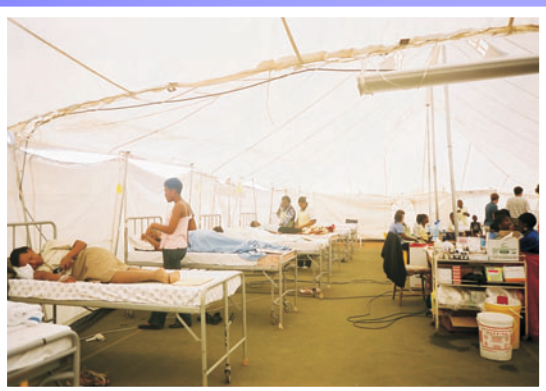
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

of the National Health Laboratory Service



NICD

NATIONAL HEALTH
LABORATORY SERVICE

Annual Report 2005

National Institute for Communicable Diseases

of the National Health Laboratory Service

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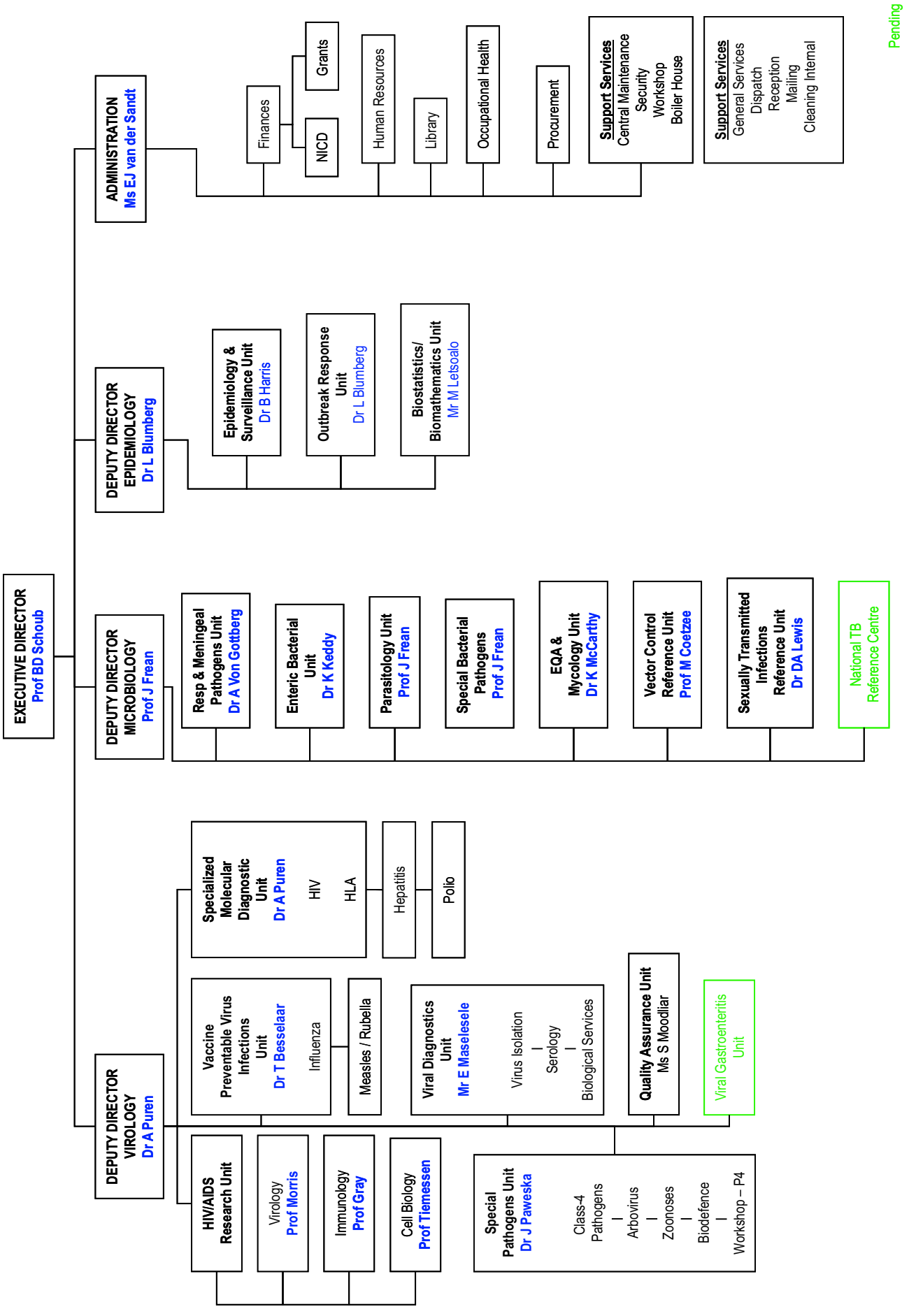
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Biostatistics/ Biomathematics Unit
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Resp & Meningeal Pathogens Unit
Dr A Von Gottberg
Enteric Bacterial Unit
Dr K Keddy
Parasitology Unit
Prof J Frean
Special Bacterial Pathogens
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EQA & Mycology Unit
Dr K McCarthy
Vector Control Reference Unit
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Mr E Maselesele
Virus Isolation
Serology
Biological Services
Quality Assurance Unit
Ms S Moodliar
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Director's Report

Executive Director:
Prof Barry Schoub

A number of exciting and gratifying developments in the growth of the NICD have taken place during 2005. As a public health institution, the NICD can now truly claim to be a world-class facility which can make a very significant contribution to the progressively expanding global network of public health institutions. The NICD is well equipped to be able to supply both National and Provincial health authorities with important data on communicable diseases crucial to the prevention and management of communicable diseases as well as contributing valuable information to international health authorities on the status of these diseases in this part of the world.

During 2005, all the microbiology laboratories were finally re-housed at the Sandringham site, thus completing the integration of all components of the Institute. This occasion was marked by the unveiling of the NICD entrance wall by the CEO of the NHLS, Mr. John Robertson, on 24 November 2005.

Two important developments took place during 2005 which will greatly enhance the Institute's responsibilities for training and capacity building. Firstly, the Poliomyelitis Research Foundation, who have for decades been the cornerstone for funding the development of virology research and capacity building in South Africa, have generously donated funds for the construction of a training centre at the NICD. The NICD has for many years trained scientists and technologists from within the country as well as from outside our borders. Because of the lack of a dedicated facility, training has had to take place in functioning service laboratories and committee rooms and boardrooms have served as seminar rooms. The new training center which will comprise 3 training laboratories (microbiology, virology and molecular technology), 3 seminar rooms, a computer room and a 240-seat lecture theatre, will be known as the PRF Training Centre. Construction will commence January 2006 and should be complete by end of 2006.

A second important training development has been the approval by the CDC (Centers for Disease Control, Atlanta, USA) to site a FELTP (Field Epidemiology and Laboratory Training Programme) at the NICD. The FELTP Programmes which have been established in 33 countries throughout the world are modeled on the highly successful EIS (Epidemiology Intelligence Service) field epidemiology training programme of the CDC. The FELTP course will be a 2-year full-time experiential and didactic course at a master's level and will commence in 2007 for the epidemiology component and 2008 for the laboratory component. During 2006 several short courses will be given.

The staff complement of NICD has grown substantially and at the end of 2005 comprised 203 permanent staff and 93 funded by grants or student bursaries. A number of our laboratories and our staff received awards and academic promotions and they are heartily congratulated on their very fine achievements which have brought great pride to the Institute. These include:-

- The poliomyelitis laboratory of the NICD was presented with the WHO Task Force on Immunization (TFI) Award for 2005 at the 13th TFI African meeting held at WHO Regional office in Brazzaville. The inscription on the plaque reads:- "… for outstanding support to the Polio Eradication Programme in Africa." The laboratory serves as the Regional Reference Laboratory for the African Polio Laboratory Network. NICD was cited as an excellent example of the commitment and success of the Network.
- Prof Maureen Coetzee was promoted to Research Professor, School of Pathology, University of the Witwatersrand
- Prof Caroline Tiemessen was promoted to Reader, School of Pathology, University of the Witwatersrand
- Prof Caroline Tiemessen also was awarded a Wellcome Trust International Senior Research Fellowship for 5 years for a project entitled "Innate and acquired cellular immunity in HIV-seropositive mothers and their infants"
- Prof David Lewis was promoted to Associate Professor at University of the Witwatersrand as well as adjunct member of the IIDMM (Institute of Infectious Diseases and Molecular Medicine, University of Cape Town) and also an Honorary Research Associate at the University of Cape Town
- Prof Lewis was also appointed as the Regional Director for Africa of the International Union against STI's at the world IUSTI meeting in Bangkok in November 2005
- Dr Nelesh Govender of the National Microbiology Surveillance Unit was awarded the Coulter Medal for the Fellowship examinations in October 2005 (The Coulter Medal is awarded to candidates who obtain excellent results in the Fellowship examination of the College of Pathologists of South Africa.)
- Dr Tonie Cilliers graduated with a PhD in June 2005
- Prof Lynn Morris, Head of the HIV/AIDS Research Unit, chaired the 2nd South African AIDS Conference, 2005
- Dr Anthony Smith, Senior Medical Scientist in the NICD's Enteric Diseases Reference Unit, was the winner of the Faculty of Health Sciences (University

of the Witwatersrand) research prize for the best research publication for the year 2005 (carried out while he was employed in the NICD's Respiratory Meningeal Pathogens Research Unit)

Visit by team from the National Institute for Allergy and Infectious Diseases (NIAID), USA to the NICD for the first NIAID/NICD colloquium on infectious diseases May 16-17 2005.

I wish to again record my sincerest thanks to all our loyal and dedicated staff who have contributed so unstintingly to making the NICD the magnificent Institution that it is and that we are all so proud of.

Sincere thanks also to Mrs. Liz Millington for putting the annual report together and Mr. Guy Hall for photographs.

BARRY D SCHOUB
EXECUTIVE DIRECTOR

NICD Microbiology Reference Units

The Microbiology Reference Units had all joined the main body of the Institute at the Sandringham site by the end of 2005. The Microbiology branch of the NICD comprises, in alphabetic order:

Enteric Diseases Reference Unit (EDRU)
Mycology and Quality Assessment Reference Units (MRU, QARU)
Parasitology Reference Unit (PRU)
Respiratory and Meningeal Pathogens Research Unit (RMPRU)
Sexually Transmitted Infections Research Centre (STIRC)
Special Bacterial Pathogens Reference Unit (SBPRU)
Vector Control Reference Unit (VCRU)

Parasitology and Special Bacterial Pathogens Reference Units

Head:
A/Prof John Frean

John Frean, Ntaba Ndou, Leigh Dini, Rita van Deventer

STAFF:

A/Prof JA Frean MBBCh, MSc MMed, DTM&H, Head of Units
L Arntzen Dip Med Tech, MSc, Laboratory Manager
L Dini BSc (Hons) MSc, Laboratory Controller
CA le Roux BSc (Hons), Medical Scientist
N Ndou BSc (Hons), Medical Scientist
M Setshedi Dip Biotech, Biotechnologist
JMG van Deventer Dip Med Tech (Parasitol), Medical Technologist
J Mathebula, Laboratory Assistant

PARASITOLOGY REFERENCE UNIT

CURRENT RESEARCH PROJECTS:

Survey and management of drug resistant *Pneumocystis jiroveci* pneumonia in South Africa

The project is a collaboration between the NICD and the Swedish Institute for Infectious Disease Control (SMI) in Sweden. Other institutions participating in this research programme are the Chris Hani Baragwanath, Tambo Memorial, and Helen Joseph Hospitals in Gauteng province, and the Karolinska Institute in Stockholm. 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. Sources of funding: Swedish-South Africa Health forum; Medical Research Council of South Africa

Assessment of malaria parasite load using digital image analysis

The load of parasites in a patient with falciparum malaria is regarded as a useful indicator of the severity of infection, correlating broadly with clinical features and prognosis. In addition, serial parasitaemia estimations give a useful objective indication of response to treatment. Recently we have investigated measurement of parasite load by means of image

analysis. Good correlation with careful manual counting is possible, especially when moderate to high numbers of parasites are present; this is precisely the situation in which manual counts tend to be difficult to perform accurately and speedily. This work has potential to substantially improve routine laboratory performance in malaria parasite load estimations, and inform clinical assessment of patients accordingly.

A study comparing the performance of 2 rapid malaria kits was completed.
A survey of stool parasites in children from the Western Cape was completed in collaboration with Stellenbosch University.
A study comparing the performance of 2 immunofluorescent antigen kits for *Pneumocystis* was completed.

TRAINING AND EXTERNAL QUALITY ASSESSMENT ACTIVITIES:

Unit staff taught the following courses:

- 2nd Workshop on Parasite Identification, Hobart, Tasmania; 11-13 November 2005; in conjunction with Professor John Goldsmid, University of Hobart Clinical School (J Frean)
- Lectures and practicals for the postgraduate Diploma in Tropical Medicine and Hygiene of the University of the Witwatersrand (all Unit staff)
- Graduate Entry Medical Programme for 3rd year medical students of the University of the Witwatersrand (J Frean)
- Registrar training course, NICD, March & September (all Unit staff)
- BSc student practicals in parasitology in September & October (R van Deventer)
- Laboratory training course in stool & urine parasite identification in November (all Unit staff)
- Coordinated 2 national Parasitology EQA surveys three times a year: Stool and Urine Parasites (159 participants) and Blood and Tissue Parasites (132 participants) (L Dini)
- Provided the first survey of the Malaria microscopy EQA programme for WHO AFRO in September (L Dini, R van Deventer)
- Trained 3 delegates from EMRO countries in the production and provision of a malaria EQA programme in April (L Dini, R van Deventer)

INTERNATIONAL MEETING PARTICIPATION:

Regional Scientific Meeting of the Australasian College of Tropical Medicine in Hobart, Tasmania, 11-13 November 2005 (J Frean)

Diploma in Tropical Medicine and Hygiene, 2005
Front: Dr G Reubenson, Dr C Moore, Dr S van den Berg
Middle: A/Prof J Frean & A/Prof M Hale (Faculty), Dr D Brink, Dr H van der Plas, Dr S Kay, Dr M Reyneke, Dr G Fletcher, Dr B Chauke, Dr R Mahfouz, Dr L Blumberg (F.)
Back: Prof M Ross (F.), Dr L Jenkin, Dr T Enslin, Dr C Venter, Dr B Prinsloo, Dr D Ngwira, Dr E Lesia, Dr Y Moosa, Dr G de Jong & Mrs L Dini (F.)

WHO SEARO/WPRO workshop on quality assurance of malaria microscopy in Kuala Lumpur, Malaysia, 15-22 April (L Dini)

WHO Regional Advisory Group meeting on EQA in Johannesburg, 17-19 August
(J Frean, L Dini, R van Deventer)

CONFERENCE PAPERS AND POSTERS PRESENTED:

Dini, L. Malaria Rapid Test Kit diagnosis: Quality Control. Annual Malaria Review and Planning Meeting, Mpumalanga, June 2005

Dini, L. Survey and management of drug-resistant *Pneumocystis* pneumonia in South Africa. Swedish/South African Health Forum, Pretoria, March 2005

Frean JA. The NICD and the ACTM: Convocation address, Regional Scientific Meeting of the Australasian College of Tropical Medicine (ACTM), Hobart, Tasmania, 11-13 November 2005

Frean JA. Medical Experiences with the South African Defence Forces. Regional Scientific Meeting of the ACTM, Hobart, Tasmania, 11-13 November 2005

Frean JA. Margaretha Isaäcson and her role with the SADF. Regional Scientific Meeting of the ACTM, Hobart, Tasmania, 11-13 November 2005

Frean JA. Anthrax the zoonosis in southern Africa. A brief review of recent outbreaks and the biology and epidemiology of anthrax in southern Africa. Regional Scientific Meeting of the ACTM, Hobart, Tasmania, 11-13 November 2005

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT

CURRENT RESEARCH PROJECTS:

RATZOOMAN is a multicountry, multidisciplinary study of disease risks linked to rodents at the rural/peri-urban interface. The project which began in 2003 will run to the end May 2006, with an International Workshop organized by the SBPRU. The SBPRU is involved in investigation of the ecology of the rodent-borne zoonoses plague, leptospirosis and toxoplasmosis. Collection sites are in Limpopo Province (Mapate), Durban (Cato Ridge) and Port Elizabeth. To date more than 5 thousand rodent and human specimens have been tested. Plague is clearly continuing its quiescent phase in the country; the substantial prevalence of both toxoplasmosis and leptospirosis show that infections are underdiagnosed in humans in South Africa.

A 3-year MRC grant has been received for a project titled 'Molecular epidemiology of plague in southern Africa'. The purpose of the project is to use modern molecular techniques to characterize southern African isolates of the plague organism, *Yersinia pestis,* and to explore its habitat in an interepidemic (quiescent) period in South Africa.

A collaborative study of *Bacillus anthracis* cultures is being set up with Dr Wolfgang Beyers from the University of Hohenheim, Stuttgart Germany. The project will look at molecular epidemiology of the *Bacillus anthracis* strains from the SBPRU culture collection.

TRAINING AND EXTERNAL QUALITY ASSESSMENT ACTIVITIES:

Lorraine Arntzen and Chantel le Roux went to Port Elizabeth to the Nelson Mandela Metro and the Coega Development Corporation to train environmental health officers to take various blood and tissue samples from trapped rodents. These samples will be used for the RATZOOMAN project (above). The training was very successful.

The SBPRU trained two students from Rwanda in the identification of *Yersinia pestis* and *Bacillus anthracis,* at the request of WHO.

Registrars were trained in the SBPRU how to identify and confirm *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 Control Unit send plague proficiency testing samples to sixteen African countries and two referee laboratories, CDC in Fort Collins and Institut Pasteur in Madagascar. Over the past year there has been an encouraging improvement in the results. Still more training is needed in some of the weaker countries.

INTERNATIONAL MEETINGS ATTENDED:

Lorraine Arntzen attended a RATZOOMAN project report back meeting at the KIT Institute in Amsterdam, The Netherlands. Current results from the four African countries were presented.

Workshop to formulate prepardness and action plans for anthrax epizootics in wildlife zones, Malilangwe Wildlife Reserve, Zimbabwe, October 2005.
Lorraine Arntzen was a discussion leader for the section dealing with laboratory diagnosis.

Plague Conference, Oslo, Norway, November 2005.
Lorraine Arntzen presented a talk on the plague situation in Africa.

External Quality Assessment and Mycology Reference Unit

Head:
Dr Kerrigan McCarthy

STAFF:

Dr K McCarthy MBBCh DTM&H FC Path (Micro), Head of Unit
V Fensham Nat Dip Med Tech Micro Nat Dip Med Tech Clin Path, Laboratory Controller EQA
R Landsberg Nat Dip Med Tech Clin Path, Senior Technologist EQA
M Smith Nat Dip Med Tech, Curator National Stock Culture Collection EQA
H Haritos Nat Dip Med Tech Micro & Clin Path, WHO EQA Technologist
S Gould BSc Nat Higher Dip Med Tech, Laboratory Controller MRU
J Patel Nat Dip Med Tech Micro, Chief Technologist MRU
Dr J Roussouw PhD MSc BSc (Hons), Medical Scientist

EQA UNIT ACTIVITIES:

The EQA Unit has continued to produce External Quality Assessment programmes for the NHLS and subscribing private laboratories in the disciplines of bacteriology (120 laboratories), tuberculosis microscopy (220 laboratories), tuberculosis culture (25 laboratories) and syphilis serology (220 laboratories). Management of EQA programmes involves technical preparation and quality control of material, documentation and shipping, evaluation and reporting of laboratory responses. The Bacteriology EQA programme is accompanied by a teaching programme which participating laboratories may use as a training resource. Results of laboratory performance are reported to appropriate NHLS management structures, and are available in summary form in a separate EQA annual report.

The Unit also produced for the fourth consecutive year, an internationally refereed bacteriology EQA programme to 64 national public health laboratories in the African Regional Office (AFRO) of the World Health Organisation. This programme is run with the assistance of the Respiratory and Meningeal Pathogens Reference Unit (RMPRU), the Enteric Diseases Reference Unit (EDRU), the Special Bacterial Pathogens Reference Unit (SBPRU) and recently the Parasitology Reference Unit (PRU). The results of this programme inform the African Regional Office of the World Health Organisation (AFRO) and the WHO pathogen- specific projects about laboratory capacity for diagnosis of epidemic-prone disease in the AFRO region. Malaria and tuberculosis microscopy were added as separate disciplines in June 2005. The NICD hosted the Annual WHO/CSR/AFRO EQA review meeting from 17-19 August 2005. A representative from the EQA Unit attended the Technical Consultancy for Laboratory Networks to support Integrated Disease Surveillance in the African region, hosted by Centres for Disease Control in Atlanta USA, 13-15 September 2005. Staff from the EQA Unit provided material for and trained technologists from all regions in Ethiopia at the Ethiopian Health and Nutrition Research Institute in September 2005 as part of a AFRO-sponsored training initiative arising out of results of EQA activities.

The EQA Unit manages the National Stock Culture Collection, which is a national resource managed according to principles established by the World Federation of Culture Collections. The collection comprises reference strains from recognised culture collections that are required for quality control purposes, and clinical strains that have been characterised and are used in EQA activities. In 2005 for the first time a collection of quality controlled reference strains was issued to all NHLS laboratories.

Figure 1. The group of laboratory technologists, scientists and technicians who received training at the Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia from 3rd to 7th October 2005

Figure 2. A practical session at the Ethiopian Health and Nutrition Research Institute

Figure 3. The set of seven lyophilized reference cultures distributed to all NHLS laboratories in 2005.

MYCOLOGY REFERENCE UNIT ACTIVITIES:

The Mycology Reference Unit commenced national surveillance for cryptococcosis in January 2005, joining the existing GERMS-SA (Group for Enteric, Respiratory and Meningeal pathogens Surveillance) surveillance network. Funding for this project was obtained from the US Government PEPFAR funds. In 2005 over 5000 cases of cryptococcosis were identified across South Africa. A molecular laboratory was set up, through funds obtained from the same agency, and Dr Jennifer Rossouw, a PhD scientist has commenced molecular analysis of isolates. Antifungal susceptibility testing of isolates has commenced and a staff member will receive training at the CDC in Atlanta in the forthcoming year. Environmental isolation of cryptococci is also being done in order to establish local reservoirs of organisms.

The Mycology Reference Unit, together with Johannesburg Hospital Microbiology Laboratory hosted an Advanced Mycology Training seminar in March and November 2005 under the NHLS School of Laboratory Medicine. Registrars in Microbiology and Clinical Pathology are taught fungal identification techniques during their rotation through the laboratories.

The Mycology Unit manages all aspects of the Mycology Basic and the Mycology Advanced EQA programme for NHLS and private subscribing laboratories. The Mycology Advanced programme consisted of three surveys sent out in 2005 to 20 laboratories. Mycology Basic programme was sent to all NHLS laboratories that participate in the bacteriology EQA programme.

Figure 4. Intrepid mycologists obtaining botanical specimens for processing in the Mycology Reference Unit in an attempt to isolate environmental strains of *Cryptococcus neoformans*

TRAINING:

- Ethiopian Health and Nutrition Research Institute, 5-day course in 'Diagnosis and susceptibility testing of pathogens causing meningitis, and quality control of antimicrobial susceptibility testing'
- School of Laboratory Medicine at the Johannesburg Hospital NHLS Microbiology Laboratory, a 2 day course on 'Antimicrobial Susceptibility Testing' offered together with the Department of Clinical Microbiology and Infectious Diseases which facilitates this course.
- School of Laboratory Medicine at the Johannesburg Hospital NHLS Microbiology Laboratory, a 2 day

course on 'Basic and Advanced Mycology' offered twice per annum, together with the Department of Clinical Microbiology and Infectious Diseases which facilitates this course.

CONFERENCE PRESENTATIONS:

- 1st Joint congress of the Federation of Infectious Diseases Societies of Southern Africa, Sun City 24-27 July 2005.
- K M McCarthy. Controversies in susceptibility testing and reporting of antifungals (invited oral presentation).
- S Gould, J Morgan, K McCarthy R Hajjeh, M Brandt. Comparative epidemiology of *Cryptococcus gattii* and non-*Cryptococcus gattii* cases in Gauteng Province, 2002-4
- K McCarthy, R Landsberg, V Fensham, H Haritos. The National Institute for Communicable Disease Tuberculosis Microscopy External Quality Assessment Programme.

Enteric Diseases Reference Unit

Head:
Dr Karen Keddy

STAFF:

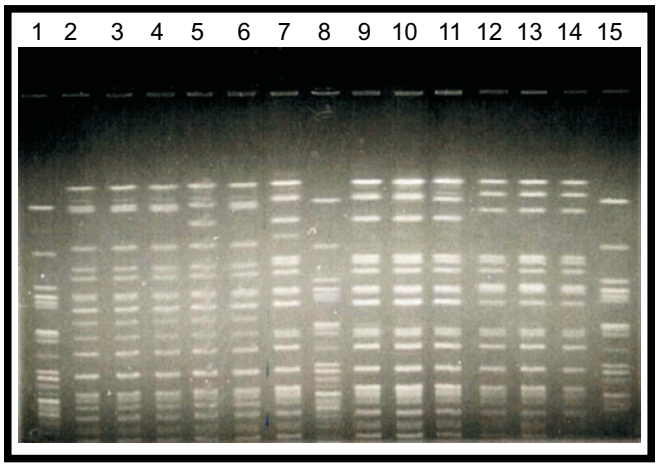
Dr KH Keddy MBBCh, BSc (Med), MMed, FCPath (SA), DTM&H, Senior consultant, Head of Unit
Dr AM Smith PhD
Mrs A Sooka MSc, Laboratory Controller
Ms S Nadan MSc, Medical Scientist
Mrs F Mnyameni Dip Biomed Tech, Senior Technologist
Ms M Ngomane Dip Biomed Tech, Senior Technologist
Mr E Khomane, Laboratory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

CURRENT RESEARCH PROJECTS:

Evaluation of *Vibrio cholerae* O1 strains from the current cholera epidemic in KwaZulu-Natal and surrounding provinces.

The organisms being worked on were from the current outbreak of cholera, which started in KwaZulu Natal and spread to the other provinces. Over 100,000 cases were notified to the Department of Health in Pretoria in 2001. The *V. cholerae* strain responsible was multidrug resistant, group 01, biotype El Tor, serotype Ogawa. Non-specific diarrhoea was present in the community, so laboratory infection was not suspected at first. PCR and PFGE were performed to check relatedness to the strains being studied.

RESULTS:



PFGE restriction patterns of selected isolates of *Vibrio cholerae* 01 serotype Ogawa and serotype Inaba. Lanes 2 to 5 represent isolates from Kwazulu Natal and lanes 6, 7 and 9 to 14 represent isolates from Mpumulanga province. Strains in lanes 2 to 6 were isolated in 2001/2002 and strains in lane 7 and 9 to 14 were isolated in 1980.

Lane 1: *Salmonella enterica* serotype Braenderup
2: K14794 serotype Ogawa
3: K15854 - serotype Ogawa
4: K1405 serotype Inaba
5: K1241 serotype Inaba
6: N8069 serotype Inaba
7: S69145 serotype Inaba
8: Salmonella *enterica* serotype Braenderup
9: S69146 serotype Inaba
10: S69147 serotype Inaba
11: S69148 serotype Inaba
12: S69173 serotype Inaba
13: S69174 serotype Inaba
14: S69175 serotype Inaba
15: *Salmonella enterica* serotype Braenderup

PFGE analysis with the restriction enzyme *Not 1* gave a suitable distribution of fragments. The analysis of the V. *cholerae* 01 isolates from the current epidemic, which included the isolate from the laboratory infection, displayed identical banding patterns.

Analysis of the peptide sequences was done by comparison with a selected reference Ogawa strain. Relative to the amino acid (aa) arrangement of the *wbeT* gene in the reference strain, a distinct difference at position 46 is noted in each of the 31 RSA strains compared. The result of this change is the replacement of the leucine in of the reference strain by tryptophan in the RSA *V. cholerae* isolates.

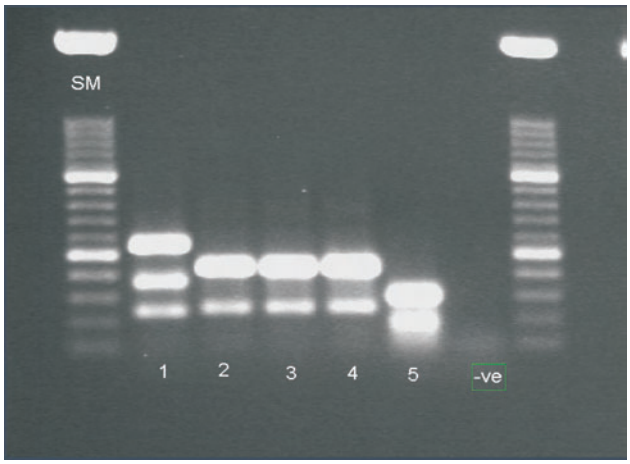
DISCUSSION

PFGE is reproducible because it looks at a stable genotype rather than at variable expressed phenotypic characteristics and is therefore a good typing method used for comparison of strains. The PFGE displayed identical banding patterns, demonstrating that the organisms are highly related in the current epidemic. Further analysis of the *V. cholerae* O1 Inaba strains suggests that they are more closely related to the *V. cholerae* O1 Ogawa strains of the current epidemic than to the *V. cholerae* O1 Inaba strains than the Inaba strains from the previous epidemic in the 1980s. The identification of cholera toxin production is an important step in the diagnosis of cholera, because only toxin-producing strains have been associated with severe, watery diarrhoea and epidemics. Data thus far suggest that the recent epidemic is a new epidemic and that the country urgently needs intervene in providing adequate water supplies for the population.

The serotype switch observed was due to a deletional mutation at nucleotide 17610, resulting in a premature stop codon at position 17618. This mutation appears to be conserved over the twenty-year period.

Multi-locus variable nucleotide tandem repeat analysis (MLVA) and *Salmonella enterica* subspecies *enterica* serotype Typhi

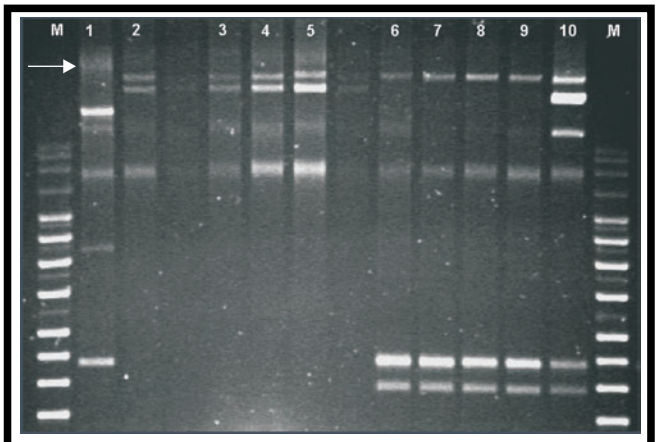
MLVA or VNTR is a new technique that assists in the epidemiological analysis of outbreak related strains. It has been shown to be very discriminatory in the analysis of non-typhoidal Salmonella and has been used to discriminate between different strains of *Salmonella* Typhi as well. In a preliminary test, EDRU utilised this method to compare outbreak strains from the recent typhoid fever outbreak in Delmas with *Salmonella* Typhi from another province, as well as *Salmonella* Enteritidis. Early analysis suggests that the technique may prove to be extremely useful as a rapid technique and will provide invaluable information on outbreak strains in future. EDRU hopes to offer this tool for future epidemiological analysis of outbreak-prone enteric diseases in South Africa.



MLVA gel of *Salmonella* strains, including from the recent outbreak in Delmas. 1= *Salmonella* Typhi (GA), 2 - 4 = *Salmonella* Typhi (MP), 5 = *Salmonella* Enteritidis, 6= Negative control, SM = Marker.

Molecular characterization of a multidrug resistant *Salmonella enterica* subspecies *enterica* serotype Isangi causing nosocomial infections in South Africa.

Salmonella spp. producing extended-spectrum ß - lactamases (ESBLs) have been reported in many countries, but there is no information on their prevalence in Africa. An apparent outbreak of infections with ESBL producing *S. enterica* serotype Isangi and *S. enterica* serotype Typhimurium, likely affecting several thousand people, has been noted in all provinces of South Africa since 2001. Isolates of *Salmonella* spp. were collected from thirteen hospitals located in different cities in South Africa over a five-month period from December 2002 to April 2003. All strains were screened for production of extended-spectrum -lactamases (ESBLs) by the double disk diffusion test, and AmpC production by assessing resistance to cefoxitin. ESBL-positive and cefoxitin resistant isolates *were* examined for bla_{SHV}, bla_{TEM}, bla_{CTX-M} and bla_{CMY-2} . This is discussed more fully in the Annual report for 2004. ESBL producing *Salmonella* spp. have become a significant public health problem in South Africa with particular implications for the treatment of serious non-typhoidal *Salmonella* infections in children, in whom third generation cephalosporins are the preferred treatment. Plasmid extraction has shown the presence of a megaplasmid, consistent with the antimicrobial resistance to multiple antibiotics.



