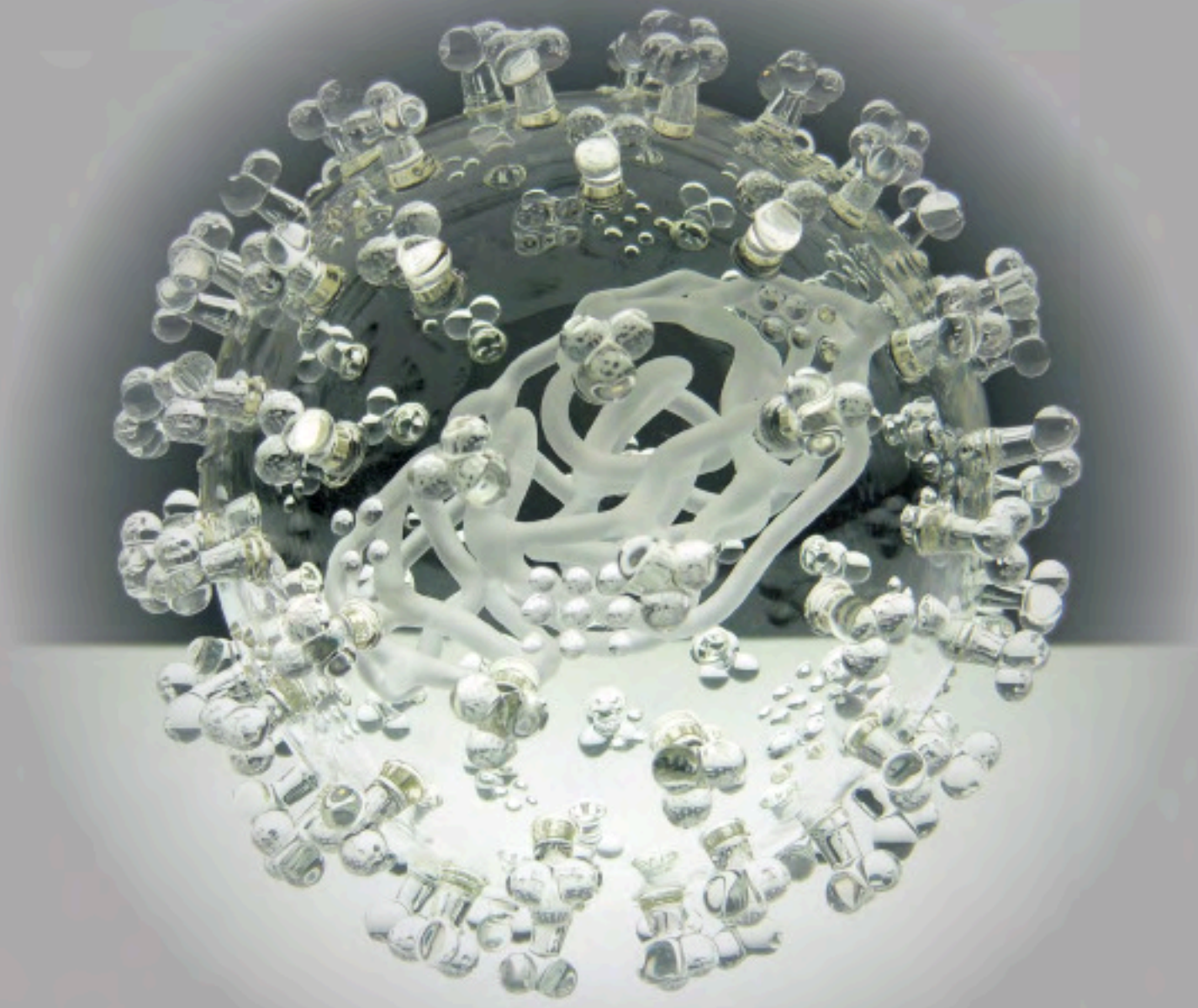
National Institute for Communicable Diseases

A division of the National Health Laboratory Service



NATIONAL HEALTH LABORATORY SERVICE

Annual Report 2009

NICD

Luke Jerram's transparent glass sculptures are created to contemplate the global impact of infectious disease while exploring the edges of perception, scientific understanding and visualization of a virus. Designed in consultation with virologist Dr. Andrew Davidson from the University of Bristol in England, using a combination of different scientific photographs and models, the sculptures were made in collaboration with a team of specialized scientific glassblowers. Through them the artist reveals the fascinating tension between something that is unusually beautiful but which is also extremely dangerous and plaguing humanity.

Luke Jerram is an inventor, a researcher and a multidisciplinary artist. Currently he is a Research Fellow at the University of Southampton, England. Jerram is the recipient of numerous awards and grants and his extraordinary projects and installations have won acclaim in cities around the world. To find out more go to www.lukejerram.com

We, at the National Institute for Communicable Diseases, are extremely grateful for having been given permission by the artist to use a picture of his glass sculpture of the H1N1 pandemic influenza virus in our 2009 Annual Report.

National Institute for Communicable Diseases

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Director's Report

The year 2009 has been a particularly successful one for the NICD. An especially large number of our personnel received prestigious awards to honour the excellence of their contributions to public health and to medical research. The research publications of the Institute continue to increase both in quantity and in quality. The Institute was privileged to host a large number of high-level international meetings. Lastly, several of the building programmes have reached finality in 2009.

Let me begin with the latter milestones. The National TB Reference Laboratory building, a new striking, modernistic structure was completed and opened in 2009 and is now fully functional. It houses the personnel of the Reference Laboratory as well as ACILT (African Centre of Integrated Laboratory Training), a joint venture with the CDC which has trained dozens of technologists and scientists from throughout the continent in applied diagnostic technologies. The upgrading of the maximum security BSL-4 laboratory has also been completed and now also includes a high containment facility for experimental bat studies. Construction work has also commenced on the influenza BSL-3 facility as well as revamped facilities for viral gastroenteritis.

What has really made this Institute proud are the extensive awards and prizes awarded to our staff. These include the following:-

- Dr Penny Moore (AIDS Research Unit) was awarded the 2009 Sydney Brenner Fellowship of the National Research Foundation. Penny was also awarded the international CHAVI (Centre for HIV/AIDS Vaccine Immunology) Young investigator of the month award.
- Dr Elin Gray (AIDS Research Unit) received a Prestigious Postgraduate Award for the best PhD graduate of the University of the Witwatersrand.
- Dr Lizette Koekemoer (Vector Control Reference Unit) was awarded the silver medal of the Southern African Association for the Advancement of Science/British Association Medal, for 2009.
- Genevie Ntshoe received the prize for the best oral presentation at the 3rd African Field Epidemiology Network (AFENET) in Mombasa.
- Professorial status was awarded to two of our senior staff during 2009 Professor Adrian Puren (University of the Witwatersrand) and Professor Marietjie Venter (university of Pretoria) in recognition of their academic contributions to their respective universities.

The 2nd cohort of the SAFELTP (South African Field Epidemiology Laboratory Training Programme) course completed their 2 year course and will graduate MPH at the University of Pretoria early in 2010.

During 2009 the NICD hosted a number of prestigious international events in the PRF Training Centre. These included:-

- The 4th Annual meeting of the International Association of Public Health Institutes
- The 1st African Influenza Scientific Symposium of WHO / CDC
- The 1st joint workshop of the NICD and the HPA (Health Protection Agency) of the UK
- 2010 FIFA World Cup Communicable Disease Control Workshop
- The 2nd Infectious Diseases in Africa symposium and
- The 3rd African Flow Cytometry Workshop

Participants in the 1st African Influenza Scientific Symposium of the WHO/CDC.

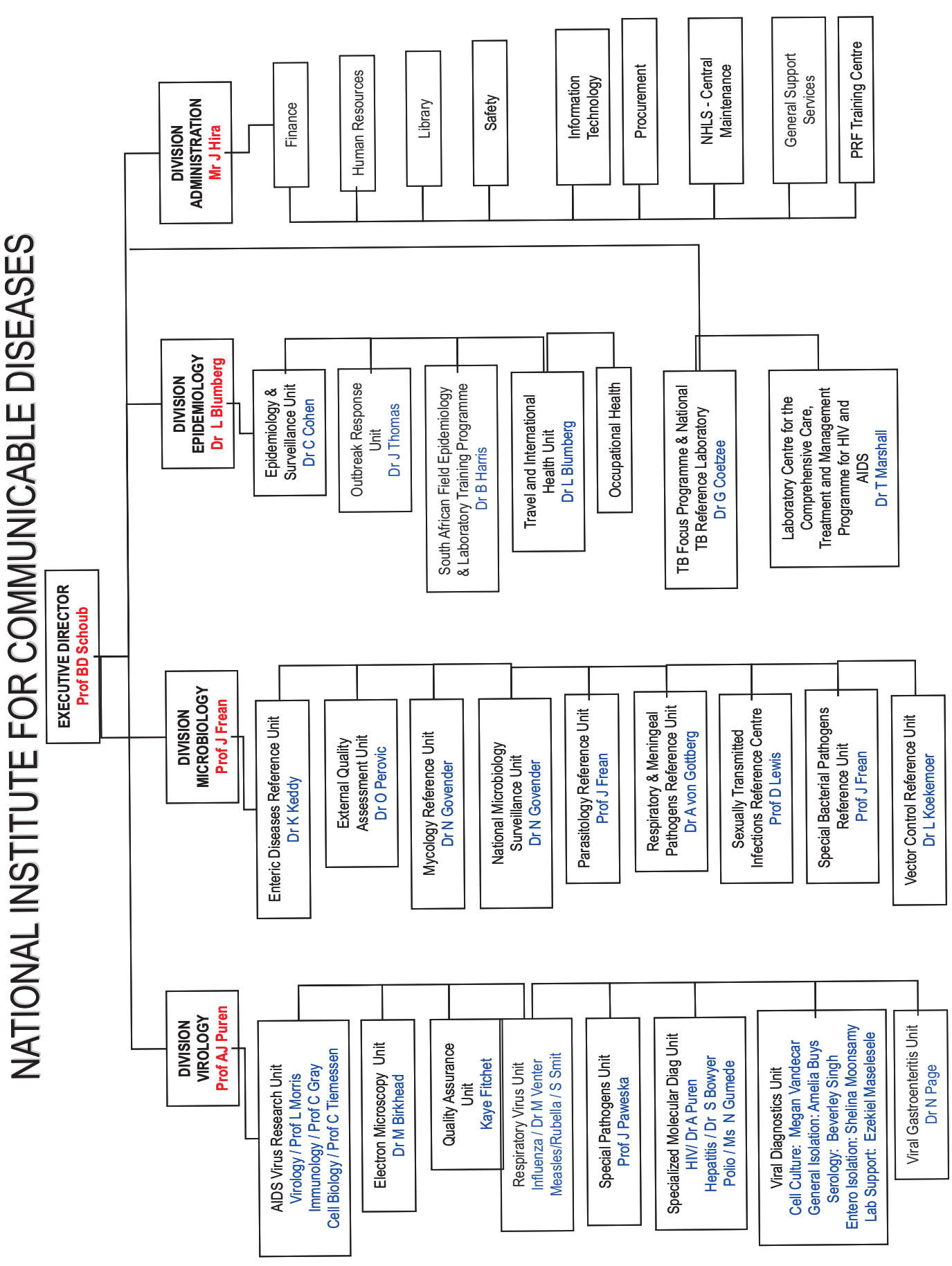
The 4th Annual James Gear Memorial Lecture for 2009 was delivered by Professor Keith Klugman and entitled "The role of bacteria in influenza-related deaths". Prof Klugman is currently Professor of Global Health at Emory University, Atlanta, USA and is also co-director of the RMPRU unit of the NICD. He has been rated as an A1 scientist, the highest recognition, of the NRF (National Research Foundation) of SA.

Prof Keith Klugman (right) with Prof Barry Schoub and Dr Anne von Gottberg.

The NICD is proud of its many achievements nationally and internationally and the Institute owes an enormous debt of gratitude to its staff who have devoted considerable dedication and enthusiasm and passion for their work. A great debt of gratitude is owed to the generous and invaluable support of our parent body the NHLS (National Health Laboratory Service) as well as the National Department of Health. Generous support has also been received from a number of donor and research bodies and their support is sincerely acknowledged:-

- Accelerated Vaccine Introduction Initiative (AVI)
- Agence Nationale de Reserches sur la SIDA (ANRS)
- Bill & Melinda Gates Foundation
- Bonn University, Bernard Nocht Institute for Tropical Medicine, Germany
- Center for HIV/AIDS Vaccine Immunology (CHAVI)
- Centers for Disease Control (CDC)
- Centre for the AIDS Programme of Research in South Africa (CAPRISA)
- Elizabeth Glaser Pediatric AIDS Foundation
- European Network for Capacity Building
- European Union
- German Research Foundation (DFG)
- GlaxoSmithKline
- HIV Vaccine Trials Network (HVTN)
- HIV Vaccine Trials Network (HVTN)
- International Atomic Energy Agency (IAEA), Vienna, Austria
- International Union against Sexually Transmitted Infections (IUSTI)
- John Hopkins
- Karolinski Institute (Sweden)
- Medical Research Foundation (MRC)
- National Institute of Health (NIH)
- National Research Foundation (NRF)
- New Partnership for African Development (NEPAD) / Biofesa
- Optimus Foundation
- PATH
- PlasmaAcute Company
- Poliomyelitis Research Foundation
- Sanofi
- South African HIV/AIDS Research and Innovation Platform (SHARP)
- The Wistar Institute
- University of the Witwatersrand (Friedel Sellschop Award)
- Virax
- Wellcome Trust
- World Health Organization (WHO)
- Wyeth

BARRY D SCHOUB
EXECUTIVE DIRECTOR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Viral Gastroenteritis Unit
Dr N Page

NICD 2009

Microbiology Division

Enteric Diseases Reference Unit

BACKGROUND

The Enteric Diseases Reference Unit at the NICD was started in 1997, under the guidance of a pathologist and a part-time technologist. Over the next few years, the capacity was increased through the hiring of extra staff members to its current complement, the unit developed capacity to fully serotype *Salmonella enterica* and *Shigella* species, as well as diarrhoeagenic *Escherichia coli* and has had training from the Centers for Disease Control and Prevention, Atlanta, USA and WHO Reference Centre for *Escherichia coli* (Statens Serum Institut, Denmark). The unit took over *Vibrio cholerae* work from the old Public Health Laboratory and has developed capacity in both phenotyping and genotyping of this group of organisms.

EDRU collects data on patients presenting throughout South Africa with both invasive and non-invasive disease caused by *Salmonella* species (including *Salmonella* Typhi), *Shigella* species, *Vibrio cholerae* and diarrhoeagenic *Escherichia coli*. In order to make these data representative and reflective of disease burden in each province in the country, we actively motivate all diagnostic laboratories throughout the country to voluntarily submit limited demographic details and isolates to us centrally. In exchange, we offer serogrouping and serotyping results free of charge (urgent results need to be requested telephonically), regular feedback (quarterly reports by province sent to every laboratory participating) and aggregated numbers are published in the NICD Bulletin. We actively contact laboratories to assess numbers of missed cases and conduct regular audits in eight of nine provinces using the DISA corporate data warehouse (CDW) to identify cases.

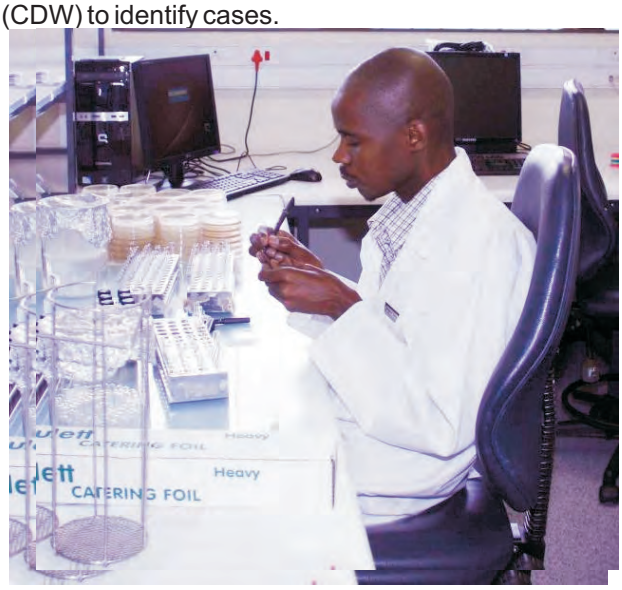

Figure 1: Mpilo Mtambo serotyping diarrhoeagenic *Escherichia coli*

In addition to serogrouping and serotyping, E-tests are used to determine the minimum inhibitory concentration (MIC) of each isolate to antimicrobial agents, according to CLSI guidelines. We also perform genotypic characterization of isolates, should this be required, such as in outbreak situations. The molecular epidemiology of these bacterial pathogens is continually being elucidated, specifically that of outbreak or epidemic-prone pathogens such as *Salmonella* Typhi, *Shigella dysenteriae* type 1 and *Vibrio cholerae* O1. A multiplex polymerase chain reaction is used to elucidate the presence of toxin genes in diarrhoeagenic *E. coli*. Our unit is developing its molecular research laboratory involved with characterising the molecular basis for antimicrobial resistance in these pathogens and has plans to further characterize the mechanism of disease due to these pathogens at a molecular and cellular level.

Figure 2: Florah Mnyameni reading E-tests

Together with collaborators from the CDC in the USA, a number of sites in the country are performing "enhanced" surveillance, where additional clinical data on all patients is being collected, by trained surveillance officers (registered nursing sisters), representing almost all the provinces. The project comprises a vibrant and energetic team of pathologists, clinicians, scientists, technologists, clerks and surveillance officers.

The unit also comprises an enthusiastic team of senior pathologists and scientists who are actively involved in

post-graduate training. The staff has a specialized programme for the training of microbiology registrars, and over a two-week period, registrars are exposed to a range of biochemical, serotyping and molecular techniques in the identification of bacterial enteric pathogens. The senior staff members are experienced in post graduate supervision of scientists and have recently started projects with epidemiology students who are examining the extensive database.

Figure 3: Mimmy Ngomane teaching EDRU students Donald van der Westhuizen and Krpasha Govindsamy

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

SURVEILLANCE ACTIVITIES

EDRU currently has the responsibility for surveillance and characterisation of bacterial enteric disease in South Africa; specifically, EDRU collects all human isolates from diagnostic microbiology laboratories in South Africa for surveillance. EDRU is an active member of GERMS-SA (a more detailed explanation of GERMS-SA can be viewed under the National Microbiological Surveillance Unit).

These isolates will be characterised at no charge to the laboratory of origin, irrespective of whether the laboratory functions in a private capacity or has a public role. This includes those isolates that may represent carriage of an enteric bacterial pathogen, rather than disease due to that pathogen

- The case definition for these pathogens for all surveillance done by EDRU includes those isolates from body sites as specified below, in both in-patients and out-patients. Specifically this includes those individuals who sought treatment at a hospital or clinic, such as outpatients who have positive stool cultures or rectal swabs, but are not admitted or discharged from casualty. In this instance carriers may be included because they add to the burden of treatment, if not the burden of disease and may represent sub-clinical cases e.g. cholera.
- The case definition for enhanced surveillance isolates includes only those *Shigella* and *Salmonella enterica* isolates that are from normally sterile body sites in “in-patients” only that is the patient should have been admitted to the hospital or enhanced surveillance site, as currently defined by the Enhanced Surveillance core, or there should have been the intention to admit, to include those patients who may expire in casualty, as established from the bed letter. This also allows for changes in the ES site, either to include new South African sites or to exclude sites which may be viewed as no longer appropriate for the study.
- EDRU initiated surveillance for diarrhoeal pathogens in children under five years old at four representative sentinel sites (see below) in 2009.
- Although EDRU does not normally do such work for other African countries, the support of the unit may be requested should one of the neighbouring countries require it.
- EDRU currently receives specimens from over 4000 human cases per annum, according to the definition above. In addition the unit undertakes to serotype *Salmonella*, *Shigella* and diarrhoeagenic *E. coli* (DEC) isolates for commercial purposes and has in the past performed a multiplex polymerase chain reaction (PCR) to diagnose DEC from veterinary specimens.
- Regular reports on the isolates received are extracted from the database for the purposes of information sharing.
- Where relevant, molecular methods may be used to establish strain relatedness in outbreaks..

GENOTYPIC CHARACTERIZATION AND CLUSTER ANALYSIS OF *SALMONELLA* TYPHI IN SOUTH AFRICA, 2005 TO 2008

In South Africa, for the years 2005 to 2008, the EDRU received 473 human isolates of *Salmonella* Typhi. Three hundred and ninety isolates were available for further analysis. The aim of our study was to investigate the level of clonality and genetic diversity of *Salmonella* Typhi in South Africa, to improve our understanding of endemic and epidemic disease in South Africa. Genotyping of isolates was investigated using pulsed-field gel electrophoresis (PFGE) analysis incorporating *Xba*I digestion of genomic DNA. PFGE patterns were analyzed using BioNumerics software. Clusters of isolates were defined by ≥3 isolates with PFGE patterns sharing ≥90% similarity on dendrogram. Using this definition, 15 clusters were identified. Nine clusters were represented by 3 to 11 isolates, 4 clusters were represented by 18 to 35 isolates, 1 cluster was represented by 92 isolates and 1 cluster was represented by 99 isolates. Our first major cluster (99 isolates) was predominated by strains isolated in 2005 from the Delmas region of the Mpumalanga Province. Our second major cluster (92 isolates) was predominated by strains isolated in 2006 from the Mthatha region of the Eastern Cape Province. The

system has proved invaluable in relating strains in an outbreak setting and may eventually be able to predict whether disease was locally acquired or imported. A representative PFGE pattern from each of the 15 clusters was submitted to the PulseNet Global Database to determine how South African genotypes compare to those from other countries. Seven of our patterns were found to be unique to South Africa and not previously reported in the global database. The other 8 patterns have previously been reported to the global database and originate from countries including India, Bangladesh, Taiwan, Kenya, Tanzania, Argentina and the USA.

Report of *qnr*-mediated fluoroquinolone-resistant *Salmonella enterica* serotype Typhi from South Africa

Salmonella enterica serotype Typhi (*Salmonella* Typhi) is a common cause of enteric fever in travellers and is associated with human-to-human transmission. We identified a case of typhoid fever in a 65 year-old woman, the contact of a traveller to Bangladesh. The isolate was multidrug-resistant, with an MIC to ciprofloxacin of 4 µg/ml. Pulsed-field gel electrophoresis analysis confirmed that the isolate belonged to a common pattern in the Global PulseNet *Salmonella* Typhi Database, rarely seen in South Africa. The presence of a single amino-acid mutation in GyrA (Ser83 to Tyr) along with a QnrS protein (encoded by *qnrS1*) and active efflux conferred fluoroquinolone resistance. We believe that this is the first report of the *qnr* gene in *Salmonella* Typhi and the first report of fluoroquinolone resistance in *Salmonella* Typhi from South Africa, in a strain that was probably imported from Bangladesh.

CHOLERA OUTBREAK IN SOUTH AFRICA: EXTENDED LABORATORY CHARACTERIZATION OF ISOLATES

Cholera is an acute intestinal infection caused by the ingestion of the bacterium *Vibrio cholerae*. Following a short incubation period (hours to 5 days), the disease typically presents as profuse watery diarrhoea that can rapidly lead to severe dehydration and death if not promptly treated. Two serogroups of *V. cholerae* cause outbreaks of cholera - *V. cholerae* O1 and O139. In November 2008, an outbreak of cholera started in South Africa and continued into May 2009. The initial cases were directly linked to cholera cases in Zimbabwe, with cases crossing the border to seek healthcare in South Africa. For the year 2009, the Enteric Diseases Reference Unit processed 570 *V. cholerae* O1 isolates associated with the outbreak. Bacteria were serotyped using standard microbiological techniques and antimicrobial susceptibility testing was performed using Etests. PCR assays were used to determine the biotype of *V. cholerae* O1 and to determine the presence of cholera toxin. The genetic relatedness of isolates was investigated using pulsed-field gel electrophoresis (PFGE) analysis. For the 570 *V. cholerae* O1 isolates, extended laboratory characterization showed the following results. Ninety-eight percent were of serotype Ogawa and 2% were of serotype Inaba, 100% were of biotype El Tor, 99.5% were positive for the cholera toxin, 100% showed resistance to cotrimoxazole, 48% showed resistance to chloramphenicol, 100% showed resistance to nalidixic acid, 0% showed resistance to ciprofloxacin, 3% showed resistance to tetracycline and 39% showed resistance to erythromycin. PFGE analysis determined that all the isolates were related with PFGE patterns clustering at >90% similarity following dendrogram analysis of patterns.

MOLECULAR CHARACTERIZATION OF MULTI-DRUG-RESISTANT CHOLERA OUTBREAK ISOLATES IN THE MPUMALANGA PROVINCE OF SOUTH AFRICA, MAY TO JULY 2008

Cholera is caused by enterotoxin-producing strains of *Vibrio cholerae* belonging to serogroups O1 and O139. Antimicrobial agents are not required in the treatment of cholera, but they can decrease the severity of the illness, duration of excretion of the pathogen and curb the transmission cycle during epidemics. However, resistance to antimicrobial agents has become a public health concern worldwide, especially in the developing regions such as Asia, Latin America and Africa. Antimicrobial resistance can be attributed to spontaneous mutations in existing genetic material or by horizontal gene transfer (HGT) through self-transmissible elements such as transposons, integrons and plasmids. Presently, we are investigating the antimicrobial resistance of thirty one isolates from the cholera outbreak that was reported from the Sheba gold mine in the Ehlanzeni district of the Mpumalanga Province of South Africa during May to July 2008. Serological and molecular identification showed that all the outbreak isolates were characterized as *Vibrio cholerae* O1 serotype Ogawa biotype El Tor, which is generally reported in Africa. Of the thirty one isolates thirty isolates were PCR positive for the cholera toxin encoded the *ctxA* gene. Minimum inhibitory concentrations (MIC's) of the isolates were determined by the E-test and disk diffusion methods. All the isolates displayed the same antimicrobial susceptibility patterns and were susceptible to ciprofloxacin and imipenem but non-susceptible to ampicillin, augmentin, sulfamethoxazole, trimethoprim, chloramphenicol, nalidixic acid, kanamycin, streptomycin and tetracycline, which was initially the antimicrobial agent of choice in the treatment of cholera in Africa. Further resistance to third generation cephalosporins (ceftriaxone and ceftazidime) was observed indicative of extended-spectrum beta-lactamase (ESBL) activity. Genomic DNA of all the isolates was typed with pulsed-field gel electrophoresis (PFGE) incorporating *NotI* restriction endonuclease. All the isolates displayed a very similar PFGE banding pattern clustering at a 95% similarity value. Molecular screening and nucleotide sequencing for ESBL genes showed that all isolates were positive for ^{bla}TEM-63. Following plasmid DNA extraction, a single plasmid of approximately 140 kb in length was identified in all the isolates. All the isolates were PCR-positive for a 592 bp internal fragment of the integrase gene of the *V. cholerae* SXT element. SXT is a

self-transmissible mobile genetic element which encodes resistance for trimethoprim, sulfa-methoxazole, chloramphenicol, tetracycline, and streptomycin. Further analysis will include: (1) nucleotide sequence analysis of SXT elements to confirm the presence resistance genes, (2) the investigation for class 1 and 2 integrons, (3) the investigation for *qnr* plasmid genes, (4) the analysis for mutations in the quinolone-resistant determining regions of DNA gyrase and topoisomerase IV, and (5) PCR-based replicon typing incorporating nucleotide sequencing to type resistance plasmids in order to enhance our understanding of the origin and family of these antimicrobial resistance plasmids.

Figure 4: Husna Ismael and Nomsa Tau in the molecular laboratory

PCR FOR IDENTIFICATION OF HUMAN DIARRHOEAGENIC *ESCHERICHIA COLI* IN SOUTH AFRICA

Enteric bacteria are major etiological agents of sporadic and epidemic diarrhoea in both children and adults. The description of etiological agents of diarrhoea is important with respect to implementing suitable control strategies as well as therapeutic initiatives. Diarrhoeagenic *E. coli* (DEC) is an important etiological agent of childhood diarrhoea and represents a major public health problem in developing countries. DEC is classified based on their virulence traits, unique clinical features and serotypes. The primary method of analysis involves a multiplex PCR assay that simultaneously detects primary virulence genes associated with multiple pathotypes of DEC. Our multiplex PCR assay detects for the presence of enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), shiga-toxin producing *E. coli* (STEC) [which includes the enterohaemorrhagic *E. coli* (EHEC)], enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* (DAEC). Multiple PCR primer pairs have been designed to target and amplify specific virulence genes situated on chromosomal and plasmid DNA. The genes targeted include the: *eae* (codes for the intimin outer membrane protein), *bfp* (codes for the bundle-forming pilus), *stx1* (codes for the shiga-toxin 1 protein), *stx2* (codes for the shiga-toxin 2 protein), *elt* (codes for the heat-labile enterotoxin), *est* (codes for the heat-stable enterotoxin), *ipa* (codes for an invasion protein), *aat* (codes for a transporter protein) and *daaC* (codes for an accessory protein involved in production of F1845 fimbriae). To extend further identification of *E. coli* serogroups O157 and O111, primer pairs have also been designed to amplify the *hlyA*, *uidA*, *rfbE* and *wbdI* genes. For the year 2009, the Enteric Diseases Reference Unit processed 891 isolates of *E. coli*, of which 371 were non-virulent (negative for all virulence genes), 322 were EPEC, 73 were EAggEC, 73 were DAEC, 39 were ETEC, 4 were EIEC, 5 were STEC and 4 were EHEC. Serogrouping of the EHEC showed that 1 isolate was untypeable, 2 isolates were of serogroup O26 and 1 isolate was of serogroup O157. Since the year 2003, this is the first report of serogroup O157 EHEC in South Africa.

FATAL CASE OF *CLOSTRIDIUM PERFRINGENS* ENTERITIS AND BACTERAEMIA IN SOUTH AFRICA

Clostridium perfringens is an important anaerobic pathogen causing foodborne and non-foodborne gastrointestinal diseases in humans and animals. Clostridial species are also a common cause of anaerobic bacteraemia, with *C. perfringens* the most commonly isolated species. Enteritis necroticans, also called pigbel, is an often fatal type of gastrointestinal infection caused by *C. perfringens* type C and is often associated with diabetics. On 14 October 2009, in the town of Tzaneen, South Africa, a previously healthy 61-year-old male, presented to a local hospital with a one-day history of fever with rigors, nausea, weakness, muscle pain, chest pain, followed by profuse watery diarrhoea. The patient deteriorated rapidly, and on admission to hospital was noted to be febrile and shocked with a decreased level of consciousness and continuous green watery diarrhoea, together with clinical evidence of disseminated intravascular coagulation. Within hours of hospital admission, he suffered cardiac arrest and died. Further inquiry determined that he had a history of untreated type 2 diabetes. The presence of a bacteraemia was diagnosed by PCR amplification of a bacterial 16S rRNA gene from the bloodstream of the patient. DNA sequence analysis of the gene identified the bacterium as *C. perfringens*. Clostridial bacteremia is a rare but nearly always fatal disease. The bacteremia usually has a gastrointestinal source and often occurs in patients with serious underlying illness, of which diabetes is very often an underlying illness. *C. perfringens* enteric infection is associated with a number of distinct clinical syndromes, depending on the strain type and toxins it produces. Although rarely encountered nowadays, enteritis necroticans is a rapidly progressive necrotizing infection of the small bowel caused by *C. perfringens* type C. It presents as an acute enteritis which may be complicated by systemic toxicity or intestinal obstruction, and carries a high mortality rate (15-45%). Fulminant disease in diabetic patients is well described, and perhaps attributable to diabetes-related gastrointestinal dysfunction allowing overgrowth of the organism and facilitating its pathogenesis. *C. perfringens* is ubiquitous, found in soil and the gastrointestinal tracts of many animals, including

humans. Outbreaks and sporadic cases of enteritis necroticans have been linked to inadequately cooked food-animal products. Affected persons usually have underlying risk factors, including the classic association with protein malnutrition and the more recently recognized association with diabetes.

MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM β-LACTAMASE PRODUCING *SHIGELLA* ISOLATES FROM HUMANS IN SOUTH AFRICA, 2003-2009

Bacillary dysentery is an acute gastroenteritis caused by *Shigella* species. It is characterized by mucoid bloody stools, abdominal cramps and tenesmus. Subsequent to invasion of the colonic mucosa, *Shigella* bacteria multiply, cause cell death and spread laterally to infect and kill adjacent epithelial cells, causing ulceration, inflammation and bleeding. *Shigella* infection is normally self-limited, however antimicrobial therapy is generally required to manage infection and reduce faecal excretion of the bacterium to prevent further transmission. *Shigella* bacteria have progressively acquired resistance to commonly recommended drugs due to extended-spectrum β-lactamase (ESBL) production and this poses a major therapeutic threat. The aim of our study was to investigate the molecular basis and molecular epidemiology of ESBL producing strains of *Shigella* in South Africa. For the years 2003 to 2009, 7842 human *Shigella* isolates were received by EDRU. Twenty of the *Shigella* isolates were ESBL positive based on the synergistic effects between clavulanate and selected β-lactams (cefotaxime and ceftazidime). Susceptibility tests revealed that all ESBL producing *Shigella* strains showed high levels of resistance to ampicillin, cotrimoxazole, trimethroprim, sulfamethoxazole and ceftazidime. One of these isolates showed resistance to nalidixic acid. ESBL and AmpC β-lactamase genes were characterized using PCR assays and nucleotide sequence analysis, and the presence of resistance plasmids was investigated by means of plasmid DNA isolation. PCR assays targeting the most common ESBL enzymes (TEM, SHV and CTX-M β-lactamase) and the most common AmpC enzyme (CMY β-lactamase), revealed that ESBL producing *Shigella* strains were positive for TEM (n=16), SHV (n=2), CTX-M (n=19) and CMY (n=6). Nucleotide sequence analysis of the PCR products revealed that these strains of *Shigella* harboured ^{bla}TEM-1, ^{bla}SHV-2, ^{bla}CTX-M-15 and ^{bla}CMY-2. Plasmid DNA of the ESBL-producing *Shigella* isolates were extracted and the molecular weight of these plasmids ranged from 42 kilobases (kb) to 166 kb with some isolates harbouring more than one plasmid. ESBL production for the *Shigella* isolates in our current study was found to be mediated by ^{bla}CTX-M-15 (for 13 isolates), ^{bla}CTX-M-15 and ^{bla}CMY-2 (for 5 isolates), ^{bla}CTX-M-15 and ^{bla}SHV-2 (for 1 isolate) and ^{bla}CMY-2 (for 1 isolate; our nalidixic acid-resistant isolate). *Enterobacteriaceae* producing ESBLs are a major problem worldwide. Previous studies suggest a spread of the ^{bla}CTX-M-15 gene amongst the *Enterobacteriaceae* and these findings predict future dissemination of resistance in the third and fourth generation cephalosporins. For this reason continued surveillance is needed to assess the incidence of ESBL producing *Shigella* isolates.

Figure 5: Tshilidzi Mazibuko and Arvinda Sooka serotyping *Shigella*

CHARACTERIZING BACTERIAL CAUSES OF DIARRHOEA IN AN UNDER-FIVE POPULATION IN SOUTH AFRICA

Diarrhoea is one of the major causes of morbidity and mortality among children under five years of age worldwide particularly in developing countries. There are various pathogens such as bacteria, viruses and parasites, which cause diarrhoeal diseases in both the developed and developing countries. The most commonly isolated diarrhoeal pathogens include: diarrhoeagenic *Escherichia coli,* Rotavirus, *Shigella* species, *Salmonella* species, *Vibrio cholerae* and *Campylobacter* species. Rotavirus is known to be the major aetiological agent of gastroenteritis. For the year 2007, approximately 527 000 cases of childhood deaths due to diarrhoea were recorded by the WHO, of which 145 000 cases were reported from Sub-Saharan Africa. The aim of this study is to describe the bacterial causes of diarrhoea in South African children under five years of age at four surveillance sites which include: (1) Chris Hani Baragwanath Hospital (Gauteng Province), (2) Dr George Mukhari Hospital (North West Province), (3) Mapulaneng and Matikwana Hospitals (Mpumalanga Province) and (4) Edendale Hospital (KwaZulu-Natal Province). Stool specimens will be collected from children under five years of age, presenting with symptoms of diarrhoea, of which patients would have presented with 3 or more episodes of loose stools over a 24-hour period. A stool swab will be transported to the Enteric Diseases Reference Unit (EDRU) using Cary-Blair transport medium. A combination of standard microbiological methods (culture, biochemical tests, serotyping, antimicrobial susceptibility testing) and molecular methods (PCR, PFGE, gene sequencing, DNA probing of bacterial colony blots) will be used for the identification and characterization of bacterial diarrhoeal pathogens from stool specimens. From April

to December 2009, 533 stool specimens were received by the EDRU, from which 408 bacterial strains were cultured. For these 408 isolates, 63 were identified as diarrhoeal pathogens and further characterized as follows: eight were *Salmonella* species (*Salmonella* Isangi [n=2], *Salmonella* Typhimurium [n=2], *Salmonella* Enteritidis [n=2], untypeable *Salmonella* [n=2]); seven were *Shigella* species (*Shigella boydii* type 2 [n=1], *Shigella flexneri* type 1b [n=1], *Shigella flexneri* type 6 [n=1], *Shigella flexneri* 2a [n=2], *Shigella sonnei* phase I [n=1], *Shigella sonnei* phase II [n=1]) and forty-eight were diarrhoeagenic *E. coli* (diffusely adherent *E. coli* [n=23], enteroaggregative *E. coli* [n=13], enteropathogenic *E. coli* [n=8], enteroinvasive *E. coli* [n=4]). In summary, we have shown an overall 12% recovery rate for bacterial diarrhoeal pathogens from stool specimens, with diarrhoeagenic *E. coli* shown to be the most commonly isolated pathogen.

A COMPARISON OF SELECTED MULTIDRUG RESISTANT GENETIC FACTORS WITHIN *SALMONELLA* TYPHIMURIUM ISOLATED FROM HUMAN AND POULTRY MEAT/PRODUCTS, 2006-2009, SOUTH AFRICA

Nontyphoidal *Salmonellae* are important zoonotic pathogens causing gastroenteritis, septicaemia and other serious illness in animals and humans in developed as well as developing countries. Foodborne salmonellosis is frequently undiagnosed and under reported in South Africa. Multidrug resistant (MDR) salmonellosis is recognised as causing opportunistic and invasive disease in immunocompromised individuals. Laboratory-based surveillance data identified *Salmonella* Typhimurium as the most frequently isolated serotype since 2006 from human clinical samples. A significant proportion of these isolates were extended spectrum beta lactamase (ESBL) producers. They also displayed pentavalent resistant patterns and resistance to nalidixic acid. *S.* Typhimurium isolates were positive for ESBL producing genes: ^{bla}TEM-1, ^{bla}SHV-12, ^{bla}TEM-63, ^{bla}CTX-M-37, ^{bla}CMY-2 and ^{bla}TEM-116 and to have genes coding for *Salmonella* Genomic Island 1 (SGI!) as well as ^{bla}PSE$_{1,}$ *aad* $_{A2,}$ ^{bla}OXA30 $_{,}$ *aad*$_{A1,}$ *drf*$_{A12}$ and *orfF*. *S.* Typhimurium is also the highest contributor (2000 to 2002) to the overall number of *Salmonella* isolates cultured from non-human sources, a large proportion being isolates from poultry. Poultry meat is the most affordable source of protein in South Africa with broilers being produced and slaughtered under intensive conditions to meet the high demands of the South African market. This research aims to investigate the phenotypic multidrug resistant profile of *Salmonella* Typhimurium in poultry meat/products as well as identifying selected MDR genetic factors such as the presence in SGI1 integrons, ESBL genes and quinolone resistant drug regions (QRDR) and comparing these results with similar data obtained from *S.* Typhimurium isolated from human samples from 2006 to 2009, using Pulse-field gel electrophoresis (PFGE).

COLLABORATIONS

Miss Florah Mnyameni travelled to Statens Serum Institut in Copenhagen, Denmark,
March 02-06 2009 the WHO reference Centre for *Escherichia coli* as well as NICD suppliers of antisera and *Escherichia coli* .

Dr Karen Keddy, Dr Anthony Smith and Mr Brett Archer attended the 7th International Symposium on Invasive Salmonelloses, Kilifi, Kenya, 25-28 January, 2009.

Dr Karen Keddy attended Foodborne Disease Burden Epidemiology Reference Group (FERG) (WHO) meeting, Food and Agriculture Organisation (FAO) Rome, Italy, 7- 10 June 2009

Dr Karen Keddy, Dr AM Smith and Ms Husna Ismail attended the FIDSSA Congress, Sun City, South Africa, 20 -23 August 2009.

Dr Karen Keddy attended the Second Annual Meeting of the Food and Waterborne Diseases and Zoonoses Surveillance Network in Europe. Corinthia Hotel and Spa, Malta, 24- 25 September 2009.

Dr Karen Keddy attended the Vaccinology meeting 19-21 October 2009

Dr Karen Keddy attended Foodborne Disease Burden Epidemiology Reference Group, FERG, WHO, Geneva, Switzerland. 26- 30 October 2009.

Dr Anthony Smith attended the 2nd PulseNet International Strategic Planning Meeting, Buenos Aires, Argentina, November 2009.

EDRU co-hosted in the Principal Investigators meeting for Enhanced Surveillance on 5 -6 November 2009.

CAPACITY BUILDING

Husna Ismail (University of the Witwatersrand) (Co-supervised with Dr Karen Keddy and Dr A Smith)
Masters Dissertation: Molecular Characterization of Cholera Outbreaks in South Africa, 2008-2009.
Submission date: 31 December 2012

Zwiithavhathu Makhari (University of the Witwatersrand) (Co-supervised with Dr Karen Keddy, Dr A Smith & Prof S Madhi)
Master of Science: Characterizing bacterial causes of diarrhoea in an under-five population in South Africa.
Submission date: 2012.

Krpasha Govindsamy (University of South Africa) (Co-supervised with Dr Karen Keddy and Prof P Kabongo
Master of Science in Veterinary Medicine Dissertation: A comparison of selected Multi-Drug resistant genetic factors within *S*. Typhimurium isolated from humans and poultry abattoirs in the Tshwane District, South Africa.
Submission date 2011

Mirriam M. Malotle (University of Pretoria) (Co-supervised with Dr Karen Keddy and Dr BN Harris)
Masters in Public Health Research Report: Factors contributing to the misidentification of *Vibrio cholerae* during an outbreak, North West Province, South Africa, November 2008 to July 2009.
Submission date: 2010.

Brett Archer (University of Pretoria) (Co-supervised with Dr Karen Keddy and Dr BN Harris)
Masters in Public Health Research Report: Epidemiology and Management of Typhoid Fever in South Africa, 2003 through 2007. Graduated 2009

Nevashan Govender (MSc, University of the Witwatersrand)
MSc dissertation: Molecular epidemiology and mechanism of resistance of invasive quinolone-resistant South African isolates of *Salmonella enterica*, 2004-2006 (Graduated 23 June 2009)

Sarika Dwarika (MSc, University of the Witwatersrand)
MSc dissertation: Molecular epidemiology of invasive isolates of *Salmonella enterica* serovar Typhimurium in Gauteng, South Africa, 2006-2008 (Graduated 23 June 2009)

EDRU has assisted in the training supervision of FELTP students

EDRU offers both short and long course to microbiology registrars from South African universities in specialised techniques that are relevant for the identification of enteric pathogens.

External Quality Assessment Reference Unit

BACKGROUND

The Unit's mission is to provide novel and educative quality assessment of microbiology laboratories, both nationally and outside our borders. Specific objectives are accurate, appropriate, timely and interpretable reports on quality system in laboratory that facilitate best care of the patient; and to establish laboratory-based antimicrobial resistance surveillance for nosocomial pathogens. The scope of work of a quality monitoring scheme includes:

- Delivery of samples that closely simulate clinical material.
- Challenging laboratories' routine methods and procedures.
- Assessment of laboratory ability to determine the 'correct' result.
- Assessment of the overall performance of a group of laboratories.

Benefits of our EQA programs are to the health system, by assessing laboratory performance; to the laboratory, as a valuable tool for education, self-assessment and confidence-builder; and to the patient to ensure individuals to trust laboratory information.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

EQA PROGRAMMES

The Unit produces external quality assessment programs for the NHLS and subscribing private laboratories in the disciplines of bacteriology (129 laboratories), tuberculosis microscopy (295 laboratories), tuberculosis culture (26 laboratories), and syphilis RPR serology (235 laboratories) and TPHA serology (49 laboratories). Surveys are sent out 3 times a year. The organization of EQA programs involves technical preparation and quality control of material, documentation and shipping, and evaluation and reporting of laboratory responses. A teaching program which participating laboratories may use as a training resource accompanies the Bacteriology EQA program. Results of laboratory performance are reported to appropriate NHLS management structures; 2009 survey results are summarized in Figures 1 to 4.

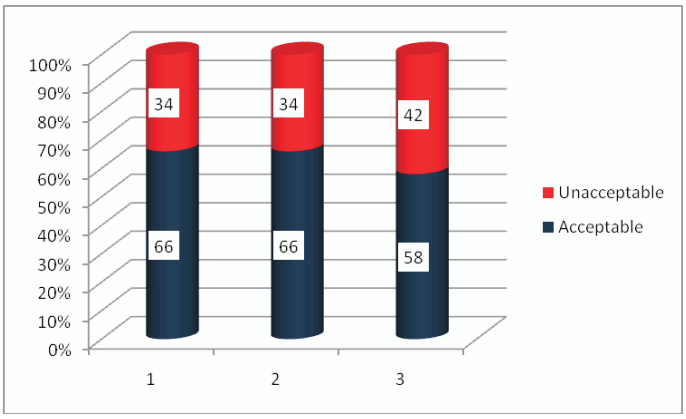

Figure 1: Results of National EQA programme (bacteriology), surveys 1-3, 2009 for responders only

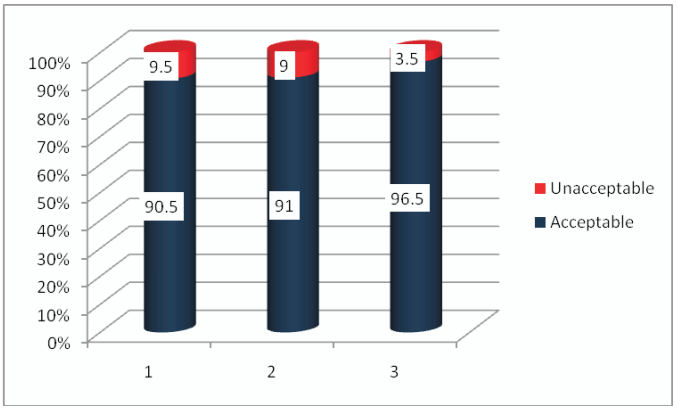

Figure 2: Results of National EQA programme (TB microscopy), average percentage, surveys 1-3, 2009 for responders only

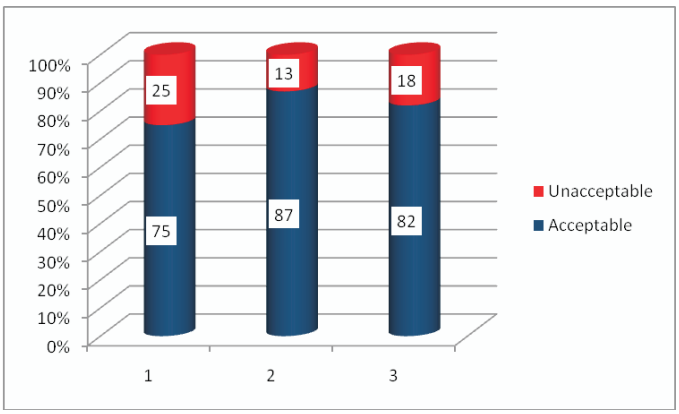

Figure 3: Results of National EQA programme (syphilis serology: TPHA), average percentage, surveys 1-3, 2008 for responders only

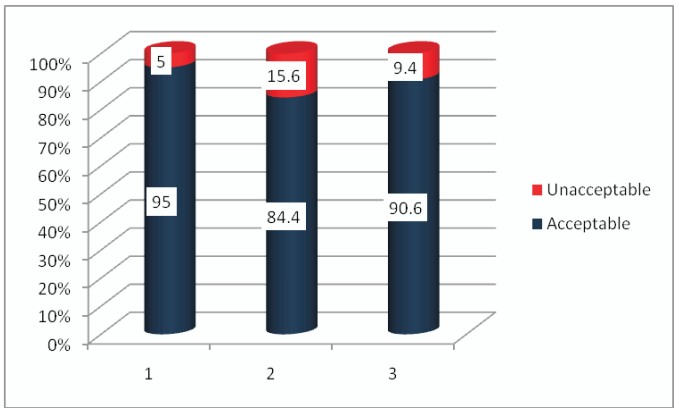

Figure 4: Results of National EQA programme (syphilis serology: RPR), average percentage for responders only surveys 1-3, 2009

The Unit also manages all aspects of a WHO grant-funded bacteriology EQA program to 77 national public health laboratories in the African Regional Office (AFRO) of the World Health Organization. Of these, 69 participants belong to malaria and 68 to TB microscopy programs. Microbiology reference units at NICD support the program. The EQA Unit has played an active role in reporting on laboratory capacity in the AFRO region, and has advised the WHO on implementation of a similar EQA program in the EMRO region. The EQA Unit is an external referee to the EMRO region proficiency testing programs. Staff from the EQA Unit acted as technical advisors on site visits.

The Unit provides a microbiology EQA program to 11 laboratories in Africa that are involved in a major GlaxoSmithKline (GSK Biologicals) malaria vaccine trial.

MEQARU administers proficiency testing programs from these NICD units: Parasitology Reference Unit, Mycology Reference Unit, and Viral Serology section.

NATIONAL STOCK CULTURE COLLECTION

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

There are a total of 436 validated strains in the NSCC comprising yeasts, moulds and bacterial strains. A total of 65 new strains were purchased from ATCC. These strains were ordered for various units for teaching, training, quality control and research purposes. Cell lines were also ordered for the NICD AIDS Unit but are not retained in the stock culture collection. A total 83 orders were received for cultures from the collection. 731 strains were prepared by lyophilisation for this purpose.

TRAINING

Elias Khomane and Vivian Fensham conducted training in the preparation and management of TB smear microscopy EQA program. The training was from the 23-28th September 2009 for delegate from Botswana, Mr. Obert Kachuwaire. He is involved in setting up a National EQA Scheme for Botswana.

SURVEILLANCE

Laboratory Antimicrobial Resistance Surveillance (LARS)

This vision of this new program is a functional, integrated, laboratory-based, antimicrobial resistance surveillance system for nosocomial pathogens that will ensure continuous and timely provision of information to the health system at all levels.

RESEARCH

The microbial aetiology of community-acquired pneumonia in adults in Johannesburg

Community-acquired pneumonia (CAP) is a common, potentially life-threatening infectious disease. Changes have occurred in the demographics of the patient population over recent years, which include an increase in the number of individuals over the age of 65 years, as well as the number of immunocompromised patients, especially in association with HIV infection. The initial empiric antibiotic choice for the management of CAP in adults is based on the likely pathogen that will be encountered in any particular geographical area, as well as the clinical situation, which depends to a large extent on age of the patient, presence or absence of co-morbid illness and the severity of the illness. The study aimed to describe aetiological agents of CAP in one distinct geographical area in South Africa. This would provide initial knowledge on etiological cause of CAP, and initiate a follow-up study with approximately 200 patients from two sites (Cape Town, Johannesburg). Such surveillance will provide appropriate etiological guidance for empirical antimicrobial treatment for the first time in South Africa. (Principal investigator: Dr Olga Perovic)

COLLABORATIONS

Department of Medicine, University of the Witwatersrand (Prof C Felman: CAP study)
Clinical Microbiology and Infectious Diseases, University of the Witwatersrand/NHLS(Prof A Duse: CAP study)
Division of Medical Microbiology, University of Cape Town/NHLS (Dr S Oliver: CAP study)
Ampath (Dr A Brink: CAP study)
WHO African Regional Office, Brazzaville, and WHO International Health Regulations Coordination, Lyon (NICD/WHO EQA Programme)

CAPACITY BUILDING

Vivian Fensham represented NICD as expert WHO/NICD EQA Program adviser at an EMRO (Eastern Mediterranean Regional Office) meeting in Cairo, Egypt, 7-8 December 2009.
All staff members have been on training for automated identification and susceptibility testing systems, and health and safety.

Mycology Reference Unit

BACKGROUND

The Mycology Reference Unit (MRU) aims to contribute to the control of fungi of public health and clinical importance by undertaking relevant laboratory-based surveillance and research projects and functioning as a reference laboratory in the field of clinical mycology.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

THE GERMS-SA CRYPTOCOCCAL SURVEILLANCE PROJECT

The cryptococcal surveillance project, managed by the MRU, is nested within a national, laboratory-based surveillance programme (GERMS-SA). The main objective of the project, ongoing since 2005, is to estimate the burden of laboratory-confirmed cryptococcal disease in South Africa. From 1 July 2008, surveillance methodology for the project was changed to reduce the reporting burden on diagnostic laboratories. Only enhanced surveillance sites (25 hospitals in 9 provinces), NHLS laboratories in KwaZulu-Natal, and laboratories in the private, mining, and military sectors were required to directly report case patients to NICD, whereas data from case patients, diagnosed with cryptococcosis at NHLS laboratories in the other 8 provinces, were obtained through an audit of the NHLS Corporate Data Warehouse. In total, 9,655 case patients with incident cryptococcosis were detected through the surveillance project in 2009. Approximately one-third of all cases (n=2,922) were reported from. Cryptococcal isolates (n=2,058), obtained from case patients at 25 enhanced surveillance sites (ESS), were characterised by phenotypic and genotypic tests at MRU. At these ESS sites, clinical data were obtained by nurse surveillance officers from 2,315 cases (79%); data were obtained from 1,361 (59%) cases through interview. A detailed report of cryptococcal surveillance findings for 2009 is contained within the GERMS-SA Annual Report 2009. Updated data from the surveillance project were presented at the International Society for Human and Animal Mycology (ISHAM-2009) Conference in Tokyo, Japan. At the request of the Dept of Health, trends in induction-phase treatment of adults with incident cryptococcosis at the 25 ESS from 2005 through 2008 were examined. These data were presented at the GERMS-SA Principal Investigator Meeting, Sandringham, 5-6 November 2009.

Antifungal Susceptibility Testing of Incident Cryptococcal Isolates, 2002-2003 and 2007-2008: There have been no South African studies examining the prevalence of fluconazole non-susceptibility amongst incident episode isolates of *Cryptococcus* species. Population-based, laboratory-based surveillance for cryptococcosis, initiated in Gauteng in March 2002 and expanded nationally in 2005, provided the opportunity for this issue to be explored further. Clinical strains of *Cryptococcus* species were submitted to the NICD, phenotypically characterised and stored at -70°C to maintain viability. We randomly chose 500 incident episode strains from surveillance case patients, diagnosed with cryptococcosis during 2 time periods (2002-2003 and 2007-2008), for fluconazole minimum inhibitory concentration (MIC) determination by various test methods. Laboratory work for the study was completed in 2009. Updated data were presented at the International Society for Human and Animal Mycology (ISHAM-2009) Conference in Tokyo, Japan and a manuscript will be prepared for submission to an international, peer-reviewed journal.

M13-PCR fingerprinting reveals identical molecular types causing recurrent cryptococcal disease amongst HIV-infected, South African patients

We aimed to compare molecular types of isolate pairs, obtained from a sub-set of patients with recurrent, laboratory-confirmed cryptococcosis in South Africa, during 2005, using M13-PCR fingerprinting. Cases of laboratory-confirmed cryptococcosis (India ink, cryptococcal antigen or culture positive) were reported to a national, laboratory-based surveillance programme in 2005. Recurrent cryptococcosis was defined as occurring in a patient if *Cryptococcus* species was detected from a specimen >30 days after the collection date of the first, positive specimen. Clinical data, including HIV-infection status, were obtained at sentinel sites. Isolate pairs, obtained from a sub-set of patients with recurrent cryptococcosis, with previously-determined fluconazole minimum inhibitory concentrations (MICs), were selected for molecular typing by M13-PCR fingerprinting. Isolates were identified to species level. Of 4356 reported, incident cases, 215 (5%) patients had recurrent disease. Of these, 73 patients had isolate pairs with previously-determined MICs; 20 pairs were selected for M13-PCR fingerprinting. For these 20 pairs, the second strain was isolated, on average, 74 days after the initial strain (median: 66 days; range: 32 to 157 days). Of 17 patients with known HIV-infection status, all were HIV-infected. All isolates were identified as *Cryptococcus neoformans*. The same fingerprinting pattern was obtained for isolate pairs from all 20 patients. These data were presented at a national conference in August 2009.

Estimation of the Current Global Burden of Cryptococcal Meningitis among Persons Living With HIV/AIDS

Cryptococcal meningitis is an important AIDS-related opportunistic infection, especially in the developing

world. In order to assist with development of global strategies and priorities for prevention and treatment, it is important to estimate the burden of disease. In collaboration with investigators at the Centers for Disease Control and Prevention, Atlanta, data from published studies were used to estimate the global burden of cryptococcal meningitis. This study was published in an international, peer-reviewed journal in 2009.

Cryptococcal meningitis in Gauteng Province, South Africa: exploring post-hospital discharge outcomes and uptake of care, 2009

We undertook to determine post-hospital discharge outcomes and uptake of care amongst a group of patients with cryptococcal meningitis, identified through GERMS-SA surveillance, at a single Johannesburg hospital in the post-HAART era. Twenty nine percent of patients died in hospital. Six-month mortality was very high (60%; range 40% to 73%). The findings of this study were summarised by Katherine Gaskell in a report submitted in partial fulfilment of the requirements for the degree of MSc in Control of Infectious Diseases at the London School of Hygiene and Tropical Medicine, and were presented at the GERMS-SA Principal Investigator meeting, Sandringham, 5-6 November 2009.

TRAC-SOUTH AFRICA (TRACKING RESISTANCE TO ANTIFUNGAL DRUGS FOR CANDIDA SPECIES IN SOUTH AFRICA): A LABORATORY-BASED SENTINEL SURVEILLANCE PROJECT FOR CANDIDAEMIA AND ANTIFUNGAL DRUG RESISTANCE IN SOUTH AFRICA, 2009-2010

During 2008, a laboratory-based, sentinel surveillance project was designed by MRU, in collaboration with 20 public and private laboratory sites across South Africa, and the US Centers for Disease Control and Prevention, Atlanta. The major objective of this project is to describe the species distribution of *Candida* spp. causing bloodstream infection at sentinel sites in South Africa, and to compare the species distribution between the public- and private-health sectors. Another major objective is to describe the prevalence of resistance to 9 antifungal drugs (including fluconazole, voriconazole, amphotericin B, caspofungin), amongst invasive *Candida* spp. in 2009-2010, and to compare antifungal drug resistance patterns between the public- and private-health sectors. Formal surveillance started on 1 February 2009. Although funding was not secured during 2009, approximately 800 cases with corresponding isolates were submitted to NICD by end of the calendar year. All isolates were stored at -70^{o}C to maintain viability. No reference laboratory work has been initiated. An MS-Access database was set up and data entry was initiated. TRAC-SA surveillance updates were presented to collaborators at the inaugural South African Clinical Microbiology Society meeting convened at the FIDSSA conference, Sun City, 20-23 August 2009 and at the GERMS-SA Principal Investigator meeting, Sandringham, 5-6 November 2009.

NATIONAL MYCOLOGY EXTERNAL QUALITY ASSESSMENT (MEQA) PROGRAMME

In 2009, the MRU coordinated 3 national MEQA surveys for 84 laboratories participating in the yeast sub-programme and 40 laboratories participating in the mould sub-programme. Results of laboratory performance were made available to NHLS management. A customer perception questionnaire was distributed to 106 laboratories on 23 June 2009, together with the second MEQA survey for 2009. Questionnaires were returned by 63 laboratories on 24 July 2009. Data were analysed, and a report, summarising the findings, was circulated to all laboratories on 28 August 2009.

SANAS ACCREDITATION

In 2009, the MRU surveillance laboratory was re-accredited by SANAS in accordance with ISO15189 standards.

COLLABORATIONS

Listing of collaborating institutions/individuals and the appropriate project/programmes:

The GERMS-SA Cryptococcal Surveillance Project

Gene Elliot, Universitas/ Pelonomi/ NHLS & University of Free State
Andrew Rampe, Dip Med Tech, Rustenberg Hospital/ NHLS
Pieter Jooste, FCPaed (SA), Kimberley Hospital
Ken Hamese, FCPaed (SA), Polokwane/ Mankweng Hospital
Jacob Lebudi, Business Manager, Rob Ferreira/ NHLS
Greta Hoyland, Dip Med Tech, Rob Ferreira/ NHLS
Yacoob Coovadia, FCPath (SA) Micro, Inkosi Albert Luthuli Hospital/ NHLS & University of KwaZulu-Natal
Nomonde Dlamini, MBBCh, Inkosi Albert Luthuli Hospital/ NHLS & University of KwaZulu-Natal
Sumayya Haffejee, FCPath (SA) Micro, Grey's Hospital/ NHLS & University of KwaZulu-Natal
Halima Dawood, FCP (SA), Grey's Hospital & University of KwaZulu-Natal
Meera Chhagan, FCPaed (SA), Grey's Hospital & University of KwaZulu-Natal
Anwar Hoosen, FCPath (SA) Micro, Steve Biko (Pretoria) Academic Hospital Complex/ NHLS & University of Pretoria
Kathy Lindeque, Dip Med Tech, Steve Biko (Pretoria) Academic Hospital Complex/ NHLS
Maphoshane Nchabaleng, MMed (Pathology) Microbiology, Dr George Mukhari Hospital/ NHLS & University of Limpopo
Bonnie Maloba, FCPath (SA) Micro, Dr George Mukhari Hospital/ NHLS & University of Limpopo
Siseko Martin, MBChB, Tygerberg Academic Hospital/ NHLS & University of Stellenbosch
Elizabeth Wasserman, MMed (Path) Microbiology, Tygerberg Academic Hospital/ NHLS & University of Stellenbosch

Olga Perovic, FCPath (SA) Micro, MMed (Micro), Charlotte Maxeke (Johannesburg) Academic Hospital/ NHLS & University of Witwatersrand
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Sandeep Vasaikar, MD, Nelson Mandela Academic Hospital/ NHLS & Walter Sisulu University
Vivek Bhat, MD, Nelson Mandela Academic Hospital/ NHLS & Walter Sisulu University
Sharona Seetharam, FCPath (SA) Micro, Chris Hani Baragwanath Hospital/ NHLS & University of Witwatersrand
Jeannette Wadula, FCPath (SA) Micro, Chris Hani Baragwanath Hospital/ NHLS & University of Witwatersrand
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David Moore, FCPaed (SA), Chris Hani Baragwanath Hospital & University of Witwatersrand
Andrew Whitelaw, FCPath (SA) Micro, Groote Schuur Hospital/ NHLS & University of Cape Town
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Xoliswa Poswa, FCPath (SA) Micro, Johannesburg (Pomona), Ampath National Laboratory Services
Inge Zietsman, FCPath (SA) Micro, Johannesburg, Ampath National Laboratory Services
Juanita Smit, FCPath (SA), Lancet laboratories
Marthinus Senekal, FCPath (SA), PathCare laboratories

Antifungal Susceptibility Testing of Incident *Cryptococcal Isolates, 2002-2003 and 2007-2008*
Shawn Lockhart, PhD, Head of Antifungal Drug Testing Unit, Mycotic Diseases Branch, Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA
Tom Chiller, MD, MPH, Deputy Chief, Mycotic Diseases Branch, Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA

Estimation of the Current Global Burden of Cryptococcal Meningitis among Persons Living With HIV/AIDS
Benjamin Park, MD, MPH, Mycotic Diseases Branch, Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA
Kathleen Wannemuehler, Biostatistics Office, Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA
Tom Chiller, MD, MPH, Deputy Chief, Mycotic Diseases Branch, Division of Foodborne, Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA
Barbara Marston, MD, Global AIDS Program, Centers for Disease Control, Atlanta, USA
Peter Pappas, MD, University of Alabama at Birmingham, Birmingham, Alabama, USA

TRAC-South Africa (Tracking Resistance to Antifungal drugs for Candida species in South Africa): A Laboratory-based Sentinel Surveillance Project for Candidaemia and Antifungal Drug Resistance in South Africa, 2009-2010
Inge Zietsman, FCPath (SA) Micro, Johannesburg, Ampath National Laboratory Services (Co-Principal Investigator)
Gene Elliot, FCPath (SA) Micro, Universitas Hospital/ NHLS
Yacoob Coovadia, FCPath (SA) Micro, Inkosi Albert Luthuli Hospital/ NHLS
Adriano Duse, FCPath (SA) Micro, Johannesburg Hospital/ NHLS
Sumayya Haffejee, FCPath (SA) Micro, Grey's Hospital/ NHLS
Anwar Hoosen, FCPath (SA) Micro, Steve Biko (Pretoria) Academic Hospital Complex/ NHLS
Ranmini Kularatne, FCPath (SA) Micro, Helen Joseph & Coronation/ NHLS
Maphoshane Nchabaleng, MMed (Pathology) Microbiology, Dr George Mukhari Hospital/ NHLS
Heidi Orth, MMed (Path) Microbiology, Tygerberg Academic Hospital/ NHLS & University of Stellenbosch
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Marthinus Senekal, FCPath (SA) Micro, Cape Town, Pathcare Laboratories
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Johan van Greune, FCPath (SA) Micro, Cape Town, Ampath National Laboratory Services
Gerhard Weldhagen, MMed (Path) (Microbiology), PhD, MASM, Pretoria, Ampath National Laboratory Services
Tom Chiller, MD, MPH, Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, USA
Shawn Lockhart, PhD, Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, USA
Guy Richards, FCP (SA), Charlotte Maxeke (Johannesburg) Academic Hospital
Mervyn Mer, FCP (SA), Charlotte Maxeke (Johannesburg) Academic Hospital

Cryptococcal meningitis in Gauteng Province, South Africa: exploring post-hospital discharge outcomes and uptake of care, 2009
Katherine Gaskell, London School of Hygiene and Tropical Medicine, United Kingdom
Kerrigan McCarthy, MBBCh, FCPath (SA), Reproductive Heath and HIV Research Unit, South Africa
Olga Perovic, MD, FCPath (SA) Micro, Charlotte Maxeke (Johannesburg) Academic Hospital/ NHLS
Alison Grant, MBBS, London School of Hygiene and Tropical Medicine, United Kingdom

CAPACITY BUILDING

Antifungal Susceptibility Testing, 12-24 July 2009
Naureen Iqbal, a CDC-based expert in the field of antifungal susceptibility testing, led an intensive 2-week training course for MRU personnel in July 2009. By the end of the workshop, all participants were able to independently perform and interpret the reference broth microdilution and Etest® method for yeasts.

