*Am. J. Trop. Med. Hyg.*, 29(5), 1980, pp. 725–737


# A LONGITUDINAL STUDY OF HUMAN MALARIA IN THE WEST AFRICAN SAVANNA IN THE ABSENCE OF CONTROL MEASURES: RELATIONSHIPS BETWEEN DIFFERENT *PLASMODIUM* SPECIES, IN PARTICULAR *P. FALCIPARUM* AND *P. MALARIAE**

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*Abstract.* The research project on the epidemiology and control of malaria conducted in the Garki District, Kano State, jointly by the Government of Nigeria and the World Health Organization included among its objectives the study of the baseline epidemiology prior to the introduction of any control measures. The present paper analyzes the project's data with respect to the relationships among the three species of *Plasmodium* present, *P. falciparum*, *P. malariae* and *P. ovale*. Parasitemia with *P. falciparum* or *P. malariae* is more likely in the presence than in the absence of the other species. Among persons positive for *P. falciparum*, those with a higher density of parasitemia are more likely to have *P. malariae* also than those with a lower density of *P. falciparum* parasitemia. There is a pronounced seasonal alternation in prevalence between *P. falciparum* and *P. malariae*.

The research project on the epidemiology and control of malaria conducted in the Garki District, Kano State, jointly by the Government of Nigeria and the World Health Organization included among its objectives the study of the baseline epidemiology prior to the introduction of any control measures. The present paper analyzes the project's data on prevalence, incidence, and parasite density by season and age, with respect to the relationships among the three species of *Plasmodium* present, *P. falciparum*, *P. malariae* and *P. ovale*.

## MATERIALS AND METHODS

The study area is a rural district of the West African Sudan savanna. There is a long dry season and a short wet season, and a very wide seasonal variation in vector density and malaria transmission. The baseline period extended from November 1970 (end of the wet season) to May 1972 (end of the dry season). Eight village-clusters (follow-up units) were surveyed every 10 weeks, for eight baseline surveys. Sixteen villages were included from survey 1; 6 villages (or sections of villages) were added at survey 5. At that survey, the 22 villages counted a total of 7,423 inhabitants. The surveys aimed at total coverage. The initial survey included a nominal de facto census, updated at each survey. Each survey included the collection of a thick blood film, linked by a code number to the person's identity. Collections were usually made in the first half of the day, except during the period of high agricultural activity (July–September), when they were usually made in the second half of the day. Further details on the demographic coverage of the surveys appear elsewhere.[1]

The blood films were stained by the Giemsa stain and examined, under oil immersion, with ×7 oculars (field number 18.5) and ×100 objective, for 200 fields, while tallying the number of fields examined and the number of fields found positive for *P. falciparum* asexual stages (trophozoites), *P. falciparum* gametocytes, *P. malariae*, and *P. ovale*, respectively. The examination of 200 fields took about 10 min and the volume of blood examined was about 0.4 $mm^3$. A systematic sample of one out of five films was examined for 400 fields; given the number of fields positive, the probability that the film would have been positive by the examination of 200 fields was calculated, and used in a random experiment to classify the film as positive or negative, before its inclusion in

Accepted 6 October 1979.

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† Supported in part by U.S. National Science Foundation grant DEB 74-13276.

the calculation of the parasite rate (PR, proportion or percentage of persons positive).[1]

The examinations were performed blindly (i.e. ignoring a person's characteristics or previous results), by junior microscopists, under supervision. A systematic sample of one out of 10 films was re-examined blindly, for the same number of fields, by senior microscopists, for the purpose of monitoring diagnostic sensitivity. The results of the senior microscopists were not used to correct the original result. In terms of positive films, the sensitivity of the juniors was equal to 0.95, 0.78, 0.79, 0.76 of that of the seniors, with respect to *P. falciparum, P. falciparum* gametocytes, *P. malariae,* and *P. ovale,* respectively, with some variation between individuals and between surveys, but no time trend. The junior microscopists detected some infections missed by the seniors and parasite rates obtained by both observations combined were higher than those obtained by either alone.

## RESULTS

### *Cross-sectional surveys*

At each survey, the prevalence of double and triple infections was compared to that expected from the prevalence of each infection under the hypothesis of independence. Tables 1 and 2 and Figure 1 illustrate this comparison in a wet season survey and in a dry season survey. In comparison with the expected, there is an excess of double and triple infections in most age-groups in both seasons. The excess may be small or moderate, but is nearly systematic. There is an obvious and systematic excess of double infections with *P. falciparum* and *P. malariae.* An excess of double infections including *P. ovale* is visible when triple infections are included.

There was also an excess of mixed infections when the results were studied within eight geographical clusters of two to four villages each, or by individual microscopists, or by number of fields examined (200 or 400). Therefore, the excess cannot be explained by a mixture of parasitologically heterogeneous populations, nor by a mixture of microscopists differing in diagnostic sensitivity, nor by a mixture of films examined for 200 and 400 fields.

Parasitemia with *P. falciparum* or *P. malariae* is thus more likely in the presence than in the absence of the other species. To determine whether the prevalence of one parasite was affected by the density of the other, the proportion of microscopic fields found positive in the thick film was used as an index of density. Figure 2 shows that, among persons found positive for *P. falciparum,* those with a higher density of parasitemia are more likely to have *P. malariae* as well in their blood film, than those with a lower density of *P. falciparum* parasitemia. The numbers examined varied, by survey, age and density class, between 125 and 487, and all differences are significant (by chi-squared test), except in the 1 to 4-year-olds in the wet season survey. On the other hand, the reverse comparison revealed no systematic or significant difference in *P. falciparum* prevalence between *P. malariae* infections of relatively low and relatively high density (less than 2% and 2% or more fields positive, respectively). In these comparisons, the numbers examined were smaller and varied by survey, age and density class, between 22 and 186.

