NUCLEAR SCIENCE FIGHTS MALARIA

RADIATION & MOLECULAR TECHNIQUES CAN PLAY TARGETED ROLES

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Malaria is the most important insect transmitted disease. Globally there are 300 to 500 million clinical cases of malaria a year. They result in two million deaths per year (one every 30 seconds), more than 90% of which occur in sub-Saharan Africa. More than 90% of those affected are children less than five years old. The economic impact of the disease is felt disproportionately by poor families who may spend a fourth of their annual income on prevention and control measures. The causative agents are parasites of the genus *Plasmodium* and they are transmitted only by female mosquitoes of the genus *Anopheles*.

Among key strategies to control malaria are the surveillance of anti-malarial drug efficacy through monitoring the levels of drug resistance, and the reduction of mosquito populations. Nuclear techniques can play important roles in these efforts to combat malaria. This article reports on IAEA activities associated with drug-resistant malaria and describes how molecular methods making use of radioactive isotopes can provide a great advantage in the diagnosis of resistance. The article further presents the IAEA's plans for initiating a research programme to assess the feasibility of developing the Sterile Insect Technique (SIT) as a complementary method to control the vector of malaria.

DETECTING DRUG-RESISTANT MALARIA

Treatment of malaria with drugs is the cornerstone of patient management and will likely remain so in the long-term. In certain regions, the parasites have developed drug resistance. Thus, the inexpensive anti-malarial drug, chloroquine, is no longer effective against many subspecies of the parasite. Resistance has been acquired through changes of the parasite's membrane such that if exposed to chloroquine the membrane will constantly pump it out.

Patients in chloroquine-resistant areas require treatment with other, more expensive drugs. Because new varieties of malarial parasites are constantly evolving and acquiring increased resistance to currently used drugs, routine surveillance for development of drug resistance is an essential activity for all malaria control programmes. Preliminary data from Kenya indicates that resistance, even to newer drugs, in some areas is as high as 30%.

Molecular methods greatly facilitate the diagnosis of drug-resistant malaria. The polymerase chain reaction (PCR) is a molecular method that can be used to show that drug resistance has developed. Use of isotopes in the DOT blot hybridization method, when combined with PCR, adds additional sensitivity and specificity in the detection of drug resistance. *(See box, page 34.)*

These molecular methods can demonstrate drug resistance to some anti-malarial drugs in a matter of hours. Conventional methods need up to 28 days to confer the same information and require large field teams.

Transfer of Molecular Methods to Member States. A 3-year IAEA technical cooperation project in Kenya, Mali, Sudan, Tanzania, Zambia, Zimbabwe, and Uganda recently introduced nuclear techniques to detect mutations in the parasite, associated with resistance to these drugs.

This project also supported the development and implementation of surveillance programmes for drug resistance, necessary for the effective management of malaria. During

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MOLECULAR METHODS IN THE FIGHT AGAINST MALARIA

The Polymerase Chain Reaction (PCR). The fundamental principle of applying the PCR in diagnostics is that, if a given DNA fragment is present, it will be multiplied by, perhaps, a million fold through the reaction, yielding so much of the substance that it can be detected easily. The process begins by heating the DNA fragment to separate it into two strands. An enzyme that copies DNA, called the DNA polymerase, is then added to create two complete copies of the original fragment. By repeating this process, millions of copies of the original DNA fragment can be made in a short time. Radiolabelled nucleic acid probes, which attach to DNA fragments, can then be used to identify the DNA fragment. This is a very sensitive and specific method requiring only a small sample of blood.

PCR-based DOT Blot Hybridization. This method can be used to characterize the DNA mutations of the parasite. The DNA is extracted from malaria infected blood that has been spotted on filter paper and amplified using the PCR. The DNA is directly "dotted" on to the nylon membrane. Hybridization, using radioactively labeled DNA probes, and autoradiography, exposing the X-ray film and viewing the results, are used to visualize the results. The advantage of this method is that the dots derived from numerous samples can be analyzed simultaneously. Another advantage is that the radioactive probe can be removed and a new one, to detect another mutation, added; thus several mutations can be screened sequentially. The method can be used to detect a minority resistant population in te patient samples, a significant proportion of which have mixed resistant and sensitive parasites.

the course of the project, 10,000 patients were treated for malaria. Out of these, 3000 were enrolled into studies and molecular analysis was carried out on 1500 finger prick blood samples collected on filter paper. Analysis of these data has shown that there was a positive relationship between the prevalence of parasite mutations and parasite resistance to antimalarial drugs Fansidar and chloroquine.

