NICD 2007

Microbiology Division

Vector Control Reference Unit

BACKGROUND

Malaria is the major vector-borne disease in Africa, killing over 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 mainly 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 collaborate 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 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. Metabolic mechanisms involved in the resistance are P450 monooxygenase enzymes and we have demonstrated that a specific CYP6 gene is responsible for pyrethroid resistance in *An. funestus.* 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.

Furthering the studies on the mechanisms of metabolic resistance in southern African *An. funestus*, in collaboration with the Liverpool School of Tropical Medicine, microsatellite and SNP markers were used to construct a linkage map and to detect a quantitative trait locus (QTL) associated with the pyrethroid resistance. The genotyping of 349 mosquitoes from second generation resistant/susceptible crosses identified a single major QTL at the telomeric end of chromosome 2R. This QTL accounts for more than 60% of the variance in susceptibility to permethrin (pyrethroids) and is genetically linked to a cluster of CYP6 cytochrome P450 genes located on division 9 of chromosome 2R.

The fitness cost to *An. funestus* mosquitoes carrying the pyrethroid resistance genes was investigated. A highly resistant selected strain was compared with a fully susceptible strain. Fitness was evaluated in terms of fecundity, fertility, egg production, developmental time and life stage progression and survival. No statistically significant differences were found between the strains and in some cases it appeared as if the resistant strain was more fit than the susceptible strain. The implications of this work are that even if insecticide selection pressure in the field is withdrawn, the resistance genes will not disappear from the population. The management of insecticide resistance in a vector control programme faced with this situation is therefore critical.

Subsequent to the severe malaria epidemic experienced by South Africa in 1999/2000 due to pyrethroid resistance in the major vector *An. funestus*, monitoring and surveillance of mosquito populations were conducted in northern KwaZulu/Natal as part of the malaria vector control programme. A sample of 269 *Anopheles funestus* group mosquitoes was collected in Mamfene using exit window traps in pyrethroid sprayed houses between May and June 2005. Mosquitoes were identified to species level, assayed for insecticide susceptibility, analyzed for *Plasmodium falciparum* infectivity and for blood meal source. Of the 220 mosquitoes identified using the rDNA PCR method, two (0.9%) were *An. funestus* and 218 (99.1%) *An. parensis*. *Anopheles parensis* is a non-vector member of the group that is fairly common in northern KwaZulu/Natal but usually found outdoors associated with cattle. Standard WHO insecticide susceptibility tests on F1 progeny from wild caught *An. parensis* females showed resistance to the pyrethroid deltamethrin. Biochemical analysis of F1 *An. parensis* showed no elevation in levels/activity of the detoxifying enzyme systems when compared with an insecticide susceptible *An. funestus* laboratory strain. Among the 149 female *An. parensis* tested for *P. falciparum* circumsporozoite infection, 13.4% were positive. All ELISA positive specimens (n=20) were re-examined for

P. falciparum infections using a PCR assay which came up negative in all cases. Direct ELISA analysis of 169 blood-fed specimens showed >95% of blood meals were taken from domestic animals. All blood fed, *P. falciparum* false positive mosquitoes had fed on animals. The combination of pyrethroid resistance and *P. falciparum* false-positivity in *An. parensis* poses a problem for vector control. If accurate species identification had not been carried out, scarce resources would have been wasted in the unnecessary changing of control strategies to combat a non-vector species found resting inside houses.

In field populations of *An. funestus*, DDT and pyrethroid resistance has been detected from Ghana and carbamate resistance from Mozambique. Strains resistant to these insecticides have been selected from wild populations and are being investigated.

Anopheles gambiae

Permethrin and DDT cross-resistance associated with target site insensitivity in the malaria vector *Anopheles gambiae* s.s. was demonstrated in southwestern Nigeria in 2002. From 2002 to 2005, changes in the proportion of *Anopheles gambiae* molecular M and S forms, their susceptibility to insecticides and the frequency of the knock down resistance (*kdr*) alleles were monitored using PCR assays. The overall collection showed that the molecular S form was predominant (>60%) but the proportions of both forms in the mosquito populations from 2002 to 2005 were not statistically different. Both forms also occurred throughout the period without apparent relationship to wet or dry season. Insecticide susceptibility tests did not show any significant increase in the resistance status recorded for either Permethrin or DDT from 2002 to 2005. Rather, an improvement in the susceptibility status of the mosquitoes to these insecticides was observed in 2004-2005 compared with 2002-2003. The *kdr* mutation was found mainly in the molecular S form and did not increase significantly from 2002. Further monitoring of this may provide information on the gene flow and reproductive barriers in these sympatric taxa.

