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On the 14th November 1953, the laboratories of the Poliomyelitis Research Foundation (PRF) were opened by the then Minister of Health, Mr A.J.A.R van Rhijn. The Poliomyelitis Research Foundation then evolved into the National Institute for Virology (NIV) in April 1976 and subsequently the National Institute for Communicable Diseases (NICD) in January 2002.

To commemorate this 50th anniversary a special academic day was held on 12 November 2003. In addition to presentations of the contemporary research activities of the Institute and guest presentations by Dr James W LeDuc, Director: Division of Viral and Rickettsial Diseases at the Centers for Disease Control and Prevention (CDC), USA and Dr David Heymann, formerly Executive Director: Communicable Diseases, WHO and recently appointed to head the WHO Global Polio Eradication program, a historical look-back at the early origins of the Institute and its subsequent evolution to the present NICD was presented. With the global polio eradication programme drawing close to its goal of certification of a world free of the virus by 2005, it is as well on the 50th anniversary of the NICD, to reflect on the early beginnings of the Institute which was born as a direct result of poliomyelitis.

The story of the NICD began in 1948 and its early beginnings are chronicled in the book “The History of the Poliomyelitis Research Foundation” by Prof James Gear, published by the Poliomyelitis Research Foundation. South Africa, in common with a number of countries throughout the world, including the United States, suffered from particularly severe outbreaks of poliomyelitis which swept through the entire country with several thousand cases and several hundred deaths. Poliomyelitis was in those days indeed a dreaded disease against which the population had little protection. Following the 1948 epidemic the Poliomyelitis Research Appeal was launched by the then Mayoress of Johannesburg, Mrs Evelyn Gordon. The appeal not only embraced South Africa, but was also supported by communities as far as Kenya. However, the appeal for funds and the drive to develop a polio vaccine was not without its controversies. From the start there was strenuous opposition from highly placed sources. The Secretary of Health of Union Health Department, Dr George Gale, opposed the Poliomyelitis Research Appeal and no support was forthcoming from the Government of the day. Sir Basil Schonland, president of the CSIR enlisted the help of Sir Edward Mellanby, Secretary of the British MRC to evaluate the appeal. Sir Edward, in consultation with British virologists, called the appeal for funds to develop a vaccine “intellectual dishonesty”. In their opinion there was no possibility of developing a vaccine against poliomyelitis in the foreseeable future. Nevertheless, over half a million pounds were collected which enabled the laboratories to be built and commissioned in 1953 under its first director, Professor James Gear.

The first batches of vaccine made in these laboratories were ready for use in 1955 – amongst the first used for human immunization in the world. They were initially tested in twelve children of members of staff of the Institute, including Prof James Gear’s sons. Vaccination was not without controversy. The South African Medical Journal in September 1955 published the following letter from the Border Branch of the Medical Association of South Africa which was subsequently reprinted in the lay press and received widespread publicity:-

“Sir, the East London division of the Border Branch of the MASA, at a recent meeting unanimously decided to ask you to make the following facts public re the present position of polio in this country. The Institute of Polio Research – to which the public of the Union of SA has donated £600,000 – has produced a vaccine which the Union Health Dept., has innoculated over 500,000 children without any trial on humans and without any knowledge at all of long term effects or the length of immunity conferred – has decided to use in mass vaccination. Without any consultation with, or information to, the medical profession, the department is almost stampeding the public into having their children vaccinated with a vaccine the effects of which – and this is admitted – may even be harmful and dangerous”.

“The gross mishandling of the whole situation and the deliberate withholding of information to the medical profession has created a set of circumstances which have thrown grave doubts on the vaccine and has decided the local body of medical men to advise their patients not to submit to vaccination with a vaccine the effects of which are questionable.”

An acrimonious exchange of correspondence took place between Prof Gear and Dr Morris Shapiro, later Director of the South African Blood Transfusion Service and Dr Hillel Shapiro, Editor of the South African Medical Journal and Medical Proceedings, who both vehemently opposed the administration of polio vaccine to children. Nevertheless by 1958 the PRF was able to vaccinate 750,000 individuals with formalin inactivated polio vaccine and in the following year the incidence of polio dropped sharply. At the same time, work in the PRF laboratories commenced on developing an attenuated live strain of polio virus to be administered orally. In 1958 the PRF received
three strains of live virus which had been attenuated by passage through cotton rats, from Dr Albert Sabin. Production of oral polio vaccine subsequently replaced that of formalin inactivated vaccine and the first mass vaccination campaign throughout South Africa began in late 1960 and was completed in 1961.

Following on these campaigns there was an immediate dramatic fall in the incidence of polio. The steep fall in polio continued through the 1970’s. In the 1980’s two large epidemics of polio occurred in under-immunised populations in Gazankulu, now Limpopo province, in 1982 and in 1988 in KwaZulu-Natal and the last case of polio in South Africa was detected in 1989.

The founding mission of the PRF had been successfully achieved. The Institute now took on the broader responsibilities of laboratory support for all virus diseases while the discipline of medical virology greatly expanded during the 1970’s. A new threat now appeared on the horizon, the viral haemorrhagic fevers, and in 1975 the first of the viral haemorrhagic fevers hit South Africa, Marburg virus disease. In 1976 the Department of Health purchased from the PRF its laboratories and renamed the Institute the National Institute for Virology (NIV). In 1979 the only BSL-4 laboratory on the continent was opened with Prof Bob Swanepoel at its head. The following decade saw the emergence of a new virus disease, the most formidable of all infectious diseases, HIV/AIDS. An MRC HIV/AIDS research unit was established in 1987 under myself and assisted by Dr Des Martin and Dr Sue Lyons and this was subsequently developed into a world-class research unit by Prof Lynn Morris together with Dr Clive Gray and Dr Caroline Tiemessen.

During the late 1990’s it became abundantly clear that there was an urgent need nationally and regionally to develop a CDC-type Institute on the African continent to monitor communicable diseases, both existing infections as well as newly emerging infections. This need was fulfilled in January 2002 by the establishment of the National Institute for Communicable Diseases (NICD) which came about with the transformation of laboratory services and the advent of the National Health Laboratory Service (NHLS). The NICD was formed by adding to the NIV, the public health oriented microbiology laboratories from the former South African Institute for Medical Research (SAIMR) and creating a new third division, that of epidemiology and surveillance.

The newly formed NICD now faces different challenges to that of its predecessor, the PRF. One vaccine preventable disease, smallpox, has been completely eradicated from the planet. Poliomyelitis itself is circulating now only in seven countries in the world (compared to a 125 at the start of the global eradication campaign in 1988). Measles has dramatically decreased and diseases such as diphtheria, pertussis and tetanus are rarely seen. These have now been replaced by HIV/AIDS, TB, malaria as major public health threats in addition to a new concern, the spectre of new and emerging infectious diseases both natural and deliberate.

The inaugural academic day of November 2003, which will now become an annual event in November of each year, showcased the sterling achievements in research and communicable diseases surveillance by the virological and microbiological units of the Institute. The academic achievements of the Institute have been most impressive. During 2003 4 PhD and 2 MSc students of the NICD graduated through the University of the Witwatersrand. The laboratories have been accredited by SANAS (South African National Accreditation System) to ISO17025. Its BSL-4 laboratory serves as a WHO Collaborating Centre and its polio and measles laboratories as WHO Regional Reference Centres. One of the HIV/AIDS laboratories serves as the only non-American site for the HVTN (HIV Vaccines Trials Network). Several WHO sponsored training courses were held at the NICD as detailed in this report.

All this has been achieved with, by international standards, a very small team. Nevertheless, they proved to be a team of highly dedicated and professional personnel. It has indeed been a privilege to serve as their Director. The NICD now looks forward with confidence to meeting the highly challenging future of monitoring communicable diseases, both regionally in southern Africa and also wider afield in the African continent. The spirit of the early scientific pioneers of 50 years ago should serve as an inspiration to the future endeavours of this Institute.

BARRY D SCHOUB
EXECUTIVE DIRECTOR
EXECUTIVE DIRECTOR
Prof B D Schoub
I Latsky, Personal Assistant

VIROLOGY DIVISION

HIV/AIDS VIRUS RESEARCH UNIT
Prof L Morris, Chief Specialist Scientist

Virology Laboratory
Prof L Morris, Chief Specialist Scientist, Head
S Herrmann, Personal Assistant
S Doig, Personal Assistant
Dr M Papathanasopoulos, Asst Dir, Medical Natural Scientist
P Moore, Postdoctoral Fellow
C Pillay, Medical Natural Scientist
T Taylor, Research Assistant
M Smith, Research Assistant
T Cilliers, Research Assistant
S Cohen, Laboratory Manager
E Gray, Research Assistant
S Loubser, Research Assistant
M Ntsala, Research Assistant
P Mthunzi, Research Assistant
J Brandful, Visiting Fellow
M P Phoswa, Chief Aux Service Officer

Graduate Students: (Supervisor)
J Nhlapo, PhD (L Morris)
I Choge, MSc (L Morris)
M Coetzer, PhD (L Morris)
S Nkosi, MSc (M Papathanasopoulos)
D Schramm, PhD (C Tiemessen)
P Walker, PhD (visitor)

SPECIAL PATHOGENS UNIT
Prof R Swanepoel, Chief Specialist Scientist
L J Dos Santos, P/T Senior Admin Clerk

Class-4 Pathogens Unit
Dr J Paweska, Chief Specialist Scientist
Dr F Burt, Specialist Scientist
Dr M Venter, Principal Medical Natural Scientist
AA Grobbelaar, P/T Principal Medical Natural Scientist
R Phili, Principal Medical Natural Scientist
P Leman, Principal Medical Natural Scientist
J Croft, Chief Medical Technologist
N B Magome, Laboratory Assistant
K L Masenya, Laboratory Assistant
R Mabilo, Laboratory Assistant
A Looyen, Artisan Foreman

Immunology Laboratory
Dr C Gray, Princ Specialist Scientist, Head
K Ihlenfeldt, Personal Assistant
S Nyoka, Chief Medical Technologist
Dr A Masemola, Post Doctoral Fellow
Dr V Morafio, Post Doctoral Fellow
G Khoury, Laboratory Manager
P Sangweni, Research Assistant
H Maila, Research Assistant
P Mohube, Research Assistant
M Rampou, Research Assistant
P Mokgotho, Research Assistant
S Xaba, Research Assistant
K Maphandu, Data Capturer

Arbovirus Unit
A Kemp, Principal Medical Natural Scientist
G Gibson, Chief Medical Technologist
S Serero, Chief Aux Service Officer
J Mahlangu, Chief Aux Service Officer
C M Chauke, Laboratory Assistant
S Modise, Laboratory Assistant
D E Mathibela, Laboratory Assistant
D Z Mnisi, Laboratory Assistant
R Nkoana, Laboratory Assistant

Cell Biology Laboratory
Dr C T Tiemessen, Princ Specialist Scientist, Head
L Short, Personal Assistant
Dr S Shalekoff, Principal Medical Natural Scientist
S Meddows-Taylor, Research Officer
F Anthony, Laboratory Manager
S Lalsab, Project Co-ordinator

HLA Laboratory
Dr A Puren, Deputy Director, Head
Dr M Paximadis, Postdoctoral Fellow
TY Mathebula, Research Student

Graduate Students: (Supervisor)
J Nhlapo, PhD (L Morris)
I Choge, MSc (L Morris)
M Coetzer, PhD (L Morris)
S Nkosi, MSc (M Papathanasopoulos)
D Schramm, PhD (C Tiemessen)
P Walker, PhD (visitor)

SPECIALIZED MOLECULAR DIAGNOSTICS UNIT
Dr A Puren, Deputy Director, Medical Services
Dr S M Bowyer, Principal Medical Natural Scientist
E Cutler, Medical Scientist
N Pradbial-Sing, Principal Medical Natural Scientist
M P Magooa, Medical Natural Scientist
A N Phungwayo, Medical Natural Scientist
E Tiale, Senior Medical Technologist
M J Sibeko, Chief Aux Service Officer
L J Mashiloane, Chief Aux Service Officer
J L Sekgobela, Laboratory Assistant
P A Kgadima, Laboratory Assistant

External Quality Assessment Unit
E Goetsch, Chief Medical Technologist
Personnel

VACCINE PREVENTABLE VIRUS INFECTIONS UNIT
Dr T G Besselaar, Senior Specialist Scientist
S B Smit, Principal Medical Natural Scientist
H N Gumede, Senior Medical Natural Scientist
S Donniger, Medical Scientist
L Botha, Biotechnologist
V Singh, Biotechnologist
M Nyuswa, Biotechnologist
J K Mokoena, Chief Aux Service Officer

Graduate Student: (Supervisor)
L Agenbach, MSc (M Venter)

VIRAL DIAGNOSTIC UNIT

Diagnostic Unit
E M Maselesele, Control Medical Technologist
S Moonsamy, Chief Medical Technologist

Virus Isolation Unit
A Buys, P/T Chief Medical Technologist
M Morgan, Chief Medical Technologist
A Oliver, Chief Medical Technologist
S Sikhosana, Senior Medical Technologist
P Ngobondwane, Senior Medical Technologist
D Lebambo, Laboratory Assistant
A Matseke, Laboratory Assistant
T Mashaba, Laboratory Assistant
L Harris, Laboratory Assistant

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

Serology Unit
S Moodliar, Chief Medical Technologist
B Miller, Chief Medical Technologist
A Mohlala, Chief Medical Technologist
H A Vilakazi, Medical Technologist
S Hloma, Laboratory Assistant

Biological/Support Services Unit

Receiving Laboratory
L M Cranston, Chief Medical Technologist
J C Franz, Chief Medical Technologist

Media
E G Mthethwa, Medical Technical Officer
A N Selepe, Chief Aux Service Officer
S E Boshomane, Principal Aux Service Officer

Admin Office
I M Hattingh, Senior Admin Clerk III
E Lemmer, Senior Admin Clerk III

Animal Section
B Mogodi, Senior Animal House Technician
T Marumo, Animal House Technician
M S Mavhungu, Laboratory Assistant
Z Zulu, Laboratory Assistant
S Sibiya, Laboratory Assistant
S S Maswanganyi, Laboratory Assistant
T E Mavhungu, Laboratory Assistant
L J Bopape, Laboratory Assistant
M P Ramoshaba, Laboratory Assistant

Diagnostic Laboratory Support
P J Masekwameng, Laboratory Assistant
E M Rathaha, Laboratory Assistant
R Ncalo, Laboratory Assistant
G Xaba, Laboratory Assistant
L F Mashangoane, Laboratory Assistant
D M Msibi, Laboratory Assistant
M M Mpyana, Laboratory Assistant

MICROBIOLOGY DIVISION

RESPIRATORY & MENINGEAL PATHOGENS
REFERENCE UNIT

Prof K P Klugman, Hon Consultant & Director
P Hyde, Personal Assistant

Dr S Madhi, Clinical Director/Consultant
Dr A von Gottberg, Senior Pathologist
Dr V Quan, Medical Officer
Dr C Cutland, Research Doctor
Dr K Soma, Medical Officer
Dr J R Heera, Medical Officer
Dr G J Jaches, Medical Officer
M L Kuwanda, Statistician
Dr A Smith, Senior Medical Scientist
Dr M du Plessis, Senior Medical Scientist
Dr P Adrian, Medical Scientist
H Ludewick, Medical Scientist
G Coulson, Medical Scientist
O de Gouveia, Laboratory Manager
A Wasas, Section Supervisor
T Rafundisani, Section Supervisor
F S Mnyameni, Medical Technologist
R M S Mphendele, Medical Technologist
O Hattingh, Medical Technologist
N van Niekerk, Medical Technologist
C Ndou, Laboratory Technologist
Sr J Appolis, Professional Nurse
Sr M Hlobo, Professional Nurse
Personnel

Sr J Mogola, Professional Nurse
Sr D V Makubire, Professional Nurse
M Moliwa, Professional Nurse
R Zabale, Professional Nurse
T D Mtshemla, Professional Nurse
L Ndweni, Professional Nurse
N E Msimango, Professional Nurse
Sr S I Fourie, Study Co-ordinator
G Nzimande, Surveillance Officer
C Miller, Surveillance Officer
A Mphokela, Surveillance Officer
W Nqovu, Surveillance Officer
M Masuku, Surveillance Officer
F Seboya, Surveillance Officer
S Matiou, Surveillance Officer
M Mokwena, Surveillance Officer
D Hlatshwayo, Surveillance Officer
K Mowasha, Surveillance Officer
P Diniso, Nursing Assistant
S Mmolawa, Research Nurse
G Senne, Nursing Assistant
S E Mudau, Research Assistant
F Butler, Data Capturer
C S Mbili, Data Capturer
T E Maringa, Data Capturer
M Hlanzi, Data Capturer

SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE
Prof H J Koornhof, Acting Head
E E M Goliath, Department Secretary
S O Odugwu, Senior Medical Officer
K Lucas, Research Co-ordinator/Trainer
J M Wright, Project Co-ordinator
F M Radebe, Laboratory Manager
P Magooa, Medical Scientist
D V Maseko, Acting Laboratory Supervisor
R R Mogoboya, Senior Medical Scientist
L Tsaaagane, Chief Medical Technologist
J F Oba, Epidemiology/Surveillance Officer
S Khumalo, Research Asst, Student Technician
D Mabaso, Research Asst, Student Technician
R Chonco, Production Assistant
F A Mngomezulu, Data Input Clerk

VECTOR CONTROL REFERENCE UNIT
Prof M Coetzee, Head
M Martheze, Department Secretary
Dr L L Koekemoer, Senior Medical Scientist
Dr B D Brooke, Senior Medical Scientist
M M Weeto, Medical Scientist
H Mafumo, Research Assistant
E Rankoe, Lab Asst, Student Technician
E Motaung, Lab Asst, Student Technician.
Prof R H Hunt, Hon Researcher
J Segerman, Hon Researcher

EPIDEMIOLOGY DIVISION

Epidemiology & Surveillance Unit
Dr B Harris, Community Health Specialist
Sr J M McAnerney, Chief Professional Nurse

Outbreak Response Unit
Dr L Blumberg, Senior Pathologist

QA Unit
E Bowers, Control Medical Technologist

GENERAL ADMINISTRATION DIVISION

J Angelides, Assistant Director

Occupational Health
Sr I Henley, Chief Professional Nurse

ENTERIC DISEASES REFERENCE UNIT
Dr K Keddy, Senior Pathologist
A Sooka, Unit Supervisor
T Kruger, Senior Medical Scientist
S Nadan, Medical Scientist
E Khomane, Student Technician

EQA & MYCOLOGY REFERENCE UNITS
Dr K McCarthy, Pathologist
V Fensham, Senior Medical Technologist
R Mogoboya, Medical Technologist
S Gould, Unit Supervisor
P Burness, Senior Medical Technologist

PARASITOLOGY REFERENCE UNIT
A/Prof J Frean, Principal Pathologist
L Dini, Section Supervisor
R van Deventer, Medical Technologist
J Mathebula, Student Technician

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT
A/Prof J Frean, Principal Pathologist
L Arntzen, Laboratory Manager
Personnel

**Library**
H Saevitzon, Principal Librarian
A Holmes, P/T Senior Admin Clerk

**Publications**
E Millington, Publications Officer

**Information Technology**
K Stead, IT Manager

**Human Resources**
P Lebepe, Admin Officer
J Murray, Senior Admin Clerk III

**Finances**
S Hutcheson, P/T Senior Admin Clerk III
M Jackman, Senior Admin Clerk III
S J Badenhorst, Senior Admin Clerk III
B Makasani, Senior Admin Clerk I
H S C van der Merwe, P/T Snr Admin Clerk II

**Procurement**
M Swartz, Chief Provisioning Admin Clerk
B Lowton, Senior Provisioning Admin Clerk III
B Motlotsi, Senior Provisioning Admin Clerk III
D Mahlangu, Senior Provisioning Admin Clerk I
A Lediga, Senior Provisioning Admin Clerk I
E Molapo, Provisioning Admin Clerk
B Meyers, Admin Clerk II
V M Mathebula, Admin Clerk
G Chaane, General Stores Assistant II

**Laundry**
L Rakau, Laundry Aid II
D Matlala, Laundry Aid II

**Dispatch**
J Maga, Senior Admin Clerk I
S Molokomme, Driver II
J Ngomo, Driver II
J Bogopa, Driver II
P M Nthoke, Groundsman

**Reception/Mailing**
B Dhlamini, Senior Messenger
E Mangena, Messenger
M Mandini, Telecom Operator

**Workshop**
A Looyen, Supervisor Maintenance
N Koegelenberg, Supervisor Maintenance
D Mintoor, Tradesman Aid I
M Ramadiro, Tradesman Aid II
E Mothobeki, Tradesman Aid II
P Mokoena, Tradesman Aid II

**Boiler House**
M Basson, Senior Operator
R Engelbrecht, Senior Operator
E Velem, Tradesman Aid II

**Security**
N Ndaba, Security Guard II
B Komape, Security Guard II
T Mooka, Security Guard II

**Cleaning**
S Mngomezulu, Senior General Foreman
M D Mphela, Cleaner II
M J Moyana, Cleaner II
M E Langrish, Cleaner II
W M Marapjane, Cleaner II
E N Malatsi, Cleaner II
S M Nhlapo, Cleaner II
M J Sape, Cleaner II
N H Selomo, Cleaner II
D Tigedi, Cleaner II
M R Mohlafase, Groundsman I
M P Makgohlo, Groundsman II
E M Mothobeki, Groundsman I
A Chuene, Groundsman II
M Lethabi, Tradesman Aid II
M B Ramatswi, General Stores Assistant II
This section of the NICD comprises the Vector Control Reference Unit and the reference microbiology laboratories and staff currently located at the central campus of the NHLS, namely:

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

Microbiology - Parasitology Reference Unit

CURRENT RESEARCH
Current research projects include:

- Operational assessment of rapid malaria antigen test kits. Analysis of Acanthamoeba spp. transport medium.
- Collaboration with RMPRU on assessment of point mutation-determined cotrimoxazole resistance in Pneumocystis jiroveci (formerly known as P. carinii), the cause of pneumocystis pneumonia (PCP). This is an important opportunistic pathogen in HIV/AIDS patients.
- A Swedish-South Africa government cooperation agreement resulted in funding of further PCP research for the period 2004-2006.
- A survey of stool parasites in children from the Western Cape was performed in collaboration with Stellenbosch University.

