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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

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MEMORANDUM

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SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3, F-34

PR 9/30/85 - 3/31/86

PR 4/1/86 - 9/30/86

PR 10/1/86 - 3/31/87

FINAL

Attachment

PN-ABA-528
3 F-34

TRANSMISSION BLOCKING IMMUNITY AND INFECTIVITY OF HUMAN POPULATIONS TO MOSQUITOES

(USAID Grant number PDC-5542-G-SS-5079-00)

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INTRODUCTION

This study had two major objectives. The first was the measurement of the malarial infectiousness of the human population in the Madang area of Papua New Guinea to mosquitoes, and the second was to determine the extent of the human immune response to gametocyte and gamete stages of malaria and how this influences infectiousness.

Infectiousness of a human population can be thought of as the proportion of the human population which at any one time is capable of infecting mosquitoes. Alternatively it can be defined as the proportion of mosquitoes biting a human population on any one night which acquire an infection. In the present paper we use the second meaning for infectiousness and define it as the mosquito infection probability (K). This term is used in preference to 'Infectious reservoir' as used in the grant application.

Three approaches were adopted to measure the mosquito infection probability K. The direct approach was to feed groups of mosquitoes on a demographically representative human population, without regard to parasitological status. A value was thus obtained for the proportion of individuals capable of infecting mosquitoes, and among such infectious individuals, of the proportion of mosquitoes which become infected. From these data a value for K was estimated.

The second approach was to determine the prevalence of gametocyte carriers in three villages in the Madang area by blood slide surveys, while data on infectivity of gametocyte carriers was independently obtained by feeding mosquitoes on known gametocyte carriers presenting to health facilities. The infection probability K was then derived by multiplying the mean probability of infection (from the gametocyte carriers tested) by the prevalence of gametocyte carriers in the human population.

The third approach was to estimate the proportion of mosquito blood meals which pick up infection using a mathematical model developed in collaboration with Dr. Allan Saul of the Queensland Institute of Medical Research. In this model mosquito infection rates together with human blood indices can be used to estimate the mosquito infection probability. Data generated in the present study has been used to investigate and validate the mathematical model. The model has the advantage that mosquitoes caugnt in biting collections as well as blood-fed in indoor or outdoor resting catches may be used to measure infection rates and hence the infection probability.

In the second major objective of this project, factors affecting the infectiousness of individuals to mosquitoes were investigated by testing sera from the endemic area in membrane feeding studies together with *Plasmodium falciparum* gametocytes grown in vitro. Gamete surface antigens recognised by the sera were investigated by immunoprecipitation from 125-I surface labelled gametes. Responses to the antigens were correlated with the effects of the

sera on infectivity.

1) Measurement of the infectiousness of the human population.

a) Direct measurement of infectivity to mosquitoes of individuals in the human population.

i. Village based infectivity studies.

During surveys in the villages of Mebat, Umun and Bahor in 1984, infectivity of individuals to mosquitoes was studied by membrane feeding. The laboratory reared mosquitoes used were from a colony of *Anopheles farauti* established from a cross between wild caught females from the village of Agan, Madang province, and males of a colony of *An.farauti No. 1* from Rabaul, East New Britain province, provided by Dr. Tony Sweeney, Army Malaria Research Unit, Ingleburn, N.S.W, Australia. At the village surveys fingerprick blood samples of at least 200 ul were drawn into heparinised containers and the blood immediately presented to starved *An.farauti* through water jacketed (37 ^{o}C) glass feeders using Baudruche membrane. Because of the reluctance of *An.farauti* to feed through membranes, in 1985 villagers in Butelgut, Mebat, Sah and Buksak were asked to allow groups of 20-30 mosquitoes to feed directly on themselves.

Immediately after being offered blood, mosquitoes were sorted and unfeds discarded. Engorged mosquitoes were held in an insectary at ambient temperature (mean 27-28 ^{o}C) for 7 to 9 days, with a continuous supply of 10% sucrose solution. Their stomachs were

then dissected, stained with 0.2% mercurochrome and examined at x400 for oocysts.

Groups of mosquitoes were dissected from feeds performed on 201 individuals (Table 1), of which 8 (4.0%) gave rise to infections in mosquitoes. The mean percent of mosquitoes infected in feeds on these 8 infectious individuals was 37.9% (range 9.1% - 75.0%). The average number of mosquitoes dissected per feed was 8.0 (s.d.= 3.5). It is possible to estimate the mosquito infection probability K by multiplying the above two proportions (0.379 x 0.04) giving 0.015. However because of the large standard error arising from such a multiplication it was preferable to estimate K from the average of the proportion infected in each group (weighted by sample size) as 0.013 ± 0.005. As a third alternative, dividing the total number of mosquitoes infected (21) by the total dissected (1610) gave an estimate of 0.013 ± 0.003 for the mosquito infection probability.

On subsequent examination of the blood slides, 17 persons (8.5%) were observed to have gametocytes (any species), and 6 of these people were infectious. However, out of the 12 persons in whose slides only *P.vivax* asexual stages were reported, two were infectious to mosquitoes, indicating that in these cases *P.vivax* gametocytes had been undetected. In no cases were infected mosquitoes obtained from persons whose slides were reported to show *Plasmodium falciparum*, *P.malariae* and/or *P.ovale* asexual stages only, or no parasites of any stage. Only one of the 54 *P.falciparum* cases (any stage) was infectious whereas five of the

20 *P.vivax* cases were infectious.

The distribution of individuals in the different age categories upon whom feeds were successfully performed was compared with the demographic profile of the population at large. Accurate, up-to-date demographic data from house-to-house surveys during 1985 was available for the villages of Butelgut, Mebat, Maraga and Sah (Table 2). There was a significant difference between the demographic profile of the population on which mosquitoes fed and the demographic profile of the population as a whole (Heterogeneity $X^2 = 8.91$, $p=0.031$). This arose from a significant underrepresentation of 0-4 year olds in feeding studies compared to their numbers in the population ($X^2 = 5.07$, $p=0.024$), due to the refusal of many young children to allow mosquitoes to feed on them. After correcting for the distribution of feeds on different age groups, the proportion of persons infectious was estimated as 3.8%.

ii. Infectivity of gametocyte carriers and measurement of gametocyte rates in the population.

Membrane or direct feeds were performed on gametocyte carriers identified amongst outpatients at Yagaum and Alexishaven Health Centres, Madang Province, and at Goroka Hospital, Eastern Highlands Province. Feeds on 31 *P.falciparum* gametocyte carriers revealed that 45.2% of them were infectious (Table 3). The average number of mosquitoes dissected per group was 10.9. The

overall proportion of mosquitoes infected in the total of 337 fed was 0.119 ± 0.018. Since there was extreme heterogeneity in the degree of infectiousness of different people, the mean proportion of mosquitoes infected was calculated by averaging the proportion infected in each group, giving a value of 0.118 ± 0.039. The mean number of oocysts per infected mosquito ranged from 1.0 to 54.2 in the different groups.

For P.vivax (Table 4), 14 out 28 feeds were infectious (50.0%). These include three feeds on P.vivax infected people in whose slides gametocytes were undetected and three feeds on persons in whom the density of gametocytes was not known. The overall proportion of mosquitoes infected was 0.147 ± 0.019, but again to allow for heterogeneity between individuals the mean proportion infected was obtained by averaging the probabilities in each group, giving an estimate of 0.203 ± 0.051. The average number of mosquitoes dissected was 12.4 and the mean number of oocysts per infected mosquito ranged from 1.0 to 31.5. The presence of a P.falciparum asexual infection in conjunction with P.vivax appeared to reduce the infectivity of P.vivax cases: only three out of 12 such mixed infections were infectious compared to 11 out of 16 of the cases with P.vivax only (X^2 = 3.65, p=0.056).

Table 5 shows data from all feeds performed on P.malariae infected cases. In all except two of them, gametocytes were detected. Four out of 11 gametocyte carriers (36.4%) produced infections in the mosquitoes; the overall proportion of mosquitoes infected was 0.072 ± 0.026. The mean proportion

infected per group was 0.111 ± 0.062. The mean number of mosquitoes fed was 10.6 and the mean number of oocysts per infected mosquito ranged from 1.0 to 7.0.

The influence of parasite density and age on infectiousness was investigated by logistic regression using the GLIM statistical package. In the case of *P.falciparum*, there was a statistically significant difference between the infectiousness of the different classes of gametocyte density ($X^2_3 = 9.3$, $p < 0.05$), but there was no trend of infectiousness increasing or decreasing with parasite density. However if the one case in which 100% of mosquitoes were infected was excluded, the effect was no longer significant ($X^2_3 = 3.85$). Analysis of the effect of age on infectiousness, which was restricted to cases in the endemic area of Madang (23 cases), showed no significant effect ($X^2_1 = 1.36$). Similar analysis of the effect of gametocyte density on *P.vivax* infectiousness was not possible because of the restricted number of gametocyte density classes. In the Madang feeds (14 cases), infectiousness of *P.vivax* increased with age ($X^2_3 = 14.84$), although the limited data available means that this result should be interpreted with caution. Similar lack of sufficient data precluded analysis of factors affecting *P.malariae* infectiousness.

There was no significant difference between the three malaria species in the proportion of gametocyte carriers who were infectious to mosquitoes, which averaged 47.5%. Nor was there a

significant difference in the proportion of mosquitoes infected per group by the three malaria species. An overall estimate of mean proportion infected, obtained by averaging the proportion infected per group, was 0.151 ± 0.029.

Of the *P.falciparum* gametocyte carriers from Madang (Table 3), the numbers in each age group, and the percentage infectious, were as follows: 0 - 4 years, 9 (56%); 5 - 9 years, 7 (29%); 10 - 19 years, 3 (33%); ≥ 20 years, 1 (0%). For *P.vivax*, the figures were as follows: 0 - 4 years, 5 (60%); 5 - 9 years, 4 (50%); 10 - 19 years, 4 (75%); ≥ 20 years, 1 (100%).

To determine gametocyte rates, six parasitological surveys were carried out in the villages of Butelgut, Mebat and Maraga between June 1983 and September 1985. Four surveys at three-monthly intervals were followed by two surveys at 9 monthly intervals. Slides were examined to determine prevalence of *P.falciparum*, *P.vivax*, *P.malariae* and *P.ovale* asexual stages and gametocytes by age group, which are presented in Fig 1. There was no significant difference between the three villages in the prevalence of *P.falciparum* asexual stages or gametocytes, which ranged from 2.2% in Maraga to 4.0% in Butelgut. Neither was there a significant difference between the villages in the *P.vivax* gametocyte rate, either overall or in any of the age groups.

The prevalence of gametocytes (all species) was 7.3% in Maraga, 7.8% in Butelgut and 10.4% in Mebat. These differences between

villages are not significant. The overall prevalence of gametocytes in the three village populations was 3.3% P.falciparum, 4.0% P.vivax and 0.7% P.malariae, giving a combined gametocyte prevalence of 8%. The gametocyte rates of P.falciparum and P.vivax were not significantly different.

The peak prevalence of P.falciparum was observed in the 5-9 years age group in Butelgut and Mebat but in the 10-19 years age group in Maraga. Prevalence rates of P.vivax peaked in the 5-9 years age group in all three villages. For P.falciparum, gametocyte rates were always highest in the 0-4 age group.

Relative ratios between infections with sexual and asexual stages were obtained from the prevalences for each age group shown in Fig.1. Despite highest asexual prevalence in 5-9 year olds, the relative ratio between P.falciparum infections with gametocytes and those with only asexuals declined sharply from 19.3% in 0-4 year olds to 8.7% in 5-9 year olds. In P.vivax the gametocyte rate was higher in the 5-9 year age group than in 0-4 year olds, but the relative proportion of P.vivax infections which had gametocytes was highest in the 0-4 age group (55.8%) and declined through the subsequent age groups to 10.4% in the over 20 age group.

Examination of 1541 blood slides taken in Butelgut, Mebat and Maraga during 1983-85 revealed a total of 51 infections with P.falciparum gametocytes, 62 with P.vivax gametocytes, and 10

with *P.malariae* gametocytes. These numbers are insufficient to investigate the distribution of parasite densities by age groups. Therefore *P.falciparum* gametocyte and asexual densities by age were analysed using a larger sample of blood slide surveys undertaken in Madang villages during 1983-1985, in which there were a total of 158 gametocyte and 1133 asexual infections (Table 6). Despite highest prevalence rates of *P.falciparum* asexual stages in 5-9 year olds (Fig 1), average asexual parasite density grade showed a decline with age from 3.7 in 0-4 year olds to 1.8 in over 20 year olds. There was a corresponding decline in gametocyte density grade through the age groups (Table 6). The decline in *P.falciparum* density with age is clear in Fig 2; in the case of *P.falciparum* gametocytes the majority of infections were in the lowest density grade. However the proportion of infections with density greater than grade 2 was significantly higher in the 0-4 year olds than in the other age groups (X^2 = 9.14, p=0.003).

The infectiousness of the population was estimated by combining the data on the average proportion of mosquitoes infected by a gametocyte carrier (0.151 ± 0.029) with the overall gametocyte prevalence in the population (0.080 ± 0.007). This leads to an estimate of 0.012 ± 0.003 for the mosquito infection probability.

b) Measurement of mosquito infection probability from wild caught mosquitoes.

