. In adults, these comparisons are compromised as the role of other behaviors and infections on these non-specific responses is difficult to control.

CC chemokines, which include CCL3, CCL4 and CCL5, have assumed center-stage in study of innate immunity because of their ability to block entry of HIV strains that use CCR5 as a co-receptor. However, it is likely that their role is more complex, and indeed may provide fundamental support of effective adaptive responses. The CC chemokines, on the basis of their unique immunomodulatory properties represent ideal candidates for fine-tuning immune responses because of their ability to attract and activate specific cells at the site of immunization. Recent published data on gene copy number of CCL3-L1 (Gonzalez et al. 2005. Science 307: 1434-1440) together with our own work on CCL3/CCL3-L1 in maternal-infant transmission of HIV-1 (Meddows-Taylor et al., J Gen Virol 87: 2055-2065) highlights the importance of CCL3/CCL3-L1 in protective immunity to HIV-1. Our data has shown that infants who are deficient producers of CCL3/CCL3-L1 (demonstrated to be genetically encoded) are at increased risk of becoming HIV-1 infected through vertical transmission. A desired immune response to HIV-1 would therefore be sufficient production of CCL3/CCL3-L1 at the site of HIV-1 encounter. It is imperative to delineate the precise roles of CCL3/CCL3-L1 in the immune response to HIV, aside from its noncytolytic inhibitory effect on HIV-1 as the ability of CCL3/CCL3-L1 to drive adaptive immunity might be the crucial factor in overall protection.

How might altered production of CCL3 achieve protection from HIV infection? We have proposed that one or more processes may be involved that include their antiviral activities, their roles in acute inflammation and enhancement of adaptive immune responses (complexities in structure of the genes and the role of the expressed proteins and implications for HIV vaccine research are discussed in Tiemessen and Kuhn, Nature Immunology, in press). We propose that given the immune enhancing effects of CC chemokines, that host genotype of CCL3/CCL3-L1 in particular, will differentiate between individuals with poor and good "natural adjuvant" abilities and so identify poor from good vaccine responders. There are major questions raised in light of these recent findings - how we would design HIV vaccines, how we identify individuals that would be poor responders to vaccines (deficient CCL3/CCL3-L1 producers), how we overcome loss-of-function that is genetically encoded, and how we find molecules that can compensate for this loss-of-function. Innate immunity needs to become a more integral component of studies on HIV vaccines, as

understanding the interplay between innate and adaptive immunity may hold the key to understanding what constitutes protective immunity to HIV. Studies of uninfected infants born to HIV-infected mothers offer a unique human experimental model to extend our understanding of these phenomena.

CELLULAR IMMUNITY

BREADTH AND MAGNITUDE OF HIV-SPECIFIC T CELL RECOGNITION AT THE ACUTE STAGE OF SUBTYPE C INFECTION DOES NOT CORRELATE WITH VIRAEMIA

To date most studies in human HIV-1 infection focus on HIV-1 specific T cell responses in chronic infection, and few data exist examining responses induced in acute infection. We hypothesized that the breadth and magnitude of HIV-specific T cell responses within weeks of primary infection correlates with viral control. We followed a cohort of 24 acutely subtype C HIV-1 infected subjects for the breadth and magnitude of T cell responses with interferon gamma ELISPOT assay using a set of 396 overlapping subtype C peptides. Subjects were followed for the first 3-6 months of infection. The median time from infection in this cohort was 6 weeks. Of the detected HIV-specific T cell responses across the genome, 74% of responses recognised epitopes in Nef, 37% in Pol, 36% in Env, 34% in Gag, 29% in Vif, 25% in Vpr, 17% in Rev, 16% in Vpu and 6% in Tat. Overall the highest cumulative magnitude of HIV-specific T cell responses was directed towards the central region of Nef, between amino acid 52 and 171. Within the central region of Nef, the most frequently recognised epitope regions were PGPGVRYPLTFGWCF (Nef peptide 33) and VRYPLTFGWCFKLVP (Nef peptide 34). Neither the breadth (1-12 peptides, r= -0.03010, ns) nor the cumulative magnitude (145-21170 spu/10^6 PBMC, r = 0.1618, p=0.0883) of the total HIV-specific T cell responses correlated with plasma viral load or CD4 T cell counts from week 1-12 of infection. There was also no correlation between the hierarchy of HIV-specific T cell responses and viraemia. From our preliminary data at this stage, this would suggest that high magnitude and recognition of multiple epitopic regions is unrelated to early viral control. These data also reflect that Nef immunodominance is established during the acute stage of subtype C infection.