Basic Mycology Workshop, 12-13 August 2009
The workshop was attended by 11 NHLS laboratory participants from the NHLS Northern Branch. Several other staff members at Steve Biko Pretoria Academic NHLS attended a few sessions. The workshop aim was to improve the capacity for diagnosis of common fungal infections encountered at NHLS laboratories in the Northern Branch. The objectives were to: (1) provide a systematic approach to identification of common pathogenic fungi, (2) ensure that participants perform systematic basic techniques to help identify common pathogenic yeasts like *Candida* species and *Cryptococcus* species, (3) demonstrate practical identification methods for common pathogenic moulds, e.g. *Aspergillus* species, zygomycetes, dermatophytes, (4) briefly discuss requests by the clinician for difficult-to-identify fungi and antifungal susceptibility testing (who to contact), and (5) briefly discuss fungal surveillance programs and EQA schemes. The focus of this workshop was on practical demonstrations and hands-on experience for the participants. The number of didactic lectures was kept to a minimum. Most participants belonged to laboratories that performed only basic mycology tests, i.e. microscopy and cryptococcal antigen testing. Very few participants had had any formal teaching in the field of clinical mycology. Day one focused on yeast identification. A theoretical introduction to mycology and the identification of yeasts was followed by a detailed practical demonstration, including India ink microscopy, cryptococcal antigen test, germ tube test, biochemical tests for yeast identification and culture of yeasts. After lunch, practical "spots" were set up to test what the participants had learnt during the morning teaching sessions. A feedback session afterwards provided a fourth opportunity for reinforcement. Day two focused on identification of a few common moulds. The format was similar to day one, starting with a brief, theoretical overview in the morning, followed by a practical demonstration on slide preparation for moulds (block culture, sellotape and tease mounts). Identification of common species within the *Aspergillus* and *Zygomycetes* groups were discussed in detail, whereas dermatophytes were discussed only briefly. Again, after lunch, practical "spots" were set up to test what the participants had learnt during the morning teaching sessions. A feedback session afterwards provided another opportunity for reinforcement. A brief outline of the mycology EQA programme was discussed to enable laboratories to better meet expectations. This programme is coordinated and evaluated by the MRU. The importance of surveillance work, performed by NICD, was discussed in detail. By the end of the workshop, participants were successfully able to perform basic techniques to identify common yeasts and moulds.

Dr Xoliswa Poswa, MBBCh, FCPath (SA) Micro, MSc Epidemiology, School of Public Health, University of the Witwatersrand, Johannesburg
Dissertation Title: In-hospital mortality of HIV-associated cryptococcal disease in patients treated with amphotericin B versus fluconazole
Supervisors: Nelesh Govender & Cheryl Cohen

Pathology Registrar Training
The MRU participated in the NICD training programme for pathology registrars. In 2009, 11 clinical pathology and mono-speciality clinical microbiology registrars were trained for a day in August 2009 as part of the short training programme. Refresher mycology courses for registrars close to the specialist exit examinations were held in February (6 registrars 2 days) and September 2009 (4 registrars 3 days). Training included practical identification of clinically important yeasts and moulds,

a review of antifungal susceptibility test methods, an introduction to molecular epidemiology and molecular diagnostic techniques as well as an overview of relevant clinical infectious disease issues.

NICD Intern Scientist Training

The MRU hosted two full-time, MSc-level intern scientists in 2009 both completed an accelerated internship programme and applied for HPCSA registration. A short workshop, held 29-31 July 2009, introduced NICD intern scientists to the field of mycology and included an introduction to identification of yeasts and moulds, an overview of antifungal susceptibility testing and a review of molecular techniques used in medical mycology. Six intern scientists attended.

Student Technician Training

Brian Nemukula joined the Unit in November 2009 and will undergo training for 2 years in preparation for technician examinations.

National Microbiology Surveillance Unit

BACKGROUND

The National Microbiology Surveillance Unit (NMSU) contributes to the control of bacterial and fungal diseases, determined to be of public health importance in South Africa, through the development and coordination of laboratory-based surveillance which provide strategic information for public health action. The objectives of the NMSU are to coordinate, direct and provide capacity for the activities of the well-established, GERMS-SA surveillance programme and coordinate and/or provide capacity for collaborative research studies to assist in the understanding of research questions arising from surveillance projects.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

GERMS-SA surveillance programme

GERMS-SA is a national, laboratory-based surveillance programme for bacterial and fungal diseases of public health importance. In 2009, 22,399 laboratory-confirmed cases were reported through this system. Eleven thousand three hundred and fifty six isolates were submitted to NICD reference laboratories for characterisation. Approximately one-third of all cases (n=5,928) were reported from 25 enhanced surveillance hospital sites. At these sites, clinical case data were obtained by nurse surveillance officers from 4,859 cases (82%); data were obtained from 2,579 (53%) cases through interview. The NMSU directed and coordinated the national surveillance programme in collaboration with other units within the Microbiology Division: Enteric Diseases Reference Unit (EDRU), Epidemiology and Surveillance Unit, Mycology Reference Unit (MRU), Parasitology Reference Unit (PRU) and Respiratory and Meningeal Pathogens Reference Unit (RMPRU). A full description of surveillance methodology, activities and results is contained within the GERMS-SA Annual Report 2009. In 2009, the cryptococcal surveillance database was transitioned from Epi Info 6.04d to MS-Access.

GERMS-SA surveillance site visits

In 2009, visits to 35 surveillance sites in 8 provinces provided an opportunity for NICD staff to engage with laboratory and hospital staff participating in the surveillance programme.

Table 1: GERMS-SA surveillance site visits, 2009

Province	Date	Surveillance site
EC	15 July 2009	Mthatha NHLS
FS	26 May 2009	Makopane NHLS
FS	26-28 August	Ampath/ Pathcare/ van Rensburg and Partners/ Universitas NHLS/ Pelonomi NHLS/ Bongani NHLS/ Manapo NHLS/ Boitumelo NHLS/ Dihlabeng NHLS
GA	19 January 2009	Charlotte Maxeke Johannesburg Academic NHLS/ Chris Hani Baragwanath NHLS
GA	2 April 2009	Dr George Mukhari NHLS
GA/LP/MP	8-9 June 2009	Dr George Mukhari NHLS (Lab workshop – 11 labs)
GA	December 2009	Dr George Mukhari NHLS (Site evaluation)
GA/LP	12-13 August 2009	Steve Biko Pretoria Academic (Lab workshop)
GA	27 July 2009	Lancet Richmond
GA	5 August 2009	Germiston NHLS
GA	24 August 2009	Ampath Pomona
GA	27 August 2009	Ampath Metalbox
GA	3 September 2009	Kalafong NHLS/ 1-Military
GA	21 October 2009	Ampath Pretoria
GA	25 November 2009	Leratong NHLS
KZ	26-27 March	Addington NHLS/ RK Khan NHLS/ Grey's NHLS/ Edendale NHLS/ King Edward VIII
LP	25-26 February 2009	Mankweng NHLS (Lab workshop – 9 labs)
MP	13 July 2009	Themba NHLS
NW	14 January 2009	Job Shimankana Tabane NHLS
NW	26 October 2009	Westvaal Orkney (AngloGold Ashanti)
WC	30 July 2009	Groote Schuur NHLS

GERMS-SA coordination meetings

The NMSU coordinated the GERMS-SA surveillance programme by convening the following meetings involving local and international collaborators in 2009:

- Surveillance Officer meeting, 12-13 March 2009
- Surveillance Officer meeting, 11-13 November 2009
- Principal Investigator meeting, 5-6 November 2009
- Steering Committee meeting, 6 November 2009

GERMS-SA publications

The NMSU compiled and distributed regular surveillance publications to the participating laboratory network and other stakeholders. These are available on the GERMS-SA website.

- Annual Report 2008
- Quarterly Laboratory Reports: Q1-2009, Q2-2009, Q3-2009, Q4-2009
- Enhanced Surveillance Site Operational Reports Q1-2009, Q2-2009, Q3-2009
- Link Newsletter: Volume 22 (April 2009), Volume 23 (October 2009), Volume 24 (December 2009

RESEARCH PROJECTS

The NMSU collaborated with other NICD units on the following projects during 2009:

- A case-control study to estimate the effectiveness of a 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South Africa
- A case-control study to determine risk factors (including HIV infection) for acquiring meningococcal disease and carriage in 3 provinces of South Africa
- Cryptococcal meningitis in Gauteng Province, South Africa: exploring post-hospital discharge outcomes and uptake of care, 2009
- Active, laboratory-based surveillance for invasive and non-invasive shigellosis in South Africa, 2003 - 2007: predominant serotypes may guide vaccine development
- Nosocomial salmonellosis: analysis of invasive cases occurring in South African hospitals over an 18-month period: 2007-2008
- Bacterial and fungal meningitis amongst children <5 years old, South Africa, 2007
- Early direct effects of two doses of the 7-valent pneumococcal conjugate vaccine (PCV7) in South Africa, 2007-2009

COLLABORATIONS

The GERMS-SA Surveillance Programme

Gene Elliot, Universitas/ Pelonomi/ NHLS & University of Free State
Andrew Rampe, Dip Med Tech, Rustenberg Hospital/ NHLS
Pieter Jooste, FCPaed (SA), Kimberley Hospital
Ken Hamese, FCPaed (SA), Polokwane/ Mankweng Hospital
Jacob Lebudi, Business Manager, Rob Ferreira/ NHLS
Greta Hoyland, Dip Med Tech, Rob Ferreira/ NHLS
Yacoob Coovadia, FCPath (SA) Micro, Inkosi Albert Luthuli Hospital/ NHLS & University of KwaZulu-Natal
Nomonde Dlamini, MBBCh, Inkosi Albert Luthuli Hospital/ NHLS & University of KwaZulu-Natal
Sumayya Haffejee, FCPath (SA) Micro, Grey's Hospital/ NHLS & University of KwaZulu-Natal
Halima Dawood, FCP (SA), Grey's Hospital & University of KwaZulu-Natal
Meera Chhagan, FCPaed (SA), Grey's Hospital & University of KwaZulu-Natal
Anwar Hoosen, FCPath (SA) Micro, Steve Biko (Pretoria) Academic Hospital Complex/ NHLS & University of Pretoria
Kathy Lindeque, Dip Med Tech, Steve Biko (Pretoria) Academic Hospital Complex/ NHLS
Maphoshane Nchabaleng, MMed (Pathology) Microbiology, Dr George Mukhari Hospital/ NHLS & University of Limpopo
Bonnie Maloba, FCPath (SA) Micro, Dr George Mukhari Hospital/ NHLS & University of Limpopo
Siseko Martin, MBChB, Tygerberg Academic Hospital/ NHLS & University of Stellenbosch
Elizabeth Wasserman, MMed (Path) Microbiology, Tygerberg Academic Hospital/ NHLS & University of Stellenbosch
Olga Perovic, FCPath (SA) Micro, MMed (Micro), Charlotte Maxeke (Johannesburg) Academic Hospital/ NHLS & University of Witwatersrand
Trusha Nana, FCPath (SA) Micro, Charlotte Maxeke (Johannesburg) Academic Hospital/ NHLS & University of Witwatersrand
Charlotte Sriruttan, FCPath (SA) Micro, Charlotte Maxeke Johannesburg Academic Hospital & University of Witwatersrand
Charles Feldman, FCP (SA), PhD, Charlotte Maxeke (Johannesburg) Academic Hospital/ NHLS & University of Witwatersrand
Sandeep Vasaikar, MD, Nelson Mandela Academic Hospital/ NHLS & Walter Sisulu University
Vivek Bhat, MD, Nelson Mandela Academic Hospital/ NHLS & Walter Sisulu University
Sharona Seetharam, FCPath (SA) Micro, Chris Hani Baragwanath Hospital/ NHLS & University of Witwatersrand
Jeannette Wadula, FCPath (SA) Micro, Chris Hani Baragwanath Hospital/ NHLS & University of Witwatersrand
Alan Karstaedt, FCP (SA), Chris Hani Baragwanath Hospital & University of Witwatersrand
David Moore, FCPaed (SA), Chris Hani Baragwanath Hospital & University of Witwatersrand
Andrew Whitelaw, FCPath (SA) Micro, Groote Schuur Hospital/ NHLS & University of Cape Town
Adrian Brink, FCPath (SA) Micro, Johannesburg, Ampath National Laboratory Services
Maria Botha, FCPath (SA) Micro, Johannesburg (Metalbox), Ampath National Laboratory Services
Suzy Budavari, FCPath (SA) Micro, Johannesburg (Sunninghill), Ampath National Laboratory Services
Mark Cruz da Silva, FCPath (SA) Micro, Johannesburg (Metal Box), Ampath National Laboratory Services
Xoliswa Poswa, FCPath (SA) Micro, Johannesburg (Pomona), Ampath National Laboratory Services

Inge Zietsman, FCPath (SA) Micro, Johannesburg, Ampath National Laboratory Services
Juanita Smit, FCPath (SA), Lancet laboratories
Marthinus Senekal, FCPath (SA), PathCare laboratories

CAPACITY BUILDING

Wits Public Health Registrar Training Programme
Student: Jacqui Mendes, MBBCh
Project: Screening antiretroviral-naive, HIV-infected adults, newly-enrolled into an urban, hospital-based clinic, with the serum cryptococcal antigen test, 2009
Duration: January to June 2009

London School of Hygiene and Tropical Medicine Student Elective
Student: Katherine Gaskell
Project: Post-hospital discharge outcomes for patients with incident cryptococcosis in Johannesburg in the early, post-HAART era, 2009
Supervisors: Nelesh Govender, Kerrigan McCarthy, Alison Grant
Duration: June to July 2009

South African Field and Epidemiology Training Programme
Student: Verushka Chetty
Project: Evaluation of a poorly-functioning GERMS-SA enhanced surveillance site, 2009
Duration: May to November 2009

Tailor-made MS-Access Training Course for GERMS-SA
Course leader: Angela Ahlquist, CDC, Atlanta, USA
Participants: 20-30 NICD personnel working on the GERMS-SA programme
Duration: 3 to 21 August 2009

Parasitology Reference Unit

BACKGROUND

The Parasitology Reference Unit (PRU) falls within the Microbiology Division of the NICD. The Unit provides reference diagnostic services for human parasitic diseases in the NHLS laboratory network, as well as outside laboratories and organizations; administers several national and international external quality assessment programmes for parasitology; undertakes national surveillance for *Pneumocystis* pneumonia as part of the Group for Enteric, Respiratory, and Meningeal disease Surveillance of South Africa (GERMS-SA); trains pathology registrars, medical scientists, and technologists; and conducts applied research in the field of human medical parasitology.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

DIAGNOSTIC SERVICE

The 2009 diagnostic workload decreased slightly compared with the previous year (Figure 1). Apart from *Pneumocystis jirovecii*, for which the laboratory offers a primary diagnostic service, most specimens are secondarily referred from other laboratories because of their unusual or diagnostically challenging nature, or for surveillance purposes.

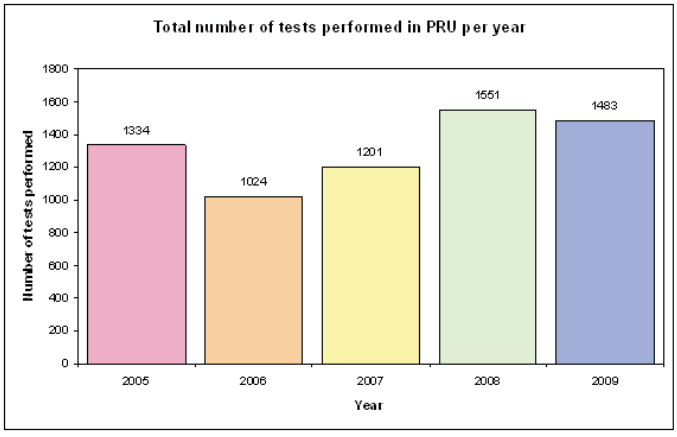


Figure 1: Parasitology Reference Unit, diagnostic workload per year, 2005-2009

ACCREDITATION AND TRAINING

The Unit was re-accredited by SANAS in June 2009. Staff attended training courses in public health, general bacteriology, ethics and informed consent, malaria microscopy, and laboratory safety and first aid.

Teaching and training in various aspects of parasitology was provided for MSc and medical students, technicians, medical technologists, intern medical scientists, pathology registrars, and SASTM travel medicine course participants, in locations including NICD, KwaZulu-Natal, and Namibia.

RESEARCH

Research programme: *Pneumocystis jirovecii*

Pneumocystis jirovecii is an unconventional opportunistic fungal pathogen which causes the important AIDS-defining infection, *Pneumocystis* pneumonia (PcP). There are several components to this programme, as follows:

Prevalence of *P. jirovecii* DHPS mutations in the population

Mutations in the *Pneumocystis jirovecii fas* gene encoding dihydropteroate synthase (DHPS) are associated with prior exposure to sulpha drugs and their appearance suggests the emergence of resistance. This study examined the frequency in South Africa of infections with *P. jirovecii* strains harbouring DHPS mutations. Respiratory specimens from 712 patients (93% >15 years of age) with suspected PCP, were consecutively received for *P. jirovecii* diagnosis during one year. *P. jirovecii* infection was confirmed by immunofluorescence microscopy in 24% of the patients. Carriage of the fungus was revealed by real-time PCR in 17% of patients with negative microscopy. The *P. jirovecii fas* gene was amplified and sequenced in specimens from 151 patients and 85 (56%) of these were found to have a mutant DHPS genotype. These findings are consistent with a high level of antibiotic pressure and underscore the importance of rational use of sulpha-drug medication, particularly TMP-SMX, either prophylactically against PCP or for other reasons. (Researcher: L Dini)

Characterization of the molecular epidemiology of *Pneumocystis* infections

DNA extraction and nested PCR targeting the ITS 1 and ITS 2 regions was successful for 51 specimens. Cloning, selection of recombinants and sequencing was completed for all of these specimens. Analysis of sequences to determine the *P. jirovecii* strains has been completed in 43 specimens and preliminary analysis has revealed Eg to be the most common ITS genotype. (Researcher: L Dini)

Incidence of PCP in a prospective patient cohort and assessment of the clinical significance of DHPS mutations

This prospective clinical, microbiological and molecular study was launched in January 2005. Patient recruitment was completed in 30 June 2009 and a total of 319 specimens was received for analysis. Patient follow-up (3 months after discharge from hospital) using a telephonic questionnaire is continuing. All specimens

have been investigated by IF microscopy and 50% (159/319) were positive for *P. jirovecii*, whilst 12 had equivocal microscopy results. Robotic extraction of DNA and real-time PCR is now in routine use. Of the 297 specimens analysed to date by qPCR, 192 (65%) were positive for *P. jirovecii*. Nested PCR for DHPS genotype analysis has been successfully conducted in 177 specimens to date and preliminary sequencing results of 82 specimens show that 61% (50/82) have a mutant DHPS genotype. (Researcher: L Dini)

Assessment of the clinical significance of *Pneumocystis* infection load

Analysis of the clinical significance of *Pneumocystis* infection load, as determined by qPCR, in these patients will be compared with a subset of patients from Addis Ababa, Ethiopia. (Researcher: L Dini)

Resolution of mixed *P. jirovecii* genotypes

Grouping of PcP cases based on the *P. jirovecii* DHPS genotype will enable us to determine whether patients harbouring different *P. jirovecii* strains, i.e., with or without mutations, have different clinical outcomes. In patients with mixed *P. jirovecii* DHPS genotypes, it is not always possible to resolve individual genotypes by direct sequencing of PCR products. These mixtures can be resolved if their PCR products are cloned into an appropriate vector, amplified by PCR and a number of clones re-sequenced. Laboratory analysis has progressed to the point where the mixed DHPS genotypes have been identified. (Researcher: B Poonsamy)

Toxplasmosis research project

Toxoplasmosis is an infection of warm-blooded vertebrates caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii*. It is one of the most common parasitic diseases of humans, infecting approximately one third of the world's population. It is a significant cause of congenital disease and an important opportunistic pathogen which has become an increasing problem worldwide due to the AIDS epidemic. Previously, the seroprevalence of *T. gondii* in samples of selected populations at risk, namely HIV-positive individuals and a more general population sample biased towards pregnant women, was therefore investigated and found to be 9.8% (37/376) and 6.4% (32/497) respectively. Further work will isolate and genotype local *Toxoplasma* strains to identify and define molecular virulence markers relevant to severe toxoplasmosis. A pilot study to isolate *Toxoplasma* strains from informally-bred chickens in Gauteng has commenced (Researcher: K Kistiah)

Assessment of malaria parasite load using digital image analysis

Quantitation of malaria parasite density is an important component of laboratory diagnosis of malaria. Microscopy of Giemsa-stained thick blood films is the conventional method for parasite enumeration. Accurate and reproducible parasite counts are difficult to achieve, because of inherent technical limitations and human inconsistency. Inaccurate parasite density estimation may have adverse clinical and therapeutic implications for patients, and for endpoints of clinical trials of anti-malarial vaccines or drugs. Digital image analysis provides an opportunity to improve performance of parasite density quantitation. Accurate manual parasite counts were done on 497 images of a range of thick blood films with varying densities of malaria parasites, to establish a uniformly reliable standard against which to assess the digital technique. By utilizing certain statistical parameters of parasite size frequency distributions, particle counting algorithms of the digital image analysis programme were semi-automatically adapted to variations in parasite size, shape and staining characteristics, to produce optimum signal/noise ratios (Figure 2). A reliable counting process was developed that requires no operator decisions that might bias the outcome. Digital counts were highly correlated with manual counts for medium to high parasite densities, and slightly less well correlated with conventional counts. Using open-access software and avoiding custom programming or any special operator intervention, accurate digital counts were obtained, particularly at high parasite densities that are difficult to count conventionally. The technique is potentially useful for laboratories that routinely perform malaria parasite enumeration. (Researcher: J Frean)

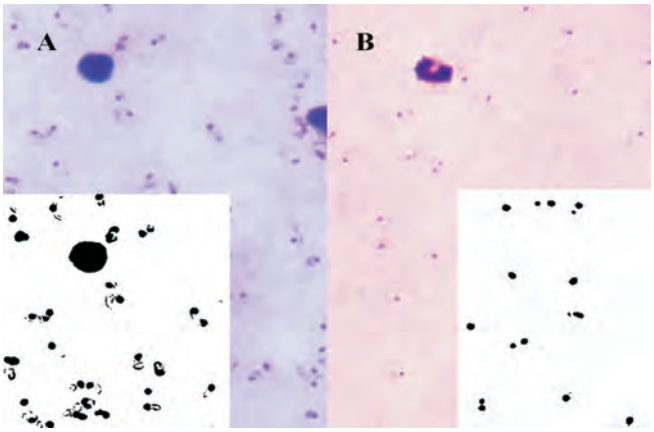


Figure 2: Giemsa-stained thick smears and corresponding digitally-processed images for counting, A, mature parasites; B, immature parasites

Quality assessment activities

The Unit provides several external quality assessment (proficiency testing) programmes. The aims of the parasitology EQA schemes are to build capacity in the field of human diagnostic parasitology in Southern Africa and to obtain an objective measure of the diagnostic ability of participating laboratories. Two national parasitology EQA schemes are offered: 'Stool parasites' and 'Blood parasites' (Figure 3); both are CPD accredited. Malaria EQA programmes are designed and specially produced for the World Health Organization (70 African laboratories) as part of a larger NICD EQA contract, and for 2 international pharmaceutical companies that are trialling antimalarial

drugs and vaccines. Another EQA programme (PCP EQA) assesses the participating laboratories' ability to correctly identify *Pneumocystis jirovecii* (Figure 4).

Figure 3: Mrs Rita van Deventer preparing malaria EQA survey challenges

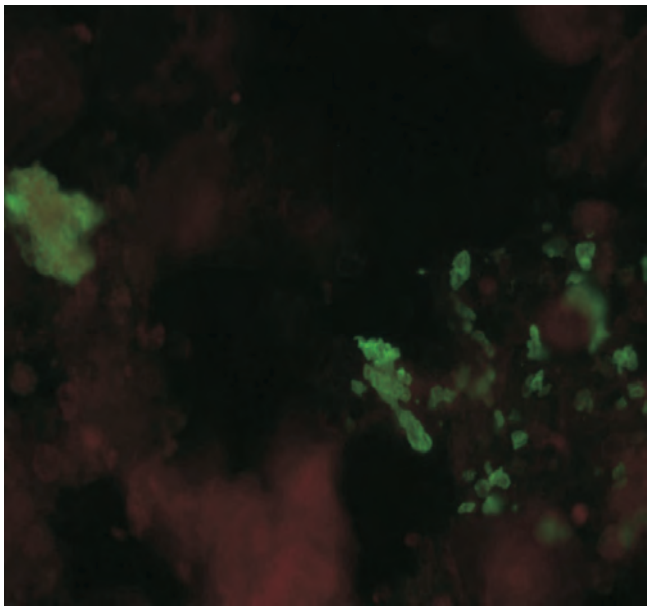


Figure 4: *Pneumocystis jirovecii*, direct immunofluorescence test

***Pneumocystis* pneumonia surveillance**

The total number of cases acquired by the GERMS-SA surveillance system in 2008 was 371, of which 341 were laboratory-confirmed. Compared with other opportunistic pathogens under surveillance, this is certainly a substantial underestimate of the burden of disease. There are several reasons for this: the condition is mainly clinically and radiologically diagnosed, and laboratory testing is only offered in a few larger centres. Strategies to better estimate the true burden of disease are being pursued with local and international collaborators.

Stool parasite surveillance

We started analysing rotavirus surveillance specimens for stool parasites in April 2009. For 2009, we processed 404 specimens, of which 20 (5%) were positive for parasites, mainly *Cryptosporidium parvum*.

COLLABORATIONS

Chris Hani Baragwanath Hospital (Drs M Wong, A Karstaedt, A Mochan): *Pneumocystis* pneumonia and toxoplasmosis studies.
Swedish Institute for Infectious Disease Control (Drs V Fernandez, A Barragan; Mrs L Dini): *Pneumocystis* pneumonia and toxoplasmosis studies.
WHO Regional Advisory Group: malaria EQA programme

CAPACITY BUILDING

Registered students

PhD: L Dini (University of the Witwatersrand)
Msc: B Poonsamy (University of the Witwatersrand)

Graduated students

MSc (Med): K Kistiah (University of the Witwatersrand)

Respiratory & Meningeal Pathogens Reference Unit

BACKGROUND

RMPRU's main function is to perform active laboratory-based surveillance for invasive disease throughout South Africa caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. The unit reports weekly data on these diseases to the Epidemiology Unit, NICD; provides data for presentation at the monthly National Outbreak Response Team (NORT) meeting, national Department of Health, Pretoria and for publication in the quarterly NICD Communicable Diseases Surveillance Bulletin. Data from RMPRU surveillance are also presented and discussed at Department of Health Expanded Programme on Immunisation (EPI) Task Group and National Advisory Group for Immunisation (NAGI) meetings.

The unit also performs national and regional reference laboratory functions for the diagnosis of meningitis and pneumonia caused by the above bacterial pathogens. Laboratory training is offered to local and international students and colleagues.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

SURVEILLANCE PROGRAMMES AND RELATED RESEARCH PROJECTS

Early, direct effects of two doses of the 7-valent pneumococcal conjugate vaccine (PCV7) in South Africa, 2007-2009

PCV7 was launched in South Africa's national infant immunisation programme in April 2009: schedule included a 2-dose primary series (6 and 14 weeks) with a booster dose at 9 months, but no catch-up vaccination. In 2009 to date, just over 1 million doses were ordered in the public sector, for a birth cohort of 1 million (estimated HIV prevalence among children <5 years, 4%). We monitored early changes in serotype distribution. We conducted active, national surveillance for laboratory-confirmed, invasive pneumococcal disease (IPD). Serotyping was determined by the Quellung reaction. Baseline IPD rates (average from 6-month winter period, weeks 15 through 40) during pre-vaccine years (2007-2008) were compared to post-introduction rates during the same period in 2009. Data for 2009 allowed for a one-month delay in reporting and included all audit cases through the 3rd quarter. For 2009, immunisation coverage among children age <1 year was at most 50%; no children had received 3 doses. Baseline rates (cases per 100,000 population) of vaccine-serotype IPD among children <1 year fell from 24 to 18 post introduction (-25%, p=0.001). Non-vaccine serotype rates in the same age group were unchanged (11, p=0.4). Rates of all IPD among children age <1 year decreased significantly: 46 to 40 (-13%, p=0.05). Rates in other age groups were unchanged. A significant decrease occurred in serotype-specific disease among infants within 6 months of PCV7 introduction in South Africa. This reduction was noted despite low coverage, no catch up, and high HIV prevalence.

RMPRU staff working with national surveillance isolates (from L to R, back) Happy Skosana, Ruth Mpembe; (front) Olga Hattingh, Mmabatho Moerane, Victoria Magomani, Maimuna Carrim

Pneumococcal bacterial loads and influenza co-infection in patients with severe acute respiratory infection in South Africa

Diagnosis of pneumococcal pneumonia is challenging, and the burden of this disease is largely underestimated. We aimed to determine the prevalence of pneumococcal DNAemia in patients presenting with severe acute respiratory infection (SARI), and to describe trends in pneumococcal and influenza co-infection. In February 2009, a surveillance system enrolling all patients presenting with SARI and collecting respiratory and blood specimens at 3 sentinel hospitals in South Africa was initiated. Blood pneumococcal bacterial loads were determined by quantitative real-time PCR detecting *lytA*. Respiratory specimens were tested for influenza by real-time reverse transcription PCR. From May 2009 through January 2010, 3037 patients were enrolled. 52% (1583/3023) were <5 years, 46% (1374/3023) were 5-64 years and 2% (66/3023) were >64 years. The overall pneumococcal detection rate was 7% (142/2125) with peaks of 23% in week 25 of 2009 and 11% to13% over weeks 35 to 38, coinciding with the two influenza peaks of seasonal influenza A H3N2 and novel pandemic influenza A H1N1. Influenza co-infection occurred in

18% (25/142) of pneumococcal cases. Bacterial loads ranged from 27 to 1.58 x 106 DNA copies/ml with a median of 932 DNA copies/ml. The pneumococcal PCR detection rate was four-fold higher compared to blood culture. Results support the synergistic interaction between these two pathogens.

Antimicrobial susceptibility of pneumococcal isolates causing bacteremic community-acquired pneumonia (CAP) in Gauteng, South Africa

Previous surveillance studies from South Africa have indicated very high levels of pneumococcal antimicrobial resistance. We determined the prevalence and patterns of antimicrobial susceptibility in pneumococcal isolates causing radiologically-confirmed bacteraemic pneumonia in Gauteng, South Africa. Susceptibility testing was performed by broth microdilution and the Etest. Macrolide resistance determinants, *ermB* and *mefA,* were detected by PCR and fluoroquinolone resistance-conferring mutations were detected by sequencing. Fluoroquinolone area-under-the-inhibitory curve ($AUIC_{90}$) was calculated from drug data obtained from healthy volunteers. Overall, 175 patients were enrolled. Penicillin non-susceptibility (NS) was documented in 33% (57/175) of the isolates when historical breakpoints were used but in only 2% (3/175) of the isolates (intermediate resistance) using the CLSI 2008 breakpoints for non-meningeal pneumococcal infections. Resistance to macrolides was documented in 13% (23/175) and to cotrimoxazole in 37% (65/175). There was no resistance to ceftriaxone, telithromycin or the fluoroquinolones and only one potentially significant first-step fluoroquinolone resistance mutation was documented. The $AUIC_{90}$ for intravenous and oral levofloxacin 500 mg b.d. and 750 mg daily, as well as for moxifloxacin 400mg daily were all above 120. This study of clinically significant pneumonia isolates of *S. pneumoniae* documented lower levels of antimicrobial resistance than has previously been reported from South Africa.

Molecular characterisation of *Neisseria meningitidis* serogroup B isolates causing invasive disease in South Africa, 2002-2006

Globally, sporadic serogroup B disease is caused by heterogeneous strains, however, prolonged outbreaks in several countries have been due to single clones, mostly of the ST-32/ET-5 clonal complex (cc). Invasive isolates, submitted to a national laboratory-based surveillance system, were characterized by pulsed-field gel electrophoresis (PFGE). PorA, FetA and multilocus sequence (MLST) typing were performed on all 2005 isolates (n=58) and randomly selected isolates from other years (n=25). 2144 invasive cases were reported. Of these, 1627 (76%) had viable isolates and 307 (19%) were serogroup B. PFGE results were available for 302/307 (98%) isolates. PFGE divided isolates into 11 clusters (189/302, 63%). The largest cluster, B1, accounted for 77/302 (25%) of isolates. Selected isolates from B1 (18/77, 23%) belonged to cc ST-32/ET-5. The most prevalent PorA:FetA types among B1 isolates were P1.19,15:F5-1(6/18, 33%) and P1.12-1,13-1:F5-1 (5/18, 28%). B1 isolates decreased over time, from 45% (21/47) in 2002 to 13% (8/62) in 2006 (p = 0.001). Isolates were present in 4/9 provinces, but were most common to Western Cape (60/77, 78%). Cluster B2 accounted for 10% (29/302) of isolates. Selected isolates from B2 (9/29, 31%) belonged to cc ST-42/44/lineage 3. P1.7-2,4:F1-5 was the dominant PorA:FetA type (9/9, 100%). B2 isolates showed no significant change over time, 6% (3/47) in 2002 to 15% (9/62) in 2006 (p = 0.649). B2 isolates were present in 4/9 provinces, but were most common to Gauteng (17/29, 59%). There was no significant change in unrelated isolates (113/302, 37%), from 2002 (19/47, 40%) through 2006 (28/62, 45%) (p = 0.577). At present, serogroup B disease in South Africa is not dominated by an epidemic clone, however, global cc's ST-32/ET-5 and ST-41/44/lineage 3 are circulating in Western Cape and Gauteng, respectively.

RMPRU scientists: (back, L to R), Chivonne Moodley, Mignon du Plessis; (front, L to R), Phumza Tikilili, Azola Fali, Victoria Magomani

PCR identification and serogroup/type confirmation of bacterial pathogens causing meningitis in South Africa, January 2007-July 2008

Bacterial meningitis is a serious disease with high morbidity and mortality. Laboratory diagnosis of meningitis can be hindered by technical and other clinical reasons. PCR is an important non-culture method which greatly improves the sensitivity, turnaround time and accurate diagnosis of bacterial meningitis. We discuss the role of PCR in confirming identification and serogrouping/typing of transport media with non-viable *Haemophilus influenzae (HI)* and *Neisseria meningitidis (NM)* strains submitted to NICD as part of national laboratory-based surveillance. All cases of *HI* and *NM* with Dorset transport media yielding no growth on culture were reviewed from January 2007 - July 2008. DNA was extracted from Dorset transport media using Roche MagNA Pure Compact Nucleic acid kit and instrument. Semi-nested multiplex PCR assay was used for organism identification and

serogroup/type confirmation. Of 637 cases of *HI* reported, 305/637 (48%) were culture negative, of which 149/305 (49%) had Dorset media tested by PCR and 101/149 (68%) were confirmed as *HI.* Additionally, 15/149 (10%) were serotyped as a, b, b mutant, e, f and non-typeable, with 8/15 (53%) being serotype b; similar to culture-confirmed cases where viable *HI* serotype b isolates were 128/332 (39%), p=0.3. Of 719 cases of *NM* reported, 297 (41%) yielded no growth and 152/297 (51%) were tested by PCR. 117 (77%) of these were confirmed to be *NM*, and 75/152 (49%) were further serogrouped as B, C, W135 and Y. W135 was the most common serogroup: 46/75 (61%). This is similar to culture-confirmed cases with viable isolates where 248/422 (59%) viable isolates were serogrouped as W135 (p=0.7). PCR for *NM* serogrouping performed better than PCR for *HI* serotyping. Molecular tests are of importance in confirming laboratory-confirmed cases of *HI* and *NM*, as well as providing additional serogroup/type information.

Multidrug-resistant *Streptococcus pneumoniae* causing invasive disease, South Africa, 2008

Infection with *Streptococcus pneumoniae (Spn)* can cause severe disease in children <5 years of age. We investigated the association between multidrug resistance (MDR) and various risk factors. *Spn* isolates causing invasive disease were received as part of ongoing national laboratory-based surveillance. Isolates were serotyped by the Quellung reaction and screened for resistance to penicillin, erythromycin, clindamycin, tetracycline, rifampicin and cotrimoxazole. Penicillin non-susceptibity was defined as an MIC≥0.12mg/L. MDR was defined as non-susceptibility to penicillin plus two or more additional antimicrobial classes. Univariate logistic regression was used for analysis of associations. In 2008, a total of 4,837 cases were reported, of which 3,327 (69%) had viable isolates. Of viable strains, 564 (17%) were MDR. The most common MDR phenotypic profile was penicillin-tetracycline-erythromycin-clindamycin-cotrimoxazole (258/564, 46%). Children aged <5 years were 2.4 times more likely to have MDR strains than cases aged 5 years or older (284/1,098, 26% <5 years vs. 260/2,077, 13% >=5 years; p<0.001). For MDR cases aged less than 5 years, PCV7 serotype 14 (163/284, 57%) and non-PCV7 serotype 6A (15/284, 5%) were the most common. For non-MDR cases aged less than 5 years PCV7 serotype 6B (130/813, 16%) and non-PCV7 serotype 6A (109/813, 13%) were the most common. Cases due to PCV7 serotypes were 15.6 times more likely to be MDR than cases with serotypes not included in the vaccine (502/1,444, 35% PCV-7 vs. 62/1,882, 3% non-PCV-7; p<0.001). There was no significant difference between HIV-positive and HIV-negative patients with respect to MDR (130/762, 17% HIV-positive vs. 61/293, 21% HIV-negative; OR 1.3; p=0.5), and between patients that survived compared to those that died, with respect to MDR (176/995, 18% survived vs. 78/425, 18% died; OR 1.0; p=0.8). MDR *Spn* are more than twice as common in children aged less than 5 years compared to individuals >=5 years. Serotypes included in the vaccine are statistically more likely to be MDR and vaccination of children at an early age may reduce the burden of pneumococcal MDR disease.

***Haemophilus influenzae* serotype b (Hib) national laboratory-based surveillance in South Africa, 2008**

The Hib conjugate vaccine was introduced into the Expanded Programme on Immunization (EPI) in South Africa (SA) in 1999, leading to a reduction in the incidence of disease in children <5 years old. Laboratories throughout SA sent strains of *H. influenzae*, isolated from normally sterile site specimens, together with patient demographics to NICD, where identification, susceptibility testing and serotyping tests were performed. When possible, culture-negative specimens were confirmed by PCR. Missed cases, identified on audit, were included in an EpiInfo surveillance database. Road to Health Card information and additional clinical data were collected from all Hib cases in children <5 years, to identify possible vaccine failures. In 2008, 392 cases of *H. influenzae* were reported to NICD; 194 (49%) cases had viable isolates and 14 (4%) specimens were non-viable on receipt but were serotyped by PCR. 111 (28%) were identified on audit. Of all *H. influenzae* cases reported, age was known in 377, and 216/377 (57%) were from children <5 years old, with 131/216 (61%) cases being from children <1 year. Of these, 100/216 (46%) were reported from Gauteng Province.

In children <5 years, serotyping data were available for 124 (57%), and 115 (53%) had viable isolates for antimicrobial susceptibility testing. 62 of 124 (50%) cases were caused by Hib; 27% (33/124) *H. influenzae* were non-typeable. Serotype f was the commonest non-b serotype (10%, 13/124). Ampicillin non-susceptibility was identified for 10/58 (17%) Hib isolates, and for 4/30 (13%) non-typeable *H. influenzae*, respectively. Vaccination history was known for 51/62 (82%) cases of Hib, of which 25/51 (49%) were identified as receiving all 3 doses of Hib, and of these 7/18 (39%) were known to be HIV infected. Hib is still the most common HI causing disease in children <5 years. Surveillance data are invaluable in providing information on incidence, serotypes and resistance, especially after implementation of new vaccines into the EPI.

TRAINING OFFERED

Anne von Gottberg, Linda de Gouveia and Ruth Mpembe led a 2-day training workshop at the NHLS Mankweng Laboratory in Limpopo, 25-26 February 2009, attended by 15 technologists and technicians from Limpopo laboratories (Polokwane, Tzaneen, Voortrekker, Jane Furse, Elim, Phalaborwa and Tshilidzini). The course consisted of hands-on basic bacteriology: microscopy, isolation, identification and susceptibility testing of bacteria.

Linda de Gouveia and Ruth Mpembe facilitating a bacteriology workshop organised with the Northern Branch, NHLS, and held at Mankweng Laboratory, Polokwane

Linda de Gouveia and Ruth Mpembe led a 2-day training workshop at the NHLS Shongwe Laboratory in Mpumalanga, 3-4 June 2009, for 7 technologists and technicians from laboratories in Mpumalanga (Themba, Malamulele, Witbank, Barberton, Ermelo, Nelspruit and Shongwe). The course consisted of hands-on basic bacteriology: microscopy, isolation, identification and susceptibility testing of bacteria.

A 4-day hands-on training workshop was held in RMPRU for 3 scientists from The Biovac Institute, Pinelands, Cape Town on phenotypic characterisation of *Streptococcus pneumoniae*, 9-12 June 2009.

Staff from the Biologicals and Vaccines Institute of Southern Africa (Biovac) being trained to work with pneumococcal isolates, from L to R, Lesetja Legodi, Cornelius Swart and Sihle Gumede

Linda de Gouveia and Ruth Mpembe, were requested by Dr Bekithemba Mhlanga, Director of the Hib-PBM Surveillance Network and Intercountry Support Team, Eastern & Southern Africa, and Dr Deo Nshimirimana, IVD Programme Manager, WHO/AFRO, to follow up on the previous laboratory assessment of the Lagos University Teaching Hospital, Microbiology Laboratory, Lagos, Nigeria, by conducting a 4-day hands-on training course for technologists in the laboratory, 22-25 June 2009.

Ruth Mpembe training technical laboratory staff in Lagos, Nigeria

Anne von Gottberg visited laboratories in the Free State to reinforce the general principles of laboratory-based surveillance for bacterial and fungal diseases of public health importance and to give feedback to laboratory personnel regarding GERMS-SA surveillance data, and using data related to pneumococcal, meningococcal and *Haemophilus influenzae* disease to highlight the work done at the NICD on isolates submitted for surveillance, 26-28 August, 2009.

- NHLS, Boitumelo Hospital, Kroonstad
- NHLS, Bongani Hospital, Welkom
- NHLS, Pelonomi Hospital, Bloemfontein
- NHLS, Universitas Hospital, Bloemfontein
- Pathcare, Bloemfontein
- Van Rensburg and partners, Bloemfontein
- AMPATH, Bloemfontein
- NHLS, Dihlabeng Hospital, Bethlehem
- NHLS, Manapo Hospital, Puthaditjaba

NHLS staff at Bongani Hospital, Welkom during a GERMS-SA surveillance feedback session

HIGHLIGHTS AND ACHIEVEMENTS ANNE VON GOTTBERG

- Expert advisor: 1st sub-Saharan African Vaccine-Preventable Diseases Regional Advisory Board Meeting, Sunday, 1 March 2009, Sandton Sun Hotel, Sandton, Johannesburg.
- Invited speaker: 4th Regional Pneumococcal Symposium, 2-3 March 2009, Sandton Sun Hotel Conference Centre, Sandton, Johannesburg, and presented South African pneumococcal disease data, "Country Spotlights: South Africa". This meeting was convened by the Sabin Vaccine Institute, Office of International Programs, Washington, DC, USA.
- Invited speaker: "Preparing for Pneumococcal Conjugate Vaccine Impact Evaluation in South Africa") at the 2009 PneumoADIP and Hib Initiative Surveillance Investigators Meeting: Evaluations, Innovations and Transitions, 4-6th March 2009, Sandton Sun Hotel Conference Centre, Sandton, Johannesburg, South Africa.
- Invited speaker: "Meningococcal disease update" FIDSSA Intercity Meeting 2009, Bytes Conference Centre, Midrand, 22 June 2009.
- Expert advisor: Pneumococcal Vaccine Economic Research Workshop called by heXor - Health Econometrix & Outcomes Research (Pty) Ltd, Intercontinental Airport Sun, OR Tambo Airport, Johannesburg, 8 July 2009.
- Expert advisor: National Advisory Group For Immunisations (NAGI) meeting, 22 July 2009, Executive Board Room, National Institute for Communicable Diseases, Johannesburg, South Africa.
- Invited expert to the Wyeth Pneumococcal Vaccines Advisory Board Meeting, 12 August 2009, Intercontinental Airport Sun, OR Tambo Airport, Johannesburg.
- Invited speaker at the Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) Congress, Sun City, North West Province, 20-23 Aug 2009. "Meningococcal vaccination update" and "Bacterial respiratory and meningitis pathogen surveillance, South Africa: update".
- External faculty: African Vaccinology Course, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, 7th-11th September, South Sun Newlands, Cape Town.
- Invited speaker: "Meningitis vaccination update" 6th Vaccinology Congress, Hermanus, Western Cape, 19-20 October 2009.

COLLABORATIONS

- Stephen Bentley, Pathogen Genomics, Wellcome Trust Sanger Institute, UK: *Streptococcus pneumoniae* Spain^{23F}-1 ST81 lineage deep genome sequencing project.
- Alan Karstaedt, Chris Hani Baragwanth Hospital, University of the Witwatersrand, Johannesburg, South Africa: Adult pneumococcal meningitis in Soweto, South Africa, 1985-2007.
- Maria Deloria Knoll, PneumoADIP, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA: Impact of statistical and modeling methods on estimates of pneumococcal serotype distribution: the pneumococcal global serotype project.
- Chrysanti Murad and Cissy B. Kartasasmita, Health Research Unit and Department of Child Health, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia: Serotype distribution and antimicrobial resistance of nasopharyngeal pneumococci among children < 5 years with non-severe pneumonia in Bandung, Indonesia, 2002-2003.
- Chris Tang, Imperial College, London, UK: Prevalence, distribution and clinical significance of insertion sequence IS*1301* in South African *Neisseria meningitidis* isolates.
- Arie van der Ende, Department of Medical Microbiology and the Netherlands Reference Laboratory for Bacterial Meningitis, Academic Medical Center, Amsterdam, the Netherlands: Evaluation of lipopolysaccharide (LPS) variants among serogroup Y meningococcal isolates from patients in South Africa.
- Xin Wang (Meningitis Laboratory), Gloria Carvalho and Lesley McGee (*Streptococcus* Laboratory), Centers for Disease Control, Atlanta, USA: Validation of real-time PCR for the detection of *S. pneumoniae*, *H. influenzae* and *N. meningitidis* from clinical specimens; and multiplex real-time PCR for detection of meningococcal serogroups.
- Heather Zar and Rendani Mamathuba, Red Cross Children's Hospital, Cape Town, South Africa: A descriptive study of the aetiology and outcome of children hospitalised with empyema.

CAPACITY BUILDING

- Chivonne Moodley Scientist intern and MSc by dissertation, Department of Virology and Communicable Disease Surveillance, Faculty of Health Science, University of the Witwatersrand, currently registered. Dissertation entitled "Molecular characterisation of *Neisseria meningitidis* Serogroup B isolates in South Africa, 2002 2006".
- Krishnee Moodley MMed by dissertation, Nelson R Mandela School of Medicine, Durban, KwaZulu-Natal, registered 2009. Dissertation entitled "Invasive pneumococcal disease in neonates in South Africa, 2000-2007".
- Siyabonga Rayise MRC intern and PhD by dissertation, Department of Virology and Communicable Disease Surveillance, Faculty of Health Science, University of the Witwatersrand, registered 2009. Dissertation entitled "Genetic characterisation of *Haemophilus influenzae* type b carriage and invasive isolates in South Africa: post-vaccination era".

- Jeffrey Ratto, Master of Public Health student at Rollins School of Public Health at Emory University, Atlanta, GA, USA. Project entitled: “Analysis of Risk Factors for *Streptococcus pneumoniae* Serotype 19A Disease and Serotype 19A Population Genetics”.
- Thembi Mthembu was accepted as a part-time student at Birnam Business College for a one-year Diploma in Gold Office Management and Project Management. She has received a bursary from the NHLS to complete these studies, and started classes on 9 May 2009.

Sexually Transmitted Infections Reference Centre

BACKGROUND

The mission of the Sexually Transmitted Infections (STI) Reference Centre is be a resource of knowledge and expertise in regionally relevant STIs 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 related to the control and effective management of STIs. Intelligence on the aetiology of major STI syndromes, as well as antimicrobial resistance data related to gonococcal infections, are communicated annually by the STI Reference Centre to the National and relevant Provincial Departments of Health in South Africa as well as to those working in public health and directly with STI patients. The STI Reference Centre also undertakes teaching and training activities, assisting with training of medical technologists, medical scientists, doctors, nurses and other healthcare staff. The unit also aims to be a centre of scientific excellence in the field of STIs by pursuing operational research relevant to public health and has established several international links with STI researchers overseas. Finally, the STI Reference Centre is the operational base for the African Region of the International Union against STIs (IUSTI).

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

MICROBIOLOGICAL AND CLINICAL SURVEILLANCE OF STIs IN SOUTH AFRICA

In 2009, microbiological surveillance for STIs was undertaken in Gauteng Province alone, at Alexandra Health Centre in Johannesburg. Negotiations and preparations were made to commence similar surveillance in the Eastern Cape, Mpumalanga and the Western Cape in early 2010.

The microbiological surveillance consists of two components:

a) Aetiological surveillance of three major STI syndromes: the male urethritis syndrome (MUS), the vaginal discharge syndrome (VDS) and the genital ulcer syndrome (GUS)

b) Antimicrobial surveillance of antibiotic resistance for isolated gonococci from MUS patients (ciprofloxacin and ceftriaxone)

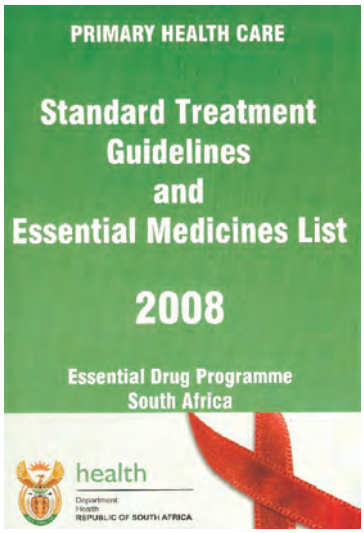
The aetiological surveillance confirmed that gonorrhoea continues to be the main cause of the MUS (71% of cases), that STIs account for only 49% of VDS presentations in women, and that genital herpes accounts for 75% of GUS cases.

Antimicrobial resistance testing demonstrated a continued high prevalence of isolation of ciprofloxacin resistant gonococci in Alexandra. In 2008, 21% of isolates were resistant to ciprofloxacin (MIC ≥ 1 mg/l).

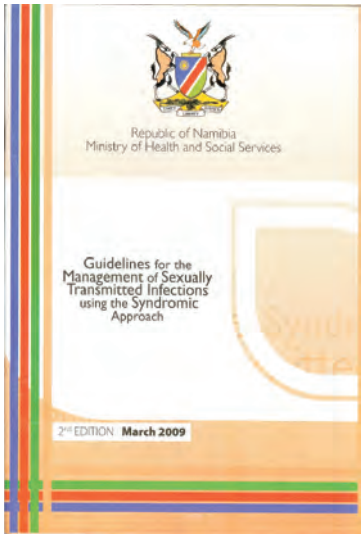
The data were presented in the form to electronic communications and a surveillance report to the National and relevant Provincial Departments of Health.

The Gauteng STI surveillance project, run by the STI Reference Centre in collaboration with the Gauteng Provincial Health Department, continued to collect data in 2009. The data for 2009 showed no major change to the data of the previous year.

REGIONAL STI GUIDELINES



A. South African Primary Health Care Standard Treatment Guidelines and Essential Medicines List, 4th Edition (2008)



B. Namibian Guidelines for the Management of STIs, 2nd edition (2009)

Figure 1: New STI Guidelines for South Africa (A) and Namibia (B)

The Essential Drugs Programme issued the 4th edition of the Primary Health Care Standard Treatment Guidelines and Essential Medicine's List (2008) in early 2009. Chapter 12 of this revised book contained the new STI guidelines that had been previously issued as a separate document by the National Department of Health in late 2008. The evidence-base for the two principal guideline changes came from a number of sources, but key among these were data from the STI Reference Centre. The national STI microbiological surveillance programme had demonstrated significant rises in quinolone resistance among cultured *Neisseria gonorrhoeae* isolates in Cape Town and Johannesburg which led to the removal of ciprofloxacin as first-line therapy for the treatment of proven or suspected gonorrhoea, and its replacement by a third generation cephalosporin (cefixime or ceftriaxone). In addition, the results from the joint STI Reference Centre (NICD/NHLS) and Centers for Disease Control and Prevention randomized controlled study on the clinical benefit of acyclovir therapy in the healing of genital ulcers among South African men was instrumental in providing the scientific rationale for including acyclovir as a first-line agent in the management of genital ulceration in primary care.

The Namibian Ministry of Health and Social Services revised their STI management guidelines during 2008 on the basis of microbiological STI surveillance, undertaken in Windhoek and Oshakati during 2007-2008. This surveillance exercise had been undertaken in collaboration with the STI Reference Centre and Professor Lewis provided technical advice in relation to STI guideline revision.

UPDATING OF THE SADC FRAMEWORK FOR THE MANAGEMENT OF STIs

The SADC secretariat requested Professor Lewis to support the updating of the SADC Framework for the Management of STIs as a technical expert. The technical meeting took place in Livingstone, Zambia between 22-25 September 2009. The revised document will be sent for approval by the SADC minister in April 2010.

Figure 2: The working team responsible for updating the SADC Framework for the Management of STIs.

IMPLEMENTATION OF GONOCOCCAL RESISTANCE SURVEILLANCE IN AFRICA

The WHO Collaborating Centre for Sexually Transmitted Diseases (Sydney, Australia), in partnership with the STI Reference Centre (NICD/NHLS) and the National Reference Laboratory for Pathogenic Neisseria (Örebro, Sweden), was awarded a WHO Agreement for Performance of Work to strengthen global surveillance efforts for antimicrobial resistance testing in Africa and Eastern Europe. Professor Lewis visited Zimbabwe at the end of September to initiate planning for a 2010 surveillance exercise in Harare. In May 2009, Professor Lewis was also invited to be a member of a new WHO Expert Advisory Panel for Global Surveillance of Antimicrobial Resistance in *Neisseria gonorrhoeae*.

SCREENING HIV-INFECTED INDIVIDUALS FOR STIs

PEPFAR funds, through the NICD:CDC co-operative agreement, continued to be used in 2009 to fund a screening programme for HIV-infected individuals attending the HIV treatment centre at Helen Joseph Hospital. The work was undertaken in collaboration with Dr. Ian Sanne and Dr. Cindy Firnhaber from the University of the Witwatersrand's Clinical HIV Research Unit. This surveillance initiative aims to determine the burden of sexually transmitted infections (STIs) among asymptomatic HIV-infected patients attending an HIV treatment centre in Johannesburg. *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Mycoplasma genitalium* infections were detected by molecular methods, microscopy was performed for bacterial vaginosis and candidiasis whilst serology was used to detect syphilis and herpes simplex virus type 2 infections. Patients were treated aetiologically for detected STIs and given partner notification slips.

EVALUATION OF SEXUALLY TRANSMITTED DISEASES CLINICAL SERVICES IN GAUTENG PROVINCE: KNOWLEDGE, ATTITUDES AND PRACTICES AMONG HEALTH CARE PROVIDERS AND CAPACITY, RESOURCES AND BARRIERS AMONGST FACILITIES

The STI Reference Centre worked closely with Gauteng Provincial Department of Health and the Division of Sexually Transmitted Disease Prevention (CDC) to complete this piece of health service research during 2009. The NICD/NHLS Principal Investigator was Professor David Lewis and the CDC Principal Investigator was Dr Susan Hariri.

The objective of the study was to evaluate the knowledge, attitudes and practices around sexually transmitted infections, particularly genital herpes, and associated risk of HIV among health care providers in Gauteng Province in order to enhance current HIV prevention efforts. A team of four data collectors undertook facility based interviews among managers and nursing staff, and among private practitioners. The findings were presented to Department of Health staff in the latter part of 2009.

DETECTION OF HIV-SPECIFIC T AND B CELL IMMUNITY IN HIGHLY EXPOSED HIV SERONEGATIVE INDIVIDUALS

Figure 3: Sr Yodwa Mzaidume at one of the Mothusimpilo recruitment areas in Carletonville

The STI Reference Centre undertook a longitudinal study, funded by a Polio Research Foundation grant. This collaborative study involves an NGO working with women at high risk in Carletonville (the Mothusimpilo Project), STI Reference Centre (NICD/NHLS) and Professor Clive Gray's Immunology Laboratory of the AIDS Virus Research Unit (NICD/NHLS). The aims were to follow up a group of HIV seropositive and HIV seronegative WAHR for one year at three monthly intervals to study T cell responses, to identify the receptor profile of natural killer cell subsets that provide protection from HIV infection, and to determine the associations between frequencies of host genes (HLA class I/II, KIR. TSG101, TRIM5αhu and APOBEC3G with immune function and clinical data.

ORANGE FARM 2 STUDY: A COMMUNITY STUDY OF MALE CIRCUMCISION

The randomized controlled trial previously conducted in Orange Farm, South of Johannesburg, demonstrated that male circumcision was able to reduce the risk of HIV acquisition by more than 50% among young men. This result was now been confirmed by two further randomized controlled trials from Kenya and Uganda.

In 2007, adult male circumcision was recognized and recommended by WHO/UNAIDS as an important additional strategy for the prevention of heterosexually acquired HIV infection in men. However, it was not known at that time whether such strategy could be successfully implemented.

The current research project, which commenced in the latter part of 2007, aims to establish a male circumcision intervention in the Orange Farm in order to evaluate its impact on knowledge, attitudes and practice regarding male circumcision, existing means of prevention (behaviour change, condom use, STI treatment-seeking behaviour and HIV VCT), and the spread of HIV, HSV-2, gonorrhoea and chlamydial infection. This project, which was tailored to low income settings, has been a success: community support and participants satisfaction with services are high and uptake is steadily increasing. It demonstrated that adult male circumcision can be rolled-out. By the end of 2009, approximately 15,000 circumcisions were undertaken at the Bophelo Pele Male Circumcision Centre. In addition, the project is now considered a model for the roll-out of comprehensive adult male circumcision services, and could be tailored for implementation in other rural and urban low-income settings in Eastern and Southern Africa.

The project is funded by the French Agence Nationale de Reserches sur la SIDA et les hépatites virales (ARNS). The STI Reference Centre is the main NICD collaborating unit. The study is being led by Professor Bertran Auvert (Professor of Pubic Health, University of Versailles). Within South Africa, Professor David Lewis is a co-Principal Investigator with Dr. Dirk Taljaard (Progressus, South Africa).

The Bophelo Pele project has been visited by the executive director of UNAIDS, Michel Sidébé, and the founder of the Gates foundation, Bill Gates. It has been endorsed by numerous health and developmental agencies such as WHO, The United States Agency for International Development (USAID), the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), Population Services International (PSI), UNAIDS, the Gates Foundation, and South African activists. The Bophelo Pele project will likely be duplicated in Southern Africa in the coming years.

An oral presentation, entitled “Roll-out of adult male circumcision in South Africa: the Orange Farm experience”, was delivered at the 4th South African AIDS conference in April 2009. A scientific paper outlining the operational methods employed in the male circumcision centre was drafted at the end of 2009 and will be submitted for publication in early 2010. Since adult male circumcision scale-up is expected to take effect within the next two years, this paper is likely to be of high interest to a wide audience, from scientists, medical professionals and public health specialists to activists, government officials and international organization officers.

ALEXANDRA MEN'S STI CLINIC

The STI clinic for men in Alexandra, staffed by the STI Reference Centre, had another successful year in 2009. Numbers of weekly attendances has risen since commencement of the clinic in 2005, from about 4-6 attendances per clinic to 12-15 attendances per clinic. Over time, there has been a trend to see young men and the proportion of those under 25 has risen substantially. The clinic has been the site of STI surveillance activities as well as a study looking at the prevalence of HPV infection among men with and without genital warts. An

oral presentation on the work of the men's clinic was made by Charles Ricketts, a nurse at the STI Reference Centre, at the 11th World Congress of the International Union against STIs in Cape Town, held in November 2009.

TEACHING AND TRAINING ACTIVITIES

During 2009, 9 intern medical scientists were trained in various aspects of STI diagnostics. The STI Reference Centre participated in the NICD training rotation for Microbiology Registrars during 2009. Ms. Crystal Jones, an MSc microbiology student from the London School of Hygiene and Tropical Medicine, undertook a 2 months' summer project at the STI Reference Centre. She worked on a human papillomavirus (HPV)-related collaborative project with the Reproductive Health and HIV Research Unit entitled "Comparison between cervicovaginal lavages and vaginal tampons for the detection of human papillomavirus DNA in genital secretions". Mr. Peter Hughes and Dr. Judith Vanderpitte, from the Ugandan Medical Research Council, visited the NICD/NHLS to learn about the STI Reference Centre's *Mycoplasma genitalium* testing capabilities.

Figure 4: Professor David Lewis presenting Dr Godfrey Muriu with the Michael Waugh Prize, for the best HIV dissertation at the 14th RDTC Conference, Tanzania

During 2009, Professor Lewis undertook STI clinical training at a number of external venues, including over 100 African dermato-venereologists on STIs at the 14th Continuing Medical Education and Graduates' Reunion Conference at the Regional Dermatology Training Centre in Moshi in January, 190 South African general practitioners attending the Refresher Course for General Practitioners and Health workers in September (organized by the University of Limpopo, Medunsa Campus), approximately 50 South African doctors at two separate HIV Management Courses run by Right to Care in May and August, and approximately 150 healthcare workers at the 2 day STI Update Course in Harare, Zimbabwe organized by the Zimbabwe Community Health Intervention Project (Zichire).

PRIZES AND AWARDS

Professor David Lewis was presented with an IUSTI Silver Medal for outstanding international contribution to the global fights against STIs by the IUSTI World President, Professor Angelika Stary (Austria) in recognition of organizing the 11th World IUSTI Congress.

Figure 5: Dr Samuel Fayemiwo (University College of Medicine, Ibadan, Nigeria) receiving a bronze medal for his oral presentation from Professor Kit Fairley (Australia)

Dr. Samuel Fayemiwo, from the College of Medicine at Ibadan University in Nigeria, received one of two runner-up prizes and an IUSTI Bronze medal for his oral presentation at the 11th IUSTI World Congress in Cape Town. He presented work undertaken at the STI Reference Centre in 2008 which involved the development of a duplex real-time PCR assay to detected plasmid-mediated high-level resistance to tetracycline and penicillin among *N. gonorrhoeae* isolates.

IUSTI-AFRICA

Professor David Lewis continued to undertake his duties as Director for the Africa Region of the International Union against STIs during 2009. During the year, the membership of IUSTI Africa grew substantially and information on STIs continues to be shared on the continent through the IUSTI-AFRICA newsletter, which is published in both English and French.

Professor David Lewis was the Chairperson for the 11th IUSTI World Congress held in Cape Town (9-12 November, 2009). The Congress comprised 7 plenary lectures, 44 symposium talks in 13 themed symposia, 48 oral presentations and 139 posters. Among the 184 free oral and poster presentations, just over 50% were by Africans.

At the opening ceremony, Dr. Francis Ndowa (WHO, Geneva) discussed the global burden of STIs and Professor David Mabey (London School of Hygiene and Tropical Medicine, UK) gave the opening plenary lecture on "STI/HIV research in Africa: what have we learned?".

The remaining plenary sessions covered rapid diagnostic tests for STIs, prevention of mother to child transmission of HIV, biological drivers of the HIV epidemic, sexual networks and the internet, male circumcision, HIV vaccines and how to use information technology (IT) in novel ways to improve STI/HIV clinical practice. The themed symposia covered STI management of men-who-have-sex-with-men, STI bacterial typing, STI/HIV public health interventions, HIV treatment approaches, condoms, STI/HIV behavioural interventions in Africa, roll out of rapid tests for syphilis screening of pregnant women, updates in STIs and IT, challenges to effective STI syndromic management, HPV vaccination and HPV clinical disease, commercial sex work, STI treatment as a component of HIV prevention, and finally IUSTI global challenges. The conference closed with a lecture by Professor King Holmes from the University of Washington (Seattle, USA) on emerging multi-component STI/HIV prevention strategies.

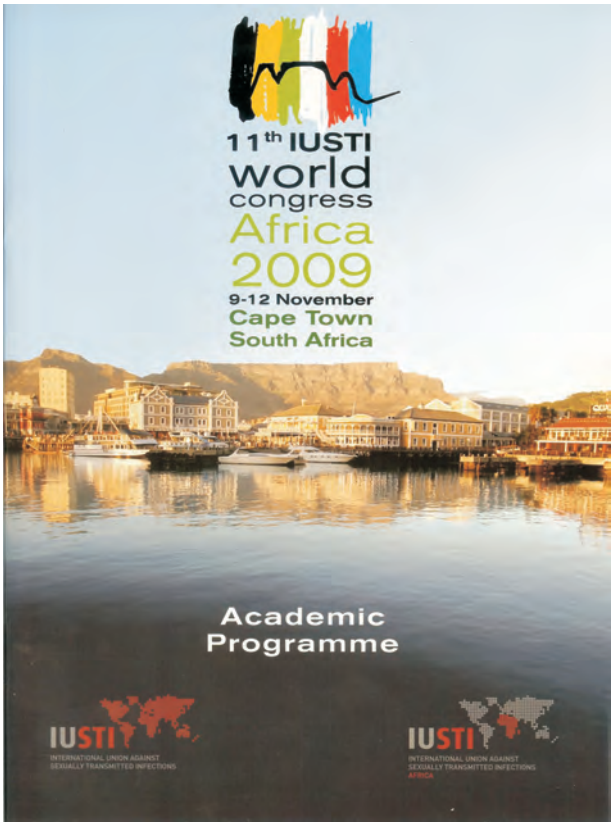


Figure 6: Conference programme for the 11th IUSTI World Congress

COLLABORATIONS

Microbiological Surveillance
Ms Eva Marumo, National Department of Health
Mr Labane Maluleke, Alexandra Health Centre

Gauteng Clinical Surveillance Providers' Survey
Dr D Moloi, Gauteng Provincial Department of Health

Episodic Herpes Trial
Dr Gabriella Paz Bailey, CDC (Atlanta)

Men's Focus Groups
Dr Jami Leichliter and Dr Gabriela Paz Bailey, CDC (Atlanta)

Youth Video and Focus Groups
Dr Mary Kamb and Dr Jennifer Mark, CDC (Atlanta)

STI Providers' Study
Dr Mary Kamb and Dr Susan Hariri, CDC (Atlanta)

STI/HIV Risk Reduction Study
Dr Mary Kamb and Dr Jami Leichliter, CDC (Atlanta)

STI Screening of HIV Patients
Dr Ian Sanne, Dr Cindy Firnhaber, Clinical HIV Research Centre

Male Circumcision Study
Prof Bertran Auvert, Paris-Ile-de-Ouest Medical School, University of Versailles

CAPACITY BUILDING

Ms Precious Magooa, Medical Scientist at the STI Reference Centre, continued working towards her MSc as a student in 2009. Her project is entitled "Detection and molecular epidemiology of ciprofloxacin-resistant *Neisseria gonorrhoeae* using real-time PCR" will be submitted in early 2010. The project is co-supervised by two STI Reference Centre staff, Professor David Lewis and Dr Etienne Müller.

Special Bacterial Pathogens Reference Unit

BACKGROUND

The Unit carries out research and diagnostics for zoonotic diseases such as anthrax, plague, leptospirosis, bartonellosis, tularemia, and botulism. The unit has a specialized biosafety level 3 (BSL-3) laboratory for handling dangerous bacterial pathogens and serves as the WHO networking laboratory for plague and anthrax in Africa. In addition, the Unit carries out plague surveillance by monitoring the susceptible rodent populations in certain areas in order to alert public health authorities to increased human plague risks, if necessary.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

ACCREDITATION AND TRAINING

The Unit retained its ISO15189 accreditation status. During the year the unit was involved in several training activities. These included: (i) training of environmental health officers from the Coega area (Eastern Cape Province) in the dissection and storage of rodent organs for surveillance purposes, (ii) training of intern scientists in the routine diagnosis of special bacterial pathogens e.g. anthrax, *Yersinia pestis*, *Bartonella* spp., *Leptospira* spp. and *Clostridium botulinum*, and (iii) introductory training of microbiology and clinical pathology registrars in BSL3 laboratory principles and activities in the Unit. Staff members were also involved in presenting lectures on the principles of PCR, PCR troubleshooting, PCR calculations and plague surveillance as part of the MSc (Biology and control of African disease vectors) degree presented by the Vector Control Reference Unit of the NICD. Staff attended various technical training courses: ethics filing, flea identification, serological diagnosis of infectious diseases, Biology in Business course, laboratory safety, first aid, and safety, health and environmental representative courses.