Mosquito collections were performed in Butelgut, Mebat and Maraga

villages by a team of four collectors who visited each village in rotation for four consecutive nights at four to six week intervals for an eighteen month period. Collectors performed biting catches between 1800 and 0600 and collections for mosquitoes resting outdoors between 0700 and 0800. Collections for mosquitoes resting inside houses were performed between 0600 and 0630 by an inhabitant or close relative of the inhabitants who searched all mosquito nets and walls in each village house. All mosquitoes were placed in an insulated box and transported back to the laboratory by 1000 hours.

Mosquitoes caught biting were used to determine the sporozoite rate by ELISA (1,2) or, in Mebat and Butelgut, the delayed infection rate. This parameter is the proportion of mosquito stomachs observed to be infected with oocysts after they have been held in the laboratory for sufficient time to allow all their oocyst infections to be visible at x400 magnification. These rates include ruptured oocysts which can be clearly seen remaining on the stomach after the sporozoites have been released. Mosquitoes caught biting were held for three days; engorged mosquitoes caught in indoor and outdoor resting collections were used either immedately to determine the human blood index by ELISA (3) or for determination of the the delayed infection rates after holding in the insectary for 5 days.

The delayed oocyst rates from the various types of catch, the sporozoite rates and the human blood indices (where available) in

the villages of Butelgut, Mebat and Maraga are given in Tables 7-9. The equations used to estimate the mosquito infection probability K from these data are given in Table 10, together with equations for the estimated variance which were derived by Dr. Richard Hayes, London School of Hygiene and Tropical Medicine. The estimates of K obtained from these equations are shown in Table 11. The mosquito infection probability was found to be significantly higher for *An.koliensis* in Butelgut (0.174 $\pm$ 0.037) than in Mebat (0.049 $\pm$ 0.012) ($z = 3.21$, $p < 0.01$). The value of K for *An.koliensis* in Butelgut was also higher than K for *An.punctulatus* in the same village (0.074 $\pm$ 0.027) ($z = 2.18$, $p = 0.03$).

To determine whether there is a differential susceptibility of the three members of the *An.punctulatus* complex to malaria parasites, the mean oocyst number in each species was compared. It was not possible to compare the proportions of mosquitoes infected, because of the differing human blood indices and species proportions in each village. Overall the geometric mean of oocyst number was 3.71. Analysis of variance on the mean log of oocyst number indicated no difference between the mosquito species (Table 12) or between the three types of collection (not shown), but a significantly higher mean oocyst number in Butelgut than in the other two villages (Table 12).

In Mebat village, there was a significantly higher proportion of mosquitoes infected with oocysts in indoor than outdoor biting catches (the two types of catch being conducted simultaneously),

(X^2 by Mantel-Haentzel test = 7.5, $p < 0.01$). In Butelgut, no such difference was observed (Table 13). In Maraga, dissection of mosquitoes from biting catches was not done. When the sporozoite rate in indoor and outdoor biting catches was compared, no significant difference was observed between the rates in the two types of catch in any of the villages (Table 13). Possible bias in the estimates of oocyst rates in Mebat due to the different infection rates in each sample was avoided by dissecting approximately equal numbers of mosquitoes from indoor and outdoor collections.

2) Immunity and parasite antigenicity in relation to infectivity of human populations to mosquitoes.

a) Antigenicity of gametocytes of *P.falciparum*.

Progress on this part of the proposed study has not been as rapid as for the rest of the project and we do not feel able to report on results at present. This situation has arisen mainly from the return of Dr. Richard Carter from the Queensland Institute of Medical Research to the National Institutes of Health, and his subsequent move to the University of Edinburgh, where these studies are still continuing.

b) Naturally occurring serum factors which affect infectivity of gametocytes to mosquitoes.

Blood samples of 5-10 ml were collected from consenting adults

attending the outpatients department at Yagaum Health Centre, Madang. Blood samples were also drawn from a cross-sectional sample of individuals over the age of 4 years attending surveys in the villages of Agan, Madang Province and Tau, East Sepik Province. At Goroka Hospital, sera were collected from adults who had recently visited a coastal area and who presented with suspected malaria. Blood was allowed to clot and the serum shipped frozen to the National Institutes of Health.

Gametocytes of strain NF54-3D7 grown in culture were allowed to activate, the gametes purified on Nycodenz gradients and then labelled with 125-I by the lactoperoxidase method. The labelled cells were extracted in SDS and Triton X-100 before immunoprecipitation with the human sera followed by separation of immune complexes with Protein-A Sepharose, SDS-polyacrylamide gel electrophoresis and autoradiography. The sera were also tested for their effects on infectivity of P.falciparum gametocytes grown in culture by feeding to mosquitoes together with fresh human erythrocytes and non-immune serum as a source of complement. Control consisted of at least two different non-immune (U.S.A.) sera included in each experiment. Nine out of 41 sera from endemic areas consistently reduced infectivity to less than 5% of control, as did three out of seven sera from the non-endemic area.

The major surface antigens recognised by the sera were of the same molecular weights (230 and 48/45 kilodaltons) as the target

antigens of antigamete monoclonal antibodies (4). One panel of 18 sera were tested together in two feeding experiments and also in a single immunoprecipitation experiment. Results from these sera are thus directly comparable with one another (Fig.3). A strong negative correlation ($r = -0.807$) was found between the total counts per minute immunoprecipitated and the average number of oocysts per gut in membrane feeds. The regression coefficient was highly significant ($t = 5.47$, $p<0.001$).

In order to compare the results obtained during different feeding experiments, in which control infectivity varied, results with individual sera (oocysts per gut) were normalised by expressing them as a percent of the mean control infectivity in each experiment. These normalised infectivities, ranked in order of magnitude of suppression, were correlated with the amount of antigen counts immunoprecipitated from the 230 and 48/45 kD proteins, measured by cutting out the relevant region of the gel and counting in a gamma counter. Spearmann's rank correlation coefficient showed there to be a significant negative correlation between infectivity and counts associated with the 230 kD protein ($rho = -0.384$, $p<0.01$).

Extensive membrane feeding studies in Papua New Guinea involving washing and resuspending of gametocytes in autologous and non-autologous sera were precluded for the following reasons:

1) Difficulties is rearing sufficient numbers of An.farauti over and above the requirements of the studies already described,

2) The extreme youth of most gametocyte carriers in Madang which

prevented venous blood being taken,

3) The lower than expected gametocyte rates in Madang, compared to Africa.

Studies still in progress involve the feeding of An.freeborni on cultured gametocytes together with sera from panels of gametocyte carriers from the Madang area, which will enable further information on the effects of transmission blocking antibodies during infections to be determined.

c) Effects of transmission blocking monoclonal antibodies on infectivity of matural infections to mosquitoes.

Three monoclonal antibodies known to have transmission blocking activity, and which recognise the three gamete surface antigens of 230, 48 and 45 kD (4), were tested against 20 isolates of gametocytes from Madang by immunofluoresence. All reacted positively and there was no evidence of strain variation in the gamete antigens, as has been previously described (5). It is to be expected therefore that they would block transmission of strains from Papua New Guinea; however this was not tested directly for the reasons mentioned above.

DISCUSSION

The proportion of bloodmeals taken on humans which are infectious to mosquitoes in the Madang area, Papua New Guinea was estimated by three methods. In the first, laboratory reared *An. farauti* were fed on individuals of all ages at village surveys. The results showed that 3.8% of people were infectious and that the mean percent of mosquitoes which became infected by feeding on these people was 37.9%. From the average proportion of mosquitoes infected, the probability that a mosquito feeding on a human would pick up infection was 0.013 ± 0.005.

These studies which involved feeding mosquitoes on large numbers of people were hampered by logistical problems including the reluctance of laboratory reared mosquitoes to feed and reluctance of the population, particularly children, to allow mosquitoes to feed on them. A second approach to estimating infectivity was therefore adopted which involved feeding mosquitoes on gametocyte carriers to measure their infectivity. The results obtained were combined with data on the prevalence of gametocyte carriers to estimate the infectivity of the population at large.

The feeding studies on gametocyte carriers indicated that 47.5% of them were infectious and that the mean probability of a mosquito becoming infected after feeding on a gametocyte carrier was 0.151 ± 0.029. Gametocyte prevalence rates in all ages measured over 18 months in three villages averaged 3.3% *P.falciparum*, 4.0% *P.vivax* and 0.7% *P.malariae*, totalling 8.0 ±

0.7%. Combining these two proportions gave an estimate of 0.012 ± 0.003 for the probability of a mosquito becoming infected following a blood meal on a member of the human population.

There was no clear evidence for a relationship between gametocyte density or age and infectivity. Highest gametocyte densities for P.falciparum were observed in the under 5 year olds. Infectious individuals were predominantly under 10 but a significant proportion were over this age. The parasitological data demonstrated that gametocyte prevalence declines with age as immunity increases. The data further indicate that gametocytes of P.falciparum and P.vivax decline in prevalence relative to asexual stages, even when the latter is rising, as in the 5-9 year age group. This suggests that some form of acquired immunity may be directly affecting the production of gametocytes and/or their survival. In P.falciparum the proportion of gametocyte carriers who were infectious was higher in the 0 - 4 year olds than in the over 5 year olds, as would be expected given the difference in gametocyte density.

Mosquito infection probability was also estimated using data on mosquito infection rates and human blood indices using the mathematical model of Allan Saul. Approximate variance formulae have been derived for the estimates from the Saul model. The estimate for K for An. koliensis was significantly higher in Butelgut village (0.174) than in Mebat (0.049), and also higher than An.punctulatus in Butelgut (0.074).

Estimates of K from wild caught mosquitoes using the Saul model are thus much higher than the measurements obtained from direct feeding studies or estimates from gametocyte prevalence rates and infectivity of gametocyte carriers. There are at least three possible explanations for the discrepancy. Firstly there may be poor estimation of some of the parameters in the Saul model, perhaps the human blood index because of the difficulty of representative sampling. Secondly the difference may be explained by non-random feeding by mosquitoes on individuals in the population, i.e. either preferential feeding on infectious people or increased feeding on a subpopulation of individuals who would thus be more likely to be infected and infectious. A final possibility is that subpopulations of mosquitoes which prefer to bite and/or rest indoors exist. There is some evidence in favour of this in the higher oocyst rates in indoor- than outdoor-biting An.koliensis observed in Mebat. A similar sub-population in An.koliensis in Butelgut could explain the higher K estimated for this species than An.punctulatus. In this study species identifications were made on a morphological basis, but it is likely that one or more cryptic species exist, possibly with different feeding and resting behaviours.

The K as measured from wild-caught mosquitoes is probably a more realistic reflection of the proportion of mosquitoes that are actually picking up infections than estimates from direct feeding studies. Sampling the wild mosquito population to estimate K also avoids logistical problems associated with direct feeding

studies.

The small size of village populations and the lack of significant variation between gametocyte rates in the three study villages prevented detailed analysis of the relationship of K to prevailing vectorial capacity and inoculation rate. Separate studies have shown the vectorial capacity to average 2.2 for *An.koliensis* in Butelgut, 9.0 for *An.farauti* in Maraga, and 29.2 for *An.koliensis* in Mebat. The inoculation rates in terms of average number of sporozoite infected bites per year were 433 in Butelgut, 353 in Mebat and 291 in Maraga (T.R.Burkot, unpublished). Given the significant variation observed in the present study between the estimates of K for the different mosquito species in a village, it is difficult at present to know how to construct a relationship between infection probability and inoculation rate for a village unit.

The fact that certain individuals with high gametocyte densities did not infect mosquitoes indicated that gametocytes are impaired or otherwise prevented from infecting mosquitoes in these individuals. It is unlikely that this is due to the sporonticidal effects of antimalarial drugs since Fansidar was not available to these study groups through local health services. Data from feeding *P.falciparum* gametocytes from *in vitro* cultures to mosquitoes by membrane feeding have shown that many sera from individuals in Papua New Guinea suppress infectivity of parasites to mosquitoes. A strong negative

correlation was shown between the infectivity of these sera and the presence of antibodies against *P.falciparum* gamete surface antigens. However the response of persons to individual gamete antigens was extremely variable and hard to explain in terms of variable degrees of exposure to different gamete antigens, suggesting that the capacity of individuals to respond was an important factor. The presence of antibodies against gamete antigens was not age related and could well account for the failure of many gametocyte carriers in any age group to infect mosquitoes.

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Table 1: Mosquito feeds at village surveys in Madang.

Slide result	Number of feeds performed (no. infectious) 0-4 yrs	5-9yrs	10-19yrs	>=20 yrs	Total
Gametocytes:					
P.falciparum	1	2	3 (1)	0	6 (1)
P.vivax	3 (1)	1	2 (1)	1 (1)	7 (3)
P.malariae	1	2 (2)	1	0	4 (2)
Asexuals only:					
P.falciparum	3	11	18	16	48
P.vivax	0	2 (1)	6 (1)	5	13 (2)
P.fal. + P.vivax	1	2	2	0	5
P.malariae	0	0	0	0	0
P.fal. + P.ovale	0	1	0	0	1
No parasites any stage:					
	10	14	30	63	117
Total:	19 (1)	35 (3)	62 (3)	85 (1)	201 (8)
Percent infectious:	5.3	8.6	4.8	1.2	4.0

Table 2: Demographic profile of study villages compared to mosquito feeds on each age group.

