VARIATION IN MALARIA TRANSMISSION INTENSITY IN SEVEN SITES THROUGHOUT UGANDA

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Abstract. Knowledge of the baseline malaria transmission in a given environment is important to guide malaria control interventions. However, in Uganda, recent information on malaria transmission intensity is lacking. Therefore, a 1-year entomological study was conducted in seven ecologically different sites throughout the country to assess spatial and temporal patterns in malaria transmission intensity. *Anopheles gambiae* sensu stricto was the main vector in five of the seven study sites, and *An. funestus* was the most important vector in the two other sites. In a peri-urban village, *An. arabiensis* contributed substantially to malaria transmission. Clear differences in annual entomological inoculation rates (AEIR) were observed between the study sites, ranging from 4 infective bites per person per year in the southwestern part of the country to >1,500 infective bites per person per year in a swampy area near the Nile River. Between villages with parasite prevalences of ≥80% in children between 1 and 9 years old, a 4-fold difference in AEIR was observed. Based on the observed behavior of the vectors, insecticide-treated bed nets will be highly effective in controlling malaria. However, in the high transmission areas, additional measures will be needed to reduce the malaria burden to acceptable levels.

INTRODUCTION

In Uganda, malaria is the leading cause of attendance at health facilities. During the period of 1991–2000, malaria incidence (confirmed and non-confirmed cases) increased from 163 to 249/1,000 persons. Similarly, an increase of malaria morbidity and mortality was observed in two district hospitals: one located in a stable and the other in an unstable malaria area. In last few years, important malaria epidemics occurred in unstable malaria regions, and this was attributed to climatic and environmental changes. Moreover, resistance to commonly used anti-malarials is widespread in Uganda.

In Uganda, according to available information collected during the 1930s to 1960s, the malaria epidemiologic situation is highly variable. Stable malaria occurs in ~95% of the country, with high transmission areas < 1,200 m altitude and low-medium transmission areas at 1,200–1,600 m altitude. Unstable malaria transmission is mainly found in the southwestern, eastern, and northeastern part of the country. A recent model based on climate suitability for malaria transmission shows similar patterns (MARA: www.mara.org.za). In areas suitable for endemic malaria, the levels of baseline transmission, as measured by entomological inoculation rate (EIR), are likely to be highly variable. Knowledge on the intensity of malaria transmission and its evolution over time is extremely important for choosing and targeting malaria control interventions. Indeed, protective efficacy of vector control measures will be dependent on the initial intensity of malaria transmission. However, recent data on malaria transmission intensity in Uganda are lacking. Therefore, a 1-year entomological study was conducted in seven ecologically different sites throughout the country. The results are reported below.

MATERIALS AND METHODS

Site description. Seven villages (one in each of the following districts: Apac, Arua, Jinja, Kanungu, Kyenjojo, Mubende, and Tororo) were involved in this entomological survey (Figure 1). The study locations are sentinel sites for the monitoring of anti-malarial drug efficacy and were selected by the National Malaria Control Program in the framework of the East African Network for Monitoring Anti-malarial Treatment (EANMAT). Such selection, based on past records and the associated ecological diversity, aimed at representing the heterogeneity of malaria endemicity in Uganda (Table 1). There were no vector control activities at the sites or in the neighboring areas either before or during the entomological survey, with few individuals owning bed nets.

Mosquito sampling and identification. For each study site, 11 entomological surveys were conducted from June 2001 to May 2002. Collections in Mubende were not correctly done during this period, so that sampling had to be done again from June 2002 to May 2003. No collections were organized during the dry month of January. Mosquitoes were collected by the human landing collection (HLC) method in three fixed houses separated by at least 300 m. Indoor collections in the three houses were made from 8:00 PM to 6:00 AM, and outdoor collections were made from 8:00 PM to 11:00 PM. Both collections were done during the same nights. Collectors were organized in teams, and replacement of workers within a team was done at 11:00 PM, 2:00 AM, and 4:00 AM. The teams rotated among houses. Distance between the house and the outdoor site was 10 m. Collections were made for 6 nights during each survey over a period of 8 days, resulting in 18 man-nights per study site per survey for the indoor HLC.

