HIGH SEASONAL VARIATION IN ENTOMOLOGIC INOCULATION RATES IN ERITREA, A SEMI-ARID REGION OF UNSTABLE MALARIA IN AFRICA

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Abstract. Entomologic studies were conducted in eight villages to investigate the patterns of malaria transmission in different ecological zones in Eritrea. Mosquito collections were conducted for 24 months between September 1999 and January 2002. The biting rates of Anopheles arabiensis were highly seasonal, with activity concentrated in the wet season between June and October in the highlands and western lowlands, and between December and March in the coastal region. The biting rates in the western lowlands were twice as high as in the western escarpment and 20 times higher than in the coastal region. Sporozoite rates were not significantly different among villages. The risk of infection ranged from zero on the coast to 70.6 infective bites per year in the western lowlands. The number of days it would take for an individual to receive an infective bite from an infected An. arabiensis was variable among villages (range = 2.8–203.1 days). The data revealed the presence of only one main malaria transmission period between July and October for the highlands and western lowlands. Peak inoculation rates were recorded in August and September (range = 0.29–43.6 infective bites/person/month) at all sites over the two-year period. The annual entomologic inoculation rates (EIRs) varied greatly depending on year. The EIR profiles indicated that the risk of exposure to infected mosquitoes is highly heterogeneous and seasonal, with high inoculation rates during the rainy season, and with little or no transmission during the dry season. This study demonstrates the need to generate spatial and temporal data on transmission intensity on smaller scales to guide targeted control of malaria operations in semi-arid regions. Furthermore, EIR estimates derived in the present study provide a means of quantifying levels of exposure to infected mosquitoes in different regions of the country and could be important for evaluating the efficacy of vector control measures, since Eritrea has made significant steps in reducing the burden of malaria based on the Roll Back Malaria initiative of the World Health Organization.

INTRODUCTION

Eritrea is situated in the horn of Africa. It lies between latitudes 12°42’N and 18°2’N and longitudes 36°30’E and 43°20’E, and has an enormous geographic diversity with high mountains, rugged plateaus, deep gorges, and expansive plains. Malaria accounts for more than 30% of outpatient morbidity and 28% of hospital admissions (Ministry of Health, unpublished data). Almost 65% of the population (3.5 million) lives in malaria endemic zones and are at risk of infection. Rainfall is scanty and highly seasonal, ranging from 400 mm to 650 mm annually in the highlands to 200–300 mm in the lowlands. Eritrea has made significant steps in implementing malaria control strategies in line with the Roll Back Malaria (RBM) initiative of the World Health Organization. The country has implemented an effective insecticide-treated bed net program and coverage currently stands at 67% of the population living in high-risk malaria areas, with each household having at least two bed nets.

There is an enormous heterogeneity in malaria transmission intensity in affected areas of Africa, and implementation of targeted malaria control operations would require an understanding of the forces that drive transmission. The assessment of indices relating to disease transmission therefore becomes central to disease control through quantifying the potential risk of infection and elucidating the patterns of disease transmission. The estimation of the entomologic inoculation rate (EIR) provides a standard and relatively simple means of quantifying levels of exposure to infected mosquitoes. The EIR uses an index of vector infectivity, the sporozoite rate, and the human-biting rate, which expresses the degree of human-vector contact. These two parameters are largely dependent on environmental conditions and would be expected to vary seasonally. It has been observed that vector species behavior, density, and the number of infective bites a person can receive per unit time is dependent on seasonal changes in environmental variables. Several studies have consequently compared transmission among different ecological zones and even among villages within the same area, and have reported significant variations in transmission intensity among villages separated only by short distances and even between rural and urban settings within the same locality. Knowledge of the magnitude of these spatial variations is critical to understanding the transmission dynamics of the disease and the evaluation of the efficacy of vector control measures. This becomes even more crucial for the semi-arid regions of Africa where malaria is generally unstable and is occasionally experienced in epidemic proportions.