	Age group				
	0-4	5-9	10-19	>=20	Total
Number of people	89	79	129	248	545
	(16.3%)	(14.5%)	(23.7%)	(45.5%)	
Number of mosquito feeds	19	35	62	85	201
	(9.5%)	(17.4%)	(30.8%)	(42.3%)	

Table 3: Results of mosquito feeds on *P.falciparum* gametocyte carriers.

Place (a)	Type of feed (b)	Age (yrs)	Parasite density(c) Pfa	Pfg	No. mosq. diss.	No. infected	Mean oocysts per infected mosquito
G	Mem	50	0	1	33	0	
* M	Dir	10	0	1	4	0	
M	Mem	8	0	1	18	1	2.0
M	Mem	2	0	1	6	0	
M	Mem	4	0	1	7	1	1.0
M	Mem	3	0	1	8	0	
M	Mem	9	0	1	7	0	
* M	Dir	16	0	1	13	0	
M	Mem	5	1	1	4	0	
G	Mem	25	1	1	11	7	3.0
* M	Mem	2	1	1	7	0	
* M	Mem	8	1	1	10	0	
M	Mem	4	1	1	7	1	1.0
* M	Mem	7	5	1	5	0	
* M	Dir	15	5	1	13	3	1.3
G	Mem	35	6	1	18	2	3.5
M	Mem	7	0	3	7	1	1.0
M	Mem	8	0	3	10	0	
M	Mem	3	0	3	4	1	1.0
G	Mem	25	4	3	12	0	
G	Mem	39	8	3	10	0	
M	Mem	30	0	4	20	0	
M	Mem	0	0	4	5	0	
M	Mem	7	1	4	5	0	
G	Mem	17	1	4	37	2	1.0
M	Mem	2	1	4	8	1	1.0
G	Mem	16	6	4	13	13	54.2
M	Mem	3	8	4	6	2	5.5
M	Mem	7	0	5	14	4	6.5
G	Dir	50	7	5	8	0	
M	Mem	7	8	5	7	1	1.0

* Feed at village survey, included in Table 1.

(a): Location: M=Madang, G=Goroka.

(b): Type of feed: Mem = membrane, Dir = direct.

(c): Parasite density: 0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961 parasites per mm3.
Pfa = *P.falciparum* asexual stages, Pfg = *P.falciparum* gametocytes.

Table 4: Results of mosquito feeds on *P.vivax* gametocyte carriers.

Place (a)	Type of feed (b)	Age (yrs)	Parasite density(c) Pfa	Pva	Pvg	No. mosq. diss.	No. infected	Mean oocysts per infected mosquito
* M	Dir	19	0	1	0	10	3	1.3
M	Mem	1	0	8	0	24	2	3.3
* M	Dir	9	8	3	0	6	2	2.0
* M	Dir	17	0	1	1	9	4	1.8
* M	Dir	54	0	1	1	4	3	1.3
* M	Mem	4	5	1	1	11	0	
* M	Dir	4	1	3	1	4	2	1.0
* M	Dir	10	3	3	1	6	0	
* M	Mem	8	4	3	1	6	0	
G	Dir	16	5	4	1	4	0	
G	Mem	16	6	4	1	16	0	
* M	Mem	3	0	5	1	11	0	
M	Dir	12	0	5	1	4	3	4.0
G	Dir	27	0	5	1	9	0	
G	Mem	17	0	5	1	10	0	
M	Mem	5	3	5	1	16	0	
G	Dir	50	0	6	1	3	2	31.5
G	Mem	30	0	6	1	9	1	1.0
G	Mem	20	0	6	1	16	2	1.0
G	Mem	14	0	6	1	17	0	
G	Dir	18	3	7	1	5	0	
G	Mem	?	6	7	1	16	0	
M	Mem	2	0	8	1	52	2	3.8
G	Mem	35	0	8	2	19	0	
G	Mem	22	8	4	2	13	0	
M	Dir	7	3	4	?	8	4	3.3
G	Mem	20	0	6	?	23	14	5.0
G	Mem	25	0	7	?	15	7	4.1

* Feed at village survey, included in Table 1.

(a): Location: M=Madang, G=Goroka.

(b): Type of feed: Mem = membrane, Dir = direct.

(c): Parasite density: 0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961 parasites per mm3.
Pfa = *P.falciparum* asexual stages, Pva = *P.vivax* asexual stages, Pvg = *P.vivax* gametocytes.

Table 5: Results of mosquito feeds on *P.malariae* infected individuals.

Place (a)	Type of feed (b)	Age (yrs)	Parasite density (c) Pfa	Pma	Pmg	No. mosq. diss.	No. infected	Mean oocysts per infected mosquito
M	Mem	4	0	3	0	30	0	
M	Dir	4	3	3	0	3	2	1.0
M	Dir	8	6	1	1	4	0	
* M	Dir	9	0	3	1	11	1	1.0
* M	Dir	7	0	4	1	14	3	7.0
M	Mem	3	0	5	1	6	0	
M	Mem	7	0	5	1	11	0	
M	Dir	8	0	4	2	4	1	1.0
M	Mem	8	0	6	2	8	0	
M	Dir	10	1	1	2	7	0	
* M	Dir	12	1	4	3	18	0	
* M	Dir	2	0	4	4	11	0	

* Feed at village survey, included in Table 1.

(a): Location: M=Madang, G=Goroka.

(b): Type of feed: Mem = membrane, Dir = direct.

(c): Parasite density: 0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961 parasites per mm3.
Pfa = *P.falciparum* asexual stages, Pma = *P.malariae* asexual stages, Pmg = *P.malariae* gametocytes.

Table 6: Average *P.falciparum* density grade by age, 1983-1985.

	Age group				
	0-4	5-9	10-19	>=20	Total
P.falciparum asexuals:					
Mean density	3.65	3.42	2.43	1.83	2.76
s.d.	2.30	2.18	1.83	1.47	2.07
N	(211)	(289)	(343)	(290)	(1133)
P.falciparum gametocytes:					
Mean density	1.46	1.16	1.20	1.07	1.26
s.d.	0.87	0.75	0.66	0.37	0.74
N	(56)	(43)	(30)	(29)	(158)

Parasite density grades: (parasites per mm^3)

0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961.

Table 7: Butelgut: Delayed infection rates, sporozoite rates and human blood indices.

	FARAUTI		KOLIENSIS		PUNCTULATUS	
Delayed infection rates:						
Db (N)	0.0645	(62)	0.0566	(601)	0.0681	(1587)
Df' (N)	0.0526	(19)	0.2000	(215)	0.1359	(206)
Sporozoite rates:						
P.falciparum	0.0161		0.0101		0.0195	
P.vivax	0.0000		0.0152		0.0150	
Total (N)	0.0161	(62)	0.0253	(792)	0.0345	(2462)
Human blood indices:						
Q (N)	-		-		0.714	(14)
Q' (N)	-		0.875	(24)	0.985	(136)

Table 8: Mebat: Delayed infection rates, sporozoite rates and human blood indices.

	FARAUTI		KOLIENSIS		PUNCTULATUS	
Delayed infection rates:						
Db (N)	0.0411	(316)	0.0414	(2536)	0.0594	(303)
Df (N)	0.0195	(154)	0.0556	(234)	0.0000	(22)
Df' (N)	0.1081	(37)	0.0820	(951)	0.0645	(31)
Sporozoite rates:						
P.falciparum	0.00528		0.00786		0.00000	
P.vivax	0.01056		0.00402		0.01234	
Total (N)	0.01584	(568)	0.01188	(5728)	0.01234	(729)
Human blood indices:						
Q (N)	0.359	(39)	0.598	(66)	0.469	(16)
Q' (N)	0.667	(9)	0.861	(83)	0.889	(9)

Table 9: Maraga: Delayed infection rates, sporozoite rates and human blood indices.

	FARAUTI		KOLIENSIS		PUNCTULATUS	
Delayed infection rates:						
Df (N)	0.0132	(2574)	0.0213	(94)	0.0000	(8)
Df' (N)	0.0556	(450)	0.0553	(1393)	0.0000	(1)
Sporozoite rates:						
P.falciparum	0.00138		0.00244		0.0000	
P.vivax	0.00063		0.00137		0.0000	
Total (N)	0.00200	(24000)	0.00382	(6547)	0.0000	(203
Human blood indices:						
Q (N)	0.071	(686)	0.364	(22)	-	
Q' (N)	0.651	(251)	0.808	(409)	-	

Table 10: Equations for the mosquito infection probability K

(1) $K = (D_f' - D_b) / (Q'(1 - D_b))$

$$Var(K) \approx k^2 \left\{ \frac{V(Q')}{(Q')^2} + \left(\frac{1}{(Df' - Db)}\right)^2 \left[V(Df') + \left(\frac{1 - Df'}{1 - Db}\right)^2 V(Db) \right] \right\}$$

(2) $K = (D_f' - D_f) / ((Q'(1-D_f) - Q(1 - D_f'))$

$$Var(K) \approx \left(\frac{K}{Df' - Df}\right)^4 \left\{ (Q' - Q)^2 \left[(1 - Df)^2 V(Df') + (1 - Df')^2 V(Df) \right] + (Df' - Df)^2 \left[(1 - Df')^2 V(Q) + (1 - Df)^2 V(Q') \right] \right\}$$

Table 11: Estimates of mosquito infection probability K.

Village	Species	Eqn.	Estimate	Estimated S.E.
Mebat	An.farauti	7	0.105	0.085
Mebat	An.koliensis	7	0.049	0.012
Butelgut	An.koliensis	7	0.174	0.037
Butelgut	An.punctulatus	7	0.074	0.027
Maraga	An.farauti	8	0.074	0.019
Maraga	An.koliensis	8	0.076	0.038

Table 12: Geometric mean of oocyst number by mosquito species and by village.

	FAR	KOL	PUN	Butelgut	Mebat	Maraga
Mean log.	0.533	0.577	0.573	0.643	0.543	0.494
Geom. mean	3.41	3.77	3.74	4.40	3.49	3.11
No. of mosquitoes	85	365	171	233	250	138

$F = 0.19$, $p = 0.85$ $F = 3.25$, $p = 0.04$

Table 13: Proportion of mosquitoes infected with oocysts and sporozoites in indoor and outdoor biting collections.

	Percent of mosquitoes infected (no. examined)					
	BUTELGUT		MEBAT		MARAGA	
OOCYSTS						
	In	Out	In	Out	In	Out
FAR	2.9(34)	7.1(28)	4.9(122)	4.0(176)	N.D.	N.D.
KOL	6.0(300)	5.3(290)	5.1(1162)	3.2(1104)		
PUN	6.6(725)	6.2(752)	9.9(132)	3.8(131)		
TOT	6.3(1059)	6.2(1070)	5.5(1416)	3.3(1411)		
	X^2 = 0.01, N.S.		X^2 = 7.5, P<0.01			
SPOROZOITES						
FAR	0.0(31)	3.1(32)	1.6(320)	1.3(308)	0.2(14023)	0.2(16417)
KOL	2.0(442)	2.6(420)	1.1(3310)	1.0(3529)	0.2(3262)	0.5(3770)
PUN	2.7(1507)	3.8(1670)	1.0(496)	0.9(668)	0.0(78)	0.6(162)
TOT	2.5(1980)	3.5(2122)	1.2(4126)	1.0(4505)	0.2(17363)	0.3(20349)
	X2 = 3.04, N.S.		X2 = 0.50, N.S.		X2 = 0.62, N.S.	

FIGURE LEGENDS

Fig 1: Prevalence of asexual stages (solid lines) and gametocytes (dashed lines) of *P.falciparum*, *P.vivax* and *P.malariae* by age group in the villages of Butelgut, Mebat and Maraga combined, 1983-1985. Bars represent binomial standard errors.

Fig 2: Frequency distribution of densities of asexual stages (A) and gametocytes (B) in *P.falciparum* infections by age group in the Madang study area, 1983-85. The number in each sample of asexual and gametocyte infections respectively was as follows: 0-4 years: 211, 56; 5-9 years: 289, 43; 10-19 years: 343, 30; $\geq$20 years: 290, 29. The density grades were as follows (parasites per mm^3): 1=0-200, 2=201-1400, 3=1401-8000, 4=>8000.

Fig.3: Relationship between infectivity in the presence of Papua New Guinea sera and counts per minute immunoprecipitated from 125-I labelled gametes. Solid circles = sera from Madang and Tau. Open circle = sera from Goroka.

