# An integrated view of entomological and parasitological observations on falciparum malaria in Gambela, Western Ethiopian Lowlands*

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## Summary

A 14-month longitudinal malaria survey was performed among the Nilote inhabitants of Gambela, a small garrison town in Illubabor Province, Ethiopia. The results are integrated with a simultaneously performed entomological study. Monthly variation in *Plasmodium falciparum* prevalence was primarily a result of seasonal fluctuation in risk of sporozoite inoculation from *Anopheles arabiensis* (= *gambiae* species B), *A. funestus*, and *A. nili*. The proportion of sporozoite inoculations actually infective was estimated, assuming a constant recovery rate, by employing Macdonald's formulae. Inoculation (incidence) rates calculated from progressive increase and decrease in *P. falciparum* prevalence were then taken as fractions of entomologically measured 'crude' inoculation rates. Among children, values of 7 to 27% were obtained, the higher occurring in the first quarter of the wet season. Estimates among adults were approximately half those for children, illustrating the more competent immune state of the older age group.

Using the methods of BEKESSY *et al.* (1976), incidence and recovery were estimated on the basis of transition rates (parasite negative to positive; positive to negative) derived from a group of inhabitants examined at 28-day intervals. Incidence rates were estimated to be two-fold greater among children than adults; recovery rates were about three-fold greater among adults than children.

## Introduction

An entomological study was performed, in 1967-69, on the dynamics of malaria transmission in the small provincial town of Gambela. The relative abundance, seasonal distribution, man-biting habits, sporozoite rates, and bionomics of the locally prevalent anopheline mosquitoes were examined (KRAFSUR, 1970; 1977). It was concluded that *Anopheles arabiensis* Patton, known heretofore as *A. gambiae* Giles species B, *A. funestus* (Giles), and *A. nili* (Theobald) were the species responsible for malaria transmission. No evidence suggested that the other common anthropophilic species, *A. pharoensis* Theo., *A. wellcomei* Theo. and *A. ziemanni* Grunberg were of importance to the epidemiology of malaria. A survey of blood parasites was simultaneously carried out in the same community among the indigenous inhabitants, the Anuak (ARMSTRONG, 1972). Blood film examinations showed that in the three-month periods

following peak sporozoite challenge in 1967-69, the average prevalence of *Plasmodium falciparum* was 58% among children (less than 15 years old) and 35% among adults (15 years of age or older). Estimates of *P. malariae* were 16% and 7% in the two age groups. *P. ovale* and *P. vivax* were found with much less frequency, as is suggested by a prevalence of ovale of 2·1% and of vivax, 0·9%, in the same children. Rates of falciparum malaria were highest in children three to nine years of age and fell off abruptly after the age of 15 years.

In the present report, the risk of contracting malaria, as derived from entomological data, is compared with parasitologically estimated infection rates and seasonal distribution of falciparum malaria in the study population. Formulae devised by MACDONALD (1950a, b) were employed to estimate the approximate proportion of potentially infective sporozoite challenges actually infective. The methods of BEKESSY *et al.* (1976) also were employed to make analogous estimations.

### The study area and population

Gambela is situated in an extensive, sparsely-settled, woodland savanna on the banks of the River Baro. The region extends from the highland escarpment, nearby to the east, west to the Sudan, and south to Kenya. The area has been described, with maps, by HUTCHINSON (1971). Distribution of rainfall is seasonal, the greater part of an annual mean of 1257 mm falling between the months of May and October. Annual flooding of the Baro occurs between July and October and, like the Nile (to which the Baro is a substantial tributary), is a consequence of the tremendous rains falling in the Ethiopian highlands. Daily average relative humidities typically vary from 64 to 74% in the wet season and 42 to 51% in the dry. Hottest and driest months are March and April.

Gambela is a small, semi-isolated administrative and marketing centre at the head of navigation on the River Baro. The preponderant ethnic group in the town, overwhelmingly so in the region as a whole, is the Anuak, a fishing and cultivating tribe of

Nilotic origin. The Anuak live in low, circular huts of a single room, made of thatch or of mud and thatch. Clusters of such dwellings make up vaguely demarcated Anuak 'villages' within Gambela. Marketing a small variety of mundane manufactured goods, maize, onion, millet, mangoes, banana, and fish, is the principal activity in town, the medium of exchange being provided by the central government payroll to police, teachers and administrators, all of whom are highlanders.

The population residing in Gambela was estimated to comprise no more than 1,600 inhabitants, of whom the majority were Anuaks. The age distribution of the population was found to be 30% of 14 years old or less, and 70% 15 years and older. The values, 0·3 and 0·7 are used to age-standardize malaria prevalence rates. The formula for age weighting is $Y = 0·3y + 0·7y'$, where $y$ and $y'$ are the respective proportions with demonstrable parasitaemia and $Y$ is the age-standardized statistic.

