LONGITUDINAL COHORT STUDY OF THE EPIDEMIOLOGY OF MALARIA INFECTIONS IN AN AREA OF INTENSE MALARIA TRANSMISSION I. DESCRIPTION OF STUDY SITE, GENERAL METHODOLOGY, AND STUDY POPULATION


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Abstract. A large-scale longitudinal cohort project was initiated in western Kenya in June 1992. The primary purpose of the project was to study Plasmodium falciparum malaria in a highly endemic area using a comprehensive and multidisciplinary approach, which included epidemiology, entomology, and immunology. Between June 1992 and July 1994, pregnant women living in 15 rural villages were identified during a monthly census and 1,164 were enrolled. The women were followed-up throughout their pregnancy and they, along with their newborn infants and direct siblings of the infants’ less than 15 years of age, were monitored over time. As of May 1995, 1,017 infants had been born to these women. This paper presents the design and general methodology used in this study and describes the initial experience with intense monitoring of a large population over a prolonged period.

Plasmodium falciparum infection can present with a wide spectrum of signs, symptoms, and history, from a fatal disease to an apparently asymptomatic infection, from a rapidly progressing, fulminant illness to a chronic insult. Many of the reasons for this wide variation in the presentation of this disease have been known for years, such as the protection afforded by some hemoglobinopathies. Recent scientific advancements and closer coordination of work done in various disciplines have begun to explain other observations for which there previously has been no satisfying explanation. For instance, application of newer genetic techniques to large populations has linked a particular human leukocyte antigen (HLA) type to increased risk of cerebral malaria, partly explaining the observation that there are some people who develop cerebral malaria while most of their neighbors, with presumably the same exposures and histories, do not. Combining the work of entomologists with that of clinical epidemiologists has demonstrated an effect of exposure pressure on disease development.

Because of the growing realization of complex interrelationships among the parasite, vector, environment, and host, advancing our understanding of falciparum malaria will require investigation of most if not all of these factors simultaneously. Conducting studies with similar designs in a variety of epidemiologic settings may also be necessary to understand the determinants of the full range of malarial illness. While a tremendous amount has been and can still be learned from cross-sectional studies, many of the questions that remain can only be addressed by monitoring larger numbers of people over longer periods of time, such as has been done in Senegal, Ghana, and Nigeria.

A study initiated in June 1992 in western Kenya was designed to address the epidemiology, entomology, immunology (both humoral and cellular responses to defined antigens), host factors, molecular biology and antigenic variation, and population genetics of P. falciparum in a large cohort of women, newborn infants, and older children monitored over an extended period. Taken as a whole, this project will provide a comprehensive characterization of malaria in a single site with intense malaria transmission. This paper describes the site, population, and methods used in this study. Additionally, we describe the initial experience with intense monitoring of a large population over time in terms of acceptance of the study methods by the population during the first two years of this project. The paper that follows presents the basic descriptive epidemiology of malaria and anemia in this population.

METHODS

Site description. The study was conducted in 15 contiguous villages in the Asembo Bay area of Siaya District in Nyanza Province in western Kenya (Figure 1). The villages are at an elevation of approximately 1,100 m and are 50 km from the provincial capital of Kisumu and 10 km south of the equator. The principal mosquito vectors in this area of Kenya are Anopheles gambiae s.s., An. arabiensis, and An. funestus. Malaria is holoendemic throughout the study site area; entomologic inoculation rates in an area similar to and immediately adjacent to the study site have been estimated to average 0.75 infective bites per person per day.

The inhabitants of the study area are predominately of the Luo ethnic group. Their principal occupations are subsistence farming, fishing, and raising cattle. Polygamy is common; different wives and their children live in separate houses within a single family compound. Many of the male heads-of-household are employed outside the village and return to their homes late in the evening or on weekends. Women and older children spend much of their time tending cattle or working in privately owned gardens, where they raise corn, beans, cassava, and other vegetables and fruit.