Plasmid extraction from *Salmonella* Isangi isolates from two different hospitals from nosocomial outbreaks. Lanes 1-5 = Hospital 1, lanes 6-10 = Hospital 2, M=marker. Despite the differing plasmid profiles, a megaplamid approximately 148 kb in size can be seen in nine of the ten isolates (arrow).

SURVEILLANCE ACTIVITIES:

- National surveillance for isolation of bacterial enteric pathogens.
- Enhancement of surveillance for trimethoprim-sulfamethoxazole resistant invasive respiratory and diarrhoeal disease in South Africa

Mimmy Ngomane subculturing Salmonella isolates submitted by NHLS laboratories

TRAINING AND QUALITY ASSURANCE ACTIVITIES:

Registrar training

- EDRU assisted in training of Microbiology registrars for their final examinations, providing specialised training in the biochemical, serological and molecular characterisation of enteric pathogens.

Quality Assurance

- EDRU participated in the Internal Quality Assessment programme offered by the NHLS, in both in an advisory role as well as submitting responses.
- EDRU participated in the External Quality Assessment programme sponsored by WHO through the NHLS, in both in an advisory.
- EDRU participated in the CAP programme for External Quality Assessment.

Site training

- Arvinda Sooka accompanied Ruth Mpembe and Elizabeth Prentice from RMPRU on a site-training visit to Pietermaritzburg from Wednesday the 30th November to Friday the 2nd of December, 2005.

International training

- Dr Karen Keddy was sponsored by Solna, Sweden on Antimicrobial Susceptibility Testing, from 6-9 September.
- Ms Arvinda Sooka, the Laboratory Controller, visited Statens Serum Institut, Copenhagen, Denmark, between 12 and 16 September, for training in the serotyping and identification of diarrhoeagenic *Escherichia coli*. The course was an introduction for the screening, identification, characterisation and serotyping of enterovirulent *E. coli*.

INTERNATIONAL MEETINGS ATTENDED:

KH Keddy: Burden of Illness Studies. Centro Nacional de Epidemiologia, Madrid, Spain, 1 - 2 June 2005.

KH Keddy: Annual Enter-net Workshop. Centro Nacional de Epidemiologia, Madrid, Spain, 2 - 4 June 2005.

NATIONAL MEETINGS ATTENDED:

Florah Mnyameni. 18th National Congress of the Society of Medical Laboratory Technologists of South Africa, Cape Town, South Africa, 29 April - 2 May 2005.

CONFERENCE PROCEEDINGS:

1. Mnyameni FS, Kruger T, Sooka A, Keddy KH. Quinolone resistance of *Salmonella* Typhimurium in South Africa, 2003-2004. 18 th national Congress of the Society of Medical Laboratory Technologists of South Africa, Cape Town Civic Centre, Cape Town, South Africa, 29 April - 2 May 2005.
2. Keddy KH. Refining *Salmonella* surveillance in South Africa - first world technology in a developing country. Annual Enter-net workshop. Centro Nacional de Epidemiologia, Madrid, Spain. 2 - 4 June 2005.

Respiratory and Meningeal Pathogens Research Unit

Head:
Dr Anne von Gottberg

STAFF:

Prof K Klugman MBBCh PhD DTM&H MMed FC Path (SA) FRC Path (Lond) FRSSAfr, Director of Research (Baragwanath and NICD)
P Hyde, Personal Assistant to Director of Research
Dr A von Gottberg MBBCh DTM&H FC Path (SA) (Micro), Senior Pathologist, Head of Unit
Dr V Quan MBBCh Dip Child Health DTM&H, Principal Medical Officer, GERMS-SA (with EDRU, MRU)
Dr E Prentice BA MBBCh DTM&H, Senior Medical Officer, GERMS-SA (with EDRU, MRU)
Dr A Smith PhD, Senior Scientist
Dr M du Plessis PhD, Senior Scientist
N Wolter MSc (Biochem), PhD Student
K Mothibeli BSc (Hons), MSc Student
L de Gouveia Nat Dip Med Tech (Micro), Laboratory Manager
T Rafundisani Nat Dip Med Tech (Clin Path), Laboratory Supervisor
O Hattingh Nat Dip Biomed Tech (Micro and Clin Path), Senior Medical Technologist
R Mpembe Nat Dip Med Tech (Micro), Senior Medical Technologist
H Skosana Nat Dip Biomed Tech (Micro), Junior Technologist
M Hlanzi, Data Clerk
E Maringa Dip Dev Health, Data Clerk

Staff of the RMPRU molecular laboratory

Staff of the RMPRU microbiology laboratory

STAFF GERMS-SA (includes NICD units: EDRU and MRU)
W Ngqovu RN, Surveillance Officer, Mthatha, Eastern Cape
K Mawasha RN, Surveillance Officer, Bloemfontein, Free State
KF Seboya RN, Surveillance Officer, Chris Hani Baragwanath Hospital, Soweto, Gauteng
KD Hlatshwayo RN, Surveillance Officer, Chris Hani Baragwanath Hospital, Soweto, Gauteng
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N Nzuza RN, Surveillance Officer, Durban KwaZulu Natal
M Mokwena RN, Surveillance Officer, Polokwane, Limpopo
M Masuku RN, Surveillance Officer, Nelspruit, Mpumalanga
D Miller RN, Surveillance Officer, Cape Town, Western Cape
N Shalabi RN, Surveillance Officer, Cape Town, Western Cape

INTRODUCTION:

In 2005 our national surveillance programme (as part of the GERMS-SA [Group for Enteric, Respiratory and Meningeal Disease Surveillance]) for invasive disease due to *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseira meningitidis* has continued to grow. Approximately 4 700 cases were reported to us nationally in 2005, with all nine provinces in South Africa taking part. Voluntary reporting from private and public

GERMS-SA Surveillance Officers' meeting, June 2005

sector laboratories has improved and regular feedback to laboratories has made the network more active. In November our activities and results from the surveillance were presented and discussed at our GERMS-SA annual meeting, bringing together the Department of Health and technologists, clinicians, pathologists from all provinces to meet and re-evaluate the network.

As a result of the surveillance, we have noted the emergence of fluoroquinolone-resistant pneumococci causing invasive disease in children, with clusters of cases in 3 tuberculosis treatment hospitals in 3 provinces in the country. In addition, due to RMPRU performing enhanced surveillance specifically investigating meningococcal disease in Gauteng province, we have documented the replacement of serogroup A *N. meningitidis* with serogroup W135 in 2005 and an increase in the total number of cases reported from this province. With ongoing surveillance of *H. influenzae* serotype b disease in children, residual disease has been described to occur in children with HIV-infection, and the strains still causing disease are more likely to be drug resistant. In 2005 the unit also published on pneumococcal mechanisms of resistance to protein-synthesis inhibiting antibiotics (macrolides, telithromycin and linezolid).

Together with other units at the NICD and our collaborators throughout the country, we have visited laboratories and hospitals in Eastern Cape, Gauteng, KwaZulu Natal, Northern Cape, and North West provinces. Specifically, the NICD GERMS-SA team conducted on-site laboratory training in Mthatha, Eastern Cape, and Pietermaritzburg, KwaZulu Natal.

Research funding in part still comes from the research unit RMPRU (MRC/NICD/WITS) under the directorship of Prof Keith Klugman (Hubert Department of Global Health, Emory University, Atlanta, Georgia, USA); and a cooperative agreement (number U60/CCU022088) from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, and the United States Agency for International Development's Antimicrobial Resistance Initiative.

GERMS-SA Pietermaritzburg training visit

SELECTED SURVEILLANCE ACTIVITIES:

The **widespread introduction of cotrimoxazole for prophylaxis** of opportunistic infections in HIV-infected people has major implications for the **development of resistance** and the selection of multiresistant bacterial clones. We have expanded our national surveillance

network to monitor the impact on resistance in all nine provinces. Ongoing analyses show dramatic increases in cotrimoxazole resistance in pneumococcal isolates. Collaborators include Drs Anne Schuchat and Stephanie Schrag at the CDC, and Allison Taylor at Emory University. As part of our surveillance, our laboratory has described the emergence of **fluoroquinolone-resistant strains** in children, mostly associated with tuberculosis hospitals in 3 provinces in South Africa. This work was presented at the annual surveillance meeting held at the NICD in November 2005. A study investigating **the clonality of South African pneumococcal serotype 3 isolates** is ongoing. CDC collaborators include Dr Lesley McGee and Dr Bernie Beall.

A **molecular epidemiology study of *Neisseria meningitidis*** in South Africa, for the period August 1999 to July 2002 has been completed and submitted for publication. Over 600 isolates have been characterized by pulsed-field gel electrophoresis (PFGE) and over 40 representative isolates were further characterized by multi-locus sequence typing (MLST). Ongoing work related to meningococci includes further molecular work on serogroup W135 strains, and evaluation of the epidemiology of W135 disease in Gauteng.

Strains of *Streptococcus pneumoniae* received from 149 **adult patients with community-acquired pneumonia** presenting from May 2004 in both the private and public sectors in Johannesburg were tested for susceptibility to beta-lactams and macrolides. There were no significant differences in the susceptibility of the isolates to ceftriaxone, chloramphenicol, tetracycline, rifampicin or ofloxacin. Significantly more resistance to erythromycin (28% vs. 4%; $P < 0.001$) and clindamycin (21% vs. 3%; $p = 0.001$) was however seen in the private sector isolates. Collaborators include Prof Charles Feldman (Johannesburg Hospital) and Dr Adrian Brink (AMPATH).

RESEARCH ACTIVITIES:

The identification of amino acid mutations in PBP 2X that confer penicillin-cephalosporin resistance in the pneumococcus
The technique of site-directed mutagenesis was used to identify which amino acid mutations in altered PBP 2X, are involved in the development of high-level penicillin-cephalosporin resistance in a Hungarian isolate with high-level resistance to penicillin (MIC 16 µg/ml) and cefotaxime (MIC 4 µg/ml). The analysis involved reversing the mutations in PBP 2X, and determining the effect on resistance levels. Of the 24 amino acid substitutions occurring in the penicillin-binding domain of PBP 2X, it was found that 6 substitutions were important. This study was published in the November 2005 issue of Antimicrobial Agents and Chemotherapy

Altered PBP 2A and its role in the development of penicillin, cefotaxime, and ceftriaxone resistance in a clinical isolate of *Streptococcus pneumoniae*
We have discovered the unusual involvement of altered PBP 2A in the development of β-lactam resistance in *Streptococcus pneumoniae*. This was investigated amid three identical serotype-14 isolates of pneumococci cultured successfully from the blood of an HIV-seropositive child with recurrent pneumonia. This study was published in the May 2005 issue of Antimicrobial Agents and Chemotherapy.

The effect of altered penicillin-binding proteins on pneumococcal cell morphology, cell division and cell growth
This project aims to investigate the biological cost that a resistant pneumococcus has to pay for its altered PBPs and development of resistance. The effect of altered PBPs on cell wall synthesis, cell morphology, cell division, and cell growth are being investigated. Experiments so far indicate that these functions are not negatively influenced in isolates with low-level or intermediate resistance. However, high-level-resistant isolates exhibit a significant reduction in growth rate (increased mass doubling time). Furthermore, we have proved that this reduced growth rate and fitness cost is the result of an altered glycosyltransferase domain (GD) in the altered PBP 1A protein. Collaborator: Dr Orietta Massidda, Università di Cagliari, Italy

Novel mechanisms of resistance to protein synthesis inhibitors in *Streptococcus pneumoniae*
Two clinical isolates of *S. pneumoniae* resistant to macrolides, linezolid and chloramphenicol were identified. Each isolate contained a 6 bp deletion, resulting in the deletion of two amino acids in a highly conserved region of the ribosomal protein L4. Transformation studies proved that these deletions confer resistance to macrolide, linezolid and chloramphenicol. The L4 mutations represent novel mechanisms of resistance to linezolid and chloramphenicol in the pneumococcus. In another study, the investigation of macrolide resistance in a clinical isolate of *S. pneumoniae* with 23S rRNA mutations was complicated by gene conversion between the wild-type and mutant 23S rDNA alleles. The isolate displayed a heterogeneous phenotype and genotype.

The impact of local fluoroquinolone use in non-responding otitis media (NROM)
Fluoroquinolones are being used as topical solution in children undergoing tympanocentesis. Pneumococci have been isolated from middle ear fluid specimens taken during surgery (167/311=54%), and then at subsequent visits (2nd visit specimens [92/163=56%], and third visit specimens [38/63=60%]). These isolates will be tested for fluoroquinolone resistance mutations once genetic relatedness to previous isolates has been determined. This study is in collaboration with Dr Adrian Brink and Dr Maurice Hockman.

Investigation of the *Streptococcus pneumoniae*-derived toxin, pneumolysin, as a target of macrolide antimicrobial agents
A significant percentage of patients with severe pneumococcal disease who receive appropriate

antimicrobial chemotherapy still die. Proposed alternative treatment strategies may be of considerable potential value in pneumococcal disease. Macrolide-sensitive and -resistant strains were used to determine the influence, if any, of macrolides on the synthesis of pneumolysin. Ceftriaxone did not alter the magnitude of pneumolysin production, while clarithromycin (both alone and in combination with ceftriaxone) inhibited production of the toxin. This work is in collaboration with Profs Ronnie Anderson and Charles Feldman.

TRAINING ACTIVITIES:

1. Practical Laboratory Training Workshop: Umtata (Mthatha), 1-2 June 2005 by Dr Elizabeth Prentice (Surveillance coordinator, RMPRU/EDRU/MRU), Ms Susan Gould (Laboratory Manager, MRU) and Ms Olga Hattingh (Technologist, RMPRU)
2. Practical Laboratory Training Workshop: Pietermaritzburg 31 November-2 December 2005 by Dr Elizabeth Prentice (Surveillance coordinator, RMPRU/EDRU/MRU), Ms Arvinda Sooka (Senior Technologist, EDRU), Ms Ruth Mpembe (Senior Technologist, RMPRU)
3. Clinical Pathology Registrar Training, on site at RMPRU 5-14 September

ORAL AND POSTER PRESENTATIONS:

1. Quality control of locally produced Trans-Isolate medium for isolation of meningeal and respiratory pathogens. **Rafundisani T, de Gouveia L, von Gottberg A, Klugman KP.** Poster presentation at 18th National Congress of the Society for Medical Laboratory Technology of South Africa, 29 April-2 May 2005, Cape Town Civic Centre, South Africa.
2. Surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* disease from 2000-2003 in South Africa. **de Gouveia L, von Gottberg A, Quan V, Soma K, Huebner R, Wasas A, Klugman K**, and Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Poster presentation at 18th National Congress of the Society for Medical Laboratory Technology of South Africa, 29 April-2 May 2005, Cape Town Civic Centre, Cape Town, South Africa.
3. Recurrent invasive pneumococcal disease episodes in South Africa in 2003 and 2004. **Quan V, von Gottberg A, de Gouveia L, Hattingh O, Mpembe R, Klugman KP** and GERMS-SA. Poster presentation at 1st Joint Congress Federation of Infectious Diseases Societies of Southern Africa, 24-27 July 2005, Sun City, North-West Province, South Africa.
4. Dual infections caused by *Streptococcus pneumoniae* and *Haemophilus influenzae*. **Mpembe R, Hattingh O, von Gottberg A, de Gouveia L, Quan V, Klugman KP** and GERMS-SA. Oral presentation at 1st Joint Congress Federation of Infectious Diseases Societies of Southern Africa, 24-27 July 2005, Sun City, North-West Province, South Africa.
5. Potential benefit of different pneumococcal conjugate vaccine formulations to prevent invasive pneumococcal disease in South Africa. **von Gottberg A, de Gouveia L, Madhi SA, Wasas A, Quan V, Klugman KP** and GERMS-SA (Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa). Oral presentation at 1st Joint Congress Federation of Infectious Diseases Societies of Southern Africa, 24-27 July 2005, Sun City, North-West Province, South Africa.

INVITED LECTURES, MEETINGS:

January 17-18, invited observer, Anne von Gottberg: Global Alliance for Vaccines and Immunization (GAVI)'s Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP) Investigator meeting: Surveillance of Laboratory Confirmed Pneumococcal Disease, Brac Center Inn, Dhaka, Bangladesh
February 1-2, invited observer, Anne von Gottberg: ABCs Steering Committee Meeting, One West Court Square, Decatur, Atlanta, Georgia, USA
August 17-19, invited speaker and participant, Anne von Gottberg: WHO Consultative Meeting on External Quality Assessment Programme in Africa, NICD Sandringham, Johannesburg, South Africa
July 24-27, invited speaker, Elizabeth Prentice: Increase in *Neisseria meningitidis* serogroup W135 in South Africa from January 2000 to December 2004, 1st Joint Congress Federation of Infectious Diseases Societies of Southern Africa, 2005, Sun City, North-West Province, South Africa
October 8, invited speakers, Dr Vanessa Quan, Dr Anne von Gottberg: Communicable Disease Surveillance, Society for Medical Laboratory Technology of South Africa (SMLTSA) Clinical day, Westvaal Hospital, Orkney
October 25, invited speaker, Anne von Gottberg: Meningococcal Disease and Meningitis, Health Promoter Workshop, Conference Centre, Witbank
October 31-November 1, invited speaker, Anne von Gottberg, Meningococcal disease: Vaccine and other control measures, Vaccinology Congress, Hermanus, South Africa

GRANTS:

1. Continuation proposal for Fiscal Year 2005, budget period 30 September 2005 to 29 September 2006: Enhancement of Surveillance for Trimethoprim-Sulfamethoxazole Resistant Invasive Respiratory and Diarrhoeal Disease in South Africa ($210 000)
2. PneumoADIP's Small Grants Program "Differences in blood culturing practices in rural and urban areas of South Africa" ($25 000)

Sexually Transmitted Infections Reference Centre

Head:
A/Prof David Lewis

STAFF:

A/Prof D Lewis MBBS FRCP (UK) DTM&H BA MSc PhD, Head of Unit
A Goliath, Secretary**
E Goliath Secretary*
Dr S Odugwu MBBS DTM&H MSc(Med) MPH, Chief Medical Scientist
Dr I Zietsman MBBCh FRCPath (SA) (Micro), Pathologist**
C Pillay BSc (Hons), Research Manager
F Radebe NH Dip Med Tech (Virology) MSc (Med), Laboratory Manager
Dr E Muller BMedSci BSc (Hons) MSc (Med Virology) PhD, Senior Medical Scientist**
M Cheyip BSc, MSc (Med), Senior Medical Scientist
L Tsaagane Med Tech (Micro), Chief Medical Technologist
P Magooa BSc (Hons) (Micro), Medical Scientist
S Khumalo, Research Assistant
D Mabaso, Research Assistant
R Chonco, Laboratory Cleaner
S Tshelane NP (EH), B Tech (EH), Surveillance Officer
N Khanyile, Data input clerk
R Maheter, Data input clerk
J Thobega, Data input clerk*
O Mohlamonyane, Professional Nurse **
Z Jele RN, Nurse Research Coordinator**
J Lethoba RN, Nurse Research Coordinator**
Z Mzaidume RN, Nurse Research Coordinator**
A Mofokeng RN, Nurse Research Coordinator**
L Mekgwe RN, Nurse Research Coordinator*
K Mmoledi RN, Nurse Research Coordinator*
M Manuel EN, Research Nurse
T Nhlapo RN, Research Nurse**
I Atlee RN, Research Nurse**
T Mosiro RN, Research Nurse**
G Khasu RN, Research Nurse**
T Makhanya RN, Research Nurse*
V Nosi, HIV VCT Counsellor**
X Siyasi, HIV VCT Counsellor**

* left during 2005
** appointed during 2005

Staff of the Sexually Transmitted Infections Reference Centre plus Mr Myron Wettrich (CDC Pretoria), 3rd top right

INTRODUCTION:

This year saw an increase in the staff complement of the STI Reference Centre (STIRC) from 16 to 28 people. With staff growth has come new challenges, new projects and a new home at the National Institute of Communicable Diseases. To detail all STIRC's activities during 2005 within the space requirements of the NICD annual report is not possible, so what follows is a brief outline of STIRC's achievements and work throughout the year. Successes in 2005 include improved national collaborative working practices with academic centres, the National Department of Health (NDoH), NGOs, primary health care clinics, our local communities and neighbouring countries. In addition, presentation of STIRC projects won both the best STI oral and best STI poster prizes at the 1st Joint Congress of the newly formed Federation of Infectious Diseases societies of South Africa in Sun City in July 2005.

CLINICAL SURVEILLANCE FOR STIs:

In 2005 STIRC successfully ran various clinical STI surveillance programmes in South Africa and other SADC countries.

The National Clinical STI Surveillance Programme for South Africa, launched in November 2003 and funded through the NICD:CDC co-operative agreement, completed its first full year of data collection from 270 sentinel sites across the country at the end of March 2005. A parallel reporting system, which was initially set up to detect data flow problems, was discontinued following a review which showed that the routine District Health information System (DHIS) was more efficient. Data for the first year has been analyzed and a draft report presented to the NDoH for comments. Overall, the reporting rate from sentinel sites was 82%. A total of 1,654,776 new episodes of STIs were recorded at primary health care clinics and level-one hospitals throughout the country, representing an incidence rate for the year of 63 per 1,000 population aged between 15 and 49 years.

The Gauteng Clinical STI Surveillance Programme is now in its 11th year. Throughout the year we received data from 21 sentinel sites, produced quarterly reports and an annual report for 2004. In that year a total of 43,697 new episodes of STIs were recorded at the 21 sentinel sites in the province.

A pilot SADC STI surveillance programme covering high transit/cross border sites in Botswana, Namibia, Lesotho and Swaziland was launched in late 2004 and has been funded by the UK Department for International Development. During the year 2005, sentinel facilities were identified in 12 regional sites within the countries. Initial training was provided to most of the sites and data collection started in 9 of them.

MICROBIOLOGICAL SURVEILLANCE FOR STIs:

In conjunction with National Department of Health (NDoH), STIRC is coordinating the national microbiological surveillance of sexually transmitted infections in South Africa through funding from the NICD:CDC co-operative agreement. STIRC and the six university departments involved aim to establish a sustainable network for national microbiological surveillance that covers all 9 provinces in South Africa and monitors 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* will be investigated.

During 2005, all six academic centres to be involved in NMS were visited by Dr Lewis and Dr Zietsman. The protocol was drafted and subsequently discussed by all key participants at a teleconference in June and a meeting in December 2005. The surveillance programme is proposed to begin in 2006.

Results from the 2004 gonococcal antimicrobial resistance survey in 2004 were presented at the 1st Congress of the Federation of Infectious Diseases Societies of South Africa in July 2005 (Figure 1). The data informed the NDoH of the need to change first line therapy for *Neisseria gonorrhoeae* from ciprofloxacin to 3rd generation cephalosporins.

We assisted with the microbiological surveillance of STIs in Swaziland and have recently been asked to similarly assist in Lesotho.

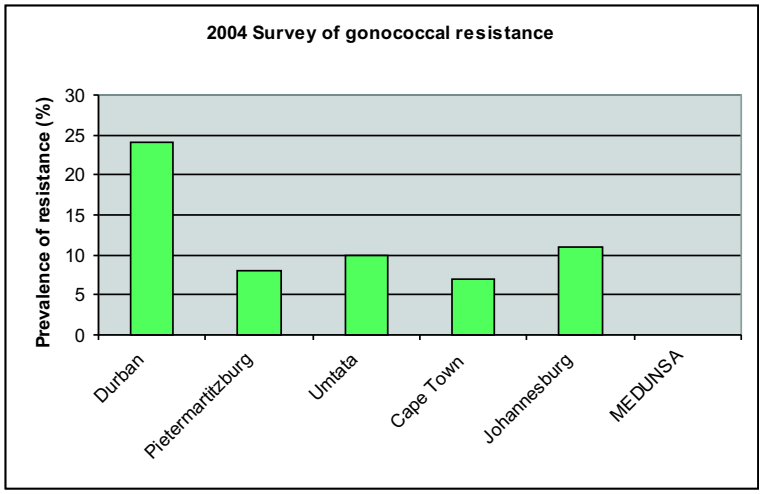


Figure 1. 2004 National Surveillance Survey on Gonococcal Ciprofloxacin Resistance (1 086 isolates) [Koornhof *et al.* Oral presentation at Sun City FIDSSA meeting, July 2005]

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

A shift in the aetiology of genital ulcer disease (GUD) towards genital herpes has been noted in many

countries, especially those with mature HIV epidemics. This study will help answer the question if acyclovir therapy for herpes should be added into the syndromic management of GUD and if it can be used as an HIV prevention strategy.

This study, funded through the NICD:CDC co-operative agreement, is a randomised placebo-control trial of acyclovir and its effect on GUD duration and HIV shedding. The trial is conducted at two clinics in Johannesburg, Eloff Street clinic and the Alexandra Health Centre under the supervision of two Principal Investigators, A/Prof David Lewis (STIRC) and Dr Gabriela Paz-Bailey (CDC Global AIDS Programme for Central America and London School of Hygiene and Tropical Medicine). A six-month interim analysis was performed in August 2005 by which stage 157 men had been recruited. The mean age of participants was 30 years and 72% were South Africa by birth. Of participants 60% were HIV positive, 32% had been tested for HIV previously and only 8% of the positives knew their status. Of GUD, 80% had herpes, 8% *Treponema pallidum,* 6% had *Haemophilus ducreyi* and in 17% no organism was identified. Participants had an ulcer for a mean of 6 days before consulting, and 12% sought care elsewhere before coming to the clinic.

We would like to thank our colleagues in Regions 7 and 8, at the two clinic sites, and all clinics who have referred patients to this study. During the course of 2005, a number of strategies were planned to increase patient recruitment, which included production of a monthly 'Herpes News' newsletter, a study leaflet, a recruitment video and posters. In addition, STIRC nurses presented the study to a large meeting of traditional healers who were very receptive to the study (Figure 2).

Figure 2. STIRC attended a traditional healers meeting in Gauteng to discuss the study.

USAID-FUNDED STUDIES:

The 3 year expanded periodic presumptive therapy (PPT) study will end in February 2006. By the end of 2005, the Tswarangaro Project based in the Westonaria and Randfontein areas of Gauteng collected more than 1,000 genital swabs from initial and follow-up attendances from women at high risk (WAHR). It is planned to analyse these data in 2006. In 2005, three new and one replacement mobile vans were delivered to NGOs involved in the PPT programme in both the Free State and Gauteng Provinces (Figure 3).

Figures 3 & 4. Peer educators working with the Lesedi-Lechabile Primary Care Project proudly demonstrate their mobile van in the Free State (Figure 3) and sing about the work of the vans in a vocal African welcome to the STIRC team during a 2005 site visit (Figure 4)

In the last quarter of 2005, preparations were made and the first wave of staff employed for a new USAID-funded study which will assess the acceptability for WAHR of HIV voluntary counselling and testing (VCT) provided by trained counsellors working in tents placed adjacent to the mobile clinic vans. STIRC has employed the first VCT counsellors in the NHLS for this project and it is anticipated, based on the demand for such a service from the WAHR themselves, that the main project will be a success in 2006. STIRC have teamed up with the Mothusimpilo Project to perform this research and are benefiting from the close working relationship built up over a number of years between the WAHR and the mobile van nurses. A short pilot of six days in December 2005 resulted in 34 WAHR testing for HIV; 24 (68%) of these women were HIV seropositive. The WAHR are also being offered STI screening and nurses working in the mobile vans will treat women with both symptomatic and asymptomatic STIs.

URETHRITIS STUDY:

Male urethritis syndrome (MUS) was investigated at Esselen Street clinic in collaboration with the Reproductive Health and HIV Research Unit (Dr Vivien Black). By the end of 2005, staff had collected endo-urethral swabs and first-pass urines from 219 patients. These patients included 165 cases of acute urethritis, 9 persistent urethritis and 45 cases without urethritis. Endo-urethral swabs were cultured for *Neisseria gonorrhoeae* and *Trichomonas vaginalis.* Urine was tested by PCR for infection with urethritis related pathogens (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis).* Among the 165 acute urethritis participants, *N. gonorrhoeae* remained the predominant pathogen isolated from patients with MUS and was isolated in 111 patients (67%). Of the atypical bacteria, *C. trachomatis* was isolated in 37 patients (22%) and *M genitalium* in 27 patients (16%). *T. vaginalis* was isolated in 15 patients (9%). No pathogen was isolated in 13 patients (7%). Dr Vivien Black won the prize for the best STI oral presentation at the 1st FIDSSA Conference in Sun City in July 2005 with her presentation of the studies findings.
Antimicrobial susceptibility testing to ciprofloxacin was performed on 69 isolates of *N. gonorrhoeae.*16% of isolates were resistant, 14% intermediately resistant and 70% susceptible. The high rates of ciprofloxacin resistance support the urgent need to change first line therapy for gonorrhoea in South Africa to an alternative effective agent, such as ceftriaxone or cefixime.

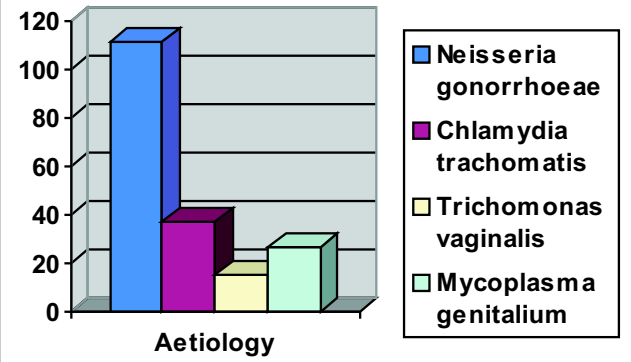


Figure 1. Aetiology of acute cases of urethritis (n=165)

PLASMACUTE STUDY:

The study has been running since 2002 in collaboration with the University of Bergen in Norway and Plasmacute. The Plasmacute assay detects the presence of anti-HIV antibodies following lysis of B-lymphocytes extracted from blood with beads. Conventional serological tests, including western blot and two different ELISA tests, are performed on B-cell lysates and on blood from each participant. Participants' blood is also tested for HIV viral load, CD4 count and the presence of the HIVB p24 antigen. Participants are followed-up to check clinically and serologically for HIV seroconversion. To increase recruitment, a research nurse has been employed to work full time on the project since August 2005. She actively recruits participants via the mine VCT program and medical stations. This has meant that recruitment has greatly increased. To date, a total of 225 sero-negative participants have been recruited to the study with only two seroconversion cases having the B-cell derived antibodies testing positive for HIV when the p24 antigen and viral load markers were negative.

MOXIFLOXACIN STUDY:

With the advent of ciprofloxacin resistance in South Africa, STIRC undertook a Bayer-funded study of the sensitivity of gonococcal isolates from women to three quinolones to ciprofloxacin, ofloxacin and moxifloxacin. Moxifloxacin, as predicted, had a lower MIC_{90} than the other quinolones tested (Table 1).

Table 1: Susceptibility results for 93 gonococci against three quinolones

Quinolone	Susceptible MIC < 0.125mg/l	Intermediate 0.125-0.5mg/l	Resistant 1mg/l	MIC_{50}	MIC_{90}
Moxifloxacin	91 (98%)	2 (2%)	0 (0%)	0.008	0.015
Ofloxacin	91 (98%)	2 (2%)	0 (0%)	0.015	0.03
Ciprofloxacin*	81 (89%)	8 (9%)	2 (2%)	0.002	0.125

* Two strains did not grow when tested against ciprofloxacin

ESTABLISHMENT OF A CLINICAL STI SERVICE FOR MEN IN ALEXANDRA:

During 2005, STIRC established in collaboration with Region 7, 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 (Mrs Mehana) and her team (Gloria Keetse and Zanele Mophosho) and the staff of 8th Avenue clinic, in particular Sr. Wendy Twalo, Reynolda Kgatuke and Ike Kekae. This clinic has been successful in encouraging younger men to attend for STIs and STIRC hopes to develop further links with the Alexandra community in 2006.

TEACHING AND TRAINING:

Several STIRC staff attended a two-day course on Good Clinical Practice and Clinical Trials run by the Wits Health Consortium in 2005.

In July 2005, STIRC staff trained personnel from the neighboring SADC countries. From the HIV/AIDS Prevention and Care programme (HAPAC) in Swaziland, four staff members, a Senior Technologist Ms Gugu Maphalala, a technologist Ms Nomcebo Makhanya and two laboratory technicians Ms Ntombi Ginindza and Fortunate Lushaba had a refresher course on Gram staining and slide reading, STI pathogen culture and identification, antimicrobial susceptibility testing, molecular detection methodology and serological techniques. Mr Raul Macuacua, a senior technician and Laboratory Manager from State and University Laboratories from Zambia, also underwent an extensive three weeks training in molecular diagnostic techniques for STIs. During these visits, both epidemiological and microbiological activities of STIRC were discussed with the delegates.