With respect to malaria prevalence, Gambela is a mesoendemic island in an hyperendemic sea. Annual sporozoite challenge was about ten-fold greater among the riverine Anuak villages typical of the region than that in Gambela (KRAFSUR, 1977). Parasite rates also were greater in the same river villages than in Gambela (ARMSTRONG, unpublished).

## Methods

### Entomological techniques

Man-vector contact was systematically measured by collection of indoor-resting *Anopheles* and all-night indoor human bait captures. Pyrethrum spray collections were made from six native dwellings daily four times weekly, and each 'village' was sampled at least twice monthly. Man-biting captures were performed indoors throughout the night twice weekly. Precipitin testing of the stomach contents of 283 engorged, indoor-resting *A. arabiensis*, 160 *A. funestus,* and 94 *A. nili* demonstrated that each had taken human blood. Mosquito collections were identified to species, age-graded, and examined for sporozoites. Further details of entomological procedures and results are given elsewhere (KRAFSUR, 1977).

### Parasitological techniques

Thick and thin blood films prepared on separate microscope slides were collected from Anuaks. Most were obtained from subjects participating in a 14-month longitudinal survey performed at four week intervals. Single-examination survey results amongst Gambela Anuaks are also included where appropriate. Malaria diagnosis and parasite counts were made, being extensively cross-checked by each of three microscopists from the thick films.

## Results

### Seasonal variation in parasite and inoculation rates

Monthly falciparum malaria infection rates among Anuak residents of Gambela varied approximately two-and-a-half-fold in children and three-and-a-half-fold in adults (Table I). An absence of homogeneity in monthly prevalence was

**Table I—Estimated mean number of sporozoite inoculations/person/month, average risk of receiving one or more inoculations/person/month, *P. falciparum* parasite rates among children and adults, and age-standardized falciparum malaria prevalence among inhabitants of Gambela, 1967-1969**

| Period | Sporozoite Inoculations | Risk* | Children < 15 yrs | | Adults ≥ 15 yrs | | Age-standardized Prevalence |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | N | Parasite Rate | N | Parasite Rate | |
| Jul | ·30 | ·26 | | | | | |
| Aug | 1·28 | ·72 | | | | | |
| Sept | 4·08 | ·98 | | | | | |
| Oct | 3·49 | ·97 | | | | | |
| Nov | 1·30 | ·73 | | | | | |
| Dec | 1·15 | ·68 | 63 | 58·7% | 96 | 22·9% | 33·7% |
| Jan | ·29 | ·25 | 45 | 46·7 | 61 | 11·4 | 27·9 |
| Feb | ·59 | ·45 | 59 | 50·8 | 63 | 12·7 | 23·2 |
| Mar | 0 | 0 | 91 | 39·6 | 131 | 16·0 | 20·0 |
| Apr | ·01 | ·01 | 89 | 34·8 | 136 | 14·0 | 15·7 |
| May | ·17 | ·16 | 58 | 34·5 | 62 | 14·5 | 19·0 |
| Jun | ·10 | ·10 | 44 | 29·5 | 67 | 16·4 | 25·8 |
| Jul | ·22 | ·20 | 37 | 48·6 | 63 | 15·9 | 24·8 |
| Aug | ·46 | ·37 | 107 | 30·8 | 101 | 12·9 | 19·5 |
| Sept | 2·48 | ·92 | 36 | 58·3 | 53 | 18·9 | 36·7 |
| Oct | 2·06 | ·87 | 33 | 57·6 | 57 | 35·1 | 45·0 |
| Nov | 2·23 | ·89 | 38 | 57·9 | 56 | 32·1 | 52·3 |
| Dec | 1·54 | ·79 | 39 | 56·4 | 57 | 40·4 | 50·6 |
| Jan | ·39 | ·32 | 34 | 73·5 | 60 | 36·7 | 42·8 |
| Feb | — | — | 93 | 60·2 | 93 | 29·0 | 39·4 |

*Risk = $1-e^{-\text{inoculations/month}}$

demonstrated by chi-square analysis (SNEDECOR & COCHRAN, 1967, p 240), and reflects the operation of seasonal phenomena. The mean number of sporozoite inoculations per person also varied greatly from month to month. Sporozoite challenge, set forth in Table I against prevalence rates of *P. falciparum,* are the sum of monthly inoculations by *A. arabiensis, A. funestus,* and *A. nili.* Most sporozoite inoculations were delivered late in the wet seasons and early in the dry season. Thereafter, sporozoite challenge declined until March, when no positive *Anopheles* were found (356 *A. arabiensis* and 660 *A. funestus* were dissected). Sporozoite rates increased perceptibly with the onset of the wet season, in May, and abruptly so in July, but very low mosquito densities ensured a relatively small chance of inoculation in the first three months of this period.

