

## STUDY ON THE NATURAL HISTORY OF MALARIA IN AREAS OF THE RONDONIA STATE — BRAZIL AND PROBLEMS RELATED TO ITS CONTROL

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Studies performed more than forty years ago in the Rondonia State, as well as in other scattered localities in the Amazon Basin indicated that the only important local vector of malaria was *Anopheles darlingi*<sup>3, 4</sup>. Since then, huge uncontrolled immigration, mostly from malaria free areas from Northeastern and Southern States, has been stimulated and remarkable change in the environment has occurred due to human activity, leading to a marked increase in the incidence of malaria in Brazil, reaching about 500 thousands cases in 1987, more than 40% of the patients being from Rondonia<sup>7, 9</sup>. Recent investigations in another Amazonian State (Para)<sup>1</sup>, have led to the incrimination of other four anopheline species besides *An. darlingi* as malaria carriers by finding them naturally infected with human malaria sporozoites, but their epidemiological importance is yet to be properly assessed.

The landscape changes in several areas in the Amazon might have led to a greater contact with man of anopheline species which previously fed on animals which disappeared or decreased with deforestation. Are there really other species, besides *An. darlingi*, which became important vectors in the Amazon, particularly in Rondonia State, due to the changes in the environment? People, natives and immigrants, living in the endemic areas are frequently in contact with infected anophelines.

How is their level of anti-sporozoite immunity? Is there a relationship between the malaria transmission rate and the degree of immunity?

To answer these questions, the most malarious Municipalities in the Rondonia State, Ariquemes and Porto Velho (capital of the State), were chosen for the implementation of a field work, which intended to determine the vector of the human malarias and their comparative importance, to study aspects of their biology which could be of help in the anti-malaria program and to evaluate the degree of anti-sporozoite immunity of the local population.

The present report intends to overview our recent findings on these subjects. The original comprehensive data underlying this report will be published elsewhere.

### THE MOSQUITO SURVEY

The mosquito study consisted of an anopheline survey to determine the local species, their prevalence and their natural infection rate by the two human plasmodia diagnosed in the area *P. vivax* and *P. falciparum*. For this purpose we used an immuno-radiometric assay (IRMA) employing species specific anti-sporozoite monoclonal antibodies<sup>10</sup>. Observations on the habits of the local species in relation to the malaria transmission were also done.

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The study has been carried out since November 1985; here we shall deal with data obtained up to August 1987. Visits to the area were done trimestrally when adults and larvae of Anophelines were collected. Adult collections included captures on human bait indoors (a) and outdoors, close (b) and far (c) from the human dwellings and in the forest (d) and on animal (cow and/or horse) outdoors (e).

Captures a, b, c and e were performed at sunset, from 6 to 9 p.m. while captures d were carried out in three schedules: 8-10 a.m., 1-3 and 6-8 p.m. Local houses are routinely sprayed with DDT by personnel of the anti-malaria campaign. The specimens collected were kept in vials with dehydratant (silica gel) up to the identification and the IRMA tests at the laboratory.

In a total of 8071 adult females of seventeen species *An. darlingi* was by far the most abundant (81.6% of the total Anophelines), followed at some distance by *An. triannulatus* (5.8%), *An. evansae* (3.4%), *An. oswaldoi* (3.2%), *An. strodei* (1.5%) and *An. braziliensis* (1.4%), all remaining species being scarce: *An. argyrtarsis*, *An. albitarsis*, *An. galvaoui*, *An. nuneztovari*, *An. rangeli*, *An. benarrochi*, *An. mattogrossensis*, *An. peryassui*, *An. minor*, *An. intermedius*, *An. fluminensis*, *An. mediopunctatus*, *An. nimbus* and *Ch. bonneae*.

Inside houses, *An. darlingi* was found in small numbers an average of 24 specimens per 10 hours of capture, but represented 98.6% of the total anophelines. Other species were collected indoors, but with a rate lower than 0.1 per 10 hours of capture: *An. albitarsis*, *An. galvaoui*, *An. benarrochi*, *An. strodei* and *An. triannulatus*.

