National Institute for Communicable diseases

of the National Health Laboratory Service

Annual Report 2002

National Institute for Communicable Diseases

of the National Health Laboratory Service

ANNUAL REPORT 2002

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Contents

Director's Report

This publication is the first annual report of the newly established NICD. The NICD is constituted from 2 predecessor institutions – the National Institute for Virology (NIV) for the virology component, and the public health microbiology laboratories of the South African Institute for Medical Research (SAIMR) for the microbiology component. During 2002 these two divisions of the NICD functioned independently of each other, largely as a result of the geographical separation – the microbiology laboratories were still housed in the ex-SAIMR headquarters in Braamfontein (near downtown Johannesburg) and had not yet moved physically to the NICD campus in Rietfontein (on the north-eastern outskirts of Johannesburg). Construction for the new laboratories to accommodate the microbiology division of the NICD is expected to commence in mid-2003. The establishment of a third division of the Institute, the Epidemiology division, is expected sometime in 2003 and this will then complete the envisaged structure of the NICD.

This being the first annual report of the NICD it is as well to again record the mission and the responsibilities of the NICD:-

Essentially, the mission of the NICD is to be a resource of knowledge and expertise in regionally relevant communicable diseases to the South African Government, to SADC countries and to the African continent at large, in order to assist in the planning of policies and programmes and to support appropriate responses to communicable disease issues.

The responsibilities of the NICD can be broken down into six major categories. Firstly, it will function as the national organ for gathering data on communicable diseases, continuously, reactively in response to events such as outbreaks, and also proactively to anticipate outbreaks. To support this responsibility both active surveillance programmes as well as passive interrogation of existing databases will be utilized. Secondly, intelligence on communicable diseases will be communicated regularly, as well as urgently in crisis situations, to the Department of Health, relevant public health institutions and the medical and general public by means of publications and electronic media. Thirdly, in respect of outbreaks, the NICD will provide specialized consultancy services as well as laboratory support as and when needed by provincial and local authorities. Fourthly, the NICD will need to function as an academic facility on a national basis. In other words it will provide training in appropriate laboratory technology as well as epidemiology and surveillance, to all institutions of higher learning throughout the country. It will seek collaborations and affiliations to all South African universities for purposes of lecturing, training, study visits by graduate students as well as providing research opportunities to students from all the country's universities. Fifthly, the NICD will aim to be a centre of scientific excellence in the field of communicable diseases by pursuing research programmes of a high international standard, and will also look to establish links with international organizations in this field. The orientation of research will, however, be geared to questions of public health relevance rather than, for example, those relating primarily to mechanisms of disease or clinical management of patients which belong more to the academic university departments of microbiology and virology. Lastly, the NICD will function as the national quality assurance body for the NHLS microbiology laboratories. It will also offer QA services to African countries and, if required, also to the private sector.

The NICD has inherited a long and very proud legacy of excellence in the field of public health communicable diseases from both of its predecessor organisations - the SAIMR and the NIV. The contents of this report will, I hope, clearly reflect how the present generation of scientists, pathologists and technologists have not only perpetuated this tradition, but have, in addition, greatly enhanced the international status of the Institute. As a major public health laboratory on

Director's Report

the African continent, the NICD plays a key role globally as a strategic sentinel institution for the emergence of new infectious diseases. It has contributed very significantly in providing diagnostic laboratory support in assisting with outbreak control and in conducting training courses and capacity building for laboratories on the African continent.

The Institute has made important contributions to the research literature, in particular in addressing regional communicable diseases problems. With respect to HIV/AIDS, the most important communicable disease on the African continent, NICD is a major component of the South African AIDS Vaccine Initiative (SAAVI) and also international programmes on HIV vaccine development such as the National Institute of Health HVTN (Vaccines trials network).

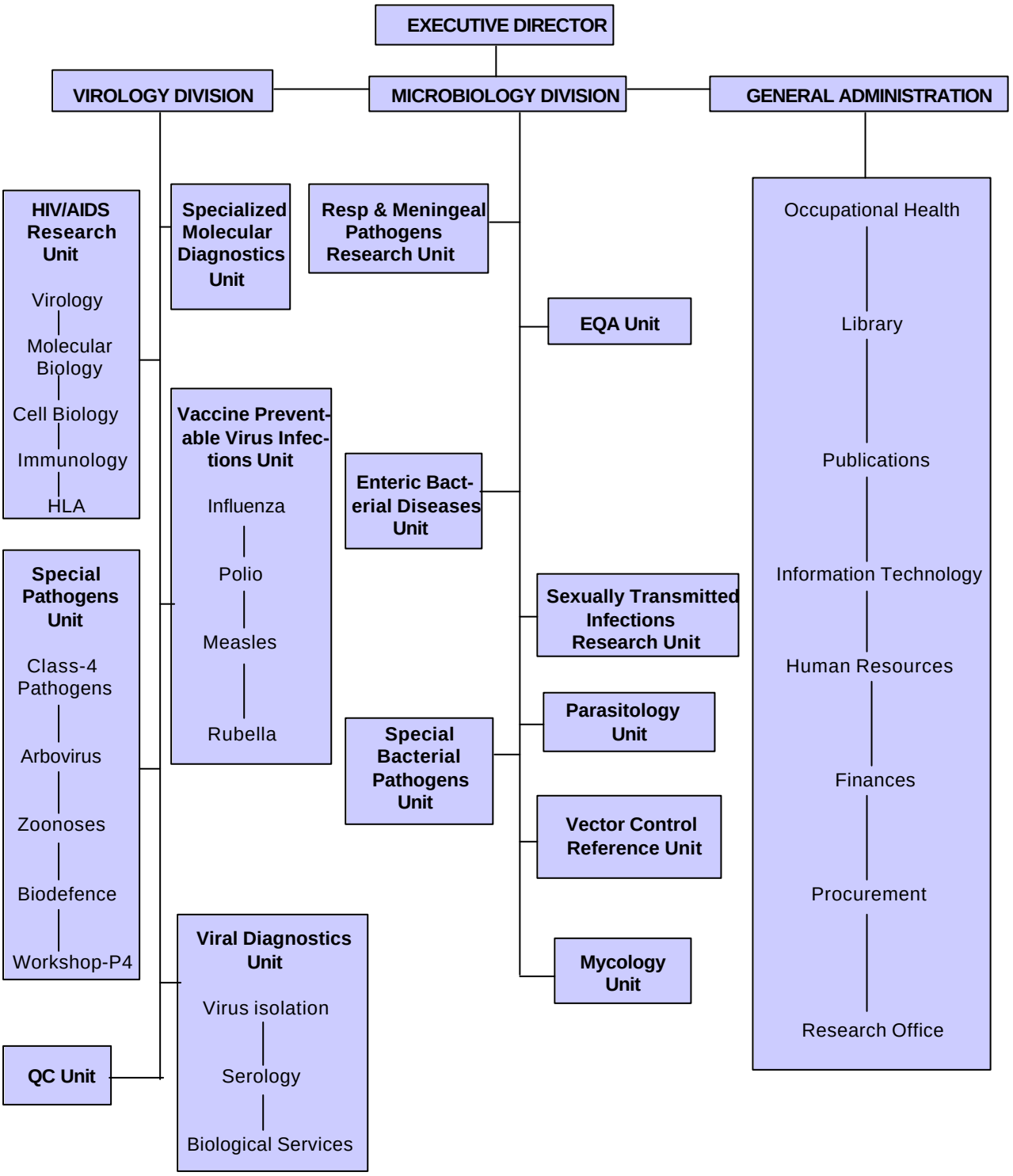
The Institute is well equipped with the most modern up-to-date technologies and infra-structural support. However, its most important asset is its human resources. The personnel of the Institute have shown exceptional dedication under conditions, which during 2002 were not always ideal. With this in mind I would like to take this opportunity of expressing my sincerest thanks for all the dedication, enthusiasm and loyalty of all our staff who have helped build this institution. I would also like to thank all those who contributed towards the production of this report - to Dr Peter Jupp for the photography and to Mrs Liz Millington for the typesetting and production.

The year 2003 will see a consolidation of the Institute, which will enable it to even more effectively meet its national and international obligations.

BARRY D SCHOUB
EXECUTIVE DIRECTOR

PERSONNEL

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES - ORGANOGRAM, 2002



PERSONNEL

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES - STAFF LIST, 2002

EXECUTIVE DIRECTOR
Prof B D Schoub
I Latsky, Personal Assistant

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

Virology Laboratory
Prof L Morris, Chief Specialist Scientist, Head
C Chezzi, Post-doc/Consultant
C Pillay, Medical Natural Scientist
N Taylor, Research Assistant
T Cilliers, Research Assistant
S Herrmann, Personal Assistant
S Cohen, Laboratory Manager
E Gray, Research Assistant
S Loubser, Research Assistant
M Ntsala, Research Assistant
M P Phoswa, Chief Aux Service Officer

Molecular Biology Laboratory
Dr M Papathanasopoulos, Asst Dir, Medical Natural Scientist, Head
T Patience, Research Assistant

Immunology Laboratory
Dr C Gray, Princ Specialist Scientist, Head
S Nyoka, Chief Medical Technologist
O Bolten, Admin Manager
Dr A Masemola, Post Doctoral Fellow
Dr V Morato, Post Doctoral Fellow
G Khoury, Laboratory Manager
T Mashishi, Research Assistant
P Sangweni, Research Assistant
H Maila, Research Assistant
P Mohube, Research Assistant
M Rampou, Research Assistant
P Mokgotho, Research Assistant
K Mafhandu, Data Capturer

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

HLA Laboratory
Dr A Puren, Deputy Director, Head
M Paximadis, Postdoctoral Fellow
J Mathebula, Research Assistant

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

QA UNIT
E Bowers, Control Medical Technologist

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

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

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

SPECIALIZED MOLECULAR DIAGNOSTICS UNIT
Dr A Puren, Deputy Director, Medical Services
S M Bowyer, Principal Medical Natural Scientist
E Cutler, Medical Scientist
M P Magooa, Medical Natural Scientist
A N Phungwayo, Medical Natural Scientist
E Tlale, Senior Medical Technologist
M J Sibeko, Chief Aux Service Officer
L J Mashiloane, Chief Aux Service Officer

Personnel

J L Sekgobela, Laboratory Assistant
P A Kgadima, Laboratory Assistant

VACCINE PREVENTABLE VIRUS INFECTIONS UNIT

Dr T G Besselaar, Senior Specialist Scientist
S B Smit, Principal Medical Natural Scientist
L Botha, Biotechnologist
H N Gumede, Senior Medical Natural Scientist
S Donninger, Medical Scientist
V Singh, Technologist
M Nyuswa, Technologist
J K Mokoena, Chief Aux Service Officer

Graduate Student:	***(Supervisor)***
L Achenbach, MSc	(M Venter)

VIRAL DIAGNOSTIC UNIT

Diagnostic Unit

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

Virus Isolation Unit

A Buys, P/T Chief Medical Technologist
M Morgan, Chief Medical Technologist
A Oliver, Senior Medical Technologist
S Sikhosana, Senior Medical Technologist
B Masuku, Medical Technologist
D Labambo, Chief Aux Service Officer
A Matseke, Chief Aux Service Officer
T Mashaba, Chief Aux Service Officer
L Harris, Chief Aux Service Officer

Serology Unit

E Goetsch, Chief Medical Technologist
S Moodliar, Chief Medical Technologist
M Mashele, Senior Medical Techologist
T Lietsiso, Medical Technologist
L P Mncube, Medical Technologist
F Ntinkinca, Medical Technologist
H A Vilakazi, Student Medical Technologist

Biological Services Unit

Media

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

Reagents

M Vandecar, Chief Medical Technologist

Cell Culture

A Mohlala, Senior Medical Technologist
A Sehata, Laboratory Assistant

Animal Section

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

Diagnostic Laboratory Support

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

EM/Support Services Unit

Specimen Laboratory

L M Cranston, Chief Medical Technologist
J C Franz, Chief Medical Technologist
S Hloma, Principal Aux Service Officer

Admin Office

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

EM

N Prabdial-Sing, Principal Medical Natural Scientist
B Miller, Chief Medical Technologist

RESPIRATORY & MENINGEAL PATHOGENS UNIT

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

Dr S Madhi, Clinical Director/Consultant
Dr A Smith, Senior Medical Scientist
Dr M du Plessis, Senior Medical Scientist
Dr A von Gottberg, Senior Pathologist
Dr V Quan, Medical Officer
Dr C Cutland, Research Doctor
Dr H Gani, Research Doctor
H Ludewick, Medical Scientist

Personnel

L de Gouveia, Laboratory Manager
A Wasas, Section Supervisor
T Rafundisani, Section Supervisor
N van Niekerk, Medical Technologist
C Ndou, Laboratory Technologist
Sr J Appolis, Research Sister
Sr S Fourie, Study Co-ordinator
P Diniso, Nursing Assistant
S Mmolawa, Research Nurse
G Senne, Nursing Assistant
E Mudau, Research Assistant
F Butler, Data Capturer
D Pillay, Data Capturer

Graduate Student
G Coulson, MSc student

ENTERIC BACTERIAL DISEASES UNIT
Dr K Keddy, Senior Pathologist

Cholera
L Arntzen, Laboratory Manager
J Mathebula, Student Technician

Salmonella, Shigella & E. coli
A Sooka, Technologist
T Kruger, Scientist
E Khumalo, Student Technician

MICROBIOLOGY EQA UNIT
Dr K McCarthy, Pathologist
V Fensham, Technologist

PARASITOLOGY UNIT
Dr J Frean, Principal Pathologist
L Dini, Medical Scientist
J Mathebula, Student Technician

SPECIAL BACTERIAL PATHOGENS UNIT
Dr J Frean, Principal Pathologist

Plague/Anthrax
L Arntzen, Laboratory Manager

MYCOLOGY UNIT
S Gould, Technologist

SEXUALLY TRANSMITTED INFECTIONS REFERENCE UNIT
Prof H J Koornhof, Acting Head
E E M Goliath, Department Secretary

S O Odugwu, Senior Medical Officer
J M Wright, Project Co-ordinator
M F Radebe, Laboratory Manager
D V Maseko, Acting Laboratory Supervisor
R R Mogoboya, Medical Technologist
J F Oba, Epidemiology/Surveillance Officer
S Khumalo, Lab Asst, Student Technician
D Mabaso, Lab Asst, Student Technician
R Chonco, Production Assistant
M N Manuel, Project Assistant
J L Mekgwe, Clinic/Projects Assistant
F A Mngomezulu, Data Input Clerk

VECTOR CONTROL REFERENCE UNIT
Prof M Coetzee, Head
M Martheze, Department Secretary

Dr L L Koekemoer, Senior Medical Scientist
Dr B D Brooke, Senior Medical Scientist
M M Weeto, Medical Scientist
H Mafumo, Research Assistant
E Rankoe, Lab Asst, Studen Technician)
E Motaung, Lab Asst, Student Technician.
Prof R H Hunt, Hon Researcher
J Segerman, Hon Researcher

GENERAL ADMINISTRATION DIVISION
J Angelides, Assistant Director

Occupational Health
Sr I Henley, Chief Professional Nurse

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

Publications
E Millington, Publications Officer
Sr J M McAnerney, Chief Professional Nurse

Information Technology
K Stead, IT Manager

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

Personnel

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

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

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

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

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

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

Workshop
N Koegelenberg, Artisan Foreman
D Mintoor, Tradesman Aid I
M Ramadiro, Tradesman Aid II
E Velem, Tradesman Aid II

Boiler House
M Basson, Senior Operator
R Engelbrecht, senior Operator
P Mokoena, General Stores Assistant

Cleaning
S Mngomezulu, Senior General Foreman
M D Mphela, Cleaner II
M J Moyana, Cleaner II
M E Langrish, Cleaner II
W M Marapjane, Cleaner II
E N Malatsi, Cleaner II
S M Nhlapo, Cleaner II
M J Sape, Cleaner II
N H Selomo, Cleaner II
D Tigedi, Cleaner II
M R Mohlafase, Groundsman I
M P Makgohlo, Groundsman II
E M Mothobeki, Groundsman I
A Chuene, Groundsman II
M Letlhabi, Tradesman Aid II
M B Ramatswi, General Stores Assistant II

Microbiology Laboratories

NICD MICROBIOLOGY LABORATORIES

This section of the NICD comprises the Entomology Unit and the reference microbiology laboratories and staff currently located at the central campus of the NHLS, namely:-

Enteric Diseases Unit (EDU)
Special Bacterial Pathogens Unit (SBPU)
Parasitology Unit (PU)
Mycology Unit (MU)
Respiratory and Meningeal Pathogens Research Unit (RMPRU)
Sexually Transmitted Infections Research Centre (STIRC)

INTRODUCTION

In 2002 the units that previously were mainly concerned with diagnostic work have generally been reorienting themselves to more fully carry out surveillance, reference and research roles, to align themselves with the main thrust of the NICD. This process has mainly involved EDU, SBPU, PU, and MU and has been constrained by the restricted number of staff. An important aspect of reference laboratory functions which is receiving increasing attention is provision of external quality assessment (EQA) services, both inside and outside the NHLS. Regarding the latter, the NHLS was contracted in 2002 by the World Health Organization to provide EQA programmes for African countries in enteric and meningitis pathogens and plague.

DR JOHN FREAN
PRINCIPAL PATHOLOGIST

Microbiology - *Enteric Diseases Unit*

Research projects in the Enteric Disease Unit are funded by grants from the Centres for Disease Control and Prevention, USA, the Medical Research Council, the NHLS/ University of the Witwatersrand Grants Committee and the Department of Health, Pretoria.

CURRENT RESEARCH PROJECTS

✍ National surveillance for isolation of bacterial enteric pathogens.-
✍ Evaluation of *Vibrio cholerae* O1 strains from the current cholera epidemic in KwaZulu-Natal and surrounding provinces.
✍ Detection of South African strains of enterovirulent *Escherichia coli* using multiplex polymerase chain reaction
✍ Molecular characterization of a multidrug resistant *Salmonella enterica* subspecies *enterica* serotype Isangi causing nosocomial infections in South Africa.
✍ Enhancement of surveillance for trimethoprim-sulfamethoxazole resistant invasive respiratory and diarrhoeal disease in South Africa

SURVEILLANCE ACTIVITIES

✍ South African representative at Enter-net – International surveillance for Enteric Pathogens.
✍ NHLS representative for diarrhoeal diseases to Biannual Outbreak Response Meetings of the Department of Health.
✍ Co-ordinator of Salm-Surv – WHO initiated surveillance for *Salmonella spp.*
✍ NHLS laboratory advisor to local departments of health and National Department of Health on *Vibrio cholerae.*

TRAINING & QUALITY ASSURANCE ACTIVITIES

✍ Co-ordinator (with QA Department, RMPRU and SBPU) for external quality asssurance for AFRO region funded by WHO.
✍ Laboratory identification of *Vibrio cholerae* and *Shigella dysenteriae* type 1 – technologist staff (all provinces), NHLS, 2-3 October 2002.

INTERNATIONAL MEETINGS ATTENDED

✍ KH Keddy: 5th Annual Enter-net Workshop, Athens, January/February 2002.
✍✍ T Kruger: Strategic Planning meeting on Salmonella, Centers for Disease Control, Atlanta, Georgia, March 2002.

Microbiology Laboratories

Staff of the Enteric Diseases Unit.

Staff of the Medical Entomology Unit.

INTRODUCTION

The Medical Entomology Department focuses mainly on malaria vector mosquitoes, being the major vector-borne disease in southern Africa, and indeed, throughout Africa. The Department houses a comprehensive collection of live mosquito colonies of the three most important vector species in Africa, including a colony of *Anopheles funestus* that is the only one in existence in the world. This places the Department in a unique position to carry out valuable operational investigations on insecticide resistance and to play a role in influencing policy decisions on vector control strategies in the region. In addition, the Department houses the largest museum collection of African arthropods of medical importance in Africa, the third largest such collection in the world.

RESEARCH

Insecticide Resistance

Anopheles funestus

Pyrethroid insecticide resistance in the major malaria vector mosquito *Anopheles gambiae* is now known to be widespread in West Africa. It also occurs in the same species in Kenya and in *Anopheles funestus* in southern Africa. This group of insecticides is currently recommended for disease control in many malarious countries for indoor residual spraying and is the only group of insecticides that can be used for treatment of bed nets. The South African malaria control programmes changed from the use of DDT to pyrethroids for their house spraying in 1996. Field and laboratory work carried out in northern Kwazulu/Natal and Mpumalanga have shown that the malaria vector species *An. funestus* can once again be found in these provinces and that it is resistant to pyrethroids. *Anopheles funestus* was eradicated from South Africa in the 1950's when malaria control became a national Department of Health programme and DDT was used for house spraying in all affected areas. The switch to pyrethroid insecticides in 1996 coincided with a series of good rainy seasons and a marked increase in the annual number of malaria cases, peaking at just under 65,000 in 2000 (Figure 1). In collaboration with the Kwazulu/Natal malaria control programme entomologist, we demonstrated that *An. funestus* could be found inside sprayed houses, was responsible for malaria transmission in the area (infectivity rates of >5%) and was resistant to permethrin but susceptible to DDT. As a result, the Kwazulu/Natal control programme implemented an emergency DDT spraying campaign targeting high risk areas at the end of 2000. Figure 1 shows the annual malaria case figures for South Africa (sourced from the Department of Health) and the dramatic decrease in malaria transmission since the reintroduction of DDT house spraying in 2001. During 2000 we also demonstrated that the large populations of *An. funestus* in southern Mozambique exhibited the same resistant patterns to pyrethroids, with evidence of cross-resistance to the carbamate propoxur. Since no malaria control had been carried out in southern Mozambique for decades before 2000, it is likely that the resistance arose through the agricultural use of pyrethroids in Mozambique, rather than through the malaria control activities in northern Kwazulu/Natal.

Currently, a resistant strain of *An. funestus* is being selected on 1.5% permethrin (double the WHO discriminating dosage to diagnose resistance) in the laboratory. This, together with a susceptible strain from Angola, will be used to investigate the P450 monooxygenases known to be involved in the resistance using molecular techniques. Collaboration with the Liverpool School of Tropical Medicine, UK, and Notre Dame University, USA, is providing us with the opportunity of utilizing the newly published *Anopheles gambiae* genome sequence to further our knowledge on resistance in *An. funestus*.

Anopheles arabiensis

Investigations carried out in collaboration with the Kwazulu/Natal malaria control programme entomologist revealed the presence of DDT resistance in *Anopheles arabiensis* near the town of Jozini. A rice-growing scheme in the area has resulted in breeding places being available throughout the year because of the draining of paddy fields onto the Makhatini Flats. Large populations of *An. arabiensis* were found and, in particular, collected in window exit traps in houses sprayed with DDT. WHO insecticide susceptibility tests showed 20% resistance to DDT, but 100% susceptibility to the pyrethroid which is sprayed in 'western'-style houses. This is diametrically opposite to *An. funestus* which is resistant to pyrethroids but susceptible to DDT. It poses a problem to the control programme because *An. arabiensis*, unlike *An. funestus*, is not an obligatory house-resting species and will

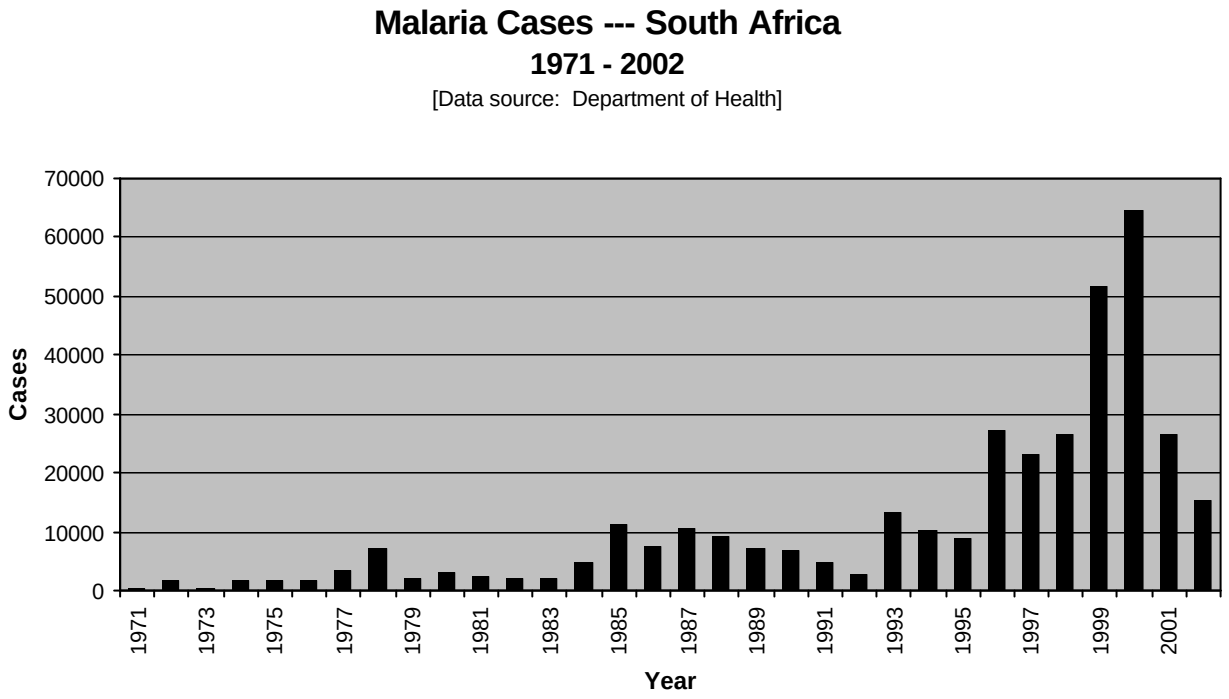


Fig 1.

frequently seek shelter outside man-made structures. Research into the resistance mechanisms involved in the DDT resistance is ongoing and may provide information on alternative chemicals that can be used.

MOLECULAR AND CYTOGENETIC STUDIES

Malaria vectors in West Africa

Collaboration with French researchers in West Africa has resulted in cytogenetic studies being carried out on two projects: association of insecticide resistance with inversion polymorphisms in *Anopheles gambiae* in Cote d'Ivoire; and species-specific polytene chromosome banding arrangements of *Anopheles nili* and *An. moucheti*, two anophelines that play an important local role in malaria transmission in the absence of the major vectors in Cameroun. Results from the first project has shown that dieldrin resistance in *An. gambiae* is linked to a common inversion on chromosome arm 2L, providing an explanation for the continued existence of dieldrin resistance in populations that have not been exposed to this insecticide for over 50 years.

The second project provided data on genetic polymorphisms in *An. nili* and *An. moucheti* using single-strand conformation polymorphism PCR. The results show distinct differences between populations of *An. nili*, indicating population structuring in this species, but not in *An. moucheti.*

Anopheles funestus

One of the three major vectors of malaria in Africa, *Anopheles funestus* belongs to a group of nine species that are morphologically very similar. Historically, the identification of the seven southern African species depended on morphological characters found in the immature stages of three, and overlapping adult characteristics for the other four species. Since only *An. funestus* is recognised as a major vector of malaria parasites, identification of these species is essential for malaria control programmes. Initial molecular studies were able to discriminate between three of the species occurring in South Africa using the restriction enzyme HpaII, and subsequently, using single-strand conformation polymorphisms (SSCP), an assay was developed that proved to be effective in differentiating between four members of the *An. funestus* group: *An. funestus, An. vaneedeni, An. rivulorum* and *An. leesoni*. However, when a fifth member of the group was collected recently and included in the analyses, overlap between it and *An. vaneedeni* occurred. As a result, a multiplex ITS-2 DNA-PCR assay for distinguishing between all the species has been developed and has proved to be reliable and cost-effective (Figure 2). This assay is now being used in many

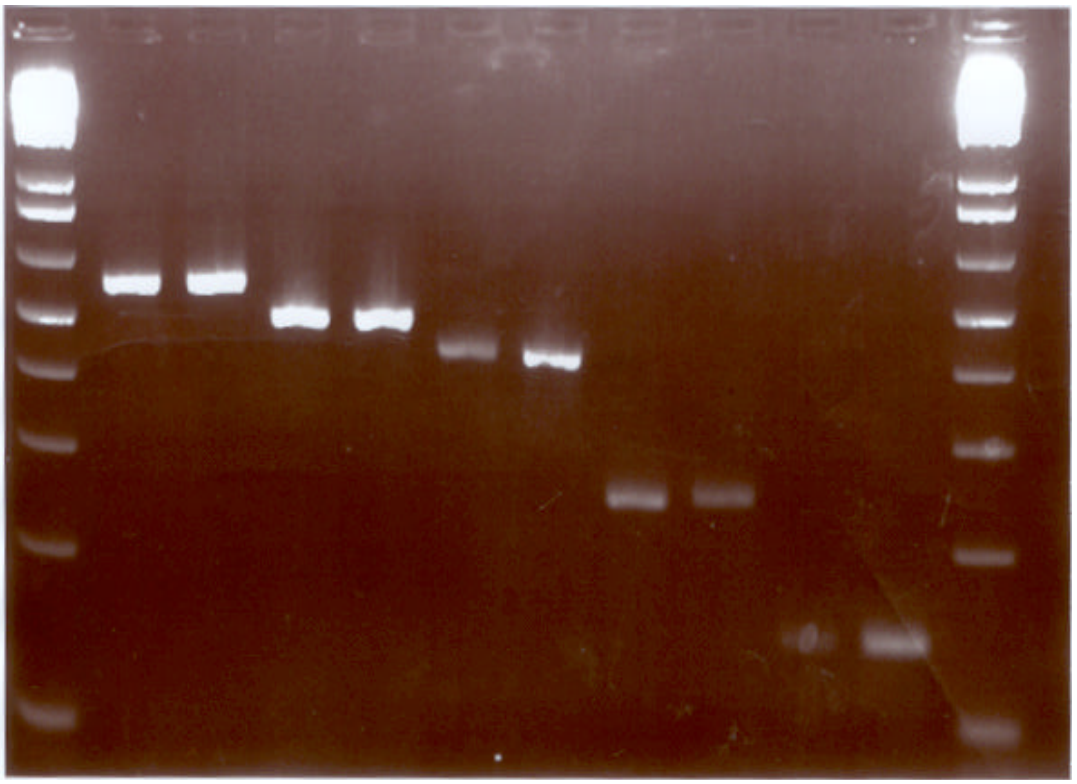


Fig 2. Species specific rDNA-PCR for identification of the *Anopheles funestus* group. Lanes 1 and 12 - 1 Kb DNA ladder; lanes 2 and 3 - *An. vaneedeni*; lanes 4 and 5 - *An. funestus*; lanes 6 and 7 - *An. rivulorum*; lanes 8 and 9 - *An. parensis*; lanes 10 and 11 - *An. leesoni.*

laboratories throughout Africa that already use molecular tools for the identification of members of the *An. gambiae* complex.

Cytogenetic studies of *An. funestus* in Kenya have revealed geographic differentiation between populations from West and Coastal Kenya based on chromosomal inversion polymorphisms. The data suggest that the populations are discontinuous, separated by the Rift Valley system.

Preliminary studies using RFLP and SSCP to compare the African *An. funestus* group of species with the Asian *An. minimus* group show that some African species are very closely related to Asian species. This supports earlier studies using cytogenetic banding sequences that showed one African species, *An. leesoni*, to be more closely related to *An. minimus* than to its African relatives.

DISTRIBUTION AND BIONOMICS

Mosquitoes of the Limpopo Province

A capacity-building project funded by the Wellcome Trust was initiated between the Department and the University of the North. The aim of this project is to collect anopheline mosquitoes from four sentinel sites in the Northern Province malarious area, identify them to species, establish their biting behaviour and their role in malaria transmission. Historical data from the malaria control programme will be analysed and compared with current findings. Technological and molecular skills have been transferred to the University of the North and one research assistant and one post-graduate student have been trained. Results have been presented at international congresses and one publication is in preparation.

Mosquitoes of Mpumalanga Province

The biting behaviour, distribution and role in malaria transmission of *Anopheles merus*, a saltwater breeder of the *An. gambiae* complex, in Mpumalanga is being studied. This species, previously rarely found in the Province, is now widespread and its role in malaria transmission needs to be determined. Larvae have been collected in areas associated with intense agricultural development, which may reflect

increased levels of agro-chemicals in the soil and thus increased salinity in the water, resulting in suitable breeding habitats for this species. While *An. merus* is not known to transmit malaria in South Africa, it is a very efficient vector in coastal areas of Tanzania.

The WHO/AFRO Multicentre Study

This was a major focus of research for 2002, involving 5 southern African countries: Namibia, Botswana, Zimbabwe, Swaziland and South Africa. Each country carried out field studies on *An. arabiensis* using the same sampling methods and WHO insecticide susceptibility tests to provide information to their malaria control programmes on vector distribution, species composition and insecticide susceptibility. The study has shown that populations of *An. arabiensis* in Namibia, Botswana and Swaziland are fully susceptible to DDT and pyrethroids. In Zimbabwe and South Africa, small foci of resistance to DDT have been found and these are being monitored. Further studies on the population structure of southern African *An. arabiensis* are being conducted using DNA microsatellite markers.

INTERNATIONAL RESEARCH COLLABORATORS

Prof J Hemingway, Director, Liverpool School of Tropical Medicine, UK
Dr H Ranson, Liverpool School of Tropical Medicine, UK
Prof D Norris, Johns Hopkins University, USA
Prof G Lanzaro, University of California, Davis, USA
Prof N Besansky, University of Notre Dame, USA
Prof F Collins, University of Notre Dame, USA
Dr M Benedict, CDC, USA and IAEA, Austria
Dr S Manguin, IRD, France
Dr D Fontenille, IRD, France

RESEARCH FUNDING FROM EXTERNAL GRANTING AGENCIES

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

TRAINING

Postgraduate Training

Departmental staff provide the medical entomology component of the Diploma in Tropical Medicine & Hygiene course run by the School of Pathology, University of the Witwatersrand. Lectures and practical demonstrations are given covering all entomological aspects of arthropod-borne diseases and arthropods of medical importance.

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

Short course in basic mosquito identification and insectary management

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

Staff Training

Dr Basil Brooke spent one month in the Entomology Branch of the Division of Parasitic Diseases at CDC, Atlanta, USA. Areas of possible collaboration were identified with respect to new technology available at CDC.

DIAGNOSTIC SERVICES

The Department of Medical Entomology 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 specimens of *Anopheles funestus* from northern Kwazulu/Natal and Mpumalanga, for species identification and to detect the presence of *Plasmodium falciparum* sporozoites. An informal quality assurance service was provided for those provinces that have the capacity to do their own DNA based identification.

Routine mosquito identifications were carried out for the Malaria Control Programmes of Namibia, Botswana, Swaziland, Zambia, Uganda and Malawi, supported financially by WHO/AFRO.

