Absence of Asymptomatic Malaria Infections in Previously High Endemic Areas of Sri Lanka

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Abstract. As the goal of malaria elimination from Sri Lanka is currently being pursued, this study was planned to determine the prevalence of asymptomatic malaria infections. Five health areas in Trincomalee and Kurunegala districts that reported high prevalence in the recent past were purposively selected. The smallest administrative units (GN divisions) having high malaria risk within each area were identified. From these divisions, 20% of the population was randomly selected for blood smear examination and in a 50% sub-sample polymerase chain reaction (PCR) assay was performed. A population of 3,730 from 13 GN divisions was sampled. Thick and thin Giemsa-stained blood smears were negative for malaria parasites. The PCR carried out in 50% of the study sample was also negative for malaria parasites. The findings illustrate the absence of asymptomatic carriers in previously high transmission areas and it appears that achieving malaria elimination in Sri Lanka by 2015 is feasible.

INTRODUCTION

Malaria has been one of the most important communicable diseases that have had devastating effects both economically and socially in Sri Lanka. After the epidemic of malaria in 1934/1935, which killed approximately 80,000 people with 3 million cases being reported, there was a gradual decline in the malaria incidence following control measures being implemented with DDT spraying. By 1963, the number of cases had declined to 17 in the island. A Plasmodium vivax epidemic was experienced in 1967–1969 and since then the number of cases have been fluctuating. Until 1994, malaria was a leading cause of hospital morbidity and a major public health problem of this country. Almost 0.3 million infections were reported in its 16 million population in 1994. Although mortality caused by malaria has been relatively low, as much as two-thirds of the entire national public health budget and 9% of the total health expenditure was spent on malaria control, as this was the fourth highest cause of hospital admission in this country over a decade ago.

Since the year 2000, malaria incidence has continued to decline in Sri Lanka and the annual parasite incidence (measuring the incidence of both Plasmodium falciparum, P. vivax, and mixed infections) has decreased from 11.2/1,000 persons to 0.05/1,000 in the year 2007. During the last 8 years (2000–2007), 334,631 malaria infections have been recorded. The district-wide morbidity pattern has undergone dramatic changes, with the majority of new infections originating mainly from the Northern and Eastern Provinces where there is an ongoing civil conflict. The proportion of cases being reported from districts in these provinces, mainly Trincomalee (Eastern Province) and Vavuniya (Northern Province) was approximately 70% of all cases reported. When the rest of the country is considered, over the past 5 years, the highest number of cases have been reported from the districts of Anuradhapura (Central Province) and Kurunegala (North Western Province) (Figure 1).

Sri Lanka has embarked on malaria elimination by controlling malaria through the targeting of adult malaria vectors through indoor residual spraying, distribution of long lasting impregnated bed nets, passive case detection, mobilization of mobile malaria clinics for active case detection of malaria patients, and administration of effective antimalaria drugs at no cost to the patient. With these measures, the number of indigenous malaria cases has reduced from 210,039 in 2000 to 196 cases reported island-wide in 2007. Of these 196 cases only seven were falciparum malaria, with four being imported from countries with multidrug-resistant falciparum. With the interruption of malaria transmission and the number of cases being reported reducing significantly, goals for elimination of the disease from the country were announced in April 2008. A new policy of antimalarial treatment with artemisinin-based combination therapy for treatment of uncomplicated falciparum malaria has now been implemented, keeping in line with the global trend of treatment to prevent the emergence and spread of drug-resistant malaria.

Studies have revealed that during high transmission times a considerable number of sub-clinical malaria infections did occur in previously endemic areas of Sri Lanka. Kodisinghe reported 31% of infections detected by house-to-house survey of blood smears in a malaria endemic area of North Western Province of Sri Lanka were sub-clinical, and Gunawardhane showed that 44% of individuals were asymptomatic in Kataragama in the Uva Province of Sri Lanka. Therefore, even with the number of malaria cases dropping to the lowest level within the past 40 years due to the prevalence of the vector mosquito and a susceptible population, transmission of both P. vivax and P. falciparum may be sustained in the country. A resurgence of transmission caused by prevalence of asymptomatic infections can result in an epidemic of malaria as risk factors; economic crisis and civil unrest are both present in the country today. If such an epidemic occurs, the entire population will be vulnerable to malaria because the immunity among the adults will be marginal and mortality may not be confined to young children and pregnant women, as tends to be the case in areas of intense malaria transmission.

With reducing malaria incidence rates to achieve elimination of malaria by 2015 in Sri Lanka, it is important to address all the barriers that exist toward elimination. In this context, it is important to acknowledge the ongoing conflict situation in the North of the country, unregulated migration of people from the neighboring country India, which has a heavy malaria disease burden and implementing a successful implementation
strategy in an ongoing economic crisis situation. However, among the technical barriers that need to be resolved, determination of the presence of asymptomatic infections in previously high-risk communities is of importance.