TRAINING AND QUALITY ASSURANCE

- Parasitology EQA programme: current participants number 133 for the Stool & Urine Parasites Programme and 138 for the Blood & Tissue Parasites Programme. 4 surveys were sent out during 2003.
- Six microbiology registrars were trained during 2003.
- The Unit was involved in lectures and practicals for the DTM&H course. First year medical students were given practicals in Parasitology for Wits University.

INTERNATIONAL MEETINGS/COURSES ATTENDED:

Leigh Dini visited the Swedish Institute for Infectious Disease Control in Stockholm, for training in laboratory diagnostic parasitology, September 2003.

Microbiology - Special Bacterial Pathogens Reference Unit

CURRENT RESEARCH PROJECTS

- RATZOOMAN is a multicountry, multi-disciplinary study of disease risks linked to rodents at the rural/peri-urban interface, which began in 2003. SBPU is involved in investigation of the ecology of the rodent-borne zoonoses plague, leptospirosis, and toxoplasmosis. Collection sites are in Limpopo Province, Durban, and Port Elizabeth. To date 858 specimens have been collected, from which 976 aliquots have been tested for one or more of the pathogens. No plague-positive sera have been found; 13% and 4% of specimens tested to date were positive for leptospirosis and toxoplasmosis, respectively.
- In vitro antimicrobial susceptibility studies in B. anthracis continued, in collaboration with Prof Keith Klugman, Emory University, Atlanta, USA.
SURVEILLANCE ACTIVITIES

- SBPRU is active in drawing up new national plague control guidelines, in collaboration with the Department of Health.
- Limited anthrax surveillance is continuing in the Kruger Park, and SBPRU will be involved in isolation and identification of *B. anthracis* from this area.
- The unit is a member of the WHO global network of experts and laboratories for anthrax and plague.

TRAINING AND QUALITY ASSURANCE ACTIVITIES

- SBPRU is part of the NHLS group contracted by the WHO for provision of external quality assessment to laboratories in African countries, and participated in a WHO-NHLS joint meeting on EQA in Johannesburg in February.
- SBPRU staff participated in a WHO-sponsored 2-week training course for African bacteriology reference laboratories in Johannesburg in September.

INTERNATIONAL MEETINGS ATTENDED


CONFERENCE PAPERS & POSTERS PRESENTED

Cohen C, Karstaedt A, Thomas J, Govender N, Hlatshwayo D, Frean J, Dini L, Crewe-Brown H,
**Microbiology - Special Bacterial Pathogens Reference Unit**


**Microbiology - Enteric Diseases Reference Unit**

**RESEARCH FUNDING:**
The Enteric Disease Reference Unit is funded by grants from the Centers for Disease Control and Prevention, Atlanta, USA, the MRC, Pretoria, the NHLS/University of the Witwatersrand Grants Committee and the Department of Health, Pretoria.

**TASK FORCE COMMITTEES:**
- South African representative at Enter-net – International surveillance for Enteric Pathogens.
- Co-ordinator of Salm-Surv – WHO initiated surveillance for Salmonella.
- NHLS laboratory advisor to local departments of health and National Department of Health on *Vibrio cholerae*.

**AFFILIATIONS:**
- Co-ordinator (with QC, RMPRU and SBPU) for EQA for AFRO region funded by WHO.

**STUDENTS REGISTERED FOR HIGHER DEGREES:**
- Mrs A Sooka – MSc (submitted)
- Ms T Kruger – MSc

**RESEARCH PROJECTS:**
- National surveillance for isolation of bacterial enteric pathogens.
- Evaluation of *Vibrio cholerae* O1 strains from the current cholera epidemic in KwaZulu-Natal and surrounding provinces.
- Molecular characterization of a multidrug resistant *Salmonella enterica* subspecies *enterica* serotype Isangi causing nosocomial infections in South Africa.
- Enhancement of surveillance for trimethoprim-sulfamethoxazole resistant invasive respiratory and diarrhoeal disease in South Africa

**TRAINING COURSES GIVEN:**
- WHO-AFRO workshop (Anglophone countries) Laboratory identification of Typhoid, Cholera and Shigella 8-12 September 2003
- WHO-AFRO workshop (Francophone countries) Laboratory identification of Typhoid, Cholera and Shigella 15-19 September 2003

**INTERNATIONAL MEETINGS ATTENDED:**

**INTERNATIONAL TRAINING:**
- T. Kruger: Division of Infectious diseases, University of Pittsburgh Medical Center, Pennsylvania, USA. 17 June 2003-1 August 2003.
**ABSTRACTS:**


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The Respiratory and Meningeal Pathogens Research Unit published in the New England Journal of Medicine its study in 39,000 children in Soweto which showed that 9-valent conjugate pneumococcal vaccine protects against 85% of invasive disease due to vaccine serotypes, reduces antibiotic-resistant pneumococcal disease by 58% and reduces episodes of pneumonia by 25%. This vaccine therefore could reduce the burden of severe pneumonia, the leading cause of death in children, by a quarter. We have expanded our national surveillance to include *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* isolated from blood or CSF. This expanded active surveillance includes the provision of a nursing officer to ten hospitals and is supported by CDC/USAID. We have shown that the introduction of *Haemophilus influenzae* type b (Hib) vaccine has been successful in reducing the burden of Hib disease in South Africa. The Unit has continued to unravel the molecular basis of amoxicillin resistance in the pneumococcus – a new threat to the most effective oral agent available. The Unit has published the first cases of fluoroquinolone resistance in the pneumococcus in Africa, including the first global case of fluoroquinolone resistance in a pneumococcus from a child. The Unit has documented the enormous burden of *Pneumocystis* infections (PCP) in HIV-infected children and has completed the first study in Africa of the burden of human metapneumovirus infection in children. We are setting up a new laboratory to study the immunological responses of HIV-infected children to pneumococcal conjugate vaccine.

**PNEUMONIAS IN HIV-INFECTED AND -UNINFECTED CHILDREN**

This study will be ongoing during 2004. Additional laboratory staff have set up *Pneumocystis jiroveci* PCR assays. The Unit embarked during 2003 on a project to determine the role of the newly discovered virus, human metapneumovirus, in pneumonia in HIV-infected and -uninfected patients. The first report on PCR positive specimens of this new virus in Africa has been published in Clinical Infectious Diseases Journal in December 2003. Project leader: Dr S Madhi. Researcher: Mr Herbert Ludewick. Collaborator: Professor Guy Boivin, University of Quebec, Canada.

**LOW DOSE HAEMOPHILUS INFLUENZAE VACCINE**

Having shown that the vaccine is highly immunogenic even in low dose – these data were published
in 2002, the analysis of low doses of two other vaccines completed in 2002, has been expanded to study the effect of booster responses and the data will be submitted in 2004. Funded by Biocine, Berna Biotech. Project leader: Dr M Nicol, Dr R Huebner.

**RISK FACTORS AND CLINICAL COURSE OF PNEUMOCOCCAL INVASIVE DISEASE IN HIV-INFECTED AND -UNINFECTED ADULTS AND CHILDREN**

A definitive study was being conducted in collaboration with Dr Victor Yu (University of Cleveland). The Unit contributed cases of pneumonia, and adults with meningitis in collaboration with Prof C Feldman. The study continued in 2002 to include pneumococcal meningitis in children. The initial study on bacteraemic pneumonia has been published in 2003 in Clinical Infectious Diseases. Further analyses of the HIV-infected population included in the study are underway, as are analyses of combination therapy and the molecular relatedness of the strains. Funded by the MRC/NHLS/Wits. Project leaders: Professor Charles Feldman, Dr Shabir Madhi.

**MOLECULAR RELATEDNESS AND MOLECULAR BASIS OF RESISTANCE IN FLUOROQUINOLONE-RESISTANT PNEUMOCOCCI**

We published in Pediatric Infectious Diseases Journal in 2003, the first global case of fluoroquinolone resistance in a child, discovered as a result of our national surveillance program. Project leader: Dr A von Gottberg. Collaborators Dr Louise Markus, Pretoria, Drs Bamber, Govind, Sturm, University of Natal. Funded by MRC/NHLS/Wits. Project leaders: Professor Charles Feldman, Dr Shabir Madhi.

**IMPACT OF HIV ON HAEMOPHILUS INFLUENZAE TYPE B INVASIVE DISEASE AND A LONGITUDINAL ANALYSIS OF THE IMPACT OF VACCINATION ON HAEMOPHILUS INFLUENZAE TYPE B INVASIVE DISEASE**

A study is in place to determine the long term level of protection afforded by Hib vaccine in HIV-infected children and to investigate the phenomenon of late failures we have observed in HIV-infected children. Funded by MRC/NHLS/Wits. Project leader: Dr S Madhi.

**BURDEN OF HAEMOPHILUS INFLUENZAE TYPE B (Hib) INVASIVE DISEASE IN SOUTH AFRICA**

South Africa implemented a program of Hib immunization in 1999. The Unit has quantified the annual incidence of Hib disease to establish the impact of immunization on the burden of haemophilus disease in South Africa. A collaboration with the EPI program and other academic laboratories in South Africa is expanded into an active program to document vaccine failures. The Unit has secured CDC/USAID support for the project for 2003-5. Reports have been prepared for NICD meetings of all the participants held during 2001, 2002 and 2003. The project has documented a decline in invasive Hib disease across the country. These data will be published in 2004. Project leader: Dr A von Gottberg. Collaborators: Dr A Schuchat, CDC, Atlanta, USA. Funding MRC/NHLS/Wits.

**HIGH LEVEL AMOXYCILLIN RESISTANCE IN PNEUMOCOCCI**

The development of high-level α-lactam resistance is complex and the involvement of MurMN in penicillin resistance appears to be dependent on specific mutations in penicillin-binding proteins (PBPs) 2X, 2B and/or 1A. An additional non-PBP-mediated resistance determinant is required for full resistance development in some pneumococci. Efforts are continuing to clone DNA fragments and conduct transformation experiments using the cloned fragments from the highly resistant strain.

**THE IDENTIFICATION OF AMINO ACID MUTATIONS IN PBP 1A AND PBP 2X THAT CONFER PENICILLIN-CEPHALOSPORIN RESISTANCE IN THE PNEUMOCOCCUS**

Pneumococcal resistance to α-lactam antibiotics is mediated by alterations in PBPs. We have used the technique of site-directed mutagenesis to identify which amino acid mutations in altered PBP 1A, are involved in the development of high-level penicillin and cephalosporin resistance in Hungarian isolate 3191 (penicillin MIC, 16i g/ml; cefotaxime MIC, 4i g/ml) and published the data in 2003. A similar analysis is continuing on altered PBP 2X from the Hungarian isolate. Project leader: Dr Anthony Smith. Funded by the MRC/NHLS/Wits.
THE EFFECT OF ALTERED PENICILLIN-BINDING PROTEINS ON PNEUMOCOCCAL CELL MORPHOLOGY, CELL DIVISION AND CELL GROWTH

Pneumococcal resistance to â-lactam antibiotics is mediated by alterations in PBPs. However, PBPs are essential enzymes which play a major role in cell wall synthesis. This project aims at investigating the biological cost a resistant pneumococcus has to pay for its altered PBPs and development of resistance. Do altered PBPs hamper normal cell wall synthesis and so affect cell morphology, cell division, and cell growth? We are investigating these aspects on laboratory strain R6, following transformation of the strain to resistance with altered PBPs 2X, 2B and 1A. Project leader: Dr Anthony Smith. Collaborator: Dr Orietta Massida, Italy. Funded by the MRC/NHLS/Wits.

EPIDEMIOLOGY & CLINICAL RELEVANCE OF EXTENDED SPECTRUM â-LACTAMASE (ESBL) PRODUCTION AND FLUOROQUINOLONE RESISTANCE IN KLEBSIELLA PNEUMONIAE

The first part of this international study in collaboration with the Unit was published in Clinical Infectious Diseases in 2000. The Nosocomial spread paper has been accepted for publication in the Annals of Internal Medicine, the risk factors for ESBLs was published in Emerging Infectious Diseases in 2002 and the impact of different antibiotic therapies has been submitted while data on virulence determinants is undergoing further experimental work by our collaborators. Project Leader: Dr A von Gottberg. Collaborators: Dr David Patterson, Dr Victor Yu, University of Pittsburgh.

IMMUNOGENICITY AND FUNCTIONALITY OF ANTIBODY INDUCED BY PNEUMOCOCCAL CONJUGATE VACCINE IN HIV-INFECTED INFANTS

We obtained initial funding for this important study from WHO. We used cohorts of children in whom we had data on immunogenicity. HIV status was determined in an unlinked anonymous way. This collection of sera was started in 2001 and completed in 2002. The analysis of the results will be completed in 2004. The Unit has sought and obtained a CIPRA grant to expand this study and a large multi-year project has been designed. An antibody functionality laboratory is being set up and Dr Peter Adrian who has joined the Unit again after a number of years in Europe attended training during 2003 with our collaborator in Finland. Project leaders: Dr Shabir Madhi, Dr Peter Adrian. Collaborator: Dr Helena Kayty, KTL, Finland. Funding: WHO, NIH, USA.

COST EFFECTIVENESS OF PNEUMOCOCCAL CONJUGATE VACCINE IN SOUTH AFRICA

This study was started in 2002 and was written up in 2003 in collaboration with WHO and Dr Gary Ginsberg from the Institute of Public Health in Israel. It has been expanded to include an analysis of the costs of treating pneumonia in the South African setting and will be submitted for publication in 2004. Project leaders Dr S Madhi, Dr G Ginsberg. Funding: WHO.

IMPACT OF COTRIMOXAZOLE PROPHYLAXIS ON RESISTANCE IN RESPIRATORY PATHOGENS

The proposed 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, with CDC and Emory University, expanded our national surveillance network to monitor the impact on resistance and the effectiveness of the intervention on pneumococcal disease. Funding has been secured from USAID via the CDC. A meeting of interested parties was held in 2002 and the appointment of epidemiological nursing assistants at the major hospitals in South Africa has taken place during 2003. Project leader: Dr A von Gottberg. Collaborator: Dr Anne Schuchat, CDC.

STUDY OF THE RELATIONSHIP OF AFRICAN STRAINS OF PNEUMOCOCCI, MENINGOCOCCI AND H. INFLUENZAE USING MUTILOCUS SEQUENCE TypING MLST

The acquisition of an automated sequencer for the Unit from the Welcome Trust (obtained in collaboration with Dr Mizrahi's Unit) allowed us to commence this project focused on pneumococci in 2001. It was ongoing in 2002 and has been expanded in 2003 to include meningococci and haemophilus strains. Mr Coulson will be going to CDC, Atlanta in early 2004 for further training. Project leaders: Dr A von Gottberg, Dr M du Plessis. Researcher: Mr Garry Coulson.
SUSCEPTIBILITY OF \textit{Bacillus anthracis} AND \textit{Yersinia pestis} TO NEW ANTIMICROBIALS

The emergence of a global bioterrorism threat in 2001 using rare respiratory bacterial pathogens led the Unit to collaborate with Dr John Frean and Lorraine Arntzen of the NHLS to investigate the activity of new antimicrobials against these pathogens. We discovered the activity of a new class of agent – the ketolides, against \textit{Bacillus anthracis}. These data and the impact of fluoroquinolones on plague were published in Antimicrobial Agents and Chemotherapy in 2003. The activity of a new fluoroquinolone, moxifloxacin against anthrax will be investigated in 2003/4.

Project leader: Dr John Frean. Collaborators Dr S Bukofzer, Abbott Laboratories, Dr Axel Dalhof, Bayer Laboratories. Sponsors: Abbott Laboratories, Illinois, USA, Bayer Laboratories, Germany.

RISK FACTORS FOR CARRIAGE OF PNEUMOCOCCI IN HIV INFECTED GOLD MINERS

This study is being conducted in collaboration with the Ernest Oppenheimer Hospital in Welkom. First results will be presented in 2004. Collaborator: Dr Gavin Churchyard. Project leader: Dr A von Gottberg.

PREVENTION OF VERTICAL TRANSMISSION OF GROUP B STREPTOCOCCI AND NEONATAL SEPSIS BY CHLORHEXIDINE SWABBING

This large intervention trial has received CDC and USAID funding and will commence in early 2004 at Chris Hani Baragwanath Hospital. A pilot trial has been completed. Project leaders: Dr Clare Cutland, Dr Shabir Madhi. Collaborator: Dr Stephanie Schrag, CDC, Atlanta, GA.

SPREAD OF PEDIATRIC PNEUMOCOCCAL SEROTYPES FROM CHILDREN TO ADULTS: THE ROLE OF GENDER IN ANTIBIOTIC RESISTANCE

An analysis of 1022 patients with pneumococcal bacteremia in Johannesburg was conducted by the Unit in 2003 to assess the role of gender in the transmission of antibiotic resistant pneumococci. This study has been accepted for publication in the Journal of Infectious Diseases. It demonstrates a preponderance of pediatric pneumococcal serotypes in HIV infected persons and also identifies for the first time a preponderance of pediatric serotypes in women compared to men.

Project leaders: Kim Buie, Rollins School of Public Health, Atlanta, Dr A von Gottberg. Collaborators: Drs Olga Perovic, Dr H Crewe Brown, Dept. Clinical Microbiology, Wits, Dr Alan Karstaedt, Prof C Feldman, Dept of Medicine, Wits.

ROLE OF PEDIATRIC SEROTYPES IN THE AETIOLOGY OF ADULT PNEUMONIA IN SOWETO

A protocol has been submitted to NIH for funding in collaboration with the CFAR Unit at Emory University to investigate the role of pediatric serotypes of pneumococci in the aetiology of adult pneumonia in Soweto. If funded this will be a 3 year project that will be further developed into an intervention trial to interrupt transmission of pneumococci from children to adults in order to decrease the burden of pneumonia and antibiotic resistance in both children and adults in Soweto.

ROLE OF THE PNEUMOCOCCUS IN VIRAL INFECTIONS

Using the vaccine as a probe the relationship of bacterial superinfection to the pathogenesis of viral pneumonia will be sought in additional analyses of the 9-valent conjugate vaccine trial during 2004.

Project leader: Dr Shabir Madhi.

USEFULNESS OF PROCALCITONIN MEASUREMENTS TO DEFINE BACTERIAL PNEUMONIA IN CHILDREN

Sera are available from a subset of children involved in the vaccine trial - procalcitonin measurements have been performed and will be analysed and presented in 2004.

Project leader: Dr Shabir Madhi. Sponsor: WHO.
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 ~12,000 cases were reported in 2003. 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 are the only ones in existence in the world. This places the VCRU in a unique position to carry out valuable research on insecticide resistance 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*

Pyrethroid insecticide resistance in the major malaria vector mosquito *An. gambiae* is widespread in West Africa and also occurs in Kenya and Zambia. It was reported for the first time in *An. funestus* in southern Africa in 2000 through work carried out in the VCRU. The pyrethroid group of insecticides is currently recommended for disease control in many malarious countries, both for indoor residual house spraying and for treatment of bed nets. In 1996, the South African malaria control programmes changed from the use of DDT to pyrethroids for their house spraying campaigns. Field and laboratory work carried out in northern KwaZulu/Natal and Mpumalanga in 1999/2000 showed that *An. funestus* had returned after an absence of nearly 50 years and was resistant to pyrethroids. The impact of this was a major increase in transmission culminating in over 60,000 cases in 2000. As a result, DDT was reintroduced for spraying traditional houses while pyrethroids were retained for western-style houses, effectively producing a “mosaic” resistance management strategy that has brought transmission back under control.