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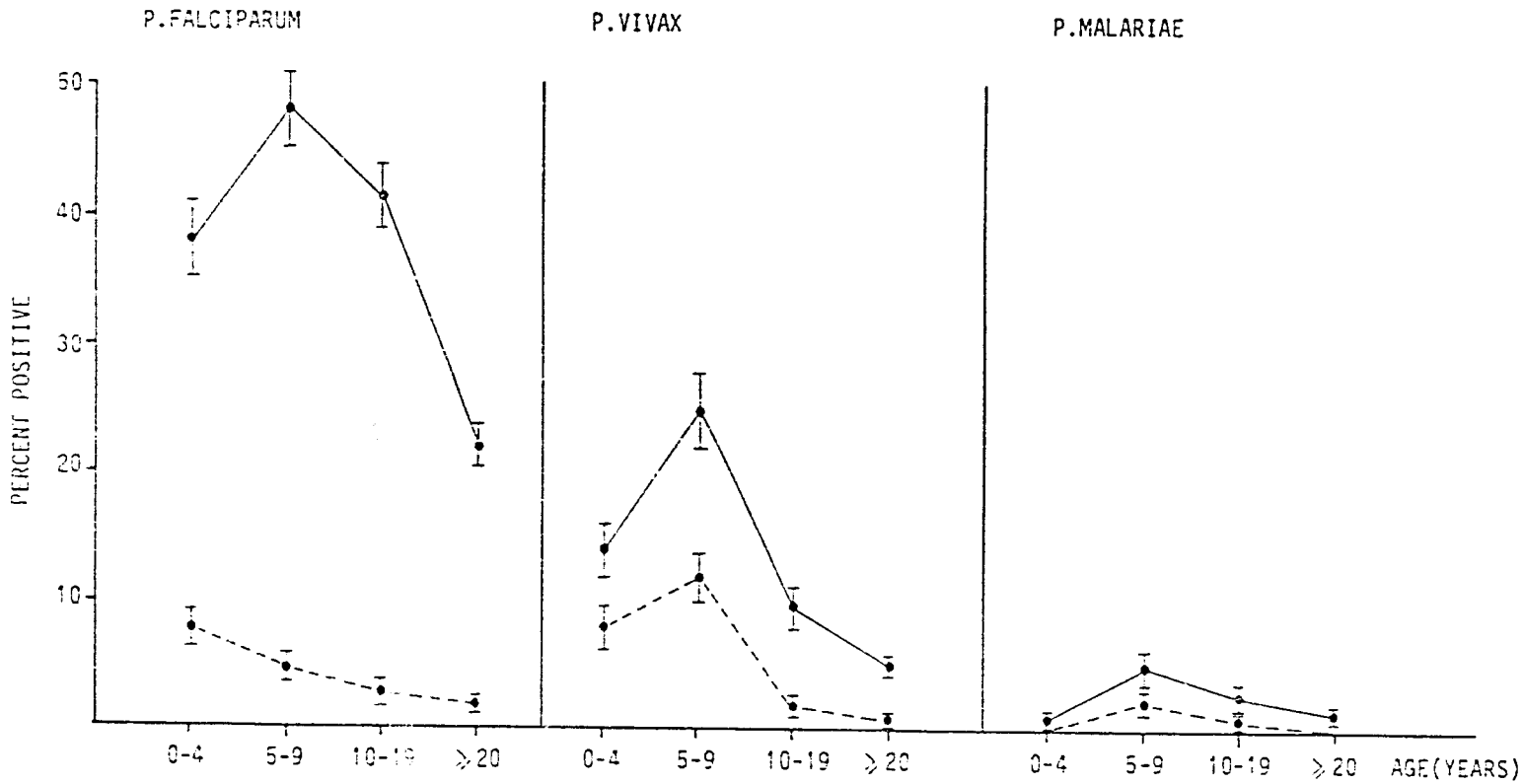

P.FALCIPARUM
P.VIVAX
P.MALARIAE
PERCENT POSITIVE
50
40
30
20
10
0-4
5-9
10-19
≥20
AGE(YEARS)

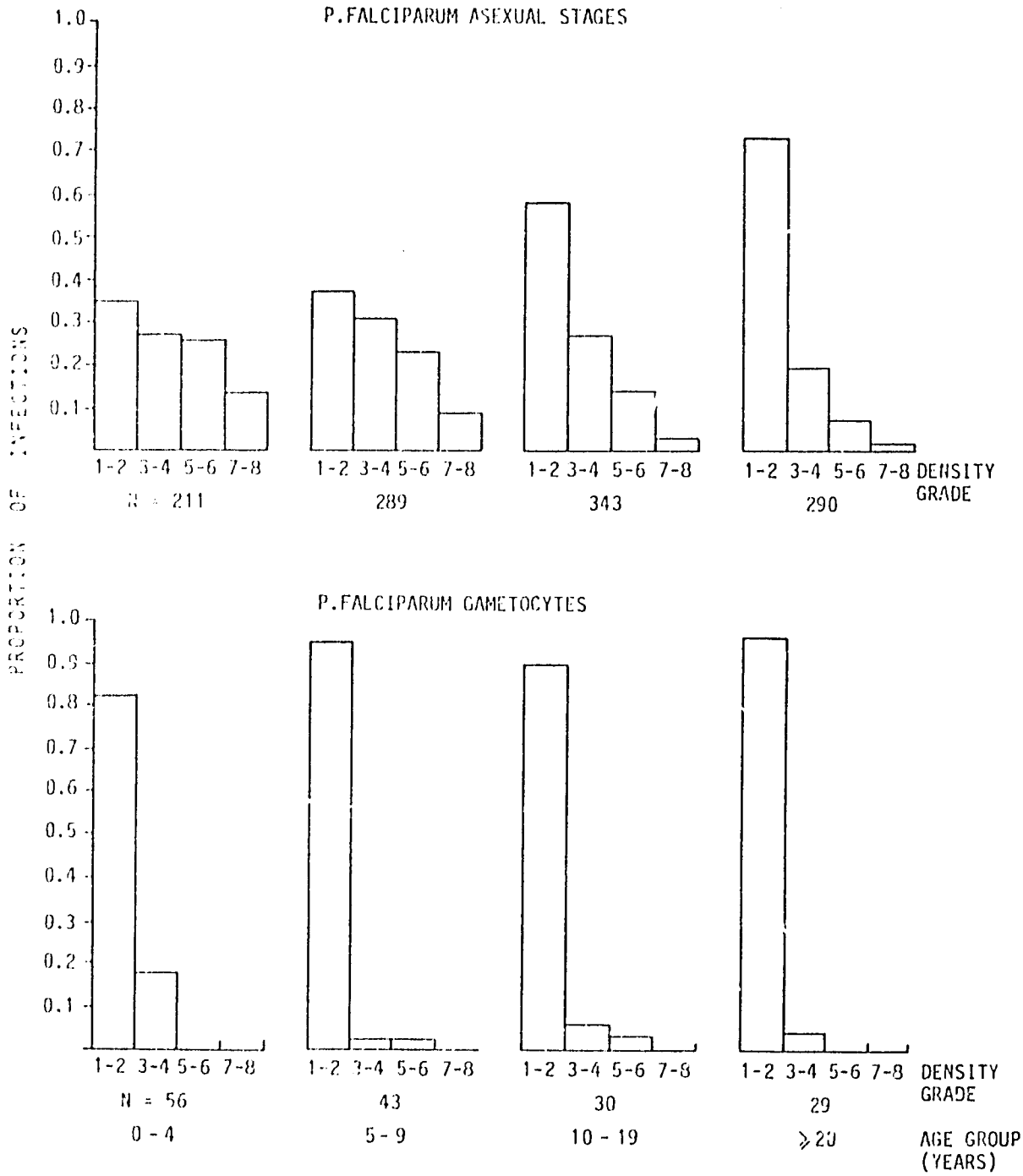
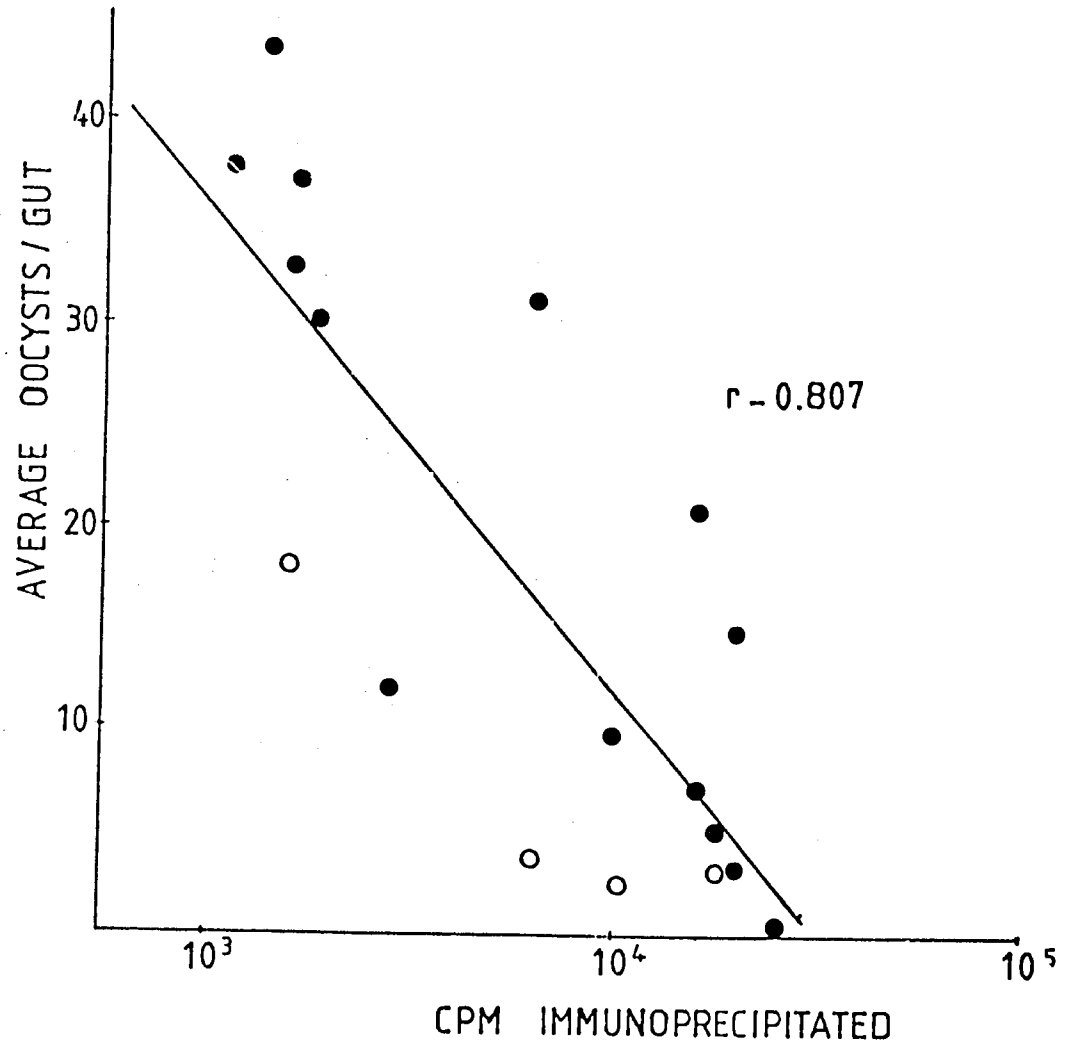

P.FALCIPARUM ASEXUAL STAGES
PROPORTION OF INFECTIONS
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1-2 3-4 5-6 7-8
N = 211
289
343
290
DENSITY GRADE
P.FALCIPARUM GAMETOCYTES
N = 56
43
30
29
0 - 4
5 - 9
10 - 19
≥20
AGE GROUP (YEARS)

Figure. 3

RELATIONSHIP BETWEEN INFECTIVITY IN THE PRESENCE OF PNG SERA AND C.P.M. PRECIPITATED FROM 125-I-GAMETES



XN-ABA-528-A
3.F. 34

TRANSMISSION BLOCKING IMMUNITY AND INFECTIVITY OF HUMAN POPULATIONS TO MOSQUITOES DURING MALARIA TRANSMISSION.

(USAID Grant No.: PDC-5542-6-55-50/9-00)

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Progress Report for the Period September 30, 1985 to March 31st, 1986.

7/28/88

43

The Papua New Guinea Institute of Medical Research (PNGIMR) has been conducting a multidisciplinary research program on malaria in approximately 70 villages around Madang for the past 5 years. On the basis of information on parasite rates and prevalence of the different species of mosquito vectors in different parts of the Study Area, three villages were selected for intensive study under the proposal. These villages are Maraga, characterised by a very large mosquito population of predominantly Anopheles farauti; Mebat, which has an intermediate mosquito population of predominantly An. koliensis; and Butelgut, in which the major vector, An. punctulatus, is present in very low numbers.

The location of each village is characteristic of the three types of environment found within the heterogeneous environs of Madang, namely swampy coastal lowland (Maraga), well-drained coastal lowland (Mebat) and inland upland (Butelgut). These three villages have been included in longitudinal epidemiological surveys for the past 2 years, the results of which are currently being analysed and will yield detailed information on the parasite rates. An additional survey was carried out in the 3 villages in September 1985 to obtain more detailed information for the present study. Overall, the parasite rate does not differ between the three villages; however, preliminary data from previous surveys indicates a differing age prevalence curve in the 3 villages, with the parasite rate peaking in a younger age group in Butelgut than in Mebat, and at an even later age in Maraga (Fig. 1).

Feasibility studies have indicated the most effective ways to

collect mosquitoes in the study villages in order to determine the infectious reservoir. The houses are of extremely open construction, and exit-trap collections are not possible. However, it was found that numerous blood-fed mosquitoes could be found resting indoors in the first hour after dawn, after which time they leave the houses. Since a large proportion of houses must be searched in order to obtain a representative sample of mosquitoes, it was found to be most productive to teach the inhabitants to search the walls and mosquito nets of their own houses shortly after arising. This also overcame taboos about outsiders entering houses. These indoor resting collections are being used to determine both the human blood index (using ELISA), and the infectious reservoir, by holding the mosquitoes in the laboratory for five days. These parameters can also be measured in blood-fed mosquitoes caught resting outdoors, but the search for these mosquitoes has proved to be productive only in Maraga, and to a lesser extent in Mebat. This reflects the relative species distributions in the three villages (since An. farauti is easier to find in the bush close to the village) as well as the overall mosquito numbers.