PROFESSIONAL APPOINTMENTS

Professor Coetzee

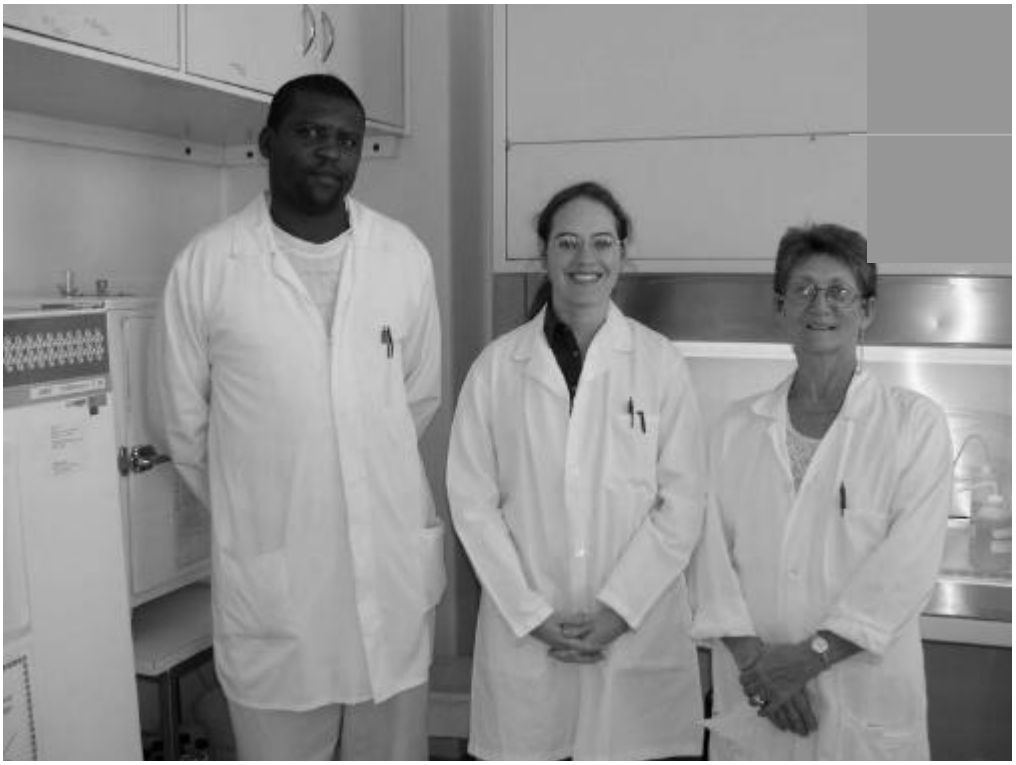
- participated in the WHO/TDR Scientific Working Group on Insect Disease Vectors and Human Health
- was appointed to the Steering Committee of WHO/TDR Molecular Entomology for 3 years
- was co-opted to the Steering Committee of WHO/TDR Research Capability Strengthening for three years.

The Department provides advice and expertise to the Department of Health both at the national and provincial levels, with the participation by Profs Coetzee and Hunt on the National Malaria Advisory Group. Prof Coetzee participates on the Kwazulu/Natal malaria advisory group.

The newly established Malaria Research Institute at the Johns Hopkins University in Baltimore, USA, held its first annual research meeting and Prof Hunt was invited to give a keynote address on mosquito species complexes.

3RD ANNUAL MEETING OF THE AFRICAN NETWORK FOR VECTOR RESISTANCE (ANVR)

Hosted by the NICD, this meeting was held in Johannesburg in October 2002 and attracted 40 delegates from 20 African countries as well as 8 international delegates from Europe and the USA. Presentations were made on country and research institution activities, with invited speakers addressing issues such as insecticide resistance management, the use of insecticide treated bed nets in resistance management and other networks that exist with a similar purpose. A list of future ANVR activities was drawn up with the specific purpose of setting direction and guiding the network for the next 3 years.



Staff of the Parasitology Unit.

Microbiology - *Parasitology*

CURRENT RESEARCH

The PRU is collaborating in a Malaria & HIV study by Dr C Cohen and colleagues, Chris Hani Baragwanath Hospital. This study examined the effect of HIV infection on malaria; it appears that a group of HIV-positive patients tends to have more severe disease.

TRAINING AND QUALITY ASSURANCE

✍ Parasitology EQA programme: Four surveys were sent out during 2002 to 115 participants. The total average score for the year was 72%, a huge improvement on previous year's scores.

✍ Two diagnostic medical parasitology courses were conducted at the Braamfontein Central campus in June 2002. There were 26 participants.

✍ A 2-day malaria laboratory diagnosis course was held in Tzaneen in November 2002. It was sponsored by the Department of Health and there were 25 participants from Limpopo laboratories.

INTERNATIONAL MEETINGS ATTENDED

✍ Leigh Dini: Global Odyssey EQA Congress in Atlanta, USA in February 2002.

✍ John Frean: Annual Scientific Meeting of the Australasian College of Tropical Medicine, Cairns, Australia, July 2002.

Microbiology - *Special Bacterial Pathogens Unit*

Research projects in SBPU are presently funded by the European Union and Abbott Laboratories.

CURRENT RESEARCH PROJECTS

✍ RATZOOMAN is a multicountry, multi-disciplinary study of disease risks linked to rodents at the rural/peri-urban interface which will run for 3 years from January 2002. The component SBPU is involved in is research on the ecology of the rodent-borne zoonoses plague, leptospirosis, and toxoplasmosis.

✍ Antimicrobial susceptibility of anthrax: the objectives were to determine the susceptibility of southern African strains of *B. anthracis* to some new, investigational agents as well as conventional antibiotics. The MICs of 26 isolates of *B. anthracis* from South Africa and Zimbabwe, as well as the Sterne vaccine strain and a type culture strain, were determined by the agar dilution method. The most active antimicrobial agents were new and conventional fluoroquinolones, and doxycycline; macrolides were intermediately active; MICs of a new ketolide were very low. The lack of activity of extended-spectrum cephalosporins against *B. anthracis*, which has been noted previously, was confirmed, in keeping with previous studies. Two new fluoroquinolones and a ketolide showed promising *in vitro* activity that would support their further evaluation in animal models of anthrax.

✍ Antimicrobial susceptibility of plague: the MICs of 28 strains of *Yersinia pestis* from a southern African plague focus were determined by agar dilution. The most active agents were cefditoren and the fluoroquinolones, both conventional and novel. The *in vitro* activity of macrolides was poor against this member of the Enterobacteriaceae. A new ketolide showed enhanced activity compared to the macrolides and further investigation of the novel quinolones in animal models of plague would seem to be justified.

SURVEILLANCE ACTIVITIES

✍ SBPU is active in drawing up new national plague control guidelines, in collaboration with the Department of Health

✍ Invited to join the WHO Global Network for Anthrax and Plague

✍ Advised Department of Health on product recall guidelines, following an botulism outbreak in early 2002

OUTBREAK INVESTIGATION : BOTULISM

Although wildfowl and domestic livestock botulism has been recognised as a problem in southern Africa, very few human cases have ever been described in the region. In late February 2002,

Microbiology - *Special Bacterial Pathogens Unit*

in Springs, a town on the East Witwatersrand near Johannesburg, two siblings aged 12 and 8 years developed acute flaccid paralysis. One child died before reaching hospital; the other died after 10 days of ventilation in ICU. Mouse bioassays revealed the presence of type A botulinum toxin in the blood of both children, and in the retrieved remains of the implicated food. Botulinum antitoxin for treatment was not available in the country. The implicated vehicle of the toxin was a tin of pilchards (fish of the sardine group) in tomato sauce, commercially produced in South Africa. Type A *Clostridium botulinum* was cultured from the food. The factory production records from the batch of tins showed no apparent deficiencies; likewise post-production testing had been uneventful. Although all unsold tins of the batch were withdrawn, none of these tins have tested positive for botulinum toxin. At this time it appears that the most likely scenario was that corrosion damage had allowed ingress of environmental organisms, including *Clostridium botulinum*, to the tinned food, where suitable conditions for growth and toxin production existed. The investigation into the provenance of the tin, and the circumstances around the consumption of its contents, revealed disturbing lapses in the enforcement of food safety-related legislation by local government health services in Greater Johannesburg. This is the first outbreak of human type A botulism in southern Africa to be documented, and the first fatal outbreak described; previous human cases in this region have involved type B botulinum toxin, which tends to produce milder disease. A few other outbreaks in elsewhere in Africa have been published, the most extensive being a type E epidemic in Egypt. Commercially tinned products have not been involved in any of these outbreaks.

TRAINING AND QUALITY ASSURANCE ACTIVITIES

✍ SBPU is part of the NHLS group contracted by the WHO for provision of external quality assessment to laboratories in African countries.

✍ Vib*rio cholerae* and *Shigella dysenteriae* type 1 training course for Health Department and NHLS staff members, October 2002.

INTERNATIONAL MEETINGS ATTENDED

✍ L. Arntzen: International Conference on Emerging Infectious Diseases (ICEID). 24-27 March 2002, Atlanta, Georgia, USA.

Microbiology - *Mycology Unit*

RESEARCH PROJECTS

The Cryptococcal Surveillance Initiative started in 2002.This project is a partnership with and funded by the CDC, Atlanta. The objective of the study is to record the incidence and prevalence of cryptococcal infection in the population of Gauteng.

Over 1000 cryptococcal isolates have been received to date from 30 participating private and NHLS Gauteng laboratories.

During February, two laboratory-training days were held. Medical technologists from the 27 participating laboratories/laboratory groups in Gauteng were invited to attend. The object of the training days was to inform participants of the objectives of the Cryptococcal Surveillance Initiative, explain the need for surveillance and to provide theoretical knowledge and practical experience in the culture, identification and confirmation of cryptococcal isolates.

Dr Mary Brandt, Chief of the Fungus Reference Unit, Mycotic Disease Branch CDC attended the training days and provided valuable input.

Three nursing sisters have been employed to liaise with the laboratories and the hospitals involved. The laboratories provide the cryptococcal isolates and the nurses follow up the clinical information of the patients. The study is expected to run for three years.

The Respiratory and Meningeal Pathogens Research Unit (RMPRU) has completed its study in 39 000 children in Soweto to evaluate the protection of a new vaccine against pneumonia and meningitis. The results of our study show that the vaccine protects against 85% of invasive disease due to vaccine serotypes, reduces antibiotic-resistant pneumococcal disease by 58% and reduces episodes of pneumonia by 32%. This vaccine therefore could reduce the burden of severe pneumonia, the leading cause of death in children, by a third. We have shown that the introduction of *Haemophilus influenzae* type b (Hib) vaccine has been successful in reducing the burden of Hib disease in South Africa. These data will be submitted for publication in 2003. The Unit has begun to unravel the molecular basis of amoxicillin resistance in the pneumococcus – a new threat to the most effective oral agent available. The unit has also described the first cases of fluoroquinolone resistance in the pneumococcus in Africa, including the first global case of fluoroquinolone resistance in a pneumo-coccus from a child. The Unit has documented the enormous burden of *Pneumocystis* infections in HIV-infected children and is embarking on the first study in Africa of the burden of human metapneumovirus infection in children. We will also be studying the functional responses of HIV-infected children to pneumococcal vaccine.

NONAVALENT PNEUMOCOCCAL CONJUGATE VACCINE TRIAL

Prof Keith Klugman and Dr Shabir Madhi presented results of the nonavalent pneumococcal conjugate vaccine trial at the Third International Symposium of Pneumococci and Pneumococcal Disease in Anchorage, Alaska in May 2002. Results have been submitted for publication. Results showed that the vaccine reduced invasive pneumococcal disease due to vaccine serotypes by 85% (95%CI, 32, 98) and by 83% (39, 97) in per-protocol and intent-to-treat analyses respectively. In HIV-infected children, the efficacy by these analyses was 58% (1, 84) and 65% (24, 86). The vaccine reduced first episodes of chest radiograph-confirmed alveolar consolidation in HIV-uninfected children by 25% (4,41) and all episodes by 32% (3,69). This vaccine therefore could reduce the burden of severe pneumonia, the leading cause of death in children, by a third.

In order to evaluate herd immunity in the community as a result of vaccination, all children admitted to Chris Hani Baragwanath Hospital are assessed for possible contact with vaccinees. Nasal swabs are being taken for assessment of nasal carriage of pneumococcus in contacts to determine whether or not this vaccine offers herd immunity.

GROUP B STREPTOCOCCUS (GBS) SEPSIS STUDY

Retrospective record review of children under 12 years of age from whom GBS was isolated at Chris Hani Baragwanath Hospital, was completed. Results published in Annuals of Tropical Pediatrics (see below). Overall burden of early-onset disease (EOD) was 2.06/1000 live births and late-onset disease (LOD) was 1/1000 live births. Overall mortality was 19.8% and 13.6% for infants with EOD and LOD respectively.

***HAEMOPHILUS INFLUENZAE* SURVEILLANCE AND VACCINATION**

We have established a surveillance program with the assistance of the Department of Health to monitor the impact of Hib vaccine effectiveness in SA. We have shown that the introduction of Hib vaccine has been successful in reducing the burden of Hib disease in South Africa. These data will be submitted for publication in 2003.

The Unit found that *Haemophilus* conjugate vaccine, currently purchased by the South African Government at a cost of more than R50m per annum, can be effectively given to children at 1/10 of the currently recommended dose. The study has the potential to save South Africa, and other developing countries, large amounts of foreign exchange. The study has been published and we propose during 2003 to continue to work with the EPI program and provincial authorities to explore the introduction of this low dose regimen in developing countries.

IMPACT OF TRIMETHOPRIM-SULPHAMETH-OXAZOLE PROPHYLAXIS ON RESISTANCE IN RESPIRATORY PATHOGENS

This surveillance program will now be expanded to monitor the impact of trimethoprim-sulphamethoxazole use in South African patients infected with HIV. The proposed widespread introduction of trimethoprim-sulphamethoxazole for prophylaxis of opportunistic infections in HIV infected people will have major implications for the development of resistance and the selection of

multiresistant bacterial clones. We have, with CDC and Emory University, set up an expanded national surveillance network to monitor the impact on resistance and the effectiveness of the intervention on pneumococcal disease. Funding has been secured from USAID via the CDC. A meeting of interested parties was held in 2002 and the appointment of 10 epidemiological assistants at the major hospitals in South Africa will take place in 2003.

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

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. We have used the technique of site-directed mutagenesis to identify which amino acid mutations in altered PBP 1A, are involved in the development of high-level penicillin and cephalosporin resistance in Hungarian isolate 3191 (penicillin MIC, 16 µg/ml; cefotaxime MIC, 4 µg/ml). The analysis involved knocking out mutations in PBP 1A, and determining the effect this has on resistance levels. Of the 43 amino acid mutations occurring in the penicillin-binding domain of PBP 1A, we found that the substitution of Leu-539 by Trp, together with 4 other consecutive substitutions (Thr-574 by Asn, Ser-575 by Thr, Gln-576 by Gly, and Phe-577 by Tyr), are important mutations which alter PBP 1A and contribute to the development of penicillin and cefotaxime resistance. This work was published in the January 2003 issue of Antimicrobial Agents and Chemotherapy. A similar analysis has been initiated on altered PBP 2X from the Hungarian isolate.

ANALYSIS OF THE MECHANISM OF AMOXICILLIN RESISTANCE IN FRENCH PNEUMOCOCCAL ISOLATES DISPLAYING AN UNUSUAL PENICILLIN/ AMOXICILLIN RESISTANCE PHENOTYPE

The recent emergence of pneumococcal isolates exhibiting an unusual resistance phenotype of higher amoxicillin MICs in relation to penicillin MICs, prompted an analysis of the *pbp* genes from nine such strains isolated in France. Transformation experiments with cloned *pbp* genes isolated from one of the resistant isolates demonstrated a step-wise development of amoxicillin resistance, involving PBPs 2X, 2B and 1A. Full resistance, equivalent to that of the donor strain, was only achieved when genomic DNA was transformed into $R6^{2x/2b/1a}$ mutants, suggesting that full resistance development in this isolate is mediated by a non-PBP determinant. Moreover, the recently identified *MurMN* resistance determinant does not appear to have any impact on resistance in this isolate. This determinant (from the French isolate) was, however, able to transform an R6 mutant harboring *pbp2x*, *2b* and *1a* genes from an extremely high-level penicillin-resistant Hungarian clone to increased levels of resistance. These results indicate that the development of high-level beta-lactam resistance is complex and the involvement of MurMN in penicillin resistance appears to be dependent on specific mutations in PBPs 2X, 2B and/or 1A. Further, an additional non-PBP-mediated resistance determinant is required for full resistance development in some pneumococci. Several other genes, known to be involved in cell wall synthesis, have been cloned and transformed into a transformant harbouring *pbp's 2x*, *2b* and *1a* from the French isolate. So far, none of them has had any impact on resistance. At the same time, efforts are being made to randomly digest the genomic DNA, clone the fragments and conduct transformation experiments using the cloned fragments. The search for this determinant is ongoing.

MOLECULAR CHARACTERIZATION OF ANTI-BIOTIC-RESISTANT PNEUMOCOCCI USING MLST

The molecular characterization of pneumococci relies on techniques such as multilocus enzyme electrophoresis (MLEE) and pulsed-field gel electrophoresis (PFGE) to assess genetic relatedness of isolates. These techniques are only adequate for identifying clusters of clones within a local population as the alleles are indirectly characterised from electrophoretic mobilities of the gene/gene product. Recently an high resolution molecular typing procedure termed, multilocus sequence typing (MLST) has been described. This technique allows for the unambiguous assignment of resistant isolates to known or new clones without the exchange of reference strains between laboratories. Briefly this technique involves the amplification and sequencing of seven housekeeping genes. Using software available at the pneumococcal MLST website, the sequences of the seven loci generated for each strain may be compared to

other sequences in the database. Sequences that are identical to sequences already presented in the MLST database are assigned the same allele number. New alleles are submitted to the curator of the database for the assignment of an allele number. The alleles at each of the 7 loci define the allelic profile and sequence type. Using the MLST technique we have currently sequenced 30 South African and African pneumococci. Preliminary results reveal that 5 of these strains are not present in the central MLST database. Further allelic profiling will be conducted and this data will be used to compare the genetic relatedness of our local isolates to other isolates that have been submitted to the central database on the www.

ANALYSIS OF MUTATIONS IN THE DIHYDROPTEROATE SYNTHASE (DHPS) GENE FROM *PNEUMOCYSTIS JIROVECI* ISOLATED FROM HIV-POSITIVE PATIENTS TREATED WITH TRIMETHOPRIM-SULPHAMETHOXAZOLE

Pneumocystis jiroveci is a common opportunistic infection associated with immunosuppressed and HIV-infected patients. Widespread use of trimethoprim-sulphamethoxazole as prophylaxis and treatment for *Pneumocystis* pneumonia (PCP) as led to concern about increased drug resistance. The aim of this study was to determine whether *P. jiroveci* dihydropteroate synthase (DHPS) gene mutations in AIDS patients with PCP are affected by trimethoprim-sulphamethoxazole prophylaxis. A total of 96 bronchoalveolar lavage (BAL) samples from HIV-seropositive patients with suspected PCP were collected for the study. No results are available at present since there have been difficulties in amplifying the DHPS gene from *P. jiroveci* DNA extracted from the samples. In order to confirm the presence of *P. jiroveci* in the samples, attempts are being made to amplify regions of the mitochondrial and 18S rRNA genes - targets known to be specific for *P. jiroveci*.

ANALYSIS OF THE GENETIC DIVERSITY OF *NEISSERIA MENINGITIDIS* ISOLATES IN SOUTH AFRICA

The objectives of this study are to evaluate the prevalence of the various meningococcal serogroups in South Africa, with particular reference to their geographical distribution and temporal variation, and to identify which clonal groups from the various prevailing serogroups in South Africa are present and circulating in the population (using genotyping methods such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)). To date, in excess of 600 South African *Neisseria meningitidis* isolates received countrywide during the period of July 1999 to July 2002 have been successfully serogrouped by latex slide agglutination. Analysis of the temporal and geographic distribution of the various meningococcal serogroups is being completed. The PFGE protocol for the genotypic characterization of meningococcal clones ("strains") has been set up and optimised. Approximately 200 of the ~600 meningococcal isolates have been analyzed by PFGE and a further 200 have been prepared and are in the process of being characterized. Preliminary results indicate that only a few clones or clonal complexes within the various serogroups being studied are responsible for meningococcal disease associated with that particular serogroup.

THE HUMAN METAPNEUMOVIRUS IN SOUTH AFRICA

A new virus belonging to the *Paramyxoviridae* family and termed the human metapneumovirus (hMPV) has recently been characterised and isolated from children with respiratory tract infections in the Europe, North America and Australia. Serological data from the Netherlands has shown that by 5 years of age all children are exposed to the virus and the virus has been circulation in human for at least 50 years. To detect and characterize the hMPV in South Africa we are currently isolating RNA from naso-pharyngeal aspirates taken from children that attended Chris Hani Baragwanath Hospital with respiratory tract infections. RT-PCR will be used to detect the hMPV using primers specific for the fusion and nucleocapsid genes. Preliminary results (3 positive) have shown that the hMPV is present in South Africa. Sequence data of one positive revealed 100% homology to a strain that was previously isolated in Canada.

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

The emergence of fluoroquinolone-resistance in pneumococci is a new phenomenon.

Fluoroquinolone-resistant strains have been found to be sporadic, but in a preliminary analysis of strains from Northern Ireland, Spain and France

we have established evidence of the clonality of these strains. The strains have been investigated from amongst isolates received from the Alexander study. The clonality of these strains has been described and were published in 2002.

We have now described the first cases of fluoroquinolone resistance in Africa in adults and the first global case in a child. These data have been submitted for publication.

Staff of the Respiratory and Meningeal Pathogens Research Unit

Staff of the Sexually Transmitted Infections Unit

The main activities of the Sexually Transmitted Infections Reference Centre (STIRC) relate to surveillance of sexually transmitted diseases (STDs), evaluation of appropriate tests for the rapid diagnosis of STDs in developing country settings, research concerning improved diagnosis and management of STDs, and community-based intervention studies. Important also is our ongoing involvement with undergraduate and postgraduate teaching as well as training of health care workers including trainers/counsellors in sexually transmitted infections (STIs) as part of combined activities of STIRC and the Reproductive Health Research Unit (RHRU) at the Esselen Street Clinic.

SURVEILLANCE OF STD SYNDROMES

Surveillance based on clinical syndromes of STDs at 21 sentinel clinics in Gauteng province is ongoing and based on vaginal discharge and pelvic inflammatory disease in females, urethritis and epididymitis in males and syndromes affecting both genders, including genital ulcer disease (GUD), bubos and genital warts.

The attendance figures at the Gauteng sentinel clinics for the period 2000 to 2002 and the number of patients diagnosed with vaginal discharge, male urethritis and GUD, the three most clinical syndromes seen at the clinics, are illustrated in Figure 1.

As can be seen, there was a fall of 16.7% in attendance from 60395 in 2000 to 50281 in 2001. Similar decreases of 19.3%, 20.3% and 13.5% respectively were observed with the three major syndromes, vaginal discharge, male urethritis and GUD between these two years. Only in the case of GUD however, was a further decrease (10.1%) evident between 2001 and 2002. Factors responsible for these changes in clinic attendance and the number of syndrome-based cases seen, are unknown but it is likely that public health measures and especially syndromic treatment may have played an important role. The marked decrease in the seroprevalence of syphilis, based on RPR results since 1985 when syndromic treatment was introduced at the clinics (Figure 2), provides compelling evidence for the success of syndromic treatment. The decrease in GUD of bacterial aetiology seen at our sentinel clinics, together with the declining seroprevalence of syphilis and the high incidence of genital herpes coupled with a lower and decreasing incidence of chancroid (for decades the most common cause of GUD in different population groups in Gauteng), further support the likely impact of syndromic treatment on the frequency and patterns of STIs in Gauteng.

MICROBIOLOGICAL SURVEILLANCE

Core activities of laboratory-based surveillance of STDs comprise a) the identification of aetiological agents responsible for STD syndromes, and b) antibiotic susceptibilities of bacterial STI pathogens.

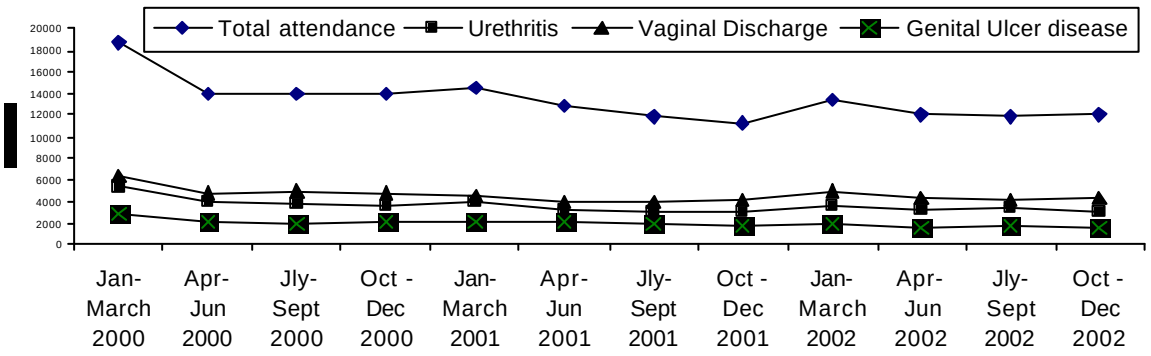


Fig 1. Quarterly reports of the number of clinic attendees and STD patients in Gauteng region, January 2000 to December 2002

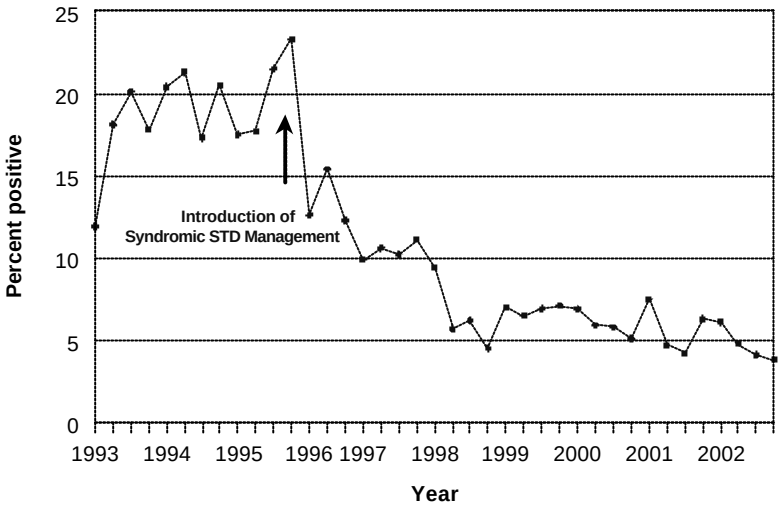


Fig 2. Prevalence of reactive syphilis serological tests (RPR) among STD patients in Johannesburg (1993 - 2002) based on data produced by STIRC and the Johannesburg Metropolitan Council

STIRC was involved in several studies concerning pathogens associated with vaginal discharge. Based on culture for gonococci and ligase chain reaction amplification (LCx®, Abbott Laboratories) for *Neisseria gonorrhoeae, and Chlamydia trachomatis,* the prevalence of these STI pathogens in women attending three clinics in Johannesburg in 2002, was determined in a collaborative study with the Program for Appropriate Technology in Health (PATH), Seattle, Washington, USA. The rates at the Esselen Street clinic for STDs, serving many high risk individuals in the Hillbrow area were 20.5%, and 18.6% for *N. gonorrhoeae* and *C. trachomatis* respectively in 533 female attendees. The corresponding figures for family planning clinics in Rosettenville were 8.0% and 15.6% in 213 women and at a general clinic in Malvern 18.0% and 23.5% of 51 women respectively. Co-infection with both pathogens occurred in 6.2%, 3.3% and 8.0% patients at the three respective clinic sites.

In a collaborative study with the RHRU on STDs in sex workers in Hillbrow during the period 2000-2002 involving 2044 visits,. *N. gonorrhoeae* infection was found in 284 episodes(13.9%), *C. trachomatis* in 238 (11.6%), and *T. vaginalis* (using PCR TV3 and TV7 primers) in 28.8% of patient visits. Using multiplex PCR for the detection of herpes simplex virus, type 2(HSV-2), *Treponema pallidum* and *Haemophilus ducreyi* and DNA in GUDs, the proportion of these agents in 29 patients in whom a specific laboratory-confirmed diagnosis was achieved, were 69%, 27.6% and 3.4% respectively.

In a cross-sectional study in Khutsong, a small mining town in the Carletonville area and conducted between September 2001-January 2002, the prevalence of *N. gonorrhoeae* and *C. trachomatis* among 2606 inhabitants using LCR was 6.3%, and 8.1% respectively, , the RPR seropositivity rate for syphilis was 8.1%, and that of the FTA-ABS test 33.2%. The HIV and HSV-2 rates were 39.3% and 63.0% respectively.

The prevalence data were similar to those obtained in cross-sectional studies involving the same town in 1998 and 2000 and formed part of a Population Council (USA) supported study to assess the effect of general intervention measures on the prevalence of STDs including education, promotion of condom use and improved clinic facilities.

ANTIMICROBIAL SUSCEPTIBILITY OF GONOCOCCAL ISOLATES

Antimicrobial resistance profiles of *N. gonorrhoeae* isolates in sex workers in Gauteng and miners with acute urethritis at Carletonville are given in the accompanying table. During the surveillance period 1999-2001 the sex workers group was subjected to periodic presumptive treatment with azithromycin.

TABLE I : ANTIMICROBIAL SUSCEPTIBILITY OF *NEISSERIA GONORRHOEAE* ISOLATES

Antimicrobial agent	Breakpoints (µg/ml)		PERCENTAGE OF ISOLATES					
			Susceptible		Intermediately resistant		Resistant	
	Intermediate	Resistant	Hillbrow[1]	Carletonville[2]	Hillbrow[1]	Carletonville[2]	Hillbrow[1]	Carletonville[2]
Penicillin G	0.12 – 1	> 2	38	15	38	40	23	45
Ciprofloxacin	0.12 – 0.5[3]	> 1[3]	100	99	0	1	0	0
Ceftriaxone	-	> 0.5	100	100	0	0	0	0
Tetracycline	0.5 - 1	> 2	62	69	3	6	35	25
Azithromycin	-	> 0.5[4]	100	100	0	0	0	0
Erythromycin	-	> 2[4]	92	94	8	6	0	0

1) Study population of Reproductive Health Research Unit of Hillbrow commercial sex workers = 60
2) Study population of Welkom and Carletonville intervention studies = 159
3) Based on response to treatment with a single oral dose of ciprofloxacin 500mg or ofloxacin 400mg
4) Decreased susceptibility due to *mtr* gene mutations occurs at MICs of > 0.5µg/ml for azithromycin and > 2µg/ml for erythromicin

In neither of these studies was evidence of the emergence of azithromycin resistance noted. Isolates remained susceptible to ceftriaxone and ciprofloxacin, with the exception of one isolate which showed intermediate resistance to the latter agent. High-level tetracycline resistance remains common

STIRC confirmed the isolation at Lancet Laboratories of a fully resistant strain with a ciprofloxacin MIC of > 1µg/ml from a patient with acute urethritis who recently returned from a visit to the Far East.

EVALUATION OF RAPID TESTS

Determine TP Rapid strip test

During the period 2001-2002, a study was conducted at antenatal clinics in the Eastern Cape to evaluate the performance of the Determine ®TP Rapid Strip Test (Abbott Laboratories) in detecting treponemal antibodies to *T. pallidum* in pregnant women. Like other *T. pallidum* antigen specific serological tests, the Determine ® test detects both currently active episodes of syphilis and inactive syphilic infection acquired in the past. As expected, this on-site rapid immunochromatographic strip test was more sensitive than the RPR test and therefore would avert more cases of congenital syphilis. It was also easier to interpret by nurses but its routine use would add to the cost of a congenital syphilis prevention programme as some cases of inactive infection would be treated unnecessarily.

STIRC was instrumental in setting up the study by training health care workers at clinics on the use of the rapid test, as well as to take responsibility for the quality assurance (QA) aspects of the study. The QA exercise demonstrated excellent agreement between the Eastern Cape regional laboratory in Umtata which was responsible for monitoring the on-site testing in the rural clinics and STIRC findings relating to the Determine ® and the *T. pallidum* haemagglutination assay (TPHA) tests. We used the fluorescent treponemal antibody absorption (FTA-ABS) test to investigate discrepancies between TPHA and Determine ® tests and found that some of the Determine ®-positive, TPHA-negative findings could be ascribed to early infections. In general, the FTA-ABS and TPHA tests confirmed the excellent performance of the Determine ® rapid strip test.

Rapid immunochromatographic strip test for N. gonorrhoeae

In a collaborative study with the PATH organization conducted at STD clinics in Johannesburg, a rapid immunochromatographic strip test for the detection of *N.gonorrhoeae*-specific antigen in endocervical and high vaginal swabs in females and urine samples in males, proved to be positive in 57 out of 120 women (47.5%) with LCR-confirmed gonorrhoea. Vaginal swabs yielded more positive results with the rapid test than endocervical swabs.(51.4% vs 47.5%). The sensitivity of the rapid test of 92.2% (154 out of 167) in males with LCR-positive gonorrhoea was appreciable greater than in women (61.4%)

LCR amplification for *C. trachomatis* (LCx ®, Abbott Laboratories) was also performed and the

prevalence of these pathogens in clinic attendees has already been given in this report. (See Microbiological Surveillance)

B-lymphocyte lysate test for the early diagnosis of HIV infection

Release of antibodies on lysis of B-lymphocytes extracted from blood with magnetic beads, forms the basis of a rapid test for the early diagnosis of HIV infection. This test, called the PlasmAcute test, was developed by Professor Lars Haaheim from the University of Bergen, Norway and is being evaluated by STIRC in HIV-negative miners attending STD clinics in Carletonville. The study started in 2002 and a total of 200 HIV-negative miners will be investigated.

To date three patients sero-converted within three weeks after initially testing negative for HIV. Patients were B-lymphocyte lysate test positive before the P24 antigen test became positive and before HIV could be detected on viral load testing. On Western blot testing of lysates and serum samples, antibodies to HIV envelope antigens appeared first, before conventional ELISA tests became positive.

Community-based intervention studies

Expanded periodic presumptive treatment programme

The Lesedi periodic presumptive treatment (PPT) project was established in the Harmony mines in the Free State in 1996. Women at high risk (WAHR) of acquiring HIV infection living around the mines were attended at mobile clinics where symptomatic women were treated with a two gram dose of metronidazole for vaginal discharge and those with reactive syphilis serology with 2.4 million units of benzathine penicillin. All WAHR in the study received, regardless of symptoms, one gram azithromycin, initially at monthly intervals at mobile clinics and subsequently every three months. Other intervention activities included training of peer educators to facilitate consistent condom use and monthly clinic attendance, as well as syndromic STD management by nurses at mobile clinics.This PPT intervention project was evaluated in 1997 and showed a marked reduction in STDs in both WAHR and local miners.