RESEARCH

Bartonella species in human and animal populations in Gauteng Province

Bartonella is a genus of fastidious bacteria responsible for a wide range of both symptomatic and asymptomatic infections. Bartonellae are often considered obligate pathogens where infection is concurrent with immunological suppression of the host. The objectives of this study were: to determine the prevalence of *Bartonella* infections in HIV-positive patients presenting for treatment at a Gauteng HIV clinic, to determine the extent of bartonellae affecting the healthy population, to determine the seroprevalence of *Bartonella henselae* and *Bartonella quintana* antibodies in HIV-negative antenatal patient sera taken from various maternity units in Gauteng public hospitals, and to investigate cats, dogs, and rodents in Johannesburg for carriage of bartonellae. A total of 382 HIV-positive patients attending the HIV clinic and 42 clinically healthy volunteers agreed to participate. Three hundred and forty-two residual sera from the national antenatal survey were selected and tested for IgG and IgM antibodies against *Bartonella.* There were 179 dogs, 98 cats and 124 rodents enrolled in this study. HIV-positive patients were found to have 32% IgG and 14% IgM seroprevalence, whereas the healthy volunteers had a lower IgG (19%) and higher IgM seroprevalence than the HIV-positive counterparts. All blood samples were cultured, but only the cat and rodent specimens yielded isolates. These were sequenced for species identification. The cat isolates were 99 and 100% similar to *B.henselae* URBHLIE 9*,* previously isolated from a patient with endocarditis, and the rat isolates were 98 and 99% similar to either RN24BJ (candidatus '*B.thailandensis*') or RN28BJ, previously isolated from rodents in China. The PCR prevalences were 22.5% in HIV-positive patients, 9.5% in clinically healthy volunteers, 23.5% in cats, 9% in dogs, and 25% in rodents. Findings of this study have important implications for HIV-positive patients in South Africa. (Student: AN Trataris)

The detection of *Burkholderia* spp. and pathogenic *Leptospira* spp. in South Africa

The genus *Burkholderia* consists of more than 45 known species, all of which occupy extremely diverse ecological niches ranging from soils to the respiratory tracts of humans and animals. They are also among the most antibiotic-resistant bacteria encountered. *Burkholderia pseudomallei,* for example, is inherently resistant to many antibiotics, requiring a large combination of antibiotics over many months; it is therefore difficult to eliminate in a clinical setting. Leptospirosis is a common zoonosis worldwide and causes a wide spectrum of disease ranging from subclinical infection to a severe syndrome which includes multi-organ failure and consequent high mortality. This study's objectives, therefore, are to determine the prevalence of pathogenic *Burkholderia* and *Leptospira* spp. in the environment at certain sites in South Africa using molecular and culture techniques; to determine the prevalence of these bacteria in the human population in selected areas using molecular, serological and culture techniques; and to determine whether the human populations in these areas are at risk from these organisms (Figure 1). (Student: AN Ruis)

Figure 1: Professor Tim Inglis, University of Western Australia, hunting *Burkholderia pseudomallei* in a Highveld dam.

Leptospirosis pilot surveillance study

Leptospirosis is a well-known cause of febrile illness in many areas of the world, but its importance in South Africa is not known. It has been recognized as a cause of sporadic disease and outbreaks elsewhere in Africa. Recent interest has been stimulated by surveys of humans and rodents in southern Africa, which showed serological evidence of previous exposure, and a small number of human cases have been recently identified locally. There is a lack of awareness of the disease amongst clinicians, and the absence of sensitive and specific laboratory tests suitable for use in routine diagnostic laboratories; in contrast, in the veterinary field, animal infections have been historically well-catered for by the reference microagglutination test (MAT). We are applying two methods of detection: a screening test (ELISA) that demonstrates the prevalence of antibodies in the patients' serum, and PCR, targeting the ribosomal gene sequences specific for pathogenic leptospires. To date, small numbers of samples have been tested, but we hope to improve awareness of this infection amongst clinicians and generate interest in sending clinically-relevant specimens.

Research programme: Immunoprophylaxis and molecular epidemiology of anthrax and the fate of *Bacillus anthracis* in living vectors and the environment of Namibia and South Africa

The frequency of outbreaks of anthrax varies from year to year and from season to season within the endemic regions of southern Africa. Although anthrax is an age-old disease, ecological conditions leading to its various epidemiological manifestations (e.g., sporadic cases vs. Epidemics, and differences in seasonality and species affected in different regions) are still not understood. Until recently the lack of reliable methods to differentiate isolates from various sources was a fundamental hindrance to understanding the epidemiology of anthrax, including the interrelationships between the disease in wild and domestic animals and humans. Programme components are:

Genotyping and epidemiology of *B. anthracis*

B. anthracis appears to be one of the most monomorphic species known; with techniques available in the past, isolates from various sources or geographical locations have been indistinguishable phenotypically and genotypically, with a few rare exceptions. Modern molecular strain typing techniques make it possible to distinguish between outbreak strains, to trace an outbreak strain back to its possible origin, and to track the routes of transmission of an outbreak strain within and between animal populations. It is becoming possible to study genotypic diversity in relation to the spatial and temporal dynamics behind the spread of the disease and possible relationships between genotype and host species.

The objectives of this study are to investigate:

- the correlation between genotypic diversity and (i) spatial and temporal distribution of outbreak strains of *B. anthracis* and (ii) the possible host specificity
- the correlation between anthrax outbreaks in wildlife and livestock in endemic areas in southern Africa
- practices of disposal of the carcasses of animals that have died from anthrax and their role in local epidemics
- the roles of vultures, flies and other insects, and other living vectors, in the epidemiology of anthrax

Fate of *B. anthracis* in natural environmental habitats and its role in the epidemiology of anthrax

Current understanding of the fate of *B. anthracis* in different environments is fragmentary. After the death of an animal the vegetative cells of *B. anthracis* are shed into the environment, mainly in the blood oozing from the orifices of the carcass or in body fluids spilled by scavengers. Sporulation of the released bacilli is induced by oxygen. The spores possess a high tenacity and, in some locations, remain viable for many decades. Where proper disposal of the carcasses is not carried out, newly contaminated regions are permanently created and become the potential sources of new infectious cycles for future years. Contaminated soil is considered the main source of infection causing a new epidemic cycle in nature. One of the important unknowns in this regard is the fate of *B. anthracis* (germination, sporulation, and replication) in natural environmental habitats, such as soil or fecally contaminated sludge. Anthrax spores are notorious for their ability to survive for decades even under harsh environmental conditions. Whether or not free-living amoebae can play a role either as a host, protecting *B.anthracis* from environmental influences, or as a vector in oral infections, has not yet been investigated. Whether or not *B. anthracis* can survive or even replicate within free-living amoebae will be investigated in this project.

The objectives of this study are to investigate:

- the fate of *B. anthracis* (germination, replication, re-sporulation) in natural habitats like soil and water holes under the different seasonal conditions
- the fate of *B. anthracis* within free-living amoebae
- the fate of *B. anthracis* in flies, after feeding on anthrax carcasses

QUALITY ASSESSMENT ACTIVITIES

A plague EQA programme is produced for WHO-AFRO as part of a larger WHO-NICD collaboration and is sent out three times a year to 18 laboratories in plague-endemic countries in Africa, India and Madagascar.

PLAGUE SURVEILLANCE

Plague in South Africa is currently in a quiescent phase, but experience from recent outbreaks in other countries (Algeria, Libya, Tanzania and Uganda) has shown that plague activity can resume unexpectedly after decades of quiescence, and cause major disruption to medical and social/economic systems and, especially, to tourism. Plague surveillance is in the process of being re-launched by the National Department of Health and the Special Bacterial Pathogens Reference Unit of the NICD. Training courses will be conducted by the staff of the SBPR Unit, for the environmental officers from historic plague-endemic regions. The Gauteng Province regions will be trained first. These courses will cover the practical and theory side of plague surveillance. The first course will commence in February, 2010. All plague surveillance samples (rodents, fleas, dog sera) will be sent to the SBPR Unit for processing.

ANTHRAX AND PLAGUE REPOSITORY

The national anthrax repository is housed within the Unit's BSL3 facility. The unit currently keeps all the historical and new *B. anthracis* isolates from the Kruger National Park as well as other isolates from the rest of South Africa and neighboring countries.

NATIONAL GUIDELINES

Staff members worked on national guideline committees, in conjunction with National Department of Health on plague (*Yersinia pestis*) and anthrax (*Bacillus anthracis*). The National Plague guidelines were printed and disseminated during 2009 (Figure 2).

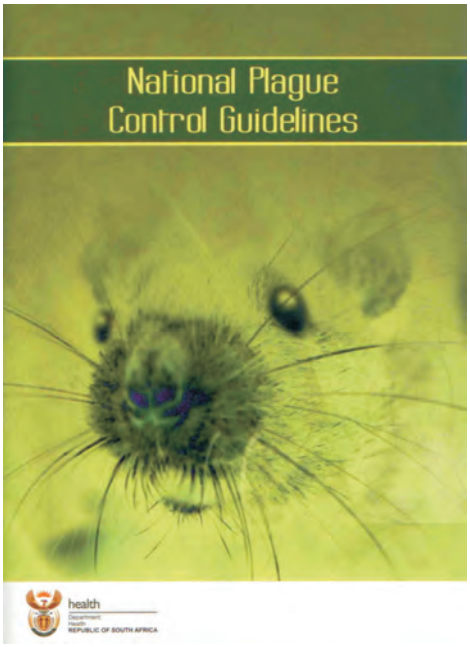


Figure 2: Plague guidelines, 2009

COLLABORATIONS

University of Hohenheim, Stuttgart, Germany (Dr W Beyer): Immunoprophylaxis and molecular epidemiology of anthrax and the fate of *Bacillus anthracis* in living vectors and the environment of Namibia and South Africa.

Protechnik Laboratory, Pretoria (Dr T Woods): Rapid dipstick detection methods for plague, anthrax and botulism.

University of Pretoria (Dr H van Heerden): Molecular epidemiology of *B. anthracis*.

CAPACITY BUILDING

Registered students

MSc (Med)	AN Trataris and AN Ruis (University of the Witwatersrand)
MSc	Ayesha Hassim (University of Pretoria)

Graduated students

BSc (Hons)	AN Ruis (University of the Witwatersrand)

Vector Control Reference Unit

BACKGROUND

Malaria is the major vector-borne disease in Africa, killing close to 1 million people annually, most of them children under five. In South Africa, malaria transmission is confined to the low-lying border areas in the northeast of the country where over 12,000 cases were reported in 2006. The Vector Control Reference Unit (VCRU) focuses on the anopheline mosquitoes responsible for malaria transmission. The Unit houses a unique collection of live mosquito colonies of the three most important vector species in Africa, *Anopheles gambiae*, *An. arabiensis* and *An. funestus*, plus the minor vector *An. merus*, and the non-vector species of the *An. gambiae* complex, *An. quadriannulatus*. Three colonies of *An. funestus* from Mozambique and Angola continue to provide us with a unique resource for research into insecticide resistance in this important malaria vector. This places the VCRU in a unique position to offer collaboration with international institutions investigating similar problems and to play a role in influencing policy decisions on vector control strategies in the region. In addition, the VCRU houses the largest museum collection of African arthropods of medical importance in Africa, the third largest such collection in the world.

RESEARCH ACTIVITIES

INSECTICIDE RESISTANCE

Anopheles funestus

Molecular research into pyrethroid resistance in *Anopheles funestus* continues to be a major focus of the VCRU. Metabolically mediated resistance is based on the up-regulation of P450 monooxygenase enzymes and a specific CYP6 gene, CYP6P9, is responsible for pyrethroid resistance in *An. funestus.* Quantitative Real-Time PCR shows that this gene is highly over expressed in the egg and adult stages of the resistant strain relative to the susceptible strain whilst the larval stages show almost no difference in expression between strains. This gene is genetically linked to a major locus associated with pyrethroid resistance and current research using micro-array analysis is being undertaken in collaboration with colleagues at the Liverpool School of Tropical Medicine, UK, to further investigate this gene and its specific functions. A positional cloning approach was used to identify the major genes conferring pyrethroid resistance in *An. funestus*. A quantitative trait locus (QTL) named rp1 explains 87% of the genetic variance in pyrethroid susceptibility in two families from reciprocal crosses between susceptible and resistant strains. Two additional QTLs of minor effect, rp2 and rp3, were also detected. A 120-kb BAC clone spanning the rp1 QTL was sequenced and identified 14 protein-coding genes and one putative pseudogene. Ten of the 14 genes encoded cytochrome P450s, and expression analysis indicated that four of these P450s were differentially expressed between susceptible and resistant strains. Furthermore, two of these genes, CYP6P9 and CYP6P4, which are 25 and 51 times over-expressed in resistant females respectively, are tandemly duplicated in the BAC clone as well as in laboratory and field samples, suggesting that P450 gene duplication could contribute to pyrethroid resistance in *An. funestus* (Figure1). Single nucleotide polymorphisms (SNPs) were identified within CYP6P9 and CYP6P4, and genotyping of the progeny of the genetic crosses revealed that these SNPs are valid resistance markers in the laboratory strains. This serves as proof of principle that a DNA-based diagnostic test could be designed to trace metabolic resistance in field populations. This will be a major advance for insecticide resistance management in malaria vectors, which requires the early detection of resistance alleles.

We investigated the insecticide susceptibility of *An. funestus* from Malawi during 2008. This revealed the presence of a new member of the *An. funestus* group. Indoor resting collections of mosquitoes from Malawi were initially identified as *An. funestus* by morphology, but failed to have this confirmed by the species-specific PCR assay. Sequence analysis of the ITS2 region identified variations within the *An. funestus* specific primer binding site and revealed a 4.5% sequence variation compared with *An. funestus*. Cytogenetic analysis of the polytene chromosome banding arrangements showed that the specimens were homosequential with *An. funestus*, with fixed inverted arrangements of the 3a, 3b and 5a inversions commonly polymorphic in *An. funestus*. The chromosomes of hybrid females showed levels of asynapsis typical of inter-species crosses (Figure 2). These molecular and cytogenetic observations support the hypothesis that this Malawi population is a new species and has been provisionally designated "*An. Funestus*-like".

Anopheles arabiensis

A population of *Anopheles arabiensis,* a major malaria vector in South Africa, was collected during 2005 from inside sprayed houses in Mamfene, northern KwaZulu-Natal, South Africa, using window exit traps. None of these specimens (n = 300 females) was found to be infected with *Plasmodium falciparum.* Insecticide susceptibility assays on 23 day old F1 progeny using WHO susceptibility kits revealed 100% susceptibility to bendiocarb. Resistance to deltamethrin (95.91%) was suspected, while resistance to permethrin (78.05%)

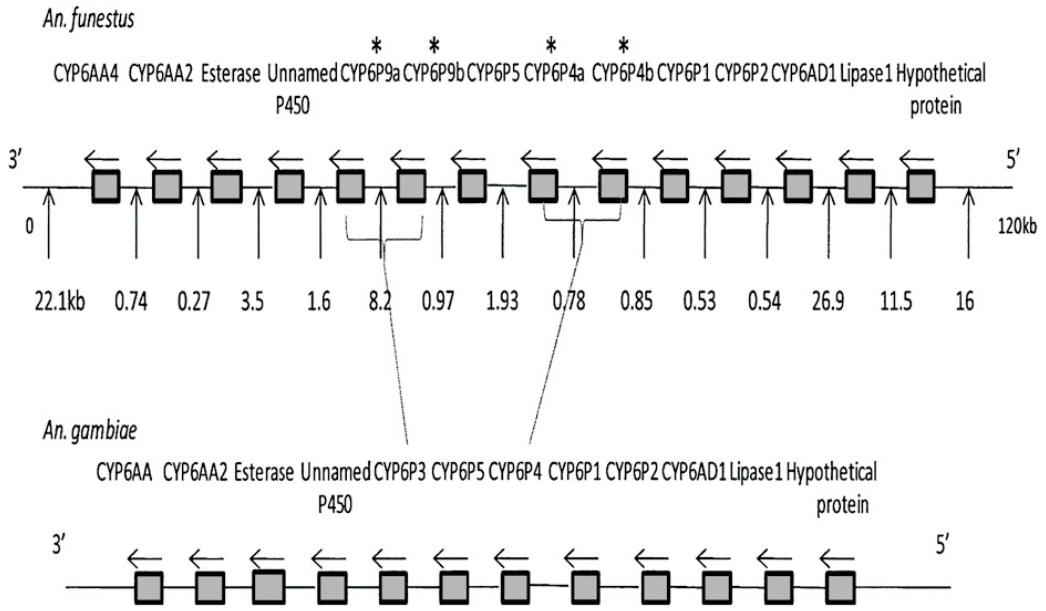


Figure 1: Schematic representation of gene organization of BAC 29F01 containing the rp1 QTL in *An. funestus* (top) compared with the organization of orthologous genes in *An. gambiae* (bottom). (Vertical arrows) Intergenic regions; (horizontal arrows) the 5939 orientation of each gene; (asterisks) duplicated genes.

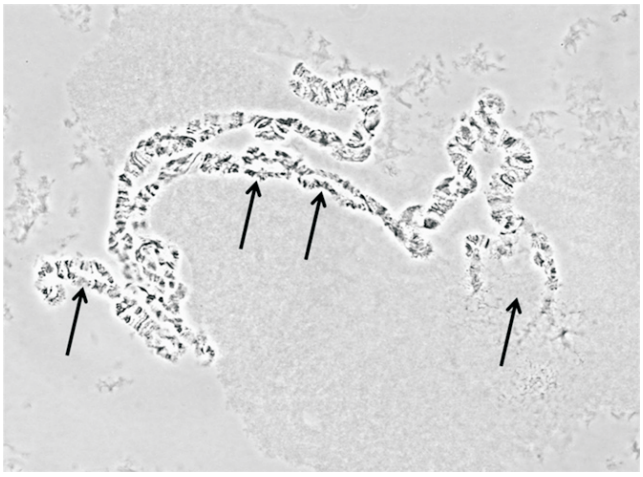


Figure 2: Asynapsis in hybrid polytene chromosomes indicated by arrows

was confirmed. The knockdown resistance (*kdr*) genotype was not found in the surviving mosquitoes. Biochemical analysis using enzyme assays showed elevated levels of monooxygenase that correlated with the permethrin bioassay data. While elevated levels of non-specific esterase were found in some families (11/12 for α- and 6/12 for β-esterases), the data did not show any correlation with the permethrin bioassay data. Analysis of permethrin and bendiocarb tolerant lines, selected in the laboratory to characterize biochemical resistance profiles, showed increased levels of non-specific esterase and monooxygenase activity in the case of the permethrin-selected cohorts, and elevated glutathione S-transferases and general esterases in that of the bendiocarb-selected line. Synergist assays, using piperonyl butoxide, confirmed the involvement of monooxygenase and glutathione S-transferase in pyrethroid and bendiocarb resistance. This study underlines the importance of routine surveillance for insecticide susceptibility in wild anopheline populations.

Anopheles gambiae

Entomological studies conducted in West Africa revealed the extent of insecticide resistance in a major African malaria vector *Anopheles gambiae* sensu stricto. Pyrethroid insecticide resistance in *An. gambiae* s.s. is a major concern to malaria vector control programmes. Resistance is mainly due to target-site insensitivity arising from a single point mutation, often referred to as knockdown resistance (*kdr*). Metabolic based resistance mechanisms have also been implicated in pyrethroid resistance in East Africa and are currently being investigated in West Africa. We reported on the co-occurrence of both resistance mechanisms in a population of *An. gambiae* s.s. from Nigeria. Molecular analysis of resistant and susceptible strains of *An. gambiae* s.s. from the same geographical area revealed >50% of the West African *kdr* mutation in resistant mosquitoes and <3% in susceptible mosquitoes. Resistant mosquitoes exposed to piperonyl butoxide prior to permethrin exposure showed a significant increase in mortality compared to the unsynergized sample. Biochemical assays showed an increased level of monooxygenase activity in resistant mosquitoes. Microarray analysis using the *An. gambiae* detox-chip showed that five resistance associated genes are over-expressed in the resistant strain compared with the susceptible strain (Figure 3). Two of these, *CPLC8* and *CPLC#*, are cuticular genes not implicated in pyrethroid metabolism in *An. gambiae* s.s., and could constitute a novel set of candidate genes that warrant further investigation.

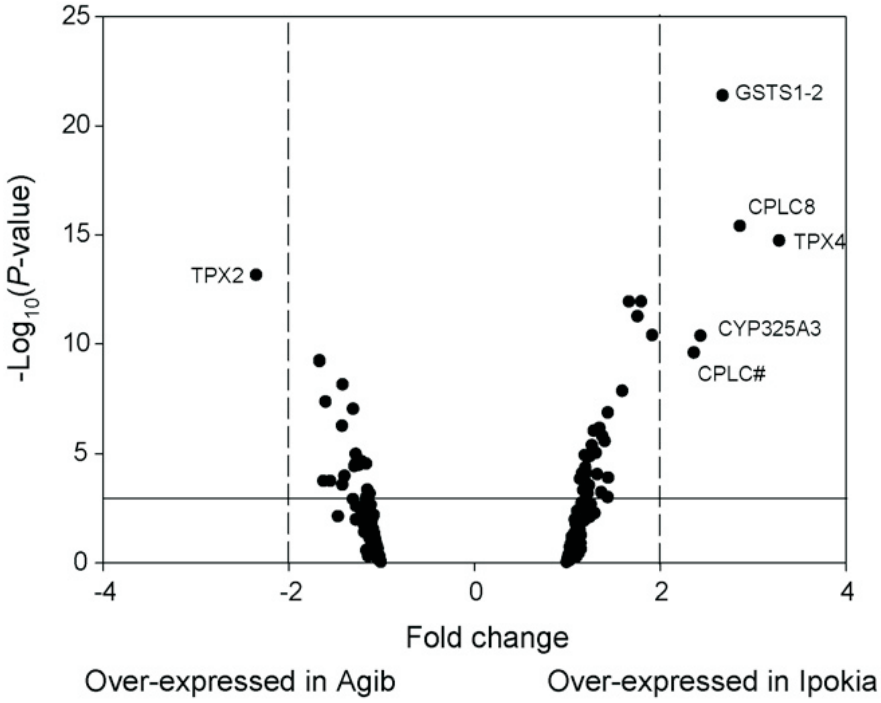


Figure 3: Microarray analysis comparing constitutive detoxification gene expression of the pyrethroid-resistant with the susceptible colony.

In addition, we conducted a study at three localities in Guinea Conakry to obtain data on malaria vector species composition and insecticide susceptibility status (Figure 4). A total of 497 mosquitoes were collected resting indoors and were morphologically identified as belonging to the *An. gambiae* complex. The majority of these were *An. gambiae s.s.* (99.6%), but a small percentage (0.4%) were identified as *An. arabiensis*. Thirty-four *An. funestus s.s.* were also collected. The molecular S form of *An. gambiae s.s.* was predominant over the M form in Siguiri (95%) and Boffa (97.4%), whereas at Mt Nimba the M form was more abundant (61.4%) than the S form (38.1%). One hybrid M/S specimen was recorded from Mt Nimba. Siguiri populations showed high levels of resistance to DDT, dieldrin and bendiocarb. *Anopheles gambiae* from Boffa were largely susceptible to the insecticides tested. At Mt Nimba, resistance to DDT and bendiocarb was detected. Biochemical enzyme analysis showed that an altered acetylcholinesterase is operating in the field at low levels. The frequency of the 1014F *kdr* allele in the *An. gambiae* S form was 0.24 at Siguiri and 0.14 at Mt Nimba. A single RR specimen was found in the M form. The heterogeneity in species composition and resistance profiles between sites requires that vector control interventions be tailored to each site based on the data collected from ongoing monitoring and surveillance. During this study we developed a new technique that is able to accurately identify the target site mutation, *kdr*, associated with DDT and pyrethroid resistance. Pyrosequencing provides a fast and accurate method for detection of this mutation.

Figure 4: Map of Guinea Conakry, West Africa. Study sites are circled.

MALARIA VECTOR CONTROL AND TRANSMISSION DYNAMICS

Mpumalanga Province, South Africa, is a low malaria transmission area that is subject to malaria epidemics. SaTScan methodology was used by the malaria control programme to detect local malaria clusters to assist disease control planning. The third season for case cluster identification overlapped with the first season of implementing an outbreak identification and response system in the area. SaTScan™ software using the Kulldorf method of retrospective space-time permutation and the Bernoulli purely spatial model was used to identify malaria clusters using definitively confirmed individual cases in seven towns over three malaria seasons. Following passive case reporting at health facilities during the 2002 to 2005 seasons, active case detection was carried out in the communities. This assisted with determining the probable source of infection. The distribution and statistical significance of the clusters were explored by means of Monte Carlo replication of data sets under the null hypothesis with replications greater than 999 to ensure adequate power for defining clusters. SaTScan detected five space-clusters and two space-time clusters during the study period. There was strong concordance between recognized local clustering of cases and outbreak declaration in specific towns. Both Albertsnek and Thambokulu reported malaria outbreaks in the same season as space-time clusters (Figure 5). This synergy may allow mutual validation of the two systems in confirming outbreaks demanding additional resources and cluster identification at local level to better target resources. Exploring the clustering of cases assisted with the planning of public health activities, including mobilizing health workers and resources. Where appropriate additional indoor residual spraying, focal larviciding and health promotion activities were all also carried out.

House-resting *Anopheles* mosquitoes are targeted for vector control interventions; however, without proper species identification, the importance of these *Anopheles* to malaria transmission is unknown. *Anopheles longipalpis*, a non-vector species, has been found in significant numbers resting indoors in houses in southern Zambia, potentially impacting on the utilization of scarce resources for vector control. The identification of *An. longipalpis* is currently based on classical morphology using minor characteristics in the adult stage and major ones in the larval stage. The close similarity to the major malaria vector *An. funestus* led to investigations into the development of a molecular assay for identification of *An. longipalpis*. Molecular analysis of *An. longipalpis* from South Africa and Zambia revealed marked differences in size and nucleotide sequence in the second internal transcribed spacer (ITS2) region of ribosomal DNA between these two populations, leading to the conclusion that more than one species was being analysed. Phylogenetic analysis showed the Zambian samples aligned with *An. funestus*, *An. vaneedeni* and *An. parensis*, whereas the South African sample aligned with *An. leesoni*, a species that is considered to be more closely related to the Asian *An. minimus* subgroup than to the African *An. funestus* subgroup. Species-specific primers were designed to be used in a multiplex PCR assay to distinguish between these two cryptic species and members of the *An. funestus* subgroup for which there is already a multiplex PCR assay.

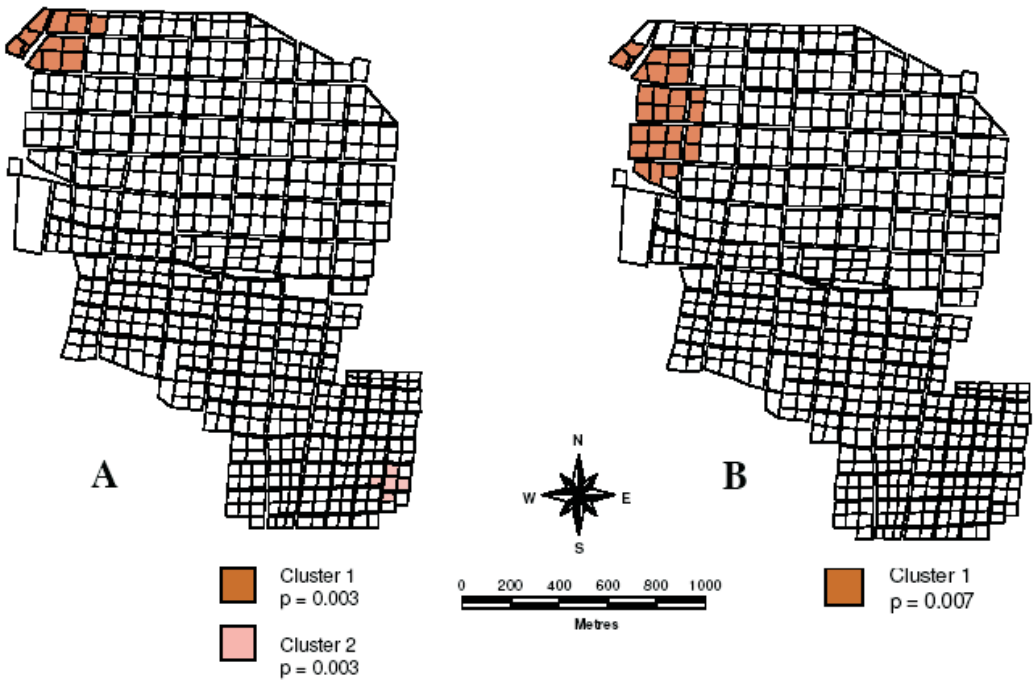


Figure 5: Spatial malaria case clusters, Albertsnek town. A.2002/2003 2004/2005 seasons B. 2004/2005 season.

NOVEL MOSQUITO CONTROL METHODS

Investigations into the effect of entomopathogenic fungi on insecticide resistant vector colonies housed at the VCRU are underway. Mosquitoes are exposed to two different fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, commonly found in soils worldwide, and analysed for survival and fungal infection. Insecticide-resistant *Anopheles* mosquitoes remain susceptible to infection with the fungus *B. bassiana*. Four different mosquito strains with high resistance levels against pyrethroids, organochlorines, or carbamates were equally susceptible to *B. bassiana* infection as their baseline counterparts, showing significantly reduced mosquito survival (Figure 6). Moreover, fungal infection reduced the expression of resistance to the key public health insecticides permethrin and dichloro-diphenyltrichloroethane (DDT). Mosquitoes pre-infected with *B. bassiana* or *M. anisopliae* showed a significant increase in mortality after insecticide exposure compared with uninfected control mosquitoes. Our results show a high potential utility of fungal bio-pesticides for complementing existing vector control measures and provide products for use in resistance management strategies.

VECTOR/PARASITE INTERACTIONS

A new facility for culturing malaria parasites and infecting mosquitoes has been established (Figure 7). This facility will be the first of its kind in South Africa to study the complex interaction between *Plasmodium falciparum* parasites and the mosquito vectors. Optimization of the techniques is currently underway and the first mosquitoes will be infected with this deadly parasite in 2010.

In addition, mosquitoes have been infected with *Plasmodium berghei*. Lab-reared mouse strains (C57/Black and Balb/c) were infected with *P. berghei* parasites by injecting 6-8 week old mice interperitoneally with infected blood. Four to five days post infection adult female mosquitoes were allowed to feed on anaesthetised infected mice. Fourteen to eighteen days after the initial blood meal, the female mosquitoes' salivary glands were dissected out and viewed under light microscopy. With the establishment of a complete parasite lifecycle between the host and vector, the parasites remain infective and cause appropriate host immune responses which provides a useful laboratory model.

DIAGNOSTIC AND OTHER SERVICES

The VCRU provides a service for the identification of medically important arthropods for entomologists, medical practitioners and health inspectors. Malaria vector mosquitoes were routinely identified by PCR for the Mpumalanga Province Malaria Control Programme. ELISA and PCR tests were carried out on the *An. gambiae* complex specimens from Mozambique, Ghana, Mali, Congo and South Africa, for species identification and to detect the presence of *Plasmodium falciparum* sporozoites. Advice and expertise is provided to the Department of Health both at national and provincial levels, with participation on the National Malaria Advisory Group.

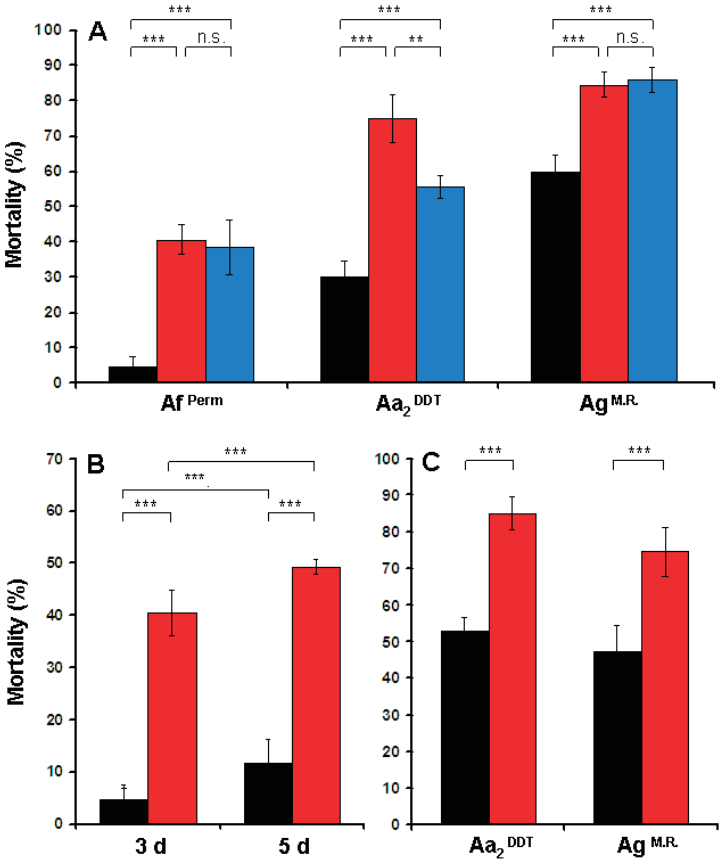


Figure 6: Effect of fungus infection on mosquito insecticide-resistance levels. (*A*) percentage mortality of uninfected (black), *Beauveria*-infected (red), and *Metarhizium*-infected (blue) mosquitoes after exposure to permethrin 3 days after fungal infection. The tested mosquito strains *A. funestus* (AfPerm), *A. arabiensis* from Sudan (Aa2 DDT), and *A. gambiae s.s.* (AgMR) exhibit high baseline levels of permethrin resistance. (*B*) Effect of permethrin exposure on *Beauveria*-infected (red) and uninfected (black) permethrin resistant *A. funestus* (AfPerm) mosquitoes at 3 days (*Left*) and 5 days (*Right*) after fungal infection. (*C*) Percentage mortality of *Beauveria*-infected (red) and uninfected (black) DDT-resistant mosquitoes [*A. arabiensis* (Aa2DDT) and *A. gambiae s.s.* (AgMR)].

Figure 7: New *P. falciparum* host/parasite facility.

INTERNATIONAL RESEARCH COLLABORATORS

Prof D Boakye, Noguchi Memorial Research Institute, Accra, Ghana
Dr M Bagayoko, WHO/AFRO, Gabon
Dr L Manga, WHO/AFRO, Brazzaville, Congo
Prof J Hemingway, Director, Liverpool School of Tropical Medicine, UK
Dr H Ranson, Liverpool School of Tropical Medicine, UK
Prof W Takken, University of Wageningen, Netherlands
Dr B Knols, University of Wageningen, Netherlands
Dr M Thomas, Penn State University, USA
Prof D Norris, Johns Hopkins University, USA
Prof K Louis, IMBB, Crete
Dr T S Awolola, Nigerian Institute of Medical Research, Lagos, Nigeria
Dr H T Masendu, University of Zimbabwe, Harare, Zimbabwe
Dr M Balkew, Addis Ababa University, Ethiopia
Mr B Ntomwa, Ministry of Health and Social Services, Namibia

CAPACITY BUILDING

POSTGRADUATE TRAINING

Postdoctoral fellowships, Masters and Doctoral students from the following countries were trained during 2009:

South Africa: M Dhoogra PhD, R Christian PhD, B Spillings PhD, M Lo PhD, L Nardini PhD, M Stradi MSc, R Norton MSc, O Wood MSc, G Kloke MSc, M Kaiser MSc
Botswana: K Waniwa MSc
Cameroon: J Mouatcho PhD, S Vezenegho PhD
Democratic Republic of Congo: C Kikankie MSc
Ghana: J Stiles-Ocran PhD
Korea: Dr K Choi, Postdoctoral fellow
Sudan: H Abdalla PhD
Zimbabwe: G Munhenga PhD

SHORT-COURSE TRAINING

Ad hoc training in morphology, insecticide resistance, PCR techniques, biochemical analysis and ELISA was given to students from the DRC and Mozambique.

Virology Division

AIDS Virus Research

BACKGROUND

The AIDS Virus Research Unit comprises 3 laboratories, namely the Virology Laboratory headed by Prof Lynn Morris who also serves as the head of the Unit, the Cell Biology Laboratory headed by Prof Caroline Tiemessen and the Immunology Laboratory headed by Prof Clive Gray. The Unit is the largest at the NICD and conducts research projects primarily on the virology and immunology of HIV. It also serves important functions for drug resistance surveillance for the National Department of Health as well as validated end-point assays for HIV vaccine trials. The Unit raises a large amount of external funding for the various projects with numerous collaborators and serves an important role in training and capacity building, including running workshops. The total number of staff and students in the AIDS Unit in 2009 was 48.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

RESEARCH PROJECTS

Virology Laboratory (Prof Lynn Morris)

Broad HIV-1 neutralization mediated by plasma antibodies against the gp41 membrane proximal external region (MPER)

We identified three cross-neutralizing plasma samples with high titer anti-MPER peptide binding antibodies from among 156 HIV-1 chronically infected individuals. In order to establish if these antibodies were directly responsible for the observed neutralization breadth, we used MPER-coated magnetic beads to deplete plasmas of these specific antibodies. Depletion of anti-MPER antibodies from BB34, CAP206 and SAC21 resulted in a 77%, 68% and 46% decrease respectively in the number of viruses neutralized. Antibodies eluted from the beads showed similar neutralization profiles as the original plasmas, with potencies comparable to the known anti-MPER MAbs, 4E10, 2F5 and Z13e1. The anti-MPER neutralizing antibodies in BB34 were present in the IgG3 subclass-enriched fraction. Alanine scanning of the MPER showed that the antibodies from these three plasmas had specificities distinct from the known MAbs, requiring one to three crucial residues at positions 670, 673 and 674. These data demonstrate the existence of MPER-specific cross-neutralizing antibodies in plasma although the ability to elicit such potent anti-viral antibodies during natural infection appears to be rare. Nevertheless, the identification of three novel antibody specificities within the MPER supports its further study as a promising target for vaccine design. *This work was published in the Journal of Virology in 2009 by Dr Elin Gray and colleagues.*

Sensitivity of HIV-1 subtype C viruses to Griffithsin, Cyanovirin-N and Scytovirin: potential HIV-1 microbicides

Griffithsin (GRFT), Cyanovirin-N (CV-N) and Scytovirin (SVN) are lectin proteins that inhibit HIV-1 infection by binding to multiple mannose-rich glycans on the HIV-1 envelope glycoprotein. Here we show that these lectins neutralize subtype C primary virus isolates in addition to envelope-pseudotyped viruses obtained from plasma and cervical vaginal lavages. Among 15 subtype C pseudoviruses, the median IC_{50} values were 0.4, 1.8 and 20.1 nM for GRFT, CV-N and SVN, respectively, similar to what was found for subtype B and A. Analysis of the envelope sequences suggested that concomitant lack of glycans at positions 234 and 295 resulted in natural resistance to these compounds, which was confirmed by site-directed mutagenesis. Furthermore, the binding sites for these lectins overlapped that of the 2G12 monoclonal antibody epitope, which is generally absent on subtype C envelopes. Thus, despite subtype-specific mannosylated glycan patterns, this data support further research on these lectins as potential microbicides in the context of HIV-1 subtype C infection. *This work forms part of Kabamba Alexandre's PhD thesis and has been accepted for publication in Virology.*

Risk factors for viremia and drug resistance among HIV-1 patients on antiretroviral treatment a cross-sectional study in Soweto, South Africa

Between March-September 2009, we conducted a cross-sectional study among patients who had been on antiretroviral treatment for ≥12 months. Genotypic drug resistance testing was performed on individuals with a viral load >400 RNA copies/ml. Multiple logistic regression analysis was used to assess associations.

Of 998 subjects, 75% were women with a median age of 41. Most (64%) had been on treatment for >3 years. The prevalence of viremia was 14% (n=139); 12% (102/883) on first-line (i.e. non-nucleoside reverse transcriptase inhibitor based regimen) and 33% (37/115) on second-line (i.e. protease inhibitor based regimen) treatment. Of viremic patients, 78% had drug resistance mutations. For NNRTIs, NRTIs and PIs the prevalence of mutations was 80%, 64% and 1% among first-line and 54%, 29% and 9% among second-line failures respectively. All sequences clustered with HIV-1 subtype C. M184V/I, K103N and V106A/M were the most common resistance mutations.

Significant risk factors associated with viremia on first-line included concurrent tuberculosis treatment (OR 6.4, 2.2-18.8, p<0.01) and a recent history of poor adherence (OR 2.7, 1.3-5.6, p=0.01). Among second-line failures, attending a public clinic (OR 4.6, 1.8-11.3, p<0.01) and not having a refrigerator at home (OR 6.7,

1.2-37.5, p=0.03) were risk factors for virological failure. Thus, risk factors for viral failure were line-regimen dependent. Second-line antiretroviral therapy recipients had a higher rate of viremia, albeit with infrequent PI resistance mutations. Measures to maintain effective virologic suppression should include increased adherence counseling, attention to concomitant tuberculosis treatment and heat-stable formulations of second-line regimens. *This work forms part of Ziad El-Khatib's PhD thesis and has been accepted for publication in AIDS.*

Viremia, resuppression, and time to resistance in HIV-1 subtype C during first-line antiretroviral therapy in South Africa

We performed a retrospective analysis of a cohort receiving zidovudine, lamivudine, and either efavirenz or nevirapine with HIV-1 RNA monitoring every 6 months. We assessed viremia (HIV RNA >1000 copies/mL after initial HIV RNA response) and resuppression (HIV RNA <400 copies/mL after viremia). Genotypic resistance testing was performed using stored plasma on a subset of patients at first detection of viremia and subsequently among patients with persistent viremia.

Between 2002 and 2006, 3727 patients initiated cART (median CD4, 147 cells/ul). Of 1007 patients who developed viremia, 815 had subsequent HIV RNA assays, and 331 (41%) of these resuppressed without regimen switch. At identification of viremia, 45 (66%) of 68 patients had HIV-1 drug resistance, 42 (62%) had nonnucleoside reverse-transcriptase inhibitor (NNRTI)resistance, 25 (37%) had M184V/I, and 4 (6%) had multinucleoside analogue drug mutations. By 12 months of persistent viremia among a subset of 14 patients with resistance testing to 12 months, 11 (78%) had nonnucleoside reverse-transcriptase inhibitor (NNRTI)resistance, 8 (57%) had M184V/I, and 2 (14%) had multi-nucleoside analogue drug mutations. Resistance was associated with a reduced probability of resuppression; however, 50% of patients with NNRTI resistance resuppressed while receiving an NNRTI.

Our findings support maximizing first-line use while minimizing risk of significant cross-resistance by implementing intensive adherence support and repeat HIV RNA testing 36 months after detecting viremia, with regimen switch only if viremia persists. *This work was done in collaboration with Dr Chris Hoffman and Aurum Institute for Health Research and is published in Clinical Infectious Diseases 2009.*

Immunology Laboratory (Prof Clive Gray)

An Early Differentiated Memory Phenotype of Gag-Specific CD4+ T-cells During Primary HIV infection Associates with Viral Control

This work investigates the association of CD4+ T-cell activation and memory maturation during primary HIV infection with viral control during the first year of infection. We hypothesize that an early accumulation of central/transitional memory CD4 cells associates with succeeding viral control. We examined a cohort of 15 subtype C HIV-infected subjects identified during primary HIV-1 infection (PHI). Polychromatic flow cytometry was used to simultaneously analyze activation and memory maturation profiles in total and antigen-specific CD4+ T cells. Isolated PBMC from each subject were stimulated for 6h with Gag, CMV (pp65) peptide pools and labeled with a cocktail of monoclonal antibodies to CD3, CD4, CD8, CD45RO, CD27, HLA-DR, CD38, Ki-67, IFN and IL2. Our results show that HIV Gag-specific CD4+ T-cells are characterized by high level of activation that is not observed on total memory or non-HIV specific cells. Assessing the maturation profile of activated CD4+ T-cells revealed that the frequency of central/transitional memory (CD27+CD45RO+) Gag-specific CD4+ T cells were significantly higher than total memory CD4+ cells ($p = 0.0392$) at 3 months post infection. The frequency of activated Gag-specific CD4+ T-cells presenting a central/transitional phenotype negatively correlated with viral load ($r = -0.65$, $p = 0.021$) at 12 months. Conversely, activated Gag-specific effector memory (CD27-CD45RO+) CD4+ T-cells at 3 months positively correlated with viral load ($r = 0.63$, $p = 0.028$) at 12 months. Our results also indicate that activation and maturation seem to be independent phenomenon-representing a dichotomous relationship between activation and memory lineage. These data show that activated and less differentiated Gag-specific memory CD4+ T-cells during PHI may play a key role in control of vireamia during the first year of infection.

Profiles of CD4+ T Cell Memory Maturation and Activation that Associate with Viral Control

Despite the ability of the host to mount strong and broad immune responses during acute and early HIV infection, most infected individuals do not control virus and are unable to maintain durable functional T cell immunity. We aimed to identify levels of T cell activation and memory maturation that associates with viral control. In early (n=15) or chronic (n=18) HIV infected cohorts, we analyzed the relationship between CD4+ T cell activation (CD38, HLADR, Ki67), memory maturation (CD45RO, CD27) and functional integrity of total and antigen-specific T cells. We additionally defined T cell memory maturation and senescence by co-expression of CD127, PD1 and CD57 and ability to phosphorylate stat-5 in response to gamma-C cytokine triggering. The lineage of T cell memory, from early, late and effector populations in CD4+ T cells showed a commensurate decline in the ability to phosphorylate stat-5 in response to exogenous IL-2, IL-7 and IL-15. Terminally differentiated cells were characterized by elevated CD57, decreased CD127 expression and hyporesponsiveness to exogenous cytokines. Memory maturation in HIV-specific CD4+ T cells at 3 months post infection was characterized as early-differentiated cells (CD45RO+CD27+). These were deemed as cytokine-responsive subsets and they inversely correlated with concurrent viral load ($r = -0.87$, $p<0.0001$). Moreover, Frequencies of highly activated total and Gag-specific $CD4^+$ T cells (CD38+HLA-DR+Ki67+) significantly correlated with viraemia at 3 months post infection

(r=0.79, p=0.0007 and r=0.58, p=0.035, respectively). Importantly, all stages of memory CD4+ T cell maturation were characterized by similar proportions of activated cells, and no association was found between activation and maturation levels of Gag-specific CD4+ T cells. Our data suggest that T cell activation, likely driven by virus, and memory maturation are independent phenomena and that the maintenance of early-differentiated memory CD4 cells during Primary HIV infection (PHI) is important for ensuing viral control.

Rapid evolution of HIV specific T cell responses within the first six months of subtype C infection

Deciphering immune events during acute HIV-1 infection is critical for understanding the course of disease. While most studies have examined HIV-1 specific T cell responses in chronic infection, consideration of how these responses evolve within the first six months of infection is critical for developing effective vaccine strategies. We have characterized HIV-1 specific T cell responses in 53 acutely HIV-1 infected clade-C subjects and followed them longitudinally from 3 to 24 weeks post infection. Comprehensive T cell recognition patterns were determined using 432 overlapping subtype C peptides using an IFN- Elispot assay. The hierarchy of epitopic regions targeted at 3-5 weeks of infection were to Nef (71%), Gag and Pol (41%), Env and Vif (29%), Vpr (24%), Rev (18%), Vpu (12%) and Tat (6%). There was also an overall increase in the frequency of individuals recognizing Nef, Pol and Gag regions from 3-5 weeks to 6-months. A closer examination showed a dynamic gain and loss of responses in Gag, Pol, Env and Nef equating with shifting peptide responses. T cell responses that were lost in the first 3-15 weeks of infection were in Gag (11%), Pol (20%), Env (27%) and Nef (42%). Increased responses were also found in Gag (43%), Nef (39%), Pol (11%) and Env (7%) and completely new responses were similarly found in Gag (23%), Pol (33%), Nef (28%) and Env (16%). Interestingly, most T cell responses lost by 6 months post infection were subdominant at acute infection. These data show the fluidity of T cell responses during acute infection, where loss of responses most likely coincides with viral mutations and escape. However, the emergent early new responses indicate the rapidity of T cell evolution and the likely unpredictable nature of T cell recognition patterns during acute to early infection.

Measurement of Killer-Cell Immunoglobulin-like Receptors (KIR) Genotypes in South Africans using Real-time PCR

The primary function of natural killer (NK) cells is the detection and destruction of malignant and virus-infected cells. NK cells express cell surface killer-cell immunoglobulin-like receptors (KIRs) that interact with human leukocyte antigen (HLA) class I ligands. The resulting receptor-ligand interaction partially regulates the cytolytic activity of the NK cell. The KIR receptors are divided into either inhibitory or activating types. To date 16 KIR genes have been identified, which include 8 inhibitory (2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2 and 3DL3), 6 activating (2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1) and two pseudogenes (2DP1 and 3DP1). The objective of this study was to identify a quick and easy method to determine KIR allele and genotype frequencies in a South African cohort using DNA extracted from whole blood. Whole blood samples were collected from 81 women recruited from Carletonville, South Africa. Leukocytes were purified from 1ml EDTA blood using a red-cell lysis method and DNA was extracted using a crude lysate method. A published real-time PCR based assay using syber-green chemistry and melting curve analysis for the detection of 16 KIR genes was modified using published sequence-specific primers. Melting curve analysis was able to separate the positive control amplicon from KIR amplicons. After KIR gene assignment using real-time PCR melt curve analysis, we found the most common KIR alleles besides the framework KIR genes (2DL4, 3L2, 3DL3 and 3DP1) were the 2DL1, 3DL1, 2DS4 and 2DP1 (>90%) and the least common KIR allele was 3DS1 (12.3%). Other KIR allele frequencies ranged from 23.5% to 74.1%. Nineteen different KIR genotypes were identified and compared to those in an online database (www.allelefrequencies.net). The AA1 genotype containing 2DL1, 2DL3, 2DS4, 3DL1 and the framework genes was the most frequently observed (27.4%). The next most common genotype was Bx21 (with all genes present except 2DS1, 2DS3 and 3DS1) at 13.6%. These genotypes were also found to be the most common in other published data from South African Xhosa and San populations. Our study also identified four genotypes (Bx6, Bx46, Bx70 and Bx118) that have not been previously been reported in South African populations. We optimised a quick and easy real-time KIR PCR method using known positive control samples. The assay was applied to a small South African sample set, in which the most frequent KIR genotypes correlated with known KIR frequencies found in the Xhosa and San populations.

Cell Biology Laboratory (Prof Caroline Tiemessen)

Understanding protective immunity to HIV using maternal-infant HIV transmission as a model

Studying the immune responses and other characteristics (viral, host) of both HIV-1 infected mothers and their infants allows us the ability to address questions of protective immunity (why some infants succumb to infection and others not), of disease progression in the HIV-1 infected mothers, and of acute infection in infants who become infected. Using this model we have described two innate immune correlates of protective immunity to HIV-1: (i) elevated production of the chemokine CCL3/MIP-1α and higher gene copy number of *CCL3L*, and (ii) unusual HIV-peptide specific natural killer (NK) cells. Innate immune responses are first to act upon initial encounter with HIV-1 and again on subsequent re-encounter/s; these early events have to be evaded by HIV-1 to successfully establish infection in the host. Our findings and very recent findings from others in mouse models show that NK cells, as major cells of the innate immune response, display features of adaptive immune responses (memory of prior exposure and remarkable specificity), drawing into question the

classical textbook definitions of these immune compartments. These are novel findings that provide an exciting new avenue for further study. To this end we are conducting various functional studies to elucidate the mechanisms responsible for the development of the HIV-specific NK cell responses, and host genes of Killer Immunoglobulin Receptors (or KIR, mostly expressed on NK cells) and HLA class I B and C molecules are being studied for associations with maternal-infant HIV-1 transmission, HIV-1 disease progression and with the development of the unusual HIV-specific NK cells.

Following from our findings of chemokines and protective immunity, we have also proposed that the role of CC chemokines may extend beyond merely blocking of HIV-1 entry via CCR5 to which they and HIV-1 bind, in that their "adjuvant" role in instructing the development of adaptive immune responses may be a key component of their protective effects. In this vein, another group has shown that gene copy number of *CCL3L* influences non-HIV cell-mediated immune responses, while our work has shown an influence on HIV-1 Gag-specific T-cell responses. To begin to understand the complexities of chemokine-chemokine receptor interactions and protective immunity, we are characterizing in detail variation in human *CCL3* and *CCL3L* genes (the two functional genes that code for CCL3/MIP1-α) as well as *CCR5* genes in both Caucasian and Black South African individuals, and assessing functionality of gene promoter regions and studying immune functional capacity to a variety of immune stimuli in relation to host gene variation. We have also cloned human *CCL3* and *CCL3L* genes for potential use as genetic adjuvants for use with HIV-1 vaccines, and are also utilizing these chemokine molecules, among other potential antiviral molecules, in an approach that utilizes bacteriophage-mediated plasmid transduction of endogenous mucosal Lactobacilli, contributing to the field of microbicides.

Work going forward builds collectively on our findings (chemokines and NK cells) and will address compound effects of the "protective" elements that we have identified by studying relationships between these immune responses and relevant host genes in HIV-1 infected individuals and in individuals who are exposed to HIV-1 but remain uninfected (adults or infants).

HIGHLIGHTS AND ACHIEVEMENTS

Virology Laboratory

Dr Penny Moore was invited by the Global HIV Vaccine Enterprise (the Enterprise) to join the Young and Early Career Investigators Committee (YECIC). One of the key challenges facing the HIV Vaccine field is the urgent need to attract, retain and nurture the careers of both young and early career investigators. During the year she attended two meetings in New York and participated in one teleconference.

The Virology Lab, AIDS Unit, was awarded World Health Organization (WHO) accreditation as a Regional HIV Drug Resistance Laboratory for the year 2008-2009.

Dr Penny Moore was awarded the CHAVI Young Investigator-of-the-month March 2009. This monthly award recognizes outstanding young investigators who have made significant contributions to the Center for HIV/AIDS Vaccine Immunology (CHAVI) mission and who have been nominated by their mentors for their exceptional leadership and valuable research.

Prof Morris and Dr Elin Gray were successful in their application for a grant from the South African HIV/AIDS Research and Innovation Platform (SHARP). Funding will be provided over 3 years for the project *"A new approach to isolating neutralizing monoclonal antibodies to the HIV-1 subtype C envelope glycoprotein"*

Prof Lynn Morris spent 3 months at Duke University, Durham, NC, USA, where she worked in Dr. Barton Haynes' laboratory at the Duke Human Vaccine Institute. She isolated monoclonal antibodies from the CAPRISA cohort. Her trip to Duke was fully sponsored by the Columbia University-southern African Fogarty AIDS Training Program.

Prof Morris attended an aids2031 meeting at the PATH office located in Washington DC on 22 and 23 June 2009. The purpose of this meeting was to review and validate the Working Group's final report and to gain consensus on the recommendations the group will put forward to the broader aids2031 initiative's final report *An Agenda for the Future*.

Immunology Laboratory

Prof Clive Gray was invited to give a guest presentation on T cell immunity and HIV at the 4th SA AIDS Conference, 31 March to 3 April 2009 at the ICC in Durban.

Prof Clive Gray was invited to give a seminar at the University of Pennsylvania entitled: A Dichotomous Relationship between CD4+ T cell Activation and Memory Maturation with Viral Control in Subtype C HIV-1 Infection on 2 July 2009.

Prof Clive Gray was invited to give a lecture on T cell response assays at the CAVD/GHRC workshop on 14 July 2009 in Cape Town.

Prof Clive Gray, Catherine Riou and Deirdre Kruger attended the IAS Conference in Cape Town from 19-22 July 2009. During the IAS Conference Clive Gray co-chaired a Poster Discussion Session on HLA Pathogen Interactions and chaired a Symposium on Hyper-immune Activation and HIV.

Prof Clive Gray was invited to chair an oral abstract session entitled "Early Events in Transmission and Infection" at the 2009 AIDS Vaccine Conference, 19 to 22 October 2009, Paris, France.

Pholo Maenetjie received a New Investigators Award and gave an oral presentation entitled: "An Early Differentiated Memory Phenotype of Gag-Specific

CD4+ T-cells During Primary HIV infection Associates with Viral Control at 12 months" at the 2009 AIDS Vaccine Conference, 19 to 22 October 2009, Paris, France.

The 2nd Infectious Diseases in Africa Symposium and the 3rd African Flow Cytometry Workshop was held from 13-15 and 13 to 20 November 2009, respectively. The Symposium was held in Parktown and the Workshop was held at the AIDS Unit, Immunology Lab. 12 Full Scholarships to attend both the Symposium and Workshop and 10 Partial Scholarships to attend the Workshop only were awarded to students who submitted high quality abstracts. A total of 45 applications were received. The symposium and workshop was successfully funded by the Office of AIDS Research, NIH, WHO (African AIDS Vaccine Programme), National Institute for Allergy and Infectious Disease (NIAID, R13 training grant), Becton Dickinson Biosciences. The events were co-organized by Clive Gray and Guido Ferrari, Duke University.

Prof Clive Gray was invited to the South African Immunology Society (SAIS) Conference in Cape Town from 9 to 11 December 2009 in order to Chair a Plenary Session on HIV infection; gave a plenary talk: "Dissecting T cell responsiveness to C-cytokines in HIV-infected subjects"**;** and was elected to serve on the re-established committee of the SAIS in the education portfolio.

Cell Biology Laboratory

A Cutting Edge paper published in Journal of Immunology on our unexpected findings of HIV-1 peptide specific responses elicited by Natural Killer cells and their association with protection against maternal-infant HIV-1 transmission, received coverage in Nature Immunology vol 10, number 6, June 2009 as a "Research Highlight".

Prof Tiemessen was invited to co-chair the session "Control of HIV by Cellular immunity" at the 5th International AIDs Society (IAS) Conference on Pathogenesis, treatment and Prevention, Cape Town, July 19-22, 2009.

Anabela Picton (PhD student, 2nd year, Witwatersrand University) was among 10 applicants selected from 50 to attend a practical course in imaging and microscopy entitled "Innovative Microscopy to understand Fundamental Biology" held at the CSIR, Pretoria May 16-22, 2009.

Anabela Picton (PhD student, 2nd year, Witwatersrand University) was awarded a scholarship to attend the AIDS Vaccine 2009 Conference, Paris, France.

Prof Tiemessen was invited to speak at the 2nd Infectious Diseases in Africa Symposium held in Johannesburg, 13-15th November, 2009. Talk title: "Innate immunity: Emerging concepts in HIV-1 infection and exposure". She also chaired the session entitled: "Role of adaptive immunity".

Dr. Leonard Damelin attended the 5th Annual Grand Challenges in Global Health Meeting, October 18-21, 2009, Ngurdoto Mountain Lodge, Arusha, Tanzania.

COLLABORATIONS

We have numerous fruitful collaborations with leading laboratories and funded networks:

Aurum Health
Canada-Africa Prevention Trials Network (CAPT)
Centre for HIV AIDS Vaccine Immunology (CHAVI)
Centre of AIDS Programme of Research in South Africa (CAPRISA).
The Collaboration for AIDS Vaccine Discovery (CAVD)
Columbia University, New York
Council for Scientific and Industrial Research (CSIR)
Duke University, NC, USA
HIV Vaccine Trials Network (HVTN)
Human Pathogenesis Program, University of KwaZulu-Natal
Institute of Infectious Disease and Molecular Medicine, University of Cape Town,
International AIDS Vaccine Initiative (IAVI)
Perinatal HIV Research Unit
The Scripps Research Institute, USA
SHARP (South African HIV/AIDS Research and Innovation Platform)/LIFElab/DST
South African National Bioinformatics Institute (SANBI)
Stanford University, USA
Torrey Pines Institute for Molecular Studies, USA
Uganda Virus Research Institute, Entebbe, Uganda
University of Alabama
University of Medicine and Dentistry of New Jersey, USA
University of Montreal
University of Oxford, UK
University of Pennsylvania
Vaccine Research Centre, NIH
Virax Holdings, Kew, Australia

CAPACITY BUILDING

Currently registered as PhD students in the AIDS Unit are: Netty Malatsi, Heather Hong, Shayne Loubser, Mandla Mlotshwa, Pholo Maenetje, Kabamba Alexandré, Hazel Mufhandu, Walter Campos and Ziad El-Khatib.

David Sacks and Kurt Wibmer are registered for their Masters degree.

Johanna Ledwaba was invited by the WHO Regional Office for Africa to attend a training workshop to pilot the HIVDR laboratory training package in Dakar, Senegal, 5 8 May 2009.

Kabamba (Alex) Alexandre was awarded a scholarship to attend the 5th International AIDS Society (IAS) Conference on HIV Pathogenesis to be held in Cape Town, 19 to 22 July 2009.

Maphuti Madiga was invited to attend a CAVD/GHRC Training Workshop on "Cryopreservation of Biological

Material for Biological and Immunological assays in HIV-Vaccine-Related Research" held at the Stellenbosch University Tygerberg Campus in Cape Town, 13-17 July 2009. The workshop is part of a series of training workshops arranged within the framework of HIV vaccine program supported by the Bill and Melinda Gates Foundation.

Dr. Adriaan Basson spent just over three weeks in the Laboratory at the Health Protection Agency (HPA) in Colindale, London, UK. The purpose of his visit to HPA was to facilitate an active collaboration between HPA and NICD and to train in the phenotypic HIV drug resistance assay using the vectors and assay being used in the HPA laboratory.

Maphuti Madiga attended a workshop in Paris which preceded the AIDS Vaccine 2009 Conference on 18 and 19 October 2009. The invitation from OCTAVE (Online Callaborative Training for AIDS Vaccine Evaluation), was entitled "Standardized Measurement of Neutralizing Antibodies to Advance HIV Vaccine Development: An OCTAVE Workshop for early Stage Investigators".

Electron Microscope Unit

BACKGROUND

The maintenance of effective disease surveillance and the successful diagnosis of infectious diseases, require the application of salient, interactive methodologies, with both modern and classic methods being integral to the rapid and accurate identification of new and known pathogens. The most obvious advantages of electron microscopy include simplicity and rapidity. A negatively-stained sample can provide a relatively quick, morphological identification and differential diagnosis of a number of varied viral agents potentially contained within the sample. Additionally, infectious particles are not necessarily required, so viral agents that cannot be cultured (either because they do not grow in tissue culture, or grow only under specialised conditions, or are no longer viable on arrival at the laboratory as the samples were not correctly transported under optimal conditions), can still be visualised and identified to family level. This can be of great value in determining the causes of certain outbreaks, particularly food-borne/viral gastroenteric outbreaks.

Electron microscopy (EM) of thin sections of tissue biopsies and fluid samples, also offers insight into new and emerging protozoan and fungal parasites, particularly microsporidia. These are increasingly observed as opportunistic infections of immuno-compromised patients, although they are known to parasitise immuno-competent hosts too. Comprehensive identification to species level is most successfully achieved using EM, as molecular techniques limit identification to previously recorded, known parasites.

Both microbiological and virological units of the NICD provide a variety of samples for diagnostic screening or research-oriented processing, and although the EM Unit has been established as a support service primarily for the NICD, assistance is also available to the wider NHLS community.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

In addition to routine diagnostic screening for the Special Pathogens, Viral Isolation and Viral Gastroenteritis Units, the EM Unit has been primarily involved in two research areas: a microsporidial study in the Vector Control Reference Unit (VCRU), and a bacteriophage research programme in the Cell Biology Laboratory of the AIDS Virus Research Unit.

Microsporidial infestations can cause serious mortality in insectaries. Preliminary investigations into larval deaths in an *Anopheles* colony of the VCRU in 2008, indicated the presence of a microsporidian parasite (Fig 1). In order to prevent further loss of mosquito colonies to pathogen infection, the VCRU initiated an insectary maintenance programme that included meticulous egg-washing procedures, baited cockroach traps and intensified laboratory hygiene. As part of a student project that ascertained the efficacy of these measures, as well as identifying and sourcing all potential pathogens, the EM Unit screened over 250 mosquito larvae, 25 cockroach intestinal tracts, bulked cockroach faeces, guinea-pig (blood-meal source) urine and faecal samples, and bulked adult female mosquito excrement. Although no microsporidia were found in any of the mosquito colonies or food sources, spores were present in a few of the cockroach faeces (Figure 1). As no other life cycle stages were observed in the cockroach gut, it has not proved possible to assign the spores taxonomically. However, as no binucleate spores have been found as yet, it is possible that they do not belong to the same genus as those that caused the 2008 mortalities. So although other insects may be a potential source of microsporidial infection, transmission from insectary cockroaches to mosquitoes does not appear to have occurred.

The use of bacteriophages to deliver expression plasmids encoding selected cytokines, to Lactobacilli that constitute normal vaginal flora, ultimately to determine whether the subsequent expression of these cytokines affects HIV infectivity, is one of the ground-breaking research hypotheses being tested in the Cell Biology Laboratory. When bacteriophages are in the lytic phase, it is possible to determine their presence using conventional plaque assays. If lysogenic stages alone are present, EM offers a simple method of confirmation of induction. Different methods of phage induction can result in different phage expression, but using mitomycin C, three morphotypes, which correspond to different taxonomic families, have been distinguished: Siphoviridae (icosahedral heads; long, flexible, non-contractile tails; Fig 2a-e)); Myoviridae (icosahedral heads; short, thick, contractile tails; Fig 2f-i); and Tectiviridae (isometric heads; tail-less, although it is possible that tails may be induced by alternative stressors; Fig 2j,k). Given that bacteriophages are specific to their hosts, it is not surprising that the phages induced from two different species of *Lactobacillus*, *L. crispatus* and *L. jensenii*, are distinctive: Siphoviridae and Myoviridae from the clinical isolates of *L. crispatus*; tail-less phages from *L. jensenii*.

EM is frequently the initial diagnostic standard for cases of neuronal ceroid-lipofuscinosis, which comprise a group of genetically inherited, neurodegenerative diseases involving lysosomal storage disorders. Before biochemical and genetic tests are conducted,

ultrastructural studies of the white blood cells need to be performed, to confirm the presence and nature of lysosomal storage material (fingerprint bodies/curvilinear profiles/granular osmiophilic deposits). The visualization of both osmiophilic deposits (Fig 3a,b) and fingerprint-like bodies (Fig 3c,d) in a blood sample from an infant, assisted the pathologist with diagnosis.

In accordance with the NHLS directive, and despite the tenuous connection between daily recording of fridge temperatures and the production of a micrograph illustrating a hitherto unknown pathogen, the EM Unit has embarked on the process of SANAS accreditation.

CAPACITY BUILDING

Registrars were trained on basic principles and techniques of transmission electron microscopy.