In this study, we examined the seasonality and spatial variations of malaria transmission at eight villages representing different ecological zones of Eritrea. As the country aims to reduce the burden of malaria based on the RBM initiative, it is important to understand the degree of malaria distribution, risk, and burden so that control options can be targeted rationally.

MATERIALS AND METHODS

Study sites and mosquito sampling. Two villages were selected from each of four zones (Anseba, Gash-Barka, Debub, and Northern Red Sea) based on recent evidence of high malaria morbidity (Figure 1). The villages represented different ecologies and variable malaria endemicity based on clinical evidence. Vector control strategies implemented at the study villages include environmental management, larviciding, and use of insecticide-impregnated bed nets. Coverage of
at least two bed nets per household has been achieved in the study villages.

Entomologic surveys were conducted one night per month for 24 months in the eight villages from October 1999 to January 2002 using human-landing catches (HLCs). Two houses that served as sentinel stations for HLCs were randomly selected in each village. Collections were made on adult volunteers from 6:00 PM to 6:00 AM. Two teams of collectors in each village conducted HLCs one night per month for 24 months at the two sentinel houses in each village. The collectors worked in pairs, one pair working from 6:00 PM to midnight and the next from midnight until 6:00 AM. One indoor collector and one outdoor collector, positioned about 20 meters away from the sentinel house, conducted the collections at each village for the specified time. The teams rotated through the sentinel houses on different sampling nights. All mosquitoes collected were identified to species based on morphologic characteristics. Indoor resting mosquitoes were concurrently sampled once a month in 10 randomly selected houses per village using pyrethrum spray catches to evaluate seasonal densities. The same houses were visited every month.

Sporozoite rates and the EIR. The head and thorax of each mosquito were separated from the abdomen and tested for the presence of *Plasmodium falciparum* circumsporozoite (CS) antigen. Mosquitoes were ground in 50 μL of boiled casein containing Nonidet 40 and final volume brought to 250 μL with blocking buffer. Fifty microliters of the triturate was used in sporozoite enzyme-linked immunosorbent assays. Positive reactions were determined visually. The EIR, expressed as the number of infective bites per unit time, was derived as a product of the sporozoite rate and the human-biting rate. The human-biting rate (HBR) was derived directly from HLCs and was expressed as the number of bites per person per night (b/p/n). The annual and per month inoculation rates were derived by multiplying the daily EIR (b/p/n) by 365 and 30 days, respectively.

Data analysis. Analysis of variance was used to analyze differences in biting rates, sporozoite rates, and EIR between seasons and among villages. Log-transformed values of mosquito densities and arcsine-transformed values of sporozoite rates and EIR was used for statistical analysis. The chi-square test was used to test the significance of variations between human biting rates with regard to host location (indoor or outdoor). Data analysis was performed using SPSS version 10 (SPSS Institute, Chicago, IL).

RESULTS

Biting density of *An. arabiensis*. *Anopheles arabiensis* accounted for 97.6% of the 2,711 anophelines collected in HLCs over the 24-month period of the study. The biting density of
this species varied significantly among the study villages (F = 11.8, degrees of freedom [df] = 7, 191, P < 0.001), giving a mean HBR of 12.4, 6.8, and 6.7 b/p/n in Hiletsidi, Maiaini, and Adibosqual, respectively. The number of An. arabiensis collected indoors (43.3%, n = 1,150) and outdoors (56.7%, n = 1,495) in HLCs among the eight study villages were significantly different (χ² = 19.29, df = 7, P = 0.007).

The HBR (b/p/n) varied significantly between months (F = 6.64, df = 10, 191, P < 0.001). The An. arabiensis biting activity was concentrated between July and November in the study villages in the highlands and western lowlands, with only peak biting density varying among sites. The highest HBRs of 37.8 b/p/n indoors and 54.8 b/p/n outdoors were obtained in Hiletsidi in September. In Maiaini, peak indoor (34.8 b/p/n) and outdoor (46.3 b/p/n) biting rates were recorded in September. In Adibosqual, the HBR in September was 32.5 b/p/n indoors and 47 b/p/n outdoors. Biting rates were generally low in the villages on the coast (range = 0.3–3.3 b/p/n), where biting activity was concentrated between December and February.