The effect of these interventions was so dramatic that an expansion of the PPT project to involve new mining areas, including 6 intervention "hotspots" around Carletonville mines was embarked upon. These interventions are ongoing and form part of the activities of the Mothusimpilo Community Outreach Project. STIRC plays a facilitating role in the PPT programme and coordinates PPT activities. Generous funding from USAID, mainly for the purchase of additional mobile clinics has been obtained from this expanded PPT programme.

The most recent report on the Mothusimpilo project, issued in 2002, showed marked decreases in STIs in WAHR clinic attendees. Gonococcal infections decreased from 20.6% for to 2.3% after 4 monthly visits, while corresponding decreases for *C. trachomatis* and GUDs were 20.8% to 8.6% and 11.1% to 5.2% respectively. The PPT interventions reduced GUDs more rapidly than was the case with genital discharge and the aetiological profile of GUDs shifted significantly from chancroid to herpes (HSV-2) infection. (Neither PPT nor syndromic management of GUDs covered genital herpes). Substantial reductions in STD episodes, similar to those in WAHR, were also documented in miners in the Carletonville region.

During 2002, section 21 companies were formed to administer both the Lesedi and Mothusimpilo projects. The staff who had been employed by the NHLS on contracts for the projects were transferred in August 2002 to these companies.

Periodic preventive treatment project in a military setting

USAID has provided funding for a mobile clinic and additional financial support for a project based on the Lesedi intervention study involving PPT of WAHR in communities in the vicinity of two South African military service (SAMS) stations. The primary objective of this proposed study is to monitor STD prevalence, HIV incidence and risk factors related to HIV acquisition following interventions to provide improved STD services involving syndromic case management and PPT, as well as risk reduction education incorporating the promotion of barrier methods to women at high risk.

Herpes intervention study in miners

Preparations for a major herpes intervention study involving several thousand miners have reached an advanced stage. The study will

assess the impact of anti-HSV-2 suppressive therapy with acyclovir on a) HIV acquisition, requiring HIV sero-negative participants and b) the effect of suppressive therapy on genital herpes infection in HIV sero-positive individuals. USAID funding for this project has been approved and additional funding from the World Health Organization and the Centres for Disease Control and Prevention, Atlanta, USA is available.

TEACHING AND TRAINING

Members of STIRC have been involved in the teaching of medical, dental, pharmacy and nursing students, as well as in postgraduate teaching. The latter includes lectures given to DTM&H students and participation in seminars. Ms Judith Wright of our centre plays an important role in the training programme in Gauteng. She has been involved with training of nurse trainers and health care providers in the West Rand, at Goldfields College of Nursing and for the West Rand Aids Project (WRAP). She has also acted as consultant for the RHRU for their STI module in their Sexual Reproductive Health training programme. The RHRU and STIRC take the lead in research and training undertaken in the field of STIs at the proposed “Esselen Street Centre of Excellence” in Gauteng.

Virology - *AIDS Virus Research Unit*

The AIDS Unit is primarily focussed on conducting research into the virology and immunology of HIV. The Unit is comprised of 5 laboratories, namely Virology headed by Professor Lynn Morris, Immunology headed by Dr Clive Gray, Cell Biology headed by Dr Caroline Tiemessen, Molecular Biology headed by Dr Maria Papathanasopoulos and the HLA laboratory headed by Dr Adrian Puren.. Collectively there are a total of 43 staff and students in the Unit of whom 6 are Specialist/ Senior scientists or post-docs, 14 post-graduate students, 15 research/technical assistants and 5 administrative support staff. The Unit collaborates closely with the Specialized Molecular Diagnostic Unit led by Dr Adrian Puren who provides significant support in terms of HIV diagnostic services. Scientists within the Unit have raised significant external funding through grant applications and collaborations. These include the South African AIDS Vaccine Initiative (SAAVI), National Institutes of Health through the Vaccine Trials Network (HVTN) and Collaborative International Program for Research on AIDS (CIPRA), The Wellcome Trust, The National Department of Health, The International AIDS Vaccine Initiative, Bristol Myers Squibb "Secure-the-Future" Program and the Poliomyelitis Research Foundation. Numerous large pieces of equipment have been purchased through grant funding including an ABI3100 Genetic Analyzer, FACSCalibur Flow Cytometer, MagNaPure extractor, LightCycler, three Elispot plate readers, Luminometer, kinetic plate reader, liquid Nitrogen storage tank and other laboratory equipment. Members of the Unit are involved in a number of international and local collaborations and have hosted a number of international visitors in 2002.

The Unit is poised to play a major role in HIV vaccine development. The HVTN has selected the NICD as the Southern African Regional Immunology Laboratory. This selection has been endorsed by SAAVI who have mandated the Unit to be the core Immunology laboratory in South Africa for HIV vaccine trials. More recently the WHO has recommended that the Unit serve as a regional reference laboratory for HIV drug resistance surveillance and as a repository for HIV subtype C viral isolates.

VIROLOGY LABORATORY

The virology laboratory is involved in studying the genetic and biologic properties of HIV-1 subtype C viruses. Over the years we have isolated in excess of 300 primary viruses from various cohorts, some of which have been used in vaccine development and in understanding the genetic and biological complexities of HIV-1 subtype C including genetic diversity and coreceptor usage. This laboratory is responsible for monitoring the humoral immune responses, in particular assessing the binding and neutralizing antibody responses to candidate HIV vaccines among sero-negative volunteers.

The Virology Laboratory has a rapidly expanding HIV Drug Resistance Program. During 2002, three new staff members, Dr Claudia Chezzi, Shayne Loubser and Matshediso Ntsala joined this group. The main focus of this program is to examine resistance mutations among women and infants following short-course anti-retroviral treatments to prevent mother-to-child transmission. Studies among South African patients failing anti-retroviral therapies are also underway to determine resistance patterns among subtype C viruses. In April, Prof Morris co-organised a WHO/AFRO-sponsored "Workshop on monitoring HIV resistance to ARV drugs in the African Region". She also gave a special lecture at the XIV International AIDS Conference in Barcelona entitled "HIV drug resistance in resource-limited settings: present and future." Below we describe some of the research highlights in 2003.

HIV-1 SUBTYPE C ISOLATES FROM SOUTH AFRICA ARE FULLY SENSITIVE TO CCR5 AND CXC4 ENTRY INHIBITORS

HIV-1 was isolated from 29 patients admitted to the Sizwe Infectious Diseases Hospital in Johannesburg with advanced HIV disease. The majority of patients had tuberculosis. The median CD4 T cell count and viral load for this group was 40 cells/ul and 322,070 RNA copies/ml respectively. Genetic analysis of the envelope gene revealed that all isolates grouped with HIV-1 subtype C, the predominant subtype circulating in South Africa. Isolates were assessed for their ability to use one or both major HIV-1 coreceptors, which are required together with CD4 for virus entry. Of the 29 isolates, 24 used the CCR5 coreceptor (R5 isolates), 2 used CXCR4 (X4 isolates) and 3 used both CCR5 and CXCR4 (R5X4 isolates). All R5 isolates were inhibited by RANTES, the natural ligand for CCR5 and PRO140 a monoclonal antibody that inhibits entry via CCR5. The R5X4 and X4 isolates were sensitive to AMD3100, a CXCR4-specific inhibitor.

Virology - *AIDS Virus Research Unit*

Staff of the Virology and Molecular Biology laboratories of the HIV/AIDS Research Unit

Staff of the Cell Biology laboratory of the HIV/AIDS Research Unit

Staff of the Immunology laboratory of the HIV/AIDS Research Unit

Results from representative isolates are shown in the Table below. These data indicate that HIV-1 subtype C isolates from South Africa are fully sensitive to inhibitors designed to block viral entry. Such agents, which can be used in both therapeutic and preventative strategies, are therefore likely to be highly effective in this setting.

Inhibition of HIV-1 subtype C viruses by CCR5 and CXCR4 inhibitors

Virus	Subtype	Phenotype	CCR5 inhibitors*		CXCR4 inhibitor*
			RANTES	PRO140	AMD3100
PCP1	C	R5	**97**	**98**	*34*
CM1	C	R5	*87*	*78*	*0*
CM7	C	R5	**94**	**97**	*3*
SW5	C	R5	**100**	**100**	*19*
SW23	C	R5	**100**	**98**	*0*
SW26	C	R5	**100**	**93**	*8*
SW29	C	R5	**98**	**98**	*13*
SW38	C	R5	**100**	**100**	*0*
SW20	C	R5X4	*0*	*79*	**100**
SW30	C	R5X4	*24*	*8*	*85*
CM9	C	R5X4	*36*	*34*	**97**
SW7	C	X4	*0*	*0*	**100**
SW12	C	X4	*0*	*0*	*86*

* Inhibition >90% is bolded and inhibition <90% is in italics

USE OF A V3 HETERODUPLEX TRACKING ASSAY TO DISTINGUISH R5 AND R5X4 OR X4 SUBTYPE C ISOLATES

The V3-loop of gp120 is the major determinant of coreceptor usage. Distinct sequence changes in this region are associated with coreceptor switching from CCR5 to CXCR4. Such changes can be monitored using a Heteroduplex Tracking Assay (HTA), which is a highly sensitive method for detecting genetic variation. Eighteen R5 (non-syncitium-inducing or NSI) and 10 R5X4 or X4 (syncitium-inducing or SI) isolates were screened with a R5 subtype C probe. All ten SI isolates migrated less rapidly in the gel indicating lower sequence homology with the R5 probe. An example is shown in the Figure below where SI samples had a higher mobility shift (separated slower through the gel) compared to the NSI isolates. In one case an increase in the number of quasispecies was associated with an SI phenotype (see lanes 1 and 2). Three of the NSI isolates however, showed a similar pattern to the SI isolates, one of which was a recombinant with subtype D and two of which were from slow progressing children (data not shown). Further sequencing analysis is necessary to determine why these 3 NSI viruses migrated less rapidly than the other 15 NSI isolates. These data indicate that the V3 HTA can be used as a rapid screening tool for detecting CXCR4-using viruses in subtype C populations.

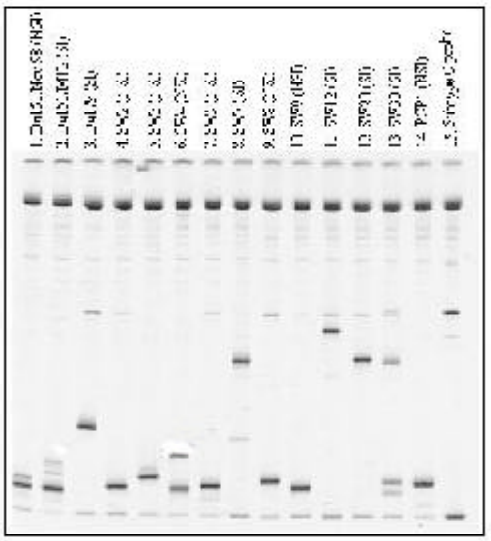


Fig 1. HTA of 6 SI isolates and 8 NSI isolates. Du151/MT-2 (lane 2) is an SI isolate grown in MT-2 cells. It is a later isolate from patient Du151 who earlier had an NSI virus (lane 1). Lane 15 is the R5 probe on its own and bands migrating at this levels should be ignored.

POLYMORPHISMS IN HIV-1 SUBTYPE C PROTEASE GENES ENCODE SECONDARY DRUG RESISTANCE MUTATIONS

The HIV-1 protease genes from 31 HIV-1 subtype C infected pregnant women in South Africa who were anti-retroviral drug naive were sequenced and analysed. Comparison of the 99 predicted amino acid consensus sequences of these samples with HIV-1 subtype B indicated 8 amino acid differences, at loci 12, 15, 19, 36, 41, 69, 89 and 93 (Figure 2). The 36I and 93I polymorphism occurred in 88% and 100% of subtype C sequences respectively and in addition 47% of sequences showed variation at position 63. These 3 loci have been shown to function as secondary resistance mutations for HIV-1 subtype B. Two sequences harboured the 46I mutation, which can confer primary resistance to Indinavir, and also functions as a secondary resistance

mutation for other protease inhibitors. No other primary mutations were found. The M36I and K41R polymorphism occurs in the flap region and may affect the conformational structure of the subtype C enzyme. These data provide important baseline data required before the introduction of protease inhibitors in regions where HIV-1 subtype C circulates. Given the absence of primary drug resistance mutations, protease inhibitors would be expected to be highly effective in this population.

titers using the SW7 TCLA strain and the primary virus Du151, a prototype subtype C vaccine strain (Figure 3). 56% of sera showed activity against SW7 TCLA compared to 43% for Du151, indicating increased sensitivity of the TCLA assay. There was also a better correlation between the subtype C TCLA and Du151 compared to the subtype B MN strain (not shown). This assay may be useful for evaluating humoral immune responses among participants in HIV vaccine trials in South Africa.

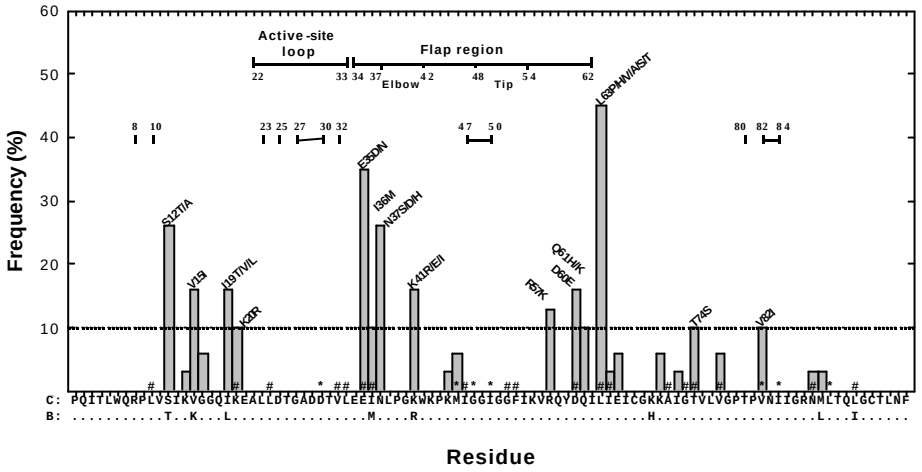


Fig 2. Frequency of predicted amino acid sequences from 31 subtype C protease sequences. Primary (*) and secondary (#) resistance mutations and changes in subtype B consensus are indicated. Active-site loop, flap region and drug binding sites are also shown (courtesy of Dr Heini Dirr).

ESTABLISHING A SUBTYPE C TCLA NEUTRALIZATION ASSAY

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. In preparation for HIV-1 vaccine trials we established a subtype C T-cell line adapted (TCLA) neutralization assay. An X4 subtype C isolate (SW7) from a patient with advanced AIDS was passaged continuously in MT-2 cells and the resulting TCLA strain was compared to the primary isolate grown in PBMC. The TCLA strain showed 10-100 fold-increased sensitivity to neutralization by HIV-1 positive sera and was also more sensitive to CD4-IgG compared to the primary isolate (0.54 ug/ml versus 25 ug/ml CD4-IgG). Sequence analysis of the primary and TCLA strains demonstrated 22 amino acid changes in gp120 and gp41 but the V3 region and CD4 binding sites were conserved.with a glycosylation site change on the gpH1 region. The SW7 TCLA strain was evaluated using 117 serum samples from HIV-1 infected individuals from southern Africa. There was a good correlation between neutralization

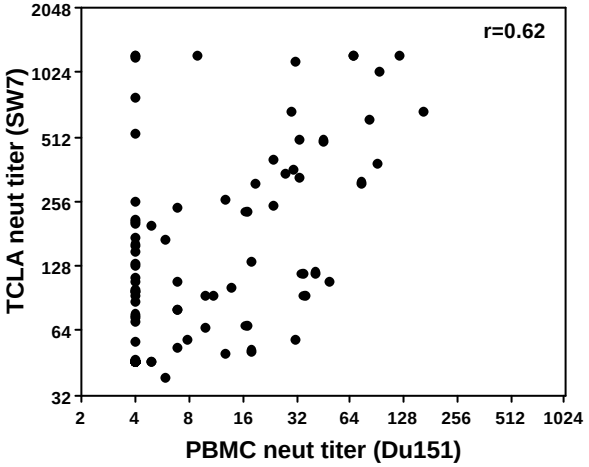


Fig 3. Comparison of antibody neutralization titres using a subtype C TCLA strain (SW7) and a subtype C primary virus (Du151)

MUCOSAL ANTIBODY RESPONSES IN HIV-1 INFECTED WOMEN AND THOSE AT HIGH RISK OF HIV INFECTION

The issue of whether mucosal immunity plays a role in the unexplained resistance of some sex workers and discordant couples to HIV infection remains controversial. Some research has shown

that highly exposed persistently sero-negative individuals (HEPS) have anti-HIV antibodies (mostly of the IgA class) in mucosal secretions, but not in peripheral blood samples, and that in some instances these antibodies can neutralise HIV. In order to explore this phenomenon in the South African context, we assayed cervicovaginal lavages (CVL) from HIV-negative sex workers participating in a microbicide trial in Durban. These women were all in sex work for at least 4 years, reported to have on average 17 partners per week, with low reported use of condoms and a clientele that was over 50% HIV-positive. CVL from HIV-positive women participating in the same trial were used as controls.

Western blot analysis using CVL from HIV-positive women showed the presence of HIV-specific IgG and IgA antibodies to all major HIV proteins. A similar analysis using CVL from 27 individuals recruited as HEPS (sex workers) showed no reactivity. Analysis of total antibody levels by ELISA showed that all HEPS women had detectable IgG and IgA and thus low antibody levels could not explain the lack of HIV-specific antibodies. These data suggest that among South Africa sex workers who remain HIV-negative despite multiple exposures to HIV, mucosal antibodies to HIV are not detectable and thus cannot account for the resistance of these women to HIV.

MOLECULAR BIOLOGY LABORATORY

The molecular biology laboratory is committed to understanding the structure/function relationship of HIV-1 proteins, in particular the envelope glycoprotein, because of the implications for both vaccine and drug development studies. We have also established an HIV-1 full-genome sequencing program, with the aim of correlating viral diversity to functional and immunologically relevant sites. Mr Sibusiso Nkosi submitted his Masters dissertation in August 2002. He was awarded a Southern African Fogarty AIDS training award to visit Dr Richard Wyatt's laboratory at the Vaccine Research Center, NIH, USA, to work on modifying HIV-1 subtype C envelope glycoproteins.

LARGE-SCALE PURIFICATION & CHARACTERIZATION OF FUNCTIONAL HIV-1 SUBTYPE C ENVELOPE GLYCOPROTEINS EXPRESSED IN INSECT CELLS

The full-length envelope glycoproteins from four South African HIV-1 subtype C isolates exhibiting different phenotypic properties were expressed in insect cells, purified, and their biological characteristics investigated. Isolates 98ZA151Du, 99ZACM9, 98ZA179Du and 99ZASW7 that utilize the CCR5, CCR5/CXCR and CXCR4 coreceptors for cell entry, respectively were selected for this study. All four full-length *env* genes were PCR amplified, cloned into a baculovirus expression system and the recombinant envelope glycoproteins were expressed in *Spodoptera frugiperda* (Sf9) insect cells. The envelope glycoproteins were purified on a large scale using *Galanthus nivalis* lectin and their biological properties were examined by their reactivity with soluble CD4 (sCD4) and a panel of monoclonal antibodies. The conformation of the four purified recombinant HIV-1 subtype C envelope glycoproteins was intact, since they all reacted with the conformational dependent monoclonal antibodies A32, C11, 17b, IgG1b12 and 7b2. However, the IgG1b12 monoclonal antibody was unable to bind to the 98ZA151Du envelope glycoprotein efficiently, suggesting that this CCR5 envelope glycoprotein exhibited lower antigenic properties compared to the CCR5/CXCR4 and CXCR4 envelope glycoproteins. The high levels of reactivity of the recombinant envelope glycoproteins with the A32 and C11 monoclonal antibodies and their lower interaction with IgG1b12 indicated they were purified as monomers. All four recombinant HIV-1 subtype C envelope glycoproteins reacted poorly with sCD4, however their high binding activity with the 17b monoclonal antibody in the presence of sCD4 confirmed that they are functional. Thus the recombinant HIV-1 subtype C envelope glycoproteins purified in this study can be used as reagents to further facilitate functional, structural, antigenicity and immunogenicity studies.

FULL-LENGTH GENOME CHARACTERIZATION OF HIV-1 SUBTYPE C ISOLATES FROM TWO SLOW PROGRESSING PERINATALLY INFECTED SIBLINGS

Isolation and characterization of HIV-1 from asymptomatic, slow progressing patients is important in studying the pathogenesis of the virus and facilitating vaccine and antiviral development. Factors that could contribute to different rates of disease progression include host immunogenetics and viral attenuations. In this study we identified two slow progressing HIV-1 infected siblings, isolated viruses and sequenced

the full viral genome to identify virus attenuations that may contribute to the altered rate of disease progression. Neither child had received antiretroviral therapy.

The 99ZATM10 and 01ZATM45 strains were isolated from two asymptomatic siblings, aged 9.5 and 11 years, respectively. Both viruses used the CCR5 coreceptor for cell entry. Proviral DNA from strains 99ZATM10 and 01ZATM45 was isolated from PBMC coculture. Virtually full-length genomes were PCR amplified and sequenced. LTR regions were amplified separately, sequenced and assembled to generate the complete genomes. A multiple alignment with all available HIV-1 subtypes was generated, and phylogenetic analysis confirmed that the two isolates were subtype C throughout their genome. Predicted amino acid sequence analysis for all the HIV-1 proteins showed that both viruses had open reading frames for all genes, and encoded for proteins of the expected length, except for the *rev* gene. The 3' end of *rev* exon 2 did not have the 16 aa truncation characteristic of subtype C viruses, and in addition, had a further 3 aa (Gly CysCys). Rev is a necessary regulatory factor for HIV expression and changes in the protein may affect viral replication. These results suggest that slower HIV disease progression in these children may be attributed to an altered Rev protein.

CELL BIOLOGY LABORATORY

CHARACTERIZATION OF NEAR-FULL LENGTH GENOME SEQUENCES OF THREE SOUTH AFRICAN HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 SUBTYPE C ISOLATES

As subtype C is the most prevalent circulating Human Immunodeficiency Virus Type 1 (HIV-1) subtype internationally as well as locally in South Africa, more information on the biological nature and molecular characteristics of these viruses is required. Proviral DNA was isolated from primary cultures of three South African R5 isolates and the near-full length genome amplified by PCR. The resultant PCR product was cloned into the pCR-XL-TOPO vector and a representative clone from each isolate sequenced by primer walking. Phylogenetic analysis showed all three clones clustered within subtype C with a bootstrap value of 100%, and no recombination with other subtypes was identified by distance scan and bootscan analysis. Analysis of the potential coding regions revealed premature truncations of the second *rev* exon but no other potential structural distortions nor frameshift mutations in the open reading frames. All the clones contained three potential NF-kB binding sites, a feature unique to subtype C viruses. The tips of the V3 loops contained the GPGQ sequence motif characteristic of CCR5-utilising subtype C strains, as well as relatively low overall net positive charge characteristic of non-syncytium-inducing isolates. This information contributes to our overall knowledge of circulating strains in South Africa and to the making of effective vaccines and chemotherapeutic agents.

CCR5 ?32 HETEROZYGOSITY IS ASSOCIATED WITH AN INCREASE IN CXCR4 CELL SURFACE EXPRESSION

CXCR4 and CCR5 are the major coreceptors utilized by HIV-1 to gain entry into CD4-bearing cells. CXCR4 is utilized by T-cell tropic or X4 and CCR5 by macrophage-tropic or R5 HIV-1 strains. Macrophage inflammatory protein (MIP)-1?, MIP-1? and regulated on activation, normal T-cell expressed and secreted (RANTES) are the specific ligands for CCR5, and stromal cell-derived factor-1 (SDF) the natural ligand for CXCR4. Individuals homozygous for a 32 bp deletion within the CCR5 gene are highly resistant to infection with HIV-1. This deletion results in an unstable truncated form of the protein that is not expressed at the cell surface. Those HIV-1 infected individuals heterozygous for the 32 bp deletion (?32/wt) present with a slower decline in CD4 T-cell counts, lower viral loads, and increased time for progression to AIDS. The expression of CXCR4 and CCR5 is reciprocally modulated *ex vivo* by incubation with interferon-and by standing time before staining, and *in vivo* in patients infected with HIV-1 and *Mycobacterium tuberculosis*. Previous work has shown that individuals heterozygous for the 32 bp deletion have decreased percentages of CCR5-expressing CD4 T-cells as compared with individuals without the mutation (wt/wt). Thus this study was undertaken to determine if lower CCR5 surface expression in CCR5 ?32/wt individuals would result in a concomitant increase in surface expression of CXCR4 in these individuals.

Cross-sectional analysis was performed on a total of 20 healthy individuals with no known disorders,

comprising 9 CCR5 ?32/wt individuals, and 11 CCR5 wt/wt individuals, median age 38 (range 26-62 years) and 39 (range 25-57), respectively. In this study the density of CCR5 and CXCR4 expression, in terms of number of antibodies bound per cell (ABC), in addition to the percentage of cells expressing these antigens was measured. The cell subsets evaluated for both CCR5 and CXCR4 expression were $CD3^+$, $CD4^+$, $CD8^+$, NK, $CD19^+$ and $CD14^+$ cells. Granulocytes were analysed for CXCR4 expression only, as these cells do not express CCR5. When data were analysed according to percentage of cells expressing either CCR5 or CXCR4, a significant reduction in CCR5 expression on $CD3^+$ ($P<0.05$) and $CD4^+$ ($P<0.01$) cell subsets from CCR5 ?32/wt individuals in comparison with CCR5 wt/wt individuals was found, with a trend to this effect on the other cell subsets. However, no difference in the percentage of cells expressing CXCR4 was observed between the two groups. When the number of ABC were analysed, the number of CCR5 ABC was found to be reduced on $CD3^+$ ($P<0.001$) (Fig. 4A), $CD4^+$ ($P=0.001$), $CD8^+$($P=0.001$), NK ($P<0.05$) and $CD14^+$ ($P<0.05$) cells from the CCR5 ?32/wt individuals when compared with CCR5 wt/wt individuals, with a trend to that effect on $CD19^+$ cells. Conversely, an increase in CXCR4 ABC on $CD3^+$ cells ($P<0.05$) was observed from CCR5 ?32/wt individuals when compared with CCR5 wt/wt individuals (Fig. 4B), with a trend to that effect on $CD4^+$, $CD8^+$, $CD19^+$ and NK cells. Interestingly, there was a negative correlation between the number of CCR5 ABC on $CD3^+$ cells ($r = -0.748$, $P<0.05$) and NK cells ($r = -0.841$, $P<0.05$) and a positive correlation between the number of CXCR4 ABC on $CD4^+$ cells ($r = 0.672$, $P<0.05$) and the age of the ?32/wt individuals. However, these correlations were not evident for the wt/wt individuals.

As we have previously found circulating levels of SDF-1 to be inversely correlated to surface levels of CXCR4 expression, the question arose as to whether such differences would be evident between CCR5 ?32/wt individuals and the CCR5 wt/wt individuals. We found significantly lower SDF-1 levels in the heterozygotes (median, 1037.32 pg/ml) than in CCR5 wt/wt individuals (median 1641.39 pg/ml) ($P<0.05$) (Fig. 4C), thus mirroring the results found for CXCR4 ABCs on $CD3^+$ cells.

In summary, CCR5 ?32/wt individuals have lower CCR5 expression with respect to both percentage of cells expressing CCR5 as well as ABC than wt/wt individuals. In addition, there was a concomitant increase in the level of CXCR4 ABC on $CD3^+$ cells in these individuals, however, this was not reflected in the percentage of cells expressing CXCR4. We anticipate that the compensatory increase in CXCR4 would be greater in individuals homozygous for the ?32 mutation than that found for CCR5 ?32/wt individuals. Of further importance was the finding of lower circulating SDF-1? levels in the CCR5 ?32/wt individuals. The mechanism underlying this reciprocal increase in CXCR4 expression associated with a lower CCR5 expression is currently unknown. The simplest hypothesis may be that lower CCR5 expression through some unknown mechanism leads to a down-regulation of the amount of circulating SDF-1? , resulting in less SDF-1? available to bind to and consequently down-regulate CXCR4, the outcome of which is increased CXCR4 expression. An important function of the chemokine/chemokine receptor system is to control leukocyte migration. SDF-1? , as well as MIP-1? , MIP1-? and RANTES induce chemotaxis of T-cells. Thus, individuals heterozygous for the CCR5 ?32, may have increased CXCR4 expression on T-cells to compensate for the reduction in CCR5 expression on these cells. Although, the CCR5 ?32 gene is associated with delayed HIV-1 disease progression, this protective effect is lost when these individuals are infected with syncytium-inducing (SI) CXCR4-utilizing HIV-1 strains, and the greater CXCR4 receptor density in these individuals begs the question as to whether more rapid disease progression is likely to occur than in CCR5 wt/wt individuals who express less CXCR4, when infected with SI viruses. Why age-related changes were found with respect to CXCR4 and CCR5 ABC in the CCR5 ?32 heterozygotes, and not the wt individuals is currently not known. This work provides evidence for a compensatory increase in CXCR4 expression (and a reduction in peripheral SDF-1? levels) in individuals heterozygous for the CCR5 ?32 mutation, identifying the existence of a common axis directly linking the regulation of cell surface expression of CCR5 and CXCR4. This may further help explain why HIV-1 pirates the use of these two particular receptors as the major coreceptors for facilitating its entry into permissive cells.

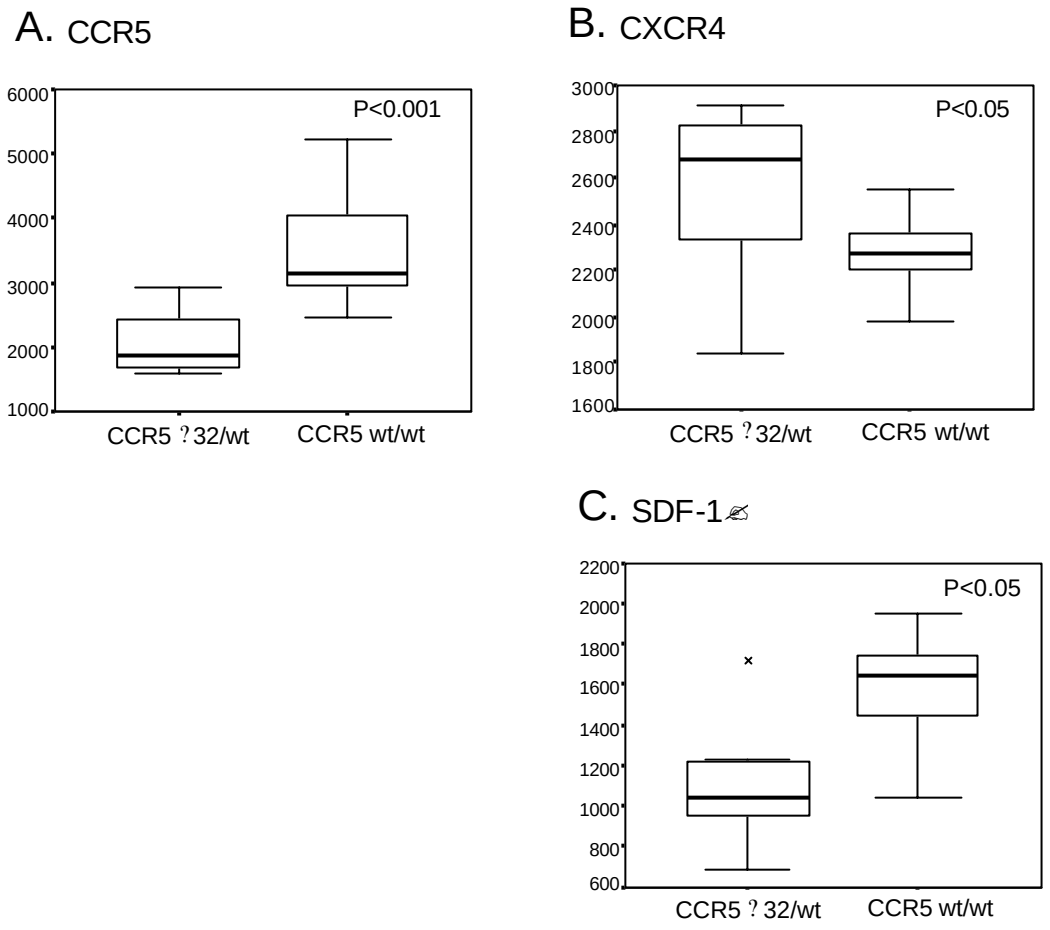


Fig 4. Antibodies bound per cell (ABC) of CCR5 (A) and CXCR4 (B) on CD3+ cells, and plasma levels of SDF-1? (C) from CCR5?32/wt individuals and CCR5 wt/wt individuals. All samples were stained within 2h of collection using highly purified PE-labelled antibodies to CCR5 and CXCR4 with a PE molecule-to-antibody ratio of 1:1, and quantitation was performed gating on coreceptor-expressing cells using the commercially available QuantiBRITE system (Becton Dickinson, San Jose, Calif.). Plasma SDF-1? levels were determined using the Quantikine human SDF-1? enzyme-linked immunosorbent assay (ELISA) systems (R & D Systems, Inc., Minneapolis, United States of America), according to the manufacturer's instructions. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Comparison of CCR5 and CXCR4 ABC and SDF-1? levels between different study groups was done using the Mann-Whitney U test with SPSSR software (SPSS Inc., Chicago, Illinois). Significant differences between the groups are indicated.

IMMUNOLOGY LABORATORY

We focused much of 2002 on validating assays for use in the forthcoming HIV vaccine trials and will continue to do so during 2003. The laboratory has been accredited by SANAS for PBMC isolation and cryopreservation and the aim for 2003 is to have the laboratory accredited for the IFN-g ELISPOT assay.