The principal factors governing dynamics of the inoculation rate were fluctuation in vector densities and their sporozoite rates. *A. arabiensis* populations were greatest when maximum flooding of the River Baro occurred in August and September. This species became, for a short period, prevalent in the dry season owing to the formation of numerous pools left by declining streams, but the mean frequency of inoculation increased only slightly, in February, because of the low infection rate shown by this species in the desiccating conditions of the season. Minimum densities of *A. arabiensis* and *A. funestus* were recorded in the first three months of the wet seasons, the result of a lack of suitable breeding sites because of rapidly fluxing stream levels. *A. funestus* populations rapidly built up once flooding of the Baro began to subside, maximum densities being observed in January and February, 1968, and December 1968, well after the onset of the dry seasons when precipitation ceased and the river level had dropped considerably. *A. nili* populations followed the distribution of rainfall, this mosquito rarely being found in the dry season but altogether common during periods of maximum precipitation.

*Estimation of risk*

The entomological inoculation rate is the product of the chance of any one mosquito bite including sporozoites (the sporozoite rate, *s*) multiplied by the estimated mean number of bites in *t* days to which a person is subject. The chance of not becoming inoculated in a single *Anopheles* bite is, $1-s$, where *s* is the sporozoite rate, and that in *n* independent bites in *t* days is, $(1-s)^{n/t}$. As *s* is small with respect to $n/t$, $(1-s)^{n/t} \simeq e^{-sn/t}$. The quantity $sn/t$ is the entomological inoculation rate, henceforward assigned the symbol $h_e$. Risk is defined as the probability of receiving one or more sporozoite inoculations per unit of time. The probability of one or more sporozoite inoculations in *t* days is therefore,

$$\text{Risk} = 1 - e^{-sn/t}.$$

Summing the estimated number of monthly inoculations per person for the period of July to December 1967 (Table I) yields 11·6. A similar estimate, 10·15 inoculations, was obtained for 1968. The chance of an Anuak resident of Gambela receiving at least one inoculation sometime in 1968 was very close to unity, as

$$1 - e^{-10} \simeq 1 \cdot 0.$$

The discrete distribution of the number of sporozoite inoculations per person among the Anuak community may be approximated by the Poisson, assuming that inoculations arrive randomly, that is, every resident is equally at risk:

$$\text{Probability } (k) = I^k e^{-I}/k! \; ,$$

where *k* is the expected number of inoculations and *I* the mean number of inoculations for the period considered, one year. Poisson expectations suggest that about 99 % of Gambela Anuaks acquire four or more inoculations, 87 % expect seven or more, while about 58 % suffer ten or more such potentially infective bites. How does the estimate of annual risk drawn above compare with the incidence of falciparum malaria?

Among children examined on five or more occasions, 50 of 52 (96%) showed evidence of falciparum infection at least once. Of 47 older subjects observed five or more times, 36 were positive (77%) on at least one examination. Considered together, 86 of 99 persons (87%) were positive at some time over the sampling period, and the age-weighted incidence was 82·4% when measured in this way. The frequencies of parasitaemia in the two age groups differ significantly ($\chi^2 = 8 \cdot 3$, P < 0·005).

*Correlation of entomological inoculation rates with parasitaemia*

Monthly estimates of *P. falciparum* frequencies varied directly with the risk of sporozoite inoculation. The slope of regression of age-standardized
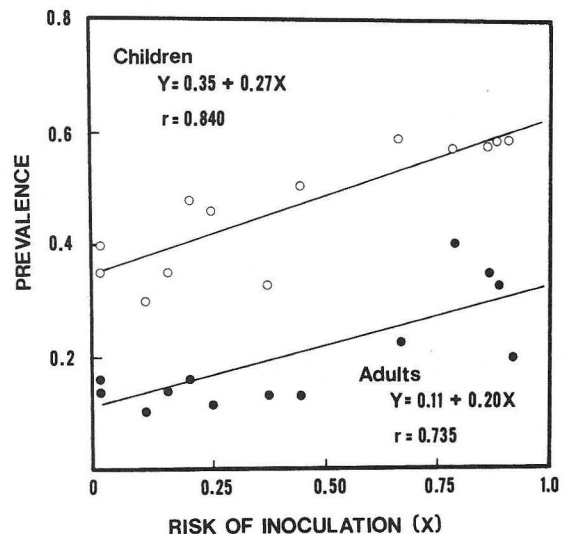


Fig. 1. Regression of age-weighted monthly proportions positive for *P. falciparum* (prevalence, Y-axis) on monthly estimates of risk, *X*. Risk is the chance of an average inhabitant receiving at least one inoculation in the course of a month.