For morphologic identification in the field, only *Anopheles gambiae* sensu lato, *An. funestus*, *An. nili*, *An. moucheti*, and *An. christyi* were caught up in a simplified illustrated key adapted from Gillies and Coetze (1987). Other anopheline species were not identified further. All the anophelines were stored individually in numbered tubes with desiccant silica gel for laboratory processing.

Mosquito processing. Morphologic identification was re-
peated in the laboratory by a different working team. Members of the *An. gambiae* complex were identified by use of a polymerase chain reaction (PCR) technique previously described. For each site and survey, 50 morphologically identified *An. gambiae* s.l. samples that tested ELISA positive for *P. falciparum* circumsporozoite protein were identified by PCR to resolve the sibling species.

**Entomological inoculation rate.** The EIR was derived as the product of the sporozoite rate and the mosquito biting rate on humans. The human biting rate was derived from human landing collections and was expressed as the number of bites per person per night.

The average daily EIR was calculated for each survey period (8 days) and for periods of ~20 days between the surveys using the mean value of the previous and following period. The number of infective bites per person was further estimated on an annual basis (AEIR). EIRs were calculated separately for indoor and outdoor collection methods.

Beier and others established a linear equation describing the relationship between prevalence of malaria infection and AEIR. This model was used to calculate the prevalence from the observed AEIR. The estimated prevalence was compared with the prevalence observed in children between 1 and 9 years old in the same sites.

**Meteorological variables.** Monthly data were obtained from the Department of Meteorology comprising rainfall totals in millimeters and the average minimum and maximum temperatures, covering the survey periods of 2001 to 2002. Three of the study sites lacked a meteorological station within the district. Data from the neighboring district located at a distance of < 50 km were used. Kanungu site (altitude 994 m) was therefore represented by Kabale station (altitude 1,841 m), Kyenjojo (altitude 1,312 m) by Kasese station (altitude 944 m), and Apac (1,064 m) by Lira station (altitude 1,170 m). For Mubende only, temperature records were available for the study period.

**Ethical considerations.** The Uganda National Council for Science and Technology and the research ethics committee of the Prince Leopold Institute of Tropical Medicine in Ant-

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![Figure 1](image-url)

**Figure 1.** Map of Uganda showing the seven study sites.

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**Table 1.** Description of the seven study sites, their geographical positions, and some meteorological indices

