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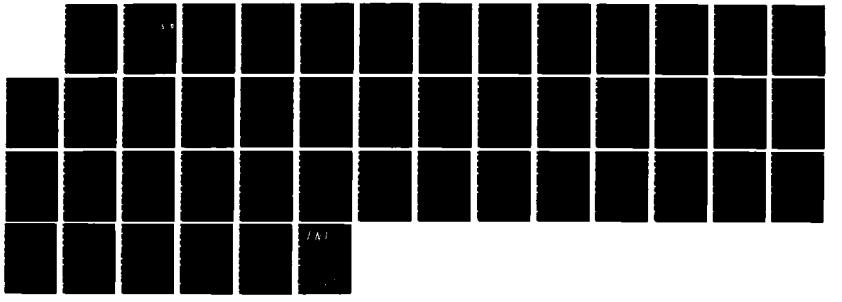

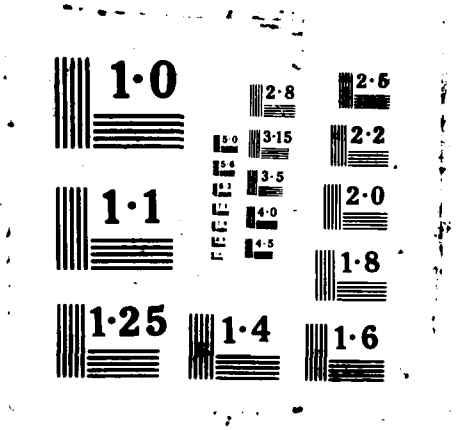
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Sporozoite Rates and Densities in the Members of the *Anopheles punctulatus* Complex

Final Report

by

Thomas R. Burkot and Michael Alpers

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Malaria sporozoite and inoculation rates were measured for 104,000 anophelines in Papua New Guinea. Comparisons of inoculation rates with parasite prevalences indicates that Plasmodium falciparum is more efficiently transmitted by sporozoites than is P. vivax. The increased efficiency of transmission may be related to the greater sporozoite densities found in P. falciparum infected mosquitoes, which were 10 fold greater than in P. vivax infected mosquitoes.

Significant correlations were found between sporozoite rates and the human blood index of the vectors and between the sporozoite rate and bed net usage. No significant correlation was found between the sporozoite rate and either the demographic profiles of the different villages or with parasite prevalences found in children in the different villages.

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SUMMARY

Malaria sporozoite rates and inoculation rates were measured over a period of at least one nine nonths in 14 epidemiologically defined villages of varying malaria endemicity in Madang Province, Papua New Guinea. Over 104,000 mosquitoes caught in landing catches were analyzed using a monoclonal antibody based ELISA to detect sporozoites of Plasmodium falciparum and P. vivax. Significant variation in average sporozoite and inoculation rates were found between the different villages, despite their close geographic proximity. Comparisons of entomological inoculation rates with parasite prevalences in children in 8 villages where detailed parasitological data was simultaneously collected with the entomology studies showed strong positive correlations for both species of malaria. However the overall prevalence rates of P. falciparum infections in children were much higher than the P. vivax prevalence rates, despite similar inoculation rates for the two species. These data suggest that P. falciparum is more efficiently transmitted from mosquito to man than P. vivax in Papua New Guinea. The increased efficiency of transmission of P. falciparum may be due, in part, to the heavier sporozoite densities in wild caught mosquitoes naturally infected with P. falciparum sporozoites which were ten fold greater than the sporozoite densities in P. vivax infected mosquitoes.

These villages were also characterized as to vector species present, densities of vectors, numbers of humans and domestic animals present, blood feeding habits and bed net usage. Regression analysis was performed to try to determine factors which result in high sporozoite and inoculation rates, which in turn lead to high parasite prevalences. Significant correlations were found between total sporozoite antigen positivity rates in An. farauti and the human blood index and bed net usage. The HBI for An. farauti was significantly related to the ratio of men to pigs in the villages. The total sporozoite antigen positivity rates in An. koliensis were also significantly related to bed net usage.

Differences in host preferences were found between members of the Anopheles punctulatus complex with An. punctulatus and An. koliensis being more anthropophilic than An. farauti.

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INTRODUCTION

Recent advances have opened up the possibility of vaccination against sporozoites of the human malaria parasites Plasmodium falciparum and P. vivax (1-6). Therefore it is essential to achieve a better understanding of the rates at which sporozoites are inoculated into the human population in malaria endemic areas and how these inoculation rates result in the parasite rates observed.

The malaria vectors in Papua New Guinea are the members of the An. punctulatus complex. The Anopheles punctulatus complex consists of Anopheles punctulatus, An. koliensis and at least three sibling species of An. farauti (Nos. 1, 2, (7) and 3 (8)). So far only An. farauti No. 1 has been found in Papua New Guinea. Members of the complex have been incriminated as the most important vectors of human malarias and periodic Bancroftian filariasis wherever they occur (9, 10). The distribution of the complex ranges from the Moluccas through New Guinea and adjoining islands to Vanuatu and north-east Australia (9). Previous studies on these vectors concentrated on determining blood feeding and resting behavior, and survivorship rates, with little work done to determine sporozoite or inoculation rates.

In the D'Entrecasteaux Islands, Spencer (11) determined the Human Blood Index (HBI) for An. farauti to be 0.75. In the Trobriand Islands, almost all the engorged An. farauti collected indoors had fed on man whereas from the outdoor resting collection only half had done so (12). Metselaar (13) from his own and other records considered the HBI of An. farauti and An. koliensis to be 0.60 and the HBI of An. punctulatus to be 0.85. However, this generalization cannot be applied to the mosquito populations around Madang where the HBI for An. farauti was found to vary greatly from one village to another, ranging from 0.09 to 0.83 (14). The variation in the HBI was ascribed to the number of animals available as alternative hosts. No mixed blood meals were reported. Earlier workers, however, had noted an interrupted feeding pattern for An. farauti and had hypothesized that if interrupted feeding is a common feature in nature, then the efficiency of these vectors of malaria could be increased (15, 16).

Mean sporozoite rates were reported as 3.3% for An. punctulatus, 2.4% for An. farauti and 2.9% for An. koliensis (17). Spencer (18) reported sporozoite rates for An. farauti as ranging from 0 to 5%.

In the past, investigations into sporozoite rates were hampered by the difficulty in determining sporozoite rates by dissecting the salivary glands of individual mosquitoes and by

the lack of morphological criteria with which to distinguish the species of sporozoite. The recent development of immunoradiometric (19) and enzyme-linked immunosorbent assays (20, 21) for the detection, identification and quantitation of sporozoites in mosquitoes has meant that large scale investigations are now feasible. As evidence to date suggests that the epitopes on the circumsporozoite proteins recognized by the monoclonal antibodies used in the assays are universally conserved, the assays should detect all sporozoite-infected mosquitoes (22).