Research into pyrethroid resistance in *An. funestus* is ongoing in the VCRU. Compelling and unusual evidence has been found that there is either linkage or cross-resistance between the gene (or genes) responsible for the pyrethroid resistance and resistance to the completely unrelated carbamate insecticide, propoxur. Currently, the resistant strain of *An. funestus* from Mozambique selected on 1.5% permethrin (double the WHO discriminating dosage for diagnosing resistance) shows extremely high levels of survival, suggesting that the resistance genes have almost reached fixation. This colony, together with the susceptible strain from Angola, are being used to investigate the P450 monooxygenases known to be involved in the resistance mechanism and to characterize the kdr domain II between segments 4-6. Research scientists and students in the VCRU have received training at the Liverpool School of Tropical Medicine, UK, in cloning cDNA, screening libraries and northern blots. Samples of *An. funestus* have been sent to Notre Dame University, USA, for production of a BAC library prior to full genome sequencing, providing us with the opportunity of utilizing the newly published *An. gambiae* genome sequence to further our knowledge on resistance in *An. funestus*.

*Anopheles arabiensis*

Investigations carried out in collaboration with the KwaZulu/Natal malaria control programme entomologist revealed the presence of DDT resistance in *Anopheles arabiensis* near the town of Jozini. A rice-growing scheme in the area has resulted in temporary breeding places being available throughout the year because of the draining of paddy fields onto the Makhatini Flats. Large populations of *An. arabiensis* were found and, in particular, collected in window exit traps in houses sprayed with DDT. WHO insecticide susceptibility tests showed 20% resistance to DDT, but 100% susceptibility to the pyrethroid which is sprayed in “western”-style houses. This is diametrically opposite to *An. funestus* which is resistant to pyrethroids but susceptible to DDT. It poses a problem to the control programme.
because *An. arabiensis*, unlike *An. funestus*, does not preferentially prefer resting inside houses and will as frequently seek shelter outside human structures. Research into the resistance mechanisms has resulted in the establishment of a partially DDT-resistant colony that shows very high levels of resistance to carbamate insecticides. Altered acetylcholinesterases have been detected. This research is ongoing.

One of the unit’s doctoral students, while conducting his field studies in Zimbabwe, detected DDT resistance in *An. arabiensis* from the Gokwe District in the north-central part of the country. Up to 25% survival on 4% DDT papers was recorded in samples collected from larval catches and inside houses in April 2002. All specimens tested on 0.25% deltamethrin were fully susceptible.

**Anopheles gambiae**

Studies on insecticide susceptibility in south-west Nigeria showed the presence of pyrethroid and DDT resistance in *An. gambiae s.s.* (76 and 81.5% mortality respectively). Surviving samples were assayed for ‘S’ and ‘M’ molecular forms using PCR and both forms were identified. The knock-down resistance (*kdr*) mutation involved in pyrethroid and DDT resistance was found in ~80% of the surviving molecular ‘S’ form. None of the surviving ‘M’ form had the mutation, suggesting that another resistance mechanism is present.

**MOLECULAR AND CYTOGENETIC STUDIES**

*Anopheles funestus* belongs to a group of nine species that are morphologically very similar. Historically, the identification of the seven southern African species depended on morphological characters found in the immature stages of three, and overlapping adult characteristics for the other four species. Since only *An. funestus* is recognised as a major vector of malaria parasites, identification of these species is essential for malaria control programmes. The multiplex ITS-2 DNA-PCR assay developed by this unit for distinguishing between five members of the *An. funestus* group: *An. funestus*, *An. vaneedeni*, *An. parensis*, *An. rivulorum* and *An. leesonii* has been evaluated on specimens from 11 African countries and found to be 98% reliable.

Collaborative cytogenetic studies of *An. funestus* in Kenya have revealed geographic differentiation between populations from West and Coastal Kenya based on chromosomal inversion polymorphisms. The data suggest that the populations are discontinuous, separated by the Rift Valley system. Large numbers of the non-vector species *An. parensis* were found resting inside houses in a village in western Kenya, underscoring the importance of correct species identification in control programmes.

Comparative studies of the *An. funestus* group using RFLP analysis, carried out in collaboration with French colleagues at IRD, Montpellier, showed distinct differences between the five most common members of the group, but, more interestingly, clear differences between samples of *An. funestus* from East, West and southern Africa. The taxonomic status of these populations are being further investigated.

The single-strand conformation polymorphism (SSCP) assay developed in the VCRU for members of the *An. funestus* group, was tested on Asian relatives in the *Anopheles minimus* group. Distinct differences were noted between *An. minimus* species A and C, *An. aconitus*, *An. pampanai* and *An. varuna*.

**DISTRIBUTION AND BIONOMICS**

Species composition of indoor and outdoor human-biting vector mosquitoes and their relative contribution to *P. falciparum* transmission were determined for two communities in Ibadan, south-western Nigeria. The three main vector species, *An. gambiae*, *An. arabiensis* and *An. funestus* were found with each playing a more or less equal role in malaria parasite transmission. Both ‘M’ and ‘S’ molecular forms of *An. gambiae* were also present in the Zambezi Valley of Zimbabwe. These molecular forms are thought to exhibit some correlation to West African chromosomal forms of *An. gambiae*, so it is surprising to find both molecular forms in the Zambezi Valley where only one chromosomal form has been found in the past.

INTERNATIONAL RESEARCH COLLABORATORS

Prof J Hemingway, Director, Liverpool School of
RESEARCH FUNDING FROM EXTERNAL GRANTING AGENCIES

Wellcome Trust
World Health Organization
PAL+, French Ministry of Research
SA Medical Research Council
SA National Research Foundation

TRAINING

Postgraduate Training

VCRU staff provide the medical entomology component of the Diploma in Tropical Medicine & Hygiene course run by the School of Pathology, University of the Witwatersrand. Lectures and practical demonstrations are 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.

Short Course in Basic Mosquito Identification and Insectary Management

This course is run on an ad hoc basis at the request of Malaria Control Programme managers, nationally and internationally, to provide technical training to staff involved in vector control. The students are given two weeks training in practical morphological identification of anophelines mosquitoes and hands-on experience of rearing mosquitoes in the laboratory. In addition to the South Africans, we have trained students from Botswana, Namibia and Zimbabwe, supported by funding from the World Health Organization.

Training Course in Malaria Vector Control with special emphasis on Insecticide Resistance Management

This 5-week WHO training course was held for the third time in 2003. Ten students came from the Gambia, Ghana, Kenya, Malawi and Zanzibar. One week was spent in the field in Limpopo Province, practical training was given in mosquito identification and insecticide susceptibility testing. Lectures and demonstrations were given in malaria vector control methods, insecticide resistance mechanisms, resistance management, molecular methods for species identification, ELISA tests for blood meal identification and parasite detection. Students produced project proposals for field research to be carried out in their home countries on completion of the course.
Microbiology - Vector Control Reference Unit

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 Malaria Control Programmes of Mpumalanga and Limpopo Provinces. ELISA and PCR tests were carried out on the An. gambiae complex specimens from Zimbabwe, Malawi, Mali, Namibia, Botswana, 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 the participation on the National Malaria Advisory Group.

Microbiology - External Quality Assessment Reference Unit

The past year (2003-2004) has seen the formal creation of this unit as a part of the NICD with the appointment of two staff members (Dr Kerrigan McCarthy, consultant microbiologist responsible for the unit, and Ms Rebecca Mogoboya, medical technologist) to complement Ms Vivian Fensham’s sole membership of the Unit.

The newly created NICD Microbiology EQA Unit produces and/or co-ordinates all the national microbiology quality assessment schemes for the NHLS, as well as a bacteriology EQA scheme for all countries of the African Regional Organisation (AFRO) of the World Health Organisation (WHO). In the national scheme, there are a total of 10 programmes that span the following disciplines: bacteriology, mycology, parasitology, HIV and syphilis serology and mycobacteriology.

The NHLS/WHO EQA programme at present covers epidemic prone diseases, such as meningitis, enteric and plague. These programmes are all produced in consultation with NICD consultants in the various disciplines. Presently 59 laboratories from 46 African countries and over 200 national laboratories (NHLS, private, Kwa-Zulu Natal and Mines) participate in NHLS EQA programmes. The EQA Unit also hosted training workshops for WHO-AFRO bacteriology laboratorians in September 2003. The EQA Unit is responsible for the National Stock Culture Collection (NSCC).

Fig 1. Training WHO-AFRO delegates in antimicrobial susceptibility testing procedures, September 2003
The Sexually Transmitted Infections Reference Centre (STIRC) remained committed to its goals centred round surveillance of sexually transmitted diseases (STDs), conducting research which addresses STD-related problems in developing countries and providing expertise in the clinical and laboratory diagnosis of STDs and their management. It continued to assist with the training of health care workers in STDs and took part in undergraduate and postgraduate teaching. At the laboratory level it is a priority of STIRC to evaluate new and advanced technologies and, when appropriate, to employ them for diagnostic and research-related activities. STIRC also serves as a referral centre for specialized testing and patients’ management and as a depository and source of laboratory-related reference material, including cultures and sera, as well as training materials in printed and electronic form.

NATIONAL STI SURVEILLANCE PROGRAMME FOR SOUTH AFRICA

Clinical Surveillance

Reliable surveillance information is crucial for effective planning, implementation and monitoring of communicable diseases control programmes. Clinical surveillance of STIs in 21 sentinel clinics in the Gauteng Province continued throughout the year as an important function of STIRC. The establishment of a national programme has long been promoted by STIRC and in 2003 funding for such an expanded programme was provided by the Centers for Disease Control and Prevention (CDC), Atlanta, USA as part of a NICD-CDC cooperative agreement. Following extensive consultations with the STI/HIV Prevention Unit of the National Department of Health and other stakeholders, a national STI surveillance programme for South Africa was designed. The original proposal from the STIRC, inputs from a technical advisory group of experts in STIs and epidemiology as well as our experience of clinical STI surveillance in Gauteng formed the basis of the programme. As a result of these consultations, it was agreed that the clinical surveillance programme be rolled out simultaneously in all 9 provinces in the country as opposed to our earlier plan to phase in implementation from province to province. A total of 270 sentinel sites, 30 from each province, were selected by a simple random process using a comprehensive list of primary health care facilities and level one hospitals as sampling frame.

The District Health Information System (DHIS) was adopted as the platform for collecting surveillance data for STIs. To this end, the Health Information Systems Project (HISP) of the University of the Western Cape has developed an STI surveillance module on the DHIS platform to be used for the national programme. The module was based on the original Gauteng STI surveillance tally sheet which was first introduced in 1998 by the Reference Centre for STDs of the then South African Institute for Medical Research.

The successful implementation of the programme requires full co-operation and essential participation of the provinces. Provincial and district health information officers (HIOs) have been identified as key players at the operational level, hence a district health information officers’ workshop (DHIOW) was held in each province. Provincial STI programme co-ordinators and other programme staff as well as Provincial STI facilitators were also in attendance as they would be responsible for training at all 30 sentinel sites in their respective provinces. The workshops were very rewarding as several issues raised at these meetings resulted in modifications to the tally and monthly summary sheets. Data flow channels, time lines and stationary supplies were also addressed.

A comprehensive monitoring and evaluation system for the programme is being developed. An essential part of this component which is taking place alongside sentinel sites training, is a baseline assessment of the quality of care. In addition to the first site training in each province, NICD personnel will continue the training process in 3 additional sites per province in order to monitor the quality of sites training.

Apart from providing training materials for the DHIOW and sites training, surveillance stationeries have been provided for all 270 sites. This assistance from STIRC was required as such provisions had not been budgeted for in 2003 and most sites expressed the need for assistance during the initial period. By the end of 2003, all provincial DHIOWs had been undertaken and at least one site trained per province.
Microbiological surveillance

For comprehensive information on the prevalence of STDs in South Africa, the aetiology of clinical syndromes as recorded in the clinical surveillance programme needs to be established. This task was undertaken by STIRC together with microbiology departments of the University of Natal, the Medical University of South Africa, Tygerberg campus University of Stellenbosch, University of the Free State, and University of the Transkei, Umtata.

As from January 2004, these six centres will determine the main aetiological agents of the three most common STD syndromes in all nine provinces. Nucleic acid amplification techniques which are not affected by delays in transport of specimens to central laboratories will be employed, using standardised methods. An external quality assessment programme to monitor proficiency will also be instituted. The laboratory based programme will cover the aetiology of 200 consecutive cases of vaginal discharge, male urethritis syndrome and genital ulcer disease respectively, as well as the antimicrobial susceptibility of 200 gonococcal isolates from each province.

COMMUNITY-BASED INTERVENTION STUDIES

Expanded periodic presumptive treatment project

Provision of effective STI preventive and curative services for women at high risk (WAHR) of infection can be an effective strategy for rapidly reducing STD prevalence in the targeted women as well as in populations served by them, and in the process reducing the efficacy of HIV transmission. The initiation of the Lesedi/Mothusimpilo projects in 1996 under AIDSCAP has demonstrated that such services including monthly presumptive treatment of WAHR with azithromycin can reduce the prevalence of STIs of bacterial origin dramatically in miners.

To build upon the successful experience of FHI/AIDSCAP and STIRC in the Lesedi/Mothusimpilo mining areas, the periodic preventive treatment (PPT) programme was extended to additional mining communities. The expanded programme incorporates new areas in the Free State and Gauteng province. Four extra mobile clinics were purchased, staffed and equipped. These mobile clinics deliver services to women at high risk five days per week, using an approach similar to that used at Lesedi and Mothusimpilo. STIRC provided the overall coordination and management of the projects. The ongoing clinical monitoring of the expanded PPT programme has been extended to include microbiological surveillance in 2004 and will involve 200 women at high risk attending the four new mobile clinics operating in four different mining regions.

Efficacy of male and female condoms in preventing STDs

In preparation for a WHO initiated study on the efficacy of female and male condoms for protection against acquisition of STIs, STIRC, in collaboration with the Reproduction Health Research Unit, is carrying out a study to determine the diagnostic accuracy of PCR when self-collected vaginal swabs stored for 4 days at room temperature are compared with clinician-collected endocervical swabs for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) and high vaginal swabs for *Trichomonas vaginalis* (TV). Female STI clinic attenders with clinical features of infection with one of the above-mentioned three agents are being recruited.

Assuming a sensitivity and specificity of 90%, 150 infected and a similar number of uninfected women are needed to evaluate the method adequately at a 95% confidence level. An assessment of performance is being done for each of the three pathogens independently. To date, 495 women have been recruited into the study. In this population the prevalence rates of GC, CT and TV based on PCR testing of clinician-collected swabs are 22.4%, 20.8% and 42.15% respectively. Eight percent of the women had co-infection with CT and GC while 35.2% were infected with either of the two pathogens. Self-collected swabs performed excellently and gave specificities for GC, CT and TV based on PCR testing of clinician-collected swabs are 22.4%, 20.8% and 42.15% respectively. Eight percent of the women had co-infection with CT and GC while 35.2% were infected with either of the two pathogens. Self-collected swabs performed excellently and gave specificities for GC, CT and TV of 96.1% (95% CI: 93.1%, 98.0%), 97.6% (95% CI: 95.0%, 99.0%) and 99.3% (95% CI: 95.7%, 99.7%) respectively. The corresponding sensitivities are 90.0% (95% CI: 83.92%, 92%, 91.55%), 90.3% (95% CI: 77.61%, 89.19%). The figures for GC and CT are not final since adequate numbers of infected women have not been recruited.
**Herpes intervention studies in miners**

Two major herpes intervention studies in miners are being planned:

An episodic treatment study sponsored by the CDC, Atlanta, will comprise 600 participants and is due to start in May 2004. In the second study sponsored by USAID, suppressive treatment with acyclovir for the prevention of genital herpes will be evaluated in 3300 miners with serological evidence of herpes simplex virus infection.

Four research co-ordinators have been appointed in 2003 and a pilot study lasting three months and designed to measure adherence to treatment, recruitment of patients and recording of herpetic lesions will start early in 2004.

**POINT-OF-CARE TESTING FOR STDs**

Problems with access to existing diagnostic tests for STDs such as long turn-around times, specimen transport and high skill requirements, as well as cost constraints, continue to make aetiological diagnosis for clinical treatment of infections impractical in resource-poor settings. In order to improve cure rates for STDs in these settings, WHO advocates the routine use of the syndromic approach to management. An efficient rapid test for the diagnosis of an STD will allow point-of-care aetiological diagnosis and reduce the frequency and cost of over-treatment. STIRC was approached by PATH (Program for Appropriate Technology in Health) an organization specifically geared to develop such tests to evaluate a rapid test of the diagnosis of gonorrhoea.

**Rapid immunochromatographic strip test for N. gonorrhoeae**

An investigation was embarked upon to determine the clinical usefulness of immunochromatographic test strips (PATH GCICS) for the diagnosis of gonorrhoeal infections in a high prevalence region. The design was that of a cross-sectional analytic study utilizing clinical specimens taken from a population of female and male adults attending two clinics in metropolitan Johannesburg.

PATH GCICS and GC LCR results were available on vaginal swabs from 536 patients. Based on LCR findings, the ICS test had a sensitivity of 61.4% (70/114), a specificity of 95.7% (404/422), a positive predictive value of 79.5% (70/88); and a negative predictive value of 90.2% (404/448). Cervical swab LCR for gonorrhoea and GCICS tests were available for 583 female recruits. Using LCR as criterion for the presence of gonorrhoea, the ICS test on endocervical swabs had a sensitivity of 47.5%, a specificity of 96.3%, a positive predictive value of 77.0% and a negative predictive value of 87.62%. First-catch urine samples from 414 males were tested for gonorrhoea with both LCR and the PATH ICS method. Of the 414 specimens, 389 yielded concordant results with the two methods. The overall sensitivity and specificity of the PATH ICS using male urine and based on LCR findings were 92.2% and 96.7% respectively.

The PATH GCICS test satisfies several of the desired characteristics of a rapid diagnostic test and would be an excellent tool for the diagnosis of *Neisseria gonorrhoeae* infection among males. Using first-void male urine specimens, the PATH GCICS test demonstrated very high sensitivity and specificity. Even though the test is less efficient among females, it represents valuable advance in the rapid diagnosis of gonorrhoea infection. At a sensitivity of 47.5%, the PATH GCICS test failed to detect gonorrhoeal infections in more than half of individuals who truly had the infection as defined by a positive LCR using randomised endocervical swabs. The use of vaginal swabs for the rapid test resulted in the detection of more cases of true infections (sensitivity, 61.4%) than with endocervical swabs. The high specificity of 96.3% - 95.7% reported indicates that the false positivity rate was very low, hence a confirmatory test on a positive sample is hardly likely to offer any significant advantage in practice.

**EVALUATION OF A B-LYMPHOCYTE LYSATE (PlasmAcute) TEST**

In an ongoing study, the PlasmAcute test conceptualised and designed by Professor Lars Haahrheim of the University of Bergen, Norway, was evaluated in HIV-seronegative miners. This test is based on the concentration of antibodies following their release from B-lymphocytes extracted from blood with magnetic beads. Conventional serological tests (Western blot and two ELISA tests) were performed on B-cell lysates and on participants’ blood.
Miners were asked to report back for follow-up testing to detect possible seroconversion. Testing for viral load, p24 antigen and CD4 counts were also performed.

A total of 114 sero-negative volunteers were recruited between July 2002 and December 2003 and of these 82 provided initial and follow-up blood samples. In two cases the B-cell derived antibodies tested positive for HIV when the p24 antigen and viral load markers were negative. These antibodies were not detected at the second visit when the patients had seroconverted, showing that lysate positivity lasts for a limited period only. These findings confirm that PlasmAcute may have value in the early detection of HIV seroconversion, thus shortening the "window period" in the serological diagnosis of HIV infection.

In 5 other participants B-cell lysates were HIV sero-positive at the first visit and were negative on follow-up. There were no other markers of HIV infection at either visit, suggesting that PlasmAcute may demonstrate resolution of HIV infection in individuals who are refractory to the HI virus.

Anomalies inherent to conventional serological testing were encountered on testing of both lysate and blood samples.

TEACHING AND TRAINING

Ms Vanessa Maseko visited Ghana in May 2003 to assist with the training of a research technologist at the Komfo Anofye Academic Hospital of the Nkruma University of Science and Technology in Kumasi. During the visit which was initiated by Professor Lars Haaheim of the University of Bergen, Norway, the procedure for the purification of B lymphocytes isolated from cord blood of babies born to HIV sero-positive mothers was reviewed in an attempt to identify problems encountered with the PlasmAcute test in Kumasi. The visit was successful and the technologist assigned to the project was trained to perform the test efficiently, producing validated results.

With regard to routine activities, members of STIRC have been involved in the teaching of medical, dental, pharmacy and nursing students, as well as in postgraduate teaching. The latter includes lectures given to DTM&H students and participation in seminars. STIRC's extensive involvement in the training of provincial staff in STI surveillance has been outlined elsewhere in this report.

Another long-standing activity of STIRC is its involvement in STI education to the private sector by means of expert advice and invitational lectures to industrial firms. This tradition was continued during 2003.
Once again our serology section participated in the annual HIV/syphilis antenatal survey for the National Department Health testing 3127 samples from the Gauteng Province. The Control Medical Technologist, Mr Ezekiel Maselesele was tasked with the national co-ordination of the survey with regard to laboratory and transportation aspects of the program in the 9 provinces. The serology section will also be responsible for performing the assays to determine the incidence rate in the population. Preparation for this new task included sending the Unit Supervisor to the Centers for Disease Control in Atlanta (USA) for training.