### *Longitudinal study of individuals for eight surveys*

Persons examined at all eight baseline surveys were classified by the number of times (0–8) they were found positive for *P. falciparum, P. malariae,* and *P. ovale,* respectively. These numbers may be called the *P.f., P.m.,* and *P.o.* scores, respectively. The correlation between different specific scores of the same person was investigated. As an example (Table 3), there is a positive correlation between the *P.f.* and *P.m.* scores in the age-group of 29–43 years. The association is significant by the chi-squared test, performed after grouping the data according to the marginal distribution. The same was done for each pair of species, in each of seven age-groups, and the results are given in summary in Table 4. All correlations are positive. Between *P.f.* and *P.m.* they are significant in all age-groups; between *P.f.* and *P.o.* in all age groups above 5 years of age; between *P.m.* and *P.o.* in four age-groups out of 7, with a suggestion that the strength of the association decreases with age (see column *r*).

A spurious correlation between scores could result from mixing villages with different average scores, in the absence of any correlation within each village. If that were the case, one would expect the correlation coefficients of different villages to scatter evenly around zero rather than around the correlation coefficient of the total pop-

TABLE 1

*The distribution of persons by age and parasitologic status in the wet season, September 1971, in 22 villages; comparison between the distribution observed (O) and the one expected (E) under the hypotheses of independence between the species*

| Parasites found | | | Age (years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | <1 | | 1–4 | | 5–8 | | 9–18 | | 19–28 | | 29–43 | | 44+ | |
| *P.f.* | *P.m.* | *P.o.* | O | E | O | E | O | E | O | E | O | E | O | E | O | E |
| + | − | − | 139 | 145.7 | 453 | 460.3 | 590 | 599.6 | 548 | 568.6 | 472 | 487.1 | 713 | 731.3 | 380 | 379.8 |
| − | + | − | 1 | 6.1 | 7 | 16.4 | 11 | 26.2 | 16 | 36.5 | 26 | 33.7 | 46 | 60.9 | 36 | 37.4 |
| − | − | + | 0 | 1.1 | 2 | 2.0 | 6 | 2.7 | 3 | 8.3 | 2 | 8.1 | 15 | 18.6 | 8 | 8.1 |
| + | + | − | 20 | 14.5 | 214 | 205.6 | 258 | 245.2 | 133 | 117.4 | 38 | 29.4 | 55 | 41.4 | 24 | 23.7 |
| + | − | + | 4 | 2.6 | 24 | 25.0 | 20 | 25.7 | 27 | 26.6 | 14 | 7.0 | 15 | 12.6 | 4 | 5.1 |
| − | + | + | 0 | 0.1 | 2 | 0.9 | 1 | 1.1 | 2 | 1.7 | 0 | 0.5 | 0 | 1.1 | 1 | 0.5 |
| + | + | + | 0 | 0.3 | 11 | 11.2 | 13 | 10.5 | 10 | 5.5 | 0 | 0.4 | 3 | 0.7 | 1 | 0.3 |
| − | − | − | 68 | 61.7 | 45 | 36.7 | 76 | 64.0 | 202 | 176.6 | 572 | 557.8 | 1,095 | 1,075.5 | 600 | 599.0 |
| Total | | | 232 | 232.1 | 758 | 758.1 | 975 | 975.0 | 941 | 941.2 | 1,124 | 1,124.0 | 1,942 | 1,942.1 | 1,054 | 1,053.9 |
| Chi-square* | | | 5.7 n.s. | | 7.8 n.s. | | 15.4 $P < .01$ | | 24.6 $P < .001$ | | 14.4 $P < .01$ | | 10.5 $P < .05$ | | 0.1 n.s. | |

* The eight parasitological combinations form a 2 × 2 × 2 table; df = 4; chi-square was computed after combining cells with small expectations until all expectations exceeded 5.

TABLE 2

*The distribution of persons by age and parasitologic status, in the dry season May 1972, in 22 villages; comparison between the distribution observed (O) and the one expected (E) under the hypothesis of independence between the species*

| Parasites found | | | Age (years) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | <1 | | 1–4 | | 5–8 | | 9–18 | | 19–28 | | 29–43 | | 44+ | |
| *P.f.* | *P.m.* | *P.o.* | O | E | O | E | O | E | O | E | O | E | O | E | O | E |
| + | − | − | 78 | 89.1 | 330 | 348.6 | 432 | 453.9 | 387 | 415.7 | 259 | 268.5 | 347 | 369.9 | 182 | 194.2 |
| − | + | − | 1 | 11.4 | 4 | 26.2 | 20 | 40.7 | 28 | 55.5 | 23 | 33.5 | 74 | 96.7 | 33 | 44.7 |
| − | − | + | 0 | 0.6 | 1 | 1.6 | 1 | 2.7 | 3 | 5.9 | 3 | 2.7 | 6 | 8.8 | 1 | 2.3 |
| + | + | − | 17 | 6.5 | 244 | 227.0 | 294 | 275.5 | 110 | 84.4 | 23 | 13.3 | 47 | 26.5 | 22 | 11.2 |
| + | − | + | 1 | 0.3 | 9 | 13.6 | 18 | 18.4 | 10 | 9.0 | 0 | 1.1 | 3 | 2.4 | 1 | 0.6 |
| − | + | + | 0 | 0.0 | 0 | 1.0 | 0 | 1.7 | 1 | 1.2 | 0 | 0.1 | 1 | 0.6 | 0 | 0.1 |
| + | + | + | 0 | 0.0 | 15 | 8.8 | 15 | 11.2 | 4 | 1.8 | 1 | 0.1 | 2 | 0.2 | 1 | 0.0 |
| − | − | − | 168 | 156.9 | 64 | 40.2 | 91 | 67.0 | 304 | 273.3 | 684 | 673.7 | 1,372 | 1,346.9 | 791 | 777.9 |
| Total | | | 265 | 264.8 | 667 | 667.0 | 871 | 871.1 | 847 | 846.8 | 993 | 993.0 | 1,852 | 1,852.0 | 1,031 | 1,031.0 |
| Chi-square* | | | 26.8 $P < .001$ | | 38.6 $P < .001$ | | 21.4 $P < .001$ | | 27.0 $P < .001$ | | 9.2 n.s. | | 23.1 $P < .001$ | | 12.3 $P < .05$ | |

* The eight parasitological combinations form a 2 × 2 × 2 table; df = 4; chi-square was computed after combining cells with small expectations until all expectations exceeded 5.

