

VECTOR INCRIMINATION AND EFFECTS OF ANTIMALARIAL DRUGS ON MALARIA TRANSMISSION AND CONTROL IN THE AMAZON BASIN OF BRAZIL

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World ecosystems differ significantly and a multidisciplinary malaria control approach must be adjusted to meet these requirements. These include a comprehensive understanding of the malaria vectors, their behavior, seasonal distribution and abundance, susceptibility to insecticides (physiological and behavioral), methods to reduce the numbers of human gametocyte carriers through effective health care systems and antimalarial drug treatment, urban malaria transmission versus rural or forest malaria transmission, and the impact of vaccine development. Many malaria vectors are members of species complexes and individual relationships to malaria transmission, seasonal distribution, biting behavior, etc. is poorly understood. Additionally, malaria patients are not examined for circulating gametocytes and both falciparum and vivax malaria patients may be highly infective to mosquitoes after treatment with currently used antimalarial drugs. Studies on the physiological and behavioral effects of DDT and other insecticides are inconclusive and need to be evaluated.

Key words: *Anopheles darlingi* - *Anopheles* sp. - quinine - tetracycline - chloroquine - malaria - control - *Plasmodium* - Brazil

A multidisciplinary approach, involving possible malaria vaccines, chemotherapy, personal protection measures, to larval and adult control, is need for an effective malaria control program. World ecosystems differ significantly and malaria control approaches must adjust to meet these requirements. To understand the epidemiology and develop effective control measures, there is a need to understand the taxonomy of the malaria vectors, their behavior, seasonal distribution and abundance, urban transmission vs rural or forest transmission, susceptibility to insecticides (physiological and behavioral), and methods to reduce the numbers of human gametocyte carriers through effective health care systems, the development of new antimalarial drugs and vaccines.

The taxonomy and geographical distribution of *Anopheles* mosquitoes in Brazil is poorly understood. In collaborative studies with the Walter Reed Biosystematics Unit, Smithsonian

Institution and information from the Instituto Oswaldo Cruz, it is known that *Anopheles albitarsis* is a complex of at least three species (Rosa-Freitas, 1989). Additionally, populations of *An. darlingi* from Costa Marques and other localities in Brazil have been separated by cuticular hydrocarbon techniques (Rosa-Freitas, unpublished data). Examination of specimens of the Arribalzagia Group, suggests that *An. mediopunctatus* does not occur in Costa Marques, Rondônia, Brazil, and those presently identified as *An. mediopunctatus*, probably represent two undescribed species (R. Wilkerson, pers. comm.). Taxonomic problems also exist with the *An. oswaldoi*, *An. nuneztovari*, and *An. triannulatus* groups as well as others. Additionally, in some areas, an *Anopheles* species is considered a vector while in other areas it is considered only an incidental vector. Presently we do not know if these differences in vector potential represent population or species differences. Because of these species problems, deductions about vector control and the epidemiology of disease and vector capacity of one species in one area cannot be construed as being the same in others.

The seasonal distribution of *An. darlingi*, the primary malaria vector in Costa Marques, is correlated positively to the Guaporé River level and shortly after the rains stop, it is not uncommon to collect >500 specimens per man-bait night in outdoor collections (Klein & Lima, 1990) (Fig. 1). *Anopheles darlingi* and *An. deaneorum*, a member of the *An. albitarsis* complex, are anthropophilic and more endophilic and anthropophilic than other species of *Anopheles* in the area (Klein et al., 1991e) (Fig. 2). Members of the *An. mediopunctatus* complex also readily bite man, but are forest species and are infrequently collected in cleared rural and urban areas. *Anopheles albitarsis*, *An. triannulatus*, *An. oswaldoi*, *An. matto grossensis*, *An. rangeli*, *An. minor*, *An. braziliensis* were much less frequently collected in human-bait collections and are zoophilic (Klein & Lima, 1990). In Costa Marques, over 90% of the *Anopheles* collected in human-bait were *An. darlingi*. The remaining 8% and 2% were *An. deaneorum* and *Anopheles* spp., respectively. Along the major graded road, the mosquito diversity is greater, but *An. darlingi* still made

