WORKING PAPERS, DISCUSSION
AND SUMMARY

WORKING SESSION V

STRATEGIES FOR FUTURE MALARIA
VACCINE TRIALS II

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METHODS FOR EVALUATION OF THE IMPACT OF VACCINATION IN CONTROL OF MALARIA: INNOVATIVE TECHNIQUES...

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Detection of malaria Infected Mosquitoes

An important parameter describing the epidemiology of malaria is the prevalence of female anopheline mosquitoes infected with malaria sporozoites. This prevalence (sporozoite rate) has been determined by dissection and microscopic observation of the salivary glands of individual mosquitoes which is an extremely time-consuming and laborious technique. Moreover, through microscopic observation, it is impossible to determine the specie of plasmodium infecting the mosquitoes, presenting a serious limitation to this technique.

We have recently reported a new technique, based on monoclonal antibodies, that can be used to determine the prevalence of naturally-infected mosquitoes, as well as their sporozoite load, and which also identifies the plasmodial species (1). In this report, we will briefly review the immunological basis of this immunoassay, recent improvements of the technique, the results of its first field applications and its potential use.

Immunological basis

The two-site immunoassay is performed by using monoclonal antibodies against the circumsporozoite (CS) proteins of malaria sporozoites. As reported elsewhere (2) these monoclonal antibodies (mAB) are species specific. The antigenic determinant, or epitope, which these monoclonal antibodies recognize, seem to be an invariant structure expressed in the CS proteins of a series of strains isolated from many of the endemic areas of the world: namely Southeast Asia, East Africa, West Africa, Central and south America (3). Within the same plasmodial species, all the sporozoites tested thus far show that they share the same antigenic determinant.

The epitope recognized by these monoclonal antibodies are repeated several times within the same molecule (4) allowing the binding of two or more antibody molecules to a single molecule of antigen. This peculiar characteristic of the CS proteins was used to standardize a two-site immunoassay. This assay consists of the binding of sporozoite antigen to immobilized monoclonal antibody, and the detection of this bound antigen by using a radioisotope or enzyme-lableld monoclonal antibody. An important feature of this assay is that it can be performed with dried mosquitoes, since the antigenic reactivity of the CS proteins remains stable for at least four months.

The monoclonal antibodies can be labeled with $^{125}$I for the two-site immunoradiometric assay (two-site IRMA) (5) or with horse-radish peroxidase for the two-site enzyme-linked immunosorbent assay (two-site ELISA) (5). When both assays were performed simultaneously, using the same batch of sporozoite antigen, the results we obtained were quite similar. However, some differences have been observed. The IRMA can detect as few as 40 sporozoites while the limit of the ELISA seems to be around
100 - 150 sporozoites. On the other hand, the ELISA assay seems to have less discriminatory power and tends to reach saturation with a smaller amount of parasites. In spite of these differences, both assays are sensitive enough to be used in the field or with experimentally infected mosquitoes.

Field Application

The two-site IRMA was recently subjected to a field trial in The Gambia (West Africa)(6). Anopheles mosquitoes were caught in different villages of this endemic area, and the prevalence of naturally-infected vectors was determined by both microscopic observation of salivary glands and the immunoassay. By dissection, a sporozoite rate of 5.5% was obtained. However, using the IRMA, this rate was determined as being 7.3%. The higher rate obtained with the immunoassay may be due to the detection of CS protein in sporozoites present in the mosquito midgut, which expresses low amounts of this antigen 24 to 48 hours before migrating to the salivary gland. The IRMA also allowed an estimation of the sporozoite load per mosquito, revealing that more than 70% of the anopheles had between 500 to 10,000 sporozoites. The maximum load detected was around 100,000 parasites per mosquito. The figures on sporozoite load we obtained were strikingly similar to those obtained in a study performed by dissection techniques, in East Africa, several years ago (7).

Our overall results indicate that the IRMA was sensitive enough to detect naturally-infected vectors, and to estimate their sporozoite load in addition to determining the species of parasites.

Potential Applications

The determination of the prevalence of infected mosquitoes and of their sporozoite load are important parameters for entomological and epidemiological studies, since it permits investigation of the relationship between inoculation and incidence rate, the two direct measures of transmission.

Vectorial capacity is another important aspect that needs to be studied since in several endemic areas these vectors have still not been clearly determined. In fact, very little is known on the vectorial capacities of different species of anophelines, or about their relative local and seasonal importance in transmission of different species of human malaria.

It should be pointed out, that by using this technique, a single mosquito can be screened simultaneously with different mAb. In this way a more comprehensive picture on the relationship between different vectors and different malaria species can be easily obtained. This information can become useful for the design or evaluation of mosquito eradication programs or other such programs directed toward malaria transmission blocking.
Experimental research on basic biological aspects of the vector-parasite relationship should also benefit by the use of this technique.
Detection of anti-sporozoite antibodies

We developed an immunoradiometric assay using as antigen the synthetic peptide H-(Asn-Ala-Asn-Pro)-OH [(NANP)₃] to study the specificity of antibodies to P. falciparum sporozoites found in the sera from humans living in an endemic area (The Gambia, West Africa). This peptide represents the repeated epitope of the circumsporozoite (CS) protein P. falciparum. The same human sera were tested by indirect immunofluorescence (IFA) using glutaraldehyde-fixed sporozoites, a method which reveals antibodies to the parasite surface membrane. We found a highly significant positive correlation (rₛ=0.087p<0.001) between the results of the two assays, and showed that most antibodies detected by IFA were in fact anti-(NANP)₃, since the IFA of every serum was strongly inhibited by the synthetic peptide. The proportion of sera containing antibodies to (NANP)₃ was age-dependent, and reached 84% in samples from adults older than 34 years who are known to be more resistant to malaria infection than younger individuals.

The findings highlight the immunodominance of the repetitive epitope of the CS proteins in man, and confirm previous similar laboratory findings in experimental malaria systems [4]. They also demonstrate the presence in humans of a B-cell repertoire recognizing the P. falciparum repeat, which can potentially respond to a synthetic peptide vaccine containing this epitope. Because of adults living in the endemic areas are already primed for the (NANP)₃ epitope, a synthetic peptide vaccine may boost their natural immunity and perhaps increase their resistance to malaria infection. On the other hand, it is conceivable that inoculation of sporozoites by mosquitoes can also boost the immune response of individuals primed by a synthetic peptide vaccine.

One question raised by these and previous findings is whether the increasing levels of antisporozoite antibodies in the serum of adults and the well-known development of resistance to malaria with age are causally related. Epidemiological studies using the IRMA with (NANP)₃ as antigen may help to clarify this important issue, since it is now possible to correlate more precisely the risk of acquiring malaria during the seasonal transmission of the disease, with the individual levels of antibodies to (NANP)₃. Such studies were never attempted in the past because of the difficulty in obtaining sufficient numbers of sporozoites to perform IFA reactions and because of the uncertainties as to the nature and specificity of the parasite antigens recognized by the serum antibodies.

Finally, it should be pointed out that P. falciparum malaria vaccines, produced either by recombinant DNA techniques or containing synthetic peptides, are likely to be tested in the near future. The precise measurement of levels of antisporozoite antibodies in endemic areas, immediately before and following the immunization, will be necessary to evaluate and compare different vaccine preparations, to determine the effects of dosage,
adjuvants, etc. The immunoradiometric assay here described seems ideally suited for this purpose provided that the candidate vaccines contain the *P. falciparum* CS protein or the repetitive epitope.
REFERENCES


ENTOMOLOGICAL TECHNIQUES TO ASSESS THE EFFICACY OF A MALARIA VACCINE TRIAL IN A COMMUNITY

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* Paper may be included as such in the final publication *
If and when a malaria vaccine fulfills the criteria of safety and efficacy in clinical trials, it will have to undergo phase III trials in natural populations where its efficacy would be tested by entomological, parasitological and immunological techniques.

**Entomological parameters to be considered**

A successful field trial of an effective malaria vaccine should decrease significantly or possibly cease the transmission of malaria in the particular locality, which may be indicated by the absence of plasmodial infections in vectors during the usual transmission season. But success of a vaccination program would not be proved unless there is an evidence of active transmission in a comparable 'control' locality during the same season. However, malaria transmission potential may inherently be different in any two localities because of the local ecological conditions. It is a common observation that malaria transmission is usually focal in nature because transmission potential varies in different localities. Therefore, vectorial capacities of vectors in the two localities (trial-locality and control locality) have to be considered. The vectorial capacity (VC) defines the average number of potentially infective bites that will ultimately be delivered by the vectors feeding upon a single host in one day. In short, it is a daily rate of potentially infective contacts caused by the vectors. The total vectorial capacity at a given time and place is the sum of the corresponding vectorial capacities of all the vector species, and reflects the transmission potential in the area. The vectorial capacity is a useful parameter which, depending upon the situation, may be used to:
- compare the transmission potential in different areas;
- compare the transmission potential of various species in an area;
- identify the peak transmission season in a locality.

Vectorial capacity is a characteristic of a mosquito population related to the ecological conditions, and may be assessed irrespective of whether any cases of malaria are present at the given place and time. A decrease in sporozoite rates in a vaccine-trial locality relative to a control locality can be interpreted as the vaccine effect only after adjustment for vectorial capacities in the two localities.

Detection of sporozoites in mosquitoes has been facilitated by a recently developed method based on immunological techniques (Zavala et al, 1982; Burkot et al, 1984). But the classical method for estimation of vectorial capacity (VC) of a mosquito species is not satisfactory. The classical method is based on the assumption that survival/mortality of mosquitoes is not related to age. Data gathered in the present study indicated that the probability of daily survival in mosquitoes decreases with age, rather than being constant as thought by earlier workers. Based on this revised concept, a new method has been proposed to estimate survivorship in a natural population and thereby the malaria vectorial capacity of a species.

Mortality pattern of mosquitoes

Review of the relevant literature indicates that there is a difference of opinion as to whether survivorship or death rate in a mosquito population is independent of age or varies with age. The classical experiment of Russell and Rao (1942), on An. culicifacies under semi-natural conditions in Madras (India), constitutes the main argument
in favour of a constant death rate hypothesis. Macdonald (1952) recognized some of the limitations of their experiment but remained convinced about the constancy of survivorship or death rate over age, an assumption which formed the central column of his extensive quantitative epidemiological analyses and mathematical models. On the other hand, a number of workers have found evidence indicating that the probability of survival in mosquitoes decreases with age; the phenomenon has been noticed in a number of species including: An. gambiae Giles (Gillies and Wilkes, 1965), An. stephensi (Reisen and Aslánkhan, 1979), An. culicifacies, An. stephensi (Reisen and Mahmood, 1980), and Cx. quinquefasciatus (Suleman and Reisen, 1979).

An effort has been made in the present study to sort out the long standing controversy concerning the mortality pattern, and if necessary, the existing procedures for estimating survivorship and vectorial capacity of mosquitoes be revised accordingly.

The survivorship or mortality pattern of Anopheles females was studied under two different sets of food conditions: (i) blood feeding without sugar solution and (ii) blood feeding with sugar solution.

Females feeding exclusively on blood exhibited a pattern of constant survivorship/mortality rate with respect to age. The survivorship curve followed a curvilinear regression pattern — the population declined along a geometric progression. The mortality pattern was similar to the one noticed by Russell and Rao (1942), which needed logarithmic transformation for make it linear. On the other hand, females feeding on blood plus supplementary food (sugar solution) exhibited a mortality rate that increased as a linear function of age (Figs. 1 & 2).
Fig. 1. Survivorship curves (lx vs. age) of An. stephensi females, provided with a source of blood-meal and sugar solution, under semi-natural conditions in the study village. Broken lines represent linear regression, and the vertical bars represent 95% confidence intervals. lx' represents expected curve if probability of daily survival (P) were constant with respect to age. (P was calculated by back-transformation of the slope of the regression of log(lx) on age.)
Fig. 2. Survivorship curves (lx vs. age) of *An. culicifacies* females, provided with a source of blood meal and sugar solution, under semi-natural conditions in the study village. Broken lines represent linear regression and the vertical bars represent 95% confidence intervals. lx' represents expected curve if probability of daily survival (P) were constant. (P was calculated by back-transform-
Both the test species (An. culicifacies and An. stephensi) exhibited similar patterns of mortality curve. The survivorship curve followed a linear regression pattern, implying that the probability of survival decreased with age. The comparison of the data in the two experiments clearly demonstrated that the survivorship or mortality pattern of mosquitoes was influenced by the type of food consumed.

These results are in agreement with Leclercq (1969) who stated that: "most of the biting flies, when they are fed exclusively on blood, rapidly die of thirst. They must always complete their blood meal by drinking water derived from various sources, by sucking in animal secretions (plant lice, Coccids) or water of vegetable origin (plant juices, the nectar of flowers). Examples of blood sucking insects which rob flowers are numerous notably among the Ceratopogonidae, the Culicidae, the Simulidae, the Rhagionidae, the Tabanidae and the Muscidae (Stomoxys)."

Clements (1963) also emphasized the requirement of plant juices and nectar as supplementary food for mosquito females.

A constant survival or mortality rate in An. culicifacies observed by Russell and Rao (1942) could be due to either or both of the following reasons: (1) obvious difficulties in catching hundreds of mosquitoes individually by inverting a test tube on each mosquito, a limitation already pointed out by Macdonald (1952), where with a decrease in population, catchability was likely to be improved toward the end of the cohort life; (ii) mosquitoes were perhaps not served adequately with a supplementary food since a few petri dishes with sugar solution in a vast field cage (40 x 20 x 10 feet) might not be enough.
Because of this conceptual change in the understanding of the mortality pattern of mosquitoes, the existing procedure for estimating probability of survival and expectancy of life over time (Macdonald, 1952) has to be revised according to the following expressions, which apply because of the linear relationship between $l_x$ and age (days):

Maximum life or age of individuals in a cohort = $1/b$ days, where $b$ is a negative slope of the regression.

When $l_0 = 1$ (i.e., when survivorship is taken as proportions), then:
- Life expectancy at the beginning of a cohort: $e_0 = 1/2b$;
- Proportion surviving through 'n' days: $l_n = 1 - bn$;
- Life expectancy after surviving thru 'n' days: $e_n = (1-bn)/2b$.

(derivation of the above expressions is illustrated in appendix-I).

Estimation of Age and Survivorship of Mosquitoes in Nature

The epidemiological significance of the adult longevity of a malaria vector can hardly be overemphasized. But correct estimation of age and survivorship in a natural population of mosquitoes is by no means an easy task. A number of indirect methods have been proposed, but each has some limitations (Suleman, 1985).

The direct method of Polovodova (Detinova, 1962) was adopted in the present study because it seems to have the potential of providing a fairly reliable estimate of the age-structure of a population at a given point in time, though it is a time consuming and laborious technique requiring numerous dissections and countings of ovariolar dilatations. This method involves the direct recording of the number of ovarian cycles (gonotrophic cycles) undergone by the individual female. The
number of gonotrophic cycles completed by the female is really a measure of its physiological age, which can easily be translated into calendar age by relating to the durations of successive gonotrophic cycles in days.

The procedure is illustrated using *Ae. culicifacies* data collected in a rural locality in Punjab during 1982-83. A representative sample of females dissected each month was classified by the number of ovariolar dilatations (gonotrophic cycles) resulting in a frequency distribution according to physiological age (Table 1). The frequencies were accumulated following a cohort approach, assuming that all age-groups were representatively sampled and the population age-structure remained relatively constant over a month (i.e. additions and losses were approximately equal). It is assumed that mortality during the first two days of adult life in the warm season, and the first three days in cold season, was negligible. Such an assumption facilitates the computational process without much loss of accuracy in results, as it is in agreement with direct observations on survivorship under semi-natural conditions (Figs. 1 & 2). To each dilatation class was assigned a chronological age corresponding to the mid-gonotrophic period in days ($X_1$ & $X_2$). The procedure for estimating age at mid gonotrophic periods is given in the footnote of Table 1.

Two alternate estimates of survivorship were made: 1) median age and constant daily loss in population, considering that survivorship decreases as a linear function of age; and 2) probability of daily survival ($p$), assuming that it remains constant over age.
The observations and results are summarized in Table 1, which consists of four blocks as follows:

- The first block contains the frequency of females dissected for age determination, by month and number of ovariolar dilatations observed. In other words, this block summarizes the monthly frequency distribution of females, by physiological age.

- The second block shows cumulative frequencies ($l_x$) classified by month, number of dilatations and the corresponding mid gonotrophic periods ($X_i$). In other words, this block summarizes the monthly cumulative frequency distribution, by physiological age as well as calendar age, on a cohort basis.

- The third block consists of results on monthly estimates of survivorship ($S_{d1}$) of the species, as obtained by linear regression of ($l_x$)$_i$ on ($X_i$)$_i$, where ($l_x$)$_i$ = the proportion surviving, and $X_i = X_1$ for warm months (Apr.- Oct.) and $X_2$ for cold months (Nov.-Jan.). Included in this block are the regression slope ($b_1$), coefficient of determination ($r^2$)$_1$ and median age ($0.5/b_1$ or $1/2b$). Median age (which equals mean age in this case) is the principal estimator of survivorship, and here survivorship is considered to vary as a linear function of age.

- The fourth block contains results on monthly estimates of survivorship ($S_{d2}$), if the probability of survival is assumed to be constant with respect to age, as obtained by curvilinear regression between the same variables (i.e., regression of $y_i$ on $X_i$, where $y = \log_e(l_x)$ and $X = X_1$ or $X_2$, depending on which month is being considered). Included in this block are the regression
Table 1. Age-grading and survivorship of females of *An. culicifacies* by month based on physiological age structure judged from number of ovariolar dialatations in representative group of females collected and dissected at fortnightly intervals.

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Cumulative frequencies (Lx) by No. dialatations and mid gonotrophic period in days (X)\(a\)

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Survivorship \((Sd_1)\)\(^b\)

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Survivorship \((Sd_2)\)\(^c\)

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Footnotes to Table 1

a X = chronological age at mid-gonotrophic period in days corresponding to the number of ovariolar dilatations/ovipositions. Gonotrophic cycle (gc) varies with temperature, and the first gc is longer than the subsequent ones. During the warm season (Apr.-Oct.) the duration of the first gc is on the average 4 days and each subsequent cycle takes approximately 2 days; therefore, the calendar age at mid-gonotrophic period is 2, 5, 7, ... days corresponding to ovariolar dilatations (gc's) 0, 1, 2, ... and so on. In the winter (Nov.-Jan.) the first gc takes about 6 days and each subsequent cycle approximately 3 days. Therefore the age at the mid-gonotrophic period is 3, 7.5, 10.5, ... days for ovariolar dilatation (gc's) 0, 1, 2, ... and so on. (e.g., Mahmood and Reisen, 1981; Reisen et al, 1982; Afridi et al, 1940; and Sulenan, 1985). X = X₁ for the period Apr.-Oct. and X = X₂ for the period Nov.-Jan.

b Estimation of survivorship considering (P) not constant, but decreasing with age, using linear regression of yi on xi; where yi = (lx₀)i, taken as proportions, and b₁ = slope of regression; (r²)₁ = coefficient of determination. Slope (b₁) which is constant over age is a measure of daily decrease in population and its reciprocal (1/b₁) gives the maximum life of females in a cohort and the expression (0.5/b₁) or (1/2b) gives a measure of median age of a cohort.

c Estimation of survivorship if it is assumed that the probability of daily survival (P) remains constant over age (a popular conventional assumption, first made by Macdonald in 1952, by applying linear regression of yi on xi where yi = [loge(1x+1)]i and xi as defined above. Probability of daily survival (P) was calculated by back-transformation of slope (b₂) i.e. e⁻ᵇ₂ ; (r²)₂ = coefficient of determination.
slope ($b_2$), coefficient of determination ($r^2$) and probability of daily survival ($P$). The value of ($P$) is derived by back-transformation (into the original scale) of the slope i.e. $P = e^b$, where $e$ = base of natural logarithms and $b$ = slope of fitted regression equation.