QUANTITATION OF CD4+ AND CD8+ T LYMPHOCYTE PROLIFERATION AT THE SINGLE CELL LEVEL USING CFSE STAINING AND FLOW CYTOMETRY

Carboxyflourescein diacetate succinimidyl ester (CFSE) is currently the best reagent available for the analysis of cellular proliferation. CFSE diffuses freely into cells where intracellular esterases convert it into a fluorescent, membrane-impermeant dye. CFSE does not adversely affect cellular function and is not transferred to adjacent cells. When cells divide, CFSE labeling is distributed equally between daughter cells, resulting in a serial halving of fluorescence intensity with successive generation, which can be readily followed by flow cytometry. Use of monoclonal antibodies conjugated to compatible fluorochromes allows immunophenotyping of the dividing cells. We wished to optimize a CFSE proliferation assay for the quantitation of CD4+ and CD8+ T cell proliferation from isolated peripheral blood mononuclear cells (PBMC). Flow cytometric analysis of CFSE-stained lymphocytes incubated with a stimulatory anti-CD3 monoclonal antibody (12F6) revealed serial halving of fluorescence being proportional to cell division. Control unstimulated cultures exhibited no stepwise fluorescence loss. Proliferation analyses using FlowJo software (Treestar) resolved 8 T cell generations with means of 44.2% CD4+ and 56.8% CD8+ T cells dividing in HIV-negative samples, compared with 27.6% CD4+ and 36.8% CD8+ T cells in an HIV-positive sample (Figure 3). In an HIV-negative sample that demonstrated a strong response to a pool of 9mer epitopes in CMV, EBV and Flu using the IFNg Elispot assay, revealed 2.7% of CD8+ T cells proliferated and no CD4+ T cell proliferation. This assay has been extended to explore the hypothesis that T cell proliferation to specific HIV antigens correlates with viral control. We found that labelling of CFSE-stained PBMC with anti-CD3 APC, anti-CD4 PerCP Cy5.5 and anti-CD8 PE after 6 days of in vitro incubation at 37 deg C, 5% CO_2 allows the analysis of T cell proliferation at the single cell level. A stimulatory anti-CD3 monoclonal antibody can be used as a positive control for CD4+ and CD8+ T cell proliferation in both HIV infected and uninfected samples and enables a quantitative method of measuring cell proliferation. Thus, in combination with other flow cytometric approaches, the use of CFSE can provide insight into the kinetics of cell proliferation and cytokine expression at the single cell level.

FUNCTIONAL AND PHENOTYPIC CHARACTERISTICS OF CD8+ T CELLS TO DOMINANT AND SUBDOMINANT EPITOPES IN SUBTYPE C HIV-1 INFECTED INDIVIDUALS

A robust cytotoxic T lymphocyte CTL response has been shown to play a critical role in the control of viral replication and slower disease progression in HIV-1 infection. Studies have focused on HIV-1 specific-CD8+ T cells secreting IFN- as an indicator of CTL response using the ELISpot assay, however, the ability and the capacity of responding CTL to function effectively and efficiently in destroying infected cells requires further examination. Our aim was to determine functional and phenotypic profiles of HIV-1 specific CD8+ T cells from chronically HIV-1 subtype C infected individuals using intracellular cytokine staining (ICS) and flow cytometry (Figure 4).

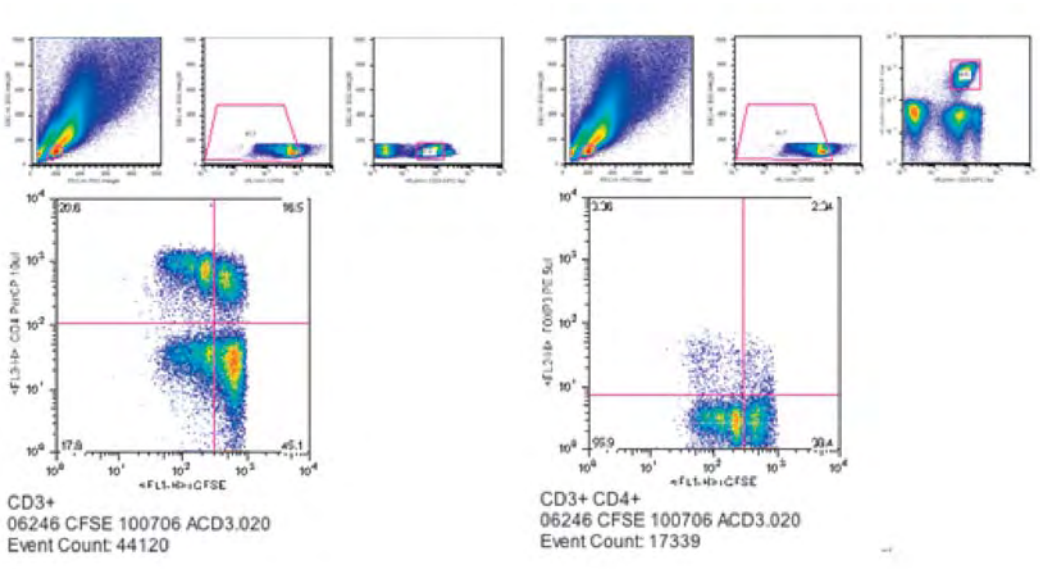


Figure 3. A representative flow cytometry plot of the gating strategies used to identify CFSE staining and daughter cell proliferation after anti-CD3 stimulation and then applied to measuring CD4+FoxP3+ Treg cell division.

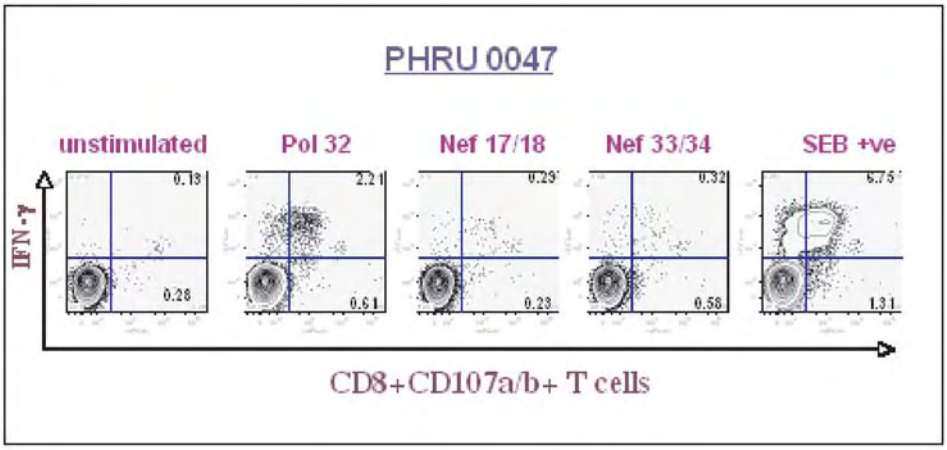


Figure 4. A representative plot of CD8+ T cell degranulation in response to HIV peptides showing differential ability to degranulate between epitopes in Pol and Nef.