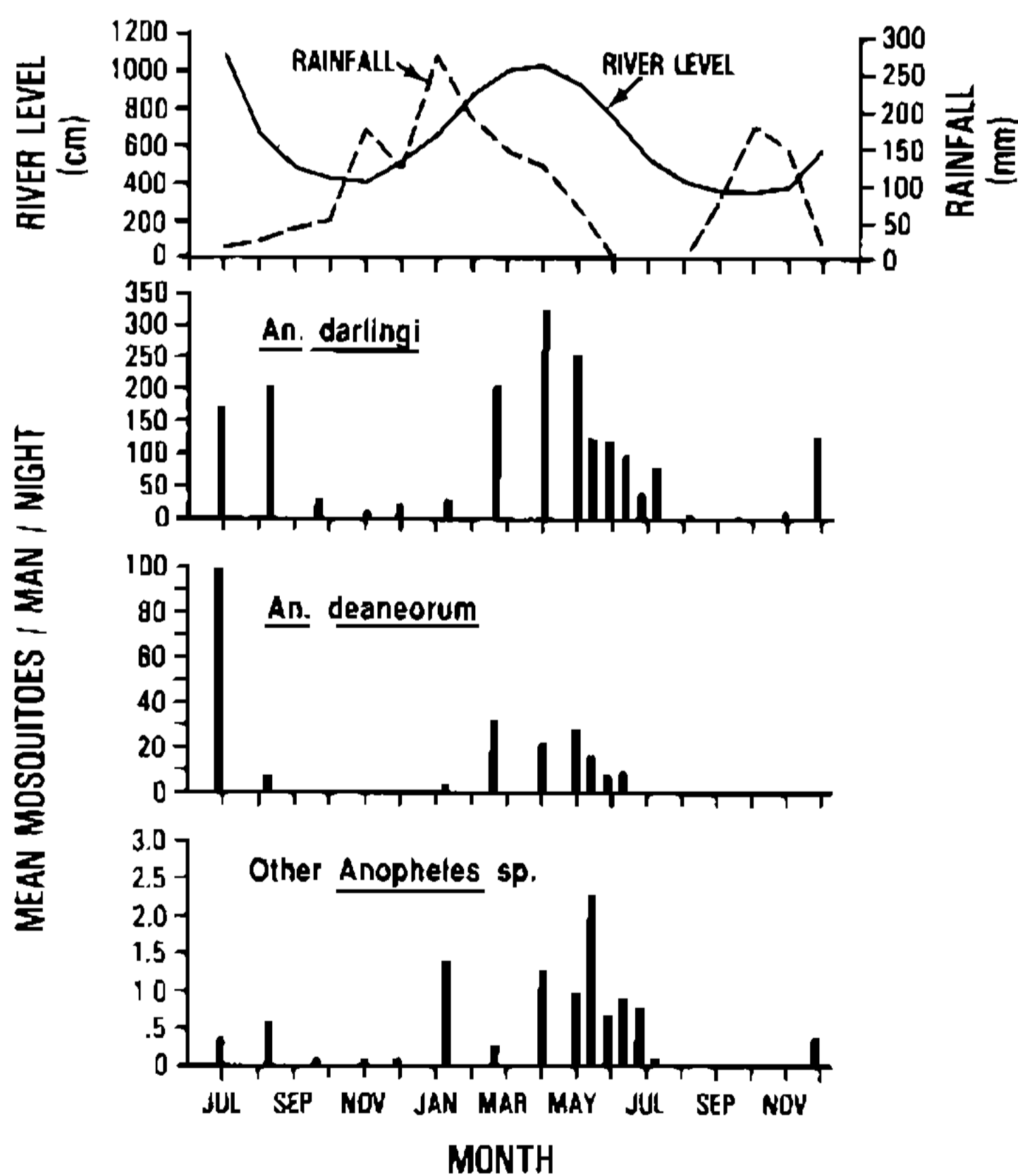


Fig. 1: rainfall, Guaporé River level and mean number of mosquitoes collected per man per night from human-bait outside 3 houses in Costa Marques from July 1986 to December 1987. "Other" *Anopheles* species include *An. oswaldoi*, *An. triannulatus*, *An. albitarsis*, *An. matto grossensis*, *An. braziliensis*, *An. rondoni*, *An. minor*, *An. mediopunctatus*, *An. nuneztovari*, *An. peryassui* and *An. benarrochi*. Meteorometric data provided by the Meteorological Military Unit, Fort Príncipe da Beira, located 26 km NW of Costa Marques.