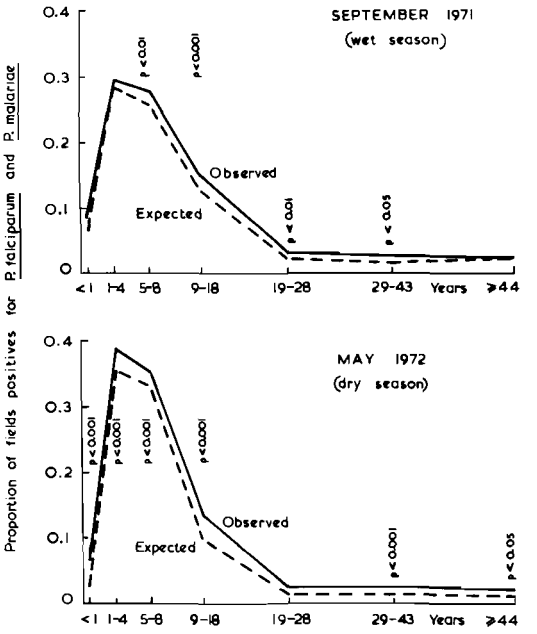


FIGURE 1. Observed prevalence of mixed infections with *P. falciparum* and *P. malariae* in the wet and dry seasons in 22 untreated villages compared with prevalence expected under the hypothesis of independence between the species.

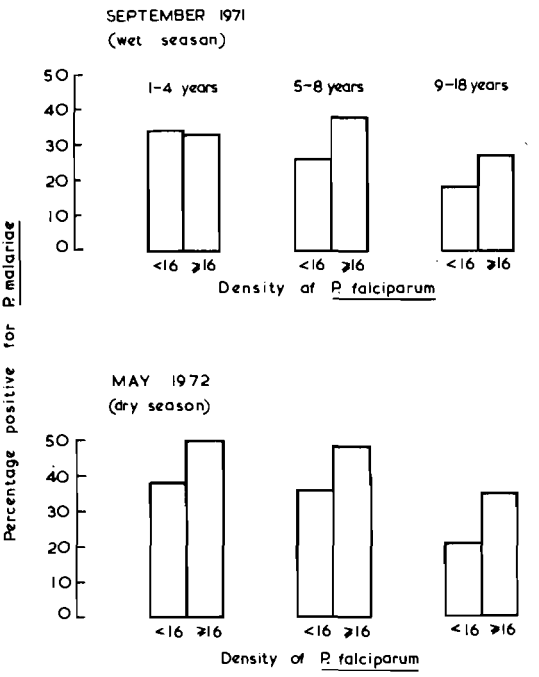


FIGURE 2. Prevalence (% positive) of *P. malariae* in persons positive for *P. falciparum*, according to the density of their *P. falciparum* infection, in 22 untreated villages (<16, ≥16 = less than, at least 16% of fields of thick film positive for *P. falciparum* asexual forms).

ulation shown in Table 4. The correlation coefficients between the *P.f.* and *P.m.* scores were calculated for each of the 16 villages available; in every age-group, the values scatter about evenly around the mean (total) correlation coefficient. Thus that particular source of bias may be ruled out.

The positive correlation between different specific scores must be due, at least in part, to the positive association between species within each survey (see previous section). In order to remove this component, surveys were classified as odd

TABLE 3

*Cross-tabulation of 823 persons, aged 29–43 years, examined at eight consecutive surveys, by the number of times each person was found positive for* P. falciparum *and* P. malariae, *respectively**

| No. times pos. for *P. malariae* | No. times pos. for *P. falciparum* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total |
| 0 | 90 | 166 | 124 | 65 | 38 | 21 | 15 | 3 | 7 | 529 |
| 1 | 27 | 42 | 49 | 37 | 25 | 13 | 13 | 2 | 3 | 211 |
| 2 | 5 | 19 | 10 | 9 | 3 | 6 | 7 | 0 | 0 | 59 |
| 3 | 0 | 1 | 3 | 0 | 1 | 3 | 2 | 2 | 2 | 14 |
| 4 | 0 | 0 | 0 | 2 | 1 | 2 | 0 | 0 | 1 | 6 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 3 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 122 | 228 | 186 | 113 | 68 | 45 | 39 | 8 | 14 | 823 |

* Correlation coefficient (ungrouped results) = +0.290; chi-square (results grouped by dotted lines) = 33.6; df = 4; $P < 0.001$.

TABLE 4

*No. of persons examined at eight consecutive surveys; correlation between the number of times each person was found positive for a given species, and the number of times the same person was found positive for another species*

| Age at survey 1 (yr) | No. persons | *P. falciparum* and *P. malariae* | | | | *P. falciparum* and *P. ovale* | | | | *P. malariae* and *P. ovale* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | r* | Chi-square† | df | P | r | Chi-square | df | P | r | Chi-square | df | P |
| <1 | 80 | +0.349 | 19.9 | 4 | <0.001 | +0.159 | <0.1 | 1 | >0.7 | +0.374 | 5.1 | 1 | <0.05 |
| 1–4 | 342 | +0.216 | 12.9 | 4 | <0.05 | +0.081 | 6.9 | 4 | >0.1 | +0.198 | 17.2 | 4 | <0.01 |
| 5–8 | 345 | +0 298 | 31.9 | 4 | <0.001 | +0.153 | 13.6 | 4 | <0.01 | +0.170 | 8.4 | 4 | >0.05 |
| 9–18 | 241 | +0.391 | 39.7 | 4 | <0.001 | +0.204 | 10.4 | 2 | <0.01 | +0.226 | 10.2 | 2 | <0.01 |
| 19–28 | 403 | +0.252 | 25.8 | 4 | <0.001 | +0.135 | 10.0 | 2 | <0.01 | +0.122 | 1.1 | 1 | >0.2 |
| 29–43 | 823 | +0.289 | 33.6 | 4 | <0.001 | +0.085 | 8.4 | 2 | <0.05 | +0.087 | 8.1 | 2 | <0.05 |
| 44+ | 551 | +0.212 | 19.7 | 4 | <0.001 | +0.113 | 8.7 | 2 | <0.05 | +0.043 | 0.8 | 1 | >0.3 |

* Correlation coefficient between the ungrouped results (0, 1, . . . , 8 for one species and 0, 1, . . . , 8 for the other).
† Data grouped, on the basis of the marginal frequencies, into 3 × 3, 3 × 2, 2 × 2 contingency tables, with 4, 2, 1 df, respectively.