In the Madang area of Papua New Guinea, there is intense year round malaria transmission of P. falciparum and P. vivax by the members of the An. punctulatus complex. Geographic variation in malaria endemicity based on consistent differences in parasite and spleen rates in children has been demonstrated in the area (23). These differences are only apparent in children since antimalarial immunity causes the overall parasite prevalence for all ages to equilibrate at 35 to 45% in all villages. Villages have been classified into three distinct stable epidemiological zones (high, intermediate and low) based on these differences. While each of these zones is characterized by a high level of endemicity, they have been designated relatively as high, intermediate and low. Differences between villages were also observed in the parasite species ratio (24). The presence of aminoquinolines could be detected in 12.7% of urine samples from residents in the study villages by the Dill-Glazko test and 81.6 and 46.6% of P. falciparum samples tested between 1979 and 1983 were resistant to chloroquine in in vitro and in vivo tests, respectively (23).

MATERIALS AND METHODS

SPOROZOITE RATES AND DENSITIES

Mosquito Populations

Entomological Surveys

Entomological surveys were conducted in each of 14 study villages for four nights each month for 9-18 months. Each member of the An. punctulatus complex, An farauti, An koliensis and An punctulatus, was present in at least one of the study villages. Since the mosquitoes in the An. punctulatus complex feed throughout the night both indoors and outdoors (18), mosquitoes were collected by indoor and outdoor man baited all-night biting catches from 1800 until 0600 hrs by a team of four collectors. The numbers of mosquitoes collected were used both as a measure of mosquito density and for later analysis by ELISA for the presence of sporozoite antigens of P. falciparum

and P. vivax. Collected anophelines were identified on a morphological basis (25) and stored frozen until assayed for the presence of sporozoite antigens of P. falciparum and P. vivax.

Ten villages (Agan, Butelgut, Dogia, Erima, Hudini, Maraga, Mebat, Panim, Sah and Umun) were characterized further as to the Human Blood Index (HBI) in order to determine the impact of the human blood index on sporozoite rates. From January 1985 through August 1985, outdoor resting mosquitoes were collected from 7 am until 8 am for four mornings each month in each of the villages. Engorged anophelines were collected inside houses in the villages of Agan, Butelgut, Dogia, Maraga and Mebat between 6 am and 7 am on the same dates.

Engorged members of the Anopheles punctulatus complex were transported back to the laboratory and identified by morphological criteria (9). Blood meals were then smeared on filter paper and stored either frozen or at room temperature with dessicant until analyzed for host blood identification.

Detection of Sporozoites

The assays used for sporozoite antigen detection were slightly modified from those described by Burkot et al. (20) and Wirtz et al. (21) as described below. The wells of polyvinyl chloride 96 well microtiter plates were prepared for detection and identification of sporozoites by coating with 50 uL of a dilution of a species-specific monoclonal antibody directed against a circumsporozoite protein in 0.01 M phosphate buffered saline (PBS) pH 7.4 overnight at room temperature. The wells were then emptied and filled with a blocking solution of PBS with 1% bovine serum albumen (BSA) and 0.5% casein.

Mosquitoes were prepared for sporozoite detection by triturating in 100 uL of PBS with 1% BSA, 0.5% casein and 0.5% nonidet P-40 (a nonionic detergent) in a 1.5 mL polypropylene microcentrifuge tube with a glass rod. The homogenate was then stored overnight at -20 C. After thawing, 400 uL of PBS with 1% BSA and 0.5% casein was added. Fifty microliters of the mosquito homogenate was then added to each well. After a two hour incubation at room temperature, the plate was washed three times with PBS with 0.5% Tween 20 and 50 uL of a dilution of the conjugated monoclonal antibody in PBS with 1% BSA and 0.5% casein was added. The wells were then washed three times as previously described and 100 uL of the enzyme substrate was added to each well. The substrate consisted of 2.2'-azino-di (3-ethyl-benzthiazoline sulfonate (6)) in buffered hydrogen peroxide. Results were analyzed after one hour at 405 nm with an ELISA plate reader.

Controls consisted of uninfected mosquitoes and a dilution of known numbers of sporozoites. Sporozoite densities were later quantitated against a standard curve of either a known amount of P. falciparum circumsporozoite antigen produced by recombinant DNA technology (5) or a known number of P. vivax sporozoites. Absorbance values corresponding to known sporozoite numbers underwent a log transformation to produce a straight line described by a regression equation. Absorbance values for mosquitoes with unknown numbers of sporozoites then underwent log transformation and their numbers of sporozoites calculated from the regression equations.

In a similar manner, sporozoite densities resulting from known numbers of ruptured oocysts (determined by first dissecting stomachs of wild caught anophelines for the presence of ruptured oocysts) were determined by assaying the remainder of the mosquito for sporozoite antigens using known numbers of sporozoites as controls.

In order to compare the proportion of mosquitoes with sporozoites in their salivary glands with the overall sporozoite antigen positivity rates, the abdomens of a sample of 1,981 mosquitoes were separated from the heads and thoraces and both were assayed individually for the presence of sporozoite antigens of P. falciparum and P. vivax.

Analysis of Mosquito Blood Meals

Blood meal sources of engorged anophelines were identified using polyclonal rabbit antiserum in an enzyme-linked immunosorbent assay modified from that described by Burkot et al.(26) through the addition of a blocking step using phosphate buffered saline with 1% bovine serum albumen and 0.5% casein. All blood meals were simultaneously tested with antisera against human, pig, dog, cat, horse, cow, rat, chicken, bird and opossum as these represent the major hosts available to anophelines in Papua New Guinea. The anti-opossum serum served as a general anti-marsupial serum.

Feeding patterns were examined using the Feeding Index of Kay et al. (27), defined as follows:

$$\text{Feeding Index} = (Ne/Ne') / (Ef/Ef')$$

where Ne and Ne' are the observed number of mosquito blood meals on hosts 1 and 2, and Ef and Ef' are the numbers of hosts 1 and 2 present in the village. Numbers of humans, dogs and pigs in a village were determined by interviewing heads of households in the villages of Butelgut, Dogia, Maraga, Mebat and Sah (Cattani, unpublished). The numbers of humans, dogs, pigs, cats and chickens in the villages of Agan, Erima, Hudin and Panim were similarly obtained. A Feeding Index greater

than one indicates an increased amount of feeding on host 1 relative to host 2.