Although the present study does not support the assumption of a constant probability of survival over age, it is included here for the purpose of comparison, in order to analyze how it would influence the estimates of malaria vectorial capacity of a species. The method adopted in this study to estimate the probability of survival ($p$) involves an improvement upon the one used by earlier workers (e.g., Reisen et al., 1982; Mahmood and Reisen, 1981) in that the regression analysis is based on cumulative frequencies, instead of original frequencies, a procedure to be desired in a cohort approach.

**Vectorial Capacity estimates**

Monthly estimates of the vectorial capacity ($VC$) of *An. culicifacies* were made, using two alternative methods as follows:

\[
VC_1 = \frac{ma^2 (1 - bn) (0.5)}{b} = \frac{ma^2 (1 - bn)}{2b} \\
VC_2 = \frac{ma^2 p^n}{-\log_e p} 
\]

New approach proposed in this study.

Commonly used formula, after Garrett-Jones (1964).

Estimation procedures for $VC_1$ and $VC_2$ differ only with respect to the survivorship/mortality pattern of mosquitoes. The procedure for $VC_1$ has been developed in the present study, considering that the probability of survival in a natural population of mosquitoes decreases with age. The
procedure for VC\textsubscript{2} is borrowed from Garrett-Jones (1964), who extracted it from Macdonald's basic reproduction rate (1952), and is based on the assumption that the probability of survival does not change with age. The individual variables were estimated as follows:

\[ m = \text{No. female mosquitoes/person} - \text{based on mosquito population estimates by pyrethrum spray method, and human population by census).} \]

\[ a = \frac{\text{HBI}}{\text{gc}}, \text{ where HBI = human blood index (anthropophagic index) estimated by precipitin tests, and gc = duration of gonotrophic cycle in days.} \]

\[ n = \text{Duration of sporogonic cycle for } P. \text{ vivax, extracted from Macdonald's temperature vs. extrinsic incubation period curve (1957), against the average figure of mean daily temperature records maintained in the study village.} \]

\[ b = \text{Constant daily decrease in population} = b_1 \text{ in Table 1).} \]

\[ P = \text{Constant probability of survival for one day} = P \text{ in Table 1).} \]

The component \( (1-bn)/2b \) for VC\textsubscript{1}, and \( P^n/-\log_e P \) for VC\textsubscript{2} are the two alternative estimates for expectation of life after surviving through 'n' days, as discussed earlier.

Estimates of the vectorial capacity in this study are made in terms of \( P. \text{ vivax} \) only; there is no need to give a parallel series of VC estimates for \( P. \text{ falciparum} \), because estimates for one species would serve as a base for the other. Naturally, the vectorial capacity (VC) estimates in terms of \( P. \text{ falciparum} \) would be slightly on the lower side (sporogonic cycle of \( P. \text{ falciparum} \) being slightly longer than that of \( P. \text{ vivax} \)) but the differences, at all levels, would be in the same direction and proportionate in magnitude to those of \( P. \text{ vivax} \) estimates.
The two alternative estimates of vectorial capacity (VC₁ and VC₂) of *An. culicifacies* by month, along with the component variables, are set out in Table 2. Similarly, vectorial capacities for the remaining four species of the common anophelines in Punjab were also estimated, but only the summary information is given here for the sake of brevity (Table 3). For all the species, except *An. annularis*, VC₁ estimates came out higher than VC₂ estimates. A comparison of VC₁ and VC₂ in different species, considering both the monthly as well as the yearly total estimates, indicates that the two series of estimates differ more widely in *An. annularis*, *An. pulcherrimus* and *An. subpictus* than in *An. culicifacies* and *An. stephensi*. These results are in line with the relationships between the two sets of monthly survivorship estimates in different species on which the VC estimates are based.

The traditional method for estimation of vectorial capacity (VC₂) gave a positive value even when the mosquito density in relation to man (m) was very small, except in very cold months when 'n' became excessively high. In other words, this method gives relatively less weight to the factor of mosquito density (m) as compared to survivorship, with the result that monthly estimates of VC₂ do not reflect (m) to the extent it is reflected in estimates obtained by the alternative procedure (VC₁). Because of the underlying assumption of a constant daily survival rate (P), and thereby an exponential relationship between survival and age, a small fraction of a mosquito population is always thought to survive beyond 'n' days, irrespective of environmental conditions. The traditional method gave a positive value
Table 2. Vectorial capacity (VC) of *An. culicifacies* by month, estimated using two alternative estimates of survival rate of females: VC<sub>1</sub>, using survivorship estimated as a linear function of age expressed by a constant daily decrease in population (b<sub>1</sub> in Table 1); and VC<sub>2</sub>, using survivorship estimated as an exponential function of age expressed as a constant probability of daily survival (P in Table 1).

<table>
<thead>
<tr>
<th>Month</th>
<th>m</th>
<th>gc</th>
<th>HBI</th>
<th>a (x10&lt;sup&gt;-3&lt;/sup&gt;)</th>
<th>temp. (°C)</th>
<th>n</th>
<th>b&lt;sub&gt;1&lt;/sub&gt; (-)</th>
<th>P</th>
<th>VC&lt;sub&gt;1&lt;/sub&gt; (x10&lt;sup&gt;-3&lt;/sup&gt;)</th>
<th>VC&lt;sub&gt;2&lt;/sub&gt; (x10&lt;sup&gt;-3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar.</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Apr.</td>
<td>0.13</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.0039</td>
<td>26.4</td>
<td>9.0</td>
<td>.154</td>
<td>.71</td>
<td>0.000</td>
</tr>
<tr>
<td>May</td>
<td>0.57</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.0172</td>
<td>30.9</td>
<td>7.0</td>
<td>.090</td>
<td>.74</td>
<td>0.035</td>
</tr>
<tr>
<td>Jun.</td>
<td>1.12</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.0339</td>
<td>33.4</td>
<td>6.4</td>
<td>.157</td>
<td>.61</td>
<td>0.000</td>
</tr>
<tr>
<td>Jul.</td>
<td>5.91</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.1788</td>
<td>32.3</td>
<td>6.9</td>
<td>.150</td>
<td>.60</td>
<td>0.000</td>
</tr>
<tr>
<td>Aug.</td>
<td>10.11</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.3058</td>
<td>31.4</td>
<td>7.0</td>
<td>.129</td>
<td>.64</td>
<td>0.115</td>
</tr>
<tr>
<td>Sep.</td>
<td>6.01</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.1818</td>
<td>30.2</td>
<td>7.3</td>
<td>.110</td>
<td>.67</td>
<td>0.163</td>
</tr>
<tr>
<td>Oct.</td>
<td>0.83</td>
<td>2</td>
<td>0.011</td>
<td>0.0055</td>
<td>0.0251</td>
<td>26.1</td>
<td>9.2</td>
<td>.067</td>
<td>.74</td>
<td>0.072</td>
</tr>
<tr>
<td>Nov.</td>
<td>5.03</td>
<td>3</td>
<td>0.011</td>
<td>0.0037</td>
<td>0.0676</td>
<td>22.8</td>
<td>12.5</td>
<td>.059</td>
<td>.82</td>
<td>0.150</td>
</tr>
<tr>
<td>Dec.</td>
<td>1.58</td>
<td>3</td>
<td>0.011</td>
<td>0.0037</td>
<td>0.0212</td>
<td>19.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20</td>
<td>.046</td>
<td>.85</td>
<td>0.018</td>
</tr>
<tr>
<td>Jan.</td>
<td>0.65</td>
<td>3</td>
<td>0.011</td>
<td>0.0037</td>
<td>0.0087</td>
<td>15.5</td>
<td>&gt;40</td>
<td>.042</td>
<td>.87</td>
<td>0.000</td>
</tr>
<tr>
<td>Feb.</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<sup>f</sup> = mean for the first fortnight only.

m = No. female mosquitoes/person.
gc = duration of gonotrophic cycle (= frequency of biting).
HBI = human blood index (anthropophagic index).
a = (HBI/gc) (= frequency of human biting).
temp. = mean daily temperature (average of the mean daily maximum and the mean daily minimum).
n = duration of sporogonic cycle in days for *P. vivax*. Thus vectorial capacity of various anophelines has been estimated in terms of *P. vivax*.
b<sub>1</sub> = daily decrease in population of females (from Table 1).
P = probability of daily survival (from Table 1).

\[
VC_1 = \frac{ma^2 (1-bn)}{b^{0.5}} = \frac{ma^2 (1-bn)}{2b} \quad VC_2 = \frac{ma^2 P^n}{-\log_e P}
\]
Table 3. Summary of the vectorial capacity estimate ($10^{-4}$) of the common anophelines, by species, month and method of estimation ($VC_1$, when probability of survival is considered to decrease with age and $VC_2$, if probability of daily survival is assumed to be constant)*.

<table>
<thead>
<tr>
<th>Month</th>
<th>An. annularis</th>
<th>An. culicifacies</th>
<th>An. pulcherrimus</th>
<th>An. stephensi</th>
<th>An. subpictus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$VC_1$</td>
<td>$VC_2$</td>
<td>$VC_1$</td>
<td>$VC_2$</td>
<td>$VC_1$</td>
<td>$VC_2$</td>
</tr>
<tr>
<td>Mar.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Apr.</td>
<td>0.00</td>
<td>0.07</td>
<td>0.00</td>
<td>0.01</td>
<td>0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>May</td>
<td>0.00</td>
<td>1.11</td>
<td>0.35</td>
<td>0.07</td>
<td>2.60</td>
<td>3.35</td>
</tr>
<tr>
<td>Jun.</td>
<td>0.00</td>
<td>2.08</td>
<td>0.00</td>
<td>0.03</td>
<td>1.57</td>
<td>1.83</td>
</tr>
<tr>
<td>Jul.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
<td>0.95</td>
<td>3.74</td>
</tr>
<tr>
<td>Aug.</td>
<td>0.29</td>
<td>3.47</td>
<td>1.15</td>
<td>0.30</td>
<td>0.00</td>
<td>0.59</td>
</tr>
<tr>
<td>Sep.</td>
<td>0.55</td>
<td>2.88</td>
<td>1.63</td>
<td>0.24</td>
<td>23.47</td>
<td>5.74</td>
</tr>
<tr>
<td>Oct.</td>
<td>0.00</td>
<td>0.46</td>
<td>0.72</td>
<td>0.05</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>Nov.</td>
<td>0.00</td>
<td>0.07</td>
<td>1.50</td>
<td>0.29</td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Dec.</td>
<td>0.00</td>
<td>0.03</td>
<td>0.18</td>
<td>0.05</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Jan.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Feb.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>0.84</td>
<td>10.17</td>
<td>5.53</td>
<td>1.14</td>
<td>28.59</td>
<td>15.86</td>
</tr>
</tbody>
</table>

Ratio
$VC_1/VC_2$ 0.08 4.85 1.80 1.56 1.03 1.29

Relative vectorial capacity of the species

<table>
<thead>
<tr>
<th>$VC_1$</th>
<th>2.15</th>
<th>14.18</th>
<th>73.31</th>
<th>3.59</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>$VC_2$</td>
<td>26.76</td>
<td>3.00</td>
<td>41.74</td>
<td>2.37</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* figures for $VC_1$ and $VC_2$ are taken from Table 2, transformed to $10^{-4}$. 


for VC even under extreme dry-hot conditions like the one prevailing during June-July. For these reasons, monthly VC estimates over the year followed a distribution with a depressed peak and a broad base, an effect which makes identification of the active transmission season relatively difficult. Under the traditional method it is taken for granted that a small fraction of the population will always survive through 'n' days, no matter how severe environmental conditions may be.

Moreover, small fractions of small populations (e.g., in March-April or in June-July), which may survive for a period longer than the length of the extrinsic incubation period in that particular season, can hardly be of any epidemiological significance, especially when the vector species under consideration are predominantly zoophagic in habits. Therefore this method tends to overestimate the VC during any period with severe climatic conditions and tends to underestimate it during any period with suitable environmental conditions.

On the other hand, the vectorial capacity estimates according to the new approach proposed in this study (VC₁) are based on proportionate weights for mosquito density and survivorship. The transmission potential of a species (VC₁) therefore has a positive value only when its density is relatively high and survivorship is above a certain threshold level, determined by the duration of sporogonic cycle in that particular season. VC₁ estimates did not give a positive value for any species during the hot-dry summer season (June-July) when survivorship was too low, or during the cold winter season (Jan.-Mar.) when mosquito density was low and survivorship threshold high. This procedure does not assume that a part of the population is going to survive beyond the
length of the extrinsic incubation period regardless of climatic conditions. For these reasons, estimates of vectorial capacity ($V_{C1}$) followed a seasonal pattern with a well marked peak and narrow base, an effect which corresponds very well to the seasonal pattern of malaria transmission in the Punjab.

It is well known fact that malaria transmission in Punjab is a seasonal phenomenon. Therefore, those VC estimates will be more reliable which would better correspond to the seasonal pattern of malaria incidence in the area. Relative merit of the two methods may be judged on this basis.

It seems appropriate to concentrate on *An. culicifacies*, which is a confirmed malaria vector in rural Punjab, in order to check the relative merits of the two alternative procedures for estimating vectorial capacity ($V_{C1}$ and $V_{C2}$). Monthly estimates of $V_{C1}$ gave low level positive values in May and higher values from August through December. $V_{C2}$ estimates gave low level positive values throughout the year, except during January through March, and no clues as to peak transmission season (Table 3). According to $V_{C1}$, this species is capable of transmitting malaria to a slight extent during late spring (May) and to a greater extent during the postmonsoon and autumn (August-December) but has zero potential of transmission during the hot summer (June-July) and cold winter or early spring (January-April). These observations are in full agreement with previous reports, based on monthly mortality and morbidity records (e.g., Swaroop, 1949) and monthly parasitological observations on infants (e.g., Marshall, 1962) regarding the malaria transmission season in Punjab. Thus the present study has provided the
entomological evidence and mechanisms to explain earlier, non-entomological findings (e.g., Swaroop, 1949; Mashall, 1962) concerning the seasonality of malaria transmission in Punjab.

Monthly estimates of malaria vectorial capacity by revised method but not by the classical method (Table 3) correlate well with seasonal incidence of malaria in the Punjab as determined by parasitological observations (Table 4). Data shown in Table 4 reflects the monthly incidence of malaria. The years 1969 and 1979 where all age-groups are included, were selected because in both of these years malaria was resurging after remaining at negligible low levels for a few years, whereby most of the cases (even vivax cases) were most probably primary infections since relapses must have died out by that time.

Similarly, high VC2 (but not VC1) estimates for An. annularis seem to be misleading because they are not consistent with previous information on the species, as this mosquito is not included in the list of confirmed or suspected vectors in the region (WHO, 1977).

Based on these evidences, it may be concluded that the revised method for estimation of vectorial capacity (VC1) provides more logical and reliable estimates than the classical method (VC2).
Table 4. Prevalence of malaria in Sheikhupura district, as recorded by the district malaria control department, Sheikhupura, by month (pv = Plasmodium vivax; pf = P. falciparum; Exam. = No. examined; +ve = No. positive; PR % = parasite rate %)

<table>
<thead>
<tr>
<th>Months</th>
<th>1982 Infants (pv + pf)</th>
<th>1981 All ages (pf)</th>
<th>1982 All ages (pf)</th>
<th>1969 All ages (pv + pf)</th>
<th>1979 All ages (pv + pf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exam.</td>
<td>+ve</td>
<td>PR %</td>
<td>Exam.</td>
<td>+ve</td>
</tr>
<tr>
<td>Jan.</td>
<td>25</td>
<td>1</td>
<td>4.00</td>
<td>4418</td>
<td>2</td>
</tr>
<tr>
<td>Feb.</td>
<td>22</td>
<td>0</td>
<td>0.00</td>
<td>4561</td>
<td>0</td>
</tr>
<tr>
<td>Mar.</td>
<td>66</td>
<td>0</td>
<td>0.00</td>
<td>4716</td>
<td>3</td>
</tr>
<tr>
<td>Apr.</td>
<td>352</td>
<td>1</td>
<td>0.28</td>
<td>6180</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>275</td>
<td>3</td>
<td>1.14</td>
<td>6154</td>
<td>0</td>
</tr>
<tr>
<td>Jun.</td>
<td>60</td>
<td>2</td>
<td>3.33</td>
<td>3287</td>
<td>0</td>
</tr>
<tr>
<td>Jul.</td>
<td>35</td>
<td>2</td>
<td>5.71</td>
<td>3532</td>
<td>0</td>
</tr>
<tr>
<td>Aug.</td>
<td>61</td>
<td>3</td>
<td>4.92</td>
<td>3594</td>
<td>7</td>
</tr>
<tr>
<td>Sep.</td>
<td>113</td>
<td>13</td>
<td>11.50</td>
<td>12064</td>
<td>45</td>
</tr>
<tr>
<td>Oct.</td>
<td>276</td>
<td>27</td>
<td>9.78</td>
<td>8927</td>
<td>60</td>
</tr>
<tr>
<td>Nov.</td>
<td>218</td>
<td>21</td>
<td>9.63</td>
<td>5757</td>
<td>49</td>
</tr>
<tr>
<td>Dec.</td>
<td>58</td>
<td>10</td>
<td>17.20</td>
<td>5685</td>
<td>18</td>
</tr>
</tbody>
</table>

Total 1561 84 5.38 70875 184 0.26 77833 1103 1.42 117709 2821 2.39 61475 1130 1.84

The reasons for looking into monthly estimates of both vivax and falciparum malaria in all ages selectively for the years 1969 and 1979 are given in the text.

Assumption: Monthly prevalence of malaria, under the given set of conditions, followed active transmission.

Conclusion: Rise in prevalence rate from September through December indicates that active transmission starts somewhere in August and continues up to mid-December.
Consideration of vectorial capacities along with the sporozoite rates is necessary for a reliable entomological assessment of the efficacy of a malaria vaccine trial. Monitoring sporozoite rates alone would not be enough.

