NATIONAL HEALTH LABORATORY SERVICE

Annual Report: 2004

NICD

National Institute For Communicable Diseases

Of The National Health Laboratory Service

National Institute for Communicable Diseases

of the National Health Laboratory Service

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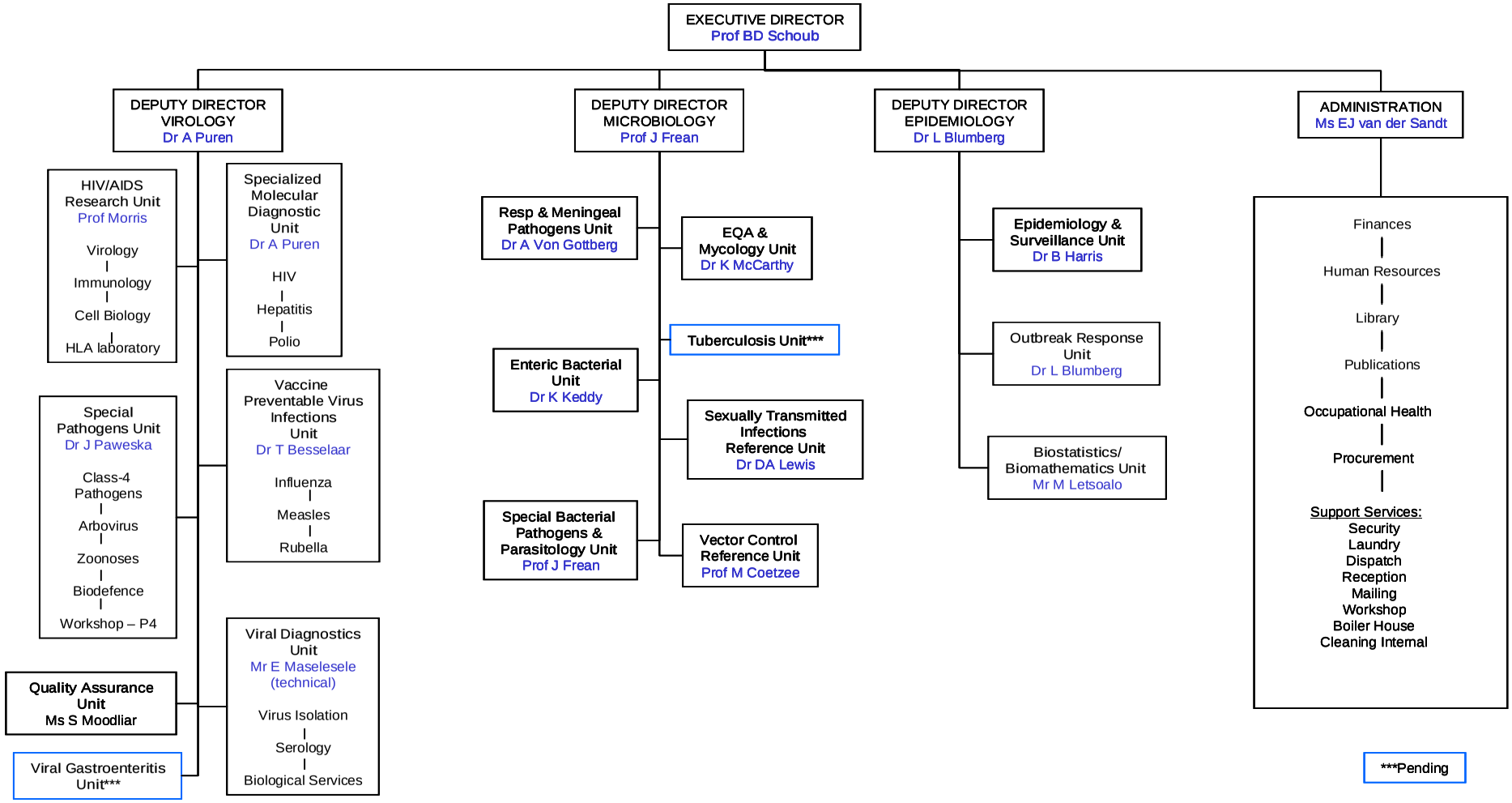
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES



Director's report

Prof Barry D Schoub
Executive Director

During the year 2004 major building operations took place on the campus of the NICD. These operations are aimed at achieving 4 main goals:-

1. The relocation of the Microbiology Division of the Institute to Sandringham from their present location in Braamfontein to physically join with the remainder of the NICD on the Sandringham site.
2. The establishment of a single building for the various laboratories comprising the HIV/AIDS Research Unit.
3. The upgrading of the BSL-4 laboratory.
4. The relocation and consolidation of the Administration section of the Institute.

Building operations have taken place in a number of phases simultaneously and this has unfortunately caused considerable operational disruption. However, the activities and the productivity of all sections of the Institute was little affected.

This year's annual report reflects how the NICD has grown in leaps and bounds and now, indeed, does occupy the position of a major resource on the African continent for monitoring, training and research into the public health aspects of communicable diseases. The Institute's laboratories, both virology and microbiology, are utilised extensively by global health bodies such as WHO for reference and for training as well as quality assurance programmes. Many of the Institute's staff now serve as technical advisors and also on expert committees of WHO in a variety of the latter's programmes. A number of collaborating research programmes are now underway with prestigious institutions and academia abroad and the Institute has also received a number of visiting scientists during the year.

The newest, and smallest of the divisions of the NICD, the Epidemiology Division has, in the very short time of its existence, taken vast strides in establishing itself as a particularly valuable resource to the country. It has assisted in several outbreaks including the avian influenza outbreak in the ostriches of the Eastern Cape. It has provided particularly valuable on-site training to rural laboratories on the laboratory support for the management of outbreaks and has been an invaluable resource for consultation for microbiological emergencies.

The NICD owes a deep debt of gratitude to its parent body the National Health Laboratory Service (NHLS) for the enormous support and encouragement as well as the considerable financial support that it receives from it. The support of the NICD by the NHLS has demonstrated that NHLS, in addition to providing the country with its diagnostic laboratory services, is also strongly committed to building capacity and research in communicable diseases in the interests of the country's public health. The NICD is also indebted to the Department of Health for its continuing major material support of the Institute as well as a number of research organisations, both internationally and nationally.

The staff of the NICD have performed sterling work over the past year and the scientific productivity, as demonstrated in this years annual report, reflects this. A record number of 12 students from the NICD graduated with 2 PhD's, 9 MSc's and 1 FFPath through the University of the Witwatersrand. The laboratories maintained their excellent standard and were fully re-accredited by SANAS (the South African National Accreditation Standard).

I would again like to thank Ms Liz Millington for her excellent production of this year's annual report.

BARRY D SCHOUB
EXECUTIVE DIRECTOR

DIRECTORATE
Professor BD Schoub MB BCh MMed MD DSc FRCPath FCPath (SA) FRSSAf
Executive Director
I Latsky, Personal Assistant
D van der Sandt BComm (Hons) MBC, Corporate Services Manager

A/Professor John Frean

Microbiology

This section of the NICD is scheduled to join the main body of the Institute at the Sandringham site early in 2005. It comprises of the following entities, currently housed at the central NHLS campus:

Vector Control Reference Unit (VCRU)
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)

PARASITOLOGY REFERENCE UNIT

CURRENT RESEARCH PROJECTS

- A research study entitled 'Survey and management of drug resistant *Pneumocystis jiroveci* pneumonia in South Africa', in collaboration with the Swedish Institute for Infectious Disease Control, is underway. The project will be funded for a total of 3 years.
- A related project, 'Utility of S-adenosylmethionine as a plasma marker of infection with *Pneumocystis jiroveci*' has also received MRC funding for 3 years.
- A study comparing the performance of 2 rapid malaria kits was completed.
- A survey of stool parasites in children from the Western Cape was completed in collaboration with Stellenbosch University.
- A study comparing the performance of 2 immunofluorescent antigen kits for *Pneumocystis* was completed.

TRAINING ACTIVITIES

- A laboratory training course in malaria diagnosis was conducted in March and 23 participants attended.
- A practical course in *Pneumocystis* diagnosis, in collaboration with the Swedish Institute for Infectious Disease Control, was conducted in October and 12 participants attended.
- 2 registrars were trained during 2004.
- The Unit was involved in lectures and practicals for the Diploma in Tropical Medicine & Hygiene course.
- PRU staff participated in the Graduate Entry Medical Programme for 3rd year medical students of the University of the Witwatersrand.
- First year medical students were given practicals in Parasitology by PRU staff.

External Quality Assessment Programmes:

- The Unit coordinated 2 EQA programmes, stool and urine parasites and blood and tissue parasites, each with 3 surveys for the year and over 130 participants in each programme.

INTERNATIONAL MEETING PARTICIPATION:

- The Unit co-hosted an international symposium, entitled *Pneumocystis* pneumonia (PCP) in South Africa, with the Swedish Institute for Infectious Disease Control. The Unit presented 2 papers. Over 50 participants from southern Africa attended, including 3 Swedish colleagues.
- The Unit participated in the African-European Conference on Travel Medicine in February in Cape Town, and the Tropical Health Innovation Forum, Cairns, Australia, in July 2004.

CONFERENCE PAPERS AND POSTERS PRESENTED:

1. Frean JA. Tick bite fever in Africa (invited paper). African-European Conference on Travel Medicine, Cape Town, February 2004.
2. Frean JA. Assessment of malaria parasite load using image analysis and capture-recapture sampling (poster). Tropical Health Innovation Forum, Cairns, July 2004.
3. Dini L. Pneumocystis diagnosis. Davies Diagnostics Research Day, Centurion, July 2004.
4. Frean J. Occurrence of malaria in non-endemic areas. Swedish-South African Medical & Health Care Dialogue, Johannesburg, October 2004.

PUBLICATIONS:

1. Frean JA, Dini LA. 2004. Unusual anoplocephalid tapeworm infections in South Africa. Annals of the Australasian College of Tropical Medicine; 5 (1): 8-11.
2. Blumberg L, Frean J. Dermatological manifestations of tropical diseases. SA Dermatology Review (2004); 4 (2): 5-14.
3. Frean J. Parasitic infections in the ICU. In: Feldman C, Sarosi GA, (eds) (2004). Tropical and Parasitic Infections in the Intensive Care Unit. Springer, New York.
4. Keddy K, Goldsmid JM, Frean J. Tropical gastrointestinal infections. In: Goldsmid JM, Leggatt PA, (eds) (2004). Primer of Tropical Medicine. Australasian College of Tropical Medicine, Townsville. (http://www.tropmed.org/tropmedprimer.htm)

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT

CURRENT RESEARCH PROJECTS

RATZOOMAN is a multicountry, multidisciplinary study of disease risks linked to rodents at the rural/peri-urban interface, 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 (Mapate), Durban (Cato Ridge), and Port Elizabeth. The results of the investigations into seroprevalence of the three diseases in trapped rodents are given in the table below. To date 3758 specimens have been tested. Plague is clearly continuing its quiescent phase in the country; the substantial prevalence of both toxoplasmosis and leptospirosis draw attention to these zoonotic infections that are almost certainly underdiagnosed in humans in South Africa.

Seroprevalence of 3 zoonotic infections in wild-trapped rodents in rural and peri-urban areas in South Africa

Disease	% of specimens positive per study site			Total
	Limpopo	Durban	Port Elizabeth	
Leptospirosis	16	10	22	18.5
Toxoplasmosis	21	4.5	17	13.5
Plague	0	0	0	0

Rat weighing, plague course, Madagascar

In vitro antimicrobial susceptibility studies in *Bacillus anthracis* continued, in collaboration with Prof Keith Klugman, Emory University, Atlanta, USA. The comparative susceptibility to 2 quinolone antibiotics of 50 southern African strains of *B. anthracis* was submitted for publication at the end of 2004 to the Journal of Antimicrobial Chemotherapy.

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

SURVEILLANCE ACTIVITIES

- SBPRU was active in drawing up new national plague control guidelines in 2004, 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 and other areas.
- The unit is a member of the WHO global network of experts and laboratories for anthrax and plague.

TRAINING ACTIVITIES

Lorraine Arntzen was a member of the faculty of a training course in plague diagnosis held in Antananarivo, Madagascar, in September 2004, under the auspices of the WHO. At home she taught registrars about plague and anthrax as part of the annual NICD training rotation.

Gram stain viewing

IF stain preparation, plague course, Madagascar

External Quality Assessment Programmes:

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 2004. 14 African plague laboratories participate in the programme (see EQA Unit report below).

INTERNATIONAL MEETINGS ATTENDED

L. Arntzen: RATZOOMAN Regional Meetings, Maputo, 11-13 February and Copenhagen, 19-25 Sept. 2004.

CONFERENCE PAPERS & POSTERS PRESENTED

Arntzen L. Zoonotic infections feedback. RATZOOMAN Regional Meetings, Maputo, 11-13 February and Copenhagen, 19-25 Sept. 2004.

PUBLICATIONS

Frean J, Arntzen L, van den Heever J, Perovic O. Fatal type A botulism in South Africa, 2002. Transactions of the Royal Society of Tropical Medicine and Hygiene (2004); 98: 290-295.

STAFF OF THE PARASITOLOGY & SPECIAL BACTERIAL PATHOGENS UNITS

Associate Professor JA Frean, Principal Pathologist
Ms L Dini, Section Supervisor
Ms R van Deventer, Medical Technologist
Mr J Mathebula, Student Technician
Ms L Arntzen, Laboratory Manager

EXTERNAL QUALITY ASSESSMENT UNIT

Dr Kerrigan McCarthy

CURRENT PROJECTS

The EQA Unit produces External Quality Assessment programmes for the NHLS and subscribing private laboratories in the disciplines of bacteriology (120 laboratories), tuberculosis microscopy (220 laboratories), tuberculosis culture (25 laboratories) and syphilis serology (220 laboratories). Management of EQA programmes involves technical preparation and quality control of material, documentation and shipping, evaluation and reporting of laboratory responses. A teaching programme which participating laboratories may use as a training resource accompanies the Bacteriology EQA programme. Results of laboratory performance are reported to appropriate NHLS management structures. The EQA unit has assisted the Northern Branch of the NHLS with training visits to laboratories in preparation for potential cholera outbreaks.

The unit also manages all aspects of a WHO grant-funded bacteriology EQA programme to 65 national public health laboratories in the African Regional Office (AFRO) of the World Health Organisation. The EQA Unit has played an active role in reporting on laboratory capacity in the AFRO region, and has advised the WHO on implementation of a similar EQA programme in the EMRO region. Staff from the EQA Unit acted as technical advisors on a site visit to Tanzania and Zanzibar, and to a Heads of Laboratories Meeting in Harare.

The EQA Unit manages the National Stock Culture Collection, which is a national resource responsible for maintenance of microbiological cultures for use in quality control procedures throughout the NHLS. The old culture collection of the SAIMR has been extensively revised, new cultures from recognised culture collections have been purchased, and storage and documentation protocols implemented. This has been done with technical input from the World Federation of Culture Collections.

TRAINING AND QUALITY ASSURANCE ACTIVITIES

Training Visit to Tonga, Shongwe and Embuhleni laboratories in Mpumalanga province, 10-12 November 2004

Site visit to Muhimbili Medical Centre, Dar Es Salaam, and Mnazimmoja Hospital, Zanzibar, Tanzania, as WHO technical advisors, September 27-October 5, 2004.

INTERNATIONAL MEETINGS ATTENDED

Training course on Culture Collection Management in Rabat, Morocco. 3-7 May 2004 World Federation of Culture Collections

WHO Meeting of Experts on Advocacy for National Public Health Laboratories, 3-5 December 2003, Lyon France

MYCOLOGY UNIT

CURRENT RESEARCH PROJECTS

The Mycology Unit completed the Gauteng Cryptococcal Surveillance project that ran from 2002-4. This entailed collection of clinical information and isolates from all cases of cryptococcosis from 2002-4. These isolates were identified and characterised by the laboratory, and their varietal status determined (*Cryptococcus neoformans var neoformans or C. neoformans var gattii*). The Unit is collaborating with the Centers for Disease Control in Atlanta who assist us with advice and training on antifungal susceptibility testing of isolates.

SURVEILLANCE ACTIVITIES

C neoformans has been included as one of the organisms in the 'Enhanced Surveillance' project that is currently managed by the RMPRU and the EDRU. Funding to cover the cost of additional surveillance officers and support personnel, and scientist has been obtained from the United States .... grant. National surveillance for *C neoformans* will commence in January 2005.

TRAINING AND QUALITY ASSURANCE ACTIVITIES

The Mycology Unit manages all aspects of the Mycology Basic and the Mycology Advanced EQA programme for NHLS and private subscribing laboratories. The Mycology Advanced

programme consisted of two surveys sent out in 2004 to 20 laboratories. From 2004 the Mycology Advanced programme has included an additional specimen of a more unusual isolate or an isolate that is difficult to identify. The results for this isolate are not marked and serve as a challenge to the laboratory personnel. This serves as an extra teaching exercise for the participating laboratories. Mycology Basic programme was sent to all NHLS laboratories that participate in the bacteriology EQA programme.

STAFF OF THE MYCOLOGY & EXTERNAL QUALITY CONTROL UNITS

Dr K McCarthy, Pathologist
Ms V Fensham, Laboratory Controller, EQA
Ms R Mogoboyo, Senior Technologist, EQA
Ms M Smith, Curator, National Stock Culture Laboratory
Ms H Haritos, Senior Technologist, EQA
Ms S Gould, Laboratory Controller, Mycology

ENTERIC DISEASES REFERENCE UNIT

CURRENT RESEARCH PROJECTS

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

The organisms studied were from the current outbreak of cholera, which started in KwaZulu Natal and spread to the other provinces. This outbreak had over 200,000 cases and included a case of laboratory-acquired infection. The *V. cholerae* strain responsible was multidrug resistant, group 01, biotype El Tor, serotype Ogawa. Non-specific diarrhoea was present in the community, so laboratory infection was not suspected at first. PCR and PFGE were performed to check relatedness to the strains being studied. *V. cholerae* 01 serotype Inaba was isolated on several occasions as well.

Results

PCR demonstrated the cholera toxin gene in all those epidemic strains tested (data not shown). PFGE analysis with the restriction enzyme *Not 1* gave a suitable distribution of fragments. The analysis of the V*. cholerae* 01 isolates from the current epidemic, which included the isolate from the laboratory infection, displayed identical banding patterns (Fig 1).

Discussion

PFGE is reproducible because it looks at a stable genotype rather than at variable expressed phenotypic characteristics and is therefore a good typing method used for comparison of strains. The PFGE displayed identical banding patterns, demonstrating that the organisms are highly related in the current epidemic. Further analysis of the *V. cholerae* O1 Inaba

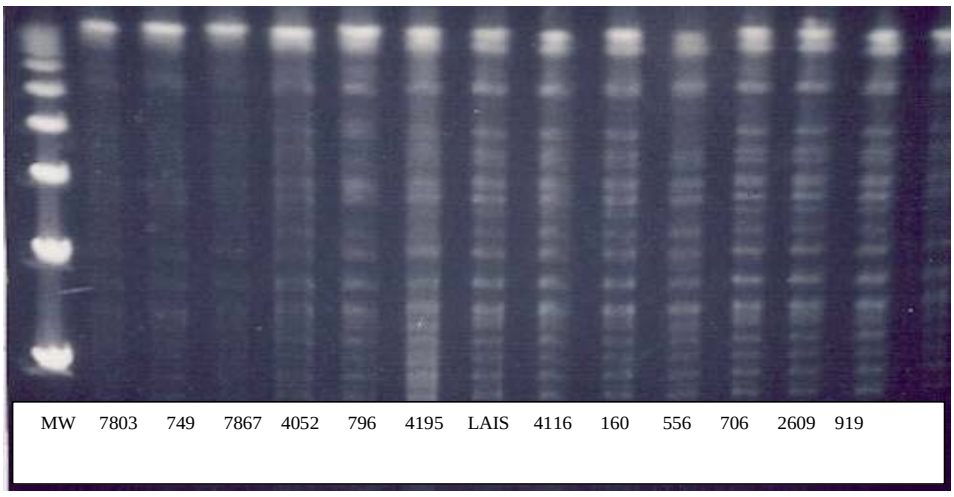


Fig 1. PFGE banding patterns of *Not 1* – digested total cellular DNA from rep-resentative *V. cholerae* strains worked on in the laboratory during the period of infection. Lane 1, Molecular weight marker. Lanes 2-6 & 8-14, strains from laboratory. Lane 7, laboratory acquired infection strain (LAIS).

strains suggests that they are more closely related to the *V. cholerae* O1 Ogawa strains of the current epidemic than to the *V. cholerae* O1 Inaba strains from the previous epidemic in the 1980s (data not shown). The identification of cholera toxin production is an important step in the diagnosis of cholera, because only toxin-producing strains have been associated with severe, watery diarrhoea and epidemics. Data thus far suggest that the current epidemic is a new epidemic and that the country urgently needs to intervene by providing adequate water supplies for the population.

Dr Karen Keddy, Senior Consultant, EDRU

Molecular characterization of a multidrug resistant *salmonella enterica* subspecies *enterica* serotype Isangi causing nosocomial infections in South Africa.
Salmonella spp. producing extended-spectrum ß -lactamases (ESBLs) have been reported in many countries, but there is no information on their prevalence in Africa. An apparent outbreak of infections with ESBL producing *S. enterica* serotype Isangi (*S*. serotype Isangi) and *S. enterica* serotype Typhimurium (*S*. serotype Typhimurium), likely affecting several thousand people, has been noted in all provinces of South Africa since 2001. Isolates of *Salmonella* spp. were collected from thirteen hospitals located in different cities in South Africa over a five-month period from December 2002 to April 2003. All strains were screened for production of extended-spectrum beta-lactamases (ESBLs) by the double disk diffusion test, and AmpC production by assessing resistance to cefoxitin. ESBL-positive and cefoxitin resistant isolates *were* examined for bla_{SHV}, bla_{TEM}, bla_{CTX-M} and bla_{CMY-2}. PCR confirmed that 15.6% (25/160) isolates produced ESBLs and 1.9% (3/160) produced CMY-2. Nine *S*. serotype Typhimurium, eight *S*. serotype Isangi and three *S*. serotype Muenchen strains produced either TEM-63 or a novel derivative of TEM-63, coined TEM-131. Both TEM-63 and TEM-131 have an isoelectric point of 5.6 and the following amino acid substitutions compared to the TEM-1 sequence: Leu21Phe, Glu104Lys, Arg164Ser and Met182Thr. Additionally, TEM-131 has an Ala237Thr substitution. ESBL producing *Salmonella* spp. have become a significant public health problem in South Africa with particular implications for the treatment of serious non-typhoidal *Salmonella* infections in children, in whom third generation cephalosporins are the preferred treatment.

Enhancement of surveillance for trimethoprim-sulfamethoxazole resistant invasive respiratory and diarrhoeal disease in South Africa

SURVEILLANCE ACTIVITIES
National surveillance for isolation of bacterial enteric pathogens.

TRAINING AND QUALITY ASSURANCE ACTIVITIES

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

Laboratory training

- A Durban site visit 29 July to 30 July 2004: Arvinda Sooka (laboratory manager, EDRU), Portia Mogale (data clerk, EDRU) included both a data report back as well as reviewing current practices in microbiology laboratories around Durban.
- Surveillance officers specifically hired for the Enhanced Surveillance programme for Enteric, Respiratory and Meningeal pathogens (the GERMS programme) representing all the sites around the country, attended a meeting at NHLS Central Institute from 1-3 November 2004. The meeting was also attended by Dr Anne Schuchat, from the CDC, Atlanta, USA, who has provided critical financial as well as operational support. The meeting encompassed both data report backs as well as educational and debriefing sessions.
- Ms T Kruger accompanied Dr G De Jong (Epidemiology) on a site visit to Maandagshoek to assist in training laboratory staff there in the diagnosis of typhoid fever and other enteric infections.

QUALITY ASSURANCE

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

INTERNATIONAL MEETINGS ATTENDED

- KH Keddy: Annual Enter-net Workshop. Berlin, Germany. 1-3 July, 2004.
- S Nadan: Pathsplash: 44th Annual Congress of the Federation of South African Societies of Pathology. Stellenbosch, South Africa. 4-7 July 2004.

CONFERENCE PAPERS & POSTERS PRESENTED

- Keddy KH. Enhanced surveillance for co-trimoxazole resistance in *Salmonella* in South Africa. Annual Enter-net Workshop. Berlin, Germany. 1-3 July, 2004.
- Nadan S, Mnyameni F, Kruger T, Keddy KH. *Salmonella enterica* serovar Wa: an outbreak in Nelspruit, South Africa (MP22). Pathsplash: 44th Annual Congress of the Federation of South African Societies of Pathology. Stellenbosch, South Africa. 4-7 July 2004.

PUBLICATIONS

1. Keddy KH, Goldsmid JM, Frean JA. Acute gastroenteritis in the tropics. In: Goldsmid JM, Leggat PA, (eds). (2004) http://www.tropmed.org/tropmedprimer.htm.
2. Sooka A, Du Plessis M, Keddy KH. Enterovirulent *Escherichia coli*. *South African J Epidemiol and Infect* (2004) 19: 23-33.
3. Kruger T, Szabo D, Keddy KH, Deeley K, Marsh JW, Hujer AM, Bonomo RA, Paterson DL. Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrob Agents Chemother* (2004) 48: 4263-4270.

STAFF OF THE ENTERIC DISEASES REFERENCE UNIT

Dr KH Keddy, Senior Consultant

Laboratory Staff

A Sooka, Laboratory Controller
S Nadan, Medical Scientist
T Kruger, Chief Biotechnologist
F Mnyameni, Senior Technologist
E Khomane, Laboratory Technician
P Mogale, Data Capturer

POSTGRADUATE STUDENTS

Arvinda Sooka

Masters Dissertation: Detection of South African strains of enterovirulent *Escherichia coli* using a multiplex polymerase chain reaction.
Graduation: November 2004

Tersia Kruger

Masters Dissertation: Molecular characterization of a S*almonella enterica* subspecies *enterica* serotype Isangi causing nosocomial infections South Africa.
Submission date: 31 December 2005

RESPIRATORY & MENINGEAL PATHOGENS RESEARCH UNIT

Professor Keith Klugmen

The Respiratory and Meningeal Pathogens Research Unit paper in Nature Medicine during 2004 showed that 30–40 % of hospitalisations associated with respiratory viral pneumonia are due to pneumococcal super–infection. Drs Klugman and Madhi contributed to the WHO guidelines for the treatment of pneumonia in HIV infected children, published in 2004. The Unit's national surveillance has been further expanded to include *Cryptococcus neoformans* thus allowing the Unit to track the spectrum of opportunistic infections associated with HIV infection, and provide a base line for interventions such as antimicrobial prophylaxis and antiretroviral therapy. Our expanded active surveillance including the provision of a nursing surveillance officer to ten hospitals continues to be supported by CDC/USAID. We continue to monitor the impact of *Haemophilus influenzae* type b (Hib) vaccine on invasive disease in South Africa. The Unit has demonstrated for the first time in Africa the important burden of human metapneumovirus infection in children. We have elucidated a novel mechanism of multiple resistance to macrolides, oxazolidinones and chloramphenicol in the pneumococcus. We will, during 2005, commence studies funded by NIH, USA in HIV infected children on their immunological responses to pneumococcal conjugate vaccine, and we will start to measure the burden of vaccine–preventable disease in HIV–infected adults.

Pneumonias in HIV-infected and -uninfected children

To follow our report on the clinical importance of human metapneumovirus in children in Africa we have documented the molecular epidemiology of human metapneumovirus over three consecutive epidemics. This paper will be submitted in 2005.
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, has been expanded to study the effect of booster responses and the findings were published in Vaccine in 2004. Funded by Biocine, Berna Biotech.
Project leader: Dr M Nicol, Dr R Huebner, Dr N Mbelle.

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 was published in 2003 in Clinical Infectious Diseases. Further analyses of the HIV - infected population included in the study will be submitted in 2005 for publication. An analysis of combination therapy showed that this was associated with decreased mortality in severely ill patients and was published in 2004. We continue to study the molecular relatedness of the strains. Funded by the MRC/NHLS/Wits.
Project leaders: Professor Charles Feldman, Dr Shabir Madhi

Mr Thomas Rafundisani performing pneumococcal serotyping

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 in South Africa

This large study remains 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 observed in HIV- infected children. An analysis of the impact of Hib vaccine on meningitis at Chris Hani Baragwanath Hospital as well as at Kalafong Hospital, and at two hospitals in Argentina was completed and published in the Pediatric Infectious Diseases Journal in 2004. Funded by MRC/NHLS/Wits.

Project leaders: Dr A von Gottberg, Dr S Madhi. Collaborator: Dr A Schuchat, Dr M Martin, CDC, Atlanta, USA. Funding MRC/NHLS/Wits, USAID/CDC.

The identification of amino acid mutations in PBP 2X that confer penicillin-cephalosporin resistance in the pneumococcus

Dr Anthony Smith

Pneumococcal resistance to **$**-lactam antibiotics is mediated by alterations in penicillin-binding proteins (PBPs). Studies have shown that high-level penicillin resistance requires altered PBPs 2X, 2B & 1A; while only altered PBPs 2X & 1A are required to confer high-level cephalosporin resistance. PBP 2X is essential to the development of resistance as it is the first PBP to undergo alteration. Furthermore, altered PBP 2X appears to be a prerequisite for the alteration of other PBPs. We have used the technique of site-directed mutagenesis to identify which amino acid mutations in altered PBP 2X, are involved in the development of high-level penicillin-cephalosporin resistance in Hungarian isolate 3191 (penicillin MIC,16 **:** g/ml; cefotaxime MIC, 4 **:** g/ml). The analysis involved knocking out mutations (reverse the mutation) in PBP 2X, and determining the effect this has on resistance levels. Of the 24 amino acid substitutions occurring in the penicillin-binding domain of PBP 2X, we have found that 5 substitutions were important, i.e., when they are reversed they result in a decreased level of penicillin-cephalosporin resistance. These substitutions are: Thr338 by Pro, Leu364 by Phe, Ala369 by Val, Ile371 by Thr, and Tyr595 by Phe. The paper will be submitted in 2005.

Project leader: Dr Anthony Smith. Funded by the MRC/NHLS/WITS.

Altered PBP 2A and its role in the development of penicillin, cefotaxime, and ceftriaxone resistance in a clinical isolate of Streptococcus pneumoniae

We have discovered the unusual involvement of altered PBP 2A in the development of **$**-lactam resistance in *Streptococcus pneumoniae*. This was investigated amid three identical serotype-14 isolates (designated isolate #1, #2, and #3, respectively) of pneumococci cultured successfully from the blood of an HIV-seropositive child with recurrent pneumonia. The passage of this strain through its human host induced several changes in the bacterium, which is typical of the adaptive and evolving nature of the pneumococcus. An efflux resistance mechanism, which conferred increased ciprofloxacin resistance, was induced in isolates #2 and #3. In addition, faster growth rates and larger capsules were also observed for these isolates, with respect to isolate #1. Notably, compared to isolates #1 and #2, isolate #3 showed a decrease in penicillin, cefotaxime, and ceftriaxone resistance. This change was associated with the replacement of an altered PBP 2A for an unaltered PBP 2A. In all likelihood, these events produced a strain, which evolved into a fitter and more virulent type, isolate #3, that resulted in an aggravated pneumococcal infection and ultimately in the patient's death. This study has been accepted for publication in AAC.

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 **$**-lactam antibiotics is mediated by alterations in penicillin-binding proteins (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 penicillin-susceptible laboratory strain R6 (MIC, 0.015 µg/ml) following transformation of the strain to resistance with altered PBPs from a highly penicillin-resistant isolate. The introduction of altered PBPs 2X and 2B results in a strain with a MIC of 0.25µg/ml, while growth rates are unaffected. When altered PBP 1A

is introduced, high-level resistance results (MIC, 4µg/ml), however there is a great reduction in growth rate (increased mass doubling time). Furthermore, we have proved that this reduced growth rate and fitness cost is the result of an altered glycosyltransferase domain (GD) in the altered PBP 1A. The GD is responsible for glycan strand elongation in cell walls. The following experiments are still planned for strain R6 and the penicillin-resistant transformants: (1) scanning and transmission electron microscopy of growing cells (2) immunofluorescence using anti-PBP antibodies to track the cellular localization of PBPs during cell division.
Project leader: Dr Anthony Smith. Collaborator: Dr Orietta Massidda, Università di Cagliari, Italy. Funded by the MRC/NHLS/WITS.

High-level amoxicillin resistance in pneumococci

The development of **$**-lactam resistance in pneumococcal strains isolated in France is complex, and the involvement of MurMN (an enzyme involved in the biosynthesis of branched-stem cell wall muropepetides) in penicillin and amoxicillin resistance appears to be dependent on specific combinations of altered penicillin-binding proteins (PBPs) 2X, 2B, and/or 1A. An additional non-PBP mediated resistance determinant is required for complete resistance development in these isolates. Efforts are underway to identify this determinant using the method of transposon mutagenesis: random insertion of a transposon into the genome of an isolate in the hope that it will insert into (and disrupt) the gene responsible for causing resistance, and therefore confer a sensitive phenotype. Furthermore, some of these isolates display the unusual phenotype of higher amoxicillin than penicillin MIC (minimum inhibitory concentration). Transformation studies have shown that this phenotype appears to be caused by alterations in PBP 2B.
Project leader: Dr Mignon du Plessis. Funded by the MRC/NHLS/WITS.

Drs Mignon du Plessis and Anthony Smith of the molecular RMPRU division

Analysis of the genetic diversity of Neisseria meningitidis in South Africa

A molecular epidemiology study of *Neisseria meningitidis* (the meningococcus) in South Africa, for the period July 1999 to July 2002 is ongoing. Over 600 isolates have been characterized by pulsed-field gel electrophoresis and over 40 representative isolates of interest were further characterized by multi-locus sequence typing. The results of this study indicate that for each of the five serogroups studied (A, B, C, W135 and Y), hypervirulent clones/clonal complexes, known to have repeatedly caused epidemics and outbreaks worldwide, are circulating in the South African population. The continuous monitoring of the changing epidemiology in South Africa of the clonal composition of meningococci in South Africa has direct implications for public health decisions, with regard to the development of effective control and intervention strategies.
Project leader: Mr Garry Coulson. Funded by the MRC/NHLS/WITS.

Novel mechanisms of resistance to protein synthesis inhibitors in Streptococcus pneumonia

The increase in the prevalence of antimicrobial resistance in *Streptococcus pneumoniae* (the pneumococcus) is of serious global concern. The ability of the pneumococcus to develop

resistance to most antimicrobial agents has lead to the development and use of alternative antibiotics for pneumococcal infections of which quinupristin - dalfopristin, the ketolide telithromycin and the oxazolidinone linezolid are examples. However, resistant isolates are emerging. These antibiotics are bacterial protein synthesis inhibitors and their site of action is the large ribosomal subunit. In this study the mechanisms of resistance in pneumococcal isolates resistant to either synercid, telithromycin or linezolid are being investigated. If the common resistance mechanisms, namely target-site modification and active drug efflux due to the acquisition of *erm*(B) and *mef*(A) genes respectively, are excluded in these isolates, then novel mechanisms are investigated. Resistance to these antibiotics has been shown to be associated with mutations in the bacterial genes encoding 23S rRNA and ribosomal proteins L4 and L22. Two clinical isolates of *S. pneumoniae* resistant to macrolides, linezolid and chloramphenicol were identified. The isolates were found to each contain a 6 bp deletion, resulting in the deletion of two amino acids in a highly conserved region of the ribosomal protein L4 ($_{64}$PWRQ$_{67}$ to $_{64}$P_Q$_{67}$ and $_{67}$QKGT$_{70}$ to $_{67}$Q_T$_{70}$). The gene encoding the mutated riboprotein L4 transformed susceptible strain R6 to macrolide, linezolid and chloramphenicol resistance, proving that the deletions confer the resistance, and indicating that these antibiotics share a common binding site. Growth studies of the R6 transformants showed increased mass doubling times, suggesting that the L4 mutations are associated with a fitness cost. The L4 mutations represent novel mechanisms of resistance to linezolid and chloramphenicol in the pneumococcus.
Project leader: Nicole Edmondson. Funded by the MRC/NHLS/WITS.

Molecular epidemiology of South African serotype 3 pneumococci
Pneumococcal serotype 3 is an important cause of invasive diseases worldwide. Patients infected with this serotype have also been shown to have higher fatality-case rates compared to those infected with other common invasive pneumococcal serotypes such as serotype 1 and 14. The aim of this study is to investigate the genetic clonality of South African pneumococcal serotype 3 isolates. For this purpose, a total of 150 serotype 3 isolates has been selected from isolates recovered from specimens of patients of all ages from Gauteng province. All specimens were collected during January 2000 to the end of 2002. To date, 49 serotype 3 isolates recovered from nasopharyngeal swabs and blood or/and CSF of children under the age of 5 years have been analysed by pulsed-field gel electrophoresis (PFGE) and multi locus sequence typing (MLST). Twelve different PFGE patterns have been identified. All but 2 isolates, shows at least 80% similarity with each other when analysed by PFGE. Five representative isolates selected according PFGE results from the 49 isolates were classified as one sequence type by MLST. The results gathered so far suggest that South African serotype 3 isolates are highly clonally closely related. Molecular typing of the remaining isolates which, were recovered mostly from adults, will confirm the clonality of this serotype within the South African adult population.
Project leader: Kedibone Mothibeli. Funded by the MRC/NHLS/WITS.

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 by Dr Peter Adrian who joined the Unit again after a number of years in Europe. He 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.

Impact of cotrimoxazole prophylaxis on resistance in respiratory pathogens
The widespread introduction of cotrimoxazole for prophylaxis of opportunistic infections in HIV- infected people has major implications for the development of resistance and the selection of multiresistant bacterial clones. We have, 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. Enhanced surveillance commenced in 2003. Currently there are

10 sites involved nationally. Data is disseminated to participants by quarterly reports and LINK newsletter. An analysis conducted in 2004 shows dramatic increases in cotrimoxazole resistance in pneumococcal isolates. This study will be submitted in 2005. Group for Enteric-Respiratory and Menigeal Disease Surveillance in South Africa (GERMS-SA).
Project leaders: Dr A von Gottberg, Dr V Quan, L de Gouveia. Collaborators: Dr Anne Schuchat, CDC, Allison Taylor, Emory University.

Susceptibility of Bacillus anthracis and 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 *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 has been investigated in 2004, and will be submitted for publication in 2005.
Project leaders: Dr John Frean, Lorraine Arntzen. Sponsor: 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 were presented in 2004 at Helsinki, ISSPD.
Collaborator: Dr Gavin Churchyard. Project leader: Dr A von Gottberg.


Dr Shabir Madhi

Role of procalcitonin in enhancing the specificity of bacterial pneumonia diagnosis in children
The usefulness of procalcitonin in improving the specificity of radiographic confirmed pneumonia to measure the efficacy of the pneumococcal conjugate vaccine against pneumonia has been accepted for publication in Plos Medicine in 2005. The study has since been expanded through funding from the Gates Foundation PneumoADIP and WHO to determine whether procalcitonin and CRP may be used as an alternative to CXRs in evaluating the efficacy of a pneumococcal conjugate vaccine in children.
Project leader: Dr Shabir Madhi. Sponsor: WHO and PneumoADIP.