We also saw the completion of the HIVNET 028 study where we have been tracking the natural course of HIV-1 infection. We developed a full-genome CTL screen using sets of overlapping peptides matching South African vaccine strains and are currently identifying correlations with viral load. We co-organised two workshops in 2002. The first was held in Cape Town, in collaboration with the University of Cape Town, where all major data-producers from the HIVNET 028 study grouped to discuss data generated and papers to be published. The first day was spent allowing young scientists to make presentations and the second day was spent discussing the best way to plan for publications. Invited speakers and participants included: Chip Sheppard, Richard Donavon, Carl Hanson (California Health Services), Guido Ferrari, David Montefiori (Duke University) and Bette Korber (Los Alamos). Local investigators from Universities of Cape Town and

Western Cape were also present. Drs Gray, Williamson and Morris collectively presented some of these data at an invited plenary session at the October meeting of the HIV Vaccine Trials Network (HVTN). The second workshop was co-organised with Duke University, Henry F Jackson Foundation and the World health Organisation and focused on "Application of the IFNg ELISPOT assay to monitor cellular immune responses in HIV-1 vaccine-related research in Africa". Participants from 16 African countries were present and wet aspects of the workshop were held in the CTL labs of the NICD. The main thrust and outcome of the workshop was to establish an ELISPOT network in Africa for sharing knowledge, expertise and reagents.

We have also continued our close collaboration with the HIV Vaccine Trials Network and Dr Gray co-chairs the Laboratory Sciences Sub-committee, and sits on the Phase III Intercurrent Infection committee and Training Executive Committee. We have continued enthusiastic support from SAAVI as the central immunology laboratory for HIV vaccine trials in South Africa.

The following description describes subtype C HIV-1 viral dynamics, CTL immune responses and HLA genotype frequencies from the HIVNET 028 study. The data generated has enabled us to 1) understand HIV-1 infection in recently infected individuals as a prelude to understanding HIV-1 vaccine responses; 2) develop the assays required for measuring vaccine-induced immune responses.

SUBTYPE C HIV-1 VIRAL DYNAMICS

As HIV viral load is an important surrogate endpoint in vaccine trials, it is important to judge the likely impact of HIV-1 vaccines by defining viral dynamics in unvaccinated infected individuals. Plotting $\log_{10}$ viral load levels over time from seroconversion in participants with a minimum of three consecutive measurements (n=50) showed the majority of participants to have viremia of 4.0 ± 0.5 $\log_{10}$ RNA copies/ml. When viral load levels were divided by time from seroconversion, the median $\log_{10}$ between 2-6 months (29 time points) = 4.01 (3.48-4.53, IQR); 7-12 months (64 time points) = 3.82 (3.43-4.4 IQR); 13-18 months (73 time points) = 4.02 (3.46-4.38, IQR); and >18 months (85 time points) = 3.84 (3.29-4.36, IQR). Using a basic model of virus dynamics we were able to use the CD4 and viral load data to fulfill estimations of viral equilibrium or set-point. Thirty-four out of 50 participants fulfilled the criteria of using the model by having five consecutive viral load measurements and four corresponding CD4 measurements. After applying the model and plotting the trajectory of viral loads it was possible to assess that viral load oscillations did not attain equilibrium until 400-500 days (12-18 months) after seroconversion and the median set-point distribution was 4.03 $\log_{10}$ (10715 RNA copies/ml). Confirmation of viral loads using different assays was used to validate the magnitude of viremia as well as allowing viral load estimations to be compared to other published cohorts. Selected samples were sent blinded to two external laboratories for measurement of RNA copies either by Nuclisens (NASBA) or ultra-sensitive Roche Amplicor 1.5. There was a significant correlation ($r=0.81$; $p<0.0001$) between bDNA and Nuclisens (NASBA), although six samples were <125 copies/ml using NASBA corresponding to a geometric mean of 2143 RNA copies/ml (range of 1241-3876) using bDNA. When samples were compared with the standard Roche Amplicor 1.5 assay there was a highly significant correlation ($r=0.87$; $p<0.0001$), although the standard Roche Amplicor 1.5 was consistently measuring a median of 0.57 $\log_{10}$ RNA copies/ml > bDNA. Comparisons between replicate subtype C viral load measurements using bDNA and Roche with subtype B viral load measurements from the San Francisco Men's Health Study (SFMHS) and Infected Participant Cohort (IPC) show that subtype C viral loads were not significantly different from recently infected subtype B HIV-1 infected male cohorts. The geometric mean of subtype C viral loads within the first 12 months from seroconversion were 27791 RNA copies/ml; SFMHS was 17542 RNA copies/ml (n=35); and the IPC was 18165 copies/ml (n=104).

CTL IMMUNE RESPONSES

We have continued to use the IFN-g ELISPOT assay using sets of overlapping peptides to cover nine gene regions; five sets being based on subtype C HIV-1 vaccine strains (Du151, Du 422 and Du179). Our aim has been to epitope map the full subtype C HIV-1 genome and to determine the breadth and hierarchy of responses in relation to viral load and genetic sequence diversity. We have screened 36 participants for responses to

each gene region where the order of cumulative responses are Nef > Pol > Gag > Env > Vif > Vpr=Tat > Rev > Vpu. Nef appears to be the most immunogenic, with Vpu the least. Fine epitope mapping in Gag revealed that 61% of epitopes fell within the p24 region, where three highly immunodominant regions were targeted (MFTALSEGATPQDLNTML, TSNPPIPVGDIYKR-WIILGL and EPFRDYVDRFFKTLRA). Thirty-four percent of responses fell within the p17 region and fewer (5%) fell within the p15 region. When responses in Gag were plotted against time after seroconversion, it was evident that one p17 epitope (RSLYNTVATLY) and two p24 epitopes(TSNPPIPVGDIYKRWIILGL and LLVQNANPDCKTILKALGPG) emerged later in infection and were absent in participants within the first 12 months. Those individuals who responded to Gag showed a 0.6 $\log_{10}$ difference ($p<0.05$) in viremia when compared with individuals who showed no response to Gag (33% of participants). The highly immunogenic nature of Nef has also been examined and 75% of all responses fell within 77 amino-acids found in the central region of the molecule. HLA restrictions have shown that two very dominant Nef epitope regions (YKAAFDLSFFLKEKG and PGPGVRYPLTFGWCF) are restricted by A30/A2 and B44 respectively.

HLA GENOTYPE FREQUENCIES

Distribution of HLA-A2, -A30, -B15, -B58, -Cw6 and -Cw7 constitutes the bulk of HLA cover in peoples found in the study. Closer inspection revealed that HLA-A2, A29, A30, A68, B15, B58, Cw2, Cw4, Cw6 and Cw7 are expressed at an allele frequency >0.1, with A29, B58 and Cw6 over-represented in HIV-1 positive participants (allele frequency of 0.154, 0.186 and 0.265 respectively versus 0.075, 0.1 and 0.125 in the HIV-1 negative group). Analysis of HLA haplotypes revealed linkage disequilibrium, most notably for HLA-A29-B42 (2.9% haplotype frequency); A29-B58 (2.9%); A74-B58 (2.9%); A74-B15 (4.8%); A30-B42 (5.8%); A30-B58 (5.8%). For extended haplotypes, HLA-A30-B58-Cw07 and HLA-A74-B15-Cw02 was the most common with a frequency of 3.5% each. These data show that subtype C HIV-1 infected individuals in southern Africa have unique HLA types, divergent from most subtype B infected cohorts studied to date.

HLA LABORATORY

The development of an efficacious vaccine for HIV-1 would have to contend with our incomplete understanding of the complex immune responses against a backdrop of a constantly evolving virus. HIV-1 evolution occurs fairly rapidly the result of high rates of replication, recombination and mutations, the result of a lack of fidelity of the reverse transcriptase. In addition, viral evolution occurs in the absence of and adapts in the presence of immune responses. The complexity of immunogenetic backgrounds, reflected in terms of haplotypes that would include HLA, in the control of HIV-1 viral replication has certainly been an area of intense research. HLA is a component albeit an important one in understanding aspects of cellular responses and viral evolution.

The Laboratory has completed the objective of performing low resolution typing of HLA to determine the allele distribution across the different South Africa population groups. Three hundred and seventy-eight individuals were typed for this project including 278 Black individuals and 100 Caucasians. There are distinct differences between the two broad groups. Thus alleles such as A*01, A*02 and A*03 account for a large proportion of the alleles in the Caucasian group and by comparison alleles such as A*02, A*30 and A*68 form the major alleles in the Black population. Similar comments apply to the B locus where distinct differences are noted for B*07, B*08 and B*42, B*44 and B*58 between the two groups (Table 2). We have demonstrated the wide diversity of alleles present in the various South African populations as well within the groups. The diversity is further emphasised when one considers the three loci haplotypes (Table 3). It is evident that there are no shared haplotypes between the two broad groups. The results will be part of the broader bioinformatics systems that will become available to interested scientists. In addition, we hope that the data will inform about decisions centred on vaccine design or expected results in vaccine trials.

TABLE 2 : HLA CLASS I ALLELE FREQUENCIES OF SA POPULATION

Allele	Caucasian	Black
A*01	19.80	3.10
A*02	29.70	16.06
A*03	11.39	6.57
A*30	4.46	22.26
A*68	2.48	12.04
B*07	15.66	5.73
B*08	11.62	7.44
B*15	13.13	18.51
B*42	0.00	11.64
B*44	12.63	6.30
B*58	3.03	16.79
Cw*03	12.38	5.88
Cw*04	9.41	12.13
Cw*06	7.92	15.07
Cw*07	35.15	23.35
	0.50	13.05

TABLE 3 : COMMON HAPLOTYPES (>2%) IN THE SA POPULATION

Caucasians (n=202)			Eastern Migration[1] (n=334)		
Haplotype	**Frequency %**	**Eastern Migration**	**Haplotype**	**Frequency %**	**Caucasians**
A*01 B*08 Cw*07	**8.91**	-[2]	A*02 B*45 Cw*16	2.39	-
A*01 B*57 Cw*06	2.97	-	A*02 B*58 CW*07	4.72	-
A*02 B*07 Cw*07	**5.23**	-	A*03 B*58 Cw*06	3.20	-
A*02 B*15 Cw*03	**6.20**	-	A*29 B*42 Cw*17	2.93	-
A*02 B*18 Cw*07	2.90	-	A*30 B*08 Cw*07	2.36	-
A*02 B*27 Cw*02	3.96	-	A*30 B*42 Cw*17	**5.95**	-
A*03 B*07 Cw*07	**6.33**	-	A*68 B*58 Cw*06	3.85	-
A*11 B*35 Cw*04	3.46	-			
A*29 B*44 Cw*16	2.97	-			

1: Eastern Migration: Zulu; Xhosa; Swati; Tsonga
2: Haplotype frequency is <1% or absent in population

There is usefulness in establishing the background HLA frequencies in terms of vaccine preparedness. However, perhaps an informative approach when considering certain vaccine approaches or applying HLA data to viral evolution high resolution data is preferred. This is particularly so given the HLA diversity observed. An example of where high resolution comes into play in is the use of supertype families in epitope vaccine design. In order to make sense of the great diversity observed between various populations, alleles have been grouped based on shared features in terms of epitope binding and in particular binding motifs. The specific residues of the epitope that influence binding are in position 2 and the C terminus. Based on this approach ten supertype families have been described viz., A1, A2, A3, A24, B7, B8, B27, B44, B58 and B62 . Thus, for example, in the A2 supertype family the alleles include A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0206, A*6802 and A*6901. The ten supertype families could cover close to 100% of the population. One possible practical value of such an approach is that a minimal number of epitopes can be identified to cover different populations despite great allelic variation. We have performed sequence based typing (SBT) on 64 individuals for Class I, A and B loci only. Our initial analysis has shown that supertype allelic coverage is

approximately 92%. Currently A*30 has not been assigned to a specific supertype family. The frequency of A*30 is ~7%.

We are currently performing high resolution HLA Class II typing using SSP-PCR. We have HLA typed 50 individuals including 25 Zulu and 25 Xhosa individuals. There are certain obvious differences between the two groups e.g. DRB1*0804 and -*1301. There is reasonable evidence to suggest that Class II alleles are implicated in the control of viral replication. The most likely scenario is the role for CD4 help for full function of CD8 cells or at least in the process of CD8 cell maturation. Once the data is complete we would integrate the data into the haplotype frequencies that we have already reported on with a view to determining the relationship between the various alleles with regard to disease protection/susceptibility and vaccine design. Interestingly DRB1*01 that has been previously reported to independently associated with resistance has a low frequency in our study population but an allele such DRB1*1301 that has also been described to be associated with resistance is well represented in our data.

The Laboratory is SANAS accredited and participates in the NEQAS Scheme for HLA typing.

Virology - *Specialized Molecular Diagnostics Unit*

The Molecular Diagnostics in terms of the remits of the NICD focused on project-based approaches to answer specific questions. An integrated approach of molecular, serological and epidemiological research was undertaken jointly by INSERM (Institut National de la Santé et de la Recherche Médicale, Paris, France) and the NICD. The model and a data set is a presented below.

BACKGROUND

Several studies have evaluated the feasibility of delivering antiretroviral therapy (ART) to patients in resource-limited settings, but despite international pressure to implement highly active antiretroviral therapy (HAART) in such countries, treatment requirements have yet to be precisely characterised. In developing countries with high HIV prevalence, such as South Africa, the fraction of the population who require HAART is not precisely known. Such information is needed to calculate the cost of scaling up antiretroviral treatments and to prepare health systems to deliver these treatments. In addition, the potential impact of the widespread use of antiretroviral drugs on the spread of HIV remains unclear and requires evaluation. There are biological and epidemiological reasons to believe that antiretroviral therapy will reduce sexual transmission of HIV. Biological studies have shown that antiretroviral drugs decrease HIV in seminal fluid and in cervicovaginal secretions. An epidemiological study of discordant couples has shown that the use of zidovudine by infected men was associated with a 50% reduction in the risk of transmission of HIV to their female sexual partner, suggesting that the wide use of HAART would slow down the spread of HIV in the countries where the route of transmission is mainly heterosexual.

The objectives of this study are firstly to estimate the proportion of the population who need HAART in a township in South Africa and secondly to estimate the short-term impact of providing anti-retroviral therapy on the spread of HIV.

METHODOLOGY

Survey

In April 2002, a population-based, cross-sectional study was conducted in a township 40 kilometres south of Johannesburg, South Africa. Households were selected by a two-stage random sampling technique. Index houses were randomly selected from a map obtained from the local municipal offices. Using each index house as a starting point, a cluster of households was identified by starting to the right of the index house and counting households around the street block and adjacent street blocks until 50 households had been reached. A self-weighting random sample of 20 households was then chosen from each cluster. All men and women aged 15 to 49 years, who slept in the selected households the night before the study team's visit, were eligible for inclusion in the study. The consent form was presented in the language of the respondent, who was invited to take part in the study, and those who agreed were asked to sign the consent form. The response rate was 68%. Eligible participants were transported to a local facility for the

interviews and the collection of blood and urine samples. If eligible participants were not at home, the study team made up to three repeat visits on different days at different times. The fieldwork was done in the late afternoon, when residents returned from work, to reach as many residents as possible. Fieldwork was conducted on Monday to Thursday and again on Saturdays to ensure that people who work during the week could be reached. Where it was not possible for participants to go directly to the interviewing points, or for household members who were not home, appointments were made at times suited to the participants and these appointments were followed up.

The questionnaire used in this study was based on a UNAIDS questionnaire. The interviewers completed the questionnaire during a private interview in the preferred language of the interviewee. Data were collected on background and behavioural characteristics. Sexual partners were divided into spousal and non-spousal partners. The spousal partners were partners to whom the respondents were married or lived with as married. The non-spousal partners were all the other partners. The questionnaire allowed for a detailed description of all the non-spousal partners of the last twelve months, including those with whom the respondent had only one sexual contact. In addition, specific questions were asked about the use of condoms in the last months with non-spousal partners.

During the survey, participants with symptoms of sexually transmitted infections (STIs) were encouraged to go to the local STI clinic for treatment. Participants who wished to know their HIV status were offered a separate free ELISA test with pre- and post-test counselling to be arranged through the normal clinical channels. Blood samples were tested for syphilis, HIV-1, CD4+ count and plasma HIV-1 RNA load. Urine samples were tested for chlamydial infection.

When results were available, a trained nurse delivered the syphilis and chlamydia infection test results directly to the participants. Participants with positive STI results were encouraged to seek treatment at the local STI clinics. Individuals with fewer than 200 CD4+ cells/mm^3 were included in a specific programme that involved voluntary counselling and testing, prevention of opportunistic infections and access to ARV therapy. Pregnant women were informed of the possibility of reducing the mother-to-child transmission (MTCT) of HIV-1 during pregnancy and childbearing. The cost of transportation to health facilities where the MTCT programme was available free of charge was carried by the study.

LABORATORY PROCEDURES

Following the interview, trained nurses collected whole blood and urine (first flow) samples. The urine samples were stored at 4°C and then transported the next day to the laboratory where they were stored at -70°C before being analysed. Two EDTA blood tubes of 20 ml of venous blood were taken and transported at room temperature to the laboratory on the following morning. One tube was centrifuged at 400 *g* for 10 minutes and 5 aliquots of plasma were then taken and frozen at -70° C. The second tube of blood was used to determine the CD4+ count.

An ELISA screen (Genscreen HIV1/2 version 2, Bio-Rad, France and Wellcozyme HIV recombinant, Abbot Murex, Dartford, UK) and ELISA confirmation (Vironostika HIV Uni-Formm II plus O, bioMerieux, Boxtel, Netherlands) were carried out on plasma to test for HIV-1 infection. Plasma HIV-1 RNA load was determined by reverse-transcription PCR using an assay designed to detect all M-group subtypes (Amplicor HIV-1 Monitor Test V1.5, Roche Diagnostic System Ins., Branchburg, New Jersey, USA).

CD4+ cell counts were determined by BD FACSOUNT analysis, BD Biosciences, Belgium. Samples with CD4+ counts lower than 500 cells/mm^3 and HIV-1 negative by serology were tested with a Western Blot test (New LAV Blot I, Bio-Rad, France) and for plasma HIV-1 RNA load as described above.

Syphilis testing was performed using a rapid plasma reagin (RPR) screen (Macro-Vue RPR Card Tests, Becton Dickinson Microbiology Systems, Becton Dickinson, Maryland) followed by a Fluorescent Treponemal Antibody Absorption (FTA-ABS) confirmatory test (FTA-ABS Test Sorbent, bioMérieux, France). A positive RPR (at any titre) and FTA-ABS were taken as evidence of recently acquired and/or untreated syphilis. Urine samples were tested for chlamydia infection using a qualitative DNA amplification method (Amplicor CT/NG Test, Roche Diagnostics, New Jersey, USA).

ETHICS

Ethical clearance was obtained from University of Witwatersrand on the 8 February 2002 (protocol study n° M020103).

DATA MANAGEMENT

Laboratory results and data generated from questionnaires were entered twice into a database (Microsoft Access, Redmond, Washington, USA) by different people. The two entries were compared and discrepancies were corrected. The data were then checked for inconsistencies. The files were then imported into the Statistical Package for Social Sciences (SPSS 8.0 for Windows, Chicago, Illinois, USA) and prepared for statistical analysis.

RESULTS

A total of 930 people agreed to participate in the survey.

PREVALENCE OF HIV-1, SYPHILIS & CHLAMYDIAL INFECTIONS

The overall prevalence of HIV-1 infection was 21.8% (19.2% – 24.6%), 17.4% (14.1% – 21.4%) among men and 25.7% (21.9% – 30.0%) among women. The highest prevalence of HIV-1 by age was 34.4% (19.2% - 53.2%) among men aged 35 to 39 years and 46.4% (34.4% – 58.7%) among women aged 25 to 29 years. The median age of HIV-1-infected people was 31 (IQR 26 – 37) years for men, and 23 (IQR 19 – 32) years for women. Among those having a spousal and those having a non-spousal partnership, the prevalence of HIV-1 was 25.6% (21.1% – 30.7%) and 23.8% (20.1% – 27.9%), respectively. The prevalences of syphilis and chlamydia were both 6.6% (5.1% – 8.4%), with a male-to-female ratio of 1:3.0 and 1:1.1, respectively.

DISTRIBUTION OF PLASMA HIV-1

The median (IQR) plasma HIV-1 RNA load was 55,750 (10,750 – 172,000) copies/ml, and the difference between men and women was not significantly different (Kruskal-Wallis test, $p = 0.59$). The median (IQR) plasma HIV-1 RNA load in participants with CD4+ counts less than 200 cells/mm^3 was 160,000 (72,900 – 410,000) copies/ml and in participants with CD4+ counts higher than 200 cells/mm^3 it was significantly lower at 46,800 (9,407 – 149,500) (Kruskal-Wallis test, $p = 0.000$). The relationship between CD4 counts and plasma viral load is presented in Figure 1.

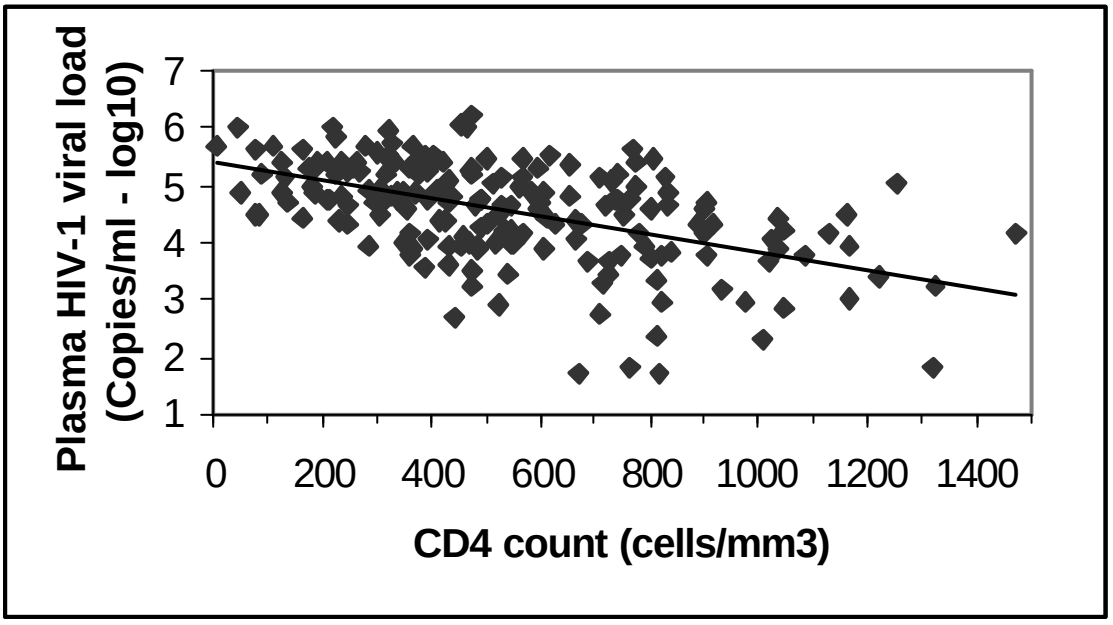


Fig 1. Plasma HIV-1 RNA load by CD4+ count among HIV-1-positive individuals. The regression line is plasma HIV-1 RNA load (copies/ml – log10) = 5.40 – 1.57 10^{-3} CD4+ count (Spearman's $r = -0.53$, $p = 0.000$).

DISTRIBUTION OF CD4+ COUNTS

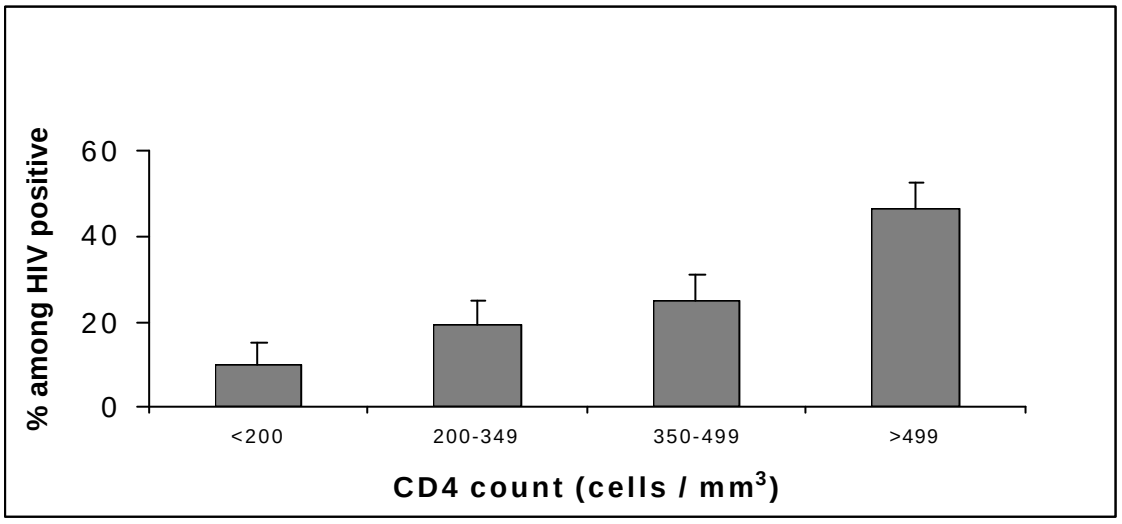
The median (IQR) CD4+ cell counts in the HIV-1-negative and -positive participants was 1,128 (911 – 1,371) cells/mm^3 and 475 (321 – 735) cells/mm^3, respectively, and the difference was statistically significant (Kruskal-Wallis test, p = 0.000). Among HIV-1-negative men, the median (IQR) CD4+ cell counts were 1,057 (850 – 1,316) cells/mm^3 and among women were slightly higher at 1,180 (963 – 1,436) cells/mm^3 (Kruskal-Wallis Test, p = 0.000). Among HIV-1-positive people, the median CD4+ counts was 488 (321 – 740) cells/mm^3 and not statistically different between men and women (Kruskal-Wallis Test, p = 0.17). The distribution of CD4+ cell counts in HIV-1 infected individuals is given in Figure 2.

CONCLUSION

The integrated approach using molecular diagnostic, serological and epidemiological tools proved useful in that a range of data was provided that could influence health policy decisions and assist in monitoring interventions.

FINANCIAL SUPPORT

Funding was received from the Agence Nationale de Recherche contre le SIDA (ANRS-2002-1265), Paris, France, from the National Institute for Communicable Diseases, Johannesburg, South Africa and from the Institut National de la Santé et de la Recherche Médicale, Paris, France.



Staff of the Specialized Molecular Diagnostics Unit

Virology - *Diagnostics & Surveillance*

The National Institute for Communicable Diseases (NICD) employs medical technologists in various departments, mainly within the routine diagnostic section (cell culture, antigen detection and serology), but also in the SPU, HIV/AIDS, and Molecular Diagnostic units. Medical technologists play a key role in providing laboratory support for the country, the region and the continent of Africa for both patient management and various research/surveillance programs.

With the changeover from the National Institute for Virology (NIV) to the National Institute for Communicable Diseases (NICD), this department will now concentrate on surveillance as opposed to diagnostic work with regard to serology. To date various ELISA techniques are in place for the following viruses – HIV, Herpes Type 2 IgG, Rubella, Measles and Syphilis. The virus isolation unit together with cell culture and media production are still intact and there are no changes to date.

In October 2002, the HIV unit participated in the Department of Health's antenatal surveillance for HIV and syphilis whereby 3200 samples from Gauteng Province were processed.

This department once more maintained SANAS accreditation.

Staff of the Diagnostics and Surveillance Unit

Virology - *Diagnostics & Surveillance*

For the past year, technologists involved in routine diagnostic testing received and processed some 22 000 samples amounting to 54 000 laboratory tests.

Five registrars were trained in various techniques and a total of 48 Technikon students visited our Institute to receive first hand information about laboratory tests as they work towards the final Biomedical Diploma.

Our Regional Polio laboratory (WHO/AFRO) trained eight technicians from Zambia, Ethiopia, Kenya, Uganda, Cameroon, and Abidjan in polio diagnosis and laboratory management. The course ran over a period of three weeks and certificates were issued at the end of the course. Mrs Shelina Moonsamy made follow-up visits to these countries and the trainees have already implemented some of the knowledge gained during training. This service together with distribution of both RD and L20B cells to various countries in Africa has improved the quality of cell lines being used and subsequent increase in isolation rate in the fight to eradicate poliomyelitis from the continent of Africa.

The NICD is proud to offer external quality assessment schemes to the National Department of Health (RSA) as well as WHO/AFRO with regard to HIV testing. The National scheme has been going since 1997 while the WHO/AFRO has only been put in place in 2002 with distribution twice a year. Analysing the results from participating laboratories one is happy to see a steady and continuous quality improvement with regard to HIV testing in Africa in general and South Africa in particular.

Lastly, HIV Rapid kit evaluation is gaining momentum with more requests every year. The results are useful to the Department of Health when deciding which rapid test kits to use within the country with regard to sensitivity and specificity.

NATIONAL AFP SURVEILLANCE - LABORATORY SUPPORT

AFP surveillance, as part of the WHO worldwide campaign to eradicate poliomyelitis, has continued throughout the year. All cases of acute flaccid paralysis including Guillain-Barré syndrome, in children less than 15 years of age, or a patient of any age diagnosed as polio by a medical doctor must be regarded as possible polio cases until proven otherwise.

National certification of polio free status depends on the following performance indicators:-

1. Completeness and timeliness of reporting i.e. at least 80% of reports received in time.
2. Sensitivity of the surveillance system i.e. at least one case of non-polio AFP detected per 100 000 children under the age of 15 years. During 2002 this translated into156 cases.
3. Completeness of investigation i.e. all AFP cases should have full clinical and virological investigation. 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).
4. All virological investigations to be conducted in laboratories accredited by the Global Polio Laboratory Network. In South Africa the only accredited laboratory is the National Institute for Communicable Diseases. The NICD also serves as national isolation laboratory for six other Southern African countries i.e. Angola, Botswana, Lesotho, Mozambique, Namibia, and Swaziland. During the year 990 stool specimens were received from patients with AFP from these seven countries. Of these 54 were from patients with onset of paralysis prior to 2002, or patients who were subsequently considered not to have AFP. Of the remainder 339 were from 189 South African cases, and 597 from the six other countries served by the NICD. (fig 1)

A further nine specimens were received in the first two weeks of January 2003 from patients with onset of paralysis in 2002. Case detection rate for South Africa ranged from 0.73 to 1.92, with the overall rate being 1.21.(fig 2) Two or more specimens taken within 14 days of onset were received from 135(71,4%) patients. The percentage of adequate stool specimens per province ranged from 41,67% to 93,332%, with an overall rate of 71,43%. (fig 3) Non-polio enteroviruses were isolated from the specimens of 63 patients, adenoviruses from five, and polio viruses from the specimens of 13 non-South African patients, all of which were identified as Sabin-like. (fig 4)

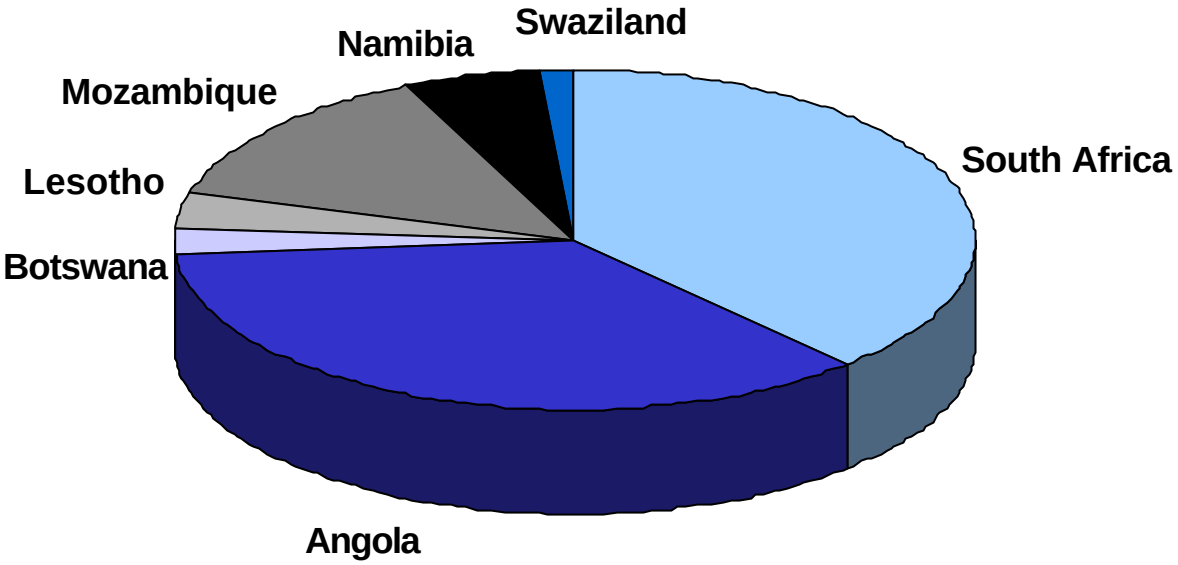


Fig 1. Stool specimens received from patients with AFP - National AFP surveillance.