The Health Systems Trust undertook training of STIRC clinical surveillance staff on the District Health information System for one week in July.

In the latter half of the year, Obed Mohlamonyane (nurse) and Lydia Tsaagane (technologist) assisted in the training and implementation of the microbiological surveillance in Swaziland at the request of the HAPAC programme. STIRC also assisted with a review of the project's SOPs prepared by the STI Reference laboratory at Mbabane Hospital. The two STIRC staff spent three days in Mbabane training local staff in the clinical and laboratory requirements of microbiological surveillance.

During October and November, four STIRC staff received training on the use of STIRC's new Aptima Combo-2 diagnostic facility for the detection of *N. gonorrhoeae* and *C. trachomatis* in urine and genital swab specimens. The course was conducted by the worldwide area manager for GenProbe through Pro-Gen S.A.. Pro-Gen S.A. staff locally subsequently did follow-up training.

Six registrars underwent 1-2 week laboratory training courses in STIs at STIRC. A comprehensive programme was designed which gave hand-on technical time in all aspects of STI diagnosis; additional lectures were given on key STI-related topics and each Registrar undertook personal library-based reading on STI pathogenesis and gave an oral presentation on their work at the end of their rotation.

CONFERENCES AND PRESENTATIONS:

Sexually transmitted infections, HIV, Gender and Violence, Lisbon, Portugal 8-9 March 2005. Lewis DA. The role of the laboratory in the diagnosis of STI in women (oral)

BHIVA and BASSH Joint Spring Meeting, Dublin, Ireland May 2005. Slater et al. Change from microscopy and culture to gonorrhoea strand displacement assay is there an impact on patient care? (Poster)

8th International Symposium on Haemophilus ducreyi pathogenesis and chancroid, Amsterdam, The Netherlands 10 July 2005. Lewis DA. Treatment of chancroid in 2005 (oral)

16th Biennial meeting of the ISSTDR, Amsterdam, The Netherlands 10-13 July 2005. Odugwu SO et al. The performance of self-administered vaginal swabs stored at room temperature on PCR detection of sexually transmitted infections (poster)
Pillay C et al. The Gauteng STI sentinel surveillance programme, South Africa 2000-2004 (poster)

1st Joint Congress of the Federation of Infectious Diseases Societies of South Africa, Sun City 24-27 July 2005
Lewis DA. Quinolone resistant gonococci cause for alarm? (oral)
Black V et al. A study to determine the aetiology of symptomatic, persistent and asymptomatic urethritis in an inner city region of Johannesburg, South Africa (oral)
Koornhof HJ et al. A national survey of antimicrobial resistance in gonococci isolated in South Africa (oral)
Prentice E et al. Characterisation of ciprofloxacin-resistant gonococci isolated in Gauteng province (poster)
Magooa PM et al. Frequency of sexually transmitted infections in symptomatic and asymptomatic commercial sex workers in Johannesburg, South Africa (poster)
Tsaagane L et al. Asymptomatic sexually transmitted infections among young people in South Africa (poster)
Cheyip et al. Measuring the prevalence and distribution of symptomatic sexually transmitted infections among mine workers in the Carletonville area of South Africa by routine syndromic surveillance (poster)
Tshelane SM et al. STI sentinel surveillance in Gauteng: 2004 (poster)

12th Priorities in Reproductive Health and HIV Conference, Spier, South Africa 18-21 October 2005
Lethoba J et al. Origins, aetiologies and sexually transmitted co-infections in men with genital ulcers presenting to primary health care clinics in Johannesburg (oral)
Tsaagane L et al. Susceptibility of gonococcal strains isolated from women in South Africa to a novel quinolone (moxifloxacin) and other commonly used antimicrobial agents (poster)

9th IUSTI World Congress, Bangkok, Thailand 15-18 November 2005. Lewis DA. The rise and fall of genital ulcer aetiologies: implications for syndromic management (oral)

Vector Control Reference Unit

Head:
Prof Maureen Coetzee

STAFF:

Prof M Coetzee MSc PhD FRES, Head of Unit
Dr L L Koekemoer BSc (Hons) PhD, Senior Medical Scientist
Dr B D Brooke BSc (Hons) PhD, Senior Medical Scientist
Ms R Naguran BSc (Hons) MSc, Medical Scientist
Ms S Oliver BSc (Hons), Medical Scientist
Mr Z Zulu, Laboratory Assistant
Mr Z Mnisi, Laboratory Assistant

Based in the Unit:

Dr R H Hunt MSc PhD FRES, Honorary Professor in the School of Animal, Plant & Environmental Sciences, University of the Witwatersrand.

POSTGRADUATE STUDENTS:

Graduated in 2005:

MSc	J Mouatcho
	N Mngomezulu

Registered as candidates for higher degrees:

PhD	D Amenya Achieng
	M Booman
	P Okoye
	T Matambo
MSc	G Nkosi
	K Hargreaves

Post-doctoral Fellow:
E. Misiani

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 13,181 cases were reported in 2004. 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 *gambiae* complex, *An. quadriannulatus*. The two 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

Research into pyrethroid resistance in *An. funestus* continues to be a major focus of the VCRU. Molecular technology used to investigate the metabolic mechanisms involved in the resistance has shown which specific P450 monooxygenase enzymes are responsible for pyrethroid resistance in *An. funestus.* Collaboration with the Liverpool School of Tropical Medicine, UK, and Notre Dame University, USA, continues and exchange of staff and students has greatly enhanced our capacity to carry out these joint research projects.

The VCRU in collaboration with AngloGold/Ashanti, carried out a baseline survey of mosquitoes at the gold mining operation in Obuasi, central Ghana. Results showed high levels of DDT and carbamate resistance in *An. funestus* and resistance to DDT, carbamates and pyrethroids in *An. gambiae* S form. Both species were 100% susceptible to the organophosphate insecticide Malathion.

Anopheles gambiae

A molecular diagnostic assay based on an allele specific polymerase chain reaction is used to detect the alanine to glycine substitution in the GABA receptor associated with dieldrin resistance in *An. gambiae* mosquitoes. We tested this assay on dieldrin resistant laboratory colonies and found good correlation with the resistant phenotype. However, subsequent testing on *An. gambiae* S form from Ghana that exhibited high levels of dieldrin resistance, showed little correlation with the mutation, indicating the presence of a second, possibly metabolic, mechanism conferring resistance to this class of insecticides.

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*, was detected in the SENN colony of *An. arabiensis* but showed no association with the multi-insecticide resistance present in this laboratory colony.

MOLECULAR STUDIES:

Comparative studies of *An. funestus s.s.* using mtDNA analysis, carried out in collaboration with colleagues at Notre Dame University, showed distinct differences in samples from southern Africa and Madagascar indicating divergence over 800,000 years ago. These populations are being further investigated using both mtDNA and RFLP analysis, together with close relatives found in southern Africa.

DISTRIBUTION AND BIONOMICS:

The collaborative studies carried out in Nigeria on the *An. funestus* group revealed the presence of at least three species, *An. funestus s.s., An. rivulorum* and *An. leesoni*, based on a multiplex polymerase chain reaction assay. *Anopheles funestus* s.s. was found in more than 80% of the collection sites with the other two species having focal distribution. Only *An. funestus* was positive for *Plasmodium falciparum* using enzyme-linked immunosorbent assays (ELISA). Analysis of blood meals by ELISA revealed varying degrees of human feeding but at each study site at least 50% of the biting was occurring on humans.

Surveys of the mosquitoes of northern Namibia showed *An. arabiensis* to be widespread. *Anopheles quadriannulatus* was recorded for the first time from the Caprivi strip but in very low numbers. No *An. funestus* was found in Namibia due to the DDT house-spraying activities, but was readily collected resting indoors in villages across the border in Angola.

Studies on the fitness cost of insecticide resistance in *An. funestus* indicated little effect on those adults carrying the resistance genes compared with their susceptible counterparts. This accounts for the high level of resistance still found in the base colony FUMOZ despite being maintained for over 50 generations in the laboratory without exposure to insecticides.

INTERNATIONAL RESEARCH COLLABORATORS:

Prof J Hemingway, Director, Liverpool School of Tropical Medicine, UK
Dr H Ranson, Liverpool School of Tropical Medicine, UK
Prof A Cornel, University of California, Davis, USA
Prof N Besansky, University of Notre Dame, USA
Prof F Collins, University of Notre Dame, USA
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:

Wellcome Trust
National Institutes for Health
World Health Organization
SA Medical Research Council
SA National Research Foundation

TRAINING:

Postgraduate Training
VCRU staff provided lectures on medical entomology for the Diploma in Tropical Medicine & Hygiene course run by the School of Pathology, University of the Witwatersrand. Lectures and practical demonstrations were given covering all entomological aspects of arthropod-borne diseases and arthropods of medical importance.

Masters and Doctoral students from all over Africa are trained, many with 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, Mali, Senegal, Zambia 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.

Viral Diagnostics and Surveillance

Head:
Ezekial Maselesele

STAFF:

E Maselesele Higher Dip Med Tech, Laboratory Controller

VIRUS ISOLATION:

Enterovirus
S Moonsamy Dip Med Tech, Chief Medical Technologist
A Oliver, Dip Med Tech Chief Medical Technologist
P Ngcobondwane Dip Med Tech, Senior Medical Technologist
E Motaung, Student Medical Technician
D Lebambo, Laboratory Assistant

Respiratory/General
A Buys Dip Med Tech, Chief Medical Technologist
N Nhlovu Dip Med Tech, Chief Medical Technologist
C Esterhyse Dip Med Tech, Senior Medical Technologist
T Mashaba Laboratory Assistant
L Harvey Laboratory Assistant

Reagents/Cell Culture
M Vandecar Dip Med Tech, Chief Medical Technologist
C Simelane Laboratory Assistant
A Sehata Laboratory Assistant

Serology
B Singh Dip Med Tech, Chief Medical Technologist
B Miller Dip Med Tech, Chief Medical Technologist
M Masango Dip Med Tech, Chief Medical Technologist
A Molefe Dip Med Tech, Chief Medical Technologist
S Majiki Dip Med Tech, Senior Medical Technologist
S Zwane Dip Med Tech, Senior Medical Technologist
M Maleka Dip Med Tech, Medical Technologist
S Hloma, Laboratory Assistant
E Kekana, Student Medical Technologist
T Modiselle, Student Medical Technologist

SUPPORT SERVICES:

Receiving Laboratory
L Cranston Spec Med Tech, Chief Medical Technologist
L Ngwenya, Data Input Clerk
R Mokoena, Data Input Clerk
E Lemmer, Laboratory Clerk
A Chauke, Data Input Clerk
E Tetseta, Driver

Media
E Mthethwa BSc, Medical Technical Officer
F Boshomane, Laboratory Assistant
A Selepe, Laboratory Assistant

Diagnostics Kitchen
J Masekwameng, Laboratory Assistant
D Msibi, Laboratory Assistant
F Mashangoane, Laboratory Assistant
M Mpyana, Laboratory Assistant
E Mathebula, Laboratory Assistant

For 2005 the serology section processed 20 120 samples in total amounting to 50 300 tests performed, a 26% increase as compared to the previous year. During October the serology section participated in the annual HIV/syphilis antenatal surveillance for the National Department of Health with 3 105 samples from Gauteng Province, a slight decrease in sample numbers from last year. Once again the section performed incidence testing for all 9 provinces in the country with a total of 6 000 samples including dried blood spot samples for the Human Sciences Research Council survey.

The Measles/Rubella Regional Reference Laboratory continued to provide an excellent service for national surveillance as well as quality control for Southern Africa as part of WHO/AFRO mandate. During 2005, the number of measles samples submitted increased sharply from 3 968 in 2004 to 5 000 as a result of vigorous surveillance following a measles outbreak in the country. Also the annual WHO/AFRO audit of the measles/rubella laboratory went very well. As part of the WHO/AFRO mandate southern African countries sent a quarter of their annual intake of samples to the serology section as part of the quality assurance process.

HIV rapid/simple kit evaluation is ongoing providing very useful information for the National Department of Health on the operational characteristics when deciding which tests kits to use within the Voluntary Counselling and Testing (VCT) program. The serology section receives dried blood spot (DBS) samples from VCT centres in Durban, Cape Town and Johannesburg as part of quality control.

The virus isolation/detection continued to provide an important service towards both patient management (CMV pp65 and shell vial, HSV shell vial, and other respiratory viruses), and surveillance (AFP, influenza viral watch and measles/rubella) with a total of 7 241 samples received for various viral investigations including urine samples for measles isolation. For patient management the method of choice is rapid viral detection whereby the turn-around times were reduced remarkably from more than 14 days to 36 hours and even less with CMV pp65.

Our Regional Polio Laboratory (WHO/AFRO) received samples from SADC national laboratories and provided training for technicians from those countries in polio diagnostics and laboratory management including quality control. The course lasted for three weeks with competency certificates issued at the end, and a follow up visit by our Regional Polio Laboratory staff to the participating country to observe implementation of the knowledge again during training. This service, together with distribution of both RD and L20 B cells by Megan Vandecar to various countries in Africa, has improved the quality of cell lines being used and subsequent isolation rate in the fight to eradicate poliomyelitis from the continent of Africa.

The Viral Diagnostic/Surveillance Unit is a registered training laboratory. Nine microbiology registrars, 60 students from the University of Limpopo, three biomedical students have received training at this laboratory, and three biomedical students received assistance in preparing for the Board Examinations in March 2005, which they all passed, and in addition three laboratory personnel from Lesotho were trained in basic quality management.

TRAINING AND VISITS BY STAFF MEMBERS:

S Moonsamy attended the Annual meeting of Directors and Data Managers of the WHO Polio Laboratory Network as well as the Technical Supervisors Meeting in Harare, Zimbabwe, September 2005.

A Buys presented a poster on Influenza Activities in South Africa at the Medical Technologists Congress in Cape Town: 29 April 3 March 2005.

M Masango attended the WHO Measles/Yellow Fever Laboratory Directors meeting in Entebbe, Uganda: 2-3 March 2005

The NICD hosted the BED ELIZA Incidence training workshop in October 2005 for the CDC.

The specimen receiving laboratory processed some 43 159 samples, a twofold increase in sample intake as a result of relocation of microbiology laboratories of the NICD from Braamfontein to the Rietfontein campus.

In conclusion, I would like to thank all staff members of this Unit including the kitchen staff for providing such an excellent dedicated service as shown by both internal and external quality control programs and continued accreditation by SANAS and WHO.

Epidemiology Unit

Head:
Dr Lucille Blumberg

Back from left: Dr Cheryl Cohen, Liz Millington, Dr Bernice Harris, Prof Hendrik Koornhof, Dr Gillian de Jong
Front from left: Benn Sartorius, Jo McAnerney, Maupi Letsoalo, Dr Lucille Blumberg

STAFF

Dr L Blumberg MBBCh MMed (Micro) DTM&H DCH DOH, Head of Unit
Prof HJ Koornhof MBChB Dip Bact DCP FRC Path, Consultant

Epidemiology & Surveillance Division
Dr BN Harris MBChB MMed (Community Health), Community Health Specialist/Epidemiologist
Dr C Cohen MBBCh DTM&H FC Path (SA) Micro MSc (Epidemiology), Specialist Microbiologist/ Epidemiologist
Sr JM McAnerney RN RM Dip Data Dip Method, Professional Nurse/Data Manager

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

Biostatistics/Biomathematics Division
M E Letsoalo BSc (Hons), HED, Msc

National Comprehensive Plan for Prevention, Care & Treatment of HIV
Dr TM Marshall MBBCh FC Path (Virol) DTM&H, Specialist Virologist, Programme Manager
N Cassim Nat Higher Dip Haematology, Project Manager

FELTP
Dr BN Harris MBChB MMed (Community Health), Community Health Specialist, Director of SAFELTP
BK Sartorius BSc (Hons) (Micro) MSc (Epidemiology & Biostatistics) EPIET Fellow

Dr I Weber MBChB DTM&H, Registrar in Community Health, on secondment from the University of Pretoria

Mrs L Millington, Publications Officer/Administration

OUTBREAK DIVISION

OVERVIEW:

During 2005 the Outbreak Division has provided support and responded to several outbreaks in various provinces in South Africa as well as outside the country where our expertise was required. The response includes technical support, advice regarding appropriate specimen collection, facilitation of laboratory confirmation, development of case definitions, recommendations regarding clinical management, outbreak investigation and advice with respect to control measures. The unit is a member of the National Outbreak and Response Team (NORT) and participates in the monthly meetings and related activities of NORT.

OUTBREAK RESPONSE 2005:

South African response to the Marburg outbreak in Uige, Angola
The Outbreak Division of the Epidemiology Unit partnered with the Special Pathogens Unit during the Angolan Marburg outbreak in 2005 to ensure South Africa was well prepared to respond to importations of disease and laboratories were able to safely and effectively handle specimens from suspected cases. There were no laboratory confirmed cases of imported Marburg in South Africa during the outbreak. A number of activities were included in this response:

- Country-wide alerts and information sheets which included case definitions and guidelines for management of suspected cases, including indications for laboratory testing, were

distributed to public and private health care facilities and laboratories countrywide
- All suspected cases were screened by the unit by means of a 24 hour hotline service
- Daily meetings were conducted to plan for possible importations and discuss each suspected patient
- Daily situation reports were issued to key role players regarding suspected cases and relevant outcomes
- Any required diagnostic testing was facilitated
- Twenty four persons with a history of travel to Angola or bordering areas within the previous 21 days with fever and/or evidence of haemorrhage were evaluated, including 13 South Africans who either worked or traveled to Angola. Two patients fulfilled the case definition for laboratory testing for Marburg and both were negative. Malaria was the most common confirmed alternative diagnosis (six patients). Five patients died, two as a result of malaria

Measles outbreak
During 2004 and 2005 outbreaks of measles were reported in Gauteng, KwaZulu Natal and the Eastern Cape Province. Low immunization coverage rates, particularly in isolated communities, were common to all the outbreaks. During January Dr Harris formed part of the NORT team who, with the WHO, investigated the measles outbreak in KwaZulu Natal.

Diarrhoeal disease outbreaks
The unit provided support to several large outbreaks of watery diarrhoea in 2005. These included 2 in the Northern Cape, and 1 across the Gauteng/ Mpumalanga border. Common to these outbreaks was the presence of faecally contaminated water sources and the challenge of obtaining suitable stool specimens for culture of viral and bacterial pathogens. Guidelines for stool collection and laboratory processing were developed and issued. The unit worked in partnership with the Communicable Disease Directorates of the respective provinces, the Enteric Disease Reference Unit of the NICD and the MRC/Medunsa Diarrhoeal Pathogens Research Unit with regard to laboratory investigation and control of the outbreaks.

Hepatitis A
A number of outbreaks of hepatitis A were reported in 2005. The first of these was an outbreak in a home for physically and mentally disabled individuals. Five cases were confirmed with one death. Investigation of the outbreak did not support a common source and person-to-person spread was most likely, given the challenges of maintenance of good personal hygiene in such facilities. The outbreak response included the provision of pooled human immunoglobulin to all residents, introduction of measures for improved hygiene and serological screening of staff. The latter showed 100% immunity to hepatitis A amongst staff members in the home. A program of hepatitis A and B immunization has now been implemented.

Additional hepatitis A outbreaks occurred at a mission station in Limpopo and in a home for HIV infected children in Gauteng.

Meningococcal Disease outbreaks
Two outbreaks of meningococcal disease were reported in 2005. The first occurred in Lindela repatriation centre in Gauteng. Thirteen laboratory confirmed cases of meningococcal disease serogroup W-135 were identified and several additional cases are suspected to have died of meningococcal disease without laboratory confirmation. The unit worked in partnership with the provincial and district Communicable Disease Directorates and the RMPRU of the NICD to investigate and make recommendations for control of the outbreak. The presence of substantial overcrowding in living areas is likely to have been the biggest contributor to the spread of disease in the facility.

An additional outbreak of meningococcal disease occurred in a community of seasonal farm workers in Beit Bridge, Limpopo. The unit carried out a site visit to the area and recommendations for control following the administration of chemoprophylaxis to close contacts included use of quadrivalent meningoccal vaccine for all workers in the compound.

Typhoid fever outbreak
A large outbreak of typhoid fever occurred in Delmas, Mpumalanga in August 2005. The outbreak was caused by faecal contamination of the water supply. The Epidemiology Unit assisted in the investigation of this outbreak and a case control study was conducted in the area in partnership with the Mpumalanga Communicable Disease Directorate.

Collecting water for testing during the Delmas typhoid fever outbreak, 2005.

One hundred and seventy seven cases of suspected typhoid fever were also reported on the East Rand of Gauteng during the Delmas outbreak. An investigation into these cases was conducted by the unit. Investigation revealed that the majority of reported cases (85%) had a history of travel or contact with Delmas. Of the remaining cases only 1 patient had a culture confirmed diagnosis of typhoid fever, 15 patients either did not meet the case definition or had an

alternative diagnosis. Thus only 12 cases were classified as probable or possible *Salmonella typhi* infection. No clear risk factors for locally acquired disease could be identified.

THE SURVEILLANCE AND EPIDEMIOLOGY DIVISION

OVERVIEW:

The division is instrumental in the management of the AFP, suspected measles and respiratory virus surveillance systems. The division provides epidemiology support to the National and Provincial departments of health and other units within the NICD through laboratory-based surveillance, data mining, special research projects, provision of up-to-date information, interpretation of epidemiological data, representation at national and sub-national communicable disease and EPI meetings, quarterly publication of the *Communicable Diseases Surveillance Bulletin*, field visits, and participation in postgraduate and operational training programmes.

SURVEILLANCE PROGRAMMES:

Suspected measles case-based surveillance
World wide, measles is still the major cause of vaccine preventable deaths and although South Africa has maintained vaccination levels above 70% for many years, measles outbreaks continued to occur. Since 1995, six southern African nations (Botswana, Malawi, Namibia, South Africa, Swaziland, and Zimbabwe) have launched measles-elimination initiatives in accordance with the recommendations of the World Health Organization (WHO) African Regional Office (AFRO). Strategies include programs to 1) achieve routine vaccination coverage of $\geq$ 95% with one dose of measles vaccine administered at age 9 months; 2) implement a one-time national catch-up measles vaccination campaign to interrupt indigenous transmission of measles; 3) implement periodic national follow-up measles campaigns to maintain interruption of measles transmission; and 4) establish case-based measles surveillance with laboratory confirmation.

The NICD is accredited by WHO to perform measles and rubella IgM testing for the national case-based surveillance and trace the molecular epidemiology of the measles virus in South Africa. Blood and urine specimens from each suspected measles case (smc) is sent to the NICD for confirmation. Case investigation forms are completed by facility or district personnel and forwarded to the National Department of Health. The numbers presented here only represent specimens received by the NICD and may differ from those presented by the National Department as they may receive information on epidemiologically-linked cases where no specimens were taken.

During 2005 the NICD tested 4 438 blood specimens from cases of rash and fever for suspected measles case-based surveillance of which 1 363 (31%) were collected in the Eastern Cape and 1108 (25%) in Gauteng. All provinces met the criteria for sufficient number of specimens collected, more than 1 smc per 100 000/population, with the Eastern Cape, Northern Cape and Mpumalanga collecting more than 16/100 000. This may however mask silent districts and sub districts (table 1). Of all specimens, 706 (16%) tested positive for measles and 1047 (24%) for rubella.

Table 1: Suspected measles case based surveillance, South Africa, 2005

Province	ECP	FSP	GAP	KZP	LPP	MPP	NCP	NWP	WCP	TOTAL
Number of SMC	1363	102	1108	580	181	469	195	215	225	4438
% of total SMC	31%	2%	25%	13%	4%	11%	4%	5%	5%	100%
cases/100 000 population	19.1	3.6	13.1	6.3	3.0	16.1	18.3	7.2	4.7	
Measles cases	565	1	42	77	2	3	0	1	15	706
Rubella cases	189	28	280	170	64	126	76	79	35	1047

Measles: 80% of all measles cases occurred in 2 districts of the Eastern Cape of whom 48.9% were 5 to 14 years of age. This epidemic peaked with 275 cases in April and tapered to 5 cases in December following a province-wide <15 years of age vaccination campaign. (Figure 1)

Small clusters of cases occurred in Gauteng in the first half of the year and only sporadic cases thereafter.

Rubella: 765/1047 (73%) of all rubella cases occurred in 4 provinces namely Gauteng (27%), Eastern Cape (18%), KwaZulu-Natal (16%) and Mpumalanga (12%). The median age of cases was 6 years of age with a range of 3 months to 77years. Most cases occurred in the spring and early summer. (Figure 2)

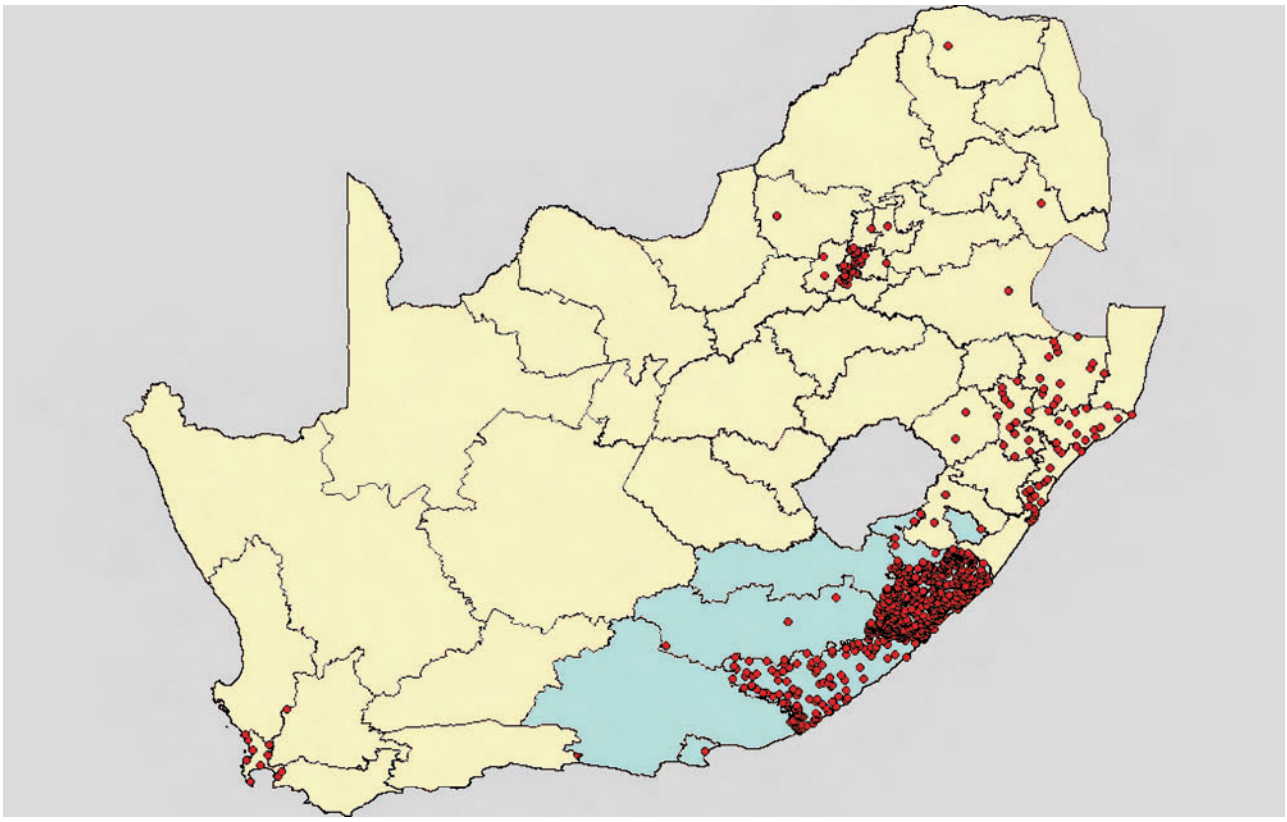


Figure 1. Measles IgM positive cases, South Africa, 2005. (Eastern Cape districts highlighted in blue.)

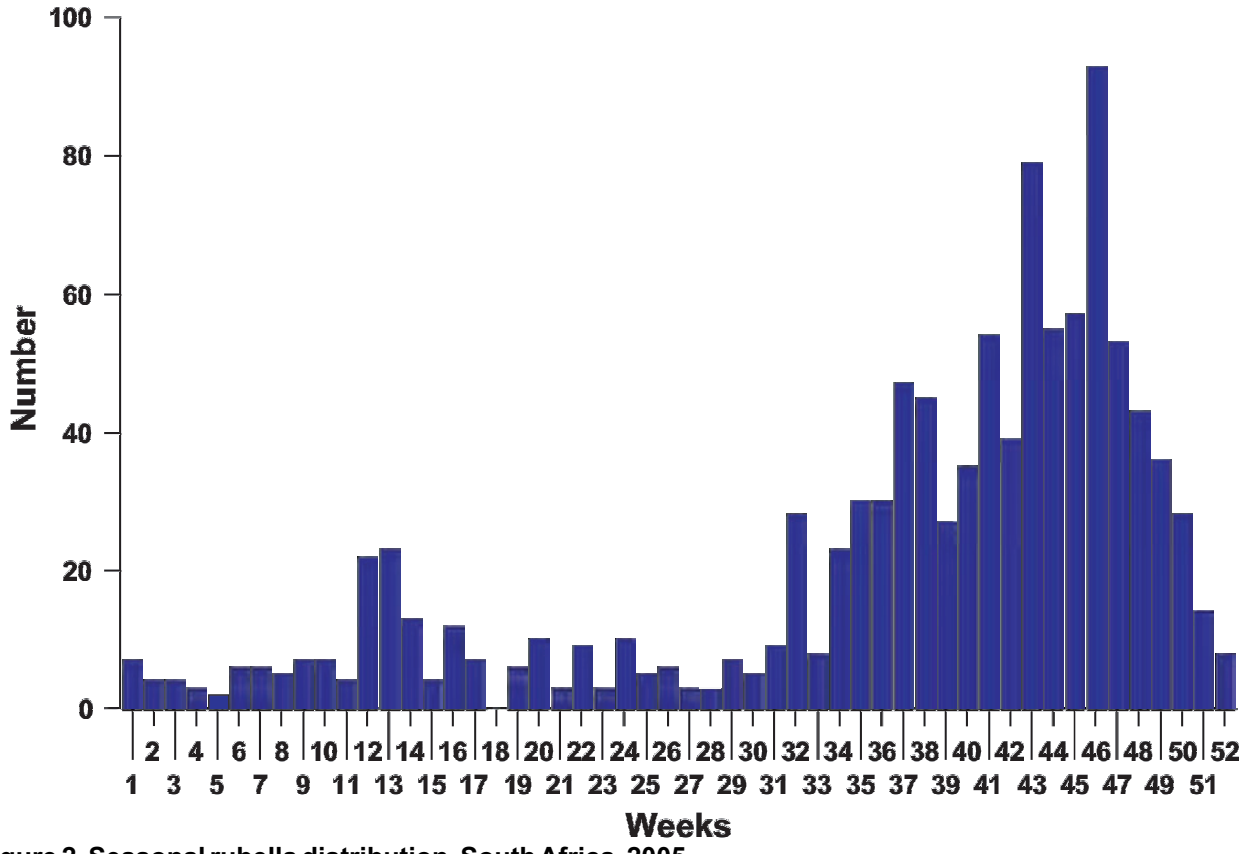


Figure 2. Seasonal rubella distribution, South Africa, 2005.

AFP Surveillance

AFP surveillance is a critical component of the programme to eradicate polio from the world. In keeping with the WHO world-wide campaign to eradicate poliomyelitis, acute flaccid paralysis (AFP) was made a notifiable condition in South Africa in April 1994. The case definition of AFP cases to be notified to the Regional Office of the Department of National Health specifies:- any case of acute flaccid paralysis including Guillain-Barré syndrome, in a child less than 15 years of age, or a patient of any age diagnosed as polio by a medical doctor. All cases of AFP must be regarded as possible polio cases until proven otherwise. All such cases require two stool specimens of sufficient quantity collected at least 24 hours apart within 14 days after onset of paralysis, and sent to the National Institute for Communicable Diseases for polio identification. During 2005, at a detection rate of one case of AFP per 100 000 children under 15 years, 157 cases needed to be identified.

The NICD also serves as national isolation laboratory for six other southern African countries i.e. Angola, Botswana, Lesotho, Mozambique, Namibia, and Swaziland.

During the year, 1 501 stool specimens were received from patients with AFP of which 953 were from patients outside South Africa, and 548 from South African cases of which 8 were from patients with onset of paralysis prior to 2005. (Figure 3)

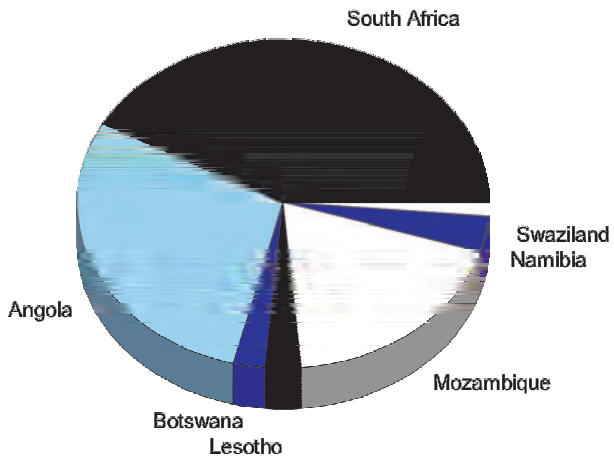


Figure 3. Stool specimens from AFP cases received by NICD, 2005.