(surveys 1, 3, 5, and 7) or even (surveys 2, 4, 6, and 8), and the score (0, 1, . . . , 4) of a person, for one species, in the odd surveys was tabulated against his score (0, 1, . . . , 4) for another species, in the even surveys. Correlations became, on the average, weaker but remained positive in 41 cases out of 42 (6 comparisons in each of 7 age-groups). For *P.f.* and *P.m.*, Table 5 gives the details.

Thus, even after removal of the effect of association within surveys, *P.f.* and *P.m.* remain significantly associated within persons. By the same test, the other associations become insignificant in a majority of cases (10/14 for *P.f.*, *P.o.*; 11/14 for *P.m.*, *P.o.*).

In persons with 1, 2, 3, . . . , 7 positive results for *P.f.* and *P.m.* respectively, the distribution of each person's eight results into double positive, double negative and single positive of each kind was compared to the distribution expected under the hypothesis of random allocation of the positive results for each species among the eight surveys. The results were summed over all persons and are presented in Table 6, for the three pairs of species. Given that a person is positive for both *P.f.* and *P.m.*, he tends to be positive for both simultaneously slightly more frequently than expected. If the computation of the expected took into account the known seasonal variation (see Storey, Brøgger and Molineaux, 1977,[2] and also below), the expected number of double positives for *P.f.* and *P.m.* would decrease, and the excess of observed double positives would be even greater.

TABLE 5

*Persons examined at eight consecutive surveys; correlation between the number of examinations positive for one species at even-numbered surveys and the number positive for another species at odd-numbered surveys**

| Age at survey 1 (yr) | All surveys (see Table 4) r | *P.f.*-odd, *P.m.*-even | | *P.f.*-even, *P.m.*-odd | |
|---|---|---|---|---|---|
| | | r | Chi-square† (1 df) | r | Chi-square† (1 df) |
| <1 | +0.349 | +0.252 | 5.00‡ | +0.380 | 9.28§ |
| 1–4 | +0.216 | +0.111 | 4.41‡ | +0.218 | 6.60‡ |
| 5–8 | +0.298 | +0.207 | 15.07‖ | +0.182 | 7.59§ |
| 9–18 | +0.391 | +0.295 | 12.29‖ | +0.280 | 18.89‖ |
| 19–28 | +0.252 | +0.099 | 3.21 | +0.152 | 9.34§ |
| 29–43 | +0.289 | +0.184 | 7.15§ | +0.189 | 4.26‡ |
| 44+ | +0.212 | +0.134 | 1.60 | +0.137 | 9.61§ |

* *P.f.*, *Plasmodium falciparum*; *P.m.*, *P. malariae*.
† Data were grouped into 2 × 2 tables for computation of chi-square.
‡ Association significant at 5% level.
§ Association significant at 1% level.
‖ Association significant at 0.1% level.

*Transition frequencies between consecutive surveys*

At a given survey, a person is either positive for *P.f.* and for *P.m.*, or positive for *P.f.* only,

TABLE 6

*Persons examined at eight surveys: distribution of results into double positive, double negative, and single positive, as observed (O) and expected (E)**

| Species† | Result | Distribution of results | | | |
|---|---|---|---|---|---|
| | | +,+ | +,− | −,+ | −,− |
| *P.f. P.m.* | O | 1,051 | 2,454 | 587 | 2,764 |
| | E | 973.2 | 2,531.9 | 664.9 | 2,686.0 |
| *P.f. P.o.* | O | 154 | 803 | 71 | 572 |
| | E | 135.8 | 821.3 | 89.3 | 553.8 |
| *P.m. P.o.* | O | 155 | 702 | 193 | 1,054 |
| | E | 149.9 | 707.2 | 198.2 | 1,048.9 |

* Under the hypothesis of random allocation of each person's positive results for each species among the eight surveys.
† *P.f.*, *Plasmodium falciparum*; *P.m.*, *P. malariae*; *P.o.*, *P. ovale*.

TABLE 7

*Transition frequencies between survey 4 (May–August 1971) and survey 5 (August–October 1971) with respect to the presence of patent parasitemia for* P. falciparum *(*P.f.*) and* P. malariae *(*P.m.*) in persons aged 29+ years*

| Survey 4 | | Survey 5 | | | | |
|---|---|---|---|---|---|---|
| *P.f., P.m.* | Result | +,+ | +,− | −,+ | −,− | Total |
| +,+ | Obs. | 9 | 38 | 9 | 40 | 96 |
| | Exp. 1* | 6.4 (1.06) | 37.1 (0.02) | 7.7 (0.22) | 44.8 (0.51) | |
| | Exp. 2† | 8.8 (—) | 38.2 (—) | 9.2 (—) | 39.8 (—) | |
| +,− | Obs. | 18 | 163 | 10 | 216 | 407 |
| | Exp. 1 | 7.3 (15.68) | 177.2 (1.14) | 8.8 (0.16) | 213.7 (0.02) | |
| | Exp. 2 | 12.5 (2.42) | 168.5 (0.18) | 15.5 (1.95) | 210.5 (0.14) | |
| −,+ | Obs. | 15 | 63 | 6 | 86 | 170 |
| | Exp. 1 | 8.0 (6.13) | 46.6 (5.77) | 16.9 (7.03) | 98.5 (1.59) | |
| | Exp. 2 | 9.6 (3.04) | 68.4 (0.43) | 11.4 (2.56) | 80.6 (0.36) | |
| −,− | Obs. | 12 | 474 | 39 | 1,061 | 1,586 |
| | Exp. 1 | 20.2 (3.33) | 489.2 (0.47) | 42.7 (0.32) | 1,033.9 (0.71) | |
| | Exp. 2 | 15.6 (0.83) | 470.4 (0.03) | 35.4 (0.37) | 1,064.6 (0.01) | |
| Total‡ | Obs. | 54 | 738 | 64 | 1,403 | 2,259 |
| | Exp. 1 | 41.9 | 750.1 | 76.1 | 1,390.9 | |
| | Exp. 2 | 46.5 | 745.5 | 71.5 | 1,395.5 | |