Our measles/rubella serology has been accorded Regional Reference Laboratory status by the WHO/AFRO and already we are receiving samples from other African national laboratories for confirmation of their results as part of quality assurance.

HIV rapid/simple kit evaluations are being processed with very useful information for the National Department of Health on the operational characteristics when deciding which tests kits to use within the country. Two nurses were trained and competency certificates were issued in the usage of these rapid/simple tests.

Our Regional Reference Polio laboratory 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 lasts for three weeks with competency certificates issued at the end. Last year 2 technicians from Kenya and Cameroon were trained. This service together with distribution of both RD and L20 B cells to various countries in Africa has improved the quality of cell lines being used and subsequent isolation rates.

We are saddened by the untimely death of our dedicated colleague (Sylvia Sikhosana) who contributed a lot to AFP surveillance in this section.

The Viral Diagnostic/Surveillance Unit is a registered training laboratory and has contributed to the training of 7 microbiology registrars, 2 medical technologists to learn CMV pp65, honours students from Rhodes University, 3 biomedical students for experimental training.

In-house training and CPD programs for medical technologists are in place together with internship for fourth year biomedical students who are preparing for the Board examination offered by the Society of Medical Laboratory Technologists of South Africa (SMLTSA).

A medical technicians course in virology is envisaged and will be put together and registered with both the SMLTSA and the Health Professionals Council of South Africa (HPCSA) in the near future.

SANAS accreditation was renewed.
AFP SURVEILLANCE - LABORATORY SUPPORT

AFP surveillance is a critical component of the programme to eradicate polio from the world. In keeping with the WHO worldwide campaign to eradicate poliomyelitis, acute flaccid paralysis (AFP) was made a notifiable condition in South Africa in April 1994. The Department of Health case definition of AFP cases to be notified to the Regional Office of National Health is as follows:- 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 2003, 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 1163 stool specimens were received from patients with AFP of which 623 were from patients outside South Africa, and 540 from South African cases. Fifty-nine of the specimens received were from patients with onset of paralysis prior to 2003. (Fig 1)

1. South African Cases

Case detection rate (only patients from whom specimens were received included) ranged from 1.03 to 2.76 (mean 1.62). Of the 255 South African cases with onset of paralysis in 2003, one specimen only was received from 30 cases, and two or more specimens from 225. The date of onset of paralysis was known for 232 cases. Two specimens taken at least 24 hours apart and within 14 days of onset were received from 200 (86.21%) of these cases (range per province 75.00% to 91.30%). (Fig 2) Non-polio enteroviruses were isolated from 56 of the 540 specimens (non-polio isolation rate 10.37%), adenoviruses from 15, and poliovirus, identified as Sabin type poliovirus from nine specimens of five patients, two of whom had been given OPV after the onset of paralysis and prior to specimen collection.

2: Other Southern African Countries

Case detection rate for the other six Southern African countries ranged from 1.30 to 2.60, with adequate stool specimens received from 63.64% to 100% of cases. (Fig 3) Non-polio enteroviruses were isolated from 126/529 (23.82%). The non-polio enterovirus isolation rate per country ranged from 0 to 25.20%. Other isolates made were 10 adenovirus, six Sabin type 1, six Sabin type 2, and nine Sabin type 3.

MEASLES AND RUBELLA SURVEILLANCE

As part of the EPI measles elimination programme, case-based measles surveillance was started in 1998. Because of the demonstrated lack of specificity of a clinical diagnosis of measles, based on the WHO clinical surveillance criteria (rash + pyrexia + one of the three C’s - coryza, cough or conjunctivitis), laboratory confirmation by ELISA IgM serology is a crucial component of the measles elimination programme. During 2003 almost five times as many specimens for measles were received as in 2002 (3957 compared to 806). Twenty-three of these were from other Southern African countries. Of the 3934 specimens from South Africa 209 were positive for measles, and 1877 for rubella. Thirty specimens were positive for both rubella and measles, 220 were equivocal for either measles or rubella, and 1653 were negative for both measles and rubella. (Fig 4) Two
Fig 2. South African AFP cases, case detection rate and percentage with adequate stool specimens per province.

Fig 3. AFP cases from other southern African countries, case detection rate and percentage with adequate stool specimens.
outbreaks of measles occurred in Gauteng and Mpumalanga respectively, with only sporadic cases in the other provinces. In Gauteng, after an isolated case in the second half of March, there was one positive case, a six month old infant, the first week in July, followed four weeks later by another positive case, a four year old boy.

From the middle of August onward positive measles cases were detected every week, totalling 156. Patients’ ages ranged from two months to 47 years (median three years) with 13.73% being nine months of age or less. In Mpumalanga measles IgM antibodies were detected in the blood specimen of a 10 month old in the first week of June. However, from the second week of August until the end of November positive patients were detected on a weekly basis, as well as three sporadic cases in December. The total number of cases for Mpumalanga was 42. Here patients’ ages ranged from four months to 30 years (median 11 years) with 15.9% being aged 18 years or older. (Figs 5 & 6)

IgM antibodies to rubella were detected throughout the year, with a marked increase in positive results from September, peaking in October. (Fig 7). Patient’s ages ranged from four months to 58 years (median seven years).

RESPIRATORY VIRUS SURVEILLANCE

Fewer specimens were received for respiratory virus isolation during 2003 i.e. 284 compared to 4400 in 2002. Just over half, 148/284 (52.11%) of the respiratory specimens received were from active surveillance programmes. One hundred-and-twenty-eight specimens were received from the Viral Watch programme, started in 1984, which was specifically designed to monitor influenza activity in the community, and detect the type of prevailing influenza strains. In 2003 the centres numbered 14, but this has ranged from 12 to 20. Centres include general medical practitioners, paediatric out-patient departments at hospitals, schools, university students as well as staff at NICD. Up to five throat swabs per week can be taken by each centre 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 NIV in viral transport medium for isolation of virus.
Fig 6. Age distribution of patients with positive measles results

Fig 7. Seasonal distribution of positive rubella IgM results: 2003
Influenza activity in 2003 was higher than in the past few years with an outbreak in a police residential college from the end of May to early June, and an outbreak amongst university students from the end of May to late June. The first influenza isolate of the season was made from a specimen collected during week 20 (mid May) and the last from a specimen collected during week 31 (end July). The ages of patients from whose specimens influenza was isolated ranged from one to 72 years (median 25). The school absenteeism programme, involving approximately 8000 children, at primary and high schools, showed a peak in absenteeism rising above the upper limit expected, from the week starting 9 June peaking two weeks later. (Fig 8)

A total of 148 respiratory virus isolates were made i.e. 73 influenza virus, 49 respiratory syncytial virus, 11 cytomegalovirus, 7 parainfluenza virus, and 6 adenovirus. The Viral Watch accounted for 51 (69.86%) of the influenza isolates. Twenty of the influenza isolates were from the police college outbreak, and only one influenza isolate was made from routine specimens submitted for respiratory virus isolation. The majority (68/73) of the influenza isolates were identified as influenza A, of which 61 were further identified as influenza A H3N2, and two as influenza A H1N2. There were five influenza B isolates.

**PUBLICATION**

The South African Virus Laboratories Surveillance Bulletin which was compiled, collated and edited at the NICD, was produced monthly from January to November 2003. The bulletin incorporated the positive laboratory results, clinical data and editorial comments from the NICD (University of the Witwatersrand) and the viral laboratories of the medical schools of the Universities of Cape Town, Free State, KwaZulu/Natal, Medunsa, Pretoria, and Stellenbosch. The circulation list included all medical virology departments, public health officials at State and local government levels, doctors providing material for routine tests and surveillance and many other interested parties in South Africa. It was also mailed to a number of institutions, international bodies, research organisations and universities abroad. This bulletin, which has been produced since 1984, has now been discontinued and will be replaced by a bi-monthly Communicable Diseases Surveillance bulletin, the inaugural issue of which was published in November 2003.

![Fig 8. Influenza isolates and school absenteeism](image-url)
HIV-1 DIAGNOSTICS

INSERM-NICD COLLABORATION

In the previous year we presented data related to a study performed in the Orange Farm area. The final analyses of that study with special reference to the proportion of HIV-1-infected individuals that require HAART. (in press, JAIDS)

Background

Calls have been made for the large-scale delivery of highly active antiretroviral therapy (HAART) to people infected with HIV in developing countries. If this is to be done, estimates of the number of people who currently require HAART in high HIV-prevalence areas of sub-Saharan Africa are needed and the impact of the widespread use of HAART on transmission and hence the spread of HIV must be assessed.

Objectives

To estimate the proportion of people who would be eligible for combination antiretroviral (ARV) therapy and hence to evaluate the potential impact of providing HAART on the spread of HIV-1 under WHO guidelines, in a South African township with a high prevalence of HIV-1.

Design

A community-based, cross-sectional study in a township near Johannesburg, South Africa of a random sample of approximately 1,000 men and women aged 15 to 49 years.

Materials and methods

Background characteristics and sexual behaviour were recorded by questionnaire. Participants were tested for HIV-1 and their CD4+ cell counts and plasma HIV-1 RNA loads were measured. The proportion of people whose CD4+ cell count was less than 200 cells/mm³ and who would be eligible to receive HAART under WHO guidelines was estimated. The potential impact of antiretroviral drugs on the spread of HIV-1 in this setting was determined firstly by estimating among the partnerships engaged in by HIV-1-positive individuals the proportion of spousal and non-spousal partnerships eligible to receive HAART, and secondly by calculating the potential impact of HAART on the annual risk of HIV-1 transmission due to sexual contacts of HIV-1 infected persons. The results were compared with those obtained when using USDHHS guidelines.

Results

The overall prevalence of HIV-1 infection was 21.8% (19.2% – 24.6%), and of these people 9.5% (6.1% – 14.9%) or 2.1% (1.3% – 3.3%) of all 15 to 49 year olds would be eligible for HAART (ranges are 95% confidence limits). In each of the next three years 6.3% (4.6% – 8.4%) of those currently infected with HIV-1 will need to start HAART. Among the partnerships where individuals were HIV-1-positive, only a small proportion of spousal partnerships (7.6%; 3.4% – 15.6%) and non-spousal partnerships (5.7%; 3.0% – 10.2%) involved a partner with a CD4+ cell count below 200 cells/mm³ and would have benefited from the reduction of transmission due to the decrease in plasma HIV-1 RNA load under HAART. Estimates of the impact of HAART on the annual risk of HIV-1 transmission show that this risk would be reduced by 11.9% (7.1% – 17.0%). When using USDHHS guidelines, the fraction of HIV-1-positive individuals eligible for HAART reached 56.3% (49.1% – 63.2%) and the impact of HAART on the annual risk of HIV-1 transmission reached 71.8% (64.5% – 77.5%).

Conclusion

The population impact of HAART on reducing sexual transmission of HIV-1 is likely to be small under WHO guidelines and reducing the spread of HIV-1 will depend on further strengthening of conventional prevention efforts. A much higher impact of HAART is to be expected if USDHHS guidelines are used.

MRC INITIATED STUDY: STEPPING STONES

Objectives

Stepping Stones, a behavioural intervention to prevent HIV transmission, is being evaluated in South Africa in a study that includes a cluster randomised controlled trial and an aspect of the evaluation using ethnographic research methods. The long term objective of the project is to determine the effectiveness of Stepping Stones in reducing the transmission of HIV among 2800 women and men aged 17-23 years in the Eastern Cape Province of South Africa. The primary outcome of the trial is therefore HIV sero-incidence after two years. The study has several secondary outcomes related to aspects of knowledge, attitudes and behaviour that are being assessed using a questionnaire. The trial design requires participants to complete a questionnaire.
and give a blood sample (tested for HIV) for a baseline measure before receiving the intervention. This is repeated at one year and two years, and a questionnaire alone is administered 6 months after the start of the study. Participants in the trial are recruited from 70 villages which have been randomly allocated to one of the two study arms. Those in one arm receive the full Stepping Stones programme of 17 sessions held over 6-8 weeks, whilst those in the other attend one 2-3 hour session on HIV and safer sex.

Role of the NICD in Stepping Stones programme

In the original proposal it was anticipated that HIV testing would be performed in the field using rapid diagnostic tests and finger prick blood specimens and the study was resourced for this level of activity only. After considerable discussions with local experts the study team decided that quality control, waste management and human subjects concerns could best be addressed by a more formal approach to specimen management. The NICD has been responsible for the advising the study on tests, provision of supplies, performing laboratory tests and handling specimens, management of waste, training staff and managing quality control. The NICD provides periodic panels to ensure test quality and site visits to inspect the organisation of the laboratory and arrangements for handling blood. Monitoring of the testing site following initiation of study has been conducted to ensure good practise. To date the results of the QA panels has been 100% and parallel testing of specimens has not revealed any discrepancies.

CAVIDI-NICD COLLABORATION

Responses to ART and Disease Progression

The mainstay of monitoring antiretroviral therapy is the HIV-1 viral load. The measurement of the HIV viral load is important in terms of assessing disease progression, initiating therapy before onset of symptoms e.g., primary HIV-1 infection (PHI), responses to therapy, the testing of new therapies, and drug failure. Currently there are three FDA-approved viral load assays that are commonly used but additional assays are available e.g. LCx HIV RNA Quantitative assay (Abbott Diagnostics). The three tests are NucliSens HIV-1 QT (Organon Technica), RT-PCR (Roche) and branched DNA (bDNA, Bayer). The RNA viral load is an important predictor of the progression to AIDS and is usually measured in conjunction with the CD4 count. In the early phase of ART the concept was to “hit early, hit hard” based on particular RNA viral load levels. However, there is the realisation that initiating
therapy based on viral load alone was not necessarily appropriate and the shift in emphasis has been to consider initiating therapy based on the CD4 count. Guidelines based on CD4+ counts vary from starting therapy at CD4+ counts of 350 cells/ml (DHSS guidelines) or the WHO guidelines of 200 cells/ml. Despite the change in guidelines, HIV RNA viral load is the standard to assess the effectiveness of therapy and disease progression. The principles of the viral load assays are either based on target amplification i.e. PCR in the case of Nuclisens HIV-1 QT and RT-PCR or signal amplification in the bDNA assay. The anticoagulant is an important in the performance of the assay. Alternative testing technologies that could be successful in terms of cost and efficacy should be given consideration in monitoring HIV-1 viral loads. Non-specific markers of inflammation such as b2 microglobulin and neopterin have not gained widespread favour as useful tools of monitoring. An example of technologies that could well fit the criteria would be the enzyme-dependent assays such as Cavidi Tech ExaVir LOAD reverse transcriptase assay where measurement of viral load is dependent on active enzyme. Enzyme activity is related to viral copies for ease of reporting. The assay would work regardless of HIV subtype. However, it would not be able to distinguish between HIV-1 and HIV-2 infections, both lentiviruses. In addition, debate around whether to have centralised laboratories perform assays to ensure quality of the results or to decentralise the testing and have "low technology" but robust assays available at peripheral complexes perform assays to ensure quality of the results or to decentralise the testing and have “low technology” but robust assays available at peripheral complexes requires attention and evaluation especially in resource-constrained countries. The NICD has undertaken initial evaluations of the ExaVir viral load assay and is currently engaged in clinical trials to establish the value of the test the suitability of the test in less high technology settings.

**QCMD-NICD COLLABORATION**

Molecular testing has certainly come to the fore and in part has outstripped the requirements for quality assurance. Molecular techniques are advantageous since they are more rapid, sensitive and specific compared to "classical" techniques. However, “best practise” needs to be defined. Implementation of national/regional/international QA programmes is a useful beginning in terms of defining what the best practise could be. An additional advantage is that panels relevant to the region can be established and tested. To this end the Quality Control for Molecular Diagnostics and the NICD entered into a collaboration to determine the feasibility of introducing a programme for HIV, HBV and HCV molecular diagnostic QA for laboratories in South Africa and the region. In addition, the programme had an unique aspect in that QA for HIV DNA PCR was introduced. The programme for 2003 included 11 laboratories (10 from South Africa and 1 from Botswana). Information as to the type and nature of the tests used by the different laboratories as well as performance against all laboratories participating in the QA programme can be established using such an approach. This type of information may well be useful in terms of monitoring the performance of laboratories that would participate in the national ART roll-out programme. Moreover, such a programme could serve as a model for other molecular diagnostic programmes.

**THE 8 KNOWN HEPATITIS B VIRAL STRAINS AND 4 PRIMATE HEPATITIS B STRAINS AND THEIR RELATION TO ISOLATES FROM AFRICA**

To date 8 genotypic groups of hepatitis B have been recognized. These are labeled genotypes A – H and are geographically distributed. Genotypes A and D are the most universal while genotypes E (East, Central and West Africa), F with H (Central and South America) and B with C (Asia) are normally confined to specific areas of the globe. When these genotypes, E, F, H, B and C, are encountered in other regions they are usually found in the same ethnic groups as populate their original region of origin and are therefore probably imported. Genotype G has been found in Europe and the United States but not in Asia but the extent of its distribution is not yet known. In 1997 we published the first sequence data from southern Africa when we reported that of 29 sequences, 24 clustered with genotype A, three with genotype D and one each with genotypic groups B and C. We also reported that of the South African group A specimens, 59.1% clustered with two global sequences to form a discrete segment which we called subgroup A’. Since this work was published not only have 2 new genotypes been recognized (genotypes G and H) but also recombination has been found to be a fairly common event during the HBV life cycle, although its mechanism is still not understood. Coinfection with HBV of distinct
genotypes has been found in about 10% of infected individuals and primate hepatitis B viruses, closely related to the human hepatitis B virus, have been identified in, among others, the chimpanzee, gorilla, orangutan, gibbon and woolly monkey.

Transmission, coinfection and possible recombination is being studied in our renal cohort (see below). In our ongoing surveillance initiative we have now sequenced and analyzed a further 53 southern African specimens over the preS2/S region of the HBV genome and 14 over the core region. This sequence data has been analyzed with GenBank sequences from all over the world in order to better characterize the southern African viral strains.

Standard methods of DNA extraction and amplification by polymerase chain reaction were used and sequence analysis was performed using the ABI prism 3100 Genetic Analyser. SEQUENCER, DNASIS, PHYLIP and SIMPLOT were used to analyze the sequence data.

There was only one specimen of each of the genotypic groups G and C in these patients with 5% genotype D, 34.6% of genotype E and 52% of genotype A. All the genotype A specimens were from the subgroup A’ of genotype A (Figure 2). In this tree all of the genotypes, with the exception of genotype A, formed a robust clade with a bootstrap value of 70 or more.

When the Namibian specimens were separated from the South African specimens the prevalence statistics of the two groups were very different (Table 1).

Table 1: Prevalence of the various genotypes in South Africa and Namibia

<table>
<thead>
<tr>
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<th>A’</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Africa</td>
<td>23</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>74.2</td>
<td>3.2</td>
<td>3.2</td>
<td>16.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Namibia</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>19.0</td>
<td>0</td>
<td>19.0</td>
<td>61.9</td>
<td>0</td>
</tr>
</tbody>
</table>

In South Africa most specimens (74.2%) were from subtype A’ of genotype A with a lower prevalence of genotypes E, G, D and C. In Namibia most specimens (61.9%) were from genotype E with a lower prevalence of genotype D and subtype A’. Although the distribution of the genotypes was disparate between the two countries, isolates from the same genotype or subgroup shared a high identity to each other and a greater than 97% identity with the various consensus groups (Table 2). Southern African specimens from the two countries which clustered in subgroup A’ were only 1.6% different from the global A’ consensus but 4.33% different from the global subgroup A specimens. The genotype D and E specimens were even more similar to their respective global consensus (0.98% and 0.82%, respectively). The South African genotype G specimen, EK46 was 1.48% different from the global G consensus while the South African genotype C specimen, ES1, differed from the C consensus by 2.64%.

A line up was then made of the consensus amino acids of each of the nine genotypes and their subgroups at each site of the preS2 and HBsAg regions which included the consensus sequences of the primate hepatitis B viruses from the Gibbon, Woolly Monkey, Orangutan, and Chimpanzee. From this analysis Subtype A’ was confirmed to differ from subtype A at the preS2 amino acids 10, 22, 32, 35, 47, 48, 53 and 54 (numbering from the start of the preS2 protein; Table 3) but no sites of difference were present in the stretch of the surface protein (amino acid 1 to 178) which was sequenced (line up not shown). There are only two full genome genotype E specimens available on GenBank. In 2001 a set of 20 unpublished partial HBV sequences from Nigerian patients was submitted to GenBank. Analysis of these Nigerian specimens, together with the 19 Southern African genotype E specimens sequenced in this study, enabled us to establish the consensus of genotype E in the preS1/preS2/S region. In particular we could distinguish the consensus of three previously ambiguous sites where the two prototype sequences differed. Surface amino acids 59, 125 and 207 were N (not S), T (not M) and N (not D), respectively.