1: South African cases

Case detection rate (only patients from whom specimens were received included) ranged from 1.48 to 2.44 (mean 1.80). Of the 273 South African cases with onset of paralysis in 2005, one specimen only was received from 40 cases, and two or more specimens from 233. The date of onset of paralysis was known for 236 cases. Two specimens taken at least 24 hours apart and within 14 days of onset were received from 191/273 (69.96%) cases (range per province 44.44% to 94.44%). (Figure 4)

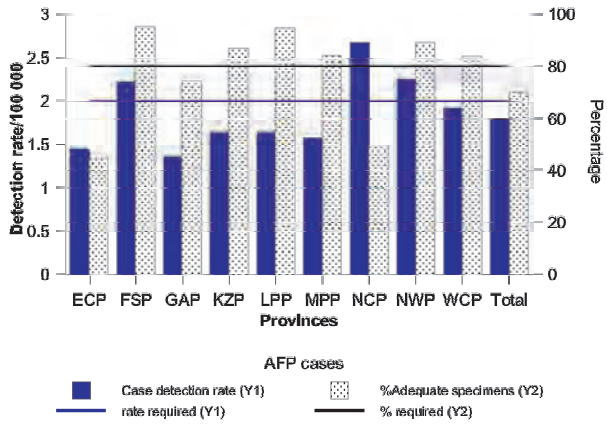


Figure 4. AFP case detection and stool adequacy rate, South Africa, 2005.

Non-polio enteroviruses were isolated from 59 of the 540 specimens (non-polio isolation rate 10.93%), and poliovirus, identified as Sabin type poliovirus from two specimens of one patient. The date of the last dose of OPV was unknown.

2: Other Southern African countries

Of the 953 specimens received from other African countries, 754 were from the six southern block countries served by the NICD, of which 54 were from patients with onset of paralysis prior to 2005. Of the 350 patients with onset of paralysis, two adequate were received from 85.71% of cases (range per country 55.56% to 90.27%). (Figure 5)

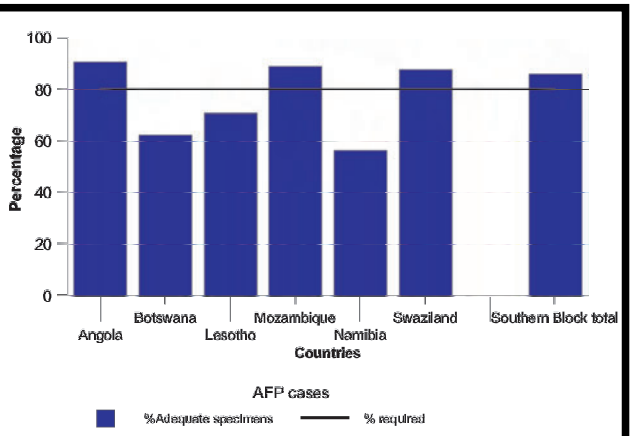


Figure 5. AFP stool specimens received by the NICD from other African countries, 2005.

Non-polio enteroviruses were isolated from 154/754 specimens with a non-polio enterovirus isolation rate of 20.42% (range per country 11.43% to 23.81%). Poliovirus was isolated from 47 specimens, 19 of which were identified as wild type polio 1, and the remainder as Sabin strains. All the wild type isolates were from patients in Angola i.e.10 patients and one contact. Date

of onset for the first case was 25 April, and for the last 13 November and originated from 7 different districts, in 4 provinces.(Figure 6) Polio was previously confirmed in Angola in 2000. The outbreak of polio was followed by a nationwide immunization campaign targeting children under 5 years of age.

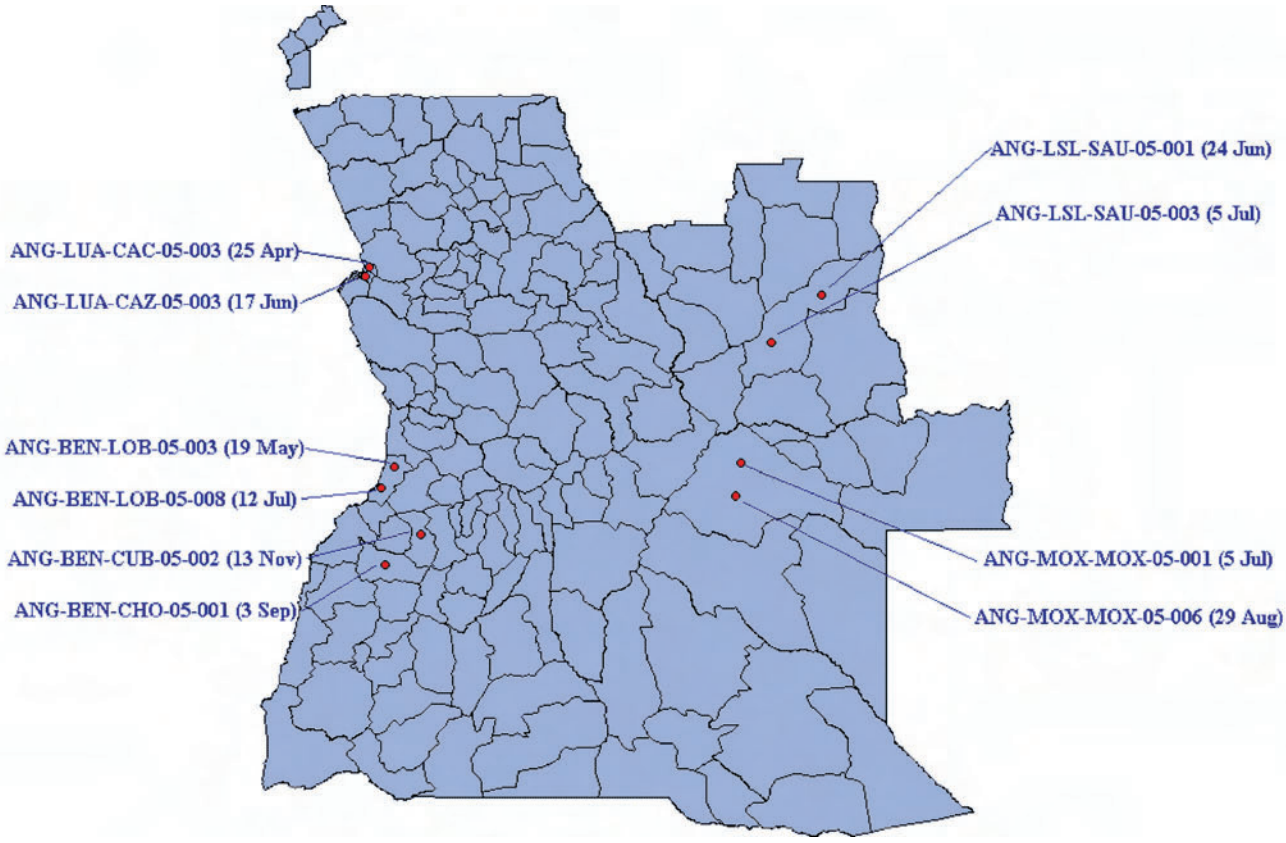


Figure 6. Distribution of polio cases, Angola, 2005.

Respiratory Virus Surveillance

During 2005 a total of 1609 specimens were received for detection of respiratory virus. Of these 1360 (84.5%) were received from the Viral Watch programme, started in 1984 and specifically designed to monitor influenza activity in the community, and detect the type of influenza strains prevalent. The number of centres was increased substantially during 2005, bringing the total to 85, mainly general medical practitioners at 65 centres. Throat swabs are submitted from these centres throughout the year from patients with respiratory tract infections of recent onset i.e. within 48-72 hours, and without obvious bacterial cause, and transported to NICD in viral transport medium for isolation of virus.

A total of 581 influenza isolations were made, of which 554 (95.4%) were from the Viral Watch The isolates were further identified as 405 influenza A, of which A H1N1 (A/NewCaledonia/20/99-like) accounted for the majority, and 116 influenza B, mainly B/Hong Kong/330/01-like. (Figure 7)

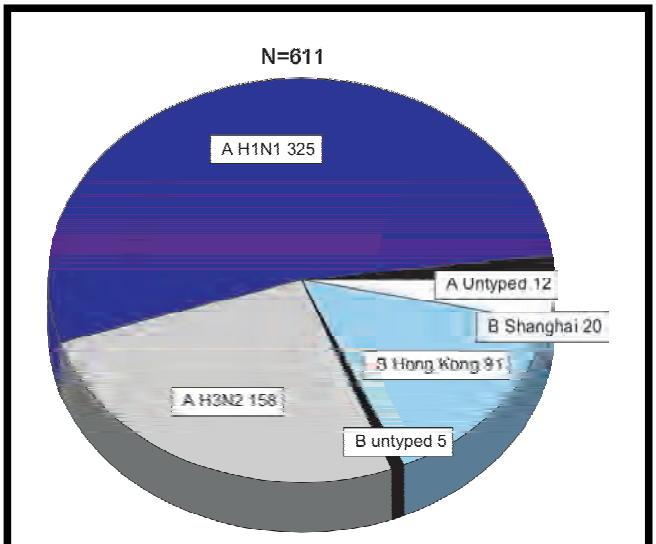


Figure 7. Influenza virus isolates, NICD, 2005.

The first influenza isolate of the season was made from a specimen collected on 20 April, and the last from a specimen collected on 29 September. (Figure 8) In addition, 30 influenza isolates were made from specimens submitted from the Seychelles.

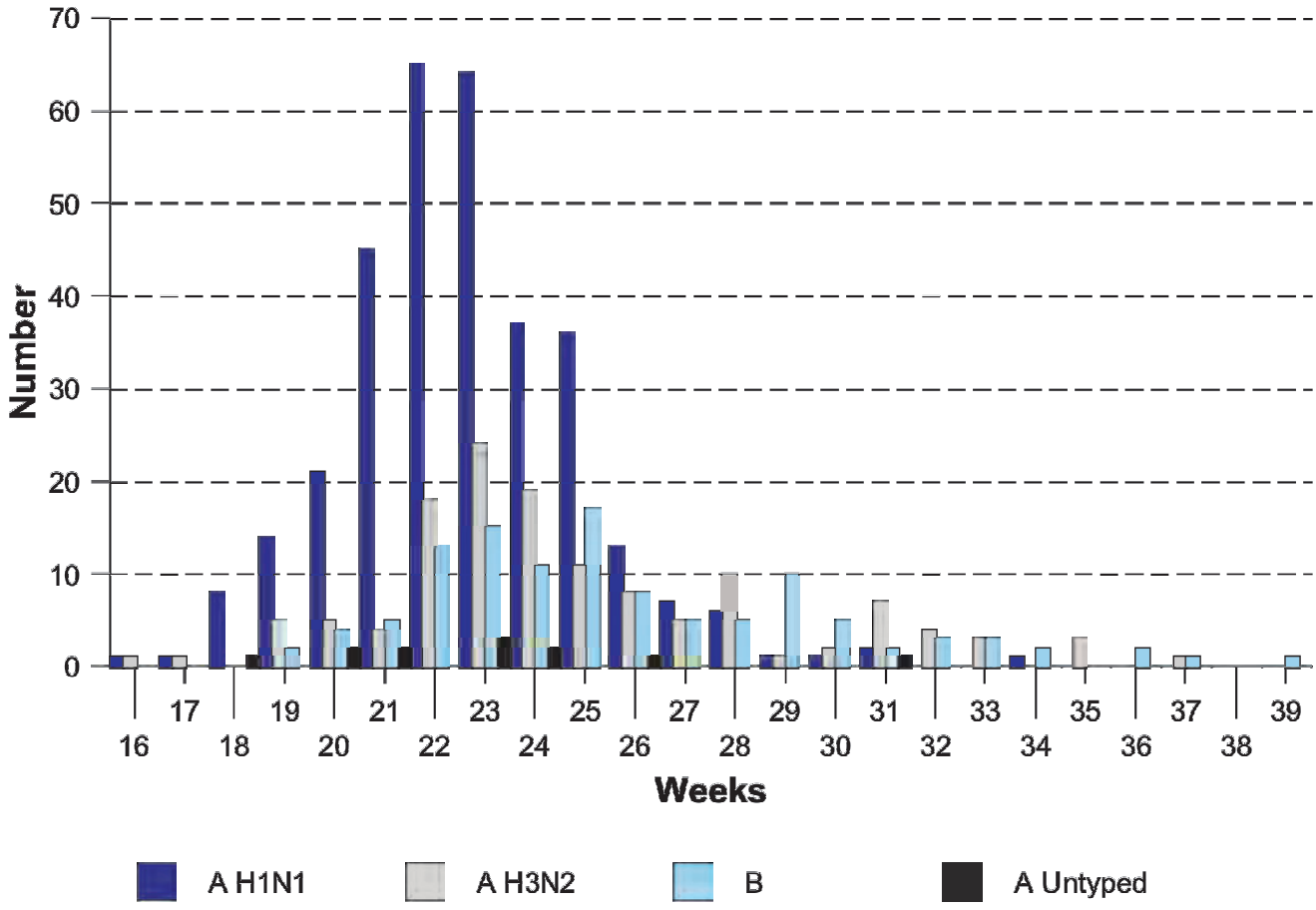


Figure 8. Seasonal variation in viral types, NICD, South Africa, 2005.

A further 79 respiratory virus isolations were made during the year, including 22 respiratory syncytial virus, 23 parainfluenza virus (8 type 1, 5 type 2, 10 type 3), and 11 adenovirus. The majority of these were made from routine specimens.

A small number of the Viral Watch centres also submitted respiratory infection morbidity data which showed an increase from week 19, peaking during week 23 and declining gradually thereafter. (Figure 9)

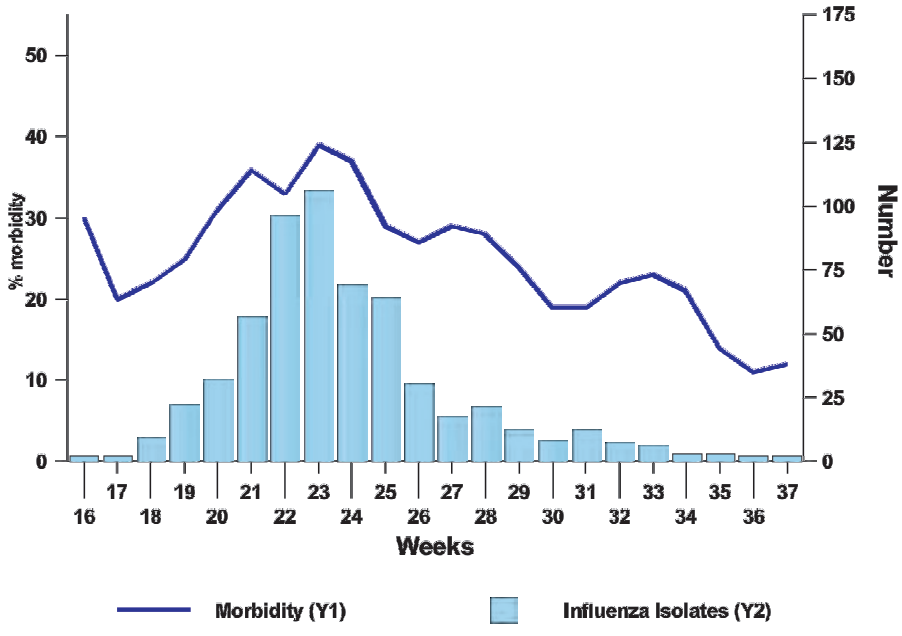


Figure 9. Influenza isolates and respiratory infection morbidity, Viral Watch, South Africa, 2005.

BIOSTATISTICS DIVISION

This division was established in 2005 to provide statistical support and expertise within the National Institute for Communicable Diseases.

Outside NICD/NHLS
This division taught an introductory course, "*Repeated Measurement Data*" at the University of the Witwatersrand Medical School, also assisted with statistical data analysis and/or sample size determinations for students at University of the Witwatersrand Medical School, University of Limpopo, and UNISA and participated in the NICD-MRC Biostatistics Sessions.

Within NICD/NHLS
Numerous consultations (data analyses & study designs) were carried out within different units, mainly Specialized Molecular Diagnostics, Immunology, and Cell Biology HIV/AIDS. An introductory course to data analysis was presented during the year.

National
Mr Letsoalo participated actively in the Medicines Control Council's Clinical Trials Committee.
He also presented a paper at the SASA conference at Rhodes University.

SOUTH AFRICAN FIELD EPIDEMIOLOGY AND LABORATORY TRAINING PROGRAMME:

The South African Field Epidemiology and Laboratory Training Programmes (SAFELTP) in collaboration with the U.S. Centers for Disease Control and Prevention (CDC) was established in 2005. The SAFELTP is designed to train field epidemiology as well as public health laboratory fellows for leadership positions in the South African national and provincial health services and the National Health Laboratory Services (NHLS). The SAFELTP will consist of both long (2-year residency) and short courses targeting different audiences within the public health system of South Africa. The first short course on Outbreak Investigation and Management is planned for May 2006 with the longer residency curriculum to begin in January 2007. Seminars were held during 2005 between the CDC and NICD role players to set up this programme.

NATIONAL COMPREHENSIVE PLAN FOR PREVENTION, CARE & TREATMENT OF HIV

On the 8 August 2003, the National Department of Health announced the launch of a new comprehensive plan for the management of HIV and AIDS in South Africa. This included providing access to anti-retroviral treatment using a phased-in approach nationally for those who had advanced HIV infection in South Africa.

The initial 8 months were spent planning the programme, getting the plan approved by the Minister of Health and the Cabinet, and the initial implementation of price negotiations with, amongst others, suppliers of pharmaceuticals and diagnostic material. From the 1 April 2004 the first clinical sites were ready to implement the programme.

One of the keys to the successful implementation of such a treatment initiative for HIV is the laboratory monitoring of people before and while on treatment. For this purpose, CD4 testing is performed as a measure of the amount of immune system destruction caused by the virus. Viral load measurements are also performed as a baseline prior to initiating treatment, and then to monitor the decrease in the amount of circulating virus present in each person once on treatment.

In the course of 2005, the number of laboratories across South Africa performing CD4, viral load and HIV PCR tests was increased, as were the test volumes (Table 1)

The following figures (1-3) indicate the numbers of CD4, viral load, and PCR tests performed nationally per month for 2005:

Table 1: Number of laboratories and tests performed per year.

	2004 (Apr-Dec)	2005 (Jan-Dec)
Number of CD4 laboratories	24	39
Number of CD4 tests performed	280 760	799 424
Number of viral load laboratories	8	10
Number of viral loads performed	37 507	145 312
Number of PCR laboratories	3	6
Number of PCR tests performed	6006 (Sept to Dec)	41 490 (Jan-Dec)

Figure 1: Number of CD4 tests per month nationally

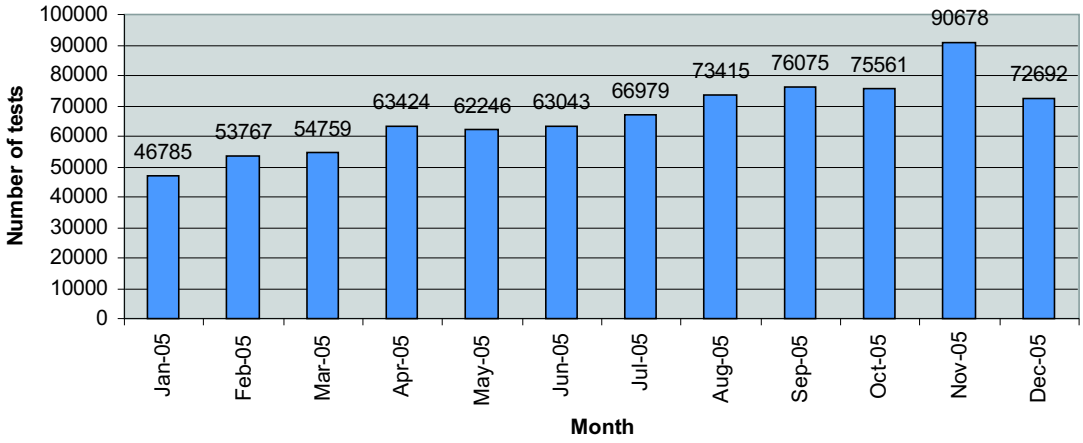


Figure 2: Number of viral load tests per month nationally

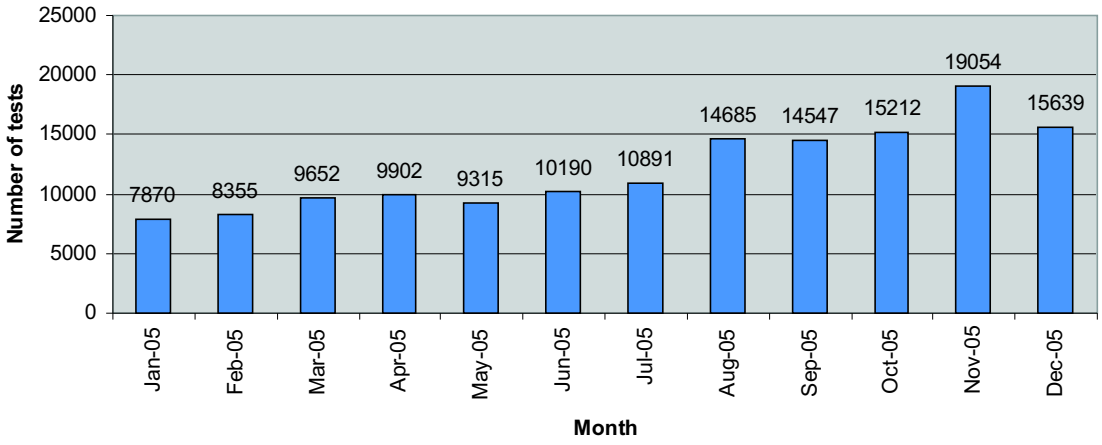
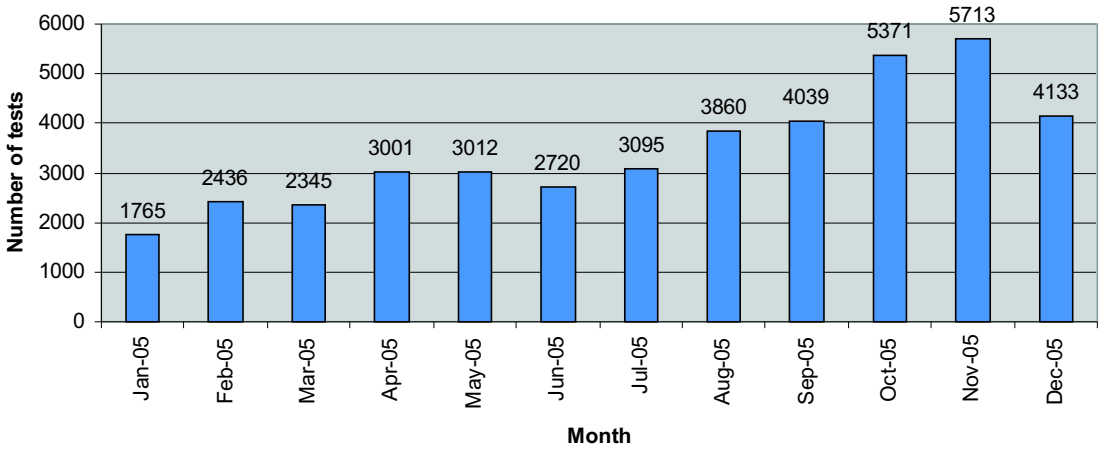


Figure 3: Number of HIV PCR tests per month nationally



It is with grateful thanks that the hard and sustained work provided by all the public service laboratories within South Africa in support of this programme is acknowledged in this report

PUBLICATIONS:

The Unit publishes a monthly Communiqué highlighting current outbreaks in South Africa and a quarterly *Communicable Diseases Surveillance Bulletin* which contains more detailed information on relevant and topical infectious disease concerns as well as current and cumulative laboratory-based surveillance data for selected infectious diseases. These are distributed electronically to a wide audience of individuals and institutions dealing with communicable diseases in the public and private sector. The publications can be accessed on http://www.nicd.ac.za.

RESEARCH PROJECTS:

The fieldwork for the serosurvey to assess the age of acquisition of rubella and the degree of susceptibility in women of childbearing age in South Africa, to assist in formulation of an immunization strategy, progressed well and is expected to be completed early in 2006. The study is funded by the American Red Cross.

A protocol was developed for malaria surveillance for imported cases in Gauteng and fieldwork started in December. The study will assess the burden of malaria disease in both the private and public health sector, evaluate diagnosis and chemotherapy of malaria in accordance with national guidelines and identify contributors to poor outcome.

A study was conducted to determine the prevalence of HIV infection and/or exposure amongst serologically confirmed measles cases. Anonymous unlinked HIV testing was performed on residual serum from specimens received by the NICD from suspected measles cases in Gauteng Province for 2004. The study is complete and will be submitted for publication shortly.

SPECIAL APPOINTMENTS:

Mr Maupi Letsoalo was appointed to the clinical trials committee of the Medicines Control Council of the National Department of Health
Drs Blumberg, de Jong and Harris are members of the National Outbreak and Response Team
Dr Harris is a member of the National EPI Task Force
Dr Blumberg continued in her capacity as a member of the national Malaria Advisory Group (MAG) and a member of the Subcommittee on Chemotherapy and Prophylaxis (SCAT)
Drs Blumberg (secretary/treasurer) and de Jong were appointed to the executive of the Infectious Diseases Society and to the executive of the Federation of the Infectious Diseases Society of South Africa. Dr Blumberg also served as a co-chairperson of the biannual conference, Sun City in July 2005. Dr Blumberg is the appointed representative of the Society to the South African Division of the International Union of Microbiological Societies.

TEACHING AND TRAINING:

The members of the Unit were involved in teaching activities to undergraduate and postgraduate health care workers in infectious diseases and public health. These activities included:

- Diploma in Tropical Medicine and Hygiene- Drs Blumberg and de Jong and Drs Harris and Weber, coordinated, taught and were internal examiners for the Diploma in Tropical Medicine and Hygiene at University of the Witwatersrand and the School of Health Systems and Public Health, University of Pretoria respectively
- Certificate Course in Travel Medicine, university of the Witwatersrand
- National Communicable Disease Coordinators workshops in a number of provinces
- Undergraduate teaching to medical students at Universities of Pretoria, Stellenbosch and the Witwatersrand
- Postgraduate teaching, within various academic institutions and the private health sector
- WHO-Afro Influenza workshop; surveillance, epidemiology and clinical issues
- Measles outbreak response and prevention to Gauteng health care workers and policy makers,
- Communicable disease control to registrars in Community Health, University of Witwatersrand
- MPH student training in managing outbreaks and use of Epi-Info for data management and analysis, University of Pretoria

Two training workshops were conducted in partnership with the Provincial Communicable Diseases Control, Limpopo Province. The workshops were conducted on site in Polokwane for doctors and communicable disease staff on the “Diagnosis and Management of epidemic prone diseases”.

Dr Harris was an external examiner for the Wits MSc (Epidemiology)) “Topics and Seminars” in July and was also an examiner for the Colleges of Medicine, South Africa.

CONFERENCES ATTENDED:

INFORAB (International Conference on Rabies), Mumbai, India, December 2005.
Dr L Blumberg, Oral presentation: “Rabies in South Africa”.

Third African Regional Training Programmes in Epidemiology and Public Health Interventions Network (TEPHINET) Scientific Conference, Accra, Ghana, 5th to 9th December 2005.
Dr C Cohen, Dr G de Jong - attended as observers.

Annual Conference: South African Statistical Association, Rhodes University, Grahamstown, November 2005.
M Letsoalo, Oral presentation: "Issues in the analysis of group randomized trials".

Vaccinology Congress, Hermanus, 31st October to 2nd November 2005.
JM McAnerney, Oral presentation: "Surveillance of vaccine preventable diseases".
Dr L Blumberg, Oral presentation: "Travel vaccines".
Dr B Harris, Dr G de Jong attended as observers.

An evening of influenza for the members of the 'Viral Watch', NICD, 6th September 2005.
JM McAnerney, Oral presentation: "Viral watch a brief history".
L Blumberg, Oral presentation: "Review of the 2005 influenza season".

Second European Influenza Conference, Malta, 11th to 14th September 2005.
Dr G de Jong attended as an observer.

TB in South Africa, Cape Town, September 2005.
Dr L Blumberg, Oral presentation: "Nontuberculous mycobacteria".

First Joint Congress of the Federation of Infectious Diseases Society of Southern Africa (FIDSSA), Sun City, 24th to 27th July 2005.
Dr G de Jong, oral presentation "Ruffled feathers avian influenza in South Africa".
Dr G de Jong, co-author, poster, "PCR detection of *Bordetella pertussis* experiences of the Division of Infection Control, NHLS, Johannesburg".
Dr G de Jong, co-author, poster, Outbreak in Maandagshoek lessons learnt".
Prof HJ Koornhof, co-author: "A national survey of antimicrobial resistance in gonococci isolated in South Africa".
Prof HJ Koornhof, co-author: "A study to determine the aetiology of symptomatic, persistent and asymptomatic urethritis in an inner-city region of Johannesburg, South Africa". (This paper won the Discovery Health Clinical Excellence Award for the best oral presentation at the congress.)
Prof HJ Koornhof, co-author: Poster "Characterization of ciprofloxacin-resistant gonococci isolated in Gauteng Province". (This poster was granted the Discovery Health Clinical Excellence Award for the best sexually transmitted infections poster at the congress.)
JM McAnerney, Dr L Blumberg, posters: "Active influenza surveillance in South Africa" and "Measles outbreak August 2003-2005".

Influenza Symposium, 16th February, NICD.
JM McAnerney, oral presentation: "Influenza surveillance in South Africa".

GERMSSA, November 2005
TM Marshall, presentation on "The Rollout of ARV laboratory aspect." Vaccinology 2005.

SMLTSA conference 2005
N. Cassim, presentation on "An introduction to the Comprehensive HIV and AIDS Care, Management and Treatment Programme in South Africa."

Specialized Molecular Diagnostics

Head:
Dr Adrian Puren

STAFF:

Dr AJ Puren BSc (Hons) PhD MBBCh, Deputy Director, Head of Unit

HIV Molecular Diagnostics Unit
E Cutler BSc, Medical Scientist
E Tlale Dip Med Tech, Chief Medical Technologist
DM Mosweu MSc, Medical Scientist
M Pillay BSc (Hons), Medical Scientist
R Mnisi Dip Med Tech, Medical Technologist
J Mokoena, Laboratory Assistant
LJ Mashiloane, Laboratory Assistant
JL Sekgobela, General Worker
PA Kgadima, General Worker

Hepatitis Unit
Dr SM Bowyer PhD, Senior Medical Scientist
N Prabdial-Sing MSc, Medical Scientist
M Vos BSc (Hons), Medical Scientist

Molecular Poliovirus Unit
HN Gumede BSc Med (Hons), Senior Medical Scientist
V Singh-Dhawakieram Dip Biotech, Senior Biotechnologist
A Mawela MSc, Medical Scientist
C Sifile BSc, Science Graduate
M Nyuswa Dip Biotech, Biotechnologist
B Guliwe Dip Biotech, Biotechnologist
O Lentsoane Dip Biotech, Biotechnologist

The activities of the Specialized Diagnostics Unit continue to be diverse in nature ranging from operational research and support e.g. HIV DNA PCR participating in a phase one microbicide clinical trial. The activities have allowed the Unit to be exposed to diverse international and local experts in various fields. Moreover, the Unit has also integrated its activities with other Units such as the Viral Diagnostics Unit in providing support or, in the case of HIV incidence, to participation in workshops.

HIV INCIDENCE TESTING:

National HIV Incidence
The availability of tests for recent HIV infection promises to be a major advance in estimating incidence in selected populations. Incidence measures are generally better than prevalence measures for assessing the dynamics of HIV transmission in different population strata. Recently, the Centres for Disease Control and Prevention (CDC) introduced the BED capture EIA (CEIA) assay to identify incident infection. The estimation of incidence is the result of a calculation that requires 3 input measures: the number classified as recent or incident infections, the “window period” (the period of seroconversion having occurred within the previous 180 days), and the number of seronegative individuals at risk for infection in the study population. The BED assay uses a multi-subtype synthetic peptide and measures the increasing proportion of HIV-IgG to total IgG after seroconversion. The BED assay algorithm is designed for seropositive specimens and has been successfully implemented in cross sectional studies. The advantage of the BED-CEIA is that it has a single window period, independent of the HIV subtype. The assay is designed to work well in populations with different HIV-1 subtypes and the testing algorithm has been successfully evaluated in populations in the United States and Thailand with B and E subtypes, as well as in cohorts from the Netherlands, Kenya, Ethiopia, Zimbabwe, and India which comprised of A, B, C, and D subtypes.