prevalence on risk (Fig. 1) departed significantly from zero ($t_{11 \text{ d.f.}} = 5 \cdot 74$, $P < 0 \cdot 001$). Bearing in mind probable differences in immune status between the broadly classed age groups, a closer look is in order. A regression coefficient of $0 \cdot 271 \pm 0 \cdot 053$ S.E. obtained among children, a lesser slope of $0 \cdot 200 \pm 0 \cdot 056$ S.E. among adults, and each (Fig. 2) was inconsistent with a null hypothesis of
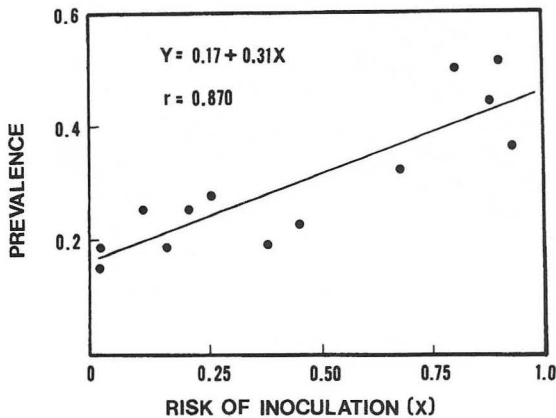


Fig. 2. Regression of falciparum malaria prevalence (Y) among children and adults on risk of inoculation, X.

slope zero (children, $t_{11} = 5 \cdot 13$; adults, $t_{11} = 5 \cdot 30$; $P < 0 \cdot 001$). These data suggest that a close relationship exists between the proportion of people with patent falciparum infection and the probability of acquiring sporozoite inoculations, and as such, warrant further analysis.

Seasonal trends in the rate of transmission and frequency of falciparum infection among the community may be evaluated to best advantage when arranged quarterly (Table II). The lag of one month between entomological and parasitological quarters compensates for the interval between sporozoite inoculation and detection of new infections in blood film surveys conducted at four-week intervals. Periods of least risk occurred in the driest quarter of the year and, surprisingly, in the first three months of the wet season. Corresponding periods of parasitaemia show broad agreement with risk, parasite and gametocyte frequencies in adults and children being least early in the wet season and greatest after periods of maximum inoculation.

Significant shifts in average quarterly frequencies of parasitaemia occurred only in those months immediately following major changes in the rate of sporozoite challenge (Table II). The parasite rates therefore suggest that equilibrium obtains between the proportion of subjects recovering from falciparum malaria and acquiring new infections of it.

*Infectivity of sporozoite challenge*

1. *Dynamics analysed by Macdonald's model.* It is common knowledge that where malaria is highly endemic only a small proportion of sporozoite inoculations result in detectable parasitaemia, even among infants (DAVIDSON & DRAPER, 1953; MACDONALD, 1955; PULL & GRAB, 1975). MACDONALD (1955) defined the coefficient $b$ as the proportion of sporozoite-positive *Anopheles* actually infective, and suggested that $b$ varied inversely with the intensity of transmission. Given the present estimates from Gambela of $h_e$ and the corresponding parasite rates, what proportion $b$ was actually infective, by age-group and season? It is necessary first to estimate $h$, the infective inoculation rate, and then take this inoculation rate as a fraction of the entomological,

$$b = h/h_e$$

We computed infective inoculation rates $h$ that satisfy the dynamics between the quarterly frequencies of patent falciparum parasitaemias shown in

Table II—Correlation of average monthly *P. falciparum* parasite rates with monthly average inoculation rates and risk of receiving one or more inoculations in Gambela, by quarter, August 1967—February 1969

| Quarter | | | | Average Monthly Prevalence | | | |
|---|---|---|---|---|---|---|---|
| | | Average | | Children | | Adults | |
| Ento-mologic | Para-sitologic | Rate* | Risk** | Rate | $\chi^2_{1\text{d.f.}}$*** | Rate | $\chi^2_{1\text{d.f.}}$*** |
| Aug-Oct | — | 2·95 | 0·95 | — | — | — | — |
| Nov-Jan | Dec-Feb | 0·92 | 0·60 | 52·7% | 10·42† | 16·8% | 0·37 |
| Feb-Apr | Mar-May | 0·20 | 0·18 | 36·6 | 0·29 | 14·9 | 0·003 |
| May-Jul | Jun-Aug | 0·16 | 0·15 | 34·0 | 15·92† | 14·7 | 11·88† |
| Aug-Oct | Sep-Nov | 1·67 | 0·81 | 57·9 | 0·46 | 28·9 | 1·23 |
| Nov-Jan | Dec-Feb | 1·39 | 0·75 | 62·0 | | 34·3 | |

*Average estimated number of sporozoite inoculations received per person in the course of a month
**Risk $= 1 - e^{-\text{inoculations/month}}$
***Chi-square contingency for homogeneity in parasite rates between successive quarters, calculated from data in Table I.
†$P < 0 \cdot 005$. Other chi-square values are not significant