* Exp. 1 (chi-square), expected under the hypothesis of independence.
† Exp. 2 (chi-square), expected under the hypothesis that having one species modifies the probability of acquiring or keeping the other.
‡ Total chi-square vs. Exp. 1, 44.17, df = 8, $P < 10^{-6}$; vs. Exp. 2, 12.33, df = 4, $P < 0.025$.

or positive for *P.m.* only, or negative for both. At the next survey, he may be in the same state, or may have moved to any of the three other states. The fate of a group of persons between consecutive surveys with respect to *P.f.* and *P.m.* can be represented by a 4 × 4 matrix. Such matrices were computed for each of the seven age-groups and for each of the seven intervals between consecutive baseline surveys. Table 7 illustrates this approach for the two oldest age-groups combined in the interval between surveys 4 and 5, i.e. the interval in which the prevalences of both *P.f.* and *P.m.* changed most rapidly. The two oldest age-groups were combined after verifying that their distributions within each row of the table were not significantly different.

The observed distribution in the 4 × 4 matrix was compared to the one expected under the hypothesis that transitions with respect to one species are independent of transitions with respect to the other species (exp. 1 of Table 7). For example, the first expected number in the first cell is [(9 + 38 + 18 + 163)/(96 + 407)][(9 + 9 + 15 + 6)/(96 + 170)]96 = 6.4. There is a great difference between observed and expected. In particular the following are more frequent than expected: single positives of either kind becoming double positives, double negatives remaining double negatives, positives for *P.m.* alone becoming positive for *P.f.* alone. Conversely, single positives remain as they are less frequently than expected. Transitions with respect to the two species are obviously not independent. In a significant majority of the 49 transition matrices (33/49 to 47/49), the estimated probability of keeping or acquiring either species is larger if the other species is initially present. The estimated conditional probabilities were used to calculate a new set of expected values (exp. 2 of Table 7). For example, the second expected number in the first cell is [(9 + 38)/96][(9+ 9)/96]96 = 8.8. This second model fits the data much better (chi-squared is much smaller even after allowing for the decrease in df and critical values). The discrepancies noted above are diminished, including the excess of persons switching from *P.m.* alone to *P.f.* alone. Again, in the 49 matrices, the second model is very consistently superior to the first over the different intervals and age-groups. The likelihood ratio is much larger than $10^{10}$. However, it is not claimed that the second model is statistically adequate to explain all of the 4 × 4 matrices; it is simply better than model 1.

### *Seasonal variation*

Figure 3 shows the seasonal variation of *P. falciparum* and *P. malariae* in terms of crude prevalence, infant conversion rate, crude conversion

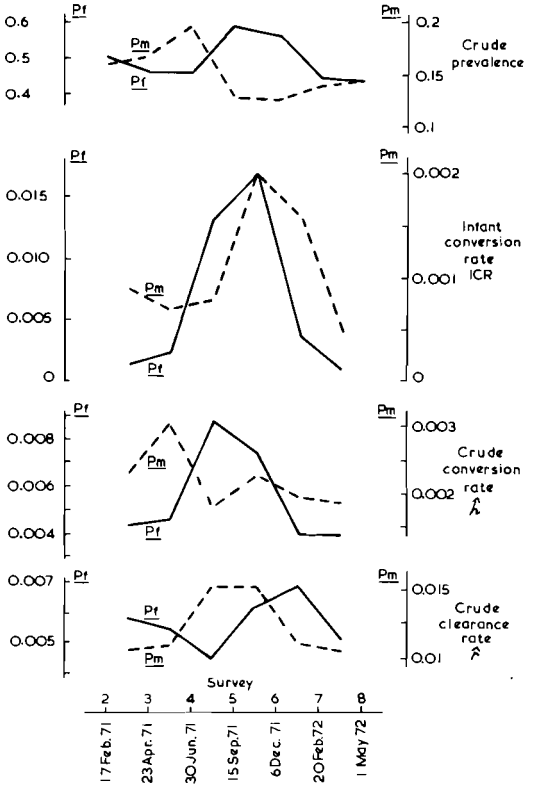


FIGURE 3. Prevalence of *P. falciparum* and *P. malariae* by survey and transition rates between consecutive surveys, in 16 untreated villages. (All curves scaled so that their seasonal peaks are equal to the same constant.)

and clearance rates, in 16 untreated villages. The prevalence is based on the examination of 4,834 to 5,586 persons per survey. The number of infants available for first parasitological conversion, for the estimation of the infant conversion rate, varied, by species and interval, between 34 and 111. The crude transition rates,[3] $\hat{h}$ and $\hat{r}$, are based on the examination of 4,235 to 5,149 persons, per pair of consecutive surveys.

Figure 3 (top) shows a strong negative correlation between the seasonal variations of the prevalences of *P. falciparum* and *P. malariae,* respectively, and in particular in the early wet season, a rapid decrease in the prevalence of *P. malariae* simultaneous with the rapid increase in the prevalence of *P. falciparum*. Both the negative seasonal association and the rapid change in opposite directions in the early wet season were also found regularly in individual villages, and in different years in the untreated villages. Figure 3 also shows, by interval between consecutive surveys, the infant conversion rate (the rate at which infants become positive for the first time), and the crude conversion and clearance rates. (The crude conversion rate is the rate at which the average negative person becomes parasitologically positive. The crude clearance rate is the rate at which the average parasitologically positive person becomes negative.) In the case of *P. falciparum,* the seasonal variations of the infant and crude conversion rates are very similar and changes in either conversion rate are closely followed by concordant changes in the prevalence. In the case of *P. malariae,* the infant and crude conversion rates behave very differently. The crude conversion rate is associated with the prevalence, as in the case of *P. falciparum*. The infant conversion rate is clearly dissociated: its seasonal variation follows, with a small delay, that of the *P. falciparum* infant conversion rate, and both follow clearly the seasonal variation in vector density. Consequently, the seasonal peak of the crude prevalence of *P. malariae* occurs about 35 weeks after the seasonal peak of the infant conversion rate.