Background: The complexities and limitations of epidemiological approaches to measure HIV incidence argues strongly for a laboratory-based method that can distinguish recent from established long-term HIV infections. The 2005 South African national household survey on HIV, Behaviour and Communication included HIV incidence testing which allowed for the first time a joint analysis of HIV prevalence, HIV incidence and HIV associated risk factors.

Methods: 23 275 individuals aged 2 years and older participated in the survey and 15 851 respondents agreed to be tested for HIV. Linked anonymous testing, i.e. HIV test result can be linked to demographic and behavioral data without revealing the identity of the tested individual, was performed using dried blood spot (DBS) specimens. The detection of recent infections in confirmed HIV positive samples was performed with the BED capture enzyme immunoassay (Calypte Biomedical Corporation). An HIV positive specimen with a normalized optical density value of ≤ 0.8 was considered to be a specimen of recent HIV infection, otherwise, the specimen was classified as long-term infection. Annualized BED HIV incidence calculation applied a window period of 180 days for HIV subtype C specimens and took into account the complex survey design.

Results: The national HIV prevalence in the population of people two years and older is estimated to be 10.8%, with a higher prevalence in women (13.3%) than in men (8.2%). HIV prevalence increases among young

females from 9.4% in the 15-19 age group to 33.3% in the 25-29 age group. In males, the increase in HIV prevalence is more protracted, and peaks at a lower level than for females, 23.3% in age groups 30-34 and 35-39. Especially alarming are the incidence rates among young females. Females aged 15-24 years have an eight times higher HIV incidence than males, 6.5% compared to 0.8%, and account for 87% of the recent HIV infections in this age group. Our incidence analysis also suggests an increased risk of HIV acquisition during pregnancy. Among African women aged 1549 years who were pregnant in the last 24 months an HIV incidence of 7.9% was found, the highest incidence rate of all analyzed sub-populations in our survey.

Conclusions: The addition of HIV incidence testing into the survey protocol enables a more precise and timely analysis of the current HIV-transmission dynamics and the impact of prevention programs in South Africa.

Collaborators: Thomas Rehle , Adrian Puren , Khangelani Zuma , Victoria Pillay , Pelisa Dana, Olive Shisana. Technical Assistance: Beverley Singh and Martin Masango.

CDC-NICD COOPERATIVE AGREEMENT ACTIVITIES: INCIDENCE TESTING:

The NICD was host to a CDC-supported HIV incidence workshop that included participants from various African countries including Mozambique, Lesotho, Namibia, Botswana, Democratic Republic of Congo and Kenya and international experts from CDC. The combined workshop of epidemiologists and laboratory participants reviewed both epidemiological and laboratory of aspects and the implementation of tools for applications to national surveillance activities.

Participants at the CDC-supported HIV incidence workshop

POLYMERASE CHAIN REACTION FOR DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN INFANCY IN LOW RESOURCE SETTINGS:

Background: Diagnosis of human immunodeficiency virus (HIV) is essential for accessing treatment. Current HIV diagnostic protocols for infants require adaptation and validation before they can be implemented in the developing world. The timing and type of HIV assays will be dictated by country-specific circumstances and experience from similar settings. The performance of an HIV-1 DNA polymerase chain reaction (PCR) test, and in particular a single test at 6 weeks of age, in diagnosing HIV subtype C infection acquired in utero or peripartum was assessed.

Methods: A retrospective review of 1825 Amplicor HIV-1 DNA PCR version 1.5 tests performed between 2000 and 2004 in 2 laboratories in Johannesburg, South Africa on 769 effectively non-breast-fed infants from 3 clinically well characterized cohorts was undertaken. The HIV status of each infant was used as the standard against which the HIV PCR results were compared.

Results: The overall sensitivity and specificity of the HIV PCR test were 99.3 and 99.5% respectively. A single test was 98.8% sensitive and 99.4% specific in the 627 infants tested at 6 weeks of age (58 HIV-infected and 569 HIV-uninfected). Repeat testing of all positive HIV PCR tests minimized false positive results.

Conclusions: In resource-poor settings where HIV PCR testing in an environment of good laboratory practice is feasible, a single 6-week HIV DNA PCR test can increase identification of HIV-infected children substantially from current levels. Further operational research on how best to implement and monitor such a diagnostic protocol in specific local settings, especially in breast-fed infants, is necessary.

Collaborators: Sherman GG, Cooper PA, Coovadia AH, Puren AJ, Jones SA, Mokhachane M, Bolton KD.

A CLUSTER RANDOMIZED-CONTROLLED TRIAL TO DETERMINE THE EFFECTIVENESS OF STEPPING STONES IN PREVENTING HIV INFECTIONS AND PROMOTING SAFER SEXUAL BEHAVIOUR AMONGST YOUTH IN THE RURAL EASTERN CAPE, SOUTH AFRICA: TRIAL DESIGN, METHODS AND BASELINE FINDINGS:

Objective: To describe the study design, methods and baseline findings of a behavioural intervention trial aimed at reducing HIV incidence. Method A cluster randomized-controlled trial (RCT) conducted in 70 villages in rural South Africa. A behavioural intervention, Stepping Stones, was implemented in 35 communities in two workshops of 20 men and 20 women in each community who met for 17 sessions (50 h) over a period of 3-12 weeks. Individuals in the control arm communities attended a single session of about 3 h on HIV and safer sex. Impact assessment was conducted through two questionnaire and serological surveys at 12-month intervals. The primary outcome was HIV incidence and secondary measures included changes in knowledge, attitude and sexual behaviours. Qualitative research was also undertaken with 10 men and 10 women from two sites receiving the intervention (one rural and one urban) and five men and five women from one village in the control arm. They were interviewed individually three times prior to the workshops and then 9-12 months later.

Results: A total of 2776 participants (1409 intervention and 1367 control) were enrolled at baseline and had an interview, and HIV sero-status was established. HIV baseline prevalence rates in women were 9.8% in the intervention arm and 12.8% in the control arm. In men the prevalence was 1.7% in the intervention arm and 2.1% in the control arm. Demographic and behavioural characteristics were similar in the two arms. In the intervention groups 59.9% of participants attended more than 75% of the sessions. In the control group 66.3% attended the control session.

Conclusion: This is the third RCT to be conducted in sub-Saharan Africa evaluating a behavioural intervention using HIV incidence as a primary outcome. It is of particular interest as the intervention in question is used in many developing countries. There is good baseline comparability between the study arms and the process data on the workshops suggested that the interventions were feasible and adequately implemented.

Collaborators: Jewkes R, Nduna M, Levin J, Jama N, Dunkle K, Khuzwayo N, Koss M, Puren A, Wood K, Duvvury N.

CAN MALE CIRCUMCISION PREVENT ACQUISITION OF HSV-2 INFECTION?

Background: Little is known about the impact of male circumcision (MC) on HSV-2 acquisition. A randomized control intervention trial to evaluate this impact was performed in a region of sub-Saharan Africa with a high prevalence of HSV-2.

Methods: 3274 uncircumcised men, aged 18-24 and wishing to be circumcised, were randomized in a control and intervention group. Men were followed for 21 months with an inclusion visit and follow-up visits at month 3, 12 and 21. Male circumcision was offered to the intervention group just after randomization and to the control group at the end of 21 month follow-up visit. Male circumcisions were performed by medical doctors. At each visit, sexual behaviour was assessed by a questionnaire and a blood sample was taken for HSV-2 serology using the Kalon HSV-2 ELISA. These grouped censored data were analyzed in an “intention to prevent” univariate and multivariate analysis using the piecewise survival model, and relative risk (RR) of HSV-2 infection (intervention vs. control) with 95% confidence interval (95% CI) and p value was determined.

Results: Loss to follow-up was <10%; <1% of the intervention group were not circumcised and < 2% of the control group were circumcised during the follow-up. We observed no effect of MC during the periods M0-M3 (RR=1.30 p=0.58) and M3-M12 (RR=1.39 p=0.32), but a protective effect in the period M12-M21 (RR=0.30 (0.13 - 0.67) p=0.0032), equivalent to a protection with an efficacy of 71%. When controlling for background characteristics and sexual behaviour, including condom use, this last RR was unchanged.

Conclusions: Male circumcision provides a high degree of protection against HSV-2 infection acquisition but one year after surgery. Further studies should examine the reason of this protective effect and long term effect of male circumcision on HSV-2 acquisition.

Collaborators: Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A.

RANDOMIZED, CONTROLLED INTERVENTION TRIAL OF MALE CIRCUMCISION FOR REDUCTION OF HIV INFECTION RISK: THE ANRS 1265 TRIAL:

Background: Observational studies suggest that male circumcision may provide protection against HIV-1 infection. A randomized, controlled intervention trial was conducted in a general population of South Africa to test this hypothesis.

Methods and Findings: A total of 3,274 uncircumcised men, aged 18-24 y, were randomized to a control or an intervention group with follow-up visits at months 3, 12, and 21. Male circumcision was offered to the intervention group immediately after randomization and to the control group at the end of the follow-up. The grouped censored data were analyzed in intention-to-treat, univariate and multivariate, analyses, using piecewise exponential, proportional hazards models. Rate ratios (RR) of HIV incidence were determined with 95% CI. Protection against HIV infection was calculated as 1 _ RR. The trial was stopped at the interim analysis, and the mean (interquartile range) follow-up was 18.1

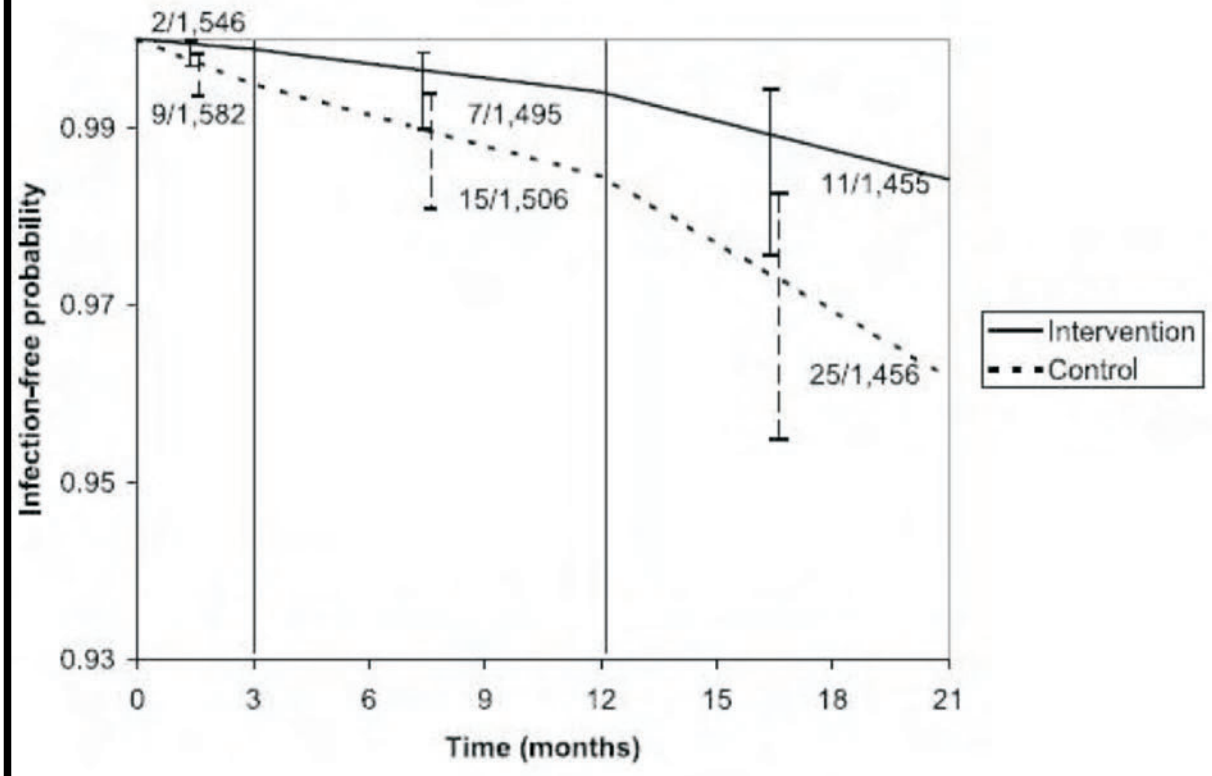


Figure: Infection-Free Probability as a Function of Time and of Randomization

mo (13.021.0) when the data were analyzed. There were 20 HIV infections (incidence rate 0.85 per 100 person-years) in the intervention group and 49 (2.1 per 100 person-years) in the control group, corresponding to an RR of 0.40 (95% CI: 0.24%0.68%; p , 0.001). This RR corresponds to a protection of 60% (95% CI: 32%76%). When controlling for behavioural factors, including sexual behaviour that increased slightly in the intervention group, condom use, and health-seeking behaviour, the protection was of 61% (95% CI: 34%-77%).

This figure represents the infection-free probability using a piecewise exponential distribution with boundaries at M3, M12, and M21 obtained with a Poisson log-linear model (see text). Each segment of exponential has been fitted to the data in each period for each randomization group.

The 95% confidence intervals have been represented in the middle of each period. x/y is the number of HIV infections observed in each period (x) and the number of persons at the beginning of the period (y).

Conclusion: Male circumcision provides a degree of protection against acquiring HIV infection, equivalent to what a vaccine of high efficacy would have achieved. Male circumcision may provide an important way of reducing the spread of HIV infection in sub-Saharan Africa.

Collaborators: Bertran Auvert, Dirk Taljaard, Emmanuel Lagarde, Joëlle Sobngwi-Tambekou, Rémi Sitta, Adrian Puren

HEPATITIS B ACTIVITIES

A COMPARISON OF UNPUBLISHED HEPATITIS B VIRUS STRAINS (HBV) FROM SOUTH AFRICA, NAMIBIA AND MOZAMBIQUE WITH VIRAL STRAINS FROM THE REST OF AFRICA AND THE WORLD:

Background: HBsAg-positive patients identified in a study initiated by the Instituto Nacional de Saude (INS), Maputo, Mozambique to establish the baseline epidemiology of viral hepatitis in Mozambique were sent to the NICD for genotyping.

Methods And Findings: Extracted DNA was amplified and sequenced in the PreS2/S region of the HBV genome and used to identify the specific genotypic groups and subgroups of the viral isolates. Nucleotide and protein alignments were then made to determine whether unique features of the virus, localized to Mozambique, existed. Fifty-one of the 97 HBsAg-positive specimens were successfully sequenced and genotyped, of which 43 (84.3%) were found to be of subgroup Aa of genotype A and 8 (15.7%) were of genotype E. No other genotypes were identified in the Mozambican cohort. Sequence data from these specimens, the first from Mozambique to be characterized, were compared with other, mostly unpublished, sequence data generated in our laboratory from 39 South African and 17 Namibian specimens)

together with other African and global sequence data from GenBank. Reports coming out of Africa, mainly in the last two years, have considerably expanded our knowledge of HBV genotypes in Africa. In the West African countries of Benin, Mali, Nigeria and Togo the occurrence of only genotype E is reported while in the East African countries of Malawi and Somalia genotype A is found exclusively. While genotype E is well conserved with only an intragenotypic difference of <2% over all specimens sequenced to date, new sequence data from Africa reveals diversity among African genotype A specimens. In Cameroon the predominant genotype was genotype E but within an HIV infected cohort 18/22 specimens were found to be from genotype A. These specimens, together with earlier specimens from Cameroon, clustered together and away from other genotype A specimens to form a third subgroup of genotype A in Cameroon which has been variously called subgroup A" or Ac (for $A_{CAMEROON}$). This is in addition to the other two subgroups of genotype A, subgroup Aa (for $A_{AFRICA/ASIA}$) and Ae (for A_{EUROPE}). The high degree of genetic diversity within Africa indicates that genotype A originates in Africa. The Mozambican subgroup Aa specimens clustered together with specimens from Malawi. The three Namibian specimens, although of subgroup Aa, showed unique sites in the preS2 region while South African genotype A specimens were found to be a mixture of those found in Mozambique and Namibia. The Namibian and South African specimens are being further characterized by full genome sequencing to establish the full extent of their diversity since subgroup Ac specimens are more similar to the European subgroup Ae over the region so far studied. The low diversity within genotype E suggests that this genotype has a short evolutionary history. This would also explain the absence of this genotype in the Americas despite the slave trade from West Africa in the late 18th and early 19th century. If this is the case, the relatively high prevalence of genotype E in Mozambique must be of recent origin. It is possible that genotype E was introduced into Mozambique during the colonial era when both Angola and Mozambique were colonized by the Portuguese.

Collaborators: [1]Sheila Bowyer, [1]Mariza Vos, [2]Jorge Barreto, [2]Isadora Sacramento, [2]Marianne Christensen, [2]Ilesh Jani and [1]Adrian Puren. [1]National Institute for Communicable Diseases, Johannesburg, South Africa [2]Instituto Nacional de Saude, Maputo, Mozambique

HEPATITIS C ACTIVITIES

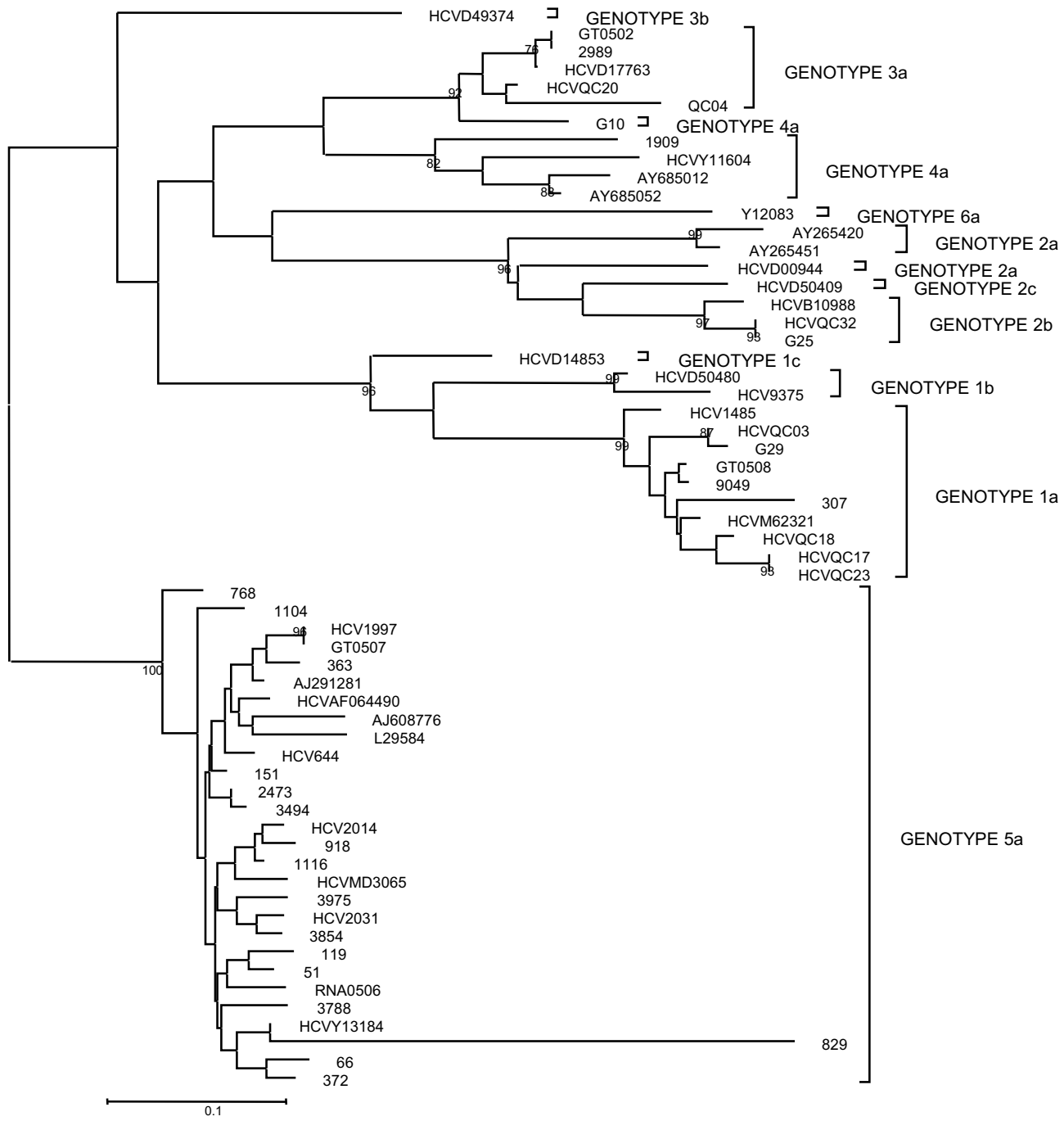
HCV EPIDEMIOLOGY IN SOUTH AFRICA:

Studies on HCV in South Africa have focused mainly on antibody seropositivity. The seroprevalence of HCV in low and high-risk South African urban blood donors is 2.6% which is comparable to low prevalence areas in Europe, U.S and Japan. Genotyping studies on HCV in South Africa have been small and limited. Genotype 5 is the most predominant genotype in South Africa. Phylogenetic analyses of the first full-length genome of HCV genotype 5 confirmed its classification as a unique genotype/subtype, genotype 5a. The present study was aimed at genotyping HCV by sequencing in the 5'untranslated (5'UTR) and subtyping in the non-structural (NS5B) regions of the genome.

One hundred and five of the 232 NAT positive specimens were amplified and sequenced in the 5'UTR region for genotyping and 51 specimens were subtyped in the NS5B region. Specimens of known genotypes are assessed in the laboratory annually in a external quality assurance program, Quality Control Medical Diagnostics (QCMD). These specimens, processed and subjected to the same tests as medical specimens, are used as standards to verify the methods used. Forty-eight specimens of the QCMD panel were tested by NAT. Thirty-seven were genotyped in the 5'UTR region and 15 were subtyped in the NS5B region. RT-PCR and amplification of cDNA was done in a one-tube assay (TITAN One-Tube, Roche) Sequencing of the nested region of a 238bp region of the 5'UTR end and 367bp region of the NS5B region was done using the Big Dye Sequencing kit, Applied Biosystems. Multiple alignments of the sequenced regions were done with CLUSTALW and phylogenetic analyses were done with the MEGA version 3.0.

Genotyping results of the 5'UTR region showed that genotype 5 was the predominant genotype in this study (60%), followed by genotype 1 (28%), genotype 3 (7%) and genotype 4 (5%). Genotype 2 and genotype 6 were not found in this study cohort. For the QCMD panel, genotype 1 (32%), genotype 2 (11%), genotype 3 (22%), genotype 4 (11%), genotype 5 (11%) were determined and 13% could not be typed.. Subtyping within genotypes was possible in the NS5B region due to a degree of variability in this region of the genome. The phylogenetic analyses of the NS5B region (Fig.1) confirmed the genotypic clusters as indicated by sequencing of the 5'UTR end. The cluster for genotype 1 had two distinct groups, indicative of genotype 1a and 1b. All results were concordant with the 5'UTR genotypic analyses. One genotype 4 specimen was successfully sequenced in the NS5B region and grouped with the genotype 4 reference sequence from GenBank. All QCMD results were concordant to results received from the program and verified the genotyping and subtyping method used in the study. The concordant results from both the 5'UTR and NS5B regions show that genotyping and subtyping using these regions is reliable and can be used as a diagnostic and surveillance tool in our laboratory.

Collaborators: Prabdial-Sing, N., Bowyer, S. and Puren, A.J.



Phylogenetic tree of HCV isolates of 367bp in the NS5B region, showing clusters of different genotypes and subtypes. Significant bootstrap values are indicated. Genbank accession numbers are as follows: M62321 (genotype 1a), D50480 (genotype 1b), D14853 (genotype 1c), D00944, AY265451, AY265420 (genotype 2a), B10988 (genotype 2b), D50409 (genotype 2c), D17763 (genotype 3a), D49374 (genotype 3b), Y11604, AY685012, AY685052 (genotype 4a), Y13184, AF064490, AJ291281, AJ608776, L29584 (genotype 5a) and Y12083 (genotype 6a). QCMD specimens are labelled as follows: GT0502, QC20, QC04, G10, QC32, G25, QC03, G29, GT0508, QC18, QC17, QC23, GT0507, RNA0506.

MOLECULAR POLIOVIRUS UNIT

MOLECULAR EPIDEMIOLOGY OF POLIOVIRUSES IN SUB-SAHARAN AFRICA:

During 2005, the Poliovirus Molecular Unit of the NICD which is a WHO Regional Reference Laboratory, received 2957 poliovirus isolates (Figure 1), which were characterized as vaccine or wild type using two intratypic differentiation methods, PCR and ELISA. These isolates were sent to the NICD from National and Regional laboratories throughout Africa namely, Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Congo, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Liberia, Mali, Malawi, Mozambique, Nigeria, Niger, Democratic Republic of Congo (DRC), Rwanda, Sierra Leone, Senegal, South Africa, Somalia, Sudan, Swaziland, Tanzania, Togo, Uganda and Zimbabwe (Figure 2). Original specimens from AFP cases were received from several southern African countries and any polio isolates were treated as above.

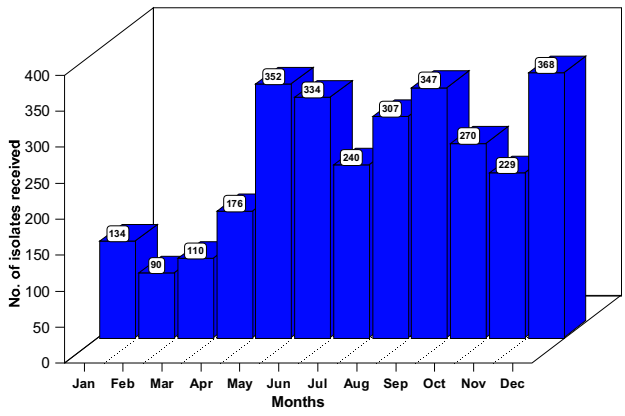


Figure 1. Poliovirus samples received in 2005

In South Africa 543 AFP cases were reported and poliovirus was isolated from nine of these. All polioviruses isolated from AFP cases in South Africa were found to be vaccine-like. The majority of the wild-type cases identified during 2005 were from Nigeria (Figure 3), 925 of which were polio type 1 and 423 polio type 3. Other cases identified in 2005, some of which were isolated late in 2004, were from Angola, Burkina Faso, Cameroon, Central African Republic, Chad, Eritrea, Ethiopia Guinea, Mali Niger, Somalia and Sudan. Ten cases of PV1 wild-type viruses and a contact were identified in Angola which belong to the SOAS genotype (Indian genotype).

Molecular sequencing of the full VP1 (900bp) can be used to answer several epidemiological questions regarding the likely location of endemic virus reservoirs and patterns of virus transmission. It also determines if an isolate is similar to endemic strains or has been introduced, i.e. closely related to viruses circulating in another country or region.

The wild-type isolates can be placed into the known genotypes using the information from the sequence

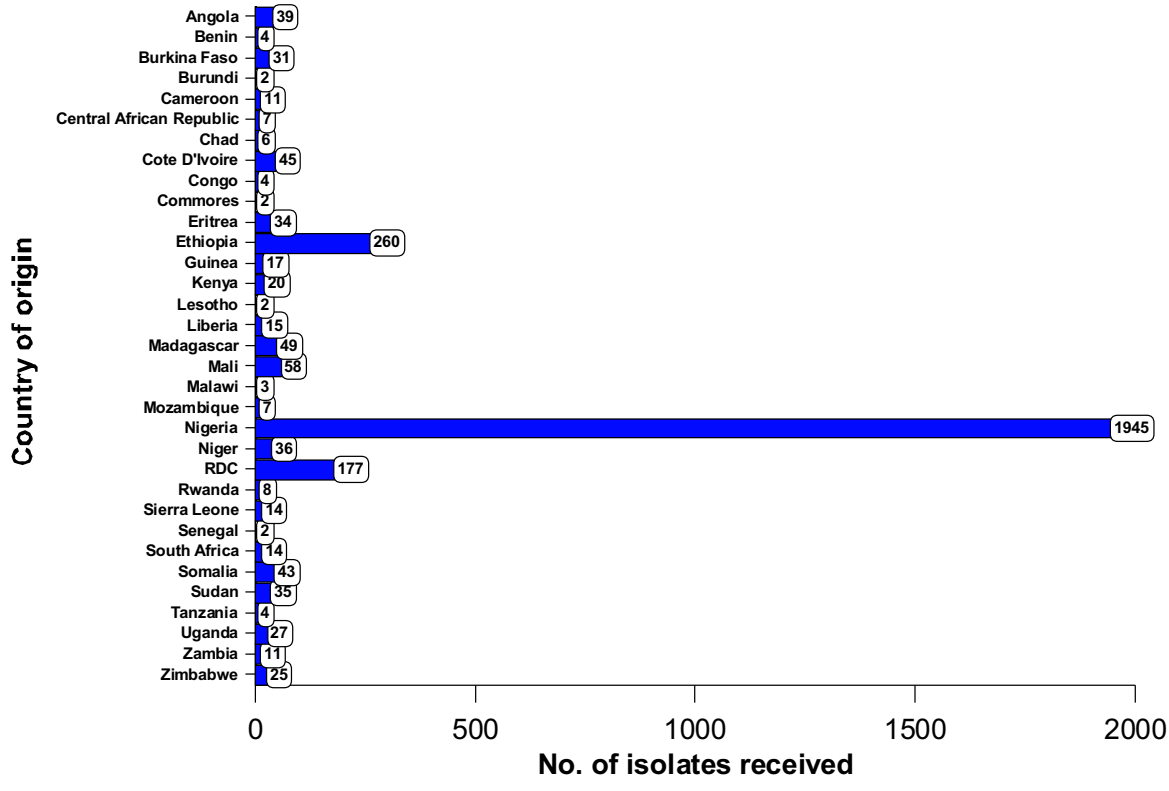


Figure 2: Poliovirus samples received from African countries

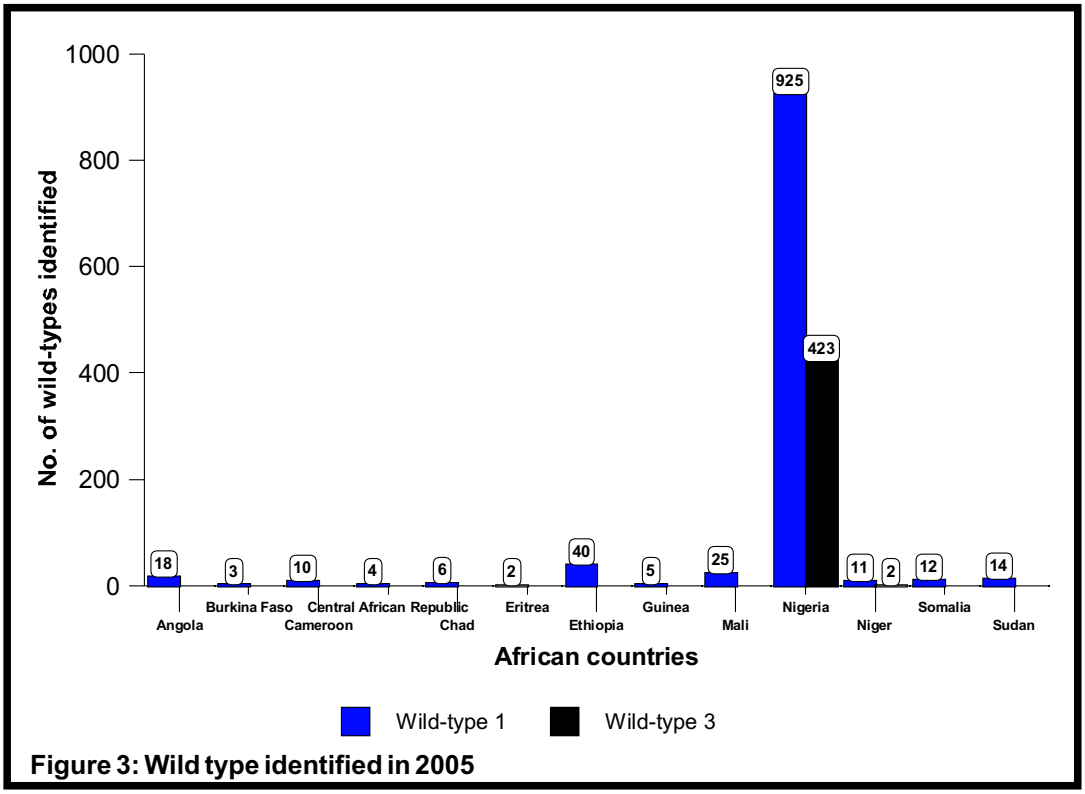


Figure 3: Wild type identified in 2005

analysis of the 900 base pairs from the VP1 region. Wild PV1 and PV3 are still endemic in Africa, major reservoirs have been found in West and Central Africa. The remaining reservoir in Africa is Nigeria (WEAF-B genotype) (Figure 4).