Biting collections are also made in the study villages to obtain mosquitoes for determination of the sporozoite rate (which is done in Madang using ELISA), and also to measure the human biting rate for determination of the vectorial capacity. Despite the fairly similar parasite prevalences in the study villages, the sporozoite rates are markedly different, being considerably lower (0.3%) in Maraga than in Mebat and Butelgut (1.7% and 3.3%

respectively). A large part of this difference appears to be due to an extremely low human blood index in Maraga. However, the inoculation rate in Maraga is much higher than would be expected given such a low sporozoite rate, due to the large biting population of mosquitoes.

Mosquitoes from the biting collection are also used to determine the infectious reservoir. In the time between the submission of this proposal and the start of funding, a mathematical model has been developed by Allan Saul (Queensland Institute of Medical Research) to determine the vectorial capacity using infection rate data from the three types of catch (biting, indoor resting and outdoor resting). This model makes significant modifications to the model formulated by Macdonald which underlay the methods originally given in the proposal for analysis of the data. The essential difference in the Saul model is that it assumes a constant mosquito survival rate per feeding cycle, rather than per day. This assumption approximates more closely to reality, and gives estimates of vectorial capacity differing from those of Macdonald under certain conditions. The data generated in the present study will be of importance in testing the Saul model.

A healthy colony of An. farauti No. 1 is now established in Madang for studies on the infectiousness of individuals. However, the overall proportion of infectious persons in the population, as measured from the uptake of oocysts in wild-caught mosquitoes, appears to be low, i.e. of the order of 5%, and random feeds performed to date on village populations have identified only a few

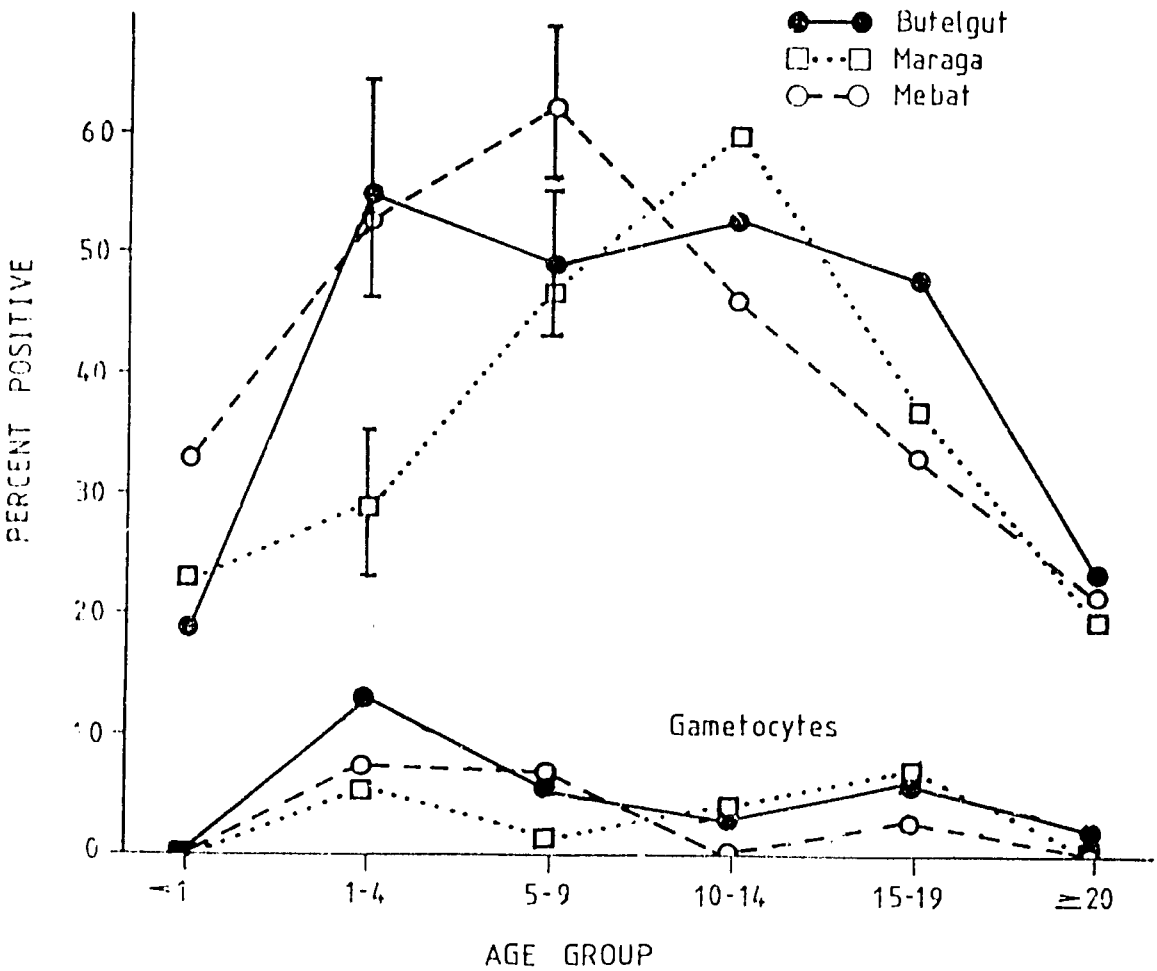
infectious individuals (Table 1). A more productive approach may be to conduct feeds on gametocyte carriers and other infected individuals identified at health-care facilities, and relate findings back to the observed prevalences of parasitemic individuals in the village populations. Such studies are being pursued at present.

Table 1: *An. farauti* fed at random on individuals in Madang.

Blood slide result	No. persons	No. batches infected
P. falciparum asexual	41	0
P. vivax	10	0
P. falciparum asexual + P. vivax	10	3
P. falciparum gametocytes	4	1
P. malariae	4	2
Negative	74	0
Total	143	6 (4.2%)

Figure 1.

AGE SPECIFIC PREVALENCE - P. FALCIPARUM



XN-ABA-528
3. F. 34

TRANSMISSION BLOCKING IMMUNITY AND INFECTIVITY OF HUMAN POPULATIONS TO MOSQUITOES DURING MALARIA TRANSMISSION

(USAID Grant No.: PIC-5542-6-55-5079-00)

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Progress Report for the period

April 1st, 1986 to September 30th, 1986.

7/21/88

1) Measurement of the Infectious Reservoir in the Human Population

a) Parasite prevalences in the study villages.

Three vilages in the Madang study area have been selected for intensive study: Butelgut, Mebat and Maraga. These villages represent the range of variation observed in this area in mosquito biting density and prevalence of the three vector species Anopheles farauti, An.koliensis and An.punctulatus. The average biting rates (all species) during 1984-1985 were 67 bites per person per night in Butelgut, 155 in Mebat and 488 in Maraga (Burkot et al, in press). The mosquito population comprised mainly An.punctulatus in Butelgut, An.koliensis in Mebat and An.farauti in Maraga.

Six parasitological surveys at three-six month intervals have been carried out in these villages, at which blood films were made for determination of the rates of infection with Plasmodium falciparum, P.falciparum gametocytes, P.vivax, P.vivax gametocytes, P.malariae and P.ovale. Average prevalence for each age group for each of these species is given in Tables 1-3. The peak prevalence of P.falciparum was observed in the 5-9 years age group in Butelgut and Mebat but in the 10-19 years age group in Maraga. P.falciparum gametocyte rates peaked in all cases in the 0-4 year-olds. P.vivax rates (asexuals and gametocytes) peaked in the 5-9 years olds. Plasmodium falciparum gametocyte rates in all age groups were significantly higher in Butelgut (4.0%) and Mebat (3.6%) than in Maraga (2.2%) ($p<0.05$). In the 0-4 years age group, the P.falciparum gametocyte rate was significantly higher in Butelgut than in Maraga. The prevalence of P.vivax infections was

significantly higher in Mebat than the other two villages; Mebat also had the highest P.vivax gametocyte rate of 5.4%. The proportion of infections of either species with gametocytes was lower in Maraga than in the other two villages. Thus in Maraga 3.3% of P.falciparum infections had gametocytes, while in Butelgut and Mebat the percentages were 10.9% and 10.3% respectively. For P.vivax, 18.8% of infections in Maraga had gametocytes whilst in Butelgut and Mebat gametocytes were present in 40.0% and 36.2% of infections.

b) Direct measurement of infectivity to mosquitoes of individuals in the human population.

Random feeding of batches of laboratory reared Anopheles farauti was conducted at the parasitological surveys in the three study villages when the supply of mosquitoes permitted. In addition random feeds were performed in the villages of Uram, Bahor, Sah and Buksak. Anopheles farauti has not proved to be an avid membrane feeder and thus direct feeds were performed where possible if individuals gave their consent. Otherwise membrane feeds were performed with fingerprick blood samples. The results of the random feeds at the village surveys are given in Table 4. On average, 6.4% of feeds gave rise to an infection in the mosquito.

Because of the difficulty of feeding on the under five year-olds, who represent the age-group with the highest rate of P.falciparum gametocyte infections, mosquitoes were transported to Goroka, in the Papua New Guinea Highlands, to perform feeds on gametocyte

carriers presenting to the Goroka Hospital. Since malaria transmission does not occur in Goroka, these patients are adults who have acquired their infections in a coastal area. Blood samples were obtained from consenting persons and offered to An.farauti by membrane feeding. Results of these feeds are shown in Table 5. In Goroka half of the P.falciparum gametocyte carriers were infectious and one third of the P.vivax cases. Serum samples have been obtained from these individuals for later assessment of transmission-blocking immunity. In Madang, 28.6% of P.vivax cases were infectious, which is not significantly different from the proportion of 33.3% observed in Goroka.

Even when a person was infectious, not all mosquitoes in a batch became infected. Table 6 shows the P.falciparum carriers from both Madang and Goroka ranked in order of density, with the percent of mosquitoes infected and the average number of oocysts observed in each case. Table 7 gives similar information for P.vivax, although in this case only the feeds on infectious persons are given.

From the data on random feeds given in Table 4, and the age of the persons on whom feeds were performed, the proportion of each age group who are infectious can be determined (Table 8). From this data and the demographic profile of the study villages (Table 9), the proportion of the overall population who are infectious may be estimated by multiplying the proportion of persons infectious in a particular age group by the proportion of the population which that age group represents. By this method it is estimated that 5.4% of the population are infectious (for all malaria species).

c) Measurement of the infectious reservoir from wild caught mosquitoes.

The infectious reservoir is defined as the proportion of mosquito feeds taken on humans which pick up an infection. Recently Dr. Allan Saul of the Queensland Institute of Medical Research has developed a model which uses infection rates in wild-caught mosquitoes to estimate mosquito survival rate and the reservoir of infection (Saul et al, submitted). Data from mosquitoes caught either biting or blood-fed in resting catches may be used as input to the model, as long as the human blood index is also known.

Mosquitoes have been collected in the study villages and their infection rates (delayed oocyst rates and sporozoite rates) determined. Human blood indices from indoor and outdoor resting collections have been measured (Burkot et al, submitted). Although the mosquito collections have been completed, the data are still being analysed using the model of Saul et al for estimation of the infectious reservoir, and results of the analysis will be given in the next progress report.

2) Immunity and parasite antigenicity in relation to infectivity of the human population to mosquitoes

a) Naturally occurring serum factors which affect infectivity of gametocytes to mosquitoes.

To assess the effect of transmission blocking activity in serum from P.falciparum gametocyte carriers, blood containing gametocytes is fed to mosquitoes in the presence of autologous serum or washed and resuspended in non-immune serum. Sera may also be tested against P.falciparum gametocytes grown in culture. In Madang it has not proved possible to perform membrane feeds involving washing and resuspension of blood from gametocyte carriers, because of the extreme youth of such carriers and their low frequency in the population. Several experiments were however performed in Goroka. The results are given in Table 10. There was evidence for the presence of transmission-blocking activity in serum in two cases: 54 (P.vivax) and 30 (P.falciparum). In the other cases, resuspension of infected blood in non-immune serum did not result in non-infectious cases becoming infectious.

In order to test the effect of sera from individuals in Madang on P.falciparum gametocytes grown in culture, sera from a cross-sectional sample of individuals of all ages living in two villages in endemic areas (Agan and Tau) were taken to Dr. Richard Carter's laboratory at the National Institutes of Health. These studies were originally planned to take place at the Queensland Institute of Medical Research, but due to delay in the start of the grant, Dr. Carter had already returned to the N.I.H.. The sera

were combined with gametocytes of strain NF54 and fed to An.freeborni mosquitoes. Controls consisted of U.S.A. sera included in each experiment. Transmission blocking activity was assessed by the reduction in average number of oocysts on the mosquito stomachs at dissection 9 days after feeding. Results of one series of feeding experiments are shown in Fig 1. Each point represents one test of each serum. Forty-two sera have been tested so far, and nine of these consistently reduced infectivity to less than 5% of control.