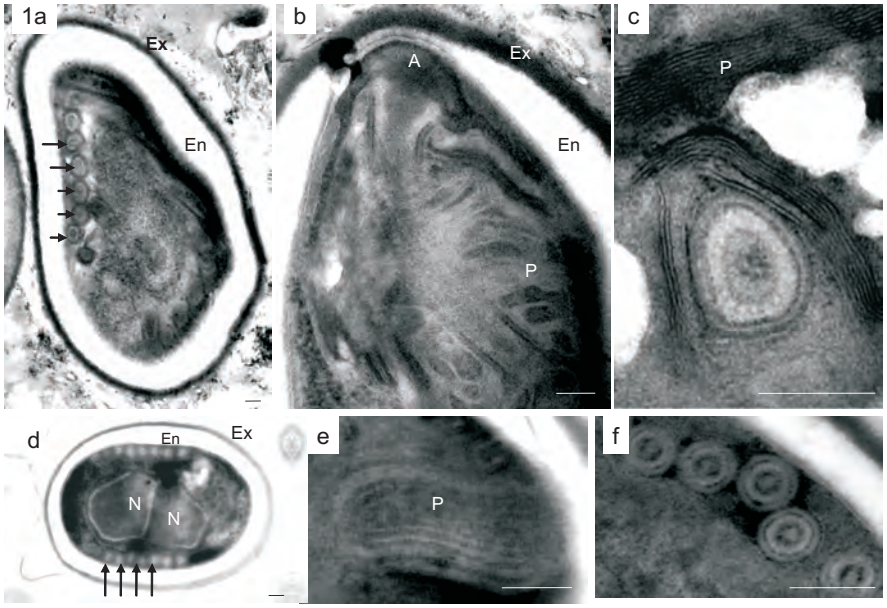


Figure 1: Ultrastructure of microsporidial spores. 1a-c from cockroach faeces, 1d-f from mosquito larvae. (A = anchoring cap; En = endospore wall layer; Ex = exospore wall layer; N = nucleus; P = polaroplast; arrows indicate gyres of transversely sectioned polar filament). All scale bars = 100nm.

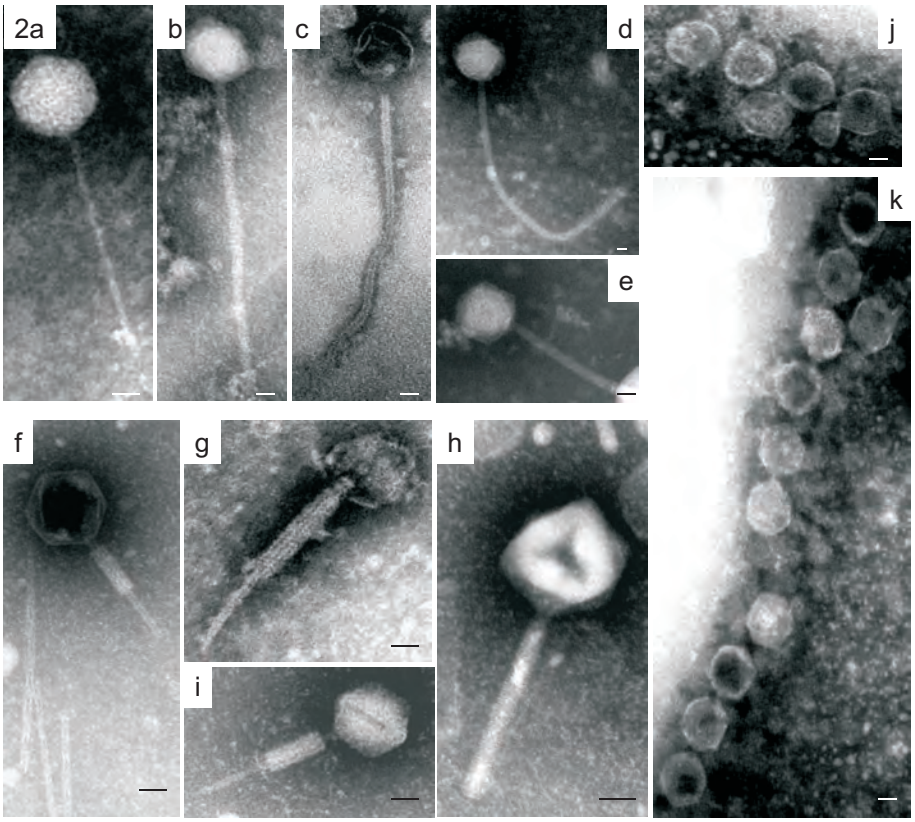


Figure 2: Bacteriophages induced from lactobacilli. 2a-e: Siphoviridae; 2f-i: Myoviridae; 2j,k: tailless phages (Tectiviridae). All scale bars = 15nm.

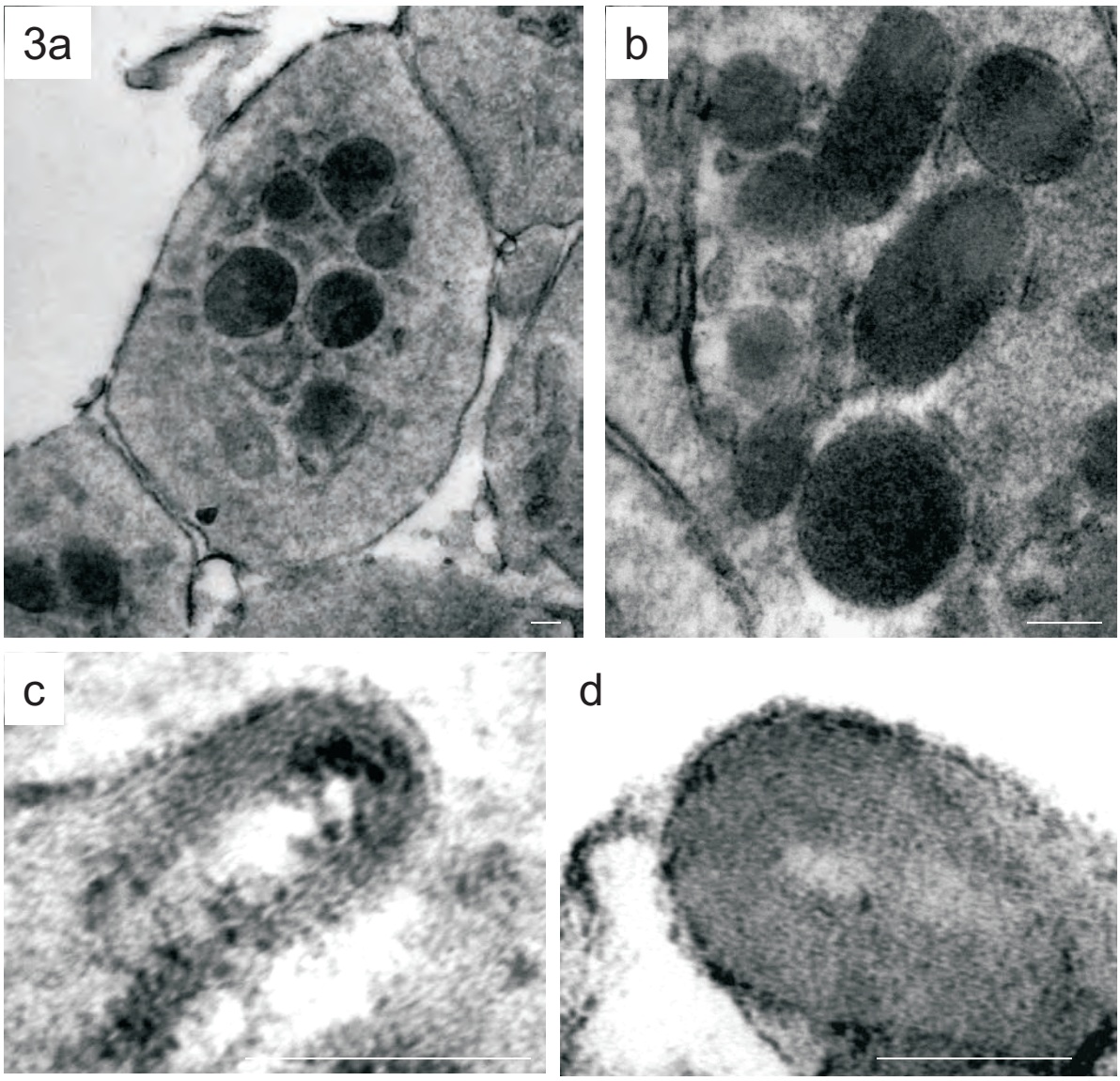


Figure 3: Ultrastructural indicators of neuronal ceroid-lipofuschinosis found in white blood cells. 3a,b: granular osmiophilic deposits within lysosomes. 3c,d: delicate, lamellar structure of fingerprint bodies. All scale bars = 1µm.

Respiratory Virus Unit

BACKGROUND

The Respiratory virus unit focuses on research, surveillance and training on respiratory viruses associated with Influenza like illness and severe acute respiratory infections (SARI). The unit is affiliated to the Respiratory and Zoonotic virus programme at the Department of Medical Virology University of Pretoria and is actively involved in research on Respiratory and zoonotic viruses as well as training of postgraduate science and medical students on projects associated with pneumonia and zoonotic diseases. It also houses **the National Influenza Centre (NIC)** which is a World Health Organization (WHO) reference laboratory and is earmarked as a WHO Regional Reference Laboratory for Influenza playing a key role in the support for the establishment of influenza laboratory and surveillance capacity in the Southern African Development Community (SADC). The unit is tasked with pandemic preparedness and response in Southern Africa. This Unit regularly characterises influenza viruses obtained from a network of sentinel physicians" and hospitals that participate in the Severe Acute Respiratory Infection (SARI) surveillance network. The RVU performs laboratory surveillance, molecular diagnosis and typing of Influenza Viruses and investigates annual Influenza molecular epidemiology, genetic drift and drug resistance. This information is shared with the WHO annually to assist in making decisions regarding the composition of the annual influenza vaccines for the southern hemisphere. Monitoring of the resistance to influenza antiviral drugs is conducted annually to determine the effectiveness of treatment with these drugs.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

INFLUENZA AND SARI SURVEILLANCE

The Unit has played a leading role in the country to detect and deal with the novel H1N1 Influenza A pandemic in 2009 and offered technical support and confirmatory testing to other Influenza Laboratories in the Region. Three main active surveillance programmes exist that are run in collaboration with the epidemiology unit from which the unit obtains specimens and provide diagnostic testing and subtyping:

- "The Viral watch programme" - a total of 246 doctors and primary health care nurses have been recruited across the country to participate in the influenza like Illness (ILI) sentinel surveillance programme from all 9 provinces. This programme focuses on mild infections seen mainly by general practitioners as well as a few pediatricians and primary health care clinics across the country.
- "Enhanced viral watch programme" - this programme was established following the emergence of the pandemic influenza A H1N1 with the aim of expanding the "viral watch" to include hospitalized patients. This includes 11 hospitals covering all 9 provinces and focuses on hospitalized patients with Severe Acute Respiratory-tract Infection (SARI) across the country.
- SARI surveillance programme - in 2009 the SARI surveillance programme was established which monitors cases of more severe disease in hospitalized patients. Detailed epidemiologic data are collected on all patients. This programme currently includes 4 hospitals in 3 provinces covering urban and rural sites.

Apart from these active surveillance sites the RVU also offers routine testing for respiratory virus disease to clinicians across the country. This service has become particularly active after the emergence of pandemic influenza A H1N1 and served as the initial diagnostic service for the country and later as diagnostic facility for severe cases and confirmation of fatal cases.

As part of the surveillance programmes and routine testing described above the unit received 9797 specimens in total in 2009, of which 9357 were appropriate for testing for Influenza. A total of 2346 Influenza A specimens were identified of which 1096 were H3N2, 1206 were pandemic H1N1, and only 6 were seasonal H1N1 while 140 Influenza B specimens were identified (Figure 1). Sporadic cases of influenza B were first detected in South Africa in week 12 and 13 but disappeared again until week 23 when case numbers again began to increase peaking in week 32 and continuing through week 36. In total there were 112 influenza B cases. The first influenza A H3N2 isolates were detected in week 18 and peaked in week 24 with 205 isolates in this week, trailing through to week 35 with a total of 1052 isolates. Only 8 cases of seasonal H1N1 were detected this year from week 22-29.

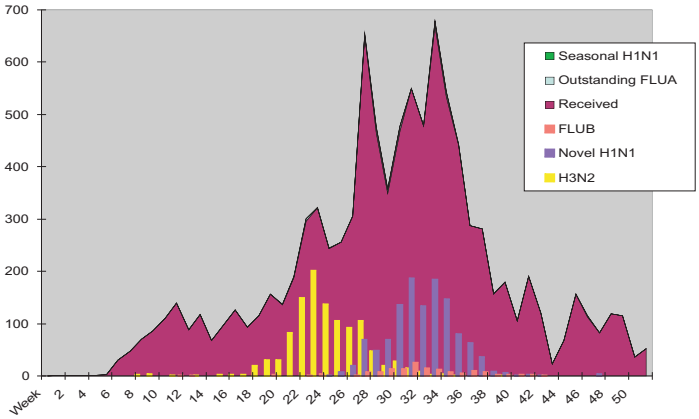


Figure 1: All surveillance and diagnostic specimens tested and subtyped for Influenza in 2009 by the National Influenza Centre

The H1N1 Influenza A Pandemic

The first cases of the pandemic influenza A H1N1 virus were detected in week 25 with a number of imported cases in travelers. The first cases of community circulation were identified in week 27 and transmission carried on until week 43. Most cases were detected in week 32, but the decrease in detected cases in the following weeks may be due to a change in the testing strategy from all cases to only severe or high risk cases. In total, 1206 cases were detected up to week 52 through the active surveillance programmes and routine diagnostic services at NICD. A clear distinction can be seen between seasonal influenza (H3N2) outbreak and the pandemic influenza A H1N1 outbreak resulting in 2 epidemic peaks this year (Figure 1). A national database of all laboratory confirmed cases of pandemic influenza A H1N1 identified by private and public sector laboratories in South Africa has been maintained by the epidemiology unit at NICD since the start of the outbreak, recording 12640 cases and 93 deaths in total nation wide. This is however likely an underestimation of the true number of cases. From mid August mainly moderate to severe cases were identified, due to a call to medical practitioners to stop testing mild cases. Apart from the local South African strains, the unit has also tested Influenza A specimens from various Southern African countries including Namibia, Angola, Botswana, Zimbabwe, Swaziland, Lesotho, Mozambique, Malawi, Zambia, Seychelles and Madagascar and identified the first novel H1N1 cases for these countries and provided training to them to establish their own testing capabilities. Weekly updates of Influenza cases have been reported to the WHO.

Strain characterization

Resistance testing:

The neuraminidase proteins of 105 pandemic H1N1 specimens have been sequenced and investigated for Oseltamivir resistance and pathogenic markers. None of the specimens had the H275Y mutation on the NA gene that is associated with resistance. Investigation of the HA D222G and PB2 E627K pathogenic markers identified in patients that died in the Northern hemisphere using sequence analysis indicated the absence of these mutations in SARI patients in South Africa. These mutations do not seem to be specifically associated with influenza disease severity in SA.

Molecular epidemiology of the H1N1 outbreak

Molecular epidemiological investigations of the South African H1N1 pandemic Influenza strains are underway. A selection of specimens from patients infected during the 2009 pandemic have been sequenced and compared with isolates from the rest of the world using Bayesian phylogenetic analysis. This included patients identified at the early stages of the outbreak, consisting mostly of travellers or their direct contact (imported strains) in June and July and patients identified after community transmission had been established (August) through to the end of the outbreak (October). The presence of 3 separate clusters could be demonstrated in the country based on the HA genes (Figure 2). Limited drift were identified over time away from the initial strain that was isolated when it first appeared in June in South Africa. Strains similar to the original introductory strains circulated for several months, although recent strains differ from original importations which may suggest positive selection and drift. The early strains that were isolated were submitted to the WHO for comparison to the vaccine strain and differed by 0.8% while by September strains had 1.3% amino acid differences to the vaccine strain. Specimens from patients with mild and severe disease had also been compared as well as geographically distinct sites across the country (Figures 3 and 4). Similar strains were identified in patients with mild and severe disease although a few unique strains were identified in patients with SARI. Specific AA changes also occur in certain SARI strains which warrant further investigation to determine any disease association.

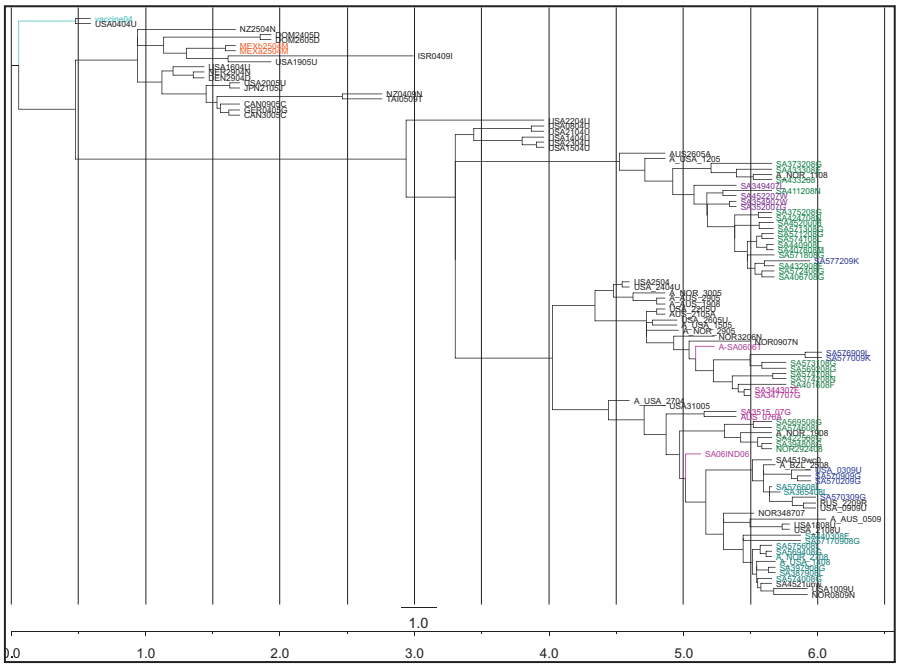


Figure 2: Bayesian evolutionary analysis over time of pandemic Influenza A H1N1 strains identified in South Africa in 2009 compared to isolates from the rest of the world. The vaccine strain is shown in turquoise on the left, the original Mexican isolates in red, the first South African isolates identified in June in in pink, South African isolates from July in purple, from August in green and from September in blue.

Differences were visible on the geographic comparison and may be the result of different imported strains seeding the different geographic outbreaks. Imported strains spread to all provinces and further drift occurred within the individual community. Molecular epidemiology of recent importations will determine if further drift away from vaccine occurred when the next wave hits. For the moment WHO Collaborating centers indicated that the vaccine strain A/California/7/2009 is antigenically similar to circulating strains. Further investigations of cases of severe disease are continuing.

Following the emergence of pandemic H1N1 Influenza Dr Venter and Dr Blumberg published a review paper to guide South African clinicians in dealing with cases in the South African Family Practice journal in August of 2009 and the RVU was part of a paper that describes the South African outbreak of the pandemic Influenza A H1N1 strain in Euro surveillance.

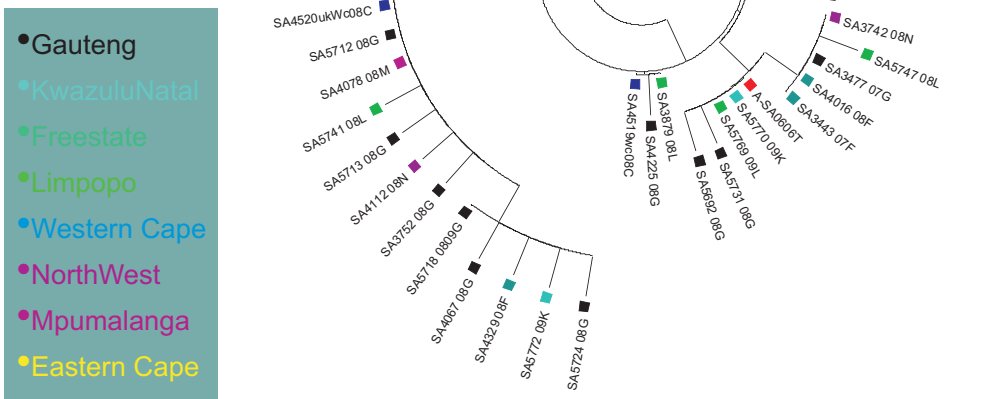


Figure 3: Comparison on pandemic H1N1 strains from different Geographic sites in South Africa.

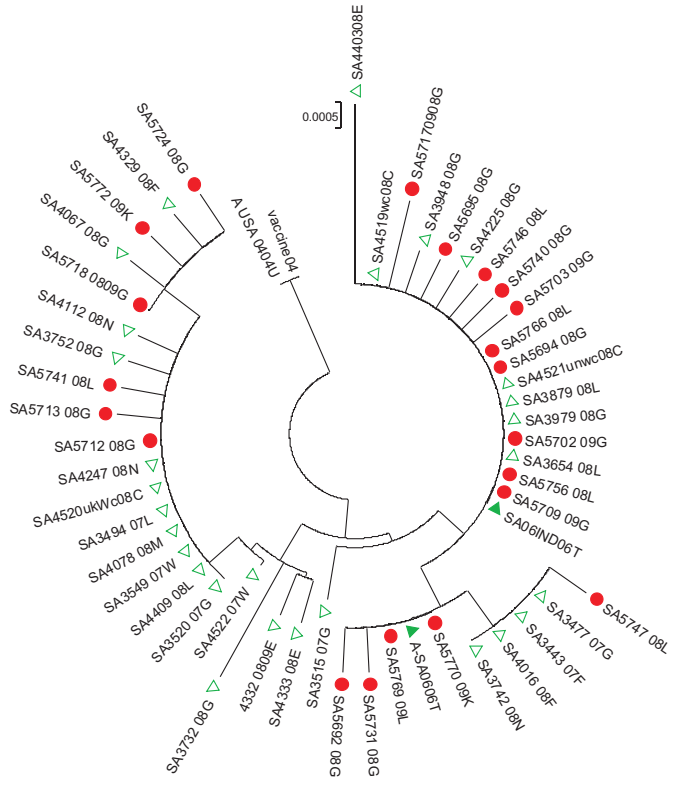


Figure 4: Comparison of strains identified in cases of influenza-like illness (ILI) (green) to severe acute respiratory infections (SARI) (red).

Other causes of SARI

A newly developed multiplex PCR was used for screening specimens received under SARI for 8 additional respiratory viruses. In total 3651 specimens have been screened from SARI surveillance the past year for Influenza A and B, RSV, Rhinovirus, Human Metapneumovirus, Enterovirus, Adenovirus, Rhinovirus and Parainfluenza virus 1-3. The test has been shown to be satisfactory in sensitivity and highly specific. The 2009 QCMD External Quality control panels from the preliminary reports showed that the assay correlates well with other laboratories using the same technology for detecting Respiratory viruses. The preliminary data suggest that in South Africa, as in the Northern Hemisphere, the RSV season precedes the influenza season with most cases of RSV occurring in week 7-21 (March to end of May). Influenza also occurred as two peaks this year, with seasonal H3N2 strains constituting the major strain detected in the first peak and pandemic H1N1 the second. Most cases of HPMV occurred in late winter and early spring, while enterovirus adenovirus and rhinovirus cases were spread throughout the year (Figure 5). Although Rhinovirus was detected most frequently, this also represented the most co-infections. Following Rhinovirus, virus detection in decreasing order included RSV, Adenovirus, Influenza A, Enterovirus, Human Metapneumovirus, PIV3, PIV1 and PIV2. For single infections Rhinovirus was detected in 33.3% of samples, followed by Influenza A (22.5%), RSV (19.2%); Enterovirus (6.7%), HPMV (6.4%); Adenovirus (5.9%); PIV3 (3.6%), and PIV 1 and 2 in < 1% (Figure 6). This data does not include pneumococcal screening, although reports from the Respiratory and Meningeal Pathogens Research Unit do not suggest that pneumococcus alone accounts for the majority of the remaining undetermined cases. This warrants investigations into additional causes of SARI.

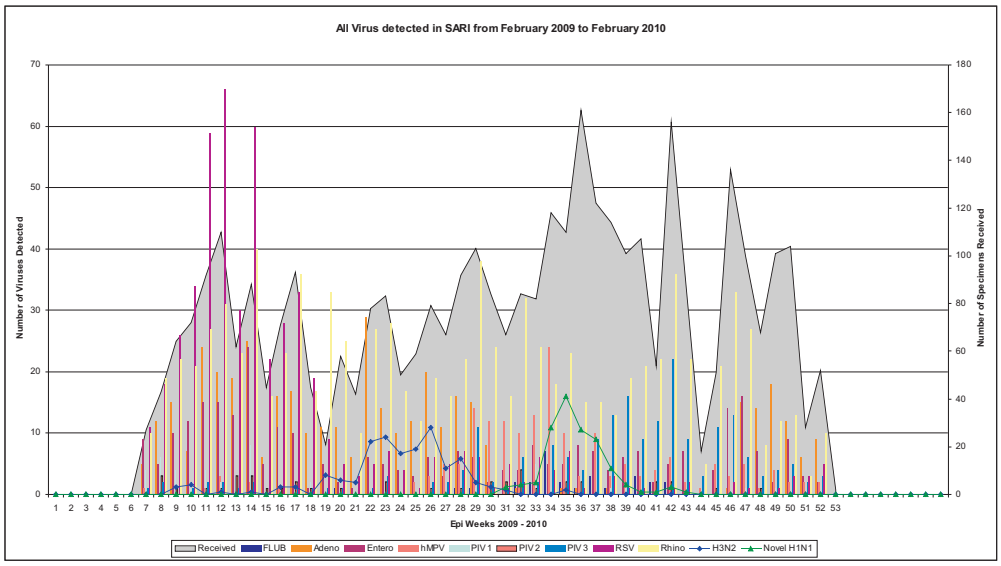


Figure 5: Screening of SARI cases for 9 different respiratory viruses.

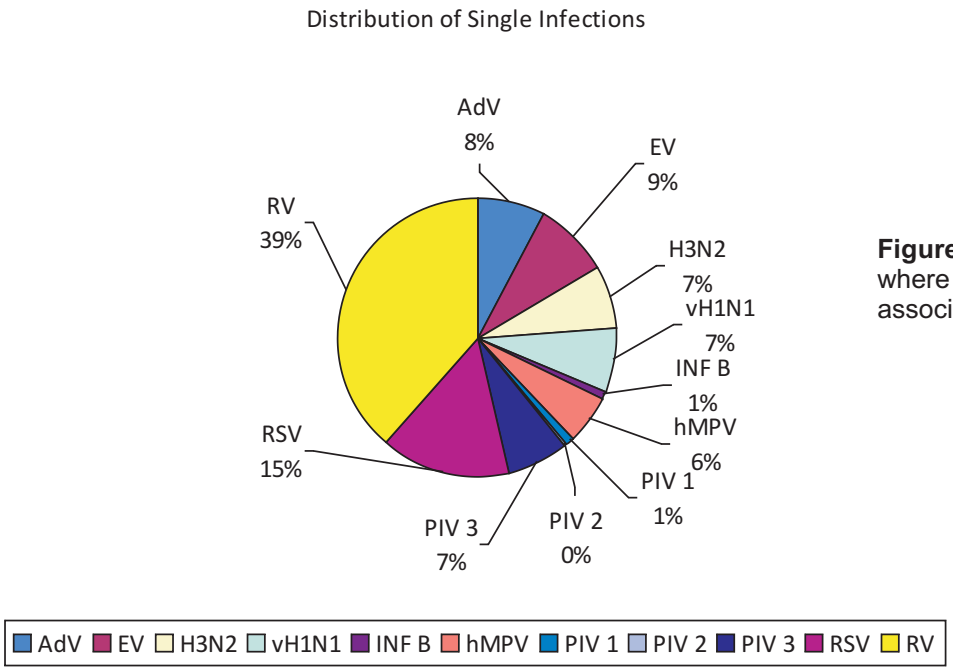


Figure 6: Percentage specimens where no other virus could be detected associated with each virus.

OTHER RESEARCH HIGHLIGHTS FROM THE RESPIRATORY AND ZOONOTIC VIRUS GROUP, DEPARTMENT OF MEDICAL VIROLOGY UNIVERSITY OF PRETORIA AND RESPIRATORY VIRUS UNIT NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

In August 2009, Dr M. Venter started as the new head of the Respiratory virus Unit in a joint appointment between the Department Medical Virology where she heads the Respiratory and Zoonotic virus programme and the NICD. This has lead to a drastic expansion of the respiratory virus unit to include 8 new MSc, PhD and postdoctoral students that work on projects related to Respiratory viruses and emerging zoonotic viruses associated with neurological disease. Postgraduate research students and postdoctoral fellows in this department that are part of the Respiratory and Zoonotic disease Programme are involved in research on the pathogenesis, molecular epidemiology, host genetics, immune response and development of novel diagnostic tools to detect respiratory and zoonotic viruses under mentorship of Prof M. Venter. The main focus of the respiratory virus group is respiratory syncytial virus (RSV) as well as newly described respiratory viruses while the zoonosis group investigates diseases in humans and animals associated with encephalitis and respiratory syndromes that may cause zoonotic infections. The main focus of the zoonosis group is West Nile Virus and virus discovery.

Respiratory virus research

The Respiratory virus group has made excellent progress over the past year in the investigation of viral causative agents of pneumonia in hospitalized children. A quantitative multiplex realtime PCR that can detect 13 different respiratory viruses in 4 realtime RTPCR reactions have been developed and validated and accepted for publication in the Journal of Virological Methods. This technique was subsequently used to screen 30-50% of all respiratory virus specimens that tested negative for conventional viruses in the Department of Medical Virology/NHLS routine diagnostic laboratory during 2006 and 2007 and identified viruses in 70% of specimens. Findings of this study suggest that Respiratory Syncytial virus remain the number one cause of severe pneumonia in children in hospitals in Pretoria. Various newly identified respiratory viruses were detected that play an important role in lower respiratory tract disease and have not previously been investigated in South Africa. Two new polyomaviruses WU and KI was detected for the first time in South Africa and the findings published in Journal of Clinical Virology while a paper of the contribution of other newly identified viruses was submitted in a combined paper to the same journal. A study of the genetics of severe pneumonia in South African children was completed and polymorphisms in the VDR and JUN genes identified that are associated with enhanced disease in children during RSV infection. Investigation of differences in the G and NS proteins of RSV strains associated with mild and severe disease is nearly completed. In this study it was shown that the Subtype A genotypes GA2 and GA5 were more likely to be associated with severe disease than the newly identified subtype B BA genotype. Strains that have most of the G-protein deleted were also identified in children with pneumonia suggesting that RSV does not require the G-protein to cause severe disease in immunocompromised children. Quasispecies were identified for the first time in the NS proteins of RSV. Investigation of the importance of a new subtype of Rhinovirus in pneumonia in South Africa is also nearly completed.

Zoonotic viruses associated with neurological disease

For the emerging neurological diseases and zoonosis programme, the pathogenesis of zoonotic viruses such as West Nile virus's (WNV) were investigated in South Africa. The group published a letter on the cytokine response during a laboratory infection of a South African lineage 2 strain in an immunocompetent patient that experienced neurological disease following a needle stick injury in the New England Journal of Medicine. In order to determine if WNV is being missed as a cause of neurological disease in South Africa, Horses were used as sentinel animals for detecting WNV disease. Horses with neurological disease and fevers that could not be explained were screened for WNV over a period of 2 years. WNV was identified in several fatal cases of severe neurological disease and we showed that up to 30% of unexplained neurological infections in horses may be due to WNV. This paper was published in Emerging Infectious Disease Journal. Virus discovery projects are carried out on the cases that tested negative for common viruses. Wesselsbron virus and a uncharacterized Bunyavirus, Shunivirus was identified as causes of neurological disease in horses. Genome sequencing of these isolates are underway. Finally a new differential diagnostic tool for aseptic meningitis have been developed and validated against clinical specimens. This low density macroarray does not require expensive equipment to use and will be invaluable in the identification of causes on aseptic meningitis outbreaks in Africa.

HIGHLIGHTS AND ACHIEVEMENTS

- The Respiratory Virus unit has been earmarked to become the regional influenza laboratory for the southern African region by the WHO Global influenza program. As a regional reference laboratory, the lab will function as a national influenza centre for neighboring countries with limited or no capacity to monitor influenza activity. This role became critical during the 2009 novel H1N1 pandemic as the unit was responsible for testing suspected cases from many neighboring countries.
- Dr M Venter and Dhamari Naidoo received a travel award from the Fogarty International Center (FIC) of the US National Institutes of Health (NIH), to give a oral presentations at the regional Multinational Influenza Seasonal Mortality Study (MISMS) meeting for Africa 21-25 April 2009 Dakar, Senegal.

- Dr M Venter visited the Vaccine Research centre at the National Institutes of Health in Bethesda, Maryland to discuss collaboration projects with Dr Nancy Sullivan, Chief of the Biodefense Laboratory, 4-9 May 2009. This followed the establishment of collaboration agreements between the NIAID and the NICD.
- Dr M Venter was invited to give a lecture at European Arbonet meeting 30 September 2009 St Raphael, France.
- Members of the RVU group were first or senior authors and co-authors of 10 publications in highly rated international peer-reviewed journals.

Post-graduate students in our group awarded prizes at the Faculty of Health Sciences Faculty Day 18-19 August 2009:

- Ms S Human (MSc student) received third prize in the category Junior Researcher: Basic Sciences [Oral] for her presentation entitled *"Molecular epidemiology and characterization of Wesselsbron virus in sentinel animals in South Africa"*
- Ms C van Eeden (PhD student) received first prize in the category Junior Researcher: Basic Sciences [Posters] for her poster/presentation entitled "*Serological and molecular characterization of flavi- and novel zoonotic vector-borne viruses associated with neurological disease in South Africa".*

- Ms A Rakgantso (BSc [Hons]) was awarded a prize for the best poster/presentation at the Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) III Congress 2009, Sun City, North West Province 20-23 August 2009 for her poster entitled *"Conformational changes in the major surface glycoprotein of different genotypes of respiratory syncytial virus (RSV) subtypes A and B".*
- Dr A Visser, was awarded a Faculty of Health Sciences' merit certificate for a publication in the category "Non-clinical publication by a Junior Researcher (<35 years)" in the Faculty in 2008 for the publication: Visser A, Delport S, Venter M. *Molecular epidemiological analysis of a nosocomial outbreak of respiratory syncytial virus associated pneumonia in a kangaroo mother care unit, South Africa. J Med Virol* 2008; 80:724-732.

COLLABORATIONS

- **Locally**: Dr Venter collaborates closely with the Department of Pathology, Faculty of Veterinary Sciences Onderstepoort, University of Pretoria; Prof Susan Delport, Department of Pediatrics, Kalafong Hospital and University of Pretoria, South Africa. Dr Wanda Markotter, Department Microbiology and Plant Pathology, University of Pretoria. Dr Gerdes, The Onderstepoort Veterinary Institute, Prof Robert Swanepoel and Dr Paweska, The Special Pathogens Unit and Dr Felicity Burt, University of the Freestate on arboviruses.
- **Internationally**: Dr Thomas Briese, Columbia University, New York, USA; Prof James E Crowe Jr, Vanderbilt University Medical School, Nashville Tennessee, USA; Dr Rinny Jansen National Institute for Public Health and the Environment, Bilthoven and Louis Bont and Jan Kimpen at the University of Utrecht, The Netherlands; Dr James Nokes, KEMRI Wellcome Trust Research Programme, Kenya; Dr Tim Myers, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; Nancy Sullivan, Vaccine Research Center, National Institutes of Health, Bethesda, Maryland, USA.

CAPACITY BUILDING

STUDENT TRAINING

Students currently registered for post graduate degrees under mentorship of Prof M Venter through the Department Medical Virology, University of Pretoria:

- A MMed student (Dr Adele Visser) participate in projects related to respiratory viruses associated with acute lower respiratory tract infections.
- 3 PhD students (Nicksy Gumede (2007-) and Dewald Zaayman (2007-); Charmaine van Eeden (2009-)
- 5 MSc students (Ria van den Bergh (2006-), Stacey Human (2008-), Yvette Westerberg (2009-), Juliete Mentoor (2009-), Stephanie Smit (2009-)
- A post-doctoral fellow, Tina Kresfelder, on a project entitled "Genetic association of respiratory syncytial virus and disease severity.

RAPID RESPONSE TRAINING FOR AVIAN AND PANDEMIC INFLUENZA

Laboratory capacity has been strengthened for the rapid diagnosis of suspected cases of novel influenza viruses. The Unit has been involved in the Rapid Response Training for Avian and Pandemic Influenza for the provinces and in providing support to a number of SADC countries for pandemic preparedness.

Pandemic preparedness

To assess the performance and sensitivity of the real time PCR assays used to identify influenza viruses and novel H1N1 influenza in particular, the unit participated in two external quality assurance panels supplied by the WHO Global Influenza Program and a panel supplied by Quality Control for Molecular Diagnostics (QCMD). The unit was successfully able to identify all influenza subtypes in all three panels.

Rapid response training

Lab technicians from Seychelles and Mauritius attended training sessions on rapid response and molecular detection of influenza and avian influenza subtypes hosted at NICD from 11 to 20 February 2009.

REGIONAL ACTIVITIES

The unit provides technical assistance to countries in the southern African region that have funding provided by CDC Atlanta.

- A lab technician from Department of Immunology at the National Institute of Public Health in Maputo Mozambique was trained on influenza virus isolation techniques from 9 to 20 February 2009
- A site visit was done at the University Teaching Hospital in Lusaka, Zambia from 1 to 3 April 2009 to assess the progress of the lab and to give recommendations to improve the quality of results from laboratory.
- Laboratory staff from Zambia and Angola were trained on RNA extractions and real time RT PCR from 11 to 15 May 2009

The unit played a vital role in developing laboratory capacity for African countries during the 2009 H1N1 pandemic on behalf of the WHO AFRO regional office. Laboratory staff was trained on techniques such as RNA extraction, real time RT PCR and the implementation of standard operating procedures. Training was given to the following countries:

- Department of Immunology at the National Institute of Public Health in Maputo Mozambique from 15 to 17 June 2009
- National Public Health Laboratory in Namibia visited the unit from 4 to 8 August 2009
- National Public Health laboratory in Gabarone, Botswana from 20 to 21 August 2009.

Specialized Molecular Diagnostics Unit

BACKGROUND

Recent advances in nucleic acid methods provided powerful diagnostic tools, by facilitating rapid, sensitive surveillance and differential diagnosis of infectious diseases. This is particularly true in areas where traditional culture-based methods alone proved insufficient. A notable advantage of molecular methods is in reducing the time from taking samples to reporting results. The specificity and sensitivity of these methods can help to improve patient care by enabling intervention when the prognosis is optimal for limiting replication, dissemination, transmission, morbidity and mortality. Additionally, from molecular surveillance perspective information such as genotyping are critical from understanding what is genotypes are in circulation, vaccine development and treatment responses.

The Specialized Molecular Diagnostics Unit has as its functions main functions clinical diagnosis, and molecular surveillance function. The laboratory aims to provide rapid and accurate diagnostic tests for patient management and research purposes as well as critical surveillance information.

The staff in SMDU are involved in reviewing new techniques for molecular detection of viruses to improve on and add to the unit's strategic role at the NICD. Challenges experienced during the implementation of new cutting-edge molecular diagnostic techniques include: to address the volume of tests (throughput), to adhere to clinically relevant turn-around times, to implement flexible platforms to handle diverse tests and sample types, to increase the efficiency of testing and to assess the need for real-time technologies, quantitative PCR or end-point assays. Any molecular test, in spite of the straightforwardness of its result or the instrument used to get the result, necessitate interpretation. Therefore training processes and competencies of staff substantially increase successful implementation of technologies. The unit's good infrastructure and investment in total quality management make indispensable tools to verify procedures against current and historical benchmarks.

Demand for simpler, point of care equipment, devices suitable for non-skilled users and fully integrated assays, necessitates the evaluation and validation of these systems within the unit. High throughput sequencing technologies and bioinformatics bring together new growth possibilities for clinical applications with major impact infectious disease genomics.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

TEST STATISTICS FOR 2009

SMDU has again noted a marked increase in testing over the months of January 2009 to May 2009. There has been a slight decrease in testing for the months of June to August 2009 when compared to the same period in 2008, but overall there has been an increase in tests of approximately 11%. Nevertheless, an increase in the trials and studies done in SMDU has increased overall.

EQA AND ACCREDITATION

A total of 7 new tests were submitted for extension of SANAS accredited scope for M0029E during late 2008 and 2009.

1. HIV-1 Viral load using Roche TaqMan
2. Hepatitis B Viral load using Roche TaqMan
3. Hepatitis C Viral load using Roche TaqMan
4. HCV Genotyping using the InnoLiPA assay
5. HCV Sequencing for genotyping
6. CD4 testing using PanLeucoGating (PLG)
7. HIV-1 DNA PCR Qualitative assay using the Roche TaqMan

SMDU was audited by PPD from the 31st August to the 1st of September 2009 based on participation in ongoing trials for the HIV Vaccine Trials Network (HVTN). SMDU participates in external quality assessment and inter-laboratory comparison coordinated by VQA, QCMD, CDC, REQAS and NEQAS. SMDU participated in 19 programs with 47 datasets submitted for QCMD during 2009. All SMDU tests include an internal quality control that is designed to confirm the validity of results obtained and to control all processes involved in PCR testing. Analytical analysis of:

HIV-1 DNA diagnosis of HIV-1 infections in infants: Early Infant Diagnostics (EID) HIV-1 DBS testing continued throughout 2009 in collaboration with the Clinton Foundation and Centre for Disease Control (CDC) for Lesotho, Liberia, Swaziland and the Caribbean. In July 2009, the Roche qualitative assay for HIV DNA Dried Blood Spots was implemented. Prior to this, the method was validated and compared to the method in use at the time. The test method was validated on two Taqman instruments. In an effort to improve diagnostic capacity, the main work input for SMDU, HIV DNA PCR, was migrated from plate detection using automated extraction, to the fully automated Roche AmpliPrep/TaqMan platform. As there is an improved sensitivity and specificity of the automated Roche AmpliPrep/TaqMan test, confirmatory testing on the LC480 is no longer required.

This has greatly increased the capacity and turnaround time for this test. Validated test is now SANAS accredited. A specific staff member was assigned to improve client liaison with sites and representatives. HIV DNA PCR testing is the major diagnostics activity performed at SMDU in support of the country programmes to expand diagnosis of HIV infants.

Multiplex Respiratory Virus PCR: A Respiratory virus multiplex PCR was introduced as part of a surveillance study in 2009 for the comprehensive identification of respiratory pathogens. RSV Multiplex PCR will continue as part of the SARI (Severe Acute Respiratory Infections Surveillance) and will be expanded to include ILI (Influenza like Illness that forms part of the normal Flu Watch Surveillance) for the for diagnosis, infection control, epidemiology, and bio-threat surveillance.

HIV-1 quantitative PCR: SMDU utilizes the Roche AmpliPrep/TaqMan platform and the Roche Ampliprep/COBAS platform of quantification of HIV-1 RNA in plasma specimens. The BioMérieux EasyMag/EasyQ instrument is used for research purposes. The Abbott m2000 and the Siemens kPCR instruments were installed at SMDU for evaluation purposes. The quantification of HIV-1 RNA using the Abbott system m2000 will be evaluated with the intention to implement it for quantification of RNA for HVTN (HIV Vaccine Trial Network) algorithms

CMV PCR: A monoplex real-time PCR assay was introduced in June/July 2009 to test for Cytomegalovirus using the Roche LC480. Over 170 specimens were analyzed since validation. This test will be submitted for SANAS accreditation and will be expanded to a quantitative CMV PCR. The recent focus to quantify viral load in relation to disease severity has come to the fore in terms of patient management and outcomes.

Aseptic meningitis multiplex PCR: Validation of the multiplex PCR for causative agents for aseptic meningitis (AM) was completed in 2009. This real-time multiplex PCR was developed for the diagnosis of aseptic meningitis targeting the following viruses: HSV 1 and 2, VZV, EBV, CMV, HHV-6, EV and Mumps. This test will be validated for diagnostics and fully incorporated into SMDU's in 2010. Evaluation of point care systems for aseptic meningitis is ongoing. A GeneXPert instrument has been placed for evaluation purposes in testing for Enterovirus. This instrument will form part of future Enterovirus surveillance studies in parallel with the in-house developed aseptic meningitis multiplex PCR. A joint aseptic meningitis sequencing project with SPU will be initiated.

HIV Vaccine Trial Network (HVTN): HVTN trials have expanded to the testing done for HVTN by SMDU. Trials include HVTN503, HVTN802, HVTN073 and HVTN403/404. HVTN repository functions have been transferred to SMDU/SVDU. A new HAWS LIMS system has been implemented in SMDU for all HVTN trials

COLLABORATIONS.

Centers for Disease Control and Prevention EID testing and training
Clinton Foundation EID testing and training
HIV-1 Vaccine Trial Network RNA testing on participants
QCMD HIV NAT EQA and IQC program

CAPACITY BUILDING

Throughout the course of the year, SMDU is actively involved in training of the following personnel - this training is done in-house on methods used in SMDU and other relevant laboratory techniques:

- Intern Medical Scientists (6)
- Registrars
- FELTP Registrars
- Student Medical Technologists

Four staff members were successfully registered with the HPSCA as a Medical Scientists.

Mark Goosen and Deirdré Greyling trained with the CDC in Atlanta, USA in IQCs for HIV-1 DNA PCR.

Mariza Vos attended training with QCMD in Scotland for report writing and data analysis for Southern Africa and NHLS/NICD participants.

Mariza Vos attended training in Seattle on the HAWS LIMS system to be implemented in SMDU for all HVTN trials.

Ewaldé Cutler is registered for an Msc.

POLIO SECTION

BACKGROUND

The molecular Polio Unit serves as a Regional Reference Laboratory with the capacity of Specialized Reference Laboratory. The unit serves as the only sequencing laboratory for polioviruses in the African region and contributes to the training of the African laboratory network through World Health Organization (WHO) workshop programmes.

Workload has dramatically increased in 2009. The laboratory has received 2963 (Figure 1) isolates by December 2009 compared to 1515 isolates received in 2008 which were characterized as vaccine or wild type using two intratypic differentiation methods, PCR and ELISA. These isolates were sent by the African laboratories that are part of Polio Laboratory Network (Figure 2). The isolates were tested by RT-PCR, ELISA, Real-time RT-PCR and Sequencing. The high volume increase was due to an outbreak experienced in the West and Central Africa and in addition all known Sabins strains were undergoing Vaccine Derived Polioviruses (VDPVs) screening using Real-Time technique.

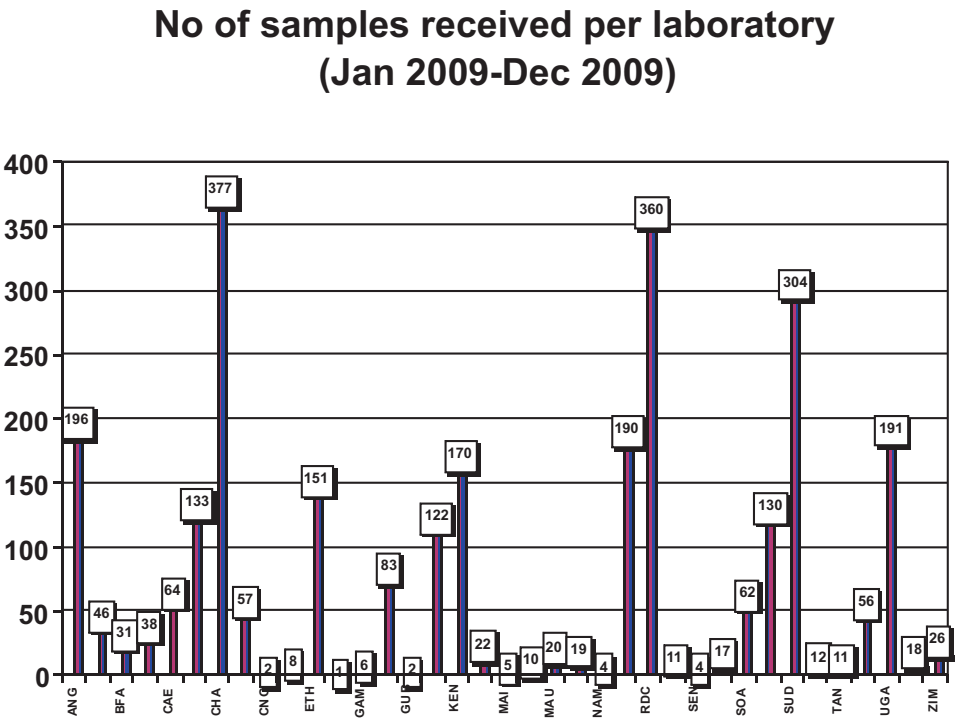


Figure 1: No of isolates received per country

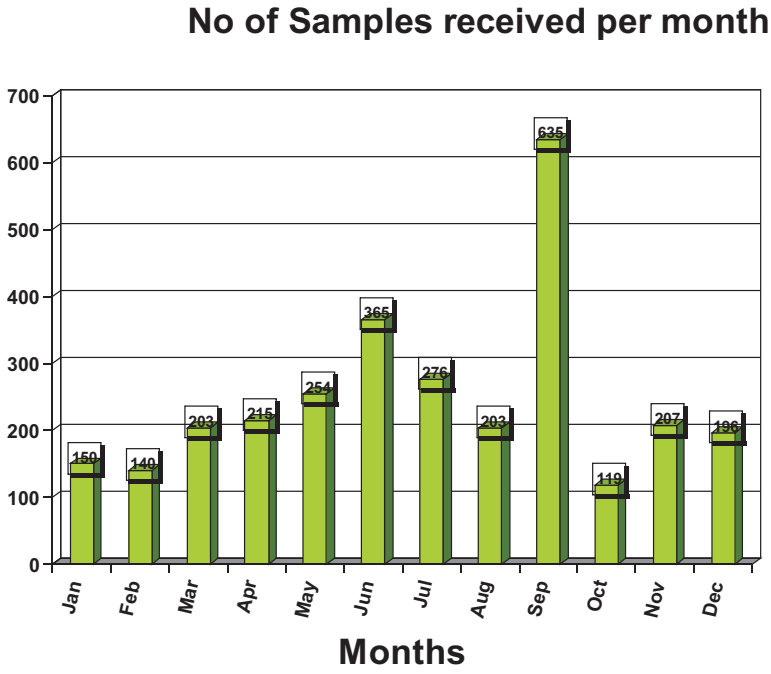


Figure 2: No of isolates received per month

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

LABORATORY ACHIEVEMENTS

The molecular polio unit has been involved in the field evaluation of real-time PCR assay. By July 2009, two new techniques were implemented within the unit i.e rRT-PCR ITD and rRT-PCR VDPV. 1278 isolates were tested by rRT-PCR, of which 399 were tested by rRT-PCR ITD assay and 879 were tested by rRT-PCR VDPV assay (Figure 3). Twenty VDPVs were identified in 5 countries namely Ethiopia, Guinea, DRC, Somalia and Malawi. Four cases representing VDPV type 2 were identified in the Democratic Republic of Congo, three with date of onset of paralysis in 2009 and one in 2008; this is a continuation of a VDPV outbreak in the DRC since 2005. In Somalia two 2009 cases and one contact were identified as VDPV type 2. In Ethiopia, two VDPV type 2 cases and 1 VDPV type were reported. The Malawi case was a VDPV type 1 while Guinea case was a VDPV type 2.

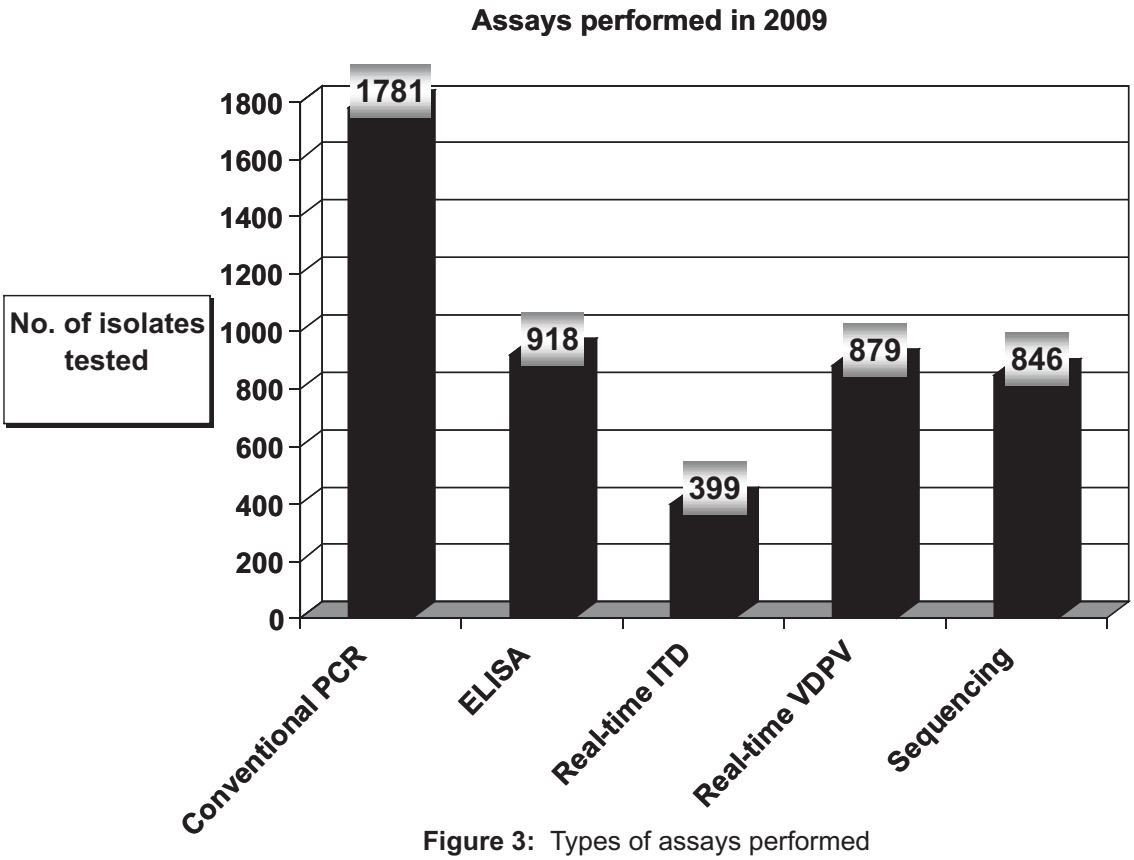


Figure 3: Types of assays performed

The implementation of Real time technique has contributed to the decision by the Global Polio LabNet (GPLN) to adopt it and replace ELISA and Diagnostic PCR for ITD and VDPV screening. This has resulted in a decrease in turn around time and increase in VDPV identification. The unit hosted two-week real time workshop in November 2009. Such trainings will also help to prevent future discordant results between Institutions. The NICD staff participated as facilitators and also some were participants.

In total, 906 isolates were sequenced of which 785 were identified as wild types and twenty as VDPVs **(**Figure 4). One hundred and one were identified as Sabin strains due to less than 10 nucleotide difference from the parental Sabin strain (data not shown).

During 2009, the molecular polio unit successfully completed proficiency panels referred by CDC, Atlanta for real-time PCR ITD and real-time PCR VDPV methods. The unit is fully accredited by both WHO and SANAS.

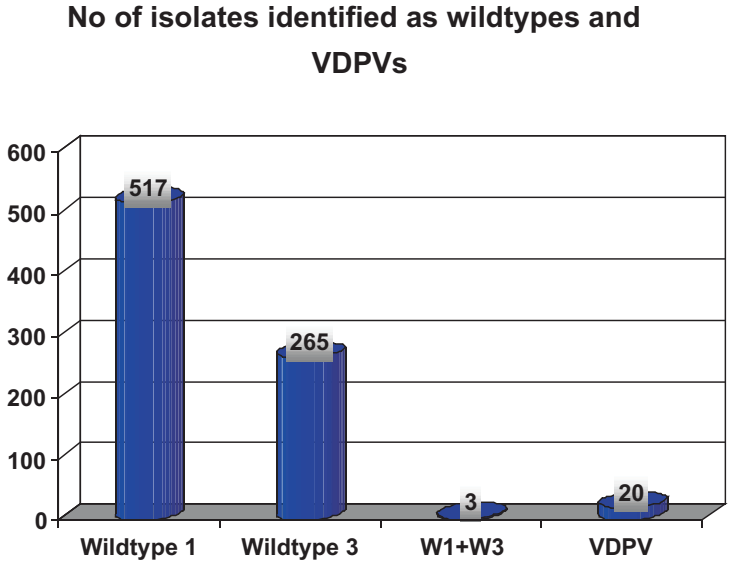


Figure 4: No of wild types and VDPVs identified in 2009

COLLABORATORS

Centers for Disease Control and Prevention, Atlanta, USA
World Health Organization, Geneva, Switzerland
World Health Organization, Brazzaville, Republic of Congo
World Health Organization, Harare, Zimbabwe
African Polio LabNet

CAPACITY BUILDING

Workshops: The molecular unit at the NICD hosted two successful workshops for Real-Time assay during 2009.

The first workshop took place from the 02 November 2009 until 06 November 2009 and was the first real-time workshop hosted in the Africa region. The second workshop took place on the 9-13 November with attendees from Francophone countries.

Registered for degrees:
HN Gumede-Moeletsi, PhD student
L Seakamela, B Tech student

HEPATITIS B (HBV) AND C (HCV) SECTION

BACKGROUND

The activities of the hepatitis unit can be divided according to the three main activities of the NHLS, namely, Service, Training and Research. In the context of the hepatitis laboratory, we aim to deliver an excellent diagnostic, clinical and laboratory service which includes the solution of operating problems and compliance with strict codes of quality management. Research can be divided into the generation of new knowledge as well as research and development, both geared toward keeping current in the hepatitis field both nationally and internationally. The latter not only feeds into the first goal, but also the last, as we aim to become a facility capable of sharing this current knowledge from registrar level to the National Department of Health, NDoH. Finally we aim to improve the quality and quantity of peer reviewed publications from this unit.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

The Unit has provided various accredited hepatitis diagnostic tests including HCV viral load testing and genotyping; for HBV: PCR, viral load. HCV genotyping by sequencing (average time of test 2-3 days) has been replaced by LiPA technology (average time of test 3-4 hrs). Advantages include higher throughput, better turn around time and detection of mixed infections.

QCMD 2009

During 2009 there were two panels (each of nine plasma specimens) for the QCMD HCV RNA and one for the Genotyping proficiency programme, respectively. Typing and subtyping of HCV mixtures still remains a challenge in the laboratory. Also over the years we have noticed that our in-house HBV PCR does not perform as well (decreased sensitivity) with genotype D specimens. Our quality assurance results have led us to chnage from HCV genotyping using sequencing to line probe assays. Ongoing validation studies of the LiPA technique indicate that the LiPA 2.0 kit (Siemens Medical Diagnostics) using the 5'UTR amplicon from the HCV Amplicor 2.0 test (Roche Diagnostics) detects mixtures better than the same test using the double amplicon (5'UTR plus Core region) obtained from the Versant LiPA Amplification 2.0 (Siemens Medical Diagnostics). The latter, however, is far better at subtyping than the former. Since subtype is not reported to affect duration of therapy, whereas knowledge of both genotypes in co-infection are crucial, we do not use the double amplicon assay unless subtype is specifically requested. Sequencing remains the gold standard against which genotyping validations are done, but only when it has been confirmed that there is no co-infection as our sequencing protocols (which target the 5'UTR and NS5b regions, respectively) only detect the major quasispecies present in the specimen.

NEW LABORATORY TESTS BEING ASSESSED

Further, toward supplying African HBV genotypes for use in future QCMD panels, methods of HBV genotyping are being assessed. The INNO-LiPA HBV genotyping assay has been validated, as far as possible (not all genotypes are available for full validation although the manufacturer's do claim that it is specific for genotypes A to H). HBV sequencing for genotyping (using a preS2/partial S amplicon) is also being re-introduced to the laboratory, and a full genome HBV assay is being optimized. Although the latter is presently part of a research project, when validated, it will be useful to be able to characterize selected HBV strains routinely as part of our surveillance initiative. This is important since most serological diagnostics are based on the surface gene of the virus only, and this is known to be mutated in occult infection, vaccine escape as well as in therapeutic resistance mutants.

SURVEILLANCE INITIATIVES

An HCV surveillance network has been established at the NICD which obtains specimens from the following sources for viral load testing and genotyping:
1. Patients attending hospitals/clinics nationwide who have positive HCV antibody which is confirmed by HCV PCR
2. Haemophiliac patients found to be HCV PCR positive in a provincial survey
3. Anonymous volunteer blood donors who are found to be HCV PCR positive by routine testing at the blood bank

Results from the survey have been, and will continue to be, used to supply current local data to the NDoH as a surveaillance activity. In addition, the collation of all results, together with patient clinical and demographic information will provide a powerful tool for the assessment of current (and historical) HCV disease burden in South Africa. The reports are collated by the NHLS Corporate Data Warehouse (CDW) and updated annually.

HIGHLIGHTS AND ACHIEVEMENTS

An ongoing collaboration with Roche Pharmaceuticals and clinicians from various participating hospitals in the Gauteng, Free State, Limpopo and KZN provinces has been initiated to monitor the response to therapy of patients receiving PEG-IFN (Pegasys, Roche) and ribavirin (Copegus, Roche). Patients are genotyped in the laboratory and results are recorded. A preliminary report of the results of this study entitled "**HCV genotypes and response to therapy in a South African study group"** was accepted for Poster presentation at **the** 3rd joint Congress of the Federation of Infectious Diseases Society in Southern Africa (FIDSSA) held at Sun City, South Africa, in August and was the winner of one of the best poster awards.

Our laboratory is providing current HBV and HCV data for inclusion in the National Guidelines for the Prevention and Control of Hepatitis in South Africa. From the first draft, the HCV guidelines were written, and then later co-ordinated and edited to their final form, by staff from this unit. The guidelines are presently being circulated prior to publication on the website of the DoH (www.doh.gov.za). Highlights of the guidelines include a new HCV testing algorithm, specifically for HCV diagnosis in South Africa, which we created, and then refined with the advice of the Hepatitis Working Group. We have also been tasked with writing and providing information for the HBV guidelines and have been involved in discussions toward the finalization of the guidelines for HAV, HDV and HEV. This exercise has proved to be extremely valuable and has brought us together with molecular biologists, epidemiologists, clinicians and DoH officials from all parts of South Africa. The resultant guidelines, even those still in their draft state, are an invaluable tool when handling the many queries referred to the unit.

The following research projects are in progress within the unit:

PROJECT 1

Molecular Characterization of unique HCV viral strains in Johannesburg

This is a PhD project which is nearing completion. This study has been funded by the Poliomyelitis Research Foundation (final report in progress) as well as the NHLS (ongoing initiatives will be continued as part of routine surveillance activities of the laboratory).

The project aims to improve and maintain HCV data collection, diagnostics (supplementary testing and method evaluation and optimization both of which are ongoing) and continued surveillance (genotyping, treatment and immunological response monitoring) in South Africa including characterization of the little known genotype 5a which is prevalent locally. A powerful database combining patient demographics, serology and molecular data has been established. The ultimate aim is to provide data which will enable health authorities to establish national awareness and provide information to clinicians leading to best prevention and treatment decisions within the local population. Most of these objectives have been achieved, however, two studies, both of which are involved with characterizing local strains are in progress, and these are outlined below.

- **To molecularly characterize genotype 5a by determining partial genomic sequences of patient specimens:** Partial sequencing of genotype 5a is being performed on all identified specimens from previous studies. This is, however, dependent on the volume and condition of the stored sera. Primers for the core, E1 and NS4B region have been designed. Sequences obtained will be used in phylogenetic analyses in order to compare local genotype 5a sequence data in these regions with other global genotypes retrieved from international databases.
- **To determine palindromic nucleotide substitutions (PNS) for determining genotypes 1 and 5a in SA:** The entire 5'untranslated end of the most common HCV genotypes in South Africa, genotypes 1 and 5a, is being sequenced. As genotype 5 remains one of the rarely described, and only partially characterized, genotypes of HCV, this data will be used to further understand the secondary structure of this genotype. The PNS informatics will be done at the Faculty of Agriculture, Iwate University, Iwate, Japan, in collaboration with Professor Giangaspero and co-workers.

PROJECT 2

Development of a high throughput hepatitis B virus (genotypic and phenotypic) variation detection assay for molecular surveillance of HBV in South Africa

Approximately 350 million people worldwide are chronically infected with the hepatitis B virus (HBV), and sub-Saharan Africa, with >8% HBsAg positivity, is rated as a highly endemic region. A safe and efficacious vaccine has been available since the early 1980s. Therapy for HBV aims at long-term suppression of viral replication, however, both nucleo(t/s)ide analogues (NAs) and interferon, the currently approved treatments and vaccine intervention, can result in viral mutation. Although data is incomplete, numerous studies have shown that both response to treatment with NAs and the progression to cirrhosis and HCC (the sequelae of HBV persistence) are closely associated with the infecting genotype. A thorough and ongoing molecular surveillance protocol with present methods is not possible. The increase in "anti-core only" profiles and occult infections in both in HIV co-infection and single infection are a warning that the presence or absence of

surface antibody is not a reliable indicator of current or past HBV disease. Sequencing is currently the gold standard for mutation detection and genotyping, but it only detects major populations as do other methods such as PCR with genotype specific primers and RFLP analysis. While hybridization and the line probe assays do detect mixtures, they can only detect known variation and no one method can look at variation across the genome.

Our aim is therefore to develop a high throughput method capable of simultaneously detecting variation, both known and unknown, across the genome of HBV.

Specific Objectives:

- To identify and characterize short conserved regions on the HBV genome
- To define and characterize target areas of interest along the genome for specific studies (resistance, genotype, core mutants etc.)
- To design primers, within the conserved regions identified, and adjacent to the target areas of interest
- To optimize and validate, individually, assay(s) capable of detecting HBV variation reliably (validated against known technologies) in the target areas
- After validating the final assay(s) for each area, they will all be combined into a protocol covering the HBV genome (for example, mutiplex/plate technology) to produce a current and adaptable assay which produces maximum information within a single "test"

This approach to primer design has yielded the highly sensitive and specific, standard and real time, HBV PCR which will replace our previous qualitative diagnostic assay.

Two other projects revolve around the Master database designed for this project:

- The first aims to programme (using Python scripting) many of the processes being used to create a robust system for automatic database population and upgrade. In addition, it aims to apply a measure of the reliability to the available information in the International Nucleotide Sequence Database Collaboration (NSDC) and in so doing determine the extent to which these databases present a skewed picture of hepatitis B viral prevalence and variation in southern Africa.
- The second uses data from the Master database to look at HBV evolution based on a Position Specific Scoring Matrix, PSSM, approach. A matrix is built using any two standardised training sets from the defined subtypes, genotypes or phenotypes of HBV and query sequences are scored according to their similarity to the two extremes used in the specific matrix.

COLLABORATIONS

South African National Blood Services (SANBS)
Dr. Johnny Mahlangu, Haemophiliac Clinic, Charlotte Maxeke Hospital
Roche Pharmaceuticals
Professor Giangaspero, Japan

CAPACITY BUILDING

Nishi Prabdial-Sing, currently registered for PhD
Shirley Muvhulawa, Intern scientist
Monalisa Kalimashe, Intern scientist

MOLECULAR MEASLES/ RUBELLA SECTION

BACKGROUND

Molecular characterization of the strain of measles virus in specimens from laboratory-confirmed cases of measles, allows genotype determination and thus inference of its origin in terms of control of measles. The genotype and sequence data is shared with the Global Measles Laboratory Network of the World Health Organization (WHO). The NICD measles laboratories (serology, viral isolation, molecular) have dual functions as a national laboratory (NL) to perform case-based surveillance and identify strains circulating in South Africa, as well as a WHO regional reference laboratory (RRL) function to assess quality of the serology results from national labs in southern Africa and to identify strains of measles and rubella viruses circulating in these countries. The molecular laboratory also provides a service to African countries outside of the RRL responsibilities that do not have access to nucleic acid sequencing technology.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

South Africa experienced a large measles outbreak in 2009 with 5857 laboratory-confirmed cases. The first cases were detected in Tshwane district, Pretoria in late March and were identified as genotype B3. The sequences were identical to those of viruses circulating in Benin in 2008/9. As the outbreak spread to other districts and eventually to other provinces, molecular characterization continued to demonstrate the presence of the same strain of genotype B3. By the end of 2009, the majority of specimens from cases from all provinces still had the same sequence as the first cases, but there were cases with a single nucleotide change at various positions in the region of interest (nucleoprotein gene). That there has been a single genotype in circulation is in keeping with the failure to maintain high vaccine coverage. Minor variations have been observed and this is expected in a RNA virus with many chains of transmission. However, a single specimen from Edenvale, collected in late November, was found to contain a virus that had 4 nucleotide changes relative to the other sequences, and although still genotype B3 it did not cluster with the main group. It has not been possible to determine whether this represents accumulated changes in the original strain (Gauteng has had the highest number of cases and therefore the most chains of transmission) or whether it might

represent a slightly different strain of virus. It will therefore be necessary to investigate more specimens from the area to determine whether there is evidence of accumulation of these changes.