Table II using MACDONALD's (1950a) formula. The relationship is,

$$x_t = L - (L - x_0)e^{-rt}, \ldots \ldots (1)$$

where $x_0$ is the initial mean quarterly parasite rate and $x_t$ the frequency of parasitaemia at time $t$, taken as 91 days per quarter. Used in the present treatment as a constant, $r$ is the daily rate of recovery from falciparum infection; it was shown to approximate $0 \cdot 005$ per day in the absence of further challenge (MACDONALD, 1950b). MACDONALD & GÖCKEL (1964) demonstrated that this value of $r$ may well be a reliable one for many geographical strains of *P. falciparum*. The $L$ in expression (1) was defined as the limiting value to which the proportion of people infected approach when equilibrium is reached. Where mortality from malaria is negligible,

$$L = h/r \text{ (MACDONALD, 1950a)} \ldots \ldots (2)$$

and given $r$, we find the inoculation rate $h = rL$. These equations, while not the most accurate of the several now available (Ross, 1916; MUENCH, 1959; DIETZ *et al*, 1974; FINE, 1975; BAILEY, 1975), have the very real advantage of allowing analysis of temporal changes in malaria prevalence on a slide rule or pocket calculator. We recognize, however, that equations (1) and (2) above do not properly reflect the effects of superinfection when $h > r$. A consequence is that $h$ and therefore $b$ may be underestimated. It should be noted also that parasitological diagnoses do not discriminate between new infections and relapses. Rate $h$ therefore comprises new infections plus relapses.

Estimates of $h$ are set forth in Table III. To give

an example of calculating $h$, the mean quarterly prevalence of falciparum malaria in children fell from $52 \cdot 7$ to $36 \cdot 6\%$ over the December-February to March-May interval. Substituting into equation (1) above, we have

$$0 \cdot 366 = L - (L - 0 \cdot 527)e^{-0 \cdot 005(91)}$$

from which $L = 0 \cdot 087$, the value of which the parasite rate would approach, under assumptions implicit to the model, if the average rate of inoculation over the three-month period was maintained indefinitely. The limit may then be used to estimate an infective inoculation rate for the March-May quarter:

$$h = r L = 0 \cdot 005(0 \cdot 087) = 0 \cdot 00043.$$

This estimate of $h$ may be taken as a fraction of the entomologically measured rate $(0 \cdot 0066)$ to yield $b$ and amounts to only $6 \cdot 6\%$ of it (Table III). Estimates of $L$ and $h$ for adults were slightly greater than for children during the March-May interval. Only $9\%$ of the sporozoite challenge was appraised to establish patent infections among adults.

The average quarterly prevalences of falciparum parasitaemia during the June-August interval showed no significant change from the previous quarter nor do they differ much from equilibria values, $L$ (Table III). Sporozoite challenge was small, but on the hypothesis that a constant recovery rate $(0 \cdot 005/\text{day})$ approximates real events, infectivity $b$ was the highest of the year, about $27\%$ among children and one-half this value among adults.

Sporozoite challenge increased ten-fold from previous levels during September to November.

**Table III—Average daily rates of *P. falciparum* inoculation by quarter in Gambela, Dec. 1967—Feb. 1969. Rates are estimated from parasitological and entomological data with Macdonald's formulae**

| Quarter | Average Monthly Parasite Rate | Limit* | Average Daily Inoculation rate | | Percentage Infective† (b) |
|---|---|---|---|---|---|
| | | | Parasit. $h$** | Ento. $h_e$*** | |
| *Children* | | | | | |
| Dec-Feb | $0 \cdot 527$ | — | — | $0 \cdot 0301$ | — |
| Mar-May | $0 \cdot 366$ | $0 \cdot 087$ | $0 \cdot 0004$ | $0 \cdot 0066$ | $6 \cdot 6$ |
| Jun-Aug | $0 \cdot 340$ | $0 \cdot 295$ | $0 \cdot 0015$ | $0 \cdot 0054$ | $27 \cdot 3$ |
| Sep-Nov | $0 \cdot 579$ | $0 \cdot 994$ | $0 \cdot 0050$ | $0 \cdot 0550$ | $9 \cdot 0$ |
| Dec-Feb | $0 \cdot 620$ | $0 \cdot 691$ | $0 \cdot 0035$ | $0 \cdot 0457$ | $7 \cdot 6$ |
| *Adults* | | | | | |
| Dec-Feb | $0 \cdot 168$ | — | — | — | — |
| Mar-May | $0 \cdot 149$ | $0 \cdot 116$ | $0 \cdot 0006$ | $0 \cdot 0066$ | $8 \cdot 8$ |
| Jun-Aug | $0 \cdot 147$ | $0 \cdot 144$ | $0 \cdot 0007$ | $0 \cdot 0054$ | $13 \cdot 3$ |
| Sep-Nov | $0 \cdot 289$ | $0 \cdot 535$ | $0 \cdot 0027$ | $0 \cdot 0550$ | $4 \cdot 9$ |
| Dec-Feb | $0 \cdot 343$ | $0 \cdot 437$ | $0 \cdot 0022$ | $0 \cdot 0457$ | $4 \cdot 8$ |