The results showed that the mutation frequency was very low in those countries (Mali and Sudan) where Fansidar resistance is low (2.5%). Regarding chloroquine resistance, a high frequency of the mutation and a concomitantly high chloroquine resistance was reported in Kenya, Mali and Tanzania.

Practical Results in the Field. In Mali, chloroquine and Fansidar molecular tests were carried out during a malaria epidemic. The assay was performed quickly on filter paper blood samples collected by finger prick where there were no facilities for microscopic analysis. Within a few days, (as opposed to the conventional test which takes 28 days) the results were available and showed that chloroquine resistant mutations were present in 75% of the samples, whereas no Fansidar resistance was present. Hence Fansidar was used and was highly effective in controlling the epidemic.

As a result of this work, a quick and robust tool for monitoring chloroquine resistance on a broad scale -- something that has not been possible before due to the high cost in time, money and staff to conduct standard clinical studies of chloroquine resistance -- is now available. Now sampling of a larger population will be possible, providing more accurate information to malaria programme managers that has not been previously possible.

Expanding the Outreach. In addition to the transfer of technology to African countries, the programme has linked with other international and regional malaria programmes. These include the World Health Organization's (WHO) Roll Back Malaria, the Multilateral Initiative in Malaria (MIM), and the East African Network for Monitoring Anti-malarial Treatment (EANMAT).

As a spin off, the IAEA's counterpart in Tanzania has been designated to genotype malaria samples from nine African countries involved in a multicentre project. The project focuses on the efficacy of multi-track therapy for the treatment of malaria which is being supported by the WHO Task Force on Anti-malarial Drug Resistance and Policy. It is hoped that the combined use of two anti-malarial drugs, namely Fansidar and artemisnin, will delay the emergence of drug resistance to both. The same molecular technology, as established in the regional African project, is

being used in the genotyping of samples.

Next Steps. Based on the successful establishment of molecular biology facilities, the IAEA decided in December 2000 to extend and widen the scope of the project and to include additional African countries in an expanded regional effort. This was widely based on proposals submitted by African Member States (Uganda, Zambia, Sudan) as well as the general interest of other Member States, including Nigeria and Ghana. The project has been formulated with the intention that more advanced and experienced institutes support less experienced institutes in the region.

While more permanent solutions to the eradication of malaria such as vaccines and vector control are at the developmental stage, the proposed methods for efficient detection of drug resistance will help in effective case management, one of the premier control strategies of WHO's Roll Back Malaria initiative. The project will be in line with the Abuja Declaration that calls, among other things, for the development of mechanisms to facilitate the provision of reliable information to decision-makers at different epidemiological levels to enable health authorities to devise appropriate control and surveillance strategies.

The overall aim of the project is that basic isotopic molecular techniques will be a stepping stone to bring the institutes to a level of confidence and capability in using more advanced, automated and possibly non-isotopic techniques to address major public health problems. To strengthen this effort, the project will be run in close collaboration with WHO.

THE CONTROL OF MALARIA MOSQUITOES

The Sterile Insect Technique (SIT) has been shown to be a very effective technology for the control and/or eradication of certain key insect pests including the vector of animal trypanosomosis, tsetse fly. As a result, over the last decade there have been repeated requests from Member States for the development of this technology for application against malaria mosquitoes.

There have been several previous attempts to develop the SIT against *Anopheles* mosquitoes and they have met with varying degrees of success or failure. In any new approach, much can be learned from these

STERILE INSECT TECHNIQUE (SIT)

Since the 1950s it has been demonstrated that insect pests can be controlled or eradicated through a "birth control" method known as the Sterile Insect Technique (SIT). The key element in SIT's application is to colonize and mass rear the target insect pest in large bio-factories, have them sterilized by ionizing radiation and then aerially release them into the field on a sustained basis and in sufficient numbers to achieve appropriate sterile to wild insect overflooding ratios. The wild females will have no offspring following mating with a released sterile male, leading to a reduction in the natural pest population.

