Army Malaria Institute - its Evolution and Achievements. Fourth Decade (1st Half): 1995-2000

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Abstract

During the 1995-2000 quinquennium, the Army Malaria Research Unit (AMRU) was re-named the Australian Army Malaria Institute (AMI) and re-located from Sydney to a modern purpose-built facility in Brisbane. Its international recognition as a centre of excellence for malaria research was further enhanced by the establishment of a molecular parasitology laboratory to investigate drug resistance. During this period AMI deployed outbreak management teams in response to the hundreds of soldiers who suffered from malaria in Bougainville and Timor Leste due to inadequate personal protection and chemoprophylactic measures. Between 10-20% of affected soldiers experienced their first attack of falciparum or vivax malaria overseas for failing to comply with doxycycline prophylaxis or, possibly, for taking doxycycline which had been degraded by exposure to adverse environmental conditions. By contrast, 80-90% of primary episodes of malaria did not occur until after return to Australia, simply because the 14-day post-exposure primaquine course was either ineffective in eradicating residual liver stages of *Plasmodium vivax* or had not been taken as prescribed. Field studies with tafenoquine, a slowly-eliminated analogue of primaquine, indicated that this drug might eventually replace primaquine and even prevent malaria while overseas. In a further field study, atovaquone/proguanil (Malarone®) proved to be just as effective as doxycycline, suggesting that it could be used as an alternative drug for malaria prophylaxis. Laboratory-based studies with Mannich base, artemisinin, and third-generation antifolate compounds provided further evidence of their potential value for the control of drug-resistant falciparum malaria. Progress was also made in evaluating drug resistance and diagnostic procedures and in identifying molecular markers of parasite resistance to antimalarial drugs, such as atovaquone. Two novel insect repellents and a self-erecting low-profile bednet provided good protection against mosquito bites. Surveys on the distribution and speciation of anopheline mosquitoes in Papua New Guinea were extended to another five provinces and, whilst DNA analysis was still in progress, early findings indicated a marked diversity of genotypes in anopheline species. Towards the end of the quinquennium, AMI became involved in efforts to provide Australian Defence Force personnel with better protection against other mosquito- borne diseases, such as Ross River virus, dengue and Japanese encephalitis.

Background

The malaria situation during the 1990s showed little improvement or worsened in many countries.[1] Although numerically Africa accounted for 90% of malaria cases worldwide, the southwest Pacific region continued to be a hotbed for malaria, with the Solomon Islands being considered one of the most malarious countries in the world. With an increased interest in regional security, and continued support for peace keeping operations throughout the world, Australian Defence Force (ADF) personnel continued to be deployed to malarious areas. As exemplified repeatedly, the ability of the ADF to operate at maximum efficiency in such areas depended on the effective protection of its personnel against this potentially fatal disease.

After its modest beginnings in the mid-1960s,[2] the Army Malaria Research Unit (AMRU) was playing an increasingly important role in providing optimum protection against malaria for ADF personnel deployed to malarious areas overseas.[3,4] Substantial progress in this regard was also made during the 1990-1995 quinquennium.[5] Based on observations by AMRU that pyrimethamine/dapsone (Maloprim®) was no longer able to protect soldiers deployed to Papua New Guinea (PNG), doxycycline became the standard drug used for malaria prophylaxis. During the deployment of almost 2000 Australian soldiers to Cambodia, Somalia and Rwanda, only 8 soldiers developed malaria, probably due to inadequate compliance with the daily prophylactic regimen. Between 1-2% of soldiers were placed on weekly mefloquine prophylaxis because of gastrointestinal intolerance, sun-sensitisation, or other side-effects

or contra-indications associated with the use of tetracyclines. Although generally effective as a malaria prophylactic drug, mefloquine was no longer able to suppress or cure falciparum malaria in some parts of Southeast Asia, as was observed by non-Australian contingents deployed to Cambodia with the United Nations Transitional Authority in Cambodia (UNTAC).

Doxycycline and mefloquine, whilst generally effective in preventing falciparum malaria and suppressing vivax malaria, did have drawbacks. Furthermore, the constant threat of the emergence of drug resistance emphasised the need to search for alternative drugs and drug regimens in collaboration with various national and international institutions. Laboratory, clinical or field investigations were carried out with doxycycline and other antibiotics, halofantrine (new drug), proguanil combined with dapsone or atovaquone (new drug combination), artemisinin compounds, PS-15 (experimental third generation antifolate compound) and Mannich bases (experimental compounds).

Although active against multidrug-resistant *Plasmodium falciparum*, none of the above- mentioned drugs were likely to have any activity against residual liver dormant stages (hypnozoites) of *P. vivax*. The 14-day primaquine eradication course continued to be the only means of preventing acute attacks of vivax malaria after return to Australia. But even with good compliance in taking this cumbersome drug regimen, hypnozoites failed to be eliminated in an increasing number of soldiers who had been infected with vivax malaria. To meet this challenge, preliminary investigations were started with tafenoquine, a long-acting analogue of primaquine.

Rapid changes in the patterns of parasite susceptibility to drugs underscored the importance of knowing the extent and degree of drug resistance in malarious areas of possible future deployment. Accordingly, new or improved methods to assess drug resistance were developed in the laboratory and then applied in the field.

Additional collaborative studies were also carried out to determine the potential value of a non-microscopic method for diagnosing falciparum malaria.

Efforts to improve personal protection against arthropod-borne diseases were continued by assessing the effectiveness of various formulations of insect repellents applied to skin, uniforms and bednets. Surveys concerning the distribution and biology of anopheline malaria vectors previously conducted in northern Australia were extended to four provinces in PNG, with the expectation that they would lead to a better understanding of the epidemiology of malaria and thereby help to control this disease in local communities as well as in military personnel deployed to these areas.

Staff and facilities

During the 1995-2000 quinquennium, AMRU was re-named the Australian Army Malaria Institute (AMI). In early 1997, it was moved from Sydney to a more appropriate purpose-built facility of about 2100 square metres in Brisbane. This was consistent with the evolution of AMI to a major centre for malaria research and training. Located at Gallipoli Barracks, Enoggera, AMI became affiliated with the Australian Centre for International and Tropical Health and Nutrition (ACITHN), a joint venture established by the University of Queensland (UQ) and the Queensland Institute of Medical Research (QIMR).

Research and other malaria activities continued under the overall leadership of Professor Rieckmann. Lieutenant Colonel Sweeney retired in December 1996 after 27 years of outstanding service to AMRU. His place as Deputy Director and Commanding Officer was filled by Lieutenant Colonel (previously Major) Michael Edstein, a member of AMRU since 1975.

The move to Brisbane was associated with the loss of 9 out of 20 full-time staff and, as may be expected, the whole re-location process interrupted research activities for quite a while. However, by the end of 1997, 6 of the positions had been filled. New staff members included Dr Qin Cheng and Major Peter Nasveld.

Dr Cheng established a molecular parasitology laboratory which enabled AMI to avail itself of recent technological advances in molecular malaria diagnosis, epidemiology and monitoring of drug resistance. After graduating in medicine, Dr Cheng had embarked on a research career in molecular parasitology, culminating with a PhD and five years post doctoral experience in malaria research at QIMR. She was joined in April 1998 by Dr Nanhua Chen, also a molecular parasitologist with post doctoral experience at QIMR.

Major Nasveld joined AMI to head up clinical studies and surveillance activities, with an emphasis on field activities in Bougainville. During his 8-year service as a medical officer with the Army, Dr Nasveld had become well acquainted with the prevention and treatment of malaria while on the Australian Defence Staff based at the Australian High Commission in PNG. In 1998, he was joined by Major Scott Kitchener who had served in the Middle East and in PNG as a medical officer until 1997. First as a reservist, and then returning to full-time Army service, Dr

Kitchener played a leading role in AMI's outbreak management team to control malaria and dengue in the forward area of operations in Timor Leste.

Other additions to the staff included Lieutenants Bruce Russell (1998), Alyson Auliff (2000) and Michael Korsinczky (2000). Captain David Kocisko (US Army) started a 2-3 year exchange posting at AMI in October 1998 to undertake drug analysis investigations.

Major Steve Frances returned to AMI in 1995 after a 3-year exchange assignment with the US Component of the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Thailand and continued his investigations with insect repellents and other personal protection measures. Major Robert Cooper, a long- standing member of AMI, carried out further ground-breaking investigations on the distribution and speciation of anopheline mosquitoes in PNG.