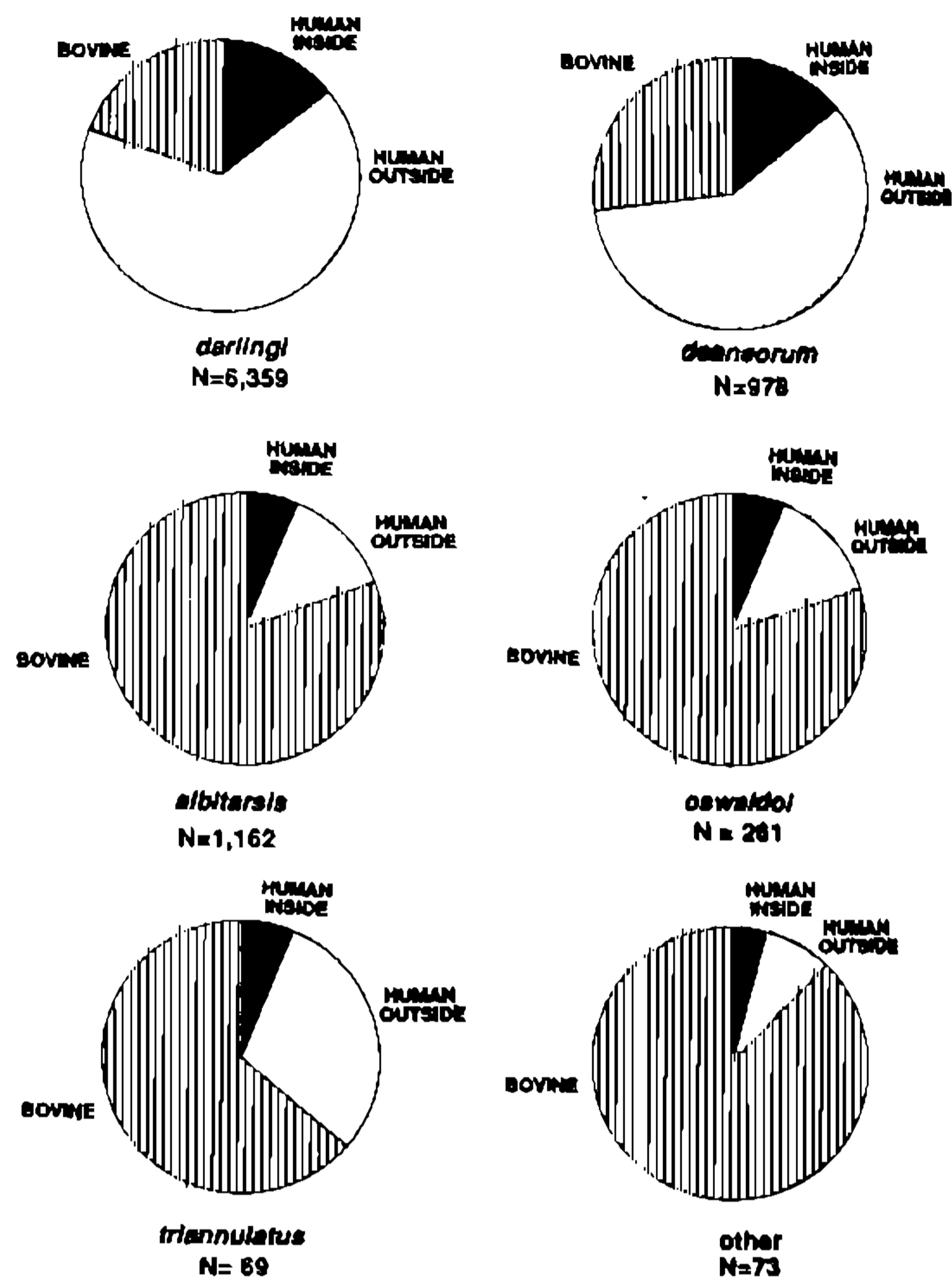


Fig. 2: comparison of feeding preference of *Anopheles* species from collections conducted during the period April through September, 1987, from bovine - and human - bait at houses 1 and 5, Costa Marques, Rondônia.

up more than 70% of the mosquitoes collected from human-bait. These observations should be made at different sites since species diversity and seasonal distribution vary. For example, in Costa Marques, *An. deaneorum* only occurs in the late rainy and early dry season, but the Malaria Division, Fundação Nacional da Saúde (formerly Superintendência de Campanhas de Saúde Pública) reports that it can be collected throughout the year in Cuiabá.

The incrimination of vector populations has, in the past, been accomplished by tedious dissections of salivary glands for sporozoites. In areas where mosquitoes are abundant, but malaria infections are low, the dissection of several thousand mosquitoes are required to identify vector populations. Additionally, numerous mosquitoes needed to be dissected before a species could be eliminated as a malaria vector. Recent advances in radio-immune and enzyme-linked immunosorbent assays (ELISAs) now enable large numbers of mosquitoes to be examined for circumsporozoite antigen which facilitates vector incrimination. Using these techniques, we and other researchers have added *An. deaneorum*, *An. albitarsis*, *An. konderi* (pre-

viously identified as *An. oswaldoi* from Costa Marques), *An. oswaldoi* and *An. braziliensis* to the list of potential vectors of *P. vivax* and *P. falciparum* (Arruda et al., 1986; Lourenço-de-Oliveiro et al., 1989). Our malaria susceptibility studies on *P. vivax* and *P. falciparum* indicate that while many of these mosquito species which are identified as malaria vectors develop circumsporozoite antigen during late sporogonic development, sporozoites are rarely seen in the salivary glands or salivary gland infections consist of only a few sporozoites (Klein et al., 1991a, c).