Role of pediatric serotypes in the aetiology of adult pneumonia in Soweto
A protocol submitted to NIH for funding in collaboration with the Center for Aids Research (CFAR) Unit at Emory University to investigate the role of paediatric serotypes of pneumococci in the aetiology of adult pneumonia in Soweto has been funded. A 3 year project is being developed to measure the potential for interruption of 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.
Project Leaders: Dr Werner Albrich, Dr Alan Karstaedt, Dr Shabir Madhi. Funding NHLS/ Wits/MRC; NIH CFAR. Collaborators: Dr Jim Curran, Dr David Stephens, Emory University.

Role of the pneumococcus in viral infections
Using the vaccine as a probe the relationship of bacterial superinfection to the pathogenesis of viral pneumonia was sought in additional analyses of the 9- valent conjugate vaccine trial during 2004. These data were published in 2004 in Nature Medicine. The results prove a major role for pneumococcal superinfection in viral pneumonia hospitalization in children. The data have led to an analysis led by Dr Klugman into the role of the pneumococcus and other bacteria in morbidity and mortality associated with influenza in 1918. The role of the pneumococcus in metapneumovirus related pneumonia will be investigated in 2005.
Project Leader: Dr Shabir Madhi.

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 was published in the Journal of Infectious Diseases in 2004. It demonstrated a preponderance of pediatric pneumococcal serotypes in HIV infected persons and also identified for the first time a preponderance of pediatric serotypes and antibiotic resistance in women compared to men.

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

Prospective bacteraemic pneumococcal community acquired surveillance study 2004
Strains of *Streptococcus pneumoniae* received from 200 adult patients with community acquired pneumonia presenting from May 2004 in both the private and public sectors will be tested for susceptibility to beta-lactams and macrolides, and will be screened for first step mutations of fluoroquinolone resistance.
Project leader: Charles Feldman, Adrian Brink. Project coordinators Dr Anne von Gottberg, Linda de Gouveia

The impact of local fluoroquinolone use in non-responding otitis media (NROM)
Fluoroquinolones are being used as topical solution in children undergoing tympanocentesis. Isolates of *Streptococcus pneumoniae* isolated from middle ear fluid will be tested for fluoroquinolone resistance.
Project leader: Dr Adrian Brink. Project coordinator: Dr Anne von Gottberg, Linda de Gouveia

Investigation of the Streptococcus pneumoniae-derived toxin, pneumolysin as a target of macrolide antimicrobial agents
A significant percentage of patients with severe pneumococcal disease who receive appropriate antimicrobial chemotherapy still die. Proposed alternative treatment strategies may be of considerable potential value in pneumococcal disease. A pneumolysin-deficient strain will be used to determine the influence, if any, of macrolides on the synthesis of pneumolysin.
Project leader Dr A von Gottberg. Collaborators: Prof R Anderson, Prof C Feldman

A rapid immunochromatographic assay for Streptococcus pneumoniae antigen (Binax NOW) on specimens additional to urine from patients presenting with community- acquired pneumonia
This assay is being increasingly used to detect pneumococcal infections. The study is being conducted at Johannesburg Hospital. Comparisons between pneumococcal antigen detection in urine and isolation of pneumococci from nasopharyngeal and oropharyngeal swabs collected on admission, from patients with community-acquired pneumonia are being performed.
Project leader Dr A von Gottberg. Collaborators: Prof Charles Feldman, Emmanuel Musabeyezu.

Long term antibody persistence and booster responses of toddlers to 9 valent pneumococcal conjugate vaccine
This study has been completed and was published in Vaccine in 2004.
Project leader: Dr Robin Huebner, Rosalia Mothupe, Dr Nontombi Mbelle. Collaborator: Dr Helena Kayhty, KTL, Finland. Sponsor: Wyeth.

Guidelines
Together with Professor Klugman, members of the unit contributed to the development of South African guidelines for the treatment of upper and lower respiratory infections in South Africa; to the WHO management guidelines for respiratory infections in HIV infected children; and to international guidelines for the treatment of pneumonia.
Project Leaders; Drs S Madhi, C Feldman.

PUBLICATIONS IN 2004

1 Paterson DL, Ko W-C, von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, Bonomo RA, Rice LB, Wagener MM, Mc Cormack JG, Yu VL (2004) International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum ß-lactamase production in nosocomial infections. *Annals Intern Med;* 140: 26–32.

2 Finch RG, Metlay JP, Davey PG, Baker LJ, on behalf of the International Forum on Antibiotic Resistance colloquium (2002) (includes Klugman KP) (2004) Educational interventions to improve antibiotic use in the community: report from the International Forum on Antibiotic Resistance (IFAR) colloquium, 2002. *Lancet Infect Dis;* **4:** 44–53 (Guideline)

3 Huebner RE, Mbelle N, Forrest B, Madore DV, Klugman KP (2004) Long term antibody levels and booster responses in South African children immunised with nonavalent pneumococcal conjugate vaccine. *Vaccine;* 22: 2696-2700.

4 Buie KA, Klugman KP , von Gottberg A, Perovic O, Karstaedt A, Crewe – Brown HH, Madhi SA, Feldman C (2004) Gender as a risk factor for both antibiotic resistance and infection with pediatric serogroups in HIV- infected and -uninfected adults with pneumococcal bacteremia. *J Infect Dis;* 189: 1996-2000.

5 File TM Jr, Garau J, Blasi F, Chidiac C, Klugman K, Lode L, Lonks JR, Mandell L, Ramirez J, Yu V (2004) Guidelines for empiric antibiotic prescribing in community-acquired pneumonia. *Chest*; 125: 1888–1901. (Guideline)

6 World Health Organization Regional Office for Africa, IMCI (2004) Management of children with pneumonia and HIV in low–resource settings. Report of a consultative meeting Harare, Zimbabwe, 30-31 January 2003. Kebede S, Mason E, Qazi S (Writing team) Klugman KP (Chairman of meeting), World Health Organization, Geneva, Switzerland. (Guideline)

7 Whitney CG, Klugman KP (2004) Vaccines as tools against resistance: the example of pneumococcal conjugate vaccine. *Semin Pediatr Infect Dis;* 15: 86-93. (Invited review)

8 Ambrose PG, Bast D, Doern GV, Iannini PB, Jones RN, Klugman KP, Low DE (2004) Fluoroquinolone – resistant *Streptococcus pneumoniae*, an emerging but unrecognized public health concern: is it time to resight the goalposts? *Clin Infect Dis;* **39:** 1554–6 (Letter**)**

9 Klugman KP (2004) Clinical relevance of antibiotic resistance in pneumococcal infections. In *The Pneumococcus*, Tuomanen EI, Mitchell TJ, Morrison D, Spratt BG (Eds.), American Society for Microbiology, Washington, DC, USA, pp 331-8. (Invited review)

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11 Madhi S, Klugman KP, The Vaccine Trialist Group (2004) A role for *Streptococcus pneumoniae* in viral associated pneumonia**.** *Nature Med;* 10: 811–13.

12 Ball AP, Bartlett JG, Craig WA, Drusano GL, Felmingham D, Garau JA, Klugman KP, Low DE, Mandell LA, Rubinstein E, Tillotson GS (2004) Future trends in antimicrobial chemotherapy: expert opinion on the 43rd ICAAC. *J Chemother;* 16: 419-36. (Review)

13 Martin M, Casellas JM, Madhi SA, Urquhart TJ, Delport SD, Ferrero F, Chamany S, Dayan GH, Rose CE, Levine OS, Klugman KP, Feikin DR (2004) Impact of *Haemophilus influenzae* type b conjugate vaccine in South Africa and Argentina. *Pediatr Infect Dis J;* 23: 842-7.

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16 Baddour LM, Yu VL, Klugman KP, Feldman C, Ortqvist A, Rello J, Morris AJ, Luna CM, Snydman DR, Ko WC, Chedid MB, Hui DS, Andremont A, Chiou CC, International Pneumococcal Study Group (2004) Combination antibiotic therapy lowers mortality in severely ill patients with pneumococcal bacteremia**.** *Amer J Resp Crit Care Med;* 170: 440-4.

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19 Klugman KP, Low D, Metlay J, Pechere J-C, Weiss K (2004) Community acquired pneumonia: new management strategies for evolving pathogens and antimicrobial susceptibilities**.** *Int J Antimicrob Agents;* 24: 411-22. (Review)
20 Paterson DL, Ko W-C, von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP**,** Bonomo RA, Rice LB, Wagener MM, Mc Cormack JG, Yu VL (2004) Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: implications of production of extended–spectrum beta-lactamases**.** *Clin Infect Dis;* 39: 31-7.
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22 Klugman KP (2004) Failures of **$**-lactam therapy for invasive pneumococcal disease. *Pediatr Infect Dis J;* 23: 980-1. (Letter)
23 Klugman KP, Madhi SA (2004) Oral antibiotics for the treatment of severe pneumonia in children. *Lancet;* 364: 1004-5. (Editorial commentary)
24 Sooka, A, du Plessis, M, Keddy K (2004) Enterovirulent *Escherichia coli*. *The South Afr J Epidemiol Infect;* 19 (1): 23-33.
25 Madhi SA, Ismail K, O'Reilly C, Cutland C. Importance of nosocomial respiratory syncytial virus infections in an African setting. *Trop Med Int Health* 2004; 9: 491-8.
26 Boivin G, Mackay I, Sloots TP**,** Madhi S, Freymuth F, Wolf D, Shemer-Avni Y, Ludewick H, Gray G, LeBlanc E. Global genetic diversity of the human Metapneumovirus fusion gene. *Emerg Infect Dis J* 2004; 10: 1154-7.
27 Kristensen IA, Thiel S, Steffensen R, Madhi S, Sorour G, Olsen J. Mannan binding lectin (MBL) and RSV-lower respiratory tract infection leading to hospitalisation in children. A case-control study from Soweto, South Africa. *Scandanavian Journal of Immunology* 2004; 60: 184-8.
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CONFERENCE PAPERS & POSTERS PRESENTED

Professor Keith P Klugman

Mar 2004 11th International Congress on Infectious Diseases, Cancun, Mexico.
May 2004 4th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD), Helsinki, Finland.
Jun 2004 7th Annual Meeting of the British Infection Society, London, UK.
Aug 2004 2004 Royal Australasian College of Physicians Joint Annual Scientific Meeting, Christchurch, New Zealand.
Aug 2004 24th International Congress of Pediatrics, Cancun, Mexico.
Aug 2004 34th National Microbiology Congress, Mexican Microbiology Association, Cancun, Mexico.
Sep 2004 42nd Annual Meeting of the Infectious Diseases Society of America (IDSA), Boston, MA
Sep 2004 4th International Symposium, Public Health: New Challenges in Infectious Diseases and Vaccines, Barcelona, Spain.
Sep 2004 70th Meeting of the American College of Chest Physicians, CHEST 2004, Seattle, WA
Dec 2004 9th Western Pacific Congress on Chemotherapy and Infectious Diseases, Bangkok, Thailand.

4th International Symposium on Pneumococci and Pneumococcal Diseases, Helsinki, Finland, May 9-13 2004:

1 **Cutland C**, Madhi SA, von Gothberg A, Wasas A, Rafundisani T, de Gouveia L, Klugman KP. Impact of age and HIV infection status on differential vaccine coverage of pneumococcal conjugate vaccines (PncCV) in African children hospitalised with invasive pneumococcal disease. (Epi 20-pg 42- poster)

2 **von Gottberg A**, Wasas A, Rafundisani T, de Gouveia L, Madhi SA, Klugman KP. Pneumococcal serotypes causing severe disease in South Africa. (9Epi 31- pg 48- poster)

3 **von Gottberg A**, Wasas A, Rafundisani T, de Gouveia L, Madhi SA**,** Klugman KP and the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). *Streptococcus pneumonia* serotype 1 causing severe invasive disease in South Africa. (Poster presentation Epi-32)

4 **Soma K**, Quan V, von Gottberg A, de Gouveia L, Madhi SA, Klugman KP. *Streptococcus pneumonia:* Outcomes of patients and antimicrobial susceptibility patterns of isolates in South Africa in 2003. (Epi-75, poster)

5 **Madhi SA**, Klugman KP. Use of a 9-valent pneumococcal conjugate vaccine (PncCV) to identify the role of bacteria in viral pneumonia. (New-07, pg 207- Oral).

6 von Gottberg A, **de Gouveia L**, Wasas A, Rafundisani T, Madhi SA, Klugman KP. Increasing beta-lactam resistance in invasive pneumococcal isolates causing disease in South Africa from 1999 to 2002. (Res-26, pg 127 - poster)

7 **Madhi SA**, Huebner RE, Mbelle N, Klugman KP. Efficacy of a 9-valent pneumococcal conjugate vaccine in preventing drug-resistant invasive pneumococcal disease. Res-32, pg 130 - poster)

8 **Smith A M** , Klugman KP. Selection of penicillin-susceptible pneumococcal strains following transformation of a penicillin-resistant strain with a penicillin susceptibility gene. (Res-43, poster)

9 **Du Plessis M,** Bingen E and Klugman KP. Analysis of clinical isolates of *S. pneumoniae* with reduced susceptibility ot amoxicillin. (Poster presentation RES 39)

10 **Ramjee Heera J**, Madhi SA, Klugman KP. Role of procalcitonin (PCT) and C-reactive protein (CRP) in augmenting efficacy DGN-05 estimates of a 9-valent pneumococcal conjugate vaccine (PncCV) against radiologically diagnosed pneumonia (DGN-05 poster).

11 **Quan V**, Soma K, von Gottberg A, de Gouveia L, Madhi SA, Schuchat A, Klugman KP and the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Surveillance of invasive *Streptococcus pneumoniae* disease in South Africa in 2003. (Oral presentation EPI-04)

12 **de Gouveia L**, von Gottberg A, Wasas A, Klugman KP. Evaluation of the Denka Seiken (Tokyo, Japan) slide agglutination test kit as a rapid routine pneumococcal serogrouping/typing method. (Poster presentation DGN-13)

13 **Rafundisani T**, von Gottberg A, Musabeyezu E, Perovic O, Feldman C, Klugman KP. Use of a rapid immunochromatographic assay for *Streptococcus pneumoniae* antigen (Binax NOW) on specimens additional to urine from patients presenting with community-acquired pneumonia. (Poster presentation DGN-17)

14 **Wasas A**, von Gottberg A, Charalambous S, Grant A, Moloi V, Magadla B, Churchyard G, Klugman KP. Comparison of oropharyngeal with nasopharyngeal swabs in detecting carriage of *Streptococcus pneumoniae* in HIV-infected mineworkers in South Africa. (Poster presentation COL-30)

NASF Meeting, Johannesburg, South Africa, May 2004
Von Gottberg A. Surveillance of invasive *Streptococcus pneumoniae* disease in South Africa in 2003. (Oral presentation)

PathSplash Conference, Cape Town, South Africa, July 2004
Von Gottberg A. Surveillance of invasive *Streptococcus pneumoniae* disease in 2003: *Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitides* antimicrobial susceptibility data. (Oral presentation)

Bayer Healthcare Pharma Meeting, Cape Town, South Africa, July 2004
Von Gottberg A. Surveillance of invasive *Streptococcus pneumoniae* disease in South Africa in 2003. (Oral presentation)

ABC's Surveillance Officer Meeting, CDC, Atlanta, USA, July 2004
De Gouveia L. Surveillance for acute bacterial infections in South Africa. (Oral presentation)

WITS Health Sciences Research Day, Johannesburg, South Africa, August 2004
Rafundisani, T, von Gottberg A, Musabeyezu E, Perovic O, Feldman C, Klugman KP. Use of a rapid immunochromatographic assay for *Streptococcus pneumoniae* antigen (Binax NOW) on specimens additional to urine from patients presenting with community-acquired pneumonia. (Poster presentation)
Von Gottberg A, **Rafundisani T**, de Gouveia L, Madhi SA, Klugman KP and the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Penumococcal serotypes causing severe invasive disease in South Africa. (Poster presentation)
Wasas A, von Gottberg A, Charalambous S, Grant A, Moloi V, Magadla B, Churchyard G, Klugman KP. Comparison of oropharyngeal with nasopharyngeal swabs in detecting carriage of *Streptococcus pneumoniae* in HIV-infected mineworkers in South Africa. (Poster presentation)

14th International Pathogenic Neisseria conference, Milwaukee, Wisconsin, USA, 6-10 September 2004
G Coulson. Genotypic characterization of invasive serogroup Y isolates from South Africa and the United States (Poster)

Vaccinology Conference, Hermanus, South Africa, October 2004
Von Gottberg A. Meningococcal disease; control and prevention. (Oral presentation)

INVITED TALKS
Professor Keith P Klugman

Mar 2004	Invited Co – Moderator of Sessions and Speaker, 11th International Congress on Infectious Diseases, Cancun, Mexico.
Mar 2004	Invited Participant, International Society for Infectious Diseases Executive Committee Meeting, Cancun, Mexico.
Mar 2004-Date	Also Washington, DC Oct 2004.
May 2004	Member of Scientific Advisory Board, Invited Speaker and Co-Chairperson of Session, 4th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD), Helsinki, Finland.
May 2004	Chairperson of the 8th Meeting of the Pneumococcal Molecular Epidemiology Network, Helsinki, Finland.
May 2004	Invited Participant, WHO Pneumonia Vaccine Trialists Group Meeting on Standardised Diagnosis of Radiological Pneumonia, KTL, Helsinki, Finland.
May 2004	Invited External Faculty, Guest Lecturer and Chairperson of Session, Symposium on Infections in the Community, Kuwait Institute for Medical Specialization, Faculty of Laboratory Medicine, Jabriya, Kuwait.
Jun 2004	Invited Lecture, Department of Infectious Diseases, Hammersmith Hospital, Imperial College, London, UK.
Jun 2004	Invited Lectures, British Infection Society Trainees' Meeting and Invited Plenary Speaker, 7th Annual Meeting of the British Infection Society, London, UK.
Aug 2004	Invited Keynote Lecture, 2004 Royal Australasian College of Physicians Joint Annual Scientific Meeting, Christchurch, New Zealand.
Aug 2004	Invited Lecture, 24th International Congress of Pediatrics, Cancun, Mexico.
Aug 2004	Invited Lecture, 34th National Microbiology Congress, Mexican Microbiology Association, Cancun, Mexico.
Sep 2004	Invited Member of Meeting as Chairperson of Wellcome Trust Tropical Interview Panel, London, UK.
Sep 2004	Invited Lecture, 4th International Symposium, Public Health: New Challenges in Infectious Diseases and Vaccines, Barcelona, Spain.
Dec 2004	Invited Lectures, 9th Western Pacific Congress on Chemotherapy and Infectious Diseases, Bangkok, Thailand.
Dec 2004	Invited Member of Review Committee, Centers for Disease Control (CDC) International Emerging Infections Program (IEIP), Bangkok, Thailand.

Dec 2004 Chairperson of Malawi Conjugate Pneumococcal Vaccine Trial Steering Group Meeting, Wellcome Trust, London, UK.

Dr S A Madhi

1. **11th International Congress on Infectious Diseases, Cancun, Mexico, March 4- 7, 2004**. The pneumococcus and vaccination - where are we in 2004?: Expectations from countries with high rates of antibiotic resistance beyond the USA. (45.004)
2. 11th International Congress on Infectious Diseases, Cancun, Mexico, March 4-7, 2004. Understanding community-acquired bacterial pneumonia in children: Challenges to diagnosing pneumonia in children. (52:001)
3. *4th International Symposium on Pneumococci and Pneumococcal Diseases,* Helsinki, Finland, May 9-13 2004. Follow-up data from the South African pneumococcal conjugate vaccine trial.
4. 35th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease. Paris, France 28Oct-1st Nov 2004. HIV and Bacterial pneumonia: Diagnosis and Prevention.

RMPRU STAFF, 2004

Prof KP Klugman, Director
Mrs P Hyde, Personal Assistant
Dr A Smith, Senior Medical Scientist
Dr M du Plessis, Senior Medical Scientist
Mr G Coulson, Medical Scientist
Dr A von Gottberg, Senior Pathologist
Ms H Skosana, Med Tech
Ms E Maringa, Data Clerk
Sr K Mawasha, Surveillance Officer
Sr A Motsi, Surveillance Officer
Sr K Mazibuko, Surveillance Officer
Sr M Masuku, Surveillance Officer
Sr C Miller, Surveillance Officer
Dr V Quan, Senior Medical Officer
Dr E Prentice, Senior Medical Officer
Ms L de Gouveia, Senior Med Tech
Mr T Rafundisani, Senior Med Tech
Ms R Mpembe, Senior Med Tech
Ms O Hattingh, Senior Med Tech
Mr M Hlanzi, Data Clerk
Sr W Ngqovu, Surveillance Officer
Sr D Hlatshwayo, Surveillance Officer
Sr R Merementsi, Surveillance Officer
Sr N Nzuza, Surveillance Officer
Sr M Mokwena, Surveillance Officer
Sr N Shalabi, Surveillance Officer

Staff at Chris Hani/Baragwanath Hospital

Dr S Madhi, Deputy Director
Dr J Heera, Research Medical Officer
Dr Z Mlokoti, Research Medical Officer
Dr M Kohler, Research Medical Officer
Ms ML Kuwanda, Statistician/Data Manager
Ms S Fourie, Study Co-ordinator, PoPS
Ms C Taylor, Administrator
Ms F Butler, Data Clerk, PoPS trial
Ms N van Niekerk, Lab Technologist
Ms M Maraba, Lab Technologist
Sr JM Mogola, Midwife, PoPS trial
Sr M Hlobo, Midwife, PoPS trial
Sr M Mpike, Midwife, PoPS trial
Sr N Msimango, Midwife, PoPS trial
Sr V Baloyi, Midwife, PoPS trial
Ms P Dineso, Nursing Assistant
Ms F Tshabalala, Nursing Assistant
Ms E Mudau, Research Asst/Councellor
Dr C Cutland, Senior Medical Officer
Dr G Jaches, Research Medical Officer
Dr K Marran, Research Medical Officer
Dr S Dittmer, Research Medical Officer
Dr P Adrian, Senior Scientist
Ms M Luttig, Study Co-ordinator, CIPRA 4
Ms M Moloi, Administrator, PoPS trial
Ms C Mbuli, Data Clerk, PoPS trial
Ms C Ndou, Lab Technologist
Sr DV Makubire, Midwife, PoPS trial
Sr L Ndiweni, Midwife, PoPS trial
Sr M Moilwa, Midwife, PoPS trial
Sr N Xaba, Midwife, PoPS trial
Sr R Zabale, Midwife PoPS trial
Ms G Senne, Nursing Assistant
Ms S Mmolwa, Nursing Assistant
Ms C Muthanyi, Nursing Auxillary
Sr J Appolis, Research Nursing Sister

SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE

Dr David Lewis
Head : STIRC

INTRODUCTION

The Sexually Transmitted Infections Reference Centre (STIRC) maintained an active programme of research, surveillance, teaching and training throughout 2004. In many ways, the work of 2004 has set the foundations for major projects to start in 2005. Kathy Lucas left in the summer of 2004, followed by Vanessa Maseko in October. Professor Hendrik Koornhof relinquished the position of Acting Head of Department in November and Dr. David Lewis arrived from the UK to take up leadership of STIRC. Dr. Lewis, who has a combined clinical and laboratory background in sexually transmitted infections (STIs), had previously studied at STIRC in both 1996 and 1997 under Professor Ron Ballard's supervision.

CURRENT RESEARCH PROJECTS

Pilot study on the efficacy of male condoms for the prevention of sexually transmitted infections

In collaboration with the Reproductive Health Research Unit (RHRU) of the University of the Witwatersrand and the WHO, STIRC undertook a pilot study among commercial sex workers to determine the efficacy of male condoms for the prevention of 3 common sexually transmitted infections (STIs) in women; gonorrhoea, trichomoniasis and chlamydial infection. The objectives of the study were to assess women's willingness to participate in a planned larger study, to assess compliance with study procedures, the frequency and pattern of client visit/sexual acts, the completion of the coital diaries and to estimate the prevalence of these infections. Pre- and post-coital vaginal/cervical swabs from female sex workers, as well as seminal fluid of male clients, were tested using nucleic acid amplification tests to determine the protective efficacy of male condoms against the different pathogens. Approximately one third of the seminal fluid samples contained at least one of the pathogens of interest. Probable male-to-female transmission of gonococci occurred in only one of 20 sexual acts. For *C. trachomatis*, transmission occurred in only one occasion out of 12, whereas no transmission of *T. vaginalis* occurred (n=12).

Pilot study for the planned herpes suppressive therapy

A pilot study was undertaken in 2004 as preparation for a planned USAID-CDC funded study to address the value of herpes suppressive therapy in reducing the risk of acquisition of HIV in HIV negative miners with serological evidence of HSV-2 infection. The objectives of the study were four-fold: (i) to evaluate the adherence of miners to a oral regimen of twice daily acyclovir, (ii) to assess the feasibility and logistics of both participant recruitment at mine clinics and directly observed treatment (DOT) administration at mine hostels, (iii) to assess miners' reactions to participating in a study which required semen collection, and (iv) to monitor possible breakthrough episodes of genital herpes. Data were collected for 17 participants over five months at Randfontein and Welkom mines. Approximately 65% of miners attended all six follow-up visits. DOT was not successfully achieved as many miners were not working at weekends and had to take their treatment away for home visits. Fourteen of the 17 miners recruited provided questionnaire data regarding semen collection; 6 miners (43%) said they would provide a sample although, overall, 50% had cultural concerns, 29% raised personal concerns and 14% had health related concerns. It was clear that the miners required more explanation as to why semen samples would be required for study purposes as well as assurances that their semen would not be used for another purpose. The miners highlighted the need for a private space to produce the sample and stressed the need for confidentiality relating to study participation.

Episodic acyclovir therapy study in men with genital ulcers

During 2004, preparations were made for the start of one of the NICD-CDC co-operative agreement projects, which will assess the impact of episodic acyclovir therapy on ulcer duration and HIV shedding from genital ulcers among 600 men in South Africa. Herpes simplex virus type 2 (HSV-2) is the primary cause of genital ulcer and one of the most prevalent STIs worldwide. In addition, HSV-2 is now recognised to be a major risk factor for HIV acquisition and transmission in multiple studies. The planned study is a randomised placebo-controlled trial of the effect of episodic acyclovir on symptomatic herpes and on

HIV shedding from genital ulcers and the genital tract (semen). Acyclovir or placebo will be added to conventional syndromic management (antibiotics) for genital ulcers. Ethical permission for the study was obtained from a number of South African and US authorities and permission to start the study was obtained from the South African Medicines Control Council (MCC). Three possible study sites were identified within inner city Johannesburg and it is planned to perform the study in two of these. In December, four nurses were appointed to commence work when the study starts in February 2005.

Expanded periodic presumptive therapy (PPT) study

STIRC, in collaboration with the mining companies and communities, have previously set up mobile clinics in the Lesedi and Welkom areas to administer monthly Azithromycin to, and treat any STIs detected in, women at high risk (WAHR). In Lesedi, this approach resulted in a reduction in STI prevalence of 70-85% and, within nine months, a reduction in both genital ulcers by 78% and gonorrhoea/chlamydia by 43% in miners living nears the intervention site.

Based on this success, USAID has funded a programme to replicate the work in other mining communities (a further site near Welkom, Westonaria and Randfontein). Four new mobile vans were bought in 2004. Baseline and follow-up genital specimens from WAHR were sent to STIRC during the latter part of 2004. In 2005, it is planned to test gonococcal isolates for antimicrobial resistance, especially Azithromycin resistance, as well as determine the effect of PPT on changing patterns of genital ulcer disease. It is hoped that by February 2006, when the project ends, that the STI prevalence in female sex workers will decrease by 90%, that there will be a 95% increase in their use of condoms and a reduction in the STIs among local mineworkers by 90%.

Plasmacute study

The study has been running at STIRC since 2002 in collaboration with the University of Bergen in Norway and Plasmacute. The Plasmacute assay detects the presence of anti-HIV antibodies following lysis of B-lymphocytes extracted from blood with beads. Conventional serological tests, including western blot and two different ELISA tests, are performed on B-cell lysates and on blood from each participant. Participants' blood is also tested for HIV viral load, CD4 count and the presence of the HIV p24 antigen. Participants are followed-up to check clinically and serologically for HIV seroconversion. To date, a total of 154 sero-negative participants have been recruited to the study. In only in two seroconversion cases have the B-cell derived antibodies tested positive for HIV when the p24 antigen and viral load markers were negative.

Urethritis study

This study represents a further collaboration between STIRC and the Reproductive Health Research Unit. The principal objectives are to determine the aetiology of symptomatic, persistent and asymptomatic urethritis as well as the association of urethritis with HIV in inner city Johannesburg. Specimens are screened for the presence of *Mycoplasma genitalium, Trichomonas vaginalis, Ureaplasma urealyticum, Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Although recruitment of men with persistent urethritis has been slow, 227 new cases of acute urethritis have already been enrolled. All gonococci isolates will be screened for resistance to first-line antimicrobial agents, including ciprofloxacin for which resistance is and emerging problem in South Africa.

Collaborative studies with Zimbabwe and Zambia

This was an assessment of targeted versus general population interventions for STI/HIV prevention. The aim of the study was to test the concept that high STI prevalence levels are sustained primarily by transmission within core groups. The studies were carried out in Zimbabwe, South Africa and Zambia among mine workers, plantation workers and sex workers. The study was conducted by the Zimbabwe/South Africa Project support group mainly funded by WHO Population Council and AIDSCAP. STIRC tested 742 urine specimens for *N. gonorrhoeae*, *C. trachomatis* and microscopically examined 800 vaginal smears for bacterial vaginosis. STIRC also provided a quality control service for some specimens tested in Zimbabwe.

Collaborative studies with Swaziland

Two studies involving partnership between STIRC, the Government of Swaziland and the European Commission on the HIV/AIDS Prevention and Care were carried out to address the issue of high rates of sexually transmitted infections and the increasing risk for HIV infection in Swaziland. STI care was improved by strengthening STI case management at public and private clinics, and by strengthening Swaziland's STI Reference Laboratory. An aetiological study of genital discharge was performed on 500 specimens, which demonstrated high prevalences of gonorrhoea (18.2%) and Chlamydial infection (10.2%). A second aetiological study was also undertaken for genital ulcer disease in 100 consecutive STI clinic attendees with genital ulcers and specimens will be tested by STIRC in 2005 using multiplex PCR technology.

SURVEILLANCE ACTIVITIES

National STI Surveillance Programme for South Africa: Clinical Syndromic Surveillance

The National STI Surveillance Programme for South Africa began in November 2003 drawing in many key stakeholders, including STIRC. By the end of 2004, 267 sites were trained in reporting clinical surveillance data. A further three sites in the Limpopo province still require training (scheduled for March 2005). In 2004, three sites in Mpumalanga required retraining due to issues such as high staff turnover. Support visits were also undertaken in Gauteng Province. Reporting rates for the period April to September 2004 were below 50% in three provinces and attempts have been made to improve this through increased training and appointment of co-ordinators in two of these three provinces (Figure 1). A Standard Operating Procedure Manual for the program was developed during 2004 by STIRC staff and is currently being reviewed using feedback and experience from training sessions.

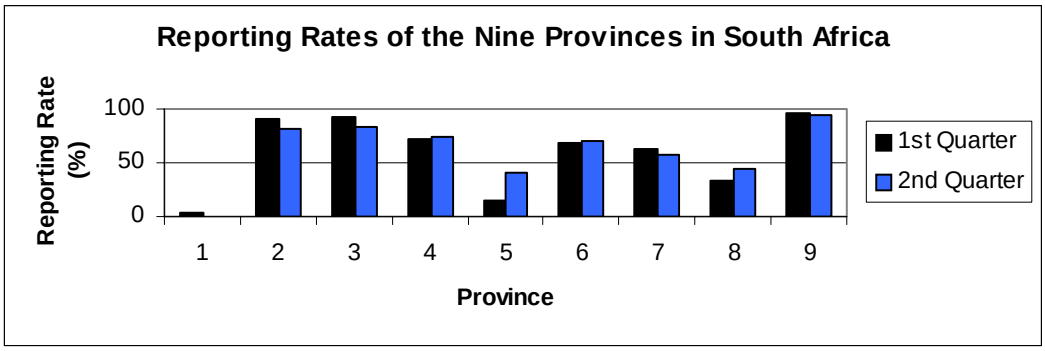

Fig 1. Reporting rates for clinical STI data amongst South Africa's nine provinces (anonymised)

While much has been learnt and progress to date, the program is already significantly behind in attaining its proposed goals and aims. The greatest challenge faced within the National STI surveillance is time adherence to the submission of the monthly provincial statistics from the provinces and the quality of reports being sent to the National Department of Health. From April 2005, it is hoped that sites will return statistics electronically rather than by fax in an attempt to improve data transfer and handling.

National STI Surveillance Programme for South Africa: Antimicrobial Resistance

In conjunction with the National Department of Health, it was decided that the main priority for 2004 was to be a survey of antimicrobial resistance for Neisseria gonorrhoeae isolates collected from patients attending STD clinics in all nine provinces. Laboratory testing would be performed in six centres; STIRC, the University of KwaZulu Natal, the University of Stellenbosch (Tygerburg), The University of the Free State, MEDUNSA and the University of the Transkei. Each centre agreed to test 200 isolates per province, some centres covering more than one province.

Professor Koornhof wrote an interim report in August 2004 that was circulated to the key stakeholders. The key finding was the alarming increase in gonococcal resistance to ciprofloxacin across South Africa: Durban (24%), Pietermaritzburg (8%), Umtata (10%),

Johannesburg (11%) and Cape Town (7%). There are still only minimal data for Mpumalanga and no data from Limpopo or the heavily populated Eastern Cape seaside region. STIRC has urged the Department of Health to meet with the participating academic centres to discuss whether ciprofloxacin should be replaced by a third generation cephalosporin as first line therapy for gonorrhoea in South Africa.

Enhancing syndromic management of STIs in cross border and high transit sites of Botswana, Lesotho, Namibia and Swaziland

In line with its HIV and AIDS Strategic Framework and Programme of Action 2003-2007 and the Maseru Declaration on HIV and AIDS, the Southern African Development Community (SADC) proposed a project which aims to reduce the transmission of STIs/HIV through building capacity for effective and prompt management and monitoring of STIs in designated high transmission areas and cross-border sites in four of its member countries. The four SADC countries where this intervention project is taking place are Botswana, Namibia, Lesotho and Swaziland. In 2004, the project was awarded to a consortium of three organizations: STIRC, the Centre for Health Policy and the Health Systems Trust. Within the tripartite consortium, STIRC has been charged with the specific responsibility of establishing and/or strengthening of routine surveillance systems for STIs at selected cross-border sites and high transmission areas. Routine STI surveillance information is not widely available in the four countries under study. Where these exist, there is need for considerable review and strengthening in order to gain timely, good quality information on a regular basis.

Gauteng provincial STI surveillance

Gauteng clinical surveillance has provided, for a number of years, detailed information on various STI syndromes presenting to 21 sentinel sites within the province. The overall reporting performance of the sites remained good with each site consistently submitting monthly summaries to STIRC during 2004. Although the relative proportion of the main three STI syndromes in both men (Figure 2) and women (Figure 3) remained relatively constant over the period 2000-2004, it was encouraging to see a decrease in the total number of STI episodes reported from the combined sentinel sites over the same time period (Figure 4). Table 1 shows the distribution of male and female STI syndromes in 2004.

Fig. 2

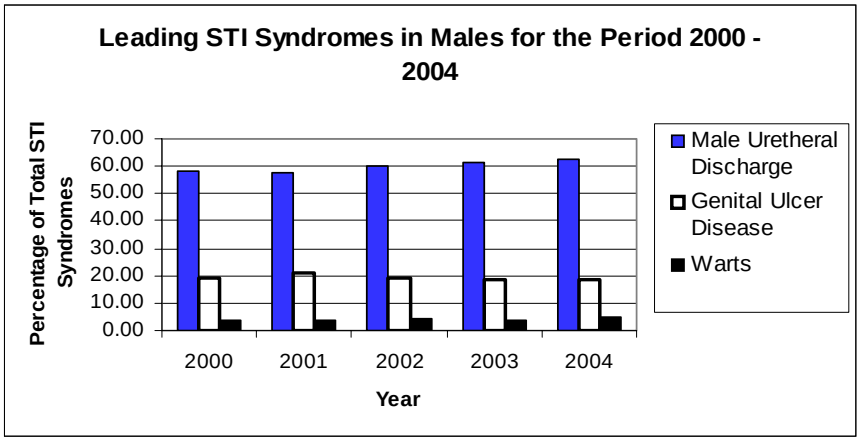

Fig. 3

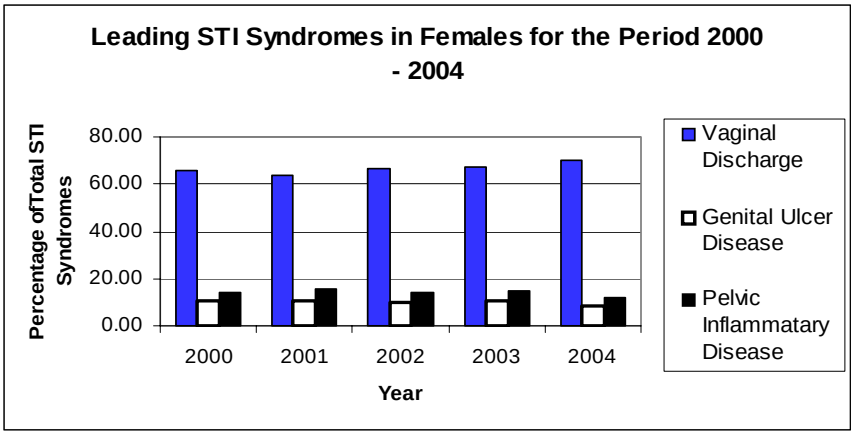

Fig. 4

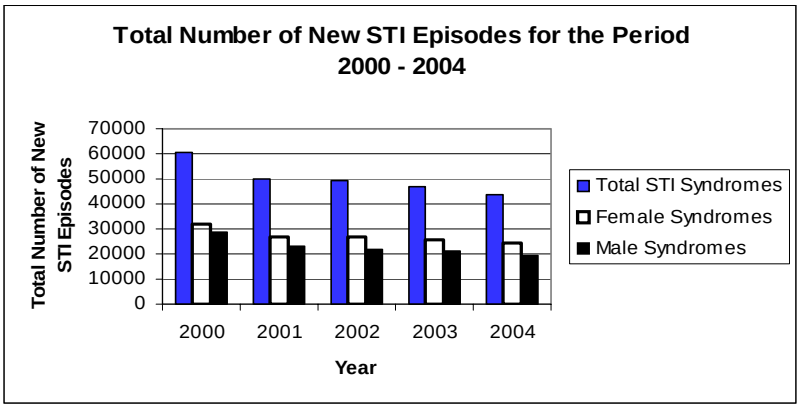

Table 1: Distribution of the main STI syndromes reported in Gauteng in 2004

STI Syndrome	Male	Female
Male Urethritis	63%	Not Applicable
Vaginal Discharge	Not Applicable	70%
Genital Ulcer Disease	18%	8%
Pelvic Inflammatory Disease	Not Applicable	12%
Warts	5%	3%
Bubo	1%	2%
Scrotal Swelling	2%	Not Applicable
Balanitis	2%	Not Applicable
Other Syndrome	8%	6%

An annual review on data received in the year 2004 was undertaken, to guide and improve the implementation, priority and target settings of the surveillance system. Reports written throughout the year influenced the smooth running of STI control and management programme activities at facility level. Improvement of the low partner notification slips issue and receipt rates remains a challenge for 2005. Integration among stakeholders was heightened through intensive collaboration systems to enhance health promotion at facility and community level. STIRC surveillance team staff continued to support health care facilities with surveillance training.