Major Ivor Harris commenced army reserve duty as veterinary officer for the animal colony in 1997, before transferring to the regular Army in 2000 to become AMI's administrative/scientific officer. Following the move from Sydney, the primate colony was re-located to 'state of the art' facilities (with an outdoor recreation area attached to the new AMI building in Brisbane). It consisted of approximately 30 owl monkeys *(Aotus griseimembra)*, descended from animals imported from the USA in 1982, and approximately 25 squirrel monkeys *(Saimiri sciureus)*, obtained from Commonwealth Serum Laboratories in the mid-1990's. After obtaining valuable additional information about the potential value of new drugs against human malaria, the squirrel monkeys were retired to a nearby zoo in 2000.

Various studies involving non-human primates were reviewed and approved by an active Animal Experimentation Ethics Committee, in conformity with national codes and state laws. Following the move to Brisbane, Ms Lynette Shanley, a leading member of the International Primate Protection League (later "Primates for Primates"), continued to visit the primate facility and provided most helpful advice on various aspects of animal husbandry and welfare.

After re-location to Brisbane, new members were selected to serve on the Army Malaria Research Advisory Board. In conformity with established procedures, all investigations involving human volunteers continued to be subject to approval by the Australian Defence Medical Ethics Committee (ADMEC).

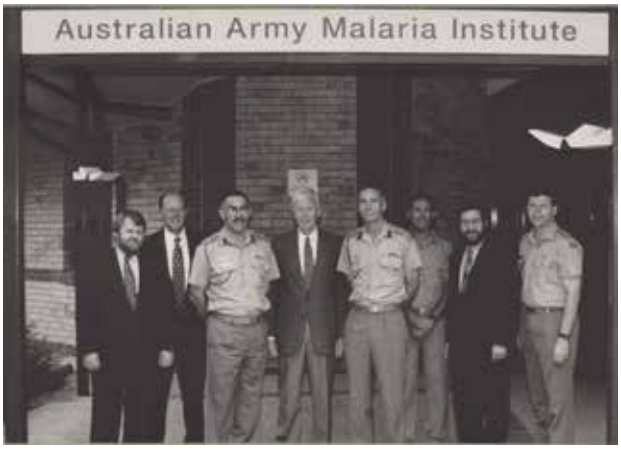

Figure 1: Army Malaria Research Advisory Board (1998)

From left: Prof John Mackenzie, Prof Tony Sweeney, BRIG Paul Buckley, Prof Karl Rieckmann, COL Wayne Ramsey, LTCOL Michael Edstein, Prof Alan Saul, LTCOL David Hutton.

Malaria situation

In May 1996, the 49th World Health Assembly (WHA) requested that increased resources be made available to enhance the World Health Organization's (WHO) action in malaria control and training programs. Shortly thereafter, the WHO Director-General's Task Force on Malaria Prevention and Control was established, Professor Rieckmann being one of its nine members.[6] Recognising WHO's leading role in providing technical guidance, support and training to member countries, the Task Force underscored the importance of WHO having the necessary resources to strengthen the ability of countries to implement the Global Strategy. However, 'the Task Force also recognised that the control of malaria is not only a difficult task, but a long one', and 'it stressed the urgent need for a comprehensive, long-term initiative to reassess the trends of the fast-deteriorating malaria situation'.[6] Since multidrug resistance was often largely responsible for the worsening malaria situation, it was obviously appropriate for AMI to intensify its efforts to monitor and investigate drug resistance, to develop improved chemoprophylactic/ treatment regimens, and to prevent exposure to malaria by conducting epidemiological and mosquito control investigations.

During the first half of the quinquennium, there were no large-scale ADF deployments to malarious areas. Consequently, the Central Malaria Register, maintained at AMI, recorded only 20 malaria infections (4 *P. falciparum*; 16 *P. vivax*) over this period. Of these, 14 were acquired in PNG, 2 in the Solomon Islands, and 4 in Southeast Asia.[7] In addition to infections acquired during short-term visits overseas, malaria attacks were also reported

among ADF personnel and their dependants during longer-term postings to PNG, particularly the Morobe Province. The ADF was confronted with many more malaria infections during the second half of the quinquennium when hundreds of infections were documented during and following the deployment of about 7000 personnel to Bougainville and Timor Leste (formerly East Timor).

Bougainville

In late 1997, about 300 ADF personnel were deployed to Bougainville to form the main component of a multi-national contingent to monitor peace-keeping after a decade of conflict on the island. Their close interaction with local communities exposed them to a particularly high malaria risk (about 40% and 10% of fever patients at 4 surveyed health clinics were infected with *P. falciparum* and *P. vivax*, respectively). Although *P. falciparum* was the predominant species in the local population, it was responsible for only 5 of the 50 malaria infections observed in military personnel during the ADF deployment (ending in 2004).[8] The remaining 45 soldiers succumbed to *P. vivax* malaria, but had their initial acute attack of malaria only after returning to Australia, with 8 of them experiencing relapses (1 to 3 further attacks) over the next 12 months.

Doxycycline prophylaxis had obviously suppressed the blood stages of both *P. falciparum* and *P. vivax* while they were in Bougainville, but had not suppressed development of the residual liver stages (hypnozoites) of *P. vivax*. Consequently, after completing prophylaxis, soldiers suffered a malaria attack every time parasites were released from the liver into the blood circulation. Fortunately, most infected soldiers would have had their hypnozoites eliminated by the post-exposure course of primaquine taken after leaving Bougainville. But these soldiers demonstrated, once again, that a more effective and less cumbersome drug regimen was needed to prevent vivax malaria after return to Australia.

The collection of blood samples for on-the-spot malaria microscopy and treatment at the 4 health clinics was well received by local communities, reminding older villagers of similar surveys conducted during past malaria control efforts. Blood samples collected by AMI staff were also used for further field assessment of a non-microscopic rapid diagnostic test (see below) and for DNA analysis (see below). Fingertip specimens of blood were also collected from malaria patients to determine the *in vitro* susceptibility of *P. falciparum* to a range of antimalarial drugs (see below). This provided both ADF and local health staff with up-to-date information regarding drug resistance patterns in these areas, and resulted in some modification of drug regimens used for malaria prophylaxis and treatment. Information was also obtained on the distribution and density of different anopheline and culicine species in close proximity to the military field sites and surrounding villages (see below). As a result, appropriate measures were introduced to lessen the risk of acquiring malaria and arboviral infections such as dengue.

In efforts to improve personal protection methods, a few soldiers participated in a study to compare the effectiveness of a new self-erecting bednet, developed by the US Army, with the current ADF bednet (see below). Many soldiers also volunteered to participate in the evaluation of two new antimalarial drugs – Malarone® and tafenoquine (see below).

Timor Leste

Between September 1999 and April 2000, over 5000 ADF soldiers led the International Force in East Timor (InterFET) in a peace-keeping role following the referendum for self determination in the former Indonesian province. Although little epidemiological information was available, both *P. falciparum* and *P. vivax* (2:1 ratio) were known to be seasonally endemic in the area. However, it soon became apparent that soldiers were suffering a significant number of non-battle casualties from malaria. At the request of Lieutenant Colonel Nasveld, who had been posted from AMI to assume the role of Senior Medical Officer for the forward Brigade (3BDE), the AMI deployed an outbreak management team led by Major Kitchener. By the end of InterFET in February 2000, 67 ADF personnel had developed malaria in Timor Leste.[10] Far fewer personnel would have been affected if more attention had been given to better compliance with mosquito protection measures and doxycycline prophylaxis.

About two-thirds of the infections were caused by *P. falciparum* and the remainder by *P. vivax*. This could not be attributed to parasites having become resistant to doxycycline because treatment with the prophylactic daily dose of 100 mg for 7 days was uniformly effective in curing falciparum infections. Obviously, these soldiers had missed taking their daily prophylaxis on more than a few occasions or, possibly, doxycycline capsules in the blister packs had been degraded by adverse environmental conditions.

Nevertheless, the vast majority of soldiers had not been seriously remiss in taking their doxycycline prophylaxis. This became quite obvious when 298 soldiers experienced their initial attack of vivax malaria following their return to Australia.[11] After completing their prophylaxis, doxycycline was no

longer present to eliminate parasites entering the blood stream from the liver. Apart from drawing attention to the urgent need for more effective drug regimens against the liver stages of *P. vivax*, the delayed onset of these infections indicated the high exposure to malaria encountered by military personnel during InterFET.