Villages in this area typically comprise a loose conglomeration of compounds separated by garden plots, grazing land, and streams. These streams and low-lying areas tend to flood during the rainy seasons in March to May (long rains) and October to December (short rains). There is one mission hospital, several government-run health clinics or dispensaries, and several private clinics in this area. While the hospital and private clinics charge for services, health care is generally free at the government facilities. Government-run district hospitals, located in Siaya and Kisumu, are approximately 50 km away.
Participant selection and field methods. Two individuals residing in each of the 15 villages were recruited and trained as village monitors to conduct interviews and collect samples from the study participants. Additionally, two trained community health workers/birth attendants (nyamrerwa) from each village were recruited and trained to conduct a monthly census of every individual residing in the study villages and to be available to attend any births occurring in their village.

Starting in June 1992, pregnant women in the 15 villages were identified during the monthly census. The study was explained and those women giving informed consent were enrolled. Participating women were visited at home once per month during their pregnancy by the village monitors. At the initial visit, the village monitors collected information regarding the participant's family and reproductive history. Individuals were considered to be family members if they spent a minimum of four nights per week in the household and, with the exception of newborns, had been present in the household for at least four weeks. Additionally, basic demographic information and information on socioeconomic indicators and the use of anti-mosquito measures was collected.

At each monthly visit the village monitors administered a standardized questionnaire, collecting information regarding illnesses that had occurred since the last visit, symptoms experienced, use of health-care facilities, and use of medicines. Axillary temperatures, thick and thin blood smears for malaria parasitemia, and capillary blood samples for hemoglobin determination and immunologic studies were also obtained. Estimated dates of delivery were calculated from each woman's reported date of last menstruation. The frequency of visits was increased to once per week beginning one month before the estimated date of delivery and continued at this frequency until delivery.

Births occurring at home were attended, when possible, by that village's nyamrerwa. Information on the birth was collected, as were maternal capillary blood samples and blood smears, cord blood samples and smears, and placental smears. Birth weights were obtained within 24 hr and gestational age was assessed by using a modified Dubowitz score within two weeks of birth.12

Mother-infant pairs were visited by the village monitors within two weeks of birth and every two weeks thereafter. Direct siblings of the infant who were less than 15 years of age were also enrolled at this point. At each visit, questionnaires as described above were administered for each individual, with information on the children's health reported by the mother or an adult (> 15 years old) sibling or guardian. Axillary temperatures were obtained by using digital ther-
The presence of gametocytes was noted but not quantified. Hemoglobin concentrations were measured using the HemoCue system (HemoCue, Anglholm, Sweden). Hematocrit (axillary temperature $\geq 37.5^\circ C$) and malaria parasitemia received a treatment dose of sulfadoxine/pyrimethamine. The village monitors were available to take temperatures and blood smears at any time between regularly scheduled visits for any participant who thought that they or a child was ill. Severe malaria, severe anemia, non-malarial illnesses, or illnesses judged to require more than oral antimalarial treatment were referred to local health facilities. Any relevant laboratory information (e.g., blood smear results, hemoglobin concentration) was supplied to the health clinic.

Mother-infant pairs and siblings less than five years of age were to be monitored in this fashion until the infant’s or sibling’s fifth birthday. Enrolled siblings between the ages of five and 15 years were followed up for a minimum of one year before being dropped from the study.

Field supervision and data management. The study site was divided into four sectors, each with a primary and assistant supervisor, who were also permanent residents of the study area and who were responsible for coordinating the activities of the village monitors and nyamwerwa. One overall supervisor was responsible for oversight of all activities and transfer of questionnaires and samples from field personnel to personnel from the main laboratory in Kisian (approximately 25 km from the study site). All data forms were reviewed on site for completeness and matched to samples and blood smears, if indicated. Supervisors ensured that at least three attempts were made to monitor participants before a scheduled visit was considered missed. Participants not at home for three consecutive months were dropped from the study. When possible, information from friends or neighbors of dropped families or participants was collected to determine reasons for absence.

All data were entered into a networked, dual-entry database system. Records with discrepancies identified through comparison of the two entries were sent back to the field supervisors for correction and verification. All hard copies of questionnaires and laboratory data were kept on file in the main laboratory in Kisian.