Human Populations

Malariometric Surveys

Eight of the villages were chosen for a more detailed study on the relationship between sporozoite and inoculation rates and parasite prevalence in children. Three of the villages studied were in the high epidemiological zone (Budup, Butelgut and Mebat), two in the intermediate (Dogia and Maraga) and three in the low zone (Umun, Sah and Hudini). Malariometric surveys were conducted at three three-monthly intervals from September, 1983 to June, 1984 (23) in six of the villages (24) and two villages, Hudini and Umun, were surveyed once. During each survey, individuals were identified according to previously obtained demographic information. Spleens were graded according to Hackett's scale and a thick and thin blood film made for determination of malaria parasite species prevalences and densities.

Examination of Blood Films

Thin blood films were fixed in methanol and stained with 4% Giemsa for 30 minutes. Thick film fields were examined at 1000 X magnification for the presence of malaria parasites. Parasite densities were recorded as the number of parasites per 200 white blood cells or in low density infections as the number of parasites in 100 fields. Species identifications were determined by examination of parasites in thin films. A random 10% samp e of the blood films was re-examined for 150 fields by a second microscopist without knowledge of the previous result. In addition, all low density infections and those in which there was doubt as to the species identification were re-examined.

Bed Net Usage

Villagers in seven villages were surveyed as to their use of bed nets. Villagers were questioned as to whether they slept under a bed net and if so, did they share the bed net with other individuals.

DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST *PLASMODIUM MALARIAE* SPOROZOITES

In order to try to make monoclonal antibodies against *P. malariae* sporozoites, a series of malariometric surveys were made in the East Sepik Province, an area highly endemic for *P. malariae*, to identify *P. malariae* gametocyte carriers. When *P. malariae* gametocyte carriers were found, groups of laboratory

reared An. farauti were fed on the carriers, with their permission. These mosquitoes were held in the insectary for 7 days when they were dissected for the presence of P. malariae oocysts. Groups of oocyst positive mosquitoes were held in the insectary until day 30 post-feeding when they were dissected to harvest P. malariae sporozoites.

BALB/c mice for inoculations of P. malariae sporozoites for monoclonal antibody production were kindly provided by Dr. Graham Brown of the Walter and Eliza Hall Institute, Melbourne.

RESULTS

Mosquito Populations

Sporozoite Antigen Positivity Rates

Average P. falciparum and P. vivax sporozoite antigen positivity rates in each of the 14 villages for An. farauti, An. koliensis and An. punctulatus are presented in Tables 1a-c. The average sporozoite antigen positivity rate was calculated from the average weekly sporozoite rate. A large range was found for sporozoite rates both within a village over time and between adjacent villages.

Relationship between Sporozoite Antigen Positives and Salivary Gland Infections

Since the ELISAs measure sporozoite antigen, the positivity rates given in Tables 1a-c include mosquitoes with sporozoite antigen present in mature oocysts as well as those with sporozoites in the salivary glands. Of the 1,981 mosquitoes whose heads and thoraces were assayed separately from their abdomens for the presence of sporozoite antigens of P. falciparum and P. vivax, 71% of the 28 P. falciparum sporozoite antigen positive mosquitoes and 76% of the 17 P. vivax sporozoite antigen positive mosquitoes contained sporozoite antigen in the heads and thoraces and therefore in the salivary glands.

Calculation of Sporozoite Densities

When sporozoite densities in naturally infected mosquitoes were quantitated against a standard curve of known numbers of sporozoites, greater sporozoite densities were found in P. falciparum infected mosquitoes (geometric mean of 4000; range of 150 - 10,000) than for P. vivax sporozoite-infected mosquitoes (geometric mean of 380; range of 150 - 4500) (Table 2).

When wild caught anophelines with known numbers of ruptured oocysts were assayed, 20 ruptured P. falciparum oocysts were

found to have produced a geometric mean of 2240 sporozoites per oocyst whereas 45 ruptured P. vivax oocysts produced a geometric mean of 220 sporozoites per oocyst.

Inoculation Rates

Plasmodium falciparum and P. vivax inoculation rates for 10 villages for An. farauti, An. koliensis and An. punctulatus are presented in Tables 3a-c. Inoculation rates were calculated from the product of the average nightly mosquito man-biting rate and the sporozoite rate (sporozoite antigen positivity rate multiplied by the proportion of positive anophelines with sporozoite antigen in the head and thorax) for each species. Sporozoite inoculation rates were seen to vary widely both within a village over time and between villages in close geographic proximity.

Blood Feeding Habits

3551 blood engorged anophelines were analyzed by ELISA, 1918 and 1633 from indoor and outdoor resting catches, respectively. Over 83% of collected anophelines yielded positive identifications to at least one antiserum with 5% of the blood meals reacting to two or more antisera.

Blood meal identifications for resting engorged mosquitoes collected either indoors or outdoors in the different villages are presented in Tables 4a-c and 5a-c. The major hosts were human, pig and dog. Positive identifications of host blood sources were found from a wider range of animals for An. farauti captured in outdoor resting collections than for either An. koliensis or An. punctulatus.

The Human Blood Indices (HBI) (proportion of blood meals containing human blood) of An. punctulatus and An. koliensis were consistently higher than for An. farauti in all villages for both indoor and outdoor resting collections (Table 6). In indoor resting collections, the HBI for An. koliensis was always greater than the HBI for An. farauti, and in Dogia and Maraga this difference was significant (S.N.D. $>$ 2; $p < 0.05$). Also, the HBIs for An. punctulatus were higher than the HBIs for An. koliensis and in Butelgut this difference was significant. In outdoor resting collections, An. koliensis had a consistently higher HBI than An. farauti; in Erima, Maraga and Mebat, this difference was significant.

Domestic host abundances in each of the villages are presented in Table 7. In addition to the hosts listed, rats are very abundant in all villages; horses, cows and buffaloes are kept on plantations near Erima and one or two horses are occasionally kept in Mebat. Marsupials are relatively scarce near villages.

In villages where large numbers of host blood source identifications of engorged outdoor resting anophelines were obtained, host feeding preferences of the members of the An. punctulatus complex were examined by calculating the Feeding Index (27) with the host abundance data presented in Table 7 and the blood meal identifications presented in Table 4. Differences in host preferences were found between the members of the An. punctulatus complex. Anopheles farauti consistently preferred feeding on dogs compared to pigs (Feeding Index Range: 1.16-3.39), and preferred pigs over humans (Feeding Index Range: 3.37-6.80) in the villages of Dogia, Erima and Maraga. An exception was seen in Agan where An. farauti preferred dogs to humans and humans to pigs. In Erima, Maraga and Mebat, An. koliensis preferentially fed on dogs rather than humans (Feeding Index Range: 1.33-2.33) and humans rather than pigs (Feeding Index Range: 1.61-3.73). In Butelgut and Mebat, An. punctulatus fed preferentially on dogs compared to humans (Feeding Index Range: 2.40-5.05) and humans compared to pigs (Feeding Index Range: 4.81-infinity).