The revised method for estimation of vectorial capacity seems to be a significant improvement upon the classical method, and would thus provide a better assessment of the malaria transmission potential in a community.

Quantitative assessment of the component variables for malaria vectorial capacity is a laborious process, which involves monitoring several variables at regular intervals by trained persons. But experience shows that two persons can handle the job in an average village of about 1500 population.

Entomological data (vectorial capacity estimates & sporozoite rates) along with parasitological data, from a vaccine-trial locality and a control locality, will hopefully lead to a fairly accurate judgement about the effect of the vaccine trial on malaria transmission.

Identification and quantification of the vaccine effect would be difficult where other control measures (e.g. insecticides) are also being used at the same time.
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158 pp. (statement from p 44).


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Giles and Anopheles stephensi Liston, with observations on
reproductive activity and survivorship during winter in
*Pakistan J. Hlth., 12*: 134-141.


Swaroop, S. (1949).

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Revised Formula for Expectation of Life after 'n' days and VC

Given a linear relationship between $l_x$ and age, and using a cohort approach, the simple linear model ($y = a + bx$) may be applied to estimate the proportion surviving through 'n' days and expectation of life after surviving through 'n' days. The procedure is illustrated below:

Maximum life in a cohort = $1/b$ days ($b$ being a negative slope).
Expectation of life at the beginning of a cohort (adult emergence) ($e_0 = T_0/l_0$ ; i.e. total time lived/ No. in the beginning) = $1/2b$ because $T_0 = (1/b)(1)(1/2)$ and $l_0 = 1$

Proportion living through 'n' days = $1-bn$
(because $y = a + bx$ ; in the present situation $a = 1$ and $x = n$).

Expectation of life after 'n' days = $\frac{(1/b - n)(1-bn)(1/2)}{1-bn} = \frac{(1-bn)}{2b}$

So the expression $p^n/-\log_e p$ is replaced by $(1-bn)/2b$ and the revised formula for the Vectorial capacity $VC_1$ differs from the classical formula ($VC_2$) as under:

$$VC_1 = \frac{ma^2 (1-bn)}{2b}$$

$$VC_2 = \frac{ma^2 p^n}{-\log_e p}$$
1 INTRODUCTION

The need for seroepidemiological survey of malaria has been well appreciated. It is in general agreed (Draper et al.; 1972, Kagan 1972a; Voller & Bruce-Chwatt, 1968; Meuwissen, 1974) that this type of survey provides information on period prevalence data concerning the total infection with malaria of an individual in a given community. The information derived therefrom is different from that obtained by parasitological examination used in the mass blood survey which provides mainly point prevalence data i.e. the frequency of people having parasitaemia on the day of blood collection. The absence of patent parasitaemia in an individual does not entirely negate that such individual has not been exposed to malaria, since patent is influenced by the immune status and by the use of anti-malarial drugs and often occurs only intermittently during malaria infections. Furthermore, seroepidemiological survey can be applied even in an area with low level of malaria transmission especially where the transmission cycle has been disturbed by the use of insecticides or drugs. It can also provide information on malaria transmission in a given area using relatively small numbers of representative samples from a given community by determination of relationship between the geometric mean reciprocal titer (GMRT) and the percentage of seropositive population. Serological surveys have nevertheless some limitations. It cannot give information on parasite density and gametocytæmia, which are important parameters in epidemiology. In addition, an individual's immune response is affected by several factors such as age, immunologic competency, cumulative exposure to malaria antigen, and the kind and amount of specific therapy. Thus a person who should become serological positive after receiving infective bites from mosquitoes may fail to do so. It is therefore important that interpretation of serological data requires detailed knowledge of the local epidemiology of malaria.

The use of seroepidemiology of malaria has been summarised by (WHO, 1972) as follows:-

In malaria endemic areas:-

a. For the establishment of malarial endemicity rates including species prevalence (in particular age-specific indices).

b. To assess changes in the degree of malaria transmission, usually during or after malaria eradication or control operations.

c. To permit specific epidemiological assessment of malaria- e.g. to
delineate malarious areas; to show the altitude delineation of malaria; and to identify and follow up foci of malaria.

d. To identify areas or individuals requiring action with regard to malaria, especially during the later stages of malaria control programmes.

In non-endemic areas.

a. For case-detection and identification, in some instances, of the species of malaria parasite responsible for the infection.

b. For the screening of blood donors.

c. To exclude the diagnosis of malaria in patients with symptoms such as pyrexia of unknown origin, hepatosplenomegaly, anaemia and nephrotic syndrome.

2. COLLECTION OF BLOOD SPECIMENS FOR USE IN SEROEPIDEMIOLOGY (WHO, 1972).

Usually finger pricked blood specimens collected in capillary tubes or filter papers are used. Venous blood can also be collected, but it should be done with caution in view of the possible unwillingness of the community to participate in the programme.

The capillary-tube method is in general recommended for the collection and transport of samples. One capillary tube with a diameter of 1.1 mm and a length of 75 mm can take up to 70 ul of whole blood. If a larger volume is needed, more capillary tubes or a larger tube like Natleson tube with capacity of 250 ul can be used. They should be centrifuged within a few hours, and cut at the plasma-cellular interface. The plasma section is sealed at both ends and stored.

The filter paper technique can be done by allowing a precisely measured quantity of blood e.g. by means of a Drummond microcap pipette on the filter paper, or simply by allowing blood to soak on the precise area e.g. a circle of 12 mm of filter paper and dried (Kagan, 1972b).

3. TESTS USED IN SEROEPIDEMIOLOGY

3.1 Serological tests for blood stages.

3.1.1 Tests indicative of malaria experience but not related to protective immunity. (Voller et al., 1980).

3.1.1.1 P. falciparum

3.1.1.1.1 Indirect fluorescent antibody test (IFA).

Both thick and thin blood smears can be used, and homologous species of parasites are recommended. For preparation of P. falciparum slides, cultured parasites using a candle jar technique (Trager & Jensen, 1976) can be used. Before the era of in vitro continuous culture of parasites,
other species of plasmodia could be used as a substitution e.g. *P. fieldi* and *P. knowlesi* but they are no longer popular.

The IFA test has merits and limitations (WHO, 1972) as follows:

**Merits of the test**

a. The preparation of comparable batches of antigen is relatively simple.

b. The whole infected cell, morphologically identifiable, is used as antigen.

c. The test can reflect experience of malaria by an individual or community.

d. The test is adequately sensitive.

e. At higher titers, the test is virtually always specific for malaria and sometimes can be used to indicate species prevalence.

**Limitation**

a. The reading of results is subjective at present.

b. Malaria parasite carriers can occasionally give negative reactions. This has been observed especially with children.

c. The necessity for an expensive fluorescent microscope and trained personnel to limit its use to well-equipped laboratories.

3.1.1.1.2 Indirect haemagglutination (IHA) test.

The antigen is prepared from parasitized cells mainly in schizont or merozoites stages. Parasitized cells can be concentrated by Percoll gradient centrifugation (Saul et al., 1983), and then freed from contaminating red blood cells by saponin lysis or hypotonic lysis with triton X-100. According to Kagan (1972a), treatment with distilled water containing 0.01% triton X-100 produced the highest recovery of the parasites. Soluble antigen from the parasites can be obtained by several techniques including pressure disruption, repeated freezing and thawing, tissue grinding and sonication.

The antigen is then used to sensitize glutaraldehyde fixed human group "O" cells (Farshy & Kagan, 1972) or sheep red blood cells (Meuwissen, 1974) with or without pyruvic aldehyde followed by the prescribed procedures (Farshy & Kagan, 1972; Meuwissen, 1974). The non-specific antibody can be removed during the test performance by mixing 50 µl of plasma or serum sample diluted 1:10 with an equal volume of control red cell suspension in double strength in the first row of the microtiter plate, followed by a brief shaking and incubation at room temperature for 1 hour. The control cells will then sediment to the bottom of the well, and an 25 µl aliquot can then be taken to initiate a 2-fold serial dilution followed by the usual procedure of the test. The IHA test has merits and limitations (WHO
1972) as follows:-

Merits

a. The test is simple to carry out and no specialized equipment or highly qualified personnel are needed.

b. The test is adequately sensitive and reproducible, and it rarely gives false positive reactions.

c. Large numbers of sera can easily be handled in this test.

d. The test reagents can be prepared in a control laboratory and can be used in many areas, thus achieving a considerable degree of comparability.

e. The test is eminently suitable for field use and action can be taken on the results the day the sera are collected.

f. The reagents can be stored in a small space and can be transported easily.

Limitations.

a) Malaria parasite carriers, especially children or individuals tested during the initial attack can show negative reactions to the test.

b) Small variations in the test procedure can drastically affect the results. The extract used contains a large number of different antigens of unknown physicochemical nature, and variations or modifications of the technique may lead to a preferential absorption of certain antigen to the red cells.

c) There are important differences in the preparation of reagents and in the performance and reading of the tests in different laboratories.

d) Antigens are available from only a few centres.

3.1.1.3 ELISA

Application of ELISA for diagnosis of malaria was first reported by Voller et al. 1975 using *P. knowlesi* antigen. Subsequently the antigen used was prepared from *P. falciparum* infected blood of aortus monkey with parasitaemia over 20% (Voller et al., 1974). Antigens from cultured parasites can also be used (Spencer et al., 1979). Parasitized cells can be concentrated by Percoll gradient centrifugation, and contaminating red blood cells removed by saponin lysis or by hypotonic solution in the presence of detergent. The test is performed using the standard technique (Voller et al., 1980). ELISA has Merits and limitations as follows:-

Merits

a) The test is very sensitive, being close to that of RIA, and specific.
b) The test is simple to perform, and thus can be carried out in the field. No highly qualified personnel are needed.

c) Large numbers of sera can easily be handled.

d) Only small amounts of antigen are required per test.

Disadvantage

a) If a single dilution is used and the result is expressed as ELISA value it is necessary to read the results using an ELISA reader which is expensive, and the equipment is too delicate to tolerate traveling in the field. If several serial dilutions are to be made, the results will be expressed in titers, reading of which can be done with naked eyes. Nevertheless it is rather difficult to read the end point without an ELISA reader.

b) The antigens are available only from a few centres.

c) Inherent problems related to plate to plate variations.

d) The extract used contains a large number of different antigens of unknown physicochemical nature, which have different binding properties to the plate. Thus small variation in the test procedure can affect the results drastically.

3.1.1.1.4 Other tests.

Gel diffusion test, counterimmunoelectrophoresis, radioimmunoassay have been used, but their inherent disadvantages have precluded their extensive uses in seroepidemiology of malaria. Published reports using these tests are limited.

3.1.1.2 P. vivax.

IFA, IHA tests and ELISA have been used for detection of antibody against P. vivax. Because of the unavailability of the continuous in vitro culture technique for this parasites, the antigens used in the published reports were derived from the blood of patients with vivax malaria (Pillay et al., 1981), from experimental animals e.g. aortus monkeys with P. vivax infections (Mathews et al., 1975, Quakyi 1980) or non-human plasmodia e.g. P. cynomolgi (Xue, 1981) and P. knowlesi (Tandon et al., 1982; Srivastava, 1983). Recently, IFA test using P. vivax infected cells from short term culture has been developed (Brockelman et al., 1984, Faculty of Science, Mahidol University, personal communication). It is foreseeable that in the near future, antigens from the short term culture of P. vivax or from parasites enriched from peripheral blood from malaria patients will be available so that improvement of techniques like IHA and ELISA will be made for a better detection of antibody against P. vivax.

3.1.2 Candidate tests for 'protective' immunity.

3.1.2.1 Tests interacting with extra-cellular merozoites.
The antibody can act by inhibition of merozoite invasion (Cohen et al., 1969; Phillips et al., 1972), merozoite clustering (Chulay et al., 1981), and inhibition of merozoite dispersal (Green et al., 1981). Merozoite invasion inhibition has been shown to be species specific, not complement-dependent, and is mediated by IgM and IgG and its (Fab')2 fragments but not its Fab (Cohen & Butcher, 1970). It is most active against homologous strain than against the heterologous strain (Wilson & Phillips, 1976). Merozoite inhibitory activity correlates with clinical immunity in 70-80% or human or monkey sera studied (Cohen, 1979).

3.1.2.2 Test of intra-erythrocytic death of malarial parasites.

Addition of human immune sera from Sudan to the culture of P. falciparum induced 'crisis' forms (Jensen et al., 1982). This parasitocidal effect of immune sera appears to be confined only to certain geographical locations since immune sera from Flores, Indonesia could not cause intra-erythrocytic death even though they possessed merozoite invasion inhibitory activity (Jensen et al., 1984).

3.1.2.3 Reversal of cytoadherence test.

Erythrocytes infected with trophozoites or schizonts of P. falciparum are not normally present in the peripheral blood but sequestered along capillaries and vascular endothelial cells (Miller, 1969). It has been shown recently that 96-100 per cent of amelanotic melanoma cells bound infected erythrocytes much better than endothelial cells of which only 4-59 per cent were bound (Schmidt et al., 1982). Immune sera revert cytoadherence (David et al., 1983) and this reaction was strain specific (Udeinya et al., 1983).

3.1.2.4 Tests for combined action of antibody and cells.

a) Opsonization. The value of opsonization in protection against malaria has been of some doubt. Passive transfer of immune serum in rodent malaria failed to enhance the in vivo clearance rate of 51-Cr-labelled parasitized cells (Wyler, 1982). Administration of silica which destroys phagocytic cells in vitro does not interfere with the recovery from malaria of vaccinated mice (Playfair & De Souza, 1979). Moreover, recovery from non-lethal P. yoelli infections coincided with decreased anti-bacterial activity of spleen, liver and peritoneal macrophages (Murphy & Lefford, 1979).

In human malaria, it has been recently demonstrated that polymorphonuclear leukocytes (PMN) from normal blood donors phagocytosed P. falciparum infected red blood cells in vitro to a greater extent than normal red blood cells. The phagocytic activity was greatly increased by immune sera but not by sera from individual recovering from a first acute P. falciparum infection. The phagocytosis enhancement was mediated by IgG (Celada et al., 1983), and was independent of complement (Celada et al., 1984).

b) Antibody dependent cell-mediated cytotoxicity (ADCC).

The observation that K cell activity towards chicken erythrocytes is
increased in malaria infection is taken as an indication that ADCC may be important in immunity to malaria (Greenwood et al., 1977). Lymphocytes from West African infected children and immune adults were found to kill *P. falciparum* in the presence of immune serum (Brown & Smalley, 1980). Immune sera have been shown to arm peripheral blood monocytes from normal unsensitized individuals to phagocytose free merozoites but not intact schizonts. A marked difference in the level of merozoite phagocytosis was observed depending on immune status of individuals whose sera were tested, but not on the antibody levels measured by fluorescence or by precipitation tests. The activity was mediated by IgG and merozoite recognition by armed macrophages was not strain specific (Khusmith et al., 1983).

3.1.2.5 Surface indirect fluorescent antibody (IFA) test against ring infected erythrocytes.

It was recently shown that sera from immune individuals reacted in the surface IFA test whereas sera from Swedish patients with primary infection was negative (Perlmann et al., 1984). Antibodies eluted from infected erythrocytes inhibit merozoite invasion in *vivo*. It has been suggested then that this test may be related to protective immunity.

Among these 5 tests, it appears that the surface IFA (if later confirmed to be related to protective immunity) can be adapted for work in seroepidemiology. Other tests, by their nature, are not easily adapted for serological surveys.

3.2 Serological tests against sporozoites

3.2.1 Tests indicative of malaria exposure

There have been relatively very limited publications on seroepidemiology of malaria based on anti-sporozoite antibody, the reason for which is obvious. It is difficult to prepare sporozoites in sufficient quantity for seroepidemiology and to overcome technical problems inherent to the tests of which IFA test using glutaraldehyde fixed cells and circum-sporozoite precipitation (CSP) test are commonly used. Using these two techniques, Nardin et al. (1979) showed that anti-sporozoite antibody against *P. falciparum* could be detected by IFA test in more than 90 per cent of sera from adults living in an endemic area in Gambia, West Africa, whereas most of the samples from children gave low or negative reactions. With CSP test, the percentage positivity was much less. Recent study in Thailand showed that anti-sporozoite antibodies against *P. falciparum* and *P. vivax* were detected by IFA but not by CSP tests in serum samples from individuals living in a village at Karnchanaburi, an endemic area of malaria with low but persistent transmission (Tapchaisri et al., 1983). Both IgG and IgM antibodies against these two species were demonstrated, with seropositive rate for IgG antibodies in all age groups of 31.6 per cent for *P. falciparum* and 20.8 per cent for *P. vivax*. The seropositive rate was low in children and increased with age. Antibody response to sporozoite infection has been considered to be a better indicator of malaria exposure than that against the blood stage, because the response will be related only to time after exposure, the frequency and doses of infective mosquito bites. The response to the blood stage on the other hand could give rise to a high antibody titer even after a single exposure,
because the parasites can increase in numbers through their cyclical development and provide sustained stimulation for antibody production.

3.2.2 Candidate test for protective immunity against sporozoites.

Only recently that an in vitro inhibition of sporozoite invasion (ISI) was developed, and this could be use for assessment of protective immunity against sporozoites (Hollingdale et al., 1982; 1984). It was shown that monoclonal antibodies (MAB), or its Fab fragments, to CS protein of P. berghei completely inhibited the entry of sporozoites into cultured human embryonic lung cells (Hollingdale et al., 1982). The ISI test was later extended to human malaria. MABs to CS proteins of P. falciparum and P. vivax were shown to block in vitro invasion of homologous plasmodia to cultured human hepatoma cells (Hollingdale et al., 1984). The ISI activity was independent of the geographical origin of each strain. The possible correlation of the ISI test and protective immunity is evident by the finding that sera from adult Gambians and a volunteer immunized with P. falciparum were ISI positive when tested against P. falciparum, whereas sera from Gambian children, a volunteer immunized with P. vivax and healthy controls were negative (Hollingdale et al., 1984).

4 APPLICATIONS AND INTERPRETATION

Seroepidemiology of malaria had been conducted in several places in the world with the goal of obtaining data on the levels and patterns of antibodies to malaria antigens in the sera of population groups in relation to relevant variables, and thus to contribute to the knowledge of the epidemiology of malaria. Seroepidemiological studies have in general been approached by 3 different types of surveys comprising single cross-sectional surveys, repeated cross sectional surveys (repeated surveys of the same population, without identification of individuals) and longitudinal surveys (repeated survey of the same population with identification of individuals). The first kind of survey is more common than the second and the second than the third. Usefulness of malaria seroepidemiology has been outlined in the introduction, and will be highlighted in this section.