Plasmodium falciparum sporozoite rates. A total of 4,886 An. arabiensis mosquitoes from HLCs and concurrent indoor-resting collections yielded an overall P. falciparum CS antigen positivity rate of 1.25% (n = 61). The sporozoite rates for the two villages surveyed in each zone combined was highest in the Gash-Barka zone (1.62%), which is also the most malarious region in the country and is located at an elevation between 500 and 1,000 meters above sea level. The sporozoite rates recorded in the Anseba and Debub zones were 0.52% and 1.23%, respectively. None of the An. arabiensis tested (n = 103) from the two villages in the Northern Red Sea zone was positive for P. falci‌parum CS antigens. Overall, the difference in sporozoite rates among villages was not significantly different (F = 1.503, df = 7, 34, P = 0.209). Of the positive An. arabiensis mosquitoes, 70.5% (n = 43) were collected from the two villages in the Gash-Barka zone. Hiletsidi (570 meters above sea level) alone comprised 63.9% (n = 39) of the total positive mosquitoes with a sporozoite rate of 1.6%, while in Dasse the sporozoite rate was 1.8%.

The data revealed significant seasonal variation in sporozoite rates in An. arabiensis. For instance, the highest proportion of infected mosquitoes in Hiletsidi was recorded in August, although infection rates tended to increase between July and October (range = 0.6–5.2%). In Adibosqual, Maiaini, and Dasse, peak mosquito infection rates were recorded from July to September, coinciding with the rainy season. Generally low sporozoite rates were recorded during the dry season between November and May in all the study villages.

**Entomologic inoculation rate.** The EIRs differed significantly among the study villages (F = 2.884, df = 7, 95, P = 0.009). The highest levels of transmission were recorded in Hiletsidi in the Gash Barka zone and in Maiaini in the Debub zone, with mean annual EIR figures of 70.6 and 32.1 infective bites per person, respectively, over the two-year period of the study (Table 1). The number of infective bites a person was exposed to annually was generally low in Dasse, Adibosqual, and Hagaz (range = 1.8–8.0 ib/p/year). The number of days it would take to receive one infective bite from An. arabiensis was also variable among the study villages, further showing the extent of geographic differences in transmission intensity. This ranged from 2.8 days in Hiletsidi to 203 days in Hagaz over the 24 months of the study.

Except for Hiletsidi and Dasse, the inoculation rates did not vary between the two years of the study among the other villages where a positive value of EIR was recorded. The annual inoculation rate in Hiletsidi was 10-fold higher in the second year of study than in the first year. The risk of infection was twice as high in the first year as in the second year in Dasse, indicating the importance of temporal variations. The EIRs were not derived in Shekaeyamo, Ghinda, and Gahtelay because none of the An. arabiensis tested were positive for CS antigens.

### Table 1

Mean annual entomologic inoculation rates by *Anopheles arabiensis* over 24 months in eight villages in Eritrea*