To determine the susceptible of potential malaria vectors, F₁ progeny of *Anopheles* species were fed on falciparum and vivax malaria patients and the levels of oocyst and salivary gland sporozoite infections compared to those observed in *An. darlingi*, the primary vector of malaria in Brazil. The susceptibility of anophelines to *P. vivax*, based on prevalence and density of sporozoites in the salivary glands can be ranked as follows: *An. darlingi* = *An. deaneorum* > *An. albitarsis* > *An. mediopunctatus* Form 1 > *An. triannulatus* > *An. oswaldoi* (Fig. 3). Since *An. triannulatus* and *An. oswaldoi* are zoophilic and the glands of infected individuals rarely contain > 100 sporozoites, they are not considered vectors, but may occasionally transmit malaria when occurring in large numbers. Although *An. albitarsis* and *An. mediopunctatus* Form 1 develop low oo-

cyst counts, high numbers of sporozoites occur in the salivary glands. However, *An. albitarsis* is zoophilic and *An. mediopunctatus* Form 1 is a forest form and both species are seasonal, so their relative importance as vectors of vivax malaria is dubious. *Anopheles braziliensis* and *An. benarrochi*, both developed oocysts and are positive by ELISA, but sporozoites have not been seen in the salivary glands.

Data for falciparum malaria have been more difficult to obtain because of the low numbers of gametocyte carriers available. Based on percentage of infected mosquitoes and the density of sporozoites in the salivary glands, the susceptibility of mosquitoes in the Costa Marques area are ranked as follows: *An. darlingi* = *An. mediopunctatus* Form 1 > *An. deaneorum* > *An. triannulatus* > *An. oswaldoi* (Fig. 4). *Anopheles mediopunctatus* Form 1, although infrequently collected outside forested habitats, are equally or more susceptible to *P. falciparum* than *An. darlingi*, and may be involved in forest malaria transmission. According to C. Golenda (pers. comm.), *An stephensi* with sporozoite counts of less than 150, did not release sporozoites when salivating in laboratory studies. It is therefore unlikely that *Anopheles triannulatus* and *An. oswaldoi* are vectors because of low sporozoite counts in the salivary glands. Sporozoites of *P. falciparum* were not seen in the salivary glands of *An. albitarsis* although oocysts were present.