In the close vicinity of the dwellings, on human bait *An. darlingi* was abundant, 105 specimens per 10 hours of capture, accounting for 92.9% of the total, followed at a long distance by *An. triannulatus* and *An. evansae* (2.9 and 1.3 specimens/10 hours and 2.6 and 1.2% of the total anophelines obtained respectively). Other thirteen anopheline species were captured in this situation, always with less than 0.5 specimens per 10 hours: *An. braziliensis*, *An. nuneztovari*, *An. rangeli*, *An. oswaldoi*, *An. peryassui*, *An. mediopunctatus* and *Ch. bonneae*, besides the species collected indoors.

Outdoors, on human baits, far from the houses and in the forest, other species were much more numerous than *An. darlingi* which

was the third in frequency in both situations, corresponding, respectively to 24.6 and 8.2% of the total (20.4 and 0.6 mosquitoes per 10 hours). *An. triannulatus* and *An. oswaldoi* were the most abundant species with 27.6 and 25.2 specimens per 10 hours far from houses (33.3 and 30.4% of all anophelines) and 4.1 and 2.5 mosquitoes per 10 hours in the forest (54.4 and 32.6% of the all anophelines), respectively. Other few species were scarce in these two modalities of capture, like *An. fluminensis* collected only in the forest (0.7% of the total).

Either on cow or on horse placed outdoors the most varied fauna was obtained — 260.9 specimens of thirteen species were collected per 10 hours of capture, but here *An. darlingi* accounted for only 29.9% of the total mosquitoes and other species were also numerous: *An. evansae* (17.4%), *An. triannulatus* (15.1%), *An. oswaldoi* (10.2%), *An. strodei* (10%), followed at some distance by *An. braziliensis* (7.6%), *An. albitarsis* (3.5%) and *An. rangeli* (2.0%), the other species being scarce.

At present, from a total of 7.686 mosquitoes of fifteen species examined by IRMA, 43, belonging to three species were positive: 37 *An. darlingi*, 5 *An. triannulatus* and 1 *An. braziliensis*. From these, 33 were infected with *P. falciparum* (32 *An. darlingi* + *An. braziliensis* and 10 with *P. vivax* (5 *An. darlingi* + 5 *An. triannulatus*).

Up to the last report on this subject by ourselves 6 only *An. darlingi* had been found infected — 8 positives out of 1.327 examined.

All these species had previously been found infected in the Amazon Region: *An. darlingi* by several workers in different areas<sup>1, 2, 4, 5, 8</sup>, *An. triannulatus* by ARRUDA et al.<sup>1</sup> and *An. braziliensis* by DEANE, CAUSEY & DEANE<sup>4</sup>. However, in Rondonia State *An. darlingi* should be considered the principal and primary vector of malaria, for being the most anthropophilic and abundant species, principally indoors and in the close vicinity of the dwellings and because it was the mosquito found infected in greater numbers with the two human plasmodia transmitted in the area. The importance of *An. triannulatus* and *An. braziliensis* in Rondonia still needs to be evaluated because of their exophilic and zoophilic habits according to the results presented above.

## SEROLOGICAL SURVEY

The study of the degree of anti-sporozoite immunity of the individuals was done by assessing sera for the presence of antibodies against the (NANP)<sup>4</sup> synthetic peptide by an immuno-radiometric assay<sup>12</sup>. The (NANP)<sup>4</sup> peptide corresponds to the repetitive epitope of the CS protein of *P. falciparum* sporozoite<sup>11</sup>. Sera were collected from all individuals present in each visited house in the four studied localities. Individuals were questioned about their age, approximate number of Years of Residence in the endemic region (YR) and of Past Attacks of Malaria (PAM), the use of prophylactic measures (chemoprophylaxis, repellents, mosquito nets, etc.) and other questions emphasising socio-economic aspects, all data being duly recorded. A total of 1476 sera collected from 1315 individuals have been obtained. From these 988 sera have been so far processed by the IRMA, both males (492) and females (496) were studied.