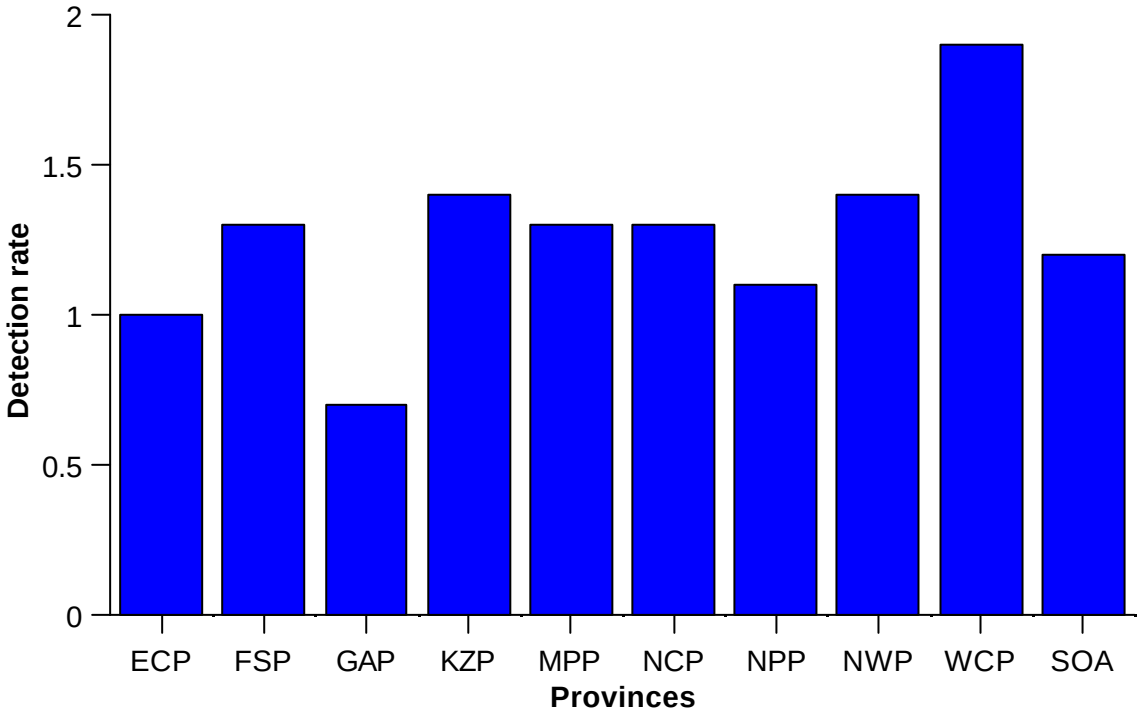


Fig 2. AFP case detection rate per province

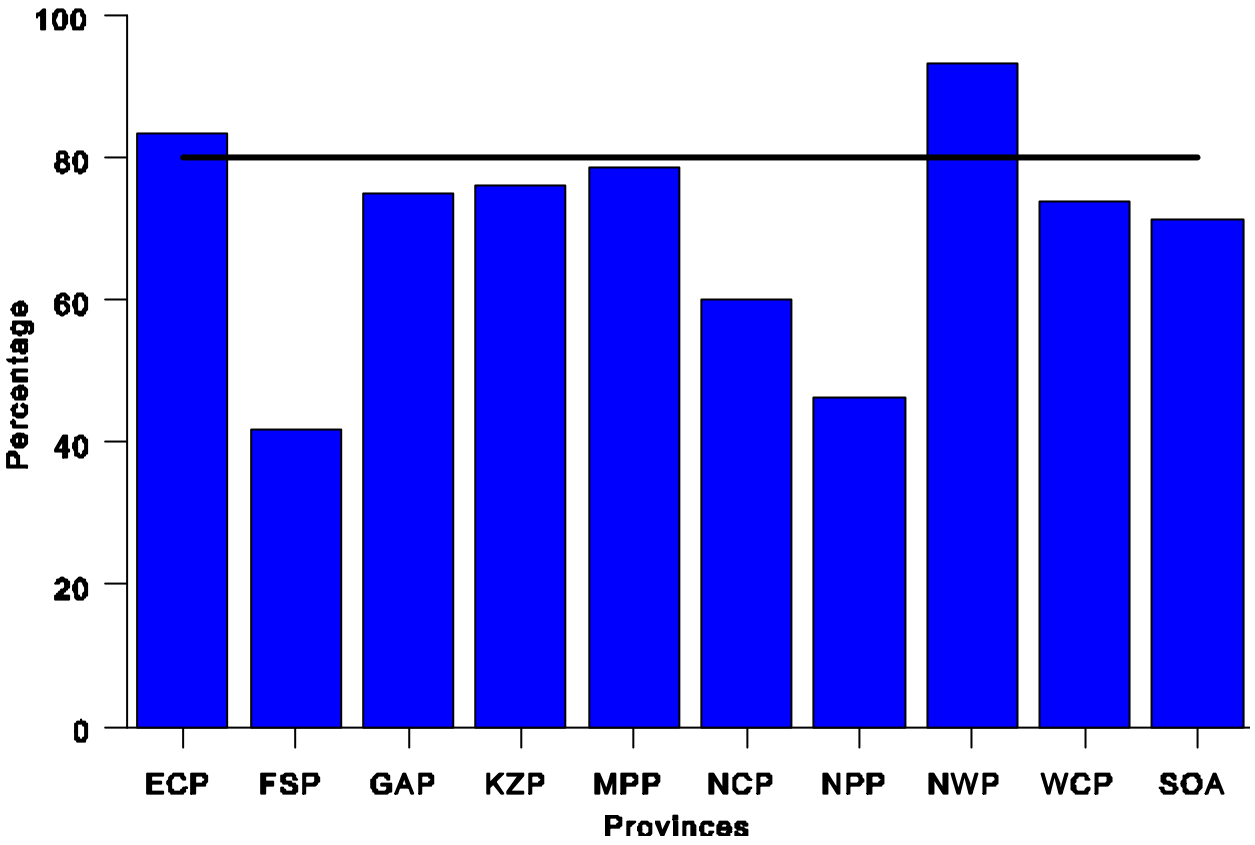


Fig 3. Percentage of cases with adequate stool specimens

RESPIRATORY VIRUS SURVEILLANCE

During 2002 a total of 4400 specimens were received for detection of respiratory virus. The majority (4181) were from an active respiratory surveillance programme. Eighty-seven specimens were received from the Viral Watch programme, started in 1984, which was specifically designed to monitor influenza activity in the community, and detect the type of influenza strains prevalent. The centres currently number 15, and has ranged from 12 to 19. Centres include general medical practitioners, a paediatrician, paediatric out-patient departments at hospitals, university students as well as staff at NIV. Up to three throat swabs per week can be taken by each centre throughout the year from patients with respiratory tract infections of recent onset i.e. within 48 - 72 hours, and without obvious bacterial cause, and transported to NIV in viral transport medium for isolation of virus. The 2002 influenza season was very quiet with comparatively low school absenteeism, showing only a short peak rising above the mean absentee rate calculated over a five year period. (fig 5) However, this was ascribed to the start of the winter school holidays, and diarrhoeal disease. A total of 136 isolations of influenza were made from specimens sent to the NICD during the course of the year. The majority 76 (55,9%) were influenza B, 54 (39,7%) were influenza A H1N1/H1N2, 5 (3,7%) were influenza A H3N2, and 1 (0,7%) was not further identified. The majority 128 (94,1%) of the isolates were made between the last week of May and the first week of September, although sporadic isolates were made both before and after this time. Other respiratory virus isolates made were 10 **parainfluenza** virus, 44 **respiratory syncytial** virus, 4 **adenovirus,** and one untyped respiratory virus. The Viral Watch accounted for 2% of all respiratory specimens with an isolation rate of 25,3%, the other active surveillance for 95% (isolation rate 2,7%), and routine specimens, mainly sent for RSV detection, for 5% (isolation rate 43,9%).

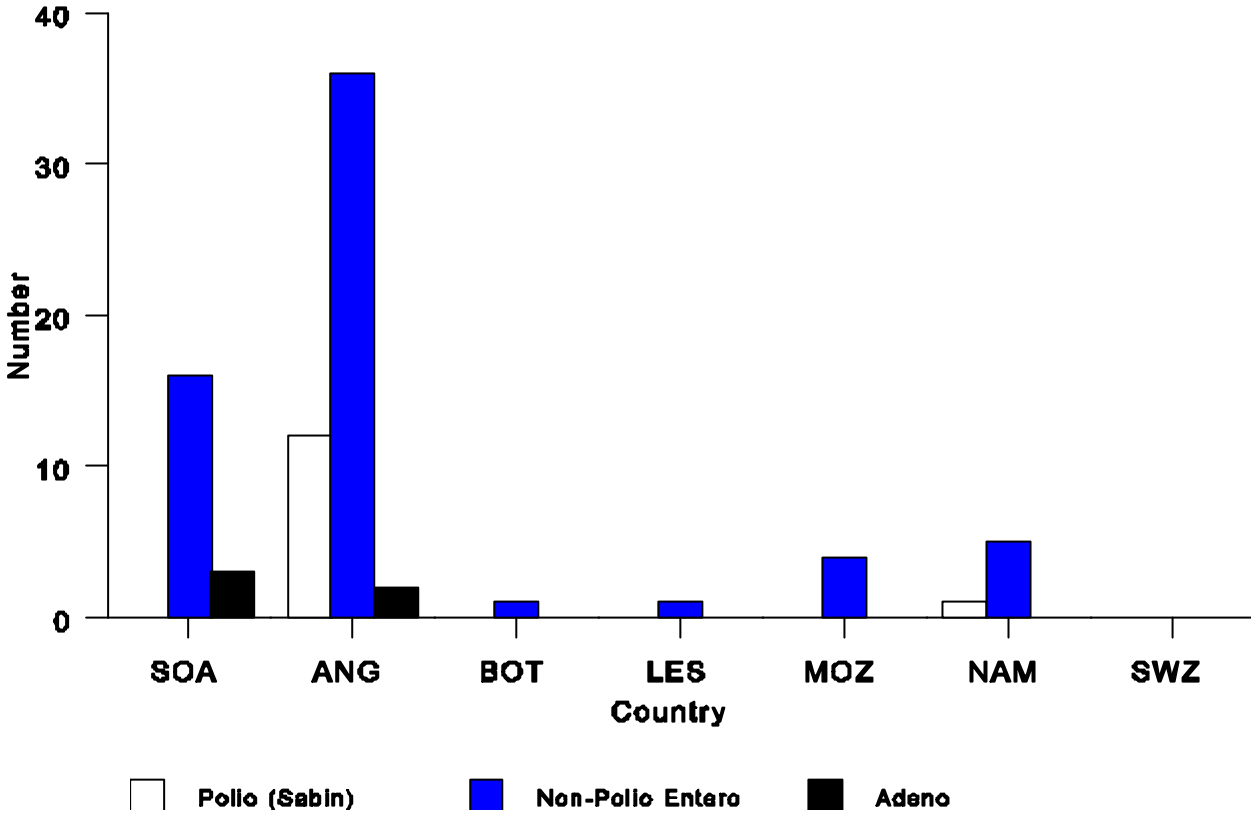


Fig 4. AFP surveillance : Isolations made

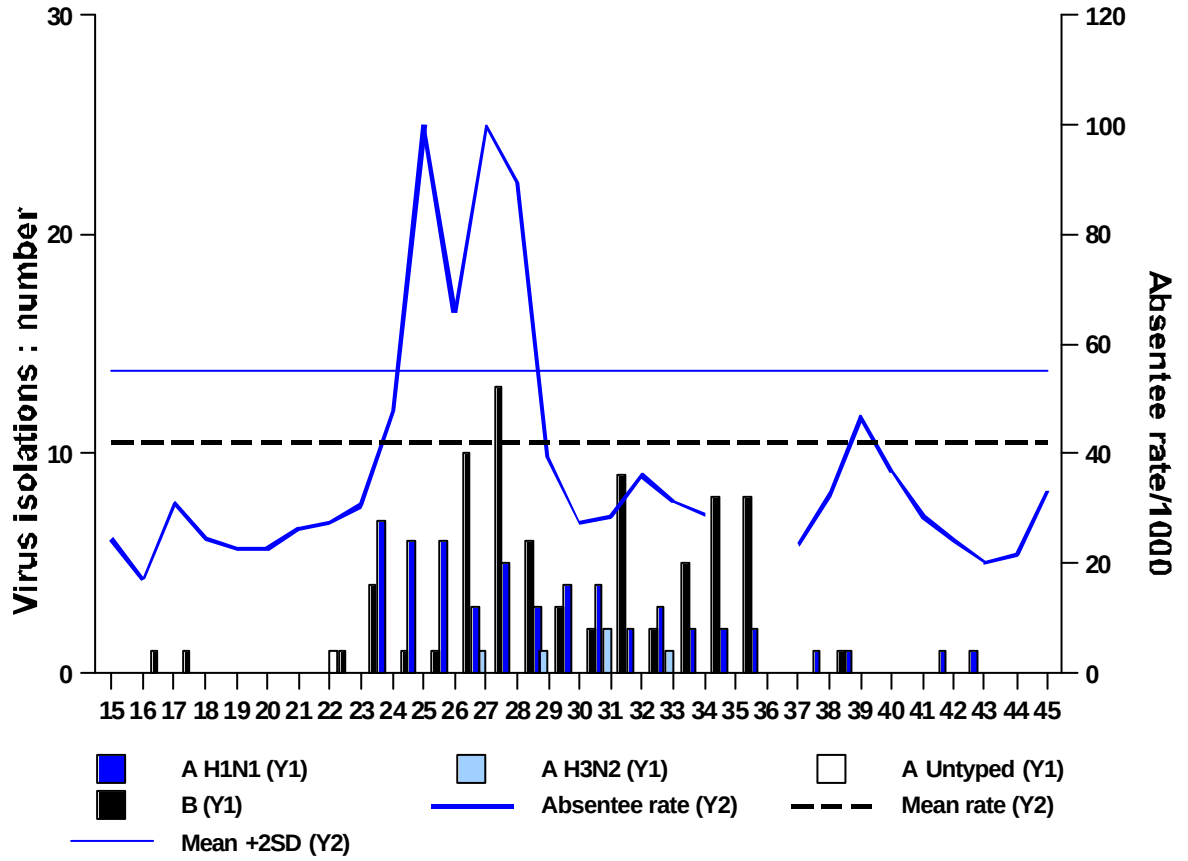


Fig 5. Influenza isolates and school absenteeism

MEASLES AND RUBELLA SURVEILLANCE

Eight hundred and six specimens were received from seven of the nine provinces for **measles** virus serology during 2002 as part of the EPI measles elimination programme. (fig 6) Patients were aged between nine months and 68 years, with a median of seven years. As part of this programme, case-based measles surveillance was started in 1998. Because of the demonstrated lack of specificity of a clinical diagnosis of measles, based on the WHO clinical surveillance criteria (rash + pyrexia + one of the three C's - coryza, cough or conjunctivitis), laboratory confirmation by ELISA IgM serology is a crucial component of the measles elimination programme. However measles IgM antibodies were only detected in 6 (0,7%) of the specimens, whereas 279 (34,6%) of these specimens were found to be rubella IgM positive. (fig 7) Patients with positive measles results were two nine month old infants, two children aged five and 12 years respectively, and two adults. The majority (97%) of those with positive rubella results were under the age of 15 with a range of two to 40 years (median seven).

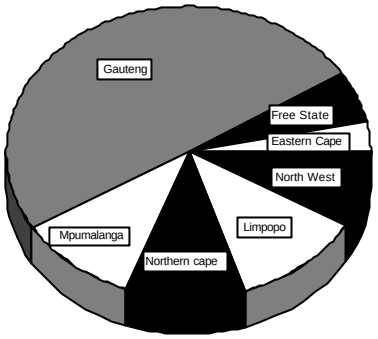


Fig 6. Specimens received for measles surveillance

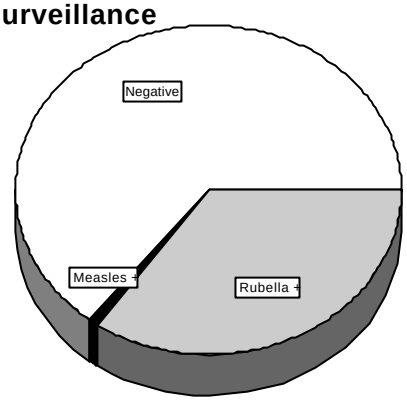


Fig 7. Measles surveillance : Results

REPORT ON DIAGNOSTIC SPECIMENS

(Excluding Special Pathogens Section)

Monitoring of all specimens received at NICD continued throughout the year. In addition to recording the number and types of viruses isolated, as well as the significant serological results obtained, as shown in tables I and II, relevant epidemiological data relating to patients and their clinical conditions were collated.
A total of 6549 specimens were received for isolation/detection of virus. The largest number, 4400 (67,2%) for respiratory viruses, followed by 1272 (19,4%) for enteroviruses and 655 (10,0%) for herpesviruses.(Fig 8a)

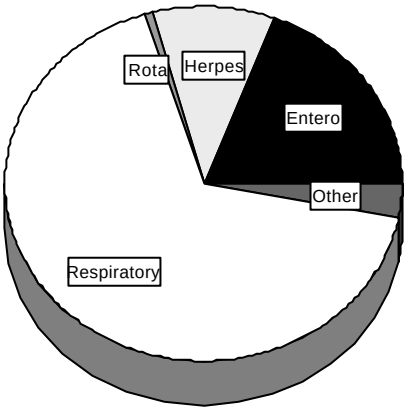


Fig 8a. Specimens received for isolation of virus

Of the 12778 specimens received for serological testing, HIV accounted for 4638 (36,3%) of all blood specimens with the next largest group 3082 (24,1) being measles, mumps, rubella and parvovirus.(Fig 8b)

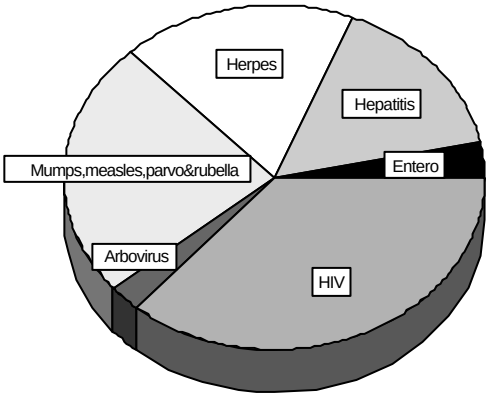


Fig 8b. Specimens received for serology

TABLE I : VIRUS ISOLATION/DETECTION NICD, 2002

	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
Polio	-	-	-	2	-	12	9	2	8	5	-	2	35
Entero, other	15	2	7	17	12	20	9	11	9	5	27	4	138
Adenovirus	2	-	-	-	1	4	-	1	3	3	-	-	14
Rotavirus	-	-	-	-	1	-	-	1	-	-	-	-	2
CMV	4	3	4	4	8	3	9	10	8	8	10	4	75
HSV	1	-	2	-	2	-	1	3	2	-	-	1	12
VZV	1	-	-	-	-	-	-	-	-	-	-	-	1
Influenza	-	1	-	2	2	35	56	33	5	2	-	-	136
Other respiratory	-	1	3	8	6	5	12	7	6	5	1	1	55

TABLE II : SIGNIFICANT SEROLOGY NICD, 2002

	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
Coxsackie B	8	8	9	5	5	4	4	5	11	2	9	3	70
CMV	5	10	7	8	7	17	14	5	12	1	-	-	86
EBV	4	3	2	6	3	6	3	3	3	-	-	-	33
HSV	2	4	4	4	3	1	2	4	1	-	-	-	25
VZV	1	-	1	1	1	1	2	3	3	-	-	-	13
HHV-6	-	-	-	-	-	-	1	-	-	-	-	-	1
Measles	10	1	2	1	1	2	1	3	2	2	-	-	25
Mumps	2	-	6	-	1	2	5	1	-	3	-	-	20
Rubella	2	3	2	11	13	16	10	16	68	66	70	40	317
Parvovirus	1	1	-	1	2	2	2	-	1	1	-	-	11
Hepatitis A	4	1	1	2	1	2	2	1	1	2	-	-	17
Hepatitis B	21	21	11	16	14	14	9	9	13	6	-	-	134
Hepatitis C	6	5	1	1	5	8	6	5	1	3	-	-	41
HIV	250	172	208	211	192	158	237	195	205	20	-	-	1848
Arbovirus	-	-	1	2	-	1	1	-	4	1	-	1	11

PUBLICATION

The **South African Virus Laboratories Surveillance Bulletin** which is compiled, collated and edited at the NICD has continued to be produced monthly from January to November. The bulletin incorporates the positive laboratory results, clinical data and editorial comments from the NIV (University of the Witwatersrand) and the viral laboratories of the medical schools of the Universities of Cape Town, Free State, KwaZulu/Natal, Medunsa, Pretoria, and Stellenbosch. The circulation list includes all medical virology departments, public health officials at State and local government levels, doctors providing material for routine tests and surveillance and many other interested parties in South Africa. It is also mailed to a number of institutions, international bodies, research organisations and universities abroad.

MOLECULAR EPIDEMIOLOGY OF INFLUENZA VIRUS

Influenza is an annual cause of morbidity and mortality world-wide. The principal way to reduce this health problem is by vaccination. Due to the continuous and extensive antigenic variation of the viral surface haemagglutinin (HA) and neuraminidase (NA) proteins, monitoring the antigenicity of viruses circulating each year is necessary to ensure the best possible match between the prevailing viruses and the vaccine strains. During the 2001/2002 winter season in the northern hemisphere, changes in the circulating viruses included the emergence of the influenza A virus subtype H1N2, a reassortant between recently circulating H1N1 and H3N2 viruses. Although the majority of influenza B viruses isolated during this period were antigenically closely related to B/Sichuan/379/99, an increase in the number of influenza B strains belonging to the B/Victoria/2/87 lineage was also seen in many countries.

The genetic drift of influenza viruses circulating in South Africa during 2002 relative to the vaccine strains was determined by sequencing the HA1 subunit of the haemagglutinin gene of representative virus isolates. The neuraminidase N2 gene of a number of the influenza A viruses was also determined. Phylogenetic analysis was performed using the PHYLIP Neighbor-Joining programme to examine the evolutionary relationships between the South African 2002 strains and influenza viruses isolated in recent years in this country as well as in other parts of the world.

Influenza activity during the 2002 season in South Africa was mild. The majority of viruses were isolated from late May until August, while two early isolates were made in April. Specimens positive for influenza were obtained from individuals in the Gauteng, KwaZulu-Natal, Western Cape, Free State, North West and Mpumalanga provinces. Both influenza A and influenza B viruses were isolated during the season. Unlike the last few years where influenza A viruses predominated in South Africa, the majority of the viruses isolated in 2002 were influenza B strains.

Antigenic subtyping of the influenza A isolates by the haemagglutination inhibition (HI) assay revealed that they were mostly subtype H1 with only ten being subtype H3N2. The H1 isolates reacted strongly with the antiserum raised against the A/New Caledonia/20/99 vaccine strain. The H3N2 virus isolates also reacted to high titres with the antiserum raised against the A/Panama/2007/99 vaccine strain. None of the South African influenza B isolates reacted with the B/Sichuan/379/99 antiserum in the HI tests, and reacted at low titres with the B/Hong Kong/330/01 antiserum. When tested with sheep serum raised against B/Shangdong/7/97, an earlier B/Victoria/2/87-like virus, higher titres were obtained. A number of the influenza isolates were sent to the WHO Collaborating Centres for Influenza Reference and Research in Melbourne and London for further serological characterisation by HI using a panel of post infection ferret sera. These assays confirmed that the influenza B viruses were antigenically similar to the B/Hong Kong/330/01-like viruses. This is unlike the situation the previous year in South Africa, where all the B strains were found to have evolved from the B/Sichuan/379/99 and B/Johannesburg/5/99-like strains.

A number of the influenza A H1 positive isolates were tested using RT-PCR assays using primers specific for the N2 neuraminidase to determine whether there were any H1N2 reassortant viruses circulating in South Africa. Both H1N1 and H1N2 viruses were identified.

Sequence analysis of the HA1 subunit of the H1 viruses revealed that they were closely related to the A/New Caledonia/20/99 vaccine strain. Four amino acid changes were observed in all the isolates sequenced; these were at residues 166 (V-A), 175 (V-I), 190 (A-T) and 215 (A-T). The valine to isoleucine substitution and alanine to threonine change at residues 175 and 215 respectively are characteristic of the H1N2 reassortant viruses seen in other parts of the world. Other substitutions observed sporadically were at residues 66 (E-K), 96 (A–T), 153 (G-R) and 187 (D-N). The results of the phylogenetic analysis of recent South African H1 HA1 sequences (979 bp) are shown in Figure 1.

The molecular characterisation of the HA1 subunit of representative influenza H3N2 isolates revealed that the viruses circulating in South Africa were very homogeneous. They all shared six common amino acid changes relative to the A/Panama/2007/99 vaccine strain at residues 21 (S-P), 53 (D-N), 106 (A-V), 144 (N-D), 183 (L-H) and 186 (S-G). Several isolates had an additional change at residue 291 (D-G). Figure 2 shows the

results of the phylogenetic analysis of representative South African H3N2 HA1 sequences (921 bp). Unlike the H3N2 viruses that were circulating in South Africa in 2001, which fell into two clades, the 2002 South African strains clustered in one clade and were very similar to several viruses isolated in Norway in January 2002.

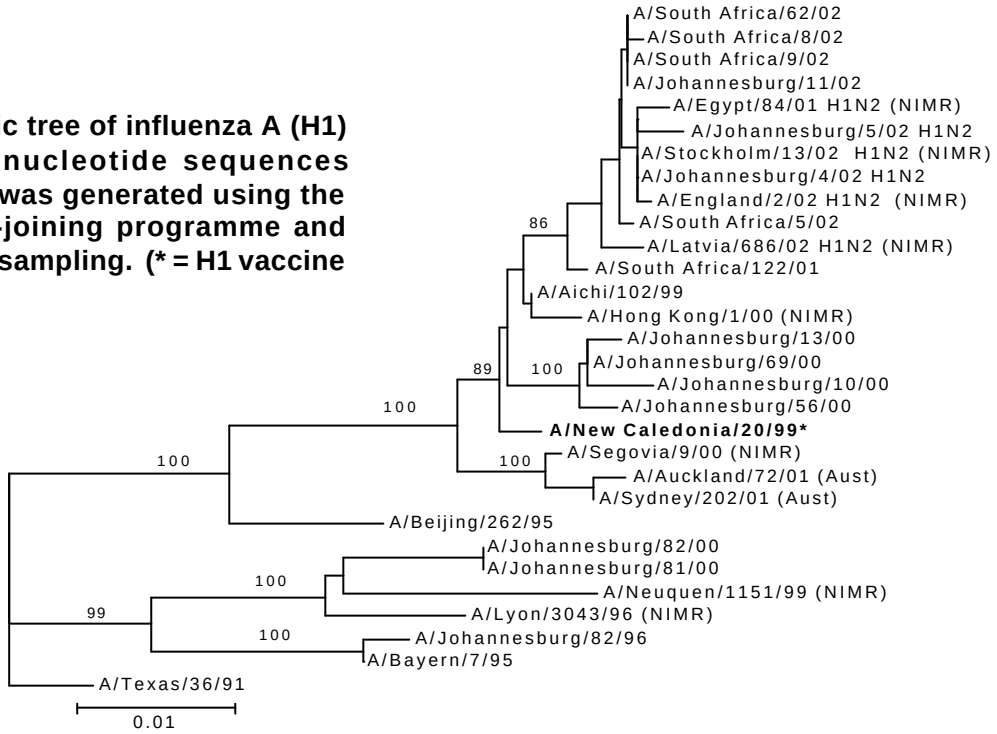


Fig 1. Phylogenetic tree of influenza A (H1) virus HA1 gene nucleotide sequences (979bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling. (* = H1 vaccine strain.

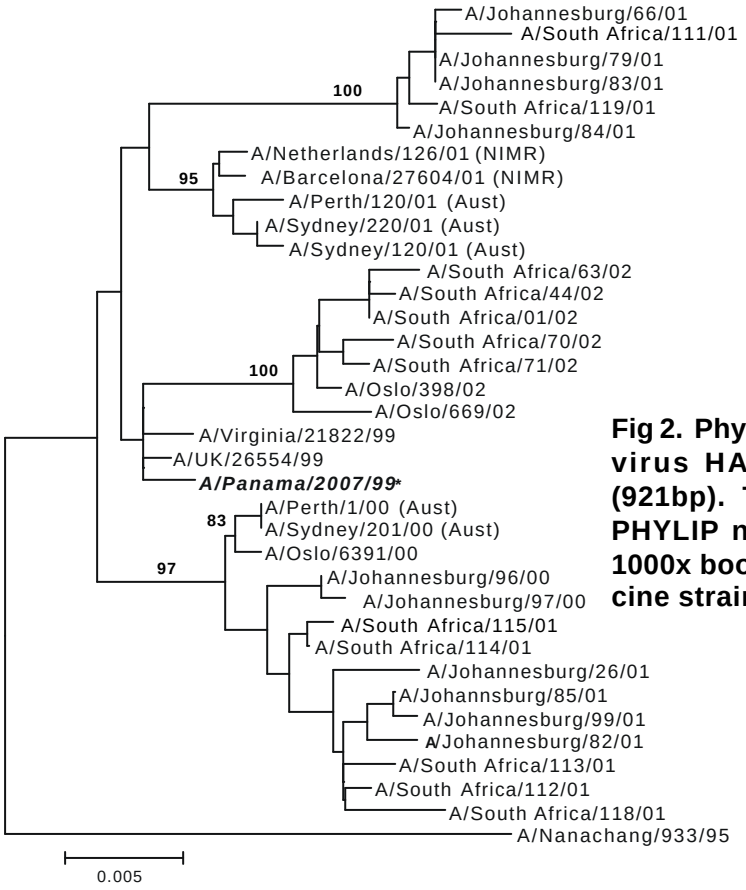


Fig 2. Phylogenetic tree of influenza A (H3N2) virus HA1 gene nucleotide sequences (921bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling. (* = H3N2 vaccine strain)

Analysis of the N2 sequences of representative H3N2 isolates demonstrated substantial genetic drift from the A/Panama/2007/99 vaccine strain. Common amino acid changes were observed at 93 (K-N), 143 (R–G), 172 (K–R), 197 (H-D), 208 (D-N), 249 (R-K), 265 (T-I), 267 (K-T), 370 (S-L), 403 (M-R) and 432 (Q-E). Sporadic substitutions were also seen at residues 256 (F-S) and 367 (S-N). Many of these changes have been reported for recent isolates from some European countries, Madagascar and Australia but the mutations at residues 256 and 367 appear to be unique to some of the South African strains. Phylogenetic analysis of the N2 gene revealed that the South African viruses were similar to H3N2 viruses isolated in Spain and France the previous year (data not shown).

The genetic characterisation of representative influenza B isolates showed that they were more closely related at the genetic level to the B/Shangdong/7/97 strain than to B/Hong Kong/330/01 virus with differences in only two amino acid residues at 121 (I-T) and 199 (N-T) compared to the Shangdong strain (Figure 3). Influenza B viruses with sequences closely matching that of B/Shangdong/7/97 have recently been isolated from Mauritius, Israel, Egypt, Tehran, France, Norway, Hong Kong, Australia and New Zealand, revealing a widespread distribution of this particular genetic subgroup. The amino acid residue changes relative to the B/Hong Kong/330/01 strain were observed at positions 116 (R-H), 121 (N-T), 164 (E-D) and 197 (S-N).

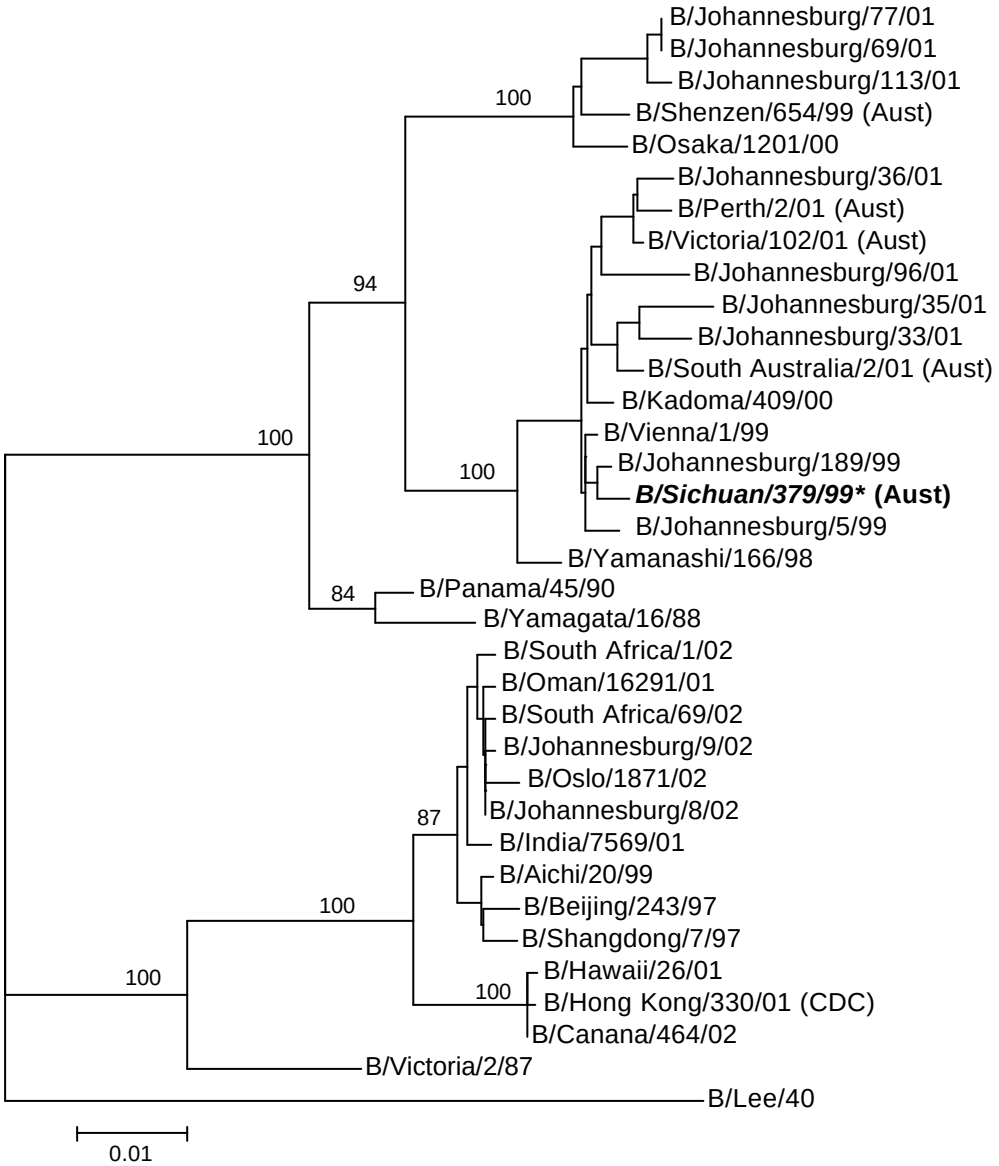


Fig 3. Phylogenetic tree of influenza B virus HA1 gene nucleotide sequences (957bp). The tree was generated using the PHYLIP neighbor-joining programme and 1000x bootstrap resampling. (* = B vaccine strain)

Virology - *Influenza*

As in the case of the 2001 winter season, the subtypes of influenza viruses that predominated in South Africa in 2002 were different to those in most other countries in the southern hemisphere. While the majority of activity was due to influenza B and A H1 strains in this country, influenza A H3N2 predominated and co-circulated with influenza B in Australia and New Zealand. Argentina and Brazil experienced mainly influenza B activity, while high numbers of influenza A H1 viruses were isolated in Chile.

The virus strains recommended by the WHO for the vaccine formulation for the southern hemisphere 2003 influenza season were the A/New Caledonia/20/99-like (H1N1), A/Moscow/10/99-like (H3N2) and B/Hong Kong/330/01-like viruses. A/Panama/2007/99 will once again be used as the A/Moscow/10/99-like strain while B/Shangdong/7/97 and B/Brisbane/32/02, both B/Hong Kong/330/01-like strains, were accepted as being suitable for use as vaccine strains for the B component.

Acknowledgements:
Sequences from other countries were obtained from the WHO Collaborating Centres for Reference and Research on Influenza, London and Melbourne, the LANL influenza database: http://www.flu.lanl.gov and from Dr Olav Hungnes, Norwegian Institute of Public Health, Oslo.

Virology - *Poliovirus*

MOLECULAR EPIDEMIOLOGY OF POLIOVIRUS IN SUB-SAHARAN AFRICA

Since 1995 the NIV (now the NICD) has been a WHO Regional Reference laboratory for the Polio Eradication Initiative. The polio molecular virology section also serves as a reference laboratory outside of the southern Africa region for many of the countries which fall under the WHO African Regional Office.