Human Population

Parasite Prevalences

Spleen rates (Hackett grade $\geq$ 2) in two to nine year olds varied from 43 to 95% and combined P. falciparum and P. vivax parasite prevalences in the one to nine year olds ranged from 32.9 to 66.6 (Table 8). Plasmodium falciparum parasite prevalences ranged from 32.0 to 56.6% and P. vivax parasite prevalences ranged from 8.0 to 25.6%. The prevalence of mixed P. falciparum and P. vivax infections ranged from 4.0 to 15.6%, and did not differ significantly from the number expected as calculated from the product of the prevalences of each infection observed (X^2 = 4.65; 0.75 $>$ p $>$ 0.50: df = 7).

Parasite Prevalences and Inoculation Rates

Analysis of more than 41,000 anophelines of the Anopheles punctularus complex captured in the same eight villages during the time that malariometric surveys were being conducted gave combined P. falciparum and P. vivax sporozoite antigen positivity rates between 0.17 and 3.88% (Table 9) with P. falciparum sporozoite antigen positivity rates ranging from 0.16 to 2.41% and P. vivax sporozoite antigen positivity rates ranging from 0.00 to 1.57%.

A comparison of malaria species prevalences in children with the sporozoite rate in each village for that malaria species showed no significant relationship. This is not surprising given the great variation observed in the average anopheline density between villages, ranging from 19 bites per

person per night in Sah to 488 in Maraga (Table 10).

The actual rate at which sporozoites are transmitted from mosquito to man is the entomological inoculation rate. The relationship between the P. falciparum inoculation rate (calculated as described above) and parasite prevalence in the one to nine year olds is described by the equation for the regression line, $Y=26.6X + 33.1$ (solid line); $df=6$; $t=3.92$; $p<0.01$. The relationship between the P. vivax inoculation rate and parasite prevalence in the one to nine year olds is described by the equation for the regression line, $Y=12.2X + 10.7$ (dashed line); $df=6$; $t=10.12$; $p<0.001$ (Figure 1). The position of the lines are different for the two malaria species with a higher parasite prevalence resulting from a given inoculation rate for P. falciparum than for P. vivax.

Demography and Bed Net Usage

Data on the age structures of ten of the villages are presented in Table 11. Analysis of the demographic structures of the villages by chi-square analysis revealed no significant differences between villages ($X^2=47.982$, $df=45$, $p=0.35$.

Presented in Table 12 is information on the prevalence of bed nets in seven of the villages as well as the average number of people sleeping under a bednet. Between 67.0% and 98.6% of people in the different villages slept under a bed net. The average number of occupants of a bed net ranged from 1.60 to 3.12.

Factors Affecting the Sporozoite Rate

Regression analysis was performed between sporozoite rates found in each mosquito species (for which greater than 500 mosquitoes were analyzed) in a village with the human blood index for that mosquito species in the same village (if greater than 10 host blood source identifications were made), anopheline biting density, bed net usage and population of men and domestic animals in the same village in order to identify factors responsible for high sporozoite rates. Significant relationships are given in Table 13 with t-values, p and regression coefficients presented.

Significant correlations were found between sporozoite rates in An. farauti and the human blood index and bed net usage, both for the proportion of the village population sleeping under bed nets, the average number of people using a bed net and the combination of the two factors. The HBI for An. farauti was also significantly related to the ratio of men to pigs in the villages. The sporozoite rates for An. koliensis was also significantly related to the average number of people using a bed net as well as the combination of the number of

people using bednets and the average number of people using a bed net.

The limited number of villages where sufficient numbers of An. punctulatus could be captured for sporozoite analysis or determination of the HBI precluded any reliable evaluation of factors affecting the sporozoite rate in this species. However when the HBIs of An. farauti and An. punctulatus were plotted simulataneously against their respective combined P. falciparum and P. vivax sporozoite rates, a highly significant relationship was found ($t=16.581$, $p<0.001$, $df=5$).

Development of Monoclonal Antibodies Against P. malariae Sporozoites

Plasmodium malariae gametocyte carriers were successfully identified during a patrol to the village of Tau, East Sepik Province in August, 1985. Laboratory reared An. farauti were fed on the gametocyte carriers. Dissection for oocysts were positive with 40% of fed mosquitoes infected with an average of 1.5 oocysts per infected mosquito. Unfortunately, due to the long extrinsic incubation period of P. malariae (30 days), all infected mosquitoes expired by 30 days post-blood feeding.

DISCUSSION

Using an immunoradiometric assay, Collins et al. (28) reported an average of 4000 P. falciparum sporozoites per infected mosquito and Pringle (29) gave geometric means of 6380 and 4570 sporozoites for An. gambiae and An. funestus when working in an area of predominately P. falciparum. In this study, sporozoite infected members of the An. punctulatus complex contained geometric means of 4000 P. falciparum or 380 P. vivax sporozoites per mosquito.

The greater sporozoite densities in P. falciparum infected mosquitoes in our studies is due not to heavier oocyst infections in P. falciparum but to greater numbers of sporozoites produced per oocyst. Plasmodium falciparum oocysts produced a geometric mean of 2240 sporozoites per oocyst, whereas P. vivax oocysts produced a geometric mean of 220 sporozoites per oocyst.

When parasite prevalences in children in each village were plotted against the entomological inoculation rate for each species of malaria, a given inoculation rate of P. falciparum was seen to result in a two and one-half fold greater prevalence of parasites in children than did the same inoculation rate of P. vivax. Plasmodium falciparum appeared to be more efficiently transmitted from mosquito to man than P. vivax.

The difference in sporozoite densities for P. falciparum and P. vivax provides a possible explanation for the difference in efficiency of transmission of the two species of malaria. Greater sporozoite densities in P. falciparum infected mosquitoes result presumably in higher inoculum doses of sporozoites while feeding than do the more lightly infected P. vivax mosquitoes. It also suggests that intervention strategies, including vaccines, directed against the sporozoite stage will more easily interrupt P. vivax malaria due to the smaller inoculated doses of sporozoites passed during blood feeding.

Other factors which might affect the prevalence of P. falciparum and P. vivax infections include the relative length of time during which a person remains parasitemic after infection, possible suppression of P. vivax infections by P. falciparum and useage of chloroquine in the area. However, the second factor appears negligible as there was no significant difference between the number of mixed P. falciparum and P. vivax infections observed and the expected number of mixed infections.