Mortality rates. For ease in comparison, crude infant and less than five years of age mortality rates were calculated by using previously described methods for Kenya. From the complete reproductive history given by women, mortality rates were calculated from information on the number of live births and subsequent survivorship during a five-year period preceding the interview and expressed as deaths per 1,000 singleton live births. Similar rates were calculated separately for twins.

Laboratory procedures. All blood smears were stained with Giemsa stain and parasite densities were quantified by counting the number of asexual parasites per 300 leukocytes. The density was calculated assuming a leukocyte count of 8,000/mm$^3$. The presence of gametocytes was noted but not quantified. Hemoglobin concentrations were measured using the HemoCue system (HemoCue, Anglholm, Sweden). Capillary blood samples were collected in heparinized tubes, kept on ice, and transferred to the main laboratory within 24 hr. Samples were then centrifuged; plasma and red blood cell pellets were stored separately at $-20^\circ C$ until analyzed in Kisian or transferred on dry ice to the Centers for Disease Control and Prevention (CDC) (Atlanta, GA) for analyses not available in Kenya. Red blood cell pellets from parasitic participants were stored in liquid nitrogen until transfer to CDC. Specific laboratory methods and entomologic methods will be presented in anticipated future papers.

This study was approved by the Ethical Review Board of the Kenya Medical Research Institute (KEMRI) and CDC.

**RESULTS**

Data presented in this paper represent information collected from women enrolled between June 1, 1992, and July 31, 1994. Follow-up data used in the analysis were collected between June 1, 1992, and May 1995.

Rainfall. In a typical year, there are two rainy seasons, the long rains from March to May and the short rains from October to December. During the study period, some deviation from this normal pattern was noted (Figure 2). Both the short rains of 1992 and the long rains of 1993 started and ended about a month later than usual. The short rains of 1992 were biphasic, with two wet months (November and January) and an intervening dry month. Overall, 1993 was a relatively dry year, with the amount of precipitation during the short rains much below that of preceding and subsequent years, while the long rains of 1994 lasted longer than usual. The first 10 months of 1995 appeared to be following a more normal pattern.

Population. The total population of the 15 study villages during this period fluctuated between approximately 17,000 and 18,000 individuals. The mean village population was 1,179 (SD = 301) and ranged from 614 to 1,686. Each village had between 75 and 196 occupied compounds (average = 133); the average compound contained 2.0 households and the average household was composed of 4.2 (SD = 2.4) individuals.

Enrollment, loss to follow-up, and completeness of data collection. As of July 31, 1994, 1,164 women had been enrolled in the study (Table 1). Of these, 995 (85.5%) were still enrolled when their infant was born. A total of 1,017 infants were born (973 singletons, 44 twins). During the period analyzed, 63 women gave birth a second time; these
Table 1

<table>
<thead>
<tr>
<th>Time point</th>
<th>No. possible</th>
<th>No. seen</th>
<th>Success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
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<td>100</td>
<td></td>
</tr>
<tr>
<td>Delivery</td>
<td>1,164</td>
<td>995</td>
<td>85.5</td>
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<tr>
<td>Infants</td>
<td>1,081</td>
<td>1,008</td>
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<tr>
<td>First visit by village monitor</td>
<td>1,081</td>
<td>1,008</td>
<td>93.3</td>
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<td>Age 3 months</td>
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<td>80.7</td>
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<td>Age 6 months</td>
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<td>805</td>
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<td>Age 18 months</td>
<td>585</td>
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<tr>
<td>Age 24 months</td>
<td>383</td>
<td>125</td>
<td>32.6</td>
</tr>
</tbody>
</table>

* The number of infants includes those from second pregnancies during the study period. Numbers of infants possible represents the maximum number of infants who could have been seen for at least that period of time given the date of birth and date of data analysis. For example, of 383 infants who were born early enough in the study to allow for at least 24 months of follow-up, 125 (32.6%) were actually monitored ≥24 months. – = unknown.