Outliers on the UPGMA tree were analysed using SIMPLOT. The outlier to genotype A, M54 was found to have evidence of D/A recombination with a breakpoint at 240 bp (Fig 3). M57 and M58 also show similar evidence but the recombinant
Fig 2. UPGMA neighbor joining tree with 100 bootstrap replicates to compare 704 bp of the preS2/S region of the HBV genome from 53 southern African specimens with the consensus sequence constructed from 9 representative specimens from each of the genotypes B – H (CONSENS. B-H), subgenotypes A and A’ (SUBCON. A and A’) and the primate hepatitis B viruses from the Woolly Monkey (W. MONKEY), Orangutan, Gibbon and Chimpanzee, all obtained from GenBank. The bold letter on the right mark the position of the 5 genotype groups into which the southern African specimens clustered. South African specimens have an EK prefix while the Namibians are prefixed with M.
Table 2: Comparison of the mean genetic distance between the southern African specimens and their relevant global consensus sequence.

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<tbody>
<tr>
<td>Subcon A'</td>
<td>1.6%</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Consensus C</td>
<td>2.6%</td>
<td></td>
<td></td>
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<tr>
<td>Consensus D</td>
<td></td>
<td>0.82%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Consensus E</td>
<td></td>
<td></td>
<td>0.98%</td>
<td></td>
<td></td>
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<tr>
<td>Consensus G</td>
<td></td>
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<td>1.48%</td>
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fragments were too small to draw meaningful conclusions, however, further sequence studies could clarify this aspect of local strain variation.

The black population of southern Africa is part of the Niger-Congo linguistic group. During the last 3 millennia BC there have been three major waves of expansion called the Bantu migration which created the North, Eastern and Western Bantu language groups. Southern African Blacks from the Xhosa and Zulu ethnic groups, known collectively as the Nguni people, formed the southern migrant group of the Eastern migration. The Western Bantu moved into Angola and Namibia (the Ovambo) and north-western Botswana (Sotho-Tswana people) and eventually occupied the entire central plateau including parts of South Africa. Because of their common history, it is interesting that the prevalence of the various genotypes in Namibians and South Africans are so different, although the viral strains themselves are closely related. HLA studies have not shown a vast difference in host genetics between South Africans of the Western and Eastern migrant groups. However, some intermingling of the two groups has occurred particularly in the central, "Highveld" region of the country. It would therefore be interesting to compare the genetics of the more geographically separated Nguni people with the Ovambo of Northern Namibia.

![SimPlot - Query: M54_____Q FileName: C:\My Documents\PROGRAMS\phylop36\Simplot1.txt](image)

**Fig 3.** Simplot showing that specimen M54 is most similar to Consensus D before the breakpoint at 240 bp and more similar to subgroup A' after the breakpoint.
Table 3: Consensus amino acid differences over the preS2 domain of the surface gene. Total consensus amino acids and numbers (columns 2 and 3) which are enclosed in a box are immunogenic epitopes and amino acids (column 3) enclosed individually are anchor residues within these.
HBV AND HCV REPORT

Patients with renal failure on haemodialysis have a high risk of blood-borne viral infections, with some of the most common being caused by hepatotropic or other hepatitis-associated viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV). Although the incidence of infection by these viruses has been decreased by the development of screening techniques for the detection of HBV and HCV in blood banks, there is still a high prevalence of hepatitis cases in these patients. Nosocomial transmission is generally the most probable cause for HCV and HBV infection in patients where parenteral transmission cannot be identified. Strict adherence to universal precautions against nosocomial infections reduces the risk of transmission without the need to isolate HCV or HBV infected patients.

Phylogenetic analysis has indicated a number of possible routes of transmission, including hand-borne transmission by health care workers and treatment with contaminated medical equipment including dialysis devices. Relatedness of viruses at the genomic level is the most convincing evidence of nosocomial transmission and provides clear documentation of patient-to-patient transmission.

We therefore selected a total of 30 specimens with markers for either HBV (N=11) or HCV (N=19) from a cohort of 83 serum samples collected from renal dialysis patients at Chris Hani-Baragwanath Hospital for this nucleic acid transmission study.

DNA and RNA extractions were simultaneously performed on the MagNA Pure LC instrument using the Total Nucleic Acid Extraction Kit.

HCV cDNA synthesis was carried out using the Titan™ One Tube RT-PCR System with primers specific for the 5’ non-coding region (5’ NCR) of the genome. Isolates that gave no positive result after the first round of PCR were then further amplified by nested PCR using a second set of inner primers.

First round (252bp) and nested (218bp) products were visualised on a 2% Metaphor agarose gel stained with ethidium bromide. For the HBV specimens, a DNA fragment of approximately 400bp was amplified by PCR with primers specific for the 5’ end of the surface region of the genome. The products were then visualised on a 2.5% agarose gel stained with ethidium bromide. The HBV and HCV amplicons were purified and sequenced using the ABI BigDye Terminator Sequencing Kit.

Fig 4. Phylogenetic tree of the early surface region of the hepatitis B virus genome (334 bp). Isolates from the renal patients are shown in bold type and have the prefix ‘HB’.
Cycle sequencing kit and then run on the ABI 3100 automated sequencer. Separate phylogenetic trees of the two regions were constructed using the PHYLIP neighbour-joining programme and 100X bootstrap resampling (Figures 4 and 5).

Only five of the specimens (45.5%) with HBV serological markers had sufficient DNA for successful amplification.

Analysis of the HBV phylogenetic tree (Fig 4) shows that the majority of the isolates cluster with the subgroup A’ of genotype A. Further sequencing will be carried out to include the preS2 region where we will be able to determine if the specimens have preS2 Arginine$^{10}$, Phenylalanine$^{32}$, Leucine$^{32}$, Valine$^{35}$, Serine$^{47}$, Threonine$^{48}$, Alanine$^{53}$ and Proline$^{54}$ which are characteristic of the subgroup A’. Arginine$^{10}$ and Threonine$^{48}$ have been found to be exclusive to South African subgroup A’ specimens.

A nucleotide alignment (Table 4) reveals four distinct sequences between the five HBV specimens. Specimens HB33, HB34 and HB38 are serotype adw (Lys at aa position 122) and specimens HB70 and HB72 are serotype ayw (Arg at aa position 122). These are the two major serotypes which are found within genotype A in sub-Saharan Africa.

Fifteen of the specimens (78.9%), all with previous serological markers, were positive for HCV nucleic acid. The majority of the HCV sequences (Fig 5, local specimens indicated in bold print) cluster with genotype 5, except for HB81 which clusters with genotype 1. The results correlate with previous reports stating that the predominant genotypes found in South Africa are 1 and 5.
Table 4: Point mutations occurring in the surface region which are characteristic of subgroup A’ and genotype A. The area highlighted in blue indicates the codon at aa position 122 that establishes the d/y determinant.

| Nucleotide Positions | 285 | 286 | 345 | 346 | 435 | 440 | 441 | 450 | 453 | 450 | 408 | 409 | 504 | 510 | 519 | 524 | 525 | 526 | 540 | 549 | 551 | 586 | 591 |
|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Consensus            | A   | A   | T   | T   | T   | C   | T   | A   | A   | T   | C   | A   | A   | A   | A   | A   | A   | C   | T   | C   |   |   |
| Genbank Numbers (Subgroup A’) |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| AF297621             | * T | C   | A   | A   | *   | A   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| AF297623             | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | *   | A   | A   | A   | T   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| AF297625             | * T | C   | A   | A   | *   | *   | *   | T   | *   | *   | *   | A   | A   | A   | T   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| M57663               | * G | C   | A   | A   | *   | *   | T   | *   | *   | *   | A   | A   | *   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| M74468               | * T | C   | A   | A   | *   | *   | T   | *   | *   | C   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | *   |   |   |
| U87728               | * T | C   | A   | A   | *   | A   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87731               | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | C   | A   | A   | T   | C   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87733               | G   | T   | C   | A   | A   | A   | *   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87734               | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87740               | C   | T   | C   | A   | A   | *   | *   | T   | C   | *   | C   | A   | A   | T   | C   | *   | *   | *   | C   | A   | A   | T   |   |   |
| Genbank numbers (Genotype A) |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| AF297623             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| AF297624             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| U87728               | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87731               | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87733               | G   | T   | C   | A   | A   | A   | *   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87734               | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| U87739               | * T | C   | A   | A   | *   | *   | *   | T   | *   | *   | *   | A   | A   | T   | *   | *   | *   | *   | C   | A   | A   | T   |   |   |
| Isolates             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| HB33                 | * T | C   | A   | A   | *   | *   | T   | *   | *   | *   | C   | A   | A   | T   | C   | *   | *   | *   | C   | A   | A   | T   |   |   |
| HB34                 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| HB38                 | G   | T   | C   | A   | A   | *   | A   | T   | *   | *   | *   | A   | A   | T   | C   | *   | *   | *   | C   | A   | A   | T   |   |   |
| HB76                 | C   | T   | C   | A   | A   | *   | *   | T   | C   | *   | C   | A   | *   | C   | *   | G   | G   | C   | A   | A   | T   |   |   |
| HB72                 | C   | T   | C   | A   | A   | *   | *   | T   | C   | *   | C   | A   | *   | C   | *   | G   | G   | C   | A   | A   | T   |   |   |
The majority of genotype 5 isolates have the classic double A mutation at positions 107 and 108 (Table 5) which is known to be a unique characteristic of this particular genotype. Although the 5’ UTR region partitions HCV specimens into genotypes it cannot differentiate subtype. The degree of heterogeneity is observed within this region and has been reported to exhibit a rate of variation three to four times higher than non-structural regions. By studying this region, the composition of quasispecies within individual patients can be determined and their sequence variations during the course of HCV infection monitored. These preliminary studies identify four unique HBV sequences among the five HBV DNA fragments analysed and seven unique HCV sequences among the fifteen HCV fragments. These results indicate that transmission within the dialysis unit is highly unlikely. Further sequencing will be undertaken to confirm these initial results. That do not appear to indicate that these specimens are epidemiologically linked.

Table 5: Point mutations occurring in the 5’ UTR which are characteristic of genotype 5. The area highlighted in blue indicates the position of the classic double A mutation of genotype 5.

<table>
<thead>
<tr>
<th>Reference (Genotype 1a)</th>
<th>Nucleotide Positions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>63</td>
</tr>
<tr>
<td>Genbank numbers (Genotype 5)</td>
<td></td>
</tr>
<tr>
<td>D10114</td>
<td>-</td>
</tr>
<tr>
<td>D10118</td>
<td>-</td>
</tr>
<tr>
<td>L28057</td>
<td>*</td>
</tr>
<tr>
<td>L29581</td>
<td>-</td>
</tr>
<tr>
<td>M84860</td>
<td>*</td>
</tr>
<tr>
<td>X78867</td>
<td>-</td>
</tr>
<tr>
<td>Y13184</td>
<td>*</td>
</tr>
<tr>
<td>Isolates</td>
<td></td>
</tr>
<tr>
<td>HB39</td>
<td>T</td>
</tr>
<tr>
<td>HB43</td>
<td>T</td>
</tr>
<tr>
<td>HB52</td>
<td>T</td>
</tr>
<tr>
<td>HB69</td>
<td>T</td>
</tr>
<tr>
<td>HB99</td>
<td>T</td>
</tr>
<tr>
<td>HB109</td>
<td>T</td>
</tr>
<tr>
<td>HB48</td>
<td>T</td>
</tr>
<tr>
<td>HB65</td>
<td>T</td>
</tr>
<tr>
<td>HB91</td>
<td>T</td>
</tr>
<tr>
<td>HB49</td>
<td>T</td>
</tr>
<tr>
<td>HB51</td>
<td>T</td>
</tr>
<tr>
<td>HB62</td>
<td>T</td>
</tr>
<tr>
<td>HB81</td>
<td>T</td>
</tr>
<tr>
<td>HB90</td>
<td>T</td>
</tr>
</tbody>
</table>

Another area of the HCV genome which is of interest is a hypervariable region (HVR1) within the second envelope gene (E2). The greatest
QCMD 2003 HEPATITIS B VIRUS (HBV) PROFICIENCY PROGRAMME

The Specialised Molecular Diagnostics laboratory participated in the QCMD 2003 Hepatitis B virus (HBV) proficiency programme. Briefly, a DNA extraction was performed on the panel of eight samples using the automated MagNA Pure LC instrument with the Total Nucleic Acid extraction kit.

The isolated DNA was amplified by PCR with primers specific for the surface region of the HBV genome, yielding a product 491bp in length. The PCR protocol used is an in-house one which was designed for the routine diagnosis of HBV.

PCR products were then run on a 2.5% agarose gel stained with ethidium bromide at 100V for one and a half hours.

The only samples to amplify were the three with the highest concentration (Table 6). To determine if the sensitivity of the protocol could be increased, a nested PCR was performed but without any modification in results. Since the current PCR could not detect DNA concentrations below 1 X 10^5 copies/ml and it is predicted that the greater part of South African patients will have less than this amount, a protocol with increased sensitivity will have to be developed for use in the Diagnostic Unit.

SYBR-green quantitative real-time PCR has been shown to have a lower detection limit of 1 X 10^3 copies/ml (Brechtbuehl et al., 2001). The sensitivity of the assay may be further increased by performing a nested PCR. This is recommended if the concentrations of DNA are below 10^5 copies/ml. Studies will be conducted in the near future to optimise this protocol for implementation in the Specialised Molecular Diagnostics Unit for routine HBV diagnosis.

Table 6: A list of the HBV proficiency panel and their corresponding expected results and actual results obtained. Specimens for which results were concordant are shown in blue.

<table>
<thead>
<tr>
<th>QCMD Code</th>
<th>Subtype</th>
<th>Matrix</th>
<th>Target Concentration (Copies/ml)</th>
<th>Expected Result</th>
<th>Actual Result Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV-01</td>
<td>A</td>
<td>Serum</td>
<td>1 x 10^6</td>
<td>Positive (++)</td>
<td>Positive (++)</td>
</tr>
<tr>
<td>HBV-02</td>
<td>A</td>
<td>Serum</td>
<td>1 x 10^3</td>
<td>Positive (+)</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>HBV-03</td>
<td>-</td>
<td>Serum</td>
<td>-</td>
<td>Negative (-)</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>HBV-04</td>
<td>D</td>
<td>Serum</td>
<td>1 x 10^5</td>
<td>Positive (++)</td>
<td>Positive (++)</td>
</tr>
<tr>
<td>HBV-05</td>
<td>A</td>
<td>Serum</td>
<td>1 x 10^4</td>
<td>Positive (+)</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>HBV-06</td>
<td>A</td>
<td>Serum</td>
<td>1 x 10^3</td>
<td>Positive (+)</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>HBV-07</td>
<td>A</td>
<td>Serum</td>
<td>200</td>
<td>Positive (+/-)</td>
<td>Negative (-)</td>
</tr>
<tr>
<td>HBV-08</td>
<td>A</td>
<td>Serum</td>
<td>1 x 10^5</td>
<td>Positive (++)</td>
<td>Positive (++)</td>
</tr>
</tbody>
</table>
MOLECULAR EPIDEMIOLOGY OF HEPATITIS C VIRUS IN SOUTHERN AFRICA

The primary aim of this project is to establish a sustained program of surveillance for HCV prevalence and genotyping in southern Africa by determining the ‘true’ prevalence of HCV infection by confirming antibody seroprevalence results with nucleic acid testing. Genotyping of local strains is presently undertaken by sequencing of the 5’ untranslated region (Fig.6) of the HCV genome. Results will be confirmed by sequencing other regions such as the NS5B regions (Fig.6).

Fig 6. A diagrammatic representation of the HCV genome flanked on either side by 5’ and 3’ untranslated regions. The structural region comprises of genes for core (C) and envelope (E1 and E2) proteins and the non-structural (NS 2-5) regions encode proteins of different functions: proteinases, replication, interferon resistance and RNA-dependent RNA polymerase.

Table 7: Summary of serology and nucleic acid test results

<table>
<thead>
<tr>
<th>TESTS</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serology +</td>
<td>308</td>
</tr>
<tr>
<td>Serology +, RNA +</td>
<td>232 (75,3)</td>
</tr>
<tr>
<td>Serology +, RNA -</td>
<td>65 (21,10)</td>
</tr>
<tr>
<td>Serology +, RNA inhibited</td>
<td>11 (3,57)</td>
</tr>
</tbody>
</table>

The results from table 7 show that for 75% of specimens, there was concordance between serology and RNA tests. Twenty-five percent of specimens gave discordant results and confirmation of such results will require other supplemental tests like the RIBA. The predictive value of EIA has been found to be low in areas of low HCV prevalence and hence a high proportion of false positives were expected in the study. However another reason for RNA being negative is that viral clearance could have occurred prior to specimen collection. Also soluble factors in serum can interfere and inhibit the Amplicor Qualitative assay and in these cases the specimen is re-tested and/or more specimen is requested.

Serum samples were obtained during the years, 2000, 2001 and 2002 from provincial hospitals around Johannesburg. Antibody screening tests were done at the NICD hepatitis serology laboratory. HCV antibody seropositive specimens were tested for HCV RNA by nucleic acid testing (NAT), as recommended by CDC, using the Amplicor HCV Qualitative assay, version 2.0, Roche. For sixty-four RNA positive specimens, reverse transcription (RT) and amplification of cDNA was done in a one-tube RT-PCR assay (TITAN One-Tube, Roche). Nested PCR was performed which yielded a 238bp amplicon in the 5’UTR region which was sequenced.
Virology - Specialized Molecular Diagnostics Unit

Fig 7. Genotypes identified in the study group

The predominant genotype in the study group was found to be genotype 5 (Fig 7). Other circulating genotypes, 1 (30%) and 3 (9%) have also been found, as reported in previous studies. In this study, genotype 4 appeared in 8% of the study population and genotype 6 was not identified.

Genotype 5, the most predominant genotype in South Africa, appears to form two distinct clusters (Fig 8, 9). Sequences, 3573,80,3788,410 form an “atypical” genotype 5 group and do not possess the characteristic double adenine (AA) at positions 106 and 107 respectively (Fig 8), as seen in previous studies. Instead a single adenine is present at position 107. Specimens 918 and 410 had a single nucleotide insertion of adenine at position 153 (Fig 8). Otherwise 918 was identical to the other typical genotype 5 specimens while 410 is part of the “atypical” cluster (Fig 9).

HCV PCR and genotyping have been included in the Quality Control for Molecular Diagnostics (QCMD) program, Europe. The results have been included in the phylogenetic analyses (Fig 9). Two HCV-QCMD panels of eight specimens each were received during 2003. One panel was tested with the Amplicor HCV Qualitative assay. Of the eight specimens, one was PCR negative. These results were concordant with the QCMD panel results. The second panel was genotyped by sequencing the 238bp amplicons of the 5’UTR. One specimen was genotype 3, two were genotype 1, two were genotype 4 and two were genotype 2 (G9-16, Fig 9). Phylogenetic analyses of the 2003 - Genotype QCMD panel compared well with expected results. However, specimen G12 was negative.

From the results it can be seen that it is necessary to establish true prevalence rates of HCV infection in southern Africa. NAT and genotyping will be used at the NICD to survey local HCV infection. From the sequencing results and phylogenetic analyses, it appears that another subtype of Genotype 5 may be circulating in the local population. Although sequencing remains the gold standard for genotyping, a more viable method is being optimized using real-time PCR. Full genome sequencing will be done to characterize local genotypes and recombinants and will clarify the true extent of the heterogeneity of the “atypical” specimens identified. This research project was funded by a PRF Research Grant.
Fig 8. Nucleotide alignment of local HCV specimens at the 5' untranslated region. The sequences are aligned with a consensus Genotype 1a (M62321) sequence and a genotype 5a (y13184) from the Genbank database. The dashes represent homology to the consensus sequence. Nucleotide positions at 44 and 45 in alignment correspond to 106 and 107 in full sequence alignment and 144 corresponds to 153 in full genome alignment.
Fig 9. Phylogenetic analyses of 64 HCV positive isolates, sequenced in the 5'UTR. The tree is rooted with Hepatitis GB virus B. Sequences in blue are from Genbank and sequences 3573,80,3788 and 410 form "atypical" Genotype 5 cluster.
Molecular Epidemiology of Influenza Virus

Influenza is a highly contagious respiratory illness that has caused epidemics of human disease for centuries. The two types of influenza viruses of public importance are influenza A and B. The subtypes of influenza A that have been circulating globally in recent years are the H1N1, H3N2 and the reassortant H1N2 viruses. Recurrent epidemics of influenza are due to the continuous and extensive antigenic variation of the viral surface glycoproteins, the haemagglutinin (HA) and neuraminidase (NA) proteins. The virus strains recommended by the WHO for the vaccine formulation for the southern hemisphere 2003 influenza season were the A/New Caledonia/20/99-like (H1N1), A/Moscow/10/99-like (H3N2) and B/Hong Kong/330/01-like viruses. The A/Moscow/10/99-like strain that was most widely used as a vaccine strain was the A/Panama/2007/99 virus strain, while either B/Shandong/7/97, B/Hong Kong/1434/02 or B/Hong Kong/330/01 were used as B/Hong Kong/330/01-like viruses.