The relationship between transition rates and prevalence is explored further in Figure 4. Ross's model was used to predict the prevalence of *P. falciparum* and *P. malariae* at surveys 3 through 8, given the prevalence at survey 2 and the transition rates in the intervals between consecutive surveys. The formula was $x_t = h/(h + r) - (h/[h + r] - x_0)e^{-(h+r)t}$, where $x_0$, $x_t$ = prevalence (proportion positive) at times 0 and t; t = the interval in days, and h, r = the daily incidence and recovery rates. The infant conversion rate was adjusted by multiplying it by the ratio between the yearly average (surveys 3–8) crude conversion rate and the yearly average infant conversion rate. This ratio was equal to 0.88 for *P. falciparum,* 1.8 for *P. malariae*. As expected, in both species, the crude conversion and clearance rates predict (E1) very well the seasonal variation of the prevalence. If the crude conversion rate is replaced by the adjusted infant conversion rate, the prediction (E2) is still qualitatively of the right shape for *P. falciparum* while for *P. malariae,* the seasonal variation is reversed.

Given the above relationship between infant and crude conversion rates, it is of interest to study the variation of the crude conversion rate $\hat{h}$, by season and broad age-groups (Fig. 5). Before combining smaller age-groups, it was verified that

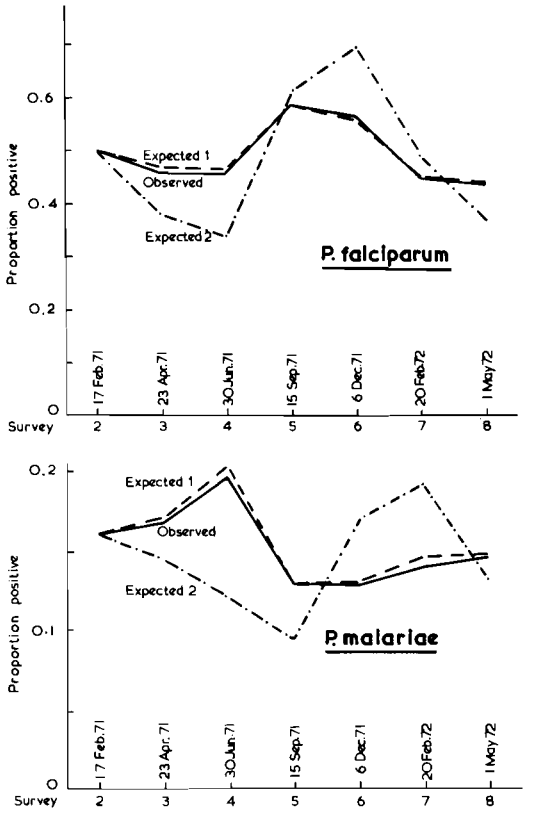


FIGURE 4. Prevalence of *P. falciparum* and *P. malariae* in 16 untreated villages by survey, as observed and as expected, from Ross's model given the initial values, the time intervals, and estimates of h and r for each interval (expected 1 uses $\hat{h}$ and $\hat{r}$, estimated from the transition frequencies, all ages combined; expected 2 uses the adjusted infant conversion rate—see text—and $\hat{r}$).

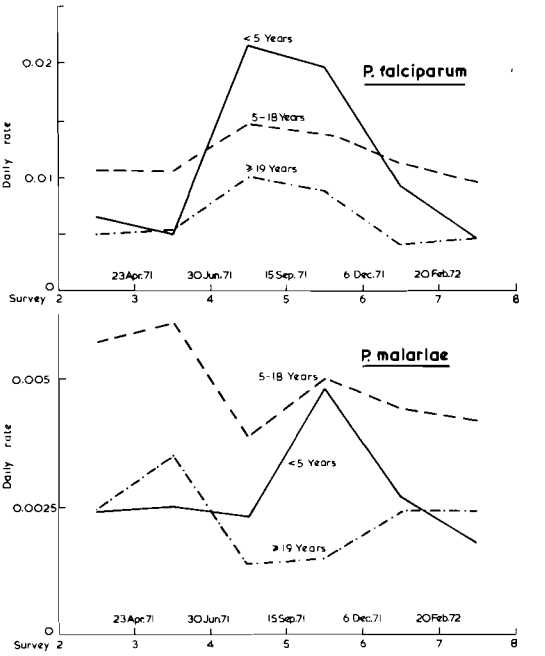


FIGURE 5. The daily rate of onset of *P. falciparum* and *P. malariae* parasitemia, by interval between consecutive surveys and by age, in 16 untreated villages.

they had a similar seasonal behavior. For *P. falciparum* the seasonal variation in $\hat{h}$ is very similar in all age-groups. For *P. malariae,* up to age 5 the pattern is about the same as that of the infant conversion rate: $\hat{h}$ is maximal in the season of high vector density. At ages 19+, it is the reverse: lowest in the wet season, highest in the dry. The age-group 5–18 shows an intermediate pattern.