The value of molecular characterization was demonstrated in patient with a travel history to India. The patient, a resident in theTshwane district, Pretoria, presented with measles shortly after returning from a short stay in India. As there was an ongoing outbreak of measles in Tshwane district, it was necessary to determine the source of the virus. Molecular sequencing showed that the virus was genotype D8 which is known to circulate endemically in India. Since the local outbreak was caused by genotype B3 it was possible to demonstrate that the patient had not acquired the infection locally.

With regard to the WHO-RRL function of the molecular laboratory, 187 specimens (clinical material/viral isolates/PCR products) were received from 5 countries for molecular analysis: Angola, Ethiopia, Senegal, Uganda and Côte d'Ivoire (who forwarded specimens from Benin, Burkina Faso, Mali, Niger, Nigeria, Cameroon and Togo). It was only possible to obtain sequences for 94 (50.3%) of these specimens. Phylogenetic analysis of the sequences revealed that various strains of genotype B3 viruses were circulating in Africa. This is interesting because historically different genotypes circulated in different regions of sub-Saharan Africa. This apparent reduction in virus diversity may be attributable to the impact of many rounds of mass measles vaccination campaigns conducted in the region and may even imply interruption of endemic transmission in these countries; however it may also reflect the relatively poor level of viral surveillance in Africa. To address the laboratory surveillance capacity a WHO-sponsored workshop was held at the NICD for both virus isolation and molecular detection by PCR.

COLLABORATIONS

WHO Measles and Rubella Laboratory Network
Measles and Rubella section, CDC
Measles section, Institute Pasteur, Coté d'Ivoire
Measles section, Uganda Virus Research Institute

CAPACITY BUILDING

The PRF centre at the NICD hosted the WHO/AFRO Measles/Rubella workshop, 14-18 September 2009. Participants from across Africa attended either the serology or virus isolation/molecular training.

Special Pathogens Unit

BACKGROUND

The Special Pathogens Unit of the National Institute for Communicable Diseases of the National Health Laboratory Service (SPU, NICD-NHLS) is tasked with the laboratory confirmation and investigation of diseases caused by biohazard class 3 and 4 viral agents. These include, amongst others, the viral haemorrhagic fevers (VHF) caused by Crimean-Congo haemorrhagic fever (CCHF); Marburg; Ebola; Lassa fever; Rift Valley fever (RVF) and hantaviruses. In addition, the Unit is also responsible for the laboratory investigation and confirmation of arboviral diseases. Arboviral diseases of public health importance include West Nile fever (WN); dengue fever (DEN); yellow fever (YF), Sindbis (SIN) and Chikungunya (CHIK). Furthermore the Unit is the only laboratory for human rabies testing in South Africa. The Unit operates high (biosafety level 3) and one of the only maximum (biosafety level 4) biocontainment facilities on the African continent. The Arbovirus Laboratory has been accredited by the South African National Accreditation System (SANAS) under ISO 15189 since 2000 (M0029B).

SPU participates and drives several projects that are aimed at the enhancement of regional capacity for outbreak response and diagnosis of VHF. Research interests of the Unit include development and improvement of diagnostic tools for the molecular epidemiology, pathogenesis and molecular biology of viruses that cause VHF, arboviral diseases, rabies and other emerging zoonoses. The Unit is also actively involved in training of international scientists on diagnosis of VHF and arboviral diseases and also contributes to the training of several post-graduate students in the field.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

DIAGNOSTIC AND SURVEILLANCE ACTIVITIES

Special Pathogens Laboratory

Comparison of specimens received in 2008 and 2009

A total of 149 clinical specimens were investigated in 2009 for suspected VHF (Table 1). This compares to 160 specimens in 2008 and 195 specimens in 2007. There was a six-fold increase in the number of specimens received for investigation of suspected VHF cases from outside of South Africa. This was related to the investigation of suspected RVF cases from previous outbreaks in Africa and response to the outbreak of Ebola in the Democratic Republic of Congo. The number of locally investigated cases decreased slightly compared to submissions in 2008 when an outbreak of Lujo virus was reported from Johannesburg. A total of 62 sera were collected from contacts during the Lujo virus outbreak at a hospital in Johannesburg. The intention is to test them to determine if any asymptomatic or mild cases went undetected during the outbreak. Development of diagnostic assays to do this is currently underway.

Table 1: Comparison of specimens received in the Special Pathogens Unit in 2008 and 2009

Specimens	Received in 2008	Received in 2009
Diagnostics		
Suspected VHF (South Africa)	138 (68 patients)	65 (58 patients)
Suspected VHF (other countries)	10 (9 patients)	67 (66 patients)
VHF contacts	7 (7 persons)	62 (62 persons)
Undiagnosed fevers	5 (4 patients	2 (2 patients)
Suspected rabies	81 (51 patients)	123 (68 patients)
Rabies immunity	108 (106 persons)	113 (108 persons)
Ticks	1 (1 accession)	1 (1 accession)
Miscellaneous	56 (13 accessions)	800 (40 accessions)
Sub total	**406**	**1 232**
Surveys		
Human	201 (10 accessions)	1 039 (8 accessions)
Livestock	7 323 (6 accessions)	869 (2 accessions)
Wildlife	666 (8 accessions)	393 (3 accessions)
Sub total	**8 190**	**2 301**
Grand total for specimens	**8 596**	**3 533**

Investigation of suspected viral haemorrhagic fevers

Only three cases of Crimean-Congo haemorrhagic fever (CCHF) were laboratory confirmed in South Africa for 2009 (Table 2). This compares to 11 cases in 2008 and a single case in 2007. These cases, I female and 2 males between the ages of 40 and 58, were reported respectively from the Northern Cape (n=1), Western Cape (n=1) and the Orange Free State (n=1). Mortality rate was 33 % (n=1). One case was confirmed in May 2009 and the other two cases during November 2009. The cases from the Western Cape and Free State had known tick bite exposures, but the source of the exposure for the case from the Northern Cape (the town of Prieska) could not be clearly determined. Nevertheless, CCHF has been frequently reported from the Northern Cape, specifically the Prieska area and in the absence of any evidence of handling of possibly tainted blood or meat, an unnoticed tick bite, is the likely source of exposure.

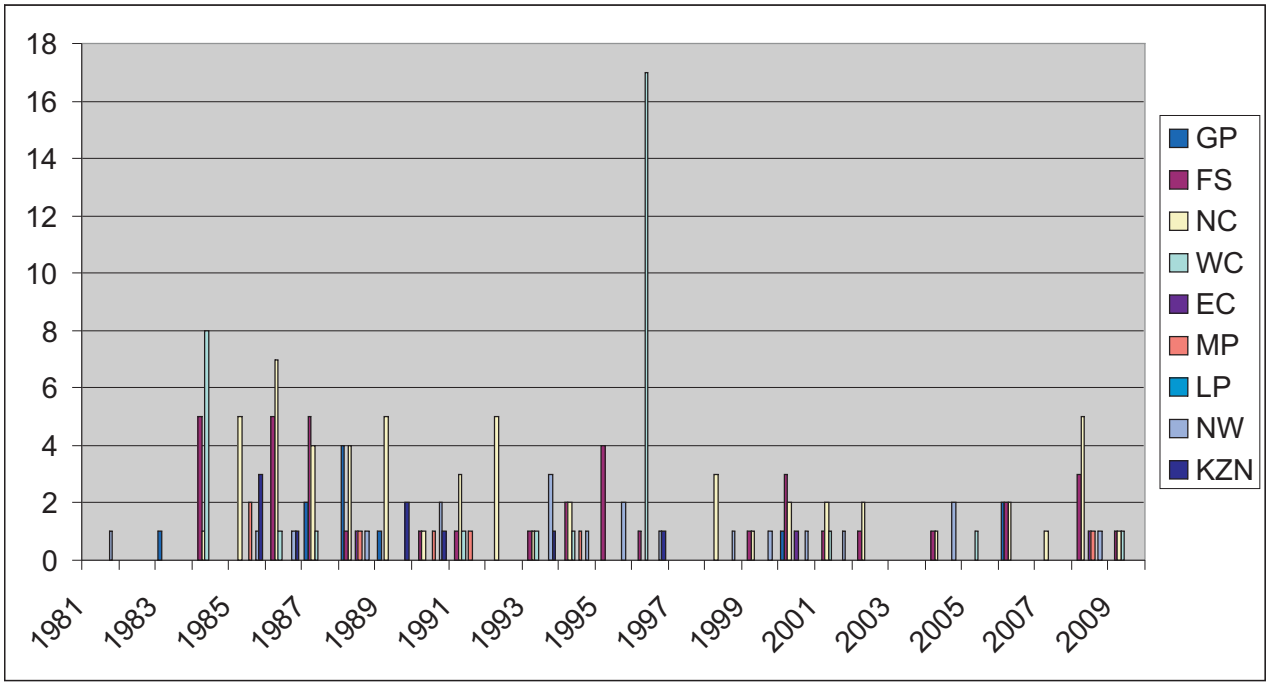
CCHF is reported from more than 30 countries in Sub-Saharan Africa, southeast Europe, the Middle East and Asia. Surveillance for CCHF is typically low and usually based on passive approaches but several countries including Turkey and Iran report between 5 and 200 cases per year (in 2007 Turkey reported 717 cases). Case-mortality rates are usually in the range of 30 % or lower. CCHF has been recognized in South Africa since 1981 and has been reported from all the provinces except the Limpopo province (Figure 1). Since it original description in South Africa up to 20 cases are laboratory confirmed annually with an average of 6.1 cases per year.

Human rabies in South Africa

A total of 15 human rabies cases were confirmed in South Africa during 2009 compared to 17 for the previous year and 14 in 2007 (Table 3). These cases where reported from the Eastern Cape (n=7); KwaZulu Natal (n=4); Limpopo (n=2) and Mpumalanga (n=2) provinces. Seven of these cases were positively linked to dog exposures but a source of exposure was not known or reported for the remaining cases. In addition, six cases were confirmed from Namibia compared with four cases during 2008.

Table 2: Laboratory confirmed cases of CCHF for South Africa, 2009

Patient	Age/Sex	Town/district/ province of exposure	Month of exposure	Source of infection	Laboratory results			Outcome
					Virus isolation	PCR	IgG/IgM	Died/survived
LV	58/F[a]	Prieska, NC[c]	May	Unknown	Positive	Positive	Negative	Died
LG	40/M[b]	Stompneusbaai, WC[d]	November	Tick	Negative	Negative	Positive	Survived
TK	52/M	Senekal, FS[e]	November	Tick	Positive	Positive	Positive	Survived



GP: Gauteng; MP: Mpumalanga; NW: North West; LP: Limpopo; KZN: KwaZulu Natal; EC: Eastern Cape; WC: Western Cape; NC: Northern Cape; FS: Free State)

Figure 1: Laboratory confirmed cases of CCHF for South Africa, 1981-2009

Since 2005, more than ten cases per annum have been consistently reported. This is in contrast to an average of less than ten cases confirmed during the 90's through 2004. The rise in the number of cases has been partially attributed to the re-emergence of canine rabies in the Limpopo province. Since confirming 22 human cases during 2006, the outbreak appears to be waning with 1-3 cases being confirmed for 2007-2009. Alarmingly a drastic rise in the number of dog rabies has been reported from the Mpumalanga province since 2008, particularly from districts where it has been effectively controlled before. Although single confirmed human cases have been reported from the province a number of suspected cases have also been investigated. Common problems with achieving laboratory confirmation for these cases are the decline of consent for the collection of post mortem specimens and/or submission of inadequate specimens for testing. Likewise there has also been an increasing number of canine rabies cases from the Free State since 2000. Only five cases of human rabies have been confirmed in the Free State since 1983, with only one case since 2000. This case was reported in 2005 and was related to a mongoose exposure. Increasing number of cases has also been confirmed from the Eastern Cape in the past 5 years.

Rabies is a largely neglected and under-reported disease in most developing countries. Factors that attribute to this are the difficulty in clinical recognition of cases and low awareness of rabies amongst medical health practitioners and the public. The SPU supports initiatives to raise awareness and educate the public and medical practitioners about rabies. Dr Jacqueline Weyer presented a paper on human rabies in South Africa as part of the World Rabies Day activities in Upington, Northern Cape in September 2009 (Figure 2).

Arbovirus Laboratory

Investigation of arboviral disease

Fewer specimens were received for 2009 than for 2008, reflecting a drop in the number of animal specimens tested for epidemiological studies (Table 4). WN, DEN and YF viruses constituted the bulk of the arboviruses

A)

B)

Figure 2:

A) Invited speakers to the World Rabies Day Scientfic Meeting held in Upington, Northern Cape. From left to right, Dr Jacqueline Weyer (SPU, NICD-NHLS), Dr Claude Sabeta (Rabies Unit, Agriculture Research Council-Onderstepoort Veterinary Institute) and Mr Kevin le Roux (Allerton Veterinary Laboratory)

B) Dr Weyer addressing medical and veterinary staff of the Upington and Kimberley areas with regards to human rabies.

Table 3: Laboratory confirmed human rabies cases, 2009

Patient	Age/Sex	Location of exposure/ district	Date of exposure and animal involved	Date admitted	Date of death	Hospital of admission	Final hospital
BG	7/Unknown	Eshowe/ KZN[c]	3 December 2008 / Unknown	2009/01/08	2009/01/08	Mbolongowane	Mbolongowane
VB	5/M[a]	Mt Frere/ EC[d]	Unknown	2009/01/10	2009/01/13	Nelson Mandela Academic	Nelson Mandela Academic
BG	6/M	Ocilwane/ KZN	December 2008 / Dog	2009/01/07	2009/01/08	Ngwelezana	Ngwelezana
AM	5/M	Tsolo / EC	Unknown	2009/02/20	2009/02/21	Tsolo	NMAH
DR 99/09	Unknown	Mdantsane/ EC	Unknown	Unknown	Unknown	Unknown	Unknown
NM	10/M	Masekane / KZN	Unknown	2009/03/09		Ngwelezana	Ngwelezana
MA	10/F[b]	Polokwane / LP[e]	April 2008 / Dog		2009/04/27	Polokwane	Polokwane
AN	8/F	Amahlathi / EC	July 2008 / Dog	2009/05/08	2009/05/09	Stutterheim	Frere
LK	11/M	Mkhulu village / MP[f]	Unknown	2009/06/30	2009/07/11	Matikwana	Sandton Clinic
KM	7/F	Mthatha / EC	Unknown		2009/08/09	Nelson Mandela Academic	Nelson Mandela Academic
LM	3/F	Nyandeni / EC	August 09 / Dog	2009/08/24	2009/08/25	Nelson Mandela Academic	Nelson Mandela Academic
AM	3/M	Libode / EC	June 09 / Dog	2009/09/19	2009/09/20	Nelson Mandela Academic	Nelson Mandela Academic
MM	4/M	Utrecht / KZN	August 09 / Dog	2009/11/02	2009/11/06	Niemeyer Memorial	Niemeyer Memorial
OR	9/F	Tshilidzini / LP	Unknown	2009/11/03		Tshilidzini	Tshilidzini
CS	18/M	Nelspruit / MP	June 09 / Dog	2009/11/27	2009/12/04	Rob Ferreira	Rob Ferreira
EN	11/M	Oshekati / Namibia	December 08 / Cat	2009/01/12	2009/01/13	Unknown	Unknown
KA	30/M	Omuntele / Namibia	August 08 / Dog	2009/01/29	2009/02/06	Onandjokwe	Onandjokwe
DS	6/F	Unknown / Namibia	Unknown	Unknown	February 2009	Unknown	Unknown
JN	9/F	Unknown / Namibia	Unknown	Unknown	April 2009	Ipinge	Ipinge
H	30/Unknown	Unknown / Namibia	Unknown	Unknown	June 2009	Ipinge	Ipinge
HV	15/F	Unknown / Namibia	Unknown	Unknown	Unknown	Unknown	Unknown
AW	7/M	Mbabane/ Swaziland	Unknown / Dog	2009/09/28	2009/10/09	Mbabane/ Rob Ferreira	Mbabane

identified serologically. Anti-flavivirus haem-agglutinating antibodies tend to cross-react in the haemagglutinating antibody inhibition test (HAI), the routine test for screening for the more common arboviruses in southern Africa. As a result, patients often tested positive for a combination of WN, DEN and YF viruses. Unfortunately, paired acute and convalescent sera were received for less than 4 % of submissions from patients. This meant that significant rise in titre could not be demonstrated and the virus responsible for the infection could not be identified in many of the positive cases. In the case of a positive HAI result, we employ IgM capture enzyme-linked immunoassays (ELISAs) or immunofluorescent antibody assays (IFAs) to distinguish between recent and past infections and it is possible that a diagnosis may not be made if the haemagglutinating antibody titre is below detection limits during the early stages of infection and if the sample volume is too small to permit virus isolation. Such cases highlight the need for a detailed clinical assessment and case history to assist in identifying the causative virus.

Positive tests were obtained for 38 % of cases in 2009 compared to 24 % for the previous year. Three isolations of RVF virus from KwaZulu Natal were made in autumn and two cases were positive for RVF virus in nucleic acid assay in the Northern Cape in the spring of 2009. Two additional cases where confirmed by IgM ELISA alone. These two cases where from the KwaZulu Natal outbreak. Several patients who had travelled in the Far East demonstrated IgM antibodies to DEN viruses or viral RNA, including 2 dengue serotype 1 (DEN-1) strains. CHIK virus is an epidemiologically rare disease in southern Africa but is responsible for extensive epidemics in south-east Asian countries; CHIK RNA was detected in a specimen from a patient, who had visited India, and at least one anti-CHIK IgM-positive case was identified from that country; another IgM-positive case was identified from Northern Province. Recent infections with SIN and WN viruses were identified in more cases than in the previous 2 years. SIN and WN are two of the most common arboviruses in South Africa and, because they normally cause mild infections, tend to be underreported and are often identified in the differential diagnosis of other arboviruses or non-specific febrile disease in cases of “pyrexia of unknown origin” (PUO). A few cases originated during the winter months, when mosquito vector activity is typically minimal; it is possible that the mild weather and sporadic rain experienced during the 2009 “dry season” allowed for mosquito-borne transmission to occur unseasonably. Only one survey was conducted by the lab this year, screening for anti-YF virus haemagglutinating antibodies on request of an International AIDS Vaccine Initiative vaccine trial in Uganda.

Table 4: Comparison of specimens received for arbovirus testing in 2008 and 2009

Suspected arbovirus infections		No. of specimens and cases	
		2008	2009
Patient submissions	South Africa	462	358
	Other African countries	15	82
	Extra-African countries	27	36
	Total submissions	504	476
Antibody/virus isolation/PCR positives*	Sindbis	24	39
	Chikungunya	4	22
	West Nile	41	79
	Rift Valley fever	18	16
	Dengue	23	42
	Yellow fever	60	101**
	Ross River		1
	Total No. of positives	171	300
	Undiagnosed fevers	375	283
	SPU referrals	54	3
Cases	Humans	474	455
	Arthropods, pooled	31	1
	Vertebrates	287	5
	Total No. of cases	792	461

* Includes multiple infections and seroconversions per submission.
** Includes a serosurvey for resting antibodies in a sample from Uganda.

Outbreak of RFV in South Africa 2008-2009

Small, focal outbreaks of RVF have been reported in South Africa since 2008. In 2008 outbreaks were reported from Mpumalanga, Limpopo, Gauteng and the North West Provinces with a total of 18 confirmed human cases. In 2009 the outbreaks extended to the KwaZulu Natal and Northern Cape provinces with a total of 7 confirmed cases (Figure 3). These cases were confirmed by nucleic acid detection (Taqman based, real time PCR and Loop mediated isothermal amplification, or LAMP-based assays) and virus isolation or IgM ELISA. All of these cases were linked to occupational exposures and included veterinarians, veterinary students, farmers and farm workers, and also a staff member from a veterinary clinical research farm. All of these cases have reportedly recuperated without sequelae.

Prior to this outbreak, RVF was confirmed nine years ago, in 1999, in aborted buffalo in the Kruger National Park. No human cases were confirmed during this outbreak. The last reported cases of human RVF in South Africa prior to the 2008 outbreak were more than 30 years ago in the 1970s. Molecular investigations of isolates of the 2008 and 2009 South Africa outbreak indicated the close relation of the outbreak strains with isolates from the 2006-2007 East Africa outbreak (Figure 3). An isolate form Northern Cape in 2009 is closely related to a 2004 Namibian isolate.

RESEARCH ACTIVITIES

REAL-TIME REVERSE TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION ASSAY: A RAPID AND SENSITIVE MOLECULAR DIAGNOSTIC TOOL

Loop mediated isothermal amplification (or LAMP) was described for the first time in 2000 and was developed to address shortcomings of widely used polymerase chain reaction (PCR) methodologies. LAMP technology allows for amplification of nucleic acids at a single temperature (typically between 60 and 65 °C) which obviates the need for automated thermocyclers typically required for PCR. This also holds a cost benefit, since only simple equipment is required for LAMP tests compared to PCR assays. The simplicity of the reaction is extended to the detection of the amplification product. Assays may be designed for simple visual detection of the amplified product (detection of pyrophosphate byproduct of the amplication reaction or through incorporation of fluorescent dyes) without the need for additional equipment (Figure 4). It is this simplicity and the relative robustness of the equipment required that renders this technology particularly attractive for use in laboratories that may be less well equipped. For the same reason LAMP is also adaptable to the field laboratory setting and therefore should be a valuable tool during outbreak responses in remote locations.

The SPU has through its collaboration with the Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan developed reverse transcription-LAMP (RT-LAMP) to detect RVF and CCHF RNA in clinical specimens. The RVF assay proved to be highly specific with no cross-reactivity with other genetically related and unrelated arboviruses, whilst still detecting isolates of RVFV collected over a 50 year period from geographically distinct areas. Results obtained with this assay were in 100 % agreement with results from a Taqman based real time PCR for RVFV and virus isolation. Similarly the assay developed for the detection of CCHF proved to be highly specific although not as sensitive in detecting low levels of infectivity (or low RNA copy numbers) as compared to a Taqman based, real time PCR for CCHF. The assay will further be optimized to increase the sensitivity of detection. Extension of the application of this assay to other known causes of viral haemorrhagic fever is underway.

EVALUATION OF A RECOMBINANT RVFV NUCLEO-CAPSID PROTEIN AS A VACCINE IMMUNOGEN IN SHEEP

The immunogenic properties of a recombinant RVFV nucleocapsid protein (recNP), in combination with two adjuvants shown to be effective in mice, were evaluated in an experimental sheep model (Figure 3). The recNP was highly immunogenic with adjuvants, resulting in detectable antibody responses within 7 days after a single immunization. Immunization of sheep with recNP resulted in reduced IgM responses after RVFV challenge, but was not able to decrease viral replication.

COMPARATIVE EVALUATION OF ELISA-BASED TECHNIQUES FOR THE DETECTION OF ANTIBODIES TO RIFT VALLEY FEVER VIRUS IN THERMO-CHEMICALLY INACTIVATED SERA

To compare the diagnostic sensitivity of a range of in-house developed Rift Valley fever serological ELISAs, a direct comparison was done of the detection of antibody in pre-inactivated sera using a simple, yet 100% effective, thermo-chemical inactivation treatment. Results in naïve and treated sera from experimentally infected sheep demonstrate that inactivation method used had no adverse effect on ELISA readings but the assays analysed differ in their ability to detect the early humoral responses to infection with RVFV (Figure 6). The IgM-capture ELISA was slightly more sensitive than the IgG-sandwich ELISA to detect early humoral response after infection. The indirect IgG ELISA, using Protein G HRPO was less sensitive in detecting seroconversion than the IgG-sandwich ELISA but this problem was alleviated when anti-sheep IgG conjugated with HRPO was used. The high concentration of viral antigen in sheep sera collected shortly after infection might contribute to false-positive results in the inhibition ELISA but its ability to detect seroconversion was comparable to that of IgM-capture ELISA.

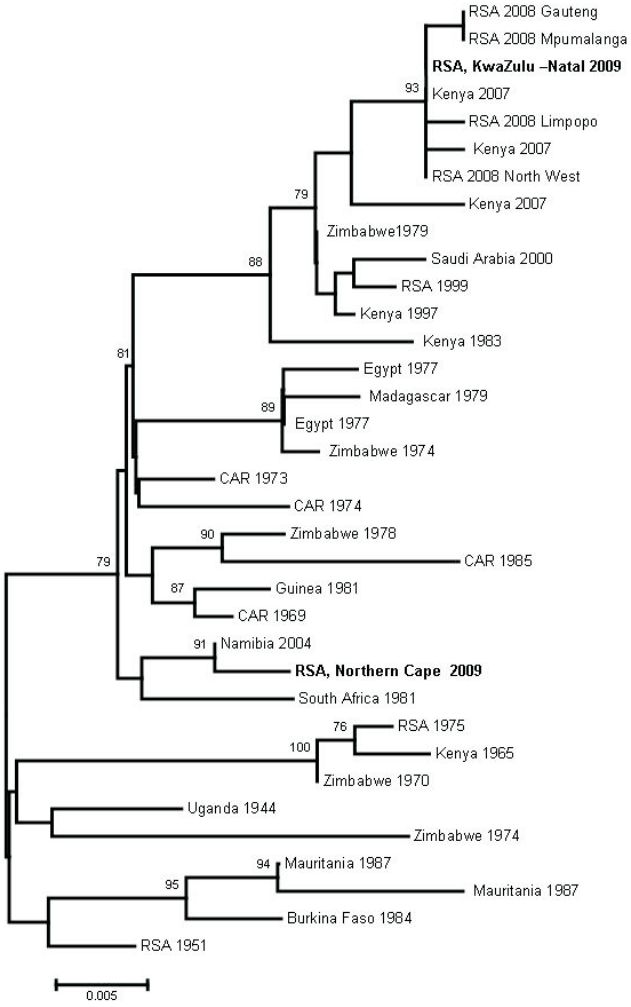


Figure 3: Neighbour-joining tree of the G2 glycoprotein of historical and recent RVF virus strains. Values at nodes indicate the level (%) of bootstrap support from 1000 replicates. South African 2009 isolates are indicated in bold.

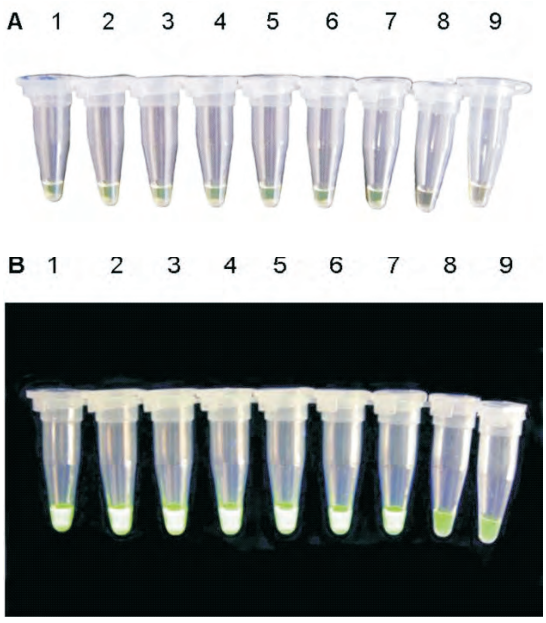


Figure 4: Visual detection of RT-LAMP amplification products in 10-fold dilutions of RVFV RNA.
A) Simple detection of pyrophosphate precipitate (white precipitate at bottom of tube) indicates a positive reaction; alternatively
B) a fluorescent dye can be added to the reactions. Tube 1 to 8 from left to right, RT-LAMP products yielded from 10-fold serial dilutions of RNA prepared from infective tissue culture supernatant containing $10^{6.8}$ $TCID_{50}$/ml of AR20368 RSA 81 isolate of RVF virus; Tube 9, negative control.

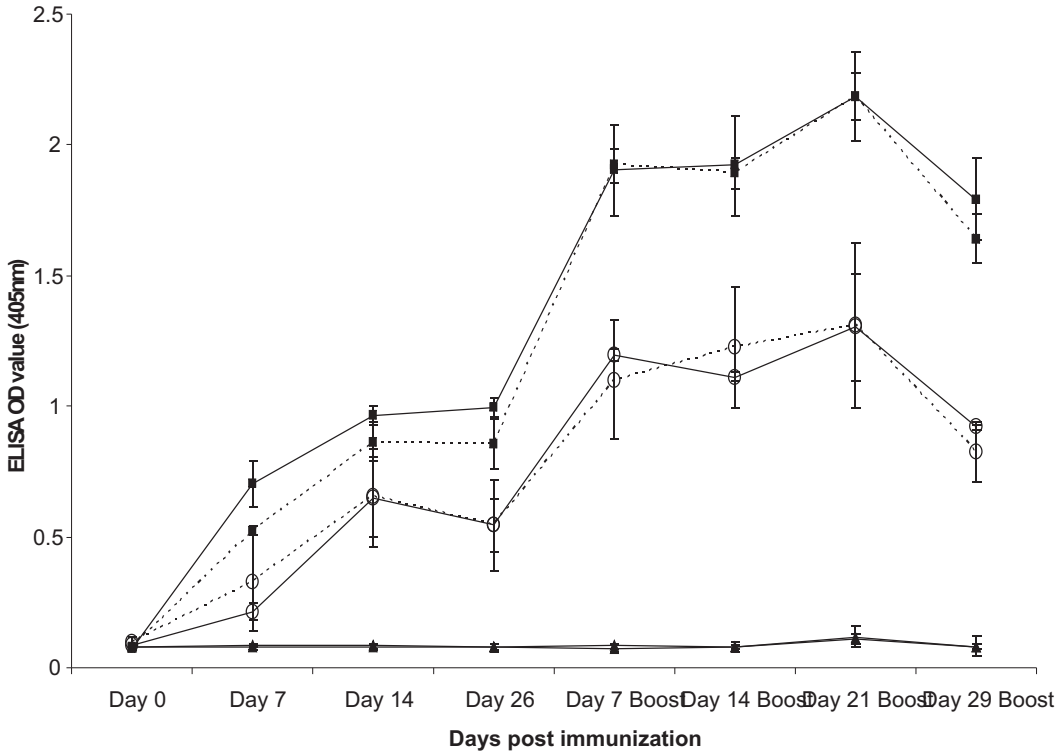


Figure 5: RVFV N-protein specific IgG responses in sheep after recNP immunization and booster immunization as measured by ELISA. The recNP/adjuvant combinations are indicated as follows: 175µg recNP/Alhydrogel (—○—), 350µg recNP/Alhydrogel (--○--), 175µg recNP/SaponinQ (—■—), 350µg recNP/SaponinQ (--■--), adjuvant and PBS controls (—▲—). The mean OD values for two sheep in each group are shown, and error bars indicate the standard deviations from the means.

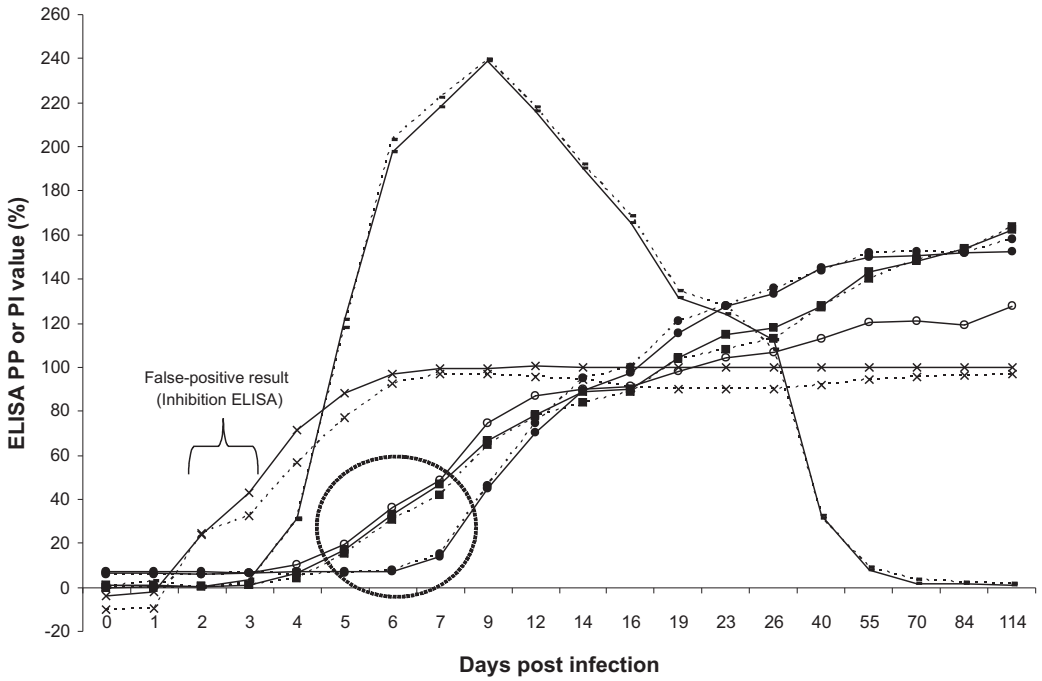


Figure 6: Comparison of immune responses in three experimentally infected sheep as measured by testing naïve (solid lines) versus thermo-chemically inactivated (dotted lines) sera by IgM- capture ELISA (—), inhibition ELISA (x), indirect ELISA Protein G HRPO (●), indirect ELISA anti-sheep IgG HRPO (○), and IgG- sandwich ELISA (■). Note level of sensitivity of indirect ELISA when using different HRPO conjugates, as indicated by the dotted circle.

BATS AS RESERVOIRS FOR EMERGING INFECTIOUS DISEASES

To date, more than 70 different viruses have been isolated or detected in tissues of different bat species. Although not all of these have been linked to disease in either animal or man, a substantial number of them have. SARS coronavirus which caused a global outbreak of severe respiratory illness in 2002-2003 has been positively linked to three species of horseshoe bats from the Orient. Since 1999 several outbreaks have been linked to Nipah virus. The latter is associated with life-threatening encephalitis in humans and animals and is carried by fruit eating bats. Likewise Hendra virus has been associated with fruit bats in Australia and outbreaks of encephalitis in equine and humans. Mounting evidence also indicates that Marburg virus is maintained in nature by cave-dwelling fruit bat species.

SPU conducts research towards elucidating the ecology, epidemiology and pathology of zoonotic agents associated with bats. As part of this goal the Unit has maintained and bred a fruit bat (*Rousettus aegyptiacus*) colony since 2004 (Figure 7). This colony represents a rare and sought after experimental animal model. Mrs Busi Mogodi, the SPU Animal Technologist reported on the breeding of these bats in captivity at the South African Association for Laboratory Animal Science conference and received the prize for the best poster presentation (Figure 8). This colony will be used in 2010 for filovirus inoculation studies which should shed light on the pathology of Marburg and Ebola viruses in this species and the study of recombinant coronaviruses.

A)

B)

Figure 7:
A) Egyptian fruit bats (*Roussetus aegypticus*) crawling along the wire-mesh of their cage.
B) A SPU staff member inspecting bats in their cage.

A)

B)

Figure 8:
A & B) Mrs Busi Mogodi being congratulated on the best poster presentation at the SAALAS conference.

Members of SPU participated in an ongoing project of the Centers for Disease Control and Prevention and the Uganda Ministry of Health to Uganda to investigate the role of Egyptian fruit bats (*Roussettus aegyptiacus)* as reservoir of Marburg virus. An outbreak of Marburg haemorrhagic fever was diagnosed in miners in Kitaka Mine, Uganda, in 2007 and the source of the infection was linked to Egyptian fruit bats during a follow-up investigation(Towner et al., 2009, PLoS Pathog. 5(7):e1000536). Based on recommendations from the WHO Outbreak Response Team, the Ugandan Ministry of Health stopped mining operations at Kitaka to interrupt transmission of Marburg virus (MARV) to humans. The following year, Marburg haemorrhagic fever was confirmed in a Dutch tourist who had visited the bat-infested Python Cave not more than 40 km from Kitaka Mine (Timen et al., 2008, EID 15:1171). Partly because the bats in Kitaka Mine were under heavy persecution, presumably in an effort to get the mine reopened, the focus of the ecological investigations shifted to the Python Cave in Maramagambo Forest. Two members of the SPU staff joined CDC-Atlanta Special Pathogens Branch (SPB) and Uganda Wildlife Authority members on three field trips in 2009 to sample the Egyptian fruit bat colony in the Python Cave at different seasons. The studies were concluded in November with the collection of pregnant females, after earlier visits in February/March and August/September to sample new-borne pups and lactating females. Bundibugyo and the Semliki Forest Reserve was visited at the end of the Maramagambo trip to investigate the feasibility of commencing a study on the role of forest bats in Ebola haemorrhagic fever and to investigate some bat caves in the foothills of the Ruwenzori Mountains (Figure 9-11). Bundibugyo was the location of an outbreak of haemorrhagic disease caused by a new strain of Ebola virus in 2007. Virological studies are being completed at SPB in Atlanta.

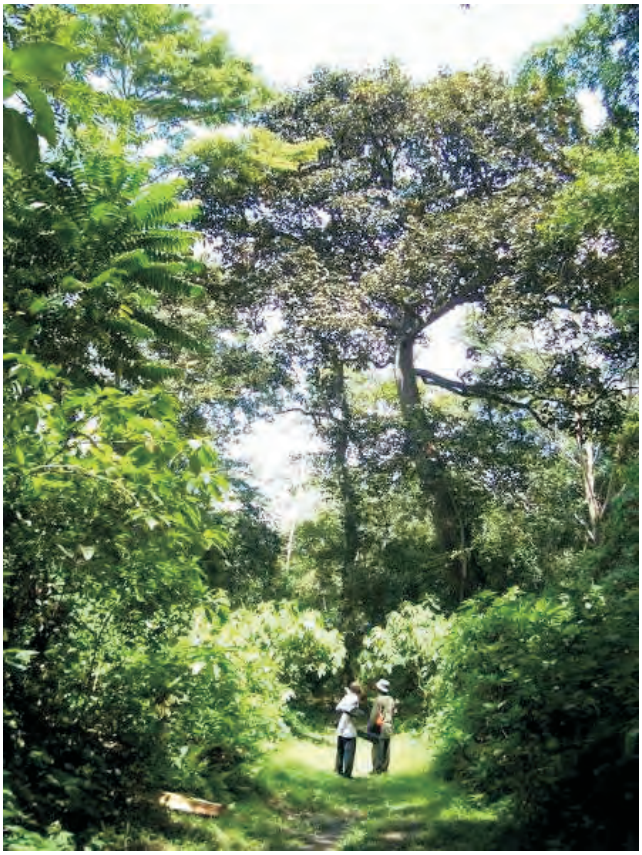


Figure 9: Mist-net site for sampling forest-dwelling fruit bats in Semliki Forest.

Figure 10: Fruit pulp spats on the forest floor, ejected by fruit bats after extracting the juice from wild figs.

Figure 11: *Epomophorus* species fruit bat collected by canopy mist-net in Semliki Forest.

EPIDEMIOLOGY OF HUMAN RABIES IN SOUTH AFRICA

Despite established and effective preventative and control measures for rabies, it is still a considerable veterinary and public health problem particularly in developing countries. The WHO conservatively estimates up to 55 000 human deaths attributed to rabies primarily from Asian and African countries annually. The public health burden is further compounded by the economic impact of the relatively expensive rabies post exposure prophylaxis with hundreds of thousands of doses administered annually globally and the anxiety coupled with animal exposures

and the possibility of rabies. In South Africa rabies is hyper-endemic in domestic dogs in KwaZulu Natal, Eastern Cape, Mpumalanga, Free State and the Limpopo provinces. The emergence of dog rabies in Free State, Mpumalanga and Limpopo provinces are more recent after 2000. In addition to the circulation of "classic" rabies viruses three other rabies-related lyssaviruses namely Duvenhage, Lagos bat and Mokola viruses have also been reported from Southern Africa. The epidemiology of these viruses remains obscure with the reservoir of Duvenhage and Mokola viruses still to be confirmed. Lagos bat virus has been associated with a fruit bat reservoir.

In collaboration with the University of Pretoria, a retrospective study of laboratory confirmed human rabies cases in South Africa, 1983-2007 was conducted. The study highlighted several features of human rabies in the developing countries using laboratory confirmed data. The majority (more than 80 %) of cases where linked to dog exposures despite the co-circulation of rabies virus in several wildlife species and also the presence of rabies-related lyssaviruses. In fact, very few cases were linked to wildlife exposures despite the known prevalence of rabies in mongoose, black-backed jackal and bat-eared foxes in the country. Rabies as a disease of children was underpinned with nearly 70 % of cases reported in under 20 year olds. The study also reported on health system failures in human rabies cases which included the failure of providing post exposure prophylaxis (PEP) and deviation of recognized PEP which results in treatment failures. This study also commented on the contribution of rabies-related viruses to the public health burden of rabies. Although the potential risks associated with infection with rabies-related viruses are appreciated, this study could not find any additional cases of human rabies due to infection with Duvenhage, or infections with Lagos bat or Mokola virus. Analysis of alternative sources of data such as dog bite registers could be an important contribution toward estimating and defining the true public health burden of rabies in countries were surveillance and clinical confirmation are low.

MOLECULAR DIAGNOSIS OF HUMAN RABIES

Human rabies remains an under-reported disease in most of the developing world for a variety of reasons. Firstly, rabies is not easy to diagnose clinically with two forms of presentation, the encephalitic and paralytic form. The encephalitic form includes most prominently hallucinations, altered behaviour but with periods of lucidity and often hydrophobia (and aerophobia). The paralytic form is not unlike poliomyelitis with a descending paralysis and coma. Clearly the list of alternative diagnosis is long and include encephalitis with a variety of etiologies, cerebral malaria, poliomyelitis and even intoxications or poisoning. There are no routine blood tests that are informative in rabies cases and specific and specialized tests are required to confirm cases. Rabies can be confirmed ante- or post mortem. Confirmation of rabies cases on post mortem brain specimens using the fluorescent antibody test remains the gold standard for rabies diagnostics. Nevertheless obtaining brain specimens is becoming increasingly challenging due to consent issues and cultural and religious beliefs. There is a trend in more of the cases being confirmed using ante-mortem specimens (including saliva, cerebrospinal fluid and nuchal biopsies) with PCR and therefore becoming a valuable source of data. Although ante-mortem confirmation is not always useful in the management of the patient it does for example exclude the possibility of treatment for other curable encephalitides.

In collaboration with the University of Pretoria, SPU has developed and validated a real time PCR assay for the detection of all rabies-causing lyssaviruses. This assay allows for sensitive and rapid (two hour turnaround time from receipt of specimen in the laboratory and result) detection of lyssavirus RNA in clinical specimens. It improves on previously reported molecular assays for rabies by including the detection of all known lyssaviruses (especially the genotypes circulating in Africa and South Africa) through the use of a single fluorescent-labelled probe and also the provision of validation data derived from a variety of clinical specimens. The assay has since been put into use for the routine diagnosis of human rabies cases in South Africa.

COLLABORATORS

Our collaborators include:

- Prof K Morita and Dr T. Kubo, Nagasaki University, Japan: Development of loop-mediated amplifications assays for rapid molecular diagnosis of VHF and arboviral disease.
- Prof C Drosten, Institute of Virology, University of Bonn Medical Centre, Bonn, Germany: Ecology and pathology of SARS an archetypal zoonosis.
- European Network for Capacity Building and the Control of Emerging Viral Vector Borne Zoonotic Diseases (ARBO-ZOONET): An international research programme funded by the European Union Fp7 Cooperation Work Programme Food, Agriculture and Fisheries and Biotechnology.
- Biological Diagnostic Supplies Limited Flow Laboratories, Scotland: Development of validation of immunoassays for diagnosis of VHF.
- Dr MT Hiese, University of North Carolina, USA. Alphavirus-derived replicon vaccines against RVF.
- Drs Louis Nel and Wanda Markotter, University of Pretoria. Lyssavirus surveillance project; Molecular epidemiology of human rabies cases in South Africa; Development and validation of a Taqman based, real time PCR assay for lyssaviruses; Pathogenesis of Lagos bat virus in a bat model.
- Prof Marietjie Venter, University of Pretoria and the National Health Laboratory Service. Investigations into flavivirus disease in South Africa.
- Prof Ian Lipkin, University of Colombia. Full genome sequencing of a emerging zoonotic viral agents.
- IAEA, Austria. Inter-Laboratory proficiency testing of a Rift Valley fever ELISA and Stage I validation of a new recombinant RVF ELISA.

CAPACITY BUILDING

Post graduate students

Various SPU staff members were pursuing post graduate qualifications during 2009. Mr Petrus Janse van Vuren was enrolled as a PhD student at the University of the Witwatersrand with a thesis entitled, "Evaluation of a recombinant RVFV nucleocapsid protein as a recombinant vaccine and an immunodiagnostic reagent". Miss Chantel A. le Roux was registered for the MSc programme of the University of Pretoria with a project entitled: "Development and validation of real-time loop mediated isothermal amplification assay for rapid diagnosis of zoonoses of public health importance." A number of postgraduate students are hosted at SPU in collaborative projects with the Universities of Pretoria and Witwatersrand.

Southern African Centre for Infectious Diseases Surveillance (SACIDS)

SACIDS is a "One Health" consortium of national academic and research institutions in the medical and veterinary field of SADEC countries (including Democratic Republic of Congo, Mozambique, South Africa, Tanzania and Zambia).

Arbo-Zoonet Technical Workshop on RVF diagnostics

Arbo-Zoonet is an international network for capacity buidling for the control of emerging vector borne viral zoonoses facilitated by the European Union. As part of these activities, SPU hosted a Technical Diagnostic Workshop on RVF diagnostics during 24-27 March 2009. The workshop was attended by 16 participants from Africa (Senegal) and several European countries (Belgium, Germany, France, Italy, Slovenia, Spain and the United Kingdom) (Figure 12). SPU staff members presented practical and theoretical sessions on various aspects of RVF diagnosis including molecular and serological assays, but also the pathology and epidemiology of the disease. A comprehensive report on this workshop is available at http://www.arbo-zoo.net.

Figure 12: Participants of the Technical Diagnostic Workshop on RVF diagnostics.

Viral Diagnostics Unit

ENTEROVIRUS SECTION

BACKGROUND

The Enterovirus Section of the Viral Diagnostic Unit provides diagnostic enterovirus isolation testing and serves as a WHO Regional Reference Laboratory for poliovirus isolation. In this capacity, the enterovirus laboratory serves as a National Laboratory serving seven countries within Africa in AFP surveillance, including South Africa, Botswana, Namibia, Lesotho, Angola, Mozambique and Swaziland. In addition, the laboratory performs confirmatory testing on samples tested in other WHO reference labs as well as parallel testing to determine accuracy of results released for countries that do not meet accreditation criteria. The Enterovirus Section also performs Coxsackie B and poliovirus 1-3 serology. The poliovirus serology testing allows for the immune status of new employees to be determined and vaccination if necessary.

ACTIVITIES

Specimens tested for poliovirus from AFP cases
Total: 2547

Table 1: Frequency by country

Country	Frequency	Percent	Comments
ANG - Angola	647	25.4%	Routine
BEN - Benin	3	0.1%	Confirmation
BFA – Burkina Faso	1	0.0%	Confirmation
BOT - Botswana	20	0.8%	Routine
CAE - Cameroon	2	0.1%	Confirmation
CAF – Central African Republic	293	11.5%	Parallel
CHA - Chad	64	2.5%	Parallel
CIV – Ivory Coast	2	0.1%	Confirmation
ETH - Ethiopia	2	0.1%	Confirmation
GUI - Guinea	4	0.2%	Confirmation
LES - Lesotho	36	1.4%	Routine
MAI - Mali	8	0.3%	Confirmation
MOZ - Mozambique	481	18.9%	Routine
NAM - Namibia	115	4.5%	Routine
RDC – Democratic Republic of Congo	1	0.0%	Confirmation
SIL – Sierra Leone	1	0.0%	Confirmation
SOA – South Africa	838	32.9%	Routine
SWZ - Swaziland	20	0.8%	Routine
UGA - Uganda	6	0.2%	Confirmation
ZAM - Zambia	3	0.1%	Confirmation
Total	2547	100.0%	

Table 2: Virus Isolated

Virus	Frequency
Non-polio enterovirus	268
Non-enterovirus	29
Vaccine polio 1	30
Vaccine polio 2	42
Vaccine polio 3	39
Wild polio 1	68
Wild polio 2	0
Wild polio 3	87

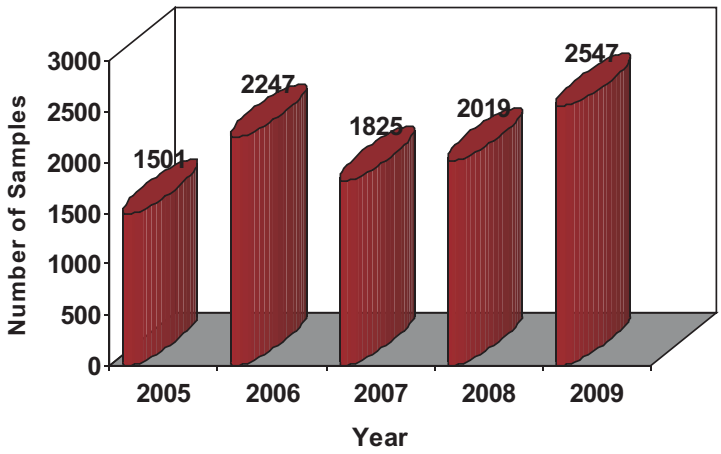


Figure 1: Comparative analysis of AFP samples tested over a 5 year period

Specimens tested for diagnostic purposes (Non AFP)
Total: 88

Specimens tested for coxsackie B and poliovirus 1-3 antibody titres
Total: 543
Coxsackie B 1-6: 361. Tests performed: 2166
Poliovirus 1-3: 187. Tests performed: 567

HIGHLIGHTS AND ACHIEVEMENTS

Joint Annual Workshop of the Measles and Polio Lab Networks, held in Entebbe, Uganda: Attended by S. Moonsamy. Objectives were to update participants on the current status and management of the networks and propose solutions to challenges and constraints, assess in-house QC procedures, and introduce new SOPs and orient participants in their use towards improved data quality and feedback.

COLLABORATIONS

WHO HQ AFP Surveillance
WHO AFRO AFP Surveillance
CDC Confirmatory testing
RIVM Proficiency testing

CAPACITY BUILDING

In Service student technologist
Microbiology/Virology registrars

VIRUS ISOLATION SECTION

(Respiratory & General)

BACKGROUND

VIRUS ISOLATION LABORATORY

- Conducts influenza surveillance in order to understand the impact of Influenza in our country. The section has been part of the Viral Watch programme since 1984, a programme that started out with only 10 sentinel sites situated in Gauteng, but has since expanded into a country wide programme with 256 sites. The programme provides data on circulating, seasonal Influenza strains and during 2009 it was actively involved in the identification of the novel pandemic Influenza A/H1N1.
- The section serves as part of the National Influenza centre (NIC) for the WHO global Influenza programme. The laboratory thus provides the regional WHO Influenza Laboratories with isolates for vaccine efficacy studies as part of the global vaccine formulation.
- In addition, the laboratory supports various African countries (including Seychelles) with outbreak diagnosis and laboratory staff training.
- Additional activities include the Severe Acute respiratory infections (SARI) project. The laboratory is responsible for culturing viruses (influenza, parainfluenza (PIV), respiratory syncitial virus (RSV) and Adenovirus) from samples testing positive by PCR to provide isolates for molecular studies.
- Provides diagnostic support for an Influenza vaccine trial that looks at the influence of HIV on vaccine response. The study is performed at Chris Hani Baragwanath hospital, Johannesburg.
- Provides primary diagnostic services for local academic hospitals and some private laboratories/ clinicians in the early detection of aetiological agents e.g. transplant units, CMV cultures, HSV, CMVpp65 antigen tests etc.)
- Is part of the WHO Measles regional reference laboratory activities. Sera and urines (or throat swabs) are sent to the NICD for Measles virus detection and surveillance. The serology Laboratory performs the IgM testing and the urines are processed by the isolation laboratory for followup molecular epidemiological studies.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

- A total of 6623 samples were received and processed by the laboratory (1779 from Viral Watch sites).
- 1828 isolates were made (isolation rate of 28.8%) For isolation results see Table 1.
- The Influenza season lasted from week 19 to 29 and peaked in week 24, with a second wave the result of the pandemic strain between weeks 29 and 39. The laboratory played a crucial role in surveillance and diagnostics See Figure 1.
- In February the NICD hosted an Influenza evening/day, inviting all the relevant parties i.e. viral watch doctors, staff members etc. to expose the clinicians to the laboratory environment, familiarize them with techniques employed and to give feedback of the successes and failures from the previous year.
- Provided inter-laboratory comparison Influenza panels for 2 other NHLS virology laboratories. (Albert Luthuli and Tygerberg).
- Provided controls for validating PCR pandemic Influenza tests used by private laboratories.
- In March Mrs Cardia Fourie presented a talk on Influenza at the Davies Diagnostics day 2009, Gauteng.
- Mrs Amelia Buys (supervisor, medical technologist) was invited as facilitator to a CDC Influenza workshop held in Nairobi, Kenya during August 2009.
- In October Mrs Cardia Fourie (Lab LQR) was invited to Kimberley NHLS laboratory to facilitate in QA training (introduction to QMS including accreditation).
- Mrs Fourie also presented an influenza talk on behalf of the Orkney SMLTSA Central-North-West branch (October).

Table 1: Isolation results

Culture Result	Total
Adeno	14
CMV	34
CMVpp65	113
HSV	4
RSV	23
PIV	17
Influenza A	1516
Influenza B	107
Total	**1828**

COLLABORATIONS

The Laboratory serves as a National influenza centre for WHO.
An African Measles Workshop was hosted in collaboration with CDC from 14-18 September 2009.

CAPACITY BUILDING

INFLUENZA TRAINING TO STRENGTHEN AFRICAN LABORATORY SURVEILLANCE

A scientist from Mozambique (Ms Noorbebi Adamo) was trained in February 2009. Techniques covered were cell culture, specimen preparation, shell vial and immunofluorescence.

ROTATING VIROLOGY REGISTRARS

Drs Sim Mayaphi and Marcelle Erasmus (from University of Pretoria) were trained during March.

An intern medical technologist student (Mduduzi Buthelezi) was also trained.

CELL CULTURE SECTION

BACKGROUND

The function of the Cell Culture laboratory is the production and banking of the different cell lines used by the Enterovirus, General and Respiratory Isolation laboratories for routine testing. These are: RD, L20B, A549, R-MIX, Vero-slam, MDCK and Vero.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

There was a marked increase in the demand for MDCK cells this winter season. Not only was there the increased routine influenza surveillance activity (Viral Watch), there was also the H1N1 influenza (Swine Influenza) outbreak around August through to November. This entailed increased ordering and distribution of viral transport medium to all participating practitioners and NHLS laboratories countrywide. There is also the ongoing measles outbreak which requires an increase in Vero-slam cells.

MEASLES TRAINING WORKSHOP

A member of the cell culture laboratory was a facilitator during the measles training workshop held at the NICD training laboratories and organised by the World Health Organisation and the Centers for Disease Control. All the cells (Vero-slam) and media used during the workshop was supplied by the cell culture laboratory.

COLLABORATIONS

The laboratory supplies WHO network laboratories within Africa with cells for measles (Vero-slam), influenza (MDCK) and polio (L20B and RD) isolation on request.

CAPACITY BUILDING

One in-service biomedical technologist student was trained in all aspects of cell culture.

SEROLOGY SECTION

BACKGROUND

The Serology Section serves various functions, including the reference laboratory for Measles and Rubella Serology testing for South Africa, while serving as the regional reference laboratory for the WHO-supported program for Measles and Rubella virus control. The NICD's other major role includes coordinating the laboratory testing for the Annual Antenatal HIV-1 Prevalence Survey, as well as Incidence testing for the survey. The laboratory supports various collaborations, such as the in end point diagnostic testing for the HIV Vaccines Trial network (HVTN). The serology laboratory also serves as the reference laboratory for the South Africa National Department of Health (NDoH) for HIV Rapid Kit evaluations and post marketing surveillance of kits selected by the NDoH. The lab provides quality assurance testing for major collaborators including the provision of HIV external quality assurance (EQA) and internal quality assurance (IQC) panels.

For 2009, the section continued to extend its activities to collaborate in various research areas for the various collaborators, described below.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

The serology section supports numerous activities related to HIV-1 and HIV-2, HSV 2 IgG, measles and rubella surveillance:

- **Measles and Rubella Surveillance:** Measles and Rubella testing forms part of routine testing. In 2009, the laboratory confirmed Measles outbreak in Gauteng Province. The outbreak was initially identified in March 2009, with a few sporadic cases. The numbers gradually increased over the months, peaking from September to December 2009. Increased number of positive cases was also seen in KZN, Eastern Cape, North West, Free State, Mpumalanga and the Western Cape Provinces. An approx. sample size of 14 772 samples were tested for Measles and Rubella in 2009.
- **National Antenatal Sentinel HIV and Syphilis Prevalence Survey in South Africa:** This survey is conducted every year in the months of October since 2002. The Serology section not only performs the testing for the Gauteng province but coordinates the national survey that includes weekly reporting to the National department of Health in terms of targets for testing. Testing is conducted for HIV Serology and RPR on pregnant women attending the antenatal clinics in the month of October. With the increase in sample size testing is now extended to the first week of November. In October 2006 the samples size for ANSUR was increased from 3 500 to approx. 7 300. Various approaches to improve management have been introduced given the magnitude of the survey. Antenatal survey Incidence Testing was performed on serum samples using the HIV-1 BED ELISA for 2008 samples from Gauteng and KwaZulu Natal Provinces. The incidence testing for the Western Cape Province is currently underway.
- **CIET BNS SURVEY: CIET** is currently determining the effect of a community-wide behavioural intervention programme to determine the effect on HIV prevalence. This survey required HIV-1/2 antibody testing on DBS samples from three countries (Botswana, Namibia and Swaziland). Incidence testing will be performed on the positive samples but this will only be done in line with CIET's next survey which is targeted for 2011.
- **HVTN Trial (HIV Vaccines Trial Network):** The HVTN 503 study involves the use of the Merck Study

Vaccine "Merck Adenovirus serotype 5 HIV-1 *gag/pol/nef* in participants enrolled in the study. The sites involved are Soweto, Cape Town, CAPRISA, KZN, KOSH and Medunsa. The Serology Unit performs the In-Study HIV Testing and Recent Exposure/Acute Infection Testing, using the *env*-based Biorad Multispot Rapid HIV1/2 test kit and the Biorad Genetic Systems HIV-1 Western Blot assay as per algorithm. In addition to the 503 study, the lab is also involved with the HVTN 073 and HVTN 204 trial. The primary tests employed for the HVTN 073 and the HVTN 204 Post Study Testing (POS) protocols include 3 Enzyme Linked Immunosorbent Assays (EIAs); namely Biorad Genscreen HIV1/2 V2, Abbott Murex HIV-1.2.0 and the Biomerieux Vironostika HIV UNI-Form 11 plus O. End of Study (EOS) testing for HVTN 503 and HVTN 073 will use the same EIA's as used for POS testing. The introduction of new LIMS will result in the HIV Serology lab will serving as the repository and receive all HVTN shipments for 503, 073, 204, 802 and 404 trials.

- **Diagnostic Services: CERG Survey:** This study is conducted on cancer patients from the NHLS Cancer Research Institute. HIV-1/2 antibody testing is performed on serum samples sent in batches of approx. 550 samples per batch, three quarters of each year. **CLS (Contract Lab Services) Western Blot Testing`:** Samples from CLS for Western Blot testing are being sent to the NICD.
- **Mozambique DBS Re-testing for QA:** This survey involved the re-testing of DBS samples for quality assurance. Approx 1 200 samples were sent in November 2009. The testing and database submission was completed in December 2009.
- **New Start - DBS Re-testing for QA:** Since the inception of New Start in 2004, the NICD Serology lab has provided a supporting role for the quality assurance of HIV rapid testing. The initial program started with four major sites in Durban, Cape Town, Johannesburg and Bloemfontein. Currently the program has expanded nationally and is operational in almost all the nine provinces. The laboratory is currently re-testing samples from 15 sites The initial sample size for re-testing started at 10% of samples tested at the site on a monthly basis when the sites opened. This percentage was decreased in 2008 to 5% for 4 of the older and major sites. In July 2009, it was decided to stop the retesting of DBS samples from the 15 sites. The objective of the re-testing was a means of monitoring each site's competence to perform HIV rapid tests and report HIV results. The objective was also to identify training needs and procedural errors that may occur during testing and to ensure the Quality of test results. Since more sites are included and the client intake increased in some of the sites, the workload at the NICD increased dramatically. In 2009, the NICD lab received an estimated 1200 DBS samples per month from all New Start sites. In order to provide data in a timely manner to monitor and improve quality of HIV rapid testing for New Start, it was decided to:
  1. Gradually phase-out the DBS retesting method for the prominent sites and then the rest of the sites. This was accomplished in August 2009.
  2. Continue the DBS retesting as a final call for "discordant' HIV
  3. Rapid test results and an option for monitoring tester performance
  4. Train and pilot Dried Tube Serum (DTS) Proficiency Testing (PT) program for each New Start lab tester. Pilot study at 4 major sites and then implement in rest of sites. This was completed in October 2009
  5. Continue the supply of IQC sera to all sites
  6. New Start to increase on site evaluation and monitoring activities
- **HIV Rapid Kit Post Marketing Surveillance (PMS):** Post marketing surveillance was introduced as a compulsory aspect of the HIV rapid test kits used by the National Department of Health and is performed on HIV Rapid test kits from kits that are selected from the NDOH RT-41 tender (see below). In 2009, the lab designed a protocol for PMS and all those companies whose kits selected from the RT-41 tender were required to submit their batches to NICD for testing before releasing for distribution. The laboratory prepared a standard set of samples that was used on all 5 kits selected from the last tender. The panel consists of two controls (one negative and one positive), a dilution series of 1:10 to 1:640, 12 known negative samples and 12 known positive samples. A HIV-2 sample is included in the panel. Reports are generated for each lot number and forwarded to the relevant companies and the NDoH.
- **HIV Rapid Kit Testing for DOH RT-41 Tender:** Test kits are sent to the NICD every two years as part of a tender process. The evaluation is performed against defined sero-negative and positive specimens using WHO criteria. The last tender ended in March 2009 and a total of sixty three kits from various manufacturers were evaluated and were included in the selection process..
- **HIV-1 Incidence Testing:** Incidence testing is conducted each year on samples from the ANSUR survey for three Provinces (KZN, GAUTENG and WCP. In 2009, both the Gauteng Province and Kwazulu Natal 2008 samples were tested and results reported. Testing on the ANSUR samples is done after the current survey which is usually the year proceeding the survey. Incidence testing was also conducted on samples from IPM (International Partnerships for Microbicides) preparedness trials. These samples were screened at IPM clinics and the positive samples were stored and sent to NICD for BED and Avidity testing. NICD also performed incidence for the HSRC SABSMM 111 study that took place between 2008 and 2009.
- **EQA Participation:** The laboratory participates in 11 different EQA schemes. The CDC MPEP HIV EQA and the Thistle EQA for HSV 2 IgG were discontinued. The lab is in the process of enrolling with the RCPA for HSV2 IgG. The lab participates in an inter-lab comparison with the SANBS for HIV P24 Ag.

- **HIV EQA Distribution:** The lab is currently involved in the characterization, preparation and distribution of EQA panels to NHLS laboratories and WHO labs. In 2009 three EQA distributions were issued to 211 NHLS labs and 105 Private labs. Two WHO distributions were issued to 43 WHO labs and 27 Private labs. To improve service delivery for 2009 the lab assessed the following:
  1. Determined how many labs respond within the time-frames allocated
  2. Determined how many labs report corrective actions
  3. Assessed which participating WHO-supported labs currently test for HIV-2 and how HIV-2 testing forms part of the testing strategy
  4. Compiled WHO summary report after closing date of each distribution.
  5. Compiled WHO comprehensive report for 2006 2008 and future reports
  6. Compiled NHLS executive summary report after closing date of each distribution (x3 year)
  7. Developed DTS (Dried Tube Serum) testing protocol and run pilot study. New Start will run a pilot scheme in 2010.
  8. Accredited the HIV EQA department, preparation for accreditation started in 2009 with monthly meetings held between the NICD QA manager and EQA department representatives.

COLLABORATIONS

The serology department liaises with:
The EPI department at the NICD and the WHO AFRO with regards to Measles and Rubella Surveillance on a national and regional level.

- The CDC in Atlanta with regards to HIV Incidence database management.
- The Human Sciences Research Council (HSRC) for HIV-1/2 results and Incidence testing, project related.
- International Partnerships for Microbicides (IPM) for procurement of HIV Rapid testing supplies and Incidence Testing.
- NEWSTART (Society for Family Health) regarding Quality Assurance at the various testing sites by providing HIV-1/2 results for every tenth client including training on HIV-1 Rapid Testing and provision of Internal Quality Control samples for HIV Rapid Testing
- NHLS Quality Assurance Unit regarding EQA distribution and reports submission to NHLS laboratories for HIV Testing
- WHO (Dr Gershy Dumeit) for WHO EQA distributions for HIV-1 testing to the WHO African laboratories.
- Contract Lab Service (CLS) HIV validation panels and HIV Western Blot testing
- CIET for HIV EIA testing
- National Department of Health HIV Rapid testing and evaluation including Post Marketing Surveillance
- Medical Research Council for upcoming HIV survey in 2010
- National Department of Immunology, National Institute of Health, Mozambique for HIV Quality Assurance Testing
- NHLS Cancer Research Unit for HIV EIA testing
- HIV Vaccines Trial Network frequent liaison with Laboratory Operations division in Seattle with regards to the HVTN 503 Trial and others.