Distribution of wild PV3 genotypes closely parallels PV1 distribution. Intergrated AFP and virologic surveillance is giving a very high-resolution picture of the patterns of wild type poliovirus circulation in Nigeria. Indigenous circulation of the WEAF-B genotype has largely stopped in southern Nigeria, The primary reason for continued circulation of both PV1 and PV3 wildtype polioviruses in Nigeria is inadequate OPV coverage, low routine coverage and insufficient quality of mass immunization campaigns.

The sequences used to construct the dendrograms were produced by the NICD and CDC (Atlanta) and the phylogenetic trees were constructed by NICD. The viruses on the trees are presented as follows: {e.g.: NIE-KTS-DRA-03-004) The first three letters represent the country, next three represent the province, next three represent the district, followed by the year of onset of paralysis and the last number is the case number.
Wild PV1 was highly endemic in northern Nigeria in 2005 and other circulations occurred in the central provinces. The immunization campaigns are much higher level in the southern provinces.

2005 PV1 wild type isolates are distributed into three genotypes, SOAS, WEAF-B and EAAF. In figure 4, WEAF-B genotype consists of viruses from Nigeria, Mali and Chad. The first case of SOAS genotype in Angola was along the coast and later cases spread all over the country. The EAAF genotype consists of the viruses from Ethiopia, Eritrea and Somalia and only one case was identified in Eritrea.

WEAF-B wild PV3 is divided into four clusters A D (Figure 5). Cluster A represents local circulation in Kebbi (KBS) province of Nigeria. Cluster B and C not represented. Cluster D resolved into two countries, Nigeria and Niger.

VACCINE-DERIVED POLIOVIRUS (VDPV):
In winter of 2005, five cases of acute flaccid paralysis (AFP) were reported in Madagascar. Stools collected from the patients were positive for the poliovirus type 2 and type 3, and the further characterization of the isolates by sequencing of the complete VP1 region (viral capsid gene 1) of the genome revealed that they were circulating vaccine-derived polioviruses (cVDPV) with lower degree of similarity (97.6% -98-8%) than in normally observed which is >99%. All the isolates from the year 2005 Madagascar outbreak were recombinants with noncapsid sequences obtained from the other species C enteroviruses.

In total, seventeen cVDPV'S were identified from the cases and contacts from Madagascar. All five Acute Flaccid paralysis (AFP) cases were from the Southern part of Madagascar from the following districts: Toliara II (MAD-TOL-TOL-05-041), Tshihombe (MAD-TOL-TSI-05-073), Beloha (MAD-TOL-BEL-05-091, Toliara I (MAD-TOL-TOL-05-082) and Sakaraha (MAD-TOL-SAK-05-113). All cases showed no history of vaccination and all developed paralysis within a five month period. The first case had seven identifiable contacts, case two had four identifiable contacts and

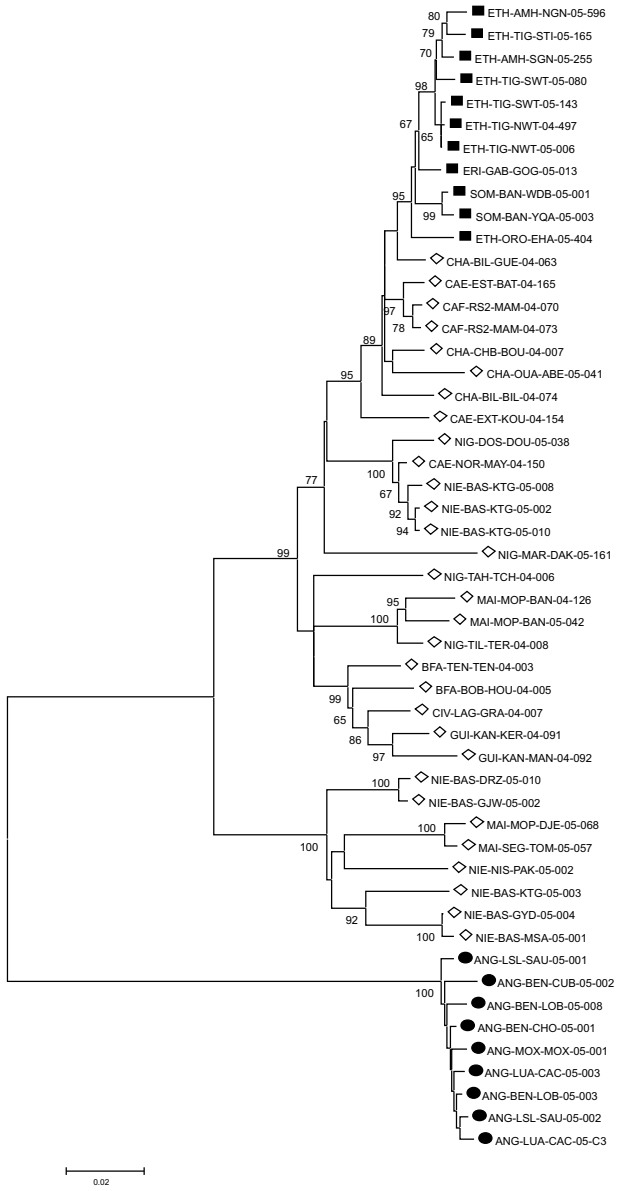


Figure 4. WEAF-B wild PV1 representative of isolates: 2004 2005. Closed represent cases that belong to SOAS genotype (Indian genotype), open diamond represent strains that belong to WEAF-B genotype and closed square represent viruses that belong to EAAF genotype.

case three had one. The forth and the fifth cases had no contacts.

CONTAINMENT OF POLIOVIRUSES IN SOUTH AFRICA:

Dr Thami Sithebe, NTF co-ordinator on behalf of the NTF / NCC South Africa

The South African Department of Health, in cooperation with the World Health Organization's (WHO) campaign to eradicate poliovirus, globally, has appointed the National Certification Committee (NCC), National Task Force (NTF) and National Polio Expert Committee (NPEC), to oversee the eradication and certification processes of poliovirus in South Africa. The primary responsibility of the NTF is to co-ordinate laboratory containment of infectious and potentially infectious poliovirus materials. The committee will therefore, be sending out laboratory survey and inventory forms requesting all biomedical/medical/pharmaceuticals laboratories and other relevant organizations and stakeholders to search all storage spaces for materials that contain infectious or potentially infectious poliovirus materials. Selected laboratories will be visited for auditing. Information gathered from this search will be used to compile a National Inventory of Laboratories containing infectious and potentially infectious poliovirus materials, which will be submitted to the WHO for certification purposes. Such laboratories will later be advised on how to appropriately work with or store poliovirus materials in the near future. The WHO, South African Department of Health, NCC, NTF and NPEC are

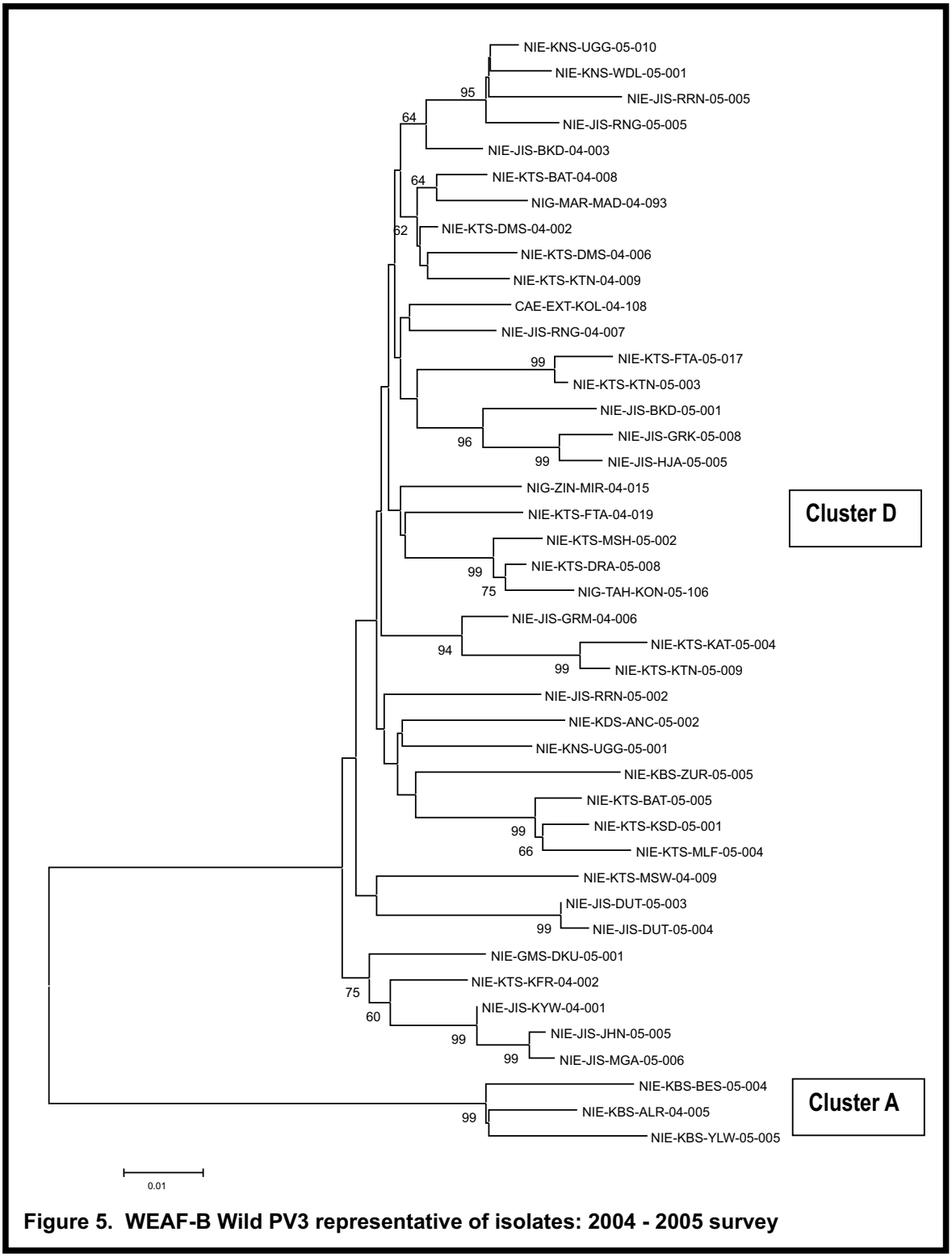


Figure 5. WEAF-B Wild PV3 representative of isolates: 2004 - 2005 survey

Table 1: Vaccine Derived Polioviruses identified in 2005

Laboratory Identity No.	EPID Number	Type	Age (yrs)	Nucleotide difference (VP1 %)	Genotype
PMOL/05/0781	**MAD-TOL-TOL-05-041**	PV3	2	1.44	**VDPV-3**
PMOL/05/0994	Contact 1	PV3	2	1.22	VDPV-3
PMOL/05/0995	Contact 2	PV3	4	1.56	VDPV-3
PMOL/05/0997	Contact 3	PV3	1.8	1.22	VDPV-3
PMOL/05/1342	Contact 4	PV3	4	1.78	VDPV-3
PMOL/05/1344	Contact 5	PV3	3	1.67	VDPV-3
PMOL/05/1345	Contact 6	PV3	0.7	1.33	VDPV-3
PMOL/05/1347	Contact 7	PV3	4	1	VDPV-3
PMOL/05/1338	**MAD-TOL-TSI-05-073**	PV2	2	2.33	**VDPV-2**
PMOL/05/1546	Contact 1	PV2	1.5	2.44	VDPV-2
PMOL/05/1547	Contact 2	PV2	3	2.55	VDPV-2
PMOL/05/1548	Contact 3	PV2	2	2.66	VDPV-2
PMOL/05/2060	Contact 4	PV2	2	0.66	VDPV-2
PMOL/05/1600	**MAD-TOL-TOL-05-082**	PV2	2	1.11	**VDPV-2**
PMOL/05/2061	Contact 1	PV2	N/A	1.77	VDPN-2
PMOL/05/1606	**MAD-TOL-BEL-05-091**	PV2	3	1.11	**VDPV-2**
PMOL/05/2055	**MAD-TOL-SAK-05-113**	PV2	2	1.33	**VDPV-2**

therefore, requesting all the laboratories which work with infectious and/or potentially infectious poliovirus materials in the country, to support the initiative by completing the questionnaire (which will be sent out from the 10th January 2006) and kindly returning to the designated address promptly so as to meet the WHO certification deadline.

CONFERENCES/MEETINGS:

Dr AJ Puren: UNAIDS Reference Group Meeting on HIV Incidence: Athens: 13-16 December 2005.

Dr AJ Puren: Quality Control for Molecular Diagnostics Advisory Board Meeting, Glasgow: 21-23 September 2005.

Dr AJ Puren: WHO Advisory Meeting to develop HPV Reference Laboratories, Geneva: 15-16 October 2005.

Dr AJ Puren: CDC Incidence Workshop, Rwanda: 9-11 June 2005.

Dr SM Bowyer: International Meeting on the Molecular Biology of Hepatitis B Viruses: University of Heidelberg, Heidelberg, Germany, 18-21 September 2005.

N Prabdial-Sing, Dr SM Bowyer: 11th International Workshop on Molecular Phylogeny and Viral Evolution: Brazil, 5-9 September 2005.

Dr SM Bowyer, N Prabdial-Sing: HCV genotyping in South Africa. International Conference for Women Engineers and Scientists (ICWES-13): Seoul, Korea, 26-29 August 2005.

Dr SM Bowyer, N Prabdial-Sing: Simunye 2005: Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) Congress, Sun City, South Africa, 24-28 July 2005.

HN Gumede: Vaccinology 2005 Congress: Hermanus, South Africa, 31 October 1 November 2005.

HN Gumede: 8th Meeting of Laboratory Directors: Harare, Zimbabwe, September 2005.

HM Gumede: Informal consultation on global Polio Laboratory Network: Buckhead, Atlanta, USA, 31 August 2 September 2005.

Vaccine Preventable Virus Infections Unit

Head:
Dr Terry Besselaar

STAFF:

Dr TG Besselaar PhD, Senior Medical Scientist, Head of Unit
S Smit BSc (Hons), Senior Medical Scientist
L Botha B Tech (Biotechnology), Chief Biotechnologist

MOLECULAR INFLUENZA LABORATORY:

Background: The ongoing outbreaks of H5N1 avian influenza in migratory waterfowl, domestic poultry and humans in Asia and most recently in Turkey have highlighted the threat of a global influenza pandemic occurring in the near future. The influenza laboratories at NICD have been involved since the mid-1980s in monitoring seasonal influenza activity and comprise one of the few WHO National Influenza Centres (NICs) in Africa. In addition to the role it plays in providing information towards the annual decision on updating the influenza vaccine formulation for the southern hemisphere, the laboratories are now also engaged in preparing for the possible H5N1 influenza pandemic by developing methods such as real time PCR for the rapid identification of this subtype of influenza virus in humans.

MOLECULAR EPIDEMIOLOGY OF THE 2005 INFLUENZA SEASON IN SOUTH AFRICA:

In contrast to 2004, where the predominant virus isolated in South Africa was influenza A H3N2, both subtypes of influenza A and B viruses circulated in 2005. Five influenza B virus isolates were also sent from the NIC in Cape Town for further characterisation. A total of 582 South African influenza isolates were made. 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 influenza viruses isolated at NICD in 2005 were influenza A subtype H1N1, which differed from Australia and New Zealand where influenza A H3N2 and influenza B viruses predominated respectively. Three hundred and twenty three specimens reacted well with the antiserum to the A/New Caledonia/20/99 H1N1 vaccine strain. Sequence analysis of the HA1 subunit of representative H1N1 isolates revealed up to seven amino acid changes compared to the A/New Caledonia/20/99 strain. While this represented the most genetic drift seen in the H1N1 viruses over recent years relative to the vaccine stain, the antigenic match of the 2005 viruses with A/Caledonia/20/99 was not compromised as shown by good reactivity in the HI test with post infection ferret antiserum (data from WHO CCs in Melbourne and London).

A further 132 influenza A isolates were identified as subtype H3N2. The majority of these isolates reacted to low titres in the HI test with the A/California/7/04-like reference antiserum. Many of the Johannesburg H3N2 isolates sent to the WHO CCs showed low reactivity in HI tests with ferret antiserum to both the A/Wellington/1/04 vaccine strain and the A/California/7/04-like reference viruses. The molecular characterisation of representative influenza H3N2 isolates revealed that the viruses circulating in Johannesburg had drifted extensively from the A/Wellington/1/04 vaccine strain with mutations in three of the five HA antigenic sites i.e. sites A, B and D. Genetic drift was also observed compared to A/California/7/04 in antigenic sites A and B. Figure 1 shows the results of the phylogenetic analysis of representative South African H3N2 HA1 sequences (921 bp).

Ninety two of the influenza B isolates were typed by HI as B/Hong Kong/333/01-like while a low percentage reacted with antiserum raised against the vaccine B/Shanghai/361/02-like virus. All 5 influenza B isolates from Cape Town reacted well with the B/Shanghai/361/02 antiserum. Sequence analysis of the HA gene of the South African 2005 influenza B viruses showed that the B/Hong Kong-like viruses had seven common changes in the amino acid residues relative to the B/Hong Kong/330/01 strain. In the B/Shanghai-like viruses, substitutions were seen at five residues.

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/California/7/04-like virus (H3N2)
A B/Malaysia/2506/04-like virus

INFLUENZA OUTBREAK IN THE SEYCHELLES:

The Seychelles experienced a large outbreak of influenza-like illness in October 2005 and influenza

H3N2 viruses were isolated from 26 of the 33 specimens sent to NICD. Molecular characterization and phylogenetic analysis of the H3 HAI subunit revealed that the viruses differed from those isolated earlier in the year in South Africa and had drifted even more extensively from A/California/7/04, the recommended H3N2 strain for the 2006 vaccine (Figure 1). This additional evolution raises some concern regarding the extent of protection that the A/California/7/04-like H3N2 strain in the vaccine will afford against H3N2 viruses circulating in 2006.

CHARACTERIZATION OF SOUTH AFRICAN OSTRICH H5N2 INFLUENZA FROM 2004 OUTBREAK:

The A/Ostrich/South Africa/1/04 virus isolated by the Special Pathogens Unit at NICD from the spleen tissue of an infected ostrich in the 2004 H5N2 avian influenza outbreak in the Eastern Cape was characterized by sequencing and phylogenetic analysis of the surface genes. The deduced amino acid sequence of the H5 cleavage site was found to have a motif of PQGEKRRKKRGLF, a sequence consistent with high pathogenicity and almost identical to the cleavage site sequence of the HPAI H5N1 strains isolated in South East Asia in 2004. Phylogenetic analysis of the A/Ostrich/South Africa/1/04 HA sequence showed that the ostrich isolate belonged to the same clade as several contemporary European H5 isolates from wild birds (Slomka, pers.comm). This suggests that the origin of the virus that caused the South African ostrich outbreak was probably due to introduction to the country by migratory birds.

Molecular characterization of the ostrich N2 gene showed that it shared the closest relationship to the H9N2 A/Pheasant/Ireland/PV/18/97 strain. It would thus appear that the N2 gene of the 2004 ostrich virus had evolved from an H9N2 virus and reassortment had occurred sometime between 1997 and 2004 to produce this particular H5N2 variant.

HIGHLIGHTS:

One of the highlights of 2005 was attending the Second European Influenza Conference in Malta in September where the latest research and aspects of pandemic preparedness were presented. Another highlight was being invited to participate as an expert panel member at the first ever WHO meeting held to discuss strengthening influenza pandemic preparedness in Africa. The “Ad hoc Expert Panel Meeting on Pandemic Influenza Preparedness” meeting was held in Harare on 12-13 October 2005. Experts were invited from five African countries i.e. Madagascar, Cote d'Ivoire, Congo, Uganda and South Africa. Presentations were given by Dr Koffi (Cote d'Ivoire), Dr Reynes (Madagascar), Dr Kaboyo (Uganda) and Dr Besselaar (South Africa). This meeting has greatly facilitated pandemic planning in the region and made it possible for Africa to play a greater role in the global influenza pandemic task force in the future.

ACKNOWLEDGEMENTS:

Virus isolation and subtyping was carried out by the Respiratory Virus Isolation section under the expert technical guidance of Amelia Buys. We would like to thank Philip Palmyre, Public Health Laboratory, Seychelles for sending us specimens for influenza isolation and characterization. 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. Information regarding unpublished avian H5 sequences was kindly supplied by Dr Marek Slomka, VLA, UK.

MEASLES/RUBELLA LABORATORY

MOLECULAR SURVEILLANCE OF MEASLES IN SOUTHERN AFRICA:

The measles outbreak that started in Mpumalanga and Gauteng in 2003 spread to the Western Cape and KwaZulu-Natal in 2004, and by 2005 hundreds of cases had been reported from the Eastern Cape. Phylogenetic analysis revealed that the two and a half year-long outbreak was caused by a single strain of measles virus, characterized as genotype D2. Molecular characterization of the viruses obtained at the start of the outbreak established that they were identical to the strain that had been circulating in Mozambique in 2003, and that these outbreaks were therefore virologically linked. Although genotypes D2 and D4 have previously been shown to circulate endemically in South Africa, the lack of viral diversity seen in this outbreak suggests an epidemic pattern of transmission rather than an endemic pattern. Standard epidemiological field investigations indicated that the outbreak had started in Mpumalanga in Mozambican citizens employed on farms along the shared border, thus also implicating Mozambique as the source of the virus. One can therefore conclude that the South African measles outbreak was due to epidemic transmission of an imported virus and that the sustained transmission was as a result of the accumulation of large numbers of susceptible individuals.

Since the NICD measles laboratory also serves as a WHO measles/rubella Reference Laboratory for 13 southern African countries (including the islands of Madagascar, Mauritius, Seychelles and Comoros), selected serum specimens are sent on a quarterly basis to the NICD for quality assurance purposes. It has been possible to characterize the genotypes present in some of these specimens using molecular methods of analysis. It was found that genotype D2 (the same strain that was circulating in South Africa and Mozambique) was present in Lesotho, Namibia, Zimbabwe, Botswana and Malawi. Measles virus strains characterized as genotype D4 (closely related to the strain of D4 circulating in Beira, Mozambique) were present in specimens from Zimbabwe, Botswana and the

Figure 1. Phylogenetic tree of influenza A (H3N2) virus HA1 gene nucleotides (923bp). (H3N2 vaccine strain is depicted in italics).

Comoros. Since measles-specific IgM-positive serum samples seldom contain free measles virus, the preceding results are based on only one or two specimens and are thus not representative of these countries. It is therefore unknown whether other genotypes are co-circulating in these countries.

A more comprehensive picture of the circulating genotypes in the WHO AFRO southern block countries would be possible if more suitable specimens (throat swabs or urine specimens) were sent in for analysis. This would have no additional cost implications if such specimens were included in the quarterly serum shipments.

CONFERENCES AND MEETINGS:

Dr TG Besselaar : Laboratory surveillance of influenza in South Africa and future pandemic challenges. Oral presentation. Virology Africa Conference, Cape Town, 9 November 2005

Dr TG Besselaar : Influenza: Surveillance and laboratory experiences in South Africa and the way forward in light of a pandemic. Oral presentation. "Ad hoc Expert Panel Meeting on Pandemic Influenza Preparedness", Harare, 12 -13 October 2005.

Dr TG Besselaar : Phylogenetic analysis of human influenza A viruses isolated recently in Africa and characterization of the South African HPAI H5N2 virus from the 2004 ostrich outbreak Second European Influenza Conference. St.-Julians, Malta, 11-14 September 2005

Dr TG Besselaar : Challenges of laboratory surveillance of pandemic influenza. Oral presentation. NICD, 12 April 2005

Dr TG Besselaar : Laboratory aspects of influenza: relevance to vaccine planning. Oral presentation. Influenza Symposium, NICD, 16 February 2005

SB Smit : Second Measles and Yellow Fever Laboratory Directors' Meeting, 2-3 August 2005, Entebbe, Uganda. Oral presentation entitled "Southern Block report on measles diagnostics".

SB Smit : Third WHO Global Measles and Rubella Laboratory Network Meeting, 25-26 August 2005, Geneva, Switzerland. Oral presentation entitled "Virological surveillance update : South Africa 2003-2005".

SB Smit : Virology Africa Congress, 8-11 November 2005, Cape Town. Oral presentation entitled "Molecular analysis of measles viruses in southern Africa".

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AA Grobbelaar MSc, Principle Medical Scientist
J Croft Dip Med Tech, Chief Medical Technologist
M Mashele Dip Med Tech, Chief Medical Technologist
NS Bodla Dip Med Tech, Senior Medical Technologist
NB Magome, General Worker
L Seema, General Worker
S Modise, General Worker

Arbovirus Section
A Kemp MSc, Principal Medical Scientist
S Serero, Laboratory Assistant
J Mahlangu, Laboratory Assistant
CM Chauke, General Worker
DZ Mnisi, General Worker
R Nkoana, General Worker

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, and operates a maximum-security laboratory (BSL-4). The Unit is recognized as a World Health Organization (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 Marburg, Ebola, Rift Valley fever, Crimean-Congo haemorrhagic fever, 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 BSL-4 laboratory was closed in April 2004 for major upgrading, upscale, and refurbishment of the facility. It is planned that BSL4 will be fully operational early in 2007. Meanwhile, research involving the use of non-infectious nucleic acid preparations of BSL-4 viral agents or less hazardous viruses and diagnostic service has continued in BSL-2 and BSL-3 laboratories of the Unit.

COMPARISON OF SPECIMENS RECEIVED IN 2004 AND 2005:

The total number of specimens tested in 2005 was lower compared to 2004 (Table 1). This was mostly due to closing down the BSL-4 in April 2004 for major upgrading and renovation. However, as a WHO Regional Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses, the Unit continued to receive specimens from other African countries and Asia, and especially from Pakistan from patients with suspected CCHF infections. Testing of other suspected cases VHF, and especially those related to the 2005 Marburg outbreak in Angola, could not be directly handled and/or investigated by the Unit but we facilitated contacts, provided guidelines regarding the collection, handling and packaging, and assisted in shipping of specimens from suspected VHF cases to other institutions housing BSL4 facilities, mostly to the Special Pathogens Branch, Centers for Disease Control and Prevention (CDC), Atlanta, USA. Compared to the last year there was an increase in a number of suspected rabies cases and undiagnosed fevers (Table 1).

INVESTIGATION OF SUSPECTED VIRAL HAEMORRHAGIC FEVERS (VHF):

Two cases of Crimean-Congo haemorrhagic fever (CCHF) were confirmed in southern Africa during 2005 (Table 2). In one case, a farmer from Namibia, there was evidence that the infection had resulted from a tickbite and the patient survived. In the second case, a farm worker in the Western Cape became ill after slaughtering a cow and subsequently died. There is no specific treatment for CCHF infections, although there is some evidence that ribavirin can improve the prognosis if administered before day 5 after onset of illness.

A total of 178 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 2005, including seventeen in Namibia, one in DRC, one in Tanzania, and 159 cases in South Africa. Marginally the largest group of cases, 78/178 (43,8%), arose from known tick bite or the squashing of ticks; a similar number, 72/178 (40.4%), arose from known or potential contact with fresh blood or other tissues of livestock and/or ticks; 7/178 (3.9%) nosocomial infections arose from contact with blood or fomites of known CCHF patients, while in 21/178 (11.8%) cases there was no

Table 1: Comparison of specimens received in Special Pathogens Unit in 2004 and 2005

Specimens	Received in 2004	Received in 2005
Diagnostic:		
Suspected VHF (South Africa)	72 (49 patients)	52 (47 patients)
Suspected VHF (other countries)	397 (391 patients)	211 (197 patients)*
VHF contacts	125 (125 persons)	55 (55 persons)
Undiagnosed fevers	74 (74 patients)	100 (100 patients)
Suspected rabies	20 (16 patients)	37 (21 patients)
Rabies immunity	165 (158 persons)	72 (68 persons)
Ticks	4 (4 accessions)	5 (4 accessions)
Miscellaneous	526 (36 accessions)	969 (44 accessions)
Surveys:		
Occupational/residential groups	0	0
Cattle goats & sheep for zoonoses	406 (4 groups) 100 (1 accession)	78 (1 group) 170 (1 accession)
Wild animals	127 (1 accession)	202 (1accession)
Ticks RSA	62 (2 accessions)	0
Ticks non-RSA	24 (1 accession)	0
Total specimens:	**2102**	**1951**

- At least 70 specimens from 45 suspected Marburg cases in Angola were sent to the Special Pathogens Branch, CDC, Atlanta, USA or the National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada for diagnostic confirmation

Table 2: List of confirmed cases of Crimean-Congo haemorrhagic fever virus infection in southern Africa, January to December 2005.

Location of exposure	Month	Age/Sex	Virus isolation	PCR	Antibody*	Died/ Survived	Source of infection
Rehoboth, Namibia	Mar	48M	Not done	Neg.	Pos.	Survived	Tick bite
Riversdale, Western Cape	Sep	44M	Neg.	Pos.	Pos.	Died	Slaughtered cow

* Demonstration of IgM and/or IgG antibody.

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 149/178 (83.7%) 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 southern Africa, but gradually declined to an overall rate of 19.9% (29/146) for a period of 1981-1998, most likely as a result of increased awareness leading to earlier recognition and institution of appropriate supportive therapy. However, the case fatality rate markedly increased to 63.3% (19/31) for a period of 1999-2005. One possibility is that there is a decline in awareness of the disease among clinicians, resulting in delayed diagnosis of cases.

THE 2005 MARBURG HAEMORRHAGIC FEVER (MHF) OUTBREAK IN ANGOLA:

On 10 March the WHO Epidemiological Focal Point for Childhood Immunization in Angola approached the SPU to test specimens from fatal VHF cases among children hospitalised in Uige Province in north-west Angola, and from a nurse who had died after taking care of some of the patients. The SPU which houses the only maximum-security laboratory (BSL-4) on the African continent could not assist with laboratory confirmation as this facility has been shut down since April last year for major renovation and upgrading. However, the Unit assisted in the shipping of specimens from Angola to the Special Pathogens Branch of the CDC, Atlanta, USA, and later on during the course of the outbreak also to the National Microbiology Laboratory (NML), Public Health Agency of Canada, Winnipeg, Canada. In addition, Dr JT Paweska (SPU) took part as a member of the WHO-GOARN (Global Outbreak Alert and Response Network) outbreak response team and visited two laboratories for diagnosis of Marburg disease in Angola, one in Luanda run by CDC, and one in Uige run by NML.

On March 21, the CDC identified Marburg virus as the cause of deaths with severe haemorrhagic manifestations in an increasing number of patients, and

linked mostly to a single paediatric ward in the main hospital in Uige Province. A retrospective epidemiological analysis, embracing the period 13 October 2004 - 23 March 2005, identified 102 cases in the outbreak. Of these, 95 had been fatal; about 75% occurred in children under 5 years of age. In adults, cases included a small number of health care workers. The predominant symptoms included fever, haemorrhaging, vomiting, diarrhoea, jaundice and headache. Diagnostic confirmation prompted a large-scale international response that began the day after the CDC's findings were publicised. The WHO outbreak response team supported the Ministry of Health in Angola in their efforts to control the outbreak, and included provision of technical support for case management, contact tracing and surveillance, infection control and raising awareness of the disease in the community. Further technical support was provided promptly by experts from the Inter-Country Programme for Southern Africa, by the Regional WHO Office for Africa, by many institutions in the GOARN, by several other organizations including Médecins Sans Frontières from Belgium, France, Holland and Spain, by UNICEF, by the World Food Programme, and by other humanitarian aid organizations. The 2005 Angolan outbreak is the largest and the deadliest of MHF on record; 374 cases and 329 deaths (case fatality rate = 88%). Of these cases, 368 (98%) occurred in Uige Province, the epicentre of the epidemic.

Early diagnosis of VHF is essential for the containment of contagion and implementation of public health measures. Diagnostic capacity worldwide is limited to selected reference laboratories, necessitating expensive and time consuming measures for safe international transportation of specimens which may result in delayed laboratory confirmation, and pose the risk of specimens deteriorating and being misplaced. Capacity for laboratory diagnosis of VHF in Africa remains a major challenge for the health community. With the BSL-4 facility at NICD at Sandringham, Johannesburg being under major renovation for more than one year now, confirmation of infections caused by class 4 pathogens must be carried out overseas. To address some of these challenges, the SPU is investigating the potential of various PCR-based diagnostic technologies which could be easily and cost-effectively adopted in the form of portable field units in developing countries.

