Collapse of Anopheles darlingi Populations in Suriname After Introduction of Insecticide-Treated Nets (ITNs); Malaria Down to Near Elimination Level

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Abstract. A longitudinal study of malaria vectors was carried out in three villages in Suriname between 2006 and 2010. During 13,392 man hours of collections, 3,180 mosquitoes were collected, of which 33.7% were anophelines. Of these, Anopheles darlingi accounted for 88.1%, and An. nunezovari accounted for 11.1%. The highest mean An. darlingi human biting rate (HBR) observed per survey was 1.43 bites/man per hour outdoor and 1.09 bites/man per hour indoor; 2 An. darlingi of the 683 tested were infected with Plasmodium falciparum. The anopheline HBR decreased to zero after the onset of malaria intervention activities, including insecticide-treated net (ITN) distribution, in 2006. Malaria transmission decreased to pre-elimination levels. It is concluded that the combination of ITN and climatic events has led to the collapse of malaria vector populations in the study sites in the interior of the country. The results are discussed in relation to the stability of malaria transmission in areas with low-density human populations.

INTRODUCTION

The human malaria parasite species prevalent in Suriname are Plasmodium falciparum, P. vivax, and P. malariae. Transmission occurs only in the interior of the country, which consists mostly of tropical rainforest. The area is sparsely settled, with people living mostly in discrete communities. Here, malaria prevalence has increased gradually since 1990. P. falciparum is predominant among the Maroon population, whereas the Amerindian and immigrant populations may also be infected with P. vivax and P. malariae.

Anopheles darlingi Root is the primary malaria vector in the interior. The role of An. darlingi in malaria transmission dynamics in Suriname was studied in the 1980s in Maroon villages along the Upper Marowijne river, along the border with French Guiana.1–4 Between 2000 and 2002, studies were done in Amerindian villages along the Upper Marowijne River and Upper Lawa River.5 An. darlingi was the most common anopheline mosquito found in or near the villages. In 2004 and 2005, vector studies were done in the same area, and this area had high malaria transmission risk.2,5,6 Both Girod and others5 and Hiwat and others6 found An. darlingi specimens infected with P. falciparum, P. vivax, and P. malariae.5,6 Among the other anopheline species collected in the villages were An. nunezovari Gabaldon, An.brazilensis Chagas, An. neivai Howard, Dyar & Knab, An. oswaldoi Peryassu, and An. intermedius Peryassu. An. darlingi is a seasonal vector with varying peak abundances depending on local (permanent and temporal) opportunities for breeding. Data available for the Upper Marowijne area suggest a year-round presence of the vector, with peak occurrences toward the end of the major rainy season or during the beginning of the major dry season.4,6

In 2006, mass distribution of long-lasting insecticide-treated nets (LLINs) was initiated in the entire interior of the country (~50,000 inhabitants) together with indoor residual spraying (IRS), active case detection (ACD), and an extensive public awareness campaign. As part of the malaria program, a longitudinal study on malaria vectors and transmission dynamics, with special emphasis on An. darlingi, was carried out in two Maroon villages and one Amerindian village in the interior from January of 2006 to April of 2010. In this paper, we present the findings of this study and relate these findings to the change in the malaria situation, climatic events, and malaria control activities in Suriname.

MATERIALS AND METHODS

Study area. Suriname is part of the northern range of the Amazon forest and borders French Guiana, Guyana, and Brazil. To the north, the country reaches the Atlantic Ocean. Most of the agriculture is located in the coastal plain, and around 90% of the country’s population of ~500,000 people live here.7 The remaining people live in the tropical rainforests of the interior. The population of the interior consists of Amerindians and Maroons living in tribal communities along the main rivers and a number of (mostly Brazilian) immigrants working in small-scale gold mines. Because of the limited road system, transportation to the villages is mostly by boat and plane. The climate is hot and humid, with an average temp of 27°C and annual relative humidity of around 80%. Four seasons are identified: the major rainy season from mid-April to mid-August, the major dry season from mid-August to November, the minor rainy season from December to January, and the minor dry season from February to mid-April.