It is important to find solutions to all the major and minor challenges on the path to malaria elimination if the country is to actually attain this goal. The solutions to these challenges in some instances are obvious while in others would need to be developed on the path toward elimination. This study addresses one such challenge and envisages determining the prevalence of asymptomatic malaria infections in two previously high transmission areas by using two methods: blood smear examination and polymerase chain reaction (PCR).

**MATERIALS AND METHODS**

**Study area.** A cross-sectional study was carried out in purposively selected previously high transmission areas within the Trincomalee and Kurunegala districts in Sri Lanka, situated in the Eastern (dry zone) and North Western (intermediate zone) provinces. With current rates of infection, a very large sample would have to be examined if probability sampling was carried out in the selected districts. Five Medical Officer of Health Areas (MOH) and 13 Grama Niladari (GN divisions, which is the smallest administrative unit), within the selected MOH areas reporting the highest prevalence of malaria infection over the past 3 years, were purposely selected for this study (Figure 2).

**Sampling.** This population in the purposively selected MOH areas was 139,367 in the Trincomalee district and 70,836 in the Kurunegala district in 2007. The selected GN divisions accounted for 7% of the population in the Trincomalee MOH areas and 13% of the population of the Polpithigama MOH areas. In the years 2000–2007, these five areas accounted for 1.41% (4,734) of the total number of cases in the country (Table 1). In the selected GN divisions, 20% of the population was randomly sampled for malaria parasites by thick and thin blood smear examination. From the above, a 50% sub-sample was examined for parasites by PCR.

**Collection of blood for parasitologic studies.** In each GN division, two households were selected randomly using the householders list available with the Grama Sevaka, and each of these households formed the starting household from where every second household was visited systematically until the required number of individuals were recruited. All people living in a household were included. The people from whom blood was taken for preparation of blood smears were numbered consecutively. In a 50% sub-sample, selected randomly from every other household included in the survey, blood was collected for PCR.

**Finger-prick blood and thick and thin blood smears were prepared.** The 0.5 mL of blood was also collected to tubes containing 0.5 mL of acid citrate dextrose for PCR. The samples were transported to the Office of the Regional Malaria Officer in ice packs and samples for PCR were stored at 4°C until dispatch.

**Measurement of auxiliary temperature.** During the visit, auxiliary temperature was measured using a digital thermometer.

**Data collection.** Six Public Health Inspectors from each of the Regional Malaria Officers in the two study areas were trained regarding identification of the population for study, collection of data, and method of collection of blood samples for smears and PCR.

**Laboratory investigations. Blood smear examination for malaria parasites.** The blood smears were stained with Giemsa stain and examined for malaria parasites by a trained microscopist at the Regional Malaria Office and cross-checked at the Department of Parasitology, Colombo, by a trained technical officer. Ten percent of the slides were checked by the Principal Investigator.

**PCR for malaria parasites.** Blood collected for PCR analysis was transported to the Central Laboratory in Colombo in wet ice. In the laboratory, the anti-coagulated blood sample was centrifuged at 3,000 rpm for 5 minutes and supernatant discarded. Plasma separated specimens were kept at −70°C for a minimum of 2 hours allowing the hemolysis of red blood cells. A portion of 20 μL each from five specimens was pooled and the malarial genomic DNA was extracted, as described by Walsh and others, with modifications by the Chelex method. The PCR was carried out in a 50-μL mixture containing 0.2 mM dNTP, 0.4 μM Plasmo F, 0.4 μM Plasmo R, 0.4 μM falc F, 0.4 μM falc R, 0.4 μM vivax F and 0.4 μM vivax R primers, 5 μL of 10× PCR buffer (50 mM KCl, 10 mM Tris-HCl,
1.5 mM MgCl₂, 0.1% Triton X-100), and 2.5 U of Taq DNA polymerase enzyme with 5-μL extracted genomic DNA, in a thermal cycler at 94°C for 4 minutes (single cycle of initial denaturation), 90°C for 1 minute, 56°C for 2 minutes, and 72°C for 1 minute (40 cycles of denaturation, primer annealing, and primer extension, respectively); with a final extension at 72°C for 5 minutes. Electrophoresis of amplified DNA products were carried out in 2% Agarose gel at 100 V in 0.5× TBE buffer containing ethidimbromide (μg/mL), and resolved DNA was visualized on the UV transilluminator. A positive control and a negative control were used with each assay to detect inhibition of the reaction and false positives, respectively.

**Data entry.** Data was entered into an SPSS (SPSS, Inc., Chicago, IL) database and analyzed using standard statistical packages.

