INDICATIONS FOR AND USEFULNESS OF SEROLOGICAL TECHNIQUES IN EPIDEMIOLOGICAL INVESTIGATION AND ASSESSMENT

by

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1. INTRODUCTION

The development of microtechniques and automation in malaria serology laboratories, the possibility of collecting and transporting blood specimens on absorbent paper, and the continuing development of more accurate and efficient methods to detect serological evidence of malaria infection have made it possible to extend the concept of seroepidemiology to malaria.

Rates of splenomegaly and parasitaemia are the classical methods of measuring the endemicity of malaria. Neither method is entirely satisfactory: spleen rates are affected by other diseases and are unreliable following chemotherapy, and the incidence of parasitaemia alone can completely fail to provide an adequate picture of the pattern of malaria in a population. The use of antimalarial drugs may affect the parasitaemia rate without a proportionate reduction of transmission. When antimalarial drugs are not used, mass blood surveys for malaria parasites provide a reasonably adequate measure of the point prevalence of the infection. In population groups, that have achieved a degree of immunity and that have scanty or periodic parasitaemia, the parasitological method alone is of limited value. When the incidence of malaria is low, mass blood surveys do not yield results commensurate with the work involved.

2. SEROLOGICAL METHODS

Serological methods can provide additional evidence of the extent and degree of malaria endemicity and reflect the period prevalence of the infection. Serological techniques have provided valuable epidemiological information, even in areas with high endemicity. McGregor et al. monitored the changes of endemicity in a rural African village, using parasitological and splenometric indices and applying the gel precipitation test. Assessment of the endemicity was found to be complicated by the curative treatment given to young malarious children. The parasite prevalence was low at the younger age levels and was greatly exceeded by the precipitin-test prevalence values. At older ages, the values for both precipitins and parasitaemia were very similar to those normally found in hyperendemic areas. The serological test results could be used to define the respective roles of the climatic variation and extensive drug use which influenced the changes of the prevalence.

Draper et al. assessed the level of endemicity in an area in the United Republic of Tanzania, 11 years 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, however, suggested that more transmission was occurring than was shown by the parasite rates: the indirect fluorescent antibody (IFA) prevalence was similar to that seen in hyperendemic areas: 84% in children one to two years of age, and 99% 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.

3. LEVEL OF EXPOSURE

Comparison of antibody profiles of communities living in different circumstances can provide a basis for estimating the level of exposure to malaria. Lelijveld compared the parasitological and splenometric indices with the serological age profiles in different areas of the United Republic of Tanzania and observed that differences in the degree of malaria endemicity were reflected in the mean IFA titres. McGregor et al. measured different serological age profiles in different villages in a small hyperendemic area, indicating different patterns of malaria endemicity.
4. MALARIA EXPERIENCE

Other studies also found that the population's antibody profile could be used as a measure of the population's malaria experience. In Senegal, the malaria antibody prevalence of persons under 20 years old in a suburb of Dakar, where malaria transmission was intermittent, was compared with that of persons of the same age category in a rural village where malaria was hyperendemic. The acquisition of antibodies in the hypoendemic suburb was much slower than in the hyperendemic village. Tin carried out a survey in schoolchildren in two villages in Burma. Despite the small sample sizes, a significantly lower serological prevalence was found in the children from the village where malaria had been eliminated than in the children from the village where the parasite rate indicated that malaria transmission persisted.

The usefulness of serological methods as tools for malaria epidemiological evaluation and assessment has been established. The widespread use of antimalarial drugs makes the application of serological techniques indispensable, to obtain information on the prevalence and extent of malaria necessary for planning control measures and evaluating their effectiveness.

5. TRANSMISSION

5.1 Altitude studies

In several areas, the altitude delineation of malaria transmission was measured by serological indices. Marked differences in the IFA responses were found in people living above and below the critical altitude for malaria transmission in the United Republic of Tanzania and in Ethiopia. Similarly, Kagan reported a high indirect haemagglutination (IHA) prevalence in individuals living below 1300 m in Nepal, whereas in persons living above that altitude the IHA prevalence was low.

5.2 Length of transmission season

Identification of the transmission season is important for optimal timing of some control measures. Use of antimalarial drugs can make the parasitaemia rate alone an unsatisfactory indicator, and other methods of evaluation may be needed, such as entomological studies or serological surveys. Temporary fluctuations of the intensity of transmission in relation to the transmission season were reflected in changes of serological parameters in the United Republic of Tanzania, Ethiopia, and Upper Volta. In India, a cohort study was carried out by the National Institute of Communicable Diseases, Delhi, in several villages where active and passive case detection had found only sporadic cases and where antimalarial drugs were widely available. The parasite rates, repeated at six-month intervals, varied from 0.5% to 0.9%, without a significant increase during the transmission season. The antibody prevalences were distinctly elevated at the end of the transmission season, in all age-groups, and had returned to the pretransmission level six months later.