CAPACITY BUILDING

- Training was done on the Measles and Rubella IgM assays from the 14th-18th September 2009. Training was facilitated by the WHO, CDC and NICD. Participants included WHO supported labs within Africa and NICD.
- The lab trained two virology registrars (one from Virology and one from SAFELTP). Training for the Virology registrar took place from May 2009-July 2009. The SAFELTP registrar was trained in July 2009
- The lab trained one student technologist from September- November 2009.
- On the 5th August 2009, B Singh, M Maleka and M Mashele presented lectures for the Microbiology registrars. On the 6th August 2009, M Mashele and M Maleka presented lectures to the SAFELTP students and basic lab requirements for ELISA testing.
- L Mahlaba presented a Laboratory Quality Control lecture to the SAFETLP students on the 29th July 2009.
- E Kekana attended the HIV Vaccines Trial Full Group meeting in Washington DC, USA in May 2009.
- D Sikosana attended the HIV Vaccines Trial Full Group meeting in Seattle, USA in November 2009.
- B Singh and M Mashele attended the WHO Measles/Rubella training in September 14th-18th 2009 as facilitators.
- M Mashele attended the annual WHO Polio, Measles and Yellow Fever Laboratory Directors Meeting in Uganda in July 2009.
- B Singh was invited to visit UW-VSL in Seattle, USA in June 2009 to visualize the LDMS-HAWS workflow.
- D Sikosana visited UW-VSL in Seattle, USA in November 2009 to gain exposure in the soon to be introduced LDMS-HAWS LIMS workflow.
- B Singh, D Sikosana, E Kekana and M Maleka attended the LDMS/HAWS LIMS training presented by Frontier Science (from the USA) from the 24th 28th August 2009.
- D Sikosana and L Mahlaba attended the WHO Measles/Rubella training in September 14th-18th 2009 as participants.
- Ushmita Patel attended the SANAS technical assessor course in September 2009.
- Training provided for D Sikosana and E Kekana in the use of Labware LIMS (HVTN data management software) by David Mokgokolo in February 2009.
- Mirriam Mashele enrolled for the B-Tech Quality Management Course in Feb 2009. She has successfully passed 4 subjects (Quality Management Systems,Quality Planning & Implementation, Quality Auditing and Continuous Quality Auditing). She still has 4 more subjects to complete her degree.

Viral Gastroenteritis Unit

BACKGROUND

The Viral Gastroenteritis Unit has been tasked with the establishment of a national surveillance system for the detection and characterization of viruses associated with gastroenteritis. This includes rotavirus, adenovirus type 40 and type 41, astrovirus, norovirus and sapovirus. In addition, the incidence of newly emerging viruses including picobirnavirus, aichivirus, torovirus and picotrirnavirus will have to be assessed in the South African population. The unit also aids the Epidemiology Department in identifying any viral aetiology involved in diarrhoeal outbreaks and characterizing the viruses isolated.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

During 2008, funding was secured to conduct diarrhoeal surveillance in sentinel sites in Gauteng, North West, Mpumalanga and Kwa-Zulu Natal Provinces. These sites included Chris Hani Baragwanath Hospital, Dr George Mukhari Hospital, Mapulaneng and Matikwana Hospitals and Edendale Hospital. The major focus of 2009 was to establish five sentinel surveillance sites and to determine baseline data at these sites prior to the introduction of the rotavirus vaccine into the national expanded program of immunization (EPI).

Between April and December 2009, 962 cases of diarrhoea were reported to the ROTA surveillance system. A total of 830 of these cases had sufficient clinical material for testing and rotavirus was detected in 48% of cases. Genotyping data from three of the sites (Chris Hani Baragwanath Hospital, Mapulaneng and Matikwana Hospitals) revealed that serotype G1P[8] strains were predominant in all three sites. However, while the globally important G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] strains were responsible for 55% of infections, unusual G and P combinations were noted in 39% of strains. These results are in line with data from previous burden of rotavirus diarrhoea studies conducted at Dr George Mukhari Hospital which indicated unusual rotavirus strains in 22-33% of cases. However, recent data from Europe shows unusual G and P combinations and potential zoonotic infections in only 2% of cases. These results emphasize the need to continue monitoring circulating rotavirus strains, especially in light of the recent introduction of the rotavirus vaccine.

Limited genotyping data was also available from Edendale Hospital showing that G2P[6] and G2P[6] strains seemed to be more common although G1P[8], G9P[8] and G12 strains were also detected.

The rotavirus vaccine was introduced into the EPI in August 2009 and is available for all South African children within a specified age range (first dose <14 weeks and the second dose >24 weeks). A case-control study investigating rotavirus vaccine effectiveness in HIV infected and uninfected South African children is being conducted in parallel with a PCV-7 vaccine effectiveness case-control study. Two additional sites at Ngwelezane Hospital (Kwazulu-Natal) and the Red Cross Children's Hospital (Western Cape) will be utilized.

In addition to the sentinel surveillance, 818 rotavirus positive stools were also received from Pathcare Private Laboratories in the Western Cape. The age distribution of the rotavirus-positive cases was similar to previous seasons in the Western Cape (2007 and 2008; Figure 1) with the bulk of the burden in children younger than two years. A concerning trend is the 27% increase year on year in the numbers of rotavirus-positive cases in children between 2 and 5 years (99 in 2007, 137 in 2008 and 189 in 2009) and surveillance in this population should be continued to monitor this phenomenon.

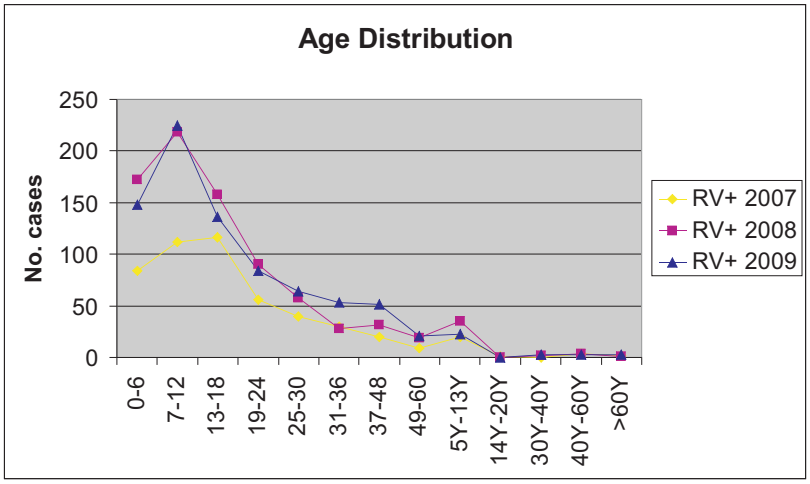


Figure 1: Age distribution of the patients infected with rotavirus diarrhoea collected in the Western Cape during 2007, 2008 and 2009

The 2009 rotavirus season in the Western Cape was relatively mild in 2009, despite the season staring early with 75 and 65 rotavirus cases seen in January and February, respectively (Figure 2). However, the number of rotavirus cases during the season from March to May 2009 averaged 100 compared to the 145 cases during the same period in 2008. In addition, the numbers of cases per month from April to December 2009 were consistently lower than 2008 levels and mostly lower than 2007 levels.

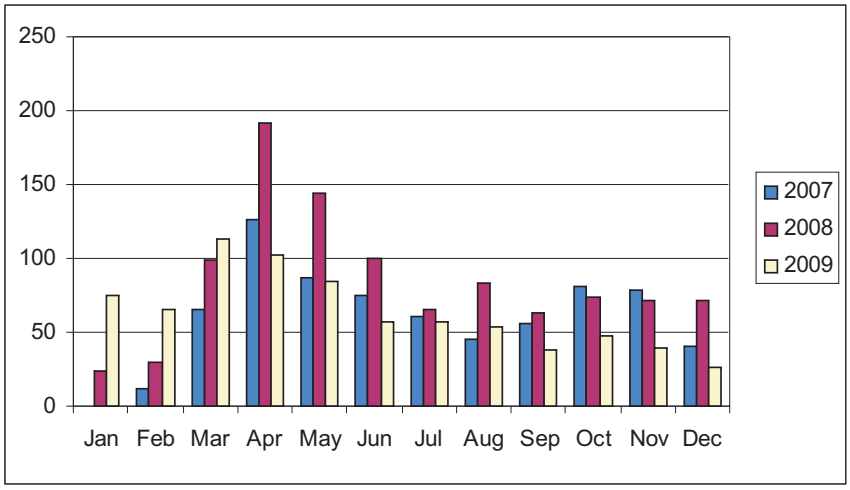


Figure 2: Seasonal distribution of rotavirus infections in the Western Cape during 2007, 2008 and 2009

A total of 62 were insufficient to conduct further analysis and 652 have been genotyped (Table 1). Genotype G1P[8] strains predominated, similarly to the rest of the country with G2P[4], G12P[8] and G9P[8] strains circulating at levels ranging from 22% to 6%. The majority of the strains (72%) were one of the globally important strains and only 18% of cases demonstrated unusual G and P combinations.

A further 230 stool samples were received from NHLS laboratories based in the Western Cape at Tygerberg, Groote Schuur and Red Cross Children's Hospitals. Genotyping data from these hospitals was similar to that seen in Pathcare patients with G1P[8] predominating and G12P[8], G12P[4], G12P[6], G2P[4], G9P[4], G1P[4] and G10P[4] strains also circulating at lower levels. A total of 29 samples had insufficient clinical material and 24 sample displayed mixed genotypes.

COLLABORATIONS

Dr Johann Görgens, Department of Process Engineering, University of Stellenbosch, Prof Emile van Zyl, Department of Microbiology, University of Stellenbosch, Prof Albie van Dyk, North-West University, Dr AC Potgieter from the Onderstepoort Veterinary Institute, Prof Ed Rybicki, University of Cape Town, Mrs Ina Peenze, Diarrhoeal Pathogens Research Unit, University of Limpopo Medunsa Campus for the Rotavirus/HPV subunit vaccine consortium funded by the South African Department of Science and Technology for the South Africa Cuba science collaboration project.

Table 1: Summary of the genotyping results from rotavirus-positive stools submitted to Pathcare Private Laboratories in the Western Cape

Genotype	n	%
Usual Genotypes		
G1P[8]	287	44
G2P[4]	140	22
G3P[8]	3	>1
G4P[8]	1	>1
G9P[8]	39	6
Total	470	72
Unusual genotypes		
G1P[4]	6	>1
G2P[6]	13	2
G2P[8]	6	>1
G9P[4]	23	4
G9P[6]	5	>1
G12P[4]	20	3
G12P[6]	2	>1
G12P[8]	44	7
Total	119	18
Mixed or not typed strains		
Mixed	18	3
G1P?	22	3
G2P?	2	>1
G9P?	11	2
G12P?	9	1
Total	62	10
Total	651	

Prof Maureen Taylor and Dr Walda van Zyl for the project titled “The development of real-time detection techniques and increased surveillance of diarrhoeal disease viruses in the South African population” funded by the PRF.

Mrs Ina Peeze, Mr Pieter Bos, Miss Mapaseka Seheri and Prof Jeff Mphahlele, Diarrhoeal Pathogens Research Unit (DPRU), University of Limpopo Medunsa Campus for projects including rotavirus antigenemia, rotavirus vaccine trials and rotavirus surveillance in South Africa and rotavirus surveillance in various other African countries.

Dr Cheryl Cohen, Dr Jocelyn Moyes and Dr Sibongile Walaza, Epidemiology and Surveillance Unit, NICD for rotavirus surveillance and case-control studies.

Prof Shabir Madhi and Dr Michelle Groome, DST/NRF Vaccine Preventable Diseases Unit, Chris Hani Baragwanath Hospital for rotavirus surveillance and case-control studies

CAPACITY BUILDING

Dr Page is co-supervisor for Mr Harry Ngoveni, Miss Leah Nemarude and Mr Phathutshedzo Ramudingana at the University of Limpopo Medunsa Campus for MSc (Med) Medical Virology degrees.

Dr Page is supervisor for Mrs Ina Peenze and Miss Mapaseka Seheri at the University of Limpopo Medunsa Campus for PhD degrees.

Mr Khuzwayo Jere graduated with an MSc (Med) degree in Medical Virology in May 2009 from the University of Limpopo Medunsa Campus.

Dr Page participated in the training of African scientists during the ninth African Rotavirus Network Workshop held at the DPRU laboratory from the 4th 15th May 2009. The workshop was organized by Dr Jason Mwenda, co-ordinator WHO AFRO and staff at the Diarrhoeal Pathogens Research Unit (DPRU), University of Limpopo Medunsa Campus and the workshop was attended by ten delegates from nine African countries. Delegates attended lectures and were trained in rotavirus analysis techniques including ELISA-detection, electron microscopy, polyacrylamide gel electrophoresis and RT-PCR genotyping.

NICD 2009

Epidemiology Division

Epidemiology & Surveillance Unit

BACKGROUND

The Epidemiology and Surveillance Unit facilitates communication and data sharing between the National and Provincial Department of Health and the NICD. The unit provides epidemiologic input to other NICD units through collaborative projects and support of surveillance and epidemiology activities. Unit staff are involved in numerous teaching and training activities and represent the NICD at meetings with the Department of Health. In 2009 the Unit co-ordinated several surveillance programmes including the Severe Acute Respiratory Tract Infections (SARI) surveillance programme, rotavirus surveillance programme, "viral-watch" respiratory virus surveillance system, the respiratory hospitalisations surveillance programme and the influenza-associated mortality surveillance programme and collaborated on several other programmes. The Unit is also responsible for publication of the quarterly Communicable Diseases Surveillance Bulletin.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

SURVEILLANCE PROGRAMMES

Detailed findings from surveillance programmes for the year 2009 can be found in the March 2010 edition of the NICD Communicable Diseases Surveillance Bulletin, available from www.nicd.ac.za. A brief summary of surveillance programmes for 2009 is presented in this report.

Suspected measles case-based surveillance

The NICD is accredited by the World Health Organisation (WHO) to perform measles and rubella IgM testing for national case-based surveillance as part of the measles elimination strategy. The case definition for suspected measles is: any patient who presents with fever ≥38ºC and rash, and at least one of the three C's (cough, coryza or conjunctivitis). Blood and urine specimens from suspected measles cases nationally are submitted to NICD for confirmation. Approximately 60% of suspected measles cases from Free State Province are tested in that province. The numbers presented here represent specimens received by the NICD and may differ from those presented by the National Department of Health as they may receive information on cases where no specimens were taken.

All blood specimens were tested by Enzygnost (Dade-Behring, Marburg, Germany) diagnostic kits for the presence of anti-measles and anti-rubella immunoglobin M (IgM). Amplification of ribonucleic acid (RNA) for genotyping was attempted in a sample of cases testing positive or equivocal for anti-measles IgM. For molecular analysis RNA was extracted directly from clinical specimens (urine if available, otherwise serum) and tested for the presence of Measles virus by reverse transcriptase polymerase chain reaction (RT-PCR).

By 31 December 2009, 15 291 specimens were collected from patients (n=15 059) who met the surveillance case definition. Gauteng province accounted for the highest proportions of specimens received (n=7 727, 50%). Data on type of specimen received was available for 13 661 patients. Of these patients, blood and urine specimens were received in 60% (n= 8 150) of the cases, blood only from 35% (n=4 821) and urine only from 0.2% (n=34). Of the suspected measles cases tested, 39% (n=5 857) were positive for measles IgM antibodies, and 20% (n=2 975) for rubella IgM antibodies.

In 2009, a large measles outbreak was experienced. The outbreak started in Tshwane district, Gauteng province in March 2009. The outbreak spread to other districts within Gauteng province despite the mass measles vaccination campaign that took place in that district from 24 August to 4 September. Within months, increases in the number of measles cases were reported in all nine provinces (Figure 1).

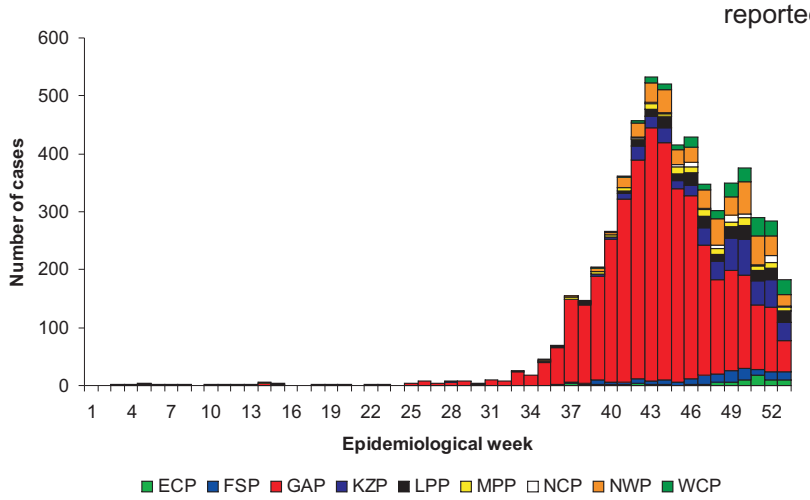


Figure 1: Measles IgM positive results per province: South Africa 2009

Of the 5 857 measles IgM positives results, majority were from Gauteng (4 109, 70%), followed by North West province (n=455, 8%). Northern Cape reported the least number of confirmed measles cases (n=62, 1%). Age and sex were known in 5 684 and 5 662 of the confirmed measles cases respectively. Age ranged from 0 month to 94 years with a median of five years. Age group 6-11 months were the most affected (n=1406, 25%), followed by 1-4 years (n=925, 16%) (Figure 2). Of the 5 662 confirmed measles cases with known sex, 53% (n=3 020) were male and 47% (n=2 642) female.

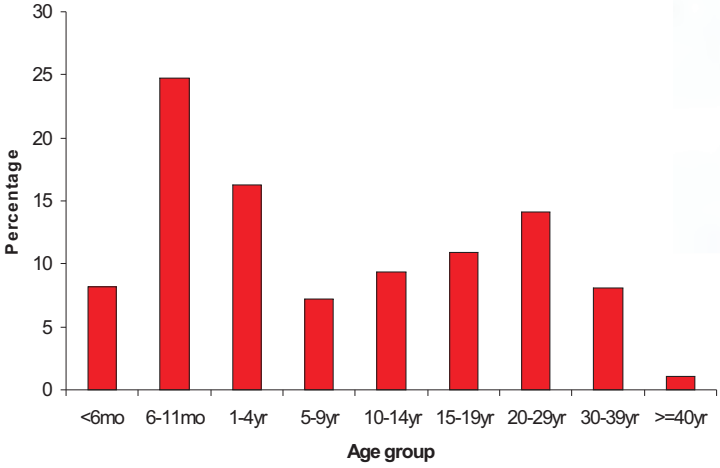


Figure 2: Age distribution of patients with measles, N=5684: South Africa 2009

Acute Flaccid Paralysis Surveillance

The NICD serves as national isolation laboratory for South Africa as well as six other Southern African countries i.e. Angola, Botswana, Lesotho, Mozambique, Namibia, and Swaziland. During the year 2156 stool specimens were received from patients with AFP from these seven countries. Of these 110 were from patients with onset of paralysis prior to 2009. Of the remainder 807 were from 413 South African cases, and 1237 from the six other countries served by the NICD. In early January a further 17 specimens were received from 8 South African cases with onset of paralysis in 2009, bringing the total number of cases in 2009 to 421.

Of the 421 South African cases with onset of paralysis in 2009, one specimen only was received from 65 cases, and two or more specimens from 356. The date of onset of paralysis was known for 358 (85%) cases. Two specimens taken at least 24 hours apart and within 14 days of onset were received from 283/421 (67.2%) cases (range per province 57% to 83%) (Figure 3). Non-polio enteroviruses were isolated from 84, and non-enteroviruses from 17 of the 837 specimens (non-polio isolation rate 12%), and poliovirus, identified as Sabin type poliovirus from four specimens of three patients.

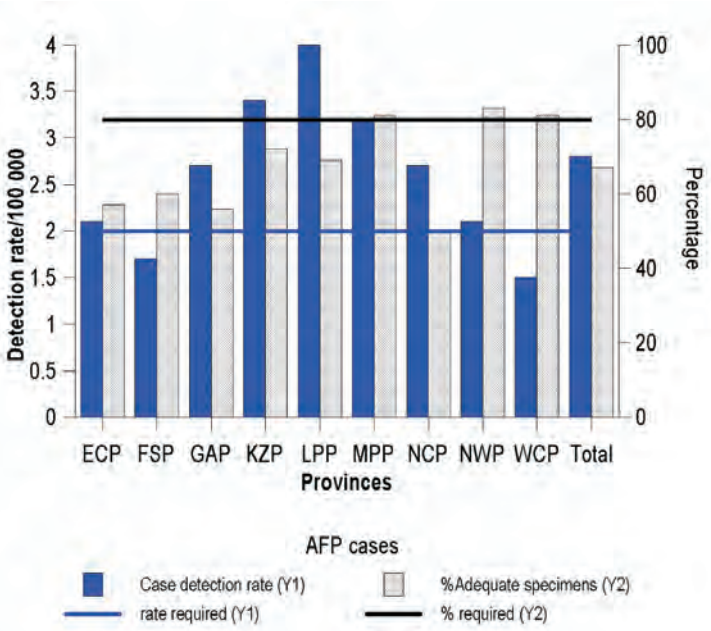


Figure 3: AFP case detection and stool adequacy rate, South Africa, 2009 (only patients from whom stool specimens were received included)

Respiratory Virus Surveillance

The ESU coordinates 4 influenza surveillance programes, each focusing on different aspects of influenza epidemiology. These are:

1. The viral watch surveillance programme
2. The severe acute respiratory infections (SARI) programme
3. The respiratory morbidity data mining surveillance system
4. Influenza-associated mortality surveillance programme

A brief summary of findings from the first 3 programmes is included in this report. As mortality data are released infrequently there was no update on excess mortality estimates conducted in 2009.

"Viral watch" surveillance system

In early 2009, 24 new sites were added in Limpopo and the Western Cape bringing the total number of sites to 243. Of these sites, 165 submit specimens directly to the NICD. Sites in the Western Cape submit specimens to NHLS Tygerberg Hospital laboratory and sites in KwaZulu-Natal submit specimens to the department of Virology at Inkosi Albert Luthuli Central Hospital/University of KwaZulu-Natal. In July, in response to the emergence of pandemic influenza A H1N1, Enhanced Viral Watch centres at 12 public hospitals in 8 provinces were enrolled to detect influenza strains in hospitalized patients.

During 2009, 3354 specimens were received at the NICD for detection of respiratory viruses from Viral Watch centres, and 123 from Enhanced Viral Watch centres. In addition another 328 positive specimens were received from centres in the Western Cape and KwaZulu-Natal for confirmation, serotyping and sequencing.

Two distinct peaks of influenza virus circulation were observed in 2009 with predominantly influenza A H3N2 circulating from week 20 through week 27 and influenza A H1N1 circulating from week 28 through week 42. Between weeks 10 (week starting 2 March) and week 42 (week starting 12 October), seasonal influenza was detected in 1054 specimens. These were identified as 59 influenza A untyped, 4 A H1N1, 866 A H3N2, and 125 influenza B virus. The detection rate increased to >10% in the week starting 11 May (week 20), and peaked at 78% in week 24 (Figure 4). The first pandemic influenza A H1N1 virus in a Viral Watch specimen was detected in a specimen collected on 22 June (week 26). A total of 743 specimens testing positive for pandemic influenza A H1N1 were identified from Viral Watch and Enhanced Viral Watch specimens.

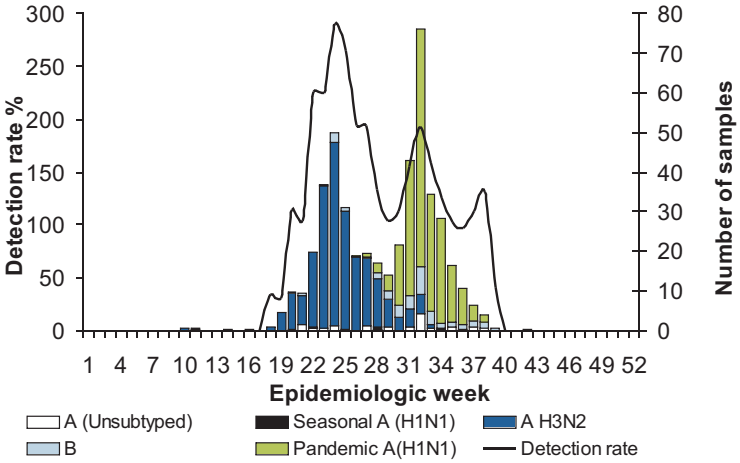


Figure 4: Number of influenza virus detections by virus type and epidemiologic week, South Africa, 2009. Detection rate for specimens tested at NICD only.

Respiratory morbidity data mining surveillance system

During 2009 there were 1 132 331 consultations reported to the NICD through the respiratory morbidity mining surveillance system. Of these 3.4% (38044) were due to pneumonia or influenza (P&I) (ICD codes J10-18).

Two peaks in P&I consultations were observed corresponding to the timing of the two peaks in influenza virus isolations (Figure 5). The second peak corresponding to the period of circulation of pandemic influenza A H1N1 was substantially higher than that associated with seasonal influenza. This may be partly related to an increased awareness of influenza, related to the pandemic.

Severe acute respiratory tract infection (SARI) surveillance

The Severe Acute Respiratory Infection surveillance (SARI) was initiated in February 2009. The aim of the surveillance was to describe the trends in numbers of patients admitted with SARI at sentinel hospitals and to determine the relative contribution of influenza and other respiratory viruses to SARI presentation in a setting with high HIV prevalence.

The first site, Chris Hani Baragwanath Hospital (CHBH, Gauteng province), started enrolling patients on the 9th of February 2009, followed by Mapulaneng and Matikwana Hospitals (Agincourt, Mpumalanga) in April 2009 and the last site, Edendale Hospital (Kwazulu-Natal) on the 2nd of September 2009. All adult and paediatric patients admitted to sentinel hospitals who met the case definition for SARI were prospectively enrolled into the surveillance programme. Detailed demographic and clinical data, in-hospital management, laboratory results and outcome data were collected. Respiratory samples (nasopharyngeal aspirates for children less than 5 years or throat and nasopharyngeal swabs for patients 5 years of age or older) and blood samples were collected from enrolled patients. Respiratory samples were tested for influenza, adenovirus, respiratory syncytial virus (RSV), human metapneumovirus, enterovirus, rhinovirus and parainfluenza virus type1, 2 and 3 using multiplex polymerase chain reaction (PCR). Samples positive for influenza were sub typed using a one step quantitative reverse transcriptase PCR real time essay. Quantitative real-time PCR (*lytA*) was used to detect pneumococcus DNA from blood specimens.

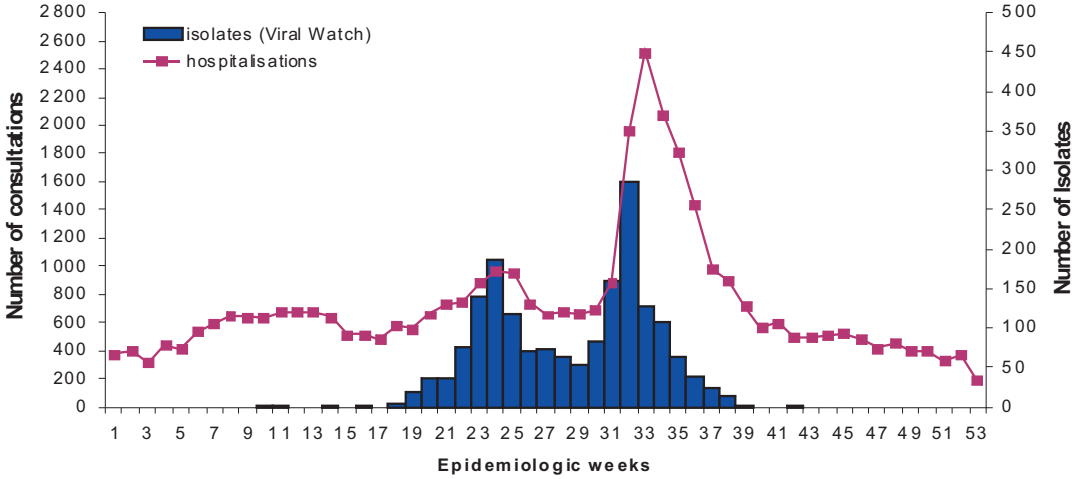


Figure 5: Number of private hospital consultations* with a discharge diagnosis of pneumonia and influenza (P&I) and viral isolates**, South Africa, 2009

*Consultations data from weekly reports of consultations at the Netcare hospital group. Discharge diagnosis is according to ICD coding by clinicians and does not represent laboratory confirmation of aetiology

** Viral isolation data from the Viral Watch sentinel surveillance programme

Between 9th February 2009 (week 7) and 3rd January 2010 (week 53), 3693 patients were enrolled into the SARI surveillance programme. The majority (2963/3693, 80%) of enrolled patients were from CHBH. Sixty percent (3141/3592) of the patients were under 5 years of age and 51% (1833/3599) were female. Of the 3693 patients enrolled, influenza results were available for 3642 (99%) patients. Of these, 391 (11%) samples were positive for influenza on multiplex PCR, 159/393 (41%) of positive samples were identified as Influenza A H1N1 (Novel), 194(49%) as A H3N2, 27(7%) as influenza B and 13 (3%) as A (Unsubtyped). Two peaks of influenza were observed in 2009. The first peak was dominated by influenza A H3N2 and the second by pandemic influenza A H1N1 (Figure 6).

Respiratory syncytial virus (RSV) was isolated in 14% (520/3647), adenovirus in 12 % (423/3647), rhinovirus 27% (993/3647), parainfluenza 1 in 1% (51/3647), parainfluenza 2 in 1% (40/3647), parainfluenza 3 in 6% (214 /3647), human metapneumovirus in 5% (177/3647) and enterovirus in 9% (316/3647) of SARI cases. The respiratory syncytial virus (RSV) season preceded the influenza season (Figure 7). Testing for pneumococcus began in week 20 and of 2023 patients with specimens tested for the presence of pneumococcal DNA 140 (7%) tested positive. An increase in pneumococcal detection rate was observed during the period when influenza was circulating (Figure 7).

In 2009, active surveillance for rotavirus infection was implemented at 5 sentinel hospitals in 4 Provinces in South Africa (Gauteng, Mpumalanga, Northwest province and Kwazulu-Natal). The programme aims to estimate the number of hospitalisations due to severe diarrhoea and laboratory-confirmed rotavirus infection in HIV-infected and uninfected children as well as determine the prevalent rotavirus strains in different geographical areas of South Africa and monitor trends in rotavirus disease following the introduction of the Rotarix® vaccine into the expanded programme on immunization in August 2009.

Stool specimens for diagnosis of rotavirus and other diarrhoeal causative agents, are collected from children < 5 years of age with diarrhoea (defined as 3 or more loose stools in 24 hours) of < 7 days duration. Testing for rotavirus is performed at the Viral Gastroenteritis Unit, NICD and Diarrhoeal Pathogens Research Unit, University of Limpopo Medunsa campus using, the ProSpecT Rotavirus ELISA kit (Oxoid, UK) and the GastroVir strip (Coris Bioconcept, Belgium), respectively. Rotavirus screening results were confirmed by RT-PCR based on standardized methods described in the WHO Manual of Rotavirus Detection and Characterization Methods. Test results of the screening were either, positive, negative or equivocal. In the case of an equivocal result, confirmatory testing was performed using RT-PCR. Case investigations forms are completed by surveillance officers at the sites, providing information on the demographics, clinical signs and symptoms and the outcome of each child enrolled into the programme.

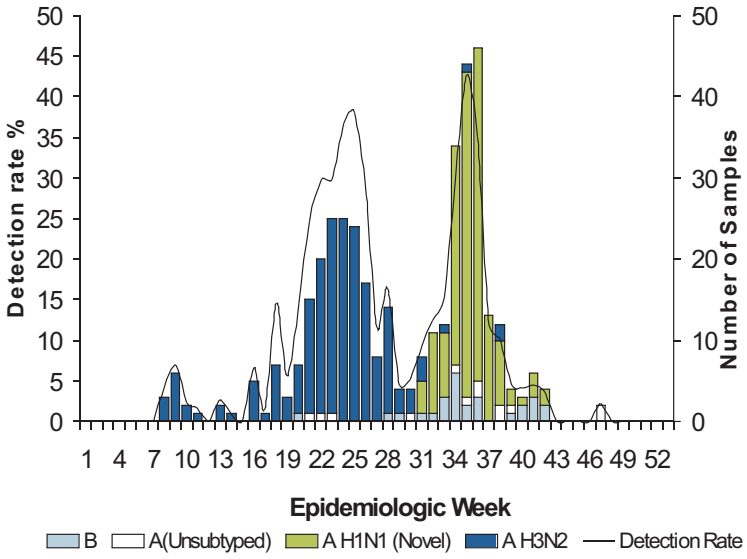


Figure 6: Number of samples testing positive for influenza by virus type and subtype and detection rate for all SARI surveillance sites, 2009.

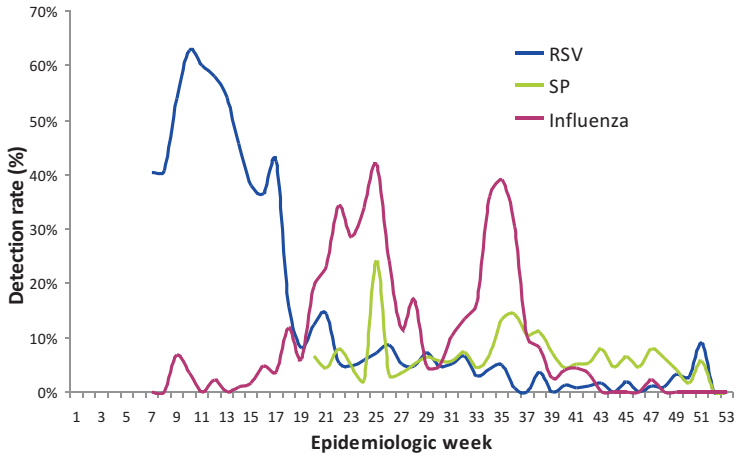


Figure 7: Detection rate for influenza, respiratory syncytial virus (RSV) and pneumococcus (SP) by epidemiologic week, for all SARI surveillance sites, 2009.

Data collection started first in Dr. George Mukhari hospital (North West) in the first week of April (05/04/2009), in Chris Hani Baragwanath(Gauteng) in the fourth week of April (23/04/2009), and in Agincourt (Mpumalanga) hospitals in May (06/05/2009 for Mapulaneng and 20/05/2009 for Matikwana). Surveillance at Edendale hospital in Kwazulu-Natal was initiated in 2010.

A total of 962 cases of diarrhoea were reported to the Rotavirus surveillance programme in 2009. Results of rotavirus testing are currently available for 830 patients, of whom 398 (48%) tested positive for rotavirus (Table1). Rotavirus circulation occurred throughout the surveillance period but 2 seasonal peaks were observed: the first from week 17-25 and the 2nd lower peak from week 29-38 (Figure 8).

Table 1: Numbers of specimens, rota cases and detection rate per hospital, South Africa, 2009

	Date of initiation	Number of specimens tested	Number positive	Detection rate
Dr. George Mukhari hospital	05/04/2009	420	182	43
Chris Hani Baragwanath hospital	23/04/2009	221	125	57
Mapulaneng hospital	06/05/2009	86	47	55
Matikwana hospital	20/05/2009	103	44	43
All hospitals		830	398	48

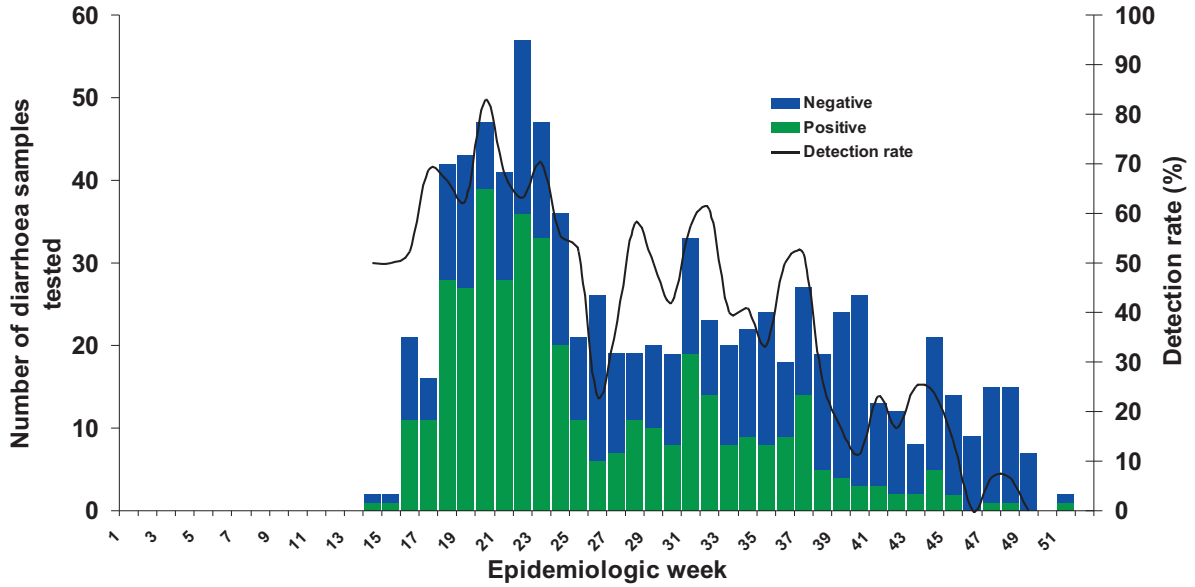


Figure 8: Epidemic curve of specimens tested and rotavirus detection rate for all surveillance sites, South Africa, 2009

GROUP FOR ENTERIC RESPIRATORY AND MENINGEAL PATHOGENS-SOUTH AFRICA (GERMS-SA) PROJECTS

The unit participated in the following collaborative projects with the National Microbiology Surveillance Unit (NMSU), Respiratory and Meningeal Pathogens Unit (RMPRU), Enteric Diseases Reference Unit (EDRU), Mycology Reference Unit (MRU) and Parasitology Reference Unit (PRU) as part of GERMS-SA:

- **Bacterial and fungal meningitis amongst children <5 years old, South Africa, 2007.** S. Meiring, C. Cohen, N. Govender, K. Keddy, V. Msimang, V. Quan, A. von Gottberg. The epidemiology of laboratory-confirmed *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae , Salmonella enterica*, and *Cryptococcus neoformans* meningitis amongst South African children was described. Findings were presented at the FIDSSA Conference in Sun City from 2-23 August
- **Comparison of patients presenting with four HIV-associated opportunistic infections, South Africa, 2006-2008.** Veerle Msimang, Nelesh Govender, Anne von Gottberg, Karen Keddy, Bhavani Poonsamy, Cheryl Cohen for GERMS-SA. The epidemiology of patients with laboratory-confirmed *Streptococcus pneumonia*, *Salmonella enterica*, *Penumocystis jiroveci* and *Cryptococcus neoformans* and was described and was presented at the 3rd FIDSSA Conference. Sun City, 2-23 August.
- **Active laboratory-based surveillance for invasive shigellosis in South Africa 2003-2008.** KH Keddy, A Sooka, P Crowther, C Cohen. The descriptive epidemiology of patients with invasive Shigella infection was described.
- **The Epidemiology of Typhoid Fever in South Africa, 2003-2007.** Brett N. Archer, Karen H. Keddy, Gillian M. De Jong, Cheryl Cohen, Bernice N. Harris. The epidemiology of patients with culture confirmed typhoid over a 5 year period in South Africa was described and findings were presented at the 7th International Conference on Typhoid Fever and Other invasive Salmonelloses in Kilifi, Kenya from January 25-28, 2009.
- **Risk factors for death amongst children of five years and younger, with invasive non-typhoidal *Salmonella* infection, 2004-2008, South Africa.** Veerle Msimang, Arvinda Sooka, Susan Meiring, Nelesh Govender, Jocelyn Moyes Cheryl Cohen, Karen Keddy for GERMS-SA. Risk factors for death in patients with non-typhoidal salmonella were described.

- **Trends in antifungal drug susceptibility of Cryptococcus species in South Africa, 2002-2008** Nelesh Govender, Jaymati Patel, Cheryl Cohen, Tom Chiller, Shawn Lockhart for GERMS-SA. Findings of relatively stable prevalence of fluconazole resistance over a 6 year period were presented at the 17th Congress of The International Society for Human and Animal Mycology 2009 (ISHAM 2009) in Tokyo, Japan from 25-29 April 2009.

Web-based surveillance reports

Web-based reporting from influenza surveillance programmes continued with weekly reports in the season and monthly reports out of the influenza season being published on the NICD web page. Weekly web-based reporting from the measles surveillance programme was introduced due to the widespread measles outbreak in 2009.

SURVEILLANCE PUBLICATIONS

The Unit continued to take responsibility for publication of the quarterly Communicable Diseases Surveillance Bulletin which aims to be a scientific publication for the regular dissemination of surveillance and outbreak data as well as relevant recent research from the NICD.

NICD TRAINING ACTIVITIES

The Unit continued to coordinate the Epidemiology Journal Club and Epidemiology Discussion Group which aim to bring NICD staff involved in epidemiology together in a forum which allows for ongoing education and discussion related to strengthening NICD epidemiology and surveillance activities. Invited speakers at the epidemiology discussion group included:

- Ms Elizabeth Zell, Statistician, Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Centre for Infectious Diseases, Centres for Disease Control and Prevention in Atlanta
- Dr Cynthia Whitney, Acting Chief the Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Centre for Infectious Diseases, Centres for Disease Control and Prevention in Atlanta
- Professor Keith Klugman, William H. Foege Professor of Global Health Rollins School of Public Health, Emory University, Atlanta, USA
- Dr Victor Fernandez, Chief Microbiologist, Head of Mycology Section at the Department of Parasitology, Mycology and Environmental Microbiology of the Swedish Institute for Infectious Disease Control

INTERNATIONAL VISITS, TRAINING COURSES AND MEETINGS ATTENDED

Dr Cohen attended the 4th Regional Pneumococcal Symposium, held at the Sandton Sun, in Johannesburg, South Africa on the 2nd and 3rd of March, 2009. The purpose of the meeting was to bring together scientific experts, key decisions makers, and public health professionals from around the world who have dedicated their efforts to pneumococcal vaccine development and control of pneumococcal disease.

Ms Locadiah Mlambo, Ms Veerle Msimang and Dr Cheryl Cohen attended the PneumoADIP and Hib Initiative Surveillance Networks Investigators Meeting: Evaluations, Innovations and Transitions 4-6 March 2009 Johannesburg, South Africa.

Dr Cheryl Cohen attended the Multinational Seasonal Influenza Mortality (MISMS) Meeting from Apr 21-25 2009 at the Meridien Conference Centre, Dakar, Senegal. The purpose of the meeting was to bring together influenza researchers from the African continent to share experiences and develop collaborations related to influenza epidemiology and genomics. Dr Cohen gave an oral presentation: “Estimation of influenza-related excess mortality in South African seniors, 1998-2005. Cheryl Cohen, Cecile Viboud, Lone Simonsen, Mark Miller, Jong-Won Kang, Terry Besselaar, Jo McAnerney, Lucille Blumberg, Barry Schoub”

Dr Cohen was an invited speaker at the Infectious Diseases Society of South Africa Intercity Meeting on 22 June 2009 at the Bytes Conference Centre. The topic of her talk was “Seasonal Influenza, Hidden Killer?”

C Cohen, L Mlambo ,S Walaza and S Tempia attended Influenza Burden of Disease Workshop and vaccine Effectiveness Meeting in Bangkok, Thailand from 26-28 August 2009. The meeting was organised by the Centres for Disease Control, USA. C Cohen gave a presentation entitled: “Influenza surveillance activities, South Africa”.

Dr Cohen and Sr McAnerney attended the Vaccinology Congress in Hermanus from 18-20 October. Dr Cohen gave two talks entitled “Influenza surveillance programmes” and “Principles of Measles Control”. Sr McAnerney gave a talk entitled “Measles outbreak South Africa 2009”.

Dr Cohen attended a joint workshop between the Health Protection Agency (United Kingdom) and the NICD (South Africa) aiming to facilitate collaborative projects between the two institutes at the PRF training centre, Sandringham, from 26-27 October 2009.

Dr Geraldine Timothy attended the Public Health Association of South Africa (PHASA) conference from 30 November to 2 December 2009 in Durban South Africa and gave a presentation “Pandemic influenza A(H1N1) 2009 in South Africa: the first 100 cases. GA Timothy, C Cohen, B Archer, Dhamari Naidoo; Stefano Tempia, L Blumberg”. This presentation summarized findings from the work conducted as part of the short report for the MMed degree in public health.

Dr C Cohen attended the International Association of National Public Health Institutes Meeting at the Poliomyelitis Research Foundation (PRF) Training Centre at the NHLS/NICD Campus from 2-3 November 2009.

Dr Cohen contributed to a World Health Organization (WHO) Workgroup on Pandemic (H1N1) Critical Care Training Module Development. She attended a meeting related to this from 3-5 December 2009 at the WHO in Geneva, Switzerland.

MEETINGS AND SYMPOSIA CONDUCTED

Start-up training for the Severe Acute Respiratory Tract Infections (SARI) surveillance programme was conducted at the NICD and Chris-Hani Baragwanath Hospital from 2-4 February 2009. The training was attended by surveillance officers and medical officers from Chris Hani Baragwanath Hospital, Gauteng as well as Matikwana and Mapulaneng Hospitals, Mpumualanga. In addition, staff from the NICD Epidemiology and surveillance Unit, Outbreak Response Unit, Respiratory Virus Unit, Respiratory and Meningeal pathogens Reference Unit and Molecular Diagnostics Unit participated in the training programme.

An Influenza Symposium was held at the NICD on 23 and 24 February 2009 at the PRF Training Centre at the NICD. Doctors participating in the viral watch surveillance system, representatives from National and Provincial departments of health and other clinicians were invited. Presentations included report back on surveillance findings to stakeholders and updates as to current developments in the field of influenza.

A startup meeting for the rotavirus sentinal surveillance programme was held at the PRF training Centre from 11-12 March. The meeting was attended by principle investigators and surveillance officers from the Chris Hani Baragwanath, Matikwana, Mapulaneng and Medunsa Sentinal Sites. The purpose of the meeting was to familiarise site teams with the planned surveillance programme and finalise plans for surveillance programme introduction. The meeting was also attended by a visitor from the Centres for disease Control (CDC) in Atlanta, USA, Dr Margaret Cortese.

The annual Severe Acute Respiratory Infections (SARI) and rotavirus surveillance programme investigators meeting was held at the Polio Research Foundation Training Centre in Sandringham from 29-30 October. The purpose of the meeting was to share preliminary surveillance findings with collaborators including clinicians and laboratorians at surveillance sites and National and Provincial Departments of Health. Operational and logistic issues were also discussed and surveillance officer training was conducted.

A Surveillance officer meeting for the GERMS-SA surveillance programme was held from 11-13 November, 2009 at the NHLS building, NHLS conference room, NHLS head office, NICD, Sandringham. The purpose of the meeting was to provide ongoing surveillance officer training, assess progress towards surveillance officer targets in 2009, and to introduce the invasive pneumococcal disease (IPD) case control study. ESU staff participated in meeting training activities including giving formal lectures, facilitating discussions and a ward-based training session on HIV staging.

The Africa Flu Scientific Symposium was held at the Poliomyelitis Research Foundation (PRF) Training Centre at the NHLS/NICD Campus from 7-9 December 2009. The NICD hosted the meeting and Dr Cohen was co-chair of the Epidemiology track. The conference brought together influenza researchers from across the African continent. More than forty oral presentations were delivered over the course of two and a half days, and an additional 20 posters were presented. Oral and poster presentations focused on seasonal influenza surveillance, pandemic H1N1 surveillance and outbreak response, anti-viral drug resistance, and animal-human interface research. The meeting was a great success and was followed by a 2 day writing workshop.

Participants at the Severe Acute Respiratory Infections and Rotavirus Surveillance Investigators Meeting at the PRF Training Centre, NICD from 29-30 October 2009

ESU staff gave the following presentations:

- Severe Acute Respiratory Infection Surveillance in South Africa: Sentinel Surveillance Programme Pilot Results: Sibongile Walaza
- Two Influenza Seasons in One Year: The Viral Watch Sentinel Influenza Surveillance Programme 2009: Jo McAnerney
- Estimation of Influenza-related Excess Mortality in South African Seniors, 1998-2005: Cheryl Cohen

Dr Cohen also co-facilitated a round table discussion session with Dr Sonja Olsen from the Centres for Disease Control and Prevention in the USA entitled "Translating Surveillance Data into Disease Burden Information and Interventions - Data for Action".

COLLABORATIONS

RESEARCH AND SURVEILLANCE COLLABORATIONS

Dr C Viboud, Dr L Simonsen, Dr M Miller, National Institutes of Health, Bethesda, Maryland, USA: Estimation of influenza-related excess mortality in South Africa.

Dr Margaret Cortese, Centres for disease Control (CDC) in Atlanta, USA, Professor Shabir Madhi The Department of Science and Technology (DST)/National Research Foundation (NRF): Vaccine Preventable Diseases (DST/NRF VPD) South Africa, Dr Duncan Steele, Programme for Applied Technologies in Health, Seattle USA. Case-Control Study to Assess the Effectiveness of Rotarix® Vaccine in HIV-infected and HIV-uninfected Children in South Africa.

Dr Laura Conklin, Dr George Nelson, Dr Cyndy Whitney, Ms Elizabeth Zell National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Case-control study to estimate effectiveness of a 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South Africa.

SARI and rotavirus surveillance programme external collaborators

- **Chris Hani Baragwanath Hospital** (SARI programme only): Andrew Black
- **Department of Science and Technology (DST)/ National Research Foundation (NRF): vaccine Preventable Diseases Unit**: Michelle Groome, Shabir Madhi
- **Dr George Mukhari** Hospital/Diarrhoeal Pathogens **Research Unit, University of Limpopo Medunsa Campus** (rotavirus programme only): Maphaseka Seheri, Ina Peenze, Marlise Sauermann
- **Edendale Hospital:** Meera Chhagan, Halima Dawood, Summaya Haffejee
- **MRC/Wits Rural Public Health and Health Transitions Research Unit (Agincourt):** Kathleen Kahn, Stephen Tollman, Rhian Twine
- **Emory University, Atlanta USA** (SARI programme only)**:** Keith Klugman
- **South African National Department of Health Communicable Diseases Directorate** (SARI programme only)**:** Frew Benson, Charles Mugero
- **South African National Department of Health EPI programme** (Rotavirus programme only)**:** Ntombenhle Ncobo, Johan van den Heever
- **United States Centers for Disease Control and Prevention (CDC)** (SARI programme only)**:** Marina Manger Cats, Stefano Tempia
- **Programme for applied technologies in health (PATH). Seattle USA** (rotavirus programme only): Duncan Steele

DEPARTMENT OF HEALTH COLLABORATIVE ACTIVITIES

Dr C Cohen is a member of the National Certification Committee for the Eradication of Polio and attended quarterly meetings of this committee.

Sr J McAnerney is a member of the secretariate of the National Polio Expert Committee and attended quarterly meetings of this committee.

Surveillance unit staff attended the quarterly EPI Task Group Meetings.

Dr Cohen was a member of the expert committee for the development of national guidelines for the management of Hepatitis A, B and C.

Dr Cohen was appointed a member of the National Pandemic Influenza Technical working group and participated in regular meetings of this group related to the emergence of pandemic influenza A H1N1.

Dr Cohen participated in regular conference calls as part of the World Health Organisation Community Epidemiological working group for A(H1N1). This is a WHO working group to facilitate exchange of epidemiologic data between WHO member countries related to pandemic influenza.

Dr Cohen represented the NICD at the Health Information Systems (HIS) Strengthening Workshop at the Sandton Convention Centre from 12-13 March 2009. The purpose of the workshop was to assess the current HIS in South Africa and identify priority areas for improvement. The meeting was convened by Statistics South Africa and the Department of Health and was attended by a variety of stakeholders.

Sr J McAnerney and Dr C Cohen attended the 13th Polio Intercountry Certification Committee (ICCC) Meeting at Emperor's Palace, Johannesburg, South Africa from 27-28 July 2009. The meeting was attended by the Deputy Minister of Health and the purpose of the meeting was to present the South Africa, Swaziland and Lesotho Annual Update PEI Reports to the ICCC.

Dr Moyes and Dr Walaza attended the 2010 FIFA World Cup Communicable Disease Control Workshop, hosted by the National Department of Health (DOH) at the PRF

training centre at the NHLS/NICD campus from 16-17 November 2009. The aims of the meeting were to develop a response plan for communicable diseases for 2010, share experience of international mass gatherings, identify risks for 2010 Fifa World Cup in South Africa, plan surveillance and response for communicable diseases for 2010 and identify roles and responsibilities and develop operational plan.

Ms Veerle Msimang attended the STATISTICS-SA seminar, with the theme "State of Measurement and Measuring the State", on 23-24 November, 2009 in the Birchwood Hotel in Boksburg. The main objective was to gain a better insight in the producing of population statistics and conducting of surveys and other products and services available to stakeholders like NICD.

CAPACITY BUILDING

Dr Cohen was course coordinator for the "Infectious Diseases Epidemiology" Module for the Master of Science in Medicine (Epidemiology and Biostatistics) at the University of the Witwatersrand School of Public Health from 13-17 July 2009.

Dr Cohen and Sr McAnerney were lecturers for the Field Epidemiology and Laboratory Training Programme.

Dr Cohen acted as an external examiner for the Masters in Epidemiology at the National School of Public Health University of Limpopo.

Mr Kevin Spicer a paediatrician studying towards his Masters degree in public health at the Ohio State University, College of Public Health, USA visited the ESU from 12 July-21 August 2009 to conduct his field practicum. Dr Spicer visited sentinel surveillance sites and spent time at the NICD. He assisted with a review of surveillance data quality and analysis of surveillance data.

Dr Elvira Singh was awarded the degree of Masters in Medicine, Public Health in May 2009. She received a distinction for her project "An evaluation of the association between mortality and HIV infection in hospitalised patients with invasive meningococcal disease in South Africa" which was co-supervised by Dr Cohen

Dr Ziyanda Vundle was awarded the degree of Masters in Medicine, Public Health in October 2009. The short report for this degree was completed during her rotation at the ESU and was entitled "Measles surveillance: Evaluation of a new laboratory testing algorithm at the National Institute for Communicable diseases, Johannesburg, South Africa".

Members of the ESU data entry team (from left to right) Lebo Motsipe, Venson Ndlhovu, Boitumelo Letlape, Rose Choeu, Debra Mathebula

Outbreak Response Unit

BACKGROUND

The Outbreak Response Unit of the Epidemiology Division is tasked with providing technical support for all aspects of communicable disease outbreaks and control in the nine provinces of South Africa, with special emphasis on optimising the role of laboratory services during these events. The unit aims to be a source of intelligence during outbreaks, and through working in close collaboration with the provincial and national Departments of Health (DoH), ensures a comprehensive outbreak response and development of systems for early detection and improved reporting. In addition, close partnerships with the NHLS diagnostic laboratories and reference units of the NICD aims to deliver appropriate laboratory diagnostic services during outbreaks and specialized diagnostic tests as required. The unit also participates actively in training public health and laboratory personnel.

2009 proved to be a challenging year for the unit with extensive epidemics observed throughout the country. During this year, the unit has worked in partnership with the provincial and national Communicable Disease Control directorates, SA-FELTP and NICD reference units in responding to several key outbreaks. The unit additionally extended collaborations with international partners, including: the World Health Organization (WHO), European Centre for Disease Prevention and Control (ECDC), Health Protection Agency (HPA), and U.S. Centers for Disease Control and Prevention (US-CDC), to strengthen response to communicable disease events during mass gatherings, in preparation for the 2010 FIFA World Cup.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

KEY OUTBREAKS IN 2009

The unit is a member of the National Outbreak Response Team (NORT) and assists with the development of provincial and national guidelines for priority communicable diseases. Our role in these outbreaks may include, but is not limited to, the following:

- Outbreak detection and reporting
- Field investigation
- Development of clinical and laboratory guidelines
- Management of laboratory surveillance data and interpretation of results
- Recommendations for prevention and control

Only key outbreaks will be highlighted here.

Diarrhoeal disease outbreaks

An outbreak of cholera, first detected in November 2008, continued until June 2009. Cases were initially detected in travellers from neighbouring Zimbabwe and Mozambique; however, the disease quickly established itself in local populations and ultimately spread to affect all nine provinces of South Africa. The unit, in collaboration with NICD and NHLS partners, played a central role in outbreak response including: the monitoring of laboratory-confirmed cases, supporting the establishment of surveillance systems for clinical cholera cases, and advising of case management and public health interventions for control. Following the last confirmed cases reported in week 23 of 2009, 1,203

Table: Cholera cases and deaths in South Africa by province, November 2008 June 2009

Province	Total clinical cases† no. (% total)	Lab-confirmed cases‡ no. (% of clinical cases)	Deaths no. (CFR%)
Mpumalanga	6855 (54)	386 (6)	30 (<1)
Limpopo	5460 (43)	671 (12)	26 (<1)
Gauteng	286 (2)	71 (24)	4 (1)
North West	91 (<1)	61 (67)	4 (4)
Western Cape	8 (<1)	8 (100)	0 (0)
Eastern Cape	2 (<1)	2 (100)	0 (0)
KwaZulu-Natal	2 (<1)	2 (100)	1 (50)
Northern Cape	1 (<1)	1 (100)	0 (0)
Free State	1 (<1)	1 (100)	0 (0)
Cumulative total	**12706**	**1203 (9)**	**65 (0.5)**

†This includes both laboratory-confirmed cases and cases meeting the clinical case definition for cholera (all individuals with acute onset of watery diarrhoea).
‡This includes all laboratory-confirmed cholera cases reported to the NICD from NHLS and private laboratories.

laboratory-confirmed cases and 65 deaths had been detected within South Africa. This equates to approximately 9% of the 12,706 clinical cases reported by the DoH, both totals likely significantly underestimating the true incidence of infection and disease. Mpumalanga and Limpopo Provinces were most affected by the outbreak (Table). Cases were detected in large numbers across all age groups; however, adults aged 20-34 years accounted for the highest proportion (n=358, 30%) of laboratory-confirmed cases.

A large outbreak of diarrhoeal disease was reported in Delmas, Mpumalanga Province in 2009. Increase in cases of diarrhoeal disease was detected during the week of August 11th 2009 through weekly diarrhoeal surveillance reports in the sub-district of Delmas. By September 11th 2009 a total of 2073 cases had been recorded on line lists at health care facilities in the area. Since Delmas previously experienced two large typhoid fever outbreaks (in 1993 and 2005), there was heightened awareness for possible typhoid fever in the area. Although *Salmonella* Typhi was isolated on two stool specimens, it had not been detected on any blood or sterile site specimens. Neither the aetiology nor source of the outbreak were identified. Awareness of appropriate laboratory testing and clinical management was heightened amongst healthcare workers, and health promotion activities were encouraged.

Foodborne disease outbreaks/illnesses

Several foodborne disease outbreaks/illnesses were investigated and managed by the unit in 2009. These include:

- An outbreak of foodborne illness was reported on 29 January from Limpopo Province. It affected a local primary school in Maruleng Sub-district, Mopani District. Approximately 300 learners and staff members presented with illness. Contamination of food served at the school on 28 January was implicated. Between the 28th and 29th January, the local hospital (Sekororo Hospital) had treated 163 cases, of which the majority (158/193, 97%) required hospitalisation. Most cases experienced symptoms for one to two days. All cases made a full recovery and were discharged. A total of 61 stool or rectal swab specimens was collected. No pathogens were isolated.
- In March, an outbreak was reported at a boarding school near Mafikeng (North West Province), primarily affecting children. Approximately 235 learners were ill and referred to Mafikeng Provincial Hospital for treatment. The majority of cases presented with mild clinical symptoms and only 9/235 (4%) required hospitalisation. All cases recovered. Clinical features, estimated incubation periods and laboratory results suggested that *Clostridium perfringens* and *Bacillus cereus* (diarrhoeal toxin) were the most likely aetiological agents in this outbreak. Ten clinical specimens (stools) and food samples (beef stew and chicken gizzards) were collected and sent to NHLS Infection Control Services Laboratory for analysis. Large quantities (innumerable) of *Bacillus cereus* and *Clostridium perfringens* were detected in both the beef and gizzard stew samples. Toxin testing was positive for *B. cereus* (diarrhoeal toxin) in the gizzard stew. *Clostridium perfringens* was isolated from 50% (5/10) of stool specimens. Interventions to ensure that knowledge of and compliance with good food hygiene practices were implemented.
- A fatal case who presented with profuse watery diarrhoea was investigated. The patient was a 61-year-old male and presented with a one-day history of fever with rigors, nausea and weakness followed by profuse diarrhoea. He also had a history of untreated type 2 diabetes. The patient deteriorated rapidly after hospital admission, was shocked with a decreased level of consciousness, and had continuous green watery diarrhoea and clinical evidence of disseminated intravascular coagulation (DIC). He died soon after admission despite intensive resuscitation. *Clostridium perfringens* was detected by PCR on a blood specimen sent to the NICD. No source was identified.

Pandemic influenza

Following the initial reports of human infection with a novel influenza strain (pandemic influenza A(H1N1) 2009 virus) in the United States and Mexico during April 2009, rapid global transmission was observed. This prompted the WHO to raise the pandemic alert level to the highest phase-6 on 11 June. Guidelines for investigation and clinical management were prepared, and updated as necessary. The "Revised Health Workers Handbook on Pandemic Influenza (H1N1) 2009 "Swine Flu" was endorsed by national DoH and widely distributed to healthcare workers countrywide.


REVISED HEALTH WORKERS HANDBOOK

ON

PANDEMIC INFLUENZA A(H1N1) 2009
"SWINE FLU"

Version 3
Last updated: 19 August 2009

Developed by:
The National Institute for Communicable Diseases (NICD)
of the National Health Laboratory Service (NHLS),

In collaboration with:
The South African National Department of Health,
The World Health Organisation (WHO), and
The Division of Infectious Diseases & HIV Medicine, University of Cape Town

NHLS

NICD

The NICD has taken a lead role in laboratory diagnostics, monitoring the extent of disease, and advising on clinical and public health response in South Africa. National Guidelines for From 28 April, the unit initiated surveillance to capture all laboratory-confirmed cases diagnosed by public and private sector laboratories throughout South Africa.

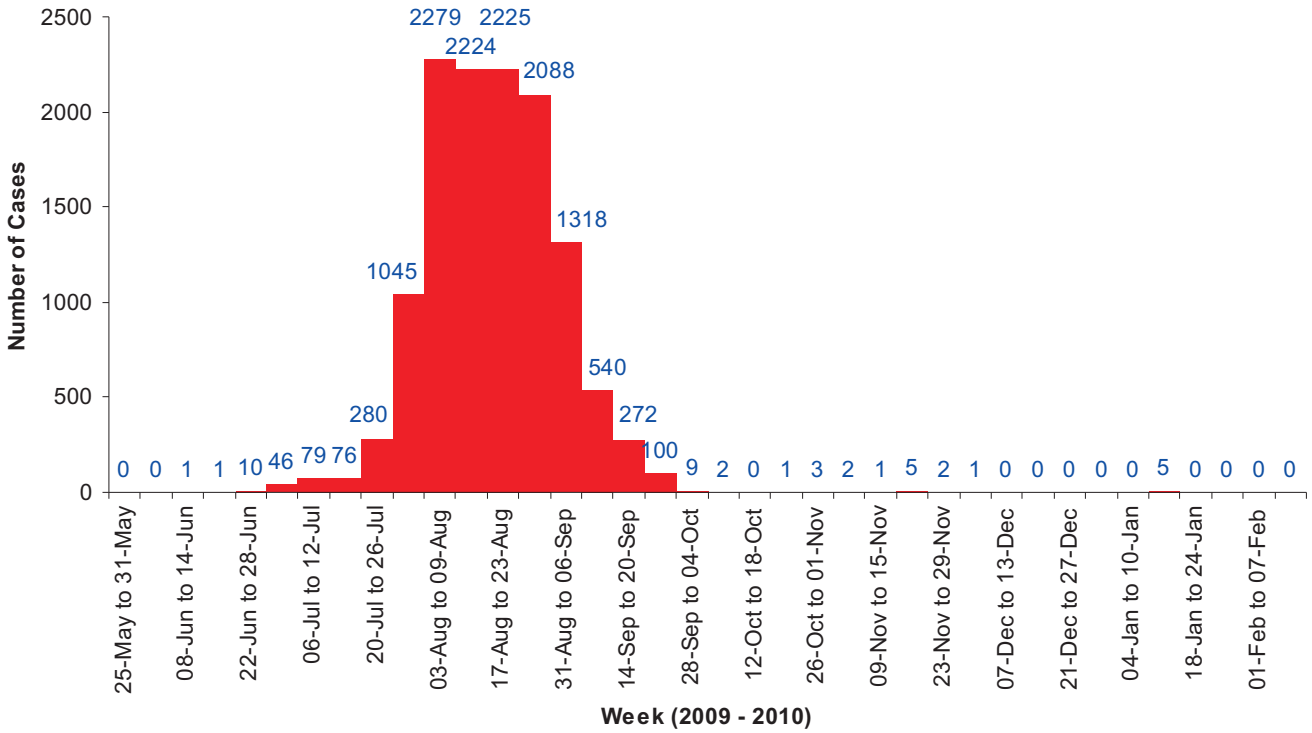


Figure: Epidemic curve illustrating the number of laboratory confirmed pandemic influenza A(H1N1) 2009 cases and deaths by week, South Africa, updated 15 February 2010 (n(cases)=12,640, of which 25 with unknown date)

Data generated by this collaborative surveillance effort has been utilised to provide regular situation reports (updated weekly and posted on the NICD website) to inform key stakeholders and international partners about the South African situation. Additionally, the unit has conducted investigations into the first 100 laboratory-confirmed cases, as well as all reported fatal cases. These data are summarised below.

The first pandemic influenza A(H1N1) 2009 infection in South Africa was confirmed on 14 June. Thereafter, the number of infections increased exponentially with sustained local transmission. During the pandemic, laboratory diagnosis and surveillance strategies shifted away from confirming all cases towards only testing severe cases where a diagnosis would assist in clinical management. Whilst the extent of the local epidemic remains unknown, the overall trends presented here are thought to be representative. The epidemic peaked during August at a rate of >2,000 confirmed-cases per week, which was sustained for four weeks (Figure). This was followed by a rapid decline in the number of newly reported cases, and from October all cases detected had been associated with international travel with no evidence of sustained transmission. As of 15 February 2010, 12,640 cases and 93 deaths had been confirmed in South Africa. The highest incidence of confirmed cases was observed in Gauteng (53 per 100,000 persons), Western Cape (39 per 100,000 persons) and KwaZulu-Natal Provinces (22 per 100,000 persons). The median age of all confirmed cases was 16 years (range <1 month 90 years), which was significantly younger than that of fatal cases (33.5 years, range <1 month 70 years). Analysis of the fatal cases is ongoing; however, preliminary analyses indicates a high prevalence of HIV- infection (50% of 38 tested), pregnancy (28%), and TB (11%), in comparison to the overall prevalence of these conditions observed in South Africa, suggesting that these comorbidities are possible risk factors associated with fatal pandemic influenza infections.

Institutional outbreaks

Hepatitis A

Two outbreaks of hepatitis A virus infection were identified during 2009. The first of these was identified in Tshwane District (Gauteng Province) Between April and June, a total of 33 suspected cases were identified, of which 22 (67%) were laboratory-confirmed. Cases detected during this outbreak were clustered around three schools and subsequently affected household members. SA-FELTP supported the investigations of this outbreak. A second outbreak starting in April was detected in an institution for drug abuse rehabilitation in Northern Cape Province, where 6 cases were identified.

Hand-foot-and-mouth disease (HFMD)

Two outbreaks of HFMD were reported and investigated by NICD in 2009. The first occurred in KwaZulu-Natal Province where 393 suspected cases of HFMD (387 learners and six educators) were reported from 16 schools (including pre-primary, primary and secondary schools) in Kwadukuza Municipality, Ilembe district, during February 2009. Control and prevention measures were initiated through health education by environmental health practitioners (EHPs) and the

school health team. A total of 33 clinical specimens (throat/oral swabs, n= 26; stool specimens, n=7) were received by NICD from suspected cases at four different schools and analysed through culture and reverse transcriptase polymerase chain reaction (RT-PCR) within the Viral Diagnostic Unit. No enteroviruses were detected in any of these specimens; however, this may have been due to deficiencies in specimen quality, timing of collection and conditions of transportation. Further investigations into 15 cases reported at a single health facility in the area revealed that the majority were female (n=10, 67%); ages ranged between one month and 10 years. Of the many signs and symptoms associated with HFMD, vesicular lesions were most commonly reported on the following areas of the body: neck, hands, trunk, face and arms. No new cases were reported as of 16 March 2009. This outbreak highlighted the importance for districts to strengthen outbreak preparedness and response in preparation for more severe epidemics.