<table>
<thead>
<tr>
<th>Zone</th>
<th>Village</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (meters)</th>
<th>Rainfall (mm)</th>
<th>Biting rate (b/p/n ± SE)</th>
<th>SR (%) ± SE</th>
<th>1/EIR† ± SE</th>
<th>Annual EIR</th>
<th>Biting rate (b/p/n ± SE)</th>
<th>SR (%) ± SE</th>
<th>1/EIR† ± SE</th>
<th>Annual EIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gash-</td>
<td>Hiletsdi</td>
<td>15.07050</td>
<td>36.39091</td>
<td>570</td>
<td>286.9</td>
<td>6.40 ± 2.60</td>
<td>0.6 ± 0.003</td>
<td>27.9</td>
<td>13.1</td>
<td>17.2 ± 6.10</td>
<td>2.0 ± 0.003</td>
<td>2.8 ± 0.009</td>
<td>128.1</td>
</tr>
<tr>
<td>Barka</td>
<td>Dasse</td>
<td>14.55346</td>
<td>37.29050</td>
<td>850</td>
<td>196.9</td>
<td>1.00 ± 0.70</td>
<td>3.0 ± 0.021</td>
<td>32.8</td>
<td>11.1</td>
<td>1.0 ± 0.29</td>
<td>1.3 ± 0.009</td>
<td>76.3 ± 4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Anseba</td>
<td>Adibosqual</td>
<td>15.41725</td>
<td>38.38956</td>
<td>1,560</td>
<td>657.7</td>
<td>6.40 ± 4.48</td>
<td>0.2 ± 0.002</td>
<td>64.2</td>
<td>5.7</td>
<td>7.0 ± 2.87</td>
<td>0.2 ± 0.002</td>
<td>71.0 ± 5.1</td>
<td>5.1</td>
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<tr>
<td></td>
<td>Hagaz</td>
<td>15.41002</td>
<td>38.16784</td>
<td>860</td>
<td>241.1</td>
<td>0.04 ± 0.04</td>
<td>1.3 ± 0.005</td>
<td>11.3</td>
<td>32.2</td>
<td>0.2 ± 0.21</td>
<td>1.8 ± 0.009</td>
<td>203.1 ± 1.5</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Maiaini</td>
<td>14.48510</td>
<td>39.05765</td>
<td>1,540</td>
<td>378.1</td>
<td>6.90 ± 3.50</td>
<td>1.3 ± 0.005</td>
<td>11.3</td>
<td>32.2</td>
<td>6.7 ± 3.89</td>
<td>1.3 ± 0.005</td>
<td>11.5 ± 3.19</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>Shekaeyamo</td>
<td>14.42410</td>
<td>38.50884</td>
<td>1,870</td>
<td>657.7</td>
<td>0.50 ± 0.45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 0.61</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NRS Gahtelay</td>
<td>15.31350</td>
<td>39.09193</td>
<td>295</td>
<td>51.6</td>
<td>0.02 ± 0.02</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 0.31</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>Ghinda</td>
<td>15.26327</td>
<td>39.06108</td>
<td>188</td>
<td>358.7</td>
<td>0.08 ± 0.06</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.1 ± 0.06</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

* b/p/n = bites/person/night; SR = sporozoite rate; EIR = entomologic inoculation rate.
† Mean rainfall over the 24 months of the study.
‡ Sporozoite rate based on enzyme-linked immunosorbent assay determination of Plasmodium falciparum circumsporozoite antigen in the head and thorax of female Anopheles arabiensis. The standard error (SE) for sporozoite rates was calculated according to a binomial distribution.
§ Number of days it would take to receive at least a single infective bite from An. arabiensis.

* b/p/n = bites/person/night; SR = sporozoite rate; EIR = entomologic inoculation rate.
† Mean rainfall over the 24 months of the study.
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§ Number of days it would take to receive at least a single infective bite from An. arabiensis.
Transmission intensity was highly variable, even among villages in the same ecologic zone. For example, transmission intensity was 15 times higher in Hiletsidi than in Dasse, although the two villages are found in generally similar ecologic zones of the western lowlands. In Anseba zone, the likelihood of infection was 17 times higher in Adigosqual than in Hagaz. A similar occurrence was evident in Maiaini, Debub zone, where the annual EIR was 33 infective bites compared with zero in Shekaeyamo. This confirms that transmission intensity is area specific and extrapolation of EIR estimates across ecologic strata in semi-arid ecosystems cannot be done with any degree of accuracy. Overall, the highest transmission intensity was observed in the western lowlands (500–1,000 meters, >200 mm rainfall per year) and the western escarpments (1,000–1,500 meters, >200 mm rainfall). Although no EIR values were recorded in the villages in the eastern es-