4.1 Establishment of rates of malaria endemicity.

In the past, malaria endemicity was rated according to splenomegaly and parasitaemia. Neither method is entirely satisfactory. Enlargement of the spleen may be caused by other common diseases, and incidence of parasitaemia alone can fail to present an adequate picture of the pattern of malaria in a population. The use of anti-malarial drugs may affect the parasitaemia rate without a proportionate reduction of transmission (McGregor et al., 1965). Introduction of serological surveys has proved to be useful in establishing malarial endemicity rates.

McGregor et al. (1965) determined fluorescent antibody levels in populations living in 4 villages in Gambia using a single cross-sectional survey and demonstrated the differences in serological age profiles in this villages indicating different patterns of malaria endemicity.

Draper et al. (1972) assessed the level of endemicity in an endemic
area in Tanzania, 11 year after discontinuation of a control programme. The vector densities and the theoretical inoculation rates had returned to the levels observed before the control programme began, but the age-specific parasite rates were much lower than expected. The serological findings suggested that more transmission was occurring than was shown by parasitological findings: 84 per cent in children one to two years of age, and 99 per cent in the two to four year age-group. The dissociation between the parasite rates and the serological prevalences was attributed to the widespread use of antimalarial drugs which reduced the level and duration of parasitaemias and therefore their immunogenicity but did not reduce the malaria transmission.

4.1.2 Assessment of changes in the degree of malaria transmission, usually during or after malaria eradication or control operations.

The effect of antimalaria measures can be monitored by serological testing to observe the progressive changes of the antibody titers, or of the antibody prevalence in repeated surveys or by cross-sectional surveys some times after implementation of the control programme.

Bruce-Chwatt et al. (1975) applied IFA test using thick blood film of P. falciparum from an infected chimpanzee in a serological survey of 4,605 individuals living in Hemathia, Greece after the introduction of an active malaria control programme in 1946. It was shown that all 2,965 individuals with the age below 20 were negative, but the antibody titers in individuals above 20 had rising antibody titer gradients with increasing age: this finding was interpreted to mean that malaria was disappearing from Greece. A similar study conducted earlier by Bruce-Chwatt et al. (1973) showed the virtual absence of positive IFA test in children below the age of 5 years in Mauritius thus providing serological evidence of eradication of malaria from this country.

Lobel et al. (1976) carried out repeated cross-sectional seroepidemiological survey of falciparum and vivax malaria in Guyana in 1973 and 1974 using IHA test. Seropositive rates (titer ≥ 1:32) were rare in people in the age groups below 30 years suggesting elimination of malaria transmission after implementation of the control programme.

Mathews et al. (1970) applied IHA test for falciparum malaria in the repeated cross-sectional serologic survey in Tobago, West Indies. The seropositive rates in samples collected from the same individuals in 1955 and 1966 dropped from 79 per cent to 10 per cent, indicating that eradication programme was successful.

Meuwissen (1974) conducted a single cross-sectional IHA field survey in Surinam in areas with different phases of the control programme. The seropositive rates (≥ 1:40) in areas in the maintenance phase or in the consolidation phase were 4 and 20 per cent respectively whereas the rates in 3 other areas in the attack phase were much higher (71, 61 and 74%).

Cornille-Brogger et al. (1978) conducted a collaborative longitudinal serological survey in conjunction with epidemiological parameters in Garki District, Nigeria covering 8 villages consisting of approximately 3,000 persons using IFA, IHA and gel diffusion tests for P. falciparum and IFA
only for \( P. \) malariae. It was shown that GMRT in individuals in protected villages was much lower than those in unprotected villages in the same age group especially those with \( \leq 26 \) years of age during the 4th and 5th survey periods (50 and 70 weeks of protection). In a corollary study, Molineux et al. (1978) showed that protected infants also had lower antibody titers than unprotected infants.

Voller et al. (1980) carried out a longitudinal serological study of \( P. \) falciparum malaria in the West Africa Savanna using ELISA technique showing a lower seropositive rate (ELISA value > 0.2) in protected individuals than that in the unprotected group of the same age.

Warren et al. (1983) continued a longitudinal serological study in population in an endemic area in El Salvador using IFA test by taking blood samples of one resident from each of 268 widely distributed houses. An increase in number of individuals with seroconversion from negative to positive or a two-fold increase in titer confirmed the continued transmission of malaria.

Jacobs et al. (1983) conducted a single cross-sectional serological surveys using IFA test in 2 districts, Sarapee and Li, in Northern Thailand covering 30 villages each. The seropositive rate in children with the age of \( \leq 15 \) year was almost zero (0.39%) in Sarapee District and 2.9 per cent in Li District which correlated well with results of malariometric survey showing absence of transmission in Sarapee District and low rate of transmission in Li District.

4.1.3 Specific epidemiological assessment of malaria.

a. Delineation of malarious areas.

Serological surveys have been shown of value in delineating foci of malaria transmission. In a longitudinal seroepidemiological studies in El Salvador, Middle America, Warren et al. (1975) showed that foci of malaria transmission existed in coastal plains and were much reduced in the interior. The seropositive rates (IFA antibody titer \( \geq 1:20 \)) in persons under 15 years in 3 coastal villages were 2.3, 7.0 and 2.5 per cent whereas these in all 3 villages in the interior were negative.

Jeffery et al. (1975) applied IFA test in the repeated cross-sectional survey of malaria in Cuiaba Sector and Campo Grande Sector of Mata Grosso State, Brazil. Higher seropositive rate (9.3-13.6%) was found in Cuiaba Sector and lower rate (4.3%) in Campo Grande Sector. The maximum positive responses in Cuiaba Sector were to \( P. \) falciparum antigen while in campo Grande, only 46 per cent of the maximum titers were for \( P. \) falciparum. Furthermore, the seropositive rate and GRMT were different in different localities even in the same Cuiaba Sector. Seroepidemiology is therefore helpful in delineating malaria endemicity.

A single cross-sectional seroepidemiological study using IFA test in 6 villages on the Pacific side of Costa Rica was conducted by Warren et al. (1975). There were no positive responses in individuals under 15 years in 3 villages, and minimal responses in the remaining 3 villages with seropositive rates of 1.1, 2.2 and 3.2 per cent. The result lend support to
the surveillance data for the absence of transmission in these villages. Positive responses in some individuals could be due to old or imported cases rather than local transmission.

b. Altitude delineation of malaria transmission.

Marked differences in the serological responses were found in people living above and below critical altitude of malaria transmission. Collins et al. (1971) compared the IFA seropositive rates and GMRT using antigens of P. falciparum, P. vivax, P. malariae and P. ovale in people living at high and low altitude in Ethiopia. 36.7 per cent of persons living at altitude of 6,000 feet or less were positive whereas only 4.3 per cent of persons living at elevation of 6,300 feet or higher were. Similar observation was made in the United Republic of Tanzania (Voiler et al., 1968).

Kagan et al. (1969) reported a high IHA prevalence in individuals living below 1,300 m in Nepal whereas in persons living above that altitude the IHA prevalence was low.

c. Seasonal changes of malaria antibody.

Mathews & Dondero (1982) carried out a follow-up survey of malaria antibody every 4 weeks for a period of one year in 62 individuals in an endemic area in Malaysia using IHA test with P. falciparum antigen. Serological responses were found to vary from month to month. In most individuals, titer increases occurred simultaneously with or 4 weeks after the diagnosis of parasitaemia by blood examination.

4.1.4 Identification of areas or individuals requiring action with regard to malaria, especially during the late stage of malaria control programmes.

It is important in the control of malaria to detect the delimiting foci of persisting or renewed malaria transmission, so that immediate action can be taken to prevent further spread of malaria.

Tikasingh et al. (1980) used IFA test to detect additional cases of malariae malaria during a recent outbreak in Grenada where malaria has been eradicated.

Ambroise-Thomas et al. (1972) could delineate two areas in Corsica to have malaria transmission even before the cases could be detected.

Using IHA test, Kagan (1972a) observed the presence of antibodies in the 1-4 year old age group in Bulla Quchi, Afghanistan but was negative in the same age group in Saidabad. Such finding predicted malaria resurgence in Bulla Quchi, which was subsequently proved to be the case.

5 CRITIQUES OF PUBLISHED REPORTS ON MALARIA SEROEPIDEMIOLOGY.

Though numerous reports on seroepidemiological surveys have been
published, thorough readings of these reports reveal some shortcomings which could be improved using more careful planning, new knowledge and technologies which have been accumulated in recent years so that more meaningful information could be obtained. Some of these shortcomings are highlighted below:

a. Most of published serological works have been concentrated only in descriptive epidemiology trying to answer 3 kinds of questions concerning: 1) the presence or absence of malaria transmission, 2) the intensity of transmission and 3) the change of transmission using three general approaches comprising single cross-sectional surveys, repeated cross-sectional surveys and longitudinal surveys (Molineaux, 1981). Repeated cross-sectional surveys provide more epidemiological information than single cross-sectional surveys, and longitudinal surveys allow answers to the same question as repeated cross-sectional surveys but has added advantage in being more sensitive.

There is however little work on application of serological surveys in analytical epidemiology to enable investigators to identify populations at risk, factors contributing to transmission and how they contribute. This type of study is considered important in the control of malaria in endemic areas with low but persistent transmission as to obviate the high cost of operation based on blood examinations which are known to be less sensitive and would yield results incommensurate with the works involved.

b. Confinement of the published works almost entirely on the blood stages and not on other stages like sporozoites or gametes. Demonstration of antibodies against these two stages is important in view of the ongoing development of sporozoite and gamete vaccines. Anti-sporozoite antibodies against human plasmodia have been demonstrated in sera from people living in endemic areas in Gambia (Nardin et al., 1979) and in Thailand (Tapchaisri et al., 1983). For reason stated earlier (3.2.1), anti-sporozoite antibody could be more closely related to the frequencies of malaria exposures than that of the blood stages, thus determination of anti-sporozoite antibody would help in assessing the relationship between man and infective mosquito contacts without interference by growth and multiplication factors occurring in the blood stages. Determination of anti-sporozoite antibody would help in dissecting only the immune response to the sporozoite in the future 'trivalent' vaccine comprising of antigens from sporozoites, blood stages and gametes. Likewise, determination of anti-gamete antibody will help in assessment of the role of anti-gamete antibodies in the population and in the highlighting only the immune response to the gamete in the future 'trivalent' vaccine.

c. All the tests so far reported in the literature are measuring only the level of antibodies signifying the presence and the magnitude of previous malaria experience but do not contain information of protective immunity. Putative tests for 'protective' immunity (outlined in 3.1.2) should be more extensively tested and verified to be used in the assessment of the level of immunity in the population before and after vaccination.
6 SEEROLOGICAL METHODS FOR EVALUATION OF THE IMPACT OF VACCINATION ON CONTROL OF MALARIA.

As availability of vaccine for use in the field trial for malaria control appears imminent, strategic planning involving the use of serological methods has to be made to effectively evaluate the impact of vaccination in the control of malaria. Serological methods should help in providing answers to following questions:

1. Do vaccinee develop adequate level of immunity after vaccination? How shall we assess protective immunity? When will protective level of immunity begin to appear and how long will it last?

2. Does vaccination has any effect on transmission of homologous as well as heterologous plasmodial species in reducing intensity (a decrease in proportion of seropositive) or causing changes in intensity of transmission (reducing the proportion positive or reducing in GMRT), or the vaccine has no beneficial effect at all?

In order to answer the above questions, steps have to be taken as follows:

6.1 Choice of the tests.

6.1.1 Tests reflecting malaria experience. The tests detecting antibody against the blood stages include IFA, IHA and ELISA for P. falciparum and IFA for P. vivax. IHA and ELISA for detecting antibodies against P. vivax can be developed in the future. The merits and limitations of these tests have been discussed in section 3.

In selecting the tests, Kagan (1972b) has laid down some guide lines as follows:

a. The test has to be simple to perform.

b. The interpretation of results must be free of subjectivity.

c. The test must be rapid.

d. The cost must be minimal.

e. The test results have to be sufficiently sensitive and specific.

6.1.2 The tests reflecting malaria exposure. The tests will be used to detect antibodies against sporozoites. Two tests comprising IFA against glutaraldehyde fixed sporozoites and CSP have been developed, among which IFA is most widely used, because it is more sensitive and technicly more practical to perform. Hence only IFA should be selected for use.

6.1.3 The tests for 'protective' antibodies.

Candidate tests for 'protective' antibodies against blood stages and sporozoites have been given in 3.1.2 and 3.2.2 respectively. Among the
tests for blood stages, it appears that the merozoite invasion (parasite growth inhibition) and the surface IFA test against ring infected erythrocytes can be used. Another test, the immunoblot should be considered as a candidate test eventhough it is not strictly related to protective immunity. The immunoblot technique could be considered as a substitute to the radioimmunoprecipitation (RIP) test, which have been used to demonstrate quantitative and qualitative differences of some precipitating bands occurring in immune sera and sera from non-immune persons after primary malaria attacks (Perrin et al., 1981, 1982: Brown et al., 1982). The immunoblot technique has some following advantages over the RIP test: - 1) Proteins which are deficient in methionine e.g. Mr 155 glycoporin binding protein, or high molecular weight S antigen (WHO, 1984) can be demonstrated by the immunoblot technique but not by the RIP test. 2) The procedure for immunoblotting is less complicated than that of the RIP test. 3) The cost to perform immunoblotting is less than that of the RIP test. Because 125-I used in the immunoblot is less expensive than 35-S-methionine used in the RIP test. Besides the non-radioactive technique can be developed for use in the immunoblot but not yet for the RIP test.

6.2 Use of serological surveys before vaccination.

Serological surveys have to be conducted along side with other descriptive epidemiology surveys to gather base line information, and at the end of the survey period, follow, , elegant information are expected:

a. Important epidemiological factors (hosts, parasites, vectors and their environment) related to malaria transmission will be known.

b. Population at risk will be identified.

c. Background immune status will be known.

6.3 Decision making before launching vaccination programme.

The base line information collected in 6.2 may help in the decision making for vaccine field trial as follows:

a. Selection of place for vaccine trial.

Only the places within the flying ranges of mosquitoes from their breeding places are good candidates. In the case of An. minimus, the distance of approximately 2 km from both banks of the running streams should be selected. Selection of housing beyond this perimeter is likely to be non-productive.

b. Selection of vaccinees.

Vaccination should be given to population at risk (identified during the base line surveys). In the study conducted by the Department of Tropical Hygiene, Bangkok Faculty of Tropical Medicine, at villages in Karnchanaburi, the population at risk were those under 30 years of age. The study in the non-risk group is unlikely to yield fruitful results.
6.4 Follow-up period after vaccination.

All population at risk in the same village should be divided into 2 groups, one receiving vaccine and the other receiving placebo. Serological surveys should be conducted at monthly intervals by taking finger tip blood samples into 1 - 2 Natleson tubes each if possible, if not collection in several regular sized capillary tubes is done instead. The plasma should be separated from blood elements within 6 hours after collection and stored in a freezer compartment of an domestic refrigerator. Attention should also be paid to the unvaccinated controls. among them some will develop malaria and the other do not. Comparison of serological findings between these 2 subgroups alone may provide information related to development of natural immunity in the populations under studied.

After the completion of the study, comparison between the vaccinated and unvaccinated groups will be compared. It is expected that the outcome would provide at least some answers if not all to the questions addressed at the beginning of this section, and whether or not the vaccine is effective will be known.

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EVALUATION OF THE IMPACT OF IMMUNIZATION ON THE CONTROL OF MALARIA

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Considerable portions of this conference have been devoted to a detailed review of the development and testing, which will lead to a powerful new local vaccines which will induce immunity to one or various stages of human Plasmodium. These vaccines will become available at a critical phase in the renewal of both conceptual and administrative/financial commitment to malaria control. Coincident with the enthusiasm for dealing with malaria with innovative epidemiologic and operational approaches there is a continuing erosion in the efficacy of currently available interventions -- specifically with insecticides and malarial drugs. The advent of vaccines represents a major opportunity, an opportunity generated by the hope that the weapons available for malaria control are not hopelessly limited.

While it may seem somewhat unorthodox, I believe that it is essential for all of us, the immunologists, genetic engineers, health planners, as well as malaria program staff, to understand the current challenges and evolving strategies for malaria control. The reason being, that, no malaria vaccine is going to radically change the issues of malaria control, nearly as much as the strategies of malaria control in the 1980's-90's will define the eventual impact of vaccines.

Malaria control has evolved as a public health strategy, to an extent in contrast to the concepts and methods of malaria eradication. Inherent in malaria control strategies are several factors:

1. Recognition that from both technical and operational standpoints, there is great heterogeneity in malaria, and rational operational efforts must be tailored to the particular local malaria epidemiology.
2. Control efforts must be formulated in most areas of the world with the understanding that malaria will not be eradicated; consequently, major emphasis has to be placed on the development of long-term commitments.
3. The public health future will de-emphasize disease-specific programs, and favor integrated health care delivery and preventive efforts with a strong focus on local health care initiative.

In the 1970's a general framework for malaria control was proposed by WHO. These tactical variants define 4 increasingly more inclusive objectives for malaria control, ranging from initiatives to control the health consequences of the disease--malaria--to programs which in addition incorporate
efforts to limit or eliminate malaria transmission.
Conceptually, the tactical variants are important to legitimate strategies for malaria control that focus on the disease, that support malaria control in areas where vector control is not feasible, and that suggests a phased inclusion of more technical and expensive interventions as resources and epidemiologic knowledge permit.

Malaria vaccines, as we currently understand them should adapt well to malaria control strategies; that is, certain vaccines may be particularly suited for blocking infection or limiting the risk of complicated disease, while other vaccines might supplement other malaria transmission control strategies.

I will review some of the epidemiologic situations which malaria vaccines will confront. We will then consider the evaluation methods which are required to plan and assess malaria control, and discuss whether those methods will be appropriate when vaccines were incorporated into the malaria control interventions.

The first epidemiologic setting to be considered relates to those areas of the world with high levels of transmission in which organized health services are limited, and malaria control efforts are rudimentary. A sizeable portion of subsaharan Africa, Papua New Guinea, and the Amazon basin have these general characteristics of uncontrolled, high levels of transmission; P. falciparum constitutes a high proportion of the malaria and in many of these areas it is believed that malaria is a major contributor to excess mortality and morbidity. The most prominent feature of malaria under these conditions is how little we know about its epidemiology.

In such circumstances, it has been proposed that controlling the more severe health consequences, malaria-related mortality and morbidity, is the priority, accepting that transmission will for the moment continue unabated. Delivery of malaria drug therapy to high risk groups—notably young children and women—must occur at the community level. Diagnosis of malaria is based on local definitions and symptoms, generally without the ability for parasitologic confirmation. Effective implementation of this quite basic strategy requires several important factors: patients or parents must recognize malaria as an illness there must be a well-supplied source of appropriate therapy and health education at the community level, and there should be a referral system for malaria too severe to be treated in the community. This is in reality the stage for primary health care—community based health programs dealing with a major health threat with simple technology, focusing on the development of a sustainable health care infrastructure.
The obvious limitations are that--

1. even this quite basic health care infrastructure with trained staff must be developed in many areas, and

2. the best intervention, drug therapy, may be compromised to varying extent by drug resistance.

