

## MALARIA. NEW TOOLS FOR EPIDEMIOLOGICAL ANALYSIS

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Parasitic diseases are still prevalent in many parts of the world today causing both human suffering and economic loss. Recent developments in biotechnology, including the use of monoclonal antibodies and recombinant DNA, have the potential for providing both more extensive and detailed information on the parasite in the infected human and in insect vectors. New methods of detection, both in man and insect vectors, have been developed for malaria. These new methodologies will be important in epidemiological studies on the prevalence and transmission of these parasitic diseases.

Parasitic diseases are still prevalent in many parts of the world, causing both human suffering and economic loss. Major efforts to control and even eradicate parasitic diseases have met with some success (for example, elimination of malaria from the southern United States, Cuba), but in many developing tropical countries, parasitic diseases remain major health problems. Such diseases can pose a significant barrier to economic development, and their control is an important goal for improved world health. Intensive research is being devoted to the development of new control measures for many parasitic diseases. These control measures include development of vaccines and new chemotherapeutic agents as well as improved vector control strategies. Previous experience has demonstrated the need for extensive baseline information before the introduction of any control program and the need for continued monitoring of the control program in order to assess its effectiveness. These diseases have complex life cycles involving both vectors of transmission and often intermediate hosts, both of which have an impact on the transmission of disease and can affect the outcome of any control measure.

Recent developments in biotechnology, including the use of monoclonal antibodies and recombinant DNA, have provided new tools for the collection of information on these diseases and have the potential for providing both more extensive and detailed information on the parasite in the infected human and in insect vectors. This article focuses on the potential impact of these new methodologies on epidemiological studies of malaria, both in man and insect vectors.

### MALARIA

Human malaria is caused by the four major *Plasmodium* species, *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. In most parts of the world, the prevailing parasite species is *P. falciparum*, which causes the most severe form of the acute disease is often fatal in children (Cohen & Lambert, 1982). *Plasmodium vivax*, the next most prevalent disease is characterized by relapses caused by parasites that remain in the liver in a latent form. The parasite is transmitted by various species of the anopheline mosquito to the human host. The sporozoite, the infectious form of the parasite released from mosquito salivary glands, initiates the exoerythrocytic cycle in the liver. Subsequently developed merozoites invade erythrocytes, and the asexual cycle continues through the course of the infection. A subset of the infected erythrocytes develop into gametocytes, the form can develop in mosquitoes, and results in disease transmission. There is no significant animal reservoir for this disease.

Malaria is among one of the major infectious diseases in the world, with acute clinical malaria affecting some 90 million to 100 million people per year according to the World Health Organization estimates (WHO, 1984a). In addition, there is a large reservoir of chronic infection. The World Health Organization estimates that more than 40 percent of the world's population is

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at risk of malaria infection and that some 365 million people live in areas where malaria endemicity has remained unchanged despite intensive world efforts at malaria eradication (WHO, 1984a).

Elimination of malaria worldwide has proved to be a difficult goal, and thus current efforts are devoted to the control of malaria (WHO, 1979). During the eradication program, several problems arose that will have an impact on any control program. Among these problems is the widespread resistance of anopheline vectors to insecticides (WHO, 1980) and the emergence of *P. falciparum* strains resistant to chloroquine, the primary chemotherapeutic agent, and the subsequent development of multidrug resistant parasite strains (WHO, 1984b). New approaches to control measures include improved conventional methods of vector control and chemotherapy and the development of innovative measures including vaccines for the malaria parasite and biological control of anopheline vectors.

The parameters of malaria transmission and disease prevalence have been studied for the last 40 years in many parts of the world. Mathematical models (McDonald, 1952, 1955; Bruce-Chwatt, 1976; Najera, 1974) of disease transmission have been developed based both on entomological factors and human factors such as immunity [for review see Molineaux & Gramiccia, 1980]. In addition, epidemiological studies have demonstrated an association of malaria prevalence with certain variants in erythrocytes, sickle cell trait (Haldane, 1949; Walker & Bruce-Chwatt, 1956; Fleming, 1979), glucose-6-phosphate dehydrogenase deficiency (Luzzato et al., 1974; Luzzato, 1979), Duffy blood-group antigens (Miller et al., 1976, 1978) and recently, alpha-thalassemia (Flint et al.). These studies have shown that malaria is a complex and dynamic disease that is varied throughout the world. Thus any control program must take into consideration the multiple factors that can affect malaria transmission and must measure these factors in each situation. For this to be achieved, it is imperative that new and efficient means of measuring both entomological and human factors on a large scale be implemented.

**Detection of malaria infection** — Both vaccine and future drug trials will require a sensitive and rapid method for detecting parasites. Currently, malaria infection is determined by the use of a thick smear stained with Giemsa. This method is both specific and sensitive for the diagnosis of malaria but has severe limitations when large numbers of samples must be handled in a timely fashion, as will be case for the collection of baseline data for many of the vaccine trials and chemotherapy studies. A trained microscopist is required for each determination, and this is time-consuming, tiring, and potentially subject to reader bias, especially when large numbers of slides must be read in a short time period. Thus alternative methods for handling large numbers of samples are necessary.