Surveillance of STIs in the Carltonville mining community

For many years, STIRC has been involved in the delivery of STI treatment to women at high risk of STIs in locations adjacent to mining communities. During 2004, STI surveillance on the Carltonville mines was analysed for the period 2000 – 2003 to identify patterns and trends of STIs. Efforts were geared towards improvement of data quality due to a high proportion of mine clinics that were not consistent in reporting through increased personal contact with mine staff. Data for 2004 were subsequently more complete compared to previous years for most Carltonville mines. Manpower compliments for 2004 have also been received which provide denominator data for STI prevalence calculations on the various mines.

TRAINING ACTIVITIES

Training of South African Personnel

In 2004, two STIRC-delivered training sessions on syndromic management for GPs were held in Randburg and Maseru along with sessions on HIV and TB management that were handled by other experts. The GP education programme, which is CPD-accredited, was sponsored by Deutshe Gesellschaft fur Technische Zusammenarbeit (GTZ), a development agency owned by the German Government.

Training of overseas personnel

STIRC is involved in training of overseas personnel as part of the capacity building and ongoing collaboration with the SADC countries. In 2004, three technical personnel from Zambia spent two weeks at STIRC to help them set up an STI laboratory in a major Zambian

University as well as a peripheral laboratory. Additionally, STIRC trained a senior technician from the Ministry of Health in the Seychelles.

Training of STIRC Staff

a) Multiplex PCR training

Dr Chen from Professor Ballard's laboratory at the CDC visited STIRC during December to assist in the implementation and quality control of Real-time PCR on STIRC's Rotorgene 3000 machine. He assisted with multiplex PCR assays for *N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, *M. genitalium* on discharge specimens and *H. ducreyi*, *T. pallidum* and herpes simplex virus on genital ulcer specimens.

b) DHIS Training Course

In July 2004, STIRC surveillance staff participated in a two-day course, run by the Health Systems Trust, outlining the District Health Information System (DHIS). Since the inception of the National STI Surveillance Program, the DHIS has been used as the tool for capturing the routine monthly data from all sentinel sites.

c) Access Training Course

Some of the epidemiological and statistical soft wares such as Epi-info and DHIS used by the surveillance team are access based. Members of the STIRC surveillance team attended one of the Access courses organized by the Central Network Services of the University of the Witwatersrand in October 2004. Knowledge derived was used to create new databases and it also made it possible for staff to work better with our current data systems.

d) HIV Management Course (Israel)

Dr Simeon Odugwu attended a three week Community Based Prevention, Management and Treatment of AIDS workshop at Galilee College, Israel in November 2004. The workshop in November/December aimed to impart advanced leadership techniques to 22 professionals from several countries.

QUALITY CONTROL ACTIVITIES

There is currently no specific national quality assurance programme for the detection and resistance testing of pathogens causing sexually transmitted infections (STIs), due in part to the small number of laboratories involved with STI-related work. In most cases, techniques vary with no accepted 'gold standard' and many STI pathogens are fastidious and difficult to keep viable for more than few days. Options available are freeze-dried specimens for culture and antimicrobial susceptibility testing, smears for bacterial vaginosis and DNA for nucleic acid amplification detection (NAAT). STIRC participates in the ongoing NHLS serology external quality assessment programme for syphilis and rapid HIV testing. It also participates quarterly in the College of American Pathologists' programme for NAATs encoding for *C. trachomatis*, *N. gonorrhoeae* and agents causing genital ulcer disease. Different technologies such as BD Probe Tec ET, Gen-Probe Aptima, Roche Amplicor, Roche Cobas and other in-house methods are compared among more that 600 participating laboratories worldwide.

INTERNATIONAL MEETINGS ATTENDED

Dr Lewis attended and presented at the following international meetings in 2004:

Joint Meeting of the British Association of Sexual Health and HIV with the American Sexually Transmitted Diseases Association held in Bath, UK (May 2004)

13th International Union against Sexually Transmitted Infections Asia Pacific Conference held in Chiang Mai, Thailand (July 2004)

CONFERENCE PAPERS AND POSTERS PRESENTED

Black K, French S, Battison T, Lewis D, Pearce V, Mann Sue. A pilot study of the use and acceptability of STI protocols and contact slips in primary care. Poster Presentation 30 at the Joint Meeting of the British Association of Sexual Health and HIV with the American Sexually Transmitted Diseases Association held in Bath, UK (May 2004)

Sturm PDJ, Moodley P, Radebe F, HJ Koornhof, Sturm AW. Diagnosis of lymphogranuloma venereum (LGV) by serology and PCR. Oral Presentation O26 at the 13th International

Union against Sexually Transmitted Infections Asia Pacific Conference held in Chiang Mai, Thailand (July 2004)

Lewis DA. Chancroid: from clinical practice to basic science. Symposium Presentation S11-2 at the 13th International Union against Sexually Transmitted Infections Asia Pacific Conference held in Chiang Mai, Thailand (July 2004)

PUBLICATIONS

1. HJ Koornhof. Screening for ß-lactam antibiotic resistance on pneumococcal isolates from patients with meningitis. Editorial comment in *South Afr J Epidemiol Infect* 2004;19(2):37-38.
2. HJ Koornhof. Probiotics – how functional are they? Editorial for *South Afr Med J* 2004;94(4): 272-273.
3. Passos MR, Barreto NA, Varella RQ, Rodrigues GH, Lewis DA. Penile myiasis: a case report. *Sex Transm Infect* 2004;80(3):183-184.
4. Lewis DA, Improving men's sexual health: a challenge for today. Editorial for *Sex Transm Infect* 2004;80(6):423-424.
5. Lewis DA, McDonald A, Thompson G, Bingham JS. The 374 clinic: an outreach sexual health clinic for young men. *Sex Transm Infect* 2004;80(6):480-483.
6. Annan TA, Lewis DA. The treatment of chancroid in resource poor countries. Accepted by *Expert Review of Anti-infective Therapy* (in press).

STAFF OF THE SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE

Dr D Lewis, Head of Department (November 2004 onwards)
Professor HJ Koornhof, Acting Head of Department (until November 2004)
EEM Goliath, Department Secretary

Laboratory Staff
FM Radebe, Laboratory Manager
DV Maseko, Laboratory Controller (until October 2004)
P Magooa, Medical Scientist
L Tsaagane, Chief Medical Technologist
S Khumalo, Research Asst, Student Technician
D Mabaso, Research Asst, Student Technician
R Chonco, Production Assistant

Surveillance & Epidemiology Staff
SO Odugwu, Medical Officer (Chief)
M Cheyip, Medical Scientist (Senior)
S Tshelane, Surveillance Officer
C Pillay, Surveillance Officer
FA Mngomezulu, Data Input Clerk
KJ Thobega, Data Input Clerk
D Thwala, STI Co-ordinator (until August 2004)

Nursing Staff
K Lucas, Research Co-ordinator/Trainer (until July 2004)
KC Mmoledi, Central Research Co-ordinator
JL Mekgwe, Research Co-ordinator
RC Husselman, Research Co-ordinator
MJ Porotloane, Research Co-ordinator

VECTOR CONTROL REFERENCE UNIT

Prof Maureen Coetzee
Head of Unit

INTRODUCTION

Malaria is the major vector-borne disease in Africa, killing over 1 million people annually, most of them children under five. In South Africa, malaria transmission is confined to the low-lying border areas in the northeast of the country where 13,181 cases were reported in 2004. The Vector Control Reference Unit (VCRU) focuses mainly on the anopheline mosquitoes responsible for malaria transmission. The Unit houses a unique collection of live mosquito colonies of the three most important vector species in Africa, *Anopheles gambiae*, *An. arabiensis* and *An. funestus*, plus the minor vector *An. merus*, and the non-vector species of the *gambiae* complex, *An. quadriannulatus*. The two colonies of *An. funestus* from Mozambique and Angola continue to provide us with a unique resource for research into insecticide resistance in this important malaria vector. This places the VCRU in a unique position to collaborate with international institutions investigating similar problems and to play a role in influencing policy decisions on vector control strategies in the region. In addition, the VCRU houses the largest museum collection of African arthropods of medical importance in Africa, the third largest collection in the world.

RESEARCH

Insecticide Resistance

Anopheles funestus

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

The VCRU in collaboration with the Nigerian Institute of Medical Research initiated a study on insecticide resistance in the *An. funestus* group in Nigeria. Results indicate that the three members of the group that are present in the study area are all 100% susceptible to pyrethroids and DDT. Other classes of insecticides are still to be tested.

Anopheles gambiae

Collaborative studies with Yale University on dieldrin resistance in *An. gambiae* were carried out. An alanine to glycine substitution in the GABA receptor was found in mosquitoes heterozygous for the dieldrin resistance allele. Analysis of the offspring derived from several backcross families confirmed that this mutation is genetically linked to resistance to dieldrin. A molecular diagnostic assay based on an allele specific polymerase chain reaction was developed to detect insecticide resistant mosquitoes and was tested on dieldrin resistant laboratory colonies.

Mosquito breeding site of *Anopheles gambiae* in Nigeria

Anopheles arabiensis

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

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

MOLECULAR AND CYTOGENETIC STUDIES

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 is being further investigated.

DISTRIBUTION AND BIONOMICS

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

INTERNATIONAL RESEARCH COLLABORATORS

Prof J Hemingway, Director, Liverpool School of Tropical Medicine, UK
Dr H Ranson, Liverpool School of Tropical Medicine, UK
Prof A Cornel, University of California, Davis, USA
Prof N Besansky, University of Notre Dame, USA
Prof F Collins, University of Notre Dame, USA
Prof D Norris, Johns Hopkins University, USA
Prof L Zheng, Yale University, USA
Dr S Manguin, IRD, France
Dr T S Awolola, Nigerian Institute of Medical Research, Lagos, Nigeria

RESEARCH FUNDING FROM EXTERNAL GRANTING AGENCIES

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

TRAINING

Postgraduate Training

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

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

DIAGNOSTIC AND OTHER SERVICES

The VCRU provides an identification service of medically important arthropods for entomologists, medical practitioners and health inspectors. Malaria vector mosquitoes were routinely identified by PCR for the Malaria Control Programmes of Mpumalanga and Limpopo Provinces. ELISA and PCR tests were carried out on the *An. gambiae* complex specimens from Ghana, Zimbabwe, Malawi, Namibia, 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.

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Temu EA, Hunt RH & Coetzee M. 2004. Microsatellite DNA polymorphism and heterozygosity in the malaria vector mosquito *Anopheles funestus* (Diptera: Culicidae) in East and Southern Africa. *Acta Tropica* 90: 39-49.

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Fettene M, Hunt RH, Coetzee M & Tessema F. 2004. Behaviour of *Anopheles arabiensis* and *An. quadriannulatus* sp. B mosquitoes and malaria transmission in southwestern Ethiopia. *African Entomol* 12: 83-87.

Masendu HT, Hunt RH, Govere J, Brooke BD, Awolola TS & Coetzee M. 2004. The sympatric occurrence of two molecular forms of the malaria vector *Anopheles gambiae* Giles *sensu stricto* in Kanyemba, in the Zambezi Valley, Zimbabwe. *Trans Roy Soc Trop Med Hyg* 98: 393-396.

Coetzee M & Fontenille D. 2004. Advances in the study of *Anopheles funestus,* a major vector of malaria in Africa. *Insect Biochem Molec Biology* 34: 599-605.

STAFF

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Dr B D Brooke, BSc (Hons), PhD, Senior Medical Scientist, Lecturer, CMID, University of the Witwatersrand
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Ms H Mafumo, BSc (Hons), MSc, Medical Scientist

Based in the Unit:

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

POSTGRADUATE STUDENTS

The following graduated in 2004:

Ph D	H Masendu (Zimbabwe)
MSc	M Weeto (South Africa)
	B Ntomwa (Namibia)
	J Chiphwanya (Malawi)

The following were registered as candidates for higher degrees:

PhD	D Amenya Achieng (Kenya)
	M Booman (South Africa)
	P Okoye (Nigeria)
MSc	N Mngomezulu (South Africa)
	G Nkosi (South Africa)
	K Hargreaves (South Africa)
	J Mouatcho (Cameroon)

Virology

Mr Ezekiel Maselesele
Laboratory Controller

VIRAL DIAGNOSTICS

October 2004 the serology section participated in the Annual HIV/syphilis antenatal surveillance for the National Department of Health with 3168 samples from the Gauteng Province. Also, for the first time, the serology section was responsible for performing HIV - 1 BED ELISA in collaboration with the Centers for Disease Control & Prevention, USA, to determine the incidence rate in the population .

The measles/rubella regional reference laboratory continued to provide an excellent service for national surveillance as well as quality control for southern Africa as part of the WHO/ AFRO mandate. During 2004, the number of measles samples submitted increased sharply to 3968 as a result of the on-going measles outbreak in the country. The annual WHO/ AFRO audit of the laboratory went very well.

HIV rapid/simple kit evaluation is on-going with 20 test kits evaluated for 2004, providing very useful information for the National Department of Health on the operational characteristics when deciding which tests kits to use within the Voluntary Counselling and Testing (VCT) program. During 2004 thirteen nurses from New Start Voluntary Counselling and Testing were trained and competency certificates were issued Also as part of our mandate the serology section receives Dried Blood Spot (DBS) samples from VCT centres in Durban, Cape Town and Johannesburg as part of quality control and the results so far are on par with NICD.

During 2004 the serology section processed 14 737 samples in total amounting to 36 939 tests performed, a sharp increase as compared to the previous year following restructuring of this Unit from point of care towards more surveillance and research projects.

Virus isolation/detection continued to provide an important service towards both patient management (CMV pp65 and shell vial, HSV shell vial, and other respiratory viruses), and surveillance (AFP, Influenza viral watch and measles/rubella) with a total of 4 823 samples received for various viral investigations. For patient management the method of choice is rapid viral detection whereby the turn-around times were reduced markedly from more than 14 days to 36 hours and even less with CMV pp65. These rapid test methods have significantly increased our virus isolation rate which is 40% at present .

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

The Viral Diagnostic/Surveillance Unit is a registered training laboratory and has trained six microbiology registrars, three medical technologists to learn pipette calibration for accreditation purposes, 22 students from Vaal Triangle Technikon, three biomedical students for experimental training, and two biomedical students preparing for the Board Examinations in March 2005. Two people from Mozambique were trained in CMV isolation in tissue culture including shell vial culture and pp65, and one person from the Seychelles Public Health Laboratory spent one week in the unit for various viral techniques applied at NICD.

WHO/AFRO training took place at NICD for two courses:-

1) **Polio Diagnosis and Laboratory Management course** was organised twice, one in March and the other in November with 4 people per session . Trainees were from Ethiopia , Abijan , Senegal , Nigeria and Uganda.

2) **Laboratory Diagnosis and surveillance for Influenza course** was organised for the first time in October and 8 people attended from Ivory Cost, DRC, Kenya, Madagascar, Nigeria, Senegal, Uganda and Zambia. Hopefully this will be on-going, like the polio course and thanks to the stewardship of Dr Terry Besselaar and Amelia Buys for the NICD and Dr Annick Dosseh for WHO/AFRO.

The specimen receiving laboratory processed some 25 590 samples for the combined Virology Division (Diagnostics, Specialized Molecular Diagnostics and Special Pathogens Unit) of the NICD.

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

VIRAL DIAGNOSTICS STAFF

Diagnostic Unit
E Maselesele, Laboratory Controller

Virus Isolation
S Moonsamy, Chief Med Technologist
M Morgan, Chief Med Technologist
A Buys, Chief Med Technologist
A Oliver, Chief Med Technologist
P Ngcobondwana, Senior Med Technologist
C Esterhyse, Senior Med Technologist
D Lebambo, Laboratory Assistant
A Matseke, Laboratory Assistant
T Mashaba, Laboratory Assistantt
L Harvey, Laboratory Assistant

Serology
E Goetsch, Chief Med Tech
B Miller, Chief Med Tech
A Mohlala, Chief Med Tech
B Singh, Chief Med Tech
S Majiki, Senior Med Tech
M Masango, Senior Med Tech
S Hloma, Student Med Technician
C Chauke, Student Med Technician
R Mnisi, Student Med Technician

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

Support Services

Receiving Laboratory
L Cranston, Chief Med Technologist
N Mpotulo, Chief Med Technologist
E Motaung, Student Med Technician
E Lemmer, Admin Officer

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

Diagnostics Kitchen
J Masekwameng, Laboratory Assistant
E Rathaha, Laboratory Assistant
R Ncala, Laboratory Assistant
J Xaba, Laboratory Assistant
D Msibi, Laboratory Assistant
F Mashangoane, Laboratory Assistant
M Mpyana, Laboratory Assistant
E Mathebula, Laboratory Assistant

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

Dr Lucille Blumberg
Head :
Epidemiology Unit

EPIDEMIOLOGY UNIT

OVERVIEW

The Epidemiology Unit provides support to the national and provincial departments of health for the control of communicable diseases through surveillance and outbreak management of diseases of public health importance. The unit also provides epidemiological support to other units within the NICD. The focus of the surveillance and outbreak functions is laboratory based.

A dedicated epidemiology unit was formally established in 2004, although infectious diseases surveillance has been carried out since 1982 at the former NIV and the NICD. Dr Bernice N Harris, a community health specialist was appointed as epidemiologist and together with Sr Jo McAnerney, runs the surveillance and epidemiology section of the unit. Dr Gillian de Jong, specialist microbiologist, joined the unit in August to expand it's capability to investigate outbreaks and develop early warning systems. She and Dr Lucille Blumberg, a specialist microbiologist and overall Epidemiology Unit head, form the outbreak response section of the unit.

The surveillance and epidemiology section provided support through laboratory-based surveillance, data mining, research projects, provision of current information, field visits and representation at national and sub-national communicable disease and EPI meetings. It is also responsible for the bi-monthly publication of the *Communicable Diseases Surveillance Bulletin* and participation in postgraduate and operational training programmes. During 2004 training was performed in influenza surveillance and measles outbreak response and prevention to Gauteng healthcare workers and policy makers; communicable disease control to registrars in Community Health; and outbreak management and use of Epi-Info for data management and analysis to MPH students. The unit was also instrumental in the management of acute flaccid paralysis (AFP), suspected measles and respiratory virus surveillance systems.

The outbreak response section provided outbreak assistance to the provinces and the NHLS laboratories concerned. There was weekly contact with provincial communicable disease coordinators and the national communicable diseases directorate to discuss notifications and other important events of the week. In addition, the unit used this opportunity to share information regarding communicable disease activities nationally and in the region and provided relevant guidelines for laboratory diagnosis, interpretation of tests and specialized information as required. Members of the unit also attended monthly National Outbreak Response team (NORT) meetings designed to coordinate responses to outbreaks and potential outbreaks in the various sectors of the National Department of Health (NDoH).

SURVEILLANCE PROGRAMMES

Suspected measles case-based surveillance

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

The NICD is accredited by WHO to perform measles and rubella IgM testing for national case-based surveillance and characterize the molecular epidemiology of the measles virus in South Africa. Blood and urine specimens from each suspected measles case (smc) is sent to the NICD for confirmation. As from mid November 2004 when KwaZulu Natal became part of our sms surveillance, specimens from all the provinces have been tested at the

NICD. Case investigation forms were completed by facility or district personnel and forwarded to the NDoH. The numbers presented here only represent specimens received by the NICD and may differ from those captured by the NDoH as they may have received information on epidemiologically-linked cases where no specimens were taken.

During 2004 the NICD tested 3 322 blood specimens from cases with rash and fever for suspected measles case-based surveillance. Of these 1 687 (51%) were collected in Gauteng and 681 (20%) in Mpumalanga. Of these specimens 732 (22%) tested positive for measles and 853 (26%) for rubella (fig 1).

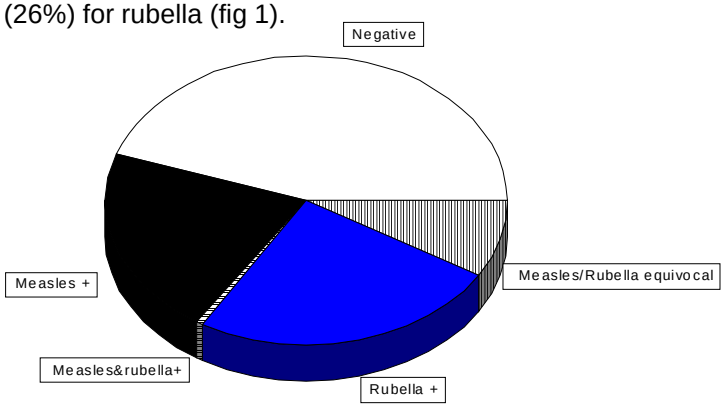

Fig 1. Specimens received for measles surveillance

Sr Jo McAnerney

Measles

At the end of August and during September 2004, a national mass poliomyelitis and measles vaccination campaign for children younger than 5 years of age was conducted in all provinces. Although South Africa experienced the biggest measles outbreak since case-based surveillance was instituted in 1998, the extent of localized outbreaks was most likely limited as a result of immunisation coverage achieved by immunisation administered routinely and during the mass campaign.

Ninety-five percent of all the confirmed measles cases occurred in 3 provinces, namely Gauteng (77%), KwaZulu-Natal (14%) and the Western Cape (5%) (fig 2).

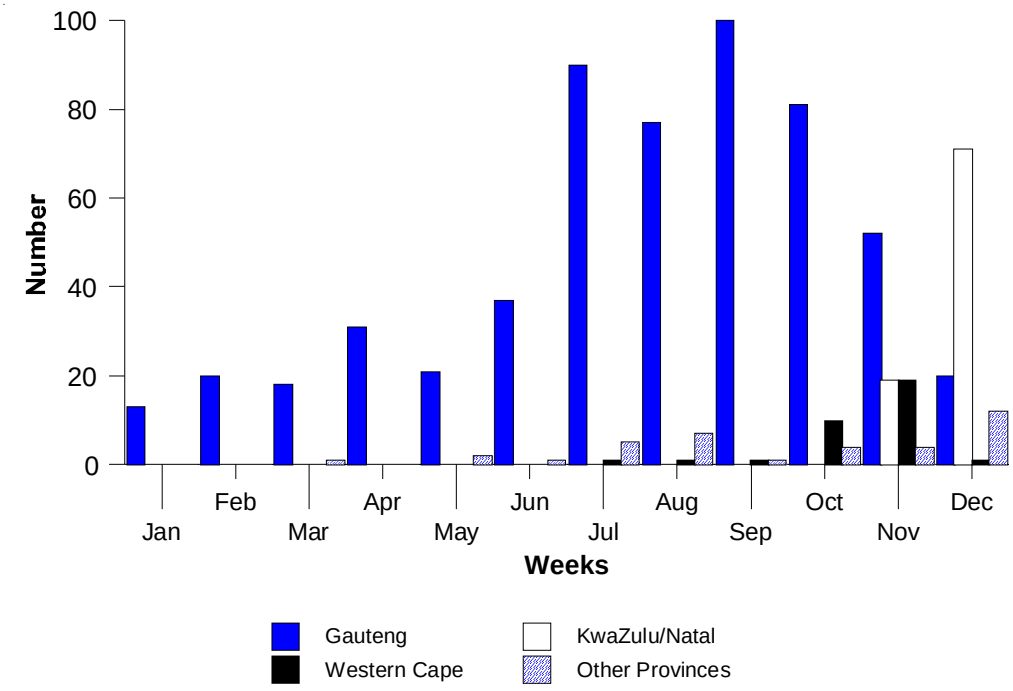

Fig 2. 2004 positive measles IgM results per month

The NICD only started receiving specimens from KwaZulu-Natal from mid November 2004. The measles epidemiology in the respective provinces was affected by the distribution of susceptibles resulting in a large prolonged outbreak in very young children in low income, high population density suburbs of Gauteng (561), two limited outbreaks in adults and older

children in the Western Cape (33 cases) and an outbreak originating in an inaccessible, low vaccination coverage area in KwaZulu-Natal (101). Sporadic measles cases occurred throughout the year in 3 provinces – Mpumalanga (11), North-West Province (10) and Limpopo (5). The Eastern Cape (7) and Northern Cape (4) experienced small isolated outbreaks and no cases were reported from the Free State.

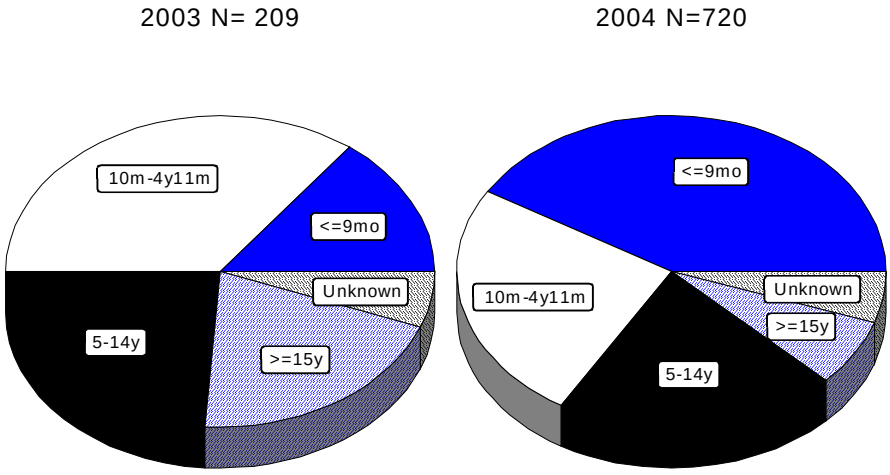

Fig 3. Age distribution of patients with positive measles results, 2003 vs 2004

Strategies employed to interrupt the circulation of the measles virus included re-introducing immunisation of all children from 6 months to 15 years of age on admission to a health facility or institution to interrupt nosocomial spread, immunisation of contacts of sporadic cases, and extensive mopping-up campaigns in areas where cases were clustered. The age of the populations targeted for mopping-up measures depended on the age distribution of cases and varied from all ages in an outbreak involving mainly non-immune adults to all children aged 6 months to 15 years in areas with widespread cases. In Gauteng, mopping-up campaigns mostly involved children aged 6 months to 5 years and the median age of cases was much lower than in other provinces. The national age distribution of cases in 2004 differed markedly from that in 2003 with significantly more cases aged 9 months and younger and fewer cases older than 5 years in 2004. (See Figure 3)

Rubella

Eighty-nine percent of all rubella cases occurred in 4 provinces, namely Mpumalanga (33%), Gauteng (29%), North West Province (14%) and Eastern Cape (12%). The median age of cases was 7 years. Most cases occurred in late winter and spring. (Figure 4)

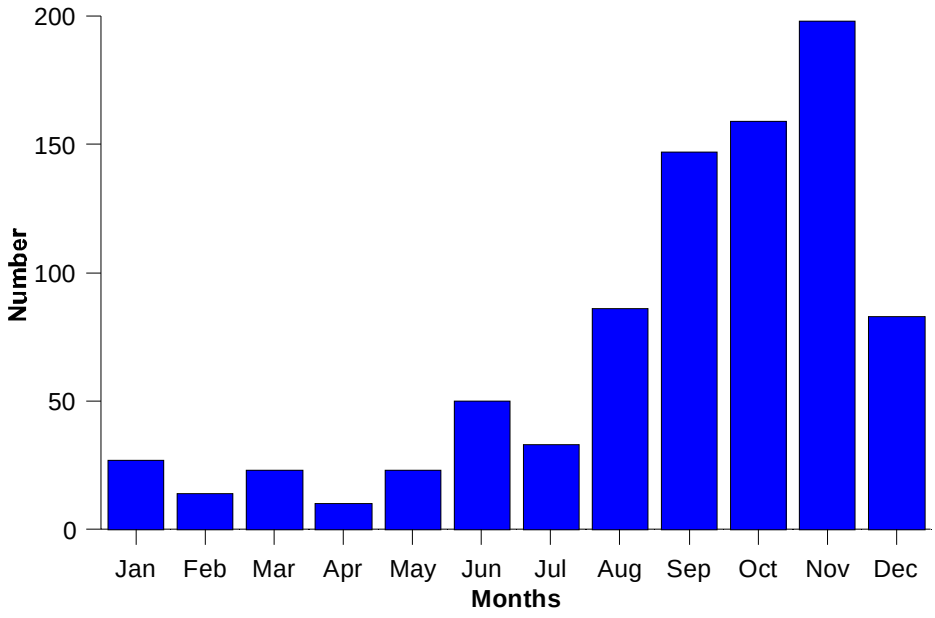

Fig 4. Seasonal distribution of positive rubella IgM results

Acute Flaccid Paralysis Surveillance - Laboratory Support

AFP surveillance, as part of the global campaign to eradicate poliomyelitis, has continued throughout the year. All cases of acute flaccid paralysis in children less than 15 years of age, including those with Guillain-Barré syndrome, and patients of any age diagnosed clinically as cases of poliomyelitis by a medical doctor, were regarded as possible poliomyelitis cases until proven otherwise.

Although the last case of South African poliomyelitis occurred in 1989, South Africa can only achieve national poliomyelitis-free certification if the country can demonstrate that adequate surveillance is in place. This implies the ability to detect 1 AFP case per 100 000 children less than 15 years of age (at least 147 cases during 2004) and to have at least 80% of reports from AFP active surveillance sites throughout the country received on time, while at least 80% of the AFP cases should have adequate stool specimens (two specimens taken within 14 days of onset of paralysis, at least 24 hours apart, reaching the laboratory in good condition). All virological investigations must be conducted in laboratories accredited by the Global Polio Laboratory Network.

The National Institute for Communicable Diseases accommodates the only accredited laboratory for AFP surveillance in South Africa and serves as the poliovirus isolation laboratory for six other southern African countries i.e. Angola, Botswana, Lesotho, Mozambique, Namibia, and Swaziland.

During the year 1 118 stool specimens were received from patients with AFP. Of these 95 were from patients with onset of paralysis prior to 2004, or patients who were subsequently considered not to have AFP. Of the remainder 480 were from South African cases and 543 from cases in the six other countries served by the NICD (fig 5). A further 32 specimens were received in the first two weeks of January 2005 from patients with onset of paralysis in 2004.

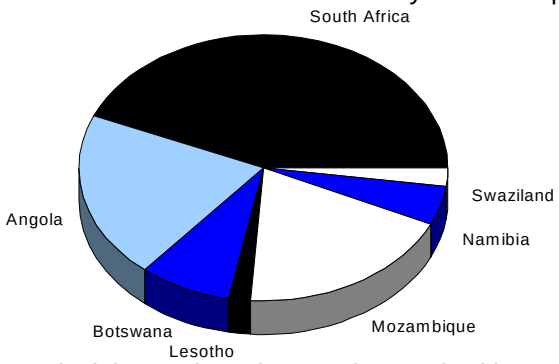

Fig 5. Specimens received for AFP surveillance

The provincial case detection rate for South Africa ranged from 1.0 to 2.7 p.a., with a national rate of 1.5. Two or more specimens taken within 14 days of onset were received from 174/230 patients. The percentage of adequate stool specimens per province ranged from 47.2% to 87.5% with a national rate of 75.7% (fig 6).

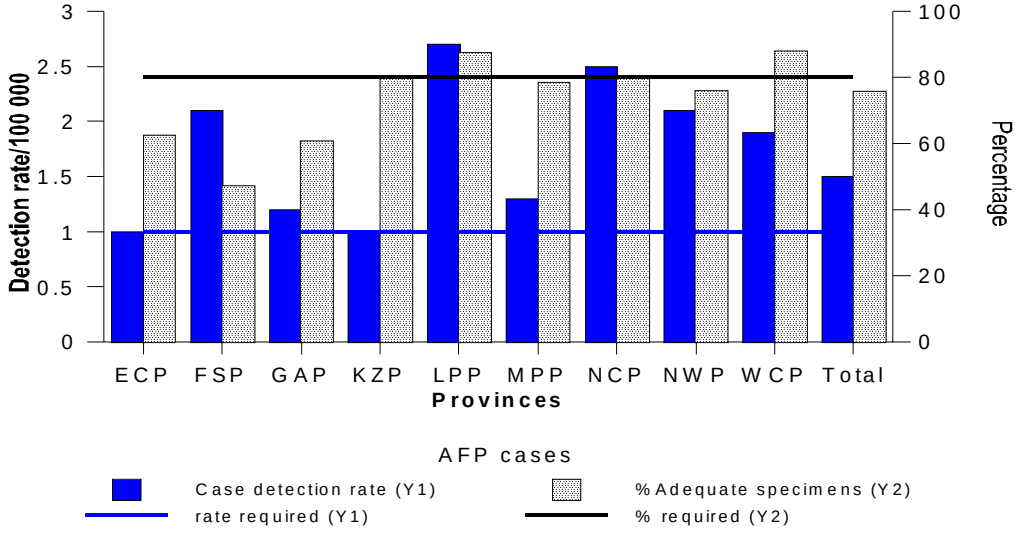

Fig 6. South African AFP cases, case detection rate and percentage with adequate stool specimens per province

Non-polio enteroviruses were isolated from one or more specimens of 27 South African and 62 non-South African cases. Poliovirus was isolated from 21 specimens of 12 South African patients, all of which were identified as Sabin-like. Poliovirus was also isolated from 12 non-South African cases, all but one were found to be Sabin-like. In one patient, a seven year old boy from Botswana, the isolate was identified as a wild poliovirus type 1 strain. (See Virology – Vaccine Preventable Virus Infections).

Respiratory Virus Surveillance

Three hundred and twenty (320) specimens were received for detection of respiratory viruses during 2004. Of these 107 were received from the Viral Watch programme. The programme was started in 1984 and was designed to monitor influenza activity in the community and detect prevalent influenza virus strains.

The Viral Watch surveillance sites currently number 12, and have ranged annually from 12 to 19. Surveillance sites include general medical practitioners, a paediatrician, paediatric out-patient departments at hospitals, university clinics as well as the staff clinic at the NICD. Up to two throat swabs per week can be taken at each site throughout the year from patients with acute respiratory tract infections of recent onset i.e. within 48 - 72 hours, and without obvious bacterial cause, and transported to the NICD in viral transport medium for isolation of virus.

The 2004 influenza season was very quiet with comparatively low school absenteeism, showing a peak rising above the mean absentee rate calculated over a five year period starting at week 26 (Fig 7). However, this was the week that winter school holidays started in government schools, and the independent schools were on a mid-term break. Influenza virus was isolated from 26 patients attending Viral Watch sites this season. The first isolate was from a specimen taken on 14 May and the last from a specimen taken on 4 August. Twenty-five of the isolates were influenza A, 19 of which have been further identified as A H3N2 (A/Fujian/411/2002(H3N2)-like). Only one influenza B virus was isolated. Patients' ages ranged from 3 to 46 years (median 21). In addition, influenza A has been confirmed in 12 specimens sent by private laboratories, 7 of which have been further identified as A H3N2. Other respiratory viruses isolated were 16 parainfluenza virus, 49 respiratory syncytial virus, 11 adenovirus while 5 were untyped. The Viral Watch accounted for 33,4% of all respiratory specimens with an isolation rate of 26,3%. It is planned to expand the viral watch network and increase respiratory diseases surveillance capacity in 2005.

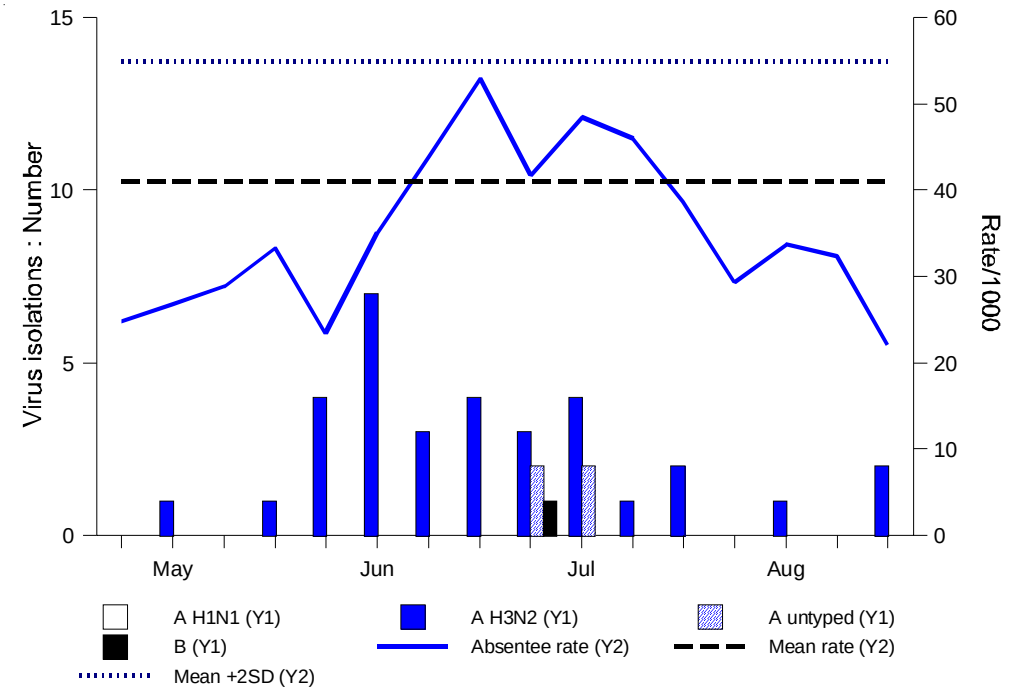

Fig 7. Influenza isolates and school absenteeism 2004

OUTBREAK RESPONSES

Avian influenza in the Eastern Cape

In September 2004, Dr Blumberg and Dr de Jong responded to the outbreak of highly pathogenic avian influenza (HPAI) H5N2 in ostriches in the Eastern Cape. Initial responses included the provision of guidelines for the laboratory diagnosis of suspected human cases and use of personal protective equipment. This was followed by a site visit to actively screen exposed personnel for evidence of human infection from the H5N2 virus infecting ostriches. The NICD team was joined by representatives of the NDoH, local district nurse managers and the Department of Agriculture. Seven farms were visited to conduct serological screening and provide information to personnel. In addition, field workers and members of the operations committee, NSPCA staff and veterinary workers were screened. The site visit was very successful. It provided an excellent opportunity to network with the NDoH, Department of Agriculture, veterinary services and local laboratories. It was of great value to interview and interact with personnel and identify areas of concern such as compliance with personal protective equipment. Ongoing support was provided by the NICD. Serological tests were performed by the Special Pathogens Unit and influenza laboratory at the NICD. Testing was conducted using the microneutralisation assay recommended for detection of human antibodies to avian influenza. Approximately 136 exposed personnel were screened. The results of this serological survey will be published shortly.

Ostriches on a farm in the Eastern Cape visited by Drs Lucille Blumberg and Gillian de Jong of the outbreak response team

This outbreak was the first recognized outbreak of HPAI avian influenza in South Africa. Outbreaks of HPAI of the H5N1 subtype have been well described in East Asia and continue to cause disease with high mortality rates in both chickens and humans. Concerns are increasing regarding the potential for the emergence of a genetic reassortant or mutation of the H5N1 virus that will produce a new influenza strain capable of efficient human-to-human spread. It is essential that South Africa prepare effectively for this potential pandemic and the unit will participate in this process together with key role players in the NDoH. Dr Blumberg and Dr de Jong currently serve as NICD representatives on the national task force for avian influenza in South Africa.