Natural populations of *Anopheles gambiae s.s.* M form and *An. melas* were assessed for susceptibility to deltamethrin, permethrin, DDT and carbosulfan insecticides in three villages in coastal Cameroon. *Anopheles melas* occurred in only two villages with all samples susceptible to the four insecticides. *Anopheles gambiae s.s. M form* from all three villages was 100% susceptible to deltamethrin and permethrin, but showed 6.3% survival on 4% DDT in one village. *Anopheles gambiae* M form was resistant to carbosulfan at all sites, ranging from 53-90% mortality. These findings provide a baseline for evidence-based planning and implementation of malaria vector control activities in the surveyed areas that can be used for comparison in vector control monitoring activities.

Anopheles arabiensis

The "knockdown" mutation (*kdr*) found in the sodium channel gene that confers resistance to pyrethroids in West African *An. gambiae*, has been detected in wild populations of *An. arabiensis* from central Sudan. A colony of *Anopheles arabiensis* from the Sennar region of Sudan was selected for resistance to DDT. Adults from the F-16 generation of the resistant strain were exposed to all four classes of insecticides approved for use in malaria vector control and showed high levels of resistance to them all (24 hr mortalities: malathion 16.7%, bendiocarb 33.3%, DDT 12.1%, dieldrin 0%, deltamethrin 24.0%, permethrin 0%). Comparisons between the unselected base colony and the DDT resistant strain showed elevated enzyme levels of glutathione S-transferase ($p < 0.05$) in both sexes and elevated esterases ($p < 0.05$) in males only associated with resistance. The Leu-Phe mutation (*kdr*) in the sodium channel gene was detected by PCR and sequencing but showed no correlation with the resistant phenotype. These results do not provide an adequate explanation for why this colony exhibits such high levels of resistance to so many insecticides and further studies are ongoing.

MALARIA VECTOR CONTROL AND TRANSMISSION DYNAMICS

Ghana

The VCRU plays an active role in the malaria control programme operating in Obuasi, Ghana. An indoor residual house spraying operation was initiated in 2005 after baseline mosquito surveys were conducted to establish the insecticide resistance profiles of the local mosquito populations. Resistance was detected to three of the four classes of insecticides authorised by WHO for malaria vector control. Only organo-phosphates were found to be fully effective on all vector species (Table 1). After two years of house spraying, the malaria case incidence at the Edwin Cade Hospital has decreased by over 70% (Figure 1).

Table 1. Mosquito susceptibility at Obuasi, Ghana, to four classes of insecticides approved by WHO for use in malaria vector control.

Insecticide	Species	Dead	Alive	Total	% Mortality
Pyrethroids (Deltamethrin)	*An. gambiae*	41	13	54	75.9
	An. funestus	53	0	53	100
Organochlorines (DDT)	*An. gambiae*	8	18	26	30.8
	An. funestus	14	9	23	60.9
Carbamates (Bendiocarb)	*An. gambiae*	22	17	39	56.4
	An. funestus	40	16	56	71.4
Organophosphates (Malathion)	*An. gambiae*	40	0	40	100
	An. funestus	45	0	45	100

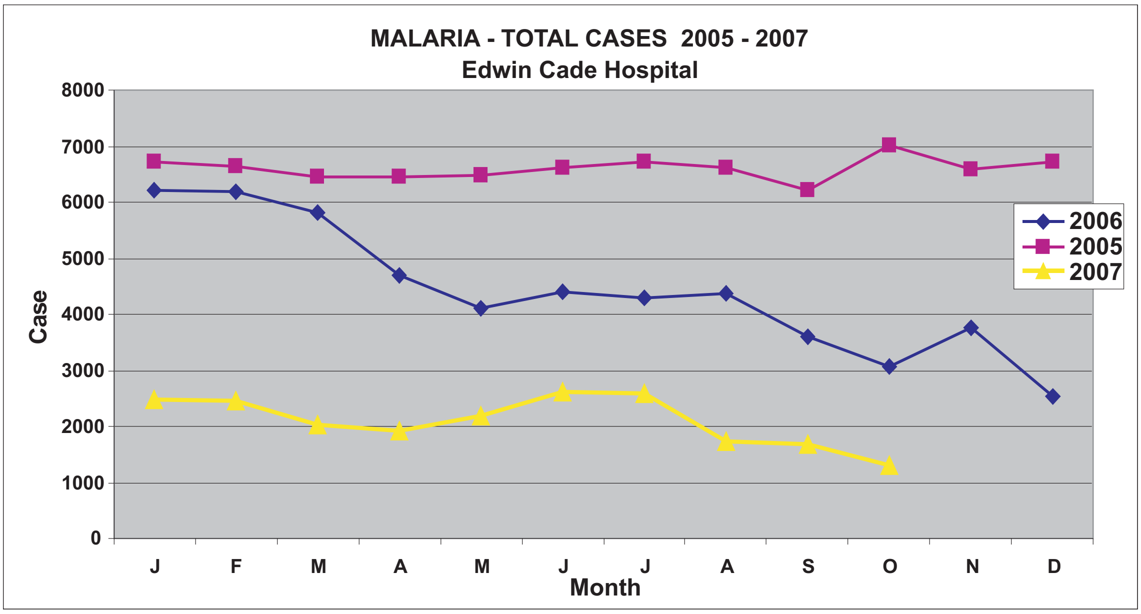

Figure 1. Malaria cases at Obuasi, Ghana, after implementation of indoor residual house spraying