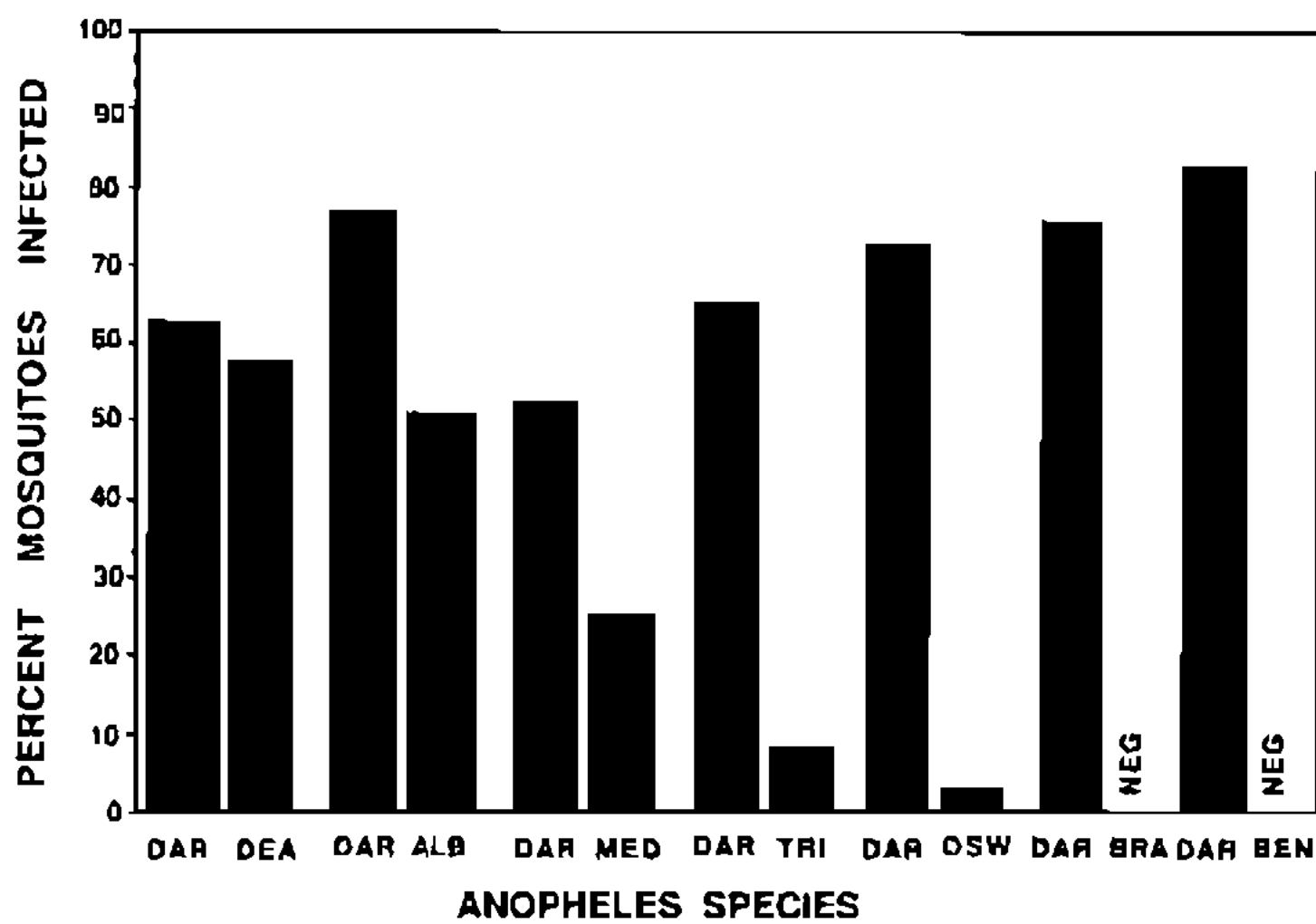


Fig. 3: percent of *Anopheles darlingi* infected with sporozoites of *P. vivax* in the salivary glands compared with 7 other *Anopheles* species. DAR = *An. darlingi*, DEA = *An. deaneorum*, ALB = *An. albitarsis*, MED = *An. mediopunctatus*, TRI = *An. triannulatus*, OSW = *An. oswaldoi*, BRA = *An. braziliensis*, BEN = *An. Benarrochi*.

