THE UNIVERSITY OF WESTERN AUSTRALIA
Achieve International Excellence

Uniview

Vol. 30 No. 1, Summer 2011

Waltzing the Wilarra

Staying a step ahead of **the cleverest parasite**

Mosquitoes that inject a parasite into human blood have turned malaria into the world's most devastating disease and one that is potentially deadly in children. We talk to two UWA researchers battling the disease on different fronts.

While we generally associate malaria with third world countries in tropical and sub-tropical regions where more than half of the world's population live, the mosquito-borne virus Murray Valley encephalitis is endemic in the Kimberley, and Barmah Forest and Ross River viruses annually affect thousands in Australia.

Mosquito-borne viruses create public health problems around the world. For example, West Nile virus was introduced to North America in 1999, and chikungunya (first described in Tanzania in 1952) has become increasingly common in Africa, India and Southeast Asia. It has been detected in people arriving in Australia from affected countries – and a mosquito capable of transmitting it is resident in Queensland.

Dengue fever is currently the subject of a major Queensland Health campaign urging residents to "Stop growing mozzies" – in pot plant containers, bird baths, gutters and ageing water tanks.

UWA's School for Paediatrics and Child Health, the Telethon Institute for Child Health Research and Princess Margaret Hospital have joined the battle against dengue, forming a group to trial a new vaccine that aims to protect against all four strains.

Study leader Associate Professor Peter Richmond says the vaccine would be of great value to Australian travellers because dengue is "an increasing scourge in Southeast Asia."

"At the moment there is no licensed vaccine and no specific treatment exists, so controlling mosquito numbers is the only effective method of prevention at this time. The vaccine, developed by one of the world's leading vaccine companies, is being trialled in eight centres around Australia," says Professor Richmond.

Researchers from this University are working in a variety of ways to keep one step ahead of what could be the world's cleverest parasite. In this article we profile the work of two researchers: an academic researcher and a PhD student.

Above: PhD student Rina Wong with a mother and child at a clinic in Papua New Guinea

UWA researchers carry out regular surveillance programs for the State's Department of Health. When they detect increased activity of Ross River and Barmah Forest viruses in mosquitoes, they inform the government. Health warnings are immediately issued and local government mosquito management programs may be intensified.

Researcher Associate Professor Cheryl Johansen of UWA's School of Biomedical, Biomolecular and Chemical Sciences is working with researchers from several universities to develop a new mosquito detection system.

Attacking the disease by studying the parasite's genetic mutations is PhD student Rina Wong of the Malaria Research Group in the School of Medicine and Pharmacology. This work has already won the promising young researcher the 2010 Western Australian AusBiotech-Glaxo Smith Kline Student Excellence Award.

Sweet success for researcher

Research A/Professor Cheryl Johansen has been involved in developing a unique surveillance strategy that could be a valuable early warning system for sometimes deadly mosquito-borne viruses causing public health problems around the world.

Tracking mosquitoes and monitoring the viruses they carry has become an important tool in the battle to keep Western Australians free of the mosquito-born viruses found in this State.

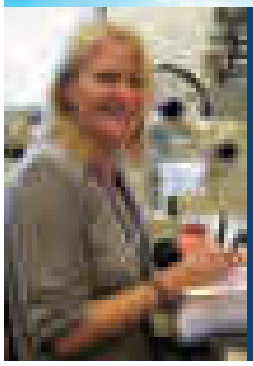
Monitoring of viruses that cause potentially fatal encephalitis is currently done through the testing of sentinel chickens, supplemented with annual trapping and testing of mosquitoes from northern WA. The sentinel chickens are kept by volunteers in some 30 locations throughout Western Australia.

Serum samples from the birds are despatched to the laboratory at UWA where they are tested for antibodies to Murray Valley encephalitis virus and the related Kunjin virus. When antibodies are detected, researchers know that the virus is active, and urgent health warnings are issued to alert people to the increased risk of potentially fatal mosquito-borne disease.

Recently, researchers at UWA, the University of Queensland, James Cook University and Queensland Health completed both laboratory and field trials for an alternative system. The team included Research Associate Professor Cheryl Johansen.

"Current detection methods are laborious, expensive and logistically complex," says A/Professor Johansen.

"Our alternative utilises the fact that mosquitoes transmit viruses in their saliva when they are either feeding on a blood source, or an energy source, such as nectar. The new detection system lets us capture



OUR TESTING INDICATES WHETHER OR NOT VIRUSES ARE PREVALENT, ENABLING RAPID DETECTION

mosquitoes in box traps where they feed on honey-soaked cards that preserve nucleic acids. While the live virus dies, its genetic material remains on the cards and can be analysed in the laboratory, and it lasts at least a week.

"So instead of testing all those mosquitoes, we can test several cards that indicate whether or not viruses are prevalent in an area, enabling rapid detection. There will still be times when standard mosquito trapping methods are required to determine which mosquito species are carrying the viruses, as this is important for targeted mosquito population management.

"However the new system for mosquito-borne virus detection may prove to be an important screening tool to see what pathogens are out there. It also allows you to sample a lot more mosquitoes at once, maximising the chances of detecting the pathogens. After testing our new system in the laboratory we did field trials and detected both Ross River and Barmah Forest viruses.