What would have happened if doxycyline prophylaxis had not been available to suppress malaria? Not only would all initial attacks of vivax malaria have occurred in Timor Leste, but medical and command personnel would also have been confronted with 500 to 600 cases of potentially fatal falciparum malaria (based on the fact that falciparum malaria was twice as common as vivax malaria among non-compliant soldiers). With up to 1000 soldiers incapacitated by malaria, this may very well have jeopardised the successful outcome of the InterFET mission.

As observed in Bougainville, vivax malaria manifested itself in a high proportion of soldiers after their return to Australia. These delayed malaria attacks indicated that the post-exposure course of primaquine (7.5 mg thrice daily for 2 weeks) had either failed to eradicate hypnozoites from the liver or that it had not been taken as prescribed. Parasite tolerance to this primaquine regimen was undoubtedly responsible for many of these infections because 63 soldiers had another attack of vivax malaria, 14 had a second relapse, and 2 had a third relapse.[11] Most of these soldiers would have received the drug under clinical supervision and, not wanting to experience another attack of malaria, would have been sufficiently motivated to comply with the prescribed primaquine regimen. Although tolerance to primaquine was a problem, many other vivax infections were due to inadequate compliance with the cumbersome primaquine regimen. This highlighted the need for increased preventive medicine and command support to improve drug compliance and for further efforts to develop more user-friendly malaria eradication regimens.

Operation Ausindojaya

In addition to substantial commitments in Bougainville and Timor Leste, AMI participated in Operation Ausindojaya between 11 May and 9 June 1998. As part of Australia's aid to the famine relief program in PNG, Joint Task Force (JTF) 108 was deployed to Wamena, Irian Jaya and played a vital role in the distribution of food to famine stricken villagers. As there was a severe malaria epidemic in some parts of the famine relief area, Major Cooper was deployed with the JTF to determine the malaria risk to its personnel and to provide assistance to District and Provincial Health authorities in the management and future monitoring of malaria in the region. Although field investigations showed that the risk of JTF personnel being exposed to malaria at Wanema was minimal, *Anopheles punctulatus* was prevalent in areas adjacent to the Wamena Valley and malaria was present in over half the villagers. Advice regarding malaria control activities was provided to local health workers and recommendations were made in a report regarding various steps that should be taken to monitor and manage future outbreaks of malaria.

Activities

AMI continued to pursue its mission to ensure that ADF personnel are able to have the best possible protection against malaria. In addition, investigations were initiated to control other vector-borne diseases (VBDs) which were jeopardizing the health of ADF personnel in Australia and overseas. As mentioned previously,[5] many investigations at AMI were carried out in collaboration with other institutions. In broad terms, activities during the 1995-2000 quinquennium were performed under one of the following headings:

1. Drug resistance and diagnostics;
2. Drug development and evaluation;
3. Vector control, biology and geographical distribution;
4. Arboviral disease control;
5. Technical advice and training;
6. Collaboration and engagement with military and civilian organisations.

Figure 2: Mr Joseph Kabui, interim Head of the Bougainville Government, visiting members of the AMI team in Bougainville (March 1999) From left: CPL Michael Reid, MAJ Peter Nasveld, MAJ Robert Cooper, WO2 John Staley, Mr Joseph Kabui, SGT Mac Hartman (Preventive Medicine), SGT Andrew Campbell, LT Bruce Russell, LTCOL Michael Edstein.

Some planned research activities were disrupted during the re-location from Sydney to Brisbane and the assignment of a substantial number of AMI personnel to field activities in Bougainville and Timor Leste. Nevertheless, the overseas deployments provided AMI personnel with the opportunity to acquire otherwise unobtainable information regarding malaria epidemiology and the effectiveness of various antimalarial measures.

1. DRUG RESISTANCE AND DIAGNOSTICS

Previously described *in vitro* tests and bioassays continued to be used to assess parasite resistance to various antimalarial drugs.[4,5] Following the establishment of a molecular parasitology laboratory, various DNA based methods were introduced which provided high sensitivity and throughput for diagnosing malaria infections. DNA fingerprinting to identify different strains of *P falciparum* could now also be used to differentiate between true treatment failures and newly-acquired infections during epidemiological and antimalarial drug studies. Moreover, molecular markers for identifying mutations and/or expressional changes in drug resistant parasites could be used to detect and monitor the development and spread of drug resistance.

Drug resistance in the Solomon Islands

The WHO *in vitro* microtest[12] once again proved useful for determining the 'true' intrinsic drug susceptibility of parasites. The results of the *in vitro* test showed a marked degree of parasite resistance to chloroquine and pyrimethamine plus sulfadoxine, strongly suggesting that these commonly-used drugs would not cure patients with little or no immunity to falciparum malaria. However, parasites were sensitive to quinine, mefloquine and atovaquone, indicating that these drugs would be suitable for treatment/prophylaxis of malaria.

Assessment of *P. falciparum* susceptibility to artemisinin and its derivatives

Artemisinin and its derivatives (artesunate, artemether, dihydroartemisinin) were starting to be used more widely because of their higher potency and faster action compared with other drugs in controlling acute attacks of multidrug-resistant malaria. However, short courses of treatment (<5 days) were not effective in curing these infections. Clearly, further investigations were required to determine the parasite susceptibility and pharmacokinetic properties of this group of antimalarials.

In vitro assessment of parasite susceptibility to various artemisinins showed a very high correlation (r value between 0.97-0.99) between the standard WHO *in vitro* microtest[12] and the radioisotopic technique.[13] Although the radioisotopic technique is a convenient method for processing a large number of samples in well-endowed laboratories, the microtest requires only finger-tip specimens of blood and can be performed in field laboratories having access to a microcope. *In vitro* tests provide information on the intrinsic parasite susceptibility to drugs without having to consider whether recrudescence of parasitaemia after treatment is due to inadequate ingestion/absorption of the medication or simply due to a new infection. As the test is not affected by a patient's degree of immunity acquired during a previous malaria infection, the influence of immunity on treatment outcome (better in immune than non-immune patients) also does not need to be considered.

Prior to adding the artemisinins to the repertoire of drugs used in the *in vitro* test, the stability of the artemisinin compounds in pre-dosed 96-well microplates had to be determined under different environmental conditions. The antimalarial activity of artesunate and dihydroartemisinin (DHA) remained stable during 52 weeks of storage at 4C, whereas artemisinin and artemether showed a significant reduction in activity during prolonged storage. Furthermore, the activity of the first two drugs was reduced only slightly when sealed wells of pre-dosed microplates were used a second time (very common under field conditions) following 48 hours at 37°C with high humidity (as during incubation).[7] Further investigations also revealed that the inhibitory concentrations (IC50 and IC90) of all the artemisinins were higher at 50% serum than 10% serum (usually employed for *in vitro* test).

In vivo assessment of the artemisinins requires the ability to measure serum/plasma drug concentrations after drug administration. As described previously,[5] good progress had been made in developing a sensitive bioassay for this purpose. Similar to other bioassays, this required determination of the inhibitory concentrations of the artemisinin and estimation of the maximum inhibitory dilutions of serum/plasma samples. Since the latter is usually determined when serum concentrations are closer to 50% than 10%, inhibitory concentrations for this bioassay was determined at serum concentrations similar to those present *in vivo*.

In order to validate the value of the bioassay, plasma samples were analysed from patients treated with artesunate. This artemisinin derivative is rapidly and completely hydrolysed to DHA after artesunate administration. In a collaborative project between the University of Western Australia and the Cho Rai

Hospital in Ho Chi Minh City, blood samples had been collected from Vietnamese malaria patients after treatment with artesunate and subjected to chemical analysis. Although specific chemical analysis had been problematical previously, it was now possible to assay the parent compound and its metabolite using a newly-developed High Performance Liquid Chromatographic (HPLC) method.[14] After completing drug analysis in Western Australia, plasma samples were forwarded to AMI for bioassay analysis. Overall, bioassay of artesunate/DHA in plasma correlated very well with HPLC analysis, and the results appeared to indicate that the combined effect of the parent compound and DHA was responsible for the antimalarial activity observed in plasma specimens after artesunate administration.[15] These findings demonstrated once again that the bioassay could be used as a convenient and sensitive means of assessing the antimalarial activity of drugs and any identified or unidentified metabolites which might not be measurable by chemical assays. Since *P. falciparum* can be cultured in basic field laboratories, the bioassay could be used to assist medical personnel in evaluating the response to treatment, either to one of the artemisinins or to a number of other antimalarial drugs.