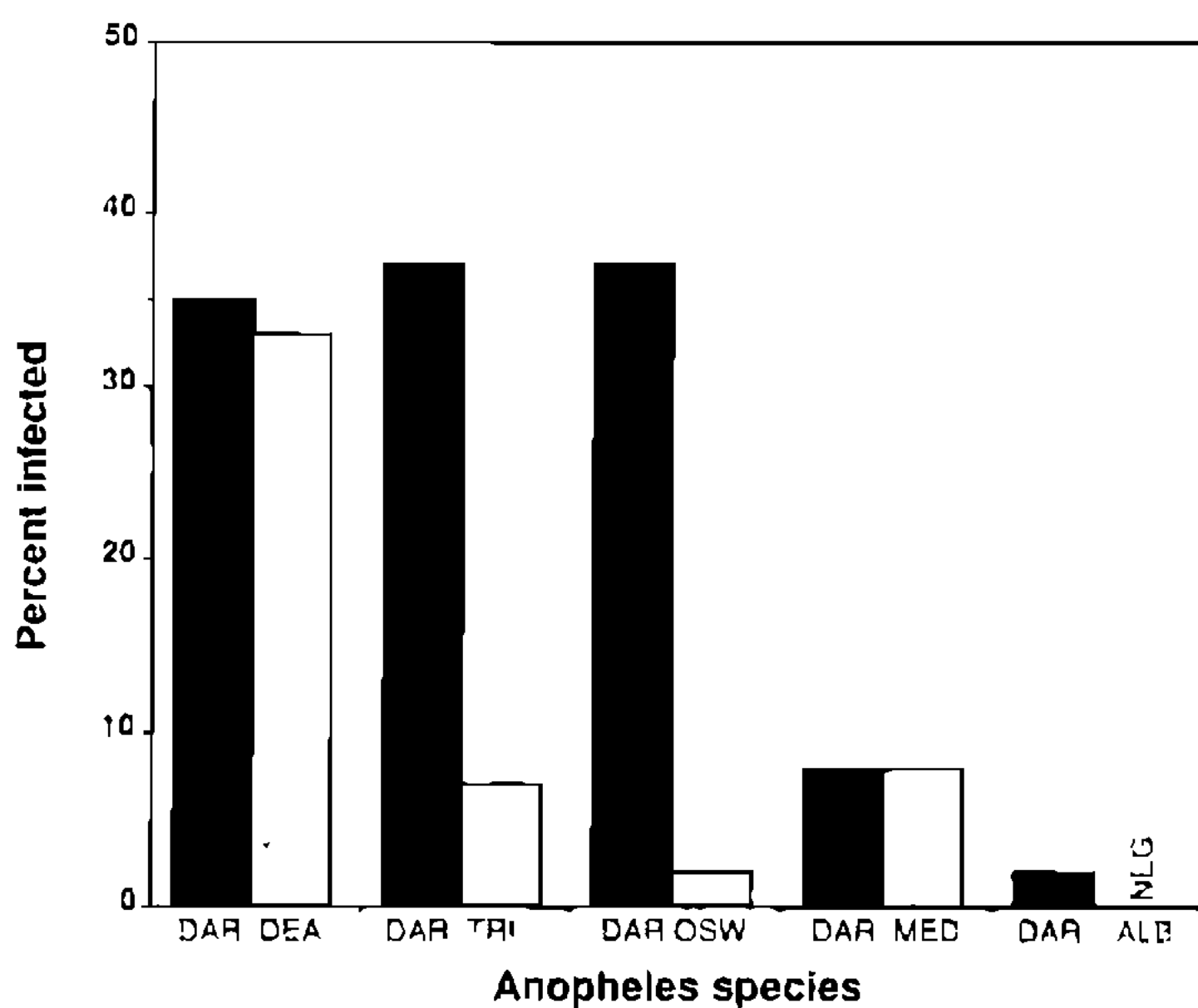


Fig. 4: percent of *Anopheles darlingi* infected with sporozoites *P. falciparum* in the salivary glands compared to percentages for 5 other *Anopheles* species. DAR = *An. darlingi*, DEA = *An. deaneorum*, TRI = *An. triannulatus*, OSW = *An. oswaldoi*, MED = *An. mediopunctatus*, ALB = *An. albitarsis*.

Additionally, malaria control relies upon the elimination of malaria carriers that continue to infect mosquitoes. When vivax malaria patients with circulating gametocytes were given 600 mg chloroquine diphosphate (initial dose) there was a dramatic decrease in infectivity 2-4 h following treatment. However, patient infectivity to mosquitoes resumed to near pre-treatment levels 4-8 h post-treatment (Klein et al., 1991d) (Fig. 5). At 24 h post-treatment, patients either did not infect or only infected a few mosquitoes. We recommend that patients with vivax malaria should be protected from *Anopheles* mosquitoes for at least 24 h to interrupt malaria transmission. However, if malaria patients are given chloroquine and primaquine concurrently, and do not vomit shortly after the initial dose, their gametocytes are not infective to mosquitoes beyond 4-6 h after the initial dose (Fig. 6).

Similar experiments to determine the effects of quinine and quinine + tetracycline on the infectivity of falciparum patients showed that many falciparum malaria patients arrived at the clinic with circulating gametocytes and subsequent to radical treatment, these patients infected *An. darlingi* for up to 21 days post-treatment (Klein et al., 1991b). Subsequent to the study, patients were given 45 mg primaquine and did not infect mosquitoes 24 h after treatment with primaquine. Nearly 20% of the falciparum patients arriving at the malaria clinic in Costa Marques demonstrated gametocytes in

the blood films. This demonstrates the need to identify falciparum patients with gametocytes and treat them with a gametocytocidal drug, such as primaquine, to reduce malaria transmission subsequent to treatment. New anti-malarial drugs that are gametocytocidal are being evaluated at the Walter Reed Army Institute of Research (W. Milhous, pers. comm.).

Anopheles darlingi from Costa Marques is infrequently collected in well constructed houses, but is frequently collected in houses without screened doors and windows or with large cracks in the walls. A major portion of malaria transmission may occur outdoors in the early evening since many people are active outdoors during the peak biting activity period or in poorly constructed houses or shelters with fewer than four walls. Insecticide susceptibility tests (WHO) and provo biológica tests (exposing mosquitoes to sprayed wall surfaces) show that *An. darlingi* in Costa Marques is physiologically susceptible to DDT and deltamethrin. At the same time, studies on *Culex quinquefasciatus* show that this species is highly resistant to DDT.