During 2002, the polio molecular virology unit of the NICD received 927 poliovirus isolates (Figure 1), which were then characterized using both intratypic differentiation methods, PCR and ELISA, as vaccine or wild-type. These isolates were sent to the NICD by national and regional laboratories throughout the African region namely, Cameroon, Cote d'Ivoire, Democratic Republic of Congo, Ethiopia, Ghana, Kenya, Madagascar, Senegal, Uganda, Zambia and Zimbabwe.

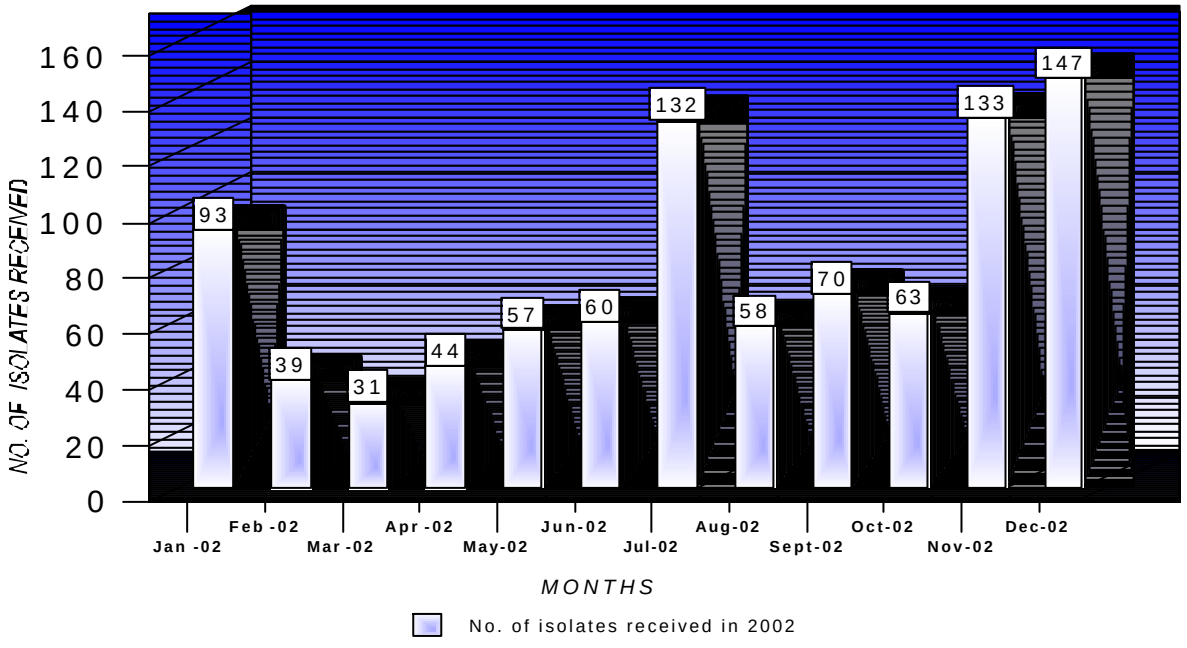


Fig 1. Isolates received in 2002

Virology - *Poliovirus*

Clinical specimens from AFP cases were sent to the NICD for poliovirus isolation and further characterization of isolates from Angola, Botswana, Lesotho, Mozambique, Namibia and Swaziland (Figure 2).

In South Africa 373 AFP cases were reported and poliovirus was isolated from seven of these. All polioviruses isolated from AFP cases in southern African countries were found to be vaccine-like.

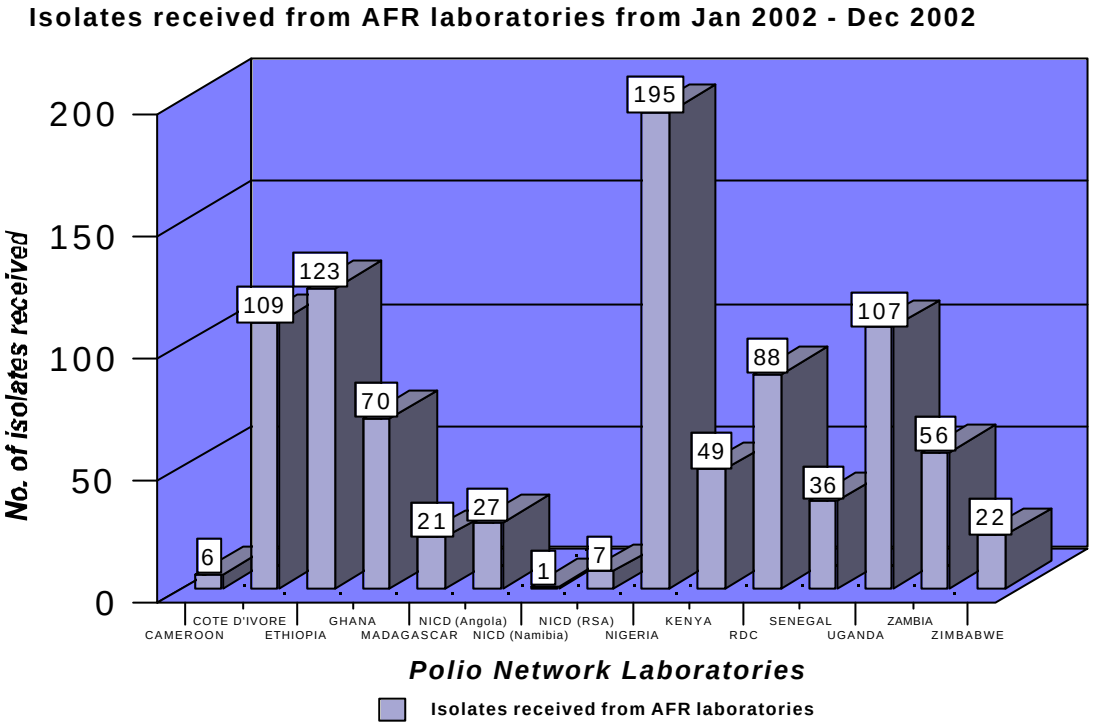


Fig 2. Samples received from African polio network laboratories.

The majority of the wild-type isolates identified during 2002 were from Nigeria (Figure 3), 109 of which were polio type 1 and 14 polio type 3. This does not represent all of the isolates from that country as many were sent to CDC (Atlanta) for ITD and sequencing. Other isolates received in 2002, some of which were actually isolated late in 2001, were Burkina Faso identified as wild-type 1, Niger wild-type 1 and 3, Somalia wild-type 3 and Zambia wild-type 1.

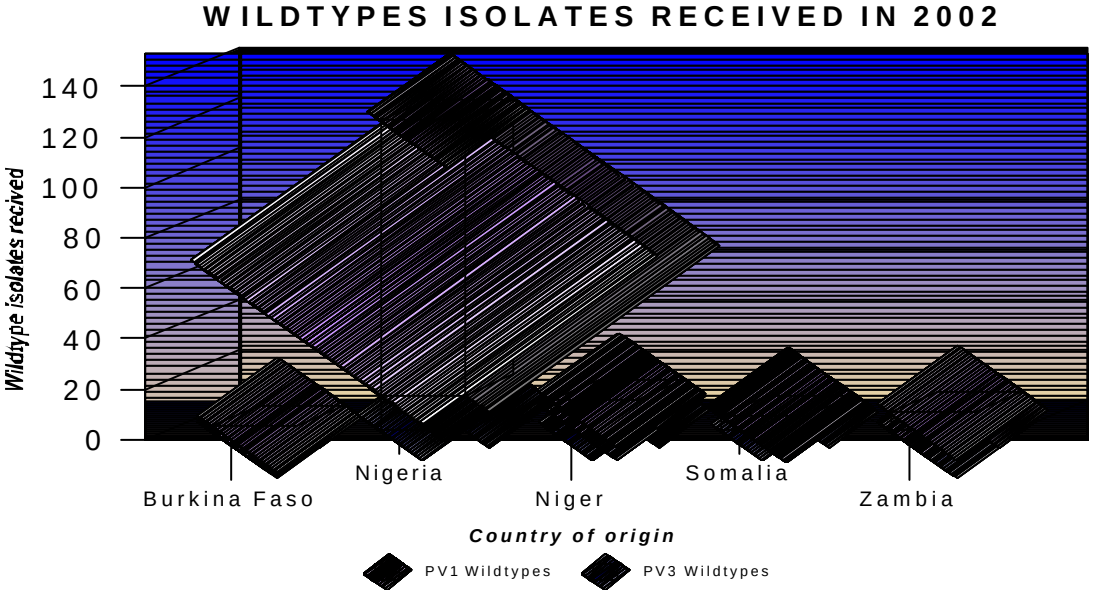


Fig 3. Wildtype isolates reported in 2002

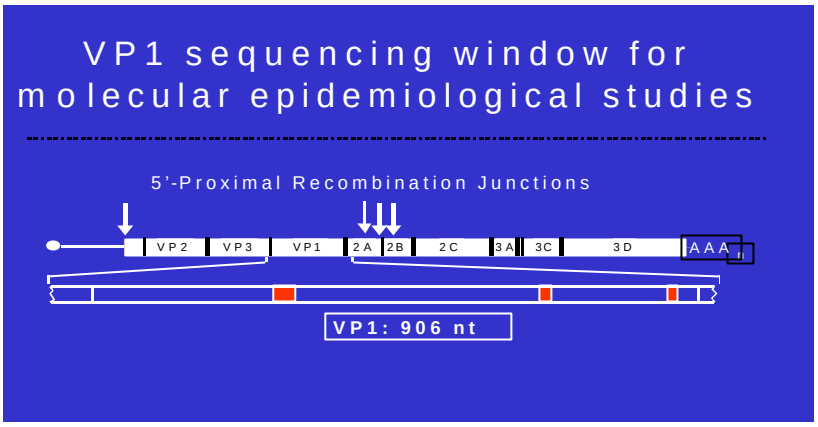


Fig 4. VP1 sequencing window for molecular epidemiological studies

Molecular sequencing of the full VP1 (900bp), (Figure 4) can be used to answer several basic 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 main African PV1 serotypes identified are WEAF-A, WEAF-B and EAAF (Figures 5, 7 and 9).

Distribution of wild PV3 genotypes closely parallels PV1 distribution, although there have been fewer isolates of wild PV3 (Figures 6, 8 and 10). The dendrograms presented were constructed by the NICD and CDC Atlanta.

The genotype in figure 5 includes viruses from Angola, Zambia, DRC, Cape Verde and Congo. The close relationships of the Angolan and Cape Verde viruses highlights the potential for the introduction of poliovirus from countries where polio is endemic to those which are polio free. This is more likely if the countries have common borders or roots thus increasing travel between the countries. In January 2002, NICD received three poliovirus isolates from a Zambian laboratory. These were from the children of the same family (1, 3 and 6 years old) with no vaccination history. Their date of onset of paralysis was 12th December 2001. All three cases were 100% identical to each other and fall in the same genotype with Angola and RDC.

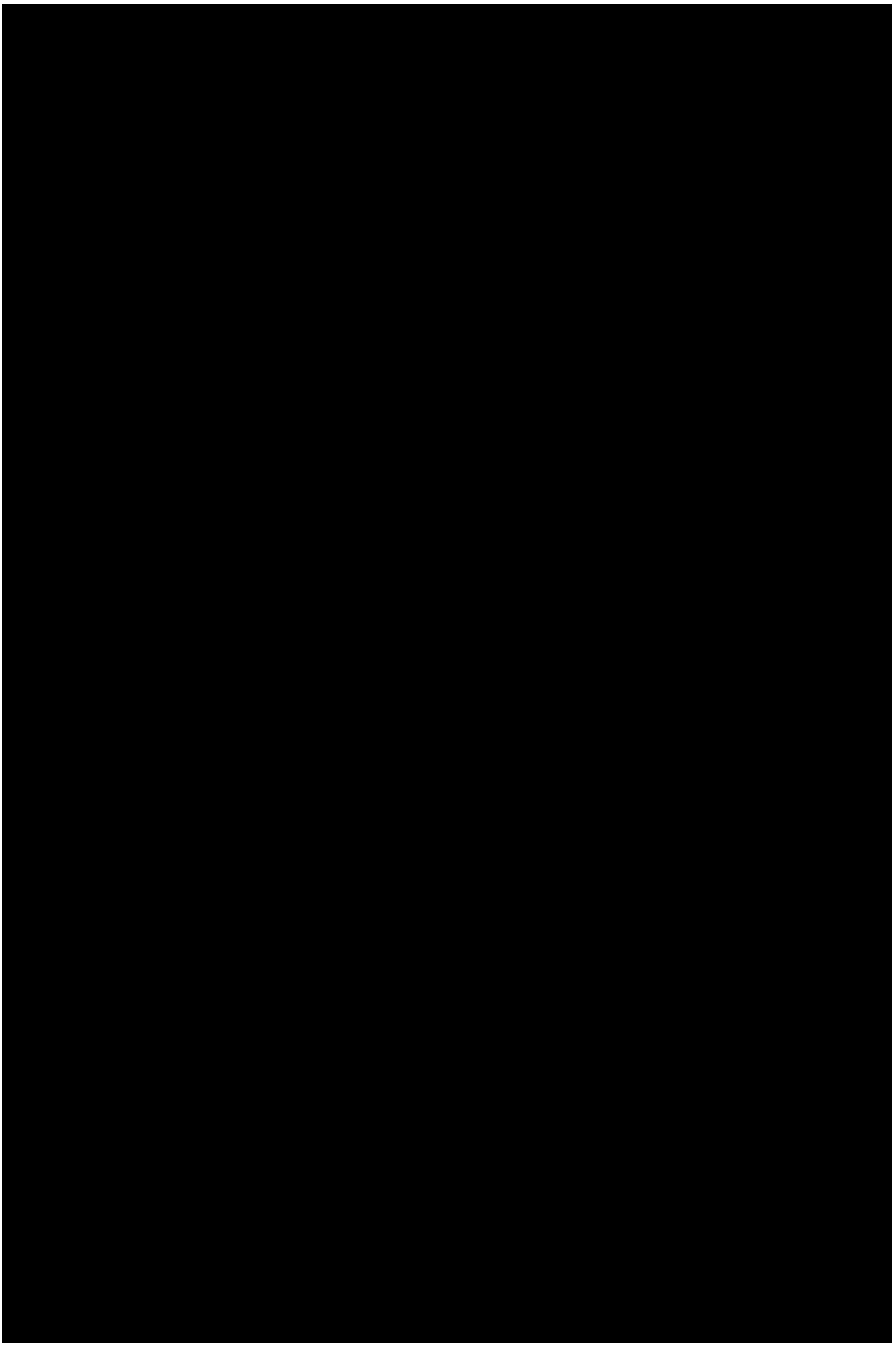
Sequence and epidemiological data suggested that viruses detected from Zambia were imported from Angola since the children were coming from Lilombo village in Cuondo province of Angola and seeking care in the Kalabo district of Zambia. In 2002, Zambia had another imported case (Figure 5).

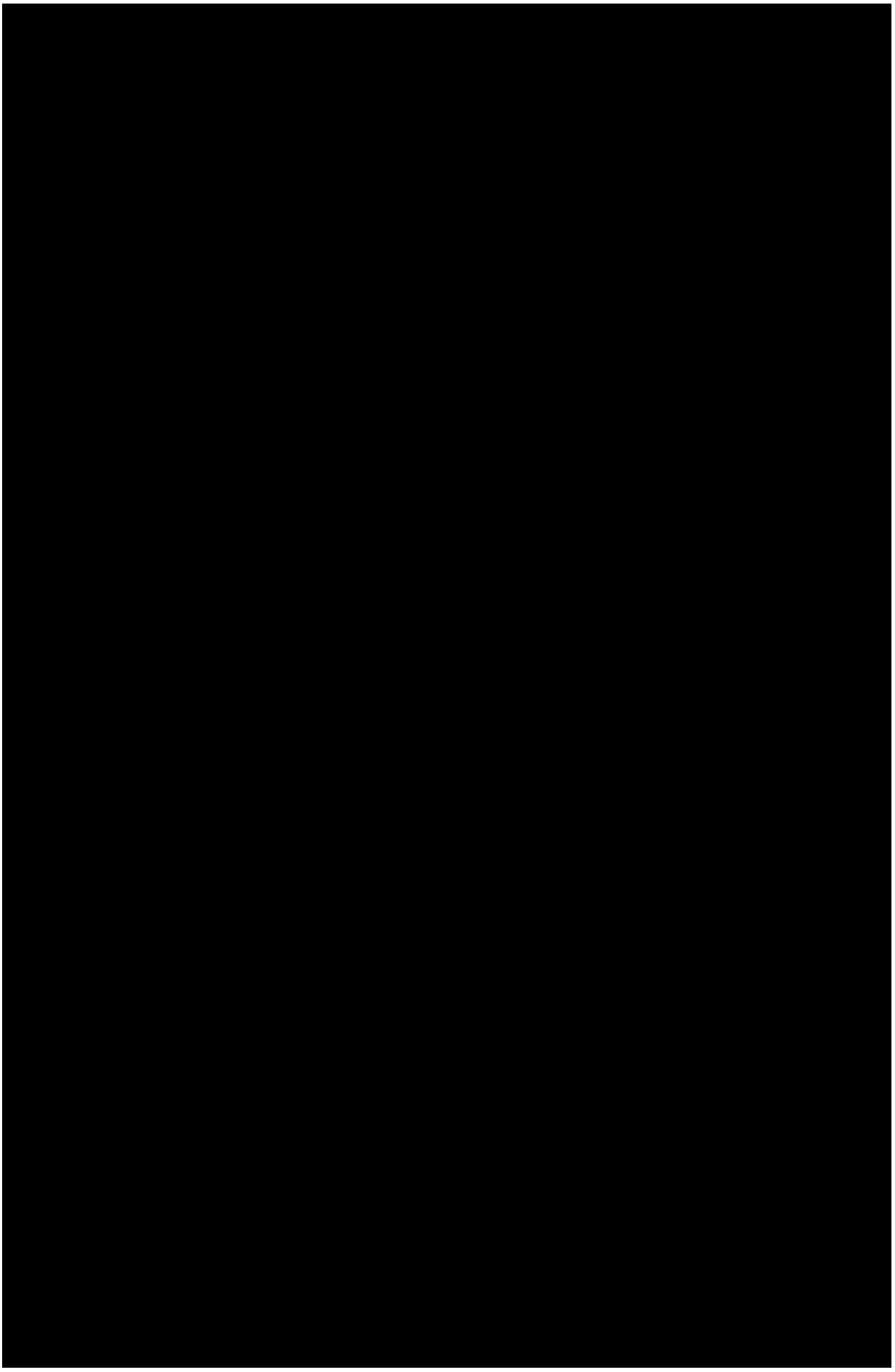
There has been no identification of WEAF-A wild PV3 since year 2000 (figure 6). The majority of the viruses in this genotype were from Angola. The Angola cluster has several lineages with Luanda being the most active lineage, which caused the local circulation and outbreak in 1999.

Wild PV1 was highly endemic in northern Nigeria in 2002 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.

2002 isolates are distributed into sixteen clusters (A to I_8), (other clusters are not shown) (figure 7). Cluster A is the most closely related group and also cluster B, C and D. 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. Cluster D has a single lineage, which reflects local circulation in Gombe (GMS) province. Only one 2002 isolate identified for cluster F and G. Cluster H consists of the local circulation in northern provinces.

Cluster I was very active in 2002 and consists of several lineages. The closely related viruses were found in Niger and Burkina Faso. In 2002 five samples from Niger were received with their date of onset occurring towards the end of 2001.





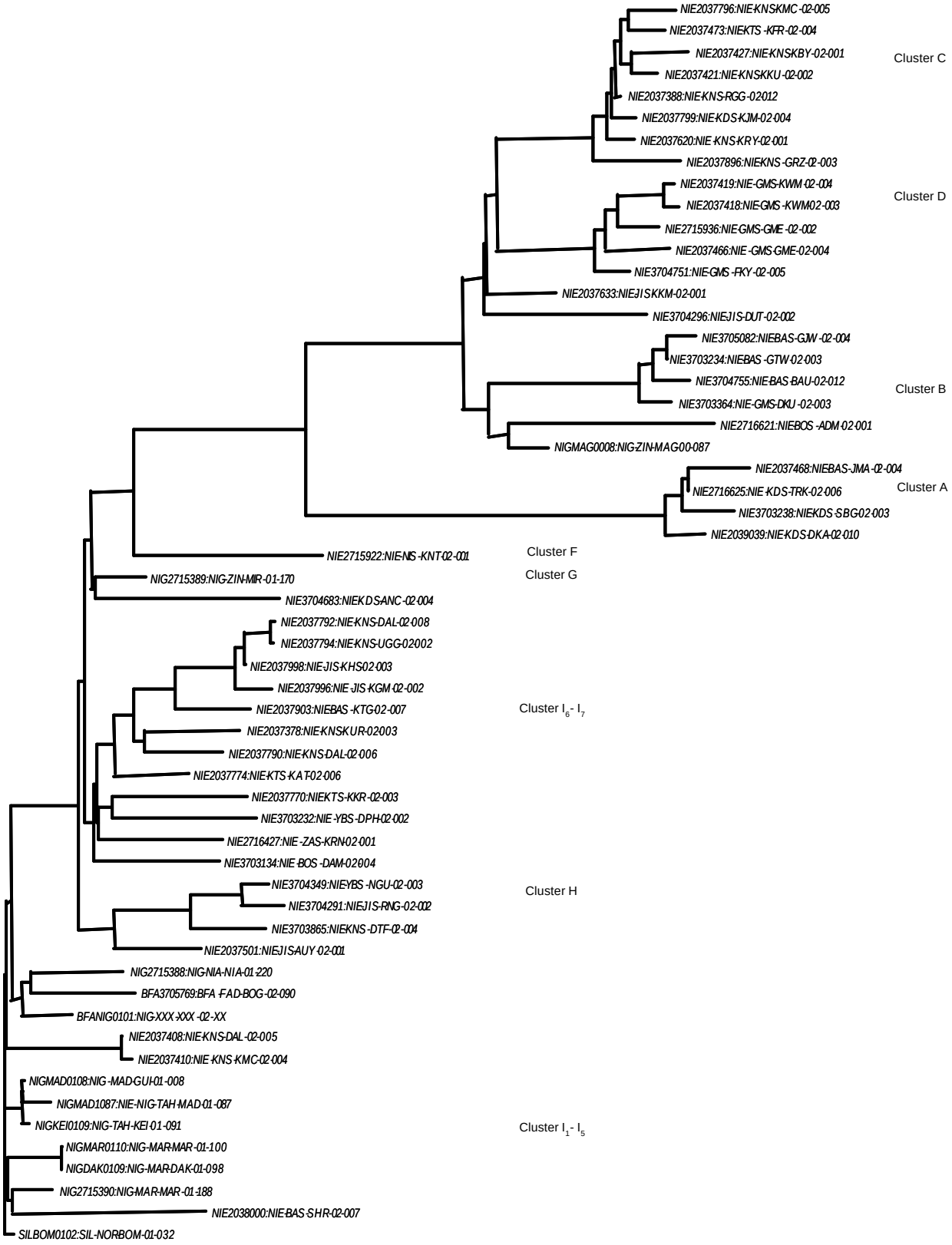


Fig 7. WEAF-B Wild PV1 representative of isolates: 2000-2002

One isolate that was previously labelled BFA-DOR-SEB-02-024 has been given a new EPID No.: NIG-XXX-XXX-02-XX, because the history of the case indicated that the child was infected in Niger.

"The child was born in Guibondi CFA (Burkina), the family established in Dogona (Niger) and travelled frequently to CFA Deba (Niger) for gold mining activities. The onset was 22 April 2002 (in Niger) and the family went to a health facility in Takatami (Burkina)."

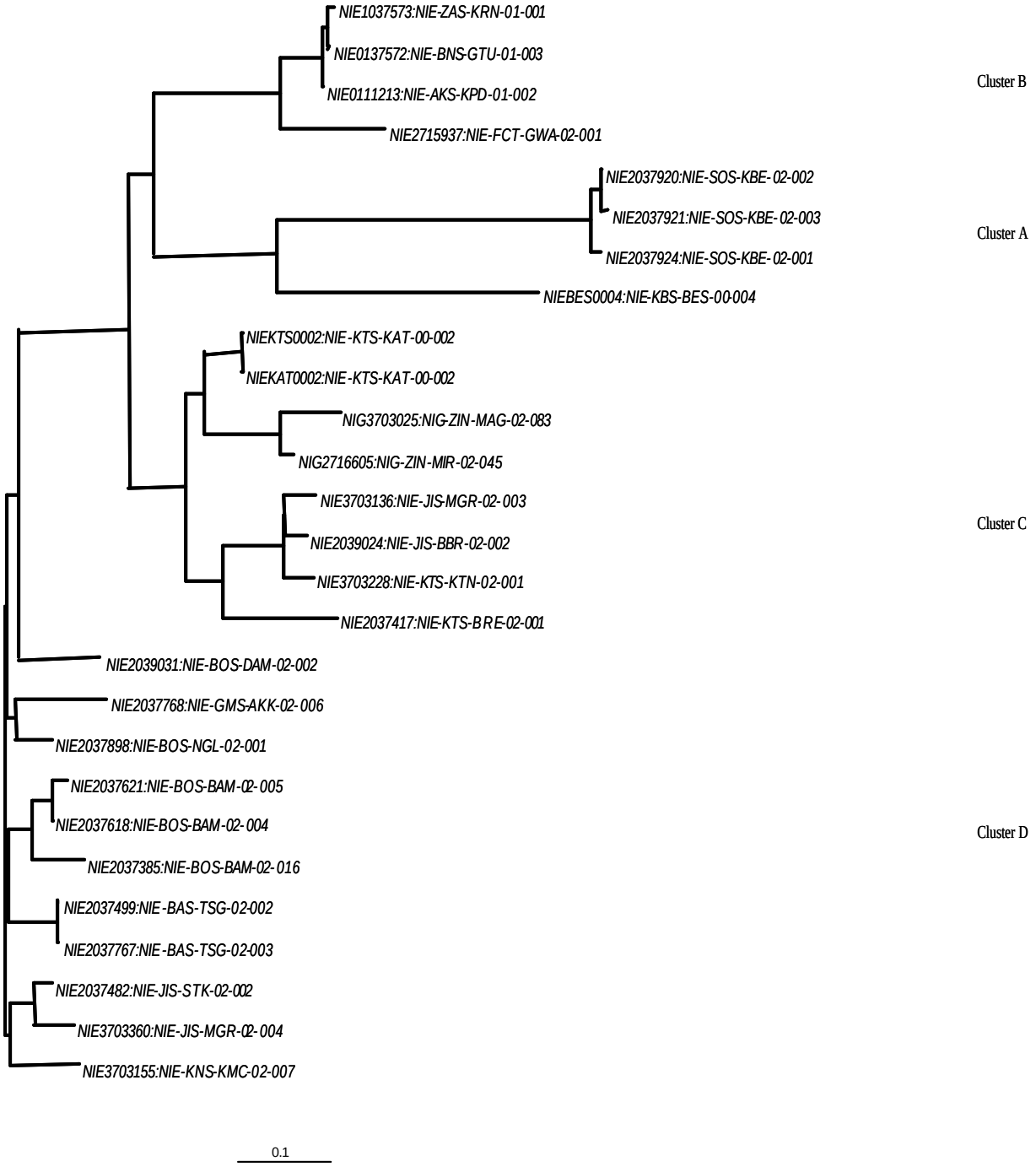


Fig 8. WEAF-B wild PV3 representative of isolates: 2000-2002 survey

WEAF-B wild PV3 is divided into four clusters (A-D) (figure 8). Cluster A represents local circulation in Sokota (SOS) province. Cluster B has a single isolate that was isolated in February 2002 and no cluster B isolates have been found since February. Cluster C is more diverse than those of clusters A, B and D. The viruses circulated in Katsina, Jigawa and the adjacent province of Zinder in Niger. Cluster D resolved into four 2002 lineages - Borno (BOS), Jigawa, Gombe and Kano. There is a great similarity among cluster D.

Fig 9. EAAF Wild PV1 representative of isolates: 1999-2001 survey

No 2002 PV1 wild isolates were identified in the EAAF genotype (figure 9). The last case was in 2001 (from Ethiopia). The EAAF PV1 genotype distributed into three clusters (A-C). Cluster A has isolates from Ethiopia and resolved into two chains of transmission (South and Amhara provinces). The South consists of the last isolate on the tree. Cluster B resolved into three lineages (BAN, LSH and WGB). BAN reflects local circulation in Banadir province. Cluster C is distributed into two lineages, SUD and CHA. The last case was in 2001 in Sud province (SUD lineage).

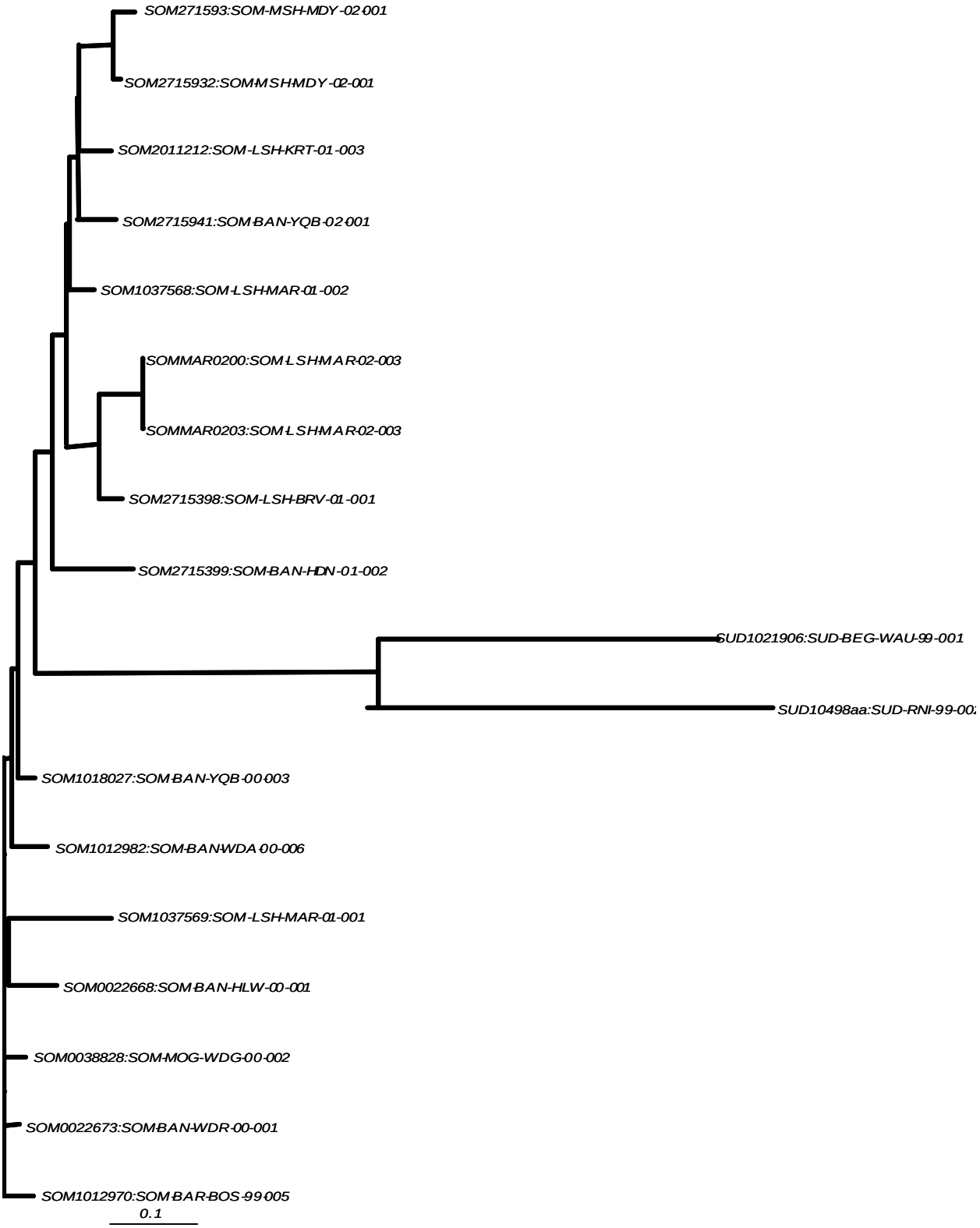


Fig 10. EAAF wild PV3 representative of isolates: 1999-2002 survey

Virology - *Poliovirus*

Five 2002 isolates were reported from PV3 EAAF genotype (figure 10). These were from MSH, LSH and BAN lineages. The last case on the tree was from Lower Shabele province of Somalia.

VACCINE-DERIVED POLIOVIRUS (VDPV)

The risk of circulating VDPV (cVDPV) is small with an identified frequency of around one episode per year, occurring in countries or regions with low vaccination cover. The more recent episodes occurred in Haiti, the Philippines and Madagascar, the latter's index case was in 2001 with several cases identified in 2002. In December a possible VDPV was identified from an isolate sent from Malawi. The assays suggested a VDPV and the sequence analysis indicated 99.12% similarity to Sabin strain. There were no reports of other cases up to the end of December 2002.

The identification is, at present, based on the results of the PCR and ELISA tests, where the PCR indicates Sabin-like and ELISA indicates wild-type. To confirm this, the WHO guidelines require a greater than 1% genetic drift from the Sabin virus as shown by the sequence data analysis. This should be further supported by demonstrating recombination with group C non-polio enterovirus or that the VDPV isolate is from a cluster of AFP cases from which a common VDPV is isolated.

The polio molecular section is looking at selected Sabin isolates from countries within the AFRO region for retrospective evidence of drift to VDPV.

CONTAINMENT

As part of the poliovirus containment programme the polio sections have completed an inventory of all polio isolates and any potentially infected specimens which are to be retained at the NICD. The pre-global eradication phase of the containment programme requires that nations:-

1. Survey all biomedical laboratories to identify those with wild poliovirus infectious materials and encourage destruction of all unneeded materials.

2. Develop an inventory of laboratories that retain such materials and report to the Regional Certification Commission.

3. Instruct laboratories retaining wild poliovirus infectious or potentially infectious materials to institute enhanced biosafety level-2 (BSL-2/polio) measures for safe handling.