Dr Lucille Blumberg with members of the Controlled Diseases Joint Operation Field Services team and Eastern Cape provincial health members

Dr Gillian de Jong interviewing personnel on the farms for collection of data on respiratory and conjunctival symptoms

Maandagshoek outbreak

On 25th October 2004, the Epidemiology Unit was informed by the Limpopo province Communicable Disease Directorate of a suspected typhoid fever outbreak in the Sekhukhune district of Limpopo province. On investigation by the unit it was determined that the diagnosis had been made based on clinical assessment and Widal serological testing only. No culture confirmation had been obtained for any of the cases. A site visit was performed in order to provide laboratory support and review the current diagnostic procedures during the outbreak. The NICD visit was conducted by Dr Gillian de Jong (Epidemiology Unit and Outbreak Response) and Ms Tersia Kruger (Enteric Diseases Reference Unit) in collaboration with members of the NDoH National Outbreak Response Team (NORT). In addition, a guideline for appropriate laboratory testing and management of suspected typhoid fever cases was issued by the Epidemiology Unit and EDRU and distributed to the laboratory and clinicians.

Outbreak Unit site visit to Maandagshoek Hospital during suspected typhoid outbreak

Based on the available laboratory data, the diagnosis of typhoid fever could not be confirmed and recommendations for use of appropriate specimens and correct laboratory processing were made. The site visit was most useful in identifying problem areas in laboratory diagnostics, educating clinicians and defining the cause of the outbreak which appeared to be related to ongoing use of contaminated water sources. As a consequence of this visit the unit will be assisting in the training of clincians in appropriate laboratory diagnostic procedures in partnership with the national and provincial departments of health.

Anthrax

Three laboratory confirmed cases of cutaneous anthrax in humans occurred in Schmidtsdrif and Delportshoop in the Northern Cape province in January 2005. One of these cases also had fatal systemic involvement with *Bacillus anthracis* identified on a blood culture by the Special Bacterial Pathogens Unit. A further 9 suspected human cases of cutaneous anthrax were identified. The Epidemiology Unit and Special Bacterial Pathogens Unit worked in close collaboration with the Communicable Disease Directorate for the Northern Cape to assist in providing information for appropriate response, diagnostic facilities and support during the outbreak.

RESEARCH PROJECTS

Following the rubella symposium organised by the NICD, a protocol was developed to assess the age of acquisition of rubella and the degree of susceptibility in women of child bearing age in South Africa to assist in the formulation of an immunisation strategy for South Africa. Fieldwork for the serological survey started in November and continued in early 2005. Mr Benn Sartorius, an EPIET student based at the Swedish Institute for Infectious Diseases (SMI), undertook a study to access absenteeism data from large South African institutions as a proxy measure of the magnitude of the influenza season.

TEACHING

The epidemiology unit is extensively involved in undergraduate and postgraduate teaching at the University of the Witwatersrand(Wits) and University of Pretoria (UP):

- Medical, nursing, pharmacy and dental students(Wits and UP)
- Diploma in Tropical Medicine and Hygiene, Wits
- Certificate Course in Travel Medicine, Wits
- Masters in Public Health, Wits and UP

- Community Medicine, Wits
- Masters in Epidemiology, Wits
- EpiInfo training at the HELP course, UP
- Training of heath care workers in malaria management in Limpopo and North West provinces

Training workshop

Members of the Epidemiology Unit joined forces with staff from RMPRU to conduct a training workshop at Mankweng, Tzaneen and Tshilidzini laboratories in Limpopo province in October 2004. Training was aimed at improving the diagnosis of enteric and meningeal pathogens, antibiotic susceptibility testing and the role of the laboratory in diagnosis and response to epidemic-prone diseases. The workshop was very successful and created an improved network of communication with the region that is ongoing.

Ms Tebogo Masebe and colleagues participating in joint RMPRU/Epidemiology Unit workshop at Mankweng Hospital, Limpopo

MEETINGS, CONGRESSES, GENERAL ACTIVITIES

During 2004, Dr Harris attended the quarterly national Communicable Disease Co-ordinators (CDC) and EPITG meetings, sharing information and knowledge gathered through the NICD surveillance systems and keeping abreast of developments within the departments of health. Dr Harris formed part of the South African delegation to the inter-country meeting on Integrated Disease Surveillance and Response (IDSR) of the southern African epidemiological block held in Lusaka from 26 – 30 April.

Dr Blumberg participated in the WHO meeting in Lyon, France 20-21st April 2004 on twinning partnerships between national laboratories in developing countries and specialized institutions. She presented an alternative model for improving laboratory services based on the polio network.

Dr Blumberg participated in Operation Medflag in Hoedspruit in May 2004. This was a joint USA and RSA military training course on infectious diseases in Africa. She delivered lectures on anthrax, rickettsial diseases and malaria.

In addition, guest lectures were delivered at the following events in 2004:

- Superbugs conference, 11th March 2004, Pretoria. Avian influenza and SARS - Dr Blumberg
- School of Military Medicine course on Viral Haemorrhagic Fevers, Pretoria - Dr Blumberg
- ICASA national congress, Pretoria 4th May 2004 Avian Influenza - Dr Blumberg
- Gauteng Infection Control Society meeting
- WHO Influenza workshop
- Sizwe Hospital infectious diseases meetings
- South African Infectious Diseases Society meetings

Members of the unit participated in NORT field visits to Gauteng and KwaZulu-Natal to help evaluate and support responses to measles outbreaks in these provinces.

The Lindela Repatriation Centre in Gauteng was visited to evaluate the potential for meningococcal disease outbreaks.

Dr Blumberg was a member of a group of provincial malaria programme managers, clinicians, communicable diseases co-ordinators, and University of Cape Town pharmacologists that conducted a confidential enquiry into malaria related deaths in KwaZulu, Limpopo, and Mpumalanga. Drs Harris and Blumberg participated in the formulation of the report of the confidential enquiry into these malaria related deaths

UNIT PUBLICATIONS

The unit produced and distributed a monthly electronic *Communique* to a wide audience of individuals and institutions dealing with communicable diseases in the public and private sectors. This publication provided an update and commentary on important communicable diseases and outbreaks of the month.

The bi-monthly publication of the *Communicable Diseases Surveillance Bulletin* is published electronically as well as in print and was distributed to a similar group of persons and institutions. It contained more detailed and formal articles on relevant and topical infectious diseases topics as well as current and cumulative laboratory-based surveillance data relating to selected infectious diseases.

PUBLICATIONS 2004

1. Blumberg L, Frean J. Dermatological manifestations of tropical diseases. South African Dermatology Review 2004; 4: 5-14.
2. Gerston KF, Blumberg L, Tshabalala VA, Murray J. Viability of mycobacteria in formalin-fixed lungs. Human Pathol 2004; 35: 571-575.
3. Johnson CL, Moonasar D, Maloba B, Blumberg L. Malaria control in South Africa. Modern Medicine 2004; 46-48.
4. Wright CA, van Zyl Y, Burgess SM, Blumberg L, Leiman G. Mycobacterial autofluorescence in Papanicolaou-stained lymph node aspirates: A glimmer in the dark? Diagn Cytopathol 2004; 30: 257-260.

Chapters In Books:

Blumberg L. Severe malaria. In: Tropical and parasitic infections in the intensive care unit. Feldman C, Sarosi GA eds, Springer. 2005: 1-16. Perspectives on critical care infectious diseases.

STAFF OF THE EPIDEMIOLOGY UNIT

Dr L Blumberg, Head of Unit
Prof HJ Koornhof, Consultant

Epidemiology & Surveillance Unit

Dr B N Harris, Community Health Specialist/Epidemiologist
Sr JM McAnerney, Nurse (Professional)

Outbreak Response Unit

Dr L Blumberg, Specialist Microbiologist
Dr GM de Jong, Consultant Microbiologist

Biostatistics/Biomathematics Unit

ME Letsoalo

National Comprehensive Plan for Prevention, Care & Treatment of HIV

Dr TM Marshall, Programme Head
N Cassim, Project Manager

SPECIALIZED MOLECULAR DIAGNOSTICS UNIT

Dr Adrian Puren, Deputy Director NICD & Head of Molecular Diagnostics Unit

HIV-1-SPECIFIC AND RELATED ACTIVITIES

The Unit members directly or indirectly support various activities including the following HIV-1-specific projects by performing various nucleic acid tests (NAT) in association with serological testing, administrative tasks, supervisory and administrative functions:

(1) HIV DNA PCR CD4 counts and viral loads for the detection of resistance in single dose nevirapine use in pregnancy and the detection resistance in second pregnancy with single dose nevirapine

(2) HIV DNA PCR in the Nestlé feeding study for the detection of HIV infection in infants

(3) Viral loads and serology in the Stepping Stones programme, a behavioural modification programme to prevent acquisition of HIV

(4) Serology and CT/NG NAT in role of ACIDFORM gel versus KY jelly in the presence of a diaphragm: a phase one trial to determine the acceptability of the gel and diaphragm in preparation for a phase II trial

(5) Serology and viral loads in the role of male circumcision in the acquisition of HIV and related STIs including HSV-2 and syphilis

(6) Assessment of the use of the Exavir viral load assay based on RT activity

(7) Development of an external quality assurance programme for blood borne viruses including HIV-1, HBV and HCV

(8) The use of dried blood spots in the diagnosis of HIV-1 infection in infants by NAT

(9) The use of DBS as an external quality assurance tool in serology

(10) The determination of HIV-1 incidence in antenatal survey specimens using serological techniques

(11) The evaluation of rapid HIV kits and field trials

(12) Assistance with the ART programme; performing viral loads and evaluations

(13) Co-organisation of III Meeting of the Regional HIV/AIDS Public Health Laboratory Network

NEW TECHNOLOGIES AND APPROACHES

The Unit has extended the range of its activities as seen above. Project support has mainly been the detection of HIV-1 using DNA PCR in infants using a standard HIV PCR detection system. Similarly viral loads have been performed using standard technologies currently present in the laboratory. An important advance in the case of the viral loads is the utilisation of automation. To this end for example the COBAS Ampliprep and Cobas now segue to ensure full automation. There are advantages in settings with high specimen throughput. The Unit has participated in the stringent Viral Quality Assurance programme as well as the QCMD programme to ensure that this approach performs well. In addition, the laboratory continues to actively support the evaluation of new technologies and thus the Exavir viral load that is based on protease activity continues to be evaluated. An example of typical results of the Exavir viral load assay versus RNA amplification is presented in the form of a Bland Altman plot (see Figure One). The laboratory evaluation will lead to a field trial planned for 2005. Additional methodologies explored included the use of AMPLISCREEN (Roche) as a tool for the detection of acute/recent HIV infections. The approach is that of the use of optimal pool sizes (primary and secondary pools) in order to make the procedure practical. The work was performed in collaboration with Isaac Choge (AIDS Virus Research Unit).

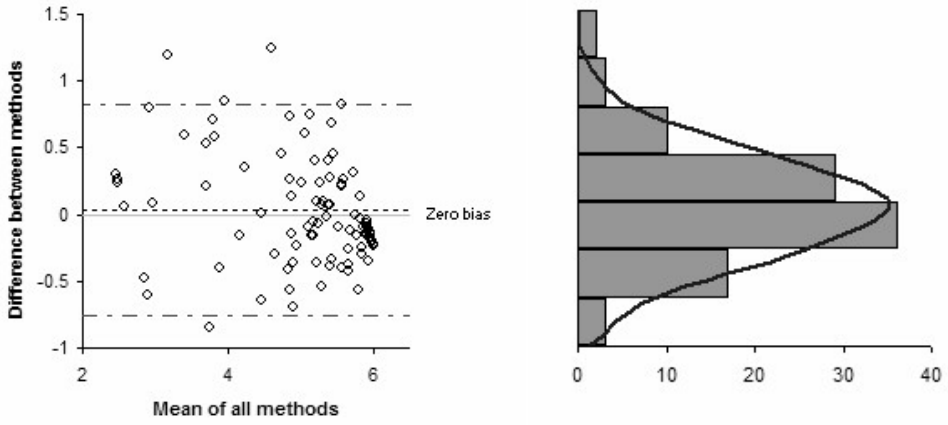

Fig 1. One Bland Altman analysis comparing two technologies

AMPLISCREEN is used in the blood transfusion settings for NAT testing. The method is well defined will a lower limit of 100 copies/ml. The primary pool size of 24 specimens/pool should have sufficient sensitivity to detect one primary infection per pool. With regard to HIV-1 diagnosis in infants, the Unit is currently exploring the use of automated technologies for DNA extraction to assist in scaling up diagnosis.

THE TIMING OF HIV-1 INFECTION IN INFANTS: THE ROLE OF HIV-1 DNA PCR

Positive antibody tests for HIV, such as the ELISA test, are not reliable for 12-18 months after birth in infants due to the potential for transplacentally acquired antibodies to cause false positive tests. Current recommendations in the public sector for the follow up of infants born to HIV positive mothers include the commencement of co-trimoxazole at the age of six weeks and follow up to 12-18 months for definitive ELISA testing. Diagnosis at a much earlier age would allow 90% of such infants who are not breast fed and expected to test negative (if nevirapine prophylaxis has been given) to be discharged immediately from follow up, thus relieving the follow up clinics of a major clinical load.

Methods

The teaching hospitals attached to the Department of Paediatrics, University of the Witwatersrand, were involved in three feeding studies starting in 2000 whereby infants of HIV positive mothers who had elected not to breast feed were enrolled into controlled feeding studies. Infants were randomised to various milks including standard starter formulas, formulas that were acidified by different methods and, in addition, may have had probiotics, prebiotics, and/or nucleotides added. The main outcomes of these studies related to growth and tolerance of the formulas up to the age of 6 months. Nevirapine was given during labour and delivery to some infants in the first study, but was routine in the subsequent two studies. According to the protocols, all infants had a PCR DNA test for HIV at the age of 6 weeks and a follow up confirmatory test at 4-6 months. If there was a discrepancy between the two tests, a third test was performed including a PCR RNA test for viral load and this test was regarded as definitive. Results from the three studies were analysed to assess the reliability of the PCR at 6 weeks of age in non breast fed infants.

Results

A total of about 450 infants were enrolled in the three studies. However, a number of infants did not complete the six month follow up period, usually due to failure to return to follow up. A total of 326 infants who had two or more PCR results were thus available for analysis.

Discrepant PCR tests results at the two time periods was observed in only 2.4% of all subjects. Of the five infants with a negative initial test at 6 weeks and a subsequent positive test, only one of these was positive on the third definitive test. Of the three who had a positive test at 6 weeks and a subsequent negative test, all were negative on the third definitive test.

The sensitivity, specificity, and predictive values of the PCR done at 6 weeks were as follows:

Sensitivity	0.97
Specificity	0.99
Positive predictive value	0.91
Negative predictive value	0.997

Discussion

The DNA PCR test for HIV at 6 weeks of age in non breast fed infants has been shown in this study to be extremely reliable. Where there has been a discrepancy between results at the two time periods, in seven of the eight cases a negative test was confirmed and, in only one case did an infant with a negative PCR at 6 weeks turn out to be positive on later testing. The negative predictive value of a PCR at 6 weeks in non breast fed infants is thus extremely high. Presented by Peter A Cooper, Keith D Bolton, Mantoa Mokhachane, Barbara J Cory and Adrian Puren*. Department of Paediatrics, University of the Witwatersrand and Johannesburg, Coronation and Chris Hani Baragwanath Hospitals and *National Institute for Communicable Diseases.

CONTINUED COLLABORATION WITH QCMD

The clinical sciences have been dramatically transformed by the rapid evolution of molecular diagnostic nucleic acid testing (NAT). One area where molecular diagnostics has had a

significant influence is clinical virology. Technologies offer advances in viral NAT quantification for therapeutic monitoring, the determination of drug resistance and facilitated the discovery of new viruses (recently SARS, avian flu). NAT has the potential of faster, accurate and reliable diagnosis over conventional culture methods. From a quality assurance perspective there are concerns that the rate of molecular diagnostic innovations has meant that issues of QC are left behind while technology advances. Concerns have been raised about quality performance in the routine clinical setting and general lack of method standardization and acceptable QC practices. The quality concerns in molecular diagnostics include: assay sensitivity and specificity, contamination, clinical significance, variable isolation/amplification procedures, lack of robustness and standardisation, lack of appropriate control material, regulations and policies.

These concerns will be applicable when considering the routine use of NAT in patient monitoring in ART programme in South Africa. We therefore propose that External Quality Assessment (EQA) be an integral part of the different laboratories quality management system (QMS) that will detect weak spots in performance as well as improve on the reliability and confidence when reporting results. An EQA programme allows comparison and benchmarking, education in good laboratory practise (GLP) and method utility. In addition, EQA provides reference where no international standards are available as well supports the validation & implementation of new methods for clinical use and helps to identify quality improvements. EQA thus complements internal QC as is a necessary part of the clinicalaccreditation process. Figure2 summarises the processes that lead to assay development and the place of an EQA programme in the quality cycle.

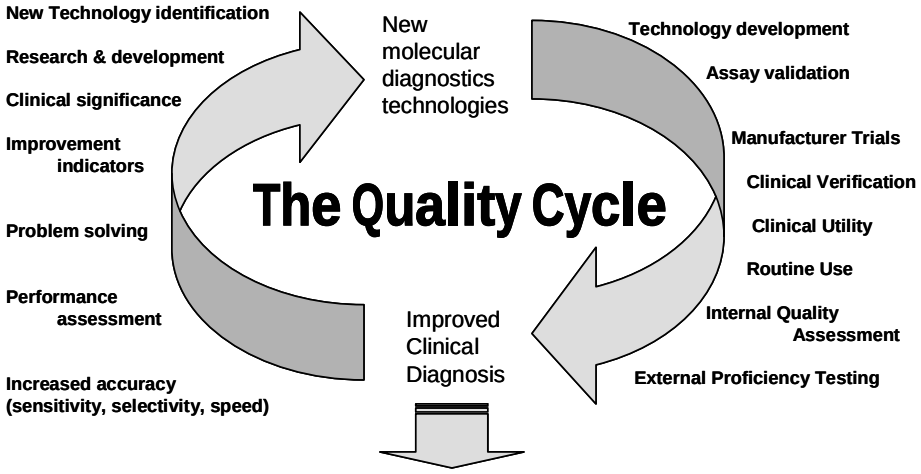

Fig 2. The place of EQA in the Quality Cycle

QCMD Blood Borne Virus (BBV) EQA Programmes for 2004: South African Participating Laboratories (AJ Puren, NICD and Paul Wallace, QCMD)

The NICD-QCMD ran an EQA programme during 2004. Eleven laboratories in total participated in the 2004 BBV (HBV, HCV, HIV) programmes and an additional HIV (DNA) pilot programme. The HIV DNA panel was an unique aspect of the programme and was specifically designed for South African participants. Table One outlines data that is useful in terms of, for example, technologies used for detection and data can be extracted for analysis purposes from a national perspective. Additional analyses include individual laboratory or group performance against world-wide performance. South Africa ranks 13th in terms of the numbers of programmes participated in. The NICD will take on more responsibilities for the 2005 programme as well as providing source material in order that panels become more relevant from a regional perspective. In addition, the NICD has made a presentation to the full executive board of the QCMD in 2004 with respect to the roles and functions of the NICD as a reference laboratory.

HIV INCIDENCE TESTING (CDC-NICD Cooperative Agreement Activity)

The antenatal survey, a point prevalence survey, is the most consistent source of HIV-1 prevalence data in South Africa. There are recognized limitations of the antenatal survey

Table 1: Technologies for NAT

key
did not participate
did not submit results

Lab code	Types of assay used			
	HBV	HCV	HIV	HIV-DNA
1			Roche Cobas Amplicor	Roche Amplicor
2			Nuclisens BioMerieux	
3	Nested PCR	Nested PCR	Nuclisens BioMerieux/Abbott LCx	Roche Amplicor
4	Real-time Lightcycler	Nested PCR	Nested PCR	Nested PCR
5	Single PCR	Roche Amplicor	bDNA	Roche Amplicor
6	Nested PCR	Roche Cobas Amplicor	Roche Cobas Amplicor/bDNA	Roche Amplicor
7			Nested PCR	
8			Roche Cobas Amplicor	Roche Amplicor
9			Roche Cobas Amplicor	Roche Amplicor
10		Nested PCR	Roche Cobas Amplicor	

e.g. there is no direct information available on infection rates among non-pregnant women, men, newborn babies and children. However, with recent intervention studies e.g. comprehensive care and treatment programme and that the prevalence may have reached a plateau there is the requirement to measure incidence of HIV to assist in the interpretation of the data as well as in determining appropriate responses i.e. whether intervention studies such as behavioural intervention studies are required, improved information presentation, improved or increased number of VCT/PMTCT facilities and the providing of antiretroviral drugs (ART). Incidence testing would not be seen as an isolated biological end-point but should be integrated with behavioural modification monitoring i.e. an integrated aspect of second generation surveillance programmes. Incidence can be mathematically modelled, however certain assumptions are required and therefore the accuracy may be questioned. Several technologies are possible to measure incidence including the detuned assay, BED technology and avidity indices. The best-studied assay is the detuned assay. The primary objective is to determine the incidence of HIV-1 in South Africa based on the annual antenatal survey. Incidence rates would be integrated into the general assessment of the HIV-1 epidemic and determine responses as described. A secondary objective is the validation of the detuned assay and BED capture EIA (BED CEIA) technology in a setting where HIV-1 subtype C virus is predominant. The CEIA is quantitative in nature. Indirectly, it measures the proportion of HIV-1 specific IgG in a given specimen with respect to total IgG. Therefore, it is very important that the protocol is strictly adhered to for high precision and reproducible results. Each plate will assay 11 controls plus 85 initial screens or 28 confirmatory in triplicate.

There has been successful implementation of the BED capture ELISA (NICD Serology Unit; Sarvashni Moodliar and Beverley Singh) and this is reflected in Figure Three where

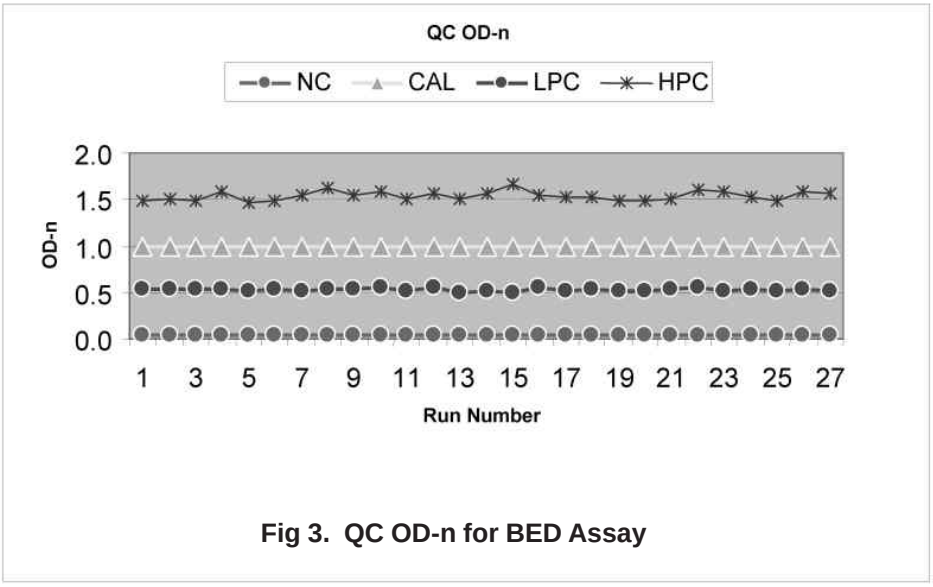

Fig 3. QC OD-n for BED Assay

the control specimens (QC OD normalised, n) have been plotted for at least 30 runs showing variation of less than 5%. In addition, the correlation between the screening and confirmatory testing shows an excellent correlation (Figure 4).

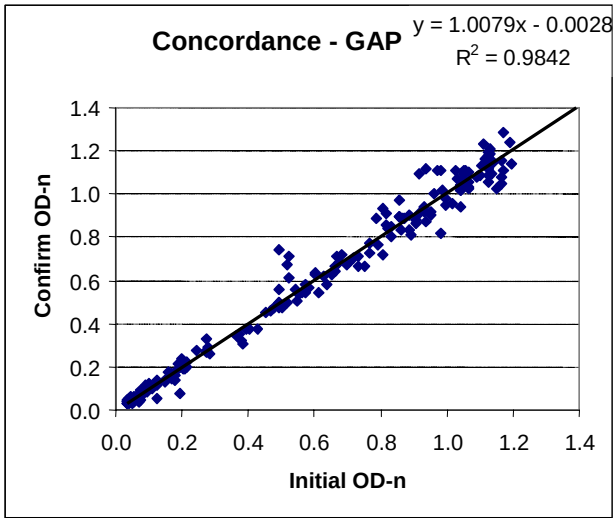

Fig 4. Correlation of Screening (Initial OD-n) versus Confirmatory testing (Confirm OD-n)

Additional quality control included testing of confirmed recent infections with an OD < 0.3 cut-off. Testing included either the Vironostika or Murex ELISA. Only 5 false positive results were observed in a total 3500 positive specimens. These results provide a measure of confidence in the quality of testing for prevalence and by implication the prevalence data.

EVALUATION OF HIV-1 RAPID KIT TESTING AND QUALITY CONTROL OF RAPID TESTS USING DRIED BLOOD SPOT TECHNOLOGY (CDC-NICD cooperative agreement activity)

HIV tests that have been rigorously tested should be used for diagnostic purposes at VCT sites. The available technologies enable specimen collection procedures that are less invasive and more acceptable than venipuncture, thus helping expand HIV testing. The decision to adopt a particular test technology in a clinical or other setting should be based on several factors, including accuracy of the test, client preferences and acceptability, likelihood of client returning for results, ease of sample collection, complexity of laboratory services required for the test, availability of trained personnel, and approval of the test following testing in a reference setting. The project goals include testing and implementation of HIV rapid kits in non-clinical settings and the development of a dried blood spot quality assurance programme for HIV rapid kits testing.

The key matters that were raised at the HIV Rapid Test Quality System Workshop Johannesburg, South Africa, May 2004 attended by Sarvashni Moodliar and Emma Goetsch. The outcome of the discussions are summarised below.

Quality Control

1. Quality control (positive and negative controls) material should be produced by national reference laboratories and distributed to rapid testing sites.
2. Controls should be run under the following conditions:
   a) New operator
   b) New test kit lot number
   c) New shipment of kits
   d) Any environmental changes e.g. Temperature
   e) At periodic intervals defined by national testing policies.

External Quality Assessment

1. EQA consists of 3 components:
   a) On-site monitoring
   b) Proficiency testing
   c) Re-testing

2. Most important of these is on-site monitoring and must be carried out even if the other 2 are not.
3. Proficiency testing can be carried out at the time of on-site visits.
4. Re-testing 10% of samples, although the common practice in most places, has no statistical significance unless the site is processing large volumes of samples. It was suggested that re-testing should only be done to detect gross errors at new sites and with new operators. If on-going re-testing is to be carried out it must have statistical significance i.e. re-testing more than 10% for medium to low volume sites.

Training

1. There must be formal training programmes in place.
2. There must be on-going training and competency certification.
3. Training of trainers.

The current strategy used at the NICD to diagnose for HIV-1 infection by serology is a screening test followed by two confirmatory tests. We therefore attempted to determine whether using a two stage strategy namely a screening and confirmatory ELISA would be sufficient to identify HIV-1 positive specimens on DBS. The approach included the initial evaluation of different elution buffers containing different detergents. The DBS specimens were prepared from known ELISA-positive specimens as well as from the study in Umata where parallel plasma specimens were available. In the latter case rapid kits were used to test the participants. The collection therefore consists of both seropositves and seronegative specimens. The primary focus was on the second Project Goal i.e. the development of the DBS as a QA tool. The initial optimisation of dilutions was performed on an initial series of experiments. The process was repeated on 998 specimens. The outcome of the exercise is encouraging in terms of the algorithm to use. The final dilution for Genscreen is 1:200 and for the confirmatory Vironostika Uniform test the dilution is 1:40. The results are as follows:

DBS Screening		**Reference data (serum)**		
Genscreen				
		P	**N**	
	P	**459**	**0**	**Sens= 99.6 %, Spec. = 100%**
	N	**2**	**531**	
		Reference data (serum)		
Uniform II				
		P	**N**	
	P	**459**	**1**	**Sens = 99.6 %, Spec. = 99.8%**
	N	**2**	**530**	

III MEETING OF THE REGIONAL HIV/AIDS PUBLIC HEALTH LABORATORY NETWORK MAY 4-6, 2004

The NICD was a co-sponsor with WHO-AFRO and CDC of the third public health laboratory network. The meeting had a range of activities for consideration.

Staff including Lynn Morris, Adrian Puren, Terry Marshall, Sarvashni Moodliar, Emma Goetsch, Debbie Glencross (NHLS) primarily participated in the work group session 3: AIDS patient management. Various key components were considered e.g. 1. Decisions on technologies to be used; 2. Procurement of instrumentation and reagents; 3. Training of testing staff ;4. Collection of specimens and transport to labs;5. Reporting of results;.6. Continuing evaluation of testing algorithms; 7. Continuing education of lab staff and clinicians on the use of the test. In addition it was important to take into account the constraints e.g. 1. Lack of trained human resources and/or training programs; 2. Lack of adequate procurement systems to maintain a steady supply of reagents and other consumables; 3. Lack of communications between national programs, clinicians, and laboratorians; 4. Lack of perception of the importance of laboratory resources in patient care and monitoring; 5. Lack of adequate infrastructure at various levels in the system; 6. Lack of objective data on the best system for conducting laboratory testing based on existing infrastructure, manpower, etc; 7. Lack of current quality systems in place; 8. Lack of current organizational structure throughout the network for support of quality systems. Recommendations included: in the case of CD4 Measurement: Use the WHO Guidelines for initiating therapy, i.e., clinical staging +/- CD4 testing; Establish a two-tiered system utilizing flow cytometry for CD4 testing based on the volume on testing to be conducted, trained staff, etc. (Manual CD4 tests cannot be recommended at this time due to their high manpower requirements, training requirements, low numbers of tests that can be run per day, and difficulties in doing QA); Use more central, high volume laboratories initially (reference lab and provincial labs) with shipment of specimens to these from more peripheral labs/sites. These would have economies of scale, be better able to retained trained manpower, be easier to supply with reagents, etc.; Use reference laboratories for training programs for other labs and for conducting QA programs; Conduct validation studies of new CD4 techniques on a regional basis with rapid dissemination of results to regional reference lab directors and national planners; WHO should lobby test manufacturers for lower pricing; Recognize that new technologies are being evaluated. Make decisions on testing for the initial roll-out of ART with the recognition that different choices may be available for later years of the program. These may be less costly, easier to use, and/or less manpower intensive; Don't require the establishment of normal ranges for each country since this is highly unlikely to impact on treatment initiation or monitoring decisions. These will likely be done by research sites on a regional basis; 9. Establish similar guidelines for monitoring paediatric patients by experts on paediatric AIDS. In the case of HIV viral load testing (and this could be based on ability to afford testing) the recommendations included the use viral load on limited basis, primarily for evaluating patients who fail to respond to ART with a rise in CD4 count or clinical indications. Moreover, for HIV Resistance Testing the following was recommended: 1. Do not do resistance testing as part of individual patient management; 2. Establish a specimen repository at the national reference lab for possible later resistance testing; 3. Consider collection from naïve patients and from those with treatment failure; 4. Do resistance testing on a regional level.

HBV QUALITY CONTROL FOR MOLECULAR DIAGNOSTICS (QMCD) PANEL RESULTS

The qualitative diagnostic results for the eight panel members, HB09 – HB16, distributed for HBV diagnostic quality control in the first half of 2004 are shown in columns 2 and 3 of Table 2. This year for the first time we tested the panel using both our in-house PCR as well as the RealArt kit (artus Biotech, USA) and an in-house SYBR-green real time PCR. Conventional PCR results can be qualitative only and we also confined our reporting of the lightcycler results to a qualitative result since in our hands the RealArt kit results did not fit onto the standard curve and could therefore not be used to confirm the SYBR-green results. Prior to participation in this QMCD exercise we made several changes to the conventional PCR which have improved our scoring as compared to evaluations from previous years. The first change was to raise the annealing temperature of the HBV PCR, we also introduced a nested PCR reaction.

It is interesting that the conventional PCR detected the low positive, <200 copies/ml, specimen (HB16) whereas the two real-time PCRs did not. The latter were of course single round PCR reactions as second round PCR does not have the requisite linear amplification curve required for qualitative estimations. Despite this, if we cannot get our sensitivity down to <200 copies/ml routinely, we should consider a nested PCR on the lightcycler even if viral load in these low copy number specimens cannot be accurately measured. As expected

this was the positive panel member most frequently identified incorrectly being missed by 25.0 % of the panel sets. The fact that one of the specimens HB-12 was missed by all three of our methods could indicate that the pellet was lost during extraction. It is also interesting that this panel member was the second most frequently incorrectly identified being missed by 6.73 % of panel sets. HB-12 was one of two low positives (both with a given concentration of 1.00E+3 copies per ml) the other, HB10, which we detected with both lightcycler protocols was missed by 2.88 % of the panel sets. The SYBR-green qualitative values were within 1.5 logs of the reported results in all cases except for specimen HB14 which was 2.8 logs higher than reported.

Our laboratory is hoping to improve the precision of our realtime PCR and to eventually use this method for routine diagnosis. The main advantages being the turnaround time and the additional quantitative information provided. Further changes which we are now implementing include using a greater starting volume and smaller re-suspension volume in the extraction to concentrate the specimens and thus increase our lower cut-off value. Also automatic dispensing of reagents prior to real-time PCR using the MagNA Pure LC instrument will be implemented for greater precision. In future we will not use the RealArt PCR kit as it is expensive. Also the standards used in this kit are not extracted with the serum specimens and we feel that to control both the extraction and the PCR will give more realistic quantitative readings. By including standards routinely in the diagnostic assay we also hope to get a better idea of the range of viral load in patients in this country and an accurate assessment of what proportion of low positive specimens have in the past been missed by less accurate methods.

QMCD panel results for 2004

	QMCD Result (copies/ml)	Reported Log range	SYBR Green	SYBR Green log range	SYBR green agreement with reported result	Real Art	Real Art log range	Real Art agreement with reported result	1st round PCR	Nested PCR	PCR agreement with reported result
HB09	1.0E+05	4,6-5.6	1,41E+05	5.15	OK	+		Low	-	+	OK
HB10	1.0E+03	2.9-3.7	5.01E+04	4.69	High	+		Low	-	L+	OK
HB11	1.0E+05	4.7-5.5	1.99E+06	6.29	High	+		Low	- *	- *	False-ve *
HB12	1.0E+03	3.1-3.9	- *	- *	False-ve *	- *	*	False-ve *	- *	- *	False-ve *
HB13	Negative	N/A	-	-	OK	-		OK	-	-	OK
HB14	1.00E+04	3.7-4.5	1.99E+07	7.29	High	+		Low	+	N/D	OK
HB15	1.0E+06	5.1-6.5	6.31E+07	7.80	High	5.12E+04	4.70	Low	-	+	OK
HB16	2.0E+02	1.9-3.4	- *	- *	False-ve *	- *	*	False-ve *	-	L+	OK
QMCD panel score					12/16			12/16			12/16

N/A = not applicable
N/D = not done
-ve = negative
* = results which do not agree with the QMCD panel results. Panel was scored 2 points for each correct result. Maximum total is thus 16.

N/A = not applicable, N/D = not done, -ve = negative
*** indicates results which do not agree with the QMCD panel results. Panel was scored 2 points for each correct result. Maximum total is thus 16.**

HEPATITIS B

Recently the Instituto Nacional de Saude (INS) in Maputo conducted a study to establish the baseline epidemiology of viral hepatitis in Mozambique. Specimens were collected from Massaca, a semi-rural community on the outskirts of Maputo in southeastern Moçambique. The rural district was divided into 12 sections (A-M), and within each section the number of infected individuals per household was identified. Blood samples, spotted onto filter paper, were sent to the INS for ELISA analysis of past or present HBV infections. Positive patients were recalled to the clinics and further samples, both whole blood and dried blood spots (DBS), were taken.

Analysis of the HBV positive samples was done by the hepatitis division of the Specialized Molecular Diagnostics unit at the NICD using a nested PCR, followed by sequencing on an ABI Prism 3100 Genetics Analyser. Phylogenetic analysis of the sequence data was used to identify the specific genotypic groups and subgroups of the viral strains infecting individuals, and nucleotide and protein alignments made to determine whether unique features of the virus, localized to Mozambique, existed and whether there is enough evidence to support intra-familial infection. DBS samples have not been tested previously at the NICD, and we were thus interested in the reliability and repeatability of this storage method for obtaining DNA for use in sequencing and genotyping studies.

Thus far 29 of the approximately 97 HBsAg-positive specimens have been successfully sequenced and genotyped, of which 22 (75.8%) have been found to be of subgroup A' (also called subgroup Aa for A[Africa/Asia], see below) and 7 (31.8%) of genotype E. No other groups or subgroups have been identified.

Although a 654 bp region covering the entire preS2 region of the hepatitis B virus, HBV, genome and 489 bp of the small surface antigen (HBsAg) gene of HBV were sequenced and analyzed, most of the definitive change was observed in the 165 bp preS2 region. An UPGMA tree of the preS2 region (Figure 1), rooted on two hepatitis B specimens from the woolly monkey taken from GenBank, was drawn using sequence data from the 29 Mozambican specimens together with other sequence data generated in our laboratory from 39 South African and 17 Namibian specimens together with a further 4 specimens from GenBank. All of the genotype E specimens from the 3 southern African countries of Namibia, South Africa and Mozambique, with the exception of 3 specimens which partition as outliers (M57 and M53 from Namibia and B1/8 from Mozambique) cluster together in the same clade. None of the Mozambican specimens cluster into genotype D but the other six southern African genotype D specimens identified also all cluster together in one clade.

Fig 1. UPGMA tree of preS2 region of HBV rooted on two woolly monkey HBV virus isolates. The names of local specimens is made up of a prefix giving the genotype or subtype of the specimen in the tree of the pS2/S region followed by a sample identifier and then an abbreviation of the country of origin (Mz = Mozambique, SA = South Africa and Na = Namibia). Sample identifiers for the Mozambican specimens consist of 2 parts a household identifier followed by a number denoting the member of that household. Genbank specimens have the same prefix followed by their accession numbers and published South African specimens from our lab have in addition an S after the accession number to denote that they too are from South Africa.

Two of these specimens are putative recombinants as they cluster in alternate clades in the tree of the full 654 bp region (not shown but indicated in the prefix of the specimen name in the preS2 tree shown in Fig. 1). One of the putative recombinants, EK30, was from a South African and this specimen clustered previously with genotype E while the other, M54, which was from a Nambian clustered previously with genotype A. In contrast, the southern African specimens partitioning in genotype A form several distinct clades. The first notable division within genotype A is the separation of the specimens into the two subgroups Aa and Ae. When we first recognized these subgroups of genotype A we called the two partitions subgroup A' and subgroup A. However, since subgroup A' appears to be found only in Africans and Asians it has been suggested that this subgroup be referred to as subgroup Aa for subgroup A[Africa or Asia] whilst the second subgroup is called subgroup Ae (for subgroup A[Europe]). Thus at the amino acid level the partitioning of the specimens into the two subgroups Aa and Ae can be seen (Figure 2 and boxed sites in Table 1) to be based on amino acids at position 32 (Aa=L/H Ae=V), 35 (Aa=V Ae=A), 47 (Aa=S Ae=A) and 54 (Aa=S/L/P Ae=T). Five other amino acid sites (highlighted in grey in Table 1), which previously we have noted to be different between the two subgroups, were not corroborated by the addition of the Mozambican data. This phenomena created a South African and Namibian clade within subgroup Aa which clustered away from the Mozambican specimens. In all five of these sites (amino acid positions 10, 11, 21, 22 and 48) the Mozambicans were more similar to subgroup Ae than to subgroup Aa. However, the opposite is seen at position 53 where the South Africans and Namibians share a valine with subgroup Ae whilst the Mozambicans have, predominantly, an alanine in this position (Figure 2 and Table 1). Thus, although the number of Namibian specimens is low (there are only 3 Namibian genotype A specimens in this cohort) the South African subgroup Aa specimens seem to be a mixture of what is seen in the Mozambicans and the Namibians (Table 1). This same trend is not reflected in the genotype E results as genotype E is rare in South Africa but makes up the majority of specimens from Namibia (61.9 %) and is found in intermediate amount (31.8 %) among the Mozambicans.