As we consider the evaluation of malaria control aimed at the limitation of mortality/morbidity, we encounter several major problems at this time. Foremost among these is the diagnosis of malaria: what symptoms, disease, or even parasitemia require therapy. There is a pressing need to systematically reassess criteria for the case definition of malaria at the local level; we need these criteria to systematize the delivery of therapy, and to evaluate the efficacy of interventions, including vaccines. A related issue is the enormous problem encountered in defining epidemiologically and measuring the magnitude of malaria-related mortality or morbidity. Particularly in those areas where the prevalence of *P. falciparum* is high, the association of a febrile illness leading to death maybe an extremely inaccurate estimate of the contribution of malaria.

One of the most critical point of evaluation for malaria-PHC does not deal with either parasitology or mortality/morbidity, but rather relates to so-called "process indicators." These statistics--measures of the function of health centers, the appropriate use of antimalarials, and even the changes in knowledge and practices by patients as a result of health education, may be the most powerful evaluation tools available to assess the success of malaria components of PHC.

How might malaria vaccines enhance our ability to control malaria-related mortality and morbidity in areas of the world with high levels of transmission and limitations on resources? Clearly, a vaccine which could induce disease-protective immunity in those segments of the population at risk of more severe *P. falciparum* illness would have the greatest attraction. Vaccines which could completely block infection would accomplish the same end, yet such absolute protection may be difficult to achieve or sustain.

The assessment methods which are used for drug-distribution strategies must apply to malaria immunization. It will be important to identify the highest risk groups, since finances and manpower restrictions mandate targeted use of even inexpensive malaria control measures. Our knowledge of the epidemiology of malaria--those factors which predispose to morbidity and mortality--must be enhanced. The new generation of antigen-specific serologic tests will permit us to understand malaria as never before. In reality, though, much of the epidemiologic assessment which is required for rational malaria control is dependent on tools of simple-minded epidemiologists and to a lesser extent on high-technology immunologists.
The 2nd epidemiologic setting to be considered are those malarious areas where there is the potential to institute programs of malaria transmission control. In the framework of current malaria control strategies, those malarious areas in which long-term commitment to vector control can be considered are generally where malaria prevalence and disease consequences are considerably less that in circumstances we have discussed in Africa. *Plasmodium vivax* is frequently the predominant malaria species. Basic diagnostic and therapeutic services extend to many malarious areas, largely in many cases due to malaria surveillance and operational activities. The disease consequences of malaria such as the retardation of socio-economic development are real, yet have proven to be quite difficult to measure or assess epidemiologically.

Those areas where transmission control appears to be at least potentially realizable are in the Americas and some areas of the Indian subcontinent and southeast Asia. These are the countries in which malaria eradication was attempted, and proved not to be totally successful, and whether sufficient political will, financial and manpower resources are currently mobilizable for widespread *Anopheles* control is unclear. How will malaria vaccines fit into these epidemiologic circumstances? We can ask the question in another way—do we anticipate that the induction of enhanced immunity could sufficiently potent epidemiologically to be cost beneficial as a tool in areas where the program objective is to limit the transmission of malaria? Such immunity would need to either dramatically and rapidly eliminate asexual replication prior to gametocyte formation or specifically attack gametocytes or parasite development in *Anopheles* vectors of malaria.

Where transmission control programs are focused on special epidemiologic situations such as transmission in urban areas, vaccines which reduced the probability of infected individuals infecting mosquitoes, combined with mosquito control efforts might be a major adjunct to malaria control. Immunization on a broader scale, with the objective of completely halting transmission—eradication in effect—would have to be considered with great caution and more knowledge than we currently possess.

In areas where *P. vivax* transmission is a major proportion of the malaria problem, it is worth considering how important *P. vivax* components to a malaria immunization program would be. We believe that *P. falciparum* infections carry a real health threat, and can understand how even a relatively expensive intervention, as immunization well may be, would be supported. With *P. vivax*, however, if there were the possibility of immunization contributing substantially to interrupting *P. vivax* transmission, then I suspect that the support would be
there. Would support be there for a \textit{P. vivax} vaccine which limits the illness (a blood-stage immunity) without measurably diminishing transmission? We must know more about the clinical epidemiology of \textit{P. vivax} infections before we can answer that question.

The entomologic assessment of malaria control programs has historically entailed relatively technically complex methods. The statistics of greatest utility describe the \textit{Anopheles} density in time and space, the human-mosquito contact, the rate of \textit{Plasmodium} infection in \textit{Anopheles} biting humans. When vaccines are incorporated into malaria control programs these same epidemiologic parameters will continue to serve as the basis by which we characterize malaria transmission, and evaluate the impact of control. Newer methods which would enhance either the sensitivity or simplicity of these assessment methods would be important.

Immunization of the human host to limit transmission, as opposed to direct attacks on the anopheline vector, would offer the opportunity to be much more selective in our control application. Field studies have indicated that not all members of a population in malarious areas are contributing equally to infecting mosquitoes. Several investigators have found that children are the principal gametocyte carriers in some areas of Africa. We need more information on this potential association, investigating not only gametocyte carriers but also the ability of individuals to actually infect the coinigenous \textit{Anopheles} vectors. This can be accomplished not only by well designed studies of mosquito infection by humans but also potentially by \textit{in vitro} assays.

If it were possible to identify a population subset which was responsible for a majority of mosquito infections then targeting of immunization might be possible. This would be particularly important if children, likely candidates for blood stage vaccines to control disease, could be immunized with effective multistage vaccines. Finally, we must recall that, despite the problems of insecticide resistance, the principal impediment to sustained attacks on malaria transmission have been the administrative and financial problems encountered in sustaining the reductions in the entomologic inoculation rates. The lesson of malaria eradication should not be missed: disease control programs must be incorporated into health services to sustain their achievements; where adequate health services do not exist we must assist in developing them with the same commitment - technical and administrative - as we are in developing vaccines.

The 3rd epidemiologic setting which confronts us today consists of various circumstances in which there is either a time-limited or geographically-local risk of malaria infection. In general there are 2 epidemiologic settings. There are those circumstances where laborers or other individuals, frequently moving from non-malarious areas, are for defined periods living
in areas of malaria transmission. In the world today, we could list many examples of this problem -- each with its own unique malaria epidemiology. A notable recent example occurred in southeast Asia, where Khmer refugees traversed western Kampuchea moving toward Thailand, passing through highly malarious areas in the process. High mortality rates of *P. falciparum* infection occurred in all age groups.

The 2nd example of transient malaria exposure is really a very special problem: that of non-immune tourists or military visiting malarious regions. In general, there is a time limited exposure. The risks are experienced by all age groups; any of the human *Plasmodium* may be present. Under these conditions, the objective is optimally to block infection, or at least to substantially decrease the risk of disease should the person become infected. Currently, antimalarial drugs used either prophylactically or, on occasion, to presumptively treat infections, are the most effective method of eliminating the risk of malaria. When prophylaxis is complied with, and the drug is effective and safe, prevention of malaria can be highly effective.

The major impediments of effective drug prophylaxis are well known: drug resistance in *P. falciparum*, toxicity of some antimalarial drugs, and compliance. With tourists or military, there are means, either education or coercion, which can to an extent, assure reasonable compliance with malarial drug prophylaxis. For labor forces, refugees, or illegal immigrants, drug prophylaxis may be completely impossible.

An effective infection blocking vaccine could potentially overcome most of these problems, even if the duration of protection was relatively short. Evaluation of a vaccine efficacy under these circumstances would be quite straightforward. Epidemiologically, it will be important to clearly identify the population at risk, and define the time period of risk. For immunization, we must know to which *Plasmodium* species protection is required. In reality the issues of protection for these groups will be resolved in phase 3 and 4 trials.

In conclusion, I believe that malaria vaccines irrespective of their potential vaccine efficacy, must fit into evolving strategies of malaria control. Those challenges are substantially organizational/financial. Equally important, is the fact that we need to know much more about basic epidemiology to effectively implement malaria control, even before we have vaccines. The evaluation of malaria immunization will be incorporated into the strategies of malaria control:

Many of our current methods of evaluation derive from malaria eradication times and require reassessment. The phenomenal success in malaria immunology will clearly result in vaccines; perhaps equally important for malaria control will be the potential for improved malaria epidemiology tools. Already we have seen the potential for much simpler and highly specific immune based tools for sporozoite detection.
The future of malaria serology will be in the use of defined antigens; soon we will be able to understand the natural acquisition of protective immunity, a topic which we must understand before marching off with a vaccine. Detection of parasitemia is a real issue -- will we even have a replacement for the microscope? I suspect that we will, but the diagnosis of malaria in many rural areas will continue to be based on symptoms alone.

The researchers and supporters of malaria vaccine research hope that their hard work will result in an intervention which will contribute dramatically to malaria control; similarly, national authorities, in their difficult daily struggle to develop effective malaria control programs, might be tempted to focus on vaccines as a final solution to their problems. In reality we all know that malaria control will not be so simple; we do not need malaria vaccination programs, rather we must work hard to build malaria control programs which will be able to use vaccines to their best advantage.
DR. ROBIN POWELL DISCUSSION

Dr. Zavala described a new species-specific means to identify and quantitate sporozoites in the mosquito host. He also described the use and potential value of new methods to determine anti-sporozoite antibodies.

Dr. Suleman discussed methodology for calculating vectorial capacity and how this index can be of value in field assessments of transmission.

Dr. Savanat provided a very insightful critique of the limitations of previously used seroepidemiological methods and the potential that now exists for new, much improved seroepidemiological assessments.

Dr. Campbell provided a perspective on current malaria control conditions and potential roles of malaria vaccine as elements of those measures, and the need for improved diagnostic methodology and for improved measures for field assessments of epidemiological and entomological variables.

These four presentations illustrate a translation of basic research progress into advances in the methodologies available for use in clinical trials and field studies. We need not only new weapons to attack malaria but also new and better methods to study malaria's epidemiology and the impact of these new weapons. We are in effect entering into a new era of research and development in field study methodologies. Time will be required to test and to learn how to use these new techniques. This changing situation with respect to study methodologies represents in itself a reason to proceed with deliberateness and no great sense of haste in field studies of malaria vaccine.

The pivotal data on the impact of vaccination on malaria control will relate to malaria's morbidity and mortality. Enroute to enlisting those data, though, we will need to proceed one step at a time to improve our abilities to obtain conclusive data, particularly for larger more complex field studies. While, as Dr. Bruce-Chwatt cautions, we should avoid trying to do too much in individual field studies, it may be useful to view the context of clinical trials and field studies as having two related elements—one to assess vaccine effect, the other to conduct basic and applied research on malaria's biology. We now have the opportunity to link basic and applied research in new ways. I think we should do all we can to foster that linkage. For example, if there are vaccine failures, we should try to be in a position to determine then and there, through basic studies involving the parasites that break through and their hosts, to pinpoint the reasons for the failures.

We will encounter substantial pressure to expedite vaccine trials and to apply instantly any positive results of such trials. Given the basic and applied R and D that remains to be done, we should consciously resist those pressures. We should exercise great care to set appropriate perspectives and expectations. In particular, we should court Delilah of the Press with considerable circumspection. We may inadvertently lead others to believe that a panacea is just around the corner, when in fact
It seems likely that with the basic research still underway, the variety of candidate vaccines, the unanswered question on adjuvants and carriers, ethical issues, and needs to improve study techniques, we may well be conducting Phase I and Phase II studies with different preparations or groups, and we may well be continuing to work on major field study methodologic questions, for at least the next 10 years.
METHODS FOR FUTURE VACCINE TRIALS II

Methods for Evaluation of the Impact of Vaccination on Control of Malaria

William Collins and Kenton Kramer, Rapporteurs

Dr. Zavala reported on the development of a two-site immunoassay for the detection of CS protein in infected Anopheles mosquitoes. Based on monoclonal antibodies against CSF, the assay (IRMA) not only determines if the mosquito is infected but also the species of Plasmodium and an estimate of the number of sporozoites present. The universality of the CSF epitope will allow for worldwide application of this assay. Information such as sporozoite rates, determining important vectorial species and vectorial capacity can be determined. There was a general agreement that this assay is a significant improvement over more classical entomological methods and will greatly contribute to the monitoring of vaccine efficacy. Questions were raised about false negative results due to populations of sporozoites not expressing the antigenic CSF epitope. Dr. Zavala responded that the CSF epitope has been found in all geographic isolates thus far examined.

In addition, Dr. Zavala presented a description of an improved assay for surveying anti-sporozoite antibodies in human populations. This solid phase immunoassay, using synthetic (NANF)₃ peptide, was described. Data was presented which showed that antibodies to (NANF)₃ was age-dependent with high titers occurring in individuals over age 25. Since this antigen [(NANF)₃] is equivalent to that proposed to be used in future vaccine trials, this assay will allow for the seroepidemiologic surveys of the study populations.

Dr. Suleman put forward a mathematical modification of Dr. McDonald's model for assessing vectorial capacity (VC). He believes that this modification takes into account more realistic estimates of a vectorial life expectancy. VC estimates, calculated by both formulas, showed that the modified formula provided a better explanation for the seasonal pattern of malaria transmission in Punjab than did the original formula. Dr. Suleman felt that VC should be determined by more classical mosquito dissection methods until evidence is obtained that it could be replaced by new methods, such as the IRMA method described earlier by Dr. Zavala. Perhaps a comparison study is warranted at this time.

Dr. Savant Tharavanij reviewed the application of serologic techniques to the epidemiology of malaria. In areas endemic for malaria serologic results can indicate: 1) the level of endemicity, including species prevalence, 2) changes in the degree of malarial transmission, 3) identification of foci of infection and 4) areas requiring special attention. In non-endemic areas, serologic studies have been used for case detection and identification, the screening of blood donors and exclusion of malaria in patient diagnosis.

For the assessment of the impact of vaccination on the control of malaria, serologic tests can be used to measure antibody response following immunization (including its appearance and persistence). Prior
to the initiation of vaccine trials, serologic tests will be used to identify the population at risk and the background immune status of the individuals who are immunized and of those who will serve as controls. After the vaccination, comparisons of serologic responses of the immunized and control groups coupled with parasitologic results will provide information on the development of immunity in both populations.

Dr. Campbell presented a thought-provoking discussion on the evaluation of the impact of immunization on the control of malaria. Malaria vaccines should blend well to malaria control strategies; certain vaccines are suited for blocking infections while others might supplement other malaria transmission control strategies.

Three epidemiologic situations were considered. The first related to areas with high levels of transmission in which organized health services are limited. Here, it is proposed that controlling the more severe health consequences (malaria-related mortality and morbidity) is the priority. In this situation, vaccines which induce disease-protective immunity would have the greatest attraction. Vaccines which could completely block infection should accomplish the same end, however this may be difficult to achieve.

The second epidemiologic setting is one in which there is a potential to institute or strengthen existing programs of malaria transmission control. In these areas, P. vivax is often the predominant malaria species. Here, vaccines would need to either eliminate asexual replication prior to gametocyte formation or to specifically attack gametocytes or gametes to stop parasite development in the mosquito. Since P. vivax is a major proportion of the malaria problem, efforts to develop an immunization program against this parasite has priority in these areas.

The third setting concerns the movement of people from areas of little or no transmission to areas of high transmission. Examples given were refugees in Southeast Asia and the movement of tourists or military into malarious areas.

It was emphasized that disease control programs must be incorporated into health services to sustain their achievements; where adequate health services do not exist, assistance must be given to develop them. It was also pointed out that malaria vaccines must fit into the evolving strategies of malaria control.

Dr. Fowell emphasized that studies on the development of malaria vaccines has resulted in the development of new field technologies and methodologies for the measurement of mosquito infections, determination of vectorial capacity and antibody responses. It was cautioned that workers move slowly into the application of the new methodologies in order to be certain of their interpretation and meaning. Pressure to apply the new technologies or to abandon them "too early" must be resisted. It was cautioned that the completion of Phase I and II trials and the development of protocols for Phase III trials may require the next 10 years.
Possible Integration of Vaccine with Other Control Antimalaria Measures in China

With the recent rapid progress in malaria vaccine research the possibility of obtaining vaccine for operational use in the near future is increasing markedly. But it will take time to solve all the scientific and technical problems encountered in producing safe and effective vaccine and to complete the clinical and field trials before the vaccine will be available. At this stage, the consideration of vaccine utilization may be somewhat premature, nevertheless, it will be of utility both to future operation and to vaccine production, for in formulating strategies, undoubtedly it will involve consideration of the potency and the immunogenic properties of the vaccine which the vaccine producer should also take into account when producing vaccine.

The role of vaccine in malaria programme

Though vaccine is often regarded as a preventive tool used to immunize individual or groups of people to prevent disease, potent vaccines, such as smallpox vaccine, poliomyelitis vaccine, measles vaccine, diphtheria vaccine etc., when strategically used can interrupt transmission and hence can be used alone to eradicate disease. The successful global eradication of smallpox is an illustrative example. Unlike these vaccine preventable diseases, malaria, although being a disease for which man is also the only source of spread of infection, will not be eradicated by vaccine alone, because the immunogenicity and potency of the malaria vaccine are not equivalent to those of potent viral or bacterial vaccines. It is well known that the immunity induced by malaria vaccine is transient, species specific, and stage specific, and it is not known yet what impact immunodepression, found in current and chronic malaria cases always met in malaria endemic areas, will have on the building up of immunity in the recipient on vaccination. However the frequent repetition of vaccination required to obtain and maintain effective immunity may not only increase the burden of the operation, but also may cause failure owing to disgust, unacceptability and poor cooperation among the target population. For those reasons, malaria vaccine should not be regarded as an alternative antimalaria tool to be used alone to combat malaria; instead, it should be used integrated with other antimalaria measures to make up each other's deficiencies to obtain desired effect in the malaria operation.

Since traditional antimalaria measures are generally effective, it will not be necessary to use vaccine extensively as is done for diseases under the Expanded Programme on Immunization; instead, malaria vaccine should be used in localities where there is strict necessity and where other measures have not proved to be satisfactory.

It is conceivable that the traditional measures will be used alone wherever feasible, but when these measures fail to achieve desired result, vaccine may be used as reinforcement to strengthen their interventional capacities to seek strategic combined effect which should have impact on the curbing of malaria transmission. In this context, vaccine will play a distinctive role in malaria programme, though the traditional measures have not proved to be satisfactory.
It is conceivable that the traditional measures will be used alone wherever feasible, but when these measures fail to achieve desired result, vaccine may be used as reinforcement to strengthen their interventional capacities to seek strategic combined effect which should have impact on the curbing of malaria transmission. In this context, vaccine will play a distinctive role in malaria programme, though the traditional measures will still play the major role.