*Calculated by arrangement from $x_t = L - (L - x_0)e^{-rt}$ (Macdonald, 1950a)
**Calculated from falciparum parasite rates using Macdonald's formulae
***$\sum$ monthly sporozoite inoculations/91 days
†Proportion infective = parasitological $h$/entomological $h_e = b$

**Table IV—Rates of becoming positive ($\alpha$) and negative ($\beta$) for falciparum malaria among a group of Anuaks sampled longitudinally at 28-day intervals. There were 32 children and 40 adults participating**

| Dates of 1st and last examination* | No. -ves becoming +/No.- $\alpha$ | | No. +ves becoming -/No. + $\beta$ | | Contingency $\chi^2_{1 d.f.}$ ** |
|---|---|---|---|---|---|
| | | *Children* | | | |
| 10 Dec— 3 Mar | 9/40 | 0·225 | 15/52 | 0·288 | 0·47 |
| 3 Mar—26 May | 7/48 | ·146 | 6/29 | ·207 | ·48 |
| 26 May—18 Aug | 9/49 | ·184 | 6/26 | ·231 | ·24 |
| 18 Aug—10 Nov | 10/29 | ·345 | 6/35 | ·171 | 2·54 |
| 10 Nov— 2 Feb | 11/25 | ·440 | 9/42 | ·214 | 3·81 |
| Totals and avgs. | 46/101 | ·241 | 42/184 | ·228 | ·08 |
| | | *Adults* | | | |
| 10 Dec— 3 Mar | 8/96 | 0·083 | 12/20 | 0·6 | 30·97†† |
| 3 Mar—26 May | 5/85 | ·059 | 4/5 | ·8 | 28·82†† |
| 26 May—18 Aug | 5/70 | ·071 | 7/15 | ·467 | 15·92†† |
| 18 Aug—10 Nov | 11/53 | ·208 | 2/18 | ·111 | ·84 |
| 10 Nov— 2 Feb | 10/42 | ·238 | 15/31 | ·484 | 4·78†† |
| Totals and avgs. | 39/346 | ·113 | 40/89 | ·449 | 54·00†† |

*There were two to four successive examinations at 28-day intervals per person per quarter, the results of which are additive

**Between estimates of $\alpha$ and $\beta$

†P <0·05

††P <0·005. Other $\chi^2$ values not significant

In the same interval, parasite rates doubled among adults and nearly did so among children. Limiting values of the parasite rate approximated to 54% in adults and 99% in children. The percentage of sporozoite inoculations resulting in detectable parasitaemia was computed as about 5% among adults and 9% in children.

Average frequencies of parasitaemia increased from previous levels in the December to February period, but not significantly (Table III). Sporozoite challenge during this period decreased about 20% from the earlier average rate, but nevertheless remained high. Equilibrium values of the parasite rate, 69% among children and 44% among adults, were less than those calculated for the previous quarter. No real change was apparent from the previous quarter in the proportion of sporozoite inoculations actually infective.

2. *Incidence and recovery rates among longitudinally followed inhabitants* DIETZ *et al.* (1974, FINE (1975), and BAILEY (1975) suggested that Macdonald's formula, used above, does not adequately account for superinfection when $h \geqslant r$. Moreover, recovery rates $r$ vary with immune status and in response to $h$, as recognized by MACDONALD (1955, p. 4) himself. Estimates of $h$, computed as above, are very sensitive to the value of $r$; where $r$ is wrongly approximated, estimates of $h$ will be seriously in error. A method now exists allowing the simultaneous approximation of $r$ and $h$ from longitudinal blood parasite surveys (BEKESSY *et al.*, 1976). The number of subjects parasite-negative on first examination positive on the second are taken as a fraction of those originally negative, to give the transition rate $\alpha$ (Table IV). The number of subjects falciparum-positive on first examination negative on the second, as a proportion of those originally positive, estimates transition rate $\beta$. Only transitions between successive 28-day intervals are included. As the subject population, comprising 30 children and 42 adults, was small and occasional absences invariably occurred within the 14-month study period, monthly transition frequencies were added to yield the mean quarterly values shown in Table IV. This sample is a subset of the larger group in Table I. Among children, the incidence of new infections plus relapses $\alpha$ was marginally less than the proportion $\beta$ becoming negative through recovery and latency in the nine-month interval between December and mid-August. When sporozoite challenge was much greater, from August to February, $\alpha$ exceeded $\beta$ in the same children. In no quarterly interval, however, was rate $\alpha$ significantly different, at the 5% level of probability, from rate $\beta$. A different picture was obtained in adults, among whom the rates of recovery and latency were significantly greater than the rates of becoming falciparum-positive in all periods except the August to December quarter. The duration of patent parasitaemia was therefore characteristically short among the adult age group.