Cameroon

Very little is known about the malaria vector population structure and transmission dynamics in the coastal areas of Cameroon. A 12-month longitudinal entomological survey was conducted in Tiko, Limbe and Idenau in coastal southwest Cameroon from August 2001 to July 2002. Mosquitoes captured indoors on human volunteers were identified morphologically and species of the *Anopheles gambiae* complex identified using the polymerase chain reaction. Mosquito infectivity was detected using the enzyme-linked immunosorbent assay and presence of sporozoites confirmed by PCR. Overall, 2773 malaria vectors comprising *An. gambiae* M form (78.2%), *An. funestus* (17.4%) and *An. nili* (7.4%) were captured. No *An. melas* was caught biting humans. Monthly sporozoite rates were higher in *An. gambiae* than *An. funestus* and *An. nili*. Entomological inoculation rates varied by locality and seasonally, increasing with rainfall. There were 287, 160 and 149 infective bites per person per year in Tiko, Limbe and Idenau respectively. *Anopheles gambiae* accounted for 72.7%, *An. funestus* for 23% and *An. nili* for 4.3% of the transmission. The prevalence of malaria parasitaemia was 41.5% in children <5 years of age, 31.5% in those 5-15 years and 10.5% in those >15 years with *Plasmodium falciparum* being the predominant species. The study showed that malaria transmission is perennial, more intense during the wet season and that *An. melas* appears not to be a vector in this region of West Africa. The findings constitute a suitable baseline for future studies and have an important implication in the planning and implementation of malaria control activities in the surveyed areas.

South Africa

Studies undertaken by the malaria control programme in Mpumalanga Province on the distribution and abundance of members of the *Anopheles funestus* group showed the presence of five members of the groups with *An. rivulorum* occurring in large numbers at one locality. The vector species *An. funestus* was recorded mainly from one locality where a minor malaria outbreak occurred and in total represented 7.8% of the total collections.

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 (Figure 2).

Figure 2. *Anopheles funestus* infected with an entomo-pathogenic fungus

VECTOR/PARASITE INTERACTIONS

A new facility for culturing malaria parasites and infecting mosquitoes has been established. Optimization of the techniques is currently underway and mosquitoes have been infected with *Plasmodium berghei*.

DIAGNOSTIC AND OTHER SERVICES

The VCRU provides an identification service of medically important arthropods for entomologists, medical practitioners and health inspectors. Malaria vector mosquitoes were routinely identified by PCR for the Mpumalanga Province Malaria Control Programme. ELISA and PCR tests were carried out on the *An. gambiae* complex specimens from Ghana, Mali, Malawi, Angola and South Africa, for species identification and to detect the presence of *Plasmodium falciparum* sporozoites. An outbreak of cutaneous myiasis, caused by the 'tumbu' fly *Cordylobia anthropophaga*, was reported from Pretoria. Patients had no history of travel and it is presumed that the infestations were obtained locally.

Advice and expertise is provided to the Department of Health both at the national and provincial levels, with participation on the National Malaria Advisory Group.

INTERNATIONAL RESEARCH COLLABORATORS

Prof J Hemingway, Director, Liverpool School of Tropical Medicine, UK
Dr H Ranson, Imperial College, UK
Prof W Takken, University of Wageningen, Netherlands
Dr B Knols, University of Wageningen, Netherlands
Dr M Thomas, CSIRO, Australia
Prof D Norris, Johns Hopkins University, USA
Prof K Louis, IMBB, Crete
Prof F Collins, University of Notre Dame, USA
Prof D Boakye, Noguchi Memorial Research Institute, Accra, Ghana
Dr T S Awolola, Nigerian Institute of Medical Research, Lagos, Nigeria
Dr H T Masendu, University of Zimbabwe, Harare, Zimbabwe
Mr B Kandeh, Malaria Control Programme, The Gambia

CAPACITY BUILDING

Postgraduate Training
Masters and Doctoral students from the following countries were trained during 2007:-

South Africa:
T Matambo, PhD student
M Coleman, PhD student
B Spillings, PhD student
M Lo, PhD student
O Wood, MSc student

Nigeria:
P Okoye, PhD student

Cameroon:
J Mouatcho, PhD student
S Vezhenego, MSc student

Zimbabwe:
G Mugenga, PhD student

Ghana:
J Stiles-Ocran, MSc student

Democratic Republic of Congo:
C Kikankie, MSc student

Sudan:
H Abdalla, MSc graduated

Short-course Training
A short course in basic mosquito identification and insecticide susceptibility was held for personnel from the provincial malaria control programmes. In addition, one participant from the Botswana malaria control programme attended this course.

Ad hoc training in PCR techniques was given to students from Zimbabwe and The Gambia.