Influenza activity during the South African 2003 winter season was characterised by a localised epidemic in Pretoria during late May – early June and mainly sporadic activity elsewhere from mid-May to July. The first influenza isolate was made from a specimen taken on 14 May (week 20) and the last from a specimen taken on 24 July (week 30). Specimens positive for influenza were obtained from individuals in the Gauteng, Mpumalanga and Western Cape provinces. Patient’s ages ranged from 11 months to 72 years (median 26 years). Both influenza A and influenza B viruses were isolated during the season. The majority of the influenza isolates were influenza A (93.5%) while 5 (6.5%) of the positive specimens were influenza B strains. Sixty one (84.7%) of the influenza A isolates were subtype H3N2 and six (8.3%) were subtype H1. Four of the H1 isolates were from Cape Town and the remaining two from Johannesburg. The remaining five (7%) of the influenza A positive specimens could not be subtyped.

The outbreak of respiratory disease in Pretoria occurred in a police residential college and was characterised by moderate to severe illness. A total of 648 students were affected, 26 of whom were admitted to hospital. Symptoms included pyrexia, severe headache and myalgia. The attack rate per dormitory building ranged from 20% to 47%, with an overall attack rate of 34%. Twenty throat swabs and bronchoalveolar lavage specimens were sent to the NICD for investigation of the cause of the outbreak. Using a multiplex nested polymerase chain reaction (PCR), the presence of influenza A RNA was detected directly in the majority of these clinical specimens. The PCR also showed that the virus was subtype H3. Virus isolates were subsequently made and characterised further both serologically and genetically by sequencing of the viral haemagglutinin and neuraminidase glycoproteins.

Antigenic subtyping of the Pretoria isolates by the haemagglutination inhibition (HI) assay revealed that these H3N2 viruses reacted poorly with ferret antisera raised against a representative H3N2 strain isolated in South Africa in 2002, suggesting significant antigenic drift from the previously circulating A/Panama/2007/99-like viruses in South Africa. HI tests on the H3N2 viruses isolated from Johannesburg and surrounding areas yielded similar findings. Subsequent data obtained from a number of South African H3N2 strains sent to the WHO Collaborating Centres for Influenza Reference and Research in Melbourne and London for further serological characterisation by HI confirmed that they had drifted away from the A/Panama/2007/99-like strains and were antigenically more related to the variant A/Fujian/411/02-like viruses. In contrast to the H3N2 viruses, the six influenza A H1 isolates reacted strongly with the antiserum raised against the A/New Caledonia/20/99 vaccine strain. All the influenza B isolates reacted well with the B/Sichuan/379/99 antiserum in the HI tests and did not react with antiserum raised against the B/Hong Kong/330/01-like viruses.

Sequence analysis of the HA1 subunit of representative viruses isolated from the Pretoria outbreak with the A/Panama/2007/99 vaccine strain sequence revealed that the isolates were identical and had 12 common amino acid differences relative to the A/Panama/2007/99 vaccine strain. The three substitutions at residues 21, 183 and 186 were the same as those seen in the 2002 South African viruses, while the changes at residues 50, 83, 202, 222 and 225 had been reported for some of the H3N2 strains isolated elsewhere in 2002. The leucine to isoleucine and
histidine to glutamine mutations at residues 25 and 75 respectively had been observed in a few 2002 isolates from Hong Kong, the Philippines and Australia.

The sequences of the Johannesburg isolates were similar to those isolated during the outbreak in Pretoria, but all had additional substitutions at residue 156 (glutamine to histidine) and at residue 275 (glycine to serine). The Johannesburg viruses did not all exhibit the arginine to glycine substitution at position 50 observed in the Pretoria isolates. The mutations at residues 131 (alanine to threonine), 155 (histidine to threonine) and 156 (glutamine to histidine) are characteristic of the A/Fujian/411/02-like variants which were seen in some of the northern hemisphere countries in the 2002-2003 winter season.

Figure 1 shows the results of the phylogenetic analysis of representative South African H3N2 HA1 sequences (921 bp) using the PHYLIP neighbor-joining programme. The 2003 isolates grouped together with the recent A/Fujian/411/02 variants in a different sub-lineage to that of the viruses that were circulating in South Africa during the 2002 season.

All six influenza A H1 isolates were shown to be the reassortant H1N2 subtype by RT-PCR assays using primers specific for the neuraminidase. Sequence analysis of the HA1 subunit revealed the H1 viruses isolated during the season showed some genetic drift from the A/New Caledonia/20/99 vaccine strain. The two isolates from Cape Town that were analysed were identical, while the two isolates from Johannesburg each differed from the Cape Town strains by one amino acid. Seven common amino acid changes were observed in all the isolates sequenced. Five of these substitutions, which were at residues 166 (V-A), 175 (V-I), 187(N-D), 190 (A-T) and 215 (A-T), had been seen in the H1N2 viruses circulating South Africa in 2002. The other two differences were observed at residues 186 (G-R) and 188 (R-M). The additional substitutions seen in the Johannesburg strains occurred at residues 237 (G-R) and 283 (Q-K). The results of the phylogenetic analysis of the South African H1 sequences are shown in Figure 2. The 2003 H1 viruses clustered together with the South African 2002 H1N2 strains as well as H1N2 viruses isolated in Europe in 2002 as represented by the A/Stockholm/13/02 strain.

The neuraminidase N2 sequences of the H1N2 reassortant viruses were very similar to the N2 sequence of the H1N2 2002 South African viruses and appear to have evolved from strains related to the reference A/Egypt/84/01 virus (Figure 3). In contrast, the N2 of representative H3N2 isolates grouped in a different sub-lineage to the 2002 South African H3N2 viruses and exhibited ten or eleven amino acid changes relative to the NA of the A/Panama/2007/99 vaccine strain.

The molecular characterisation of representative influenza B isolates showed that they were fell within the B/Shenzhen/654/99 sub-lineage of the B/Yamagata/16/88 lineage (Figure 4). The South African strains were most closely related to the B/Texas/3/02 strain with only one amino acid difference at residue 179. This is unlike the situation the previous year in South Africa, where all the B strains were found to be similar to the B/Hong Kong/330/01-like viruses which had evolved from the B/Victoria/2/87 lineage.

With regard to the influenza vaccine strains for the southern hemisphere 2004 season, the significant drift observed in the influenza A H3N2 strains from the A/Panama/2007/99 vaccine strain has made it necessary to update the H3N2 strain component. The WHO has recommended an A/Fujian/411/02-like virus with both A/Kumamoto/102/02 and A/Wyoming/3/02 being acceptable for use as the actual vaccine stains. The influenza A H1N1 and B strains remain unchanged from the 2003 formulation.

Acknowledgements:
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.
Fig 1. Phylogenetic tree of influenza A (H3N2) virus HA1 gene nucleotide sequences (921 bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling. (H3N2 vaccine strain is depicted in bold).
Fig 2. Phylogenetic tree of influenza A (H1) virus HA1 gene nucleotide sequences (979 bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling. (H1N1 vaccine strain is depicted in bold).
Fig 3. Phylogenetic tree of influenza A (H1 and H3) virus N2 neuraminidase gene nucleotide sequences (960 bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling (H3N2 vaccine strain is depicted in bold).
Fig 4. Phylogenetic tree of influenza B virus HA1 gene nucleotide sequences (957 bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling. (B vaccine strain is depicted in bold).
MOLECULAR SURVEILLANCE OF MEASLES AND RUBELLA IN SOUTHERN AFRICA

A) Measles

After several years of very low measles activity in South Africa, we experienced two outbreaks, and identified a few sporadic cases by routine case surveillance.

In early November 2002, measles was diagnosed in three Angolan citizens living in Hout Bay, Cape Town, at least one of whom had a recent history of travel from Angola. A total of 26 measles cases were confirmed in the following six weeks, mostly from Hout Bay, but with sporadic cases around the peninsula. A single measles virus genotype, B2, was detected at the end of the outbreak (Fig 1). This unusual genotype has only been detected before (Gabon, 1984) and has never been shown to be circulating in southern Africa. Accordingly, this measles outbreak was probably caused by the introduction of measles virus from elsewhere in Africa, most likely Angola.

In March 2003 (week 12), three suspected cases of measles were identified in Boksburg, but only one of these could be confirmed. A virus belonging to genotype D3 was identified. This is most unusual, because it has only been shown to circulate in Japan and Taiwan in recent years.

A measles outbreak occurred late in March 2003 (week 13) in Luanda, Angola, and four urine specimens from IgM positive cases were sent to the NICD for serological confirmation and genotyping. A portion of the measles virus genome could be amplified by RT-PCR from one of the specimens, and again, only one single genotype was present. Interestingly, the outbreak was also caused by a B2 genotype virus, but differed by 2 nucleotides (in the partially sequenced nucleoprotein {N} gene) from the virus that caused the outbreak in Hout Bay (Fig 1). The outbreak in Luanda provided direct evidence for the circulation of genotype B2 measles virus in Angola. The recent detection of genotype B2 viruses that are closely related to a virus isolated nearly 20 years ago in Gabon (differed by only 1.5 to 2%) illustrates the gaps in viral surveillance in southwestern Africa. It is likely that these B2 viruses have been continuously circulating in Angola, and possibly other neighbouring countries, and were only detected when they were introduced into a country with active viral surveillance.

Towards the end of May (weeks 20-21), there was a suspected measles outbreak in Maputo, Mozambique, and again, sera and a few matched urines were sent to NICD for laboratory confirmation and genotyping. Genotype D2 was identified, which was not unexpected because this genotype has previously been shown to circulate endemically in southern Africa, and was the predominant genotype in South Africa during the late 1980s and early 1990s. Some time later, identical D2 viruses were detected in the Gauteng and Mpumalanga provinces of South Africa in an outbreak that continued until the end of the year. D2 viruses that differed by one or two nucleotides at various positions in the N gene were also detected during the outbreak (Fig 1).

Two unrelated genotype D4 viruses were also detected. One of these viruses, obtained from the Bushbuckridge area in Limpopo province (week 29) was not closely related to any of the currently described D4 viruses. It is also particularly interesting that the other D4 virus (Boksburg,SOA/38.03, Fig 1) was obtained from a child that became symptomatic in Boksburg a few days after arriving in South Africa from the Beira area in Mozambique (week 38), indicating that D4 viruses were active in Mozambique. It therefore appears that at least 2 genotypes of measles virus were circulating in Mozambique during 2003.

During the first few weeks of the D2 outbreak in Gauteng and Mpumalanga, it became apparent that health care practitioners were unsure of the procedures relating to specimen collection, which made it difficult to perform molecular characterization of the virus. It should be noted that 2 specimens should be always be collected from suspected cases: a serum sample that is required for serological confirmation, as well as a urine specimen (or even a throat swab in viral transport medium if urine is difficult to obtain, as in the case of young infants) needed for molecular epidemiology. This information is used for tracking transmission pathways of measles.

B) Rubella

Currently, molecular information is only available for 17 countries worldwide; no surveillance has been done on the African continent. Two rubella genotypes (RGI, RGII) have been identified, and so far RGI seems to predominate and have a wider global distribution, while RGII seems to be...
limited to parts of Europe and Asia. However, the geographic ranges and distributions are not yet well defined because of limited sampling. To date, only a small number (15) of rubella viruses from five South African provinces have been examined, and all have grouped as genotype II. However, many more specimens need to be analyzed before one can draw conclusions about the distribution and transmission patterns of rubella virus in South Africa.

Figure 1. Phylogenetic analysis of measles virus N gene sequences. WHO reference strains are shown in italics, South African viruses are in bold print, and Mozambican and Angolan sequences are shown in blue.
Molecular Epidemiology of Poliovirus in Sub-Saharan Africa

During 2003, the polio molecular unit of the NICD a WHO regional Reference Laboratory received 1185 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 the Africa namely, Benin (BEN), Burkina Faso (BFA), Burundi (BUU), Cote d’lvoire (CIV), Congo (CNG), Eritrea (ERI), Ethiopia (ETH), Ghana (GHA), Guinea (GUI), Kenya (KEN), Nigeria (NIE), Niger (NIG), Democratic Republic of Congo (DRC), Rwanda (RWA), Senegal (SEN), South Africa (SOA), Somalia (SOM), Sudan (SUD), Tanzania (TAN), Togo (TOG), Uganda (UGA) and Zimbabwe (ZIM) (Figure 2). Original specimens from AFP cases were received from several southern African countries and any polio isolates were treated as above.

In South Africa 543 AFP cases were reported and poliovirus was isolated from nine of these. All polioviruses isolated from AFP cases in southern African countries were found to be vaccine-like.

Fig 1. Isolates received in 2003

Fig 2. Samples received from African countries
The majority of the wild-type cases identified during 2003 were from Nigeria (Figure 3), 174 of which were polio type 1 and 154 polio type 3. Other cases identified in 2003, some of which were isolated late in 2002, were from Benin, Burkina Faso, Ghana, Niger and Togo.

Molecular sequencing of the full VP1 (900bp) (Figure 4) 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 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 reservoirs in Africa is Nigeria (WEAF-B), and possibly southern Somalia (Figures 5). The last case for WEAF-A genotype was in November 2002 for wild type 1 and in April 2002 for both WEAF-A wild type 3 and EAAF.

Distribution of wild PV3 genotypes closely parallels PV1 distribution. Integrated 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, but northern Nigeria, with Kano, Katsina and Kaduna as the main source reservoir, has a large ongoing endemic (Figure 6).

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 dendrograms presented were constructed by the NICD and CDC (Atlanta) and are presented as follows: (e.g. NIE4700350: NIE-KTS-DRA-03-004: KATSINA) The first three letters and a number represent the Lab ID, followed by EPID No. and lastly the province or district.

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**Fig 5.** WEAF-B wild PV1 representative of isolates: 2000-2003
Wild PV1 was highly endemic in northern Nigeria in 2003 and very active circulation occurred in the northern provinces of Kano (KNS), Kaduna (KDS), Katsina (KTS) and others. Other circulations occurred in the central provinces. Immunity levels are much higher in the southern provinces.

2003 isolates are distributed into sixteen clusters (A to I), (other clusters are not shown). Cluster A (not shown) is the most closely related group and also cluster B, C and D. In figure 5, cluster C consists of major lineages, KNS, KDS and KTS which are clearly defined and reflect intense local, point source outbreak in Kano province. The last case in cluster C was on the 29 of October 2002.

Cluster D has a single lineage, which reflects local circulation in Gombe (GMS) province. Only two 2003 isolates identified for cluster F and the last case for cluster G was on the 15 of October 2002. Cluster H consists of the local circulation in northern provinces.

Cluster I was very active in 2003 and consists of several lineages. The closely related viruses were found in Togo, Ghana and Burkina Faso and all belong to cluster I3. In 2003 nineteen cases were identified from Niger, seven from Burkina Faso, eight from Ghana, one from Togo and with the hot case being identified in Benin. The Benin case is closely related to Nigeria cases.

Fig 6. WEAF-B Wild PV3 representative of isolates: 2000-2003 survey
WEAF-B wild PV3 is divided into four clusters A – D (Figure 6). Cluster A represents local circulation in Sokoto (SOS) province. Cluster B has a single isolate that was isolated in February 2002 and no cluster B isolates have been found since February. Cluster C is more diverse than those of cluster A, B and D. The viruses circulated in Katsina, Jigawa and the adjacent province of Zinder in Niger. Cluster D resolved into several 2003 lineages Borno (BOS), Jigawa, Gombe, Katsina, Kaduna and Kano compare to 2002 where only four lineages were observed. There is a great similarity among cluster D.

Fig 7. EAAF Wild PV3 representative of isolates: 2000-2002 survey
Virology - *Vaccine Preventable Virus Infections*

Five 2002 isolates were reported from PV3 EAAF genotypes (figure 7). These were from MSH, LSH and BAN (not shown) lineages. The last case on the tree was from Lower Shabele province of Somalia.

**VACCINE-DERIVED POLIOVIRUS (VDPV)**

No VDPV was identified in 2003.

**CONTAINMENT**

The National Task Force (NTF) for Polio Laboratory Containment in South Africa, appointed in 2001, has to date managed to create a database for most of the country’s biomedical laboratories (include hospitals, laboratories, academic institutions, scientific research organizations, environmental research organizations, water and sewage facilities, waste technologies and vaccine producers) suspected with infectious or potentially infectious material.

The NTF has designed a laboratory survey questionnaire enabling the facilities to perform an inventory of materials that may contain wild poliovirus. These inventories should be forwarded back to the NTF after being signed by the head of the facility.

The inventory forms should help NTF to create a database/national inventory of facilities housing potentially infectious material.

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**Virology - Special Pathogens Unit**

The Special Pathogens Unit of the National Institute for Communicable Diseases is responsible for the diagnosis and investigation of diseases associated with the so-called formidable (biohazard class 4) viruses in southern Africa, and operates a maximum security (biohazard containment level 4) facility with two suit laboratories where workers are protected in all-enclosing plastic suits with breathing air supplied through hoses. Class 4 viruses known or considered likely to occur in Africa include Marburg, Ebola, Rift Valley fever, Crimean-Congo haemorrhagic fever, Lassa fever, and hantaviruses. The Special Pathogens Unit is also responsible for the diagnosis of rabies and rabies-related infections in humans and it incorporates the Medical Ecology Unit, which is responsible for monitoring bubonic plague activity in small mammals in South Africa. The Unit is recognized as a World Health Organization (WHO) Regional Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses.

**COMPARISON OF SPECIMENS RECEIVED IN 2002 AND 2003**

The total number of specimens tested in the Unit during 2003 was low in comparison with 2002, mainly because the previous year’s figures were inflated by the large number of cattle sera tested in a survey of antibody to Crimean-Congo haemorrhagic fever virus (Table 1). As in recent years, the number of specimens received from South Africa and its immediate neighbours for the investigation of suspected viral haemorrhagic fever has continued to decline.

In addition to the above specimens, VHF antibody screening tests were applied to 203 sera from 188 patients submitted to the Arbovirus Section of the Unit for the investigation of suspected infection with arthropod-borne viruses without positive result.

**INVESTIGATION OF SUSPECTED VHF**

For the first time since 1981 no cases of suspected viral haemorrhagic fever (VHF) were confirmed in southern Africa during the report year. In particular, no cases of Crimean-Congo haemorrhagic fever (CCHF) were detected, and this does not appear to be related to decreased tick vector or virus activity. There were no marked climatic deviations during the year and veterinary acarologists did not observe any decrease in *Hyalomma* tick activity. Moreover, the high prevalence of CCHF antibody detected in the sera of cattle of all ages including yearlings during the survey of 2002 indicates that virus activity remains at the same high levels detected in surveys two decades ago. Soon after the initial recognition of the presence of CCHF virus in southern Africa in 1981 we estab-
Table 1: Comparison of specimens received in Special Pathogens Unit in 2002 and 2003

<table>
<thead>
<tr>
<th>Specimens received in 2002</th>
<th>Specimens received in 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnostic:</strong></td>
<td></td>
</tr>
<tr>
<td>Suspected VHF (South Africa)</td>
<td>75 (54 patients)</td>
</tr>
<tr>
<td>Suspected VHF (other countries)</td>
<td>214 (209 patients)</td>
</tr>
<tr>
<td>VHF contacts</td>
<td>113 (113 persons)</td>
</tr>
<tr>
<td>Undiagnosed fevers</td>
<td>148 (106 patients)</td>
</tr>
<tr>
<td>Suspected rabies</td>
<td>22 (17 patients)</td>
</tr>
<tr>
<td>Rabies immunity</td>
<td>176 (150 accessions)</td>
</tr>
<tr>
<td>Ticks</td>
<td>2 (2 accessions)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>174 (28 accessions)</td>
</tr>
<tr>
<td><strong>Surveys</strong></td>
<td></td>
</tr>
<tr>
<td>Occupational/residential groups</td>
<td>250 (2 groups)</td>
</tr>
<tr>
<td>Cattle &amp; sheep for zoonoses</td>
<td>8905 (2 accessions)</td>
</tr>
<tr>
<td>Dogs for plague</td>
<td>656 (21 districts)</td>
</tr>
<tr>
<td>Rodents for plague</td>
<td>569 (21 districts)</td>
</tr>
<tr>
<td>Wild animals</td>
<td>590 (13 accessions)</td>
</tr>
<tr>
<td><strong>Total specimens:</strong></td>
<td><strong>11 494</strong></td>
</tr>
</tbody>
</table>

Established that there is widespread occurrence of infection in livestock, with the potential for transmission of infection to humans to occur through tick bite or contact with fresh infected tissues of livestock. The implication was that the infection had long been present in southern Africa, and that failure to recognize human disease had been due to lack of clinical awareness. Once the presence of the virus was established there was increasing recognition of human disease, with 5-20 cases of CCHF being confirmed each year in southern Africa.