Table 1: Amino acid differences which partition the southern African specimens of genotype A

aa		7	10	11	21	22	32	35	37	38	41	45	46	47	48	53	54
subgroup A' (Aa)	Mozambique	A	Q	A	Y	F	L	V	N	I|T	H	I|T	S	S	R	A	L|S
	South Africa	A	Q|R|K	A|D	Y|H	P|F|L	L|H	V	N|I	I|T	H	I	S	S	T|K|R	V|A	P|L
	Namibia	A	R|K	D	H	P|H	L	V	N	I	H	I	S	S	T	V	P
subgroup A (Ae)		A	Q	A	Y	F|L	V	A	N	I	H	I	S	A	R	V	T

The 4 boxed amino acid positions partition the subgroup Aa from the subgroup Ae. Shaded amino acid positions are responsible for the Mozambican specimens forming their own clade within subgroup Aa. Other amino acids shown differentiate genotype A from the other genotypic groups.

Of the four households found to have multiple infections (see the 4 household groups B4, B16, G2 and C15, demarcated by horizontal lines in Figure 2), only household B4 has identical amino acid sequence in all three infected household members. Little work has been done re quasispecies, rate of mutation and transmission so it is difficult to judge whether, for example, the C15 household, all of which have an unusual variation at amino acid 34 (Figure 2), are all infected with a different virus or whether the same virus has been transmitted between them and the variations observed evolved later.

The Blacks of Africa descend from West Africans who traveled southward 3 to 5 thousand years ago in three major waves collectively called the Bantu Expansion. Each of the major waves of migration moved along a separate route to southern Africa. The first and earliest wave formed the North-Western Bantu while the second wave, believed to have started well before the third wave of the Eastern Bantu-speakers, formed the Western Bantu. The mixture of the Namibian and Mozambican genotype A characteristics in the South African specimens could, however, be the result of recent mixing as South Africa is host to migrant labourers from throughout the subcontinent. But since genotype E is rare in South Africa

this same recent origin is not reflected in the incidence of this genotypic group. It is likely that the mixing of Western and Eastern migrations resulted in the 33% incidence of genotype E in the Mozambicans since a movement of migrant workers from the West of southern Africa to the relatively poor and Portuguese speaking Mozambique is not likely. Finally it is of interest that genotype E which shows characteristics of being a recombinant of genotype D and an unknown second parent is much more conserved than genotype A. If this recombination is a recent event then this could account for the lack of variation in the genotype E specimens but it still remains for this hypothesis to be proven by finding the missing parent.

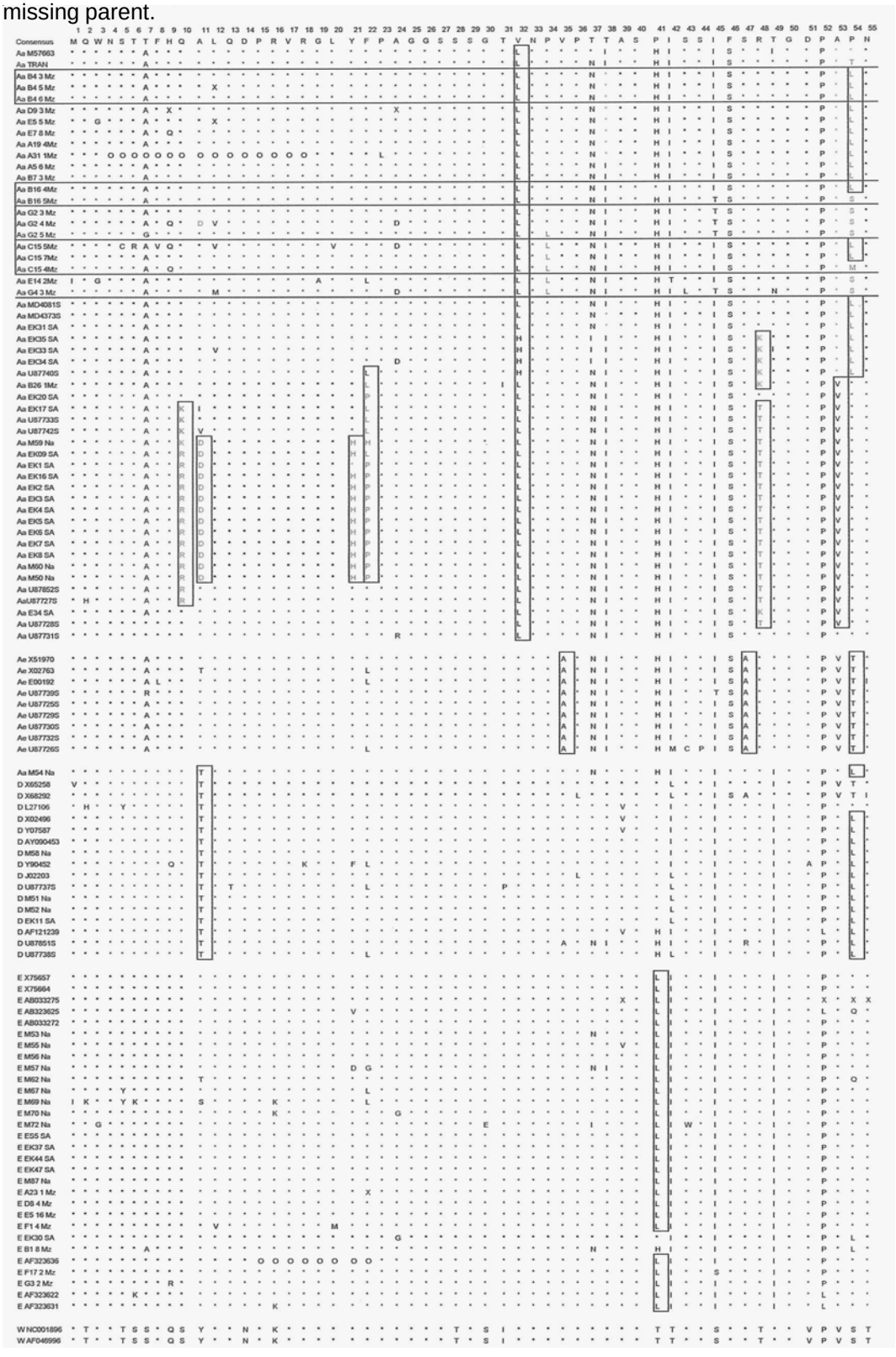

Fig 2. Amino acid alignment of southern African specimens with Genbank specimens (see legend to Fig 1 for labeling convention used). Horizontal lines group together specimens from the same household and double horizontal line separates the Mozambican subgroup Aa specimens from the rest of the subgroup. Chosen definitive amino acids are boxed for clarity (see text for full explanation).

Dr Sheila Bowyer in the Post-PCR laboratory analyzing the results of a quantitative hepatitis B real-time polymerase chain reaction (PCR) run on one of the three light cyclers available on campus.

HEPATITIS C

Genotyping using the 5'untranslated (5'UTR) region of the HCV genome was continued during the year. Of the 308 RNA positive specimens, 113 specimens were genotyped in this region. Sixty-three (56%) were genotype 5, 31(27%) were genotype 1, 7(6%) were genotype 4, 8(7%) were genotype 3 and 2(2%) were genotype 2. None were genotype 6. From the phylogenetic analyses, it was evident that more specimens of Genotype 5 grouped with the "atypical" cluster (Fig.1). These sequences did not have the characteristic double adenine (AA) at positions 106 and 107.

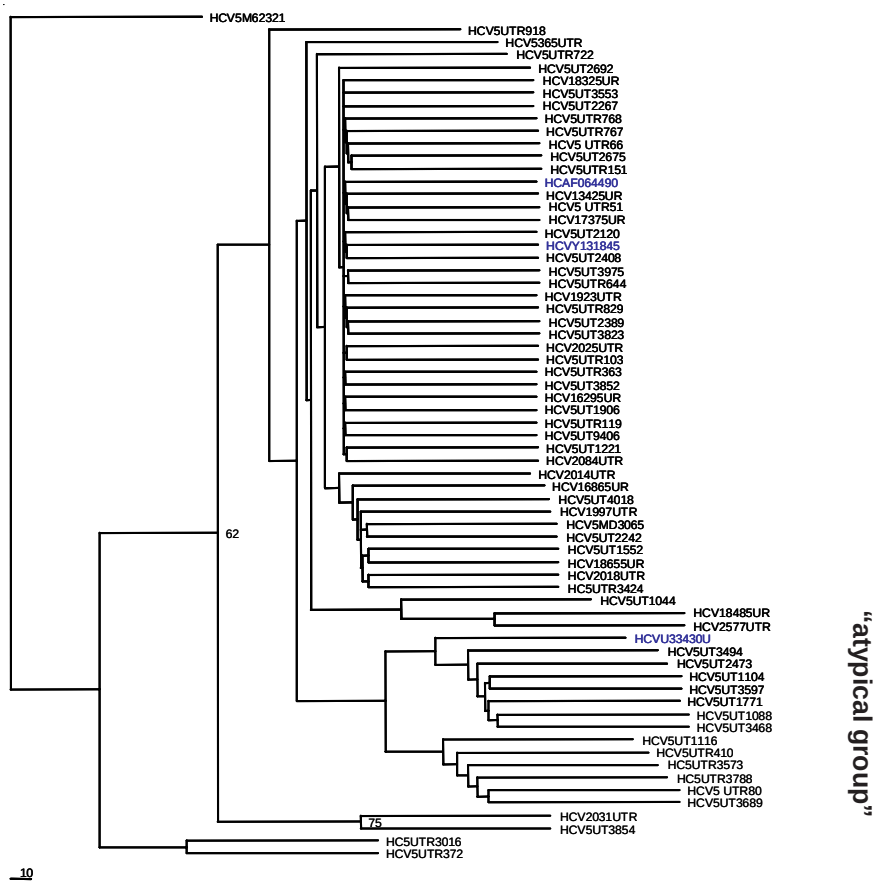

Fig.1. Phylogenetic analyses of 63 HCV genotype 5 isolates, sequenced in the 5'UTR. The tree is rooted with HCV Genbank Genotype 1 M62321 isolate. Sequences in blue are Genbank genotype 5 and 20 sequences at the bottom form the "atypical" cluster.

From previous studies the nonstructural 5b protein region (NS5B) was found to be more variable and allowed for further characterization and subtyping of HCV. It was used to confirm genotyping results of the 5'UTR region. Eighteen specimens were randomly chosen to be sequenced in the NS5B region. Most of these specimens were genotyped in the 5'UTR region previously. Twelve of the 18(66%) of the specimens were successfully nested and sequenced using published primers. Phylogenetic analysis of this region compared well with the 5'UTR region, with only one subtyping discrepancy for specimen 9375, which was subtyped as a genotype 1a in the 5'UTR region and is genotype 1b in the NS5B region (Fig.2). Specimens 1108 and 1909 still remain outliers as in the 5'UTR region of the genome (not shown). Quality control specimen QC4 could not be amplified in the 5'UTR region but grouped correctly with genotype 3a, as expected from QCMD results given. Four of the 6 specimens that could not amplify were of genotype 4 in the 5'UTR region. The primers used for the NS5B area may have been non-specific to that specific genotype. Genotype 5 specimens from the "atypical cluster" (Fig.1) will be further studied in the NS5B region.

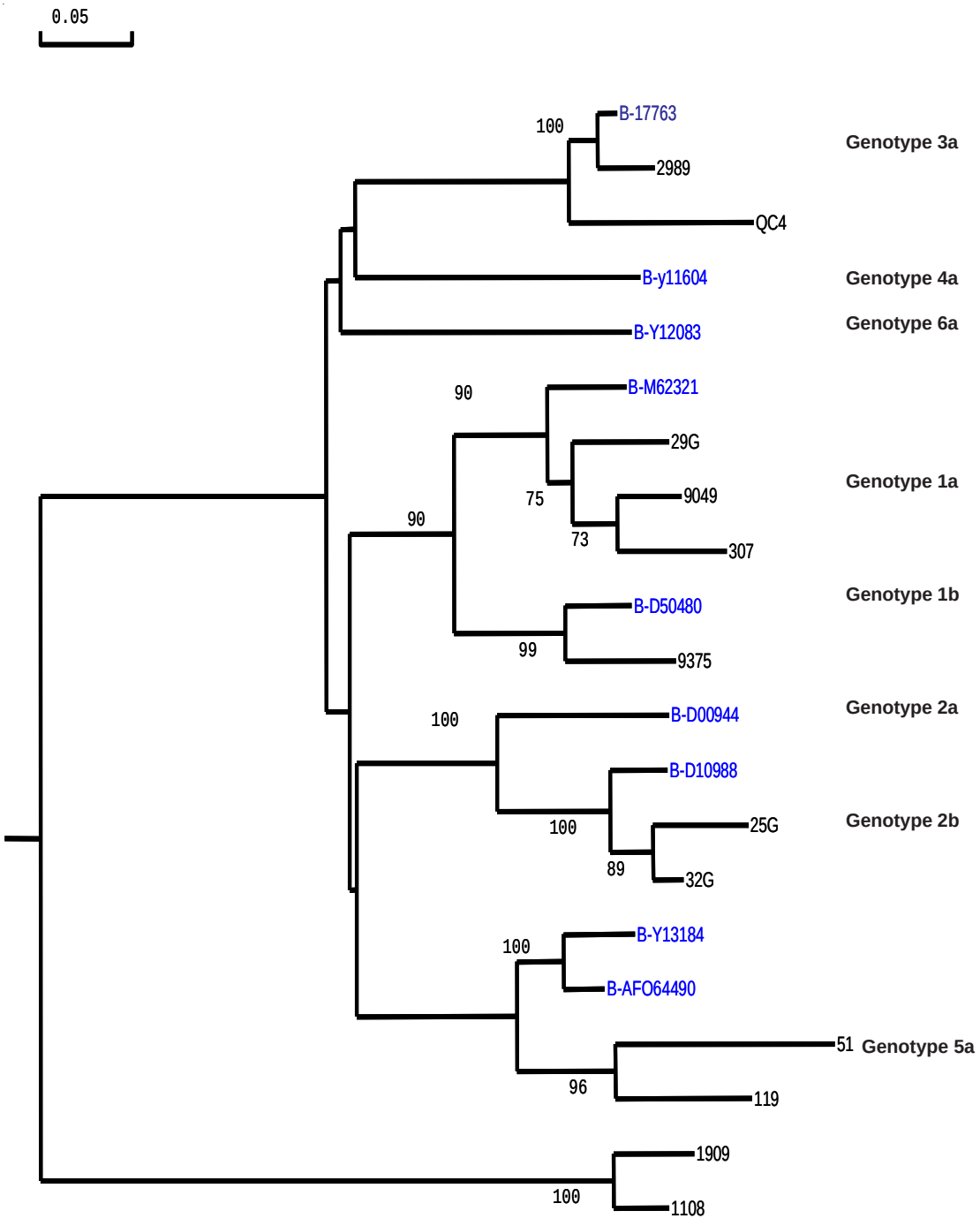

Fig. 2. HCV NS5B gene phylogenetic tree for genotypes previously studied in the 5'UTR region. Sequences in blue are from the Genbank database.

HCV Qualitative PCR and genotyping of the 5'UTR region have been used in assessing the annual QCMD panel. Two HCV-QCMD panels of eight specimens each were received during 2004. 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. Two specimens were genotype 2, two were genotype 1, subtyped 1a and 1b, one was genotype 3 (subtype a), two were genotype 4 and one was negative. The phylogenetic analysis of the 2004 - Genotype QCMD panel compared well with expected results.

CONFERENCES/MEETINGS

Dr Puren: Meeting at CDC on HIV diagnostics in Infants: the use of real time PCR, October 2004

Dr Puren: Board meeting of QCMD: the role of EQA in the molecular diagnostics lab, October 2004

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MOLECULAR EPIDEMIOLOGY OF INFLUENZA VIRUS

Influenza viruses pose significant challenges to both human and animal health. In the human population, frequently occurring point mutations in the viral surface haemagglutinin (HA) and neuraminidase (NA) proteins allow these viruses to escape existing immunity to previously circulating influenza viruses. Through this process of antigenic drift, new variants evolve in humans throughout the world, causing epidemics almost every year. The subtypes of influenza A that have been circulating globally in humans in recent years are the H1N1, H3N2 and the reassortant H1N2 viruses, while two distinct lineages of influenza B virus have also circulated since the late 1980s.

Unlike influenza B viruses which occur in humans only, influenza A viruses have an animal reservoir and can cause global pandemics in humans. All 15 different HA subtypes and nine different NA subtypes of influenza A virus are found in influenza A viruses in wild birds, the primary natural reservoir for influenza A virus. Infection with certain avian influenza A viruses can cause widespread disease and death among some species of wild as well as domestic birds. Human pandemics occur due to an abrupt major change in the viruses, resulting in new HA and/or new HA and NA proteins not present in the previously circulating human influenza viruses. This antigenic shift results in a novel influenza A subtype with most people having little or no protection, and subsequent high levels of high morbidity and mortality worldwide.

Recently, fears of an imminent pandemic have arisen due to the historically unprecedented situation that has occurred since December 2003 where large outbreaks of highly pathogenic avian H5N1 influenza in domestic poultry were reported in several Asian countries simultaneously. Of the nine countries (Cambodia, China, Indonesia, Japan, Laos, Malaysia, South Korea, Thailand and Vietnam) affected, two reported human cases of avian influenza A H5N1. The human cases of avian H5N1 influenza occurred in Vietnam and Thailand with 44 cases being laboratory confirmed. Of these 44 patients, 32 died. More cases of avian H5N1 human infection have recently been reported in Vietnam since late December 2004. It has been speculated that it is highly likely that additional human cases have occurred in other affected countries, but have remained unrecognised due to a lack of clinical awareness or diagnostic facilities.

To date, no evidence of sustained person-to-person transmission has been identified, although a probable instance of limited person-to-person transmission in a family cluster was identified in Thailand earlier in 2004. The concern nevertheless exists that the H5N1 viruses have the potential to reassort with existing human influenza viruses to produce a strain with high virulence and efficient transmissibility, thereby initiating a new influenza pandemic. Many countries have accelerated their pandemic preparedness planning as a result of the recent avian H5N1 outbreaks in Asia.

Influenza activity in South Africa in recent years has been mild. An exception occurred in 2003 when a localised epidemic affecting 646 individuals in Pretoria during late May – early June took place. The epidemic was shown to be due to the introduction of influenza A H3N2 viruses that were antigenically distinct to those that had circulated in the country in previous years. These 2003 strains were closely related to the A/Fujian/411/02 variant viruses that were first detected in Asia in 2002. These variants were quite distinct from the A/Panama/2007/99 vaccine strain used for the 2002 influenza season in the southern hemisphere. A/Fujian/411/02-like viruses were also isolated in other regions in the southern hemisphere, necessitating an update of the H3N2 vaccine strain from A/Panama/2007/99 to an A/Fujian/411/02-like strain. The A/Fujian/411/02-like virus that was most commonly used as the actual H3N2 vaccine strain during the 2004 season was A/Wyoming/3/02. The virus strains recommended by the WHO for the influenza A H1N1 and influenza B vaccine formulation for the southern hemisphere 2004 influenza season were the A/New Caledonia/20/99-like and B/Hong Kong/330/01-like viruses. The latter included either the B/Shangdong/7/97 or B/Brisbane/32/02 strains.

Influenza activity was sporadic during the South African 2004 winter season. Influenza virus was isolated between May to August from 38 patients attending Viral Watch Centres and private pathologists. The school absenteeism programme, monitoring approximately 8 000 children at primary and high schools, rose to above the mean (calculated over a five year period), on weeks 26 and 29. However, these were the weeks that winter school holidays started and ended in government schools.

The vast majority of the South African viruses isolated in 2004 were influenza A viruses (97,3%) with only one influenza B virus identified. In addition to analysing the South African influenza isolates, nineteen specimens received from the Institut Pasteur, Abidjan, Cote d'Ivoire, were also tested and subtyped for influenza.

Antigenic subtyping of the Johannesburg influenza A virus isolates by the haemagglutination inhibition (HI) assay revealed that 33 were subtype H3N2 while the remaining four could not be subtyped. Some of the H3N2 viruses reacted well with the A/Fujian/411/02-like reference antisera in the WHO HI Kit while others reacted at lower titres. Subsequent data obtained from a number of South African H3N2 strains sent to the WHO Collaborating Centres (WHO CCs) for Influenza Reference and Research in Melbourne and London for further serological characterisation by HI showed that they were antigenically similar to the more recent A/Christchurch/28/03 and the A/Wellington/1/04 reference strains.

With regard to the specimens received from the Institut Pasteur, Abidjan, Cote d'Ivoire, seven specimens were positive for influenza A and six positive for influenza B. Subtyping by HI revealed that the majority of the influenza A positives were H3N2 A/Fujian/411/02-like with one being subtype H1N1 A/New Caledonia/20/99-like.

Figure 1 shows the results of the phylogenetic analysis of representative South African influenza A H3N2 HA1 sequences (921 bp). The 2004 isolates exhibited genetic drift from the A/Fujian/411/02 and A/Wyoming/3/02 strains and the majority grouped with the more recent A/Wellington/1/2004 reference strain.

Amino acid analysis of the HA1 subunit of the Johannesburg influenza A H3N2 viruses with the A/Fujian/411/02 strain sequence revealed that the isolates shared three common amino acid changes at positions 159 (tyrosine to phenylalanine), 189 (serine to asparagine) and 227 (serine to proline). The former two positions map to the influenza antigenic site B and the latter to the receptor binding site of the HA. These mutations were also seen in many 2004 isolates from other areas of the world (WHO data). Other substitutions were observed in some of the South African isolates at position 188 (aspartic acid to tyrosine) in antigenic site B and 199 (serine to proline). Several H3N2 isolates had the same amino acid changes at 145 (lysine to asparagines) and 226 (valine to isoleucine) that were seen in some of viruses isolated from New Zealand, Australia and Asia isolated later in the 2004 season (WHO data, Melbourne). One of these isolates (A/Johannesburg/31/04) was recovered from a patient who returned ill from a trip to Australia and Singapore.

Sequence analysis of representative H3N2 viruses isolated from the specimens sent by the Institut Pasteur, Abidjan, Cote d'Ivoire, showed that some strains were similar to the A/Wellington/1/04-like and the majority of the 2004 Johannesburg viruses. One of the viruses from Abidjan, on the other hand, lacked the characteristic S227P mutation seen in the A/Wellington/1/04-like viruses and shared a higher homology with viruses like the A/Norway/88/03 isolate.

The 2004 B/Johannesburg/22/04 isolate reacted with the B/Hong Kong/330/01-like reference antiserum in the HI test and was confirmed to be antigenically similar to the B/Shandong/7/97 and B/Brisbane/32/02 strains by the WHO CCs. Five of the six influenza B positive specimens from Abidjan reacted with the reference B/Sichuan/379/99 antisera while the remaining one could not be typed by HI.

The molecular characterisation of the Johannesburg influenza B isolate showed that it was closely related to a 2004 strain from Israel and was typical of other recent viruses of the B/Victoria/2/87 lineage (Figure 2). This is unlike the situation the previous year in South Africa, where all the B strains had evolved from the B/Yamagata/16/88 lineage.

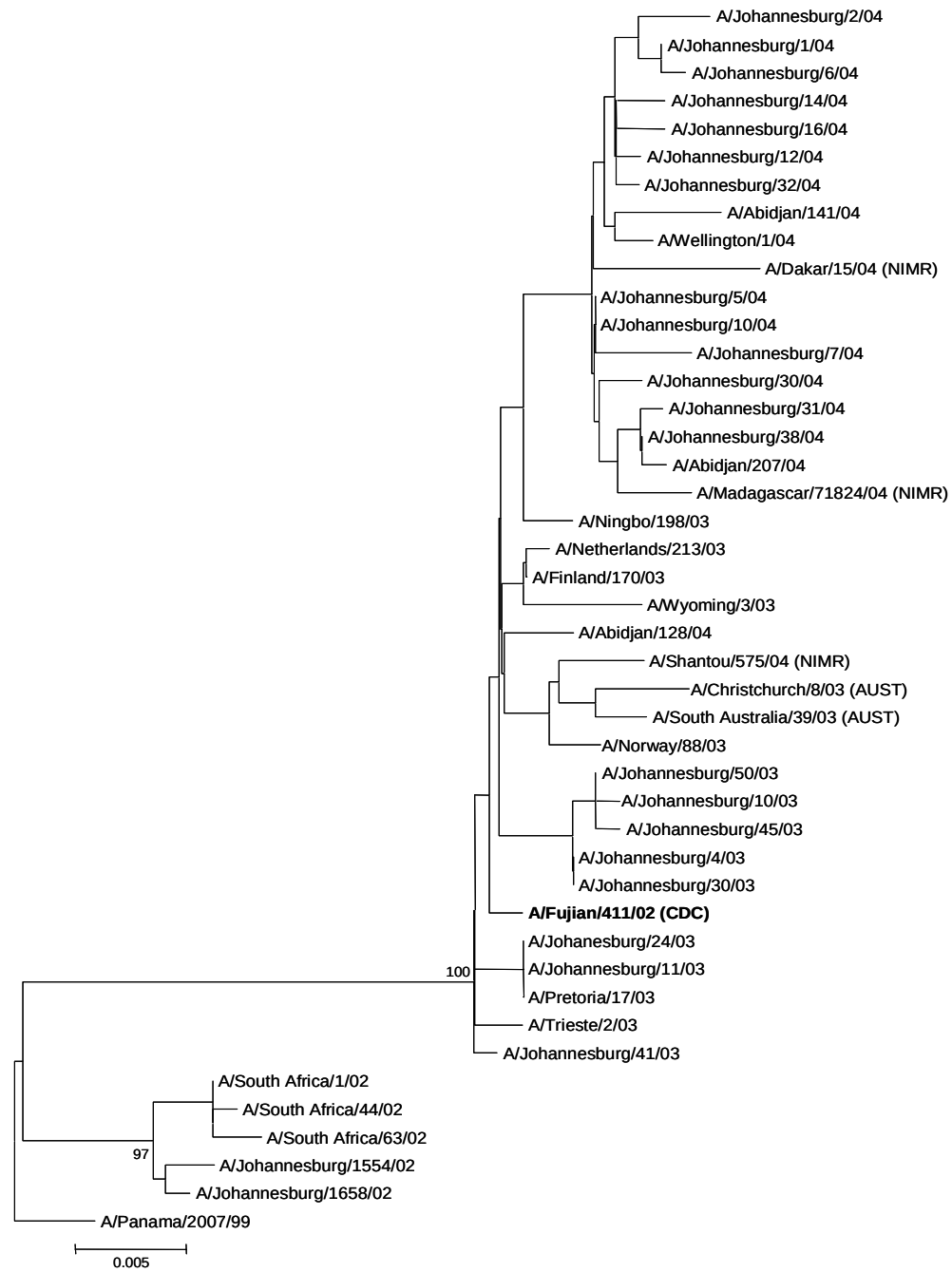

Fig 1. Phylogenetic tree of influenza A (H3N2) virus HA1 gene nucleotide sequences (923 bp). (H3N2 vaccine strain is depicted in bold)

Sequence analysis of the 2004 isolate from Abidjan that could not be typed by HI revealed it to clearly belong to the B/Victoria/2/87 lineage. It clustered with strains isolated from Madagascar in 2004 (NIMR, London). The other Cote d'Ivoire B isolates had evolved along the B/Yamagata/16/88 lineage and were related to Italian 2004 viruses as well as Johannesburg viruses isolated in 2003.

Based on the antigenic drift observed in the influenza A H3N2 strains observed in South Africa and other areas in the world during March – October 2004, the H3N2 vaccine component was updated to an A/Wellington/1/2004(H3N2)-like virus for the 2005 southern hemisphere vaccine. The recommended influenza B strain was a B/Shanghai/361/2002-like virus where the actual vaccine viruses included the B/Shanghai/361/2002, B/Jilin/20/2003 and B/Jiangsu/10/2003 strains. The recommended H1N1 strain remained unchanged.

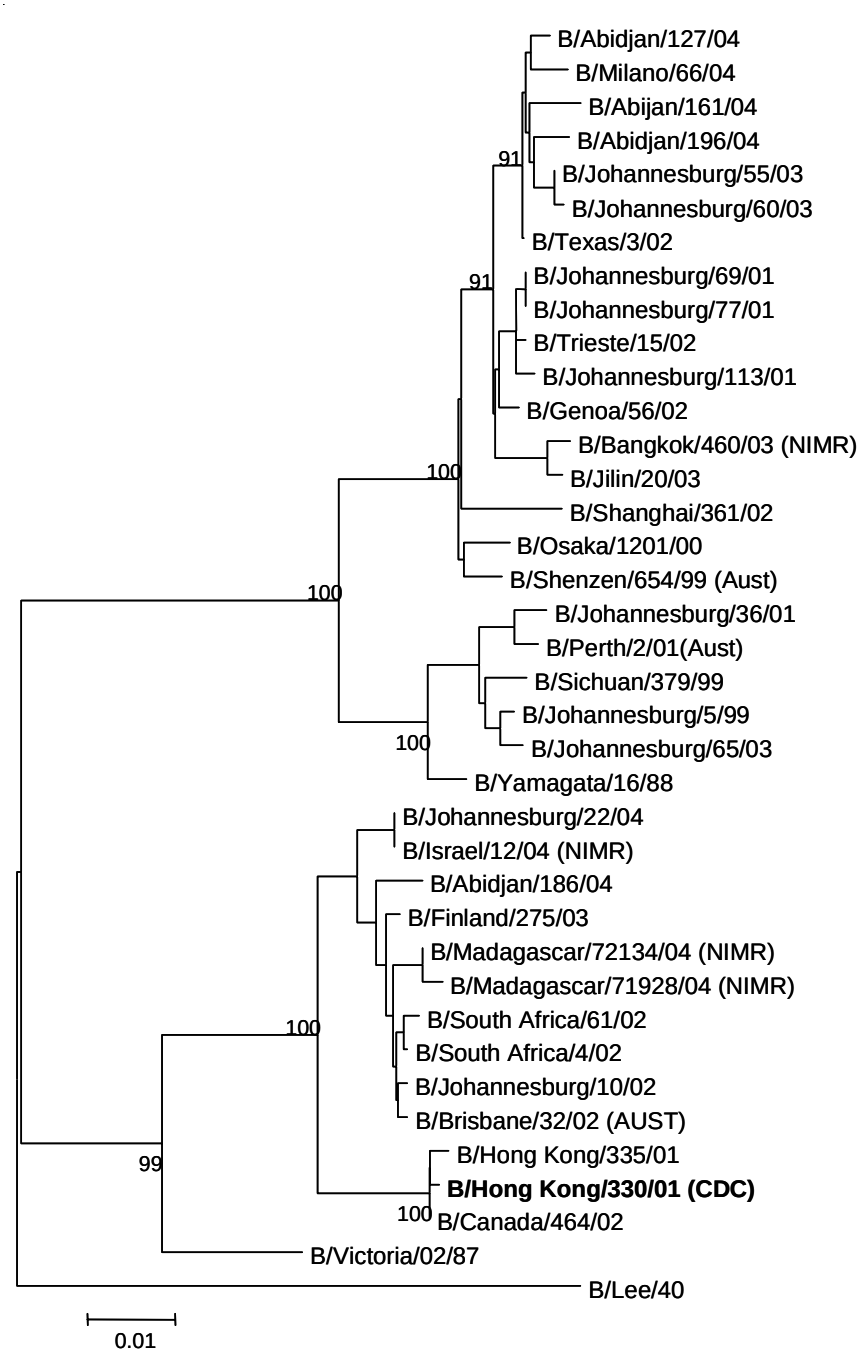

Fig 2. Phylogenetic tree of influenza B virus HA1 gene nucleotide sequences (957 bp). (B vaccine strain is depicted in bold).

Given the current pandemic threat posed by the highly pathogenic avian influenza H5N1 which is now widely entrenched in Asia, it is important that measures to taken to reduce the impact of the pandemic. These include establishing a BSL-3 laboratory to allow for the rapid detection of highly pathogenic strains of influenza, updating the South African influenza pandemic preparedness plans and ongoing collaboration with the WHO Global Influenza network.

Acknowledgements:
We would like to thank the Institut Pasteur, Abidjan, Cote d'Ivoire, for sending us influenza specimens for characterization. Sequences from other countries were obtained from the WHO Collaborating Centres for Reference and Research on Influenza, London and Melbourne, the LANL influenza database: http://www.flu.lanl.gov and from Dr Olav Hungnes, Norwegian Institute of Public Health, Oslo.

MOLECULAR EPIDEMIOLOGY OF POLIOVIRUS IN SUB-SAHARAN AFRICA

Nicksy Gumede
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Molecular Polio Laboratory

During 2004, the Poliovirus Molecular Unit of the NICD, which is a WHO Regional Reference Laboratory, received 2716 poliovirus isolates (Figure 1), which were characterized as vaccine or wild type using two intratypic differentiation methods, PCR and ELISA. These isolates were sent to the NICD from National and Regional laboratories throughout Africa namely, Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Congo, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Liberia, Mali, Malawi, Mozambique, Nigeria, Niger, Democratic Republic of Congo (DRC), Rwanda, Sierra Leone, Senegal, South Africa, Somalia, Sudan, Swaziland, Tanzania, Togo, Uganda and Zimbabwe (Figure 2). Original specimens from AFP cases were received from several southern African countries and any polio isolates were treated as above.

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.

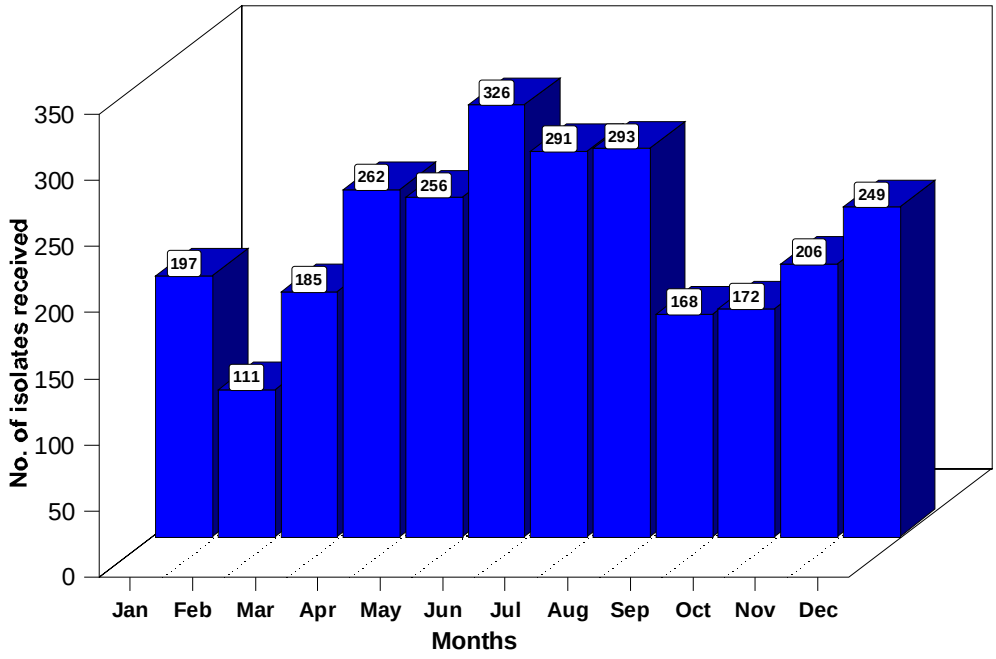

Fig 1. Poliovirus samples received in 2004

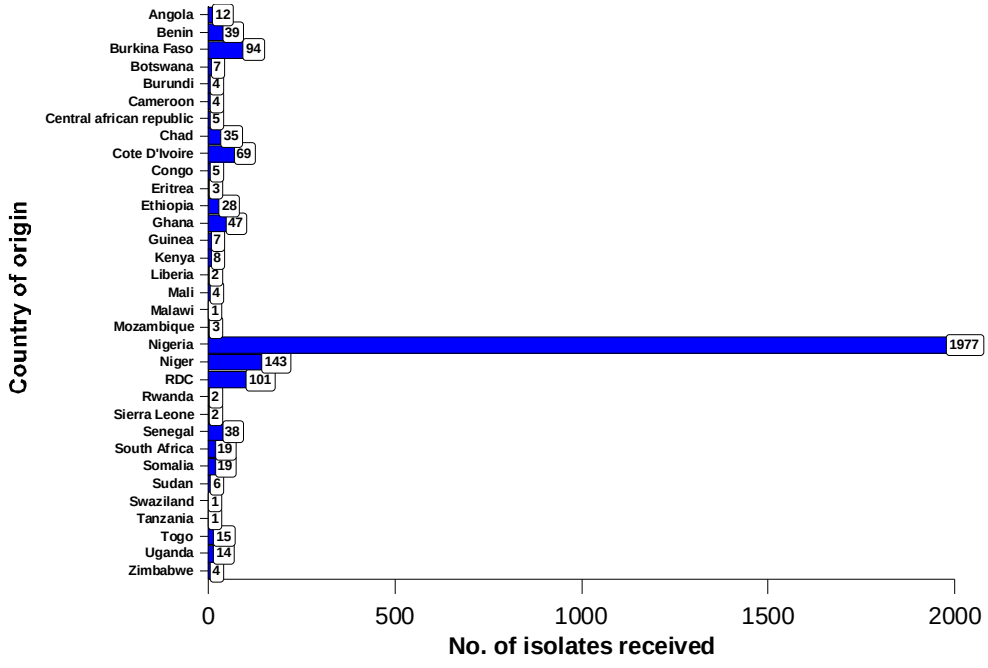

Fig 2. Poliovirus samples received from African countries

The majority of the wild-type cases identified during 2004 were from Nigeria (Figure 3), 594 of which were polio type 1 and 179 polio type 3. Other cases identified in 2004, some of which were isolated late in 2003, were from Benin, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Cote D'Ivoire, Guinea, Mali and Niger. One case of wild type 1 poliovirus was isolated from a stool specimen of a 7 year old male child in the Ngami district in North-Western Botswana in March 2004. The child developed paralysis on 8 February 2004, and adequate stools were collected on 18 and 19 February. As of January – April 2004, Botswana has an annualised AFP detection rate of 3.0 and stool adequacy of 100%.

