

## Parasitology Reference Unit

### BACKGROUND

The Parasitology Reference Unit (PRU) provides reference diagnostic services for human parasitic diseases; 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) network; 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 2008 diagnostic workload increased compared with the previous 3 years (Figure 1) and is at its highest level since the Parasitology Reference Unit moved to the Sandringham site. 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.

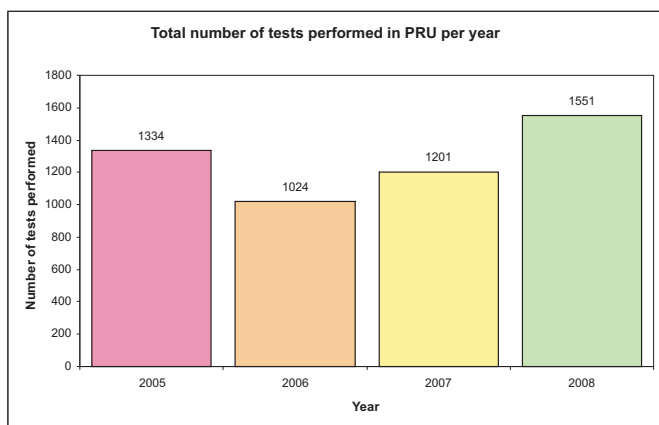


Figure 1: Parasitology Reference Unit, diagnostic workload per year, 2005-2008

#### ACCREDITATION AND TRAINING

The Unit was re-accredited by SANAS in October 2008. Staff attended training courses in good laboratory practice, research techniques, introduction to the public health laboratory, Microsoft & EpiInfo computer programs, malaria molecular biology, fire warden duty and first aid.

Training performed by unit:

- There were 16 participants on the Unit's stool parasite diagnosis course (Figure 2)
- 20 microbiology and clinical pathology registrars, 1 intern medical scientist and 1 medical technologist received training in parasitology
- Staff at Groote Schuur, Tygerberg, and Chris Hani Baragwanath NHLS laboratories were trained in the diagnosis of *Pneumocystis jirovecii*, through GERMS-SA
- 2 delegates from Oman and 1 from Nigeria were trained on how to co-ordinate a malaria EQA Programme, sponsored by WHO
- 2 GSK EQA sites (Burkina Faso and Malawi) received training in malaria microscopy



Figure 2: Parasitology course in the NICD's PRF Training Centre laboratory

#### RESEARCH

##### *Pneumocystis jirovecii* research 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. Sulfonamides inhibit a step in the folate pathway, by acting as a false-substrate inhibitor for the enzyme dihydropteroate synthase (DHPS). An increased frequency of point mutations in the *fas* gene, which codes for DHPS, are associated with exposure to sulfonamides and there is the potential for drug resistance. The project is a collaboration between the NICD, clinicians at Chris Hani Baragwanath Hospital, and the Swedish Institute for Infectious Disease

Control. A laboratory-based study to determine the prevalence of *P. jirovecii* DHPS mutations was completed and a manuscript is being submitted for publication. A mutant DHPS genotype was found in 54% (98/181) of respiratory specimens. A clinical study to determine the significance of these mutations is in progress. To date, the *P. jirovecii* fungal load of 244 specimens has been determined using quantitative real-time PCR targeting the mitochondrial gene coding for the large ribosomal subunit (mtLSU) of the fungus. All real-time PCR positive specimens are analysed for DHPS polymorphisms using nested PCR and Sanger sequencing. The prevalence of DHPS polymorphisms so far is similar to that found in the laboratory-based study. *P. jirovecii* strains are being typed at the ITS locus using nested PCR, cloning and Sanger sequencing. (Student: 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. (Student: B Poonsamy)

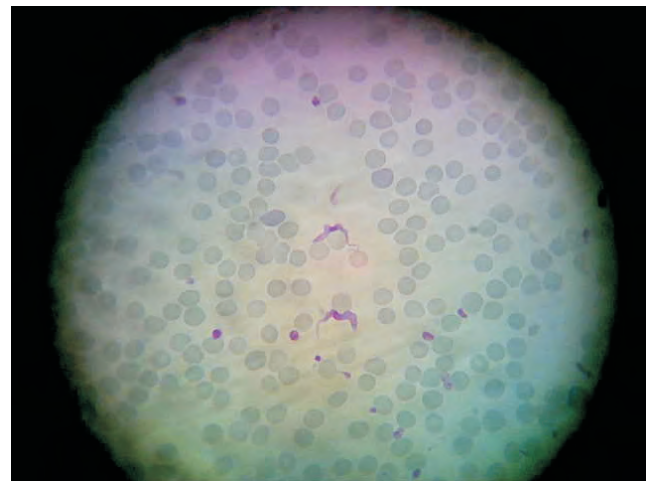
#### **Toxoplasmosis research project**

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. There is limited historical information about the disease in South Africa. More knowledge is needed at a regional level to properly consider solutions aimed at reducing the risk for this disease. 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) in the respective samples. The Pastorex Toxo latex agglutination test was evaluated and found to be a cheap, reliable method to screen for *T. gondii* exposure. PCR-based diagnostics were developed for direct diagnosis on tissue samples. Rodent *T. gondii* infection prevalence was investigated, but did not yield any positive results. This study helped to answer questions relating to the seroprevalence and diagnosis of *T. gondii* in South Africa. Further work will isolate and genotype local *Toxoplasma* strains 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. An NICD medical scientist intern is undertaking the

research in South Africa and Sweden, in collaboration with the Swedish Institute for Infectious Disease Control. (Student: K Kistiah)

#### **Assessment of malaria parasite load using digital image analysis**

The load of parasites in a patient with falciparum malaria is regarded as a useful indicator of the severity of infection, correlating broadly with clinical features and prognosis. In addition, serial parasitaemia estimations give a useful objective indication of response to treatment. Manual quantitation of parasites under the microscope is tedious and difficult to do accurately and consistently. Currently we are investigating measurement of parasite load by means of digital image analysis. Good correlation with careful manual counting is possible, especially when moderate to high numbers of parasites are present; this is precisely the situation in which manual counts tend to be difficult to perform accurately and speedily. One problem for resource-poor laboratories is the cost of a digital microscope camera. We have shown that it is possible to use an ordinary cellphone camera to take acceptable pictures, which can then be transmitted locally (e.g., to a PC) or anywhere in world via the cellphone system or internet, for further interpretation (Figure 3). If applied successfully to malaria blood films, this has potential to substantially improve routine laboratory performance in malaria parasite load estimations, and inform clinical assessment of patients accordingly. (J Frea)



**Figure 3: Cellphone photomicrograph showing blood film with trypanosomes**

#### **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 (116 participants in 9 African countries). In 2008, a new EQA programme (PCP EQA) was launched; it assesses the participating laboratories' ability to correctly identify *Pneumocystis jirovecii*.

### **PNEUMOCYSTIS PNEUMONIA SURVEILLANCE**

The total number of cases acquired by the GERMS-SA surveillance system in 2008 was 339, of which 299 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.

## **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 rapid diagnostic test evaluation

## **CAPACITY BUILDING**

Registered students

PhD L Dini

MSc K Kistiah, B Poonsamy