## DISCUSSION

### *The main findings*

The epidemiology of the relationship between *P. falciparum* and *P. malariae* in Garki may be summarized as follows. 1) There is a positive association between *P. falciparum* and *P. malariae* parasitemias within persons within specific age-groups. Persons having the one are more likely to have the other, both at the same time and at other times. Among persons positive for *P. falciparum,* those with a higher density of parasitemia are more likely to be simultaneously positive for *P. malariae.* 2) Between consecutive surveys, the two species are not independent with respect to acquisition or loss of patent parasitemia. Persons showing either species at the initial survey are more likely to keep or acquire the other species. 3) There is seasonal alternation between the two parasites in the population. For *P. malariae* in individuals above 5 years of age, the peak of the crude conversion rate is shifted from the wet season (season of high vector density, and high transmission, as measured by the infant conversion rate) to the dry season (season of low vector density and low transmission). There is a regular coincidence, in the early wet season, between a rapid increase in *P. falciparum* prevalence and a rapid decrease in *P. malariae.*

There is also a relative excess of persons with simultaneous parasitemia with *P. falciparum* and

*P. ovale* or with *P. malariae* and *P. ovale* or with all three species simultaneously, in comparison with the frequencies expected under the hypothesis of independence. No significant association was found between *P. ovale* at a given time and other species at other times, in the same person.

### *Possible sources of bias and comparison with the findings of others*

Possible sources of bias in the findings should be carefully considered. A positive association between two species, at the same or at different surveys, could result from mixing villages or ages, within each of which there might be no association between the two species. It was shown above that, in this particular study, the positive associations observed did not result, to any appreciable extent, from mixing villages. The effect of age was only partially controlled by subdividing the population into age-groups: they are, and, for practical reasons, have to be rather broad by definition, and their actual contents may be even broader because of misclassifications. The variation of prevalence, as a function of age, was very similar in the three species. Therefore, mixing ages produces automatically a positive association between species.

The importance of this effect varies with the age-groups. Here, prevalence of all three species varies greatly between the four youngest age-groups, but hardly at all between the three oldest.[2] Therefore the mixing of ages within age-groups may explain, at least in part, the positive association between species observed in the younger age-groups (e.g. the relatively strong association observed in the age-group of 9–18 years, a range of ages during which prevalence of all three species decreased rapidly), but not the positive associations observed in the oldest age-groups.

A positive association between species at the same survey could also be spurious if certain blood films were better prepared or better examined, or if a fraction of the parasites actually belonging to a given species were attributed to another species. Any of these biases would also increase the correlation between the numbers of times a person is positive for two different species in the same group of surveys. The comparison between one species in the odd surveys and another in the even surveys was free from this bias. Such a comparison still demonstrated a significant positive association between *P. falciparum* and *P. malariae,* but not between the other pairs of species. The risk of misclassification was probably greatest between *P. falciparum* and *P. malariae,* but it could not explain the observed seasonal alternation.

We may thus consider as genuine the positive association between *P. falciparum* and *P. malariae* within persons, at least in the oldest age-groups, and the seasonal alternation between the two species, in the population. On the other hand, we did not demonstrate a genuine association between *P. falciparum* and *P. ovale,* or between *P. malariae* and *P. ovale.*

The positive association between species observed within persons may contradict some previous findings. Cohen reviewed the literature for surveys allowing tests of hypotheses re interaction between species of human plasmodia.[4] In most of the 14 surveys he retained, there was a deficit of mixed infections, and the deficit increased with immunity. Conflicting findings could result from differences between methods of blood examination. The examination of a fixed volume of blood (approximated in the present study by a fixed number of microscopic fields) should make the sensitivity of the examination with respect to one species independent of its sensitivity with respect to another species. On the other hand, as Cohen pointed out,[4] a flexible stopping rule (such as the examination of 100 fields of all films, and an additional 100 fields of the films negative after 100 fields) may easily produce a spurious deficit of mixed infections. As immunity causes a decrease in the density of infection (and in the probability of diagnosis by the examination of a given volume of blood), the spurious deficit of mixed infections may decrease with immunity. Among the publications reviewed by Cohen some do not state the stopping rule used, but several were using an elastic stopping rule which is, however, not fully described. It is not possible to compare the density of infection in the present study with the densities in the studies reviewed by Cohen because the densities were observed in only one of these studies. In that study, "parasite densities were judged very approximately", and provide no reliable basis for comparison.

### *Possible explanations of the positive association between* P.f. *and* P.m. *in persons*

Accepting as genuine the positive association between *P. falciparum* and *P. malariae* within persons, a simple explanation would be that the

two infections are transmitted by the same vectors and any differences in exposure between persons would apply automatically to both species. In Garki, however, after early childhood there was a negative association between *P. falciparum* parasitemia and several serological indicators of the immune response: the level of IgM, the number of bands of precipitation in the Ouchterlony-*P.f.* test, and the IHA-*P.f.* titer.[5,6] This suggests that, in Garki, after early childhood parasitological differences between persons are related to differences in their acquired immunity status rather than to differences in current exposure. It was also found that the variation of IgM within each person decreased with increasing age, while the variation of IgM between persons increased with age, as if each person were tending towards his characteristic maximum level of immune response.[5,7] The differences in immunity status may be due either to differences in past exposure or, more probably, to constitutional, probably genetical, differences in immune response to a given exposure. The positive association of *P. falciparum* and *P. malariae* suggests that persons that have a weaker immunity to the one also have a weaker immunity to the other and vice-versa. The latter would be expected not only if immunity is partly nonspecific or heterologous, but also if differences in past exposure or in immune response to a given exposure apply to both species.

In the above discussion, it is implied that heterologous immunity, if it existed, would produce an excess of mixed infections and not a deficit, as expected by Cohen from different premises.[4] Obviously, the expected parasitological effect of heterologous immunity will depend on the parasitological effect of homologous immunity. With increasing immunity to malaria, episodes of patent parasitemia become somewhat less frequent and markedly shorter; this was suggested in Garki by the estimation of transition rates from the longitudinal observations for both *P. falciparum* and *P. malariae*.[2,3] If there is heterologous immunity, persons more likely to have patent parasitemia of one species, because of a lower immunity, would also be more likely to have patent parasitemia for the other species, and there would be an excess of mixed infections. On the other hand, if there is competition between the two species, in the sense that the presence of the one would tend to prevent the presence of the other, one would expect a deficit of mixed infections, and the situation might be described as heterologous premunition.