Transition frequencies $\alpha$ among children did not agree with an hypothesis of homogeneity between successive quarterly estimates ($\chi^2_4 = 10·44$, $0·05 > P > 0·025$). Rates of becoming falciparum

negative between quarterly intervals, on the other hand, were homogeneous ($\chi_4^2 = 1 \cdot 83$, $0 \cdot 7 > P > 0 \cdot 5$). Amongst the adult age group, transition frequencies $\alpha$ and $\beta$ each differed significantly between successive intervals ($\alpha\chi_4^2 = 15 \cdot 86$, $P < 0 \cdot 005$; $\beta\chi_4^2 = 12 \cdot 81$, $0 \cdot 025 > P > 0 \cdot 01$). Deviation in $\alpha$ and $\beta$ between the two age groups was highly significant ($\alpha\chi_1^2 = 15 \cdot 16$, $\beta\chi_1 = 13 \cdot 96$, $P << 0 \cdot 005$).

The chi-square contingency analysis brings forth very real differences between age groups in response to sporozoite challenge. Rates of becoming positive were greater in children than in adults. Recovery rates were uniformly less in the younger age group. It is probably significant that quarterly recovery rates were statistically homogeneous among children even in the face of seasonally fluctuating inoculation rates.

The data from which the transition rates were calculated were based on thick blood smears. The low mean densities of parasitaemia among adults and significant parasite losses during dehaemoglobinization (DOWLING & SHUTE, 1966) should exaggerate $\alpha$ and $\beta$. That is, the statistic $\beta$ comprises an uncertain proportion of false negatives and $\alpha$ an unknown proportion of apparent relapses, especially among adults. The exaggeration of transition rates $\alpha$ and $\beta$ are compensated to an unknown degree by superinfection, which goes unrecognized by the diagnostic procedure. Bearing in mind the foregoing qualifications, the instantaneous transition probabilities $\hat{h}$ and $\hat{r}$ may be computed according to the formulae set forth by BEKESSY et al. (1976):

$$\hat{h} = [-\alpha/t\,(\alpha + \beta)] \log_e (1 - \alpha - \beta) \ldots (3) \text{ and}$$
$$\hat{r} = [-\beta/t\,(\alpha + \beta)] \log_e (1 - \alpha - \beta) \ldots (4).$$

$\hat{h}$ is defined as a daily incidence rate, representing infective inoculations plus relapses. $\hat{r}$ is the daily recovery rate, comprising actual recoveries plus latencies. $t$ is the interval in days between successive parasitological examinations ($t = 28$); $\alpha$ and $\beta$ are defined as before.

Mean daily incidence/relapse and recovery/latency rates for five successive quarters are shown in Table V. Incidence rates $\hat{h}$, on the whole, were about twice as great among children than adults. Rates of recovery $\hat{r}$ were nearly three-fold higher among adults than children. Estimates of $\hat{r}$ were least in the quarter of maximum sporozoite challenge; this was a natural consequence of superinfection, newer infections masking the loss of older ones. Standard error of $\hat{h}$ and $\hat{r}$ were of sufficient magnitude, owing to sample sizes, to make inappropriate the explicit comparisons of parasitological with entomological inoculation rates. Incidence/relapse rates, moreover, were greater among both age groups than the estimates afforded by working of Macdonald's formula for the parasite rate. Similarly, estimates of recovery/latency rates were considerably higher than assumed to prevail in the earlier treatment. The greater recovery/latency rates lead quite naturally to higher estimated inoculation rates.

Equilibrium values (Table V) of the parasite rate, given by the expression, Limit = $\hat{h}/(\hat{h} + \hat{r})$ (Ross, 1916; MUENCH, 1959; BEKESSY et al., 1976), seemed reasonably close to the observed parasite rates with due regard to the large standard errors attached to $\hat{h}$ and $\hat{r}$.

## Discussion

There are assumptions beyond those already stated underlying the present analytical treatment given the parasitological and entomological data. Among these is the possibility that adults and children did not share a common risk to inoculation because frequencies of man-vector contact varied with host age. We have no entomological evidence to support or deny the proposition, but suspect man-biting rates were not greatly different between the two age groups, for where *P. falciparum* existed in epidemic form, as in Surdator, Indonesia, and Puerto Rico (SCHÜFFNER, 1938; EARLE, 1939, data reproduced by MACDONALD, 1951) the age distribut-