4. Plan for implementation of the Post Global Eradication phase.

Staff of the Molecular Virology laboratories

SOUTHERN AFRICAN HEPATITIS B VIRUS GENOTYPE STUDIES

To date eight genotypes, A through H, of hepatitis B virus (HBV) have been described. Recently recognised primate hepatitis B viruses, including those of the orangutan, gibbon, gorilla, chimpanzee and the New World woolly monkey, have been found to cluster with the human hepatitis B viruses. Since the early reports of D/A and B/C recombinants in HBV, A/C (Vietnam), A/D (southern Africa) and C/D (Tibet) recombinants have been reported and the general consensus is now that recombination is fairly common in HBV. With the exception of genotype strains from the New World (genotypes F and H), representatives of all the major genotypes have been found in southern Africa (Figure 1). Although the exact percentage of the different genotypic groups varies in different studies (Table 1a and b), specimens are predominantly from genotype A (50 – 90 percent) and genotype D in South Africa and genotype E and genotype D in Namibia. The endemic Asian genotypic groups, less frequently encountered in this part of the world, namely genotype B and C, are normally found locally in Asians and thus were probably imported. We recently found a specimen from the newly described genotype G in a South African patient and it remains to be seen how common this genotype is in the subcontinent.

HBV sequence data from two groups of patients (studies 5 and 6 in Table 1) from South Africa and Namibia, respectively, are presently being determined in what is to be an ongoing surveillant initiative of hepatitis strains in southern Africa. The South African specimens (N = 685) were obtained from a group of industrial workers from sites across South Africa and tested for past and present HBV infection using the Monolisa core antibody and surface antigen (HBsAg) tests, respectively. Two hundred and twenty-two specimens (32.4 %) were found to be positive for either or both of the HBV markers while 58 (8.5 %) were positive for HBsAg alone. Twenty-eight of these specimens have so far been sequenced over a 761 base pair fragment which extended from position 3154 of the preS1 region to position 687 of the surface gene. Twenty-two (78.6 %) of these were found to be from genotype A, five from genotype D (21.4 %) and one from genotype G. Genotype G has been reported from France and the USA but this is the first report of an African genotype G. All the genotype A specimens had preS2 Valine[22], Leucine[32], Valine[35], Serine[47], which distinguished them as members of the subgroup A' of A, as well as preS2 arginine[10] and threonine[48], which were found previously to be characteristic of South African subgroup A' specimens only. Previously we found 69.6 % of genotype A in urban Gauteng (study 2 which incorporates study 1, Table 1a and b) and 89.2 % in a rural area of the Eastern Cape (study 3, Table 1a and b) of which 64.1 and 97 %, respectively, were found to be of subtype A'. Genotype D was found in 16.1 % of the Gauteng specimens and 10.8 % of the Eastern Cape specimens. Small numbers of genotype B (3.6 %), C (7.1 %) and E (3.6 %) were also found amongst the Gauteng specimens (Table 1b). It is hoped that by collecting specimens from a more representative cross section of South Africans we will be able to ascertain if the apparent geographical distribution of the genotypes in South Africa is real, particularly with respect to the reported frequency of 44.4 % of genotype D in the Western Cape (study 4, Table 1).

A random sample of 240 HBsAg-positive first time blood transfusion Namibian specimens (N = 2709) were genotyped by HBV genotype-specific ELISA last year. The method uses genotype-specific monoclonal antibodies to the preS2 antigen to partition specimens into the seven genotypes A to G. Since the preS2 antigen was found to be low in many of the specimens, where possible (sufficient serum remains for only 81 of the specimens) the results are being checked by sequencing PCR amplicons from the preS2/S region. Eighteen of these specimens have been sequenced of which 11 (61.1 %) were found to be from genotype E, 4 (22.2 %) from genotype D and 3 (16.7 %) from genotype A (study 6, Table 1). Discrepant results in the specimens typed to date mainly indicate the HBV genotype-specific ELISA over-reports the incidence of genotype A at the expense of genotype D and E. Specimens which could not be typed because of insufficient preS2 antigen have amplified, and have therefore been included in this follow up study. Results obtained in this sequencing study agree with earlier Namibian studies where the majority of specimens examined were found to be of the two *ayw* serotypes, genotype D (6.2 %) and genotype E (93.8 %) although a percentage of specimens from genotype A, subgroup A', is also being found.

Sequence data from the surface gene will also be examined in an attempt to explain the apparent partial failure of HBV EIA to effectively partition the Namibian specimens and to determine the degree to which local variation is responsible for this. Completion of the two studies has been delayed as more sensitive amplification methods are sought. A spin-off study to check viral load in these and other specimens studied in the molecular hepatitis research laboratory is also being instituted. It is hoped that this will enable us to determine the sensitivity of different genotyping methods, and how this too is affected by local variation.

All specimens will also be sequenced in the core gene region and examined using Simplot for evidence of recombination. In particular, representative specimens from genotype E will be sequenced completely in order to characterize this African genotype. This group is interesting as it does not partition as an independent genotype in the core gene region but partitions as a subgroup of genotype D. It is possible that genotype E is a recombinant of genotype D and an as yet unidentified genotype.

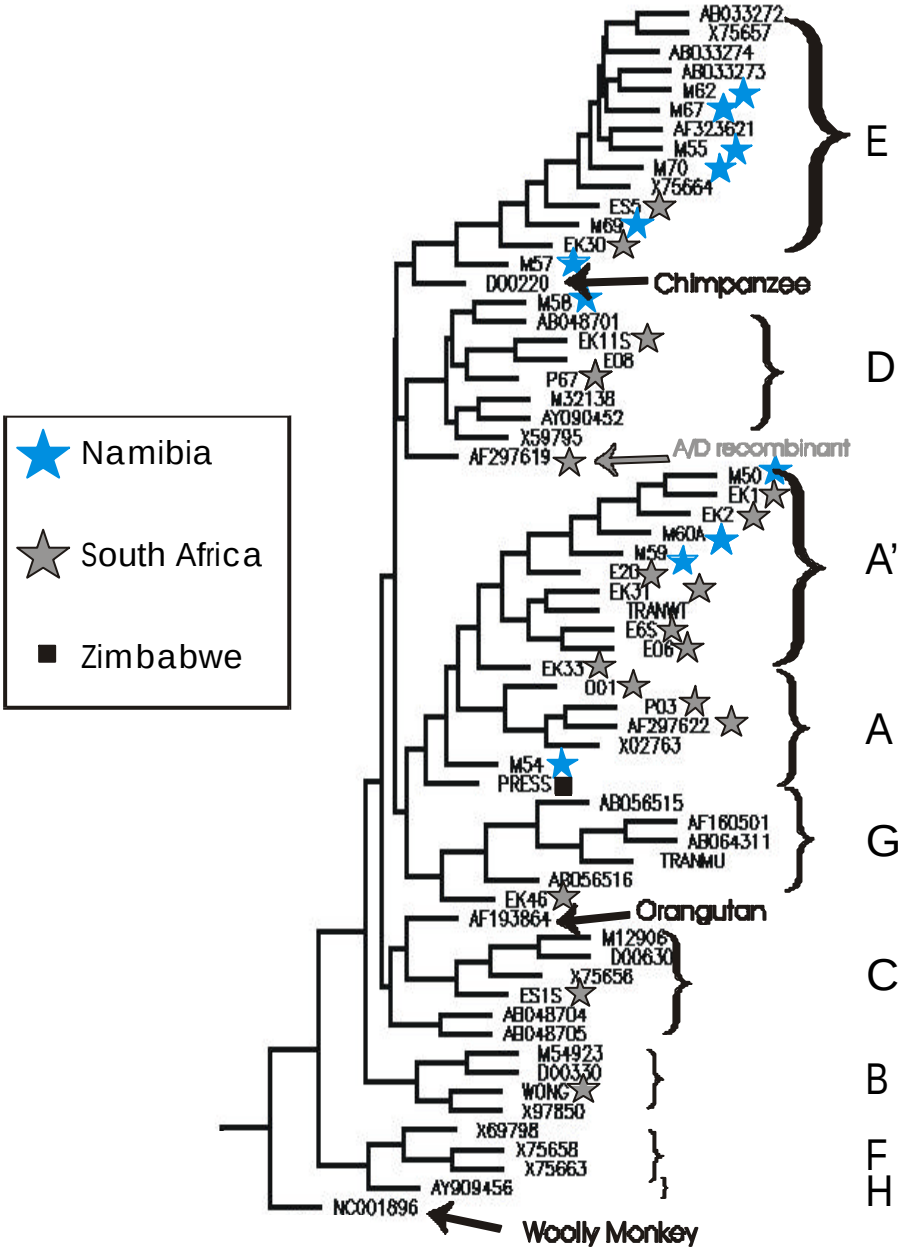


Fig 1. Isolates from all except the two New World genotypic groups, F and H, have been found in southern Africa

TABLE 1 : GENOTYPE STUDIES IN SOUTHERN AFRICA
(a) Source and size of patient groups

	Country	urban/rural Province	Source	N	HBsAg positive	Selection Strategy	No. tested	Method/s used
1	South Africa	urban Gauteng (subset of study 1)	HBV DNA extracted and amplified from 11 acute hepatitis B and 18 chronic carriers from the Gauteng province of South Africa were sequenced & compared with 8 global sequences from GenBank	29	29	all specimens tested	29	Seq
	South Africa	urban Gauteng	In the past, 40 to 50,000 diagnostic specimens were sent annually from doctors, hospitals and clinics in Gauteng Province to the NICD. Of these 8 to 9,000 specifically request hepatitis studies	50,000 or 5 years stored at NICD	about 5,000 (10%) of these will be HBsAg positive	random sampling of NICD stored HBsAg-positive diagnostic specimens	37	RFLP, Seq HBV EIA
2	Namibia	rural NW & NE province	High school children, all first time blood donors from the NW and NE provinces of Namibia	230	39	all specimens with sufficient volume tested	16	RFLP, Seq HBV EIA
		rural Ovamboland	Existing cohort from a previous study collected from state hospital and regional clinic patients in	1338 children 1272 mothers	201 children 140 mothers	random sample of HBsAg- and HBeAg-positive	24	RFLP, Seq HBV EIA
		rural Rundu NE province	Chronic carriers identified among first time blood donors	8	8	all specimens tested	8	EIA
3.	South Africa	rural Eastern Province from	Age stratified sample of children 0-6 years of age the Eastern Cape Province of South Africa	2299	238	random sampling	37	RFLP
4	South Africa	urban Western Cape	14 'community acquired' patients resident in Cape Town, 5 HBV strains identified in an outbreak of HBV in an oncology unit, 8 liver transplant patients	27	27	all specimens tested	27	Seq
5	South Africa	urban Nationwide	Stored sera from a cross-section of industrial labourers collected and tested for an HIV study	690	58	all PCR positive sera to be tested	28*	Seq
6	Namibia	urban & rural Nationwide	Stored sera from a cross-section of first time blood donors collected for a baseline pre-immunisation study of hepatitis B markers in Namibia	2805	304	184 had sufficient serum for HBV EIA but only 81 remain for sequencing	18*	HBV EIA, Seq

* Studies still be be completed, RFLP = Restriction fragment length polymorphism assay, HBV EIA = HBV Enzyme immunoassay, Seq = PCR fragment sequencing

TABLE 1 : GENOTYPE STUDIES IN SOUTHERN AFRICA
(b) Results and study references

	Country	urban/rural province	A	A'	B	C	D	E	F	G	H	Reference
1	South Africa	urban Gauteng	**37.9**	**44.8**	**3.5**	**3.5**	**10.3**	0	0	0	0	Bowyer SM *et al* (1997). A unique segment of the HBV group A genotype identified in isolates from South Africa. *J Gen Virol;* **78:** 1719-1729.
2	South Africa	urban	**25.0**	**44.6**	**3.6**	**7.1**	**16.1**	**3.6**	0	0	0	Bowyer SM (2002). *Molecular characterization of HBV in South Africa.* A thesis submitted in fulfilment of the requirements for the degree PhD (Virology). Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.
	Namibia	rural Northern Province	0	0	0	0	**6.2**	**93.8**	0	0	0	
3	South Africa	rural Eastern Province	**2.7**	**86.5**	0	0	**10.8**	0	0	0	0	Vardas E (1999). *The epidemiology and molecular characteristics of HBV infection in children from a hyper-endemic area of South Africa, including a field trial of the hepatitis B immunisation programme and its impact on infection in this population.* A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Medicine (Virology), Faculty of Medicine, University of the Witwatersrand, Johannesburg
4	South Africa	urban Western Cape	**3.7**	**48.2**	0	**3.7**	**44.4**	0	0	0	0	Hardie & Williamson (1997). Analysis of the preS1 gene of HBV to define epidemiologically linked and unlinked infections in South Africa. *Arch Virol*; **142:** 1829-1841.
5	South Africa	urban Nationwide	0	**78.6**	0	0	**17.8**	0	0	**3.6**	0	Ongoing surveillance in the NICD hepatitis research laboratory.
6	Namibia	urban & rural Nationwide	0	**16.7**	0	0	**22.2**	**61.1**	0	0	0	Ongoing surveillance in the NICD hepatitis research laboratory.

PHYLOGENETIC ANALYSIS OF THE CARBOXY TERMINAL OF HEPATITIS E VIRUS

Prevalence studies based on serological assessment from different populations have suggested activity by hepatitis E virus (HEV) in South Africa but the virus has never been detected in materials from patients with clinical episodes of hepatitis.

All HEV strains studied to date appear to comprise a single serotype. Known HEV strains cluster into four major genotypes: genotype I which includes Afro-Asian isolates, genotype II including North American isolates, genotype III including the recently discovered African isolates together with the Mexican isolate and genotype IV including the recently discovered Chinese and Taiwanese isolates.

Previously we reported the development of a nested reverse transcriptase polymerase chain reaction (RT-PCR) for both the polymerase region in open reading frame 1 (OFR1) and the carboxy terminal end of open reading frame 2 (ORF2) of the HEV genome and these have been used to amplify RNA extracted from clarified stool and serum specimens obtained from an outbreak of non-A non-B non-C hepatitis in Rundu, Namibia, in 1995.

The ORF2 RT-PCR amplified a 727 bp region coding for an HEV structural gene. Sequence data was obtained for five of the Namibian specimens in this carboxy terminal region, of which 419 bp of unambiguous data were used in the analysis. A consensus sequence was generated from these five specimens and used for phylogenetic analysis together with the GenBank sequences listed in Table 1.

TABLE I : HEV SEQUENCES USED FOR ORF2 ANALYSIS

Name	GenBank Accession No.	Reference
80[a]-Algeria	U40046	van Cuyck-GandrÁ et al. (1997)
82-Burma-B1	M73218	Tam et al. (1991)
95-Central African Republic	AF228532	van Cuyck-GandrÁ et al. (2000)
83-Chad	U62121	van Cuyck-GandrÁ et al. (1997)
84-Chad T3	U40191	van Cuyck-GandrÁ et al. (1996)
87-China-A-Xinjiang	D11092	Aye et al. (1992)
87-China-B-Hebei	M94177	Bi et al. (1993)
87-China-C-K52-87	L25595	Yin et al. (1994)
87-China-D-Uigh179	D11093	Unpublished data
93-Egypt	AF051351	Tsarev et al. (1999)
94-Egypt	AF051352	Tsarev et al. (1999)
92-Fulminant (India?)	X98292	Donati et al. (1997)
90-India-Hyderabad	AF076239	Panda et al. (1995)
93-India-Madras	X99441	Unpublished data
87-Mexico	M74506	Huang et al. (1992)
94-Morocco	AF065061	Meng et al. (1999)
89-Myanmar (Burma-B2)	D10330	Aye et al. (1993)
92-Nepal-TK15/92	AF051830	Gouvea et al. (1998)
83-Namibia	AF105021	He et al. (2000)
97/98-Nigeria 1	AF172999	Buisson et al. (2000)
97/98-Nigeria 4	AF173000	Buisson et al. (2000)
97/98-Nigeria 5	AF173001	Buisson et al. (2000)
97/98-Nigeria 6	AF173230	Buisson et al. (2000)
97/98-Nigeria 7	AF173231	Unpublished
98-Nigeria 9	AF173232	Buisson et al. (2000)
88-Pakistan-Abb-2B	AF185822	van Cuyck-GandrÁ et al. (1999)
87-Pakistan-SAR-55	M80581	Tsarev et al. (1992)
95-USA-1	AF060668	Schlauder et al. (1998)
95-USA-2	AF060669	Erker et al. (1999)

[a]Year of isolation

The analysis revealed that the consensus sequence clustered together in genotype III with the Mexican isolate. Also clustering in this genotype were closely homologous Nigerian isolates from the 1997/98 outbreak in that country. The Namibian consensus shared 88.8 to 91.5 % homology with the Nigerian isolates and 85.4 % homology with the Mexican isolate (Figure 1). The consensus sequence shared only 61.6 to 76.0 % identity with the African isolates in genotype I and 75.3 to 77.6 % identity with the Asian isolates in genotype I.

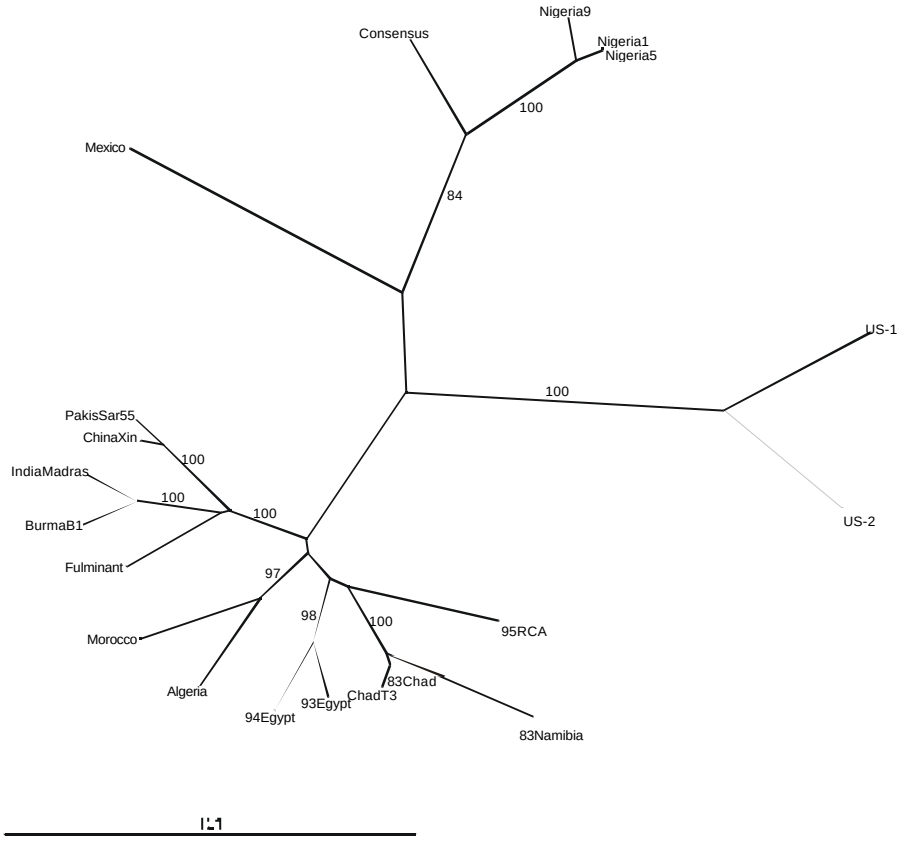


Fig.1: Unrooted phylogenetic tree of HEV isolates from the carboxy terminal end of ORF2

A histogram was constructed in which the frequency of evolutionary distances calculated between the consensus and the GenBank sequences listed in Table 1 was plotted in each 0.05 range between zero and 0.4 was plotted (Figure 2 and Table 2). The Namibian sequence differed from the other genotype III isolates by distances ranging from 0.0848 to 0.1641.

Analysis of the ORF2 distances suggests that isolates within a single genotype would have genetic distances of less than 0.1641, while distances between genotypes are greater than 0.1874 (Table 2). This can also be seen in the histrogram which has two slightly overlapping peaks. The first peak represents the intra-genotypic distances of 17 % or less and the second represents the inter-genotypic distances of 18 % or more.

The first sequence data from Southern Africa was obtained from specimens collected during an outbreak which occurred in Namibia in 1983. These isolates were reported to cluster in genotype I. The 1995 Namibian sequences analysed in this study cluster together with the 1997/98 Nigerian isolates in genotype III. Both the 1983 and 1995 Namibian outbreaks were from the same area, Rundu, in the North-Eastern part of the country. We were therefore surprised to find a different strain in our study. However, our result correlates with other reports from Africa in the late 1990s.

TABLE II : PHYLOGENETIC DISTANCES WITHIN AND BETWEEN GENOTYPES FOR ORF2

The range given represents the minimum to maximum genetic differences within and between groups of sequences derived from the genotypes shown.

	Phylogenetic distance (substitution per position)		
ORF2 sequences	**Genotype I**	**Genotype II**	**Genotype III**
Genotype I	0.0110-0.1394	0.2004-0.2779	0.1874-0.3837
Genotype II		0.0-0.0717	0.2308-0.2500
Genotype III			0.0848-0.1641

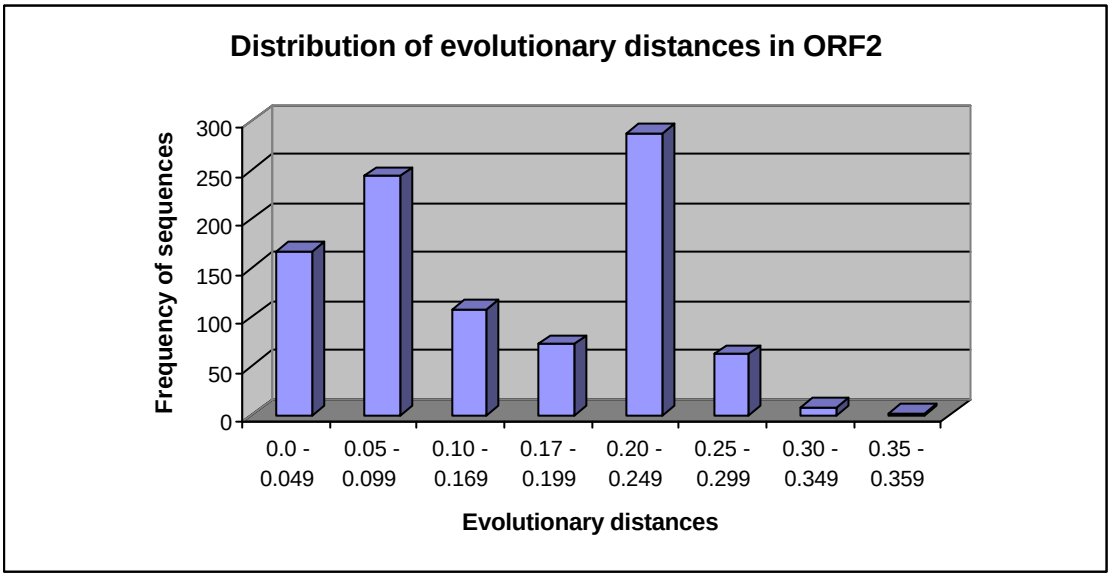


Fig 2: Histogram representing the distribution of genetic distances calculated for the ORF2 region.

Virology - *Respiratory Syncytial Virus*

During the year 2002 exciting progress was made in characterizing RSV molecular biology and immunology in South Africa. The RSV lab received two PRF research grants as well as a travel grant from the NHLS to present our work at the international Virology conference. Two papers on the molecular epidemiology of RSV in South Africa were accepted for publication in international journals and the results of a study about the CTL-response of RSV in South Africa was presented at the 12th International Virology congress in Paris. A MSc student joined the lab and we initiated a project looking at the rate of molecular evolution in the RSV antigenic proteins. At the end of 2002, a Fogarty international collaboration grant was awarded to the RSV CTL project, which is a collaboration between M Venter and Dr Caroline Tiemmesen of the NICD and Dr. James E Crowe Jr of Vanderbilt University Medical School, Nashville Tennessee. The following report describes the progress made in the two fields of research carried out at the RSV lab:

RESEARCH REPORTS

Molecular epidemiology of RSV in South Africa

Two projects described in the 2001 report were completed this year and accepted for publication in international journals. These included:-

1. Molecular epidemiological analysis of community circulating respiratory syncytial virus in rural South Africa: comparison of viruses and genotypes causing different disease manifestations. M. Venter, M. Collinson, B.D. Schoub. *Journal of Medical Virology,* J Med Virol. 2002 Nov;68(3):452-61.
2. Respiratory syncytial virus associated illness in high risk children and national characterisation of the virus genotype in South Africa. S. A. Madhi; M. Venter, R. Alexandra, H. Lewis, Y. Kara, M. Greef, C. Larsen. *Journal of Clinical Virology*, In press.

A new project was initiated entitled:-
The role of molecular evolution in Respiratory Syncytial Virus antigenic proteins during annual epidemics in South Africa.

This project forms part of Ms Elizabeth Agenbach's MSc. project. In a previous study we conducted at Chris Hani Baragwanath hospital over four consecutive seasons (1997-2000) we found switching of the predominant genotype between individual seasons, with genotypes appearing and disappearing from one year to the next. However in 1999 genotype GA2 became the dominant genotype with 42% of isolates falling in this group, and increased even further in 2000 when 78% of all isolates belonged to this genotype. When considering other studies, it appears to be unusual for one genotype to predominate for more than one season. Furthermore this genotype was isolated across the whole country during 2000. This suggests that highly effective and fit strains may have the ability to spread nationwide. However, we hypothesized that heard immunity will eventually overcome the effectiveness of this strain to re-establish epidemics. These observations were made based on genotyping and phylogenetic analysis of the G-protein a major neutralisation antigen of RSV. It is thought that variability in the G-protein may play a role in reestablishment of annual epidemics. Investigations of antigenic differences in genotypes that cocirculate and re-establish epidemics may elucidate the importance of the variability observed between and within G-protein genotypes. We have shown that positive selection occurs over consecutive seasons in the G-protein, however the transmission dynamics and rate of evolution in the G-protein during a single outbreak has not been determined in a confined study. Few studies have yet looked at a correlation between the currently identified genotypes and previously identified G-protein escape mutations, or at changes in some of the more conserved antigens. We have recently shown that positive selection does not occur in the CTL epitopes of the highly conserved N-protein of RSV. However, no study has yet determined if selection occurs in the CTL and neutralisation epitopic domains of the more conserved F-protein which is considered to be the major protective antigen of RSV.

To look at these questions, the first aim of our study was to look at the importance of G-protein variability in re-establishment of annual epidemics. We conducted a molecular epidemiological analysis of an outbreak of RSV at Chris Hani Baragwanath hospital during the 2001 RSV season and compared this data to the previous four seasons. A total of 81 isolates were typed and sequenced from this outbreak. Only 9 out of 81 specimens that were selected for typing were subtype A (11%), while 72/88 of isolates (88.8%) were subtype B. Figures 1 A and B show the phylograms of subtype A and B, respectively. Three subgroup A genotypes (GA2, GA5 and SAA1) were identified and 3 subgroup B genotypes (GB3, SAB1 and SAB3). Table 1 summarises the genotypes identified in this outbreak, indicating the number of unique sequence groups identified, the number of identical isolates in each of these groups, and the date of first and last isolation. A total of 24 unique sequence groups were identified in this outbreak, many circulating concurrently. We found that the genotype GA2 that was dominant for the previous two seasons were clearly replaced and that genotype SAB3 dominated this new epidemic with 69% followed by SAB1 with 18%. Only 3 GA2 isolates were identified. The SAB1 genotype has not been very prominent in previous outbreaks, in fact; only one isolate has been identified before in Soweto. This may confirm the notion that rare strains that first appear in a particular season may become prominent outbreak genotypes in future epidemics in an immunological naive population. Most strains only infected one or two individuals; however, four strains (one SAB1 and three SAB3 strains) were clearly dominating the outbreak and infected many individuals throughout the epidemic (March to May for strain ix (SAB1) and March to June for SAB3 strains xiii, xiv and xv). We hypothesised that these three strains may have been the initial SAB1 and SAB3 outbreak strains since they were isolated first and circulated up to

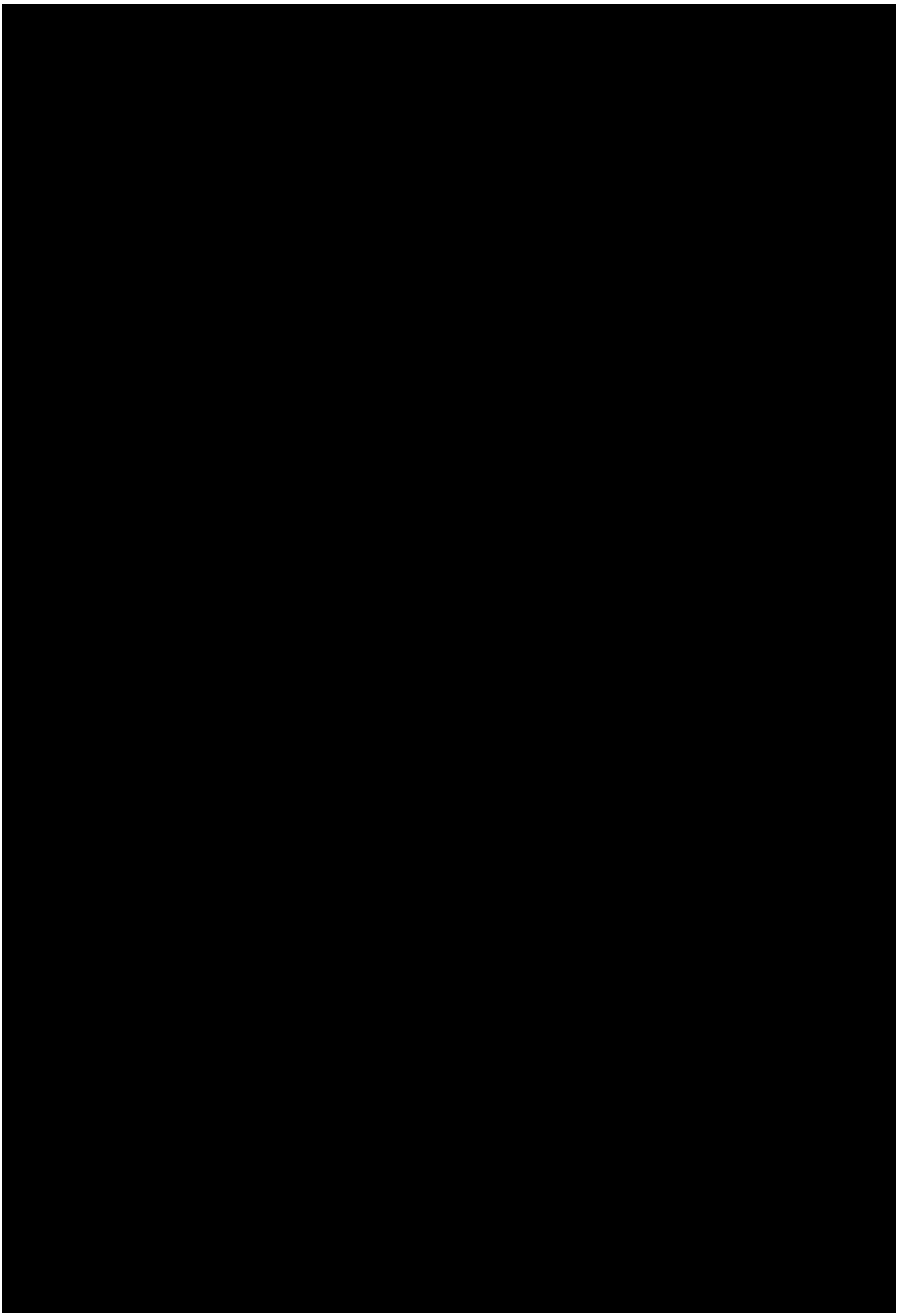
the end of the outbreak ,and that the other SAB1 and SAB3 strains may have resulted from these strains possibly by positive selection. It has been suggested that each of the individual genotypes can be seen as an independent epidemic and that a RSV outbreak like this can in fact be seen as multiple concurrent epidemics. This may suggest that positive selection within each genotype may account for some of the different strains that are later observed in each genotype.

To determine the role of selection in this outbreak, the nucleotide P distances and amino acid P-distances were compared as well as the percentage of synonymous substitutions per synonymous site (Ks) and the non- synonymous substitutions per non-synonymous sites (Ka). We found that for both subgroup A and B the mean amino acid P distance (0.13 and 0.06 for subgroup A and B, respectively) exceeded the mean nucleotide P distance (0.07 and 0.03 for subgroup A and B, respectively). The Ka/Ks values were 1.75 and 2 for subgroup A and B respectively, suggesting positive selection in both cases. This suggests that even within a single outbreak of RSV positive selection occurs which may account for the large number of strains that are identified throughout an outbreak. Figure 2 shows the differences in amino acid sequences between the individual sequence groups and genotypes. Single amino acid changes can be identified between the early SAB1(ix) and SAB3 strains (xiii,xiv,xv) and later isolates from the same genotypes. For subgroup A, the positions of amino acid changes are indicated that were previously identified in escape mutants or natural occurring strains that failed to react with monoclonal antibodies to prototype strains. In subgroup B, differences in the protein lengths can be seen in SAB3 which is associated with changes in strain specific epitopes. These indications of positive selection and changes in the antigenic structure

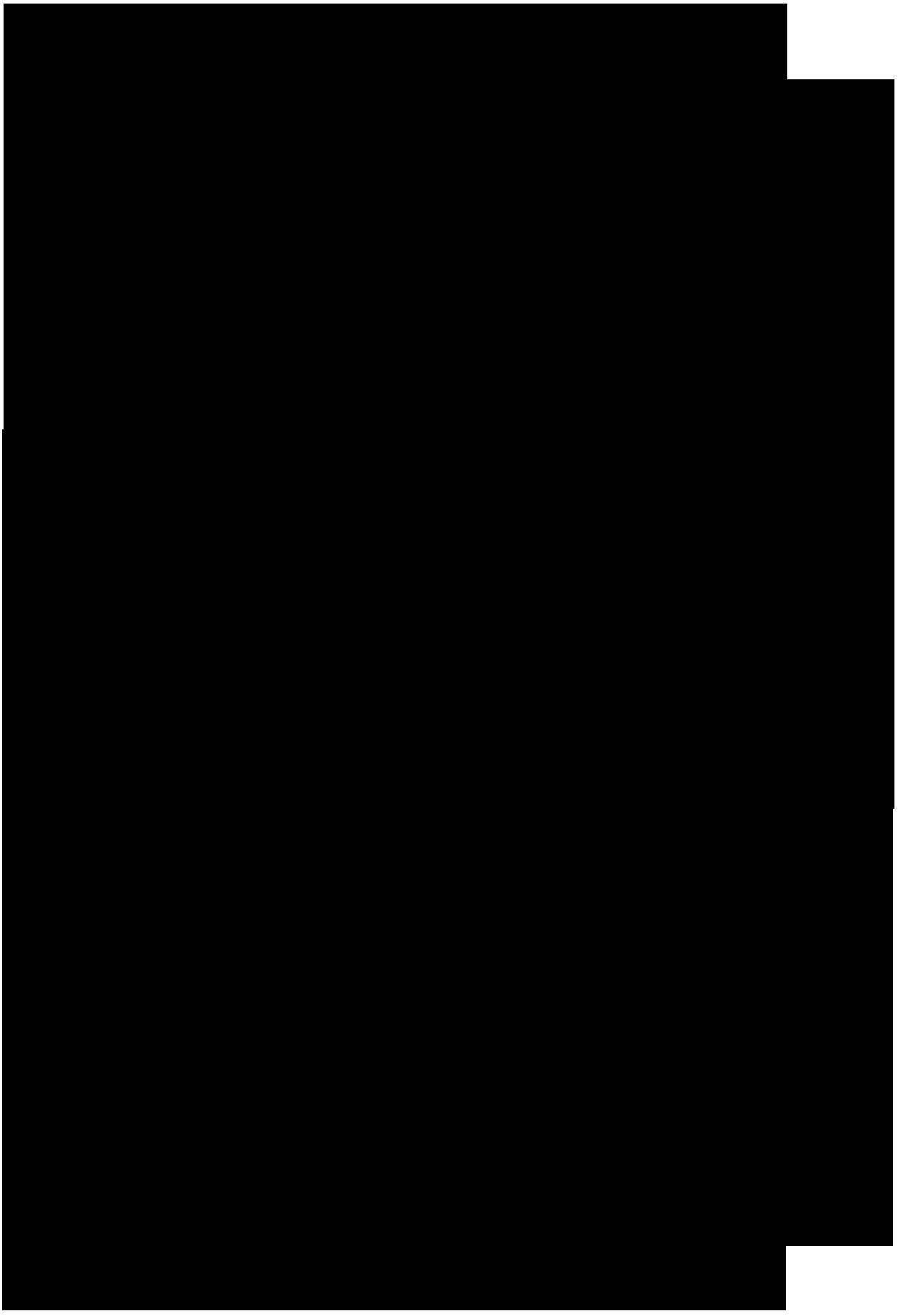
TABLE I : RSV STRAINS IDENTIFIED DURING THE 2001 OUTBREAK AT CHRIS HANI BARAGWANATH HOSPITAL. THE STRAIN NUMBERS ARE INDICATED WITH ROMAN FIGURES.