Single reactive peptides across the subtype C proteome were identified in 33 chronically HIV infected individuals using the IFN- ELISpot assay. Using multi-parameter ICS flow cytometry, antigen-specific CD8+ T cells were assessed for functional ability to express IL-2, IFN- and CD107a/b (degranulation). Responses were correlated with CD4 counts, range: 305-1367.4 cells/mm^3 There was preferential targeting of epitopes in Nef 75.8%, Gag 67%, Pol 48.5%, Env 33% and Tat, Rev, Vif, Vpr, Vpu (TRVVV) 30% by ELISpot assay. We identified, using ICS, CD8+ T cells that were IFN-+IL-2- expressing confirming epitope recognition in Vif, Env, Nef and Gag (range: 0.144% - 1.274%). Recognition of the less frequently targeted epitopes in Vif and Env resulted in IFNg+CD107a/b+ degranulating cells (Figure 4). We have identified a phenotype of antigen-specific CD8+ T cells that expressed IFN- and functionally possessed cytolytic potential to less dominantly recognized epitopes. Our data suggest that CD8+ T cells that recognize sub-dominant epitopes in subtype C HIV-1 infection may have greater functional capacity than those recognizing immunodominant epitopes.

CHARACTERISATION OF REGULATORY T CELLS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND TUBERCULOSIS (TB) INFECTED ADULTS IN SOUTH AFRICA

Regulatory T cells (Tregs) are CD4+ T cells expressing the gene *FOXP3* that suppress other cellular immune responses. We measured the frequency of Tregs in HIV and TB infected patients in South Africa. Fresh peripheral blood mononuclear cells were isolated from patients with HIV, with HIV and TB co-infection, with TB infection alone and from healthy controls (n=35). The frequency of FoxP3 positive cells was measured by flow cytometry before and after 96 hr culture with anti-CD3, purified protein derivative (PPD), tetanus toxoid, HIV-1 derived Gag and Nef peptide pools. We examined CD4 and CD8 gamma interferon responses and CD4, CD8

and Treg proliferation. We found elevated levels of Tregs in the peripheral blood of HIV patients (median 9%, range 5.03-26.27%) compared with control (median 3.55%, range 2.07-5.60% p=0.0097). There was no significant difference between control and TB infected patients. Treg levels were inversely correlated with CD4 count. CTLA-4 and GITR frequency correlated with FoxP3 frequency with no significant difference between groups. Overall, these data show that FoxP3+ CD4+ Treg cells are elevated in HIV-infected patients and are related to lower CD4 counts, thereby possibly contributing to clinical immune suppression.

HLA

ASSOCIATIONS BETWEEN HIGH RESOLUTION CLASS I HLA ALLELES AND DISEASE STATUS IN INDIVIDUALS FROM SOUTHERN AFRICA INFECTED WITH HIV-1 SUBTYPE C.

Genetic variation at the Human Leukocyte Antigen (HLA) Class I loci plays an important role in determining host immune response to human immunodeficiency virus type 1 (HIV-1) infection. A cohort of 127 HIV-1 subtype C-infected was studied to identify HLA associations with HIV viral loads and CD4 counts. HIV+ individuals from southern Africa were recruited as part of the HIVNET 028 and CAPRISA studies. Comparative HLA data were from an HIV- ESKOM cohort. HLA-A and HLA-B genotyping was performed at high resolution using DNA sequencing. Viral loads were determined using commercial kits. CD4 counts were measured using the BD FACSCOUNT assay. Viral loads and CD4 counts were stratified as high, medium or low using set criteria.

A total of 32 different HLA-A and 34 different HLA-B alleles were observed. The most common HLA-A alleles in this HIV+ cohort were A*2301 (10.3%), A*3002 (9.9%) and A*6802 (8.7%), and the most common HLA-B alleles were B*5802 (11.8%), B*1503 (11.0%), B*4403 (11.0%) and B*1510 (9.8%). Alleles A*3001 and B*1801 were significantly more frequent in HIV- samples than in HIV+ samples (P<0.05). Allele A*2902 was significantly associated with low viral load (<10 000 RNA copies/ml) and alleles A*4301, A*0101 and A*2902 were significantly associated with high CD4 counts (>500). Therefore these Class I alleles may have a protective role in host immunity to HIV infection. Alleles A*6601 and B*4403 were significantly more frequent in HIV+ samples than in HIV- samples (P<0.05). Alleles A*3004, B*0801, B*5801 and B*4101 were significantly associated with high viral loads (>50 000 RNA copies/ml), whilst alleles A*2301, A*3004 and B*0801 were significantly more frequent (P<0.05) in individuals with low CD4 counts (<250); these alleles may represent potential susceptibility variants. We have identified HLA Class I alleles that significantly associate with control of HIV viraemia and higher CD4 counts. These unique associations underscore the different HLA distribution profile of individuals in southern Africa.

OPERATIONAL ACTIVITIES

2006 was a very busy year with regards to our training and operational activities. Training was performed by our lab members at both local and international centres (including sites in the USA, UK, Uganda, Malawi, Tanzania and Zimbabwe). Training took place for the following methods: HLA typing, PBMC isolation, cryopreservation, ELISPOTs, shipping, sample handling, usage of Laboratory Information Systems, and quality control.