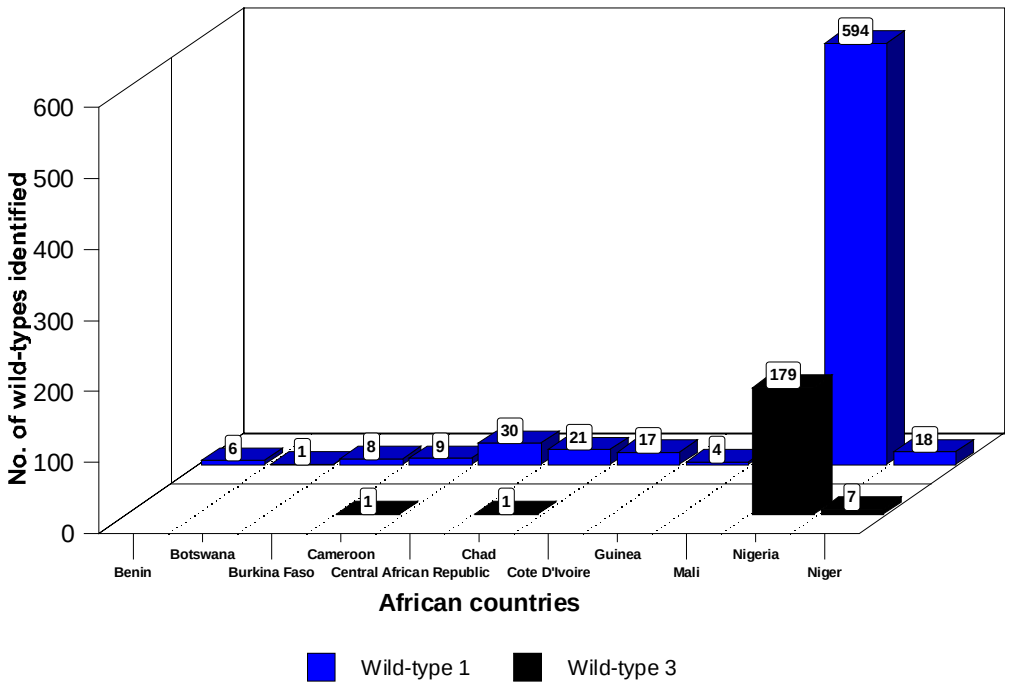

Fig 3. Wild type cases reported in 2004

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

The wild-type isolates can be placed into the known genotypes using the information from the sequence analysis of the 900 base pairs from the VP1 region. Wild PV1 and PV3 are still endemic in Africa, major reservoirs have been found in West and Central Africa. The remaining reservoir in Africa is Nigeria (WEAF-B genotype) (Figure 4).

Distribution of wild PV3 genotypes closely parallels PV1 distribution. Intergrated AFP and virologic surveillance is giving a very high-resolution picture of the patterns of wild type poliovirus circulation in Nigeria. Indigenous circulation of the WEAF-B genotype has largely stopped in southern Nigeria, but northern Nigeria, with Kano, Katsina and Kaduna as the main reservoir source, has a large ongoing endemic (Figure 5).

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) The first three letters and a number represent the Lab ID and EPID Number.

Fig 4. WEAF-B wild PV1 representative of isolates: 2002 - 2004

Wild PV1 was highly endemic in northern Nigeria in 2004 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.

2004 isolates are distributed into sixteen clusters (A to I_8), (other clusters are not shown). In figure 4, 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. Cluster I was very active in 2004 and consist of several lineages. The closely related viruses were found in Togo, Ghana and Burkina Faso and all belong to cluster I_3. In 2004, 896 cases were identified in Africa with Nigeria having the largest number.

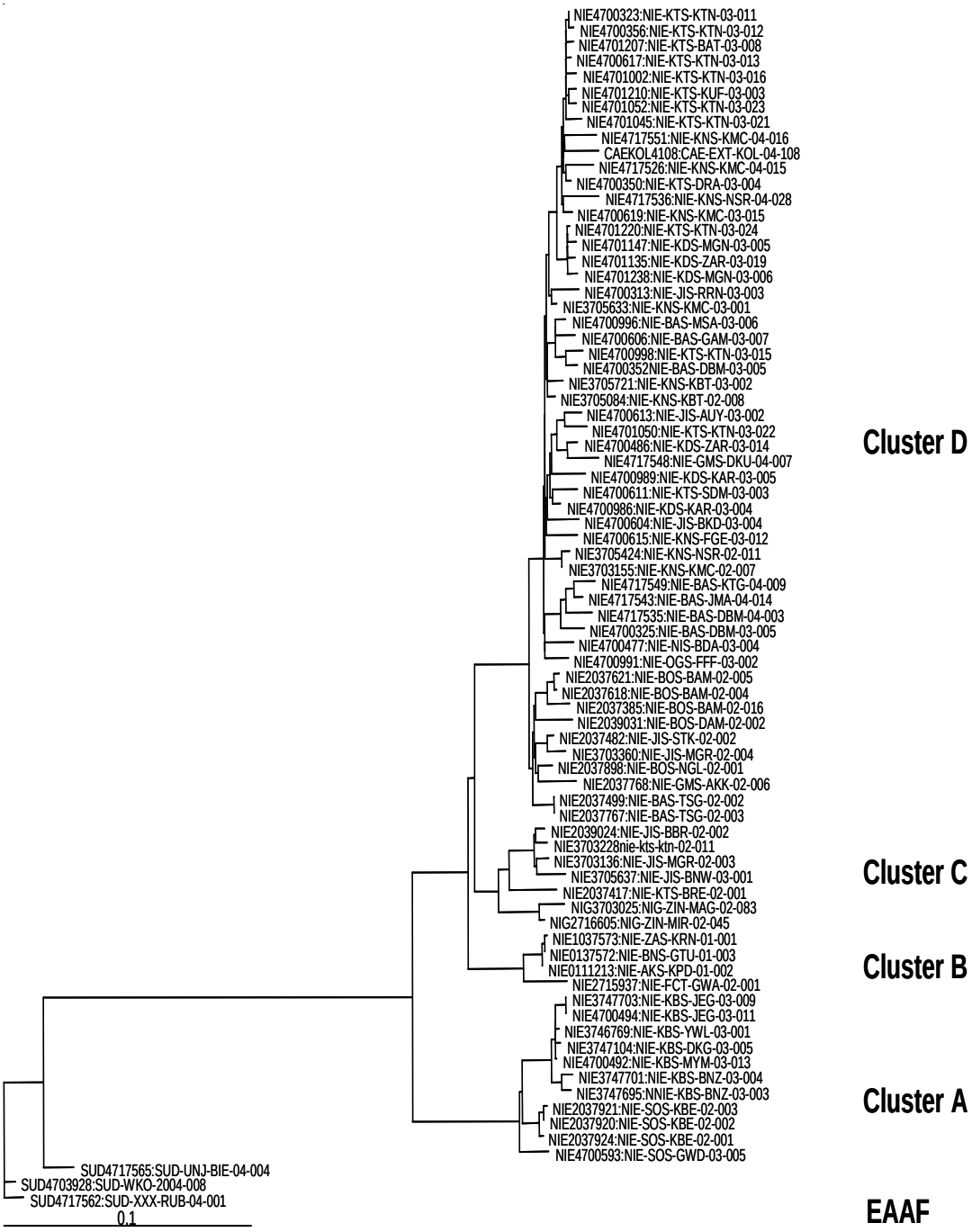

Fig 5. WEAF-B Wild PV3 representative of isolates: 2000 - 2004 survey

WEAF-B wild PV3 is divided into four clusters A – D (Figure 5). Cluster A represents local circulation in Sokoto (SOS) province of Nigeria. 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. Viruses from three countries, Nigeria, Niger and Cameroon, fell into Cluster D, compared to 2003 where viruses from only Nigeria and Niger grouped into Cluster D. Three 2004 isolates were reported from PV3 EAAF genotypes (figure 5). These were from Sudan lineages.

VACCINE-DERIVED POLIOVIRUS (VDPV)

No VDPV was identified in 2004.

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 questionnaires 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 the NTF to create a database/national inventory of facilities housing potentially infectious material.

MOLECULAR SURVEILLANCE OF MEASLES AND RUBELLA IN SOUTHERN AFRICA

Sheilagh Smit
Senior Medical Scientist
Molecular Measles Laboratory

Measles

There were fairly large measles outbreaks in 2 provinces of South Africa, Mpumalanga and Gauteng, starting in June and July of 2003 respectively, that have continued throughout 2004 (cases are still being confirmed in February 2005) with the virus basically spreading throughout the country. This sustained transmission indicates the existence of large numbers of susceptible individuals either in the general population or in some specific high-risk population groups (for example several nursery schools and children's homes were involved in Gauteng, plus there was evidence of nosocomial transmission). The majority of viruses in this outbreak were identical to the D2 genotype detected in Maputo, Mozambique earlier in 2003, but as the outbreak progressed, there were accumulated nucleotide changes especially in the 3rd codon positions that therefore did not result in amino acid changes in either the nucleoprotein (N) or hemagglutinin (H) genes. Considering the general view that RNA viruses have a high mutation rate because their polymerases do not have a proof-reading function, it is surprising that there was very little variation in either the N or H genes during the more than 18 month period of the outbreak.

At the start of the outbreak in Gauteng and Mpumalanga, no urine specimens were sent to the NICD, so it was necessary to use sera as specimens for genotypic analysis. Because such cell-free specimens contain less measles virus than specimens containing cells, it was necessary to resort to nested PCR procedures. This has various drawbacks (e.g. contamination issues) but was nevertheless technically possible. Measles genotyping was performed on 192 specimens (1 CSF, 6 throat/nasal swabs, 25 sera, 159 urines) from IgM-positive measles cases. 110 specimens (57%) were positive by RT-PCR or nested PCR, and all were identified as genotype D2.

It is likely that the WHO will recommend the use of filter paper cards for collection of blood (dried blood spots, DBS) in countries that have difficulty in maintaining a cold chain, as the DBS cards can then be mailed using ordinary postal services. We have evaluated the use of DBS for the purposes of molecular epidemiology. It was found that the RNA was remarkably stable as long as the cards were kept dry. When the methodology was applied to DBS collected from confirmed measles cases, it was found that nested PCR was required to obtain sufficient material for genotypic analysis.

Rubella

There has been a tendency for vaccine manufacturers to move away from the production of monovalent measles (M) vaccines in favour of combination vaccines such as MR (measles, rubella) or MMR (measles, mumps, rubella) because although the production costs of monovalent and combination vaccines are similar, there is a significant difference in their market prices. Since the EPI program in South Africa makes exclusive use of M vaccine, while combination vaccines are used in the private sector, we need to understand the molecular epidemiology of rubella virus in the context of vaccine availability, natural immunity and vaccine-induced immunity.

Currently, molecular information is only available for 17 countries worldwide, and 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 of rubella viruses from five South African provinces have been examined. All 16 viruses were clearly genotype II and formed 4 groups. This is unusual and many more specimens need to be analyzed before one can draw conclusions about the distribution and transmission patterns of rubella virus in South Africa.

TRAINING

Dr TG Besselaar: Facilitated the WHO AFRO training course on influenza surveillance and diagnosis at the National Institute for Communicable Diseases, Johannesburg, South Africa, 11-15 October 2004.

Dr TG Besselaar was invited to spend 5 months working as a scientist in the Global Influenza Programme at WHO, Geneva, May - October 2004. Topics covered: Avian influenza, pandemic prepared-ness, pandemic vaccines, antivirals, biosafety risk assessments, coordination of a WHO AFRO laboratory training course for African countries (above) to be held in October at NICD.

CONFERENCES/MEETINGS

Global Vaccine Research Forum, Montreux, Switzerland, 7-10 June 2004

Dr TG Besselaar: Influenza: The modelling of a potential pandemic, the H5N1 avian influenza outbreak in 2004 and international response, the development of several vaccines and the industry perspective.

Meeting : Informal consultation on global polio laboratory network, Geneva, Switzerland, September 2004

HN Gumede: Molecular epidemiology of poliovirus in sub-Saharan Africa.

Meeting: 7th Meeting of Laboratory Directors, Nairobi, Kenya, September 2004

HN Gumede: Molecular epidemiology of poliovirus in sub-Saharan Africa.

Vaccinology Congress, Hermanus, South Africa, October 2004

HN Gumede, S Smit.

Workshop on ITD, Dakar, Senegal, 1-12 November 2004

HN Gumede: Molecular epidemiology of poliovirus in sub-Saharan Africa and Quality Assurance.

PUBLICATIONS

Besselaar TG, Botha L, McAnerney JM, Schoub BD. Antigenic and molecular analysis of influenza A (H3N2) virus strains isolated from a localized influenza outbreak in South Africa in 2003. *J Med Virol* 2004; 73: 71-78.

Besselar TG, Botha L, Schoub BD, McAnerney JM. Phylogenetic studies of influenza B viruses isolated in southern Africa: 1998-2002. *Virus Research* 2004; 103: 61-66.

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SPECIAL PATHOGENS UNIT

The Special Pathogens Unit of the National Institute for Communicable Diseases is primarily responsible for the diagnosis and investigation of biohazard class 4 viruses, and operates maximum security laboratory (BSL-4) 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-related arenaviruses, and hantaviruses. The Special Pathogens Unit is also responsible for the diagnosis of rabies, rabies-related, and other biohazard class 3 viruses. The Unit is recognized as a World Health Organization (WHO) Regional Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses.

SPECIAL PATHOGENS UNIT – 25 YEARS ANNIVERSARY

The Poliomyelitis Research Foundation (PRF) in Johannesburg, which was the main centre for medical virology in South Africa, was consulted by scientists abroad when Marburg disease first appeared in Europe in 1967 in association with monkeys imported from Africa, and again in 1969 when the rodent-borne Lassa fever first appeared as a fatal disease of missionary nurses in West Africa. In 1975, two young Australian tourists became sick in South Africa with what proved to be Marburg haemorrhagic fever after they had been hitch-hiking in Zimbabwe, and a nurse became infected while attending the patients in Johannesburg. The sudden emergence of these dangerous new viruses in Africa prompted the development of the concept of maximum-security laboratories, and health authorities in South Africa felt the need to construct such a laboratory in the country.

In April 1976, the laboratories of the PRF were transferred to the State Department of Health and reconstituted as the National Institute for Virology (NIV), with Professor OW Prozesky being appointed as the first Director of the new Institute. Professor Prozesky, plus the Secretary for Health, Mr J de Beer, as well as an architect from the Department of Public Works, Mr CF Beyers, and a consultant engineer, Mr AP Thompson, undertook a tour of microbiological institutes abroad, and on the basis of the information they gathered a biosafety level 4 (BSL4) laboratory was planned and built at NIV. Construction was completed in 1979, and in January 1980 Dr R Swanepoel from Zimbabwe was appointed as the head of a new Special Pathogens Unit to operate the BSL4 laboratory for the purpose of providing a diagnostic and investigatory service for viral haemorrhagic fevers in southern Africa. Within months, a Department of Health Medical Ecology Unit, responsible for monitoring plague activity in rodents, was incorporated into the SPU. In 1981, the Arbovirus Unit at NIV was made part of the SPU, and in 1983 responsibility for the diagnosis and investigation of rabies was allocated to the Unit.

In its second month of operation, February 1980, the SPU became involved in the investigation of an outbreak of Marburg haemorrhagic fever in Kenya in association with the Special Pathogens Branch of the Centers for Disease Control in Atlanta, USA, leading to lasting cooperation between the two laboratories. In 1981, the first case of Crimean-Congo haemorrhagic fever (CCHF) was recognized in South Africa, and for the next decade work on this disease dominated the research programme of the SPU.

In 1995 the SPU was given recognition as a World Health Organization (WHO) Regional Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses. By the end of 2003, the SPU had investigated 4 570 blood and other samples from 2 788 suspected cases of viral hemorrhagic fever in South Africa and its immediate neighbours, plus a further 4 687 specimens from 3 091 patients from 22 countries in the rest of Africa, and 10 countries in Asia and Europe. Important diseases diagnosed included Marburg haemorrhagic fever, Ebola haemorrhagic fever, CCHF, Lassa fever, Rift Valley fever, yellow fever, dengue, West Nile fever, chikungunya and o'nyongnyong. However, the majority of the suspected cases of haemorrhagic fever proved to be more common vaccine-preventable or treatable diseases, and the number of patients investigated does not reflect the actual extent of the epidemics involved. In the most extreme example 4 600 people are estimated to have died in southern Sudan in 1999 from what the SPU diagnosed as louse-borne relapsing fever, which could readily be controlled through the use of an inexpensive antibiotic and insecticide. SPU staff has participated in many missions abroad, sometimes

as members of international teams organized by the WHO to respond to epidemics, or to conduct ecological studies to determine the source of infectious agents in nature, such as Marburg and Ebola viruses. Many thousands of specimens from domestic and wild animals, and insect vectors have been tested in the course of such investigations. Major missions included trips to the Democratic Republic of the Congo, Gabon, Sudan, Kenya-Somalia, Uganda, Sierra Leone, Guinea, Liberia, Afghanistan and Saudi Arabia.

The busiest time for the Unit occurred during the last 2 months of 1996, when a major outbreak of CCHF among workers at an ostrich abattoir caused the European Union to ban importation of all ostrich products from South Africa, thereby threatening the continued existence of a major industry. While still testing samples from hundreds of abattoir workers, the SPU had to study the nature of experimental CCHF infection in ostriches to provide information so that the Department of Veterinary Public Health could promulgate regulations designed to prevent the virus getting into ostrich products, thus making it possible for the ban to be lifted. Meantime, Ebola haemorrhagic fever was diagnosed in a nurse in Johannesburg, and a frantic search ensued to locate the source patient who proved to be a doctor from Gabon who had sought treatment for his illness in South Africa. This in turn led to a major exercise to identify and monitor health care workers and others who had been in contact with the 2 patients. Tragically, the nurse died but further spread of the infection was prevented.

In 2003 the SPU became involved in performing diagnostic tests on suspected cases of severe acute respiratory syndrome (SARS), but fortunately the disease was not confirmed in southern Africa. Most recently the Unit has played an important role in investigating the occurrence of human infections with highly pathogenic avian influenza A H5N2 virus followed by an outbreak of the disease in ostrich population in the Eastern Cape in May 2004.

In April 2002, the NIV and the South African Institute for Medical Research (SAIMR) were amalgamated to form a new National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service (NHLS). Professor BD Schoub who had succeeded Professor Prozesky as Director of NIV in 1983, became the first Director of the NICD.

Staff numbers remained relatively constant over the years, at approximately 7 scientists, 2 technologists, 3 general assistants, a secretary, plus a maintenance artisan with 1-2 lay assistants. Post-graduate degrees obtained by staff members included 1 DSc, 3 PhDs, and 3 MScs. The Institute has an association with the Medical School of the University of the Witwatersrand, with some staff members holding joint appointments, and Dr Swanepoel was appointed as an Assistant Lecturer in 1983, and a Reader in Viral Haemorrhagic Fevers and Zoonsoses in 1990, with the title Professor. Over the years staff members of SPU have presented their research findings at numerous international meetings, and have served on various international committees. The Unit has produced over 100 publications in international scientific journals. Professor Swanepoel received the Theiler Trust Memorial Award in 2002 in recognition of his contributions to veterinary and medical science in Africa.

Fig 1. Professor Robert Swanepoel and his successor, Dr Janusz T Paweska.

Professor Swanepoel is still one of the NICD's most productive and internationally active scientists and provides a great deal of mentorship to the Special Pathogens Unit which he ran successfully for 24 years (1980-2003).

Professor Swanepoel retired in 2001, but remained in employment on contract, and handed over management of the SPU to his successor, Dr JT Paweska, in January 2004, which was the 25th year of operation of the Unit (Fig 1). By 1999, the BSL4 laboratory had become difficult to maintain. Planning was undertaken to replace it, but sufficient funds could not be allocated. In April 2004 the BSL4 was finally closed for major upgrading, upscale, and refurbishment of the facility. It is planned that BSL4 will be fully operational late in 2005. Research on less hazardous agents and diagnostic service has continued in BSL2 and BSL3 laboratories of the Unit. Meanwhile, field studies on the ecology of viral haemorrhagic fevers continue in association with our colleagues at the Centre International pour la Recherche Médicales de Franceville (CIRMF) in Gabon, and the Centers for Disease Control, Atlanta, as shown in the photographs of activities during a recent trip to investigate potential reservoir of Ebola fever in eastern Gabon (Figs 1A-F).

Fig 2. Ebola ecology investigations in eastern Gabon, Ogoue-Ivindo Provice: joint field expedition of CIRMF (Franceville, Gabon), NICD-SPU (Johannesburg, SA) and CDC (Atlanta, USA).

A - Funeral of Ebola patient in a village - health officials dressed in protective clothing are fetching cadaver from a hut and disinfecting coffin; B – The preparation of the field laboratory about 5 km from Ekata village: Dr Pierre Rouquet (CIRMF), Prof R Swanepoel and Dr JT Paweska (NICD); C - Prof R Swanepoel and Dr Pierre Rouquet dissecting bats – disposable protective clothing, two pairs of gloves, battery operated respirators with positive-pressure filtered air; D - Mr Alan Kemp (NICD) spraying insecticide up into the forest canopy E - Dr Darin Caroll (CDC) and Mr A Kemp entering data of dissected animals and arthropods; F - Dr A Killbourne (ECOFAC) collecting skull of gorilla thought to have died of Ebola infection.

COMPARISON OF SPECIMENS RECEIVED IN 2003 AND 2004

Although the BSL4 facility was closed down at the end of April 2004 for major renovation and refurbishment, the total number of specimens tested in the Unit during the period under review was slightly higher than in 2003 (Table 1). This was mainly due to the increased number of specimens received early in 2004 from South Africa, other African countries and Asia for the investigation of suspected viral haemorrhagic fevers (VHF), including specimens received for investigation of suspected Lassa fever in Sierra Leone and Nigeria, and Crimean-Congo haemorrhagic fever in Pakistan. The number of ostrich and primates sera for export/import testing and human sera received for screening for post-vaccination antibody to Rift Valley fever was also higher compared to those received in 2003. In addition, following an outbreak of highly pathogenic avian A H5N2-influenza on commercial ostrich farms in the Somerset East area of the Eastern Cape in May 2004, the Unit become involved in testing sera and other clinical specimens as a part of the outbreak response carried out by the Epidemiology Unit of NICD which was concerned with the health risks for farm workers, persons directly involved in controlling the epizootic and veterinary laboratory staff handling the specimens. A number of requests for testing of suspected VHF cases which were received after April 2004 from South Africa and other African countries could not be investigated by the Unit but we assisted in the shipping of specimens to other suitable institutions, notably to the Special Pathogens Branch, Centers for Disease Control, Atlanta, USA.

Table 1: Comparison of specimens received in Special Pathogens Unit in 2003 and 2004

Specimens	Received in 2003	Received in 2004
Diagnostic:		
Suspected VHF (South Africa)	35 (30 patients)	72 (49 patients)
Suspected VHF (other countries)	195 (195 patients)	397 (391 patients)
VHFcontacts	146 (146 persons)	125 (125 persons)
Undiagnosed fevers	153 (93 patients)	74 (74 patients)
Suspected rabies	33 (20 patients)	20 (16 patients)
Rabies immunity	93 (49 accessions)	165 (158 patients)
Ticks	0	4 (accessions)
Miscellaneous	185 (46 accessions)	526 (36 accessions)
Surveys:		
Occupational/residential groups	300 (1 group)	406 (4 groups)
Cattle goats & sheep for zoonoses	190	100 (1 accession)
Dogs for plaque	369 (14 districts)	0
Rodents for plague	216 (14 districts)	0
Wild animals	258 (17 accessions)	127 (1 accession)
Ticks RSA	0	62 (2 accessions)
Ticks non-RSA	0	24 (1 accession)
Total specimens:	**1 983**	**2102**

INVESTIGATION OF SUSPECTED VHF

Five cases of Crimean-Congo haemorrhagic fever (CCHF) were confirmed in southern Africa during 2004 (Table 2). In four instances the infection was the result of a tick bite. The fifth case was a farmer who became ill after dipping sheep on his farm in the Northern Cape Province. Three of the patients died shortly after being admitted to hospital. There is no specific treatment for CCHF infection, although there is some evidence that ribavirin can improve the prognosis if administered before day 5 after onset of illness. A total of 176 cases of CCHF have been diagnosed in southern Africa from the time the disease was first recognized in 1981 up until the end of 2004, including sixteen in Namibia, one in DRC, one in Tanzania and 158 cases in South Africa. Marginally the largest group of cases, 77/176 (43.7%), arose from known tick bite or the squashing of ticks; a similar number, 71/176 (40.3%), arose from known or potential contact with fresh blood or other tissues of livestock and/or ticks, 7/176 (3.9%) nosocomial infections arose from contact with blood or fomites of known CCHF patients, while in 21/176 (11.9%) 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 147/176 (83.5%) of all cases of the disease diagnosed to date.

Table 2: List of confirmed cases of Crimean-Congo haemorrhagic fever virus infection in southern Africa, January to December 2004.

Location of exposure	Month	Age/Sex	Virus isolation	PCR	IgG and/or IgM antibody	Died/ survived	Source of infection
Lichtenburg, NW Province	Jan	69/M	yes	yes	yes	survived	tick bite
Bloemfontein, Free State	Jan	35/M	yes	yes	yes	died	tick bite
Mafikeng, NW Province	Mar	41/M	no	yes	no	died	tick bite
Rehoboth, Namibia	Apr	67/M	yes	yes	yes	died	tick bite
Warrenton, N Cape	Jun	58/M	yes	yes	yes	survived	sheep/ticks

The case fatality rate for CCHF in southern Africa fluctuated around 30% during the first few years after the disease was initially recognized here, but gradually declined to an overall rate of 19.9% (29/146) for a period of 1981-1998, probably as a result of greater awareness leading to earlier diagnosis and institution of appropriate therapy. In contrast, the case fatality rate has increased to 60% (18/30) for most recent period from 1999-2004. One possibility is that there has been a marked decline in awareness of the disease among clinicians, resulting in delayed recognition of cases. In this respect it is notable that failure to confirm CCHF during the previous reported year, 2003, was associated with the lowest number of requests from clinicians to investigate suspected cases of haemorrhagic fevers since 1982.

Dr Felicity Burt

RABIES

A total of 9 cases of human rabies were confirmed by the SPU during 2004 (Table 3). The number of rabies cases confirmed was still low compared to the number of cases observed prior to 1997. The majority of patients contracted rabies from contact with rabid dogs in Kwa-Zulu Natal. In seven instances there was no history of receiving post-exposure prophylaxis, or details of history were not available to determine if appropriate treatment had been administered. One patient received partial post exposure treatment and in one case there was evidence that the patient had received correct and appropriate post exposure prophylaxis but contracted the disease and died. This patient had a severe bite on the wrist from a water mongoose in Standerton, Mpumulanga. Within 5 hours of being bitten the patient received rabies vaccine and immunoglobulin, and although the patient completed the vaccination schedule, 3 weeks after exposure was admitted to hospital with symptoms of rabies infection and died.

There are two biotypes of rabies circulating in southern Africa, the canine biotype, which is associated with dogs, black-backed jackals and bat-eared foxes, and the mongoose biotype associated mainly with viverrids. Phylogenetic analyses of partial nucleotide sequences determined from RNA extracted from post mortem specimens obtained from humans infected by the canine biotype have shown that the isolates are closely related genetically. In comparison, partial nucleotide sequences of rabies isolates obtained from humans infected with mongoose rabies form a phylogenetically distinct group and are easily distinguished from the canine group. RNA obtained from specimens submitted from the patient bitten by the mongoose was amplified using primers specific for the G and L intergenic region of the virus genome. A comparison of the nucleotide sequence obtained and data retrieved from Genbank confirmed that the patient had been infected with a biotype of rabies that is found circulating within the mongoose population in South Africa. Further studies are in progress.

Table 3. Confirmed cases of rabies, 2004

Name	Age/Sex	District of exposure	Exposure: bitten by	Onset	Admitted hospital	Died	Final hospital
KM	4/M	Greytown	Dog, Jan	Feb	26 Feb	4 Mar	Grey's
IN	15/M	NW Namibia	Dog, Jan		11 Apr	14 Apr	Ondandjokwe
MN	9/M	Mthunzini	Dog, Mar	April	April	10 Apr	Eshowe
ND	3/M	Nkabla	Dog		Mar	Unknown	Charles Johnson Mem.
ZK		Mtunzini	Dog			June	
AT	57/M	Standerton	Water mongoose (8 July)	July	24 July	4 Aug	Pretoria East
JR	16/M	Pinetown	Dog?	8 Aug	8 Aug	23 Aug	Entabeni
PD	12/M	Harding,KZN	Puppy (August)	Oct	5 Oct	5 Oct	Murchison
SN	11/M	Harding,KZN	Puppy (August)	Oct	20 Oct	21 Oct	Murchison

ARBOVIRUS SECTION

A total of 442 specimens submitted to the Arbovirus Section during 2004 included: 257 serum samples from 247 patients in southern Africa and 47 serum samples from 41 patients in Pakistan, DRC, Kenya and the Seychelles, with suspected arbovirus infections, and 130 from NICD and Onderstepoort laboratory staff members which were submitted for Rift Valley fever (RVF) or yellow fever immunity tests. In addition, serum samples form 8 primates were submitted for yellow fever immunity tests. After routine arbovirus tests had been completed, 240 serum samples from 236 patients were screened for antibody to CCHF virus by indirect immunofluorescence, and none were found positive.

Among the specimens from southern Africa, 98 sera from 90 patients had demonstrable haemagglutination inhibition (HAI) antibody to one or more arbovirus antigens as shown in Table 4. HAI positive sera were tested by ELISA for IgM antibody activity to the relevant viruses, and five patients were found to be IgM positive for Sindbis virus and one patient was IgM positive for West Nile. Yellow fever virus was isolated from one patient with a recent history of vaccination. Seroconversion was demonstrated in a second specimen submitted from the patient. Partial nucleotide sequence determination of a region of the viral genome confirmed that the isolate obtained was vaccine-related.

Dengue virus was isolated from specimens submitted from two patients during the acute phase of illness. Sera from two additional patients had demonstrable HAI and IgM antibody to dengue virus. The patients with dengue infection all had a recent history of travel to areas where dengue virus is endemic. Nucleotide sequence determination of PCR products identified the two isolates as belonging to dengue virus types 1 and 2.

In August 2004, 22 serum samples were submitted from the Kenya Medical Research Institute (KEMRI) in Nairobi to confirm a presumptive diagnosis of chikungunya (CHIK) or o'nyong nyong (ONN) among villagers in a coastal Kenyan town of Lamu. CHIK and ONN are antigenically closely related and indistinguishable serologically. Outbreaks of CHIK have frequently been identified in southern, West, Central and East Africa, while ONN has been isolated from more sporadic outbreaks in Kenya, Tanzania, Uganda, Malawi, Mozambique, Central African Republic and Senegal. Most of the samples submitted from the recent outbreak had demonstrable HAI and IgM antibodies to both CHIK and ONN. One isolate was obtained from the samples and the partial nucleotide sequence was determined for a region of the genome. A comparison of the sequence data confirmed that the aetiologic agent of the outbreak was CHIK virus. Three months later an outbreak of CHIK in the Kenyan coastal town of Mombasa, approximately 250 kms south of Lamu, was identified by KEMRI and samples were submitted to the SPU for confirmation. Preliminary studies suggest that there were small nucleotide differences between the strains of CHIK circulating during the Mombasa outbreak and the strains circulating in Lamu.

Table 4: Numbers of diagnostic sera positive to one or more arboviruses antigens

SIN	CHIK	WN	RVF	DEN	YF	No. positive sera
pos						7
	pos					19
		pos				17
					pos	26
		pos			pos	3
				pos	pos	2
		pos	pos			1
		pos		pos	pos	14
		pos		pos		2
pos		pos				3
pos		pos		pos	pos	2
pos					pos	1
					Sera	**98**
					patients	**90**

SIN=Sindbis, CHIK=chikungunya, WN=West Nile, RVF=Rift Valley fever, DEN=dengue, YF=yellow fever

DEVELOPMENT, PRODUCTION AND VALIDATION OF DIAGNOSTIC ASSAYS AND IMMUNOREAGENTS

Serological diagnosis of viral haemorrhagic fevers and arboviral infections is entirely based on in-house produced diagnostic reagents. During 2004 the members of the Unit spent much time on production, preservation, and standardization of traditional and new generation immunoreagents, optimization of assay protocols, and validation of old and newly developed tests. One of our major challenges was to satisfy an increasing international demand for standardized and safe immunoreagents for diagnosis of Rift Valley fever (RVF) and to assist in improvement of regional diagnostic capacity for this disease. RVF virus is an important zoonotic and potential biothreat agent. The occurrence of the first confirmed outbreaks of RVF among humans and livestock outside Africa, in the Arabian Peninsula in 2000-2001 carries the implication of further spread of infection into non-endemic RVF areas since the virus is capable of utilizing a wide range of mosquito vectors. Considerable efforts have been made recently to develop nucleic acid techniques for rapid detection and identification of RVF virus. However, traditional and molecular procedures for diagnosis of RVF may be beyond the resources and capabilities of many laboratories, particularly in developing countries. Therefore, we undertook intensive development and validation studies aiming at expanding the repertoire of standardized, safe and accurate assays for diagnosis of RVF which could be easily adapted for wider use both in medical and veterinary laboratories in southern Africa and elsewhere.

IgG-sandwich and IgM-capture ELISA for the detection of antibodies to Rift Valley Fever (RVF) virus in human sera

Both ELISAs were based on gamma-irradiated antigens which were produced in a suckling mouse system. Validation data sets derived from testing field-collected sera from Africa (n = 2400) were dichotomised according to the results of a virus neutralisation test. In addition, sera from laboratory workers immunized with inactivated RVF laboratory workers immunized with inactivated RVF vaccine (n = 93) and serial sera from a single RVF case were used.

An Inhibition ELISA

This is a single test format which allows rapid detection of antibody to RVF virus in humans, domestic and wild animals using the same set of diagnostic reagents. The inhibition ELISA was based on tissue culture derived gamma-irradiated antigen. Validation data sets for the inhibition ELISA were derived from field-collected sera in Africa (humans = 1367, cattle = 649, goats = 806, sheep = 493, buffalo = 258, camels = 156), individual sera from 93 laboratory workers immunized with inactivated RVF vaccine, 136 serial bleeds from eight sheep experimentally infected with wild type of RVFV, and from 200 serial bleeds from 10 sheep vaccinated with the live-attenuated strain of the virus. Sera were dichotomized and ELISA data analysed as described above.

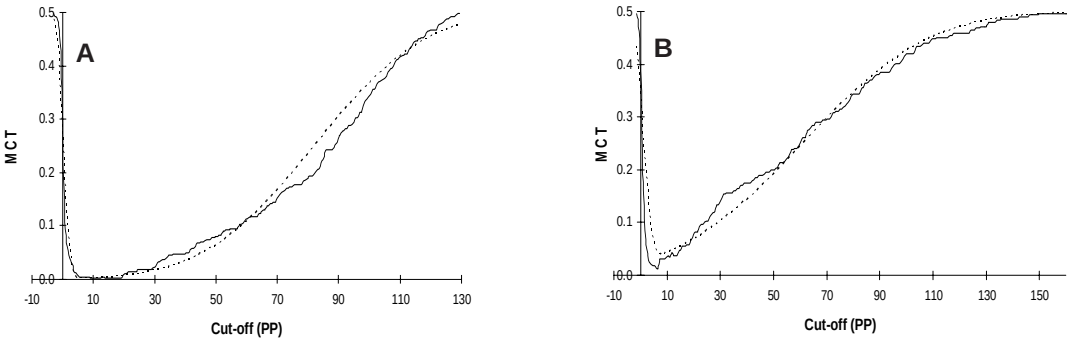


Fig 3. Optimisation of cut-offs for RVF IgG-sandwich and IgM-capture ELISA in humans using the misclassification cost term (MCT) option of the two-graph receiver operating characteristics analysis. At cut-off value of 13.2 PP for IgG ELISA (A) and of 7.1 PP for IgM ELISA (B) the overall misclassification costs become minimal (0.002 and 0.013, respectively) under assumption of 50% disease prevalence and equal costs of false-positive and false-negative test results. The two curves represent MCT values based on non-parametric (smooth line) or parametric (dashed line) estimates of sensitivity and specificity derived from data sets in field-collected sera. Optimisation of cut-off values was based on the non-parametric program option due to departure from a normal distribution of data sets analysed.

Our results demonstrate that these assays will be useful for early diagnosis of infection, disease-surveillance and control programmes, import/export veterinary certification, and for monitoring the immune response in vaccinees.

An Indirect Sandwich ELISA (IS-ELISA) for the Detection of RVF Virus Antigen

The IS-ELISA utilizing a serum pool from sheep experimentally infected with RVF virus was used to capture viral antigen in test samples. Using various specimens of known virus titres, the IS-ELISA detection limit was determined as ~$10^{3.5}$ $TCID_{50}$ of virus/1 ml of test sample. RVF virus replicates in susceptible vertebrate species, competent vectors, and in vitro systems to significantly higher levels than the diagnostic threshold established. Thus, the IS-ELISA could be useful in diagnosis and monitoring an epidemic of RVF and vector surveillance and be the method of choice in RVF non-endemic areas.

Molecular Cloning and Expression Laboratory

During 2004 a cloning and expression laboratory was established in the Unit. This highly specialized laboratory focuses specifically on the production of recombinant antigens which could be used as immunoreagents for the detection and production of antibodies as well as safe vaccines for immunization against viral haemorrhagic and arbovirus infections. The recombinant technology for the production of antigens should contribute to the improvement of biosafety in the production of diagnostic reagents by eliminating the need for large-scale production of BSL3 and BSL4 viruses in *in vivo* or *in vitro* systems and enables production of antigens of highly pathogenic viruses outside high-biocontainment laboratories. The first project undertaken was to clone the glycoproteins (G1 and G2) of RVF virus in a mammalian expression vector. Secondly, the PrM-E protein of West Nile (WN) virus was cloned into a mammalian expression vector in collaboration with the Microbiology Department of the University of Pretoria.

Microneutralisation Assay for the Detection of Antibody to Avian Influenza A H5N2 virus

An extensive outbreak of highly pathogenic avian A H5N2-influenza on commercial ostrich farms in the Eastern Cape during early summer of 2004 raised concerns of possible spread of the outbreak virus amongst farm workers, personnel involved in controlling the epizootic and laboratory staff handling clinical specimens. The virus neutralization test (VNT) is thought to be a sensitive and specific assay for detecting virus-specific antibody to avian influenza A H5N1 virus in human sera and potentially for detecting antibody to other avian subtypes. The Unit has successfully established and standardized the VNT microtitration procedure in BSL3 laboratory using the local virus isolate adapted to MDCK cells. The egg passage of the outbreak virus was provided by Dr James Kitching, Regional Veterinary Laboratory, Stellenbosch. The results of VNT and other tests in human sera will be reported by the NICD elsewhere.

Multiplex Discrimination of Haemorrhagic Fever Viruses using Mass Tag PCR
The capacity of current multiplex assays to simultaneously detect numerous targets is limited and they often require cumbersome post amplification processing. To address the need for sensitive multiplex assays in diagnostic molecular microbiology, Dr Ian Lipkin's group from Columbia University, New York, USA, developed a polymerase chain reaction platform in which microbial gene targets are coded by a library of 64 distinct Masscode tags (Qiagen Masscode technology). Primers, synthesized with a 5' C6 spacer and aminohexyl modification are covalently conjugated by a photocleavable link to Masscode tags. Forward and reverse primer sets are labeled with distinct molecular weight tags; therefore, amplification of a pathogen gene target produces a dual signal allowing evaluation of specificity. Most recently the Mass Tag PCR has been successfully used for detecting 22 respiratory pathogens in clinical samples. This technology has a number of potential applications: differential diagnosis of infectious diseases, blood product surveillance, forensic microbiology, and biodefense. In collaboration with our colleagues from Columbia University, New York, the feasibility of the Mass Tag PCR has been evaluated for the diagnosis of viral haemorrhagic fevers (VHF) using cDNA templates of viral RNA derived from gamma-irradiated preparations of BSL4 viruses including Ebola, Marburg, CCHF, RVF, and Hantaan viruses. It is planned that in 2005 further validation of the Mass Tag PCR will be continued using RNA extracts from clinical specimens collected during the major outbreaks of VHF in Africa.