The number of suspected cases of VHF in southern Africa referred to the laboratory for investigation has fallen progressively in recent years, and the 30 cases investigated during 2003 (Table 1) represents the lowest total since 1982. Consequently, it seems that the apparent decrease in incidence of CCHF may relate to underdiagnosis due to diminished clinical awareness of the disease. It is also notable that for the first time since 1981 we did not receive any ticks removed from human hosts for identification or virus studies.

The total number of cases of CCHF diagnosed in southern Africa from the time that the presence of the disease was first recognized in 1981 up until the end of 2002 remains at 171, with one infection having occurred in the Democratic Republic of the Congo, one in Tanzania, fourteen in Namibia and the rest in South Africa. Marginally the largest group of cases, 74/171 (43,3%), arose from known tick bite or the squashing of ticks; a similar number, 69/171 (40,3%), arose from known or potential contact with fresh blood or other tissues of livestock and/or ticks; 7/171 (4,1%) nosocomial infections arose from contact with blood or fomites of known CCHF patients, while in 21/171 (12,3%) cases there was no direct evidence of contact with livestock or ticks, but the patients lived in or visited a rural environment where such contact was possible. Most patients were employed in the livestock industry, and males constitute 142/171 (83%) of all cases of the disease diagnosed to date. The case fatality rate fluctuated around 30% in the first few years after CCHF was initially recognized in southern Africa, but has declined to 25,7% (44/171) probably as a result of better case management. Ribavirin is believed to improve the prognosis if administered before day 5 after onset of illness.

Alternative diagnoses established in suspected cases of VHF in southern Africa included the usual admixture of malaria, viral hepatitis including three cases of fulminant herpes simplex hepatitis, bacterial septicemias, tick-borne typhus, Q fever and HIV/AIDS with opportunistic infections.

In contrast to southern Africa, we continued to receive many specimens from suspected cases of VHF elsewhere in Africa and the Middle East, and we confirmed 41 cases of CCHF in Pakistan in association with colleagues at the National Institute of Health in Islamabad. This brings the total to over 100 cases of CCHF confirmed in Pakistan and neighbouring Afghanistan over the
past two years, where it appears that migrant people are being exposed to tick bite on a large scale. We also became involved in the investigation of an outbreak of yellow fever in southern Sudan which had been diagnosed by colleagues at the Kenya Medical Research Institute in Nairobi, as discussed below in relation to the Arbovirus Section of the Special Pathogens Unit.

INVESTIGATION OF UNDIAGNOSED FEBRILE ILLNESSES

Early in the year we received approximately 150 enquiries concerning patients in whom the severe acute respiratory syndrome (SARS) was suspected, and after careful screening of case histories it was decided that laboratory investigations were warranted in 8 instances only. We had received serological reagents from colleagues at the Centers for Disease Control (CDC) in Atlanta, USA, and performed reverse transcriptase polymerase chain reactions (RT-PCR), cell culture, enzyme-linked immunoassay and indirect immunofluorescence tests as appropriate on nasopharyngeal aspirates and serum samples from the 8 patients without obtaining a positive result. The most likely case of SARS occurred in a 62 year old businessman who became ill shortly after returning from a visit to Hong Kong during the epidemic. He was admitted to hospital in South Africa and recovered from pneumonia before succumbing to myocardial infarction on day 28 of illness. He remained virus and antibody negative throughout and our negative findings were confirmed on specimens referred to CDC. Nevertheless, he had received corticosteroid therapy and criteria established by the World Health Organization stipulated that a diagnosis of SARS can only be excluded in patients who receive such treatment if they remain antibody negative for at least 30 days after onset of illness. Consequently, this patient has been recorded as a probable case of SARS.

Later in the year we tested blood samples from outbreaks of dengue fever in Pakistan and the Seychelles as discussed below in relation to the Arbovirus Section of the Special Pathogens Unit.
RABIES

The 11 cases of human rabies confirmed during 2003 (Table 2) is low by comparison with the 20-30 cases confirmed annually 10-15 years ago, and this probably reflects improved dog vaccination coverage. However, it is invariably found that patients who succumb to the disease have received nil or incomplete treatment post-exposure, and this indicates that education of the public remains inadequate. Most cases of the disease continue to occur in children in KwaZulu-Natal Province where dog rabies is most prevalent. One case that occurred in a 9 year old child who was bitten by a jackal in North West Province, was unusual in that the patient, who had received a single dose of vaccine on day 3 post-exposure, survived for over 3 weeks after onset of symptoms. Most patients in South Africa succumb within less than a week after admission to hospital, frequently within 24-48 hours, and the only other patients observed to have undergone a protracted morbid period were two children in KwaZulu-Natal who also received incomplete courses of immunization post-exposure.

Table 2: Confirmed cases of rabies, 2003

<table>
<thead>
<tr>
<th>Name</th>
<th>Age/Sex</th>
<th>District of exposure</th>
<th>Exposure: bitten by</th>
<th>Admitted hospital</th>
<th>Died</th>
<th>Final hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>5f</td>
<td>Lower Umfolozi</td>
<td>Dog, 17 Dec</td>
<td>7 Jan</td>
<td>8 Jan</td>
<td>Ngwelezaan</td>
</tr>
<tr>
<td>KN</td>
<td>8m</td>
<td>Durban</td>
<td>Dog, 2 Jan</td>
<td>13 Jan</td>
<td></td>
<td>RK Khan</td>
</tr>
<tr>
<td>MSS</td>
<td>13m</td>
<td>Cofimvaba</td>
<td>Dog, Nov</td>
<td>15 Feb</td>
<td>11 Feb</td>
<td>Cecilia Makiwane</td>
</tr>
<tr>
<td>MB</td>
<td>3m</td>
<td>Eshowe</td>
<td>Dog, 19 Feb</td>
<td>DOA</td>
<td>3 Mar</td>
<td>Eshowe</td>
</tr>
<tr>
<td>NK</td>
<td>3f</td>
<td>Port Shepstone</td>
<td>Dog, Feb</td>
<td>24 Mar</td>
<td>27 Mar</td>
<td>Port Shepstone</td>
</tr>
<tr>
<td>KM</td>
<td>55f</td>
<td>Klerksdorp</td>
<td>Jackal, 25 May</td>
<td>1 Jul</td>
<td>Aug</td>
<td>Klerksdorp</td>
</tr>
<tr>
<td>VG</td>
<td>7f</td>
<td>Unrecorded</td>
<td>No history</td>
<td>22 Aug</td>
<td>24 Aug</td>
<td>Port Shepstone</td>
</tr>
<tr>
<td>ZS</td>
<td>12m</td>
<td>Tugela Ferry</td>
<td>Dog</td>
<td>25 Aug</td>
<td>2 Sep</td>
<td>Greys</td>
</tr>
<tr>
<td>NZ</td>
<td>7f</td>
<td>Bizana</td>
<td>Dog, Sep</td>
<td>2 Oct</td>
<td>3 Oct</td>
<td>Port Shepstone</td>
</tr>
<tr>
<td>BM</td>
<td>12m</td>
<td>Manguzi</td>
<td>Dog, June</td>
<td>28 Sept</td>
<td>31 Sept</td>
<td>Manguzi</td>
</tr>
<tr>
<td>DM</td>
<td>59m</td>
<td>Port Shepstone</td>
<td>Dog contact</td>
<td>21 Nov</td>
<td>22 Nov</td>
<td>Port Shepstone</td>
</tr>
</tbody>
</table>
PLAGUE SURVEILLANCE IN DOGS AND RODENTS

The Department of Health ceased formal surveillance of plague activity in small mammals during 1999, but provincial health authorities continue to collect small numbers of samples as opportunity permits. Serum samples from 369 dogs and 216 rodents from 14 districts in the Free State Province were tested for antibody to *Yersinia pestis* F1A antigen by enzyme-linked immunoassay, and there were no positive results.

RESEARCH

Research activities were severely curtailed by staff shortages and by disruptions caused by building operations associated with converting our cabinet line laboratory into a second suit laboratory, and converting a separate suite of rooms into a BSL3 facility. The Special Pathogens Unit has a small staff complement, 7 scientists and technologists, and for most of the year we had two vacancies, and one senior scientist was on protracted leave while another spent five months abroad on a training course.

Nevertheless, we managed to complete virological testing of a further collection of specimens from wild animals as part of ongoing Ebola virus ecology studies, and made good progress in phylogenetic studies of some of the animals involved, as well as in phylogenetic studies on rabies virus isolates from human patients in South Africa. Late in the year we conducted a course on SARS diagnosis and outbreak management for 16 laboratory workers, epidemiologists and clinicians from 6 African countries on behalf of the World Health Organization, and we were assisted by 2 epidemiologists from Geneva and 2 scientists from Germany.

JC VIRUS

The Unit received requests to test for the presence of JC virus nucleic acid by PCR on cerebrospinal fluid samples from patients with progressive multifocal leukoencephalopathy (PML). JC is a polyomavirus that causes benign infection in 70-90% of people worldwide, but is associated with the fatal disease PML in immunocompromised individuals. There are at least 7 genotypes of JC virus which appear to have evolved in separate geographic regions conforming to global patterns of human migration. PML is a fairly common complication to AIDS in patients abroad, but the paucity of reports from Africa led to the hypothesis that PML is rare here because of the absence of virus genotypes associated with the condition. Genotypes 3 and 6 have been identified in East and West Africa but the distribution of types across the rest of Africa is unknown. We determined full genome sequences of 5 JC isolates from cerebrospinal fluid samples from PML patients in South Africa. Three isolates from African AIDS patients grouped with type 3 and one from an African AIDS patient in KwaZulu-Natal Province grouped with type 7, while one from a Caucasian leukaemia patient grouped with type 2 (Figure 1). Widespread distribution of type 3 on the continent may reflect African migration patterns in antiquity, but this is the first report of type 7 in an African individual. Genotype 7 is known from Asia, and since large numbers of Indians have settled in KwaZulu-Natal province over the past 2 centuries, it cannot be excluded that this case resulted from a recent transmission event. The type 2 isolate differed from known subtypes and may reflect evolution of JCV in European settlers in South Africa.

ARBOVIRUS SECTION

The total of 369 specimens tested in the Arbovirus Section during 2003 included 255 serum samples from 229 patients in southern Africa with suspected arbovirus infections, 33 from NICD staff members tested for immunity to Rift Valley fever (RVF) and yellow fever, plus 81 specimens from 79 patients which had been submitted to the Special Pathogens Unit from countries elsewhere in Africa and the Middle East for the investigation of undiagnosed fevers.

Among the specimens submitted from patients in southern Africa, 97 sera had demonstrable haemagglutination inhibition (HAI) antibody to one or more arbovirus antigens as shown in Table 1. HAI positive sera were tested by ELISA for IgM antibody activity to the relevant viruses, and 7 patients were found to be IgM positive for Sindbis virus. Dengue virus was isolated from serum samples taken during the acute phase of illness from 3 patients in South Africa, and nucleic acid only was demonstrated by RT-PCR in serum from a fourth patient. The patients had histories of
Fig 1. Phylogenetic comparison of the full coding region of JC virus isolates from South Africa with representative sequences of all genotypes. The midpoint rooted minimum evolution tree was reconstructed with Mega version 2 using the Kimura 2-distance parameter and the close neighbour interchange with search level 2 options under 100 bootstrap iterations. Reference sequences were obtained from Genbank.

recent travel to India, Bangladesh, and Malaysia-Vietnam, and nucleotide sequencing of RT-PCR products identified 3 viruses as belonging to dengue type 2 and the fourth virus as dengue type 3.

Among the specimens submitted from countries abroad, 32 samples came from 27 military personnel with a history of a febrile illness while stationed on peacekeeping duties in the Democratic Republic of the Congo. Antibody reactions provided evidence of recent infection with Sindbis virus in two patients.

The Kenya Medical Research Institute (KEMRI) in Nairobi identified yellow fever virus as the causative agent of an outbreak of suspected haemorrhagic fever in the Imatong region of Torit county, southern Sudan. Specimens were submitted to the Special Pathogens Unit to confirm the diagnosis and in a collaborative study with KEMRI isolates were genetically characterized. A region of the polyprotein gene was amplified using RT-PCR and the nucleotide sequence determined. Five genotypes of yellow fever virus have previously been identified in Africa. The virus circulating during the recent outbreak in southern Sudan belonged to the East African genotype and was closely related to an isolate from a large outbreak which occurred in Kenya in 1993 (Figure 2). The fact that the present virus was closely related to an isolate obtained 10 years ago, supports the contention that yellow fever is now endemic in East Africa, with the potential to cause large outbreaks when circumstances favour transmission to humans.

Antibody to dengue virus, including IgM positive reactions, were detected in sera from outbreaks of febrile illness in two areas of Pakistan, and 5 isolates of virus were obtained. Sequencing of RT-PCR products indicated that the isolates belonged to dengue virus type 2. Antibody to dengue virus was also detected in sera from the Seychelles, and although virus could not be isolated nucleic acid was detected by RT-PCR in one sample. Nucleotide sequencing of the amplicon indicated that the virus belonged to dengue type 1.
**Fig 2.** Phylogenetic tree showing the relationship between yellow fever virus circulating during an outbreak in southern Sudan in 2003 and isolates from previous outbreaks in Africa (data from Genbank), determined using a 572 base pair region of the polyprotein gene, a weighted parsimony method and Phylogenetic Analysis Using Parsimony (PAUP) software. Node values indicate bootstrap confidence values generated from 100 replicates (heuristic search).

**Table 3 :** Numbers of diagnostic sera positive to one or more arbovirus antigens

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ENTOMOLOGY SECTION

CCHF ecology studies were continued on 3 farms which had been selected for high, low and zero prevalence of antibody to the virus in cattle sera. Despite reports from veterinary acarologists that tick prevalences were normal during the year, and despite the fact that our studies were conducted principally during the winter and early spring seasons when immature tick activity is highest for most ixodid species, especially in the genus *Hyalomma*, the number of immature ticks recovered from small vertebrates at the study sites was well below recoveries in previous years. No ticks were recovered from 42 shrews and rodents collected by Sherman live traps over a total of 960 trap-days. The most common small mammal on all 3 farms was the rodent, *Mastomys coucha* (67% of total catch), while the shrew, *Crocidura mariquensis*, was collected only at Olifantsvlei, which has permanently wet pasture as a result of its proximity to a large waste water-treatment facility. Only 2 immature ticks, both *Rhipicephalus* sp. larvae, were recovered from 201 birds collected by mist-net, woosh-net (rubber bungee-powered catch net) and bal-chatri (noosed raptor trap) on the study farms. The 2 immature ticks were collected from a redbilled quelea and a whitewinged widow bird. The low tick recoveries from small vertebrates are probably a consequence of hemi-monthly or monthly acaricide treatment of cattle on the 3 study farms, as all the associated cattle herds were found to be relatively free of ixodid tick infestation. In the past, infestations tended to be heaviest on rodents rather than on birds on the study farms.

A further 91 birds were collected in various parts of North West, Free State, Gauteng, Mpumalanga and Limpopo Provinces, and only 3 ticks, including a *Haemaphysalis* sp. larva and nymph and a *Hyalomma* sp. larva, were recovered from passerine birds in the Molopo Game Reserve near the far western boundary of North West Province. Attempts to isolate virus from all ticks collected from birds during the year were negative.

In order to obtain material to establish colonies for experimental infection studies, 351 ticks were collected from sheep and eland antelope, or as questing, unengorged adults, at Bloemhof (SA Lombard Nature Reserve & Bloemhof Dam Nature Reserve), Strydenburg (Dwaalhoek Farm) and Molopo Game Reserve. Among the *Hyalomma* species, *H. truncatum* constituted the bulk (>95%) of the collection with small numbers of *H. marginatum rufipes* and *H. m. turanicum* being obtained. Other species collected were almost exclusively *Rhipicephalus evertsi evertsi* plus single specimens of *Amblyomma marmoreum* and *Rhipicephalus theileri*. Those *Hyalomma* ticks that survived the sampling and transport procedures were used in attempts to establish laboratory colonies, but success was achieved only with *H. truncatum*. Efforts are being made to obtain further material of the remaining two species of *Hyalomma*.

CONFERENCES ATTENDED

Dr Lucille Blumberg attended the International Chemotherapy Conference in Durban from 7th to 9th June 2003 and also the Medicines Control Council’s Drug Resistance Symposium in Durban from 27th to 28th October 2003. She also chaired a session at the National Malaria Conference which was held on 3rd to 4th November 2003 and presented a paper on malaria mortality audits.

The Medical Virology in South Africa Conference in the Kruger National Park was attended by the following members of staff of the Special Pathogens Unit: Professor Bob Swanepoel, Dr Janusz Paweska, Dr Maria Venter, Antoinette Grobbelaar and Roger Phili.

DEGREES AND AWARDS

Maria Venter was awarded a PhD degree by the University of the Witwatersrand for her thesis ‘Molecular epidemiology and cellular immunology of respiratory syncytial virus in South Africa’.

She received the prize for the best scientific presentation by a young scientist at the Medical Virology in South Africa Congress, Berg-en-Dal, and was awarded a post-doctoral fellowship by the National Institutes of Health of the USA to visit the National Institute of Allergy and Infectious Diseases for 5 months for training in microarray technology and research on West Nile virus.
The AIDS Unit is the largest Unit at the NICD and is primarily focused on research into the virology and pathogenesis of HIV. Members of the Unit continue to play a leading role in HIV research in South Africa. Funding of research is largely through grants from the South African AIDS Vaccine Initiative (SAAVI), National Institutes of Health including CIPRA (Collaborative International Program for Research on AIDS), HVTN (HIV Vaccine Trials Network) and RO1 grants, The Wellcome Trust, Bristol Myers Squibb Secure-the-Future Program and the Doris Duke Human Pathogenesis Program.

The Immunology Laboratory, headed by Dr Clive Gray was made a Central Immunology Laboratory of the HIV Vaccine Trials Network (HVTN) and is the first and only international laboratory outside the USA. Within the USA there are three domestic laboratories at Duke University, California Health Department, Richmond and the Fred Hutchinson Cancer Research Center, Seattle. In this capacity, the South African Immunology Laboratory (SAIL) commenced immunology end-point measurements of South Africa’s first HIV vaccine trial which started in November 2003. Furthermore the Immunology Laboratory also embarked on establishing a SAAVI MHC/tetramer Core Facility for southern Africa, which aims to synthesize an array of novel class I tetramers to newly identified epitopes, restricted by common HLA class I alleles in the South African population. The Laboratory was also externally audited by PPD, the NIH and HVTN.

Three PhD degrees were awarded: Dr Sharon Shalekoff, Dr Stephen Meddows-Taylor and Dr Gillian Hunt, all supervised by Dr Caroline Tiemessen who heads the Cell Biology Laboratory. Sibusiso Nkosi was awarded his Masters degree and Isaac Choge and Natasha Taylor submitted their Masters dissertations in 2003.

Dr Penny Moore, a new post-doctoral Fellow, joined the Unit in 2003. Other new staff members are Patience Mthunzi, Priscilla Xaba, Melene Smith, Polly Walker, Sheila Doig, Lesley Short and Kate Ihlenfeldt. Also a farewell to Tumelo Mashishi, who left the Unit in August and is now reading for his DPhil at Oxford University with Professor Sarah Rowland-Jones. The Unit is also hosting a SAAVI visiting fellow from Ghana, Jim Brandful as well as Melissa Ketunuti, a summer student from Stanford University. A number of staff and students had the opportunity of spending time in overseas laboratories: Sibusiso Nkosi was hosted by Dr Richard Wyatt at the Vaccine Research Center at the National Institutes for Health, Elin Gray spent a month with Dr David Montefiori at Duke Medical Centre and a week with Dr John Mascola at the Vaccine Research Center, Dr Agatha Masemola visited Dr John Altman at Emory University with our ongoing collaboration of formation of an MHC tetramer Core Facility for South Africa. Greg Khoury spent time at Duke Medical Center and Stephina Nyoka was hosted by Dr Marcella Sarzotti-Kelsoe, Duke University where she acquired skills to measure T cell receptor repertoire.

Members of the AIDS Unit also participated in major international meetings including the 2nd IAS Conference on HIV Pathogenesis and Treatment in Paris, France; two HVTN full-group meetings in Washington and Seattle; Keystone Symposia in Banff, Canada; Fifth Congress of the Federation of African Immunological Societies (FAIS), Victoria Falls, Zimbabwe; International Conference of AIDS and Sexually Transmitted Infections in Africa, Nairobi, Kenya. In addition a large contingent of staff attended the South African AIDS Conference in Durban where they were involved in the organization as well as presentation of data.

The Unit had a number of visitors in 2003 that included Dr Barney Graham, Dr Guido Ferrari, Dr Mike Betts, Dr Larry Corey, Dr Julie McElrath, Dr Ann Deur, Prof Alan Landay, Prof Louise Kuhn and Dr John Moore.