Study sites. The three sentinel sites of this study are Drietabiki (4°06’ N, 54°40’ W), a Maroon village along the Tapanahony River (3,028 registered inhabitants in 2001; data from Medical Mission), Kwamalasamutu (2°21’ N, 56°47’ W), an Amerindian village along the southern border of Suriname with Brazil (1,068 registered inhabitants in 2001; data from Medical Mission), and Jamaica (4°20’ N, 54°23’ W), a small island with Maroon settlements (about 50 people) in the Lower Lawa River along the border of Suriname with French Guyana (near the medical clinic of Stoelmans Island with 3,111 registered inhabitants in 2001; data from Medical Mission; www.medischezending.sr). Kwamalasamutu is a Riverside village surrounded by tropical rainforest. Most houses have an open structure. A significant number of houses are built on stilts, with the people spending a lot of time underneath the house during the day. Drietabiki is part of a conglomerate of small islands in the Tapanahony River. The island has a

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surface area of 0.4 km². It is partly vegetated and surrounded by tropical rainforest. The Maroon houses in Drietabiki and Jamaica are mostly ground-level, closed structures. Jamaica is a settlement on a small island in the middle of the Lawa River situated just south of the larger Stoelmans Island. The island is partly vegetated and has a small swampy area on the southwestern side, a presumably ideal breeding site for *An. darlingi*. In all study sites, the people’s livelihood depends for a large part on hunting, fishing, and slash and burn agricultural activities. The people of each study village had access to a medical clinic, where suspected malaria cases were examined and if positive, treated appropriately.

**Human landing collections.** All-night human landing collections were conducted, with indoor and outdoor (peridomestic) collections simultaneously, during 3 consecutive nights during each survey. In 2006 and 2007, the surveys were done at two sites per village, with two collectors indoors and two collectors outdoors. Collections were done monthly in the first quarter of the year and one time in the subsequent quarters. In 2008 and 2009, the collecting effort was decreased to one site per village, with one collector indoors and one collector outdoors. Collections were done one time per 3 months. The collectors were trained local residents teamed up with or supervised by entomology technicians of the Bureau of Public Health. Collectors (males and females of variable age and body mass) were rotated based on availability in two 6-hour shifts between 18:00 and 06:00 hours. All mosquitoes landing on the lower legs of the collectors were collected with aspirators.

**Processing of samples.** The collected mosquitoes were counted, identified, labeled, and stored per hour of collection. Identification was done using the keys in the works by Faran and Linthicum, Linthicum and Gorham. Ovaries of female anophelines were dissected to determine parity rate by observing the tracheolar skeins as described in the work by Detinova. Heads and thoraxes were then stored in tubes with silica gel for additional processing in the laboratory. In the laboratory of the Bureau of Public Health, anopheline females were tested individually for the presence of *P. falciparum, P. vivax* 210, *P. vivax* 247, and/or *P. malariae* using the sandwich (modified) enzyme-linked immunosorbent assay (ELISA) for *P. falciparum, P. vivax* (VK210 and VK247 variant epitopes), and *P. malariae* circumsporozoite protein (CSP) detection according to the process by Wirtz and others.

**Malaria case detections.** In the three study sites, malaria incidence was established through passive case detection by the Medical Mission health clinics (for Jamaica, the reference clinic was Stoelmans Island). Malaria diagnostics at the local Medical Mission clinics were done by microscopic analysis of blood slides or rapid diagnostic tests (RDTs) cross-checked at a later date with microscopic analysis of the blood slides. Paracheck-Pf (Orchid Biomedical Systems, Verna, Goa, India) has been routinely used as an RDT for diagnosis of *P. falciparum* malaria. In 2005, Pf/Pan-specific tests were introduced as national policy in the country (BinaxNOW Malaria, Inverness Medical Innovations, Inc.). Malaria incidence data originate from the national malaria database maintained by the Ministry of Health.

**Data analysis.** The human biting rate (HBR) was calculated as the number of female anopheline bites per person per hour. The CSP index was calculated as the proportion of mosquitoes found to be positive for CSP in the ELISA analysis. The entomological inoculation rate (EIR) was calculated as the

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### RESULTS

Between January of 2006 and April of 2010, a total of 13,392 man-hours of collection (5,185 in 2006, 6,048 in 2007, 864 in 2008, 864 in 2009, and 432 in 2010 [until April]) resulted in 3,180 mosquitoes, including *Anopheles* (33.7%), *Culex* (43.3%), and *Aedes* (22.1%) species. The most abundant anopheline species were *An. darlingi* (88.07%) and *An. nuneztovari* (11.09%), which together, accounted for 99.16% of the anophelines collected. Other anopheline species found were *An. oswaldoi*, *An. albimanus* Wiedemann, and *An. intermedius*. Of the *An. darlingi* mosquitoes caught in 2007 almost all originated (363 of 372) from one survey at Jamaica in July of that year (Table 1). As of late 2007, anophelines were no longer found in any of the three villages. The mean hourly HBR of *Aedes* spp. and *Culex* spp. also decreased during the study from 0.581 and 0.200, respectively, in 2006 to 0.067 and 0.047, respectively, in 2009.