**Ethical considerations.** Ethical clearance was obtained from the Ethics Committee Faculty of Medicine, University of Colombo. Written, informed consent was obtained from all participants. In the case of minors less than 12 years of age, consent was obtained from the parents or guardians.

**RESULTS**

Examination of blood smears for malaria parasites was carried out in 1,905 individuals from the four MOH areas of the Trincomalee district and PCR carried out in 930 of these individuals. Similarly, in the Kurunegala district blood smears were examined in 1,827 individuals from the Polpithigama MOH area and PCR carried out in 909 of these individuals. None of the individuals were positive for malaria parasites either by blood smear examination or by PCR (Table 2). Eight individuals from the Kurunegala district and 15 individuals from the Trincomalee district had an auxiliary body temperature above the normal (98.4°F). Over the past 2 years, no history of malaria infection was given in any of the individuals (Table 2).

The sex distribution in the districts was similar, with approximately 60% of the study population in each area being females. The age ranged from 5 months to 90 years of age (mean age in the Trincomalee district 25.8 and in the Kurunegala district 33.6).

**DISCUSSION**

The results of the study show that there are no asymptomatic malaria cases in this population. This confirms the common observation that asymptomatic parasite densities decrease and disappear as symptomatic malaria infections decrease in a community. The sample size required to estimate a prevalence of 0.02%, with a precision of ±0.01, and a error of 0.05 was 56,935 persons living in approximately 11,387 households, assuming the average number per household to be five persons. The cost of testing this number of individuals would have been approximately US$200,000 and was clearly beyond available resources. Hence, the present sampling method was adopted.

No asymptomatic malaria infections were detected by the gold standard for malaria diagnosis, which still remains as light microscopic examination of Giemsa-stained thick and thin blood films or by PCR, which was performed in 50% of the study population. Polymerase chain reaction-based methods have been used since the early 1990s for the detection of *Plasmodium* parasites in human patients and have been reported as being more sensitive and specific than examination of thick or thin blood smears. Compared with antibody detection tests for malaria diagnosis, which have a limited sensitivity, the antigen capture tests have permitted rapid diagnosis of malaria, but are accepted to have a lower sensitivity and specificity than microscopy, especially for the diagnosis of vivax malaria. By carrying out PCR, we assumed that we did not miss parasitaemia at sub-patent levels not detected by microscopy. It may be assumed that the fever cases detected during this study were caused by an agent other than the malaria parasite.

The strategy for elimination of malaria in Sri Lanka is based on three zones: namely, the non-conflict zone, transitional
This study was carried out in two districts situated in the non-conflict and transitional zones where a high incidence of malaria has been reported over the past few years. The problem of asymptomatic carriers and their importance in the pre-elimination phase has been recognized by the National Anti Malaria Campaign. One of the greatest concerns of the pre-elimination phase is the re-introduction of malaria into areas that have not reported malaria cases for the past 3 years.

Although no asymptomatic carriers were found in these high-risk malaria endemic areas, it is not possible to predict the number of asymptomatic carriers in the country, especially with the number of imported cases being on the increase. Ideally, mass blood surveys combined with PCR examination of a sample of the population should be carried out in all districts, especially the conflict zones from where the highest number of malaria cases continues to be reported. When considering the cost of PCR examination, the less sensitive antigen/antibody detection methods may have to be used as we progress toward elimination. Already, the National Malaria Program has used these methods in some localities in the screening of febrile patients to determine/exclude malaria infections.

With the introduction of artemether-lumefantrine combination therapy, the distribution of long-lasting impregnated bed nets, indoor residual insecticide spraying, and the absence of any asymptomatic carriers, in this study it is envisaged that the current national objective of declaring the non-conflict and transitional zones free of malaria by the year 2015 will be achieved.

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### Table 1

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<th>MOH area</th>
<th>2000</th>
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<td>Malaria cases in the selected Medical Officer of Health (MOH) areas</td>
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<td>Pop rate*</td>
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<td>Mal cases (Pv:Pf)</td>
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* Number of malaria cases in 100,000 population.

### Table 2

**Clinical and laboratory investigations**

<table>
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<th>MOH area</th>
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<th>Trincomalee</th>
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<tr>
<td>Number (%)</td>
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<tr>
<td>Number of blood smears positive for malaria parasites</td>
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<td>0 (0.0)</td>
</tr>
<tr>
<td>Number of samples positive for malaria parasites by PCR</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
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<td>Normal body temperature at the time of blood filming</td>
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<td>1,519 (80.0)</td>
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<tr>
<td>Body temperature &gt; 98.4°F at the time of blood filming</td>
<td>8 (0.5)</td>
<td>15 (20.0)</td>
</tr>
<tr>
<td>Number of malaria attacks during last 2 years</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

* PCR = polymerase chain reaction.
REFERENCES