Some highlights of the data produced in the AIDS Unit in 2003 are summarized below:

**LOW FREQUENCY OF THE V106M MUTATION AMONG HIV-1 SUBTYPE C INFECTED PREGNANT WOMEN EXPOSED TO NVP**
A recent report described a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation among HIV-1 subtype C viruses exposed to efavirenz (EFV), but not nevirapine (NVP) or delavirdine (DLV). This mutation, which encodes a valine to methionine change at position 106 (V106M) causes high-level cross-resistance to both NVP and DLV. Subtype C viruses appear to be pre-disposed to developing this mutation
probably due to differences in codon usage between various subtypes. None of the current drug resistance algorithms highlight the V106M mutation as a drug resistance mutation.

Nevirapine (NVP) used in single doses to prevent mother-to-child transmission (MTCT), has been shown to be associated with the development of transient resistant mutations. We examined 141 pregnant women who received a single dose of 200 mg NVP at the onset of labor to prevent HIV-1 transmission to their infants and found that 7 (5%) had V106M and five (3.5%) had the V106A mutation 6 weeks post-partum. Analysis of the pre-NVP samples from ten of these women showed them all to have a wild type valine at this position suggesting that the V106M and V106A mutations were selected by NVP. It is expected that women who developed V106M following a single dose of NVP would be resistant to all NNRTI during the time that this mutation is present.

NVP has been shown to induce a number of drug resistance mutations, including K103N, V106A, Y181C, Y188C and G190A after a single dose. It will now be necessary to add V106M to this list of mutations for subtype C and databases will need to be updated accordingly. The implications of the induction of the V106M mutation for MTCT are unclear, particularly the impact on a second pregnancy and on subsequent NNRTI-containing HAART regimens. It is likely that this mutation will fade in the absence of drug pressure, as do other mutations selected by single dose NVP.

Increasingly, HIV-infected people in developing countries are accessing anti-retroviral therapies with EFV and NVP included in first-line regimens because of their cost and efficacy. Continual monitoring of NNRTI resistance, including the presence of novel subtype-specific mutations, is needed to ensure the effectiveness of long-term therapy and MTCT programs.

SENSITIVITY OF SUBTYPE C VIRUSES TO THE ENTRY INHIBITOR T-20
T-20 is the first in a new class of anti-retroviral drugs targeting the entry stage of the virus life cycle. It is a 36 amino acid peptide which binds to the HR1 region of gp41 preventing gp41-mediated fusion with the host cell membrane. T-20 was designed based on the HR2 sequence of HIV-1 subtype B gp41, a region which shows significant genetic variation with HIV-1 subtype C sequences. In order to assess the efficacy of T-20 to inhibit subtype C isolates, a total of 23 isolates were tested for their ability to replicate in the presence of T-20. This included 15 isolates that used CCR5, five that used both CCR5 and CXCR4 and three that used CXCR4. Five of these were from patients failing other anti-retroviral therapies. Sequence analysis of the HR2 region indicated that there were 10-16 amino acid changes in the region corresponding to T-20. However, all isolates were effectively inhibited by T-20 at 1 mg/ml. There were no significant differences between viruses that used CCR5 or CXCR4 to enter cells. All isolates, except one, had GIV at positions 36-38 in the HR1 region. One isolate had a GVV motif but this did not affect its sensitivity to T-20. Therefore, T-20 inhibited subtype C viruses despite significant genetic differences in the HR2 region and there was no evidence for baseline resistance to T-20. These data suggest that T-20 would be highly effective in patients with HIV-1 subtype C infection, including those failing existing anti-retroviral drug regimens.

SINGLE-CYCLE FLOW CYTOMETRIC ASSAY FOR MEASURING ANTIBODY NEUTRALIZATION
A new approach for measuring HIV-1 neutralization by flow cytometric detection of intracellular p24 antigen in first round infected cells was recently described by Mascola and co-workers for subtype B isolates. With the assistance of Dr John Mascola we explored the utility of this assay for subtype C viruses and sera. We used CD8-depleted PHA-stimulated PBMCs infected with HIV-1 Du151 isolate at a MOI=0.1 in the presence of the protease inhibitor indinavir. Cells were fixed and stained for intracellular p24-Ag using KC-57 MAb. The percentage of infected cells varied proportionally with the viral input and the number of infected did not increase between days 2 and 3 demonstrating that a single round infection is being measured. In the absence of indinavir, the percentage of positive cells increased 2- to 3-fold by day 3 (data not shown). In the presence of neutralizing sera the number of infected cells decreased and this effect could be titrated out (Figure 1A). The titer at which sera inhibited 80% of virus infection could be calculated by plotting serum dilution against the number of p24 antigen positive cells (Figure 1B). This assay was slightly less sensitive than the standard Ag-capture assay that measures multiple rounds of
Fig 1. Antibody-mediated neutralization measured in a single cycle intracellular p24 antigen assay (ICp24 Ag). Panel A shows flow cytometric plots of HIV-infected PBMC in the presence of different concentrations of HIV-positive serum. There were no p24-Ag positive cells in mock infected cultures while the virus control (no serum) had 2.06% infected cells (boxed). Sera tested at a 1:5 dilution showed a high level of inhibition with only 0.32% cells staining for p24 antigen. The number of infected cells increased as the serum was diluted out. These data are plotted graphically in Figure 1B and show that the serum titre at which 80% of the virus is inhibited (ID80) is 1:6. Figure 1C shows a correlation between the titres obtained using the ICp24 Ag assay and the standard PBMC extracellular p24 antigen assay for 79 serum samples. There was a highly significant correlation between these 2 assays.

infection. There was however, a highly significant correlation between this assay and the standard PBMC assay when using the same virus (Du151) (Figure 1C). Therefore the IC-p24 assay is suitable for high-throughput and rapid measurement of neutralizing antibody titers in HIV-1 subtype C infected persons.

STUDIES ON MOTHER-TO-CHILD TRANSMISSION OF HIV-1: AGE-RELATED CHANGES IN IMMUNE CELL PHENOTYPES
The past year has seen the completion of projects that addressed receptors important in functions of immune cells involved in both the innate arm and the acquired immunity arm of the immune response. Immune responses differ between adults and infants, and studying functional competence of infant immune cells is pivotal to understanding the susceptibility of infants to perinatally acquired HIV-1 infection, their immune responsiveness to other infectious organisms and to vaccines.

AGE-RELATED CHANGES IN POLYMORPHONUCLEAR NEUTROPHIL CHARACTERISTICS IN INFANTS BORN TO HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 SEROPOSITIVE MOTHERS
In infants, the major components of the innate immune system appear weakened, and it has been shown that both polymorphonuclear neutrophil (PMN) production and function are immature. This study was conducted to assess the expression of a number of receptors important
to normal PMN function and the integrity of PMN degranulation in cord blood and in uninfected children of varying ages born to human immunodeficiency virus type 1 (HIV-1) seropositive mothers. Although the expression of L-selectin (CD62L) on PMN did not differ between the infants aged 12, 15, and 18 months, the expression of the interleukin-8 (IL-8) receptors CXCR1 and CXCR2, and the complement 5a receptor CD88 displayed a similar pattern, with the highest levels expressed on PMN from infants in the 12 month old age group, and declining with age. It was also observed that PMN from a substantial proportion of the younger infants were unresponsive to a variety of stimuli including IL-8, complement 5a (C5a), stromal cell-derived factor (SDF)-1α and phorbol 12-myristate 13-acetate (PMA), with the proportions of children showing positive (adult-like) PMN degranulation responses increasing with age. Exposure to HIV-1 did not appear to be the cause of impaired degranulation responses, since a similar proportion of cord blood PMN from uninfected infants born to HIV-1 infected and HIV-1 uninfected mothers were unresponsive. The altered expression of these important receptors and inefficient agonist-induced degranulation in early life may contribute to the increased susceptibility of infants to secondary microbial infections.

AGE-RELATED CHANGES IN EXPRESSION OF CXCR4 AND CCR5 ON PERIPHERAL BLOOD LEUKOCYTES FROM UNINFECTED INFANTS BORN TO HIV-1 INFECTED MOTHERS

Chemokines and their receptors are involved in controlling lymphocyte trafficking, which is a critical component of systemic immunity. HIV-1 has evolved the means to utilize chemokine receptors to facilitate its entry into CD4+ cells, the major ones being CXCR4 and CCR5 that mediate infection with T cell-tropic and macrophage-tropic viral strains, respectively. The immune system of the infant, functionally less mature at birth, undergoes a process of sequential maturation and development that is reflected in qualitative and quantitative changes in a number of leukocyte subsets. Infants have increased numbers of lymphocytes compared with adults, as well as different distributions of the major lymphocyte subsets.

The purpose of this particular study was therefore to assess whether age-related changes exist in the expression of CXCR4 and CCR5 in infants, as this could have relevance for infant immune responses to microbes, and for differing disease susceptibility and progression found between HIV-1 infected adults and children. We monitored coreceptor expression in cord blood (n=27) and in infants 4.5 months (n=9), 9 months (n=16) and 15 months (n=9) of age that were born to HIV-1 seropositive mothers, none of whom breastfed, recruited as part of the PETRA trial. All 61 infants included had been determined HIV-1 seronegative by ELISA at 18 months of age, and hence defined as HIV-exposed-uninfected infants. T-lymphocyte maturation abnormalities have been detected in uninfected HIV-exposed infants, and so results here must not be seen as necessarily representative of uninfected infants that are not exposed to HIV-1. However, a direct comparison of cord blood subsets of infants born to HIV-uninfected mothers showed no significant differences to those uninfected infants born to HIV-infected mothers. This would suggest that at least in utero exposure is not sufficient to result in substantial alterations in the cell subsets studied. Healthy HIV-uninfected adults (n=18; mean age 37.8 years; range 26-57) were included for comparison.

The proportion of cells expressing CXCR4 was highest in cord blood. Thereafter, proportions of CXCR4-expressing cells declined at 4.5 months and then increased to adult levels, a pattern similar amongst the different cell types. CXCR4 expression was significantly higher in cord blood than in adult blood, although differences were very modest. The most dramatic modulation of CXCR4 with respect to age occurred on NK cells, CD14+ monocytes and granulocytes. In contrast to that found for CXCR4, the proportions of cells expressing CCR5 were initially very low and gradually increase with age. Similar patterns were observed for all cell types except for CD19+ lymphocytes, where there was no significant age-related increase in CCR5 expression, and the CD14+ monocytes where there was only a significant increase in expression in the 9 month (P<0.05) and adult group (P<0.01) when compared with the cord blood samples.

Virus populations in the infected infant have been shown to be more homogeneous than those of their mothers, which suggest that either a single genotype is transmitted or is initially replicating in the child. Whether such a restriction in the virus
transmitted, is in any way related to the coreceptor expression on permissive cells remains largely unknown. However, HIV-1 has been shown to primarily infect CXCR4-expressing cells in placentae from nontransmitting HIV-1-infected mothers, whereas infection of predominantly CCR5-expressing cells was demonstrated in placentae from transmitting women. Macrophage-tropism of mother’s HIV-1 isolates has been shown to be associated with an increased risk of transmission of HIV-1 to the infant. Taken together, these data are consistent with transmission of a population of nonsyncytium-inducing (NSI) isolates that use CCR5 as coreceptor.

It is therefore unlikely that peripheral blood receptor expression is a restricting factor for susceptibility to infection by CCR5-utilizing strains of HIV-1, as these strains are very readily transmitted from mother to child. An explanation for this may be that CCR5 can be upregulated by cellular activation, thereby allowing increased viral entry, or that entry of even small amounts of virus can lead to active virus turnover through some postentry enhancement of replication promoted through cellular activation.

Results further showed that differences in cell maturity are reflected in the differential expression of CXCR4 and CCR5 on infant and adult peripheral leukocytes that are also mirrored by alterations in the naïve and memory T-cell phenotype with age. These findings have implications for cellular trafficking in response to the specific ligands of CXCR4 and CCR5, in that these capabilities would be expected to improve as the infant ages. Such differences may in part underlie differences in HIV-1 disease progression between adults and children and have implications for age-dependent susceptibility to other microbial infections in early infancy. In the latter regard, it is particularly noteworthy that CXCR4-expressing NK, CD14, and granulocyte populations increase remarkably with age, whereas CCR5-expressing NK and CD14 cells show a more gradual increase. These findings suggest that CXCR4 may play a more prominent role in the innate arm of the immune system, as opposed to CCR5, which appears to be more strongly associated with cells of the acquired arm of the immune system. How persistent exposure to HIV (intrapartum and postpartum exposure through breastfeeding) might alter these immune parameters and so alter predisposition to infection, or alter the disease profiles of other infecting organisms, remains an important future question for study.

Taken together, the data show that studies in children need to take into account the differences in expression of these two receptors in relation to age. These data add to our knowledge of the developing immune system of the infant that will provide valuable understanding with respect to the pathogenesis of neonatal disease, especially with respect to perinatal HIV-1 infection.

VIRAL DYNAMICS AND CD4 COUNTS IN SUBTYPE C HIV-INFECTED INDIVIDUALS FROM SOUTHERN AFRICA

We describe the course of viral loads and CD4 counts and the use of mathematical modelling to predict the time taken to reach relatively stable viremia. Sixty-five individuals were recruited into the HIVNET 028 study, a four-country study to investigate the natural history of subtype C HIV-1 infection in Zimbabwe, Malawi, Zambia and South Africa. Fourteen participants were excluded from the analysis due to indeterminate times of seroconversion. The median interval time between last antibody negative and first antibody positive was 6.5 months with an interquartile range of 2.3-9.3 months. Participants were followed at 2, 4, 7 and 9 months after enrolment. Fifty-eight HIV-1 seronegative individuals were recruited as controls. Viral loads were assayed by bDNA (version 3.0, Bayer, Emeryville).

Median viral loads between 2-6 months was 4.01 (3.48-4.53, IQR); 7-12 months was 3.82 (3.43-4.4 IQR); 13-18 months was 4.02 (3.46-4.38, IQR); and >18 months was 3.84 (3.29-4.36, IQR). These values indicate that the majority of participants at the earliest time analysed after seroconversion attained a level of viremia that remained relatively stable. A smaller number of individuals showed persistently increased or decreased viremia, relative to baseline values and some showed transient changes that returned to within the baseline range. The overall median viral load of 4.0 log_{10} copies/ml was similar to other subtype C cohorts followed in Ethiopia and was also within range of viremia measured in CRF02-infected female sex-workers without a sexually transmitted disease in west Africa.

Set point was defined as viral load changes not exceeding 0.5 log_{10} variation on at least two
Fig 2. Curve fitting of log_{10} viral load trajectories using a basic mathematical model for 44 of 51 participants who had the minimum requirements for parameter estimation, where viral set point was solved by: \( V^* = \frac{d(ks/dc - 1)}{dc} \). The plot shows the interquartile ranges for both the time to set point and the final set point. The Nelder-Mead search method was used to find the best fit of the model for each individual data point. Curve fitting was done and the best fit was defined in terms of square distance between actual data and modeled data, while taking into account two further assumptions: a) CD4 count upper limit never exceeded 1200 cells/ml and b) death rate of infected cells (\( c \)) was less than the death rate of virus (\( c \)). A curve for each participant was rooted by the first actual viral load data point.

consecutive time-points. Curve fitting of the modeled data showed the trajectory of viral loads (Figure 2), where the calculated median time to set point was projected to be 16.57 (12.7-22.5, IQR) months and the median set-point distribution was 4.08 log_{10} (3.59-4.42, IQR) RNA copies/ml.

The fluctuations observed within the first 17 months after seroconversion was a product of viral load and CD4 changes for each participant. These data have implications for designing future phase III vaccine trials in South Africa, where our viral load set-point values may be used as benchmark values.

FULL GENOME SCREENING OF SUBTYPE C HIV-1 INFECTED INDIVIDUALS

Understanding the relationship between the breadth and magnitude of T cell epitope responses with viral load is important for designing effective vaccines. We screened a cohort of 46 subtype C HIV-1 infected individuals recruited as part of HIVNET 028 cohort for T cell responses against a panel of peptides corresponding to the complete subtype C genome. We used the IFN-γ ELISPOT assay to explore the hypothesis that patterns of T cell responses across the expressed HIV-1 genome correlates with viral control. The estimated median time from seroconversion in the cohort was 13 months and the order of cumulative T cell responses was Nef > Gag > Pol > Env > Vif > Rev > Vpr > Tat > Vpu. Nef was the most intensely targeted protein where 97.5% of epitopes were clustered within 119 amino-acids, constituting almost one third of responses across the expressed genome. The second most targeted region was p24, comprising 17% of responses. There was no correlation between viral load and the breadth of responses, but there was a weak positive correlation (\( r=0.297; \ p=0.034 \)) with the total magnitude of response, implying that the magnitude of T cell recognition did not contribute to viral control. When hierarchical patterns of recognition were correlated with viral load, preferential targeting of Gag significantly (\( r=0.445; \ p=0.0025 \)) associated with viral control. These data suggest that preferential targeting of Gag epitopes, rather than the breadth or magnitude of response across the genome, may be an important marker of immune efficacy. These
Distribution of HIV-1 Specific T Cell Responses across Subtype C: Forty-four of 46 (95.6%) subtype C HIV-1 infected individuals responded to one or more of the 396 peptides used in this study. Figure 3 shows the distribution of sfu/10^6 PBMC from the 44 responders across the expressed genome, with 87% recognizing Nef; 83% recognizing Gag; 74% recognizing Pol; 63% Env; 28% Vif; 22% Vpr; 17% Tat; 15% Rev and 2% recognizing Vpu. When median sfu/10^6 PBMC values were compared across different regions of the genome, the median response to Nef was highest. No significant differences existed between Gag, Pol, Env and Nef sfu/10^6 PBMC. However, these responses were all significantly higher than Vif, Vpr, Tat, Rev and Vpu (Figure 3).

Correlation between HIV-1 specific T cell responses and plasma viremia: There was a low (r=0.297), but significant (p=0.034) correlation between total cumulative sfu/10^6 PBMC responses from all protein regions with plasma viral load analyzed in 44/46 participants. A more detailed analysis of region-specific T cell responses and viral load, revealed no significant associations, except a highly significant (p=0.002) positive correlation of 0.424 between Nef-specific responses and viral load. When total responses were correlated with CD4 count, there was no significant correlation (r=-0.134; p=0.57).

Correlation between the hierarchy of HIV-1 specific T cell responses and plasma viremia: When the cumulative sfu/10^6 PBMC of the 44 individuals who responded to the peptide pools was ranked in order of magnitude, the overall order of recognition was Nef > Gag > Pol > Env > Vif > Rev > Vpr > Tat > Vpu (Figure 4A) and when adjusted for protein length, the order became Nef > Gag > Rev > Vif > Vpr > Tat > Pol > Env > Vpu (Figure 4B). Combined, these data reflect the dominance of Nef and Gag HIV-1 specific T cell epitope responses within this cohort. As there appeared to be a clear hierarchy of responses within the cohort, we wished to identify on an individual basis whether hierarchical rankings were associated with plasma viral load. Thus, for each individual, the total sfu/10^6 PBMC per protein was ranked in order of magnitude from 1-9 (corresponding to the gene regions). When these resulting hierarchies were plotted with viral load, a significant positive correlation existed for Gag (unadjusted for protein length: r=0.445; p=0.0025 and adjusted for protein length: r=0.428; p=0.0027, Figure 4C), suggesting that when Gag is the preferred target by CD8+ T cells, there was an association with a significantly lower viral load.
A similar positive association was not found for any other region (Figures 4D-F). However, there was a significant negative correlation with the unadjusted Nef hierarchy and viral load, which when adjusted for protein length was no longer significant (unadjusted for protein length: \( r = -0.317; p=0.025 \) and adjusted for protein length: \( r = -0.203; p=0.162 \), Figure 4F). There was also no significant association between the hierarchy of Gag recognition with overall immune status of individuals, as measured by CD4 count \( (r=-0.07; p=0.57) \).

**IDENTITY OF NOVEL AND PROMISCUOUS CTL EPITOPES IN EARLY HIV-1 INFECTION**

As a follow-up to showing the potential importance of Gag recognition in reference to viral control, we have characterized optimal CTL epitopes in Gag that may directly inform vaccine development and indicate the universal nature of Gag recognition. We screened 38 individuals with recent subtype C HIV-1 infection using IFN-γ ELISPOT assay and HLA restrictions were defined using CTL killing assays. Seventy-four percent of individuals recognized at least one Gag peptide pool and ten epitopic regions were identified across p17, p24 and p2p7p1p6. Over two-thirds of targeted regions were directed at: TGTEELRSLYNTVATLY (p17, 35%); GPKEPFVRDVYDFFKTLRAEQATQDV (p24, 19%) and RGGKLDKWEKLRPGKKHYMLKHL (p17, 15%). When the epitopic regions were aligned with sequences derived from the autologous infecting viral populations, consensus subtype C from southern Africa and consensus M, it was evident that the regions targeted were highly conserved. Fine epitope mapping revealed that 5/9 identified optimal Gag epitopes were novel: HLVواسر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، ليفر، L
epitopes from subtype B HIV-1 infection. In conclusion, these data emphasize the promiscuous nature of epitope binding and supports our hypothesis that HLA diversity between populations can shape fine epitope identity, but may not represent a constraint for widely shared recognition of Gag in highly conserved domains.

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