Fig 1: A

Fig 1: B

Fig 1: C

Fig 1: D

Fig 1: E

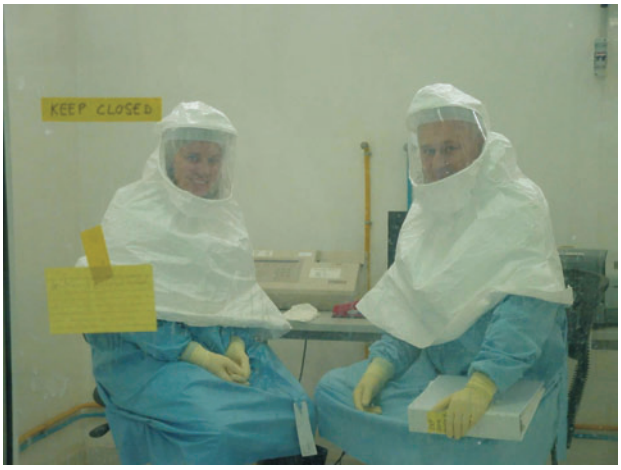

Fig 1: F

Figure 1. Marburg haemorrhagic fever epidemic in Angola, 2005
A - Infection control measures in the provincial hospital in Uige, Northern Angola where the 2005 Marburg outbreak was first recognized: health care workers in protective clothing are burning disposable waste in field "drum" incinerator using diesel fuel; B - Community facilitator and health care workers explaining procedures for specimen collection to Uige residents ; C - Collection of nasal swab from a diseased patient with severe epistaxis; D - Health care workers preparing for burial; E - CDC-Atlanta Marburg laboratory in Luanda: Ms. K. Slaughter and Dr JT Paweska in ELISA "hot" laboratory; F - Mobile PCR laboratory set up by Dr Heinz Feldmann and his colleagues (NML-Winnipeg) at the provincial hospital in Uige.

RABIES:

A total of 8 cases of human rabies were confirmed by the SPU during 2005 (Table 3). The number of rabies cases confirmed was still low compared to the number of cases observed annually prior to 1997. The majority of patients contracted rabies from contact with rabid dogs in Kwa-Zulu Natal or the Eastern Cape. In one case the patient was bitten by a caracal in the Free State and did not receive any post exposure prophylaxis (PEP). Two patients from KZN had no history of receiving PEP. It was not possible to determine if the patient from Sterkspruit received PEP. The remaining patients had a history of incomplete PEP with vaccine but with no documented record of anti-rabies immunoglobulin.

Table 3: Confirmed cases of rabies, 2005

Name	Age/ sex	District of exposure	Exposure: bitten by	Admitted hospital	Died	Final hospital
PR	12 f	Jagersfontein, Free State	Caracal Aug 04	21 Jan	22 Jan	Pelonomi
MK	20m	Sterkspruit, Eastern Cape	Dog 20 Dec	31 Jan	3 Feb	Lady Grey
SN	12 m	Ngqamakwe, Eastern Cape	Dog 9 June	27 June	28 June	Died on way to Cecilia Makawane
LS	12 m	Ngqamakhwe, Eastern Cape	Dog 9 June	28 June	11 July	Cecilia Makawane
ST	8	Umzimkulu, Eastern Cape	Dog 28 Aug		17 Sept	Died at home
MS	4 m	Mathuhini, KZN	Dog Aug	3 Nov	4 Nov	Port Shepstone
CM	12 f	Kwabangibizo, KZN	unknown	14 Nov	15 Nov	Port Shepstone
RJ	9 m	Namibia	Dog 12 May		June	RS Hospital

ARBOVIRUS SECTION:

A total of 505 specimens submitted to the Arbovirus Unit during 2005 included: 281 serum samples from 258 patients in southern Africa, 2 samples from 2 patients in the Seychelles, 61 serum samples submitted to the SPU-NICD from Pakistan with suspected dengue infections, 21 sera from NICD laboratory staff members which were submitted for Rift Valley fever (RVF) or yellow fever immunity tests, serum samples from 128 primates submitted for yellow fever immunity tests and 12 samples from an external quality assurance programme.

An outbreak of chikungunya in Lamu, Kenya was confirmed in collaboration with the Kenya Medical Research Institute (KEMRI) in Nairobi in August 2004. Three months later chikungunya was identified in Mombasa 250 kms south of Lamu. In July 2005 samples were submitted from two patients in the Seychelles

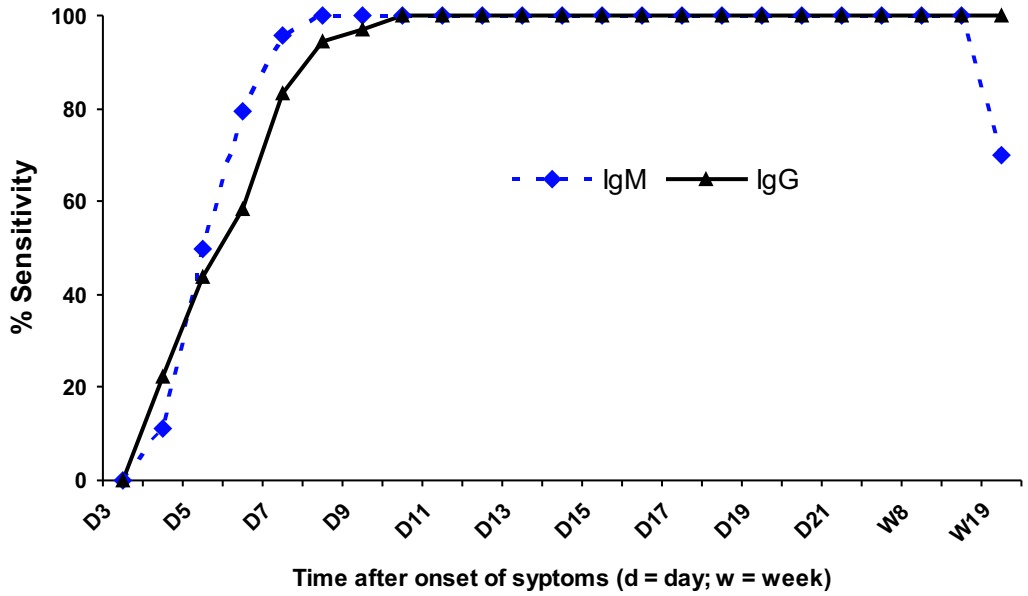

Fig. 2. Diagnostic sensitivity of IgG-sandwich ELISA for detection of IgG antibody and IgM-capture ELISA for detection of IgM antibody to CCHF virus in human sera

where an outbreak of chikungunya was suspected. Chikungunya infection was confirmed in both samples by RT-PCR and antibody detection. A large outbreak of chikungunya has also been reported in Reunion and the genetic relatedness of the strains circulating in Kenya, the Comores, the Seychelles and Reunion in 2004 and 2005 will be investigated by the SPU in collaboration with KEMRI.

RESEARCH AND DEVELOPMENT OF DIAGNOSTIC ASSAYS:

Traditional and molecular diagnostic procedures for VHF may be beyond the resources and capabilities of many laboratories, particularly in developing countries. Therefore, we conduct intensive development and validation studies aimed at expanding the repertoire of accurate and cost-effective assays for diagnosis of VHF which could be easily adopted for wider use both in medical and veterinary laboratories in southern Africa and elsewhere.

IgG-sandwich and IgM-capture ELISA for the detection of antibodies to CCHFv

Sera collected at different times after onset of disease symptoms from CCHF patients confirmed by PCR and virus isolation were used to determine ELISA diagnostic sensitivity and sera from CCHF negative objects were used to determine ELISA diagnostic specificity. We demonstrated that in non-fatal cases IgG and IgM antibody to CCHF virus are detectable in 50% of patients between days 6-7, and in all patients, on day 10 after the onset of symptoms (Fig. 2).

Cloning and expression of Rift Valley fever virus nucleocapsid (N) protein and evaluation of an N-based indirect ELISA for the detection of specific IgG and IgM antibodies

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 Utrecht University, the Netherlands, Eduardo Mondlane University, Maputo, Mozambique, and University of Pretoria, we tested 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 ruminant sera. Our results indicate that the I-ELISA based on recombinant N protein has the potential to complement the traditional assays for serodiagnosis of RVF. In addition, RVFV glycoprotein gene and West Nile virus (WNV) envelope gene were amplified and cloned in frame with the histidine tagged transfer vector (pFastBac) and recombinant Bacmids for protein expression - their potential as diagnostic antigens will be evaluated in different immunoassay formats.

Multiplex differential diagnosis of haemorrhagic fever viruses using Mass Tag PCR

In collaboration with Jerome L. and Dawn Greene Infectious Disease Laboratory, Mailman School of Public Health and Columbia Genome Center, Columbia University, New York, Mass Tag PCR was validated for the multiplex molecular diagnosis of selected VHFs. The sensitivity and specificity of PCR multiplex primers targeting conserved genomic regions of Ebola Zaire, Ebola Sudan, Marburg, Lassa fever, Rift Valley fever, Crimean-Congo hemorrhagic fever, Hantaan, yellow

fever, and Kyasanur Forest disease was assessed using calibrated synthetic standards as well as tissue culture - derived viral nucleic acid. Diagnostic accuracy with clinical materials was tested using blood, sera or oral swabs from human victims of VHF, including cases of Ebola hemorrhagic fever from the 1995 Kikwit outbreak, Democratic Republic of the Congo (DRC); cases of Marburg hemorrhagic fever collected in 2000 during the Durba outbreak, DRC, and in 2005 in Uige, Angola; cases of Lassa fever obtained in 2004 from Sierra Leone; cases of Rift Valley fever from Namibia in 2004 and Kenya in 1998; and cases of Crimean-Congo hemorrhagic fever from South Africa collected from 1986-93. The results showed that MassTag PCR offers a rapid, sensitive, specific and economic approach to the differential diagnosis of infectious diseases.

Bats as potential reservoirs of filoviruses

Since the first outbreaks of Marburg in 1967 in Germany and of Ebola in 1976 in Zaire, until very recently, epidemics caused by filoviruses were rare. However, simultaneous Ebola outbreaks in humans, great apes, and other primates, have occurred each year since 2001 in Gabon and the Republic of Congo. In collaboration with Centre International de Recherches Médicales de Franceville, Gabon and the CDC, Atlanta, we completed testing a total of 1 030 animals, including 679 bats, 222 birds and 129 small terrestrial vertebrates, which were captured in 2002 and 2003 in Gabon, in areas close to outbreaks of Ebola in human, gorilla and chimpanzee populations. The serological and PCR results suggest that at least three species of fruit bats, namely *Hypsignathus monstrosus, Epomops franqueti* and *Myonycteris torquata* may act as potential carriers of Ebola virus.

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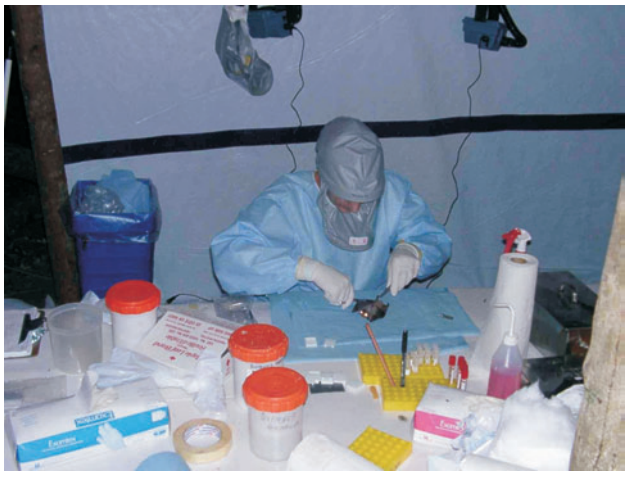

Figure 3. Ebola ecology study investigations in eastern Gabon.
A - Mandemba village, eastern Gabon, Ogoue-Ivindo Province: house of first patient to die of Ebola haemorrhagic fever in the 2001-2002 epidemic. This household ate meat from a dead duiker; B - Funeral of Ebola patient in a village: health officials dressed in protective clothing are loading coffin on vehicle; C - Research camp about 5 km from Ekata village: main bedroom structure with mosquito nets over bed; D - Mr Alan Kemp spraying the camp with insecticide, E - Skull of gorilla thought to have died of Ebola infection, F - Bat (*Epomops franqueti*) captured in net, G General view of the field laboratory: Prof R Swanepoel (NICD, SA) and Dr Pierre Rouquet (CIRMF, Gabon) dissecting small terrestrial vertebrates, gas stove in foreground used for sterilizing instruments for dissecting; H - Dr JT Paweska (NICD, SA) dissecting bat: disposable protective clothing, two pairs of gloves, battery operated respirator with positive-pressure filtered air.

In order to further address the potential of bats to harbour filoviruses, the SPU successfully established a colony of *Rousettus aegyptiacus leachi* (Fig. 4). Since 2003 the colony produced 14 babies in its first and 24 babies in the second reproductive season in captivity. It is intended to allow the *Rousettus* colony to increase to approximately 90 bats before experimental work with the filoviruses will commence. It is worth mentioning here that the 1998-1999 MBG outbreak in north-eastern DRC broke out amongst gold miners and their immediate contacts in Gorumbwa mine in Durba which gave shelter to a population of an estimated 30 000 Egyptian fruit bats (*Rousettus aegyptiacus leachi)*, plus large colonies of insectivorous bats.

Figure 4. Bat colony at SPU-NICD: female Egyptian fruit bat with pup and attentive male

Studies on the natural transmission cycles of filoviruses are hampered by the unavailability of validated assays for antibody detection in candidate vertebrate reservoir species, including bats. Unfortunately, the virus neutralisation assays have not been well established for filoviruses. Although Protein A/G conjugates offer a wide spectrum of binding reactivity with various serum species, they have different species-specific affinities and have not been rigorously tested against many vertebrate genera and families, especially in small terrestrial vertebrates. In conjunction with our colleagues at the CDC in Atlanta we collected sera from many species of bat and arranged for commercial production of anti-bat immunoglobulin conjugate which now allows us to develop a wide range of specific serological assays. We applied an indirect ELISA for testing bat sera for antibody to Marburg and Ebola viruses. The diagnostic cut-offs for these assays were derived from results obtained in bat sera collected in the Kruger National Park. Antibody to Marburg virus was found in bat sera from northeastern DRC where an outbreak of the disease occurred in 1998-2000.

Serological survey for Ebola-Zaire antibody in Pygmy population in the DRC

In collaboration with the Institute of Tropical Medicine, Antwerp, Belgium; Institut de Recherche Biomédicale, Kinshasa, DRC; Hôspital Général de Kilo-Moto, Watsa, DRC; and the Ministry of Health, DRC, we tested Pygmy population inhabiting the Watsa area of the DRC for the presence of IgG antibody to Ebola virus. The Watsa area is situated in the North East of the DRC. Its vegetation is dominated by woodland savannah and gallery forest, its climate is tropical humid with a short dry season from November to January. The population is about 180,000, including 4,000 pygmies. The Pygmies live a semi-nomadic life in the forest, organised in clans with a clan leader. Their settlements are mobile to a varying degree, composed of huts from branches and leaves that can be easily torn down and re-erected in case of need. Contact with Pygmies was made with the help of health workers based in health facilities in villages close to the Pygmy settlements and therefore known to the Pygmies and familiar with their customs. After obtaining informed verbal consent, the study participants were interviewed and bled at three meeting points in 35 to 105 km distances from Durba, the epicentre of the Marburg disease epidemic in 1998-2000. Most study participants reported acivities (hunting, handling, preparing bush meat, entering caves) and contacts with wild animals (rodents, bats, monkeys, or apes) considered to be risk factors for human infection with filoviruses. Of 300 Pygmies tested at least 60 (20%) had IgG antibody to Ebola virus (Fig. 5). In contrast 100 individual sera collected in Western Cape Province, SA (Ebola control negative population), were all negative. Our results seem to support earlier findings indicating that exposure to Ebola virus in some populations in central Africa is not uncommon and suggest the existence of less or non-pathogenic strains of the virus.

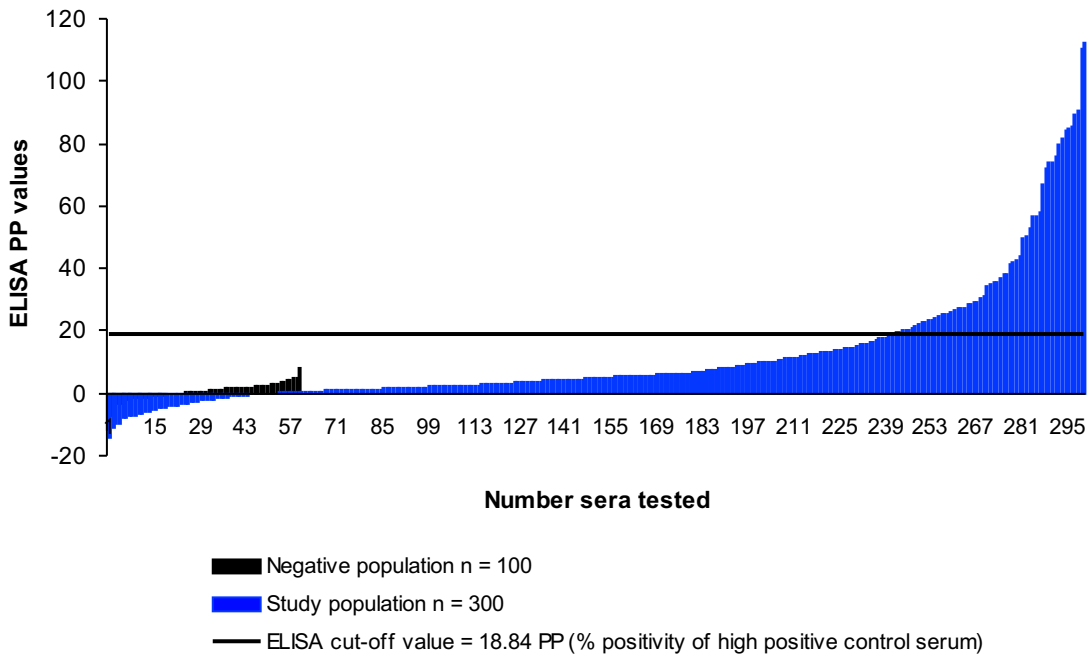

Figure 5. Distribution of PP values in Pygmies in the Watsa area, north-eastern Democratic Republic of Congo (study population) and in a control negative population of Western Cape Province, South Africa as measured by Ebola-Zaire indirect ELISA.

Replicon based vaccines against Rift Valley fever virus (RVFV)

Vaccination of livestock would provide the most practical and effective means of controlling infections with RVFV in humans and preventing spread of the virus by livestock trade to non-endemic areas. 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. An alphavirus-based vaccine vector for RVFV has been developed in collaboration with our colleagues at the Carolina Vaccine Institute. The vaccine-delivery systems are based on Sindbis and Venezuelan equine encephalitis virus replicons encoding the glycoproteins of RVFV. After very promising results in a mouse model (full protection against lethal challenge following vaccination with RVFV and demonstrating of neutralizing antibodies) the efficacy of the vaccine is currently being evaluated in sheep.

CONFERENCES ATTENDED

Dr JT Paweska, Prof R Swanepoel, Mr A Kemp, European Union Food Safety Authority Working Group on Rift Valley fever, Rome, Italy, 13-14 January 2005.

Prof R Swanepoel, Universities of Manitoba and Nairobi Symposium on Collaborative Research, Nairobi, Kenya, 5th February 2005.

Dr F Burt, Dr M Venter, Mrs A Grobbelaar, International Virology Congress, San Francisco, USA, 23-28 July 2005.

Dr JT Paweska, Prof R Swanepoel, Veterinary Epidemiology & Preventive Medicine, Pretoria, South Africa, 11-12 August 2005.

Dr JT Paweska, Rift Valley Fever Conference, Dakar, Senegal, 3-7 October 2005.

Dr JT Paweska, Technical Assistance in ELISA and PCR Diagnostic Activities, Luanda, Angola, 30 October to 5 November 2005.

Dr JT Paweska, Prof R Swanepoel, Dr F Burt, Mr A Kemp, Virology Africa 2005, Cape Town, South Africa, 8-9 November 2005.

Prof R Swanepoel, 7th Meeting of the WHO Advisory Committee on Variola Virus Research, WHO, Geneva, Switzerland, 10-11 November 2005.

Prof R Swanepoel, Steering Committee Meeting of the WHO Global Outbreak Alert & Response Network, Singapore, 7-9 December 2005.

HIV/AIDS Virus Research Unit

Head:
A/Prof Lynn Morris

STAFF
A/Prof L Morris DPhil, Chief Specialist Scientist (Head of Unit)

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A/Prof L Morris DPhil, Chief Specialist Scientist, Head
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S Herrmann, Admin Assistant
Dr P Moore PhD, Senior Medical Scientist
Dr A Basson PhD, Senior Medical Scientist
Dr V Pillay PhD, Senior Medical Scientist
Dr T Cilliers PhD (June 2005), Research Assistant
S Cohen Btech, Laboratory Manager
E Cave MSc, Research Assistant
I Choge MSc, Research Assistant
E Gray MSc, Research Assistant
J Ledwaba MSc, Research Assistant
N Leseka MSc, Research Assistant
S Loubser MSc, Research Assistant
J Nhlapo MSc, Research Assistant
S Nkosi MSc, Research Assistant
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N Taylor MSc, Research Assistant
M Smith BSc, Research Assistant
M Phoswa, Lab Technician
M Coetzer, PhD Student
P Walker BSc (Hons), Visiting PhD Student

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L Short, Secretary
Dr S Shalekof PhD, Senior Medical Scientist
Dr S Meddows-Taylor PhD, Senior Medical Scientist
F Anthony BSc, Laboratory Manager
S Lalsab BSc, Project Director
B Mathebula BSc, Student Scientist
D Schramm, Postgraduate Student (PhD)
J Mans, Postgraduate Student (PhD)
S Donninger, Postgraduate Student (MSc)
F Anthony, Postgraduate Student (MSc)
S Smit, Postgraduate Student (MSc)

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Dr D Barkhan PhD, Senior Medical Scientist
Dr M Suchard BSc MBBCh, MMed Student
G Khoury BTech Med Tech, Operations Manager
P Mokgotho HED BSc (Hons), Repository Manager
S Xaba Nat Dip Med Tech, Laboratory Manager
MK Mufhandu Nat Dip Med Tech, Project Co-ordinator
H Maila MSc, Research Assistant
P Mohube HD Psychology, Research Assistant
M Mlotshwa MTech Biomed Tech, Research Assistant
N Malatsi MTech Biomed Tech, Research Assistant
V Dyer, Laboratory Assistant
S Nyoka NH Dip Med Tech, Chief Technologist, QA

KOSH Satellite Laboratory
Dr S Roux MBBCh, Site Director
O Seabi MSc, Laboratory Manager
I Kgotlagomang BSc (Hons), Research

In February 2005 the AIDS Unit relocated to a new building on the NHLS campus. The facility covers over 2,000 square meters on two levels and includes laboratory space for Prof Morris (Virology), Prof Tiemessen (Cell Biology), Dr Puren (HLA) and Prof Gray (Immunology) and their staff. Research conducted in the Unit continues to be funded by the South African AIDS Vaccine Initiative (SAAVI), the Center for the AIDS Programme of Research in South Africa (CAPRISA, an NIH funded study), the HIV Vaccine Trials Network (HVTN), the Centers for Disease Control, The Wellcome Trust, Bristol Myers Squibb "Secure the Future" plus other smaller funding sources. More recently the NICD has become involved in the Center for HIV and AIDS Vaccine Immunology (CHAVI), a new NIH initiative under the leadership of Dr Bart Haynes. This new grant dovetails well with existing efforts funded under CAPRISA. In 2005 the Unit acquired large items of equipment including a new ABI3100 Genetic Analyzer and an LSRII flow cytometer, capable of analyzing up to 19 different cell parameters. The AIDS Unit continues to provide high quality research data and have fully accredited laboratories (both local and international accreditation) as well as ongoing participation in a number of external quality assurance programs and performing proficiency testing.

Eleanor Cave, Dr Visva Pillay, Nana Leseka, Johanna Ledwaba, Mpho Rakgotho and Dr Adriaan Bassoon all joined the Unit in 2005.

Prof Caroline Tiemessen was promoted to Reader at the University of the Witwatersrand in February 2005. She was awarded a Wellcome Trust International Senior

Research Fellowship for 5 years for the project entitled "Innate and acquired cellular immunity in HIV-seropositive mothers and their infants".

Tonie Cilliers was awarded his PhD and two other students, Jabulani Nhlapo and Mia Coetzer submitted their PhD's in 2005. Sarah Cohen completed a BTech Degree in Laboratory Management at the Pretoria University of Technology.

Sibusiso Nkosi spent 4 months at the Vaccine Research Centre with Dr Richard Wyatt funded by a Fogarty Fellowship. Isaac Choge spent 1 week in Prof Carolyn Williamson's laboratory and Sibusiso Nkosi spent 2 weeks in Dr Colleen Flanagan's laboratory, both at the University of Cape Town. Dr Vivian Morafo spent 8 weeks at the Weatherall Institute of Molecular Medicine, John Radcliffe Hospital to perform experiments on dendritic cells as antigen presenting cells in ELISPOT assays.

Prof Lynn Morris chaired the 2nd South Africa AIDS Conference held in June at the ICC in Durban. The Conference brought together 4,000 delegates to present the latest findings and make recommendations for dealing with the HIV/AIDS epidemic in South Africa. Professor Gray, in collaboration with Dr Tammy Myers and Professor Brian Eley, co-organized a paediatric satellite session at the Conference on Treatment and Research options for paediatric HIV infection in South Africa: towards improving the care of HIV-infected children. This was held under the auspices of the Elizabeth Glaser Pediatric AIDS Foundation International Leadership Award.

Professor Clive Gray organised a week-long 1st African Flow Cytometry Workshop on Detection of Antigen-Specific T Cells by Intracellular Cytokine Staining (ICS). This was the first attempt at gathering some the World's leading T cell immunologists and flow cytometry-based experts in ICS and students from 13 African countries.

A number of scientists spent time in NICD laboratories in 2005. They are Dr Zenda Woodman from the University of Cape Town, Dr Peter Balfe from the University of Birmingham, Sudeb Dalai, a medical student from Stanford University and Ziad El Khatib, an epidemiologist from the Karolinska Institute in Sweden.

Local visitors to the Unit included Dr Peter Manyike from SAAVI, Dr Debra Meyer from University of Johannesburg, Dr Colleen Flanagan from University of Cape Town and Dr Rachel Chikwamba from the CSIR in Pretoria. International visitors included Dr. Louise Kuhn from Columbia University, Dr. David Katzenstein and Dr. Matthew Rabinowitz from Stanford University, Dr. Meg Doherty from Johns Hopkins University, Dr Luis Montaner from the Wistar Institute, Dr Rami Kantor from Brown University, Dr John Hural and Constance Ducar from the HVTN in Seattle, Dr John Altman from Emory University, Dr Rafick-Pierre Sékaly, University of Montreal, Dr Guido Ferrari, Duke University, Dr Mike Betts, University of Pennsylvania, Dr Richard Koup, Vaccine Research Center, Dr Marty Bigos, Gladstone Institute for Virology, Dr Steve de Rosa, University of Washington.

Below is a description of some of the research projects completed in 2005

GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF VIRAL ISOLATES FROM HIV-1 SUBTYPE C INFECTED CHILDREN WITH SLOW AND RAPID DISEASE PROGRESSION:

The biological genotypes, phenotypes and coreceptor usage of HIV-1 isolates obtained from forty perinatally infected infants in South Africa were analyzed. This included fifteen infants who had HIV-related symptoms most of whom died within two years of birth (rapid progressors) and twenty five children who survived between four and nine years with varying signs of disease (slow progressors). Heteroduplex Mobility Assays and sequence analysis confirmed that within the *env* and *gag* regions, all isolates were HIV-1 subtype C. Viral isolates from fourteen of the fifteen rapid progressors were NSI and used the CCR5 coreceptor, whilst one (RP1) was SI and used both the CXCR4 and CCR5 coreceptors. Among the twenty-five slow progressors, twenty-two isolates used CCR5 only, two used CXCR4 only, and one used both CCR5 and CXCR4. Two of the slow-progressing children who harbored CXCR4-using viruses had AIDS. All 4 CXCR4-using viruses had genotypic changes in the V3 region previously shown to be associated with CXCR4 usage. This cross-sectional study shows that HIV-1 subtype C viruses from both rapid and slow progressing perinatally infected children predominantly use CCR5. Although CXCR4-usage was rare in subtype C infection it was more frequently associated with a longer duration of infection; however, the isolation of a CXCR4-using virus from a rapid progressing infant suggests that CXCR4-using viruses may also be vertically transmitted in subtype C infection. This study is currently in press in AIDS Research and Human Retroviruses.

THE NATURE OF NON-FUNCTIONAL ENVELOPE PROTEINS ON THE SURFACE OF HIV-1:

HIV-1 neutralizing antibodies (nAbs) are thought to have the unique ability to recognize functional gp120/gp41 envelope glycoprotein (Env) trimers. All HIV vaccine candidates tested to date as well as natural infection appear to induce anti-Env Ab responses that comprise a large fraction of non-neutralizing Abs. This is a paradox, particularly for HIV particle immunogens that are thought to bear only functional trimers, and might therefore be expected to only stimulate nAbs. The observation that non-neutralizing Ab can specifically capture HIV-1 led to the proposal that alternative form(s) of Env may exist on virus surfaces. We have shown that HIV-1 bears non-functional gp120/gp41 monomers and gp120-depleted gp41 stumps. Furthermore, using a native electrophoresis band shift assay, we show that trimer binding predicts neutralization and that non-functional Env may account for virus capture by non-neutralizing Abs. We hypothesize that the non-

Table 1: Sensitivity of HIV-1 subtype C pseudovirions to anti-HIV Mabs

Env clone	IC_{50} (mg/ml)*						
	2G12	IgG1b12	2F5	4E10	TriMAb[1]	TriMAb+ 4E10[2]	sCD4
RP1.12	>45	>45	>45	**13.2**	>50	**8.9**	**16.4**
RP4.3	>45	**0.9**	>45	**17.1**	**1.6**	**1.0**	**8.4**
RP6.6	>45	**11.9**	>45	**45.8**	**20.1**	**11.1**	**27.0**
COT6.15	>45	>45	>45	**3.0**	>50	**0.9**	**8.3**
COT9.6	>45	**3.4**	>45	**35.9**	**5.0**	**2.6**	**0.4**
TM7.9	>45	**0.2**	>45	**34.5**	**0.2**	**0.2**	**7.3**
TM3.8	>45	>45	>45	**21.6**	>50	**13.5**	**26.0**

* Concentration of each MAb alone or in combination that achieves 50% inhibition (bolded)

[1]TriMAb: equimolar combination of 2G12:2F5:IgG1b12

[2]TriMAb: equimolar combination of 2G12:2F5:IgG1b12:4E10

functional forms of Env serve to divert the Ab response, allowing the virus to evade neutralization. This work was conducted at the Torrey Pines Institute for Molecular Studies in collaboration with Dr James Binley. It is currently in press at the Journal of Virology.

INSENSITIVITY OF PAEDIATRIC HIV-1 SUBTYPE C VIRUSES TO BROADLY NEUTRALIZING MONOCLONAL ANTIBODIES RAISED AGAINST SUBTYPE B:

A phase I clinical trial using neutralizing monoclonal antibodies (MAbs) as passive immunoprophylaxis to prevent mother-to-child transmission of HIV-1 in South Africa has been proposed. In order to assess the suitability of such an approach we determined the sensitivity of paediatric HIV-1 subtype C viruses to the broadly neutralizing MAbs IgG1b12, 2G12, 2F5 and 4E10. The gp160 envelope genes from 7 HIV-1 subtype C infected children were cloned and used to construct Env-pseudotyped viruses which were tested in a single-cycle neutralization assay. The epitopes defining three of these MAbs were determined from sequence analysis of the envelope genes. None of the 7 HIV-1 subtype C pseudovirions were sensitive to 2G12 or 2F5, which correlated with the absence of crucial N-linked glycans that define the 2G12 epitope and substitutions of residues integral to the 2F5 epitope. Four viruses were sensitive to IgG1b12 and all of them were sensitive to 4E10 (Table 1). Only 4E10 showed significant activity against HIV-1 subtype C isolates while both 2G12 and 2F5 were ineffective and IgG1b12 was partly effective. It is therefore recommended that 2G12 and 2F5 not be used for passive immunization experiments in southern Africa and other regions where HIV-1 subtype C viruses predominate.