Molecular markers for chloroquine resistance

With the establishment of a molecular parasitology laboratory at AMI, investigations were carried out to determine whether molecular markers carrying genetic mutations could be used to determine the presence of drug resistance. Using polymerase chain reaction (PCR)-based methods, a chloroquine resistance transporter gene (*pfcrt*) had recently been identified that correlated perfectly with chloroquine resistance phenotype in culture-adapted parasites originating from Southeast Asia, Africa and South America. To validate this candidate marker for chloroquine resistance in the South Pacific region, 33 parasitised blood samples, collected in Bougainville (see above), were analysed at AMI. All parasite isolates determined to be sensitive to chloroquine by the microtest had the wild type *pfcrt*, while all those determined to be resistant had a mutated *pfcrt* or a mixture of wild type and mutated *pfcrt*.[16] The study also showed that 87% of *P. falciparum* parasites were resistant to chloroquine and had mutation patterns that were similar to those observed in chloroquine-resistant parasites from PNG.[16] This PCR-based method, requiring only finger-tip specimens of blood, could thus be used as a convenient surveillance tool for monitoring the prevalence or spread of chloroquine resistance in local communities. However, the degree of chloroquine resistance was likely to be modulated by mechanisms on genetic loci other than just *pfcrt*.[17]

Using PCR-based genotyping, DNA fingerprinting of malaria parasites also made it possible to determine whether parasites recurring after chloroquine treatment were different from those before treatment. If they were different, a recurrence of parasitaemia was obviously due to a new infection and not due to treatment failure of the original infection. During the ADF deployment to Timor Leste, AMI collaborated with the Merlin/WHO Roll Back Malaria group in assessing the response of 48 malaria patients to chloroquine treatment over a period of 4 weeks. Although 32 patients had a recurrence of parasitaemia (uncorrected failure rate of 66.6%), all 48 patients were infected with parasites carrying mutant *pfcrt* gene. Presumably the patients who were cured had a significant degree of partial immunity and/or a low level of resistance to chloroquine. Interestingly, the mutation pattern in Timor Leste parasites was identical to that seen in Bougainville and PNG, indicating a common origin for chloroquine-resistant parasites in these countries.[18] In determining how many of these treatment failures were caused by the original infection, genotyping revealed that re-infection had occurred in only 4 of the patients.[19] With such a poor response to chloroquine treatment, national malaria treatment policy was changed, and pyrimethamine/sulfadoxine replaced chloroquine as standard treatment for uncomplicated falciparum malaria.

Identification of molecular markers for atovaquone resistance

Atovaquone was being increasingly recognised as a potential antimalarial drug, but many treatment failures were observed unless it was combined with other drugs, such as proguanil.[5] Given the widespread interest in the further development of atovaquone, studies were undertaken to elucidate the mechanism by which parasites became resistant to it and to identify molecular markers for atovaquone resistance. After producing 5 atovaquone-resistant parasite lines in the laboratory, a single (M133I) and several double amino acid substitutions (M133I+P275T, M133I+G280D, M133I+K272R and L283I+V284K) were identified in cytochrome *b* of parasites' mitochondrial electron transport chain. In addition, a single amino acid substitution (Y268S) was identified in the cytochrome *b* of parasites obtained from a patient who had failed atovaquone treatment. Significant correlation was observed between these substitutions and *in vitro* susceptibility to atovaquone, with Y268S conferring the highest degree of resistance (>9000-fold increase in IC50 value), followed by L283I+V284K (76-fold increase in the IC50 value).

In order to better understand how these amino acid changes affected atovaquone binding to parasites, a *P. falciparum* cytochrome *b* model was constructed with a view to predicting atovaquone binding site by using molecular modeling technology. These investigations predicted that amino acid changes such as Y286S, M133I and V284K resulted in changes to contact residuals responsible for atovaquone binding to parasite cytochrome *bc*1. This, in turn, reduced the binding of atovaquone to parasites and resulted in resistance to atovaquone.[20] The estimated parasite mutation rate was 1 in $5x10^8$ parasites per generation. Such a high mutation rate explained the rapid development of resistance observed in clinical trials and emphasised the importance of always using atovaquone in combination with other antimalarial drugs. Since then, these mutations and resistant parasite lines have been used as molecular markers for atovaquone resistance and in the development and evaluation of new drugs, respectively.

Non-microscopic malaria diagnosis

Examination of blood films by competent microscopists is the definitive way to establish malaria diagnosis. Because this is not always possible, a novel non- microscopic test was developed by ICT Diagnostics, Sydney. Earlier field evaluation of the ICT Malaria Pf test card had indicated that it could be used as a rapid diagnostic test (RDT) for falciparum malaria when microscopic examination of blood films was impractical.[5] During the field deployments in Bougainville and Timor Leste, another RDT incorporating both *P. falciparum* and *P. vivax* antigens - AMRAD/ICT Pf/Pv test – correlated well with microscopic findings for *P. falciparum* (even at low parasite densities). However, the sensitivity of the test was less than optimum for *P. vivax*, with many infections being missed at densities below 500 parasites/μL of blood. As most primary vivax infections in non-immune individuals present with parasite densities below this level, it was decided to recommend the use of the Pf test card in preference to the Pf/Pv card.

Could the Pf test card, based on detecting *P. falciparum* histidine rich protein 2 - PfHRP2, be used to verify cure of a malaria infection following treatment? This was answered by a soldier who had developed falciparum malaria shortly after returning from the Solomon Islands. Microscopic examination of thick blood films detected no parasites within 3 days after starting treatment with quinine and doxycycline nor in follow-up blood films examined weekly for 4 weeks. However, RDTs remained positive up to 2 weeks after treatment, probably because residual malaria antigen continued to circulate in the blood stream after parasites in red blood cells had been destroyed. Similar findings observed in other patients established the fact that this RDT could not be used to monitor the response to treatment. Conversely, this test might be very useful for confirming a malaria diagnosis in patients who may have been treated presumptively before a definitive diagnosis was able to be established.

In addition to RDTs, novel, highly-sensitive DNA-based methods could now also be used by AMI for estimating the incidence of different malaria species during field surveys. In a collaborative study with AFRIMS, PCR analysis of 230 blood samples from Thai soldiers detected 26 *P. falciparum*, 39 *P. vivax* and 1 *P. malariae* infections. This represented a 100% specificity and sensitivity for *P. falciparum* and a 100% specificity and 89% sensitivity for *P. vivax*, with PCR analysis being accomplished in a fraction of the time required for microscopic examination.[7]

2. DRUG DEVELOPMENT AND EVALUATION

Efforts were continued to ensure that ADF personnel were provided with the best possible protection against malaria. The three most commonly used drugs – doxycycline, primaquine and mefloquine – were not ideal because 1) they all had side-effects that limited or precluded their use by some individuals; 2) the frequency of drug intake and/or duration of prophylaxis did not encourage compliance; 3) malaria parasites were becoming increasingly resistant or tolerant to mefloquine and primaquine. As a possible alternative to doxycycline, further studies were conducted with atovaquone/proguanil

Figure 3: Dr Qin Cheng and MAJGEN Bui Dai during visit to AMI in 1998.

(Malarone®), including a field study in Bougainville. Following earlier investigations with tafenoquine (WR238605; Etaquine),[5] clinical/pharmacological studies were conducted in Thailand, Bougainville and Timor Leste to determine whether this drug might be a suitable replacement for primaquine. In addition, non-clinical investigations were continued with third-generation antifolates, Mannich bases, and artemisinin compounds.

Doxycycline concentrations to validate drug compliance

Since doxycycline was the main prophylactic drug used by the ADF, AMI collaborated with the United States Army Malaria Research Unit (USAMRU) in Kenya to validate drug compliance in 30 volunteers taking 100 mg doxycycline daily for malaria prophylaxis. Analysis of plasma samples for doxycycline revealed mean trough concentrations of 660 ± 339 ng/mL in 26 samples. No measurable doxycycline was detected in the 4 'prophylaxis failures'. These observations provided useful baseline data for monitoring the prophylactic efficacy of doxycycline and confirming drug ingestion.