Greg Khoury and Sarah Cohen were appointed by the HVTN as project co-ordinators for the HVTN 503 trial, where the NICD will represent the primary laboratory and operations hub for South Africa's first HIV vaccine efficacy trial.

CHAVI International Sample Repository
A sample processing and data management workshop was held in January 2006 for all laboratory sites involved in CHAVI studies. The course was attended by participants from South Africa (Johannesburg, Durban and Klerksdorp), Uganda (Entebbe), Malawi (Lilongwe and Blantyre), Tanzania (Moshi), USA (Durham, NC) and the UK (Oxford and London). The programme included a 2 day wet workshop in the laboratory where participants were trained in the theoretical and practical aspects of PBMC isolation, cryopreservation, shipping and sample handling and a 1 day training workshop for the Laboratory Information System, LDMS. Thereafter additional follow-up training was provided to each of these sites before they were activated to begin CHAVI clinical trials. The sample repository has begun to receive samples from 6 of these clinical sites with the initiation of the CHAVI001 protocol.

HVTN Central Laboratory and Sample Repository
The Repository was involved in staff training, qualification and site initiation at the Perinatal HIV Research Unit (Soweto), Contract Laboratory Services (Cape Town), Aurum Health Research (Klerksdorp), Medunsa (Pretoria), and CAPRISA (Durban) in preparation for Phase II vaccine trials. The HVTN 204 trial began enrolment in July at the Johannesburg, Cape Town and Klerksdorp sites with the sample repository receiving weekly shipments of samples from these sites for long-term storage. The HVTN 503 trial is scheduled to start in January 2007. The sample repository has initiated a quality control programme for ensuring sample integrity and viability for PBMC samples isolated and cryopreserved at these sites.

VISITORS

Visiting students
Victoria Eastham is a University of Bath Master of Biochemistry Undergraduate currently visiting for undertaking a six-month work placement at the HIV Immunology Laboratory as part of her studies. Victoria began at the NICD in February and is developing a

carboxyflourescein diacetate succinimidyl ester (CFSE) proliferation assay for the monitoring of CD4+ and CD8+ T lymphocyte proliferation in response to HIV peptides. This will be an important assay for measuring vaccine-induced T and B cell responses.

Dr Melinda Suchard is a Clinical Pathology Registrar doing the research component of her MMed degree. The focus of her research is the role of regulatory T cells (Tregs) in HIV. Her work to date has included establishing the methods for quantitative detection of Tregs for use in this laboratory. This involves primarily flow cytometry with intracellular cytokine staining.

Dr Elizabeth Mayne is a Registrar from the Department of Haematology and Molecular Medicine who is visiting the HIV Immunology Laboratory on research rotation. Elizabeth is currently developing a method for accurate quantitation of regulatory T cells using flow cytometry as part of her Master of Medicine Degree. After validation of the assay, she intends to use it to quantify the response of regulatory T cells to active infection with Mycobacterium tuberculosis both before and after commencement of standard therapy and in the presence and absence of co-infection with HIV in the hope of a better understanding of the immunoevasive strategies utilized by these pathogens.

Visiting scientists

In early May 2006, Dr Guido Ferrari from Duke University spent two weeks in the laboratory helping to optimize assays and experiments for CHAVI.

The NICD was visited by Drs Julie McElrath and Pat D'Souza on 23 January 2006, where the NICD is the only regional Central Lab for measuring immunogenicity and end-points. Discussions and plans were made for South Africa's first HIV efficacy trial and the role of the NICD for measuring humoral, T cell and viral endpoints.

Dr Catherine Riou started working in Prof Gray's lab in November 2006. Dr Riou is a post-doctoral fellow from Dr Rafick-Pierre Sekaly's lab, CHUM Saint-Luc, University of Montreal. She will be staying a year as a visiting scientist at the NICD and will work on assessing phosphorylation profiles in antigen-specific T cells and mentoring staff.

Visitors who attended the Becton Dickinson Biosciences LSR II Multicolor Flow Cytometry Workshop from 27 November to 1 December 2006 workshop were Jennifer Serwanga from Uganda, Nazma Mansoor from UCT and Michael Liu from Oxford University.

Dr Ron Veazey gave a talk at the NICD on 23 November 2006 and was a visitor invited to attend the ASSAf Nutrition Panel the previous day. He gave a talk on the HIV/SIV pathogenesis and the role of Gut Immunity.

CONFERENCES, WORKSHOPS AND MEETINGS

Prof. Lynn Morris participated in a Bristol-Myers Squibb Secure the Future Learning and Sharing Conference held on 19 March in Johannesburg. The title of her talk was: HIV drug resistance in resource-limited settings.

Sibusiso Nkosi attended the AAVP HIV Immunology Workshop from 18-19 March in Dakar, Senegal and gave 2 oral presentations: (1) The current status of B cell immunology and antibody correlates of protection; and (2) Assays to measure neutralizing antibodies in HIV infected individuals, vaccinated animals and in vaccinees.

Elin Gray attended the Keystone Symposia in Keystone, USA from 27 March 2 April and presented a poster entitled: Restoration of glycan 295 in subtype C viruses partially renders sensitivity to 2G12 neutralization.

Mia Coetzer attended the HIV Dynamics and Evolution Meeting from 1 7 April in Woods Hole, USA and gave a poster presentation: Molecular and biological heterogeneity in sequential HIV-1 subtype C isolates from a patient that acquired the ability to use CXCR4.

