



Microbiology Division

Parasitology Reference Unit

BACKGROUND

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

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

The Unit was accredited by SANAS in November.

***Pneumocystis jirovecii* PROJECT**

Pneumocystis jirovecii is an unconventional opportunistic fungal pathogen which causes the important AIDS-defining infection, *Pneumocystis pneumonia* (PcP). Trimethoprim-sulfamethoxazole (cotrimoxazole) is the drug of choice for treatment and prophylaxis of PcP. Sulphonamides inhibit a step in the folate pathway, by acting as a false-substrate inhibitor for the enzyme dihydropteroate synthase (DHPS). Point mutations in the *fas* gene, which codes for DHPS, have been linked to sulphonamide antifolate (sulfa) drug resistance. The project is a collaboration between the NICD, clinicians at Chris Hani Baragwanath Hospital, and the Swedish Institute for Infectious Disease Control. In the laboratory-based part of the work, a wild type DHPS genotype was found in 46% (83/181) of respiratory specimens, while 54% (98/181) had a mutant genotype. Quantitative real-time PCR was performed on samples from 95 patients enrolled in the clinical study, and the *P. jirovecii* fungal load was quantified. The IFA-positive specimens had a median fungal load of 8663 gene copies/assay. 37.5% (18/48) of the IFA-negative specimens were QPCR-positive with a median fungal load of 65 copies/assay. There was a significant difference in the fungal loads from the 2 groups of specimens. Specimens from 51 patients have been analysed for DHPS polymorphisms to date. 57% of patients were infected with *P. jirovecii* of a mutant DHPS genotype. This prevalence is similar to

that found in the laboratory-based study. This high prevalence of *P. jirovecii* DHPS polymorphisms in Gauteng Province contrasts with two studies from the Western Cape Province that found much lower prevalences of 1.9% and 13.3%, respectively. Provincial HIV prevalence rates show geographic variations in HIV prevalence, which may account for the considerable geographical differences in the prevalence of DHPS mutations in South Africa. A national laboratory-based surveillance programme for *Pneumocystis jirovecii* was launched by the unit in May 2006. This programme serves as another source of geographically diverse clinical specimens, in which to detect DHPS polymorphisms. All of the specimens were analysed by QPCR and DHPS PCR and sequencing. It was found that 34% of specimens (n = 34) contained mutant DHPS genotypes. (Student: LDini)

TOXOPLASMOSIS PROJECT

Toxoplasmosis is a common opportunistic infection in HIV infection and a potentially serious problem when primary infection occurs in pregnancy. Recent reports indicate that seroprevalence of toxoplasmosis (latent infections) is probably high in Africa but systematic demographic and epidemiological data is lacking. To fill this knowledge gap, we are investigating the seroprevalence of toxoplasmosis in Johannesburg among healthy individuals and populations at risk for severe complications, ie HIV-positive individuals and pregnant women. Furthermore, we will isolate and genotype local *Toxoplasma* strains from humans and rodents to identify and define molecular virulence markers relevant for severe toxoplasmosis. The results will have direct implications for establishing strategies to combat and prevent this potentially lethal opportunistic disease. A South African Masters degree student is undertaking the research in South Africa and Sweden, in collaboration with the Swedish Institute for Infectious Disease Control. (Student: K Kistiah)

***In vitro* MALARIA GAMETOCYTE PRODUCTION**

As part of a collaborative research project with the Vector Control Reference Unit of the NICD, the PRU established *in vitro* cultures of a laboratory-adapted *Plasmodium falciparum* strain. While cultures have been successfully maintained for many months, it has proved very difficult to reliably infect vector mosquitoes with malaria parasites and these techniques are being refined. (Researcher: G Venkatesan)

QUALITY ASSESSMENT ACTIVITIES

The Unit provides several external quality assessment (proficiency testing) programmes, the flagship being the national EQA programme (in which over 200 laboratories from southern Africa participate). 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 NICD parasitology EQA schemes are offered: 'Stool and urine parasites' and 'Blood and tissue parasites'. Surveys are issued 3 times per year and survey challenges encompass parasite identification and laboratory techniques. A teaching series is included in each survey to encourage participants to learn more about medically important parasites. Both Parasitology EQA programmes are CPD accredited. Malaria EQA programmes are designed and specially produced for the WHO (65 African laboratories) as part of a larger NICD EQA contract, and for GlaxoSmithKline Biologicals, specifically for their malaria vaccine trial sites (9 African countries).

***Pneumocystis pneumonia* surveillance**

The total number of cases acquired by the GERMS-SA surveillance system in 2007 was 286, of which 252 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 diagnosed and laboratory diagnosis is only offered in a few larger centres.

TRAINING PERFORMED BY UNIT

Laboratory diagnosis of malaria (16 participants); 7 registrars received training in parasitology

Staff attended training courses in molecular biology, SANAS accreditation, good laboratory practice, and fire and safety.

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): *Pneumocystis pneumonia* and toxoplasmosis studies.

WHO regional advisory group: malaria EQA programme

WHO: basic malaria microscopy manual, bench aids, slide bank SOPs and QA manual.

Research Institute for Tropical Medicine, Philippines (Ms J Luchavez): quality control of malaria microscopy and RDT testing

CAPACITY BUILDING

Registered students

PhD	L Dini
MSc	K Kistiah
BSc Honours	V Ntigwa

Postdoctoral fellow: G Venkatesan



PRU malaria course, PRF Training Centre Laboratory, July 2007.