In order to be successful, the combination of measures should be tactically maneuvered on the basis of the epidemiological conditions existing in the locality so that obstacles not overcome before may be surmounted by the new tactics in the malaria campaign.

It can be foreseen that vaccine will be integrated into the malaria surveillance system, under which it will be brought into full play. The effectiveness and the efficiency of surveillance operations will also improve by the reinforcement provided by the vaccine. In conjunction with other measures, vaccine will have application in the elimination of stubborn residual malaria foci, particularly where vectors are not amenable to control, in areas under consolidation or in areas where malaria has been kept under control. Vaccine will provide condition for quicker interruption of transmission, despite the vectors not being significantly affected by mosquito control measures. Coordinated by other measures, it may be applied in the highly vulnerable localities and in the highly recepable localities to raise the immunity level of their residents so that they may deal better with the menace of imported malaria. It may find use also in the operation to suppress malaria outbreak in areas where malaria has been kept under control or eradicated, for timely immunization of the people living in the surrounding neighborhood of the outbreak area will prevent further spread of malaria and enable malaria operation to be concentrated in limited area, whether at the original site of the outbreak or in affected localities in the immediate neighborhood.

Possible operational use of vaccine in China

The present status of malaria is quite different from that in the preliberation days and during the earlyliberation period. The condition of hyperendemicity formerly existed in many malarious areas no longer exist. Malaria endemic areas in the country are continually decreasing in extent. Except during outbreak, the transmission intensity in most endemic areas has greatly reduced. Such situation together with the existence of the well organized peripheral health services throughout the country provide favorable condition for utilization of vaccine in localities where it will be needed. It can be expected, therefore, when safe and effective vaccine will be available for operational use, it will be integrated with other antimalaria measures to be employed where it will be necessary in the malaria programme to surmount problems impeding its progress.

As P. Vivax is predominantly prevalent in China, over 95% of the total malaria cases reported in the country are due to this species, and its endemic areas are far more bigger than that of P. falciparum, where P. Vivax also exist. Moreover, particularly in the vast P. vivax areas in central and northern China An. sinensis, which is not amenable to control,
has made the Huanghai and the Jianghan plains the stress of the operations of malaria campaign in China, for the malaria cases reported in these regions accounted to about 80% of the total cases in China. Therefore, it is clear that *P. vivax* vaccine will be much more in need than the *P. falciparum* vaccine. However, *P. falciparum* vaccine may be brought into full play in the localities where it will be needed, for example, in localities where chloroquine resistant *P. falciparum* strain exists and in localities where there is distribution of *An. dirus*, notorious vector difficult to control.

Besides immunization of individuals or of selected groups of people, going to work or travelling in malarious areas at home or abroad, such as frontier troop and other personnel working in malarious frontier; dam, road, and railway constructors, other big construction workers, and prospecting team etc. In malarious localities, vaccine will be chiefly used in conjunction with other control measures to resolve problem not solved before to achieve further reduction or suppression of malaria. As malaria is by nature a focal disease, and malaria in most parts of the country is unstable, opportunity should be taken, when malaria recede to localities where it is firmly entrenched, to attack them with the combination of vaccine and other measures to eliminate them when possible to ensure consolidation and further expansion of the gains already achieved.

Strategic attack on strategic sites by this combination of measures may be conducted to clean out malarious sites in low endemic areas.

Undoubtedly, vaccine will be integrated into the malaria surveillance system and play important role in the later and final stages of malaria programme and during the maintenance phase.

Therefore, vaccine may find application in the following operations:

1. Immunization of people from nonendemic or low endemic areas going to places still malarious, particularly in chloroquine resistant *P. falciparum* areas, to give them protection while staying in the endemic area and to prevent them from spreading the disease agent when they have left the area.

2. Integrated with other antimalaria measures, vaccine will be tactically manoeuvred in coordination with other measures to attack malaria in localities where traditional measures did not provide desired result. Such strategy will find application in areas where chloroquine resistant *P. falciparum* exists, or where vectors are not amenable to control, or where both conditions exist.

3. Integrated into the surveillance system, it will be used in operations for elimination of residual malaria foci in areas where malaria has been kept under control.

4. In coordination with other emergency measures in malaria surveillance, it may be used in operations for suppression of outbreak, particularly post eradication outbreak or outbreak in areas where malaria has been kept under control.
Concluding remarks

What have been said above do not imply that the integration of vaccine with other control measures will be the final solution of the problems existing in malaria control or eradication, instead, only the possibilities of vaccine utilization in malaria campaign have been discussed. Vaccine, when available, will be one of the valuable weapons in the armament in against malaria which must be used appropriately to win the fight against the disease. To bring vaccine into full play, it should be integrated with other control measures and most probably also integrated into the malaria surveillance system to strengthen interventional capacities and resolve problems already existed and not solved by traditional measures to achieve further reduction or suppression of malaria. With this end in view, vaccine when used in malaria campaign should have a considerable impact on the curbing of malaria transmission to be of operational value.
INTEGRATION OF MALARIA VACCINES IN THE HEALTH DELIVERY SYSTEM IN INDIA

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ABSTRACT

Malaria control and the extended programme of immunization (EPI) are integral to the primary health care system in India. As and when mass immunization against malaria becomes feasible, the delivery of malaria vaccines should be merged with the EPI. This may require additional inputs and strengthening of the existing infrastructure to develop malaria vaccination as a dedicated system at the village level. Priority groups for vaccination and the integration of malaria vaccines as an adjunct to other methods of malaria control have been discussed.

INTRODUCTION

In ancient India malaria was known as the king of tropical diseases. Malaria was the single most important cause of the biggest misfortune degrading the life of people in every possible way. Till 1952, malaria was directly responsible for about 75 million cases and 800,000 deaths annually, with considerable increase in the morbidity and mortality due to malaria during the epidemic years. Malaria was nearly eradicated from the country in the early 1960's as a result of the nationwide anti-malaria campaign launched by the National Malaria Control/Eradication (NMCP/NMEP) of the Government of India. Still in 1965 there were at least 100,000 parasite positive cases of malaria scattered all over the country. This followed a period of resurgence for various administrative faults, financial constraints and technical reasons (Sharma and Mehrotra, 1985). Initially for several years malaria cases started to multiply quite insidiously, and at that time NMEP and the general health services were not prepared to tackle the situation effectively. As a result over the years malaria resurgence and focal outbreaks occurred even in areas that were at one time freed from the disease. To combat malaria the Government of India in 1977 implemented the modified plan of operations (MPO) through the NMEP, thus converting the malaria eradication programme to that of control and containment (Pattanayak and Roy,
The implementation of MPO resulted in the steady decline of malaria cases from 6.4 million in 1976 to 2 million cases in 1984. It may be noted that although there was reduction in total malaria cases in the country but the incidence of *P. falciparum* has remained at about 0.5 million cases annually for several years now (Sharma, 1984a).

Control of malaria in the country is beset with many formidable problems e.g., insecticide resistance in malaria vectors, exophilic and exophagic behaviour of mosquitoes, escalating cost of insecticides, drug resistance in the malaria parasite *P. falciparum* and operational failures etc. As a result the available methods of malaria control have had limited success. It is commonplace to find sudden spurt of malaria where insecticidal spraying was withdrawn (e.g. Haryana State, Sharma et al., 1983) or in areas where vector species developed high degree of resistance (e.g., Shahajahanpur U.P., Chandrahas and Sharma, 1983). Use of replacement insecticides can control malaria for some time (e.g., malathion spraying in Kharkhoda PHC, Haryana, Subbarao et al. 1984), but vectors have the ability to develop multiple resistance resulting in operational failures (e.g. areas in Gujarat and Maharashtra, Sharma, 1984a). While bio-environmental methods to control malaria are being
developed, malaria vaccines are emerging as the most powerful tools to help control malaria. The availability of three types of malaria vaccines are being contemplated i.e., (i) the sporozoite vaccine (ii) asexual blood stage vaccine, and (iii) the transmission blocking vaccine. In the coming decade or so, all the three vaccines may be available, but sporozoite vaccine is emerging as the first most likely vaccine to be made available for clinical trials. Therefore, immunization against malaria is now becoming a reality. It is against this background that there is an urgency to make appropriate preparations to undertake trials and plan for the integration of malaria vaccines with the primary health care system to help realize the Alma Ata declaration of health for all by 2000 A.D.

IMMUNIZATION AGAINST MALARIA

India is a thickly populated country. Latest census (1981) reported 684 million population with a density of 221 persons/sq.km. and 2.47% growth rate. Estimates in 1985 place India's population to about 700 million. The current estimates are that India's population would increase to 799 million in 1991 and 917 million in 2000 A.D. (476 million population in rural and 243 million population in
urban areas). It may also be mentioned that 78% population lives in 57,936 villages and half of these villages have a population of 500 or less. Some villages are located in remote and inaccessible areas. There are at least 1,643,100 problem villages which have no facility of potable drinking water. Against this background, malaria vaccination is a formidable task. Even the immunization of children (up to 14 years) would be an uphill task as this group alone constitutes about 39% of the total population (273 million).

In India, _P.vivax_ is the most dominant infection (70%) followed by _P.falciparum_ (30%) and only small number of _P.malariae_ cases occur every year. _P.falciparum_ is widely distributed throughout the country with great variation from one region to another. About 40% of all _P.falciparum_ infections occur in one state (Orissa) alone. The entire northeastern region has very high incidence of falciparum malaria. To contain/liquidate _P.falciparum_ and prevent its spread to other areas the _Plasmodium falciparum_ Containment Programme (PfCP) was launched in 1977 (Ray, 1979). PfCP is currently working in 94 million population living in 79 districts (Sharma, 1984a). As a result, while there is a considerable improvement in areas under PfCP, falciparum malaria is on the increase in rest of the country (A.P. Ray, personal communication). Limited surveys conducted by Malaria Research Centre (MRC) revealed that the incidence
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other immunizations. The EPI work of MPWs is supervised by an EPI supervisor. At the PHC level medical officer coordinates and executes the programme. Immunization against malaria would have to follow the same pattern as set out for other immunizations under the EPI. In many malaria endemic areas, work load may increase considerably and therefore additional staff may be required. To strengthen primary health care a village health guide scheme is being implemented. Under the scheme it is proposed to train one health guide for every 1,000 population or for each village. Depending on the ease with which malaria vaccines could be delivered, the village health guide could also be trained and assigned the duties of malaria vaccination. The present difficulties faced by EPI in the maintenance of cold chain must be removed, if vaccination is to be ensured at the periphery and cold chain is an essential requirement for malaria vaccines.

The scope of the integration of malaria vaccines with other methods of malaria control may be examined with reference to the control of malaria in urban and rural areas. In 1971-72, urban malaria scheme (UMS) was launched in the country. Under UMS the mainstay of malaria control is by anti-larval methods. At present 125 towns with a population of 43 million are covered under the scheme (Pattanayak et al. 1981). The rural malaria control is mainly achieved by spraying residual insecticides to
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urban malaria as the vector breeding sites are well defined. Therefore integration of malaria vaccines with the urban malaria scheme would be the best answer to the control of urban malaria in India.

Similarly, a study during 1981-82 in Kharkhoda PHC revealed extremely high incidence of malaria, although villages were under BHC spray for several years. During the same period, out of 9124 A. culicifacies dissected, 83 specimens were found positive for the sporozoites. In such a situation immunization of entire community may be very difficult and expensive. Also if the protection from malaria is short lived, there may be very little value of immunization. In contrast, if malaria vaccines are administered in an area where transmission is interrupted, malaria can be very much reduced or even eliminated. For example in Kharkhoda villages where malaria transmission was intense, three rounds of malathion spraying (2 g/m²) in 1982 produced dramatic reduction in malaria cases. And out of 1245 specimens dissected, only one mosquito was found positive for the sporozoites (Choudhury, 1984). Malathion is still being sprayed in this area. During 1983 in 18 trips, and 66 man hour collections, only 6 A. culicifacies could be collected. Similarly in 1984 only 5 A. culicifacies could be collected (D.S. Choudhury, personal communication). The vector A. culicifacies which was fully susceptible to malathion has been nearly eliminated and the transmission of
malaria completely interrupted. During 1984, there was no case of malaria as against a few hundred cases per thousand population per month during the transmission season. Such malaria free areas need to be protected from the introduced malaria. In achieving this objective malaria vaccines would have immense value, if applied judiciously. In such areas spraying may be terminated and surveillance strengthened to detect every possible case of malaria. All malaria positive cases and people living in neighbourhood may be vaccinated to prevent the buildup of secondary cases. Withdrawal of spraying would drastically reduce cost and retain the susceptibility status of the vectors against the replacement insecticides which are more toxic and many times more expensive. These insecticides could be held in reserve to tackle any sudden spurt of malaria in areas freed from the disease.
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INTEGRATION OF MALARIA VACCINES IN THE HEALTH DELIVERY SYSTEM IN INDIA

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ABSTRACT

Malaria control and the extended programme of immunization (EPI) are integral to the primary health care system in India. As and when mass immunization against malaria becomes feasible, the delivery of malaria vaccines should be merged with the EPI. This may require additional inputs and strengthening of the existing infrastructure to develop malaria vaccination as a dedicated system at the village level. Priority groups for vaccination and the integration of malaria vaccines as an adjunct to other methods of malaria control have been discussed.

INTRODUCTION

In ancient India malaria was known as the king of tropical diseases. Malaria was the single most important cause of the biggest misfortune degrading the life of people in every possible way. Till 1952, malaria was directly responsible for about 75 million cases and 800,000 deaths annually, with considerable increase in the morbidity and mortality due to malaria during the epidemic years. Malaria was nearly eradicated from the country in the early 1960's as a result of the nationwide anti-malaria campaign launched by the National Malaria Control/Eradication (NMCP/NMEP) of the Government of India. Still in 1965 there were at least 100,000 parasite positive cases of malaria scattered all over the country. This followed a period of resurgence for various administrative faults, financial constraints and technical reasons (Sharma and Mehrotra, 1985). Initially for several years malaria cases started to multiply quite insidiously, and at that time NMEP and the general health services were not prepared to tackle the situation effectively. As a result over the years malaria resurgence and focal outbreaks occurred even in areas that were at one time freed from the disease. To combat malaria the Government of India in 1977 implemented the modified plan of operations (MPO) through the NMEP, thus converting the malaria eradication programme to that of control and containment (Pattanayak and Roy,
The implementation of MPO resulted in the steady decline of malaria cases from 6.4 million in 1976 to 2 million cases in 1984. It may be noted that although there was reduction in total malaria cases in the country but the incidence of *P. falciparum* has remained at about 0.5 million cases annually for several years now (Sharma, 1984a).

Control of malaria in the country is beset with many formidable problems e.g., insecticide resistance in malaria vectors, exophilic and exophagic behaviour of mosquitoes, escalating cost of insecticides, drug resistance in the malarial parasite *P. falciparum* and operational failures etc. As a result the available methods of malaria control have had limited success. It is commonplace to find sudden spurt of malaria where insecticidal spraying was withdrawn (e.g. Haryana State, Sharma et al., 1983) or in areas where vector species developed high degree of resistance (e.g., Shahajahanpur U.P., Chandrahas and Sharma, 1983). Use of replacement insecticides can control malaria for some time (e.g., malathion spraying in Kharkhoda PHC, Haryana, Subbarao et al. 1984), but vectors have the ability to develop multiple resistance resulting in operational failures (e.g. areas in Gujarat and Maharashtra, Sharma, 1984a). While bio-environmental methods to control malaria are being
developed, malaria vaccines are emerging as the most powerful tools to help control malaria. The availability of three types of malaria vaccines are being contemplated i.e., (i) the sporozoite vaccine (ii) asexual blood stage vaccine, and (iii) the transmission blocking vaccine. In the coming decade or so, all the three vaccines may be available, but sporozoite vaccine is emerging as the first most likely vaccine to be made available for clinical trials. Therefore, immunization against malaria is now becoming a reality. It is against this background that there is an urgency to make appropriate preparations to undertake trials and plan for the integration of malaria vaccines with the primary health care system to help realize the Alma Ata declaration of health for all by 2000 A.D.

**IMMUNIZATION AGAINST MALARIA**

India is a thickly populated country. Latest census (1981) reported 684 million population with a density of 221 persons/sq.km. and 2.47% growth rate. Estimates in 1985 place India's population to about 700 million. The current estimates are that India's population would increase to 799 million in 1991 and 917 million in 2000 A.D. (476 million population in rural and 243 million population in
urban areas). It may also be mentioned that 78% population lives in 575936 villages and half of these villages have a population of 500 or less. Some villages are located in remote and inaccessible areas. There are at least 164310 problem villages which have no facility of potable drinking water. Against this background, malaria vaccination is a formidable task. Even the immunization of children (up to 14 years) would be an uphill task as this group alone constitutes about 39% of the total population (273 million).

In India, \textit{P. vivax} is the most dominant infection (70%) followed by \textit{P. falciparum} (30%) and only small number of \textit{P. malariae} cases occur every year. \textit{P. falciparum} is widely distributed throughout the country with great variation from one region to another. About 40% of all \textit{P. falciparum} infections occur in one state (Orissa) alone. The entire northeastern region has very high incidence of falciparum malaria. To contain/liquidate \textit{P. falciparum} and prevent its spread to other areas the \textit{Plasmodium falciparum} Containment Programme (PfCP) was launched in 1977 (Ray, 1979). PfCP is currently working in 94 million population living in 79 districts (Sharma, 1984a). As a result, while there is a considerable improvement in areas under PfCP, falciparum malaria is on the increase in rest of the country (A.P. Ray, personal communication). Limited surveys conducted by Malaria Research Centre (MRC) revealed that the incidence
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REFERENCES


INTEGRATION OF VACCINE WITH OTHER MALARIA CONTROL MEASURES IN NORTHERN THAILAND

Udoa Chitpraprap
Malaria Center
Chiangmai, Thailand

In Northern Thailand malaria was previously the number one cause of death. In the 1930's the annual malaria death rate sometimes reached 1,500 per 100,000 population. The malaria control program of the 1950's and the eradication program of the 1965's succeeded in lowering the death rate to its present level of 0.8-1.0 per 100,000. Malaria remains a major public health problem, because of transmission in forested and foothill areas. About 20-30,000 cases are detected annually. Of these, 98% come from forested and foothill areas. These are mainly forest working villagers, such as wood cutting, hill farmers, etc.

The two main vectors, An minimus, and An dirus (An balabacensis), bite and rest mostly outdoor. P. falciparum is the predominant parasite species (60-70%). The combination of the villagers' temporary migration to the forests and neighboring countries, outdoor transmission, and drug pressure caused P. falciparum to develop and spread drug resistance, first to chloroquine, then sulfadoxine-pyrimethamine (Fansidar), and now quinine (some reported cases).

The current main control measures are residual DDT spraying in high receptivity villages, case detection, and treatment. Supplementary measures include promotion of community self-protection using larvivorous fish, mosquito nets, and repellents.