Kabamba (Alex) Alexandré attended the Microbicides 2006 Conference from 23 26 April in Cape Town where he presented a poster: Sensitivity of HIV-1 subtype C viruses to Griffithsin and Scytovirin: potential HIV microbicides.

Prof Lynn Morris and Sarah Cohen attended the HIV Vaccine Trials Network (HVTN) Full Group Meeting from 22 24 May in Washington DC, USA.

Prof Lynn Morris attended the HIV Drug Resistance Meeting from 13 16 June in Sitges, Spain.

Prof Lynn Morris, Dr Visva Pillay, Dr Adri Basson, Dr Gillian Hunt, Johanna Ledwaba, Mpho Rakgotho and Sarah Cohen attended the Southern Africa Treatment and Resistance Network (SATuRN) planning Meeting from 4 6 July at NICD.

Prof Lynn Morris, Dr Penny Moore, Elin Gray, Isaac Choge and Natasha Taylor-Meyer attended the AIDS Vaccine 2006 Conference from 29 August 2 September in Amsterdam, Netherlands. Presentations included:

- PL Moore, ES Gray, IA Choge, N Leseka, K Mlisana, SS Abdool Karim, C Williamson, L Morris and the CAPRISA 002 Study Team. Neutralization profiles of HIV-1 subtype C viruses from acute infection (Oral presentation).
- J Binley, PL Moore, E Crooks, J Robinson, M Franti, L Morris, D Richman and D Burton. Mapping the neutralizing and non-neutralizing fractions of plasmas from HIV infected donors (Oral presentation).

- ES Gray, PL Moore, IA Choge, T Meyers and L Morris. Characterisation of naturally occurring 4E10 resistant viruses in a subtype C HIV-1 infected child (Poster presentation).
- IA Choge, PL Moore, N Leseka, ES Gray, F Treurnicht, K Mlisana, SS Abdool Karim, C Williamson, L Morris and the CAPRISA 002 Study Team. Generation of functional envelope clones from HIV-1 subtype C plasma RNA for use in pseudovirion assays (Poster presentation).

Prof Lynn Morris and Natasha Taylor-Meyer attended a NeutNet meeting in Amsterdam on the 2 September where Natasha gave a talk: Validation of the pseudovirion neutralization assay.

Prof Lynn Morris, Dr Penny Moore and Elin Gray attended the Annual Center for HIV/AIDS Vaccine Immunology (CHAVI) Meeting from 3-6 October in Durham, North Carolina, USA. Talks were given by Dr Moore: Evolving neutralizing antibody response in acute HIV-1 subtype C infection; and Elin Gray: Anti-MPER antibody responses in subtype C acute HIV-1 infection.

Dr Visva Pillay gave a talk at a CDC/PEPFAR Meeting on 3 October at Centers for Disease Control (CDC), Pretoria entitled: HIV-1 Antiretroviral Drug Resistance Surveillance.

Sarah Cohen attended the HIV Trials Network (HVTN) Conference from 18-20 October in Seattle, USA.

Prof Lynn Morris attended the Vaccinology 2006 Conference from 23-24 October in Hermanus and gave an oral presentation: Antibody responses to candidate HIV vaccines.

Prof Lynn Morris attended the First African Structural Biology (FASB) Conference from 25-27 October in Wilderness, South Africa.

Prof Lynn Morris attended the Full Group Meeting for the Collaboration for AIDS Vaccine Discovery/Comprehensive Antibody Immune Consortium (CAVD/CA-VIMC) from 15-16 November in Durham North Carolina, USA. She gave an oral presentation on the NICD Regional Humoral Immunity Laboratory.

Prof Caroline Tiemessen attended the 13th Conference on Retroviruses and Opportunistic Infections, Denver, Feb 5-9, 2006 and presented a poster entitled "Reduced ability of newborns to produce CCL3 is associated with increased susceptibility to perinatal HIV-1 transmission".

Prof Caroline Tiemessen was invited to give a Session Lecture entitled "CC chemokines and protective immunity : insights gained from mother-to-child transmission of HIV-1" at the 27th African Health Sciences Congress, Durban, December 3-6, 2006.

Samantha Donninger attended the MCBG Symposium, Witwatersrand University, Johannesburg, 5 October, 2006,and gave an oral presentation (for which she was awarded second prize) entitled "CCL3L1 gene copy numbers in South African populations, and associations with maternal-infant transmission of HIV-1".

Prof Gray attended a 2 day workshop (14-15 January) at the Weatherall Institute of Molecular Medicine at the John Radcliffe Hospital, Oxford, to discuss details of the flow cytometric analysis to identify T cell epitope-specific responses in the CHAVI consortium. The NICD is to play a lead role in this analysis along with Duke University, where joint scientific hypotheses are being explored with Oxford University.
Prof Gray was invited to attend the scientific review panel for the European Commission STREP proposals on HIV and AIDS from 16-18 January. These are innovative grants from European partners.

Prof Clive Gray and Mandla Mlotshwa attended the CAPRISA Scientific Advisory Board meeting on 19-21 April 2006. Data was presented showing T cell responses during the acute stage of infection in 24 enrolled HIV positive individuals.

Dr Vivian Morafo and Prof Clive Gray attended the SAAVI induction meeting on 30 May 2006. Vivian is to head the SAAVI National Working Group on Immunology.

A second SAAVI meeting 19-20 July 2006 was attended by Prof Clive Gray, Dr Vivian Morafo, Dr Debra de Assis Rosa and Mandla Mlotshwa.