<table>
<thead>
<tr>
<th>Study sites (district–village)</th>
<th>Position</th>
<th>Altitude (m)</th>
<th>Rainfall* (mm)</th>
<th>Minimum temp. (°C)</th>
<th>Maximum temp. (°C)</th>
<th>Landscape</th>
<th>Dominant ethnic group and economic activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arua–Cilio</td>
<td>03°11’42.2&quot; N 31°01’56.2&quot; E</td>
<td>930</td>
<td>1,289</td>
<td>17.3</td>
<td>28.7</td>
<td>Rural, north-western Uganda: savannah grassland interrupted by rocky hills.</td>
<td>Lugbara, Kakwa, and Madi people growing tobacco and cotton. Fishing.</td>
</tr>
<tr>
<td>Apac–Olami A</td>
<td>01°59’33.2&quot; N 32°43’10.1&quot; E</td>
<td>1,064</td>
<td>1,474</td>
<td>12.2</td>
<td>30.6</td>
<td>Rural, northern Uganda: savannah grassland with woodland and thickets, interrupted by extensive swamps and a few hills.</td>
<td>Lango people, peasant farming with cotton growing, fishing.</td>
</tr>
<tr>
<td>Tororo–Namwaya Central</td>
<td>00°46’10.6&quot; N 34°01’34.1&quot; E</td>
<td>1,125</td>
<td>1,459</td>
<td>17.8</td>
<td>29.9</td>
<td>Rural, eastern Uganda: dry savannah grassland interrupted by bare rocky hills.</td>
<td>Jopadhola, Iteso, Basamia, Bagwere, Banyioli people, peasant farming with cotton growing.</td>
</tr>
<tr>
<td>Jinja–School</td>
<td>00°26’33.2&quot; N 33°13’32.3&quot; E</td>
<td>1,162</td>
<td>1,523</td>
<td>17.1</td>
<td>28.1</td>
<td>Peri-urban, southern Uganda near Lake Victoria: hilly grassland.</td>
<td>Basoga people, growing coffee, cotton, sugarcane. Fishing.</td>
</tr>
<tr>
<td>Mubende–Nkrumah</td>
<td>00°22’31.4&quot; N 31°13’38.4&quot; E</td>
<td>1,228</td>
<td>–</td>
<td>15.5</td>
<td>26.6</td>
<td>Rural, southwestern Uganda: hilly grassland.</td>
<td>Baganda, Banyoro people growing coffee, cotton, tea. Batuoroor and Bakiiga people growing coffee and tea.</td>
</tr>
<tr>
<td>Kyenjojo–Kasina</td>
<td>00°37’02.4&quot; N 30°37’58.0&quot; E</td>
<td>1,312</td>
<td>918</td>
<td>17.8</td>
<td>30.4</td>
<td>Rural, southwestern Uganda: hilly grassland.</td>
<td>Bakiga, Batuoro, Banyankole people growing tobacco and coffee. Fishing.</td>
</tr>
<tr>
<td>Kanungu–Kihihi</td>
<td>00°45’03.1&quot; S 29°42’03.6&quot; E</td>
<td>994</td>
<td>1,071</td>
<td>11.9</td>
<td>24.5</td>
<td>Rural, southwestern Uganda: hilly grassland.</td>
<td></td>
</tr>
</tbody>
</table>

* Rainfall data for Mubende were not available for 2001–2002.
RESULTS

Anopheline biting rates. A total of 34,699 anophelines were collected indoors and outdoors; 96.9% were *An. funestus* and *An. gambiae* s.s. Four *An. nili* specimens were caught in Arua. No *An. moucheti* or *An. christyi* were collected during the study period. The category of "other Anopheline species" varied between 0% (Kyenjojo) and 13% (Jinja). Only *An. gambiae* s.l. and *An. funestus* were further considered in the analysis. *An. gambiae* s.s. was the predominant vector in five of the seven study sites: this species was dominant in Arua (69%, of 1,164 anophelines caught), Kanungu (96%, N = 1,320), Kyenjojo (64%, N = 107), Mubende (84%, N = 257), and Tororo (82%, N = 10,127). In Apac, *An. funestus* was the dominant species, representing 92% of the 21,353 anopheline mosquitoes caught. *An. arabiensis* was found in Apac, Arua, Jinja, and Tororo. In Jinja, it represented 45% of the collected anopheline mosquitoes (N = 371) and was the dominant species in this study site.

In Apac, *An. funestus* showed high indoor biting rates with almost 190 bites per man per night (BMN) in August and September. In May 2002, 160 BMN of *An. gambiae* s.s.s were observed in Tororo. Indoor biting rates were generally lower in Jinja, Mubende, Kyenjojo, and Kanungu, rising to a maximum of 36 BMN in Kanungu (Figure 2).

Entomological inoculation rate. In total, 9,070 *An. gambiae* s.l. and 6,244 *An. funestus* were tested by ELISA. Transmission intensity was highest and perennial in Apac, Tororo, and Arua (Table 2). *An. gambiae* s.s.s was responsible for > 80% of the infective bites in Tororo and Arua, whereas *An. funestus* was the main vector in Apac, contributing up to 88% of the AEIR. The daily EIR showed seasonal fluctuations depending on the study site. The lowest values were observed at the end of the long dry season (February). In the other study sites, Kyenjojo, Kanungu, Jinja, and Mubende, a much lower AEIR was observed (Table 2). Often the sporozoite rate could not be estimated in these low transmission sites, and we attribute a value of 0 for the EIR.