No difference was observed in the average age between individuals from the studied localities however some differences were recorded concerning the mean numbers of PAM and YR. Individuals from Machadinho presented the highest rate of PAM ( $8.3 \pm 7.8$ ) followed by individuals from Cujubim ( $6.3 \pm 5.5$ ), Itapoa do Oeste ( $6.2 \pm 8.2$ ) and Ariquemes ( $5.8 \pm 7$ ) ( $p < 0.001$  for Machadinho vs Cujubim and for Machadinho vs Ariquemes). In the same way individuals from Itapoa do Oeste and Machadinho have been living in the area for longer periods ( $7.2 \pm 7.3$  and  $5.8 \pm 5.1$  YR) than those from Ariquemes and Cujubim ( $4.5 \pm 5.3$  and  $4.0 \pm 4.4$  YR) ( $p < 0.001$  for Itapoa vs Cujubim, Machadinho vs Ariquemes, Machadinho vs Cujubim and  $p < 0.01$  for Itapoa vs Ariquemes). According to these data and to data from SUCAM (Ministry of Health) showing the very high prevalence of positive thick smears in the localities of Machadinho (185 positive smears per 100 inhabitants) and of Cujubim (125 positive smears per 100 inhabitants) it could be unexpected that the Machadinho inhabitants present one of the lowest percentages of individuals positive for the presence of anti-sporozoite antibodies (3.3% comparing to 10% in Cujubim, 2.7% in Itapoa do Oeste and 4.9% in Ariquemes). Although the mean titer of anti-(NANP)<sup>4</sup> Ab (calculated for all four localities) increased with

the number of PAM ( $p < 0.01$  for 9 vs 0 PAM and  $p < 0.001$  for 9 vs 1-3 PAM), the relationship between these parameters was only evident in Ariquemes and Cujubim. These would indicate that the numbers of PAM and YR are subjective parameters to predict the degree of anti-sporozoite immunity. Indeed, recrudescence of *P. vivax* malaria or of chimo-resistant *P. falciparum* malaria could be equivocally interpreted as new attacks of malaria induced by anopheline bites. In the same way the number of YR would reflect the degree of exposure to the risk of malaria infection only if we assume that individuals are submitted to comparable living conditions and that they are not using, at different degrees, measures of preventions such as repellents and mosquito nets. Since in the population studied here we can not exclude that these variations occur, the YR does not seem to be a very accurate parameter to evaluate the risk of exposure of individuals to the bites of infected mosquitoes. The anti-sporozoite Ab titers seem to be however related to the rate of infected **Anopheles**.

In fact Cujubim presented not only the highest percentage of Ab positive individuals as well as the highest mean Ab titer but also the highest rate of anopheline carrying *P. falciparum* sporozoites. The percentage of Ab positive individuals (2.7% in Itapoa do Oeste, 3.3% in Machadinho, 4.9% in Ariquemes and 10% in Cujubim) increased with the increasing rates of sporozoite carrying mosquitoes (0.02% for Itapoa, 0.27 for Machadinho, 0.45 for Ariquemes and 0.88 for Cujubim). However the reasons for the discrepancy between the high rate of positive blood thick smears and the low frequency of anti-sporozoite positive and the low rate of sporozoite infected mosquitoes in some localities remain to be determined.

In summary two kinds of conclusions can be drawn, up to the moment, from the serological survey: a) In the population studied, that corresponds to migrant people from non endemic areas of malaria, more time of exposure to malaria infection and several PAM seem to be necessary for the development of anti-sporozoite immunity; and b) It seems that the development of the anti-sporozoite immunity is related to the degree of exposure of individuals to infected mosquitoes that is a reflect of the rate of infected vectors, the

density of anophelines as well as of factors that affect this exposure such as individual prevention measures.

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