Mandla Mlotshwa and Netty Malatsi attended the XVI AIDS Conference, Toronto, Canada (13-19 August 2006) and presented posters as follows:

- Mandla Mlotshwa, Greg Khoury, Francois van Loggerenberg, Koleka Mlisana, Carolyn Williamson, Salim Abdool Karim and Clive M Gray and CAPRISA 002 study team. Breadth and magnitude of HIV-specific T cell recognition at the acute stage of subtype C infection does not correlate with viraemia.
- Netty O. Malatsi, Galit Alter, Greg Khoury, Stephina Nyoka, Debra Barkhan, Marcus Altfeld, and Clive M. Gray. Novel CTL Epitope Identity in the EBNA-3A Region from Epstein Barr Virus Infected Individuals Living in South Africa and USA: Relevance Immunogenicity Measurements.

Vivian Morafo was awarded an African Scientist scholarship to attend the MASIR (Measuring Antigen Specific Immune Responses) conference in Santorini, Greece from 14-17 June 2006. Dr Morafo presented a poster and an oral presentation entitled: "Characterization of CD8+ T cell responses to dominant and sub-dominant epitopes in subtype C HIV-1 infected individuals".

COMPREHENSIVE CARE, MANAGEMENT & TREATMENT PROGRAMME FOR HIV & AIDS

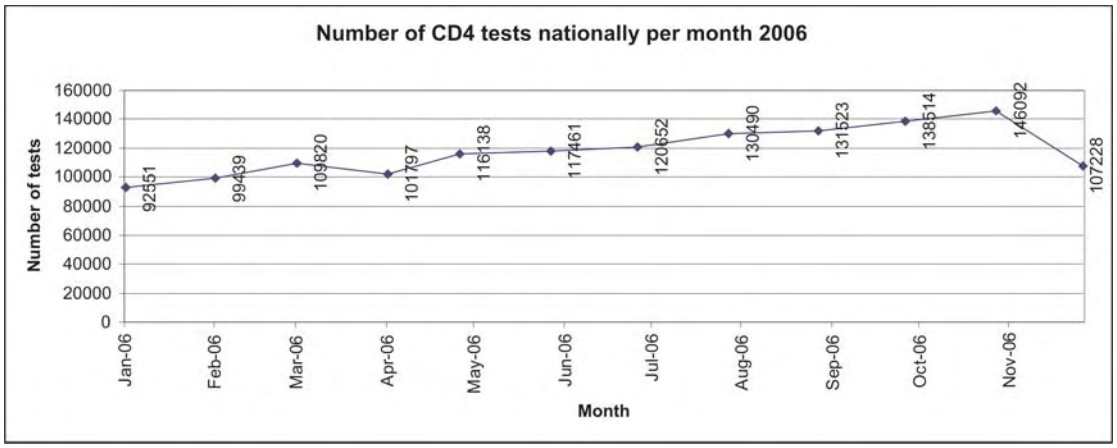
STAFF

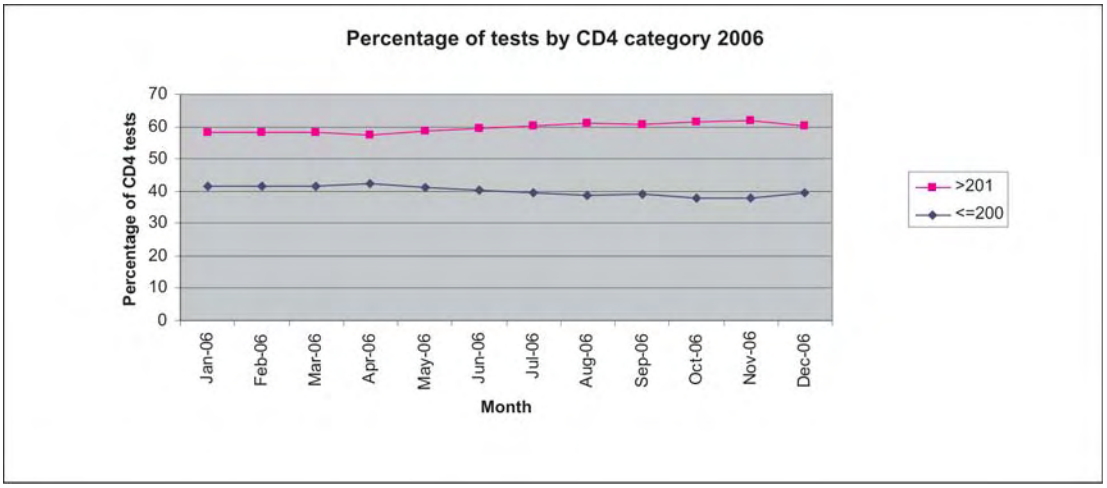
Dr Teresa M Marshall MBBCh FC Path (Virol) DTM&H, Specialist Virologist, Programme Manager
Mr Naseem Cassim Nat Higher Dip Haematology, Project Manager
Mr Riyahd Maheter Bsc (Hons), Admin Assistant

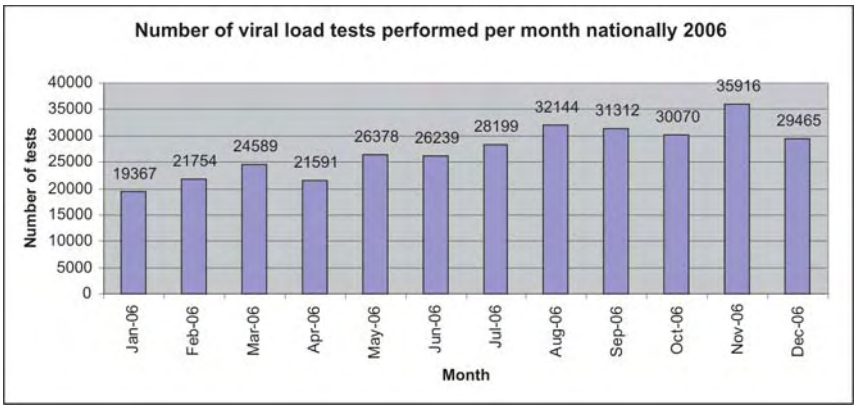
As of December 2006, there were approximately 245 000 people on antiretroviral therapy in the public health sector. There was at least one accredited clinic in each health district, totalling 392 clinics in South Africa administering antiretroviral therapy to people living with HIV infection.