**Table V—*P. falciparum*. Parasitologically estimated mean daily incidence/relapse rates $\hat{h}$ and daily recovery/latency rates $\hat{r}$ among Anuaks longitudinally sampled at 28-day intervals. Gambela, December, 1967—February, 1969**

| Dates of 1st and last exam. | Incidence $\hat{h} + $ S.E. | Recovery $\hat{r} + $ S.E. | Limit $\hat{h}/(\hat{h}+\hat{r})$ | Avg. monthly parasite rate |
|---|---|---|---|---|
| *Children* | | | | |
| 10 Dec— 3 Mar | $\cdot 0113 \pm \cdot 004$ | $\cdot 0144 \pm \cdot 004$ | $\cdot 438$ | $\cdot 544$ |
| 3 Mar—26 May | $\cdot 0064 \pm \cdot 005$ | $\cdot 0091 \pm \cdot 006$ | $\cdot 413$ | $\cdot 396$ |
| 26 May—18 Aug | $\cdot 0085 \pm \cdot 008$ | $\cdot 0106 \pm \cdot 012$ | $\cdot 443$ | $\cdot 387$ |
| 18 Aug—10 Nov | $\cdot 0173 \pm \cdot 006$ | $\cdot 0086 \pm \cdot 005$ | $\cdot 668$ | $\cdot 567$ |
| 10 Nov— 2 Feb | $\cdot 0255 \pm \cdot 009$ | $\cdot 0124 \pm \cdot 007$ | $\cdot 673$ | $\cdot 618$ |
| *Adults* | | | | |
| 10 Dec— 3 Mar | $\cdot 0050 \pm \cdot 002$ | $\cdot 0361 \pm \cdot 012$ | $\cdot 122$ | $\cdot 154$ |
| 3 Mar—26 May | $\cdot 0048 \pm \cdot 003$ | $\cdot 0651 \pm \cdot 043$ | $\cdot 068$ | $\cdot 083$ |
| 26 May—18 Aug | $\cdot 0037 \pm \cdot 002$ | $\cdot 0239 \pm \cdot 010$ | $\cdot 138$ | $\cdot 170$ |
| 18 Aug—10 Nov | $\cdot 0089 \pm \cdot 003$ | $\cdot 0048 \pm \cdot 004$ | $\cdot 651$ | $\cdot 383$ |
| 10 Nov— 2 Feb | $\cdot 0151 \pm \cdot 006$ | $\cdot 0306 \pm \cdot 010$ | $\cdot 330$ | $\cdot 372$ |

ion of parasitaemia and gametocytaemia was remarkably homogeneous, suggesting equal risk among all age groups.

The *Anopheles* man-biting rates and sporozoite rates were established, we believe, with reasonable accuracy, as the entomological investigation was intensive and of sufficient duration to explore spatial and temporal variation. So too were the parasitological investigations. Indeed, the seasonal features of the parasite rate outlined here were amply confirmed in the subsequent years. Efforts quantitatively to integrate parasitological with entomological parameters include the classical studies of DAVIDSON & DRAPER (1953) in Tanzania, DAVIDSON (1955) in Uganda, and the comparative consideration of their data given by MACDONALD (1955). Their approach, in each instance with surveys limited to three months, was predicated on lines suggested by MACDONALD (1950a). A heavy surplus of inocula was detected by these observers, $0 \cdot 3$—$0 \cdot 95$ sporozoite challenges per day, and estimates of $b$ were correspondingly low. A similar surplus of sporozoite inoculations was detected by PULL & GRAB (1974) among longitudinally followed infants in Kenya. The proportion $b$ of sporozoite inoculations that established falciparum infection was only $1 \cdot 2$ to $2 \cdot 6\%$. The estimate was based on MUENCH'S (1959) reversible catalytic equation. The intensity of transmission in Gambela clearly was much less, of the order of $0 \cdot 03$ sporozoite challenges per day, but even here it seemed that no more than a third of the estimated inoculations generated patent infections. The chief assumption made was that a constant rate of recovery from falciparum infection prevailed. Were actual values of $r$ greater than assumed, then estimates of inoculation rates $h$ would have proved greater, as would the estimated proportions of sporozoite challenge establishing infection.

The foregoing estimates of 'actual' (versus 'crude', entomologically derived) rates of inoculation in Gambela rest entirely on average quarterly frequencies of *patent* parasitaemia. Owing to low densities of infection and parasite losses in dehaemoglobinization, estimates of prevalence were somewhat less than those actually prevailing, particularly among adults.

Several inferences may be drawn from the present exercise. Two fundamentally different methods of analysis suggested that sporozoite inoculations were nearly twice as infective to children than to adults. Surely this is as good a measure as any of the relative states of immunity between the two age groups. Analysis of the parasite rate according to Macdonald's formulae suggested that for much of the year most sporozoite injections fail to 'take'. But when community resistance is least (assuming a constant recovery rate), early in the wet season, the estimated proportion of bites establishing infection increases three to five fold, only suddenly to drop again.

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# New Fellows

**Elected 20th April, 1978**

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