The mean duration of follow-up before delivery of their child remained fairly constant over the first 18 months (13% [53 of 408] in 1992 and 14.9% [69 of 462] in 1993); however, the percentage decreased in the first half of 1994 (4.4% [13 of 294]; P < 0.01 compared with 1993, by χ² test). While moving away from the study area as a reason for leaving the study remained fairly constant over time (60.4% [32 of 53] in 1992 to 65.2% [45 of 69] in 1994), voluntary withdrawals decreased from 18.9% [10 of 53] in 1992 to 7.7% [1 of 13] in the first half of 1994 (P > 0.1, by χ² test). Of the 1,081 births to women enrolled in the study (including second pregnancies), 838 (77.5%) were attended by study personnel. Birth attendance improved over time as well, from a low of 58.2% (149 of 256) in 1992 to 85.5% (329 of 385) in 1994.

Between delivery and the first follow-up visit by the village monitors, an additional 70 women left the study, leaving 1,008 infants with at least one post-delivery visit. Delivery of a stillborn child accounted for one-third of these women leaving the study. Voluntary withdrawal and moving out of the study area were the next most common reasons for leaving the study.

The mean duration of follow-up before delivery of the child (or loss to follow-up) was 2.9 months (SD = 2.2). As of the time of analysis (May 1995 or approximately 35 months after the start of the project), there were a total of 13,037 person-months of post-delivery follow-up for enrolled mothers and 13,317 person-months of follow-up for infants. The mean duration of follow-up after delivery was 11.3 months (SD = 9.0, range = 0.07–32.7 months). The dropout rate averaged 2.8% per month over the first 24 months of the infant’s life. Dropout rates decreased with increasing length of participation; dropout rates averaged 5.6% per month between 0 and 6 months of age, decreasing to an average of 1.6% per month between 18 and 24 months.

The protocol required participants to be interviewed at least twice a month during the time they were monitored. Given the actual length of time in the study for each of the enrolled mothers, 72% (18,635 of 26,074) of expected interviews were actually conducted (for families with more than two visits in a given month, only the two routine visits were counted). For infants, 73% (19,418 of 26,634) of expected interviews were successfully completed.

Demographic characteristics and reproductive history of the study population. The mean age of women at enrollment was 26 years (SD = 7.1); 17.8% (206 of 1,156) were currently in their first pregnancy, 16.0% (185 of 1,156) had one previous pregnancy, 66.1% (764 of 1,156) had two or more previous pregnancies (one woman had no reported data on previous pregnancies). Of those not in their first pregnancy (n = 949) at the time of enrollment, the mean number of previous pregnancies was four (SD = 2.6) and ranged from one to 12.

The 949 multiparous women reported a total of 3,825 children born of previous pregnancies, 95.5% of which were singleton births, 3.1% were twins, and 1.8% were indeterminate (abortions/miscarriages). Of 3,652 singleton births, 97% were born alive and of these singleton live births, 79.5% were alive at the time of enrollment. Of the 59 sets of twins, 100 (84.8%) children were born alive and of those, 61% were alive at time of the woman’s enrollment. The mean age (± SD) at death was 1.3 (± 2.0) years and 0.5 (± 0.8) years for singleton and twin live births, respectively. Among live-born children from previous pregnancies who were reported as having subsequently died, 61.9% of the singleton births and 83.9% of twin births died before one year of age. When information collected from the reproductive history of women at the time of enrollment was used, the crude infant mortality rates for births occurring in the 10-year period before the interview date (n = 2,462) was 109 per 1,000 singleton live births; the crude under-five years of age mortality rate was 174 per 1,000 singleton live births. The corresponding rates for twins (n = 72) were 250 per 1,000 twin live births and 319 per 1,000 twin live births.

Housing, education, and socioeconomic indicators. Most houses belonging to study participants were constructed of traditional materials. Eighty-five percent (974 of 1,152) of houses had mud walls, 82% had dirt floors, and 80% had a thatched roof. Most houses (52%) did not have windows. When present, most windows (44%) were closed by either glass windowpanes or wooden shutters; 72% of the doors were wooden. Houses of completely modern construction accounted for only 10% of the houses in the study area.

Of women interviewed on enrollment, 91% (1,049 of 1,153) reported having some formal education; 64% attended school for at least seven years and 5% reported attending for 11 years. Overall, 72% of the women reported that they could read a newspaper and 87% reported that they could write a letter.