Atovaquone/proguanil (Malarone®) given in combination with artesunate for malaria treatment

Earlier studies by AMI had provided a good deal of evidence supporting the efficacy of atovaquone/proguanil against falciparum malaria.[5] In May 1998, Malarone® was included in the Australian Register of Therapeutic Goods (ARTG) for the treatment of malaria. In all likelihood, the potential value of this drug combination for treating multidrug-resistant malaria would be increased even further by the addition of an artemisinin derivative as it would enhance rapid clearance of symptoms and prevent/delay the development of parasite resistance. But would there be a drug-drug interaction between artesunate and atovaquone/proguanil? In collaboration with the Wellcome Mahidol University Oxford Tropical Medicine Research Programme in Bangkok, healthy Karen adults on the Thai-Cambodian border participated in a randomised, two-period crossover study during which they received atovaquone/proguanil (1000/400 mg) with or without artesunate (250 mg).[21] The results of this WHO-sponsored study revealed that the triple drug combination was well tolerated and that the pharmacokinetic properties of atovaquone/proguanil and artesunate were comparable to those reported previously for the individual drugs, suggesting a lack of drug-drug interaction.

Atovaquone/proguanil (Malarone®) compared to doxycycline for malaria prophylaxis

In view of the efficacy of atovaquone/proguanil for treating drug-resistant falciparum malaria, could this drug combination also be used as an alternative to doxycycline for malaria protection? This was investigated during the deployment of 148 ADF personnel to Bougainville (see above) when half of them volunteered to receive a daily dose of one tablet of Malarone® (atovaquone 250mg; proguanil 100mg) and the other half, the standard one tablet of doxycycline 100mg.[22] Weekly examination of blood films indicated that both drug regimens suppressed malaria completely. No significant haematological or biochemical changes were observed, and side-effects were comparable and relatively minor in both groups. Gastrointestinal complaints were reported slightly more frequently in the doxycycline group, whereas headaches were more common in the Malarone group. Photosensitivity was reported by only the odd soldier in the doxycycline group. The findings indicated that Malarone® could potentially be used as an alternative to doxycycline for malaria prophylaxis. Health Policy Directive HPD 215 was subsequently amended to reflect the fact that Malarone® could be used as an alternative antimalarial drug for ADF personnel who were intolerant to doxycycline.

Effectiveness of a higher dose of primaquine against vivax malaria

The southwest Pacific area had long been considered to harbour liver stages (hypnozoites) of *P. vivax* that were more difficult to eradicate by standard courses of primaquine (15 mg base/day for 14 days) than in other parts of the world. This led to a higher total daily dose of primaquine – 22.5 mg (7.5 mg thrice a day) - being used for post-exposure prophylaxis.[23] Despite this, an increasing number of soldiers deployed to PNG in 1988[24] and in 1998 (see above) were experiencing relapses of vivax malaria after return to Australia. Following the outbreak of malaria among ADF personnel during the InterFET operation in Timor Leste, 267 vivax malaria cases and relapses were reported to the Central Malaria Register within six months after completion of the operation.[10] Might a higher total daily dose of primaquine 30 mg/day (15 mg twice a day for 14 days), given in combination with a standard course of chloroquine (1500 mg over 3 days), be more effective than primaquine 22.5 mg/day in curing these infections? Retrospective cohort analysis of cases receiving one or other of these treatments indicated a relapse rate of 7% in 71 patients receiving the higher primaquine dose, whereas 47% of 75 patients receiving the lower dose experienced a relapse (RR 6.63; CI 2.75-15.96).[25]

These findings indicated that primaquine-tolerant strains of *P. vivax* were present in Timor Leste and provided further support to the view that higher doses of primaquine should be used for the prevention and cure of vivax malaria.[23]

Potential replacement of primaquine by tafenoquine (also known as Etaquine; WR238605)

Earlier studies with tafenoquine at AMRU had shown it to have greater *in vitro* and *in vivo* antimalarial activity than primaquine.[5] Since this 8-aminoquinoline drug is eliminated from the body much more slowly than primaquine (half-life of 14 days vs 6 hours),[26] patient compliance with taking a short treatment course of tafenoquine for vivax malaria would be expected to be much better than was currently the case with the 14-day primaquine eradication course. Tafenoquine administration at regular intervals might also protect military personnel and travellers against vivax and falciparum malaria during their stay in malarious areas. As it killed gametocytes of *P. falciparum*, tafenoquine might eventually also be used to limit the spread of drug-resistant malaria and to eliminate malaria from epidemiologically isolated communities.[27]

Tafenoquine prophylaxis during deployment to malarious areas

Between April and November 1998, several AMI staff members participated in a joint clinical field trial with Thai and US Components of the Armed Forces Research Institute of Medical Sciences (AFRIMS). The purpose of the trial was to evaluate the effectiveness of tafenoquine in Thai Marine soldiers stationed in a malarious area along the Thai-Cambodian border. Loading doses of 400 mg base tafenoquine daily for 3 days, followed by single monthly doses of 400 mg tafenoquine, were given to over 200 non-glucose 6 phosphate dehydrogenase (G6PD) deficient Thai soldiers for 6 months. This prophylactic regimen was effective in preventing both vivax malaria and multiple-drug resistant falciparum malaria.[28-30] It was also well tolerated, with only mild and transient side effects (headache and diarrhoea) being reported. Using a simple, rapid and accurate HPLC method developed at AMI,[31] plasma tafenoquine concentrations indicated that trough concentrations of 80-100 ng/mL suppressed both vivax and falciparum malaria, whereas concentrations of <40 ng/mL were unable to do so.[32] The findings suggested that tafenoquine needed to be taken only once a month to prevent malaria. Although good compliance with this dosing interval might provide adequate protection against malaria, weekly prophylaxis with lower tafenoquine doses is expected to reduce the risk of 'breakthroughs' from 'missed doses' or of possible adverse drug reactions.

Tafenoquine post-exposure prophylaxis after return from malarious areas

In military personnel returning to a malaria-free area (e.g. Australia), might it be possible to replace the 14-day post-exposure primaquine eradication course with a less cumbersome 3-day course of tafenoquine? This was investigated in 1989/1999 when 592 Australian soldiers volunteered to take either a 14-day primaquine course of 7.5 mg base thrice daily or a 3-day tafenoquine course (400 mg daily or 200 mg twice daily) after the end of their deployment to Bougainville (see above).[33] About 7 hours after the last dose of tafenoquine, their mean plasma tafenoquine concentrations (584ng/mL) were substantially lower (730 ng/mL) than had been observed in the prophylactic study involving Thai soldiers.[32] Apart from Australian soldiers weighing more than Thai Marines (mean body weight 77 kg vs 60 kg), ethnic differences in the metabolic disposition of tafenoquine could also have contributed to these findings. Within 12 months after post-exposure prophylaxis, acute attacks of vivax malaria were observed in 6 of the 214 Australian volunteers who received primaquine and in 7 of the 378 volunteers who received tafenoquine.[33] Mean plasma tafenoquine concentrations were comparable in soldiers who developed and did not develop malaria. Relatively more gastrointestinal disturbances, such as nausea and abdominal cramps, were observed in the tafenoquine groups (single or split dose) than in the primaquine group, and these symptoms tended to be more common in female volunteers than in their male counterparts. However, these adverse events were transient in nature and generally not sufficiently troubling to interfere with performance of daily activities.

The deployment of the ADF to Timor Leste (see above), commencing in September 1999, provided a further opportunity to assess the value of tafenoquine in another area with falciparum and vivax malaria. Volunteers from the Third Battalion, Royal Australian Regiment preparing to return to Australia following InterFET service in February 2000, were randomly allocated to receive a 3-day tafenoquine course (either 400mg or 200mg daily) or the standard 14-day primaquine course. As observed in Bougainville, comparable episodes of vivax malaria were documented over the ensuing 12 months in the 3 groups of volunteers.[34] Soldiers preferred the 3-day tafenoquine course to the longer 14-day primaquine course. In an unsupervised setting, this shorter post-exposure prophylaxis would have the advantage of improving drug compliance, thereby reducing the

number of vivax infections after return to Australia. In contrast to the lengthy primaquine course, it might be operationally feasible to administer the short tafenoquine course under direct supervision before the departure of personnel from a malarious area, with an even better outcome for returning ADF personnel.