In all sites except Jinja, the indoor AEIR estimated for the period between 8:00 PM and 11:00 PM was only a small proportion (0–13%) of the indoor AEIR estimated from whole night surveys, indicating that the bulk of malaria transmission occurs later at night (Tables 2 and 3). The indoor and outdoor AEIR were compared for the 3-hour period of 8:00–11:00 PM. During that period, the AEIR was higher indoor than outdoor, except in Jinja and Kanungu. In Jinja, this was because of the transmission of both *An. arabiensis* and *An. gambiae* s.s.

It is important to notice that *An. gambiae* s.s.s contributed to the outdoor transmission relatively more than *An. funestus*, although in Arua, their relative contribution was almost equal (Table 3). Indoor and outdoor sporozoite rates for the 3-hour period were not significantly different (Fisher exact tests; P > 0.05), suggesting that differences in AEIR were determined by different biting rates in the indoor and outdoor collections.

In Jinja, the parasite rate in children 1–9 years old was much lower than that estimated by the model on the basis of the observed AIER. In all other sites, the observed and predicted values were similar (Table 4), although in Kyenjojo and Mubende, the observed parasite rates tended to be higher that those predicted by the model of Beier and others.9

DISCUSSION

Previous studies implicated *An. christyi*, *An. moucheti*, *An. hancocki*, *An. costalis*, and *An. pharoensis* in malaria transmission.16–18 In this study, we focused on five anopheline species, *An. gambiae* s.l., *An. funestus*, *An. nili*, *An. moucheti*, and *An. christyi*. All other anopheline mosquitoes were classified as other. *An. gambiae* s.l. and *An. funestus* were the omnipresent vectors, representing between 87% and 100% of the collected anopheline mosquitoes depending on the study site. No *An. moucheti* and *An. christyi* were collected during the study period. It is very unlikely that the other *Anopheles* species play an important role in malaria transmission, although they can be of local importance. *An. bwambae*, a malaria vector belonging to the *An. gambiae* complex, is unique to a small area around geothermal springs on the northwestern fringe of the Ruwenzori Montains.19

The EIR estimates the level of exposure to the malaria parasite-infected mosquitoes and is a commonly used index for assessing the malaria endemcity and the transmission intensity. Quality control was ensured by carrying out twice and by two different teams the morphologic identification of the main vectors collected, *An. gambiae* s.l. and *An. funestus*. Weak ELISA reactions were excluded to avoid overestimation of the sporozoite index.13 Random distribution of mosquitoes among houses of the same village is rare as adult wild mosquito populations often exist in clusters so that changing the houses sampled would have increased the chances of not capturing the seasonality of the biting rates and of the EIR.20 Therefore, in each sentinel site, to estimate the biting rates’ seasonality, mosquitoes were sampled from the same three houses throughout the project, the major disadvantage being that only a limited number of houses was followed during the study period. Because no vector control activities before or during the study period were implemented, the estimated intensity of malaria transmission could be used as a reference for any vector control program.

Important differences in AEIR were observed between the study sites, ranging from 4 infective bites per person per year in Mubende to > 1,500 infective bites per person per year in Apac. The values of AEIR found in Uganda cover the range observed throughout Africa,21 although Apac had one of the highest AEIR reported in Africa. This site, located between Kwania Lake and the Victoria Nile, is characterized by wetlands, where *An. funestus*, responsible for > 87% of the malaria transmission, is predominant. In two other sites, Tororo (eastern part) and Arua (northwestern part) with AEIRs > 395, *An. gambiae* s.s.s prevailed. Three sites had an AEIR < 10 and were all located in the hilly region west-southwest of Kampala and can be classified as unstable malaria areas.9,22 This part of the country shows large differences in climate suitability for endemic malaria (MARA: www.mara.org.za).