RSV subtype	Specimens	Genotype	Strain	Number of identical isolates	Date of first isolation	Date of final isolation 1
A	**0069KS01**	GA2	I	1	24 Apr 01	
	157KNSB01	GA2	II	2	11 May 01	15 May 01
	0240KS01	GA5	III	1	31 May 01	
	G148S01	GA5	IV	1	15 Jun 01	
	G160S01	GA5	V	1	22 Jun 01	
	G58S01	GA5	VI	2	10 Apr 02	30 Apr 01
	VG134S01	SAA1	VII	1	12 Jun 01	
	Total type A		7	9		
RSV subtype B	**0137KS01**	GB3	VIII	1	03 May 01	
	G19S01	SAB1	IX	9	27 Mar 01	11 May 01
	V0041KS01	SAB1	X	2	05 Apr 01	04 May 01
	193KS01	SAB1	XI	2	17 May 01	08 Jun 01
	0227KS01	SAB1	XII	2	01 Jun 01	15 Jun 01
	G36S01	SAB3	XIII	12	04 Mar 01	19 Jun 01
	G7S01	SAB3	XIV	22	22 Mar 01	22 Jun 01
	G31S01	SAB3	XV	11	30 Mar 01	28 Jun 01
	V2004KS01	SAB3	XVI	1	02 Apr 01	
	G42S01	SAB3	XVII	1	04 Apr 01	
	G68S01	SAB3	XVII	1	12 Apr 01	
	VG72S01	SAB3	XIX	1	17 Apr 01	
	0112KS01	SAB3	XX	1	25 Apr 01	
	G102S01	SAB3	XXI	3	26 Apr 01	07 Jun 01
	0140KS01	SAB3	XXII	1	03 May 01	
	0219KS01	SAB3	XXIII	1	23 May 01	
	G154S01	SAB3	XXIV	1	19 June 01	
	Total type B		17	72		
Overall Total			**24**	**81**		

Virology - *Respiratory Syncytial Virus*



Virology - *Respiratory Syncytial Virus*



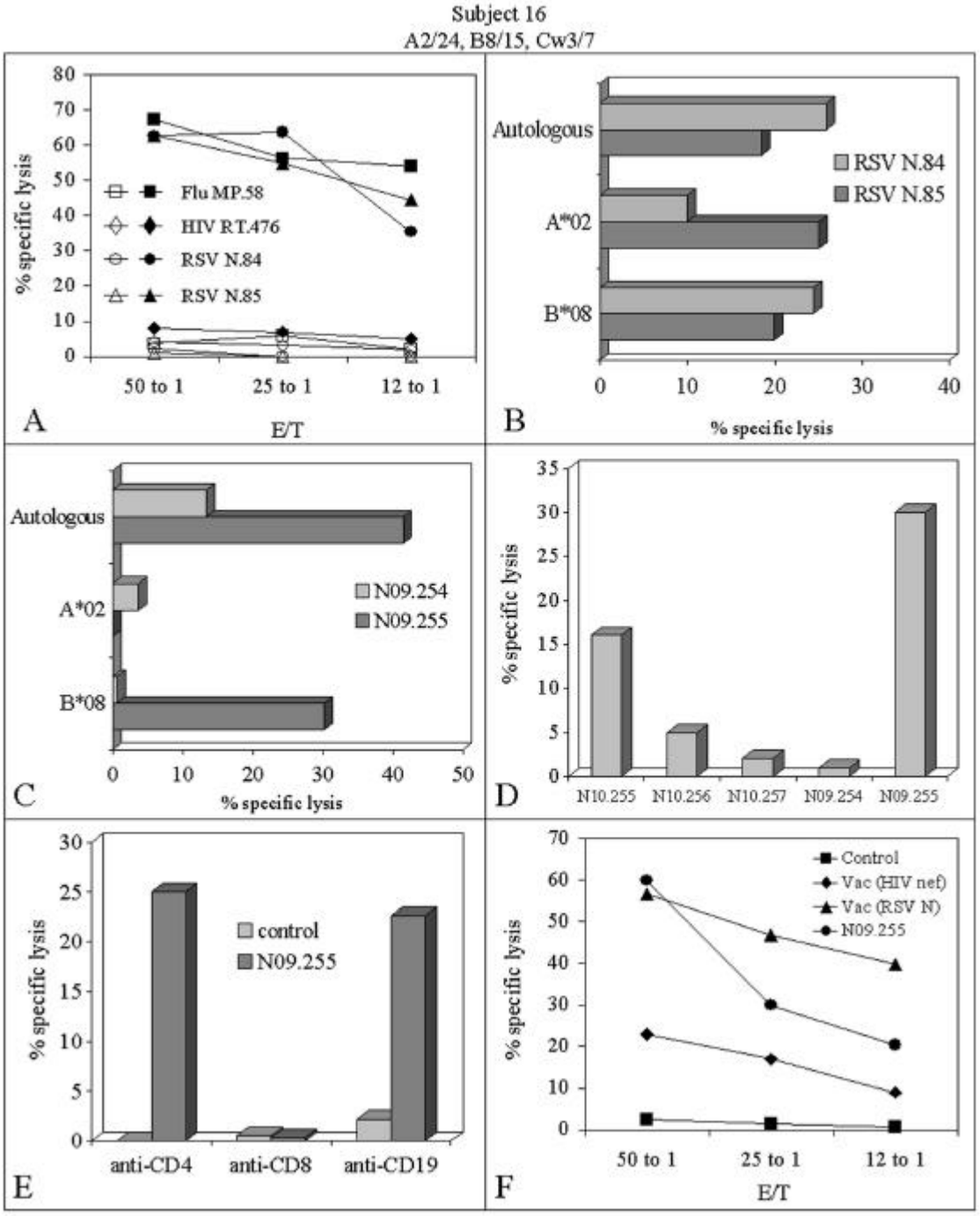


Fig 3. Recognition of viral epitopes by peptide-specific effector cells generated from subject D16 (HLA-type indicated) in standard 51Cr-release assays. (a) Effectors were Flu MP.58 (¾), HIV RT.476 (t), RSV N.84 (¯), or RSV N.85 (p) peptide-specific cell lines. Target cells were autologous BCL pulsed in the presence (closed symbols) or absence (open symbols) of the indicated peptides. (b, d) Effector cells were RSV N.84 or RSV N.85 (b), or RSV N09.254 or RSV N09.255 (d) peptide-specific cell lines. Target cells were autologous or HLA single-matched BCL (corresponding HLA-alleles are indicated) pulsed in the presence of the indicated peptides. (c) Peptide-specific effector cells were generated and assayed for cytotoxicity against HLA-B*08-matched BCL target cells pulsed with the indicated peptide. (e) RSV N09.255 peptide-specific effector cells were depleted of CD4+, CD8+, or CD19+ cells by paramagnetic beads and assayed for cytotoxicity against HLA-B*08 restricted BCL pulsed in the presence or absence of peptide RSV N09.255. (f) Effector cells were RSV N09.255 peptide-specific cell lines. Target cells were HLA-B*08-restricted BCL that were pulsed in the presence (¯) or absence (¾) of peptide RSV N09.255, or infected with recombinant vaccinia virus expressing the HIV nef protein (t) or the RSV N protein (p). The data are presented as the percent specific lysis at an effector to target ratio (E/T) of 25/1 (b-e) or at the indicated E/T ratios (a and f).

that may result in escape of the immune response may suggest that the later strains may result because of mutations and possibly selection in the early strains. This variability may give rise to new strains that may again overcome heard immunity in future outbreaks.

To determine if this positive selection is reflected in the other antigenic proteins, the F-protein of each of the genotypes identified in this and previous South African outbreaks have been amplified by RT-PCR and will now be sequenced.

Characterization of the cytotoxic T-cell response to RSV in South Africans

The second part of the research carried out in the RSV laboratory, entails a study of RSV Cytotoxic T-cell immunology. Studies in mice suggest that the CTL responses are crucial for the disease free control of RSV. Although CTL activity has been shown to be important for viral clearance, it has also been shown to be capable of causing immunopathology when passively transferred in large amounts into the lungs of mice (Zinkernagel & Hengartner, 1994). This emphasises the fine balance that exists between protection and disease-enhancement, and the importance of a thorough surveillance of the immune response during testing of experimental vaccines. In the 2001 report we described the first part of this project which aimed at identifying cytotoxic T-cell epitopes in South African adults which can be used as peptide reagents in CTL assays in future studies or vaccine trails to monitor the CTL response in infants. In short, to characterise the RSV-specific CTL response in South Africans, IFN-gamma ELIspot was used to screen 37 healthy adults with diverse HLA backgrounds for memory CTL responses to peptides covering the complete N-protein of RSV. Responses of more than 40 spot forming units per million (SFU/million) cells were detectable in 21 individuals. The dominant responses were further characterized and two 14-mer peptides identified with cytolytic activity restricted to HLA B*08, A*02 and B*15. During 2002 the epitopes on these 14mers were mapped using truncated peptides and confirmed as CD8+ CTL epitopes. Finer mapping identified a novel 9-mer B*08-restricted, RSV-specific, CD8+ CTL epitope (Figure 3). These HLA types are common in the South African population and will be useful for subsequent studies of CTL responses to RSV in infants. This is the first time that a dominant CTL response has been characterised within a complete RSV protein, and is only the fourth human CTL epitope to be identified in RSV, and the first to these particular HLA types. The identified CTL epitope was then shown to be completely conserved in the nucleoproteins of all genotypes identified in South Africa to date by sequencing representatives of each genotype and comparing the amino acid sequences. No indications of positive selection could be found in the selected epitopes or on the N-protein, suggesting that regular updating would not be required if this epitope is used to monitor the CTL response in RSV vaccine trials. This work has been accepted for publication in the Journal of Virology.

At the end of 2002 an International Fogarty collaboration grant was awarded to this project. This work will be continued by Gillian Hunt under the supervision of Dr. Caroline Tiemessen and in collaboration with Dr. James E. Crowe Jr. at Vanderbilt University Medical School in Nashville Tennessee. Marietjie Venter will remain an advisor to this project but has moved to the Special Pathogen Unit upon completion of her PhD. The Molecular Project will be completed by Ms. Agenbach under the supervision of Ms. Venter. Final investigations into the Molecular biology and immunology will include an analysis of the F-protein of RSV to determine if CTL and B-cell epitopes remains conserved in this protein which is an important target for recombinant vaccine development.

Virology - *Special Pathogens Unit*

Staff of the Special Pathogens Unit, including entomology and arbovirus sections

Virology - *Special Pathogens Unit*

The Special Pathogens Unit of the National Institute for Communicable Diseases is responsible for the diagnosis and investigation of diseases associated with the so-called formidable (biohazard class 4) viruses in southern Africa, and operates a maximum security (biosafety containment level 4) laboratory with two sections: a cabinet-line laboratory where work with the viruses is done under negative air pressure in enclosed (class 3) gloveboxes and a suit laboratory 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 and rabies-related infections in humans and it incorporates the Medical Ecology Unit, which is responsible for monitoring bubonic plague activity in small mammals in South Africa. The Unit is recognized as a World Health Organization (WHO) Regional Collaborating Centre for Reference and Research on Viral Haemorrhagic Fevers and Arboviruses.

COMPARISON OF SPECIMENS RECEIVED IN 2001 AND 2002

The total number of specimens tested in the Unit during 2002 was higher than in recent years, mainly because a large number of cattle sera were tested in a survey of Crimean-Congo haemorrhagic fever antibody prevalence in North-West Province, and because wild vertebrate specimens were received in connection with the search for reservoir hosts of Ebola and Marburg haemorrhagic fever viruses in Gabon and the Democratic Republic of the Congo (Table I).

We continued to receive specimens from many countries in Africa and the Middle East for the investigation of suspected cases of viral haemorrhagic fever (VHF), and we confirmed 36 cases of Crimean-Congo haemorrhagic fever in an Asian country. A field trip was undertaken to Gabon to perform ecological studies on Ebola fever in association with the Centre International pour la Recherche Médicales de Franceville (CIRMF), Gabon, and the Centers for Disease Control and Prevention (CDC), Atlanta.

TABLE I : COMPARISON OF SPECIMENS RECEIVED IN SPECIAL PATHOGENS UNIT, 2001 & 2002

	Specimens received in 2001	Specimens received in 2002
Diagnostic:		
Suspected VHF (South Africa)	112 (75 patients)	75 (54 patients)
Suspected VHF (other countries)	140 (128 patients)	214 (209 patients)
VHF contacts	57 (57 persons)	113 (113 persons)
Undiagnosed fevers	40 (39 patients)	148 (106 patients)
Suspected rabies	15 (13 patients)	22 (17 patients)
Rabies immunity	109 (109 accessions)	176 (150 accessions)
Ticks	3 (3 accessions)	2 (2 accessions)
Miscellaneous	658 (56 accessions)	174 (28 accessions)
Surveys:		
Occupational/residential groups	228 (3 groups)	250 (2 groups)
Cattle & sheep for zoonoses	320 (3 accessions)	8 505 (2 accessions)
Dogs for plague	1 101 (20 districts)	656 (21 districts)
Rodents for plague	712 (20 districts)	569 (21 districts)
Wild animals	275 (13 accessions)	590 (13 accessions)
Total specimens:	3 770	11 494

* In addition, VHF antibody screening tests were applied to 256 sera from 256 patients submitted to the Arbovirus Unit for the investigation of suspected infection with arthropod-borne viruses, without positive results.

INVESTIGATION OF SUSPECTED VHF

Five cases of Crimean-Congo haemorrhagic fever (CCHF) were confirmed in southern Africa during 2002 (Table II). In three instances there was evidence that the infection was the result of a tick bite. One patient visited a farm in northern Namibia and found a tick on himself a few days before onset of illness. A farm worker from the Northern Cape Province noticed a tick bite the day before onset of illness, and a rural resident gave a history of being bitten by a suspected *Hyalomma* tick while processing animal hides in a tanning plant in a Free State town before presenting with a febrile illness.

A total of 171 cases of CCHF have been diagnosed in southern Africa from the time that the presence of the disease was first recognized in 1981 up until the end of 2002, with one infection having occurred in the Democratic Republic of the Congo, one in Tanzania, fourteen in Namibia and the rest in South Africa. The total of 165 cases reported last year should have read 166 cases. Marginally the largest group of cases, 74/171 (43,3%), arose from known tick bite or the squashing of ticks; a similar number, 69/171 (40,3%), arose from known or potential contact with fresh blood or other tissues of livestock and/or ticks; 7/171 (4,1%) nosocomial infections arose from contact with blood or fomites of known CCHF patients, while in 21/171 (12,3%) cases there was no direct evidence of contact with livestock or ticks, but the patients lived in or visited a rural environment where such contact was possible. Most patients were employed in the livestock industry, and males constitute 141/170 (82,9%) of all cases of the disease diagnosed to date. The case fatality rate fluctuated around 30% in the first few years after CCHF was initially recognized in southern Africa, but has declined to 25,9% (44/170) probably as a result of greater awareness, earlier diagnosis and appropriate therapy. Ribavirin is believed to improve the prognosis if administered before day 5 after onset of illness.

The majority of specimens submitted from patients with suspected VHF were negative, and alternative diagnoses included malaria, viral hepatitis including a number of cases of fulminant herpes hepatitis, bacterial septicaemias, tick-borne typhus, Q fever and HIV/AIDS with opportunistic infections. None of the other VHFs were diagnosed in humans within South Africa during the report year.

CCHF was confirmed in 36 patients from an Asian country where the arid climate is well suited to the tick vectors of the disease, and where nomadic herdsmen and refugees are regularly exposed to ticks and fresh blood of slaughtered livestock.

RABIES

Despite intense efforts to improve dog vaccination coverage and the availability of post-exposure prophylactic treatment to humans in recent years, 10 cases of human rabies were confirmed by the SPU during 2002 (Table 3). Nevertheless, the position remains much improved as compared to the years before 1997. In addition, one patient who died in 2001 was diagnosed in 2002, bringing the total number of cases in 2001 to eight. As in the past, the majority of patients acquired infection from contact with rabid dogs in KwaZulu-Natal. No histories of post-exposure prophylactic

TABLE 2: LIST OF CONFIRMED CASES OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS INFECTION IN SOUTHERN AFRICA, JANUARY TO DECEMBER 2002

Location of exposure exposure	Month	Age/Sex	Virus Isolation	PCR	Antibody*	Died/ Survived	Source of Infection
Mangeti, Namibia	Jan	47M	+	+	+	Survived	Tick bite
Niekershoop, Nn Cape	Feb	22M	+	+	+	Survived	Tick bite
Rehoboth, Namibia	Sep	65M	+	+	+	Died	Sheep
Kennard, Nn Cape	Nov	49M	+	+	+	Died	Sheep
Heilbron, Free State	Dec	33M	+	+	+	Survived	Tick bite

***** Demonstration of IgM and/or IgG antibody responses.

TABLE III : CONFIRMED CASES OF RABIES, 2002

Name	Age/ Sex	District of exposure	Exposure: bitten by	Onset	Admitted to hospital	Died	Final Hospital
MM*	70M	Unknown	Dog 16/9/01		10/12/01	10/12/01	Manguzi
SN	8M	Umhlanga	Dog, Jan	15/02		18/02	King Edward
CJvH	51M	Lesotho	Dog			02/03	Bloemfontein
SNX	18M	Kranskop	Dog, Jan	09/03		14/03	Untunjambili
KL	8	Ndwedwe	Dog, Feb	12/04		15/04	Madadeni
NN	5F	Paulpietersburg	Dog, Apr	14/04	15/04	15/04	Vryheid
ES	35M	Umfolozi	Unknown		03/08	03/08	Ngwelezana
KT	46M	Maputo	Dog, May	22/09	26/09	28/09	Rob Ferreira
BM	14	Eshowe	Dog, Aug	13/09	16/09	21/09	Ngwelezana
MG	11M	Augrabies	Cat, Sept		03/11	09/11	Kimberley
NB	6M	Nkampini	Dog?	09/11	11/11	13/11	Port Shepstone

* Rabies case from 2001, specimen received and diagnosed in 2002.

treatment were obtained for any of the confirmed cases of rabies.

PLAGUE SURVEILLANCE IN DOGS AND RODENTS

Serum samples collected from 656 dogs and 569 rodents from 21 districts in three provinces were tested for antibody to *Yersinia pestis* F1A antigen by ELISA, and there were no positive results.

RESEARCH

EBOLA FEVER ECOLOGY STUDIES IN GABON

An outbreak of Ebola haemorrhagic fever occurred in eastern Gabon and adjacent parts of the Republic of Congo (Brazzaville) towards the end of 2001 and continued into 2002. The outbreak was unique in that human chains of infection arose independently at scattered separate foci where people found and consumed meat from dead animals, mainly gorillas and chimpanzees, but also duiker antelope. Previous outbreaks of Ebola fever involved spread of infection from a single or very few primary human cases. The local people were hostile towards medical teams coming from outside of the affected districts to try and help control the outbreaks and infection smouldered on into early 2002. The low density of the human population in the affected areas probably contributed towards the fact that the outbreak remained at low intensity. In February, 2002, members of our Unit joined staff of the CIRMF laboratory in Gabon, and CDC Atlanta in trapping and collecting samples from animals and insects in eastern Gabon, as part of a study to try and identify the reservoir hosts of the virus. It was intended that a second field trip would be undertaken late in 2002 or early 2003, but testing of the specimens we had collected consumed the major part of our research efforts for the remainder of the year. The results will be made known in due course.

REASSORTMENT IN RIFT VALLEY FEVER VIRUS

Dr M Sudhanva, a Specialist Registrar at the West of Scotland Specialist Virology Centre, Gartnavel General Hospital, Glasgow, visited the SPU 2002, and investigated the possible occurrence of natural reassortants among 27 Rift Valley fever (RVF) isolates collected over a period of 29 years from 1969-1998 in Zimbabwe, where the virus appeared to have been endemic. The RVF virus genome consists of three single stranded RNA segments designated L (large), M (medium) and S (small). The segmented nature of the genome theoretically facilitates the occurrence of reassortment. Partial genomic sequencing was performed on all three RNA segments of the 27 isolates, and phylogenetic analysis of the sequence data suggests the existence of two closely related but distinct groups, one of which can be subgrouped. The phylogenies were similar for each RNA segment, but the assignment of 3/27 isolates to a particular subgroup did not remain constant within the three phylogenies (Figures 1-3), and hence it could be assumed that these three isolates were naturally occurring genetic reassortants.

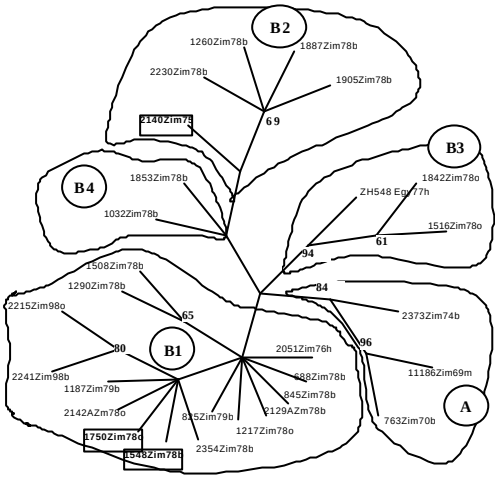


Fig 1. Maximum likelihood phylogram of 284-bp L segment region from 27 Zimbabwean RVFV isolates (1969 – 1998) and the Egyptian isolate ZH548. Values at nodes indicate the level (%) of bootstrap support using weighted parsimony (ts/tv = 7/1) after 100 replicates in PAUP. The tree is unrooted. Reassortants are bold and boxed.

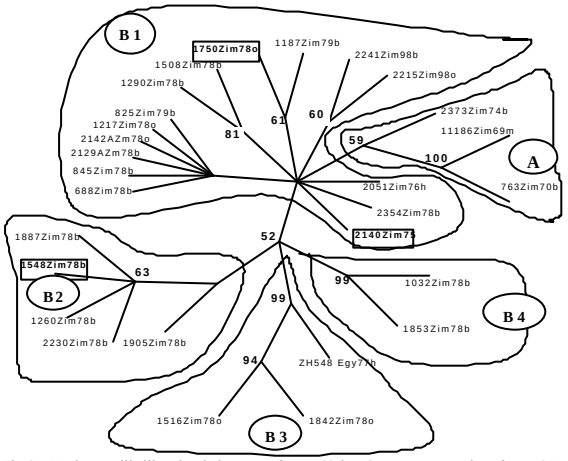


Fig 3. Maximum likelihood phylogram of a 569-bp S segment region from 27 Zimbabwean RVFV isolates (1969 – 1998) and the Egyptian isolate ZH548. Values at nodes indicate the level (%) of bootstrap support using weighted parsimony (ts/tv = 3/1) after 100 replicates in PAUP. The tree is unrooted. Reassortants are bold and boxed.

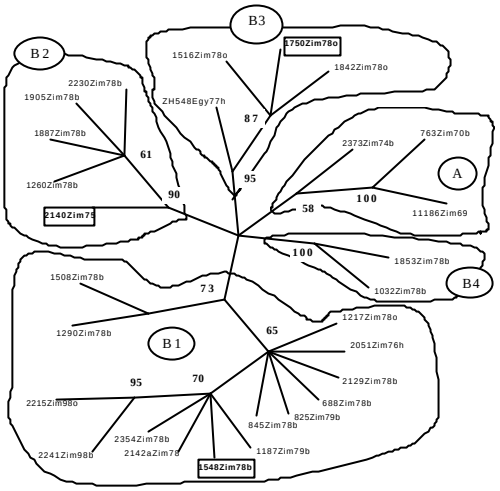


Fig 2. Maximum likelihood phylogram of a 535-bp M segment region from 27 Zimbabwean RVFV isolates (1969 – 1998) and the Egyptian isolate ZH548. Values at nodes indicate the level (%) of bootstrap support using weighted parsimony (ts/tv = 8/1) after 100 replicates in PAUP. The tree is unrooted. Reassortants are bold and boxed.

DEVELOPMENT AND VALIDATION OF ELISA FOR RIFT VALLEY FEVER

In recent years Rift Valley fever has caused large epidemics in East and West Africa, as well as on the Arabian peninsula, with the loss of hundreds of human lives. Consequently, there is a demand for properly validated antibody test kits for diagnosis of the disease, and for the testing of import and export livestock. The Unit developed and validated IgG-sandwich and IgM-capture ELISA kits for use in testing human and domestic ruminant sera. Large numbers of sera were tested in parallel by virus neutralization, and IgG and IgM ELISAs, and it was found that the kits provide a safe and highly accurate means of testing sera.

CCHF SURVEY IN NORTH-WEST PROVINCE

Over the past few years there have been several cases of CCHF in the North-West province, and consequently an IgG antibody survey was conducted on cattle in the province in collaboration with the Onderstepoort Veterinary Institute at the request of provincial veterinary authorities. A total of 8 496 sera from 100 randomly selected farms was tested. Antibody was detected on all farms, and the prevalence on individual farms ranged from 31.9% to 100% (mean 84.5%). This is in keeping with the findings in a nationwide survey conducted more than a decade previously. Considering the high prevalence of antibody in livestock, and the high seroconversion rate previously observed in young animals, it remains surprising that so few human infections are diagnosed each year. Possible explanations include the facts that the principal vectors, *Hyalomma* ticks, prefer livestock and seldom feed on humans, and that infected animals are viraemic for a very short period only, usually less than a week. Humans can acquire infection from contact with the blood of infected animals only during the short viraemic period.

Virology - *Special Pathogens Unit - arboviruses*

A total of 721 specimens were submitted to the Arbovirus Unit during 2002, including: 271 serum samples from 256 patients in southern Africa with suspected arbovirus infections, 23 sera from NICD staff members to be tested for immunity to Rift Valley fever (RVF) and yellow fever, 29 sera for quality assurance tests, 252 serum samples from a sheep RVF vaccine experiment, and 146 samples referred from other countries in Africa and the Near East.

Among the specimens from southern Africa tested for suspected arbovirus infections, 93 sera from 77 patients had demonstrable haemagglutination inhibition (HAI) antibody to one or more arbovirus antigens as shown in Table 1. HAI positive sera were tested by ELISA for IgM antibody activity to the relevant viruses, and four patients were IgM positive for Sindbis virus, two were IgM positive for dengue virus and one was IgM positive for West Nile. Dengue virus was isolated from early serum samples collected from the two patients in which IgM antibody to dengue virus was demonstrated. In addition dengue virus was isolated from an early, antibody-negative, serum sample collected from a patient for which no follow-up sample was available. The 3 patients with dengue infection all had a recent history of travel to eastern countries where dengue virus infection is endemic. West Nile virus was isolated from one patient in South Africa and yellow fever virus was isolated from a febrile patient with a recent history of vaccination. Sequence analysis of the yellow fever isolate confirmed that it was of 17-D vaccine origin.

There were five HAI reactors to the alphavirus antigens, o'nyong-nyong and chikungunya, among samples submitted from Uganda. No viruses could be isolated, but two samples were positive using PCR with genus-specific alphavirus primers, and sequence analysis of the amplicons was used to confirm that o'nyong-nyong virus was the aetiological agent of an outbreak of febrile illness in Uganda.

After routine arbovirus tests had been completed, 256 serum samples from 256 patients were screened for antibody to CCHF virus by indirect immunofluorescence, and none were positive.

TABLE I : NUMBERS OF DIAGNOSTIC SERA POSITIVE TO ONE OR MORE ARBOVIRUS ANTIGENS

SIN	CHIK	WSL	WN	RVF	DEN	YF	Number of positive sera
pos							10
	pos						0
		pos					4
			pos				10
				pos			3
					pos		0
						pos	29
pos		pos				pos	2
		pos	pos				8
		pos	pos		pos	pos	7
	pos					pos	1
	pos	pos	pos		pos	pos	1
	pos		pos				1
pos				pos			1
pos		pos	pos				3
pos		pos					2
			pos		pos	pos	7
pos			pos				2
			pos			pos	1
pos						pos	1
							93

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

CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS ECOLOGY

A long term project is being conducted on the ecology of Crimean-Congo haemorrhagic fever (CCHF) virus in the Highveld region of South Africa. One of the principal aims is to determine why virus activity, as evidenced by livestock immune rates, is highly prevalent on some farms and absent on others. CCHF is an arthropod-borne virus transmitted principally by ticks of the genus *Hyalomma* throughout its geographic range in Africa, Eastern Europe and Asia. The ticks are ectoparasites of mammals and birds, with the immature stages (larvae and nymphs) parasitizing small vertebrates (hares, rodents, shrews, and birds) while the adults parasitize large vertebrates (sheep, cattle, antelopes and ostriches). Other tick species are also known to be susceptible to CCHF virus in the laboratory and are capable of transmitting the virus, although most transmission experiments were performed after artificial inoculation of ticks with virus, and need to be repeated by allowing the ticks to become infected by feeding on viraemic hosts. The *Hyalomma* species are uncommon on the South African Highveld, but they are occasionally introduced to the Highveld through cattle and wild herbivore movements, or possibly by bird migrations, to establish focally viable populations.

Over the past four years, field studies were conducted on tick parasitization rates and CCHF antibody prevalence in livestock and wild vertebrates on selected properties, but these were scaled down during the past year to concentrate on collecting *Hyalomma* and other ticks in order to establish colonies on which transmission experiments can be performed. Ticks are being maintained in the laboratory by feeding on rabbits.

INTRODUCTION OF *AEDES ALBOPICTUS* INTO PORT ELIZABETH IN A USED TYRE SHIPMENT FROM TAIPEI

A container-load of used tyres, received in November from Taipei by an importing company in Port Elizabeth, was found to contain wet tyres. The tyres were treated with an anti-mosquito larval agent before being forwarded to a vulcanizing plant. Workers complained that they were being bitten inside the container while unpacking the tyres, and the container was resealed and fumigated, after which the remainder of the tyres were removed. Mosquito samples were identified by Dr R. Hunt and Prof M. Coetzee of the Entomology Unit, NICD, NHLS Central, as belonging to the species *Aedes (Stegomyia) albopictus*, the so-called Asian tiger mosquito. Members of the subgenus *Stegomyia* include important vectors of yellow fever and dengue fever viruses, such as *Aedes aegypti*, *Aedes bromeliae* (formerly classed as *Aedes simpsoni*) and *Aedes africanus*. Two species, *Ae. aegypti* and *Ae. albopictus*, have been shown to be capable of breeding in used tyres and being transported between continents in used tyre shipments.

The SPU entomologist was asked to visit Port Elizabeth to assess the situation, to set up a surveillance programme and to advise port and provincial health authorities on suitable control methods. An inspection of the site and its surroundings revealed a distinct lack of potential oviposition sites/larval habitats for *Ae. albopictus*. Housekeeping practices in and around the industrial site were of an exceedingly high standard so that artificial containers were almost completely lacking and the few trees present in the area were species that do not normally support tree holes. Human-baited collection attempts were fruitless; *Ae. albopictus* is a day-time biting species and should have been detected if present. Surveillance based on ovitraps and gravid traps was undertaken in December, but no evidence of mosquito breeding was detected. It was concluded that *Ae. albopictus* had not become established in Port Elizabeth, largely due to the inhospitable environment where the introduction took place, plus the efficient manner in which the Port Health Authority fumigated the container and treated the wet tyres with larvicide.

Several introductions of *Aedes albopictus* have occurred in South Africa previously and procedural guidelines have been in place since 1993 to minimize the risks of introduction of exotic mosquitoes in used tyres, and to monitor mosquito populations in our ports. Unfortunately, the procedures are apparently not being implemented fully for various reasons but mainly because of staff shortages. *Ae. albopictus* is continually colonizing new areas of the globe and it is probably only a matter of time before it is detected as a breeding population in South Africa, most likely along the east coast with its harbours and sub-tropical climate providing ideal conditions for introduction and successful maintenance of tree-hole breeding mosquitoes.

Poliomyelitis Research Foundation Library

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lent	0
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lent	87
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from our library	94

Eighty three research papers written by the staff of the NHLS were published in British, American and South African journals during 2002. These were added to the already large number of articles in the Reprint Collection. Fourteen chapters for books which will be published shortly, were written by invitation.

Users still enjoy the weekly displays of new journals, and are able to photocopy immediately articles of interest to themselves, not having to wait for the journals to be sent to each person. This reduces the distribution of journals from the library.

As the need for the rapid spread of research information has become more obvious, more and more publishers are making their journals available online. We are able to access articles from certain journals that were previously available only by requesting them through interlibrary loans, which delayed receipt of the information. We are now able to provide users with the articles much more quickly, sometimes on the same day that they are requested from the library. In this way the service rendered by the library is more efficient.

Hazel Saevitzon and Sandy Holmes - staff of the Poliomyelitis Research Foundation Library

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