Behavioral studies are needed to determine the repellency effect of DDT and other insecticides to estimate their effectiveness as a control measure. Our preliminary studies indicate that both DDT and deltamethrin kill a majority of the bloodfed mosquitoes that were released inside a sprayed house.

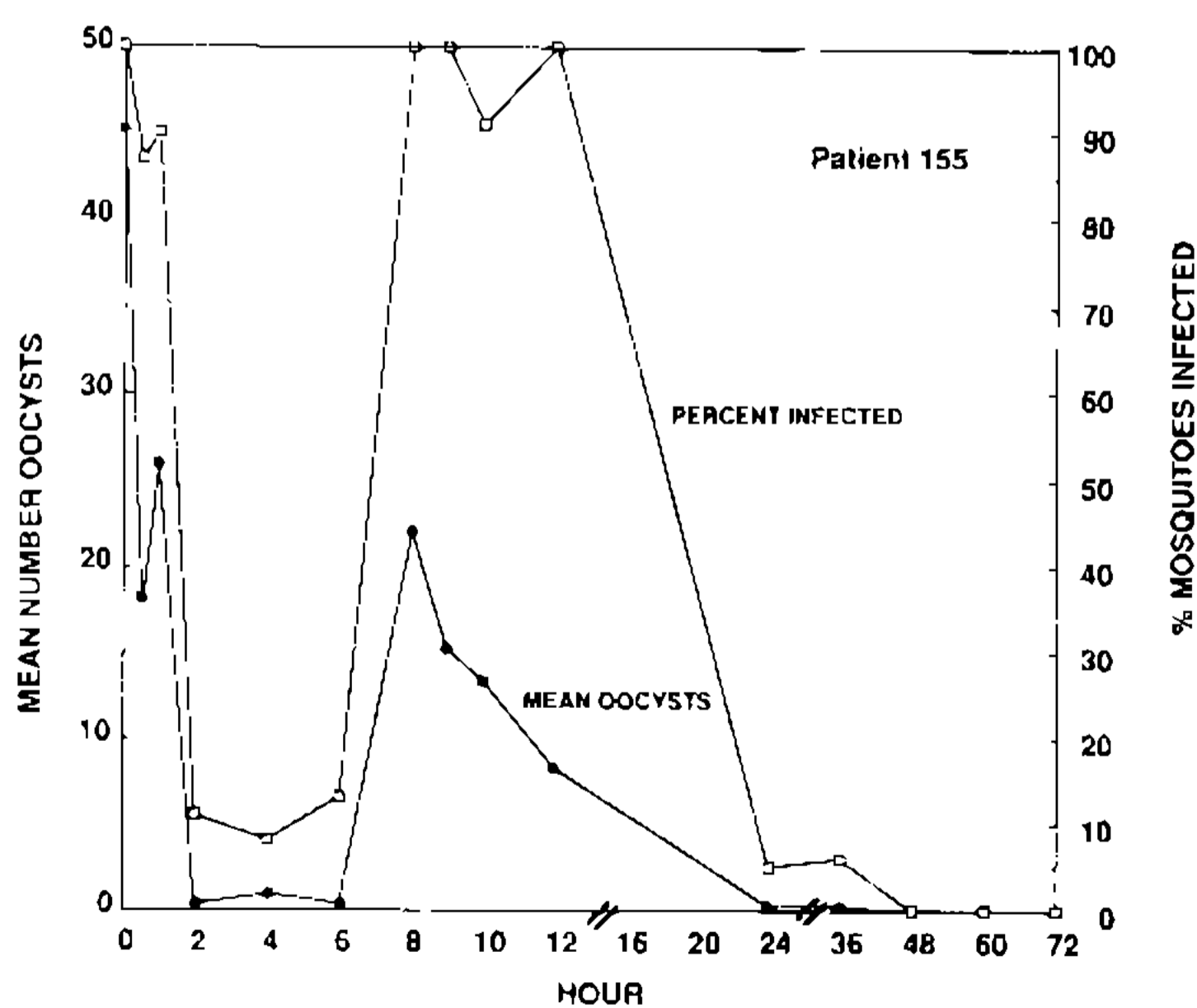


Fig. 5 mean number of oocysts (no. oocysts/no. mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient before and during treatment with chloroquine diphosphate.