RESEARCH

Hunt for Filovirus Reservoirs and Bat Colony
There is evidence to suggest that bats are epidemiologically associated with Ebola and Marburg viruses. In order to further address the potential of bats to harbour filoviruses, the SPU team collected a sample of 30 Egyptian fruit bats, *Rousettus aegyptiacus leachi*, from the Limpopo Province to establish the first of several bat colonies in the SPU in November 2003. The process of colonisation was ably guided by Ms Pauline Hawkins of the South African Vaccine Producers (SAVP) Small Animal Unit. The new colony, consisting of equal numbers of males and females, has produced 14 pups in its first reproductive season in captivity. It is intended to allow the *Rousettus* colony to increase to approximately 60 bats before experimental work with the filoviruses will commence.

Molecular Epidemiology of African and Asian Crimean-Congo Haemorrhagic Fever Isolates
The widespread geographic distribution of CCHF virus, its ability to produce severe human disease with high mortality rates, and fears about its intentional use in a bioterrorism attack make CCHF virus an extremely important human pathogen and a worldwide public health concern. Initially it was thought that African strains of CCHF virus were less pathogenic for humans than Asian strains, although this is no longer believed to be correct.

To determine the genetic diversity of the virus, partial nucleotide sequences were determined for 35 isolates from southern Africa plus 11 isolates obtained from serum samples received from human patients in Iran and Pakistan, and sequence data for a further 40 isolates from other locations were retrieved from GenBank for inclusion in the phylogenetic analysis. Tree topology supports the existence of three groups of genetically related isolates, A, B and C. Within group A there are two clades: an African clade and a predominantly Asian clade comprising isolates from Pakistan, China, Iran, Russia and Madagascar. Group B includes isolates from southern and West Africa and Iran, and group C includes a single isolate from Greece. Despite the potential which exists for dispersal of the virus between Africa and Eurasia, it appears that circulation of the virus is largely compartmentalized within the two land masses. The tree topology shows no obvious correlation between the grouping of isolates and source of infection, year of infection, or pathogenicity for humans, but the geographic distribution of phylogenetic groups appears to relate to the distribution and dispersal of vectors of the virus.

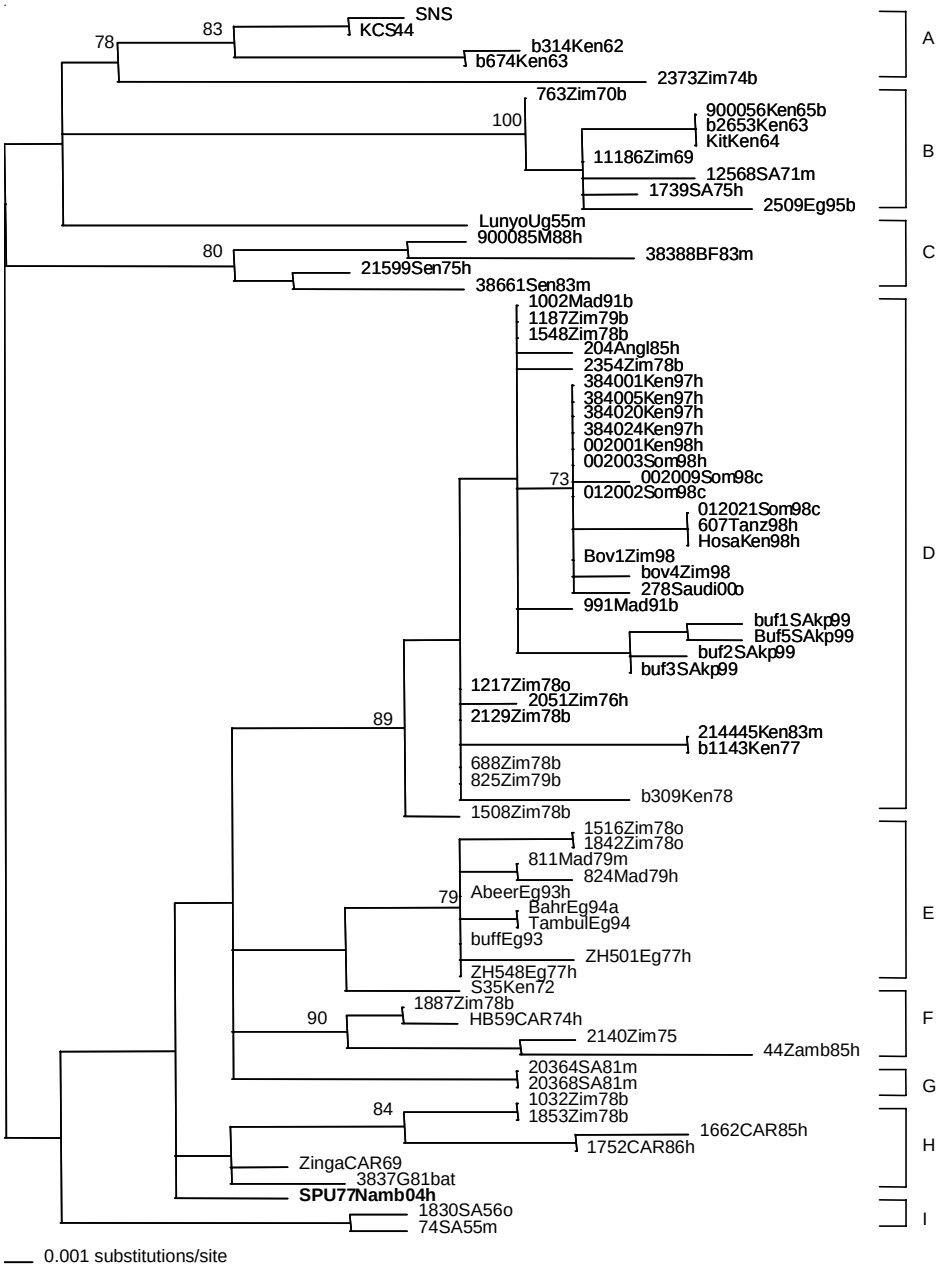
Mechanisms for the dissemination of the tick vectors and CCHF virus must have operated for millenia, and include bird migration and the movement of livestock and wild animals. Trade in livestock along the Asian and east African coasts has been associated with outbreaks

of human disease in the Near East. However intercontinental spread of CCHF virus may be a slow process, taking place over centuries, which accords with the observation that the lineages of CCHF strains appear to circulate largely within the continents of Africa or Eurasia, despite mechanisms for movement of ticks between continents. It can be concluded that the phylogenetic evidence supports the concept of the evolution of CCHF virus strains within specific geographic regions, probably as a consequence of association with particular tick vector species. Further investigations focusing on tick species associated with CCHF virus strains would help to identify the role of ticks in genetic variability of the virus.
of ticks in genetic variability of the virus.

Fig 4. Phylogenetic relationship among 70 geographically distinct CCHF virus isolates was determined for a 450 nucleotide region of the S segment of the viral genome using a weighted maximum parsimony method, PAUP. Two nairoviruses, Dugbe and Hazara, were included as outgroups. Numbers at each branch indicate the percent bootstrap support for that node generated from 100 replicates (heuristic search). Tree topology indicates the existence of three groups of genetically related isolates, A, B and C, with two clades within group A: an African clade and a predominantly Asian clade, which includes isolates from Pakistan, China, Russia, Iran and Madagascar (shown in italics).

MOLECULAR EPIDEMIOLOGY OF RIFT VALLEY FEVER: 1994-2004

Large outbreaks of Rift Valley fever (RVF) occur at irregular intervals of years in association with population explosions of floodwater-breeding aedine mosquitoes following heavy rains. We investigated the molecular epidemiology of RVF to determine whether epidemics of the disease result from spread of a single virus strain or are triggered by increased activity of multiple strains circulating at endemic levels within the country where the epidemic occurs. A total of 102 field isolates of RVF virus recovered over a period of 60 years from various hosts, and during endemic and epidemic periods of disease in 15 African countries, Madagascar and Saudi Arabia were characterized by partial genomic sequencing and analysis of a 535-nucleotide segment of the G2 glycoprotein coding region of the M segment.



Zim=Zimbabwe, Mad=Madagascar, Ken=Kenya, Som=Somalia, Tanz=Tanzania, kp=Kruger National park, South Africa, Saudi= Saudi Arabia, Ang=Angola, CAR= Central African Republic, Zamb=Zambia, G=Guinea, SA=South Africa, EG= Egypt, Ug=Uganda, M=Mauritania, BF=Burkina Faso, Sen=Senegal, Namb=Namibia, b=bovine, h=human, c=caprine, o=ovine, m=mosquito, SNS=Smithburn neurotropic strain.

Fig 5. Phylogenetic tree of RVF virus isolates determined using a 490 base pair segment of the G2 glycoprotein-coding region of the M segment. The tree was generated using a maximum likelihood method and Phylogenetic Analysis Using Parsimony (PAUP) software. Node values indicate bootstrap confidence values generated from 100 replicates.

Although phylogenetic studies indicate that RVF virus strains circulating in Africa, Madagascar and Saudi Arabia over the past sixty years cannot clearly be compartmentalised geographically, they do cluster into 9 major groups (A-I) supported by bootstrap values indicated on the phylogenetic tree (Fig 5). Group E comprises mostly Egyptian isolates and group C comprises West African strains. Group G and I comprise only South African isolates and groups A, B, D, F and H comprises isolates from other African countries, Madagascar and Saudi Arabia. We found that an outbreak precipitated by abnormally heavy rain may result from spread of a single strain of virus as observed during the 1997-98 East African RVF outbreak. Isolates from Kenya, Somalia and Tanzania separated by vast distances were found to have identical or very closely related sequences. The same virus strain that appears to have been active in Zimbabwe in 1998, South Africa in 1999, and in Saudi Arabia in 2000, indicating that there was rapid spread of the strain down the subcontinent and across to Saudi Arabia. Mechanisms which have been suggested for the rapid spread of the virus over vast distances include: 1- carriage of infected mosquitoes by high winds, 2 - carriage of infected mosquito eggs by migratory birds, and 3 - movement of infected livestock.

Results obtained on isolates from the 1978-79 outbreak in Zimbabwe indicate that outbreaks can also result from the intensification of activity of multiple strains of the virus already operating at endemic levels within the country where the epidemic occurs. The Zimbabwean isolates belong to one of four groups (D, E, F, and H) indicating that multiple strains of RVF virus were active during single outbreak.

Interestingly, the most recent isolate of RVF virus isolated from a blood specimen of a person infected in the Caprivi Region, Namibia 2004, does not cluster with any group. The Namibian 2004 isolate is the only known field isolate of RVF virus recovered in southern Africa since a limited epizootic of the disease occurred in African buffalo in the Kruger Park in 1999. This suggests that during prolonged unfavorable enviroclimatic conditions cryptic circulation of the virus is maintained within isolated geographical areas.

INVESTIGATION OF REPLICON BASED VACCINES AGAINST RIFT VALLEY FEVER AND CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUSES

Rift Valley fever virus (RVFV) is a mosquito-transmitted virus that causes outbreaks of lethal disease in young sheep, goats and cattle, and abortions in pregnant animals in Africa. Humans acquire infection from mosquito bite but more frequently from contact with infected livestock. Most people suffer a benign febrile illness but a proportion succumbs to fatal haemorrhagic disease and/or encephalitis. Vaccination of livestock would provide a practical and effective means of controlling RVFV infection in humans and for preventing spread of the virus by livestock trade to areas where it is not endemic. Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne virus that causes a disease in humans with a 30% fatality rate. Humans become infected by tick-bite or contact with infected blood or other tissues of livestock and abattoir workers, farm workers and slaughtermen are considered at high risk for CCHFV infections.

Outbreaks of CCHF and RVF infections are always associated with fatalities and the development of suitable vaccines is an important public health issue. Antibody responses against the viral glycoproteins are believed to mediate protective immunity against RVF and possibly CCHF viruses. Previous studies using recombinant RVF G1 and G2 proteins have demonstrated that protective immunity can be achieved by immunization with both G1 and G2 proteins or G2 proteins only. Alphavirus-based vaccine vectors for RVFV and CCHFV have been developed in collaboration with our colleagues at the Carolina Vaccine Institute. The vaccine-delivery systems are based on Sindbis and Venezuelen equine encephalitis virus replicons encoding the glycoproteins of RVFV and replicons encoding the glycoproteins of CCHFV. The efficacy of the vaccines is being evaluated by monitoring the antibody response and the survival rate from challenge experiments. Preliminary studies performed in mice have shown that the vaccine against RVF virus does provide protection against lethal challenge and elicits neutralizing antibodies against the virus.

IDENTIFICATION OF GENETIC DETERMINANTS OF PATHOGENICITY OF WEST NILE (WN) VIRUS STRAINS

This work was conducted in collaboration with our colleagues from the University of Columbia, NY, USA and from the Microbiology Department, University of Pretoria. Studies conducted in mice in SPU demonstrated differences in pathogenesis among strains of WNV from lineage I and II correlate with genotype rather than lineage. The WN virus +RNA genome consist of a single long open reading frame that translates into a large polyprotein that is cleaved into at least 10 proteins. The N-terminus encodes the structural capsid-premembrane-envelope protein followed by the non-structural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5). The host signal peptidase is responsible for cleavage between C-prM; prM-E, E-NS1 and near the terminus of NS4A. It has been demonstrated in other flaviviruses that mutations in this region that improve or decrease the signal signalase strength change the pathogenicity or the immunogenic properties of the virus. For example, mutagenesis that enhances the C-prM signalase cleavage of yellow fever virus is lethal for virus production. Also, inefficient cleavage of prM further results in deficiency in secretion of the prM-E heterodimer which causes a lack of immunogenicity if such constructs are used for immunization. To investigate the potential role of signalase cleavage efficiency in WN virus pathogenesis, we sequenced the signalase cleavage regions of 3 highly neuroinvasive lineage 2 WN virus strains and 1 mild strain, and compared the translated amino acid sequence with the highly neuroinvasive lineage I strain (NY385/99) as well as a poorly neuroinvasive Kunjin strain. We found that the C-preM cleavage region in all lineage 2 strains were completely conserved and had a higher predicted cleavage efficiency (+9) than the Kunjin strain (+7) and the highly neuroinvasive lineage 1 strain NY385/99 (+5). This could suggest that less efficient cleavage of this region in the NY385/99 strain may elicit a less vigorous immune response to the prM-E heterodimer, or that lineage 2 strains may replicate less efficiently than the NY385/99 lineage 1 strain. However, conservation of the region in both highly pathogenic and mild lineage 2 strains suggests that additional determinants exist that determines pathogenic potential of genotypes in this lineage.

GENE EXPRESSION IN MICE INFECTED WITH WEST NILE VIRUS STRAINS OF DIFFERENT NEUROVIRULENCE

To identify host genes that modulate WNV pathogenesis and protection, DNA microarrays were used to compare gene expression in mice infected peripherally with seven lineage 1 and 2 strains of WNV, including 4 that had been associated with severe disease and 2 with benign infection in humans and birds, and confirmed to be of high or low neuroinvasiveness in mice, plus a mosquito isolate with low neuroinvasiveness in mice.

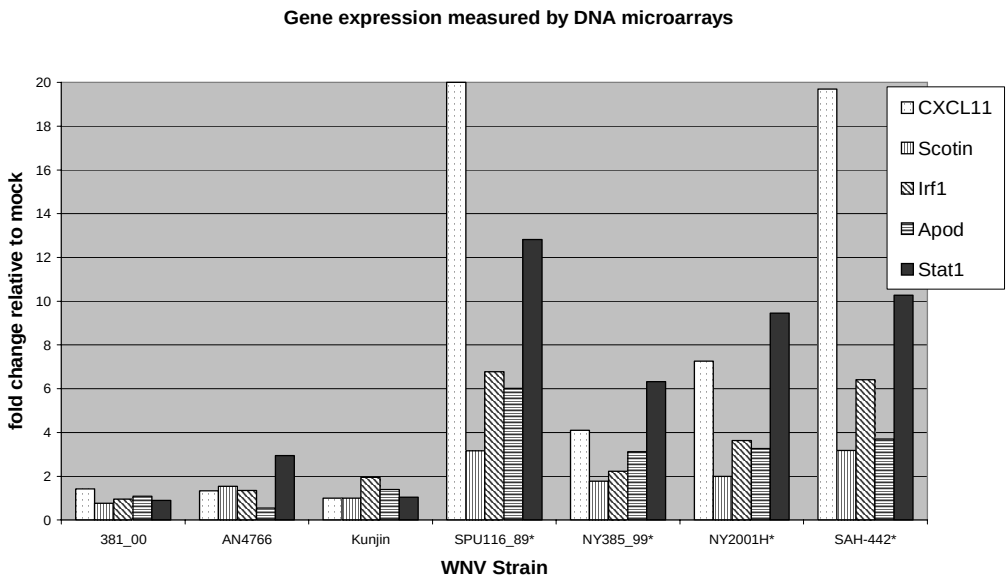


Fig 6. A selection of genes identified with DNA microarrays that were expressed significantly higher in the brains of mice infected with the highly neuroinvasive WN virus strains relative to the less neuroinvasive strains. Fold change were measured relative to mock infected mice. Highly neuroinvasive WN virus strains (Lineage 1: (NY385/99, NY2001Hu), Lineage 2: (H442 and SPU116/89) less neuroinvasive strains (Lineage 1: An4766 and MRM16 (Kunjin) and Lineage 2: AR381/00 clustered together.

The 4 strains with highest neuroinvasiveness were found to be associated with significant ($p<0.001$) up-regulation of 52 genes in the central nervous system, 104 genes in the liver and 81 genes in the spleen, relative to the 3 least neuroinvasive strains. Increased expression of genes involved in interferon signaling pathways, protein degradation, T cell recruitment, MHC class I and II antigen presentation and apoptosis were identified which may help control viral infection, but could also contribute to the pathogenesis of encephalitis and hepatitis. Increased expression of acute proteins, central nervous system specific proteins and proteins associated with T cell hepatitis, were also identified that may be more specifically related to_exalted virulence. Increased expression of these genes may help explain pathogenic mechanisms which distinguish the strains and have potential implications for treatment of the infection.

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MAJOR MEETINGS, CONFERENCES AND WORKSHOPS

1 Paweska JT, Swanepoel R: 2004 meetings of Biological Weapons Working Committee (BWWC) of the South African Council for the Non-proliferation of Weapons of Mass Destruction, Pretoria.
2 Swanepoel R: Plenary speaker, African European conference on Travel Medicine, Cape Town, 8-11 February 2004.
3 Burt F, Paweska JT, Swanepoel R: Organizers and Lecturers of WHO Training Course on Laboratory Diagnosis of Crimean-Congo Haemorrhagic, NICD, Sandringham, South Africa, 16 February- 05 March, 2004.
4 Paweska JT: International Conference on Women and Infectious Diseases: From Science to Action, Atlanta, Georgia, USA, 27 - 28 February 2004.
5 Paweska JT: International Conference on Emerging Infectious Diseases, Atlanta, Georgia, USA, 29 February – 03 March 2004.
6 Swanepoel R: Session moderator, International Conference on Emerging Infectious Diseases, Atlanta, 29 February – 3 March, 2004.
7 Paweska JT, Swanepoel R: Second Meeting, International High Security Laboratory Network, Atlanta, 4 -5 March, 2004.
8 Swanepoel R: World Health Organization Global Outbreak Alert and Response Network Meeting, Atlanta, 5 March, 2004.
9 Paweska JT: Study visit to Centers for Disease Control and Prevention, Special Pathogens Branch, Atlanta, Georgia, USA, 09 – 19 March 2004.
10 Kemp A, Swanepoel R: First Meeting of the European Food Safety Authority Scientific Working Group on the Assessment of the Risk of a Rift Valley Fever Incursion and its Persistence within Certain Parts of the Community, London, 16 August, 2004.
11 Swanepoel R: Meeting, World Health Organization Ad Hoc Committee on Orthopox Infections, Geneva, 31 August - 1 September, 2004.

12 Swanepoel R: World Health Organization Workshop on Ebola Fever, Paris, 7-8 September, 2004.
13 Kemp A, Paweska JT: Second Meeting of European Food Safety Authority Scientific Working Group on the Assessment of the Risk of a Rift Valley Fever Incursion and its Persistence within Certain Parts of the Community, London, UK, 11-12 October 2004.
14 Paweska JT: WHO Consultation on a Coordinated Response for the Fast-Track Development Tools for New and Re-emerging Infectious Diseases, Kobe, Japan, 20-22 September 2004.
15 Swanepoel R: Meeting, World Health Organization Ad Hoc Committee on Orthopox Infections, Geneva, 31 August - 1 September, 2004.
16 Venter M: Invited speaker at the 2004 Molecular and Cell Biology group Symposium. Title of presentation: "Identification of genes involved in the host response to neurological and hepatic West Nile virus infection using DNA microarray technology". University of the Witwatersrand Medical School, 6th October, 2004.
17 Swanepoel R: World Health Organization Workshop on Ebola Fever, Paris, 7-8 September, 2004.
18 Swanepoel R: United Nations Mission in Sierra Leone and World Health Organization to investigate facilities for laboratory diagnosis of Lassa fever in the Mano River Countries: Sierra Leone, Guinea-Conakry and Liberia, 24 October - 1 November, 2004.
19 Paweska JT, Swanepoel R: Steering Committee Meeting, World Health Organization Global Outbreak Alert and Response Network, Johannesburg, 15-16 November, 2004.
20 A. Kemp, JT Paweska, R. Swanepoel: Third Meeting of European Food Safety Authority Scientific Working Group on the Assessment of the Risk of a Rift Valley Fever Incursion and its Persistence within Certain Parts of the Community, London18-19 November, 2004.

STAFF OF THE SPECIAL PATHOGENS UNIT

Dr JT Paweska, Chief Specialist Scientist, Head of Unit
Professor R Swanepoel, Consultant
LJ Dos Santos, Senior Admin Clerk
Dr FJ Burt, Specialist Scientist
Dr M Venter, Principal Medical Scientist
PA Leman, Principal Medical Scientist
J Jardine, Medical Scientist
AA Grobbelaar, P/T Principle Medical Scientist
J Croft, Chief Medical Technologist
M Mashele, Chief Medical Technologist
NS Bodla, Senior Medical Technologist
NB Magome, Laboratory Assistant
L Seema, Laboratory Assistant
S Modise, Laboratory Assistant

Arbovirus Unit

A Kemp, Principal Medical Scientist
S Serero, Chief Auxillary Service Officer
J Mahlangu, Chief Auxillary Service Officer
CM Chauke, Laboratory Assistant
DZ Mnisi, Laboratory Assistant
R Nkoana, Laboratory Assistant

HIV/AIDS VIRUS RESEARCH UNIT

A/Prof Lynn Morris
Head of Unit

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 both within South Africa and internationally. Major activities within the Unit during the year have contributed towards the immunological evaluation of candidate HIV vaccines, addressing issues of HIV drug resistance in mother-to-child prevention programs, and understanding mechanisms of mother-to-child transmission. The Unit trains junior scientists and has a number of post-graduate students and also contributes to training local scientists and those from other African countries through laboratory-based training and workshops. Members of the Unit are involved in a number of networks including the HIV Vaccine Trials Network (HVTN), WHO Regional HIV/AIDS Public Health Laboratory Network and the WHO Global HIV Drug Resistance Network. Funding of research is largely through grants from the South African AIDS Vaccine Initiative (SAAVI), National Department of Health, National Institutes of Health including CIPRA (Collaborative International Program for Research on AIDS), RO1's, USAID and HVTN, The Wellcome Trust, The Fogarty International Centre, Bristol Myers Squibb Secure-the-Future and the Doris Duke Human Pathogenesis Program.

A major project during the year has been the design and planning of new laboratories to house the AIDS Unit. The area is over 2,000 square meters on two levels and includes a BSL3 laboratory for HIV culture and dedicated areas for flow cytometry and molecular biology. Occupation is scheduled for February 2005. The AIDS Unit laboratories are accredited with the South African National Accreditation Society (SANAS) and also participate in a number of external quality assurances programs.

Immunology end-point measurements of South Africa's first HIV vaccine trial which started in November 2003 were conducted in 2004. This candidate vaccine is a viral vector containing an HIV-1 subtype C *gag* gene and both cellular and humoral immune responses are being monitored. Assays used in these assessments have undergone an extensive program of validation and will be used in additional trials due to start in 2005.

A major project funded by the National Department of Health has been the study of the incidence and persistence of nevirapine (NVP) resistance mutations following a single dose to prevent mother-to-child transmission. These data were presented by the Prof Morris at a MinMEC meeting held in Pretoria on 25th June and the implications of these findings for the mother-to-child prevention programs.

Natasha Taylor and Isaac Choge were awarded their Master's degrees from the University of the Witwatersrand and Sarah Cohen obtained a degree in Laboratory Management from the University of Pretoria. Pascal Bessong who was co-supervised by Prof Morris obtained his PhD from the University of Venda. A number of staff and students had the opportunity of spending time in overseas laboratories: Jabulani Nhlapo spent 4 months in the laboratory of Dr James Robinson at Tulane Medical Centre in New Orleans; Mia Coetzer spent 1 month in Dr Jim Mullin's laboratory in Seattle; Dr Penny Moore spent 7 weeks in Dr James Binley's laboratory at the Torrey Pines Institute for Molecular Studies, San Diego, followed by a one week visit to Dr David Montefiori's laboratory at Duke University; Shayne Loubser had a 3 week laboratory visit to Columbia University; Dr Agatha Masemola visited the Vaccine Research Center at the NIH, Washington DC and Dr Vivian Morafo visited the Henry F Jackson Foundation of the Walter Reed Institute.

Members of the AIDS Unit presented their research and participated in major international meetings including the 11th Conference on Retroviruses and Opportunistic Infections (CROI) held in San Francisco; the Keystone Symposia at Whistler, Canada; the XV International AIDS Conference in Bangkok, Thailand; the HVTN full-group meetings in Washington and Seattle; the XIII International HIV Drug Resistance Workshop in Tenerife; the AIDS Vaccine Meeting in Laussane; the International Society for Analytical Cytology (ISAC) Congress in Montpellier, France.

New staff members in the Unit in 2004 included Dr Debra Barkhan, a new post-doctoral Fellow, Netty Malatsi who has joined the HIV Immunology Laboratory, Samantha Donninger

who joined the Cell Biology Laboratory and Janet Mans, a PhD student based at the NIH. Short-term visitors included Jim Brandful from the Noguchi Memorial Institute in Ghana who was a one year SAAVI visiting fellowship.

Dr Clive Gray was the recipient of an Elizabeth Glaser Pediatric AIDS Foundation International Leadership Award for 2004. This is a 3 year grant to fund the Program for the Enrichment of Paediatric HIV Immunology in South Africa (called PEPHISA). Dr Gray was appointed as visiting Professor at the University of the Western Cape until March 2005. Dr Vivian Morafo was awarded the Albert Beyers Traveling Fellowship (Immunology) that will enable her to spend two weeks in a laboratory at Oxford University. Mary Phoswa was recognized for giving 25 years of service at NIV/NICD at a Unit function. Prof Morris was invited to participate in the Laboratory Sciences Working Group of the Vaccine Enterprise.

The Unit had a number of visitors in 2004 that included Dr Makobetsi Khati, Matthew Stremlau, Dr Don Sutherland, Dr Austin Denby, Dr Analise van Rie, Dr Louise Kuhn, Dr David Katzenstein, Dr Mike Betts and Dr Guido Ferrari.

Some highlights of the data produced in the AIDS Unit in 2004 are summarized below:

INCIDENCE AND PERSISTENCE OF RESISTANCE AMONG WOMEN AND INFANTS EXPOSED TO SINGLE-DOSE NEVIRAPINE

A single-dose of nevirapine (NVP) has shown to be efficacious in the prevention of mother-to-child HIV-1 transmission (pMTCT). However, studies have shown that this intervention is associated with the development of NVP resistance mutations. In order to examine this within South African populations we followed two large pMTCT cohorts in Johannesburg and Durban for the development of NVP resistance mutations using a commercial genotyping assay. At 7 weeks post-NVP, 38% of women and 42% of infected infants had high-level resistance (Figure 1). Mutations in mothers included K103N (31%), Y181C (12%), Y188C (8%), V106M (4.3%), G190A (5%) and V106A (2%) while in infants these were Y181C (32%), K103N (12%), Y188C (5%) and V106A (3%). The majority of women (21%) had a single mutation, 13% had two mutations and 5% had three or four mutations. More than 99% of women were infected with HIV-1 subtype C viruses. Baseline samples (pre-NVP) showed that in almost all cases these mutations developed as a result of NVP exposure. Persistence of NVP resistance mutations was evident in 14% of women at 24 weeks. All had the K103N mutation and 2 also had the G190A mutation. Viral load levels were higher among women who still had mutations at 24 weeks compared to those who had lost the mutation (113,000 versus 43,550 HIV-1 RNA copies/ml, $p<0.05$). Similarly CD4 counts were significantly lower among women who still had resistance mutations at 6 months (306 versus 363 cells/ul, $p<0.05$) suggesting that persistence is associated with more advanced disease. In infants 26% still had resistance at 24 weeks which was predominantly Y181C. These data indicate that NVP resistance mutations develop among a significant proportion of South African women and infants following single-dose NVP. Persistence of K103N and Y181C in some women and infants up to 24 weeks suggests that both are relatively fit viruses. The impact of these mutations on future treatment options, including nevirapine in a subsequent pregnancy, remains to be explored.

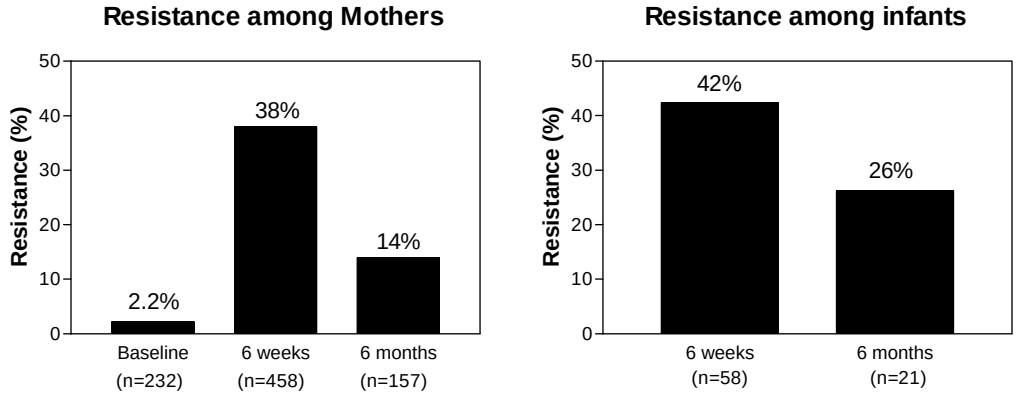

Fig 1. Incidence and persistence of NVP resistance mutations following exposure to single-dose of nevirapine

NEVIRAPINE RESISTANCE MUTATIONS AMONG HIV-1 INFECTED INFANTS FOLLOWING SINGLE DOSE NEVIRAPINE

One of the potential obstacles to the success of NVP to prevent mother-to-child transmission of HIV-1 is the emergence of drug resistant variants. This study assessed the rates of resistance among infants enrolled in the standard regimen (SR) where both mothers and infants receive a single dose of NVP (sd-NVP) compared to those infants who received sd-NVP as post exposure prophylaxis (PEP). Among the infants on the PEP regimen 3 of the 23 (13%) had mutations conferring high level resistance to NVP 6 weeks after receiving NVP. These were Y181C, Y181C plus K103N and Y181C plus V106M. A fourth infant had the K101E which is reported to confer low-level resistance to NVP. The Y181C mutation was found in 8 of the 24 (33%) infants on the SR at 6 weeks. All infants were infected with HIV-1 subtype C viruses. This preliminary data suggest that the PEP regimen is associated with a lower frequency of resistance compared to the standard regimen probably as a result of less exposure to NVP.

COMPARISON OF GENOTYPIC AND PHENOTYPIC ASSAYS FOR MEASURING NEVIRAPINE RESISTANCE AMONG WOMEN PARTICIPATING IN MOTHER-TO-CHILD PREVENTION PROGRAMS

Nevirapine (NVP) used in single doses to prevent mother-to-child transmission is associated with the development of genotypic resistance mutations. In order to determine if these mutations confer phenotypic resistance to NVP we tested the sensitivity of HIV-1 reverse transcriptase (RT) in the presence and absence of NVP using a low cost and rapid phenotypic resistance assay ExaVir™ Drug Susceptibility assay. RT extracted from plasma from 10 women pre-NVP were phenotypically sensitive to NVP with IC_{50} values <1.5 µM NVP in agreement with a lack of evidence of genotypic mutations. Samples from the same women 6 weeks after a single 200 mg dose of NVP all had evidence of genotypic mutations. In all cases the mutant population was either mixed or minor. All, except 1 of the women who had K103N plus Y181C, had evidence of phenotypic resistance. The IC_{50} values ranged from 3.3 µM (2.2 fold increase in resistance) to 49.5 µM (33-fold increase). These data suggest that this small group there was a good correlation between phenotypic and genotypic resistance. This assay may be useful for monitoring resistance in women who have received single dose NVP and are candidates for HAART.

In vitro GENERATION OF HIV-1 SUBTYPE C ISOLATES RESISTANT TO ENFUVIRTIDE

Enfuvirtide (ENF) is the first in a new class of antiretroviral agents targeting the fusion process of the viral life cycle. It is a synthetic 36 amino acid peptide that binds to the HR-1 region of gp41 preventing fusion of viral and cellular membranes. Resistance to ENF among subtype B HIV-1 isolates is associated with amino acid changes mainly in the HR-1 region, although other regions of envelope have also been implicated. We previously showed that HIV-1 subtype C viruses are highly susceptible to this drug. In order to determine whether subtype C viruses developed similar resistance mutations to subtype B viruses, 11 subtype C and 4 subtype B viruses were passaged in the presence of increasing concentrations of ENF. The subtype C isolates showed varying levels of replication at 1 mg/ml ENF by day 18, but by day 29 all replicated efficiently at 10 mg/ml ENF. All subtype C isolates showed evidence of genotypic changes in gp41 HR-1 following exposure to ENF that included G36S/E/D, I37T, V38M/A/L/E, N/S42D, N43K, L45R/M and A50T/V (Table 1).

Table 1: The IC_{50} and IC_{90} values with corresponding resistance sequences for subtype C and B isolates from S2 day 29.

	Biotype	Inhibitory concentration (ug/ml)		HR-1 amino acid sequence																
		IC_{50}	IC_{90}																	
Subtype C																				
Consensus C				G	I	V	Q	Q	Q	S	N	L	L	R	A	I	E	A	Q	Q
Du151a	R5	9.52	14.08	**S/G**	.	.	.	.	.	.	.	.	.	.	.	.	.	**T**	.	.
Du422a	R5	15.24	23.55	.	.	.	.	.	.	.	**K**	.	.	.	.	.	.	.	.	.
SW5	R5	24.99	>25	.	.	**L**	.	.	.	.	.	.	**R**	.	.	.	.	.	.	.
CM7	R5	15.75	23.87	**S**	.	.	.	.	.	N	.	.	.	.	.	.	.	**T**	.	.
SW29	R5	19.79	>25	**S**	.	.	.	.	.	**D**	.	.	.	.	.	.	.	.	.	.
SW38	R5	19.89	>25	.	.	**A**	.	.	.	.	.	.	.	K	.	.	.	T	.	H
PCP1	R5, X6	>25	>25	**G/E**	.	**A/V**	.	.	.	.	.	.	.	.	.	.	.	.	.	.
RP1	R5, X4	>25	>25	.	.	.	.	.	.	.	**K**	.	.	.	.	.	.	**V**	.	.
CM9	R5, X4, X6, R3, Bob	22.62	>25	.	.	**E**	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SW7	X4	>25	>25	.	V	**A**	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DR28	X4	16.69	23.7	.	**T**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Subtype B																				
Consensus B				G	I	V	Q	Q	Q	N	N	L	L	R	A	I	E	A	Q	Q
SM1	R5	17.21	24.91	.	.	**M/E/K**	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SM2	R5	22.31	>25	**D**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DS3	R5	NR	NR	**S**	.	.	.	.	.	.	.	.	.	**M**	.	.	.	.	.	.
DS12	R5, X4	17.12	>25	.	.	.	.	.	.	.	**N/K/S**	.	.	.	.	.	.	.	.	.

NR, no replication; Mutations arising from ENF treatment are in bold and highlighted; Dots indicate no change from subtype consensus sequence; Residues not in bold represent differences from consensus sequence present in parental and ENF-resistant viruses

Five viruses had compensatory changes in the HR-2 region, which corresponds to the ENF sequence, and two isolates had changes in the V3 region. Mutational patterns among the 4 subtype B viruses were similar to those for subtype C and those previously published in the literature. These data indicate that *in vitro* resistance to ENF develops rapidly among HIV-1 subtype C isolates. In general, mutational patterns for subtype C were similar to those described for subtype B, suggesting that the mechanism of action for ENF is similar for HIV-1 subtype B and C isolates.

PREDICTED GENOTYPIC RESISTANCE TO THE NOVEL ENTRY INHIBITOR, BMS-378806, AMONG HIV-1 ISOLATES OF SUBTYPES A TO G

BMS-378806, a small molecule inhibitor of HIV-1, targets virus entry by inhibiting the binding of HIV-1 gp120 to CD4. BMS-378806 exhibits potent antiviral activity against subtype B HIV-1 viruses, including both R5 and X4 viruses. Analysis of a limited number of viruses of non-B subtypes suggest that the drug may be less efficacious in these viruses. HIV-1 viruses resistant to BMS-378806 have been selected *in vitro,* and mutations within the virus envelope conferring resistance to BMS-378806 have been identified. Mutations include M475I, M434I/V, M426L, D350K, D185N, K655E, I595F, V68A and S440R. We analysed 1226 Env sequences of subtypes A-D, F-G and CRF01_AE, obtained from the Los Alamos database in order to determine the frequency of naturally-occurring resistance mutations to BMS-378806. The occurrence of resistance mutations, either individually or in combination, was expressed as a percentage for each subtype. In line with sensitivity data, the level of background resistance to BMS-378806 among HIV-1 subtypes other than B, including subtype C which is now responsible for the majority of new infections worldwide, was found to be considerably higher than that observed in subtype B. These results suggest that reduced efficacy in non-B viruses may be due to high levels of genotypic resistance within the drug-naïve population. These high levels of background genotypic resistance to BMS-378806 suggest that these resistance mutations do not confer a fitness disadvantage that is characteristic of viruses resistant to the RT and PI inhibitors and possibly also Enfuvirtide. BMS-378806 is a prototype drug likely to be followed by derivatives that may have broader specificity. Nevertheless, the high genotypic resistance to BMS-378806 may limit its usefulness in non-B populations, particularly in subtype C and CRF01_AE viruses.