In this low transmission sites, the main malaria vector was *An. gambiae* s.s., except in Mubende, where *An. funestus* was predominant. In the peri-urban village near Jinja, only *An. gambiae* s.s.was observed. *An. arabiensis* played on important role...
in malaria transmission, but not proportional to its relative abundance. It contributed up to 45% to the collections, but it was only responsible for 23% of the malaria transmission. *An. arabiensis* is favored by drier environments, and the adaptation of this species to peri-urban environments has been described elsewhere in Africa. However, in Jinja, other environmental conditions (e.g., thermal breezes from the lake) probably explain the dominance of this species in this area. Indeed, in the rural Kisumu area, also along the Victoria Lake, *An. arabiensis* is dominant on the valley floor when *An. gambiae* is abundant on the foothills. Similar patterns were observed around the Tanganyika lake, with predomination of

**Figure 2.** Observed indoor bites per man per night (BMN) over the study period in the seven study sites for the three vector species *An. funestus, An. gambiae* s.s., and *An. arabiensis* and the observed rainfall data for the same period. In Mubende, no rainfall data were available for the study period.
An. arabiensis in the northern valley and An. gambiae s.s. along the southern foothills.\textsuperscript{25,26}

The observed AEIR in the seven study sites are in line with the past findings\textsuperscript{8} and recent predictions (MARA) of malaria endemicity. However, extremely variable AEIR corresponded in Apac, Tororo, and Arua to similar malaria prevalence among children 1–9 years old. By looking at the relation between prevalence and AEIR in Africa, Beier and others\textsuperscript{9} observed little variations in the prevalence of malaria infection at the highest AEIRs. Likewise, the incidence of infection in infants did not increase when the daily EIR exceeded one infective bite per person per night.\textsuperscript{27} Hence, malaria prevalence and incidence of infection are poor indicators of the intensity of malaria transmission. However, it is important to establish how the observed differences in AEIR in Apac, Tororo, and Arua translate in disease burden and what control effort is needed to decrease such burden to acceptable levels. The incidence of clinical \textit{P. falciparum} malaria in children < 18 months of age increases with increasing EIR.\textsuperscript{28} Furthermore, mortality in children < 1 year of age strongly increased with EIR.\textsuperscript{29}

In the sub-urban area of Jinja, located along the Victoria Lake, a lower parasite prevalence than expected by the estimated AEIR was observed. This might be because of the widespread use of anti-malaria drugs in the community.\textsuperscript{15} In this site, in contrast to other sites, the risk of being bitten by a positive An. gambiae s.s. or arabiensis is higher outside than inside. This could be explained by the close house constructions and the use of domestic insecticides inside the houses.

In Kyenjojo and Mubende, the observed prevalence tended to be higher than the predicted values. The prevalence of the 1999 survey was compared with the prediction based on AEIR measured in 2001–2002. The El Niño phenomenon of 1998 remarkably prolonged rainfall seasons and caused a malaria epidemic in Kyenjojo district.\textsuperscript{3,30} In these epidemic prone areas, yearly changes in transmission intensity are likely to occur so that malaria prevalence can vary substantially from 1 year to the other.

The insecticide-treated net (ITN) coverage, one of the key interventions for malaria control, is limited in Uganda (estimated at 13\%), with the majority of users in lowland and urban areas (RBM, http://rbm.who.int/wmr2005/profiles/uganda.pdf).\textsuperscript{31} ITN will be highly effective in controlling malaria because of the endophagic and late biting behavior of the two main vectors in Uganda. Indoor residual spraying (IRS) can be effective in response to changing transmission patterns in low transmission areas of southwestern Uganda.

Control measures against mosquito bites have a beneficial impact on malaria morbidity and mortality.\textsuperscript{32} The degree of control needed to obtain a public health impact would clearly vary between the sites, and control strategies should take the initial transmission levels into account. Indeed we know that the protective efficacy (relative decrease in mortality) of ITNs decreases with increasing malaria transmission. Protective efficacy of ITNs on child mortality was 33\% on the Kenyan coast (AEIR: 30)\textsuperscript{33} but only 16\% in the intense perennial transmission area of Western Kenya, Asembo (AEIR: 300).\textsuperscript{34}