The second suspected HFMD occurred during May 2009 in a crèche in the Frances Baard District, Sol Plaatje Municipality, in Kimberley during May 2009. Within a period of four days there were a total of 11 cases mainly presenting with oral lesions and a vesicular rash on the hands. Further investigation identified a total of 13 cases who developed signs and symptoms in keeping with HFMD. Cases were clinically diagnosed as no laboratory specimens were taken. Of the 13 suspected HFMD cases, seven were female and six were male. Age ranged from one to five years (median of two years). The majority (n=6, 46%) of cases were aged two years. Two household clusters were identified; each household had two cases. All 13 cases recovered; duration of symptoms lasted less than seven days in five of the eight cases interviewed.

OTHER EPIDEMIC-PRONE DISEASES

Measles

The unit has supported both the Epidemiology and Surveillance Unit (NICD) and DoH in monitoring and responding to an extensive measles outbreak which began in June, and continues to affect all provinces. A description of this outbreak has already been given (see pg*??? Liz to complete*). Activities during the outbreak included:

- collation and analysis of measles serology data by means of a weekly situation report which was posted on the NICD website
- participation in measles-focused DoH NORT and Expanded Programme on Immunisation task team meetings
- preparation of fact sheets for both healthcare workers and lay public regarding measles vaccination and posting of these on the NICD website
- assisting the City of Johannesburg DoH in managing surveillance for clinical cases

Viral haemorrhagic fevers

A total of three laboratory-confirmed cases of Crimean-Congo haemorrhagic fever (CCHF) were identified in 2009. These cases were investigated by members of the unit in partnership with the Special Pathogens Unit, NICD. Cases were reported from Free State (n=1), Northern Cape (n=1) and Western Cape (n=1) Provinces.

A total of 5 human cases of Rift Valley fever (RVF) were confirmed in South Africa during 2009, all of which had occupational exposure to infected animals, and included veterinarians and farm workers. These cases were a result of multiple epizootic foci identified during the year. The first occurred in dairy herds on farms in the Ixopo and Underberg areas (KwaZulu-Natal Province), and subsequently resulted in 3 confirmed human cases. The second was among sheep in Mbombela District (Mpumalanga Province); however, no human cases were identified. A third epizootic was identified on farms along the Orange River around Kakamas (Northern Cape Province). Although multiple human exposures were identified here, only 2 cases were confirmed; SA-FELTP supported the investigation of this outbreak.

Meningococcal disease clusters

Two laboratory-confirmed meningococcal disease cases were reported from the same school in Gauteng Province within a 10-week period. This prompted concerns of an institutional outbreak. The index case was an 18-year-old matric learner who was admitted on 4 December 2008. *Neisseria meningitidis* was confirmed on culture of cerebrospinal fluid. The second case was a 15-year-old learner who was admitted to hospital on 16 February 2009 and subsequently died. *N. meningitidis* was isolated from a blood culture and later confirmed as serogroup W135. Post-exposure prophylaxis was widely administered in response to this case. Retrospectively, further definitive serogrouping for the index case could not be performed as the isolate was no longer available, but bacterial latex agglutination performed at the time of presentation on a cerebrospinal fluid specimen was positive for *E. coli*/*N. meningitidis* serogroup B suggesting serogroup B disease. In response to the first case, post-exposure prophylaxis was administered to 500 learners all of whom had allegedly attended the same matric dance 72 hours prior to onset of illness in the index case. Following the review of laboratory results, these cases were no longer considered to represent an institutional outbreak and were likely sporadic.

To allay anxiety, NICD prepared and disseminated a special communiqué reassuring healthcare workers, health officials and the public that there was no confirmed meningococcal disease outbreak in Gauteng Province. Sporadic cases were occurring, but there was no increase in the expected number of laboratory-confirmed cases of meningococcal disease at that time of the year as compared to the same period in previous years.

Pertussis
A cluster of pertussis (whooping cough) was identified in Gauteng Province in January 2009. The index case was a ten-week-old infant who presented on 9 January. Secondary cases included her mother (who had been symptomatic during the puerperium), grandmother (asymptomatic) and a 27-year-old healthcare worker who nursed the index case during admission (and who presented on the 22 January). All secondary cases were identified during follow-up of household contacts. Symptomatic cases presented with a history of persistent cough for approximately two weeks. Cases were confirmed by a single-target PCR for *Bordetella pertussis* performed on nasopharyngeal swabs/aspirate. Post-exposure prophylaxis was provided to immediate household and "household-like" contacts of the confirmed cases.

Diphtheria
A case of diphtheria was notified to local health authorities in the Eastern Cape Province in March 2009. The patient was a 10-year-old child who presented with clinical features suggestive of diphtheria, was severely ill with marked neck swelling (bull-neck sign), a pharyngeal membrane and serosanguinous nasal secretions. The patient was admitted to the Intensive Care Unit (ICU), in isolation, and treatment with intravenous penicillin was initiated. He subsequently died due to renal and cardiac complications. The immunization history could not be confirmed. Diphtheria anti-toxin, which was indicated, could not be accessed as it is not available in South Africa. A throat swab was obtained for culture and *Corynebacterium diphtheriae* was isolated. Confirmation of the presence of diphtheria toxin using the Elek method will be performed. In response to the notification of this case, public health officials traced the close contacts (family, close friends at school and in the neighborhood), post-exposure chemoprophylaxis was given and immunization was offered to both adults and children. All close contacts were monitored for symptoms of diphtheria.

Enteroviral meningitis
A suspected viral meningitis outbreak was reported from Western Cape Province in 2009. Suspicion was based on a cluster of 17 people hospitalised with similar clinical syndromes in the Kannaland Local Municipality area (Eden District Municipality, Western Cape Province) since the second week of November, prompting an outbreak investigation. Cases ranged in age from 4 months to 20 years, with a median age of 7 years; in three instances siblings from the same family were affected. Most cases presented with fever, vomiting and/or headache. At least 10 cases had aseptic meningitis (lymphocyte-predominant pleocytosis) which resolved rapidly. Enterovirus PCR was positive in 2/2 CSF and 7/13 stool or throat swab specimens sent to the Diagnostic Virology Laboratory, Groote Schuur Hospital, and the enterovirus characterised as echovirus 13.

AUTOMATED LABORATORY ALERT SYSTEMS
During 2009, the Unit strengthened collaborations with the NHLS Corporate Data Warehouse (CDW) in the development and validation of automated systems for alerting response personnel to the diagnosis of priority communicable disease infections. The current system provides the Outbreak Reponses Unit with timely notifications and patient information following the confirmation of the following infections by NHLS laboratories throughout South Africa: *Salmonella* Typhi, *Vibrio cholerae*, and *Neisseria meningitidis*. A similar system designed to send automated SMS alerts was also piloted during 2009. Furthermore, the NHLS-CDW served to provide compressive line-list datasets of disease conditions as required during outbreaks to support investigations. We hope to further develop these systems during 2010.

AVIAN INFLUENZA (AI) AND PANDEMIC INFLUENZA PREPAREDNESS
The unit continues to play a role in national AI and pandemic influenza preparedness. In 2009, activities included:

- Distribution of regular AI situation reports to key health personnel
- Screening of suspected imported AI cases and liaison regarding decision making for laboratory testing conducted by the Influenza Reference Laboratory at NICD
- Training of Rapid Response Teams (RRTs) for Avian and Pandemic Influenza in collaboration with the national DoH at the provincial level.
- Support for training of RRTs at district level
- Assisting provinces in operationalising plans for pandemic preparedness

2010 PREPAREDNESS
During May 2009, Brett Archer attended a two day workshop at the European Centres for Disease Control (ECDC Stockholm), to consult on tools for mass gathering preparedness and response. The meeting focused on defining communicable disease risks expected during mass gatherings, as well as developing algorithms for predicting these risks.

Dr A. Cengimbo visited the ECDC Stockholm from 7-12 September 2009. The visit was hosted by the Preparedness and Response Unit (PRU), Epidemic Intelligence section, whose activities include early detection activities within a general framework of Epidemic Intelligence. The ECDC has experience with supporting Epidemic Intelligence activities during mass gathering events (including the 2008 FIFA World Cup), and the purpose of the visit was to exchange experiences on event based surveillance and other epidemic intelligence activities linked to early warning and response activities, as well as to strengthen collaboration with the ECDC.

Daily round table meeting at ECDC, Stockholm. L to R: Denis Coulombier (ECDC), Sybille Rehmet (ECDC), Ayanda Cengimbo (NICD) and Lara Payne Hallstrom (ECDC)

In September 2009, the Unit participated in an international simulation exercise run by the ECDC and the HPA (Exercise Purple Octagon), which tested communication channels between health institutions throughout the world. The network developed here will be utilised should the need arise during 2010.

During November 2009, and in collaboration with the DoH, the unit hosted the national FIFA 2010 World Cup Communicable Disease Control Workshop. This workshop brought together provincial and national Communicable Disease Control directorates and international experts from the WHO, ECDC, HPA, US-CDC, NHLS and NICD with the aim of discussing potential risks around this mass gathering, and developing preparedness and response plans for the event. The workshop also served to strengthen collaborations between the Unit and its international and national partners.

THE "OUTNET" PROGRAMME

This programme is a laboratory-based Outbreak Network for SA, which has been running since 2005 with the nomination and training (in collaboration with SA-FELTP) of 9 provincial laboratory "OutNet' representatives. These individuals continue to act as the key points of contact for provincial public health staff and facilitate the role of the laboratory for detection and response to outbreak in collaboration with the Outbreak Response Unit. Regular updates and contact with these representatives is maintained via monthly teleconferences and direct contact during outbreaks. In addition, the OutNet representatives are actively involved in training activities at provincial level. During 2009, the OutNet representatives participated in the provincial food poisoning incidences workshops hosted by the DoH.

NATIONAL GUIDELINES

- Revised Health Workers Handbook on Pandemic Influenza (H1N1) 2009 "Swine Flu"

NATIONAL WORKING GROUPS

- Member of the National Outbreak Response Team (NORT)
- Member of the National Working Group for the development of guidelines for South Africa
- Member of the National Multi-sector Taskforce on Cholera and Pandemic Influenza A(H1N1) 2009

COLLABORATIONS

- Communicable Disease Control Directorates, national and provincial DoH
- Department of Agriculture, Forestry and Fisheries
- Department of Water Affairs
- Onderstepoort Veterinary Institute
- World Health Organization
- European Centre for Disease Prevention and Control (ECDC)
- Health Protection Agency (HPA, UK)
- US Centers for Disease Control and Prevention (US-CDC)

CAPACITY BUILDING

EPIDEMIC PREPAREDNESS AND RESPONSE (EPR) TRAINING

In 2009 the unit continued to assist the national and provincial Departments of Health in training provincial public health personnel and doctors in EPR with an emphasis on case management and appropriate laboratory diagnostic tests.

"CASE OF THE MONTH" SERIES

This is a laboratory capacity-building activity that has been distributed on a quarterly basis to all NHLS laboratories in South Africa since 2005. The series aims to train staff in diagnostic laboratories in basic principles of epidemiology as applied to the role of the laboratory in communicable disease control. Approximately 200 NHLS laboratories continued to participate regularly in

this activity in 2009, for which they earn professional development credits.

TRAINING WORKSHOPS FOR THE INVESTIGATION OF FOODBORNE DISEASE OUTBREAKS

The unit partnered with the national DoH in training provincial DoH representatives in the investigation and response to foodborne disease outbreaks. Workshops were conducted in 8 of the 9 provinces in 2009.

Dr Chuma Makunga, Dr Waasila Jassat and Dr Ayanda Cengimbo of the NICD with (second from right) Riensie Vellema, the Communicable Disease Control Co-ordinator for Mpumalanga Province, after conducting a workshop for both foodborne disease outbreaks and epidemic preparedness and response training.

EXTERNAL LECTURES

The unit presented lectures for various groups in 2009, including: general practitioners, Hospital-based healthcare staff, medical specialists, private healthcare workers and allied medical workers.

ARTICLE

The following article was included in the Communicable Diseases Communiqué of the National Institute for Communicable Diseases:-

Archer BN, Cengimbo A, De Jong GM, Keddy KH, Smith AM, Sooka A, Makunga C, Ntshoe G, Blumberg L. Cholera outbreak in South Africa: descriptive epidemiology of laboratory confirmed cases, 15 November 2008 to 30 April 2009. Communicable Diseases Surveillance Bulletin. 2009; 7(2): 3-8.

SA-FELTP

The Unit continues to support the training of future epidemiologists and public health experts through the SA-FELTP. The unit provides supervision to residents during outbreak investigations, and additional gives lectures during both short and long courses offered by the programme. In 2009, the unit served as a field post for a second year SA-FELTP resident.

WITS PUBLIC HEALTH REGISTRAR TRAINING PROGRAMME

The unit continues to support the training of public health specialists, by hosting 6-month placements for registrars to gain experience in both outbreak response activities and communicable diseases-related public health.

COMMUNICATIONS

The Unit publishes a monthly Communicable Diseases Communiqué, which reports recent outbreak and communicable disease cases/issues of relevance. This is distributed to a wide audience including: general practitioners, specialists, infectious diseases and travel medicine societies, and national and provincial public health personnel.

In addition the Unit published special urgent advisories and Communiqués in response to acute events requiring immediate dissemination of information.

South African Field Epidemiology & Laboratory Training Programme

BACKGROUND

Over the past 25 years, Field Epidemiology Training Programmes (FETPs) have been established in over 35 countries worldwide. There are now 11 such programmes in Africa. South Africa launched its Field Epidemiology and Laboratory Training Programme (SAFELTP) in May 2006, the second programme to have a laboratory component, Kenya being the first. Most new programmes are now FELTPs.

The South African Department of Health, National Health Laboratory Services, the National Institute for Communicable Diseases, the US Centres for Disease Control and Prevention and the University of Pretoria, established this programme to build epidemiological capacity and strengthen public health laboratory practice in South Africa.

The programme's main output is graduates with a Masters in Public Health (MPH) and two years supervised work experience and training aimed at strengthening practical skills and knowledge. The residents participate in several core modules at the University of Pretoria and NICD and work under a supervisor for the remainder of the two years at field placement sites at national, provincial, district or municipal level within the Department of Health and the NHLS. On completion of all the requirements of the training programme, the field epidemiology fellows take up positions as national and provincial epidemiologists, public health laboratorians, surveillance officers or other relevant positions in the South African public health system.

Two applied field epidemiology short courses are presented annually aimed at public health professionals from national and provincial Departments of Health, local and municipality metro city councils who are involved in communicable diseases control, disease surveillance, outbreak investigations and data management.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

- Assisted in 17 outbreak investigations including cholera, measles and the novel Influenza A H1N1 outbreaks
- Undertook 11 surveillance system evaluations
- Conducted 8 analyses of large databases
- The SAFELTP conducted its first Scientific Meeting.
- Staff and residents contributed to 6 articles published in the NICD's Communicable Diseases Bulletin and 21 in the NICD's monthly Communicable Diseases Communiqué
- Genevie Ntshoe received the prize for the best oral presentation at the 3rd African Field Epidemiology Network (AFENET) Scientific Conference held in Mombasa. This biannual conference is an opportunity for residents and graduates of the Field Epidemiology Training Programmes in Africa to present the work they have done during their training. It was attended by over 350 participants from Africa, USA, Germany, India, Belgium and France. 45 oral and 100 poster presentations were made. SAFELTP residents and graduates delivered 6 oral and 20 poster presentations at this conference. The SAFELTP delegation comprised 1st and, 2nd years residents, field supervisors (including members of the Northwest and Gauteng Provincial Departments of Health, the National Department of Health and Mrs Mkhize, Executive Manager: NHLS KwaZulu-Natal Region), 2 resident advisors and 2 staff members. 24 research abstracts were submitted.
- Mr Kavi Velen delivered an oral presentation at the 5th TEPHINET Southeast Asia and Western Pacific Bi-Regional Scientific Conference, 2 -6 November 2009. This is the biannual conference of the field epidemiology programmes in this region.
- Two residents and 1 staff member attended the EIS conference in Atlanta where the residents presented a poster on a diarrhoeal outbreak after a white river rafting trip by a school group.

The 2009 residents with Prof Elliot Churchill, Dr Bernice Harris, Dr Faustine Ndugulile, Lazarus Kuonza and Mmampedi Huma during the "Excellence in Scientific Writing" module

- SAFELTP now hosts CDC/TEPHINET FELTP Fellowship programme. Dr David Mutonga from Kenya is the first fellow to be with us for six months.
- Two residents from the 2007 SAFELTP cohort became staff members of the SAFELTP as AFENET Fellowship recipient and NHLS staff member respectively.
- Dr Tshimanga participated in the initial CDC-WHO joint assessment for the establishment of the new regional FELTP for French speaking Central Africa, including Cameroon, DRC, Central Africa Republic.
- The SAFELTP conducted a field supervisors meeting and an advisory committee meeting during the scientific meeting.
- Dr Khin San Tint participated in teaching activities of the Steve Biko Centre for Bioethics and the School of Public Health at Wits University.
- Dr Ndugulile participated in the Mozambique and Angola FELTP outbreak short courses.
- Dr Harris coordinated the Diploma in Tropical Medicine and Health (DTM&H) and lectured on MPH modules of the School of Health Systems and Public Health (SHSPH) of the University of Pretoria. She also participated in the Wits University's School of Public Health assessment of research protocols for the fulltime MSc Epidemiology and Biostatistics and the MSc Field Based Epidemiology students.

ARTICLES

The following articles were included in the Communicable Diseases Surveillance Bulletin of the National Institute for Communicable Diseases:-

- Archer BN, Cengimbo A, De Jong GM, Keddy KH, Smith AM, Sooka A, Ntshoe GM, Blumberg L. Cholera outbreak in South Africa: Preliminary descriptive epidemiology on laboratory-confirmed cases, 15 November 2008 To 30 April 2009. Comm Dis Surveill Bull. May 2009;7(2):3-8.
- Motladiile TW, Malaza AL, Archer BN, Maimela E, Moetlo P, Khin San Tint, Harris BN. Trends and Characteristics of cholera outbreak In Limpopo Province, South Africa, 15 November 2008 to 01 February 2009. Comm Dis Surveill Bull. May 2009;7(2):8-12.
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COLLABORATIONS

The South African National and Provincial Departments of Health
National Health Laboratory Services
Other units in the National Institute for Communicable Diseases
United States Centres for Disease Control and Prevention
University of Pretoria
African Field Epidemiology Training Network

CAPACITY BUILDING

- 12 residents were enrolled in the 1st year of the MPH accredited programme including 2 residents from the newly formed West African Field Epidemiology and Laboratory Training Programme
- 9 residents completed the 2nd year of the SAFELTP MPH accredited programme
- 64 public health professionals from national, provincial and local Departments of Health from 5 provinces (KwaZulu-Natal, Eastern Cape, Mpumalanga, Limpopo and the Free State Provinces)) were trained in 2 two-week *Applied Field Epidemiology* short courses. One course was run in Pietermaritzburg and the other at the NICD. 61 participants undertook 47 field projects in the 3 months between the 2 contact weeks and presented their findings in the second week. Participants were from a number of district and sub-district departments including communicable disease control, surveillance and infection control. A member of MSF Malawi and the SA EPI national manager also attended the course.
- The SAFELTP conducted its first Scientific Meeting. The theme of the meeting was “Providing Evidence for Public Health Transformation.” It was an opportunity for residents to deliver 9 oral and 26 posters presentations on a range of public health investigations conducted during their training. The residents showcased their work in key public health areas such as planning for the Soccer World Cup 2010, typhoid, Rift Valley Fever, tuberculosis, and other priority diseases in South Africa. The impact of these residents is already evident as they were actively involved in the investigation and identification of the new arenavirus entitled “LuJo” (named after the connection between Lusaka, Zambia and Johannesburg, South Africa). In addition to the residents' presentations, plenary session were held with South African and CDC subject matter experts in zoonotic and vector borne diseases, traveller and refugees health, drug-resistant TB, and HIV/AIDS. These sessions engaged visiting representatives from South Africa's national and provincial health departments and laboratories, various local universities, the Ministries of Health of Zimbabwe and Mozambique, CDC, and the

residents in productive discussions addressing solutions to problems that exist nationally and internationally in these areas. Participants of the meeting came from the NICD, NHLS, National and Provincial Departments of Health, Universities of Pretoria, KwaZulu-Natal and Stellenbosch, HSRC, Department of Agriculture, CDC Atlanta, FELTP Zimbabwe and MOH Mozambique.

- Dr Harris participated in the measles panel at the Vaccinology Conference in Hermanus.
- Dr Harris attended the Training Programs in Epidemiology and Public Health Interventions NETwork (TEPHINET) Directors' meeting in Lyon. The purpose of this meeting was to improve collaboration between programmes across the world and improve the network. The meeting was very useful to learn from experiences elsewhere in the world and be exposed to tools and solutions to address common challenges.

SAFELTP staff, residents and graduates join participants from Provincial Departments of Health during the first week of the field epidemiology short course.

Travel Medicine & International Health Unit

BACKGROUND

This unit was established in April 2008 within the Epidemiology Division with the aim of being a centre for travel health-related activities and liaison and consulting for international health matters.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

CONSULTATIONS

Provision of pre- and post-travel health advice and consultations for travel health practitioners and for staff of the National Health Laboratory Service, in particular those of the National Institute for Communicable Diseases on work-related travel for field activities and conferences.

Consultation with international focal points, institutes and health practitioners for South African travelers presenting with infectious diseases after travel to southern Africa. Expert consultations both locally and internationally for travel-related diseases including trypanosomiasis, severe malaria and rickettsial disease.

Consultation for the elimination of malaria in South Africa and other countries in the southern African region.

Malaria control programme camp with practice wall for insecticide sprayers, Mpumalanga Province.

GUIDELINES

National Guidelines for the Treatment of Malaria 2009 and the National Guidelines for the Prevention of Malaria 2009 were published.
Travel Advisory for the FIFA 2010 World Cup was updated.

CAPACITY BUILDING

Dr Blumberg is a lecturer and examiner for the Certificate in Travel Medicine, University of the Witwatersrand, an annual course that trains 100 practitioners, in collaboration with the South African Society of Travel Medicine. The 2009 course was held at the NICD.

EXPERT COMMITTEE MEMBERSHIP

Dr Blumberg is a member of the following:
Malaria Advisory Group to the National Department of Health and the subcommittee for Chemotherapy and Therapy
National Rabies Advisory Group
National Outbreak and Response Team
National Institute for Communicable Diseases Committee for preparation for the 2010 FIFA World Cup

INTERNATIONAL MEETINGS

Dr Blumberg attended the following meetings during 2009:-
The East Asian Rabies meeting, September 2009, in Vietnam.
The Tropical Diseases Network meeting in Oxford, United Kingdom, in September 2009 where she presented on African viral haemorrhagic fevers and risks to travelers.
A WHO Clinical Consultation in Washington, USA, 14-16 October 2009 where she presented on H1N1 in HIV-infected persons - the South African experience.

Dr Blumberg organized a workshop at NICD entitled 'Communicable Disease Risks for World Cup 2010' on 16 and 17 November. Speakers included a number of international guests from organizations such as the European Centre for Disease Control and the WHO Mass Gatherings Unit as well as from the national and provincial Departments of Health and from both the National Health Laboratory Service and private laboratories.

In December 2009 Dr Blumberg organized the African Influenza meeting, which she co-chaired with CDC. Other partners were from WHO and the Pasteur Institute. 130 delegates from 31 African countries attended the meeting and presented on experiences of H1N1 and seasonal influenza in Africa.

PUBLICATIONS

Dr Blumberg contributed the Beyond our Borders: Infectious diseases risks for travelers section of the monthly Communicable Diseases Communiqué sent out by the Outbreak Response Unit of the National Institute for Communicable Diseases.

Special Programmes

Comprehensive Care, Management & Treatment of HIV & AIDS (CCMT) Laboratory Support Unit

BACKGROUND

The Comprehensive Care, Management and Treatment (CCMT) Unit was created in 2003 after the Operational Plan for Comprehensive HIV and AIDS Care, Management and Treatment for South Africa was approved by Cabinet in November 2003 and the National Health Laboratory Service (NHLS) required a vehicle to prepare for laboratory support for the programme. The CCMT Unit was the first instance of a vertical laboratory programme unit and was formed at the National Institute for Communicable Diseases (NICD). The CCMT Unit works very closely with the NDOH, and provincial HAST directorates to identify and respond to policy changes, new guidelines and HIV related campaigns. The CCMT unit works directly with the NHLS at various levels to ensure that the CD4, HIV viral load and HIV DNA testing service is provided equitably and accessibly. The CCMT unit currently has been integral to the laboratory expansion of South Africa's HIV management programme, providing support to the CD4, HIV viral load and HIV DNA PCR testing laboratories across the country. These laboratories form part of the NHLS testing network and provide support for the national rollout program. The table below summarizes the state of laboratories supported by the CCMT unit and their testing systems:-

Current estimates indicate that South Africa has approximately 947 026 patients on antiretroviral therapy (ART) and with the implementation of the new Presidential Mandate ART guidelines and HCT campaign, an additional 490 000 will be placed on ART in 2010/11, 420 000 in 2011/12 and 370 000 in 2012/12. A massive HIV Counselling and Testing (HCT) campaign targeting 15 million individuals will facilitate this expansion in the 2010/11 financial year. In addition Tuberculosis (TB) and Cervical Cancer screening have for been included for the first time in the ART guidelines and are key aspects of the HCT campaign. The NHLS will face significant testing demands in these three areas as the CCMT programme scales up.

Table 1: Current laboratory capacity

Test	Number of Laboratories	Number of Instruments	Current Max Tests p/month	Current Annual Test Capacity
CD4	69	99	283k	~ 4 Million
HIV Viral load	16	29	113k	~ 1.6 Million
HIV DNA PCR	11	14	23k	~ 0.3 Million

Table 2: Current laboratory capacity per NHLS Branch

Branch	CD4		HIV Viral load		HIV DNA PCR	
	Number of Laboratories	Number of Instruments	Number of Laboratories	Number of Instruments	Number of Laboratories	Number of Instruments
Central	13	22	4	7	4	5
Coastal	14	21	4	8	4	4
KwaZulu-Natal	25	29	3	7	1	2
Northern	17	27	5	7	2	3
Total	**69**	**99**	**16**	**29**	**11**	**14**

The projected CD4, HIV viral load and HIV DNA PCR volumes for the CCMT Programme are as follows:-

Table 3: Projected CCMT test volumes

Period	Projected CD4 Volumes	Projected HIV Viral load Volumes	Projected HIV DNA PCR Volumes
2009/10	2,901,910	1,138,733	248,489
2010/11	3,330,000	1,540,000	320,000
2011/12	4,300,000	2,500,000	320,000
2012/13	4,750,000	2,980,000	320,000
2013/14	5,250,000	3,450,000	320,000

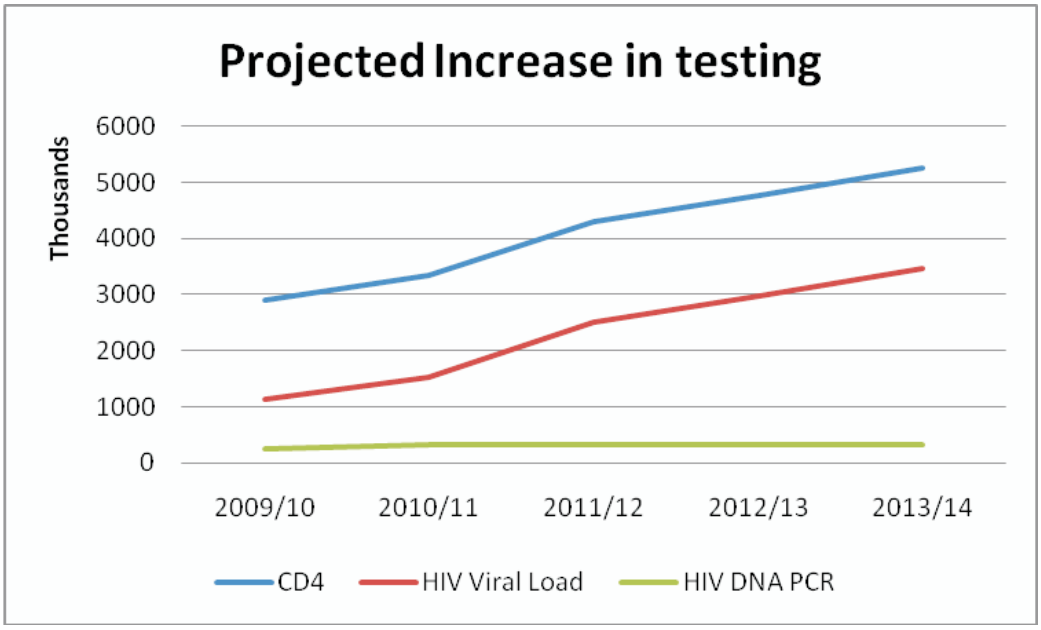

Figure 1: Projected demand for laboratory support of the CCMT Programme

CCMT REPORTING 2006 TO DATE

CD4

The CCMT Unit has been involved in expanding CD4 testing since April 2004 and by April 2005 the CCMT Programme had built up the capacity to provide CD4 testing at 18 laboratories and a monthly test volume of 50 000. Since then, the unit has assisted with the development of 51 more laboratories to bring the total to 69 laboratories.

The annual increase in CD4 tests has been in the region of 40% per annum since 2006 (2007: 1.7million, 2008: 2.3million and 2009: 2.9million). This reflects the rapid scale up in the CCMT Programme for both staging and monitoring patients on ART and in the pre-ART Wellness Programme. With introduction of new ART guidelines, the reduction in CD4 requesting frequency will be compensated for by increased ART enrolment, resulting in a projected increase to ~ 3.4 million tests in the 2010/11 financial period.

The proportion of patients with a CD4 absolute count greater than 200 has always been larger than the proportion of patients with a CD4 absolute count below 200. However, the percentage of patients with a CD4 absolute count below 200 has been steadily decreasing each year from 40% in 2006 to 31% in 2009. This is most likely due to the ART enrolment scale up in South Africa from 2004.

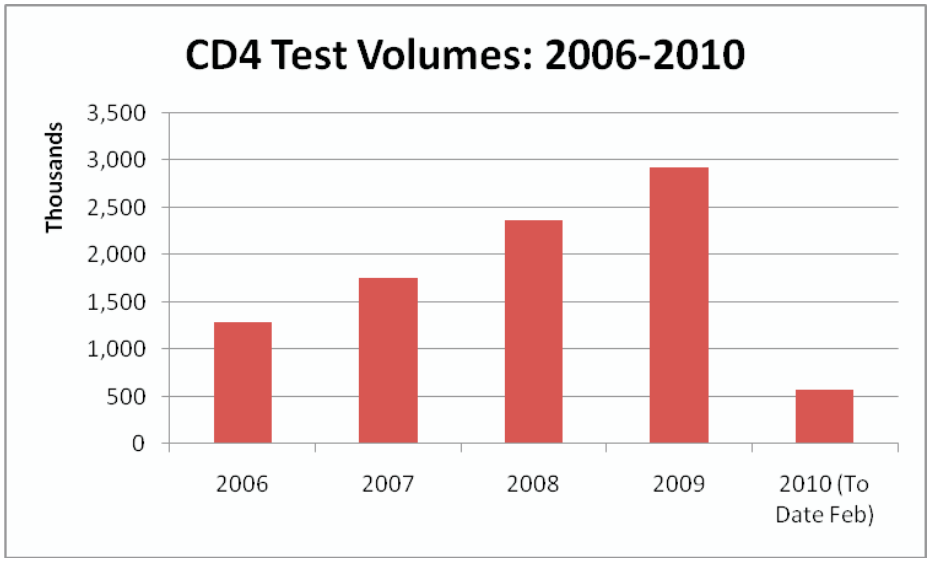

Figure 2: CD4 test volumes per annum

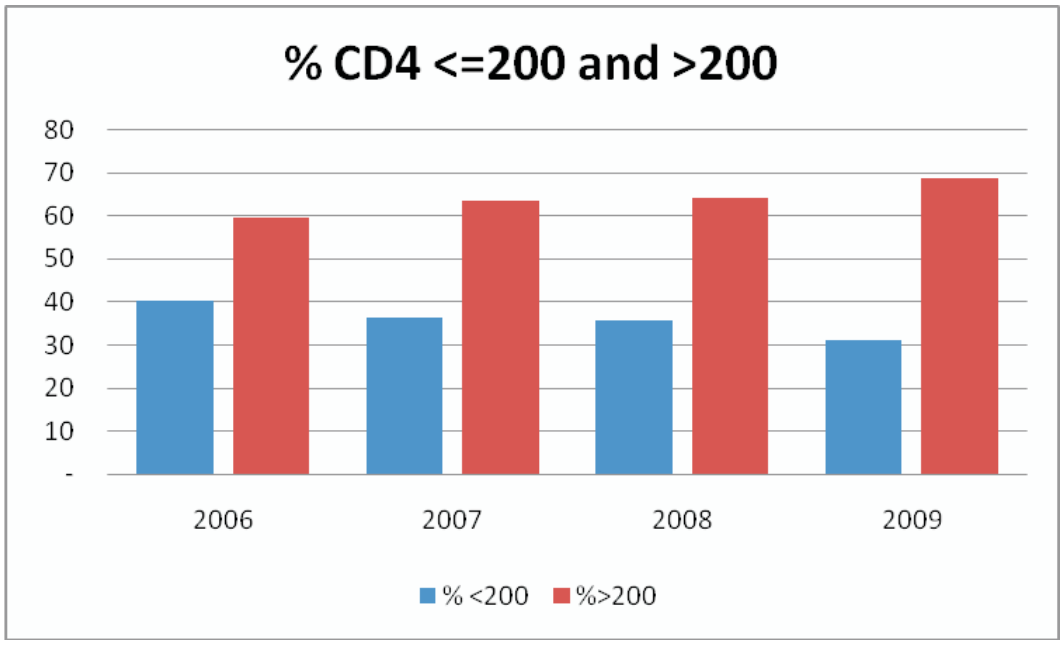

Figure 3: CD4 test volumes by result range per annum

HIV Viral load

The CCMT Unit has been involved in expanding HIV Viral load testing since April 2004. By April 2005 the CCMT Programme had built up the capacity to provide HIV Viral load testing at 7 laboratories with a monthly test volume of 8000 samples. The total number of NHLS laboratories providing HIV viral load testing has grown to 16 laboratories.

HIV Viral load test volumes have increased three-fold between 2006 and 2009. The total HIV Viral load tests performed in the first 2 months of 2010 is equated to 50% of the HIV Viral load testing volumes for the 2006 calendar year. This increase has taken place as changes in the ART guidelines removed the baseline HIV Viral load for all new patients on ART. The new ART guidelines implemented from 1 April 2010 will see existing patients on ART being monitored for CD4 and HIV viral load only once every 12 months.

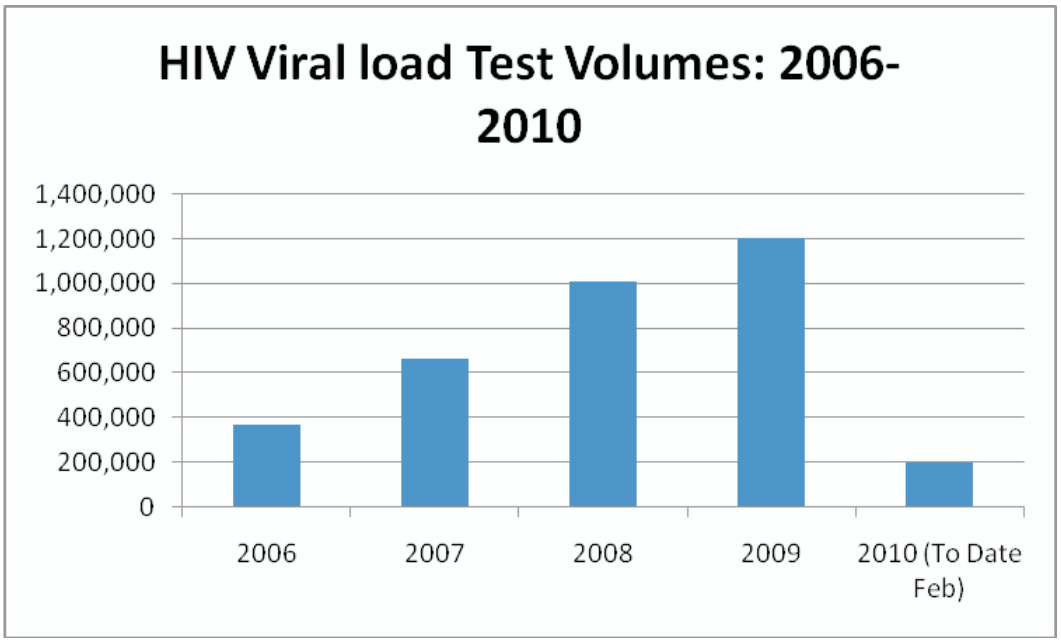

Figure 4: HIV Viral load test volumes per annum

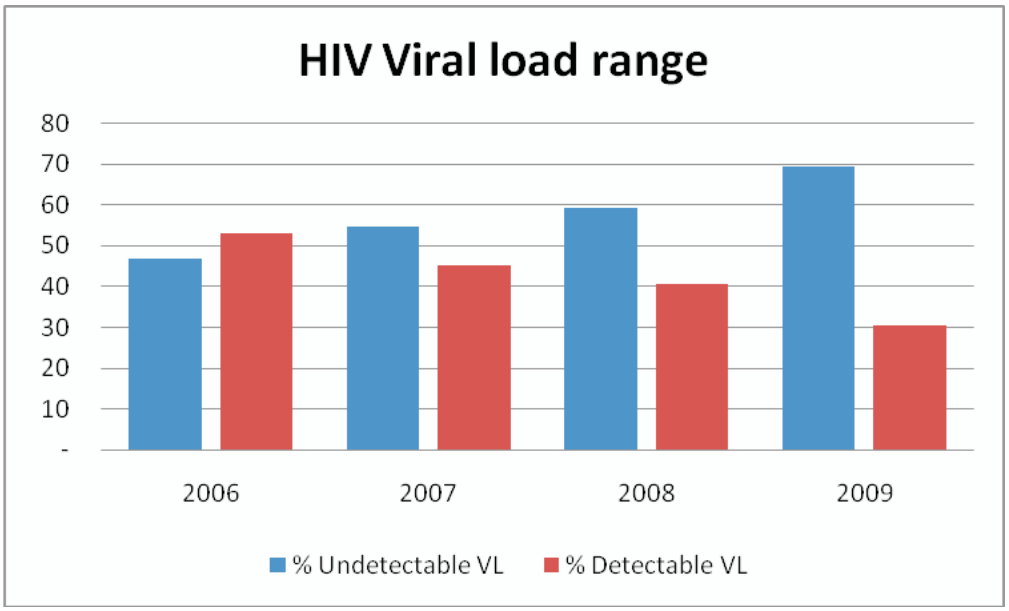

Figure 5: HIV Viral load test volumes by result range per annum

In 2006 the majority of patients had a detectable HIV viral load (53.14%), reflecting that the CCMT programme was still in its early days. However between 2007 and 2009 the majority of patients had an undetectable viral load, with 54% reported in 2007, 59% in 2008 and 69% in 2009. This confirms the progress made in the enrolment of patients on ART with good virological response. The rapid uptake in ART enrolment from 15 April 2010 as part of the HCT campaign should further increasing undetectable HIV viral load proportion.

HIV DNA PCR

By April 2005, the CCMT programme had built up the capacity to provide HIV DNA PCR testing at 3 laboratories with a monthly test volume of 2000 tests. The CCMT Unit has been involved in expanding HIV DNA PCR testing since April 2004 and has assisted with the development of 14 laboratories over that period.

HIV DNA PCR volumes have increased three- fold from 2006 to 2009. This is confirmation of a significant uptake in the PMTCT programme and improved access to HIV DNA PCR testing. With the changes in the new paediatric ART guidelines, the annual HIV DNA PCR volumes are expected to increase from 245 000 p/annum to 320 000 p/annum.

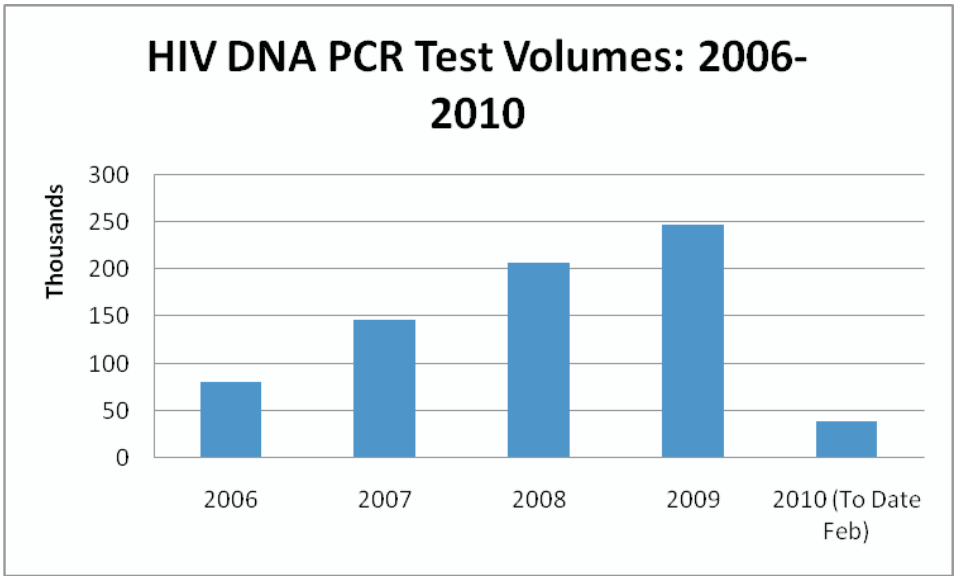

Figure 6: HIV DNA PCR test volumes per annum

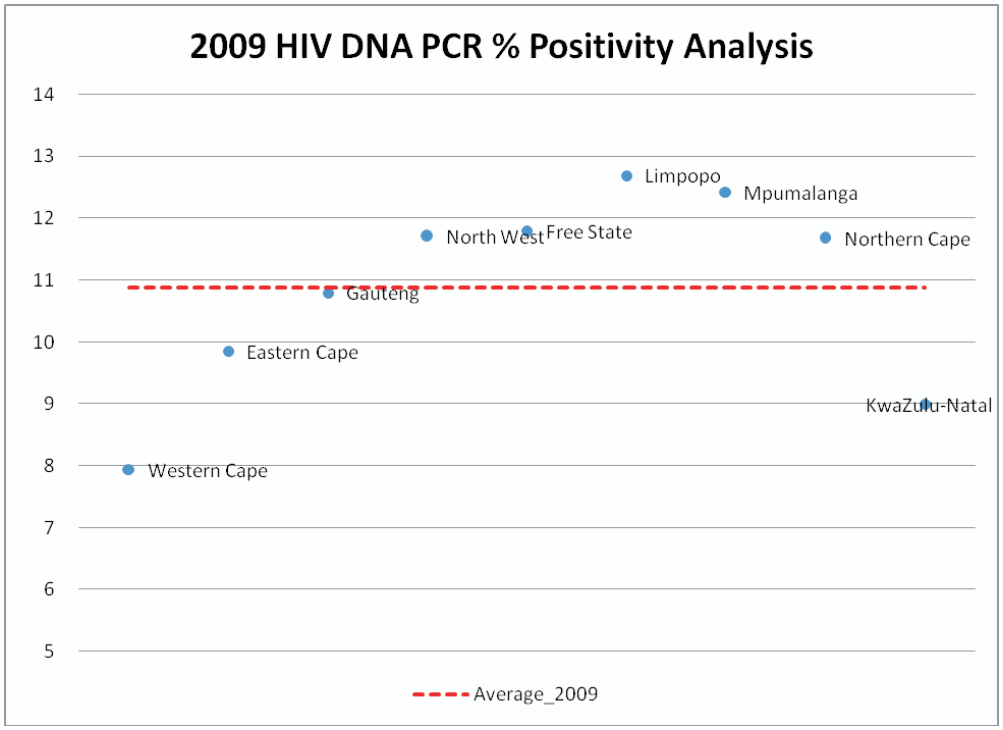

Figure 7: HIV DNA PCR national and provincial positivity rate: 2009

In 2009, the national HIV DNA PCR positivity rate was 10.87%. The Gauteng, Eastern Cape, Western Cape and KwaZulu-Natal provinces were below the national average. However despite being above the national average, the other provinces have made significant progress, for example the Limpopo Province HIV DNA PCR positivity rate declined from 37% in 2006 to 12.7% in 2009 (refer to graph below).

Between 2006 and 2009 the national HIV DNA PCR positivity rate has decreased by 15%. Most babies tested for HIV by DNA PCR are non reactive (2009: 86% negative), indicating the effectiveness of the PMTCT programme.

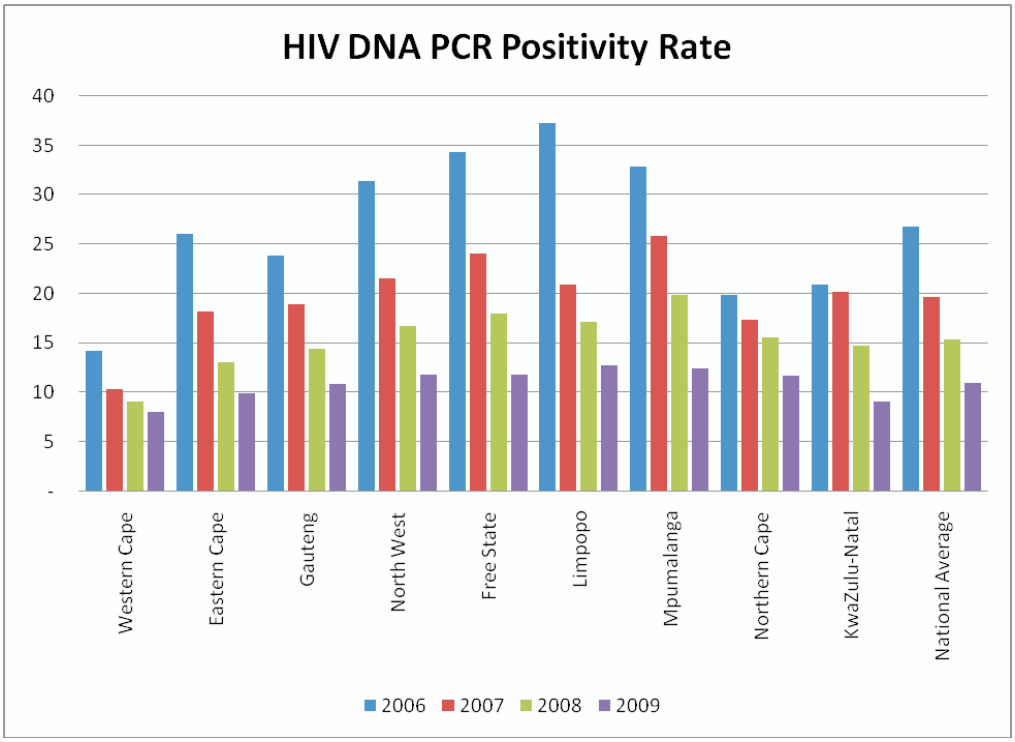

Figure 8: HIV DNA PCR national and provincial positivity rate: 2006-2009

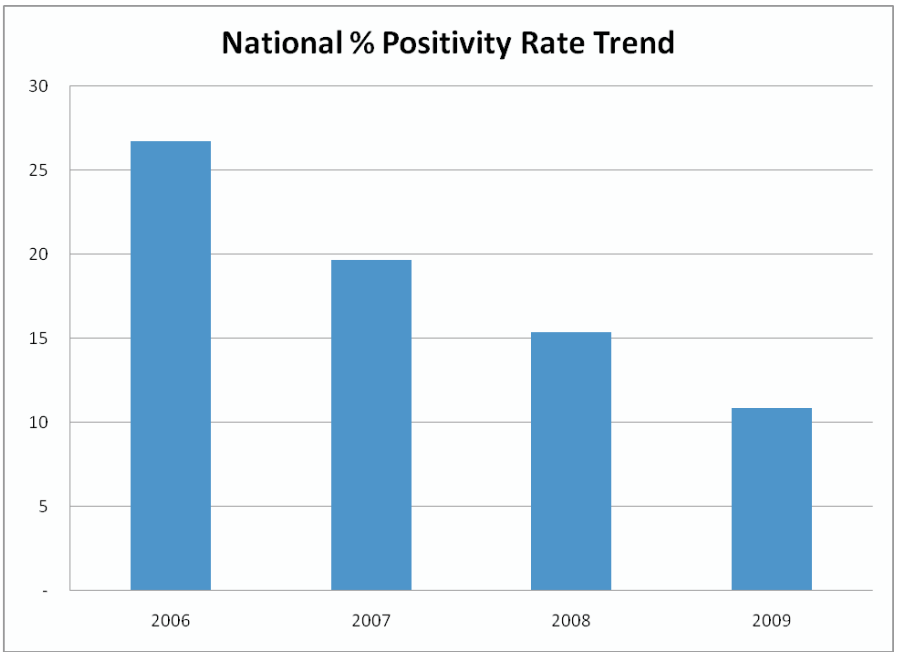

Figure 9: HIV DNA PCR National positivity rate: 2006-2009

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

- The development of the Flow Count Rate (FCR) reporting system for CD4 PLG testing using Disa*Lab and TrakCare to provide data to the Corporate Data Warehouse (CDW). Provides an automated email based reporting system that distributes weekly reports to subscribing laboratories.
- Further development of CD4 EQA scheme: African Regional External Quality Assessment Scheme for CD4 Proficiency Testing CD4 AFREQAS).
- Developed HAST automated reporting from the CDW to provincial HAST coordinators.
- Deployment of CD4, HIV viral load and TB direct SMS results printing system in KwaZulu-Natal.
- Development of TrakCare LIMS (Laboratory Information Management Systems) CCMT reporting tools.
- Incorporation of KwaZulu-Natal aggregate CD4, HIV viral load and HIV DNA PCR data into the CDW as from January 2006.

COLLABORATIONS

NHLS Corporate (Sandringham): Finance, IT and Procurement
NHLS Branch Management and individual CD4, HIV Viral load and HIV DNA laboratories
Clinton Health Access Initiative (CHAI): involvement in the planning for the Presidential Mandate announcements culminating in the revised ART and PMTCT guidelines and HCT campaign.
CDC (Centers for Disease Control) South Africa: Various projects with CDC including GIS development, spatial data sharing and Presidential Mandate planning.
UNAIDS (The Joint United Nations Programme on HIV/AIDS): Presidential Mandate planning and HCT campaign.
SANAC (South African National AIDS Council): Presidential Mandate planning and HCT campaign.
NDOH (National Department of Health): HAST meetings, National Strategic Plan (NSP) discussions, CCMT data reporting, programme management.
Provincial DOH (Department of Health): HAST meetings, data reporting and programme management.
CD4, HIV Viral load and HIV DNA PCR testing systems suppliers: supply management, data reporting and stock management.

CAPACITY BUILDING

The CCMT Unit will continue building CD4, HIV Viral load and HIV DNA PCR capacity within the NHLS to support the anticipated needs as part of the introduction of the new ART guidelines, HCT campaign and PMTCT expansion. The implementation of the CD4 and HIV Viral load tender will continue to increase our testing capacity. The CCMT Unit is planning to expand testing capacity as follows:-

Table 4: New laboratory sites for CCMT testing

Test	New Sites Planned	Upgrade Testing Capacity at existing Sites
CD4	7	2
HIV Viral load	6	2
HIV DNA PCR	1	1

National Tuberculosis Reference Labororatory

BACKGROUND

Tuberculosis (TB) remains one of the foremost causes of death and morbidity in South Africa and on the African continent. The HIV-TB co-epidemic has had a negative impact on TB control with profound consequences for South Africa which has now been classified by the WHO among the 22 high burden countries. Collectively these countries contribute to over 80% of global cases of TB. In addition HIV co-infection has created major diagnostic challenges. The importance of the laboratory and laboratory-based research in TB control has been recognised in South Africa and culminated in the establishment of the National Tuberculosis Reference Laboratory (NTBRL) in 2006 to serve as a resource and reference facility within the NICD and NHLS. The prime function of the NTBRL is to strengthen and support National Tuberculosis Control Programme (NTBCP) of the Department of Health through capacity building of TB laboratory services within the NHLS. In the process the NTBRL provides a national TB referral service, quality assurance (QA) and training programmes and the validation and introduction of state-of-the-art technology. Provision of a laboratory service for the rapid diagnosis of drug-resistant TB enjoys a high priority, while the NTBRL plays a critical role in surveillance of TB and drug-resistant disease. The Corporate Data Warehouse (CDW) has markedly improved laboratory-based surveillance of TB, in particular MDR- and XDR-TB

The eagerly awaited move of NTBRL staff to the new architecturally beautiful building on the Sandringham campus of the NHLS/NICD, housing modern and sophisticated microbiology and molecular laboratories, will take place early in 2010

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

STRATEGIES IN SUPPORT OF NATIONAL TB CONTROL PROGRAMME

Background

The enormous and escalating burden of TB, including MDR-TB and XDR-TB requires aggressive strategies for its control. Volumes of microscopy and culture tests for TB continued to increase during 2009 but at a lower rate compared with previous years. These increases are a continuation of trends observed during the previous few years. (Tables 1and 2)

Table 1: TB microscopy specimens per province per year (excluding KwaZulu Natal)

PROVINCE	2004	2005	2006	2007	2008	2009	Grand Total
EASTERN CAPE	238,510	334,278	458,145	541,227	800,791	819,503	**3,192,454**
FREE STATE	159,394	187,084	203,328	206,602	207,182	201,468	**1,165,058**
GAUTENG	338,055	447,206	510,399	474,086	601,320	624,567	**2,995,633**
LIMPOPO	92,606	130,233	169,883	195,445	273,134	328,219	**1,189,520**
MPUMALANGA	148,625	168,950	198,747	209,521	247,425	270,340	**1,243,608**
NORTH WEST	141,385	191,879	222,724	220,070	273,157	282,526	**1,331,741**
NORTHERN CAPE	71,313	90,003	98,343	94,875	106,076	103,990	**564,600**
WESTERN CAPE	360,891	394,054	428,689	442,723	488,653	497,715	**2,612,725**
Grand Total	**1,550779**	**1,943687**	**2,290258**	**2,384549**	**2,997738**	**3,128328**	**14,295339**

Table 2: TB culture specimens per province per year (excluding KwaZulu Natal)

PROVINCE	2004	2005	2006	2007	2008	2009	Grand Total
EASTERN CAPE	35,132	47,866	69,229	96,496	140,559	154,600	**543,882**
FREE STATE	18,240	22,434	29,288	37,072	36,629	31,569	**175,232**
GAUTENG	79,481	124,193	155,780	172,968	223,785	225,889	**982,096**
LIMPOPO	4,128	5,315	8,407	13,504	16,884	19,550	**67,788**
MPUMALANGA	13,246	13,813	15,616	21,047	34,880	46,237	**144,839**
NORTH WEST	14,312	17,864	24,036	36,134	44,388	41,842	**178,576**
NORTHERN CAPE	20,016	25,062	31,949	35,133	41,362	44,250	**197,772**
WESTERN CAPE	115,300	135,106	158,584	177,710	211,902	230,228	**1,028,830**
Grand Total	**299,855**	**391,653**	**492,889**	**590,064**	**750,389**	**794,165**	**3,319,015**

Strategies in support of the NTBCP include the roll out of line probe testing for the rapid detection of rifampicin and isoniazid resistance. Improved and extended QA programmes, including validation of existing and new technologies have been put in place and expanded during 2009

Roll out of line probe assay sites
A line probe assay (LPA) roll out project for early detection of MDR-TB was initiated in January 2009. Twenty NHLS sites located in 8 of the 9 South African provinces were identified as targets for the first roll out cycle of this test. Funding for this project is provided by the Centers for Disease Control and Prevention (CDC).

The business and TB Control Programme goals and objectives of this LPA project are to provide rapid diagnosis of MDR-TB in order to

- Expeditiously effect appropriate treatment to prevent further development of resistance to anti-TB drugs
- Prevent further transmission of TB
- Ultimately cut down on the cost of diagnosing TB by using the LPA to screen out drug-susceptible TB specimens from conventional drug susceptibility testing (DST)
- Decrease the cost of treating TB by reducing transmission through early diagnosis, as well as preventing development of drug-resistant TB which results in more expensive treatment.

It is planned to initially roll out the assay to 20 laboratories by December 2010 and to implement LPA with as little disruption to the normal work flow as possible, concentrating on standardising and streamlining performance, including reading, interpretation and reporting of results by all newly established laboratories.

Effect of chemical and heat treatment on PCR performance on sputum samples.
With the introduction of line probe testing into the NTBCP, many laboratories will be required to perform LPAs on sputum without the need for culture examinations on specimens which, by definition require preservation of live *M. tuberculosis* organisms. Safety considerations are important and ideally specimens should be rendered safe without undue loss of sensitivity of the LPA. Operational research on preserving DNA integrity for PCR on chemical treatment of sputum samples submitted for line-probe testing was therefore conducted during 2009. Treatment of sputum samples with peracetic acid at a final concentration of 2% initially showed promising results on MGIT cultures but on testing the effect on LPA directly on smear microscopy positive sputum specimens, some loss of band definition was observed, resulting in an increase in inconclusive results and loss of sensitivity of the assay compared with conventional NALC-NaOH treated samples. Studies are ongoing and include the option of heat treatment. Alternative strategies to address this important issue are also being considered.

Quality Assurance

Quality assurance (QA) is key to attaining internationally recognised standards of excellence.

External Quality Assessment programmes
External Quality Assessment programmes (EQAs) are an important component of QA and the NTBRL liaises with the Microbiology Division of the NHLS EQA Unit which provides both smear microscopy and TB culture proficiency programmes, as well as with the MRC, supporting their culture and DST programme. All NHLS laboratories are now enrolled with the above-mentioned programmes

Smear microscopy re-checking programme
To augment TB EQA, the NTBRL introduced a quarterly smear microscopy re-checking programme in 2008. Sample sizes of smears for re-checking were calculated according to a Lot Quality Assurance Sampling (LQAS) method based on smear positivity rates and numbers of AFB-negative smears per business unit during quarterly evaluation periods. Smears with specific sequence numbers identified on a random basis using the CDW, were submitted centrally for blind re-checking by a NTBRL technologist (controller). All smears with discrepant results were re-examined by a second controller whose assessment was accepted as final. Smears were evaluated on appropriate surface area , smear thickness, quality of staining, test status (positive or negative) and quantification of degree of smear positivity and a detailed report with appropriate recommendations was sent to the business manager concerned.

Following successful pilot studies in Mpumalanga and Limpopo provinces, the programme has now been extended to the Doctor George Mukhari Hospital Laboratory and the Rekopane and North-West business units. The programme has been well received and was able to identify areas for improvement. When requested, NTBRL staff assisted with a site visit to help identify and correct microscopy problems and to retrain laboratory staff. A plan has been developed to extend the programme to all provinces during 2010, but roll-out to all nine provinces depends on capacity as NHLS laboratories which process more than 3 million TB smears annually.

During 2009 the NTBRL staff involved with the smear rechecking programme worked closely with IT to further improve and standardise electronic handling and analysis of data, reporting of performance and corrective action recommendations.

EQA Programme for LPA
As there is currently no international EQA system for TB LPAs, the NTBRL established an interim EQA programme for LPA. LPAs are currently in use for the early diagnosis of MDR-TB in NHLS laboratories across the country. DNA prepared from susceptible, mono-resistant and MDR-TB organisms as well as non tuberculosis mycobacteria (NTM) was prepared, distributed and collated. A quarterly EQA round of DNA

material will be distributed in this fashion until an independent EQA system is established.

Audits were carried out in the three TB culture laboratories in Gauteng. In addition, the newly established culture laboratory in Ermelo was visited. Training at an ACILT course was organised for one of the Ermelo staff members. Audits of the culture laboratories will be extended to other provinces in the future.

WHO Review of NTBCP

The external review by WHO of the RSA TB control programme took place from 6-17 July 2009.

NHLS laboratories were reviewed as an integrated part of the NTBCP. This included vertical audit (i.e. interface with the clinics/hospitals, transport system, turn-around time and flow of information) as well as laboratory review, where accreditation documentation, quality assurance (internal as well as EQA) and safety were reviewed.

The review team comprised international technical experts, as well as independent local peers. A report produced at the end of the review had many complimentary references to the laboratory system imbedded in the general text that will be very widely circulated and read. The review illustrated good insight by the review team and advice was constructive and relevant.

Validation studies

Validation of second-line DST

The NHLS laboratory in Kimberley recently introduced routine second-line DST and the NTBRL assisted with training laboratory staff and provided this laboratory, as well as Braamfontein TB Referral Laboratory which also requested validation, with cultures with different drug resistance profiles against second-line drugs for simultaneous testing at the NTBRL and the routine laboratory concerned.

Evaluation of the Roche LightCycler Mycobacterium Detection Assay

Two NHLS laboratories in Kimberly and the Department of Medical Microbiology, Pretoria University respectively and the Department of Microbiology, University of Regensburg, Germany, participated with the NTBRL in the evaluation of the Roche LightCycler Mycobacterium Detection Assay, using real-time PCR and melting curve analysis combined with fluorogenic hybridisation probe to detect *Mycobacterium tuberculosis*, *Mycobacterium kansasii* and *Mycobacterium avium* in sputum. Evaluation of the analytical performance of the participating laboratories showed excellent results: The assay shows high sensitivity, with detection limit of 28 copies per ul for *M.tuberculosis*. Non-mycobacterial organisms analysed showed no cross-reactivity and no substance interference was observed. Additional non-tuberculous mycobacteria other than *M. kansasii* and *M. avium*, were detected and may be identified by specific melting temperatures. Overall accuracy and precision were also excellent (coefficient of variation around 1%) in medium to high concentration samples, further evaluation of clinical samples showed a sensitivity of 75% and specificity of 97% for detection of *M. tuberculosis*.

Validation of iLED microscopy performance

Three laboratories in Northern Kwa-Zulu Natal participated with the NTBRL in a Foundation for Innovative New Diagnostics (FIND) study to evaluate iLED microscopes. Following initial training by FIND, the microscopes were introduced into the three laboratories. Re-checking of smears examined by iLED microscopy continued for nine months with ACILT organising a follow-up training workshop.

QUALITY ASSURANCE AND SUPPORT PROJECT FOR SWAZILAND TUBERCULOSIS LABORATORY NETWORK

Following an approach by the President's Emergency Plan for AIDS Relief (PEPFAR) team in Swaziland for the NTBRL to assist with the upgrading of TB laboratories in Swaziland and specifically with quality assurance (QA) aspects of the laboratory diagnosis and treatment monitoring of TB, the NTBRL in close collaboration with the CDC in Swaziland and Dr Sukati, clinical pathologist in charge of laboratory services of that country initiated a programme to achieve these goals. The TB laboratory network in Swaziland comprises a referral laboratory for TB culture and DST involving first-line drugs in Mbabane and 12 TB microscopy laboratories scattered throughout the country. During 2008 several visits were made to Swaziland laboratories, initially to explore facilities and to meet the microscopists and assess their working environment. Esther Tsheola subsequently gave an introductory talk to key laboratory staff, including Dr Sukati together with QA and laboratory managers and supervisors to explain the envisaged role of the NTBRL and the importance of QA. She also presented a work plan for the implementation of a laboratory support programme for Swaziland. Subsequently visits were undertaken during 2008 and 2009 by Esther Tsheola, Neeshan Ramdin and Professor Koornhof to introduce and monitor QA practices in the laboratories.

The initial objectives of the NTBRL support programme for Swaziland were to:

a) introduce a concentration method linked to fluorescence microscopy in order to increase the sensitivity of TB smear microscopy, and

b) promote good QA practices, also in the performance of TB culture and DST at the TB Referral Laboratory in Mbabane and

c) facilitate acquisition of additional MGIT equipment for DST on second-line drugs

During 2009 a 9-month support programme from March 2009 to November 2009 funded by the USAID-linked University Research Company, LLC (URC) under the direction of Dr Samson Haumba was conducted by the

NTBRL The programme was an extension of activities started in 2008 and included periodic visits by Professor Koornhof and regular visits by Neeshan Ramdin during which adherence to QA procedures was assessed by check list, including recording of daily positive and negative batch controls, confirmation of scanty positive microscopy findings by a second microscopist, recording of daily or weekly positivity rates and monthly recording of laboratory statistics which included total volume of smear microscopy tests performed, total positive and negative findings, as well as smear positivity rates. Other documentation of laboratory activities initiated by the programme, including establishment of standard operational procedures (SOPs), documentation of instrument use and care and adherence to safety practices were monitored. Examples of NHLS SOPs as well as relevant safety documentation for TB laboratories from the NHLS Safety Manual were provided to assist laboratories with the compilation of their own unique documents

All equipment and reagents required for fluorescent microscopy on concentrated specimens were purchased and delivered to Swaziland.

A workshop dealing with the sputum concentration and fluorescence staining method, QA and laboratory safety was conducted by ACILT from 28-09-2009 to 02-10-2009 in Swaziland and was followed up by a second 4-day training workshop conducted by Neeshan Ramdin. Participants were required to compare their findings, using to new technology with their current (to be replaced) method.

The NTBRL team advised Dr Sukati and the URC on computerization of the Swaziland Laboratory Network and facilitated meeting experts relating to the introduction of a laboratory information system (LIS) for Swaziland. The team also inspected the new TB reference laboratory in Mbabane and assisted the URC on design modification required for laboratory safety and the inclusion of PCR rooms for TB line-probe testing to be introduced in the near future. The URC was advised to seek expert opinion from biosafety engineers on optimal design features and the NTBRL arranged for a South African firm to provide such advice

A joint application by the NHLS involving the NICD HIV/AIDS Unit and the NTBRL to provide HIV/AIDS and TB related laboratory support and technical assistance services in the Kingdom of Swaziland under PEPFAR to the value of $400000 was unsuccessful and regretfully the NTBRL assistance programme for Swaziland will be terminated by the end of 2009.

SURVEILLANCE

Corporate Data Warehouse- Based Surveillance

Access to data from NHLS laboratories performing DST against first- and second-line anti-TB drugs through the CDW has improved management of the TB control programme. MDR/XDR-TB data extracted from the CDW (excluding KwaZulu- Natal), is used to maintain registers utilised by provinces to identify new MDR cases in the periphery for referral to MDR centres. In addition, it has enabled the NTBRL to provide annual figures of MDR-TB and XDR-TB cases, which is useful for monitoring the effectiveness of the National TB Control Programme as well as for establishing strategies for TB management.

Tables 3 and 4 summarise the number of MDR- and XDR-TB patients diagnosed by the NHLS by province by year as extracted from the CDW.

Electronic Drug-Resistant TB Register

A project with the NTBRL, DoH, CDC and Wamtechnology CC was initiated with the goal to develop an interface that could, on a daily basis, transfer data relating to MDR-TB and XDR-TB patients into the EDRWeb (Electronic Drug-Resistant Tuberculosis Register), developed by Wamtechnology CC. The interface for the NHLS CDW went live during the year and has been running successfully.

National Drug Surveillance Survey

Protocol development for the National Drug Surveillance Survey has been completed. Population-proportionate cluster sampling will ensure that specimens are representative of TB patients in South Africa. Fieldwork commences in 2010, after finalisation of funding has been achieved. Prevalence of MDR/XDR-TB in new and re-treated cases will be established and used in rational management planning.