5.3 Interruption of transmission

Serological indices have been used in investigations to assess the interruption of malaria transmission, e.g. in Greece, Guyana, Mauritius, the Moldavian Soviet Socialist Republic, Tobago, and Tunisia. Several of these investigations concentrated on areas where any residual malaria infections would most likely have remained undetected by the health services, or on localities where some transmission might have persisted. Persons, born after the risk of infection had been eliminated, did not have malaria antibodies, so the absence of transmission was confirmed. A cohort study in Tunisia found that the annual regression rate of seropositivity resembled the natural regression of the parasite reservoir after interruption of transmission, indicating the continued absence of malaria transmission.

When a large area needs to be assessed, a multistage cluster sampling method permits a survey to be concentrated on a relatively small number of localities. Such surveys can be carried out at low cost within a short period of time and with relatively little personnel. Limiting the size of the study often results in more precise observations.
5.4 **Delimitation of transmission foci**

Delimiting foci of persisting or renewed malaria transmission is very important. In the investigation of a recent outbreak of *Plasmodium malariae* on the island of Grenada, where malaria had been eradicated, use of the IFA test proved to be helpful in detecting additional cases. Ambroise-Thomas et al. showed the absence of malaria on the island of Corsica, France, and were able to delineate two areas where malaria transmission had occurred, even before cases had been detected. Kagan suspected the occurrence of malaria in a locality in Afghanistan because of elevated serological indices, before any malaria cases had been found. An outbreak of malaria later supported the serological observations.

The use of serological indices to delineate and characterize malaria foci has also been described in Middle America and in South America. The Peruvian studies showed the advantage of obtaining sera in investigations in remote areas and of testing such sera, also, for the presence of antibodies to other etiological agents. In one area with a low antibody prevalence for malaria, most individuals with a recent history of malaria-like symptoms had antibodies against leptospirosis.

6. **EFFECT OF CONTROL MEASURES**

The effect of antimalaria measures can be monitored by serological testing; and the progressive changes of the antibody titres, or of the antibody prevalences, in repeated surveys can indicate whether the measures are successful or not. Lelijveld found the mean antibody titre a useful variable for evaluating the progress in antimalaria programmes. In two localities in Suriname, serological data, in addition to the parasitological and splenometric data, indicated that an antimalaria campaign was successful. In another area of Suriname, where control measures had been ineffective, the serological indices did not indicate a change in the intensity of transmission. A large-scale longitudinal study in Tunisia also demonstrated that the decline of malaria transmission could be measured by serological indices and that such indices are especially useful when the parasite rate becomes too low to be a reliable parameter.

7. **SURVEILLANCE**

Serological testing has been used effectively in Costa Rica since 1974 by the National Malaria Eradication Service to strengthen the malaria surveillance, especially in high risk populations and in areas considered vulnerable or receptive.

Najera has suggested that efforts should be made to identify the real positive localities and even the malarious house so that available resources for malaria control can be used more efficiently. The importance of this suggestion is demonstrated by a study in a suburban location in Pakistan where, during the course of one year, 66% of all cases occurred in only 13% of the households. The inhabitants of these "malarious" houses were also at higher risk for other diseases such as diarrhoea, tuberculosis, and upper respiratory infections than the inhabitants of the other houses (D. Nalin, 1981, personal communication).

An unpublished observation in Guyana illustrates the potential for better identification of positive and negative localities by also using serological indices. Several years ago, a parasitological and serological investigation was conducted within a focus of *falciparum* malaria near the Brazilian border in Guyana. A total of six "positive" and five "negative" villages was included and 338 people were examined, representing 84% of the population. The mean village size was 36 inhabitants and the sampling ratio was highest in children under five years of age. Intensive active case detection had been carried out during the preceding year and a village was considered "positive" if any case of malaria was detected during the preceding year.

No malaria parasites were found in any of the thick blood films. The serological prevalence was high in all age-groups in each of the "positive" localities, confirming the malaria surveillance information. A high seroprevalence in young children in two of the
five "negative" villages suggested recent malaria infections, despite the negative parasitological findings and the absence of detected cases during the preceding year (Table 1). The serological observations in the other three "negative" villages supported the impression from the malaria surveillance that malaria infections had probably not occurred there recently.

Improvement of immunological surveillance techniques is an important research subject in the development of malaria control methods. Before serological tests are used in a new area, pilot studies should be carried out to prevalidate the tests. Similarly, the epidemiological methods need to be evaluated in each new area before seroepidemiological investigations of malaria are implemented.

Surveillance of blood donors. In nonendemic countries, malaria serology is used mostly in investigating induced cases of malaria. An infective blood donor can be identified by testing the sera of donors with a history of possible past exposure. In the sero- logical screening of 33 donors with a compatible history, two infective donors were identified, one of whom transmitted P. falciparum 47 years after apparent clinical illness, which is the longest reported period between probable clinical malaria and the subsequent transmission of that infection by transfusion.