Vaccination, if effective and available, would be included as an additional measure in this integrated control program. Vaccines would be used only with the high risk group of villagers who work in forested and foothill areas. We estimate this group to comprise about five to ten percent of the total population. By system of working, all of the malaria cases were investigated so we got information already about who, where and when these group of villagers going to work in the forest outside the village. The only problem that may arise is that when the number of malaria cases was reduced to a low level, it is very difficult to conclude that it was from vaccination or from another one from the integrated control program.
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<th><strong>MALARIA CASES, 1984</strong></th>
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<td><strong>NORTHERN THAILAND (CHIANGMAI REGION)</strong></td>
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<td>1.</td>
<td><strong>POPULATION</strong></td>
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<td><strong>P. falciparum</strong> 68.7%</td>
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<td><strong>P. vivax</strong> 30.9%</td>
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<td><strong>P. malariae</strong> 0.1%</td>
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<td><strong>Mixed</strong> 0.3%</td>
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<td>10+ yrs. 92.37%</td>
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<td>-In the village 3.3%</td>
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<tr>
<td></td>
<td>-In the forest 89.0%</td>
</tr>
<tr>
<td></td>
<td>-Neighboring countries 7.7%</td>
</tr>
</tbody>
</table>
## TABLE II: MEASURES FOR CONTROL MALARIA IN NORTHERN THAILAND (CHIANGMAI REGION)

<table>
<thead>
<tr>
<th>Measures</th>
<th>receptivity of area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Residual Spray</td>
<td></td>
</tr>
<tr>
<td>2. Case Detection and Treatment</td>
<td></td>
</tr>
<tr>
<td>- Village Volunteer</td>
<td>X</td>
</tr>
<tr>
<td>- Midwifery Center</td>
<td>X</td>
</tr>
<tr>
<td>- Health Center</td>
<td>X</td>
</tr>
<tr>
<td>- Hospital</td>
<td>X</td>
</tr>
<tr>
<td>- Malaria Clinic</td>
<td>X</td>
</tr>
<tr>
<td>- Special Case Detection</td>
<td></td>
</tr>
<tr>
<td>3. Additional Measures</td>
<td></td>
</tr>
<tr>
<td>- Larvivorous Fishes</td>
<td>X</td>
</tr>
<tr>
<td>- Self Protection</td>
<td>X</td>
</tr>
<tr>
<td>4. Vaccination ?</td>
<td>Only high risk groups</td>
</tr>
</tbody>
</table>
INTEGRATION OF VACCINE WITH OTHER MALARIA CONTROL PROGRAMS

1. INTRODUCTION

Our population now is 54 million. Around 15.4 million lives in areas still endemic more or less of malaria. Of the 15.4 million, 55% or 8.47 million are children below 14 years old; 5% or 770,000 are women of child-bearing age; and another 5% lives in developing areas such as mining, logging, irrigation and road construction camps, and agricultural resettlement areas.

2. MORBIDITY AND MORTALITY OF MALARIA

The number of malaria cases, deaths by age-group, sex and rates per 100,000 population in 1977 is shown in Table 1. There were 7,597 (45.7%) malaria cases and 15 (27%) deaths in males below 14 years old as compared with 5,224 (39.2%) cases and 137 (35%) deaths in females.

<table>
<thead>
<tr>
<th>AGE &amp; SEX</th>
<th>Male Cases</th>
<th>Female Cases</th>
<th>Both sexes Cases</th>
<th>Male Deaths</th>
<th>Female Deaths</th>
<th>Both sexes Deaths</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL AGES...</td>
<td>16625</td>
<td>13330</td>
<td>29955</td>
<td>66.5</td>
<td>585</td>
<td>389</td>
<td>974</td>
</tr>
<tr>
<td>Under 1 yr...</td>
<td>382</td>
<td>373</td>
<td>755</td>
<td>46.9</td>
<td>29</td>
<td>30</td>
<td>59</td>
</tr>
<tr>
<td>1 - 4 yrs...</td>
<td>2394</td>
<td>1586</td>
<td>3980</td>
<td>67.0</td>
<td>47</td>
<td>46</td>
<td>93</td>
</tr>
<tr>
<td>5 - 9 yrs...</td>
<td>2361</td>
<td>1599</td>
<td>3960</td>
<td>61.9</td>
<td>46</td>
<td>42</td>
<td>88</td>
</tr>
<tr>
<td>10-14 yrs...</td>
<td>2460</td>
<td>1666</td>
<td>4126</td>
<td>74.6</td>
<td>33</td>
<td>19</td>
<td>52</td>
</tr>
<tr>
<td>15-19 yrs...</td>
<td>2361</td>
<td>1586</td>
<td>3947</td>
<td>86.8</td>
<td>39</td>
<td>38</td>
<td>77</td>
</tr>
<tr>
<td>20-24 yrs...</td>
<td>1546</td>
<td>1506</td>
<td>3052</td>
<td>77.1</td>
<td>63</td>
<td>23</td>
<td>86</td>
</tr>
<tr>
<td>25-29 yrs...</td>
<td>931</td>
<td>1239</td>
<td>2170</td>
<td>61.8</td>
<td>65</td>
<td>31</td>
<td>96</td>
</tr>
<tr>
<td>30-34 yrs...</td>
<td>964</td>
<td>804</td>
<td>1768</td>
<td>62.2</td>
<td>40</td>
<td>36</td>
<td>76</td>
</tr>
<tr>
<td>35-39 yrs...</td>
<td>831</td>
<td>746</td>
<td>1577</td>
<td>68.9</td>
<td>40</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>40-44 yrs...</td>
<td>632</td>
<td>586</td>
<td>1218</td>
<td>64.6</td>
<td>31</td>
<td>16</td>
<td>47</td>
</tr>
<tr>
<td>45-49 yrs...</td>
<td>399</td>
<td>493</td>
<td>892</td>
<td>57.9</td>
<td>29</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>50-54 yrs...</td>
<td>299</td>
<td>319</td>
<td>618</td>
<td>46.4</td>
<td>30</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>55-59 yrs...</td>
<td>316</td>
<td>186</td>
<td>502</td>
<td>41.0</td>
<td>28</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>60-64 yrs...</td>
<td>299</td>
<td>200</td>
<td>499</td>
<td>52.8</td>
<td>16</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>65-69 yrs...</td>
<td>216</td>
<td>199</td>
<td>415</td>
<td>65.3</td>
<td>15</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>70+ &amp; over...</td>
<td>201</td>
<td>213</td>
<td>414</td>
<td>50.5</td>
<td>23</td>
<td>17</td>
<td>40</td>
</tr>
</tbody>
</table>

Not Stated... 33 22 62 - 11 2 13 -

There were also 9,028 (54.3%) malaria cases and 430 (74%) deaths in males as against 8,106 (60.8%) cases and 252 (65%) deaths in females in the 15 years old and over. In all the ages there were 585 (60%) deaths in males as against 389 (40%) deaths in the females.
In 1984, the annual parasite incidence of malaria was 7.2/1000 population with \textit{P. falciparum} (65\%) predominating.

3. PROBLEMS

3.1 DDT now costs US $2.80 per kilo as against US $2.00 in 1983 and US $1.30 per kilo in 1982;

3.2 Reluctance of the people now to DDT residual house spraying;

3.3 Some environmentalists are questioning DDT residual house spraying;

3.4 Spraying operation is now costly due to increased cost of freight and fuel oil;

3.5 Difficulty of spraying operation due to lack of transport;

3.6 There is a high turnover of volunteer village spraymen under the Primary Health Care (PHC) approach;

3.7 Incomplete treatment of malaria cases due to frequent movement of population;

3.8 Preference of injectable antimalarial drugs to the tablets by some people especially those traders to Sabah;

3.9 Widespread \textit{P. falciparum} resistance (70\%) to the 4-aminoquinoline drugs.

4. RECOMMENDATIONS

Integration of vaccines with some malaria control programs is therefore a welcome additional approach. At present vaccination is given by the expanded program on immunization (EPI) of the Ministry of Health. Malaria Control is already integrated into the Field Health Services and the malaria vaccines will also be given in the Barangay Health Stations, Primary Health Centers, Dispensaries, Clinics; in the District Hospitals and in the Provincial and Regional Hospitals. In the event that the vaccines may not be adequate then the priority would be the 1) children below 14 years old comprising 55\%; 2) the women of child-bearing age comprising 5\%; 3) the people living in developing areas such as mining, logging, irrigation and road construction camps, and agricultural resettlement areas.

For the clinical field trials to evaluate first the vaccines, we welcome and will gladly join the team to implement it according to formulated guidelines. At the moment, we are proposing either Tayabas or Palawan as possible site. The epidemiological profile of Tayabas is shown in Table 2. There were 3,311.0 (50.04\%) cases in 1984 in the 14 years old and below as against 3,301 (49.95\%) cases in the 15 years old and over with 25\% \textit{P. falciparum}. Tayabas is 100 miles south of Manila and it is very accessible throughout the year by vehicle on concrete road.
The epidemiological profile of Palawan is shown in Table 3. In 1984, there were recorded 1,762 (59.56%) cases in the 14 years old and below as against 1,196 (40.5%) cases in the 15 years old and over with 71% *P. falciparum* cases. Palawan is southwest of Manila and it is one hour flight by plane. There are very good electric, water and housing facilities in both sites.

**Table 2. Malaria Cases, Tayabas, Quezon by Age-Group, Sex, Species and Rates, 1984**

<table>
<thead>
<tr>
<th>AGE-GROUP</th>
<th>P. f.</th>
<th>P. v.</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>0 - 11 mos.</td>
<td>(1.44)</td>
<td>(1.7)</td>
<td>(1.8)</td>
</tr>
<tr>
<td>12 - 23 mos.</td>
<td>(1.06)</td>
<td>(2.4)</td>
<td>(2.0)</td>
</tr>
<tr>
<td>2 - 9 yrs.</td>
<td>(26.9)</td>
<td>(32.8)</td>
<td>(28.4)</td>
</tr>
<tr>
<td>10-14 yrs.</td>
<td>(17.79)</td>
<td>(16.3)</td>
<td>(16.7)</td>
</tr>
<tr>
<td>15 yrs.</td>
<td>549</td>
<td>296</td>
<td>1478</td>
</tr>
<tr>
<td>&amp; Over</td>
<td>(52.8)</td>
<td>(46.9)</td>
<td>(51.2)</td>
</tr>
<tr>
<td>TOTAL =</td>
<td>1040</td>
<td>632</td>
<td>2888</td>
</tr>
<tr>
<td>%</td>
<td>(15.72)</td>
<td>(9.56)</td>
<td>(43.66)</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>0 - 11 mos. N=</td>
<td>20</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(1.9)</td>
<td>(2.4)</td>
<td>(3.1)</td>
</tr>
<tr>
<td>12-23 mos. N=</td>
<td>40</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>(3.7)</td>
<td>(3.5)</td>
<td>(4.3)</td>
</tr>
<tr>
<td>2 - 9 yrs. N=</td>
<td>364</td>
<td>389</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>(34.1)</td>
<td>(37.6)</td>
<td>(31.9)</td>
</tr>
<tr>
<td>10-14 yrs. N=</td>
<td>194</td>
<td>173</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>(18.2)</td>
<td>(16.7)</td>
<td>(18.2)</td>
</tr>
<tr>
<td>15 yrs. &amp; over</td>
<td>449</td>
<td>412</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>(42.1)</td>
<td>(39.8)</td>
<td>(42.5)</td>
</tr>
<tr>
<td>TOTAL N=</td>
<td>1067</td>
<td>1035</td>
<td>419</td>
</tr>
<tr>
<td></td>
<td>(36.1)</td>
<td>(34.8)</td>
<td>(14.2)</td>
</tr>
</tbody>
</table>
While the vaccines against the sporozoites, gametes and the blood asexual stages (merozoites) especially of *P. falciparum* will have their respective indications, advantages, probably limitations as well as difficulties of application, our greatest constraints will be lack of funds, transports and other logistics to support the program.
Friday, April 26, 1985
Abstract No. 61

Dr. Bruce-Chwatt Discussant
Friday morning, April 26th

Before I start, will you allow me, Sir, to say a few words about my intervention of the previous meeting. I had a second thought about what I said about precipitin tests. And I realize now that while precipitin tests of the old kind may not be applicable any more, newer methods of gel electrophoresis for finding out the origin of blood by Anopheles and other mosquitoes have still a very important place in entomology generally, and especially in entomology related to distributional viral diseases. And I'd like to apologize to my dear friend, Dr. Meuwissen, for having upset him. Thank you.

While the problem of integrational vaccine with other antimalaria measures as part of an antimalaria program has been most ably assessed by our colleagues from China, from Thailand, from India and from the Philippines respectively, and the opinions that were expressed by them were very close, really. Our colleague from China expressed himself forcibly, and I quote, "Vaccine should not be viewed simply as an alternative to, but must be utilized on the basis of its distinctive properties to seek strategic combined effect."

This is a very important and a very valid statement, with which few would disagree. Dr. Senqui limits the use of prospective vaccine to chloroquine resistant areas for immunization of stubborn resistant foci, or for a suppression of an epidemic focus.

The same is the theme of our colleague from India who pointed out the situation in India at the present time and limited the same type of activity for India. I'm not sure when he said that vaccines should be used for protection of tourists, whether he meant, really, the use of vaccine for prophylaxis of tourists exposed to malaria. I doubt very much if one million tourists who come to India at the present time, would let themselves to these prophylactic measures. I can't help feeling that other, perhaps, less drastic methods of protection of tourists, which is booming in India at the present time, are perfectly adequate.

In northern Thailand, our colleague who presented most ably the situation in Chiangmai area, once again proposed the vaccines in drug-resistant areas; specifically, in those areas where transmission is carried by Anopheles dirus and limited to high-risk groups in forested and foothills areas, which are operationally extremely difficult to cover. These groups combine 5% of the population, and quite certainly, this is probably the best method for malaria control in those areas.
In the Philippines, once again, the use of vaccines will be restricted to areas such as mining, logging, road construction groups and areas that are difficult of access. I cannot help feeling that those four presentations pinpointed very neatly the specific role of malaria vaccines for malaria control in particularly tricky areas. I'm referring to what they said, especially our colleagues from the Philippines, about malaria control within the primary health system. And there is not the slightest doubt that the support of health services in malaria control in this respect is paramount. This really refers to all four areas that we have heard about. This support will depend on the structure of the primary health system, on the epidemiological situation, on the aims of the control strategy and on the availability of resources. The selection and the definition of specific antimalarial measures should be based on a good knowledge of epidemiology of the area and of the communities involved. As you will probably remember, one of the latest reports of the WHO on malaria control within the matrix or within the orbit of primary health care, stressed again and again the importance of involvement of the community and no better example of the involvement of the community on malaria control could be found today than in China. In China, this is a supreme example of very close cooperation between the vertical services and between the horizontal services of primary health care.

This study group defined and classified the most important antimalarial measures and stressed that their use should not be a study but a dynamic process continuing hand-in-hand with the development of socioeconomic conditions within those areas. While the relationship of objectives of malaria control to selection of technological resources is very closely related and very often, new technological advances dictate the operational changes of malaria control. That is what I mean by stressing the dynamic relationship between the two. This has been seen during the past few years in many countries, which made adjustments of their programs in the course of transition from malaria eradication down to the level of malaria control. And once again, I'd like to say what I said a few years ago that it is very wrong to consider malaria control as a poor man's malaria eradication. Not at all. Malaria control in many, many ways is a much more difficult, and much more intelligent, and much more productive activity than malaria eradication, which went by the rules by one book - let us pray and hope for the best. In malaria control, the situation is very different. You need people who know intimately the area involved, who know the socioeconomic, the epidemiological, the political conditions, and who know how to adjust specific malaria control to the situation of the area. So, in many ways, we are coming back, and perhaps, just as well, to the situation before malaria eradication, where much more intelligent approach to malaria control - a tailor-made approach to malaria control, is needed. And this can be done only if there is very close cooperation.
with the communities involved, with the national government and let's not forget it, with the training facilities for the new generation of both malariologists and community workers.

The availability of anti malaria vaccines will also change many tactical approaches to malaria control, but the actual trends of these changes will become fully evident only when all the characteristics of new vaccines are better known. At the present time, we really don't know what are the characteristics of new vaccines. We don't know the specificity of this or that vaccine, we don't know the duration of effect of this or that vaccine, we don't know the possibility of using them in hot countries and whether there is any need of cold chain. And these are technical problems that will have to be looked into very closely. Nevertheless, one could presume that anti-sporozoite vaccine will be used chiefly for protection of non-immune immigrant populations, or particularly vulnerable groups of indigenous community exposed to high degree of transmission, with few, if any, possibilities of control, in the same way as antimalarial drugs were used on a wide scale before resistance to those drugs became evident. The prospective gamete-reactive vaccine will find its use for hopeful interruption of transmission during epidemics or at the start of an intensive control program using, perhaps, other methods, very likely, in conjunction with some drugs or with environmental control methods or many other methods that are available.

On the other hand, the merozoite or asexual erythrocytic vaccine will probably be used depending on its specificity, on its duration of action, as an alternative, or perhaps an addition to schizonticidal drugs for intensive control of epidemic malaria and for large scale treatment of seasonal waves of malaria. Much of the future use of vaccines for these or other ways of attack or prevention of malaria, will depend in part from biological characteristics of these products. They will have been on their cusp. This was stressed recently by Don Henderson when talking at the meeting on Expanded Program of Immunization, and I'd like to add a few words to this point.

While any reflections on the cost of the vaccine are purely speculative at the present time, and, really, they have nothing to do with practicability of its introduction. However, what I propose to say may serve as an indicator of the realities. And it's based on information from the WHO Expanded Program of Immunization, which is now in its fourth year. Now, this Program, as you probably know, refers to six target diseases; namely, diphtheria, pertussis, tetanus, measles, poliomyelitis and tuberculosis. When it comes to costs, we know that none of the current vaccines are ideal.

Now, when it comes to tuberculosis, it is assessed that the TB vaccine is relatively expensive. It costs US$0.50 per dosage when bought through UNICEF in large quantities. It requires only one dose and can be administered from birth. Its inconvenience lies that there must be very great care when using it, because in some cases, it induces side effects. And, there is still the persisting concern about the efficacy of this vaccine in other groups, and perhaps, in some parts of the world, as India, as you probably know.
Now, let's come to measles. Measles vaccine is still the most expensive of vaccines, although its price has come down quite considerably during the last few years. The recent cost was US$0.07 per dose when purchased through UNICEF on international tender. The major drawback - in developing countries - is that they need to wait until maternal antibodies have subsided before giving it to the child, while in developing countries, the average age of onset of measles is often above the age of three years. In developing countries, we have only a narrow window of opportunity after the age of 9 months, when the maternal antibodies have disappeared and when, during the very brief period of, perhaps, 6 months or 1 year, this vaccine can be given. A further problem is that, by the time a child in developing countries has reached the age of 9 months, the mother or other guardian might find it very difficult to come to a health facility because, as you say, the child is too young to walk and too heavy to carry. So, this very simple problem may be a drawback for measles vaccination.