A RELIABLE PHENOTYPE PREDICTOR FOR SUBTYPE C BASED ON ENV V3 SEQUENCE

The use of both CCR5 and CXCR4 as coreceptors during host cell entry in HIV-1 subtypes B, A and D is well known. In these subtypes, there is a strong association between CXCR4 usage and disease progression. However, HIV-1 subtype C, that is responsible for 42% of global infections, rarely uses CXCR4. The ability to screen large subtype C-infected cohorts for X4 viruses, and relate their presence to disease status, is vital to understanding this important difference. However, *in vitro* determination of coreceptor usage is not always feasible, especially in those developing countries where subtype C predominates. A reliable phenotype prediction method, based on genetic sequence data, could provide for rapid and less expensive screening. Existing methods using subtype B V3 loop data do not perform well when applied to other subtypes. We hypothesized that, using V3 sequences of subtype C isolates of known phenotype, we could develop a reliable subtype C-specific phenotype predictor. We derived predictors from position specific scoring matrices (PSSM) based on a subtype C training set of 229 R5 V3 sequences (from 200 subjects) and 51 X4 V3 sequences (20 subjects). The C-specific predictor (C-PSSM) had a specificity of 91% and sensitivity of 84% based on leave-one-out bootstrap using all sequences. Analysis based on single sequences per individual gave comparable specificity (94%) but somewhat lower sensitivity (75%). These are similar to the data obtained when subtype C sequences are scored using a subtype B-specific PSSM (specificity of 93% and sensitivity of 88%). The C-PSSM is significantly more sensitive than the B-PSSM applied to subtype C sequences (sensitivity of 37%). We also performed V3-based heteroduplex tracking assays (V3-HTA) using a R5 probe and a subset (n=21) of the training set as targets. V3-HTA mobilities correlated with C-PSSM score ($p < 0.0001$; $R^2=0.56$). In summary, we derived a C-specific phenotype predictor that performs nearly as well on subtype C V3 loops as do existing B-specific methods on subtype B V3 loops.

USE OF ALTERNATE CORECEPTORS ON PRIMARY CELLS BY TWO HIV-1 ISOLATES

Two HIV-1 isolates (CM4 and CM9) previously shown to use receptors other than CCR5 or

CXCR4 on transfected cell lines were tested for their sensitivity to inhibitors of HIV-1 entry on primary cells. Both isolates were from patients with cryptococcal meningitis, a severe AIDS defining condition. CM4 is a complex recombinant comprised of five subtypes, and CM9 is subtype C throughout its genome. CM4 was able to use CCR5 and Bob/GPR15 efficiently in transfected cells. This isolate grew in D32/D32 CCR5 PBMC in the presence of AMD3100, a CXCR4-specific inhibitor, indicating that it uses a receptor other than CCR5 or CXCR4 on primary cells. It was insensitive to the CCR5 entry inhibitors RANTES and PRO140, but was partially inhibited by vMIP-1, a chemokine that binds CCR3, CCR8, GPR15 and CXCR6. The coreceptor used by this isolate on primary cells is currently unknown. CM9 used CCR5, CXCR4, Bob/GPR15, CXCR6 and CCR3 on transfected cells and was able to replicate in the presence of AMD3100 in D32/D32 CCR5 PBMC. It was insensitive to eotaxin, vMIP-1 and I309 when tested individually, but was inhibited completely when vMIP-1 or I309 was combined with AMD3100. Both I309 and vMIP-1 bind CCR8, strongly suggesting that this isolate can use CCR8 on primary cells. Collectively these data suggest that some HIV-1 isolates can use alternate coreceptors on primary cells, which may have implications for strategies that aim to block viral entry.

SUSCEPTIBILITY OF SUBTYPE C VIRUSES TO NEUTRALIZATION BY MONOCLONAL ANTIBODIES RAISED AGAINST SUBTYPE B

Subtype C is one of the most prevalent genetic subtypes of human immunodeficiency virus type 1 (HIV-1) in the world and the most common in southern Africa. Few broadly neutralizing monoclonal antibodies (MAbs) have been described, all of which were raised against subtype B isolates. Passive transfer studies in animals have shown that these MAbs can protect against HIV-1 challenge although high concentrations are needed and the challenge strain needs to be sensitive to neutralization by these MAbs *in vitro*. The use of these neutralizing MAb in humans has been proposed, including their use to prevent mother to child transmission. Although this could be a useful strategy, resistance of circulating strains to these MAbs is likely to compromise the outcome. There is conflicting data regarding the sensitivity of subtype C isolates to these broadly neutralizing MAbs raised against subtype B viruses. In this study we have investigated the ability of these MAbs to neutralize subtype C isolates. Eight subtype C viruses from South Africa were tested in a PBMC based neutralization assay against 4 broadly neutralizing MAbs. IgG1b12 targets the CD4 binding site while 2G12 recognizes a cluster of mannose residues on gp120. 2F5 and 4E10 recognize linear epitopes in gp41 defined as ELDKWA and NWFDIT respectively. sCD4 was used as a positive control. Only one of the 8 subtype C isolates was sensitive to 2G12 and this correlated with the absence of 295N that is required for 2G12 neutralization. Two of the subtype C isolates were neutralized by IgG1b12. 2F5 failed to neutralize any of the subtype C isolates and this correlated with the absence of the ELDKWA epitope in gp41. 4E10 neutralized all the subtype C viruses and all had the crucial FW amino acids in the target sequence recognized by this MAb. Neutralization of subtype B isolates was more effective with the majority showing sensitivity to the MAbs tested. All subtype B and C viruses were sensitive to sCD4. In summary, with the exception of 4E10 the broadly neutralizing MAbs raised against subtype B viruses failed to neutralize a significant number of subtype C isolates. In general this correlated with the absence of the target sequence (epitope) in the envelope gene. This suggests that these MAbs would not be highly effective in a subtype C infected population.

CIRCULATING LEVELS OF SDF-1α AND IL-7 IN HIV-1 INFECTION AND PULMONARY TUBERCULOSIS ARE RECIPROCALLY RELATED TO CXCR4 EXPRESSION ON PERIPHERAL BLOOD LEUKOCYTES

In sub-Saharan Africa the coincidence of HIV-1 and *Mycobacterium tuberculosis* co-infection is ever-increasing, this being associated with increased progression to disease and reduced patient survival. Raised plasma levels of stromal cell-derived factor (SDF)-1α and interleukin (IL)-7, cytokines important in T-cell development, and in the modulation of surface CXCR4 expression, have been reported to be associated with HIV-1 disease progression but their role has not been questioned in the context of coinfection with HIV-1 and *M. tuberculosis*.

SDF-1 is a CXC chemokine that was first cloned from a bone marrow stromal cell line, and identified as a pre-B-cell growth stimulating factor. SDF-1, unlike most chemokines, is constitutively expressed in a broad range of tissues, is a potent chemoattractant for early-stage B-cell precursors, monocytes and lymphocytes, and plays a vital role in development.

Mice deficient in SDF-1 die perinatally with major defects in cardiac septal formation, gastrointestinal vasculogenesis, cerebellar development, myelopoiesis and B-cell lymphopoiesis. SDF-1 is the natural ligand for the seven-transmembrane G-protein-coupled receptor, CXCR4, which also serves as the major HIV-1 coreceptor utilized by T-cell tropic, syncytium-inducing (SI) HIV-1 strains. SDF-1, by binding to CXCR4 has been shown to inhibit virus entry and replication by SI HIV-1 strains that are usually isolated at late, symptomatic stages of HIV-1 infection.

Interleukin (IL)-7, a cytokine produced predominantly by the stromal cells of the bone marrow and thymus, is essential for the development of the immune system. IL-7 was initially described as a growth factor for precursor B-cells, and was later demonstrated to play a key role in modulating T-cell development, and in enhancing the function of mature T-cells. The role of SDF-1 in HIV-1 disease progression has not been well established. A single nucleotide polymorphism, found in a conserved part of 3'-untranslated region of the SDF-1 gene that encodes the ligand for CXCR4, had been reported to delay the onset of AIDS. The authors postulated that the mutation could increase the amount of SDF-1 available to bind to CXCR4, consequently preventing the emergence of late stage X4 viruses. However, these results were contradicted in other studies. A positive correlation between SDF-1 levels and $CD4^+$ T-cell count was found in HIV-1-infected patients. In addition, these patients had lower levels of SDF-1 than uninfected controls. Contrary to this report, another study found higher plasma SDF-1 levels in HIV-1-infected individuals than in uninfected controls, and an inverse correlation with $CD4^+$ T-cell count, while another found no significant differences in SDF-1 levels between HIV-1- seropositive patients and healthy controls. Higher SDF-1 levels have been shown to be associated with the presence of NSI HIV-1 strains and interestingly, IL-7 levels in HIV-1 seropositive individuals, with $CD4^+$ T-cell counts of < 200, were higher in those patients infected with SI isolates.

IL-7 is able to upregulate HIV-1 replication *in vitro*. Moreover, significantly higher plasma IL-7 levels have been found in HIV-1-infected individuals when compared with healthy individuals. In addition, recent studies have demonstrated an inverse correlation between circulating IL-7 levels and numbers of $CD4^+$ T- cells in HIV-1-infected individuals, and a positive correlation with HIV-1 viral load.

We have previously found that the *in vivo* expression of CXCR4 expression was significantly reduced on peripheral blood leukocyte subsets in persons infected with HIV-1, those with pulmonary tuberculosis and those coinfected with HIV-1 and *Mycobacterium tuberculosis*, when compared with healthy control individuals. Given the functions described above for SDF-1α and IL-7, in particular their effects on T-cells and their possible role in HIV-1 disease progression, and the ability of SDF-1 and IL-7 to modulate CXCR4 expression *in vitro,* led us to determine their peripheral levels in similar disease groups, and to question whether there was any relationship between circulating levels of SDF-1α and/or IL-7 and CXCR4 expression on different leukocyte subsets from patients infected with HIV-1 and/or *M. tuberculosis*. In addition, whether a relationship exists between plasma levels of these cytokines and HIV-1 disease progression was evaluated. Therefore, cross-sectional analysis was performed on a total of 59 individuals, comprising 19 healthy normal donors (ND or control group), 16 HIV-1-seropositive patients without active tuberculosis (HIV group), 13 patients with pulmonary TB (TB group), and 11 patients co-infected with *M. tuberculosis* and HIV-1 (HIV/TB group). All but one of the patients with pulmonary TB was on standard anti-TB therapy. None of the patients in the HIV and HIV/TB groups had received any antiretroviral treatment. SDF-1α and IL-7 plasma levels were significantly elevated from normal in similar disease groups ($P<0.001$). Both SDF-1α and IL-7 plasma levels correlated negatively with the percentage of all subsets of leukocytes expressing CXCR4, across the study groups regardless of the presence or absence of disease. This suggests that CXCR4 receptors are likely modulated in similar ways by increased levels of these cytokines, even though the underlying mechanism of their increased production is likely to be different. Additionally, plasma levels of SDF-1α correlated negatively with percentage of $CD4^+$ T-cells, and both SDF-1α and IL-7 levels correlated positively with plasma HIV-1 viral load. Collectively, these results confirm a role for SDF-1α and IL-7 in HIV-1 disease progression, and suggest that these cytokines play a role in the modulation of CXCR4.

In conclusion, this study has shown that (i) overproduction of SDF-1α and IL-7 is a consequence of immune processes in response to infection with HIV-1 and *M. tuberculosis* infection, alone or in combination; tuberculosis had greater modulatory effects on SDF-1α production whereas HIV-1 influenced IL-7 production to a greater extent, (ii) circulating levels of these cytokines increase with advancing HIV-1 disease, and (iii) in general increased levels of both cytokines are associated with correspondingly reduced expression of CXCR4 on various leukocyte subsets irrespective of the presence or absence of infection. Results presented provide additional support for the role of both SDF-1α and IL-7 in the pathogenesis of HIV-1 disease, and further suggest a role in the pathogenesis of pulmonary tuberculosis. The relationship between SDF-1α and IL-7 and their respective roles in disease pathogenesis remains intriguing, and the role these cytokines play individually or in concert in HIV-1 and *M. tuberculosis* infected persons awaits further confirmatory studies on larger cohorts.

REDUCED ABILITY OF NEWBORNS TO PRODUCE CCL3/MIP-1a IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PERINATAL HIV-1 TRANSMISSION – A LINK TO HOST GENETICS?

The role of CC chemokines CCL3, CCL4 and CCL5 in protection against mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission is not well understood. These chemokines can act as HIV-1 suppressor factors on the basis of their ability to block cell entry via CCR5 of R5-utilizing HIV-1 strains. One study has shown that HIV-1 Env specific T-helper cell responses detected in uninfected infants born to HIV-1 seropositive mothers were associated with the enhanced expression of CC chemokines, and it was postulated that these factors could mediate non-cytolytic inhibition of infection during vertical transmission of HIV-1. We have observed in a nested case-control study that *in utero* priming by HIV-1 increased mitogen-induced production of CCL3/MIP-1 and CCL4/MIP-1 by cord blood mononuclear cells, but that a deficiency in the production of CCL3 was associated with increased susceptibility to intrapartum HIV-1 infection. The production of CCL5/RANTES showed no relationship with HIV-1 infection outcome in the infant, but was partially inhibited by HIV-1 exposure and infection. Intrapartum transmitting mothers presented with the same deficiency in mitogen-induced CCL3 production, suggesting that the underlying nature of this deficient response was genetically encoded (Figure 2 shows data for CCL3; groups stratified according to HIV-1 exposure and infection outcome of the infant). Sequencing of the promoter and first inton of the two functional CCL3 genes (CCL3 or LD78-alpha and CCL3-L1 (LD78-beta), and determination of CCL3-L1 copy numbers (which are variable in most populations) has revealed a complex array of genetic changes that underlie levels of production that warrant further clarification.

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Overall findings suggest that infants who display a deficient production phenotype of CCL3 are at increased risk of acquiring HIV-1, supporting the hypothesis that CCL3 in particular plays an important role in non-cytolytic inhibition of vertical HIV-1 transmission. It was further evident that increased CCL4, induced by HIV-1 exposure, was not sufficient to circumvent acquisition of HIV-1 in those infants who became infected. This would imply the presence of a susceptibility factor in our populations and would have far-reaching implications for HIV-1 infection and disease progression, response to HIV vaccines and antiretroviral therapies in South Africans, and very likely other African populations. Furthermore, it is likely that such an increased susceptibility to HIV-1 in our population would also be fuelling the TB epidemic in this country particularly in the presence of coexisting HIV-1 infection.

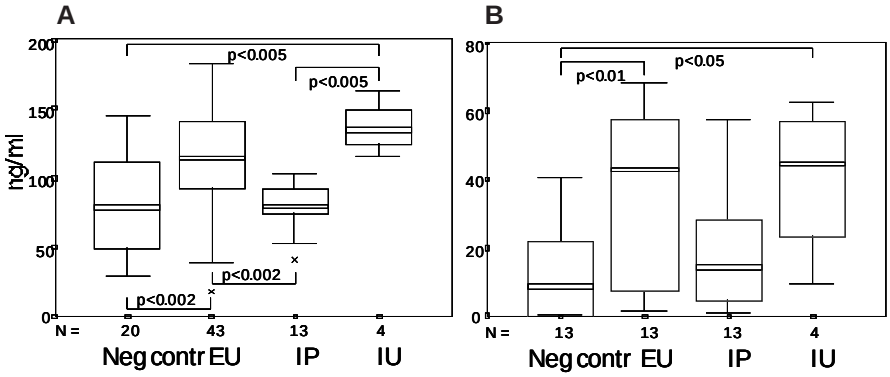

Fig 2. PHA-induced production of CCL3 in uninfected (Neg contr), exposed-uninfected (EU), intrapartum infected (IP) and *in utero* infected (IU) infants (A), and in a selection of uninfected (Neg contr) and non-transmitting (EU) mothers and all (IP and IU) transmitting mothers (B)

HLA FREQUENCIES IN SOUTH AFRICAN POPULATIONS

The primary objectives of this study is to determine the background frequencies at high resolution of HLA class I and II alleles in different sub-populations in South Africa in the context of HIV-1 vaccine design. The sub-populations were identified by parental cultural/ linguistic identity. In addition, the data may be useful in the light of associations between rare alleles/supertypes and disease outcome. An example of so-called rare alleles/supertypes is HLA B57 and disease control. Individuals with this specific allele have been shown to control HIV viraemia. Identification of the equivalent alleles as part of the HLV B57 supertype family and the relative frequencies is important in the context of population-based outcomes of viral control and viral evolution. The progress thus far is outlined below.

Black population (200 individuals): We have completed the high resolution (4 digit) typings for the HLA-Cw* allele. This data together with the completed HLA-A* and HLA-B* locus data and DRB1* has been assembled for analysis.

Caucasian population (100 individuals): We are currently completing the high resolution typing of the Caucasian population including the HLA-A*, HLA-B*, HLA-Cw* and HLA-DRB1* alleles. To date all the individuals have been typed for the Cw* allele, 80 individuals have been typed for the A* and B* alleles and 100 individuals have been typed for the DRB1* allele. Upon completion we will attempt to resolve ambiguous alleles and use the frequency data of the Caucasian population to compare with the Black population.

Resolution of HLA Ambiguity:

Ambiguous alleles for both class I A* and B* were resolved using either SSP-PCR allele-specific kits or sequencing using primers specifically designed to bind and sequence one of the alleles present. In summary, for the A* locus, eight ambiguous allele combinations (13 individuals) were resolved using SSP-PCR, while six ambiguous allele combinations (24 individuals) were resolved using sequencing. For the B* locus, seven allele combinations were resolved using SSP-PCR (20 individuals) and five ambiguous allele combinations (8 individuals) were resolved using sequencing.

Ambiguity in Class II DRB1 was resolved using SSP-PCR. In total, out of ten ambiguous allele combinations (18 individuals), we managed to resolve five (12 individuals).

New South African Class I HLA-A* and HLA-B* alleles:

We have completed sequencing all of the six alleles that were cloned into the pCR®4-TOPO® cloning vector. The recommended guidelines for identification of new alleles were followed: 2000 bp of four clones of each allele were sequenced in both the forward and the reverse directions using four pairs of designed sequencing primers to span the entire region. Sequences were analysed, aligned and a resulting consensus sequence for each allele was determined and analysed for the presence of the 'new' polymorphisms as well as for additional polymorphisms in the intron sequences. For our four HLA-A* alleles, sequence for exons 1, 2, 3 and 4 and introns 1, 2, 3 and 4 was obtained whilst for our two HLA-B* alleles, sequence for exons 2, 3 and 4 and introns 1, 2, 3 and 4 was obtained (the PCR fragment using the HLA-B*-specific primers does not yield the complete exon 1 sequence).

Sequences were subsequently submitted to the GenBank database as well as to the official HLA Informatics Group (Anthony Nolan Research Institute) for the application for official acceptance and assignment of a new allele by the WHO Nomenclature Committee. Five out of the six submitted alleles were accepted as 'new alleles' and designated the following names: A*300102; A*300202; A*6827; B*4206 and B*4507. The sixth allele has been accepted as a confirmatory report of an allele already described in December 2003, namely A*2911. All six alleles will be listed in the next Full Nomenclature Report and will be published in Tissue Antigens, Human Immunology and the European Journal of Immunogenetics.

Data Analysis

The initial data was reviewed and transformed using Python for Population (PyPop), a programme designed to generate parameters such as frequencies, haplotypes and linkage disequilibrium (LD). The LD format on PyPop was not entirely practicable and the programme may have to be reviewed for ease of accessing the data. In addition, the data was analysed based on subgroups using currently available data to determine whether the subgroups

using currently available data to determine whether the subgroups were linked or distinct using Phylip. Initial analysis appears to be useful (Figure 3). The figure indicates that using primarily data from various African populations for Class I A and B locus that the Sotho and Nguni populations are distinct. These types of analyses are dependent on what HLA databases are available and what group(s) to use as an outlier in the analysis. It was agreed that the South African Caucasian data would be a useful source for this purpose to root the trees and determine whether the two broad populations are truly distinct. Whether such differences will influence areas such as vaccine design and responses to HIV and HIV vaccines remains to be determined in planned longitudinal studies.

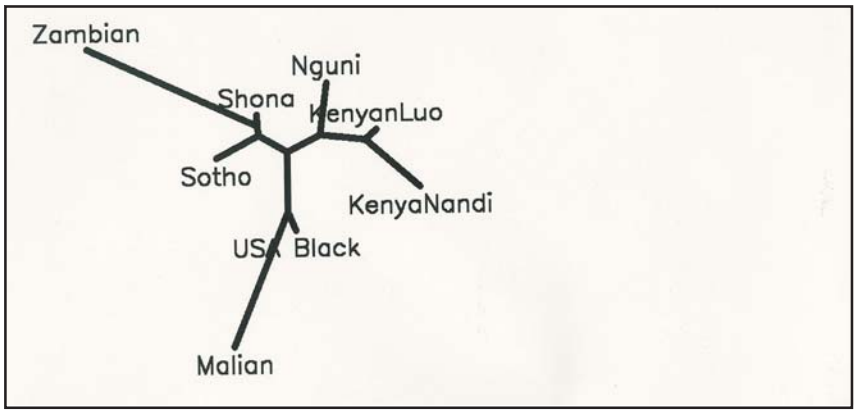

Fig 3. Phylip analysis of HLA-A* and HLA-B* using various databases.

Quality Assurance

We have continued to participate in the NEQAS UK external quality assurance programme for HLA typing for both high and low resolution with 100% concordance being achieved so far this year. We have also recently undergone an internal audit and continued accreditation, including sequence-based HLA typing, with the South African National Accreditation Schemes (SANAS).

ASSOCIATIONS BETWEEN CLASS I HUMAN LEUKOCYTE ANTIGEN (HLA) TYPES AND DISEASE STATUS IN INDIVIDUALS FROM SOUTHERN AFRICA INFECTED WITH HIV-1 SUBTYPE C INFECTION

Genetic variation at the Human Leukocyte Antigen (HLA) Class I loci plays an important role in determining host immune response to human immunodeficiency virus type 1 (HIV-1) infection. Data derived from a cohort of 65 HIV subtype C-infected and 20 uninfected individuals was studied to define the HLA phenotype in all individuals, and to identify HLA associations with HIV viral loads and CD4 counts. Individuals from Zimbabwe, Malawi, Zambia and South Africa were recruited as part of the HIVNET 028 study and DNA was isolated from peripheral blood mononuclear cells. HLA-A, B and Cw genotyping was performed at low resolution using SSP-PCR kits (Pel-Freez) and at high resolution using DNA sequencing (Applied Biosystems / Abbott Laboratories). Viral loads were determined using bDNA assays (Bayer) or RT-PCR (Roche), and CD4 levels were measured using a FACscount assay (Becton Dickinson). A total of 18 HLA-A, 21 HLA-B and 13 HLA-Cw different alleles were observed in total (n=85) using low-resolution (two-digit) nomenclature. Alleles A*30, B*18 and Cw*08 were significantly more frequent in HIV seronegative individuals than in HIV seropositive individuals; where HLA-A*29 was significantly associated both with <10 000 RNA copies/ml viral loads and with CD4 counts >500. HLA- B*07 was significantly associated with viral loads >50 000 RNA copies/ml, and alleles B*53 and A*30 were significantly associated with low CD4 counts (<250), suggesting that these alleles may represent potential susceptibility variants. These are unique associations and underscore the different HLA distribution profile of disease in individuals from southern Africa.

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IMMUNOLOGICAL REACTIVITIES OF OPTIMAL SUBTYPE B-DERIVED CTL EPITOPES IN SUBTYPE C HIV-1 INFECTED INDIVIDUALS

Cytotoxic T lymphocytes (CTL) directed by class I HLA alleles, are considered important for killing virally infected cells and have been shown to be required for control of SIV infection.

Eliciting CTL is thus considered a requirement for an HIV vaccine and it is thought that specific HLA class I alleles within a given population may shape and direct the fine specificity of CTL epitope recognition. We tested a series of subtype B-derived optimal CTL epitopes for recognition by subtype C HIV-1 infected individuals. These epitopes are known to bind several HLA alleles belonging to various supertype families and have been predicted to cover >80% HLA alleles in a given population. Forty six peptides known to bind 5 HLA supertype families (A1, A2, A3, A24 and B7) spanning Pol, Gag, Env, Nef, Vif, Vpr and Rev HIV genomic regions were used in the study. Seventy-four subtype C HIV-1 infected individuals were randomly chosen without regard for HLA background and screened using the IFN-g ELISpot assay. We used a pool/ matrix approach to assess epitope-specific responses. Single epitope confirmations were done in some individuals and only responses above 100 sfu/106 PBMC were considered positive. Our results show that epitope-specific responses were observed in 35/74 (47%) individuals, where the bulk of responses were directed to Integrase. Of the 20 individuals who were HLA typed at high resolution, 50% responded to epitopes that did not conform to the HLA supertype family. Those individuals who did not recognize any of the tested subtype B optimal epitopes, responded to subtype C epitopes and epitopic regions in areas of the genome distal to the optimal epitopes. There were significant differences in the HLA supertype families between responders and non-responders for A2 (p=0.014), A3 (p=0.035) and B7 (p=0.0012). The observed responses between responders and non-responders could not be attributed solely to the differences in HLA supertype families but most likely to recognition and restriction by unpredicted HLA molecules. These data indicate that many subtype B-based CTL epitopes in our study, designed to bind five supertype families, do not conform to the expected supertype binding. This would suggest that a high degree of epitope degeneracy exists and that many subtype B-based epitopes are not recognized in natural subtype C HIV-1 infection. Our data indicate that a vaccine candidate based on CTL epitopes may yield unexpected or low responses in the target population.

ANALYSIS OF ANTIGEN SPECIFIC IFN-GAMMA-SECRETING T CELLS IN INDIVIDUALS INFECTED WITH MYCOBACTERIUM TUBERCULOSIS AND HIV-1

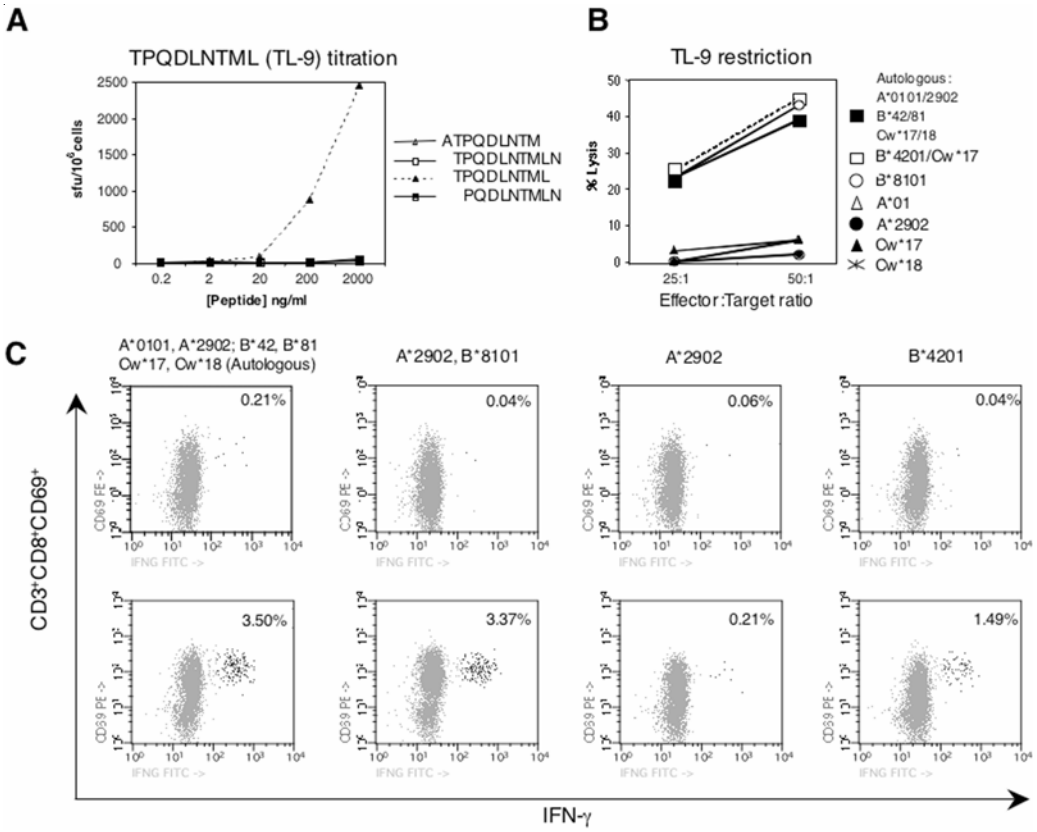
Worldwide an increased proportion of cases of MTB infection occur in individuals with HIV-1 infection. An understanding of T cell responses to both these intracellular pathogens is essential for the development of effective vaccines. We wished to quantify T cell responses to Esat-6, a secreted antigen specific for Mycobacterium tuberculosis (MTB), and absent from BCG, in dually MTB/HIV-1 infected and single MTB infected patients. We aimed to assess a) the magnitude of T cell responses to Esat-6: b) the impact of standard anti-MTB therapy on the frequency of antigen-specific T cells: c) whether responses are CD4 or CD8 T lymphocytes mediated and d) whether immunodominant epitopes were targeted in Esat-6. A series of 21 peptides were pooled into a matrix design and used as stimuli in the IFN-gamma ELISPOT assay. This was used to quantify the frequency of Esat-6-specific T cell responses. Twenty-six dually MTB/HIV-1 infected individuals, 19 single MTB infected and 10 control individuals were screened. The IFN- intracellular cytokine assay, using flow cytometry, was used to confirm whether responses were CD4 or CD8 mediated and the frequency of epitopic recognition within the Esat-6 protein. In the control group only 1/10 individuals had detectable Esat-6 specific T cell responses, whereas 15/19 and 24/26 of single MTB and dually MTB/HIV infected individuals, respectively, responded to Esat-6. No epitopic region was dominantly targeted within Esat-6, although the frequency of responses were significantly higher in the MTB group (p=0.027). The responses were mediated by a mixture of both CD4+ and CD8+ T cells and there was a progressive decline in the frequency of responses over 52 weeks of follow-up in both groups, where anti-MTB treatment was administered in the first 6 months. There was a highly significant decline (p=0.007) of anti-MTB specific responses over time in the dually MTB/HIV infected group, even after adjusting for viral load and CD4 counts. Our data indicate that the magnitude of T cells targeting Esat-6 were elevated during active MTB and decline with response to therapy, significantly so in the dually MTB/HIV-1 infected group. These data shed light on the dynamics of anti-MTB T cell responses to secretory proteins in the context of MTB disease resolution and antigenic stimulation in vivo. Loss of responses to Esat-6 may represent a mark of successful disease outcome with standard anti-TB treatment.

T CELL RECEPTOR (TCR) REPERTOIRE SKEWING AS MEASURED BY SPECTRATYPING IN HIV-1 UNINFECTED NEONATES BORN TO HIV-1 INFECTED MOTHERS

Mother-to-child transmission is one of the most common forms of HIV-1 infection in South Africa, occurring either *in utero*, during birth or through breastfeeding. Primary infection with HIV-1 in adults frequently results in expansion and subsequent exhaustion of antigen-specific T cells. These disruptions can be measured by investigating T cell Receptor (TCR) variable beta (Vb) families used by both CD4+ and CD8+ T cells. To extend our adult studies, we examined the TCR profiles in cord blood of HIV-1 uninfected neonates born to HIV-1 infected mothers. Our hypothesis is that exposure of HIV-1 antigens *in utero* can cause priming of neonatal T cells. Twelve (from a total of 24) HIV+ mothers, 18 (from a total of 24) HIV-1 negative neonates and 10 (from a total of 41) HIV-1 negative and unexposed control cord blood samples have been analyzed to date. The samples were recruited from mothers and babies attending Coronation Women's and Children's Hospital, Johannesburg. Spectratyping was used to analyse CDR-3 length variations after amplification of Vb TCR cDNA corresponding to 23 Vb families in paired cord blood and maternal PBMC, CD4+ and CD8+ T cells. TCR profiles were classified as either Gaussian (no antigen engagement), polyclonal skew (antigen engagement) or oligoclonal skew (clonally expanded T cells). The TCR profile observed from 18 HIV-1 negative babies born to HIV-1 positive mothers showed a 5% mean Gaussian distribution; 68% polyclonal skewing and 27% oligoclonal skewing. Ten control cord blood samples showed a mean of 18% Gaussian distribution, 79% polyclonal skewing and 3% oligoclonal skewing. There was 0% Gaussian distribution in HIV-1 positive mothers, where 35% was polyclonal and 65% was oligoclonal. The relatively lower Gaussian distribution and higher frequencies of polyclonal and oligoclonal skewing of the TCR repertoire observed in uninfected neonates born to HIV-1 positive mothers is suggestive of antigen priming in utero. The same degree of skewing was not observed in cord blood samples from HIV-1 uninfected babies born to HIV-1 negative control mothers. These data indicate that there was selective use of the TCR in utero, and in some cases the oligoclonal nature of the TCR repertoire indicates expanded T cell populations in the absence of overt HIV infection. We are currently investigating the causal relationship between HIV antigen exposure and TCR skewing.

APPLICATION OF THE INTRACELLULAR CYTOKINE STAINING ASSAY FOR DETECTION OF ANTIGEN SPECIFIC INTRACELLULAR CYTOKINES

The generation and maintenance of immunity to most viral antigens involves a complex balance of humoral and cellular immunity. Quantitation of T cell responses is a focus of interest in our understanding of what constitutes protective immunity to HIV-1 infection. Intracellular cytokine staining (ICS) combines cell phenotype with the functional readout of cytokine production and is used to characterize and quantify antigen-specific T cells. We have used the ICS assay to identify CD4+ or CD8+ T cell populations recognizing Gag specific responses from subtype C HIV-1 infected individuals. We have also used a modified assay to identify the HLA restricting allele of optimal epitopes within a peptide stretch. Intracellular IFN-gamma, was detected following a 6 hour in vitro stimulation of cryopreserved PBMC samples. 1 x 10^6 PBMC were incubated with HIV-1 Gag derived peptide pools, anti-CD28 and anti-CD49d monoclonal antibodies at 37 ºC and 5 % CO_2 for 1 hour. Brefeldin A, a secretion inhibitor that enhances the accumulation of intracellular cytokine, was added after 1 hour and cells incubated for a further 5 hours. Cultured cells were stored at 4 ºC overnight and analyzed the next morning. After washing, cells were permeabilized and stained with monoclonal antibodies against CD8 (PerCP), CD3 (APC), CD69 (PE) and IFN-gamma (FITC) for 30 minutes at room temperature in the dark. Cells were washed, fixed and acquired on a four color flow cytometer. Analysis for IFN-gamma positive cells was performed using Paint-A-Gate and 15 HIV-1 clade C infected individuals were screened for HIV-1 Gag peptide pool responses. For HLA restriction, B cells expressing a single matched HLA allele with autologous B cells were pulsed with HIV-1 Gag peptide for 1 hour and the B cells were then mixed with antigen specific cells (effectors) and incubated with CD28/CD48d monoclonal antibodies and the assay continued as described for ICS. An example of an ICS HLA restriction is shown for the epitope TL-9, restricted by both HLA-B*42 and B*81:



Fine epitope mapping of the TL-9 epitope (A) and HLA restrictions using the ^{51}Cr release assay (B) and intracellular cytokine staining (C). HLA restriction from bulk cultured cells shows that HLA-B*42 and -B*81 are the restricting alleles for TL-9 in the same individual. This was confirmed using a direct *ex vivo* ICS assay showing the response to be CD3+CD8+ T cell mediated. IFN-γ positive cells were gated from the CD3+/CD8+/CD69+ T cell population and 50 000 gated CD3+ events were counted. The HLA class I types of the partially mismatched targets in Fig 4B: (□) A*2301/2601, B*0801/8101, Cw*04/07; (○) A*30/34, B*4201/4501, Cw*06/17; (△) A*01/11, B*07/52, Cw*07/12; (●) A*2902/8001, B*4901/5801, Cw*07/-; (▲) A*3201/68011, B*4101/5802, Cw*06/17; (✶) A*3002/-, B*45/57, Cw*16/18 (matching HLA alleles are shown in boldface).

NOVEL AND PROMISCUOUS CYTOTOXIC T LYMPHOCYTE EPITOPES IN CONSERVED REGIONS OF GAG TARGETED BY INDIVIDUALS WITH EARLY SUBTYPE C HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 INFECTION FROM SOUTHERN AFRICA

Characterization of optimal CTL epitopes in Gag can provide crucial information for evaluation of candidate vaccines in populations at the epicenter of the HIV-1 epidemic. We screened 38 individuals with recent subtype C HIV-1 infection using overlapping consensus C Gag peptides and hypothesized that unique HLA restricting alleles in the southern African population would determine novel epitope identity. Seventy-four percent of individuals recognized at least one Gag peptide pool. Ten epitopic regions were identified across p17, p24 and p2p7p1p6 and greater than two-thirds of targeted regions were directed at: TGTEELRSLYNTVATLY (p17, 35%); GPKEPFRDYVDRFFKTLRAEQATQDV (p24, 19%) and RGGKLDKWEKIRLRPGGKKHYMLKHL (p17, 15%). After alignment of these epitopic regions with consensus M and a consensus subtype C sequence from the cohort, it was evident that the regions targeted were highly conserved. Fine epitope mapping revealed that 5/9 identified optimal Gag epitopes were novel: HLVWASREL, LVWASRELERF, LYNTVATLY, PFRDYVDRFF, TLRAEQATQD and were restricted by unique HLA-Cw*08, -A*30/B*57, -A*29/B*44 and –Cw*03 alleles, respectively. Notably, three of the mapped epitopes were restricted by more than one HLA allele. Although these epitopes were novel and restricted by unique HLA, they overlapped or were embedded within previously described CTL epitopes from subtype B HIV-1 infection. 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 universal recognition of Gag in highly conserved domains.

CONFERENCES/MEETINGS 2004

A/Prof Lynn Morris

4 – 7 February 2004:
Stanford University : San Francisco, USA
HIV RestNet Meeting; and
Enterprise Laboratory Working Group Meeting on Lab Standardisation
8 – 11 February 2004:
San Francisco, USA
11th Conference on Retroviruses and Opportunistic Infections ((CROI)
24 – 26 March 2004:
Bangalore, India
The Wellcome Trust International Fellows Meeting
11 – 16 July 2004:
Bangkok, Thailand
XV International AIDS Conference
1 – 2 October 2004:
University of KwaZulu-Natal, Durban
HIV Pathogenesis Programme (HPP)
13 – 15 October 2004:
Seattle, USA
HIV Vaccine Trials Network (HVTN) Full Group Meeting
18 – 19 October 2004:
Atlanta, USA
Meeting with Walid Heneine, Chief: Drug Resistance and Retroviral Zoonoses Laboratory, Centers for Disease Control and Prevention (CDC) to discuss in detail expanding collaborative studies on drug resistance emergence following intrapartum single dose nevirapine interventions.
26 October 2004:
Johannesburg
Speaker at the Swedish-South African Medical & Health Care Dialogue Conference-Workshop.
"Ensuring Success of the HIV Treatment Program by Restricting the Development of Anti-Retroviral Drug Resistance".
8 – 11 November 2004:
Vienna, Austria
International Atomic Energy Agency (IAEA)
Project Coordination Meeting on Initiatives in HIV-1 Molecular Epidemiology and Immunology in Support of the UNAIDS-WHO African AIDS Vaccine Programme.
30 November – 8 December 2004
Cape Town
Co-organiser of the Wellcome Trust/EMBO Workshop on HIV/AIDS and TB involving over 50 participants from Africa and elsewhere.

Prof Clive M Gray

30 August - 1 September 2004:
Lausanne, Switzerland
AIDS Vaccine 2004

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M Ramela, Admin Clerk

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