Although the impact of chloroquine usage on P. vivax in the area, especially in view of chloroquine resistant P. falciparum malaria, cannot be ignored when gauging the efficiency of transmission of these two species of malaria, we feel that a 12.7% presence of aminoquinolines in the urines sampled is insufficient in itself to explain the nearly two and one half fold difference in parasite prevalence for a given inoculation rate. In fact, the discrepancy in efficiency of transmission of disease between the two species is even greater than that shown by Figure 1 because the parasite prevalences for P. vivax includes both new infections and relapses whereas P. falciparum is incapable of relapsing.

The extremely large variation in sporozoite rates and inoculation rates in a small area stresses the importance of adequately sampling the mosquito population in a number of villages in order to assess the relative risk of infection and therefore to be able to measure the impact of intervention strategies including vaccines against malaria.

In the Madang study area there is extensive variation between villages in host selection by the members of the An. punctulatus complex. Although the numbers of available hosts differ greatly between villages, in many villages two or three of the vectors occur together, and thus relative feeding preferences can be determined. Our findings indicate that An. punctulatus and An. koliensis are more anthropophilic than An. farauti, since the HBI for An. farauti is consistently lower than the HBI for the other two species in all villages for both indoor and outdoor resting collections. Analysis of blood

feeding patterns with the Feeding Index revealed that An. punctulatus and An. koliensis preferred dogs to humans and humans to pigs while An. farauti preferred dogs over pigs and pigs over humans.

Overall the proportion of patent mixed blood meals was 5%. The proportion of patent mixed blood meals involving humans ranged from 0.0% for An. punctulatus in Butelgut to 7.8% for An. koliensis in Dogia. Such variation is expected given the variation in host selection patterns for the different species in the different villages.

Whether the interrupted feeding patterns observed are epidemiologically important is not immediately obvious. It may be that, as hypothesized by Garrett-Jones and Grab (30), the increased number of feeds taken as a result of interrupted feeding increases the vectorial capacity of the population by increasing the chances of acquiring and transmitting the disease agent. Despite this, we believe that a strong argument can be made that interrupted feeding habits resulting in mixed blood meals involving man will not increase (and might even reduce) transmission of malaria from man to mosquito. Barber and Rice (31) demonstrated that partial blood meals by Anopheles on known gametocyte carriers resulted in a lower mosquito infection rate. Boreham and Garrett-Jones (32) also presented the possibility that under certain conditions, the taking of a mixed blood meal may diminish the chances of a malaria vector becoming infected by requiring the ookinetes to transverse a layer of disparate blood and a second peritrophic membrane in order to reach the gut wall.

Although it has been shown that malaria can be transmitted through probing alone (33), it has also been demonstrated that the severity of a malaria attack, as measured by the prepatent period and the duration of clinical disease, is related to the number of sporozoites inoculated (34). Probing and partial blood meals on man by sporozoite-infected mosquitoes could therefore diminish the severity of the resulting disease should the inoculum of sporozoites be proportional to the amount of blood ingested.

The blood feeding habits of the An. punctulatus complex are an important component in determining the sporozoite rates found in a particular village. A significant correlation was found between the HBIs for An. farauti and the sporozoite rates in An. farauti. Insufficient blood meal identifications for An. punctulatus precluded the same analysis for this species. However, when regression analysis was performed using the HBIs for An. punctulatus and An. farauti against their respective sporozoite rates, a significant correlation was again seen. Other factors having significant correlations with the sporozoite rate were the prevalence of bed nets in the villages, the

average density of persons using a bed net or a combination of the two measures of bed net usage. Obviously, bed net usage will affect the HBI, particularly for An. farauti, which prefers to feed on pigs or dogs rather than man. This is reflected by the significant relationship seen between the HBI for An. farauti and the ratio of the number of men to pigs in a village. The numbers of dogs in a village was generally too few to be expected to have a substantial impact on the HBI. Interestingly, although there was a wide range of parasite prevalences in the human population and a wide range in sporozoite rates and inoculation rates in the vector population, there was no significant differences in the demographic profiles between different villages. It therefore appears that the demographic composition of a village does not predispose the village to a higher malaria parasite prevalence. Variables that have an impact on entomological parameters, particularly on the HBI (ie. domestic animal populations and usage of bed nets), would appear to exert a significant influence on the magnitude of the sporozoite rate. The sporozoite rate, in turn, in conjuction with the anopheline man-biting density produces the entomological inoculation rate which will determine what the eventual parasite prevalence in an area will be, within the constraints of host immunity.

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The research project described in this paper was approved by the Medical Research Advisory Committee of Papua New Guinea which acts as the National Ethical Clearance Committee for Papua New Guinea. In addition, the study procedures were explained in detail to the participants of the malariometric surveys or if less than 15 years of age, their guardians. Those who consented were entered into the study.

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Table 1a. Average sporozoite antigen positivity rates for *An. farauti*.

Village	Anophelines Analyzed (No.)	Sporozoite Antigen Positive Rate *P. falciparum* (X±S)	*P. vivax* (X±S)
Agan	10,520	0.0003±0.0006	0.0019±0.0052
Budup	562	0.0390±0.0451	0.0199±0.0292
Butelgut	63	0.0111±0.0471	0.0000±0.0000
Dogia	8,354	0.0009±0.0011	0.0010±0.0012
Erima	4,750	0.0062±0.0106	0.0012±0.0013
Gonoa1	148	0.0000±0.0000	0.0000±0.0000
Gonoa2	172	0.0016±0.0042	0.0000±0.0000
Hudini	550	0.0005±0.0012	0.0000±0.0000
Kyiumbun	64	0.0000±0.0000	0.0000±0.0000
Maraga	30,440	0.0015±0.0017	0.0005±0.0006
Mebat	628	0.0034±0.0088	0.0119±0.0231
Panim	3	0.0000±0.0000	0.0000±0.0000
Sah	40	0.0000±0.0000	0.0000±0.0000
Umun	111	0.0242±0.0616	0.0182±0.0603
Total	56,405		

Table 1b. Average sporozoite antigen positivity rates for *An. koliensis*.

Village	Anophelines Analyzed (No.)	Sporozoite Antigen Positive Rate *P. falciparum* (X±S)	*P. vivax* (X±S)
Agan	776	0.0008±0.0027	0.0002±0.0005
Budup	1,741	0.0236±0.0421	0.0278±0.0360
Butelgut	862	0.0060±0.0133	0.0075±0.0137
Dogia	2,353	0.0096±0.0232	0.0002±0.0006
Erima	7,491	0.0075±0.0051	0.0018±0.0036
Gonoa1	2,309	0.0040±0.0046	0.0005±0.0015
Gonoa2	1,264	0.0029±0.0040	0.0016±0.0029
Hudini	567	0.0034±0.0057	0.0000±0.0000
Kyiumbun	977	0.0015±0.0025	0.0000±0.0000
Maraga	7,032	0.0015±0.0024	0.0016±0.0024
Mebat	6,839	0.0068±0.0055	0.0048±0.0066
Panim	270	0.0195±0.0388	0.0062±0.0131
Sah	246	0.0034±0.0084	0.0058±0.0100
Umun	2,682	0.0055±0.0103	0.0008±0.0015
Total	35,409		

Table 1c. Average sporozoite antigen positivity rates for *An. punctulatus*.