Search for alternative third-generation antifolate compounds

After demonstrating the remarkable antimalarial activity of PS-15 and its metabolite - WR99210,[5] further studies were carried out to identify other possible antimalarial agents in this class of third-generation antifolate compounds.[35] Results obtained after administration of 4 analogues to *Saimiri sciureus* monkeys showed that their *ex vivo* antimalarial activities were similar or lower than those observed with PS-15. When the *in vitro* antimalarial activities of 26 analogues of WR99210 were compared with one another, 11 of them were slightly more active than WR99210, 8 were slightly less active, and 2 showed very poor activity. These findings indicated that alternative antifolate compounds were available as possible candidates for further development.

Influence of parasite stages and densities on activity of Mannich base compounds

Earlier *in vitro* and *ex vivo* antimalarial studies with various Mannich bases had indicated that these compounds had greater antimalarial activity than standard antimalarials such as chloroquine, amodiaquine and pyronaridine.[5,36] Further observations highlighted the importance of parasite developmental stages and parasite densities when assessing the *in vitro* activity of antimalarial drugs.[7]

Using tightly synchronised *in vitro* cultures, the blood stage-specific sensitivity of drug-resistant parasites to four of the most potent quinoline Mannich bases changed markedly during parasite maturation, with the highest inhibitory effect being observed against young ring stages. Asynchronous cultures, in which the proportion of different parasite stages varied at the start of culture, not only yielded variable results from one experiment to another, but also failed to provide vital information regarding selective drug action against different parasite stages. In view of the stage-specific sensitivity of Mannich bases, combining them with companion drugs having different blood stage sensitivity patterns might improve the treatment response and delay the onset of drug resistance.

The *in vitro* studies also showed that increased concentrations of normal and infected erythrocytes were associated with a reduction in parasite growth and that the inhibitory effects of Mannich bases (including amodiaquine and pyronaridine) were reduced at higher parasite densities. These findings indicated that (a) *in vitro* concentrations of normal and infected erythrocytes must be carefully controlled during further studies with Mannich base compounds; (b) increased drug activity at lower parasite densities may be related to selective concentration in parasitized cells by this group of antimalarials; (c) malaria patients may respond poorly to standard chemotherapy due to elevated parasite densities and not due to suboptimum drug concentrations or drug-resistant parasites.

Artemisone - a new artemisinin compound

The artemisinin compounds were becoming increasingly important in countering the global threat of multidrug-resistant malaria (see above).[5] However, there was some concern about the safety of these compounds because some artemisinins, particularly DHA, had exhibited neurotoxicity in animal models. In the meantime, a new semi-synthetic more water soluble artemisinin compound – artemisone – had been synthesised which displayed low lipophilicity and negligible neuro- and cyto-toxicity in *in vitro* and *in vivo* assays.[37] With the support of Medicines for Malaria Venture (MMV), Switzerland, and Bayer AG, Germany, *in vitro* studies at AMI during 1999 showed that artemisone was more active than artesunate against a number of multidrug-resistant strains of *P. falciparum*. These studies were followed up by oral administration of a single dose of 30mg/kg of artemisone or artesunate to non-infected *Saimiri sciureus* monkeys, the collection of blood samples over a period of 6 hours, and determining the *ex vivo* serum activity[5] of both compounds against drug-resistant isolates of *P. falciparum in vitro*. The results clearly indicated that the efficacy and duration of activity was significantly greater for artemisone than for artesunate.[37] These findings suggested that further studies should be carried out to determine the efficacy of artemisone in *Aotus griseimembra* monkeys infected with drug-resistant *P. falciparum* malaria.

3. VECTOR CONTROL, BIOLOGY AND GEOGRAPHICAL DISTRIBUTION

Insect repellents

Deet (diethylmethylbenzamide; diethyltoluamide), first released in 1954, soon became the main chemical component in topical applications used to repel biting insects. Although Deet preparations were quite effective, efforts were continued to improve personal protection measures for soldiers in the field.[4] Previous collaborative studies with

AFRIMS in Thailand had indicated that two novel chemicals – the piperidine AI3-37220 and the lactone CIC-4 – were equivalent or superior to Deet against anopheline mosquitoes. Following the return of Major Frances to Australia in 1995, further tests were carried out to compare the effectiveness of these repellents against *Anopheles farauti*, the main malaria vector in Australia and the southwest Pacific region. In laboratory tests with insectary-reared mosquitoes, all three repellents exhibited activity, with AI3-37220 being slightly less protective than the other two repellents. Since results obtained in the laboratory may differ from those subsequently observed in the field, tests were conducted at Brown Range, Cowley Beach Training Area, northern Queensland during 1996. At a concentration of 25% (in ethanol), Deet and CIC-4 provided substantial protection against *An. farauti* for 7 hours after skin application, and AI3-37220 continued to provide greater than 95% protection up to the last observation at 9 hours. As observed in Thailand, AI3-37220 showed greater protection when tested under field conditions, highlighting the importance of assessing repellent efficacy in volunteers exposed to natural populations of mosquitoes.[38] Additional tests, conducted in the Morobe and Central Provinces of PNG during Operation Anopheles 1998, showed repellents provided a greater than 95% protection for only 3 hours. This was probably due to the very high anopheline densities encountered in these areas. Furthermore, unlike earlier studies in Thailand and Australia, AI3-37220 was not uniformly more effective than the other two repellents.[39-41] In summary, these studies showed that these repellents were generally not superior to the standard ADF repellent formulation, containing 35% Deet in a gel, which was introduced in 1992.[42]

Self-erecting, low profile bednet

The effectiveness of a new self-erecting, low profile bednet was assessed in March 1999 during the ADF deployment to Bougainville. The primary aim of the study was to compare the effectiveness of this US Army prototype bednet with the current ADF bednet and to assess permethrin impregnation under field conditions. The prototype net was more effective in preventing the entry of mosquitoes during the night because, unlike the ADF net, it has a taffeta floor with a zippered opening on the side. After impregnation with permethrin, both types of nets provided >97% protection, although some mosquitoes were able to obtain a blood meal biting through the nets.[43]

Distribution and speciation of anopheline mosquitoes in Papua New Guinea (1996-1999)

Studies on the identification, distribution and speciation of malaria vectors in PNG, started in1992,[5] were continued during this quinquennium. Jointly conducted with the PNG Defence Force, Operation Anopheles was extended to Morobe

Table. Anopheles species collected in Papua New Guinea and their vector status

Species	Distribution and abundance	Vector status	No. positive for CS protein/no. tested
Anopheles koliensis	widespread/common	major	41/8600
Anopheles punctulatus	widespread/common	major	3/245
Anopheles farauti	widespread/common	major	41/9692
Anopheles farauti 2	widespread/common	major	10/1189
Anopheles farauti 4	limited/common	major	15/1535
Anopheles longirostris	widespread/uncommon	minor	62/793
Anopheles farauti 8	limited/uncommon	minor	2/308
Anopheles bancroftii	widespread/uncommon	minor	1/476
Anopheles farauti 6	limited/common >1500m	minor	0/180
Anopheles subpictus s.l.	limited/uncommon	minor	0/116
Anopheles karwari	limited/uncommon	minor	0/13
Anopheles meraukensis	limited/uncommon	none	0
Anopheles novaguinensis	limited/uncommon	none	0
Anopheles farauti 3	limited/uncommon	none	0
Anopheles sp near punctulatus	limited/uncommon	none	0

Province (1996), Central Province (1997), Gulf, Milne Bay and Northern Provinces (1998). These activities were supported by rotary wing aircraft from 162 Reconnaissance Squadron and fixed wing aircraft from 173 Surveillance Squadron. Information obtained from these surveys would enhance our knowledge of the epidemiology of malaria in PNG and assist in its control.

During these operations, over 25,000 anopheline specimens were collected from larval breeding sites, CO2-baited light traps and adult biting catches. By using morphological and DNA techniques,[4] 18 anopheline species were identified from these collections (Table).[44-47] In addition, specimens were examined for their vectorial capacity by employing species-specific monoclonal antibodies and ELISA to detect circumsporozoite (CS) protein of human malaria parasites.[48] Based on field observations on the distribution and abundance of these species, and their ability to develop human malaria parasites, 5 species were considered to be major vectors of malaria, 7 minor vectors of malaria, while 7 were considered as unimportant with regards to malaria transmission (Table).[48] *An. farauti* 2 and *An. farauti* 8 (a newly recognised species) were both incriminated here for the first time as malaria vectors.