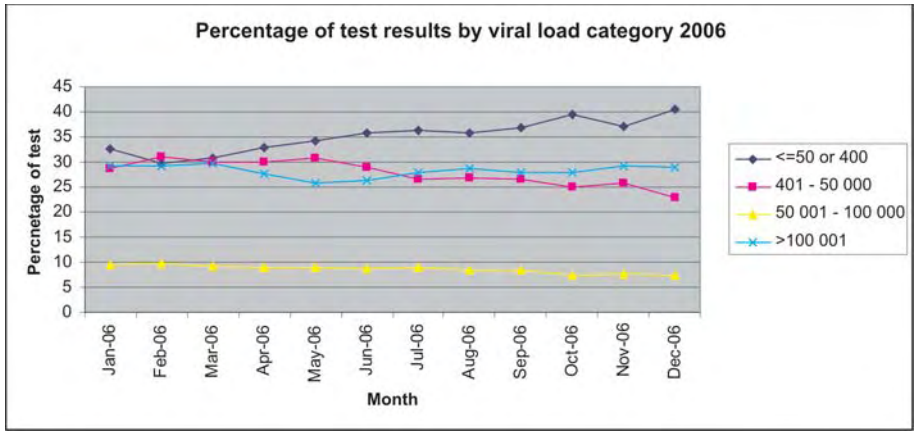
At the start of the programme in April 2004, the NHLS tested 14 135 specimens for CD4 count and percentage. By December 2006, this number had increased to an average of 118 000 CD tests per month. Initially the percentage of people presenting to the services with CD4 counts < 200 cells/mm^3 was in excess of 50%. By December 2006, this had decreased to 40% due in part to patients responding well to therapy, and also greater accessibility of services permitting HIV infected people access to clinics while still enjoying healthier CD4 counts.

In May 2004 we performed 2654 viral load assays in support of this programme, with fewer than 5% of these patients having undetectable viral loads in response to privately acquired treatment. By the end of December 2006, the number of viral load tests performed had increased to an average of 27 200 tests per month. The percentage of viral loads measured that were undetectable had increased to 40%. This is an encouraging response to anti-retroviral therapy.