Village	Anophelines Analyzed (No.)	Sporozoite Antigen Positive Rate *P. falciparum* (X±S)	*P. vivax* (X±S)
Agan	20	0.0909±0.3015	0.0000±0.0000
Budup	61	0.0022±0.0079	0.0128±0.0462
Butelgut	3,177	0.0166±0.0222	0.0133±0.0139
Dogia	213	0.0018±0.0050	0.0000±0.0000
Erima	1,500	0.0114±0.0125	0.0000±0.0000
Gonoa1	162	0.0000±0.0000	0.0000±0.0000
Gonoa2	268	0.0000±0.0000	0.0019±0.0051
Hudini	611	0.0036±0.0058	0.0000±0.0000
Kyiumbun	159	0.0000±0.0000	0.0000±0.0000
Maraga	240	0.0022±0.0101	0.0000±0.0000
Mebat	1,164	0.0009±0.0037	0.0161±0.0298
Panim	1,286	0.0386±0.0573	0.0169±0.0234
Sah	828	0.0195±0.0173	0.0062±0.0107
Umun	2,508	0.0053±0.0088	0.0035±0.0052
Total	12,197		

Table 2. Geometric means for sporozoite densities per wild caught infected mosquito in Madang Province, 1984.

Anopheline Species	Sporozoites			
	P. falciparum		*P. vivax*	
	Density	(No.[1])	Density	(No.[1])
An. farauti	3,700	(26)	320	(6)
An. koliensis	4,400	(42)	270	(12)
An. punctulatus	3,500	(23)	580	(13)
Overall Geometric Mean	4,000	(91)	380	(31)
Range	150 - 10,000		150 - 4500	

[1]Number of sporozoite-infected mosquitoes analyzed.

Table 3a. Average sporozoite inoculation rates for An. farauti.

Village	Anopheline Density Per Night	Sporozoite Inoculation Rates P. falciparum (X±S)	P. vivax (X±S)
Budup	10.7	0.282±0.393	0.146±0.162
Butelgut	1.3	0.006±0.025	0.000±0.000
Dogia	284.4	0.153±0.130	0.252±0.319
Erima	57.1	0.113±0.112	0.074±0.090
Hudini	1.0	0.015±0.036	0.000±0.000
Maraga	429.6	0.389±0.434	0.188±0.256
Mebat	8.3	0.025±0.056	0.069±0.117
Panim	0.3	0.000±0.000	0.000±0.000
Sah	0.3	0.000±0.000	0.000±0.000
Umun	1.4	0.016±0.036	0.009±0.029

Table 3b. Average sporozoite inoculation rates for An. koliensis.

Village	Anopheline Density Per Night	Sporozoite Inoculation Rates P. falciparum (X±S)	P. vivax (X±S)
Budup	35.3	0.504±0.825	0.479±0.852
Butelgut	16.2	0.092±0.207	0.134±0.283
Dogia	100.0	0.144±0.174	0.018±0.049
Erima	70.3	0.248±0.286	0.052±0.086
Hudini	1.1	0.050±0.079	0.000±0.000
Maraga	80.6	0.093±0.178	0.123±0.201
Mebat	86.9	0.487±0.697	0.280±0.467
Panim	4.0	0.042±0.083	0.024±0.049
Sah	6.2	0.047±0.115	0.050±0.078
Umun	33.5	0.061±0.083	0.048±0.090

Table 3c. Average sporozoite inoculation rates for *An. punctulatus*.

Village	Anopheline Density Per Night	Sporozoite Inoculation Rates *P. falciparum* (X±S)	*P. vivax* (X±S)
Budup	1.1	0.017±0.060	0.013±0.048
Butelgut	42.4	0.541±0.834	0.412±0.707
Dogia	12.2	0.031±0.087	0.000±0.000
Erima	13.3	0.071±0.088	0.000±0.000
Hudini	0.9	0.032±0.049	0.000±0.000
Maraga	2.3	0.003±0.016	0.000±0.000
Mebat	10.2	0.006±0.022	0.101±0.149
Panim	20.3	0.239±0.159	0.110±0.219
Sah	13.6	0.229±0.190	0.084±0.153
Umun	29.4	0.043±0.050	0.038±0.054

Table 4a. Blood meal sources of An. farauti from outdoor resting collections.

Host	Village					
	Agan	Dogia	Erima	Hudini	Maraga	Mebat
Single Host						
Human	51	17	35	0	43	10
Pig	1	84	79	12	343	8
Dog	17	89	32	0	202	3
Cat	0	0	1	0	0	0
Cow	0	0	1	0	0	0
Horse	0	0	2	0	0	1
Chicken	0	21	0	0	62	0
Others[1]	0	0	0	0	0	0
Mixed Blood Meals						
Human/Pig	1	5	1	0	3	0
Human/Dog	1	0	3	0	9	0
Human/Chicken	0	2	0	0	0	0
Dog/Pig	1	3	4	0	13	0
Dog/Chicken	0	1	0	0	2	0
Chicken/Pig	0	0	0	0	8	0
Human/Dog/Pig	0	0	1	0	1	0
Total Positive	72	222	159	12	686	22
Total Tested	88	272	209	12	741	27

[1]Bird, rat, and marsupial

Table 4b. Blood meal sources of An. koliensis from outdoor resting collections.

	Village						
Host	Agan	Dogia	Erima	Hudini	Maraga	Mebat	Umun
Single Host							
Human	6	0	34	0	8	28	11
Pig	0	2	4	5	6	0	2
Dog	1	3	7	2	8	6	0
Others[1]	0	0	0	0	0	0	0
Mixed Blood Meals							
Human/Pig	1	0	1	0	0	1	0
Human/Dog	0	2	2	0	0	2	0
Dog/Pig	0	0	1	1	0	1	0
Pig/Chicken	0	0	0	1	0	0	0
Total Positive	8	7	49	9	22	38	13
Total Tested	8	9	84	12	28	49	16

[1]Cat, cow, horse, chicken, bird, rat and marsupial.

Table 4c. Blood meal sources of An. punctulatus from outdoor resting collections.