### *Possible explanations of the seasonal alternation between* P.f. and P.m. *in the population*

The seasonal alternation between *P. falciparum* and *P. malariae* in populations has been described previously but not analyzed in detail. The increase in the rate of onset of *P. malariae* parasitemia in the latter part of the dry season and the immediately following increase in prevalence may be due in part to new infections, in part to relapses. The time-lag of 30 weeks between transmission, measured by the infant conversion rate, and the crude conversion rate suggests an incubation period of about 30 weeks. The time-lag appears only after the age of 5 years and is therefore unlikely to be a genetically determined characteristic of the parasite. Among other explanations to be considered are immunity and suppression of *P. malariae* by *P. falciparum*. Immunity to *P. malariae* increases rapidly with age, but it is not known whether this immunity would prolong the incubation period. Suppression of one species of *Plasmodium* by another is known from clinical observations,[8] and suppression of *P. malariae* by *P. falciparum* is also suggested by the timing of observed events. The rapid increase in vector density after the onset of the wet season is rapidly followed by a marked increase in the prevalence of *P. falciparum* at the same time as the prevalence of *P. malariae* decreases markedly. The same sequence is observed very regularly, although the rapid increase in vector density occurs at somewhat different times in different villages and years.

If *P. falciparum* suppresses *P. malariae,* it is not obvious why suppression becomes visible only at an age and immunity level at which both species become relatively scarce. Suppression due to a toxic effect might explain this alternation. If suppression were due to competition for a common resource, this alternation would be expected only if the resource itself became scarce simultaneously with the two species.

The findings could be explained by combining the concepts of competition and heterologous or nonspecific immunity. Let us suppose that either species activates "immune slots" (e.g., macrophages) effective against both. Parasites of both species, present in the same host, compete for these "slots." If there are more slots than parasites of either species, but fewer than the total number of parasites, as in a host with a relatively high level of immunity, then each species has a certain

probability of disappearing from or becoming undetectable in the blood film, while the other persists. The addition of parasites of one species, e.g., by inoculation, will have the following consequences: 1) an increase in the number of activated "slots"; 2) an increase in the ratios of the number of that species both to the number of slots and to the number of the other species, hence a decrease in the probability of disappearing; 3) for the other species, a decrease in the corresponding ratios, hence an increase in the probability of disappearing. At an intermediate level of immunity, the number of slots lies between the numbers of the two species, and only the less abundant species can disappear. Its probability of disappearing decreases when its own number increases, and increases when the number of the other species increases. Such a conceptual model could explain both that there is a seasonal alternation between *P. falciparum* and *P. malariae* and that the seasonal alternation is limited to the older, more immune, age-groups.

This conceptual model can be formalized by the hypergeometric probability law. Out of N parasites contained in the small volume of blood that will eventually be examined, n are *P.m.*, (N − n) are *P.f.* A sample of size, s, is drawn at random, where s is the number of activated nonspecific, or heterologous, immune slots, available to the small blood volume considered. The probability p(n) that all n *P.m.* parasites are in the sample which will occupy the s immune slots, and therefore the probability that no *P.m.* parasites eventually appear in the blood film, is:

$$p(n) = \binom{n}{n}\binom{N-n}{s-n}\Big/\binom{N}{s}$$
$$= (N-n)!s!/[(s-n)!N!], \qquad \text{if } n \leq s \leq N.$$

p(n) decreases when parasites of the same species are added (e.g., when n, N, and s increase by an equal amount). p(n) increases when parasites of the other species are added (e.g., when (N − n), N and s increase by an equal amount) and successive additions of the other species will have a cumulative effect. The probability that an increase in one species causes the disappearance of the other becomes significantly large when s approaches N, i.e. when the immunity level is high.

If *P. falciparum* and *P. malariae* are competing, through whatever mechanism, it is easy to explain why *P. falciparum* suppresses *P. malariae,* and not the reverse. 1) A given vector population, physiologically capable of transmitting both species, transmits *P. falciparum* much more rapidly, because its incubation interval between uptake of infective gametocytes from one human host and appearance of infective gametocytes in the next human host is much shorter, and because the proportion of vectors surviving the shorter extrinsic incubation period is much larger. 2) In the human host, *P. falciparum* multiplies faster than *P. malariae.*

Also if suppression of *P. malariae* by *P. falciparum* is of epidemiological importance, as suggested here, then it may be a factor in the geographical distribution of *P. malariae,* which has been puzzling investigators for a long time.[9] In particular, the very high prevalence of *P. malariae* found by Sulzer et al.[10] in an isolated community of the Amazonian forest might be due in part to the remarkable absence of *P. falciparum* from the same community.

The model of nonspecific, or heterologous, immunity outlined above could explain an excess of mixed infections, given a sufficient proportion of persons sufficiently immune to suppress both species simultaneously.

Observations on the relationship between *P. falciparum* and *P. malariae* have been made in the past in the school children of Freetown, Sierra Leone. Between 1925–1926[11] and 1931, a marked increase in the prevalence of *P. malariae,* "at the expense of *P. falciparum,*" was reported;[12] this was confirmed in 1932,[13] and 1935.[14] The absolute increase in the prevalence of *P. malariae* is easily explained by the use of thin films in 1925–1926, thick films in 1931, 1932, and 1935. Its increase in relation to *P. falciparum* is more difficult to explain but may be due, at least in part, to the fact that in 1925–1926 the examinations were made mostly in the wet season while the later examinations were made largely in the dry season, and in part to the implementation of a relatively effective program of source reduction.[14]

## CONCLUSIONS

We offer this interpretation of all the epidemiological observations: 1) there is a positive correlation within persons between the level of immunity to *P. falciparum* and the level of immunity to *P. malariae.* This correlation exists even when persons of the same age are compared. It explains why double infections are more common than expected under the hypothesis of independence. The

correlation is both direct, through the nonspecific or heterologous part of the immune response, and indirect, through differences in the constitutional, probably genetic, ability to respond, specifically or otherwise, to a given antigenic stimulus; 2) there is a partial suppression of *P. malariae* by *P. falciparum* in the individual host which explains the seasonal alternation between the two species. The suppression results from the partly nonspecific or heterologous nature of the immune response. Suppression is observed in only one direction because *P. falciparum* multiplies and is transmitted more rapidly.

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