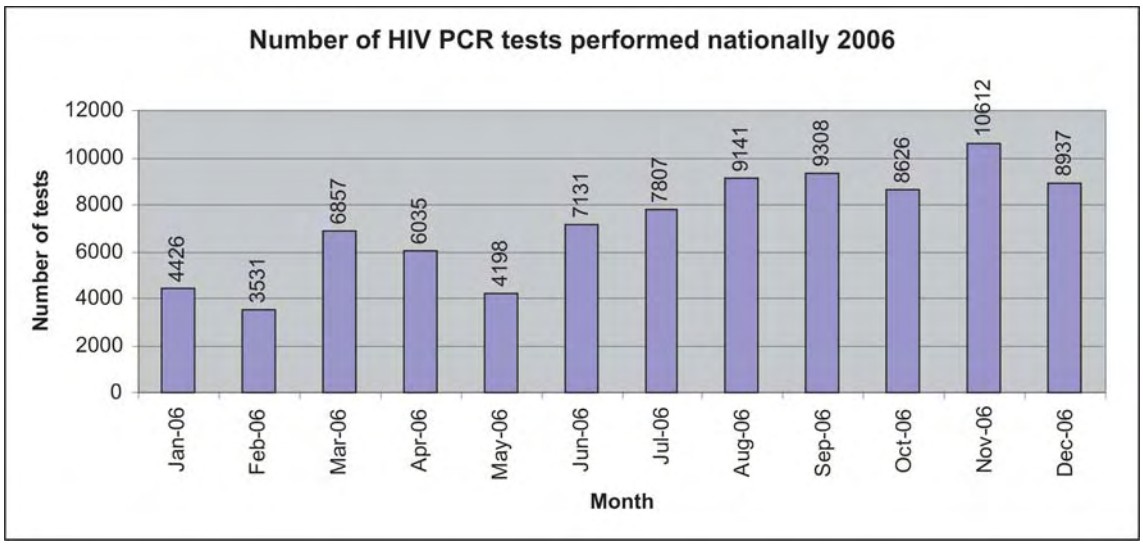


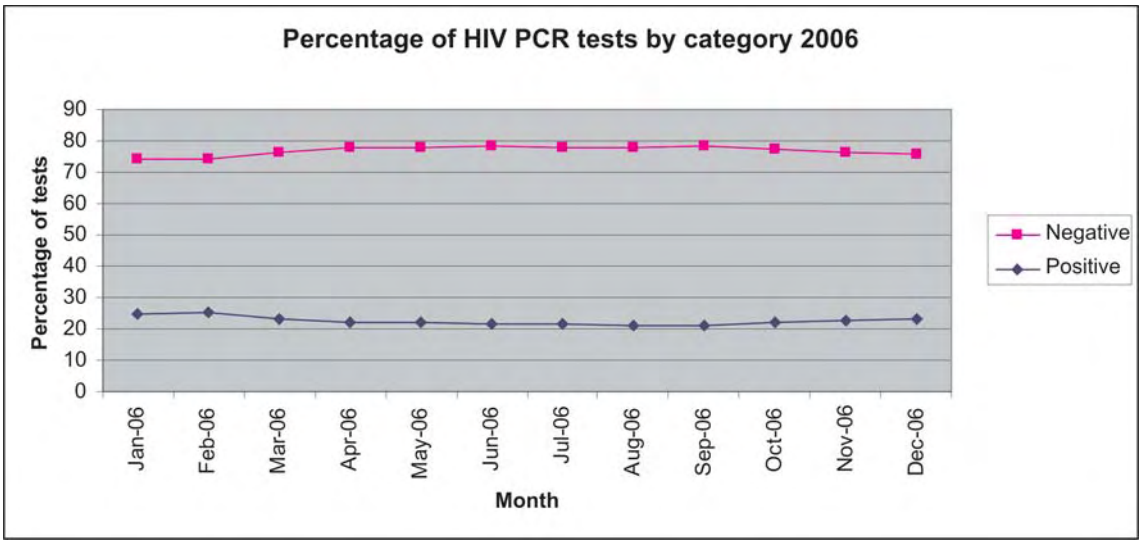




In April 2004, depressingly few children were tested for HIV infection using the HIV PCR assay. Until September 2004, the number tested monthly was fewer than a thousand children. As slightly less than a third of our annual birth cohort is exposed to HIV infection, it was clear that little progress was being made in detecting and managing HIV in children on a national level. As confidence grew in the management of HIV infection and awareness of the needs of children began to increase, so testing became more widely used to the extent that by December 2006, we were performing an average of 7000 tests per month, with some months in excess of 10 000 tests for infant diagnosis. This number is expected to increase in the coming years as PCR testing using dried blood spots is now available in all laboratories in NHLS currently performing this assay. As venipuncture in 6 week old babies is complicated and requires special skills not always available in community care clinics, a simple heel prick and collection of blood on specially designed filter paper immediately lifts some of the limitations previously encountered in children accessing proper care for HIV infection.

The graphs below show an HIV positivity rate of 20% in the assays performed in NHLS. This data is somewhat skewed as testing is performed not only on babies emerging from the pMTCT programme, but also on children entering hospitals who are already ill with HIV related conditions.





This unit is currently ensuring that there are sufficient resources available within NHLS to support the National Department of Health in realizing their goals set forth in the National Strategic Plan for HIV and AIDS 2007-2011.

PRF LIBRARY

LIBRARY STAFF

Mrs Phindile Bekwa B.Bibl.Ed, B:Tech,
Lib & Info, Cert: KM,
Supervisor: Library Services

Ms Antonia Sehunoe,
Library Assistant

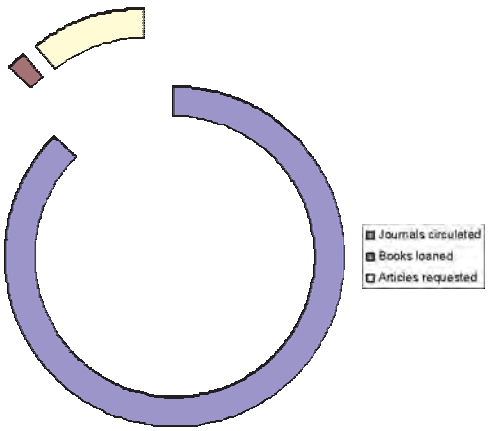
LIBRARY ACTIVITIES

The PRF Library has continued to support the staff with information sources for their administrative and research duties. The year 2006 has seen the library being part of the opening of the PRF Training Centre, which is a success story to all stakeholders. With the facility we can now make a significant contribution to the attainment of the organisational objectives. In August 2006 Ms Antonia Sehunoe was appointed as Library Assistant after the resignation of Ms Yolande Schroder. As part of her responsibility Ms Phindile Bekwa managed to create a sound working relationship with all Library users, and created a conducive environment for study within the library. Four computers were purchased to allow users to access their e-mails and Internet whilst working in the library. We purchased InMagic Library Management System software to improve on efficiency in providing services to our users. Services in the library include journals circulation, inter-library loans, current awareness, and article requests. We have subscribed to 39 journal titles with 5 free online access, including 11 titles that are donated by staff members and other organisations.

STATISTICS

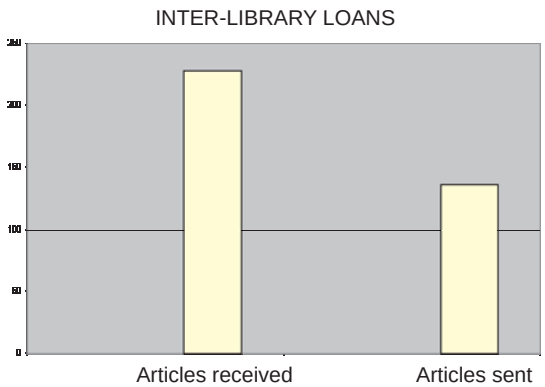
- CIRCULATIONS

Journals	7178
Books	123
Articles requested	891



- INTER-LIBRARY REQUESTS

Articles received from other libraries
228
Articles sent to other libraries
135



CHALLENGES

1. The integration of the services of the NHLS, NICD, and NIOH libraries for resources sharing and to save space, time, and costs. That will assist to cover costs for online subscriptions
2. Automation of the library management system

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