The highest mean HBR for *An. darlingi* was found in Jamaica during the survey in July of 2007. For this survey, the mean HBR indoors was 1.09 bites/man per hour, whereas for outdoor biting, the mean HBR was 1.43 bites/man per hour. The overall percentage of indoor versus outdoor biting of *An. darlingi* was similar in all three study sites: Kwamalasamutu, 40.0% versus 60.0%; Jamaica, 37.2% versus 62.8%; Drietabiki, 34.6% versus 65.4%. The pattern of indoor versus outdoor biting during the nightly hours of collecting for Drietabiki and Jamaica is shown in Figure 1. In Jamaica, the percentage of outdoor biting showed a peak during the eighth hour of collecting, which is between 01:00 and 02:00 hours. In Drietabiki, biting steadily increased toward 06:00 hours. By contrast, 71.4% of the total number of *An. nuneztovari* was collected between 19:00 and 20:00 hours.

Table 2 shows the mean hourly HBRs of *An. darlingi* over the years per study site, and Figure 2 shows the mean HBRs in 2006, 2007, and 2008 for Drietabiki and Jamaica. In Kwamalasamutu, the HBR was very low in 2006; one specimen of this species was found in 2007, and since 2008, the HBR has been zero. In Drietabiki, the HBR decreased

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### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Drietabiki</th>
<th>Jamaica</th>
<th>Kwamalasamutu</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human nights</td>
<td>372</td>
<td>372</td>
<td>372</td>
<td>1,116</td>
</tr>
<tr>
<td>Culicidae</td>
<td>1,331</td>
<td>1,422</td>
<td>400</td>
<td>3,153</td>
</tr>
<tr>
<td><em>Anopheles</em> spp.</td>
<td>241</td>
<td>803</td>
<td>29</td>
<td>1,073</td>
</tr>
<tr>
<td><em>An. darlingi</em></td>
<td>220</td>
<td>705</td>
<td>20</td>
<td>945</td>
</tr>
<tr>
<td><em>An. nuneztovari</em></td>
<td>19</td>
<td>93</td>
<td>7</td>
<td>119</td>
</tr>
<tr>
<td><em>An. oswaldoi</em></td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><em>An. albimanus</em></td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><em>An. intermedius</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>An. spp.</em></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Culex</em> spp.</td>
<td>686</td>
<td>324</td>
<td>368</td>
<td>1,378</td>
</tr>
<tr>
<td><em>Aedes</em> spp.</td>
<td>404</td>
<td>295</td>
<td>3</td>
<td>702</td>
</tr>
</tbody>
</table>

*Not identified to species because of loss of wings and/or legs.*
between January and February 2006, it was zero in January of 2007, and only three specimens have been collected during subsequent surveys until April of 2010. In Jamaica, the HBR increased between January and February of 2006 but decreased again after July of 2006. In October of 2006, the HBR was zero. It remained near this level, except during the surveys in July of 2007 and April of 2008. From 2009 on, no An. darlingi or other anophelines were found in any of the study sites, except for one An. nuneztovari in Drietabiki in 2010. Culex and Aedes population densities were also very low.

Worth mentioning is that, during short-term collections in an isolated small village about 100 km southwest of Drietabiki in 2008 and 2009, An. darlingi was still found, although the population had received and were using ITNs (Hiwat H, unpublished data).

Rainfall data from Drietabiki show that the rainfall pattern was in line with the annual seasons during the first 3 years of the study but a bit off during 2009; in that year, the month of March had a higher rainfall, and the month of May had a much lower rainfall than the previous years (Figure 3). Rainfall data from Jamaica and Kwamalasamutu are incomplete, unfortunately, which makes it difficult to relate peaks in HBR to rainfall and availability of mosquito breeding sites.

The overall parity rate of the An. darlingi females was 57%. The number of mosquitoes collected and dissected per survey or location was too low to determine seasonal patterns in parity rate. Combining all females of 2006 and 2007, we found a parity rate of 73.1% for January (N = 201), 56.7% for February (N = 164), 54.6% for March (N = 183), and 89.7% for April (N = 29). Considering the rain data available for Drietabiki (Figure 3), the numbers suggest a higher parity rate during the wetter months, which may indicate a (partial) dependence of the population on temporal (seasonal) breeding sites.

A total of 683 of the anophelines collected were tested for malaria infection. Of these 683, 642 were An. darlingi. Two anophelines were found to be malaria-infected: both were An. darlingi females, and both were infected with P. falciparum. One specimen originated from Jamaica and was collected in February of 2006. The other specimen originated from Drietabiki and was collected in January of 2006. This finding results in an An. darlingi CSP in Drietabiki of 1.02% in January of 2006 and 0.68% for the whole year. Combining this finding with the HBR data results in an EIR in Drietabiki of 1.7 infected bites per human in January of 2006 and 2.2 infected bites per human for the year of 2006.