Central to SIT's application is the area-wide concept in which the total population of the pest in an area, or region, has to be managed. SIT is also not a stand-alone technology. To be effective it has to be integrated in a package together with other pest control methods. However, SIT has the unique attribute of increased efficiency with decreasing target population density and as a result can lead to eventual eradication if applied systematically and on an area-wide basis over many generations. SIT is also the most environment-friendly pest management method as it is completely *species specific*: the sterility is induced exclusively in the target species, thereby impacting only the population of the pest insect.

Field Experiments with SIT Against Mosquitoes. There have been several attempts in the past to develop the SIT against mosquitoes, with varying degrees of success. They include the following field experiments.

Culex fatigans	India, 1962
C. pipiens quinquefasciatus	Florida USA, 1970
C. p. fatigans	India, 1975
C. tarsalis	California USA, 1965
C. tarsalis	California USA, 1980
Aedes aegypti	Florida USA, 1962
Anopheles quadrimaculatus	Florida USA, 1962
Anophleles albimanus	El Salvador, 1975

earlier experiences together with the major improvements that have been made in SIT technology since these very early field trials. Two consultant reports commissioned by the IAEA have recommended several candidate *Anopheles* species for SIT together with potential target sites for initial field trials. However, they stressed that before this stage, important technical constraints relating to several key components of SIT technology must first be removed.

To this end the IAEA -- as requested by many Member States and a General Conference Resolution (GC-44/24) in September 2000 -- has decided to conduct a feasibility study of the use SIT for one important vector of malaria. This research and development effort will focus initially on *Anopheles arabiensis*, a major malaria-transmitting species that is the only vector in large parts of its distribution in Africa.

Feasibility Study. The feasibility study will address the following technical constraints:

■ *Development of efficient methods of mass-rearing.* SIT relies on the efficient mass production of good quality insects for sterilization and release. For *Anopheles* mosquitoes major improvements will need to be made in larval rearing and pupal collection. Both these stages are aquatic and do not lend themselves easily to mass production technology. Maintenance of large numbers of adult mosquitoes for egg production should not present major problems and membrane-feeding systems are already available.

■ *Improvement of sterilization, handling and release methodology.* Radiation procedures will need to be developed to produce sterile male mosquitoes of the right quality. In the past, this has presented some problems and innovative ways are needed to effectively sterilize male pupae or adults. An area-wide SIT programme for mosquitoes will require that sterile insects be dispersed over large areas requiring aerial release. It remains to be seen whether this is possible with fragile insects such as mosquitoes or whether techniques involving the release of sterilized pupae need to be considered.

■ *Design of genetic and molecular methods for the production of male mosquitoes.* Any SIT programme for mosquitoes will require the release of males only to prevent increased transmission by released sterile females, as only females can transmit malaria. In the 1970s genetic sexing systems were developed for many *Anopheles* species using classical Mendelian genetics and experience with the Mediterranean fruit fly, *Ceratitis capitata*, has demonstrated that this approach is feasible for the mass production of male insects. Molecular approaches to develop genetic sexing systems for *Anopheles* mosquitoes are currently under way in many laboratories but as yet no proven techniques have been demonstrated. Without a robust and secure sexing system the use of the SIT would be severely compromised.

■ *Integration of the SIT with other Anopheles control approaches.* SIT is not a stand-alone technology and has to be integrated with other methods of population suppression. Insecticide treated bednets, which are being promoted by WHO, are currently widely deployed for mosquito control and major improvements are being made in their effectiveness and cost. The nets act as a lethal barrier between humans and female *Anopheles* mosquitoes. As this method targets female mosquitoes, it is perfectly compatible with the concurrent release of sterile males so that a target population would be subject to both interventions. Even the use of house spraying is directed to the female mosquito, so here again there is a possibility to integrate the release of sterile males.

Future Directions. The search for genetically manipulated mosquitoes that are unable to transmit malaria, with the purpose of population replacement, has a high priority in many agencies, including the WHO, despite some scepticism from medical entomologists. It is proposed that this possible replacement be accomplished by seeding a target population with a relatively small number of the genetically manipulated mosquitoes, which are then able to actively spread the refractory characteristic through the population.

As yet there is no proven mechanism that will accomplish this replacement and it is conceivable that mass releases of fertile mosquitoes will be required. The rearing and release methods outlined in this article will then become essential.

There are also serious considerations being given to using sterile female mosquitoes to deliver vaccines. Here, too, mass rearing and release technology would be required. ❐