Further entomological investigations were carried out in PNG during 1999 in 90 different sites located in Buka Island, and the Arawa/Kieta and Tonu areas of Bougainville. Preliminary observations revealed both *An. farauti* and *An. farauti* 2 to be present on the main island of Bougainville whereas *An. punctulatus* could only be found on Buka Island.[49] The apparent absence of *An. punctulatus* in typical breeding sites around Tonu was surprising, given its proximity to the Solomon Islands were *An. punctulatus* was found to be quite common. The other interesting finding was that *An. farauti* 2 was not observed biting humans on Buka and Bougainville Island, despite the abundance of its larval stages on Buka Island.[49] This suggested that this species may play no role in malaria transmission on this island which is in marked contrast to previous observations on this species in PNG.[48] *An. koliensis*, a common malaria vector in PNG, was not observed during the survey. It was reported to be present the last time a comprehensive survey of Buka Island was carried out in 1960, and its absence 40 years later could possibly be due to the widespread spraying of residual insecticides during the early 1960s.

In 1998 an anopheline faunal survey was conducted on the north coast of Guadalcanal in the Solomon Islands. Although this region has the highest malaria transmission rates in the country, the identity and distribution of the anopheles species was poorly understood. *An. farauti* was the most widespread and abundant species in the area, with *An. farauti* 2 and *An. farauti* 7 being commonly collected as larvae but not found biting humans and unlikely to play a role in malaria transmission. Similar findings were made for *An. farauti* 2 on Buka and Bougainville Islands which lie in the same archipelago. Unlike the findings on Bougainville, *An. punctulatus* was fairly common in the inland coastal plain of Guadalcanal.[50]

Commencing in 1997, material collected from PNG and earlier surveys in northern Australia[4,5] was examined using a range of molecular tools made available as a result of recent advances in DNA-based technologies. In collaboration with Dr Nigel Beebe of the University of Technology, Sydney, both internal transcribed spacer regions (ITS2 and ITS1) of the ribosomal DNA gene were characterised and found to be suitable for studying the inter-phylogenetic relationships of these species and their population genetics.[51-55] This work revealed that several genotypes within the *An. farauti* taxon occur in discrete isolated independently evolving populations separated by overt physical and climatic barriers, such as the mountains of the Central Range in PNG, the arid region of the Gulf of Carpentaria and the sea gaps between PNG and the Solomon Islands.[52,56] Several genotypes also exist within the *An. farauti* 2 taxon; of epidemiological interest is the fact that the genotype which occurs in mainland PNG (and is a vector of malaria feeding on humans) is different from the one found in Buka, Bougainville and the Solomon Islands which does not feed on humans.[53] Molecular analysis of the ITS2 and ITS1 regions of *An. bancroftii* and *An. longirostris*, both of which are minor malaria vectors in PNG, revealed four cryptic species in the *An. bancroftii* taxon occupying discrete areas across northern Australia and PNG, and 9 cryptic species in the *An. longirostris* taxon spread across PNG.[57,58]

4. ARBOVIRAL DISEASES

Ross River virus and Barmah Forest virus

In 1995 several ADF personnel developed Ross River (RR) and Barmah Forest (BF) virus infections during Exercise Ready Soldier at the Shoalwater Bay Training Area (SWBTA) in Queensland. The following year there were further infections in both Australian and US soldiers following Exercise Tandem Thrust at SWBTA. Moreover, the likelihood of soldiers being exposed to these viruses was highlighted in the Environmental Impact Statement on the recently-established Bradshaw Training Area in the Northern Territory. In view of these threats to the health of ADF and other personnel, Brigadier Paul Buckley, the Director General of Defence Health Services

(DGDHS), encouraged AMI to conduct investigations relating to the epidemiology and control of mosquito-borne infections not transmitted by anopheline mosquitoes.

In March 1998 a longitudinal mosquito survey of the SWBTA was started to assess the potential threat of arboviruses to the combat readiness and efficiency of ADF personnel. With the logistical support and technical assistance of 4th Preventive Medicine Company, the main aim of the survey was to monitor the distribution, seasonal prevalence, densities and larval habitats of mosquito vectors and determine the activity of RR and BF viruses. This information would clarify the current and potential threats to the health of ADF personnel and enable the implementation of effective and sustainable control measures. Monthly collections from 15 sites for 2 years revealed that the predominant species were the saltmarsh mosquitoes *Aedes vigilax* (36%), *Culex sitiens* (17%) and the freshwater species *Culex annulirostris* (30%). After homogenising 1350 lots of mosquitoes (25 mosquitoes per lot, pooled by species and sex), each lot was screened for the presence of the RNA of RR and BF viruses using a highly sensitive and specific PCR technique established and optimised at AMI. With the ability to detect a single virus-infected mosquito per lot, a number of *Ae. vigilax* lots were found to be positive for RR virus.[59,60]

The importance of RR virus at SWBTA was documented during 1999 when 3 out of 169 mixed military and civilian personnel developed a debilitating illness and were IgM positive for RR virus after participating in a 10-day exercise. Subsequent investigations highlighted various problems in obtaining meaningful information about the local epidemiology of RR virus disease. Single serum specimens for IgG and IgM analysis proved to be of limited value, and it drew attention to the importance of collecting specimens both during the acute and convalescent phases of the illness. Only by instituting this procedure for all symptomatic cases reported during post-exercise surveillance would it be possible to identify the RR virus disease risks of training in this area.[61]

Dengue virus

Dengue is an important mosquito-transmitted infection prevalent in areas to the north of Australia, such as Timor Leste. During the period of the INTERFET Operation from September 1999 to February 2000, AMI confirmed 160 cases of dengue among ADF personnel.[62] The outbreak peaked in January 2000, with the risk of exposure to the virus being particularly high in the area around the Dili airfield. Although the likelihood of acquiring dengue was increased by the proximity of internally displaced (and infected) local people to ADF personnel, the epidemic could have been prevented by better application of personal protection (bednet usage, etc.) and vector control measures.

All 4 serotypes of dengue were identified, but serotype 3 was responsible for most of the infections. During its serosurveillance studies AMI successfully field-tested a new IgM ELISA card. Most ADF personnel were found to have no immunity to dengue before their deployment to Timor Leste, despite many of them having undergone prior training in dengue-endemic north Queensland. In the minority of soldiers with some pre-existing immunity acquired after a previous dengue infection, there was of course always the risk that some of them might experience serious clinical complications following exposure to a different serotype of the virus.[63]

Medical personnel were also aware of the possibility that soldiers returning to northern Australia might introduce the dengue virus into the local population of *Aedes spp.* – an efficient mosquito vector of the dengue virus. In 2000, AMI successfully managed virus containment when 9 soldiers infected with dengue serotypes 2 and 3 were medically evacuated to Lavarack Barracks, Townsville.[64] No cases of dengue were reported during the four months following their arrival in Townsville.

In addition to defining the epidemiology and managing the outbreak of dengue, work began on identifying potential vaccine candidates. AMI's involvement in the Australian development of quadrivalent dengue vaccine candidates was facilitated by its close collaboration with the US component of AFRIMS, Bangkok, and Aventis Pasteur.

Japanese encephalitis

Mosquito-borne Japanese encephalitis (JE) is the leading cause of viral encephalitis in Asia, but there has been a progressive eastward movement of the virus crossing the Wallace line into the Western Province of PNG. It was first reported in the Torres Strait islands in 1995 and then in the mainland of Australia in 1998. Due to the shortage and cost of JE vaccine (Biken) in Australia, the first of a series of studies was undertaken in 1998 using one tenth or one fifth the volume of the vaccine administered intradermally.[65] As preparations for deployment to East Timor escalated in 1999, the high cost of the limited supply of [then] CSL JE vaccine consumed half the ADF vaccine budget. A second proof of concept study was conducted in 2000 comparing the safety and efficacy of subcutaneous and intradermal vaccination in soldiers of the Sixth Battalion RAR.[66] This confirmed the dual intradermal method of

vaccination (0.1 ml in two injections) to be effective and well tolerated. By using less vaccine, it was possible to extend the vaccine stockpile life. The requirement for a replacement JE vaccine was to become a key research interest of the Institute during the next 5 years.