The longitudinal entomological study presented here started just before the onset of the main interventions of the national malaria control program. As part of this program, vector control measures were introduced in the study sites in the second quarter of 2006. The malaria control program distributed free LLINs (PermaNet 2.0; Vestergaard-Frandsen, Switzerland) to people living in communities in the interior; 55,100 LLINs were distributed between 2006 and 2007, covering almost all stable communities in the interior (83%). In 2008 and 2009, an additional 14,508 and 386 LLINs were distributed, respectively, to replace used nets in high-risk areas and supply small communities that had not previously received them. Impregnation kits containing deltamethrin WT 25% (KO-Tab 123; Bayer Pty., Ltd) were used for impregnation and reimpregnation of nets since 2006. ITNs, including LLINs, were reimpregnated in high-risk areas. IRS with α-cypermethrin (Fendona; BASF) was conducted only along the Upper Marowijne and Tapanahony Rivers areas (including Drietabiki and

### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Drietabiki</th>
<th>Jamaica</th>
<th>Kwamalasamutu</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>0.1256 ± 0.4157</td>
<td>0.2146 ± 0.4577</td>
<td>0.0094 ± 0.0782</td>
</tr>
<tr>
<td>2007</td>
<td>0.0005 ± 0.0158</td>
<td>0.1606 ± 0.6265</td>
<td>0.0006 ± 0.0170</td>
</tr>
<tr>
<td>2008</td>
<td>0.0069 ± 0.0832</td>
<td>0.0903 ± 0.3525</td>
<td>0.0</td>
</tr>
<tr>
<td>2009</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2010*</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Overall</td>
<td>0.0428 ± 0.2487</td>
<td>0.01410 ± 0.5015</td>
<td>0.0039 ± 0.0499</td>
</tr>
</tbody>
</table>

* Until April of 2010.
Jamaica), which were the areas with the highest Annual Parasitic Index (API) in the country during two rounds in 2006. An overall coverage of 71% was achieved.

The number of malaria cases reported by the Medical Mission clinics in Drietabiki, Kwamalasamutu, and Stoelmans Island (nearby Jamaica) decreased to zero in 2006 and 2007 and remained around this level until the end of the study (Figure 4).

**DISCUSSION**

Within months after onset of this study, the population densities of *An. darlingi* and other mosquitoes dropped in the three study sites. Except for what seem incidental, possibly seasonal, occurrences, the HBRs observed in all three sentinel sites have been zero bites per man per night since 2007. Although this finding does not necessarily mean a complete disappearance of anophelines, the long-term absence of anophelines in human landing collections and the decrease in number of malaria cases suggest a collapse of the populations, which has not been reported in the country before. Although the quarterly observations do not provide a complete image, the overall long-term change in population densities compared with the data of 2006 and previous studies (Stoelmans Island area3,4,6) is clear. The (most likely) seasonal peaks recorded in Jamaica in 2007/2008 indicate a variability in population density that may be better understood with monthly surveys. One important constraint to doing monthly surveys would be costs of logistics.

The most interesting site in this study is Jamaica, not only because this site is situated in (what used to be) the heart of the high malaria risk area but also because mosquito data

![Figure 2. *An. darlingi* HBR. Mean HBR of *An. darlingi* per survey between January of 2006 and April of 2010 in Drietabiki (gray circle) and Jamaica (black circle).](image1)

![Figure 3. Monthly rainfall (millimeters) in Drietabiki from 2005 to 2009.](image2)
from Jamaica and locations nearby, from previous studies, exist. One of the study sites of Rozendaal\textsuperscript{2–4} was Aselikamp, a location almost 7 km south of Jamaica along the Lawa River. Rozendaal\textsuperscript{2–4} collected mosquitoes during selected hours of the night. The HBR in Aselikamp found during the studies by Rozendaal\textsuperscript{2–4} varied from monthly means of less than 1 to 5 bites/man per hour. Hiwat and others\textsuperscript{6} included Jamaica in a 1-year mosquito study with all-night collections in 2004/2005 and found an overall mean HBR over this period of just over 1.5 bites/man per hour compared with HBR values < 0.22 and decreasing during the current study.\textsuperscript{6} This finding indicates relatively high mosquito densities in the years before the study and interventions.