When the sera were reacted with 125-I-surface labelled gametes, many (although not all) of the blocking sera immunoprecipitated a 230 KD gamete surface protein, and one reacted with the 230 KD and 48/45 KD proteins. A negative correlation was observed between the total c.p.m. immunoprecipitated and the average oocysts per gut (mean of two experiments). The data obtained in one series of immunoprecipitations is shown in Fig. 2. The relationship is described by the regression line $y = 106.8 - 23.76\ x$ ($t = 5.47$, $p<0.01$). Reaction of sera with particular proteins was investigated by counting the radioactivity associated with particular bands cut out of the polyacrylamide gels. Because of the differing intensity of infection in experiments not performed concurrently, results from such experiments cannot be combined. However, infectivity in the presence of different sera can be ranked, and Spearmann's rank correlation coefficient showed a strong negative correlation between infectivity and counts associated with the 230 KD protein (rho = -0.384, $p<0.01$) but not with the 48/45 KD doublet (rho = -0.142, $p=0.17$).

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Table 1: Average parasite prevalence by age in Butelgut.

Species	Age Group 0-4	5-9	10-19	over 20	Total
P.falciparum:					
asexuals	41.8	50.6	46.9	24.3	36.6
gametocytes	9.0	6.7	3.4	2.0	4.0
P.vivax:					
asexuals	10.4	22.5	7.6	4.2	9.1
gametocytes	9.5	13.0	2.0	0.0	3.6
P.malariae:	0.0	1.1	2.1	0.0	0.7
P.ovale:	0.0	1.1	0.7	0.0	0.4
Overall:	49.3	65.2	51.0	29.1	43.1
N :	67	89	145	251	552

Table 2: Average parasite prevalence by age in Mebat.

Species	Age group 0-4	5-9	10-19	over 20	Total
P.falciparum:					
asexuals	45.6	52.0	30.8	22.5	34.3
gametocytes	7.8	5.0	1.9	2.0	3.6
P.vivax:					
asexuals	22.2	26.0	10.6	8.5	14.9
gametocytes	10.5	11.4	2.8	1.4	5.4
P.malariae:	1.1	10.0	4.8	2.5	4.2
P.ovale:	1.1	0.0	0.0	0.0	0.2
Overall:	56.7	69.0	42.3	33.3	46.6
N :	90	100	104	204	498

Table 3: Average parasite prevalence by age in Maraga.

	Age group				
Species	0-4	5-9	10-19	over 20	Total
P.falciparum:					
asexuals	28.8	42.6	44.3	17.6	30.5
gametocytes	5.8	1.1	1.9	1.1	2.2
P.vivax:					
asexuals	8.7	25.5	11.3	1.1	9.6
gametocytes	1.4	4.6	1.4	0.8	1.8
P.malariae:	1.0	3.2	0.9	0.5	1.2
P.ovale:	0.0	0.0	0.0	0.0	0.0
Overall:	33.7	62.8	53.8	19.3	38.1
N :	104	94	106	187	491

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Table 4: Mosquito feeds on individuals living in Madang.

Slide result	Total feeds	Total feeds, >=5 mosq.*	No. pos feeds	% persons infectious
Negative	151	103	0	
Pf asex	78	46	0	
Total, Neg + Pf asex	229	149	0	0.0
Pf asex + Pfg	7	4	1	
Pfg only	2	0	0	
Total Pfg	9	4	1	25.0
Pv only	32	21	7	
Pf asex + Pv	20	14	3	
Total Pv	52	35	10	28.6
Pm only	15	10	2	
Pf asex + Pm	7	4	0	
Total Pm	22	14	2	14.3
TOTAL	312	202	13	6.4

*No. of feeds in which at least 5 mosquitoes were dissected.

Table 5: Mosquito feeds on patients attending Goroka Hospital.

Slide result	Total feeds	Total feeds, >=5 mosq.*	No. pos feeds	% persons infectious
Pf asex + Pfg	7	7	4	
Pfg only	1	1	0	
Total Pfg	8	8	4	50.0
Pv only	12	10	5	
Pf asex + Pv	6	5	0	
Total Pv	18	15	5	33.3
TOTAL	26	23	9	39.1

* No. of feeds in which at least 5 mosquitoes were dissected.

Table 6: Feeds on *P.falciparum* gametocyte carriers.

ID No.	Location	Age	Density*	% inf	No.diss.	Mean oocysts
E/156-40	Madang	8	1/100	0.0	10	
E/168-23	"	7	1/100	0.0	5	
E/156-7	"	2	2/100	0.0	7	
E/180-26	"	15	5/100	23.1	13	1.3
JMS/G76	Goroka	35	6/100	11.1	18	3.5
JMS/G13	"	25	12/100	63.6	11	3.0
JMS/G231	"	33	16/100	0.0	33	
JMS/G36	"	25	10/200	0.0	12	
JMS/G49	"	39	10/200	0.0	10	
JMS/G83	"	17	16/200	5.4	37	1.0
JMS/G222	"	16	29/200	100.0	13	54.2
JMS/G11	"	50	44/200	0.0	8	

* Density measured per 200 wbc or (for scanty infections) per 100 fields.

Table 7: Feeds on infectious P.vivax cases.

ID No.	Location	Age	Density*	% inf	No.diss.	Mean oocysts
E/188-14	Madang	17	6/100	44.4	9	1.8
E/188-26	"	54	11/100	75.0	4	1.3
E/184-2	"	4	12/200	14.3	14	1.0
E/188-212	"	9	12/200	33.3	6	2.0
E/180-82	"	7	31/200	50.0	8	3.3
LFN 72	"	12	62/200	75.0	4	4.0
JMS/G54	Goroka	20	87/200	12.5	16	1.0
JMS/G26	"	20	118/200	60.9	23	5.0
JMS/G29	"	30	171/200	11.1	9	1.0
JMS/G3	"	50	190/200	66.7	3	31.5
JMS/G8	"	25	213/200	46.7	15	4.1
LFQ 70	Madang	1	H	8.3	24	1.0
LFQ 55	Madang	2	VH	3.8	52	1.0

*Density measured per 200 wbc or per 100 fields
H = Heavy (50-100 parasites per field)
VH = Very Heavy (more than 100 parasites per field)

Table 8: Proportion of infectious feeds by age group.

	Agegroup			
	0-4	5-9	10-19	over 20
No. of feeds	19	45	60	74
No. infectious feeds:				
P.falciparum:	0	0	1	0
P.vivax:	3	3	2	0
P.malariae:	0	2	0	0
Total:	3	5	3	0
Percent	15.8	11.1	5.0	0.0

Table 9: Demographic profile of the study villages.

Village	Agegroup 0-4	5-9	10-19	over 20	Total
Butelgut	16	14	33	48	111
Mebat	36	54	80	123	293
Maraga	38	24	34	87	183
Total	90	92	147	258	587
Percent	15.3	15.7	25.0	44.0	

Table 10: Membrane feeds of washed and resuspended blood from patients attending Goroka Hospital.

Species from:	Case No.	Percent of mosquitoes infected (no. dissected)		
		Whole Blood	Resuspended in Aust. serum	Resuspended in cam plasma
P.VIVAX	54	12.5 (16)	3.8 (26)	0.0 (9)
	115	0.0 (7)	0.0 (13)	0.0 (13)
	136	0.0 (6)	0.0 (10)	0.0 (2)
	161	0.0 (9)	0.0 (12)	0.0 (5)
	242	0.0 (8)	0.0 (12)	0.0 (5)
P.FALCIPARUM	36	0.0 (12)	16.7 (12)	0.0 (15)
	49	0.0 (10)	0.0 (11)	0.0 (5)
	76	n.d.	0.0 (13)	11.1 (18)
	83	7.1 (14)	5.3 (19)	3.6 (28)
	222	100.0 (8)	72.7 (11)	87.5 (8)
	231	0.0 (23)	0.0 (14)	0.0 (15)

Figure. 1

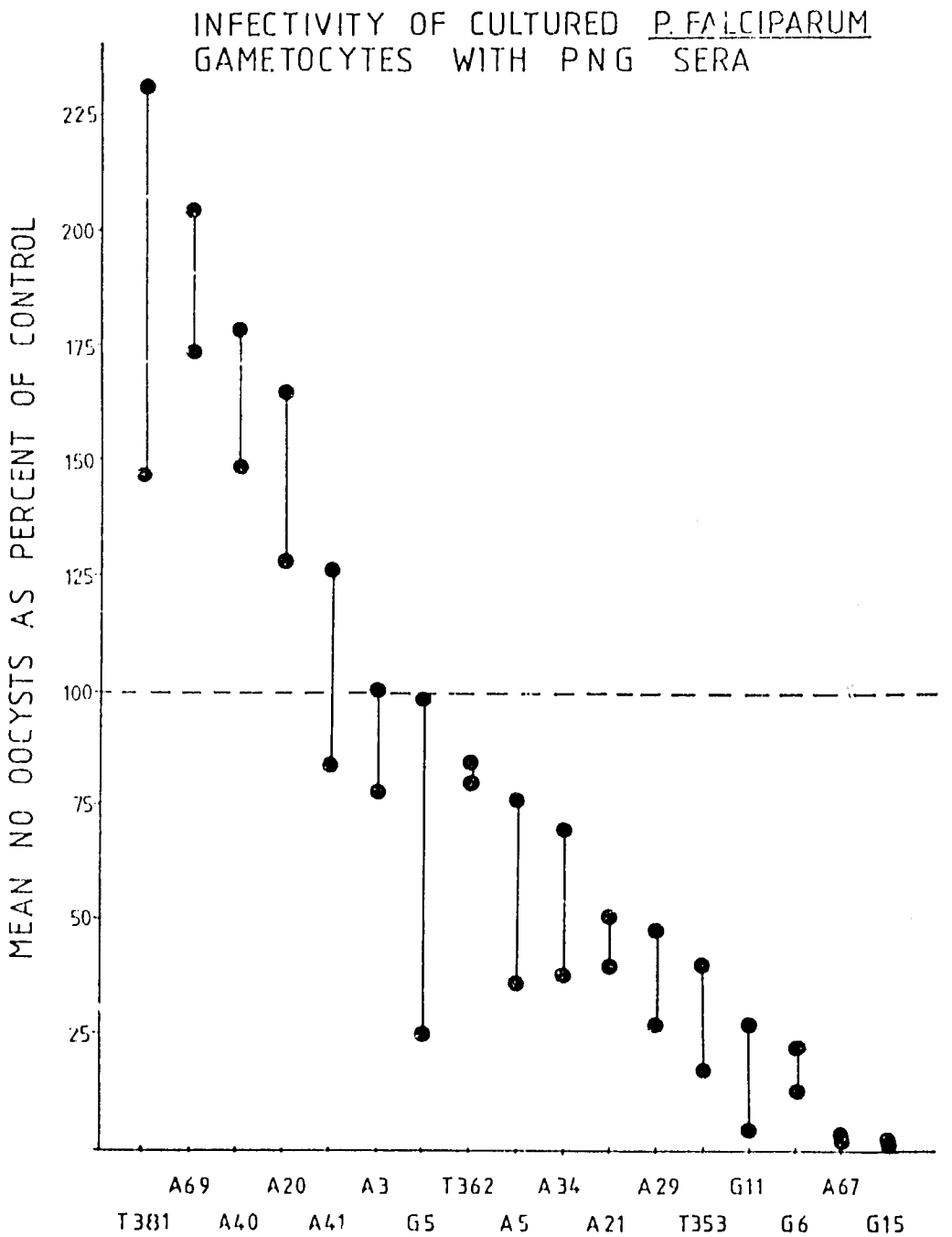
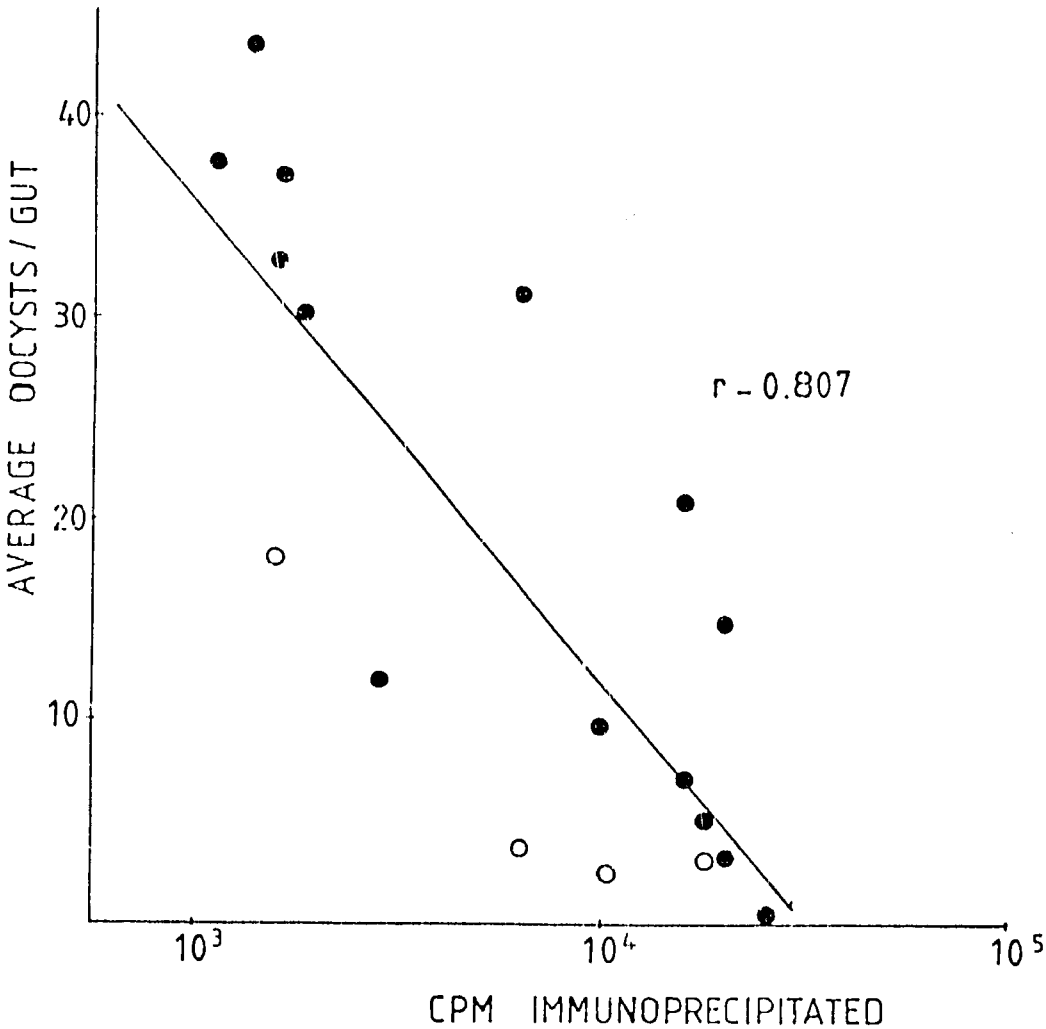