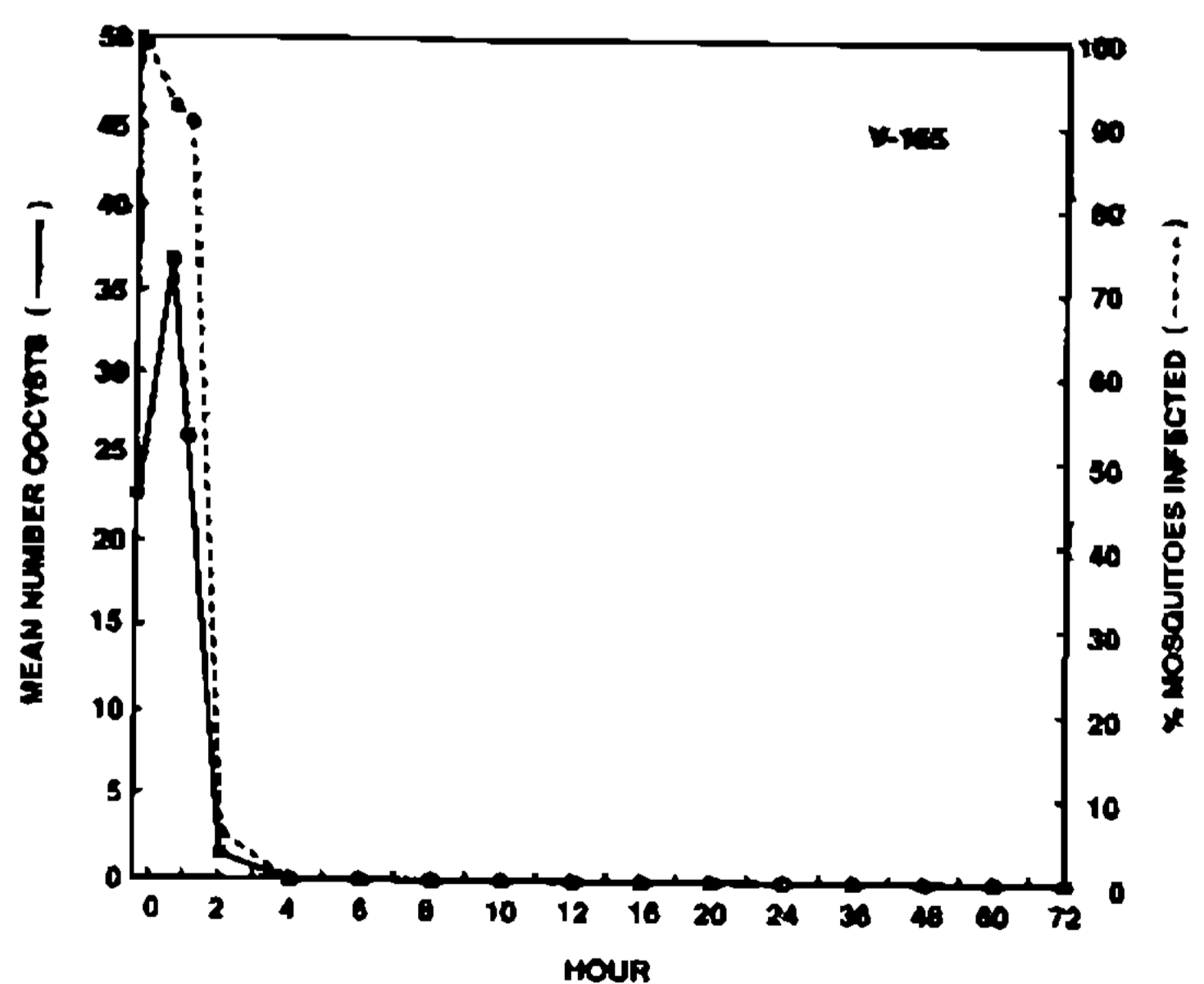


Fig. 6: mean number of oocysts (no. oocysts/no. mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient before and during treatment with chloroquine diphosphate + primaquine.

In summary, malaria is endemic in Brazil with approximately 99% of the malaria cases reported from the Amazon Basin where the great expanse of water and numbers of suitable habitats make larval control in many areas impractical. *Anopheles darlingi* is the primary vector of malaria in Costa Marques based on its seasonal distribution and abundance, biting behavior and natural and laboratory sporozoite infection rates. *Plasmodium falciparum* patients with circulating gametocytes who develop mature gametocytes after treatment for asexual stages need to be treated with gametocytocidal drugs such as primaquine. Chloroquine treated patients are infective to mosquitoes for up to 24 h after treatment and should be treated concurrently with primaquine or protected from mosquitoes to prevent further malaria transmission. Data on the effect of DDT and other insecticides in malaria control are preliminary and critically need to be evaluated under natural field conditions rather than artificial methods that will not be implemented or accepted by the community. Repellent studies and other personal protective measures, such as insecticides treated curtains and bed nets, should be evaluated to determine their effectiveness in reducing the malaria incidence in the Amazon Basin.

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