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Hiletsidi</th>
<th>Dasse</th>
<th>Adigosqual</th>
<th>Hagaz</th>
<th>Maiaini</th>
<th>Shekaeyamo</th>
<th>Galtelay</th>
<th>Ghinda</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBR (×1000)</td>
<td>1.8</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>SR (×1000)</td>
<td>0.1</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>EIR (×1000)</td>
<td>0.1</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*HBR = human biting rate; SR = sporozoite rate; EIR = entomologic inoculation rate. The sporozoite rate was derived from indoor resting collection and human-landing catches. EIR = infective bites/person/month.*

**Figure 2.** Seasonal variation in entomologic inoculation rates (top) and mean monthly rainfall totals (bottom) over the two-year period of the longitudinal study in five villages in Eritrea. The entomologic inoculation rate and rainfall data are monthly means over the two-year period.
carrpents (200–900 meters, rainfall >200 mm per year), the dry coastal lowlands (<500 meters, rainfall <200 mm), and the highlands (>1,800 meters), it is likely that transmission occurs below the entomologic threshold for detection (Table 3).

DISCUSSION

The entomologic assessment carried out in eight villages provides important information on malaria transmission risk throughout Eritrea. *Anopheles arabiensis* represented more than 97% of the anophelines caught in HLCs, but the density of this species differed significantly among villages. The biting densities fluctuated with the seasonal rainfall among all study villages. Overall, the number of bites due to *An. arabiensis* was nine times higher in the wet season than in the dry season. These spatial and seasonal variations in biting density may be accounted for by rainfall and temperature patterns. Recent studies indicate that vector production in Eritrea is associated with roadside ditches, ponds, and drainage channels, which form as soon as the rains begin, and streambed pools that persist into the dry season on a number of intermittent streams that drain through or close to the study villages.12

The mean sporozoite rates for the combined villages per zone were two times higher in the villages in the Gash Barka zone than in the Debub and Anseba zones, and were significantly higher during the wet season (June to September) and the period soon after the rains (October to December). It is likely that the scarcity of rainfall during the dry season and the cold temperatures associated with the high altitude areas could have influenced geographic variations in survival rates and parasite development.13 Studies conducted at the Mwea-Tebere irrigation scheme in Kenya have shown that the survival rate of *An. arabiensis* was twice times higher during the rainy season than in the dry season.14 It is therefore likely that due to prevailing environmental conditions at each of the villages by season, some *An. arabiensis* did not live long enough to complete sporogony. Although the present study did not measure house-level variations in sporozoite rates, it is also likely that differences on a microecologic scale may exist and would be important in determining the variability in malaria transmission intensity.1

The mean sporozoite rates for the eight sites displayed enormous geographic variations (range = 0–70.6 ib/p/year). This confirms that malaria transmission is heterogeneous, consistent with the high degree of ecologic diversity in Eritrea. Since the EIR uses estimates of mosquito biting densities and sporozoite rates, it is likely that variation in the EIR was influenced by environmental determinants of vector populations and survival associated with different ecologic scenarios.15 The annual EIR derived in the present study for Hiletsidi and Maiaini would seem high, if one considers that these sites received only a mean annual rainfall of 286.9 mm and 378.1 mm, respectively, over the two years of study. More than 90% of the total rainfall was received between June and September, and more than 95% of the transmission risk was also restricted to the same period. The relatively small differences in rainfall and soil moisture content in these dry regions could have led to pronounced changes in ecology and consequent differences in the composition and population of mosquito vector species.16 The same factors would explain the variation in the EIR during the two years of the study. For instance, in Hiletsidi, the annual EIR was 10 times higher in the second year than in the first year, while in Dasse, the inoculation rate was twice as high in the first year as in the second year. Similar year-to-year variations in inoculation rates have been reported in two villages in Senegal (Dielmo and Ndop).3