Host	Village						
	Butelgut	Erima	Hudini	Mebat	Panim	Sah	Umun
Single Host							
Human	10	2	1	7	1	3	2
Pig	0	1	4	0	0	0	3
Dog	3	1	0	6	1	1	2
Horse	0	0	0	2	0	0	0
Others[1]	0	0	0	0	0	0	0
Mixed Blood Meals							
Human/Pig	0	1	0	0	0	0	0
Human/Dog	0	0	0	1	0	0	0
Dog/Pig	1	0	0	0	0	0	0
Total Positive	14	5	5	16	2	4	7
Total Tested	16	9	8	31	2	5	7

[1]Cat, cow, chicken, bird, rat and marsupial

Table 5a. Blood meal sources of An. farauti from indoor resting collections.

Host	Village			
	Agan	Dogia	Maraga	Mebat
Single Host				
Human	122	138	164	6
Pig	1	2	1	2
Dog	42	66	79	0
Horse	0	0	0	1
Chicken	1	0	2	0
Rat	0	0	1	0
Others[1]	0	0	0	0
Mixed Blood Meals				
Human/Pig	4	0	1	0
Human/Dog	2	4	9	0
Human/Cat	1	1	2	0
Human/Chicken	1	2	0	0
Dog/Pig	0	2	2	0
Dog/Chicken	0	1	0	0
Total Positive	174	216	261	9
Total Tested	222	227	314	13

[1]Cat, cow, bird and marsupial.

Table 5b. Blood meal sources of An. koliensis from indoor resting collections.

Host	Village				
	Agan	Butelgut	Dogia	Maraga	Mebat
Single Host					
Human	26	21	178	323	71
Pig	0	0	4	4	0
Dog	6	3	7	63	10
Cat	0	0	0	2	0
Chicken	0	0	1	0	0
Rat	0	0	0	0	1
Others[1]	0	0	0	0	0
Mixed Blood Meals					
Human/Pig	0	0	7	1	0
Human/Dog	4	0	4	14	1
Human/Chicken	0	0	4	0	0
Human/Cat	0	0	1	0	0
Dog/Pig	0	0	0	1	0
Dog/Cat	0	0	0	1	0
Total Positive	36	24	206	409	83
Total Tested	52	34	252	509	123

[1]Cow, horse, bird and marsupial.

Table 5c. Blood meal sources of An. punctulatus from indoor resting collections.

Host	Village		
	Butelgut	Dogia	Mebat
Single Host			
Human	134	3	8
Pig	0	0	1
Dog	2	0	0
Others[1]	0	0	0
Mixed Blood Meals	0	0	0
Total Positive	136	3	9
Total Tested	153	3	11

[1]Cat, cow, horse, chicken, bird, rat and marsupial.

Table 6. The human blood indices (HBI[1]) for the members of the An. punctulatus complex for both indoor and outdoor resting collections.

Village	Location	Anopheline An. farauti HBI (No.[2])	An. koliensis HBI (No.[2])	An. punctulatus HBI (No.[2])
Agan	Indoor	0.75 (174)	0.83 (36)	----
	Outdoor	0.72 (72)	0.81 (8)	----
Butelgut	Indoor	----	0.88 (24)	0.99 (136)
	Outdoor	----	----	0.71 (14)
Dogia	Indoor	0.67 (216)	0.94 (206)	1.00 (3)
	Outdoor	0.09 (222)	0.14 (7)	----
Erima	Outdoor	0.23 (159)	0.72 (49)	0.50 (5)
Hudini	Outdoor	0.00 (12)	0.00 (9)	0.20 (5)
Maraga	Indoor	0.67 (261)	0.83 (409)	----
	Outdoor	0.07 (686)	0.36 (22)	----
Mebat	Indoor	0.67 (9)	0.87 (83)	0.89 (9)
	Outdoor	0.45 (22)	0.78 (38)	0.47 (16)
Yevin	Outdoor	----	0.85 (13)	0.29 (7)

[1] HBI Number of blood meals containing human blood/ Total number of blood meals identified

[2] Total number of blood meals identified

Table 7. Domestic host abundances in selected villages in Madang.

Village	No. of Households Surveyed	Number of Hosts				
		Man	Dog	Pig	Cat	Chicken
Agan	13	59	8	8	9	60
Butelgut	19	108	18	52	nd[1]	nd
Dogia	16	101	17	57	nd	nd
Erima	20	118	24	59	25	69
Hudini	15	98	16	33	9	57
Maraga	23	105	45	127	nd	nd
Mebat	13	104	18	25	nd	nd
Panim	13	41	10	2	5	78
Sah	25	142	11	110	nd	nd

[2]Not done

Table 8. Results of 3 malariometric surveys in Madang Province, 1984-1985.

Village	Spleens grade 2 (2-9 yr. olds) (%)	Parasite Prevalence (1-9 yr. olds) *P. falciparum* (%)	*P. vivax* (%)	Combined (%)
Hudini[1]	58	32.0	8.0	32.9
Umun[2]	43	36.0	12.0	44.0
Sah	62	40.9	13.6	48.7
Maraga	79	44.7	18.7	53.5
Dogia	79	51.5	13.2	60.3
Butelgut	80	54.1	13.9	62.4
Mebat	87	54.4	25.6	64.4
Budup	95	56.5	15.9	66.6

[1]Surveyed in February, 1985.
[2]Surveyed in October, 1984.

Table 9. Sporozoite antigen positive rates in Madang Province during malariometric surveys, 1984-1985.

Village	No. Anophelines Analyzed	Sporozoite Antigen Positive Rates		
		P. falciparum	*P. vivax*	Combined
Hudini	4,830	0.17	0.00	0.17
Dogia	9,646	0.16	0.10	0.26
Maraga	13,649	0.22	0.07	0.29
Umun	3,881	0.34	0.18	0.52
Mebat	3,756	0.67	0.77	1.44
Sah	1,095	1.64	0.55	2.19
Butelgut	2,609	1.57	1.34	2.91
Budup	2,294	2.31	1.57	3.88
Total	41,760			

Table 10. Entomological inoculation rates in Madang Province, during malariometric surveys 1984-1985.

Village	Average Anopheline Density	Entomological Inoculation Rates		
		P. falciparum	*P. vivax*	Combined
Hudini	109	0.129	0.000	0.129
Umun	69	0.165	0.095	0.260
Sah	19	0.217	0.078	0.295
Dogia	374	0.415	0.297	0.712
Maraga	488	0.766	0.274	1.040
Budup	47	0.783	0.570	1.353
Butelgut	67	0.753	0.693	1.446
Mebat	155	0.738	0.916	1.654

Table 11. Demographic profiles for villages in the Madang Study Site, 1985.

Village	Age Class (years) 0-9	10-19	20-29	30-39	40-49	50+	Total Population
Budup	33	27	19	10	7	6	102
Butelgut	30	33	18	9	9	12	111
Dogia	40	30	21	18	6	5	120
Erima	113	90	47	36	21	24	331
Hudini	53	41	27	17	9	17	164
Maraga	62	34	35	23	11	18	183
Mebat	90	80	32	31	29	31	293
Panim	94	46	43	29	14	22	248
San	46	40	26	17	18	11	158
Umun	119	93	65	36	23	20	356

Table 12. Survey of bed net useage in selected villages in the Madang Study Site.