Based on an informal review of perceptions of residents in the area, three indicators were used to estimate relative socioeconomic status of families: ownership of more than one building within a compound, ownership of a bicycle, and ownership of a pressure lamp. Forty-four percent (507
of 1,151) of families owned two or more buildings, 35% owned at least one bicycle, and 17% owned at least one pressure lamp. Twenty-two percent of the families owned two or more buildings and either a bicycle or a pressure lamp and would be considered of high socioeconomic status for this area; 77.6% of the families were, therefore, judged to be of low socioeconomic status.

**Use of anti-mosquito measures.** In response to questions on the use of anti-mosquito measures, 8.5% (98 of 1,153) of the women reported using a bed net regularly, 17.7% burned mosquito coils, 2.7% used an insecticide spray, and 12.1% reported burning dung or leaves. Overall, 67% of the women reported not taking protective measures on a regular basis, and only 5% reported using more than one method regularly.

**DISCUSSION**

Measuring the public health impact of interventions for malaria, from antimalarial drugs to bed nets to vaccines, requires identifying and using endpoints or outcomes that can serve as markers for protection from the effects of malaria. For example, endpoints used to evaluate antimalarial drugs should represent some definition of therapeutic success, such as duration of favorable clinical response or adequate hematologic recovery. To choose endpoints that are relevant and interpretable, the complex interrelationships among malaria parasite, host, vector, and environment need to be clarified. Large, population-based longitudinal studies using a multidisciplinary approach may provide the best method for achieving this goal. The study described here was designed to begin to address these interrelationships in an area of intense *P. falciparum* transmission.

The primary difficulty encountered in this study was maintaining the intense follow-up schedule in a large population over time. Two factors played a large role in this difficulty: the general mobility of the people living in this area of Kenya, and voluntary withdrawal. Much of the movement was determined by economics and cultural practices and was beyond our control. Voluntary withdrawals occurring early in the follow-up schedule were anecdotally linked to a poor understanding of the project; voluntary withdrawals occurring late in the follow-up schedule were more closely linked to participants tiring of the study. A concerted effort to explain the study in greater detail and to involve the participants more directly in discussions of the study goals, and a modest incentive program (e.g., provision of free nyamrerwa services and a small gift consisting of a few baby-care items at the birth of the infant) helped reduce withdrawals for all reasons from 13% in 1992 to 4.4% in the first half of 1994 and refusal to continue participation from 18.9% in 1992 to 7.7% in 1994. Additionally, a better understanding of the study may have accounted for the increase in the proportion of births attended by study personnel from 58% in the first year to 85% in the second. Although a better understanding of the goals of the project probably decreased study fatigue, the mean duration of participation as of May 1995 was only about 11 months.

Completeness of data collection, as measured by the percentage of expected routine interviews actually being conducted, was approximately 72%. Given the intensity of follow-up, frequency of blood sampling, requested length of participation, and mobility and size of the population monitored, we were satisfied with this completion rate. Again, reasons for missed interviews were most commonly related to the mobility of the population (short-term moves away from the study site).

The crude infant mortality rate for this population (109 per 1,000 live births), estimated from women’s reproductive history over 10 years before enrollment, was nearly twice the national rate of 59.6 per 1,000 live births during the period 1979–1989 and was higher than the provincial rate of 94.2 per 1,000 live births. Published infant mortality rates for specific towns close to the study site were slightly higher (150–162 per 1,000 live births). The overall less than five years of age mortality rate in this area was 174 per 1,000 live births, again higher than the rates reported for either Kenya as a whole or for Nyanza Province (89.2 and 148 per 1,000 live births, respectively), perhaps reflecting the excessive burden of mortality being experienced by rural populations living in highly malarious areas.

While many of the interventions being developed to decrease the burden of malaria-associated morbidity and mortality hold tremendous promise, in theory at least, they are years away from realization. As new interventions are developed and tested in the field, evaluation using relevant endpoints becomes crucial. However, new methods for managing malaria as it currently exists in sub-Saharan Africa are seriously needed. Not only can a detailed description of the natural history of malaria in a population assist in identifying relevant endpoints, it can also provide insights into how disease states develop, define more clearly who is at risk of developing those disease states and when, and suggest new interventions that can be tested and applied immediately.

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