DNA probes specific for human malaria have been developed by several groups (Franzen et al., 1984; Pollack et al., 1985; McLaughlin et al., 1985) and recent work by Barker et al. (1986) has demonstrated that the DNA probe specific for *P. falciparum* can be used to detect malaria infection directly in finger-stick blood of infected patients. The DNA probes specific for *P. falciparum* are dispersed, highly repeated DNA sequences isolated from the *P. falciparum* genome using recombinant DNA technology. Both in laboratory and field testing, the pPF14 probe (Barker et al., 1986) is specific for *P. falciparum* and does not react with *P. vivax*, the other major human malaria. The methodology compares favorably in sensitivity with the routine microscopy, detecting parasite densities as low as 40 parasites per microliter of blood. As can be seen in Table I, the DNA probe method detects *P. falciparum* infection in 129 of 632 patients compared with the 121 detected by routine examination of Giemsa stained thick smears. Subsequent examination of duplicate slides by expert microscopists confirmed the DNA probe diagnosis in those patients missed by routine microscopy. The DNA probe method offers the advantage of a standardized procedure that can be used in a batchwise fashion on large numbers of samples. An important feature of this methodology is that it is reproducible over a large number of samples and should be less subject to reader bias. In addition, in our recent work, we have been able to correlate the intensity of DNA hybridization with parasite density and thus the DNA probes may also provide information on the intensity of infection. One limitation of the correlation of hybridization intensity with parasite density is the potential for variation in the number of repeated target sequences in different *P. falciparum* strains. The DNA probe methodology now must be tested in an epidemiological study to assess its general usefulness.

Methods for the detection of malaria specific antigens or antibodies have also been developed and tested (Machey et al., 1980; Avraham et al., 1982). A major problem with these assays has been the presence of both antigen and antibody after the malaria parasites have disappeared from the bloodstream. Malaria-specific antibody can persist for long periods and this is useful to assay initial infections in naive individuals, especially young children (this is complicated by the presence of maternal antibody) but cannot be used to determine present infection with the

TABLE I

Comparison of DNA/probes specific for *P. falciparum*

Diagnostic Test	No. of patients *
Clinic microscope positive	121
DNA positive	129
Clinic negative	511
DNA negative	503

\*Patient population consists of 632 patients examined at malaria clinics of the Malaria Division of the Thailand Ministry of Public Health in either Bangnamron or Chatiburi, Thailand in July 1985. Blood was collected by digital puncture into a heparinized capillary and treated as previously described (Barker et al., 1986). Malaria thick smears were prepared in the routine manner for diagnosis at the clinic. A separate set of slides, both thin and thick smears, was prepared and subsequently analyzed by malaria experts (Barker et al., 1986)

parasite in individuals previously infected (Avraham et al., 1983). Extensive recent work with monoclonal antibodies has led to the identification of many different malaria antigens, and a major area for research in the future should be in the testing of specific antigens or antibodies and their correlation with active infection or protective immunity.

**Detection of infective mosquito vectors** – An important parameter in malaria transmission is inoculation rate which is the number of infective mosquito bites per unit time. This rate is based both on the man-biting rate of the vector species and the fraction of infective mosquitoes. Zavala et al. (1982) and Collins et al. (1982) have developed an immunological method for the determination of infective mosquitoes that uses a monoclonal antibody specific for the major protein of the malaria sporozoite, the circumsporozoite protein. This method has been field tested and compared with the existing method, which is the capture and dissection of mosquitoes to determine infection. The advantage of this new methodology is that it can determine the species of sporozoite in the infected mosquito. Another advantage is that large numbers of mosquitoes can be tested easily. Thus vectors that have a low rate of infection and have previously been overlooked are now being discovered, and their contribution to malaria transmission is being determined (Arruda et al., 1986). A similar approach should be tried using DNA probes for the detection of malaria infected mosquitoes.

**Determination of human genetic parameters** – Epidemiological studies have demonstrated an association malaria prevalence and certain variants in erythrocytes. Recent advances in the detection of human genetic variants with the use of restriction site polymorphisms of specific DNA fragments should allow more extensive investigation both of these genetically inherited diseases and perhaps, new diseases that have not yet been associated with malaria prevalence. An elegant study by Flint et al. demonstrates the potential of this technology. A single variant of alpha-thalassemia has been correlated with malaria prevalence in Melanesia.

## CONCLUSION

The application of new methods of biotechnology to the epidemiology of malaria is in its initial phases. The new tools offer distinct advantages with regard to specificity, sensitivity, and easy to use in large numbers of samples when compared to existing methodologies and have an enormous potential for their contribution to new knowledge on the transmission and prevalence of this disease. Before these methods are generally accepted for use, they must be extensively tested under field situations and modified to provide the relevant information important for epidemiological analysis.

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