Above: Mosquitoes feeding on honey-baited card with blue food dye. Inset: Researcher A/Professor Cheryl Johansen

"We're still modifying the trap so it is more user-friendly and we've recently used it in a parallel study with sentinel birds. However, given that mosquito-borne viruses are a global public health problem that could be increasing, this system is an important way of screening."

This collaborative research was published in *Proceedings of the National Academy of Sciences of the United States of America* in June.

>>CONTACT

A/Professor Cheryl Johansen
School of Biomedical, Biomolecular and Chemical Sciences
Phone: +61 8 9346 4656
Email: cheryl.johansen@uwa.edu.au

On the front line of the war against malaria

UWA's Rina Wong is a frontline fighter in a global war against one of the world's deadliest parasites. Plasmodium falciparum kills one million annually and scientists are working to get one step ahead of the malaria parasite.

The malaria parasite *Plasmodium falciparum* is complex and cunning. It grows in red blood cells, replicates every 48 hours, has a seemingly endless capacity to mutate and is able to radically alter its DNA composition.

Thus armed and deadly, malaria has been able to spread to new areas in the wake of population movements and to re-emerge in areas where it was thought to have been eradicated.

The World Health Organization sees anti-malarial drug resistance as one of the greatest health challenges we face today. Scientists working to get one step ahead of the parasites complain of insufficient funding in a battle that mainly affects poor countries – at a time when malaria parasites have demonstrated some level of resistance to almost every anti-malarial drug available.

Left: Rina Wong in the laboratory in Cleveland, USA

Rapid detection of drug resistance has become a vital tool in selecting the right treatments and this is the focus of research by Rina Wong.

"We can no longer use a single drug as treatment: we need to mix and match and use combinations to slow down the development of drug resistance," says Rina. "I work with the most deadly species of the parasite, *Plasmodium falciparum*, which is responsible for the highest mortality. It causes cerebral malaria that can kill, and while most of the research focus is on adults, it is children and pregnant women who are the most vulnerable due to their low immunity. That's why my project focuses on malaria in kids."

Malaria research crosses the boundaries of several science disciplines: as a blood-borne disease, it falls into the category of haematology, as a parasite into microbiology while its drug resistance sees it studied in biochemistry laboratories.

When Rina completed her Honours degree at Curtin University of Technology and received an Australian Postgraduate Award scholarship, she opted to study malaria and contacted one of Australia's foremost malaria specialists, UWA's Winthrop Professor Tim Davis. He is collaborating with the Papua New Guinea Institute of Medical Research in clinical trials for new drug treatments and Rina became part of his research team.

She spent five months in the Madang Province collecting malaria-infected blood samples for laboratory culturing and drug sensitivity testing. "I learned basic Tok Pisin language and that gave me the opportunity to help with screening babies and kids who presented with malarial fever as part of a WHO-sponsored treatment trial," she recalls.

"From the remote Alexishafen Health Centre we would take a bumpy ride in a 4WD back to the jungle hospital where research facilities are situated. Electricity is a luxury and black-outs are common, so I got used to working, cooking and showering by candlelight!"

The clinical trial resulted in changes in the way antimalarial drugs were tested and allowed resistance to be identified more speedily and at an early stage. The researchers froze samples of malaria-infected blood and shipped them to the United States where Rina travelled to study parasite DNA mutation under the supervision of Dr Peter Zimmerman at the Case Western Reserve University. Here she extended a new high-throughput molecular method to screen 10 additional mutation sites in the parasite's

multidrug resistance gene. This work won her a WA Department of Health New Investigator Award in 2009. She used the prize money and some additional funding from her supervisor to present this work at the 14th International Congress of Infectious Diseases in Miami in March last year.

"My research focuses on the parasite's genetic mutations and how that affects treatment outcomes," explains Rina.

Growing inside red blood cells where they replicate every 48 hours, the parasites synthesise DNA that can be detected by the binding of inexpensive DNA-specific fluorescent dyes. Using the flow cytometry facilities at UWA's Centre for Microscopy, Characterisation and Analysis, Rina is working in collaboration with PhD student Stephan Karl (who recently won the 2010 Western Australian AusBiotech-Glaxo Smith Kline Student Excellence Award). They aim to detect early signs of resistance more rapidly, in a cost effective way.

Rina's malaria research has also uncovered some promising leads for potential new drug treatments, including the use of the cholesterol-lowering drug atorvastatin.

The UWA student says that malaria parasites are very fussy to breed, demanding special (and costly) laboratory accommodation and daily attention.

"If they stress they die and you have to start again," she says. "That means going to the lab every day, every weekend, during holidays or when you're sick, to keep them alive, fed and happy – before I kill them off in my experiments! It's been very challenging, but I have to say my PhD journey has been most memorable."

CHILDREN AND PREGNANT WOMEN ARE THE MOST VULNERABLE TO MALARIA DUE TO THEIR LOW IMMUNITY

Rina's research with Professor Tim Davis on the potential of statins as antimalarial drugs was published in *Antimicrobial Agents and Chemotherapy*. Her research on the *in vitro* sensitivity of *Plasmodium falciparum* to conventional and novel antimalarial drugs in PNG was published last year in *Tropical Medicine International Health*. Her collaborative research with Stephan Karl is supervised by Professor Tim St Pierre.

>>CONTACT

PhD student Rina Wong
Malaria Research Group
School of Medicine and Pharmacology
Phone: +61 8 9431 2358
Email: rina.pm.wong@gmail.com