However the numbers of lives saved (5.53/1,000 children) is

\begin{table}
\centering
\caption{Overview of the daily and annual EIR for the seven sentinel sites}
\label{tab:overview}
\begin{tabular}{lcccccccccccc}
\hline
\hline
Arua & 0.21 & 0.12 & 1.38 & 2.80 & 3.35 & 2.16 & 1.61 & 0.06 & 0.50 & 0.87 & 0.72 & 397 & 11.1 & 88.5 & 0.4 \\
Apac & 6.93 & 9.70 & 6.15 & 6.91 & 5.45 & 2.02 & 6.49 & 0.58 & 4.69 & 5.06 & 3.99 & 1,586 & 87.8 & 12.2 & 0 \\
Tororo & 1.18 & 5.25 & 1.62 & 0.46 & 0.86 & 1.38 & 2.61 & 0.35 & 1.60 & 3.01 & 1.03 & 562 & 18.5 & 80.0 & 1.5 \\
Jinja & 0.06 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.11 & 0.07 & 0.00 & 0.00 & 6 & 0 & 8.0 & 76.9 & 23.1 \\
Kyenjojo & 0.11 & 0.11 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.11 & 0.11 & 0.00 & 0.00 & 6 & 0 & 100.0 & 0 \\
Kanungu & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0.00 & 0 & 0 & 0.0 & 0 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{1} The EIRs are based on the indoor collections. The contribution of \textit{An. funeitus}, \textit{An. gambiae} s.s., and \textit{An. arabiensis} to malaria transmission is given in percentages.

\textsuperscript{*} The estimated AEIR for Mubende is based on the period March 2002–December 2002. The EIRs for the surveys June 2002–December 2002 were all zero, and are not given in the table.

\begin{table}
\centering
\caption{Observed and predicted parasite prevalences}
\label{tab:prevalences}
\begin{tabular}{lccc}
\hline
\textbf{Site} & \textbf{Observed AEIR} & \textbf{Observed \textit{P. falciparum} prevalence in children 1–9 years old from the 1999 survey 95\% CI} & \textbf{Predicted parasite prevalence 95\% CI} \\
\hline
Arua & 397 & 80.5 (71.7–89.4) & 87.6 (61.1–100.0) \\
Apac & 1,586 & 79.3 (70.8–87.8) & 100.0 (72.0–100.0) \\
Tororo & 562 & 90.6 (85.5–95.9) & 91.2 (63.9–100.0) \\
Jinja & 6 & 14.9 (6.4–23.5) & 42.9 (27.7–58.1) \\
Kyenjojo & 4 & 57.3 (46.6–80.0) & 40.3 (25.8–54.8) \\
Kanungu & 7 & 67.8 (58.0–77.6) & 45.8 (29.7–61.3) \\
\hline
\end{tabular}
\end{table}

\textsuperscript{1} The observed malaria prevalence data are from a cross-section survey done in 1999. The predicted values are based on the model of Beier and others.\textsuperscript{9}
similar in all areas, independent of the transmission level, and this paradox is explained by the higher overall mortality in high transmission areas.\textsuperscript{10} Effectiveness of ITNs is not only determined by the coverage, adherence using nets properly, and periodic re-treatment of the nets, but also on the vector species involved in the malaria transmission. In Western Kenya, \textit{An. funestus} was strongly affected by just the presence of at least one treated net within the house, and compliance seems to be less important when \textit{An. funestus} is the predominant vector,\textsuperscript{35} which is the case in Apac and in Mubende. In Apac, the transmission is considerably higher (five times) than in Western Kenya, and additional measures (e.g., environmental management of the swampy areas around the Nile River) may be required.\textsuperscript{36,37} In Western Kenya, indoor resting \textit{An. gambiae} s.l. was not or poorly affected when residents did not sleep under a net or if bed nets had not been retreated within 6 months.\textsuperscript{35} When \textit{An. gambiae} s.l. is the predominant vector, situation occurring in all other sites of this study, ITNs will only have an impact when nets are retreated and used consistently. Long-lasting insecticidal nets (LLINs) are now available and are the appropriate response for low re-treatment rates of conventional ITNs.\textsuperscript{38} Educational activities should be an integral part of the malaria control strategy to assure the use of ITNs every night. Scaling up coverage of ITNs is now the absolute priority for the control of malaria in Uganda.

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