Table 3: Number of MDR-TB patients diagnosed by the NHLS by province per year

PROVINCE	2004	2005	2006	2007	2008	2009	Grand Total
EASTERN CAPE	379	545	836	1,092	1,501	1,858	**6,211**
FREE STATE	116	151	198	179	381	253	**1,278**
GAUTENG	537	676	732	986	1028	1,307	**5,266**
KWAZULU-NATAL	583	1,024	2,200	2,208	1,573	1,773	**9,361**
LIMPOPO	59	40	77	91	185	204	**656**
MPUMALANGA	162	134	139	506	657	446	**2,044**
NORTH WEST	130	203	225	397	363	520	**1,838**
NORTHERN CAPE	168	155	188	199	290	631	**1,641**
WESTERN CAPE	1,085	1,192	1,179	1,771	2,220	2,078	**9,525**
Grand Total	**3,219**	**4,120**	**5,774**	**7,429**	**8,198**	**9,070**	**37,810**

Table 4: Number of XDR-TB patients diagnosed by the NHLS by province per year

PROVINCE	2004	2005	2006	2007	2008	2009	Grand Total
EASTERN CAPE	3	18	61	108	175	123	488
FREE STATE	1	6	3	4	3	3	20
GAUTENG	5	14	19	38	30	65	171
KWAZULU-NATAL	59	227	336	241	181	254	1,298
LIMPOPO		2	5	2	2	6	17
MPUMALANGA				12	14	18	44
NORTH WEST	1	5	9	4	4	13	36
NORTHERN CAPE	4	10	3	7	19	40	83
WESTERN CAPE	12	16	28	42	60	72	230
Grand Total	**85**	**298**	**464**	**458**	**488**	**594**	**2,387**

SADC consensus meetings

Members of NTBRL attended three SADC meetings during the year. Topics of discussion included harmonisation of services and development of SADC supra-national laboratories and reference laboratories for TB, HIV and malaria in the SADC region.

COLLABORATIONS

Guardian Technologies and Arum Health are involved in a collaborative project with the NTBRL to evaluate digital imaging technology for improved smear microscopy and possible automation.

The Centres for Disease Control and Prevention (CDC) have contributed to the financing of several projects, including the development of the integrated TB/HIV management system, automation of processing of specimens for smear microscopy, the slide re-checking EQA programme and others.

Various partners contributed to the establishment of ACILT, and some are participating in the governance of the centre. Stakeholders include the South African National Department of Health, CDC South Africa, NHLS, NICD, WHO AFRO, USAID, Becton Dickenson (US), ASM and JICA. Funding of the centre is by PEPFAR through the CDC South Africa.

The South Africa FIND iLED microscopy demonstration project has been described earlier in the report and is a collaborative effort between the NTBRL, FIND and NHLS KZN. The aim of the investigation is to establish the feasibility and impact of the implementation of rapid concentrated fluorescent iLED microscopy screening for TB.

The NHLS collaborates with the Department of Health on an ongoing basis to improve laboratory services and patient management and members of the NTBRL staff liaise regularly with staff at Sizwe Hospital. Members of the NTBRL staff as well as one of the microbiologists at the Braamfontein TB Referral Laboratory regularly attend XDR-TB management meetings at the hospital and play a valuable role in the management of XDR-TB patients.

The NTBRL established NIH funded collaboration with Prof Gilla Kaplan's team from the International Center for Public Health, New Jersey, USA in a cross-sectional population-based study as well as a 5-year longitudinal study aimed at exploring host involving mainly nutritional and immune status, and pathogen factors that contribute to the failure of treatment of MDR-TB, ultimately leading to the emergence of XDR-TB strains. The identification of factors contributing to poor treatment outcomes in MDR-TB, could prove to be useful in formulating future TB control strategies.

CAPACITY BUILDING

AFRICAN CENTRE FOR INTEGRATED LEARNING (ACILT)

ACILT was established in 2008 on the Johannesburg campus of the South African NICD and the NHLS. The aim of this new centre is to build a new generation of laboratory experts, particularly in the fields of HIV, TB and malaria throughout Africa. The vision of ACILT is *"A healthier Africa through quality laboratory practices that support efforts to combat major infectious diseases",* while the mission is *"To provide integrated hands-on training courses to expand laboratory capacity in Africa for the diagnosis and monitoring of major infectious diseases including HIV/AIDS, TB and malaria."*

Various partners contributed to the establishment of ACILT, and some will partake in the governance of the centre. Stakeholders include the South African National Department of Health, CDC South Africa, NHLS, NICD, WHO AFRO, USAID, APHL, ASCP, Clinton Foundation, FIND, Roche Diagnostics, Becton Dickenson (US), ASM and JICA. PEPFAR funds the centre through the CDC South Africa.

Courses focus on TB culture/DST, microscopy and molecular diagnostics, HIV Early Infant Diagnosis PCR, BED, laboratory management and accreditation, quality management systems (QMS) and commodity management. By request from the CDC, a limited number of participants from non-African countries were included in 2009.

Up to December 2009, 21 courses have been offered by ACILT to 317 participants. The shortest courses were 3 days, while technician comprehensive training was for 20 work days. Participants included laboratory staff from Nigeria, Uganda, Egypt, Mali, Ethiopia, Mozambique, Namibia, Angola, Rwanda, Botswana, Malawi, Ivory Coast, Tanzania, Ghana, Sierra Leone, Sudan, DRC, Barbados, Vietnam, Brazil, Zambia, Cameroon, Kenya, India, Zimbabwe, Lesotho, Swaziland and South Africa.

Entrance to the new National TB Reference Laboratory at Sandringham, Johannesburg

Staff List

Executive Director, Prof BD Schoub MBBCh MMed MD DSc FRCPath FCPath FRSSAf MASSAf
I Latsky, Personal Assistant

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Deputy Director, Prof J Frean

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A Sooka Dip Med Tech, MSc, Laboratory Manager
F Mnyameni Dip Biomed Tech, Senior Technologist
M Ngomane Dip Biomed Tech, Senior Technologist
R Kganakga Dip Biomed Tech, Technologist,
T Mazibuko Dip Biomed Tech, Technologist Laboratory
M Mtambo, Laboratory Technician
V Moonallal, Laboratory Technician
N Tau BSc (Hons), Intern Scientist
H Ismail MSc Med, Medical Scientist
D van der Westhuizen BSc (Hons), Intern Scientist
Z Makhari, MSc Student
P Mogale, Data Capturer
M Dickmolo, Secretary

EXTERNAL QUALITY ASSESSMENT UNIT
Dr O Perovic MD DTM&H FCPath (Micro) SA MMed (Micro), Head of Unit (from November 2009)
VE Fensham Nat Dip Med Tech (Clin Path, Micro), Laboratory Manager
RR Landsberg BTech (BiomedTech), Medical Technologist
M Smith Nat Dip Med Tech (Micro), Medical Technologist and Curator, National Stock Culture Collection
P Botes Nat Dip Med Lab Tech (Micro), Medical Technologist (until May 2009)
E Khomane SMLTSA, Medical Technician
Qulu Y, Data Clerk

MYCOLOGY REFERENCE UNIT
Dr N Govender MBBCh, FC Path (SA) Micro MMed (Micro) DTM&H Dip HIV Man (SA), Pathologist, Head of Unit
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TG Zulu Dip Med Tech, Medical Technologist
M van Wyk MSc, Intern Medical Scientist
N Govender MSc, Intern Medical Scientist (until October 2009)
M Phadagi BSc (Hons), Intern Medical Scientist (until April 2009)
D Mlotshwa, Laboratory Clerk
B Zigana Dip Office Admin, Laboratory Clerk
B Nemukula, Student Medical Technician

NATIONAL MICROBIOLOGY SURVEILLANCE UNIT
Dr N Govender MBBCh FC Path (SA) Micro MMed (Micro) DTM&H Dip HIV Man (SA), Pathologist, Head of Unit
Dr VC Quan MBBCh DTM&H Dip Child Health (SA), Medical Officer
Dr ST Meiring MBChB DTM&H, Medical Officer
Nkosi D MBChB, Medical Officer (until December 2009)
M Fortuin-de Smidt MBChB, Medical Officer
P Crowther MSc (Med) Epidemiology & Biostatistics MSc (Med) Molecular Biology, Data Manager
J Appolis RN, Surveillance Officer
D Hlatshwayo RN, Surveillance Officer
Z Kgaphola RN, Surveillance Officer
S Joyi RN, Surveillance Officer
M Masuku RN, Surveillance Officer (until June 2009)
K Mawasha RN, Surveillance Officer
K Mazibuko RN, Surveillance Officer
B Mbatha RN, Surveillance Officer
R Merementsi RN, Surveillance Officer
C Miller RN, Surveillance Officer
N Mngceke RN, Surveillance Officer (until May 2009)
L Moapese RN, Surveillance Officer
M Mokwena RN, Surveillance Officer
LJ Morapeli RN, Surveillance Officer
A Motsi RN BA (Hon) Nursing Science, Surveillance Officer
I Naidoo RN, Surveillance Officer
S Njikho RN, Surveillance Officer
S Nkomo RN, Surveillance Officer
N Nzuza RN, Surveillance Officer
M Rakhudu RN, Surveillance Officer
F Seboya RN, Surveillance Officer (until November 2009)
N Shalabi RN, Surveillance Officer
G Moyo, Administration Officer
E Dloboyi, Laboratory Clerk
A Makgoga, Laboratory Clerk
T Mthembu, Laboratory Clerk
P Mogale, Laboratory Clerk
D Mlotshwa, Laboratory Clerk
B Zigana, Laboratory Clerk
N Ganaakgomo, Laboratory Clerk (until February 2009)

PARASITOLOGY REFERENCE UNIT
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K Mogoye BSc (Hons), Medical Scientist
K Kistiah BSc (Hons) Msc (Med), Medical Scientist
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Dr M du Plessis PhD, Senior Medical Scientist
Dr N Wolter PhD, Senior Medical Scientist
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C Moodley BSc (Hons) (Microbiology), Medical Scientist
P Tikilili BSc (Hons) (Medical Microbiology), Medical Scientist, started 01.12.2009
A Tshangela BSc (Hons) (Human Genetics), Medical Scientist, started 01.12.2009
VN Magomani BSc (Hons) (Molecular Biology), Intern Medical Scientist
A Makokga, Laboratory Clerk**,** started 01.08.2009
T Mthembu, Laboratory Clerk
M Carrim BSc, Medical Technician, resigned 08.01.2009
P Naidoo BSc, Medical Technician**,** started 01.11.2009
G Shirindza, Student Technologist**,** started 01.11.2009
S Rayise MSc, MRC Intern, started 01.02.2009
Prof KP Klugman MBBCh PhD DTM&H MMed FCPath (SA) FRCPath (Lond) FRSSAfr, Director of Research Unit (RMPRU Bara and NICD)
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L Collins, Secretary (Agency)
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Laboratory Team:
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N Gaanakgomo, Clerk (Laboratory)
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V Chiloane RN, Research Nurse
A Mothle RN, Research Nurse
T Nhlapo RN, Research Nurse
I Atlee RN, Research Nurse
N Metsing RN, Research Nurse
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S Mabogwane, HIV VCT Counsellor

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M Lo, PhD student

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M Dhoogra, PhD student
L Nardini, PhD student
Abdalla H, PhD student
C Kikankie, MSc student (graduated)
J Stiles-Ocran, MSc student
O Wood, MSc student
G Kloke, MSc student (graduated)
M Kaiser, MSc student
M Stradi, Msc student
R Norton, MSc student
K Waniwa, Msc student

VIROLOGY DIVISION

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M Madiga MSc, Medical Scientist
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N Malatsi MTech, PhD Student
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R Lassauniere BSc (Hons) (MSc student, respiratory virus programme)
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M Mashiloane, Laboratory Technician
T Maseko, Laboratory Technician

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AA Grobbelaar MSc, Medical Scientist
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CT Ndou BSc, Medical Technician
T Taleng Dip Med Lab Tech, Medical Technologist
NB Magome, General worker
SG Nkomo, General worker

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TE Chaane, Laboratory Assistant, Intern
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D Tigedi, General Worker

Animal Section
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J Maseko, General Worker
L Seema, General Worker
S Sibiya, General Worker
MS Mavhungu, General Worker

BSL-4 Workshop
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R Mabilo, Artisan
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E Motaung Cert Med Tech, Laboratory Assistant
D Lebambo, Laboratory Assistant

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N Ndlovu Nat Dip Med Tech (Microbiology), Medical Technologist
C Fourie Nat Dip Biomed Tech (Clinical Pathology & Virology), Medical Technologist
X Stuurman Nat Dip Biomed Tech (Virology), Medical Technologist
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L Harvey, Laboratory Assistant
P Makutu, Data Clerk
G Nkosi, Data Clerk
L Mangena, Data Clerk
H Hlanzi, Data Clerk

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A Sehata, Laboratory Assistant
R Simelane, Laboratory Assistant

Viral Serology Section
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B Miller Nat Dip Med Tech (Virology), Medical Technologist
MM Maleka Nat Dip Biomed Tech, Medical Technologist
M Mashele Nat Dip Med Tech (Virology), Medical Technologist
DS Motshegwa Nat Dip Biomed Tech, Medical Technologist
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SM Koruakae, Clerk Administration
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LM Cranston Higher Dip Med Tech (Microbiology, Parasitology & Virology), Medical Technologist
F Boshomane, Laboratory Assistant
S Moloto Dip Data Capture, Data Clerk
G Nkosi NQF4 (Business Administration), Data Clerk
S Lee, Data Clerk
I Khwane, Data Clerk
E Tseta, Data Clerk
J Kana, Data Clerk
E Mnguni, Messenger

Quality Assurance
K Fitchet Nat Dip Med Tech & Datametrics, Quality Manager
T Nhleko, Dip Man Asst, Quality Officer

Laboratory Support Services (General)
A Selepe, General Worker Leader
J Masekwameng, General Worker
M Mpyana, General Worker
F Mashangoane, General Worker
J Sekgobela, General Worker

VIRAL GASTROENTERITIS UNIT
Page NA, BSc (Agric); BSc (Agric) Hons; MSc (Med); PhD, Senior Medical Scientist, Head of Unit
Kruger T, BTech (Biotech), MSc (Med), Chief Biotechnologist
Nadan S BSc BSc Hons MSc (Med), Medical Scientist
Naidoo N, BSc (Hons), Intern Medical Scientist

EPIDEMIOLOGY DIVISION
Deputy Director, Dr LH Blumberg

EPIDEMIOLOGY & SURVEILLANCE UNIT
Dr C Cohen MBBCh DTM&H FCPath (SA) Micro MSc (Epidemiology), Specialist Microbiologist/Epidemiologist, Head of Unit
Dr J Moyes MBBCh MSc (Epidemiology) DTM&H, Medical Officer
Dr S Walaza BSc Hons (Occupational Therapy) MBChB, Medical Officer (appointed June 2009)
T Ginindza MSc (Epidemiology), Epidemiologist (resigned 30 September 2009)
V Dermaux-Msimang Bio-IR MSc (VEPH), Epidemiologist/Medical Scientist (appointed January 2009)
L Mlambo MSc (Epidemiology), Database manager/Statistician (appointed January 2009, resigned November 2009)
JM McAnerney RN RM Dip Data Dip Method, Nurse Epidemiologist
K Shangase RN, Surveillance officer (appointed June 2009)
W Ngubane RN, Surveillance officer (appointed August 2009)
S Kashe RN, Surveillance officer (appointed March 2009)
A Sambo RN, Surveillance officer (appointed February 2009)
N Malinga, Research assistant (appointed May 2009)
M Letyane, Research assistant (appointed May 2009)
J Mapalane, Research assistant (appointed May 2009)
N S Ndam, Secretary (appointed April 2009)
R Choeu, Data clerk (appointed August 2009)
B Letlape, Data clerk (appointed July 2009)
T Mathebula, Data clerk (appointed July 2009)
V Ndhlovu, temporary data clerk (appointed July 2009)
L Motsipe, temporary data clerk (appointed August 2009)

OUTBREAK RESPONSE UNIT
Dr G de Jong MBBCh DTM&H FCPath (Micro), Specialist Microbiologist, Head of Unit (resigned May 2009)
Dr J Thomas MBBCh DTM&H DipHIVMan(SA) FCPath (Micro), Specialist Microbiologist, Head of Unit (appointed September 2009)
Dr A Cengimbo BSc HDE MBChB DTM&H, Medical Officer
Dr C Makunga Bsc Hons MBChB, Medical Officer (resigned December 2009)
BN Archer BMedSc PGCertPH MPH, Field Epidemiologist (appointed January 2009)

TRAVEL & INTERNATIONAL HEALTH UNIT
Dr LH Blumberg MBBCh Mmed (Micro) DTM&H DCH DOH FFT (Glasgow), Specialist Microbiologist

L Millington, Publications Officer/Administration

PUBLIC HEALTH REGISTRARS (until end December 2009)
Waasila Jassat MBBCh
Muzimkhulu Zungu MBChB
Geraldine Timothy MBChB

SOUTH AFRICAN FIELD EPIDEMIOLOGY & LABORATORY TRAINING PROGRAMME
Dr BN Harris MBChB MMed (Community Health), Director

Dr F Ndugulile MD MMed (Microbiology /Immunology) Dip Health System Management, Laboratory Resident Advisor
Dr Khin San Tint MBBS MMed Sc (P&TM) MPH MMed Sc (Bioethics), Field and Epidemiology Track Coordinator
Dr M Tshimanga MBBS MPH, Epidemiology Resident Advisor
M Huma BSc Hons MPH, Field Epidemiologist
MM Malotle BSc Hons, 2nd year resident
MP Modise BSoc, 2nd year resident
TW Motladiile BSc BSc Hons Human Genetics Msc (Med), 2nd year resident
MD Nteo Nat Dip Biotech BTech Biotech, MTech Biotech, 2nd year resident
VM Ntlebi BSc Hons, 2nd year resident
GM Ntshoe Dip Med Tech (Chem Path) NHD Med Tech (Chem Path) BSc Med Hons (Envir Health), 2nd year resident
TT Sigudu BTech, Masters degree entrance programme, 2nd year resident
K Velen Bsc (Biological Science) BSc Med Hons (Medical Microbiology), 2nd year resident
PCB Zondo BSc Nursing Hons, AFENET Fellowship
V Chetty BSc (Biotechnology) BTech, 1st year resident
AG Dlomu BSc MSc (Medical Science), 1st year resident
BG Hottie BCur, 1st year resident
Dr MM Mathonsi BVSc, 1st year resident
MP Matsaneng BMed Sci Hons BSc (Micro), 1st year resident
AL Mazala PhD, 2nd year resident
L Mbata BSc BSc Med Hons, 1st year resident
RL Mokethe BCur BTech (Occup Health) Dip Nursing, 1st year resident
MA Phungwayo BSc Hons MSc (Med), 1st year resident

LR Quntana BCur Dip Clin Nursing, Dip Advanced Nursing, 1st year resident
B Temane, Administrative Support

SPECIAL PROGRAMMES

COMPREHENSIVE CARE, MANAGEMENT & TREATMENT PROGRAMME FOR HIV & AIDS

Dr TM Marshall MBBCh FCPath SA DTM&H, Head of Programme
N Cassim Higher Dip Med Tech, Diplomas in Business and Project Management, Project Manager/Business Analyst
EKM Tlale Dip Med Tech Cert Project Management, Project Manager
MT Mamahlodi BSc Med Sci, Project Manager
GN Mbenenge MBChB, Virology Registrar
MV Muthambi MBChB, Virology Registrar
SCT Sibeko, Secretary

TB FOCUS PROGRAMME & NATIONAL TUBERCULOSIS REFERENCE LABORATORY

Dr GJ Coetzee MBChB MMed MSc (London), Head of Unit
Prof HJ Koornhof MBChB DCP (London) Dip Bact (London) FRCPath, Consultant
Dr L Erasmus MBBCh MMed DTM&H, Pathologist
Dr L Matsoso BSc Hons MSc PhD, Scientist
Y Gardee Bsc Hons (Microbiology), Medical Scientist
Z Bhayat Nat Dip Biomed Tech, Medical Technologist
E Tsheola Nat Dip Biomed Tech BTech, Medical Technologist
N Ramdin Cert Med Tech (Microbiology), Medical Technician
Thlako H, Nat Dip Biomed Tech (Clin Path), Medical Technologist
Pepu B Nat Dip Biomed Tech (Clin Path and Micro), Medical Technologist
A Axcell BSc Hons (Physiology), Student Intern Scientist
R de Villiers, Controller (administration)
Q Motaung, Departmental Secretary
S Masne, Administration Officer

Staff & Student Achievements

MICROBIOLOGY DIVISION

ENTERIC DISEASES REFERENCE UNIT

Brett Archer (University of Pretoria)
Masters in Public Health Research Report: Graduated with Distinction, 2009.

Nevashan Govender (University of the Witwatersrand) MSc dissertation: Molecular epidemiology and mechanism of resistance of invasive quinolone-resistant South African isolates of *Salmonella enterica*, 2004-2006. Graduated 2009.

Sarika Dwarika (University of the Witwatersrand)
MSc dissertation: Molecular epidemiology of invasive isolates of *Salmonella enterica* serovar Typhimurium in Gauteng, South Africa, 2006-2008. Graduated 2009.

PARASITOLOGY REFERENCE UNIT

B Poonsamy attended the Malaria Microscopy Training Course, Malaria Diagnostic Centre of Excellence, in Kisumu, Kenya. This is a Walter Reed Army Institute of Research-affiliated organisation.

K Kistiah graduated MSc (Med), University of the Witwatersrand.

Prof J Frean spent sabbatical leave at the Australian Army Malaria Institute, Brisbane, Australia.

The Unit's 3 intern scientists met the requirements for registration as medical scientists in 2009.

RESPIRATORY & MENINGEAL PATHOGENS REFERENCE UNIT

Mmabatho Moerane passed the Professional Board for Medical Technology examination in the category of Microbiology on 11 March 2009, and registered as a Medical Technologist with the Health Professions Council of South Africa (HPCSA).

Azola Fali and Chivonne Moodley successfully registered as medical scientists with the HPCSA.

Chivonne Moodley was awarded the best poster prize in the infectious diseases category for her poster "Molecular characterisation of *Neisseria meningitidis* serogroup B isolates causing invasive disease in South Africa, 2002-2006" at the Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) Congress, Sun City, North West Province, 20-23 Aug 2009.

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT

Dr Jenny Rossouw visited the laboratory of Dr Wolfgang Beyer at the University of Hohenheim, Stuttgart, Germany, in March 2009 to work on the molecular diagnostics and typing (MLVA and SNP) of *B. anthracis* in South Africa.

Lorraine Arntzen attended the train-the-trainer workshop on Biosafety and Biosecurity in the Laboratory, hosted by the African Biological Safety Association held in Nairobi, Kenya, March 2009.

Lorraine Arntzen attended a grant writing workshop hosted by the International Association of National Public Health Institutes (EMROY Global Health Institute) held in Kampala, Uganda, March April 2009.

Natasha Trataris attended the Methods in Surveillance Workshop hosted by Veterinary Laboratory Association (UK) held in Durban, KZN, August 2009.

SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE

Prof David Lewis was presented, at the 11th IUSTI World Congress in Cape Town, with an IUSTI Silver Medal for outstanding international contribution to the global fights against STIs.

Dr Samuel Fayemiwo, from the College of Medicine (Ibadan, Nigeria), who undertook an attachment at the STI Reference Centre in 2008, received an IUSTI Bronze medal for his oral presentation.

The STI Reference Centre was awarded a 5 year co-operative agreement with the Department of STD Prevention at the Centers for Disease Control and Prevention, Atlanta, USA.

Prof David Lewis was appointed to be a member of a new WHO Expert Advisory Panel for Global Surveillance of Antimicrobial Resistance in *Neisseria gonorrhoeae*.

VECTOR CONTROL REFERENCE UNIT

Dr Lizette Koekemoer was selected as a member of the WHO/TDR Research Strengthening Group (RSG) steering committee.

Dr Lizette Koekemoer received the S2A3 2009 British Association Medal.

Prof Maureen Coetzee was the 2nd runner up in the DST/NRF Women in Science Awards.

Prof Richard Hunt received the Elsdon Dew Medal from the Parasitological Society of Southern Africa.

The VCRU was recognised by the University of the Witwatersrand as a Research Unit within the School of Pathology. The new unit is known as the Malaria Entomology Research Unit (MERU). Prof. Maureen Coetzee is the director of MERU.

Dr Basil Brooke was elected to the Editorial Board of the journal *African Entomology.*

Ms Luisa Nardini received the prize for the best "first time presenter" at the 39th Annual Conference of the Parasitological Society of Southern Africa.

VIROLOGY DIVISION

AIDS VIRUS RESEARCH UNIT

Virology Laboratory

Prof Lynn Morris received a promotion to Research Professor, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand. The promotion took effect from 1 April 2009.

Dr Elin Gray received a Prestigious Postgraduate Award for 2008. This is an annual award from the University of the Witwatersrand for the best PhD degree. Prof Bev Kramer, Assistant Dean, Research and Postgraduate Studies, University of the Witwatersrand presented this to Elin on 19 June 2009.

Dr Penny Moore was awarded a Sydney Brenner Fellowship. Dr Sydney Brenner is a South African-born and trained molecular biologist who was awarded the Nobel Prize in 2002.

Immunology Laboratory

Prof Clive Gray, Pholo Maenetje and Netty Malatsi were awarded Scholarships to attend the 4th SA AIDS Conference, which took place from 31 March to 3 April 2009 at the ICC in Durban.

Pholo Maenetjie received a DH Clinical Excellence Award for winning the 4th prize for Best Paper Presentation at the 4th SA AIDS Conference, 31 March to 3 April 2009 at the ICC in Durban.

Heather Hong was awarded a Fogarty Scholarship to the Global Infectious Diseases Research Training Program for a short-term training visit Professor Gilla Kaplan's laboratory at the University of Medicine and Dentistry of New Jersey, Public Health Research Institute Center. The training visit will be in preparation for her role on the upcoming NIH-funded project: "A Prospective Observational Study to Analyze Host and Pathogen Contributions in the Emergence of Extensively Drug Resistant Tuberculosis, RO1 AI080737" where she will measure plasma cytokines in patients experiencing episodes of MDR and XDR-resistance to TB drug treatment.

Cell Biology Laboratory

Prof Caroline Tiemessen and Dr Leonard Damelin each received a research award from the Faculty of Health Sciences, University of the Witwatersrand for "dedication and achievement in research" on the occasion of the 2009 Research awards dinner, August 6, 2009.

RESPIRATORY VIRUS UNIT

Dr Marietjie Venter received the University of Pretoria's Outstanding Young Researchers award at an awards ceremony to recognize Exceptional Academic Achievers which took place on 25 March 2009: Edoardo Villa Museum, Hatfield Campus, University of Pretoria.

Dr Venter was promoted to Associate Professor in the department Medical Virology, University of Pretoria in November 2009.

Dr Venter was nominated for the L'Oréal Women in Science awards, category Distinguished Women Scientist in the Area of Natural Sciences, Department of Science and Technology.

SPECIAL PATHOGENS UNIT

The following appointments and awards were received:

Dr Jacqueline Weyer was appointed as Extraordinary Lecturer to the Department of Microbiology, University of Pretoria in November 2009.

Mrs Busi Mogodi received the award for "Best poster presentation by a technologist" with her poster entitled "Colonization and breeding of Egyptian fruit bats (*Roussettus aegyptiacus*)" at the South African Association for Laboratory Animal Science (SAALAS) in Umhlanga, September 2009.

Dr Janusz T Paweska was appointed as Extraordinary Professor to the Department of Microbiology, University of Pretoria for his ongoing and outstanding contribution to postgraduate student education at the University in January 2009.

Dr Janusz T Paweska was appointed as Senior Researcher to the Department of Pathology of the University of the Witwatersrand in April 2008.

As from January 2009 Dr hab. Janusz T Paweska has been appointed as Extraordinary Professor in the Department of Microbiology, Faculty of Natural and Agricultural Sciences, University of Pretoria, and by virtue of the decision of December 30, 2009, the President of the Republic of Poland conferred upon him the title of Professor of Veterinary Science. In the Polish education system the title of professor is granted to holders of the degree of *doctor habilitatus,* who have proceeded to both outstanding academic attainments and important educational achievements. The professor title is officially conferred by the President of Poland upon a motion of the Central Commission for Academic Degrees and Titles.

Dr hab. Janusz T. Paweska, Head Special Pathogens Unit, NICD/NHLS, receiving the title of professor from the President of the Republic of Poland, Lech Kaczyński, 03 March 2010, Presidential Palace, Warsaw, Poland,

The following students finished their degrees:

Ms Ania Szmyd-Potapczuk was awarded a MSc degree (University of Pretoria) with a project entitled: "Molecular epidemiology of human rabies diagnosed in South Africa between 1983 and 2007". Prof Janusz Paweska and Dr Jacqueline Weyer were co-supervisors for this study.

The following workshops/training courses were successfully completed:

Mr Petrus Jansen van Vuuren completed the Certificate Course in Laboratory Animal Science, University of Pretoria in June 2009. He received his certificate with distinction.

Dr Jacqueline Weyer successfully completed the BSL4 Science and Safety Training Program held at the Emory University, Atlanta, Georgia in February 2009.

Mrs Phumza Lekhuleni (former employee) successfully completed the "Serological diagnosis of infectious diseases course", NHLS, Braamfontein in July 2009.

Mr Chaka Ndou successfully completed the "Advanced QMS including accreditation course", NHLS, Braamfontein in October 2009.

EPIDEMIOLOGY DIVISION

EPIDEMIOLOGY & SURVEILLANCE UNIT

Mr P Nyasulu was awarded the prize for best oral presentation at the University of The Witwatersrand School of Public Health Biennial Research Day for his talk entitled "Increased risk of death in HIV-infected patients with pneumococcal meningitis, South Africa, 2003-2005". The research presented was the subject of his report for the MSc Epidemiology conducted under the co-supervision of Dr Cheryl Cohen.

Dr Elvira Singh was awarded her MMed degree in public health in May 2009. She received a distinction for her project "An evaluation of the association between mortality and HIV infection in hospitalised patients with invasive meningococcal disease in South Africa" which was co-supervised by Dr Cheryl Cohen

Dr Ziyanda Vundle was awarded the degree of Masters in Medicine, Public Health in October 2009. The short report for this degree was completed during her rotation at the NICD and was entitled "Measles surveillance: Evaluation of a new laboratory testing algorithm at the National Institute for Communicable diseases, Johannesburg, South Africa".

Sr Jo McAnerney was presented with an award on her retirement after 29 years of service on 24 July 2009.

OUTBREAK RESPONSE UNIT

Brett Archer graduated from the SA-FELTP and obtained a Masters degree in Public Health (MPH) in the speciality of Epidemiology and Biostatistics.

THE SOUTH AFRICAN FIELD EPIDEMIOLOGY & LABORATORY PROGRAMME

Genevie Ntshoe, a Field Epidemiology and Training Programme resident was awarded the prize for best oral presentation at the Fifth African Regional Tephinet and Third Afenet Scientific Conference. August 31 September 4, 2009: Mombasa, Kenya. Her presentation related to work conducted in collaboration with ESU and was entitled: "The Viral Watch influenza surveillance programme in South Africa: Its attributes and viral Influenza patterns, 2005-2008." Genevie M Ntshoe, J McAnerney, L Blumberg, F Ndugulile, KTint

SPECIAL PROGRAMMES

NATIONAL TUBERCULOSIS REFERENCE LABORATORY

E Tsheola obtained her certificate in Advanced Project Management (Damelin)

COMPREHENSIVE CARE, MANAGEMENT & TREATMENT PROGRAMME FOR HIV & AIDS

Miss E Tlale obtained a Certificate in Laboratory Management for Diagnosis and Monitoring of HIV infection in Japan 2009.

International Conferences

Anyangu AS, Gould LH, Sharif SK, Nguku PM, Omolo J, Mutonga D, Rao CY , Lederman E, Schnabel D, Sang R, Paweska JT, Katz M, Hightower A, Njenga MK, Feikin DR, Breiman RF. Risk factors for severe Rift Valley fever infection in Kenya, 2007. OIE Report Regional Seminar on Re-emergence of Rift Valley fever in Southern Africa: how to better predict and respond, Bloemfontein, South Africa February 16-18, 2009.

Archer BN, Keddy KH, De Jong GM, Cohen C, Harris BN. The epidemiology of typhoid fever in South Africa 2003-2007 (oral presentation). 7th International Symposium on Invasive Salmonelloses, Kilifi, Kenya, 25- 28 January, 2009.

Archer BN, Keddy K. Evaluation of the enhanced site surveillance system for invasive disease caused by Salmonella spp. 7th International Symposium on Invasive Salmonelloses, Kilifi, Kenya, 25-28 January 2009. (Poster Presentation)

Auvert B, Lissouba P, Cutler E, Zarca K, Puren A, Taljaard D. High-risk Genital Human Papillomavirus Infection as a Risk Factor with HIV Incidence. Abstract W-1003. 16th Conference on Retroviruses and Opportunistic Infections (CROI), Montreal, Canada, February 8 -11, 2009.

Auvert B, Lissouba P, Cutler E., Zarca K, Puren A, Taljaard D. Is Genital Human Papillomavirus infection associated with HIV incidence? 5th IAS Conference on HIV Pathogenises, Treatment and Prevention (IAS), Cape Town, South Africa, July 19-22, 2009.

Barnard M, Tsheola E, Coetzee G, Erasmus L, Van Schalkwyk E, Koornhof HJ, Bosman M. Inter-laboratory comparative analysis and accreditation for the MTBDR*plus* LPA in South Africa Abstract PS-94629-06 40th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease, Cancun, Mexico, 3-7 December 2009.

Barnard M, Tsheola E, Erasmus L, Koornhof HJ, Bosman M, Coetzee G. Inter-laboratory comparative analysis of the performance of the TB line probe assay in South Africa. Abstract PS-95428-06. 40th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease, Cancun, Mexico, 3-7 December 2009.

Barnard M, Bhayat Z, Coetzee G, Azevedo A, Cox H, Mc Dermot C, Bosman M, Koornhof HJ. Low level isoniazid and ethionamide co-resistance:preliminary findings in the Western Cape. Abstract PS-95400-05 40th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease, Cancun, Mexico, 3-7 December 2009.

Blumberg L. African viral haemorrhagic fevers and risks to travelers. Oral presentation. Tropical Diseases Network meeting, Oxford, UK, September 2009.

Blumberg L. H1N1 in HIV-infected persons - the South African experience. WHO Clinical Consultation, Washington, USA, 14-16 October 2009.

Cele LP, Khin San Tint, Cooper L. Malaria Surveillance data analysis (2003-2007) KwaZulu-Natal, South Africa. Poster presentation. 3rd African Field Epidemiology Network (AFENET) Scientific Conference, 31 August to 4 September 2009 in Mombasa, Kenya.

Cele LP, Khin San Tint, Khoza C. HIV and Syphilis Antenatal Seroprevalence survey, Kwa-Zulu Natal province, South Africa, 2007. Poster presentation. 3rd African Field Epidemiology Network (AFENET) Scientific Conference, 31 August to 4 September 2009 in Mombasa, Kenya.

Choi KS, Koekemoer LL, Coetzee M. Population genetic structure of *Anopheles funestus* group in Southern Africa, 5th MIM Pan-African Malaria Conference, Nairobi, Kenya. 2-6 November 2009.

Coetzee M. Malaria vector mosquitoes-new species still being discovered and the implications for malaria control in Africa. The African malaria vector *Anopheles funestus* a species complex. Europe-Africa Frontier Research Conference Series, Infectious Diseases: from basic to translational research, Cape Town, South Africa. 4-9 April, 2009.

Coetzee M. New Species In The Genus Anopheles: Do They Matter For Genetic Control Of Vectors? European Molecular Biology Organization Conference, Crete, Greece. 20-24 July 2009.

Coetzee M. Insecticide resistance in malaria vector mosquitoes. 7th Congress of Toxicology in Developing Countries, Sun City, South Africa. 8-10 September 2009.

Coetzee M. Tracking insecticide resistance in Africa. Pan-African Malaria Vector Control Conference, Zanzibar. 25-29 October 2009.

Cohen C, Viboud C, Simonsen L, Miller M, Kang J-W, Besselaar TG, McAnerney JM, Blumberg L, Schoub BD. Estimation of influenza-related excess mortality in South African seniors, 1998-2005.. Multinational Seasonal Influenza Mortality (MISMS) Meeting, 21-25 April 2009, Meridien Conference Centre, Dakar, Senegal.

Cohen C. Influenza surveillance activities, South Africa. Influenza Burden of Disease Workshop and Vaccine Effectiveness Meeting. 26-28 August 2009, Bangkok, Thailand.

Cox H, Mc Dermid C, Coetzee D, Goemaere E, Kasne N, Hall L, Xiniwe S, Bosman M, Barnard M, Coetzee G, Simpson J, Azevedo V. Prevalence of drug resistant tuberculosis and association with HIV in Khayelitsha, South Africa. World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease, Cancun, Mexico, 3-7 December 2009.

Crowther P, Cohen C, Govender N, Keddy K, von Gottberg A for for the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA). Predictors of non-reporting to a national laboratory-based surveillance programme. Emerging Infections Program Network: Active Bacterial Core surveillance (ABCs) Surveillance Officer Meeting, San Francisco, USA, 1-2 June 2009.

du Plessis M, Mothibeli K, von Gottberg A, Murphy E, Andrew L, Hoiseth SK, Zlotnick G and Klugman KP. Factor H binding protein diversity among *Neisseria meningitidis* isolates causing invasive disease in South Africa, 2005. Oral presentation. Tenth European Monitoring Group for Meningococci (EMGM) Meeting, The Radisson Hotel, Manchester, UK, Jun 17-19, 2009.

Dwarika S, Smith AM, Keddy KH. Characterization of *Salmonella* in chickens isolated in Gauteng South Africa, 2007-2008. 7th International Symposium on Invasive Salmonelloses, Kilifi, Kenya, 25-28 January, 2009.

El-Khatib Z, Katzenstein D, Ledwaba J, Laher F, Maphuti M, Kassaye S, Mohapi L, Petzold M, Ekstrom AM, Morris L on behalf of the South African Adherence & Virologic Evaluation (SAVE) study team. Adherence and drug resistance in an HIV treatment program in South Africa. Abstract 668 (Poster Presentation). Conference on Retroviruses and Opportunistic Infections (CROI) Montreal, Canada, 8-11 February 2009.

Fayemiwo S, Müller E, Gumede L, Lewis D. Plasmid-mediated antibiotic resistance among gonococci in South Africa. Oral presentation at the 11th International Union against STIs World Congress, Johannesburg, November 9-12, 2009.

Feldman C, Brink AJ, von Gottberg A, Wolter N, de Gouveia L, Perovic O, Klugman KP. Antimicrobial Susceptibility of Pneumococcal Isolates Causing Bacteremic Community-Acquired Pneumonia in Gauteng, South Africa. Poster Board #B59. American Thoracic Society International Conference, San Diego, May 15-20, 2009. *Am J Respir Crit Care Med* 179;2009:A5962.

Firnhaber CS, Sello M, Maskew M, Williams S, Schilze D, Williamson AL, Allan B, Sanne I, Lewis D. Determination of human papillomavirus (HPV) types in HIV seropositive men with genital warts in Johannesburg, South Africa. Poster presentation at the 25th International Papillomavirus Conference, Malmö, Sweden, 8-14 May 2009.

Friedman A, Leichliter J, Paz Bailey G, Habel M, Sello M, Vezi A, Lewis D. Identifying and addressing men's STI-related knowledge, beliefs, experiences and information needs in Gauteng, South Africa. Poster presentation at the 18th Meeting of the International Society for STD Research, London, June 28 to July 1, 2009.

Govender N, Smith AM, Karstaedt AS, Keddy KH for GERMS-SA. First report of plasmid-mediated quinolone resistance in Enterobacteriaceae from South Africa. 7th International Symposium on Invasive Salmonelloses, Kilifi, Kenya, 25- 28 January, 2009.

Govender N, Patel J, Cohen C, Chiller T, Lockhart S for GERMS-SA. Trends in antifungal drug susceptibility of Cryptococcus species in South Africa, 2002-2008 The 17th Congress of The International Society for Human and Animal Mycology 2009 (ISHAM 2009). 23-29 May, Tokyo Japan.

Govender N. Cryptococcosis in Sub-Saharan Africa (invited speaker). In: Abstracts and Program Book (Abstract EP-01-3), International Society for Human and Animal Mycology (ISHAM)-2009, Tokyo, Japan, 23-29 May 2009.

Gray C. Dichotomous associations between CD4+ T cell activation, memory differentiation and viral control during primary HIV infection. Poster Presentation at the HIV Acute Infection Meeting, Boston USA, 23-24 September 2009.

Gray ES, Moore PL, Madiga M, Mlisana K, Abdool Karim SS, Binley JM, Shaw GM, Mascola J, and Morris L. Broadly cross-reactive anti-MPER plasma antibodies define novel epitopes. (Poster Presentation) Keystone Symposia HIV Immunology: From Infection to Immune Control, Keystone Resort, Colorado, USA, 22-27 March 2009.

Gray E. Evolution of an anti-MPR gp41 antibody response that mediates broad HIV-1 cross-neutralization. (Oral Presentation) CHAVI (Center for HIV/AIDS Vaccine Immunology) 5th Annual Retreat, Durham, NC, USA, 4-7 October 2009.

Grobbelaar A, Swanepoel R, Paweska J. Molecular epidemiology of Rift Valley fever virus based on genetic analysis of the virus isolates recovered in 1944-2008 from distinct geographic regions. FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health, Vienna, Austria, 8-11 June, 2009.

Habel MA, Leichliter JS, Paz Bailey G, Friedman A, Sello M, Lewis DA. We only get *one* day": facilitators and

barriers to STI/HIV health care among South African men. Poster presentation at the 18th Meeting of the International Society for STD Research, London, 28 June 28 to 1 July, 2009.

Huma M, Khosa E, van der Gryp R. Hepatitis A outbreak in a Primary school, Claremont-Tshwane, Gauteng Province, March to October 2008. Poster presentation. 3rd African Field Epidemiology Network (AFENET) Scientific Conference, 31 August to 4 September 2009 in Mombasa, Kenya.

Hunt G, Taylor B, Coovadia A, Abrams EJ, Sherman G, Meyers T, Morris L, Kuhn L. Development of drug resistance among a cohort of HIV-infected infants exposed to nevirapine for pMTCT initiating protease-inhibitor-based antiretroviral therapy in South Africa. Abstract 957 (Poster Presentation). Conference on Retroviruses and Opportunistic Infections (CROI) Montreal, Canada, 8-11 February 2009.

Hunt G, Ledwaba J, El-Khatib Z, Coovadia A, Seoighe CX, Kuhn L, Katzenstein D, Morris L. Drug resistance patterns among HIV-infected children and adults failing Kaletra-based regimens in South Africa. (Oral and Poster Presentation) XVIII International Drug Resistance Workshop, Fort Myers, Florida USA 9-13 June 2009.

Jacobs CA, Argent A, Cohen C, Cameron NA, Whitelaw A, Harris BN, Blumberg L. Evaluation of the Public Health Response to an Isolated Diphtheria Death in Cape Town, South Africa, 2008. Poster presentation. 3rd African Field Epidemiology Network (AFENET) Scientific Conference, 31 August to 4 September 2009 in Mombasa, Kenya.

Jacobs CA, Harris BN, Cameron NA. Risk factors for diarrhoea in young children in Delft, Cape Town, South Africa, 2008: A Case-Control Study. Poster presentation. 3rd African Field Epidemiology Network (AFENET) Scientific Conference, 31 August to 4 September 2009 in Mombasa, Kenya.

Jacobs CA, Harris BN, Khin San Tint, Cameron NA, Naledi T, Roux I. Cervical Cancer Screening: An analysis of the Cytology and District Health Information Systems in the Western Cape Province, South Africa. Poster presentation. 15TH Maternal and Child Epidemiology (MCH EPI) Conference, Grand Hyatt Hotel, Tampa Bay, Florida, USA , 9- 11 December 2009.

Jansen van Vuren P, Tiemessen CT, Paweska JT. Evaluation of a recombinant Rift Valley fever virus nucleocapsid as a vaccine immunogen in combination with four adjuvants. International Meeting on Emerging Diseases and Surveillance, Vienna, Austria, February 13-16, 2009.

Jansen van Vuren P, Paweska JT. A comparative evaluation of ELISA-based techniques for serodiagnosis of Rift Valley fever. Arbo-Zoonet Annual Meeting, St. Raphaël, France, 30 September, 2009.

Jansen van Vuren P, Paweska JT. Safe detection of Rift Valley fever virus in human and animal specimens by a sandwich ELISA. International Meeting on Emerging Diseases and Surveillance, Vienna, Austria, February 13-16, 2009.

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Acknowledgement of Funders

MICROBIOLOGY DIVISION

ENTERIC DISEASES REFERENCE UNIT
- Medical Research Council
- National Health Laboratory Service
- PEPFAR
- World Health Organization

EXTERNAL QUALITY ASSESSMENT UNIT
- WHO African Regional Office, Brazzaville, and WHO International Health Regulations Co-ordination Programme (NICD/WHO EQA Programme)
- GlaxoSmithKline Biologicals (malaria vaccine trial)

MYCOLOGY REFERENCE UNIT
- National cryptococcal surveillance project (GERMS-SA): NHLS/CDC Cooperative Agreement
- Antifungal susceptibility testing of South African isolates of *Cryptococcus neoformans* against fluconazole and amphotericin B: NHLS Research Trust.

NATIONAL MICROBIOLOGY SURVEILLANCE UNIT
- GERMS-SA surveillance programme: NHLS/CDC Cooperative Agreement

PARASITOLOGY REFERENCE UNIT
- Medical Research Council
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- National Health Laboratory Service Research Trust
- World Health Organization
- GlaxoSmithKline Biologicals
- Pfizer South Africa

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- Centers for Disease Control and Prevention (CDC/NHLS cooperative agreement)
- Global Alliance for Vaccine and Immunization Initiative (GAVI)
- Medical Research Council (MRC), South Africa
- National Research Foundation (NRF), South Africa
- Wyeth
- Sanofi Pasteur

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT
- German Research Foundation (DFG), GZ:BE2157/3-1

SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE
- Agence Nationale de Reserches sur la SIDA et les hépatites virales (ARNS), France.
- National Health Laboratory Service
- PEPFAR through the CDC:NICD and the STIRC: DSTDP Co-operative Agreements, USA
- Poliomyelitis Research Foundation, South Africa
- International Union against STIs

VECTOR CONTROL REFERENCE UNIT
- UBS Optimus Foundation, Switzerland
- World Health Organization
- National Research Foundation
- Medical Research Council of South Africa
- NHLS Research Trust
- South African Malaria Initiative (SAMI)

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- Bill & Melinda Gates Foundation
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- Department of Science and Technology/LIFElab
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- HIV Vaccines Trial Network (HVTN)
- National Institutes of Health (NIH)
- Office of AIDS Research, National Institute of Health
- Poliomyelitis Research Foundation (PRF)
- South African AIDS Vaccine Initiative (SAAVI)
- University of Pennsylvania
- Virax Holdings
- Wellcome Trust International Senior Research Fellowship (CT Tiemessen)
- WHO-UNAIDS-African AIDS Vaccine Programme

RESPIRATORY VIRUS UNIT
- Department of Education: to establish a new high containment (BSL-3) laboratory in the Faculty of Health Sciences, University of Pretoria to facilitate research on zoonotic diseases.
- National Research Foundation: development of molecular and immunological tools for diagnosis, prevention and control of West Nile virus in South Africa: Impact and immune control of neurological cases in humans and horses.
- Medical Research Council: Association of HIV infection and genetics with severe lower respiratory tract infection in children in South Africa.
- Poliomyelitis Research Foundation research grant for the project: Identification and characterization of host and viral factors associated with immunopathogenesis of respiratory syncytial virus

and newly identified viral causes of acute lower respiratory tract infections in children in South Africa.
- National Health Laboratory Services Pathology Research Award: Characterization of pathogenic flaviviruses, alphaviruses and other arboviruses in South Africa.
- Department of Education: to establish a new high containment (BSL-3) laboratory in the Faculty of Health Sciences, University of Pretoria to facilitate research on arboviruses.
- Centers for Disease Control and Prevention: Co-operative agreement grant: preparedness and response to avian and pandemic influenza in South Africa (CDC-RFA-CI07-702).

SPECIALIZED MOLECULAR DIAGNOSTICS
- ANRS
- Bill and Melinda Gates Foundation
- PEPFAR
- National Health Laboratory Service
- World Health Organization

SPECIAL PATHOGENS UNIT
- Biological Diagnostic Supplies Limited Flow Laboratories
- European Union
- International Atomic Energy Agency
- National Health Laboratory Trust Grant Fund
- Poliomyelitis Research Fund
- Rheinische Friedrich Wilhelms-Universitat, Bonn University

VIRAL GASTROENTERITIS UNIT
- PRF 07/10 for the project titled “The development of real-time detection techniques and increased surveillance of diarrhoeal disease viruses in the South African population”.
- GSK grant for Rotavirus Sentinel Surveillance in South Africa

EPIDEMIOLOGY DIVISION

EPIDEMIOLOGY & SURVEILLANCE UNIT
- Centers for Disease Control and Prevention. Cooperative Agreement, Preparedness and Response to Avian and Pandemic Influenza
- Centers for Disease Control and Prevention. Provision of Strategic Information through Laboratory-based Surveillance for AIDS-associated Bacterial and Fungal Opportunistic Infections in South Africa

SOUTH AFRICAN FIELD EPIDEMIOLOGY & LABORATORY TRAINING PROGRAMME
- President's Emergency Plan for AIDS Relief (PEPFAR)
- The South African National and Provincial Departments of Health
- National Health Laboratory Services
- National Institute for Communicable Diseases
- United States Centrers for Disease Control and Prevention
- United States Health and Human Services Pandemic Influenza Fund
- The African Field Epidemiology Network (AFENET)

SPECIAL PROGRAMMES

NATIONAL TUBERCULOSIS REFERENCE LABORATORY
- Centers for Disease Control & Prevention (CDC)
- Foundation for Innovative New Diagnostics (FIND)
- University Research Company, LLC (URC), USA
- National Institutes of Health (NIH)

Visitors to the NICD

MICROBIOLOGY DIVISION

ENTERIC DISEASES REFERENCE UNIT

Prof Kryoshi and Prof Taro Yamamoto, Department of Science and Technology visited NICD EDRU February 2009.

Dr Patricia M. Griffin was an invited guest to EDRU at the PI meeting 5 & 6 November 2009. Dr Griffin is Chief Medical epidemiologist from the Foodborne Enteric Diseases Epidemiology Branch, Division of Foodborne, Bacterial, and Mycotic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Centers for Disease Control and Prevention (CDC) in the US.

Dr John Wain was hosted by EDRU on 26 October 2009, as part of the HPA-NICD collaboration meeting.

MYCOLOGY REFERENCE UNIT

Ben Park, MD, MPH, Epidemiologist, Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA

Naureen Iqbal, Antifungal Drug Susceptibility Unit, Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA

Angela Ahlquist, Epidemiologist, Mycotic Diseases Branch, Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, USA

NATIONAL MICROBIOLOGY SURVEILLANCE UNIT

Cynthia Whitney, MD, Branch Chief, Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, USA

Victor Fernandez, Microbiologist, Swedish Institute for Infectious Disease Control, Sweden

Katherine Gaskell, MD, London School of Hygiene and Tropical Medicine, United Kingdom

Patricia Griffin, MD, Chief, Enteric Disease Epidemiology Branch, Centers for Disease Control and Prevention, Atlanta, USA

Angela Ahlquist, Epidemiologist, Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, USA

Benjamin Park, Epidemiologist, Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, USA

PARASITOLOGY REFERENCE UNIT

Dr Tim Inglis, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, Australia.

RESPIRATORY & MENINGEAL PATHOGENS REFERENCE UNIT

Visitors from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA visited RMPRU and spent an intensive 3 days (21-23 September 2009) planning a case-control study to evaluate the pneumococcal conjugate vaccine as being used in the Expanded Programme on Immunisation (EPI) of the Department of Health, South Africa. The CDC staff that visited: Laura Conklin MD, Medical Officer; Jennifer Rabke Verani, MD MPH, Medical Epidemiologist; and Ms Elizabeth Zell, Mathematical Statistician. All are from the Respiratory Diseases Branch of the CDC.

Dr Cynthia Whitney, Chief, Respiratory Diseases Branch, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, held meetings with NICD staff related to ongoing RMPRU research projects during her visit to the NICD, 4-6 November 2009.

SPECIAL BACTERIAL PATHOGENS REFERENCE UNIT

Drs Wolfgang Beyer and Judith Lazak, Institute of Environmental and Animal Hygiene, University of Hohenheim, Stuttgart, Germany

Dr Peter Turnbull, executive editor of 'Anthrax in humans and animals', 4th edition (WHO guidelines), Salisbury, United Kingdom.

Drs Gisela Eberle and Massimo Scacchia, Central Veterinary Laboratory, Windhoek, Namibia.

Professor Tim Inglis, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Crawley, Australia.

Delegation from American Embassy: Dr Kimberly Ottwell, Dr Paul McOmber, Dr Mary Awantang, Susara Dahms, and Special Agent Paul Sanchez.

Members of the Anthrax Working Group: Dr R Bengis, Dr H Pienaar and A Dekker from DAFF, Dr H van Heerden, Dr J Picard, Dr A Michel, and A Hassim from UP (Onderstepoort), Dr A Galaw and Dr A van Zyl from ARC-OVI, Dr W Ramkrishna from NDoH (Communicable Disease Control), Col P van der Merwe from SANDF (Division Animal Health), and D Govender from DEAT (SANParks).

SEXUALLY TRANSMITTED INFECTIONS REFERENCE CENTRE

Professor Bertran Auvert, Professor of Public Health, University of Versailles, Paris, France

Dr. Sarah Alexander, STI Bacteria Laboratory, Health Protection Agency, UK

Professor Magnus Unemo, National Reference Laboratory for Pathogenic Neisseria, Örebro University Hospital, Sweden

Dr. Mary Kamb, Capt. US Public Health Service, International Coordinator, Division of STD Prevention, Centers for Disease Control & Prevention (CDC), Atlanta, USA

Dr. Jami Leichliter, Centers for Disease Control and Prevention (CDC), Atlanta, USA
Dr. Susan Hariri, Centers for Disease Control and Prevention (CDC), Atlanta, USA
Dr. Ye Htun, Centers for Disease Control & Prevention (CDC), Atlanta, USA
Dr. Cheng Chen, Centers for Disease Control and Prevention (CDC), Atlanta, USA

VECTOR CONTROL REFERENCE UNIT
Dr Glynn Vale, SACEMA, Zimbabwe
Mr Keith Hargreaves, KZN Malaria Control Programme
Prof Belinda Bozolli and Mr Iain Burns, DVC Research Office, University of the Witwatersrand
Prof Daniel Boakye, Noguchi Memorial Institute, Accra, Ghana
Dr John Govere, WHO AFRO, Harare, Zimbabwe

VIROLOGY DIVISION

AIDS VIRUS RESEARCH UNIT
Dr. David Katzenstein, Professor of Medicine, Stanford University, USA
Dr Chris Seebregts, Medical Research Council
Dr Chris Hoffmann, Division of Infectious Diseases, The Johns Hopkins Hospital, New York, USA
Prof. Debra Meyer, Biochemistry Department, University of Pretoria
Dr. Guido Ferrari, Duke University
Dr. Steve Perfetto, Vaccine Research Centre, NIAID, NIH
Dr. Mike Betts, University of Pennsylvania
Professor Gilla Kaplan, University of Medicine and Dentistry of New Jersey
Dr Dorothy Fallows, University of Medicine and Dentistry of New Jersey
Dr Louise Kuhn, Columbia University, New York, USA

SPECIALIZED MOLECULAR DIAGNOSTICS UNIT
Dr Francis Kasolo, WHO-AFRO, Cite Du Djoue, BP06 Brazzaville, Republic of Congo
Dr Charles Byabamazima, WHO-Intercountry, Harare, Zimbabwe
Dr Mark Pallansch, Centers for Disease Control & Prevention, Atlanta, USA
Dr Karen Ching, Centers for Disease Control & Prevention, Atlanta, USA
Dr Esther de Gourville, WHO-HQ, Geneva, Switzerland
Dr Diop Ousmane, Institut Pasteur de Dakar, Senegal
Dr Annick Dosseh, WHO, IST, Ougadougou, Burkina Faso
Dr Adjogoua Edgard Valery, Institut Pasteur de Cote d'Ivoire, Cote d'Ivoire
Dr Kofi Odoom, NMIMR, Legon, Accra-Ghana
Mr Joshua Dawurung, WHO National Polio Lab, Maiduguri, Borno State, Nigeria
Ms Chipo Berejena, Zimbabwe National Virology Lab, Parirenyatwa Hospital, Zimbabwe
Ms Prossy Wamanga, UVRI, Entebbe, Uganda
Mr Keith Shaba, WHO-AFRO, Cite Du Djoue, Brazzaville, Republic of Congo
Mr Riziki Yogolelo, INRB, Democratic Republic of Congo
Dr Fall Hamet, Institut Pasteur de Dakar, Senegal
Mr Arthur Mazitchi, Institut Pasteur, Bangui, Central African Republic
Dr Nelson Seta Adriamamonty, Institut Pasteur de Madagascar

SPECIAL PATHOGENS UNIT
Professor Mark M Rweyemamu, Executive Director, Southern African Centre for Infectious Disease Surveillance (SACIDS), Sokoine University of Agriculture (SUA), Morogoro, Tanzania
Professor Dominic Kambarase, Sokoine University of Agriculture (SUA), Morogoro, Tanzania
Jeroem Kortekaas, Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands
Riank Vloet, Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands
Chandre Gould, Senior Researcher, Crime and Justice Programme, Institute of Security Studies
Dr Brian Rappert, Exeter University, Exeter, England
Dr Emanuelle Tuerlings, Biorisk Reduction for Dangerous Pathogens (BDP), Dept of Epidemic and Pandemic Alert and Response (EPR), World Health Organization
Dr Michael Selgelid, Head: WHO Collaborating Centre for Bioethics, Centre for Applied Philosophy and public Ethics, Australian National University, Canberra

VIRAL DIAGNOSTICS UNIT
Constance Ducar, International Lab Program Manager, HIV Vaccines Trial Network
Dr. Charles R Byabamazima, Polio & Measles Laboratory Coordinator, WHO Inter Country Support Team/EPI, Eastern & Southern Africa (ESA)
Dr David Featherstone, WHO Head Quarters
Travis Behm, B.S. Laboratory Data Coordinator, Frontier Science and Technology Research Foundation, C/O HIV Vaccines Trial Network
Jason Carr, LDMS User Support, Frontier Science and Technology Research Foundation, C/O HIV Vaccines Trial Network
Ms Jeanette Twell, Inspections, Diagnostics and Laboratory Technology, Essential Health Technologies,World Health Organization
Dr Gabby Vercauten, Essential Health Technologies, World Health Organization
Ameena E Goga, Paediatrician and Specialist Scientist, Health Systems Research Unit, Medical Research Council

EPIDEMIOLOGY DIVISION

EPIDEMIOLOGY & SURVEILLANCE UNIT
Dr Margaret Cortese, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC) in Atlanta, USA
Dr Deborah Atherley, senior health economist and policy officer, Programme for Appropriate Technologies in Health (PATH)
Dr Richard Davis, senior program analyst, Office of Global Health, Centers for Disease Control in Atlanta

Dr Duncan Steele, Rotavirus Vaccine Programme, Programme for Applied Technologies in Health (PATH) in Seattle
Dr Jennifer Verani, Medical Epidemiologist, Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention in Atlanta
Dr Laura Conklin, Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention in Atlanta
Ms Elizabeth Zell, Statistician, Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention in Atlanta
Dr George Nelson an Epidemic Intelligence Service Program, Office of Workforce and Career Development, Centers for Disease Control in Atlanta, USA
Dr Cynthia Whitney, Acting Chief, Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention in Atlanta

OUTBREAK RESPONSE UNIT
Dr Lara Payne-Hallstrom and Dr Denis Coulombier, European Centre for Disease Control, Stockholm, Sweden
Dr Mauritzio Barbeshi and Dr Michael Hopmeier, World Health Organization
Dr Marc Mendelson, Division of Infectious Diseases and HIV Medicine, University of Cape Town
Ms Nadine Sunderland, Centers for Disease Control and Prevention, Atlanta, USA
Mr Peter Fuhri, Mr Dries Pretorius, Ms Tsakane Furumels and Mr Johan van Heerden from the National Department of Health
Prof Adriano Duse, Department of Clinical Microbiology, School of Pathology, University of the Witwatersrand

SOUTH AFRICAN FIELD EPIDEMIOLOGY AND LABORATORY TRAINING PROGRAMME
US CDC, Division of Global Public Health Capacity Development, Coordinating Office for Global Health
- Mr Eric Gogstad, Public Health Advisor
- Mr Bassam Jarrar, Deputy Director
- Ms Juliette Mannie, Management and Programme Analyst
- Dr Peter Nsubuga, Branch Chief
- Dr Italia Rolle, Epidemiologist
- Dr. Jim Vaughan, Division of International Health

US CDC, South Africa
- Dr Okey C Nwanyanwu, Country Director, CDC, Global AIDS Program, South Africa
- Dr Thurma M Goldman, Country Director, CDC, Global AIDS Program, South Africa
- Dr Katherine Robinson, Epidemiologist/Surveillance Officer, Acting Branch Chief, Epidemiology and Strategic Information Branch

US CDC, Other
- Dr Ingrid Weber, Medical Epidemiologist, Arboviral Diseases Branch, DVBID

AFENET
- Dr Gerald Shambira, Zimbabwe FETP
- Dr Notion, Gombe, Zimbabwe FETP
- Dr Ousmane Badolo, Chief Surveillance Officer, Ministry of Health, Burkina Faso
- Prof Lassana , Sangare, Head, Bactériologie-Virologie, Ouagadougou University, Burkina Faso
- Dr Danladi Nassoury, Chef Division de l'Epidémiologie, Coordonnateur National du PEV, Ministry of Health, Togo
- Prof Laurent, Ouedraogo, Publique Health Prof at University of Ouagadougou (Burkina Faso)
- Dr Henry Akpan, Director, Nigerian FELTP
- Dr Patrick Nguku, Resident Advisor, Nigerian FELTP

Universities
- Prof Kuku Voyi, Chair, School of Health Systems and Public Health, University of Pretoria
- Dr Neil Cameron, Department of Community Health, Stellenbosch University
- Dr Stephen Knight, Department of Community Health, University of KwaZulu-Natal
- Professor John Matjila, Head, Department of Community Health, University of Pretoria
- Dr Goedele Louwagie, Senior Specialist, Department of Community Health, University of Pretoria
- Prof Dan Kayongo, Director, Eastern Cape Regional Training Centre, Walter Sisulu University, Mthata, Eastern Cape
- Prof Cheryl McCrindle, Veterinary Public Health, Faculty of Veterinary Science. University of Pretoria
- Prof. Isabella Quakyi, Dean, School of Public Health, University of Ghana
- Dr TM, Chandra, Senior Manager, Health Services Research Unit, and Department of Community Health, Faculty of Health Sciences, University of Free State Free State
- Dr John Tumbo, Bojanala Platinum District, and Family Physician, Department of Family Health

Department of Health
National Department of Health
- Dr Yogan Pillay, Deputy Director General: Strategic Health Programmes
- Dr Frew Benson, Cluster Manager, Communicable Disease Control
- Dr Lindiwe Makubalo, Cluster Manager, Health Information Evaluation Epidemiology and Research
- Ms Stephina Tshelane, Deputy Director: Epidemiology and Surveillance
- Dr Dan Kibuuka, Data Manager, Child & Youth Health, National
- Mr Wayne Ramkrishna, Deputy Director: Public Health, Communicable Diseases

Gauteng Province
- Dr M L Likibi, Medical Specialist, Gauteng
- Ms Thandi, Chaane, Chief Director Health Programmes, Gauteng
- Ms Joy, Mnyaluza, CDC/Outbreak/EPI Coordinator, Gauteng

- Ms Mpho Moshime, Tshwane/Medwsiding EPI/CDC Coordinator, Gauteng
- Dr Hannelie Venter, Deputy Director, Clinical Support, Tshwane District, Gauteng
- Dr Baski, Desai, Deputy Director: Public Health, City of Johannesburg
- Ms Antonia , Barnard, Deputy Director: Public Health, Communicable Diseases, City of Johannesburg

Mpumalanga Province

- Mr Molefe Machaba, Strategic Planning, Information, Research and Epidemiology,
- Ms Anne-Marie Gouws, Cluster Manager : INP/MCWYH/EPI, Mpumalanga
- Ms Jaqui Kekana, Delmas District CDC, Mpumalanga
- Ms Riensie Vellema, Provincial CDC Surveillance Manager, Mpumalanga
- Mr Mandla Zwane, Provincial CDC Programme Manager, Mpumalanga

Eastern Cape Province

- Ms Elmarie, Claasen, District Information Manager, Port Elizabeth, Eastern Cape
- Mr Thomas, Dlamini, Communicable Disease Control, Eastern Cape
- Dr E Hoosain, Director: Public Health, Nelson Mandela Metro, Eastern Cape

Free State Province

- Mr MW, Fikizolo, Executive Manager Human Resource, Free State
- Dr S Kabane, Executive Manager Head of Clinical services, Free State
- Mrs Laché Katzen, General Manager: Strategic Health Programs, Free State
- Dr N Kgasane, Senior Manager Strategic Health Programs, Free State
- Mrs SRO, Khokho, Executive Manager Strategic Health Programs, Free State
- Dr AS Sesing, CEO National Hospital, Free State
- Mr Toli Moeketsi, Assistant Manager Health Information System, Free State

KwaZulu-Natal Province

- Ms Gugu Mabaso, Surveillance/EPI Ethekwini District, KZN

Limpopo Province

- Mr Eric Maimela, LP, Manager: Epidemiology Services, Public Health Programmes
- Ms Pauline Moetlo, Public Health Programmes

Northen Cape Province

- Ms Noreen Crisp, Provincial CDC Programme Manager, Northern Cape
- Dr PS Kegakilwe, Director, Veterinary Services, Northern Cape Department of Agriculture

North West

- Mrs Mmule Rakau, Chief Director of Health, Bojanala District, North West

Western Cape

- Mr Stephen Titus, Public Health Programmes, Western Cape

Other

- Dr. Albie de Frey, Director, Worldwide Travel Medical Consultants

SPECIAL PROGRAMMES

NATIONAL TUBERCULOSIS REFERENCE LABORATORY

Prof EC Böttger, National Centre for Mycobacteria, University of Zurich

Prof Dorothy Fallows and Professor Gilla Kaplan from the International Center for Public Health, New Jersey, USA

Dr Linda Parsons, Team Leader, Clinical and Opportunistic Infections Monitoring Team, International laboratory Branch, National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention, Global AIDS Program, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Bill Donnovan, Richard Borrelli, John Paganini; Fleming Lure from Guardian Technologies, USA

Giorgio Roscigno, Dr. Catharina Boehme, Dr. Richard O'Brien; Dr. Ákos L. Somoskövi from FIND

Dr Chris Gilpin, International Organisation for Migration.

Publications

Ahmed J, Bouloy M, Ergonul O, Fooks AR, Paweska J, Chevalier V, Drosten C, Moormann R, Tordo N, Vatansever Z, Calistri P, Estrada-Pena A, Mirazimi A, Unger H, Yin H, Seitzer U. International network for capacity building for the control of emerging viral vector-borne zoonotic diseases: ARBO-ZOONET. *Eurosurveillance* 2009; 14 (12): 1-4.

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