Village	Percentage of Villagers Using Bed Nets	Average Number of Persons per Bed Net
Budup	72.8	3.12
Butelgut	67.0	1.82
Dogia	95.3	1.83
Maraga	98.6	1.75
Mebat	98.4	2.10
Panim	84.2	1.60
Sah	82.5	1.90

Table 13. Regression analysis of variables related to sporozoite rates in villages in the Madang Study Site: Significant Relationships.

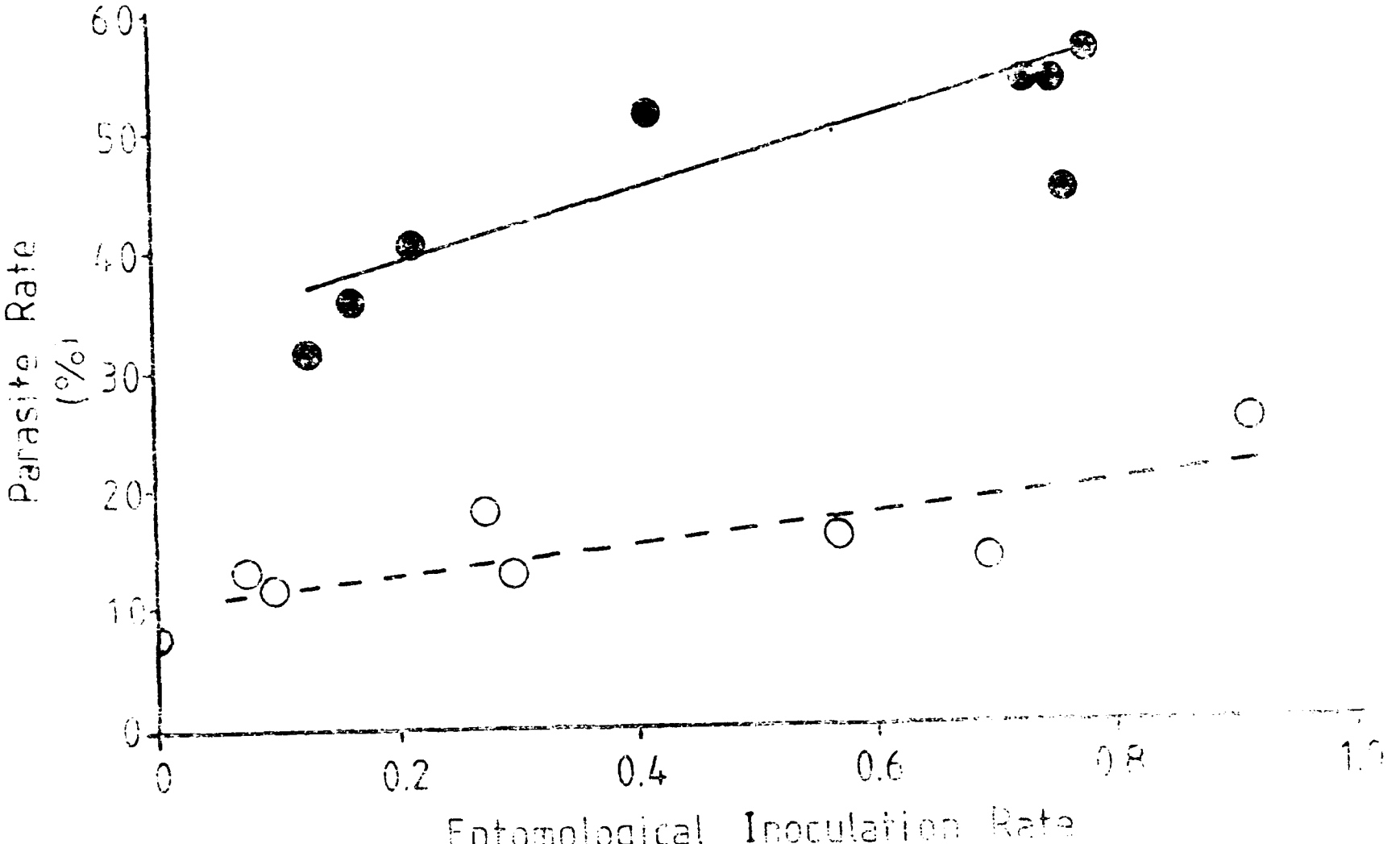
Anopheline Species	Variables Dependent; Independent	Correlation Coefficient	df	t-value	p
An. farauti					
	Sporozoite Rate[1]; HBI	0.9986	3	26.561	0.0014
	Sporozoite Rate; Ave. Under Nets[2]	0.9986	3	26.515	0.0014
	Sporozoite Rate; Ave. Under Nets/(% Nets[3])	0.9914	3	10.726	0.0086
	Sporozoite Rate; % Nets	0.9522	3	-4.408	0.0478
	HBI; No. Man/(No. Pigs)	0.9596	3	4.826	0.0404
	HBI; No. Man/(No. Pigs + No. Dogs)	0.9228	3	3.387	0.0772
An. koliensis					
	Sporozoite Rate; Ave. Under Nets/(% Nets)	0.9757	4	7.715	0.0045
	Sporozoite Rate; Ave. Under Nets	0.9751	4	7.620	0.0047
	Biting Density; % Nets	0.9598	4	5.923	0.0096
	HBI; No. Man/(No. Dogs)	0.9939	2	9.045	0.0701
An. punctulatus					
	Sporozoite Rate; No. Man/(No. Pigs)	0.8506	4	2.802	0.0677
	Sporozoite rate; % Nets	0.9073	3	-3.052	0.0927

[1] "Sporozoite Rate" is the combined sporozoite antigen positivity rate for *P. falciparum* and *P. vivax*.

[2] "% Nets" is the percentage of the village population that sleeps under a bed net.

[3] "Ave. Under Nets" is the average number of people that sleep under the same bed net.

Figure 1. Relative efficiency of transmission of P. falciparum and P. vivax from mosquito to man. Each point represents the parasite prevalence in 1 to 9 year olds for either P. falciparum (●) or P. vivax (o) with the corresponding entomological inoculation rate for that species of malaria parasite in one village.


60
50
40
30
20
10
0
Parasite Rate
(%)
0
0.2
0.4
0.6
0.8
1.0
Entomological Inoculation Rate

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Graves, P.M., T.R. Burkot, A. Saul and R. Carter. Estimation of anopheline survival rate and vectorial capacity from mosquito infection rates.

In preparation:

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