5. TECHNICAL ADVICE AND TRAINING

AMI provided the DGDHS with information regarding the latest laboratory, clinical and field investigations to assist his staff in updating health strategies and policies to prevent/treat malaria and other VBDs. Various publications and instructions on malaria and other VBDs were drafted and reviewed by AMI staff, such as Health Policy Directive 215 on Malaria and ADFP 705, Pesticides Manual guidelines for the use of Deet and permethrin by the ADF. As might be expected, health personnel at various command levels frequently (often several times a week) sought advice regarding personal protection measures, prophylaxis and treatment. In addition, AMI assisted ADF personnel in PNG and the Solomon Islands when problems arose regarding correct diagnosis and appropriate treatment of malaria.

AMI continued to conduct several malaria training courses for ADF personnel in laboratory and field methods and procedures. It also continued to train foreign military and civilian health personnel in malaria and other VBDs. Sponsored by IPDiv, WHO or AusAID, more than a dozen professional and technical personnel spent between 1 to 6 months at AMI; many of them subsequently occupied leading positions in PNG, Solomon Islands, Thailand, Vanuatu and Vietnam.

6. COLLABORATION AND ENGAGEMENT WITH MILITARY AND CIVILIAN ORGANISATIONS

Collaboration with military and civilian organisations, both in Australia and overseas, continued to be promoted during this quinquennium. This was considered to be the most efficient way to successfully develop and evaluate effective measures and tools to control malaria and other VBDs in the ADF. Apart from advantages derived from being a WHO Collaborating Centre for Malaria, AMI benefited from its association with many institutions identified previously.[5] Additional institutions with whom collaboration was established during this quinquennium included the University of Queensland, the Queensland Institute of Medical Research, the University of Technology Sydney, the University of Western Australia, the Papua New Guinea Defence Force, Bayer AG, Germany, and the Secretariat of the Pacific Community.

Further research activities were also being planned in collaboration with Vietnam. As early as 1986, the Australian Development Assistance Bureau (ADAB) had sponsored Professor Rieckmann as WHO Malaria Consultant to Vietnam to review the current malaria situation and identify potential areas of cooperation in field research, particularly with regard to drug-resistant malaria. Subsequent visits by various teams, including AMRU staff members, led to the establishment of the Vietnam Malaria Control Project in 1994. This project was funded by the Australian International Development Assistance Bureau (AIDAB) which replaced ADAB and subsequently became AusAID in 1995. Although information about various aspects of the epidemiology and control of malaria was mutually beneficial to Vietnamese public health research personnel and AMI, only relatively minor cooperative research projects were established.

In September 1991, Professor Bui Dai, a malaria specialist from Vietnam in the Ministries of Health and Defence, had visited AMRU for 2 weeks on a WHO Fellowship. Further consultation between him and Professor Rieckmann eventually led to a proposal for cooperative studies on malaria between the Vietnam People's Army (VPA) and the ADF. In April 1996, ministerial endorsement for the proposal was obtained by the International Policy Division (IPDiv) of the Department of Defence. After further planning and consultation, an official invitation was received by IPDiv from the Ministry of Defence in Hanoi to discuss possible collaborative activities on malaria of mutual interest to both armies.

In March 1998, Professor Rieckmann and Lieutenant Colonel Edstein had a series of meetings with senior medical personnel led by Major General (Professor) Bui Dai. They also included a meeting with the Vice Minister for Defence who expressed the view that cooperation in the field of medicine was an effective way of initiating a defence relationship between Vietnam and Australia. At the conclusion of the 4-day visit, the Australian Embassy commented that "The visit by the Australian delegation has successfully achieved real progress for the first time in our efforts to enhance direct cooperation between the defence forces of Australia and Vietnam". Following several meetings of a joint steering committee in Vietnam and Australia, a Memorandum of Understanding (MoU) was signed in Hanoi on 22 March 2000 for a 5-year collaborative project on malaria control – the Vietnam Australia Defence Malaria Project (VADMP). Funded by IPDiv, its main purpose was to improve malaria control measures in the military forces of both countries through various training and research activities.

Figure 4: The Vietnam Australia Defence Malaria Project initiated at Hanoi on 22 March 2000 by signing of Memorandum of Understanding between MAJGEN Nguen Van Thuong (Director of Military Medicine,Vietnam People's Army) and BRIG Wayne Ramsey (Director General Defence Health Services, Australian Defence Force).

Conclusions

The first half of the fourth decade (1995-2000) was characterised by many significant events and achievements which included:

1) Re-location of the Army Malaria Research Unit from Sydney to Brisbane and its re-designation as the Australian Army Malaria Institute (AMI).
2) Establishment of a molecular parasitology laboratory at AMI to address issues relating to drug resistance, including identification of a molecular marker for parasite resistance to atovaquone, a new antimalarial drug.
3) Deployment of AMI field teams to monitor local malaria situations and to assess effectiveness of various antimalarial measures during large-scale ADF peace-keeping operations in Bougainville and Timor Leste.
4) Demonstration of the continuing efficacy of doxycycline for malaria prevention and of the potential value of atovaquone/proguanil (Malarone®) for back-up prophylaxis during deployment to malarious areas.
5) Field investigations with tafenoquine indicating that it might not only replace primaquine to prevent vivax malaria after return to Australia but also be used for prophylaxis while in malaria-endemic areas.
6) Further laboratory-based evidence of the potential value of Mannich bases, artemisinins, and third-generation antifolate compounds for treatment/prevention of drug-resistant malaria.
7) Completion of mosquito surveys in PNG, with ongoing analysis of population genetics using DNA-based technology.
8) Commencement of investigations to provide better protection of ADF personnel against arboviral infections.
9) Consolidation and extension of collaboration with other institutions to maximise AMI achievements.
10) Initiation of the Vietnam Australia Defence Malaria Project.

Highlights

1996

- Standardisation of *in vitro* assays to determine susceptibility of *P. falciparum* to the artemisinins
- Several third-generation antifolates active *in vitro* and *ex vivo* against multidrug- resistant falciparum malaria
- Activity of Mannich base compounds greater against ring stages and lower densities of *P. falciparum*
- Non-microscopic rapid diagnostic test (based on PfHRP2) unsuitable for monitoring drug resistance
- Two new skin repellents not uniformly more effective than Deet for mosquito protection
- Mosquito survey in Morobe Province, PNG

1997

- AMI re-located from Sydney to purpose-built facility at Enoggera Barracks, Brisbane.
- Lieutenant Colonel Michael Edstein appointed as Commanding Officer following retirement of Lieutenant Colonel Tony Sweeney.
- Major (Dr) Peter Nasveld joins AMI as team leader for clinical studies
- Atovaquone / proguanil (Malarone®) pharmacokinetics not altered by administration of artesunate
- Mosquito survey in Central Province, PNG

1998

- Dr Qin Cheng joins AMI and establishes molecular parasitology laboratory
- Monthly doses of tafenoquine protect against malaria in Thailand
- AMI field team deployed to Bougainville Province, PNG
- Major (Dr) Scott Kitchener joins clinical studies team
- Non-microscopic test more useful for diagnosis of *P. falciparum* than *P. vivax*
- Molecular markers and DNA fingerprinting used to assess chloroquine resistance
- Bioassay used successfully to determine pharmacokinetics of artesunate
- Mosquito surveys in Gulf, Northern and Milne Bay Provinces, PNG

1999

- Atovaquone/proguanil (Malarone®) prophylaxis as effective as doxycycline in Bougainville, PNG
- Molecular markers identified for atovaquone resistance
- Tafenoquine post-exposure prophylaxis at least as effective as primaquine in Bougainville, PNG
- Artemisone, a new artemisinin compound, has greater *ex vivo* activity than artesunate
- Prototype of a low-profile, self-erecting bednet more effective than ADF bednet
- Mosquito survey in Bougainville Province, PNG

2000

- AMI field team deployed to Timor Leste to carry out risk assessment of VBDs
- Doxycycline prophylaxis prevents large-scale outbreak of malaria in Timor Leste
- Tafenoquine 3-day course preferred to 14-day primaquine eradication course in Timor Leste
- Vietnam Australia Defence Malaria Project established

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