The onset of the malaria control interventions unexpectedly coincided with unusual, extensive flooding of the major rivers in the interior of the country. Population density and distribution area of \textit{An. darlingi} often have a positive relation to the availability of breeding sites.\textsuperscript{14–17} The combination of people on the move as a result of the flood and increased availability of mosquito breeding sites because of the rains was expected to trigger an increase in malaria transmission. As a preventive measure, additional bed nets were distributed.

Immediately after the floods, the mosquito densities, as established by HBR, in the sentinel sites were down to zero. This finding was thought to be a result of the flooding. Pajot\textsuperscript{18} found similar events in French Guiana, where the occurrences of heavy rains were followed by a decrease in and sometimes, a total absence of the mosquitoes. Heavy rains can flood breeding places and create flood currents that carry away immature mosquito stages.\textsuperscript{14,15} Having the floods coincide with malaria control activities makes the impact of target interventions difficult to measure. Drops in mosquito densities may have been caused by a combination of both factors. The continued low density (or even absence) of anophelines as well as \textit{Culex} spp. and \textit{Aedes} spp. in these sites over the course of several years is another matter. This finding cannot be attributed to the effect of the floods, especially because incidental increases in populations of these mosquito species were observed since that time. The role of other macroclimatic factors is not clear. Also, the role of the LLINs is not clear. A mass effect of the introduction of LLINs on the mosquito populations can be hypothesized. Decreased mosquito population densities and biting pressure have been found after use of insecticide-treated materials elsewhere, and (local) mass effects have been reported.\textsuperscript{20,21} The fact that, in a small isolated village 100 km southwest of Drietabiki, \textit{An. darlingi} can still be found, whereas along the much more populated Upper Marowijne River, the collectors return empty-handed rather supports the notion that a large-scale distribution of LLINs could have an impact on mosquito densities. Having an impact does not necessarily mean that local anopheline populations are eliminated (even if we cannot find them), which is shown by the peak occurrences of \textit{An. darlingi} in Jamaica in July of 2007 and again in April of 2008; these occurrences may have been related to the temporal availability of breeding sites.

Considering the vigorous washing behavior of the Maroon population, the impression is that the durability of the nets in terms of insecticide levels over time is much lower than advertised by the manufacturers (more information in the work by Atieli and others\textsuperscript{22}). Although this information will need to be confirmed, the nets may even reach the critical level of insecticide within 6 months of use. Redistribution and reimpregnation activities were executed in anticipation of the possible lower durability of the nets because of washing.

\textit{An. darlingi} is assumed capable of maintaining a high malaria transmission rate even when found in low densities, and it may keep up the transmission by a high human biting rate combined with a relatively high susceptibility to \textit{Plasmodium} infection.\textsuperscript{23–28} Finding the asymptomatic malaria cases in the human population is an important aspect of malaria control. Asymptomatic cases with very low parasitemias can be infective to \textit{An. darlingi}, even if the infections occur at a much lower rate than symptomatic cases.\textsuperscript{29} The ACDs performed within the malaria control program in Suriname showed that asymptomatic cases were present in the Jamaica and Drietabiki populations.
areas. By eliminating those sources of infection through treatment, continued transmission was prevented. Clearing of the infectious reservoir of malaria through ACD at times when mosquito densities are low is considered an effective strategy for the prevention of the return of malaria, because it is likely that the mosquito populations might increase again should the intensity of vector control lessen.

The *An. darlingi* biting data from Jamaica show a relatively high biting pressure in the early morning hours. A similar pattern was found by Harris and others in the Amazon forest in Bolivia. In contrast, Rozenaal et al. found a decreasing biting pressure toward the morning hours in a location not far from Jamaica. Early morning biting peaks have implications for malaria control through impregnated bed nets. Depending on the activity pattern of the local human population, it can present a considerable risk of transmission, which needs to be taken into account. Alternative complementing measures for vector control or prevention of malaria transmission need to be considered. These measures may also be of use in the control of malaria transmission in the gold mining areas, where the highly mobile human population is known to have a different daily activity pattern than the village population.

Epidemiological data show that current malaria transmission in Suriname is especially a problem of gold mining areas. Most Maroon and Amerindian villages have been free of malaria for a number of years. The absence of *An. darlingi* from our study sites since 2008 suggests a collapse of the local vector populations, and it will most certainly have had its effect on local transmission. Considering the events and interventions, this collapse is because of either the introduction of LLINs or a combination of climatic events and bed nets. The challenge will be to prevent malaria transmission in the village populations and ensure that anophele populations from elsewhere cannot become established in the former endemic malaria zone(s). In addition, work on decreasing transmission in the mobile gold mining populations should receive high priority.

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Reference