The diptheria, tetanus and pertussis vaccine costs only US$0.02 per dose, and considering the primary immunization consists of three doses, the cost of a fully immunized child costs about the same as measles vaccine. That's about US$0.07 per dosage.

The trivalent oral poliomyelitis vaccine is very cheap. It costs only US$0.02 per dosage, and the cost, therefore, is the same as DPT. However, this is the least stable of vaccines and it has been found in the field that it can withstand only one day at the temperature of 37° before losing potency. Of course, there is a new inactivated vaccine against poliomyelitis, Professor Salk's vaccine, which is much more expensive. It costs at the present time US$0.50 per dosage, which is tenfold the price of three doses of the oral vaccine. I'm quite sure that the cost of it is going to decrease, and I'd like to stress what Dr. Luc Perrin has said at one of our meetings, that those costs today mean very little the more the vaccines are going to be developed, the more widely they will be used, the cheaper they will become.

But this brings me to another rub. While the vaccines themselves pound for pound, so to speak, are cheap, the costs of their use, the cost of their application is quite high. And it has been assessed that the cost of any of those vaccines that are used when the transport and especially the cost of labor because the cost of their use with the health personnel is included, those vaccines come to a very much higher figure - on the order of $5.00 per protected child. Now, this is a very high figure. If you remember, that in developing countries today, especially in Africa, which I know best, the cost of all health and hospital services for any of the African countries is on the order of not more than $2.00 per person per annum. Well, this is going to be one of the considerable problems that we are facing.
The other point that I would like to stress is that most of those vaccines are usually sold to the countries either by UNICEF or they are donated by a number of countries. Which means that, while the low cost of vaccine comes from the outside, the cost of its utilization in the field, the cost of its application, falls on the shoulders of the developing countries themselves, and this is one of the major problems in expanded immunization. And, when it comes to malaria, the same sort of problem will come to our attention and will have to be solved in one or the other way.
INTEGRATION OF VACCINE WITH OTHER MALARIA CONTROL PROGRAMS

David Clyde, Rapporteur

While it has been stressed that clinical trials of malaria vaccines should preferably be undertaken in the absence of other anti-malarial measures, particularly chemotherapy which might confuse the evaluation, nevertheless in respect of operational use of proven vaccines there is unanimous agreement that this will complement and not replace standard control measures. The use of vaccination together with other methods for the control of malaria is justifiable, provided that the overall methodology is appropriate to the epidemiological situation in the locality and community.

To achieve malaria control within primary health care systems requires the support of the expert malaria unit of the health service. This support will depend on the structure of the primary health care system, on the epidemiological situation, on the aims of control strategy and on availability of resources. Selection and definition of specific antimalaria measures should be based on knowledge of the communities involved, and these measures should be adapted dynamically to the changing situation. The relationship of objectives of malaria control to selection of technological resources is interrelated, and new advances may dictate operational changes. This has been seen in recent years in many countries whose programs were adjusted from eradication to control: thus, case detection and presumptive treatment have been taken up by primary health care workers instead of the former surveillance staff, and large-scale residual spraying has been replaced in many situations by focal spraying.

The availability of vaccines will change many tactical approaches to malaria control, but the changes will of course depend upon the particular characteristics of the vaccines in respect to stage and species protection and duration.

Nevertheless, it may be assured that an anti-sporozoite vaccine will be used mainly to protect non-immune immigrants or particularly vulnerable groups of the indigenous community, exposed to high degrees of transmission. The gamete-reactive vaccine will be intended for interruption of transmission, during epidemics or at the start of intensive control programs using other methods. The asexual erythrocytic vaccine would be used (depending on its specificity and duration of action) as an alternative or addition to schizontocidal drugs for intensive control of epidemic malaria or for large scale treatment of seasonal waves of the disease.
Much of the use of vaccines for these purposes will depend on their cost. This may become a critical element in deciding the feasibility of malaria vaccination in the developing countries. It should also be appreciated that the cost of the vaccine is only a small part of the utilization—the cost of administrations of the vaccine is much greater and falls on the national administrations.

The diversity in development of malarious countries (in respect of managerial abilities, public awareness, budgets) will affect implementation of all malaria integration measures including vaccination. Care should be taken that combining of several procedures and methods will not lead to a confused inability to implement and evaluate each.

A consideration of integration of vaccine with other anti-malaria methods in China, India, and the Philippines and Thailand follows:

In China, it is felt that vaccine will play a distinctive role in malaria programs when integrated with other control measures, although the latter are expected to continue to be the main approaches in the future. Vaccination should, however, not be viewed simply as an alternative tool, but must be utilized with other control measures to seek a strategic combined effect.

Malarious areas in China are continually diminishing and transmission intensity, except during outbreaks, has greatly reduced. Such conditions are favorable to the utilization of vaccine. As P. vivax is predominantly prevalent in this country, vaccine with P. vivax immunogens will be greatly desirable, although P. falciparum vaccine may be brought into full play in falciparum malarious areas, particularly in areas with chloroquine resistant strains. Polyvalent vaccine will be needed in the future malaria program. No intensive deployment of vaccine will be necessary, but besides vaccination of selected groups designed to be protected, vaccine should be used in conjunction with other control measures, according to local epidemiological conditions, to curb transmission and to solve problems formerly unsolved whenever possible. In China, vaccine may be used in the following situations:

1. Vaccination of people from non-malarious or hypoendemic areas going to places which are still malarious, especially in chloroquine resistant P. falciparum areas, to give them protection while staying in the area and to prevent them from spreading the disease agent when leaving the area.

2. Integrated with other control measures, it may be used as reinforcement measure in localities where traditional measures did not provide desired results. The combined measures should be well-planned to effect strategic attack aiming at interruption of transmission when feasible. Such strategy may be used in chloroquine resistant P. falciparum areas, especially where local vectors are difficult to control.

3. Integrated into the malaria surveillance system, it may be employed as a reinforcement tool to expedite elimination of
stubborn residual foci. Immunization of the inhabitants in the focus and in the surrounding neighborhood will provide better conditions for quicker elimination of the focus by the other measures.

4. It may be used in conjunction with other control measures to suppress malaria outbreaks, especially post-eradication outbreaks which may occur sometimes. Vaccination of the population living in the neighborhood will prevent malaria spread, and provide favorable conditions for concentrating effort in suppressing the outbreak at its original site with appropriate measures.

In India, malaria control and the extended program of immunization (EPI) are integral to the primary health care system. When mass immunization against malaria becomes feasible, the delivery of malaria vaccines should be merged with the EPI. This may require additional inputs and strengthening of the existing infrastructure to develop malaria vaccination procedures at the village level.

During 1985 the estimated target population under EPI is 89 million (60-70% coverage), and this will increase to 140 million in 1990 when full coverage is aimed for. Although the national malaria program is implementing vector control measures in areas having Annual Parasite Incidences of 2.0 (per thousand population) or more---i.e. 342 million rural and 43 million urban inhabitants---nevertheless malaria affects all age groups, and more than 600 million people are at risk. Therefore the task involved in immunization against malaria is much greater than protection against communicable diseases under the EPI. The priorities of malaria may be considered in order of priority:

(i) PHCs showing high levels of \textit{P. falciparum} resistance to chloroquine;
(ii) Migratory labor populations which may introduce new parasite strains, particularly between N-E India and the central-eastern states;
(iii) Areas of green (agricultural) revolution, and industrial areas;
(iv) Areas of high \textit{P. falciparum} incidence;
(v) Infants and children

Primary health care is regarded as the main system for the delivery of health services in India, including the immunization program. The FHC service extends into the community, family, and to the individual. At the periphery, a health sub-center is staffed by one male and one female multipurpose worker (MPW). Among the other duties, the MPW female is responsible for the immunization of pregnant women and infants, while the male MPW has the responsibility of all other immunizations.
The EPI work of MPWs is supervised by an EPI supervisor. At the PHC level a medical officer coordinates and executes the program. Immunization against malaria would follow the same pattern as set out for other immunizations under EPI. In many malaria endemic areas, work load may increase considerably and therefore additional staff may be required. To strengthen primary health care a village health guide scheme is being implemented. Under the scheme it is proposed to train one health guide for every 1,000 population or for each village. Depending on the ease with which malaria vaccines could be delivered, the village health guide could also be trained and assigned the duties of malaria vaccination. The present difficulties faced by EPI in the maintenance of cold chain must be removed, if vaccination is to be ensured at the periphery and cold chain is an essential requirement for malaria vaccines.

The scope of integration of malaria vaccination with other methods of malaria control may be considered in respect of malaria control in urban and rural areas of India. In towns, anti-larval methods are the mainstay, while in rural areas spraying residual insecticides is the rule. In both of these strategies, integration of vaccines would produce dramatic results, as is suggested by observations being made in two studies in progress.

In the first study, an integrated vector control methodology has succeeded in interrupting transmission among 25,000 population in 7 villages. The study has now expanded to cover 350,000 people in 100 villages. Immunization of malaria positive cases, and vulnerable groups, would prevent reestablishment of transmission. Thus the villages would remain malaria free by integrating environmentally safe methods with malaria vaccines. By extension, it may be pointed out that when anti-larval methods succeed in interrupting transmission in such highly endemic and well irrigated rural areas as these, a good urban malaria program using similar methods will also succeed because the vector breeding sites are more readily defined and accessible. Therefore integration of malaria vaccines with the urban program would be likely to be highly effective.

In the second study, during 1981-82 an extremely high incidence of malaria prevailed in Kharkhoda PHC area, despite long-standing spraying of BHC(HCH). In this situation immunization of the entire population would be very difficult and expensive, and of little value if the duration of protection is brief. In contrast, if the vaccines are administered in an area where transmission has been interrupted, the disease would be greatly reduced if not eliminated. Such would occur in Kharkhoda PHC area now, since in 1982 intensive spraying of malathion produced a dramatic reduction in malaria cases. The freed area would be protected from reintroduction of malaria if vaccines were applied judiciously following termination of spraying and strengthening of surveillance. All malaria
positive cases, and people living in the vicinity, would be vaccinated thus preventing buildup of secondary cases.

In the Philippines, vaccination is the responsibility of the EPI of the Ministry of Health. Priority targets for anti-malaria vaccination would be children under 14 years of age (comprising 55% of the population in endemic malarious areas), women of childbearing age (5%), and people living in development areas (mining, logging, irrigation, road construction camps) and settlement areas (comprising 5%); high risk areas and vulnerable groups. Malaria control is integrated with the peripheral health services, permitting early utilization of malaria vaccination procedures. Such procedures could first be tested in Phase III and IV in Tayabas and Palawan areas.

Integration of malaria vaccines would be a useful additional malaria control measure, because 65% of the cases are P. falciparum, and existing measures are encountering major constraints, namely (1) increasing spread of P. falciparum resistant to 4-aminoquinolines, (2) increasing cost of insecticides, (3) difficulty in applying the insecticides by volunteer spray-men, (4) movements of population to and from malarious areas.

In Thailand, in the northern parts of the country malaria was previously the leading cause of death; control programs have, however, lowered this to its present level of 0.8-1.0 per 100,000 population. Malaria remains, nevertheless, a major public health problem due to transmission in the forested and foothill areas. About 20,000 to 30,000 cases are detected annually, 98% coming from the forested and foothill areas. P. falciparum is the predominant parasite, and the two main vectors, A. minimus and A. dirus are particularly difficult to control by insecticides as they rest outdoors.

The combined problems of villagers moving back and forth to the forests and neighboring countries where transmission is unchecked, outdoor transmission by A. minimus and A. dirus, and general resistance by P. falciparum to choloquine and now to replacement drugs, interfere with the efficacy of standard malaria control measures, which include DDT spraying in high receptivity villages, case detection, and treatment.

Malaria vaccination would be important as an additional measure in this integrated control program. Vaccines would be used only among the high risk group of villagers who work in forested and foothill areas, a group estimated to comprise 5-10% of the population.
C. STRATEGIES FOR FUTURE MALARIA VACCINE TRIALS - PANEL DISCUSSION

Moderator: Leonard J. Bruce-Chwatt
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Friday, April 26, 1985
Panel Discussion
Workshop Session V. Part C.
Strategies for Future Malaria Vaccine Trials

Moderator: Leonard Bruce-Chwatt

GENERAL DISCUSSION

The moderator, Dr. Bruce-Chwatt, assembled and outlined a tentative list of criteria that may merit consideration in selecting sites for Phase III malaria vaccine trials. He grouped the prospective criteria in the following 6 categories.

1. The site should involve a high level of malaria endemicity, a low level of in/out migration, at least two distinct epidemiological stratifications, reasonable ethnic homogeneity, a usual age distribution of the populace and, roughly, a total population of 10,000 to 15,000.

2. The site should offer reasonable land/air access, a reasonable level of basic health services, basic laboratory support capabilities and, if possible, proximity to a pertinent malaria research unit or center.

3. Circumstances should include approval of national, provincial, and local health authorities, cooperative relations with the study population, and assurances of an absence of political or comparable interference.

4. International, national, and local commitments for at least a 3-year period.

5. Availability or potential availability of requisite epidemiological and other baseline data.

6. Concurrence with utilization of a standardized "con" study protocol or set of protocols.

The ensuing general discussion dealt with the following 10 matters: 1) At present, there appears to be no reason to anticipate an association between parasite drug resistance and susceptibility to malaria vaccine effect; 2) the discussions at this and recent related other conferences on malaria vaccine development make a powerful case for coordination of efforts to orchestrate the plans for and conduct of different clinical studies to achieve reasonable overall coherence and prospective comparability/elements of standardization that might result could relate, for example, to the delineation of certain "core protocols" which than could be elaborated on to meet local needs and to central or common elements of epidemiological and other baseline data; 3) consideration of island populations to meet needs in relative isolation of study populations may be warranted; 4) costs should be a major factor in consideration of the vaccine development potential utility; 5) if one potential use of a malaria vaccine is to assist in malaria control in
specific areas in which drug resistance presents large problems, in such instances combining a sporozoite or merozoite vaccine with a gamete-blocking agent may merit special consideration to curtail or contain transmission; 6) initial and during-study use of chemotherapy in the study population and others in the area continues to deserve close attention; 7) elaborating explicit criteria for Phase III trials deserves high priority; 8) the role of various actual or potential commercial interests merits consideration; 9) pressure for premature adoption or utilization of vaccine preparations that appears to be effective will occur mainly after Phase III trials as "registration" of an agent occurs; 10) field studies of malaria vaccine preparations will entail challenges far more complex and difficult than those associated with other types of vaccine.
Rapporteur's Report

Initial Comments from Panel

1. Dr. Shou-pai Mao  People's Republic of China

Vaccine would be very useful to malaria control programs. Each country would have a definite target population in mind. For instance, in the People's Republic of China, there may be needs for use in military travelers and laborers moving from non-endemic to highly endemic areas. If this is a major need, then the vaccine should be prepared for their purpose, with attention to the appropriateness of the antigen for the specific parasite strains of the endemic area. A question was posed as to the possibility of strain differences (e.g. drug resistant vs. drug sensitive strains) which would affect the efficacy of the vaccine. There was concern that all early pre-clinical and clinical studies would utilize non-immune populations subjects. This would not be typical of the human populations utilizing the vaccines.

2. Dr. V.P. Sharma  India

Trials of vaccines could be planned for India, and the government would welcome such trials. The true value of the vaccines will be in areas where there are severe problems of drug and insecticide resistance. There is a question as to the acceptability of a vaccine which affects only one species of parasite in an area where multiple species exist. A number of human factors (e.g. genetic population variants) still need to be investigated. Troops, migrating labor, etc., would provide appropriate populations.

3. Dr. Natth Bhamarapravati  Thailand

It is felt that vaccine development is crucial for Thailand, and that the country should do everything possible to facilitate this development. Expertise exists in several institutions in the country, such as the Malaria Division, the University and the Ministry of Health. It is felt that a vaccine trial would provide a good development model for a country such as Thailand. It would be advantageous to have established a malaria vaccine policy study group, drawing resources from a number of appropriate institutions. This would be a "center without walls", a coordinating group with a small permanent nucleus and a network of expertise and resources.

4. Dr. Ezaddin Mohamed  Malaysia

In Malaysia, initiation of vaccine trials would involve three national committees: A Medical Research Committee, the Drug Biological Authority, and the Ethics Committee. A number of pertinent factors relating to conduct of the trial would be considered by these committees. There are appropriate population groups in Malaysia for field trials, but characterization and final selection, the most appropriate areas would be difficult. The one large issue in determining eventual vaccine introduction would be whether there is a real necessity for adding a vaccine to a current control program.
The introduction of vaccines will not necessarily alter present field strategies of malaria control. Initial prime candidate groups would be trans-migrants and infants and under-fives. The major questions which must be considered in testing a vaccine would include: 1) Characteristics of the vaccine and its activity, 2) target groups proposed, 3) characteristics of the implementor institution, 4) characteristics of the site chosen for the trial, and 5) the duration of vaccine effect.

Each country should have the opportunity to conduct vaccine trials, and support for such trials should be assured. Vaccine cohorts should be identified for each type of vaccine, according to the different objectives of vaccine administration. Protocols for testing vaccines must be reviewed carefully within the country where the trials are to be conducted.

In Papua New Guinea, careful studies have been done. The area studies may indeed not be the best area for testing vaccines because of problems which have been detected -- but all areas well characterized will have a range of similar problems. Careful studies are essential for early characterization of prospective trial areas, but only a limited number of parameters should initially be examined. Initial small scale studies can be followed by more intensive investigations if an area is eventually selected for vaccine trials. It is strongly felt that vaccine trials must be truly an international effort -- not just a national or bilateral effort.

Government policy (PNG) is now toward a malaria central program, with continual exploration of alternative measures appropriate for local conditions. Operational vaccine would be integrated into the control program. The government would welcome a vaccine trial in PNG.

It would seem important to conduct Phase II trials in each country where the vaccine will be used. There is still a need to develop and test new technology which will be essential to the field testing of vaccines. The accumulation of pre-trial data base is highly important, and some field trials need to be done to clarify issues. Late Phase III trials must take into consideration on-going control measures in the trials area. To the extent possible, protocols and procedures should be standardized. A number of important questions will emerge as the vaccine passes into and through the later trial Phases. Are the national resources and facilities adequate? Are the testing institutions powerful enough to resist demands by national groups for premature expansion of vaccine use? Are commercial interests willing to postpone premature distribution of the product.
There are a number of appropriate and differing epidemiologic situations in WPR which would provide the potential for vaccine evaluation -- including all 3 types of vaccine. The selection of countries and localities as trial sites would be governed by: 1) malaria characteristics; 2) population behavioral characteristics; 3) the will of the government to support the trial, avoiding over-enthusiasm and over-expectations; 4) logistical considerations; 5) ethical considerations; and 6) the existence of appropriate health delivery systems.