INFECTIVITY OF CULTURED P. FALCIPARUM
GAMETOCYTES WITH PNG SERA
MEAN NO OOCYSTS AS PERCENT OF CONTROL
225
200
175
150
125
100
75
50
25
T381
A69
A40
A20
A41
A3
G5
T362
A5
A34
A21
A29
T353
G11
G6
A67
G15

Figure. 2

RELATIONSHIP BETWEEN INFECTIVITY IN THE PRESENCE OF PNG SERA AND C.P.M. PRECIPITATED FROM 125-I-GAMETES



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3. F.34

TRANSMISSION BLOCKING IMMUNITY AND INFECTIVITY OF HUMAN POPULATIONS TO MOSQUITOES DURING MALARIA TRANSMISSION

(USAID Grant No. : PDC-5542-G-SS-5079-00)

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Progress Report for the period

October 1st, 1986 to March 31st, 1987

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During this six-month period data collected in the project has been extensively analysed. Data on mosquito feeds at village surveys described in the last progress report have been corrected and reanalysed. The results are combined with additional information on the gametocyte rates in the persons on whom feeds were performed to provide more accurate estimates of the proportion of infectious bloodmeals taken by mosquitoes on humans (the mosquito infection probability, K). Computer analysis of malaria survey data from the three study villages Boteloni, Medat and Maraga have clarified the prevalence rates of asexual stages and gametocytes of the three malaria species, Plasmodium falciparum, P.vivax and P.malariae in the population around Madang. Larger samples of blood slides from the Madang area during 1983-1985 have also been analysed to obtain average densities of P.falciparum asexual stages and gametocytes by age group. Further results from feeding studies on known gametocyte carriers are described, and these data (combined with the gametocyte prevalence data) are used in a second method of estimating the proportion of mosquito feeds on humans which pick up an infection.

Feeds at village surveys

Feeds of laboratory reared Anopheles farauti were successfully performed on 201 individuals (Table 1), of which 8 (4.0%) gave rise to infections in mosquitoes. The mean percent of mosquitoes infected in feeds on these 8 infectious individuals was 37.5% (range 9.1 - 75.0%). The average number of mosquitoes

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dissected per feed was 8.0 (s.d.= 3.5). Only those feeds in which a minimum of four mosquitoes fed and survived to dissection were included. On subsequent examination of the blood slides, 17 persons (0.5%) were observed to have gametocytes (any species), and 6 of these people were infectious. However, one of the 17 persons in whose slides only *P.vivax* asexual stages were reported, two were infectious to mosquitoes, indicating that in these cases *P.vivax* gametocytes had been undetected. In no cases were infected mosquitoes obtained from persons whose slides were reported to show *P.falciparum*, *P.malariae* or *P.ovale* asexual stages only or no parasites of any stage. Thus 8 out of 19 gametocyte carriers were infectious (42.1%) and the mean number of mosquitoes infected per batch was 37.9%, giving a probability of 0.159 for a mosquito to become infected after feeding on a gametocyte carrier.

The distribution of individuals in the different age categories upon whom feeds were successfully performed was compared with the demographic profile of the population at large, as reported in the last progress report. There was a significant difference between the demographic profile of the population on which mosquitoes were fed and the demographic profile of the population (X^2 = 8.91, p=0.031). This arose from a significant under-representation of 0-4 year olds in feeding studies compared to their numbers in the population at large (X^2 = 5.07, p=0.024), due to the refusal of many young children to allow mosquitoes to feed on them. After correcting for the distribution of feeds on

different age groups, the proportion of persons infectious was estimated as 3.0%.

Since not all mosquitoes feeding on an 'infectious' person become infected, estimates of the mosquito infection probability I must take account of both the non infectious members of the population and the proportion of mosquitoes which fail to become infected after feeding on an 'infectious' person. An estimate of I may be made from the overall proportion of mosquitoes which became infected out of all those fed on the population at large, which was 0.013 ± 0.003 (N = 1610).

Gametocyte prevalence rates

The prevalence of gametocytes (all species) was 7.7% in Maraga, 7.8% in Butelgut and 10.4% in Mebat. These differences are not significant (χ^2 = 3.51, p=0.173). The overall prevalence of gametocytes in the three village populations combined was 3.3% *P.falciparum*, 4.0% *P.vivax* and 0.7% *P.malariae*, giving a combined gametocyte prevalence of 8%. The gametocyte rates of *P. falciparum* and *P.vivax* were not significantly different (χ^2 =0.93, p=0.33).

Considering the villages separately, gametocyte rates of *P.falciparum* were 2.2% in Maraga, 3.6% in Mebat and 4.0% in Butelgut; *P.vivax* gametocyte rates were 2.9% in Maraga, 5.6% in Mebat and 3.6% in Butelgut. There was no significant difference between the villages in the *P.falciparum* or *P.vivax* gametocyte

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rates, either overall or in any of the age groups.

The peak prevalence of P.falciparum asexual stages was observed in the 5-9 years age group in Butelgut and Mubit but in the 10-19 years age group in Maraga, while prevalence rates of P.vivax peaked in the 5-9 year age group in all three villages. In P.falciparum, gametocyte rates were always highest in the 0-4 age group. Despite highest asexual prevalence in 5-9 year olds, the relative proportion of gametocyte and asexual infections with P.falciparum declined sharply from 19.2% in 0-4 year olds to 8.7% in 5-9 year olds (Fig.1). In P.vivax the gametocyte rate was higher in the 5-9 year age group than in 0-4 year olds. However the relative proportion of P.vivax infections which had gametocytes was highest in the 0-4 age group (55.6%) and declined through the subsequent age groups to 10.4% in the 20-29 age group. (Fig 1.)

In the three villages studied intensively during 1983 to 1985, surveys of 1541 blood slides revealed a total of 51 infections with P.falciparum gametocytes, 62 with P.vivax gametocytes, and 10 with P.malariae gametocytes. These numbers are insufficient to investigate the distribution of densities of parasites in the different age groups. Therefore gametocyte and asexual densities of P.falciparum by age have been analysed using a larger sample of blood slide surveys undertaken in Madang during 1983-1985, in which there were a total of 158 gametocyte infections and 1173 asexual infections (Table 2). Despite higher prevalence rates of

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P.falciparum asexual stages in 5-9 year olds than 0-4 year olds (Fig 1), average asexual parasite density grade declined from 5.7 in 0-4 year olds to 3.4 in 5-9 year olds and down to 1.8 in the over 20 year olds. There was a corresponding decline in gametocyte density grade through the age groups (Table 2). The distribution of density grades by age group for P.falciparum asexuals and gametocytes is shown in Fig.2. The decline in parasite density through the age groups is clear for P.falciparum asexuals; in the case of P.falciparum gametocytes, the majority of infections were in the lowest density grade. It is only in the 0-4 year olds that there was a significantly higher proportion of infections in the higher density grades (χ^2 = 7.14, p=0.005).

The parasitological data demonstrate that gametocyte prevalence declines with age as immunity increases. They further indicate that gametocytes of P.falciparum and P.vivax decline in prevalence relative to asexual stages, even when the latter is rising, as in the 5-9 year age group. This suggests that some form of acquired immunity may be directly affecting the production of gametocytes and/or their survival.

Mosquito feeding on known gametocyte carriers

Feeds of laboratory reared An.farauti on P.falciparum gametocyte carriers identified at outpatient clinics in Madang and Goroka revealed that 45.2% of them were infectious (n=71) (Table 3). However the proportion of mosquitoes infected in each case varied from 5.4% to 100%, averaging 26.1%. The average number of

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mosquitoes fed on each person was 10.9 and the mean number of oocysts per infected mosquito ranged from 1.0 to 54.5.

In the case of *P. vivax* (Table 4), 14 out 28 feeds were infectious (50.0%). These include three feeds on *P. vivax* infected people in whose slides gametocytes had not been detected and three feeds on persons whose slides were not examined for gametocytes. The percent of mosquitoes infected per batch averaged 40.6% (range 3.8% to 75%) and the average number of mosquitoes dissected was 12.4. The mean number of oocysts per infected mosquito ranged from 1.0 to 31.5. The presence of a *P. falciparum* asexual infection in conjunction with *P. vivax* appeared to reduce the infectivity of *P. vivax* cases: only three out of 12 such mixed infections were infectious compared to 11 out of 16 of the cases with *P. vivax* only (X^2 = 7.65, p=0.056).

Table 5 shows data from all feeds performed on *P. malariae* infected cases. In all except two of them, gametocytes were detected. Four out of 11 gametocyte carriers (36.4%) produced infections in the mosquitoes; the average proportion of mosquitoes infected was 30.6% (range 9.1% to 55.2%). The mean number of mosquitoes fed was 10.6 and the mean number of oocysts per infected mosquito ranged from 1.0 to 7.0.

There was no significant difference between the three malaria species in the proportion of gametocyte carriers who were infectious to mosquitoes (X^2 = 0.6, p=0.74) which averaged 45.7%.

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Of mosquitoes which fed on infectious gametocyte carriers, an average of 33.7% became infected, although there was a very large variation in this proportion. Therefore an average of 0.337 x 0.457 = 0.154 of the mosquitoes which fed on gametocyte carriers became infected. Taking into account the complete non-infectious gametocyte carriers, the feeding data was used to estimate that 0.12% ± 0.012 of the mosquitoes feeding on a gametocyte carrier (any species) would pick up an infection. Since the gametocyte rate (all species) was 0.08% ± 0.007, the mosquito infection probability I was estimated as the product of these two proportions, i.e. 0.010. Thus two methods of estimating the mosquito infection probability arrive at the figure of approximately 1% of mosquitoes feeding on humans that will become infected.

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Table 1: Mosquito feeds at village surveys in Madang.

Slide result	Number of feeds performed (no. infectious) 0-4 yrs	5-9yrs	10-19yrs	>20 yrs	total
Gametocytes:					
P.falciparum	1	2	3 (1)	0	6 (1)
P.vivax	3 (1)	1	2 (1)	1 (1)	7 (3)
P.malariae	1	2 (2)	1	0	4 (2)
Asexuals only:					
P.falciparum	3	11	18	16	48
P.vivax	0	2 (1)	6 (1)	5	13 (2)
P.fal. + P.vivax	1	2	2	0	5
P.malariae	0	0	0	0	0
P.ovale	0	1	0	0	1
No parasites any stage:	10	14	30	63	117
Total:	19 (1)	35 (3)	62 (3)	85 (1)	201 (8)
Percent infections:	5.3	8.6	4.8	1.2	4.0

Table 2: Average *P.falciparum* density grade by age, 1983-1985.

	Age group				
	0-4	5-9	10-19	>20	Total
P.falciparum asexuals:					
Mean density	3.65	3.42	2.43	1.83	2.76
s.d.	2.30	2.18	1.83	1.47	2.07
N	(211)	(209)	(343)	(290)	(1133)
P.falciparum gametocytes:					
Mean density	1.46	1.16	1.20	1.07	1.26
s.d.	0.[illegible]	0.75	0.66	0.32	0.74
N	(56)	(43)	(30)	(29)	(158)

Parasite density grades: (parasites per mm^3)

0=negative, 1=1-80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961.

Table 3: Results of mosquito feeds on *P.falciparum* gametocyte carriers.