SILENCING OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 SUBTYPE C PRIMARY ISOLATES BY EXPRESSED shRNAs TARGETED TO *gag*:

Discovery of sequence-specific silencing by activating the RNA interference (RNAi) pathway has led to exciting new strategies for treating infection with human immunodeficiency virus type 1 (HIV-1). Of the HIV-1 subtypes, C is especially common in areas of the world that are worst affected. Although prone to mutation, genome plasticity of this subtype is limited in functionally important regions. We identified conserved sequences within the HIV-1 subtype C *gag* open reading frame and assessed whether they are suitable targets for inhibition of viral replication by RNA Pol III-driven small hairpin RNAs (shRNAs). Initially, efficacy of each of a panel of 10 shRNAs against HIV-1 was determined using a reporter assay. shRNAs A and B, which targeted the 5 end of *gag*, were most effective and were used to assess inhibition of replication in cultured cells of two R5 isolates (Du151 and Du422) and one X4 virus (SW7). These shRNAs diminished intracellular HIV-1 *gag* RNA and HIV-1 protein concentrations as well as p24 secretion by up to 80% without inducing an interferon response. However, shRNA-mediated knockdown efficacy against each of these viral isolates varied slightly. These data show successful activation of RNAi to inhibit the replication of biologically distinct HIV-1 subtype C isolates. The effector shRNAs described here are potential candidates for gene-therapy applications against the most common global subtype of HIV-1. This study was performed in collaboration with Prof Patrick Arbuthnot and Dr Marco Weinberg and is current in press at AIDS Research and Human Retroviruses.

DECAY OF K103N MUTANTS IN CELLULAR DNA AND PLASMA RNA AFTER SINGLE-DOSE NEVIRAPINE TO REDUCE MOTHER-TO-CHILD HIV TRANSMISSION:

Single-dose nevirapine (sd-NVP) for prevention of mother-to-child HIV-1 transmission is associated with selection of resistant viral variants, particularly the Lysine (K) to Asparagine (N) mutation at codon 103 (K103N) of reverse transcriptase. As this may influence subsequent treatment responses, a better understanding of the dynamics of decay and persistence of drug resistant variants is needed. We

therefore measured the frequency of K103N mutants among a cohort of HIV-1-infected pregnant women recruited at an out-patient clinic in Johannesburg, South Africa. Samples taken 6 weeks, 3, 7 and 12 months after delivery from 67 HIV-1-infected women who received sd-NVP during labor to prevent transmission were analyzed. Quantification of K103N mutants in maternal plasma viral RNA and cellular DNA was done using an allele-specific PCR assay capable of detecting codons AAC and AAT if their frequency was >0.002 of the total viral population. Using this assay, 87.1% (27/31) of RNA samples and 52.3% (23/44) of DNA samples collected 6 weeks after sd-NVP had detectable K103N variants.

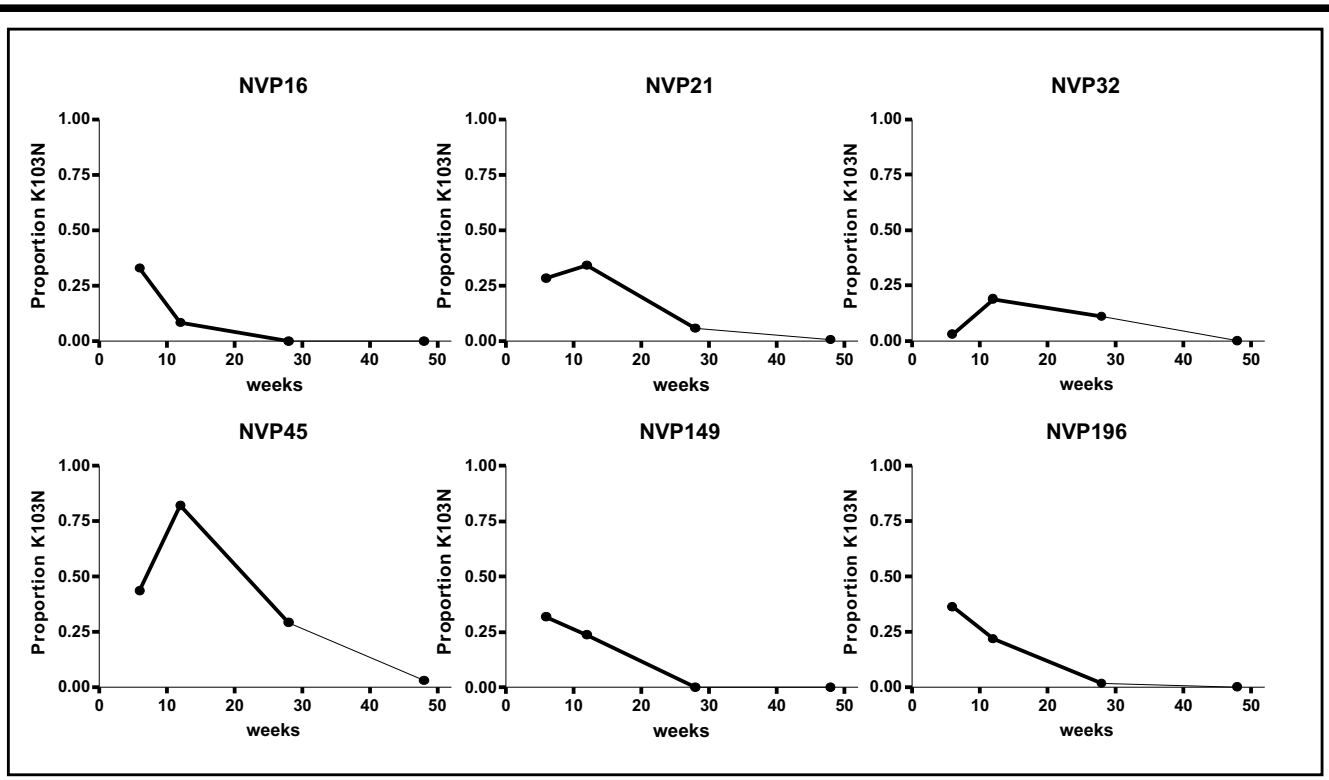

Figure 1. Longitudinal examples of the relative frequency of K103N variants in maternal plasma viral RNA at time-points after single-dose nevirapine for individual women to illustrate different patterns of decline. NVP16, NVP149 and NVP196 showed a decline typical of the whole group of 16. All three women (NVP21, NVP32 and NVP45) with atypical patterns are shown.

This declined to 65.4% (17/26), 38.9% (14/36), and 11.3% (6/53) in RNA at 3, 7 and 12 months respectively, and to 4.2% (2/48) in DNA at 12 months. Thus, K103N resistant variants were present in almost all women 6 weeks post-sd-NVP but decline rapidly over time (see Figure 1 for analysis of individual patients). Resistant variants were detected less frequently in cellular DNA with persistence in this compartment by 12 months post-sd-NVP among a minority. This study is in press at AIDS.

WHAT GENETIC CHANGES IN V3 ARE ASSOCIATED WITH CXCR4 USAGE IN HIV-1 SUBTYPE C ISOLATES?

CXCR4 coreceptor usage appears to occur less frequently among HIV-1 subtype C viruses. The aim of this study was to investigate the genetic determinants within the V3 region of subtype C isolates able to use CXCR4. Thirty-two subtype C isolates with known phenotypes (16 R5, 8 R5X4 and 8 X4 isolates) were assessed using a subtype C specific V3-heteroduplex tracking assay. Results indicated that there were sufficient genetic differences to discriminate between R5 viruses and those able to use CXCR4 (both R5X4 and X4). In general, R5 isolates had a mobility ratio >0.9 whereas CXCR4-using isolates were usually <0.9. Multiple bands were more frequently seen among R5X4 isolates, which on clonal analysis were identified as R5-like or X4-like variants. Sequence analysis of the V3 region showed that CXCR4-using viruses were often associated with an increased positive amino acid charge, insertions and loss of a glycosylation site, similar to HIV-1 subtype B (Figure 2). In contrast, where subtype B consensus V3 has a GPGR crown motif irrespective of coreceptor usage, all 16 subtype C R5 viruses had a conserved GPGQ sequence at the tip of the loop, while 12 of the 16 (75%) CXCR4-using viruses had substitutions in this motif, most commonly arginine (R) (Figure 3). Thus, the rare occurrence of CXCR4-using viruses in subtype C may be due to the highly conserved nature of the GPGQ crown that may limit the potential for the development of subtype C X4 viruses.

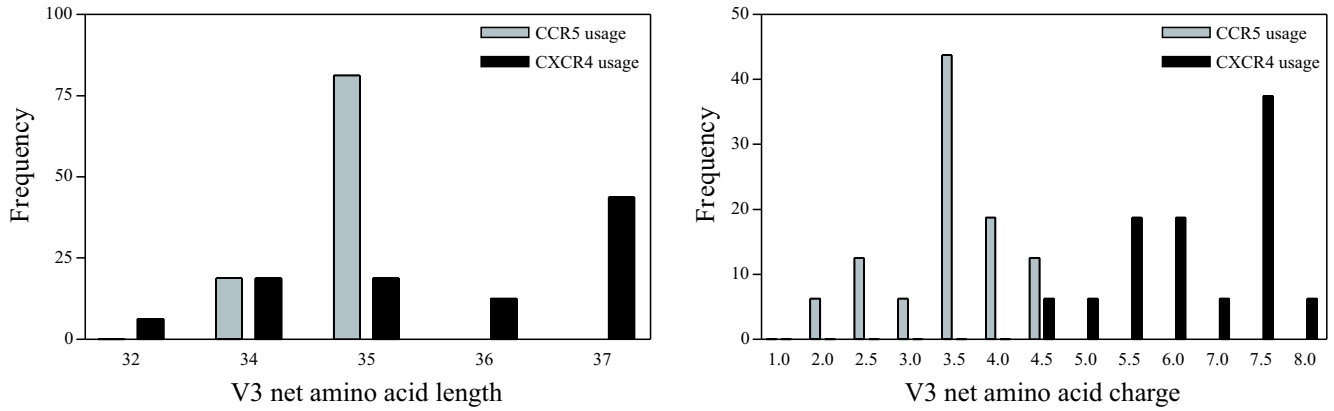

Figure 2. Comparison of V3 length and V3 charge of HIV-1 subtype C isolates able to use CCR5 and CXCR4.

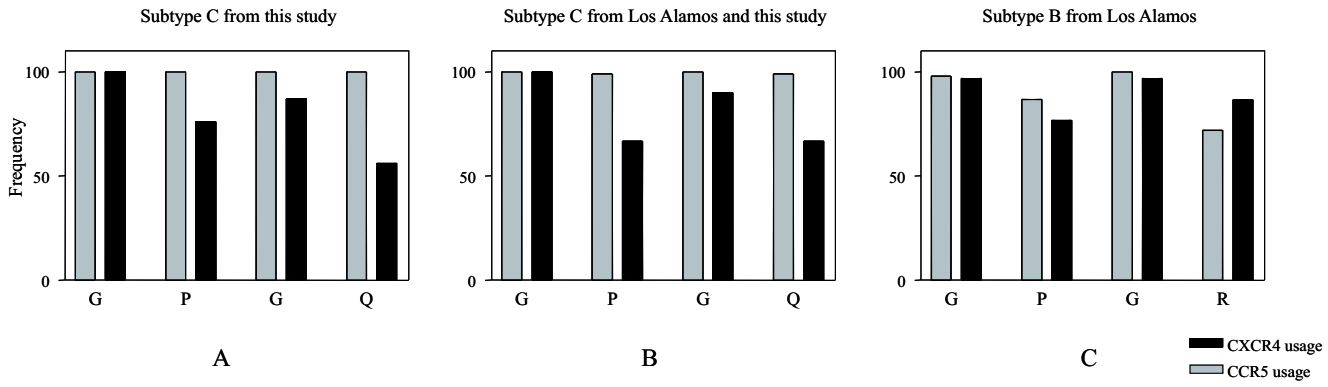

Figure 3. Comparison of amino acid variation in the V3 crown of subtype C isolates from this study (16 CCR5 and 16 CXCR4) (p>0.0001) (A); subtype C isolates from Los Alamos and this study (106 CCR5 and 33 SI) (p<0,0001) (B) and subtype B isolates from Los Alamos (99 CCR5 and 30 CXCR4) (C).

PROTECTIVE IMMUNITY TO HIV-1: INSIGHTS GAINED FROM STUDIES ON MOTHER-TO-CHILD TRANSMISSION OF HIV-1:

Our research over the past several years has focused on utilizing maternal-infant transmission as a model for the study of protective immunity to HIV-1. More basic research is required to provide insight into the mechanisms of escape employed by HIV-1, and the mechanisms of defence used by the host as this is the basis for designing effective and better targeted interventions of protection. Understanding and defining the components of the immune response to HIV-1 that constitute protective immunity remains an ever-important goal for HIV vaccine research. Much can be learnt from natural HIV-1 infection and experimental vaccine studies. There is consensus that the desired immune responses for prevention of chronic infections such as HIV-1 would be a combination of neutralizing antibodies and cell-mediated immune responses (CD4+ and CD8+ T-cell responses), and it has become clear that mere detection of these responses is unlikely to be sufficient and more detailed qualitative and functional characterization is required. It also stands to reason that the innate immune system would represent a very important component of an immune response to HIV-1, given that such responses are first to act upon initial encounter with HIV-1 and on subsequent recall, and furthermore it is the innate immune system that instructs the development of adaptive immunity. There is a large body of evidence accumulating that supports a role for innate immune factors such as the CC chemokines (CCL3, CCL4 and CCL5) in protective immunity to HIV-1.

A large proportion of infants born to HIV-1 infected mothers escape HIV-1 infection even in the absence of any antiretroviral intervention, providing testament to the presence of protective immune processes. Our recent work set out to test prospectively if foetal CC chemokine production primed by HIV-1 *in utero* can protect against HIV-1 transmission with subsequent exposure at delivery. The main findings were as follows: (i) HIV-1 exposure *in utero* (i.e. being to born to an HIV-positive mother) resulted in increased infant production of CCL3/CCL3-L1 and CCL4 but decreased CCL5 production; (ii) HIV-exposed infants who failed to respond with elevated CCL3/CCL3-L1 production

(suggesting a deficiency in their capacity to produce CCL3/CCL3-L1) were more vulnerable to acquiring HIV-1-infection at delivery; (iii) mothers transmitting intrapartum (IP) also presented with a deficient CCL3/CCL3-L1 production phenotype, evident in plasma, suggesting that the underlying nature of deficient infant CCL3/CCL3-L1 production was genetic and not due to a difference in *in utero* exposure between EU (exposed-uninfected) and IP infants (supported by similar levels of soluble immune activation markers in cord blood plasma of EU and IP infants and by the fact that differences in CCL3/CCL3-L1 production between EU and IP infants were not attenuated after adjustment for maternal viral load or CD4 count); (iv) copy number of CCL3-L1 was lower in IP infants and their mothers than in EU infants consistent with an influence of copy number on induced protein production in culture. However, copy number did not entirely account for the deficiencies in CCL3/CCL3-L1 production associated with risk of transmission, suggesting the presence of inactive copies of CCL3-L1 in IP infants; (v) mutations in CCL3 gene regions or lack of appropriate transcription factors or differential mRNA stability may be contributing factors to reduced production. Our results highlight that copy number of CCL3-L1 per se does not always dictate levels of CCL3/CCL3-L1 production and that copies of CCL3-L1 or CCL3 in different individuals represent different abilities to be induced to produce protein. Whatever the contributing factors, it is clear that the association with susceptibility to HIV-1 lies at the level of induction of gene expression of CCL3/CCL3-L1, culminating in quantitative differences in protein production.

The reduced abilities of mothers and infants in the IP group to produce CCL3/CCL3-L1 in response to mitogen, in part but not entirely due to reduced copy numbers of CCL3-L1, suggested that, other genetic variants may exist amongst the genes that code for this chemokine. The promoter regions, exon 1 and most of intron 1 of both CCL3 and CCL3-L1 genes were sequenced for the mothers who had CCL3 production levels determined and their matched infants (i.e. a total of 86 sequences each for CCL3 (1240 bp) and CCL3-L1 (1550 bp). These regions were selected based on former descriptions of associations with HIV/AIDS (32), and on likelihood of effects on gene transcription. Data revealed the presence of 4 single nucleotide polymorphisms (SNPs) in CCL3 and 3 in CCL3-L1. A number of these sites are earmarked as potential candidates for altered CCL3 production. Characterization of SNPs in the regions outside of those sequenced to date is underway.

In vivo EFFECTS OF HIV-1 EXPOSURE IN THE PRESENCE AND ABSENCE OF SINGLE-DOSE NEVIRAPINE ON CELLULAR PLASMA ACTIVATION MARKERS OF INFANTS BORN TO HIV-1 SEROPOSITIVE MOTHERS

Short-course antiretroviral drug regimens reduce the risk of mother-to-child transmission of HIV-1 but mechanisms affording protection of such interventions remain poorly defined. Since T-cell activation is an important factor in productive HIV-1 infection, we tested the hypothesis that single-dose nevirapine (NVP) reduces immune activation which in turn reduces the likelihood of transmission. We compared concentrations of cord and maternal blood plasma immune activation markers, neopterin, $_2$-microglobulin ($_2$-m) and soluble L-selectin (sL-selectin), in two groups of HIV-1-exposed newborns whose mothers either received NVP at the onset of labour or who only received NVP as post-exposure prophylaxis (PEP) within 72 hours of birth and among HIV-unexposed controls. *In utero* exposure of the infant to HIV-1, regardless of NVP exposure, led to demonstrable increases in immune activation markers, this being most notable in the presence of pre-existing infection. Contrary to what was hypothesized, immune activation was increased by pre-birth exposure to single-dose NVP, with this effect being enhanced in infants already infected at birth. Our data suggest that reductions in immune activation do not explain transmission prevention effects of single-dose NVP. Our data also suggest a biological explanation for why HIV-1 infected infants exposed perinatally to antiretroviral drugs might experience hastened disease progression, namely that in some HIV-1 infected individuals NVP may synergize with HIV-1 to enhance an environment that favours increased HIV-1 replication.

PROMISCUOUS EPITOPE REACTIVITIES THAT DO NOT CONFORM TO HLA SUPERTYPES IN SUBTYPE C HIV-1 INFECTED INDIVIDUALS:

The human leukocyte antigen (HLA) molecules found on the cell surface of nucleated cells, present virally-derived short peptides (epitopes) for recognition by CTL. Cytotoxic T lymphocytes (CTL) have been shown to be an important arm of the immune response for controlling HIV-1 infection and specific HLA class I alleles will shape and direct the fine specificity of CTL. In a given population, the combined polymorphisms of HLA class I genotypes and the infecting HIV-1 subtype can influence the effectiveness of the CTL responses and recognition of epitopes. We tested a series of optimal subtype B-based CTL epitopes having been shown to bind in vitro to HLA molecules belonging to five supertype families and predicted to cover >80% HLA alleles in a given population. Forty six peptides shown by binding assays to be restricted by 5 HLA supertype families (A1, A2, A3, A24 and B7) spanning Pol, Gag, Env, Nef, Vif, Vpr and Rev were used in the study. One hundred and eight subtype C HIV-1 infected individuals, randomly chosen without regard for HLA background were screened using the gamma-interferon ELISpot assay, utilizing a pool/ matrix approach to assess epitope-specific CTL responses.

Responses to optimal peptides were observed in 44% (48/108) of subtype C HIV infected individuals and the bulk of the responses were directed at Integrase. When the high resolution HLA background was unblinded, we found that 42% (14/33) of individuals recognizing epitopes failed to conform to the expected supertype family. These data suggest that epitope reactivity in individuals not conforming were promiscuous in nature

and were likely to be novel HLA restrictions. The HLA-B7 superfamily was significantly over-represented (p=0.0465) in those individuals responding to any of the peptides.
Our results indicate a limited response elicited by subtype B-derived epitopes in subtype C HIV-1 infected individuals and additionally highlight the existence of a high degree of epitope promiscuity. Subtype B-based HIV-1 vaccines containing multi-epitopes would a) not elicit expected epitope reactivities and b) not match with epitope reactivities generated during subtype C HIV-1 infection. This would suggest that preventative vaccine immunogens based on subtype B-based optimal epitopes would elicit reactivities that have <50% match with T cell reactivities in natural subtype C HIV infection, implying that clade-specific vaccines might be more appropriate. Our data also highlight the pleotropic response of T cells to epitopes restricted by multiple HLA alleles.

NOVEL EPITOPE IDENTITY IN THE Ebna-3a REGION FROM EPSTEIN BARR VIRUS INFECTED INDIVIDUALS LIVING IN BOSTON AND JOHANNESBURG: RELEVANCE TO GLOBAL HIV VACCINE IMMUNOGENICITY MEASUREMENTS:

Epstein Barr virus (EBV) is carried by a majority of individuals as a life-long and asymptomatic infection. Studies using B95.8 (type 1) EBV isolate peptides have shown that dominant major histocompatibility complex (MHC) class-I restricted cytotoxic T-lymphocyte (CTL) responses identified in latently infected EBV carriers are directed to epitopes derived from the Epstein Barr nuclear antigen-3 (EBNA-3) family of proteins. The aim of this study was to identify CTL epitopes to the EBNA-3A region of the Ag876 (type 2) EBV isolate that are targeted across different populations for inclusion as positive control peptides in immunogenicity end-point assays. Peripheral blood mononuclear cells (PBMCs) were isolated from 34 HIV-negative blood-bank donors from Johannesburg, South Africa and 20 from Boston, USA. PBMCs were screened in duplicate, using the interferon-gamma (IFN-g) ELISPOT assay, for responses against pools of EBNA-3A peptides; 15-20 mers in length overlapping by 11 amino-acids. Single peptide responses were subsequently confirmed using the IFN-g ELISPOT assay. Thirty eight percent (13/34) of donors from Johannesburg and 75% (15/20) of donors from Boston responded to one or more peptide pools with responses targeting the N-terminus of the EBNA-3A region for Johannesburg donors and a dispersed response across the EBNA-3A region for the Boston donors. Clustered responses were identified in the two populations recognizing epitopic regions in aa 76-163; 163-234; 366-437; 495-579 and 721-801. Collectively, 35 novel epitopes were identified that resulted in greater number of responses identified in the existing positive control pool of CMV, EBV and Flu peptides. No immunodominant responses to the EBNA-3A region of EBV were identified in either population. Only three epitopic regions of the novel epitopes were shared by both populations thus suggesting that a selection of EBNA-3A epitopes can be used to supplement the existing positive control relevant for global use in HIV vaccine immunogenicity measurements.

CD8+ T RESPONSES ASSOCIATED WITH CONTROL OF HIV-1 SUBTYPE C INFECTIONS:

CD8+ T cells are important in the immune response to acute and chronic HIV infection. Recent evidence suggests that functional impairment, exhaustion and physical deletion of antigen specific CD8+ T cells accompany ineffective viral control during chronic HIV infection. We have recently shown that the preferential recognition of Gag is associated with subtype C viral control in a cohort of early HIV-1 infected individuals. We have extended this study to examine the quality of CD8+ T cells recognizing an epitope in Gag (TL9) in two contrasting individuals with slow and fast HIV-1 disease progression profiles. The slow progressor had a focused mono-specific response to TL-9 whilst the more rapid progressor had a broader response across the HIV genome, with an immunodominant response in Nef. Comparison of antigen-specific T cell cytokine profiles revealed that in both individuals, TL-9 specific CD8+ T cells expressed IFN-, MIP-1, TNF- and low frequencies of IL-2; although the frequency of cytokine producing cells was ≥2fold higher in the more rapid progressor. Consistent with other reports, the majority of antigen specific cells expressed effector (E) or effector memory memory as TL-9-specific CD8+ T cells that co-expressed CD27+CD45RO+ and effector memory as CD27-CD45RO+.

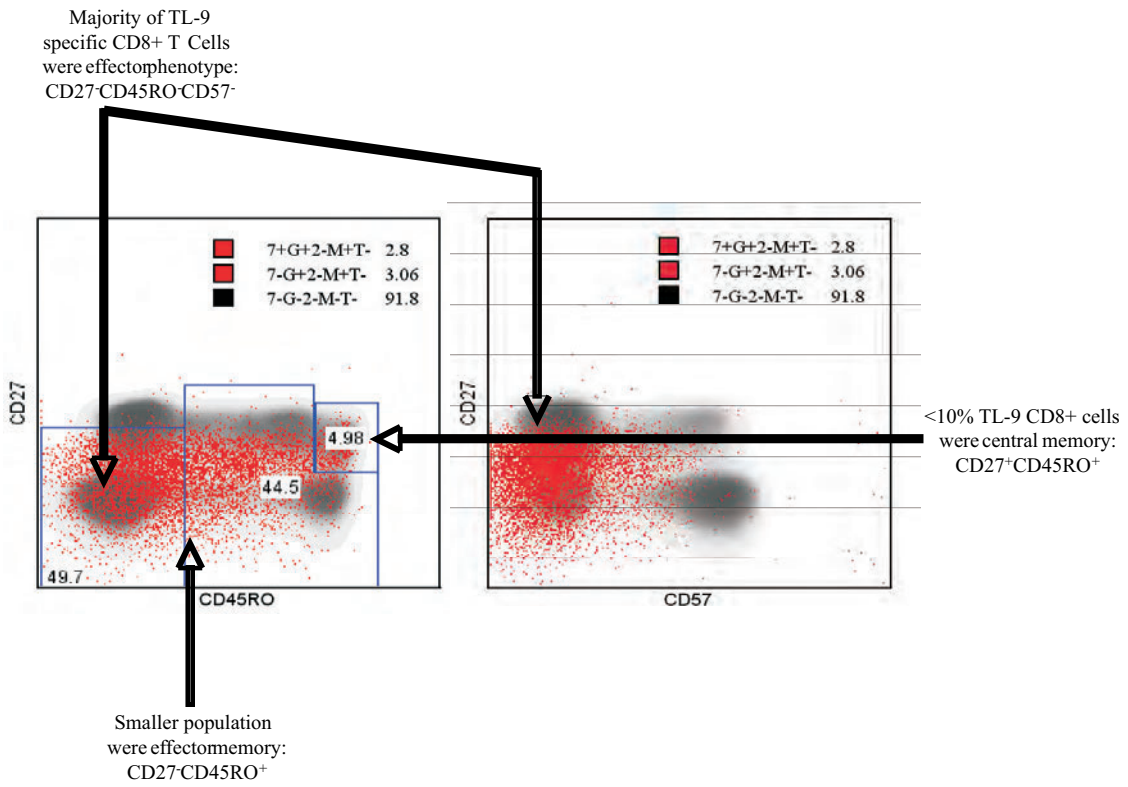

Figure 4: Multiparameter flow cytometry, involving 13 colour ICS analysis, of TL-9-specific CD3+CD8+ cells and examined the cells for memory lineage. In this example, we defined central (EM) phenotypes based on differential CD27, CD45RO and CD57 staining. Whilst >85% of TL-9 specific E/EM CD8+ T cells from the rapid progressor expressed the degranulation marker CD107, less than 50% was observed in the slow progressor. We suggest that the divergent distribution of E/EM phenotypes reflect differences in HIV antigen load for the two individuals. Additionally, the frequency of cells expressing the IL-7 receptor -chain were investigated to determine the number of TL-9 specific long-lived memory cells at two different time points. We observed diminished to absent long-lived memory CD8+ T cells in the rapid progressor during both early infection and at the onset of antiretroviral treatment. To conclude, our data suggest that HIV disease progression is characterized by a broadly targeted response to HIV with a high turnover of antigen specific cells that produce antiviral cytokines and have cytotoxic activity. Viral control is most likely achieved by the quality of response to HIV from a pool of long-lived memory CD8+ T cells.

CONFERENCES ATTENDED

Prof Lynn Morris attended and gave a presentation "*Use of sensitive real-time PCR assays to detect NVP resistance mutations in women following sd-NVP*" to the Elizabeth Glaser Pediatric AIDS Foundation held in Washington DC, USA on Monday 21 February 2005.

Prof Lynn Morris attended the the 12th Conference on Retroviruses and Opportunistic Infections (CROI) in Boston, USA from 22 to 25 February 2005 and was invited to be a moderator for a session on oral abstracts - "*HIV Drug Resistance: Selection, Persistence and Impact of Response*".

Prof Lynn Morris attended and gave a presentation to the Laboratory Sciences Advisory Committee (LSAC) at the HIV Vaccine Trials Network (HVTN) in Boston on Saturday 26 February 2005.

Shayne Loubser attended the 12th the CROI conference in Boston from 22 to 25 February 2005 and gave a presentation: Shayne Loubser, P. Balfe, G. Sherman, S Jones, S. Cohen, L. Kuhn, S. Hammer, L. Morris. "*Sensitive Real-time PCR Quantification of 103N Resistance Mutants following Single-dose Treatment with Nevirapine"*

Mia Coetzer also attended the CROI conference and presented a poster: M. Coetzer, M.A. Jensen, A.B. van 't Wout, L. Morris, J.I. Mullins. *A Reliable Phenotype Predictor for Subtype C Based on EnvV3 Sequence*"

Prof Clive Gray organized the First African ICS Workshop held at NICD from 6-12 March 2005, and Vivian Morafu and Stephina Nyoka were facilitators at the workshop.

Prof Lynn Morris attended the opening conference "Understanding the Major Infectious Diseases of Africa"

and the official launch of the Institute of Infectious Diseases and Molecular Medicine (IIDMM) at the University of Cape Town from 22-24 March, 2005.

Prof Clive Gray attended the Keystone Symposium in the USA from 8-16 April, 2005.

Prof Lynn Morris attended the Keystone Symposia held in Banff, Alberta, Canada from April 9-15, 2005. She chaired a Joint Session (Protective Immunity I: Neutralizing Antibodies and gave a talk "*Neutralizing Antibody Response to Clade C HIV-1 Infection in Africa"*.

Dr Penny Moore attended the Keystone Symposia held in Banff, Alberta, Canada from April 9-15, 2005 and presented a Poster "*Predicted Genotypic Resistance to the Novel Entry Inhibitors, BMS-378806 and BMS-488043, among HIV-1 isolates of subtypes A to G."*

Sarah Cohen attended the 18th National Medical Technology Congress in Cape Town from 28th April to 3rd May 2005.

Prof Clive Gray attended a meeting at the University of Rochester, New York, USA, from 5-6 May, 2005.

Prof Clive Gray, Greg Khoury, Stephina Nyoka and **Pauline Mokgotho** attended the HVTN Full Group Meeting, Washington, USA, 9-11 May, 2005.

Prof Clive Gray attended the NIAID Colloquium at the NHLS on 16 and 17 May, 2005.

Vivian Morafo, Mandla Mlotshwa, Debra Barkhan, Netty Malatsi and **Stephina Nyoka** attended the SA AIDS Conference, Durban from 7-10 June, 2005.

Shayne Loubser attended the XIV International HIV Drug Resistance Workshop in Quebec, Canada from June 7-11, 2005 and presented a poster - *Increased Sensitivity of Detection of K103N Resistance Variants by Real-Time PCR in RNA and DNA after Single-Dose Nevirapine.*

Eight members of the laboratory attended the SA Aids Conference in Durban namely **Prof Lynn Morris, Dr Penny Moore, Natasha Taylor, Elin Gray, Sarah Cohen, Mary Phoswa, Dr. Visva Pillay** and **Eleanor Cave**. Posters were presented by Dr. Penny Moore, Natasha Taylor, Isaac Choge. Elin Gray and Polly Walker gave oral presentations.

Prof Clive Gray attended the SAAVI Strategic Discussion Meeting on 2-3 August, 2005.

Greg Khoury and **Vivian Morafo** attended the CHAVI Meeting, Durham, North Carolina from 22-25 August, 2005.

Prof Lynn Morris attended the AIDS Vaccine 2005 Conference in Montreal, Canada from 69 September 2005. She was invited to be Chairperson of one of the plenary sessions entitled "Major Scientific Obstacles in the Field of Vaccine Development".

Elin Gray received a full scholarship to attend the AIDS Vaccine 2005 conference in Montreal, Canada and presented a poster "Susceptibility of subtype C viruses to neutralizing monoclonal antibodies raised against subtype B".

Prof Clive Gray attended the EGSA/ILA Think Tank, White Oak, USA from 25-29 September, 2005.

Vivian Morafo attended the AAVP Conference, Cameroon, 17-19 October, 2005.

Prof Clive Gray attended the HVTN Reverse Site Visit, USA, 7-9 November, 2005.

PRF Library

STAFF

H Saevitzon, Librarian
P Nkosi, Librarian
Y Schroder, Admin Clerk

The library provides information sources to staff for research and academic purposes, and general information to all staff members. In October 2005 a new Librarian, Ms Phindile Nkosi, was appointed with the retirement of Mrs Hazel Saevitzon in December 2005.

Subscription to a number of print version journals with others including free online access make it possible for the library to meet its objective of providing accurate information. It is a challenge for the library to subscribe to online journals with the drastic change in technology. A circulation of current journals is done according to the need of staff members.

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STATISTICS

- ***CIRCULATION***

Journals	7115
Books	123

- ***SUBSCRIPTION***

Titles on current subscription	47
Free titles	14
Issues on loan for display	497
Issues lent for display	204

- ***INTERLIBRARY REQUESTS***

Articles received from other libraries	228
Articles sent to other libraries	48
Books borrowed	1
Books loaned	0

- ***COLLECTION***

Books	1813
Staff reprints	1160
Slides	927
Tapes	34
Videos	10

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