8. OTHER USES

Malaria serological indices may be useful to clarify the etiology of other diseases. For instance, an investigation in Nigeria indicated that P. falciparum was of pathogenetic significance in only about a third of the children with nephrotic syndrome.

9. SAMPLING TECHNIQUES

Even when more valid, reliable, and comparable laboratory techniques are developed, the usefulness of the test results to improve the statistical information on malaria depends on the data collection method. The collection of data may be regular or irregular through population sampling. Unless probability sampling techniques are used in surveys, prevalence data are produced that are difficult to interpret and impossible to compare.

Statistically valid sampling techniques can and should be used even when detailed demographic information may be absent or inaccurate. Further refinements of such methods will facilitate standardization and comparability of serological survey results.

10. TRAINING

As researchers have shown, the technology for serological testing can be transferred to laboratories in endemic countries. But only if training is provided for the malaria epidemiologists on how to use these tools for epidemiological studies and assessment, will this transfer be productive. When serology laboratories are established, studies should be developed simultaneously to establish the epidemiological expertise and to develop guidelines for the operational use of these immunological tools.

SUMMARY

Serological indices can be useful to complement parasite and spleen rates for estimating the extent and degree of malaria endemicity, even in areas with high endemicity. Use of antimalarial drugs can affect the parasite and spleen rates without a proportionate reduction of transmission, and other methods of evaluation, including serological surveys, are then also needed.

Comparison of antibody profiles of populations living in different circumstances can provide a basis for estimating the level of exposure to malaria. Serological indices are especially useful when the parasite rate is too low to be a reliable indicator and in investigations to assess the interruption of transmission. The effect of antimalaria measures can be monitored by such indices. Progressive changes of the antibody titres, or prevalences, in repeated surveys can indicate whether the measures are successful or unsuccessful.
Better identification of malaria occurrence is important for optimal application of malaria control measures. Serological data can be useful for the identification and delineation of foci, the delineation of the maximum altitude of transmission, the identification of the transmission season, the surveillance of high risk population groups, and the better identification of positive and negative localities. Serological data can also be useful to clarify the etiology of other diseases.

Statistically valid sampling methods should be used in serological surveys and training of malaria epidemiologists is essential when serological techniques are being introduced in malarious countries.

**RESUME**

**INDICATIONS ET UTILITE DES TECHNIQUES SEROLOGIQUES**

**DANS LES ETUDES EPIDEMIOLOGIQUES ET EVALUATION**

Les indices sérologiques peuvent servir à compléter les indices plasmodique et splénique dans l'évaluation de l'étendue et du degré de l'endémicité paludéenne, et ceci même dans les zones à haute endémicité. L'emploi de médicaments antipaludiques peut influer sur les indices plasmodique et splénique sans qu'il y ait une réduction proportionnelle de la transmission; d'autres méthodes d'évaluation supplémentaires deviennent alors nécessaires, y compris les enquêtes sérologiques.

La comparaison des profils d'anticorps de populations vivant dans des conditions différentes peut servir de base pour estimer le degré d'exposition au paludisme. Les indices sérologiques sont particulièrement utiles dans les cas où l'indice plasmodique est trop bas pour être un indicateur fiable et dans les enquêtes destinées à établir l'interruption de la transmission. L'effet des mesures antipaludiques employées peut être évalué par des contrôles sérologiques. Des changements progressifs dans le taux ou la prévalence d'anticorps observés au cours d'enquêtes répétées peuvent être une indication du succès ou non des mesures antipaludiques appliquées.

Une meilleure connaissance de la fréquence du paludisme est importante pour obtenir une application optimale des mesures antipaludiques. Les données sérologiques peuvent aider à l'identification et à la délimitation des foyers, à la délimitation de l'altitude de la transmission, à l'identification de la saison de transmission, à la surveillance des groupes de population à haut risque, et à une meilleure identification des localités positives et négatives. Les données sérologiques peuvent aussi être utiles pour élucider l'étiologie d'autres maladies.

Des méthodes d'échantillonnage statistiquement valables devraient être employées pour les enquêtes sérologiques, et la formation d'épidémiologistes du paludisme est essentielle lorsque les techniques sérologiques sont introduites dans des pays impaludés.
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<table>
<thead>
<tr>
<th>Age-group (years)</th>
<th>Six positive villages</th>
<th>Two negative villages</th>
<th>Three negative villages</th>
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<tr>
<td></td>
<td>Number examined</td>
<td>Percentage seropositive</td>
<td>Number examined</td>
</tr>
<tr>
<td>0-4</td>
<td>36</td>
<td>38.9</td>
<td>21</td>
</tr>
<tr>
<td>5-14</td>
<td>48</td>
<td>37.5</td>
<td>48</td>
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<tr>
<td>15-39</td>
<td>58</td>
<td>55.2</td>
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<tr>
<td>40 and older</td>
<td>17</td>
<td>82.4</td>
<td>15</td>
</tr>
</tbody>
</table>

* One or more malaria cases detected by active case detection (ACD) during preceding year.

** No malaria cases detected by ACD during preceding year.