Place (a)	Type of feed (b)	Age (yrs)	Parasite density(c) Pfa	Pfg	No. mosq. diss.	No. infected	Mean oocysts per infected mosquito
G	Mem	50	0	1	33	0	
* M	Dir	10	0	1	4	0	
M	Mem	8	0	1	18	1	2.0
M	Mem	2	0	1	6	0	
M	Mem	4	0	1	7	1	1.0
M	Mem	?	0	1	8	0	
M	Mem		0	1	7	0	
* M	Dir	16	0	1	13	0	
M	Mem	5	1	1	4	0	
G	Mem	25	1	1	11	7	3.0
* M	Mem	?	1	1	7	0	
* M	Mem	8	1	1	10	0	
M	Mem	4	1	1	7	1	1.0
* M	Mem	7	5	1	5	0	
* M	Dir	15	5	1	13	3	1.3
G	Mem	35	6	1	18	2	3.5
M	Mem	?	0	3	7	1	1.0
M	Mem	8	0	3	10	0	
M	Mem	3	0	3	4	1	1.0
G	Mem	25	4	3	12	0	
G	Mem	39	8	3	10	0	
M	Mem	30	0	4	20	0	
M	Mem	0	0	4	5	0	
M	Mem	?	1	4	5	0	
G	Mem	17	1	4	37	2	1.0
M	Mem	2	1	4	8	1	1.0
G	Mem	16	6	4	13	13	54.2
M	Mem	3	8	4	6	2	5.5
M	Mem	?	0	5	14	4	6.5
G	Dir	50	7	5	8	0	
M	Mem	7	8	5	7	1	1.0

* Feed at village survey, included in Table 1.

(a): Location: M=Madang, G=Goroka.

(b): Type of feed: Mem = membrane, Dir = direct.

(c): Parasite density: 0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19950, 8=>19951 parasites per mm3.
Pfa = *P.falciparum* asexual stages, Pfg = *P.falciparum* gametocytes.

Table 4: Results of mosquito feeds on *P.vivax* gametocyte carriers.

Place (a)	Type of feed (b)	Age (yrs)	Parasite density(c) Pfa	Pva	Pvg	No. mosq. diss.	No. infected	Mean oocysts per infected mosquito
* M	Dir	18	0	1	0	10	3	1.3
M	Mem	1	0	8	0	24	2	8.3
* M	Dir	9	8	3	0	6	2	2.0
* M	Dir	17	0	1	1	9	4	1.8
* M	Dir	54	0	1	1	4	3	1.3
* M	Mem	4	5	1	1	11	0	
* M	Dir	4	1	3	1	4	2	1.0
* M	Dir	10	3	3	1	6	0	
* M	Mem	8	4	3	1	6	0	
G	Dir	16	5	4	1	4	0	
G	Mem	16	6	4	1	16	0	
* M	Mem	3	0	5	1	11	0	
M	Dir	12	0	5	1	4	3	4.0
G	Dir	27	0	5	1	9	0	
G	Mem	17	0	5	1	10	0	
M	Mem	5	3	5	1	16	0	
G	Dir	50	0	6	1	3	2	31.5
G	Mem	30	0	6	1	9	1	1.0
G	Mem	20	0	6	1	16	2	1.0
G	Mem	14	0	6	1	17	0	
G	Dir	18	3	7	1	5	0	
G	Mem	2	6	7	1	16	0	
M	Mem	2	0	8	1	52	2	3.8
G	Mem	35	0	8	2	19	0	
G	Mem	22	8	4	2	13	0	
M	Dir	7	3	4	2	8	4	3.3
G	Mem	20	0	6	2	23	14	5.0
G	Mem	25	0	7	2	15	7	4.1

* Feed at village survey, included in Table 1.

(a): Location: M=Madang, G=Goroka.

(b): Type of feed: Mem = membrane, Dir = direct.

(c): Parasite density: 0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961 parasites per mm³.
Pfa = *P.falciparum* asexual stages, Pva = *P.vivax* asexual stages, Pvg = *P.vivax* gametocytes.

Table 5: Results of mosquito feeds on *P.malariae* infected individuals.

Place (a)	Type of feed (b)	Age (yrs)	Parasite density (c) Pfa	Pma	Pmg	No. mosq. diss.	No. infected	Mean oocysts per infected mosquito
M	Mem	4	0	3	0	30	0	
M	Dir	4	3	3	0	3	2	1.0
M	Dir	8	0	1	1	4	0	
* M	Dir	9	0	3	1	11	1	1.0
* M	Dir	7	0	4	1	14	3	7.0
M	Mem	3	0	5	1	6	0	
M	Mem	7	0	5	1	11	0	
M	Dir	8	0	4	2	4	1	1.0
M	Mem	8	0	6	2	8	0	
M	Dir	10	1	1	2	7	0	
* M	Dir	12	1	4	3	18	0	
* M	Dir	2	0	4	4	11	0	

* Feed at village survey, included in Table 1.

(a): Location: M=Madang, G=Goroka.

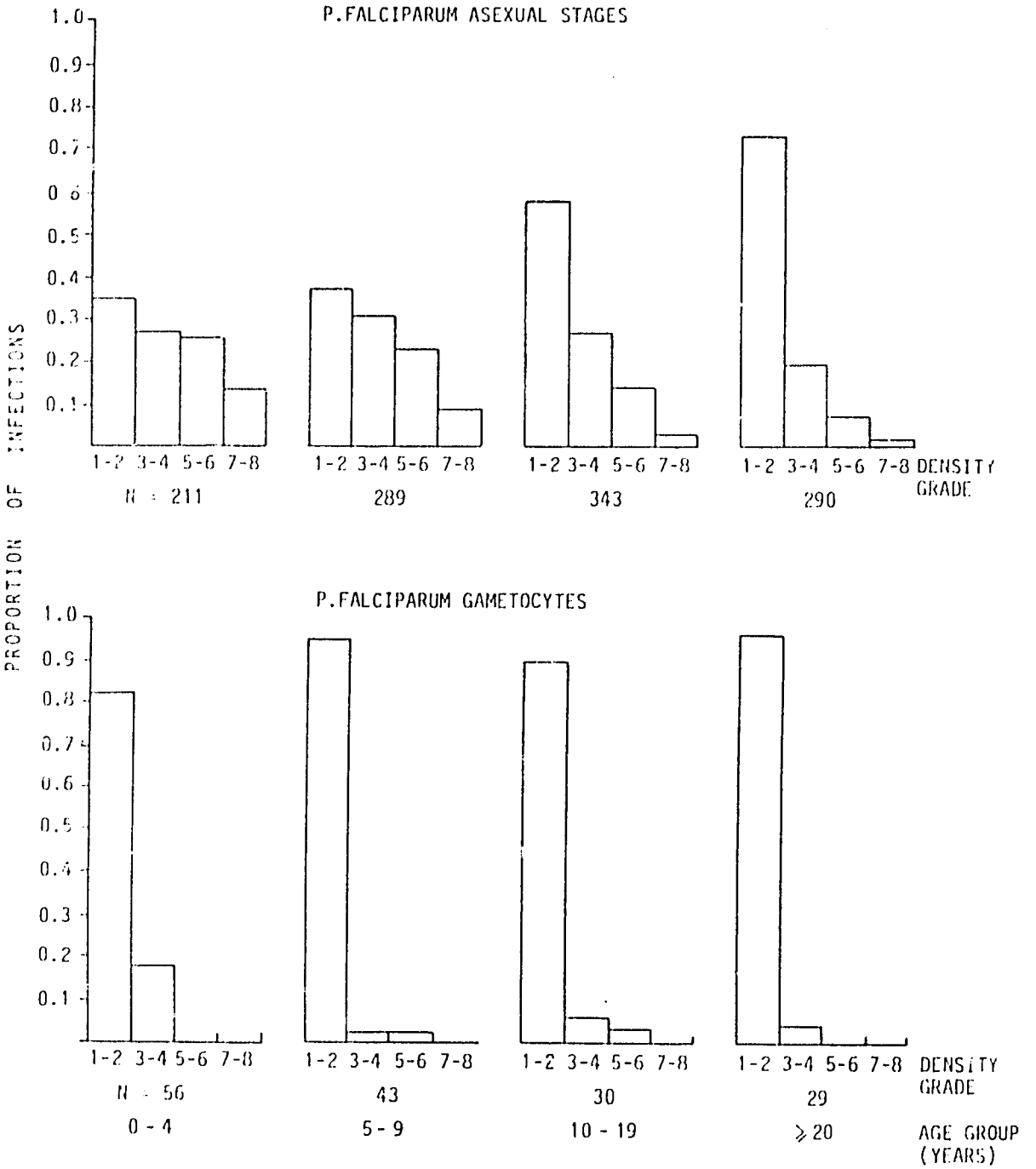
(b): Type of feed: Mem = membrane, Dir = direct.

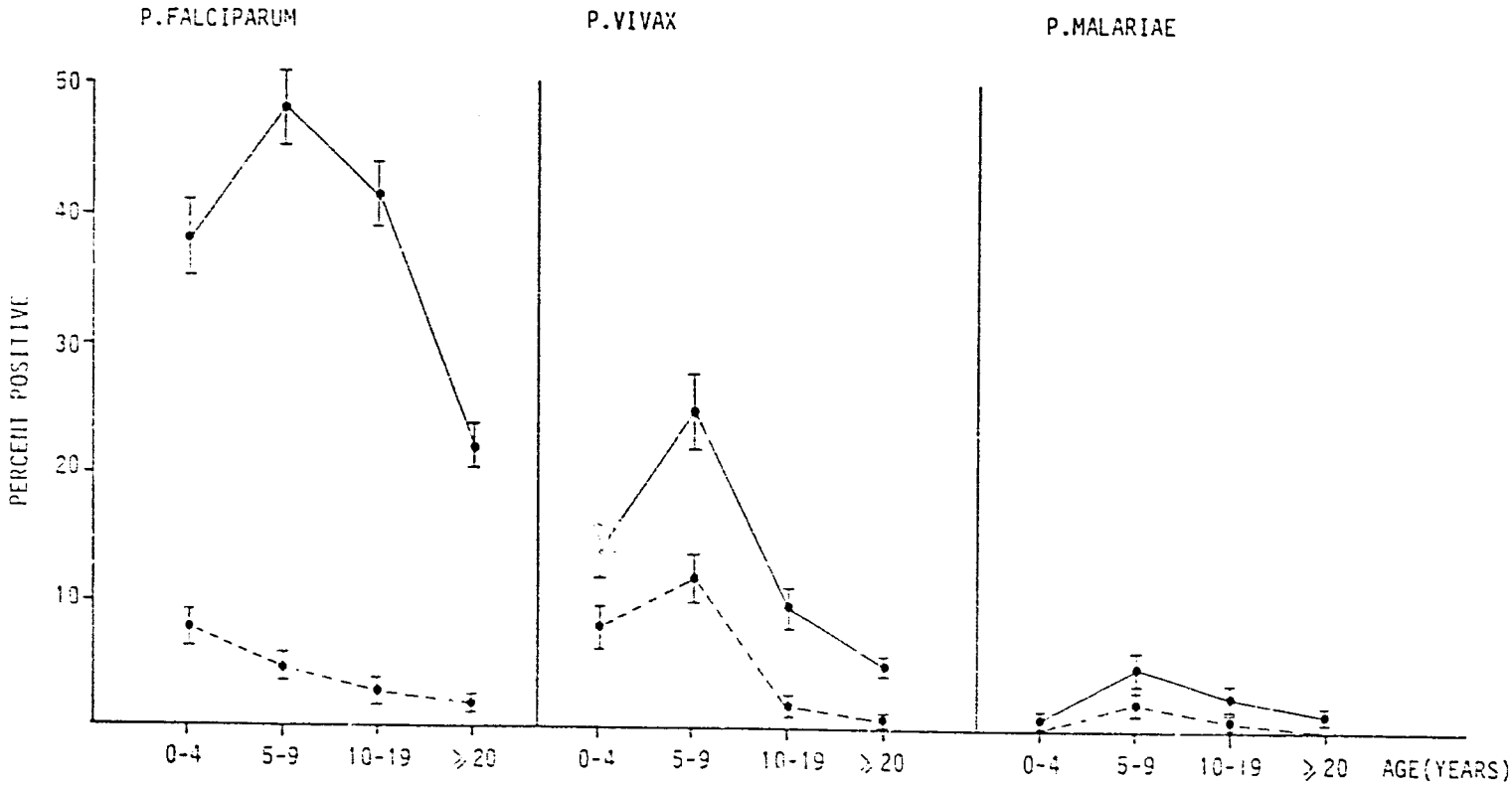
(c): Parasite density: 0=negative, 1=<80, 2=81-200, 3=201-600, 4=601-1400, 5=1401-3400, 6=3401-8000, 7=8001-19960, 8=>19961 parasites per mm3.
Pfa = *P.falciparum* asexual stages, Pma = *P.malariae* asexual stages, Pmg = *P.malariae* gametocytes.

FIGURE LEGENDS

Fig 1: Prevalence of asexual stages (solid lines) and gametocytes (dashed lines) of *P.falciparum*, *P.vivax* and *P.malariae* by age group in the villages of Butelgut, Mebat and Maraga combined, 1983-1985. Bars represent binomial standard errors.

Fig 2: Frequency distribution of densities of asexual stages and gametocytes in *P.falciparum* infections by age group in the Madang study area, 1983-85. For density grades see footnote to Table 7.


P.FALCIPARUM ASEXUAL STAGES
PROPORTION OF INFECTIONS
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1-2 3-4 5-6 7-8
N = 211
289
343
290
DENSITY GRADE
P.FALCIPARUM GAMETOCYTES
N = 56
43
30
29
0 - 4
5 - 9
10 - 19
≥ 20
AGE GROUP (YEARS)


P.FALCIPARUM
P.VIVAX
P.MALARIAE
PERCENT POSITIVE
50
40
30
20
10
0-4
5-9
10-19
≥20
AGE(YEARS)