Despite the scarce rainfall in Eritrea, the inoculation rates obtained in the present study were generally comparable with records from most entomologic surveys across Africa, where transmission is short and restricted to approximately three months.17 However, the EIRs were generally lower compared with data from The Gambia, Kenya, Senegal, and Tanzania, where malaria is highly endemic. For example, annual inoculation rates in Tanzania ranged from 93.7 to 702.6 infective bites per person, with 75% of the transmission occurring in more than six months.17 Overall, entomologic assessment of transmission risk in semi-arid to arid regions of the continent where malaria occur mostly in severe epidemic proportions is generally lacking. Although EIRs = 0 were recorded in Shekaeyamo, Ghinda, and Gahtelay, it is likely that transmission is occurring below entomologic thresholds for detection. Some degree of variation in the EIR among study villages could also be attributed to inherent bias in selection of the villages because this was based on numbers of clinical malaria in recent years.

The study further demonstrated that EIR was significantly correlated with monthly rainfall amounts. In villages where transmission was detected entomologically, transmission was

<table>
<thead>
<tr>
<th>Ecologic zone</th>
<th>Study villages</th>
<th>No. tested</th>
<th>CS positive</th>
<th>Mean annual EIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western lowland wet zones, 500–1,000 meters, &gt;200 mm rainfall/year</td>
<td>Hiletsidi, Dasse</td>
<td>2,657</td>
<td>43</td>
<td>1.61 (0.012–0.022)</td>
</tr>
<tr>
<td>Western escarpments, 1,000–1,600 meters, &gt;200 mm rainfall/year</td>
<td>Adibosqual, Hagaz, Maiaini</td>
<td>2,053</td>
<td>18</td>
<td>0.88 (0.005–0.014)</td>
</tr>
<tr>
<td>Highlands above 1,800 meters, &gt;400 mm rainfall/year</td>
<td>Shekaeyamo</td>
<td>73</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eastern escarpments, 200–99 meters, &gt;200 mm per year</td>
<td>Ghinda</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dry coastal lowlands, &lt;500 meters, &lt;200 mm rainfall/year</td>
<td>Gahtelay</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*EIR = entomologic inoculation rate.
†Sporozoite rates based on enzyme-linked immunosorbent assay determination of the *P. falciparum* circumsporozoite (CS) antigen in head and thorax of female *An. arabiensis*.
‡Exact 95% confidence intervals (in parentheses) calculated according to a binomial distribution.
restricted to 1–3 months between July and December, except for Hiletsidi where transmission occurred also between March and May. The high wet season inoculation rates could be attributed to the fact that EIR estimates were based on HLCs, which increased with the onset of rains, and also may be a consequence of increased use of insecticide-impregnated bed nets in the study villages. The excito-repellency properties of pyrethroid insecticides could divert host-seeking mosquitoes to the exposed collectors, leading to an overestimation of the HBR. Eritrea has a successful bed net implementation program with coverage of at least two bed nets per household in at least 67% of the high-risk malaria regions (Ministry of Health, unpublished data).

Our study demonstrates that malaria transmission in Eritrea shows strong spatial and seasonal variations that have to be accounted for in planning for malaria control. For instance, the timing of bed net re-impregnation and selective indoor residual spraying would effectively be planned based on the onset of transmission in respective villages or regions. Eritrea is currently undertaking the implementation of vector control based on the RBM initiative, and the estimation of the EIR in the present study provides the malaria control program with means of quantifying levels of exposure to infected mosquitoes in different regions of the country. The data also provides important means for evaluating the efficacy of vector control measures, including use of impregnated bed nets. Despite the large differences in EIRs observed, it must be emphasized that annual EIRs, even as low as 5 or less, would still be responsible for substantial malaria prevalence and a high incidence of severe life-threatening malaria.

Therefore, malaria control programs must also consider clinical and parasitologic assessments of disease to complement entomologic component in defining and further stratifying malarious areas in a country for effective disease control. Monitoring of rainfall amounts under similar semi-arid conditions experienced in Eritrea could provide a sensitive surveillance tool for forecasting malaria transmission and epidemics. Rainfall monitoring is currently being undertaken at a number of sentinel surveillance sites in Eritrea.

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