

INFECTION OF *ANOPHELES DARLINGI* FED ON PATIENTS INFECTED WITH *PLASMODIUM VIVAX* BEFORE AND DURING TREATMENT WITH CHLOROQUINE PLUS PRIMAQUINE IN COSTA MARQUES, RONDÔNIA, BRAZIL

TERRY A. KLEIN/⁺; MAUROS. TADA*^{*}; JOSE B. P. LIMA*^{**} & AMAZONIA TODA TANG*^{***}

U. S. Army Medical Research Unit-Brazil, American Consulate-Rio, APO Miami, FL 34030, U. S. A. *Núcleo de Medicina Tropical e Nutrição, Universidade de Brasília, Caixa Postal 15-3121, 70910-900 Brasília, DF, Brasil

Five patients with asexual and sexual parasites of *Plasmodium vivax* were treated orally with 600 mg chloroquine diphosphate (hour 0) followed with 300 mg at 8, 24 and 48 h later. Primaquine phosphate, 15 mg, was administered concurrently at h 0 and at 24 h intervals for 14 days. *Anopheles darlingi* were fed before the first dose (h -0.5) and 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60 and 72 h later. Mosquitoes were examined for oocysts on day 8 and for sporozoites on day 15 after infection. Four of the five patients studied were still infective to mosquitoes from 1-5 h after the first dose of chloroquine plus primaquine. One of these and one other patient, who vomited 15 min after the first dose, became ineffective again at hours 10 and 12, respectively. Once produced, oocysts in mosquitoes fed on patients before, during and after chloroquine plus primaquine treatment appeared normal and produced sporozoite infected salivary glands. In view of these data, it is concluded that primaquine demonstrated rapid gametocytocidal activity and should be administered concurrently with chloroquine to reduce *vivax* malaria transmission.

Key words: *Anopheles darlingi* - chloroquine - primaquine

The principal aims of chemotherapy of *vivax* malaria are the elimination of clinical symptoms and of asexual blood and exoerythrocytic stage parasites. An additional goal is the reduction of malaria transmission by elimination of infective gametocytes. Chloroquine is a 4-aminoquinoline which suppresses the maturation of young gametocytes by interfering with haemoglobin digestion (Wernsdorfer, 1980; Desjardins et al., 1988). Chloroquine, the drug of choice for treating *Plasmodium vivax* erythrocytic stages, fails to rapidly eliminate the infective gametocytes, allowing for a

continuation of malaria transmission from patient to mosquito hosts (Jeffery, 1958; Klein et al., 1991a). For example, after treatment with chloroquine alone, patients with circulating *P. vivax* gametocytes infrequently infected mosquitoes 2-6 h after treatment. However, these same patients became more infective after 6 h and continued to infect mosquitoes for 24+ h following treatment (Klein et al., 1991a).

Primaquine is a 8-aminoquinoline which likely interferes with the mitochondrial electron transport chain or the metabolites may act as competitive inhibitors of specific enzymes (Wernsdorfer, 1980; Desjardins et al., 1988). Primaquine, a gametocytocidal drug, is administered for the exoerythrocytic stage. It is either given concurrently with chloroquine or administered at 72 h, after initial treatment with chloroquine because of its contraindications in G6PD deficiency patients and gastrointestinal side effects.

The objective of the present study was to determine the effect of chloroquine diphosphate plus primaquine phosphate on the duration of infectivity of circulating *P. vivax* gametocytes to *An. darlingi*, the principal malaria vector in Brazil (Klein & Lima, 1990;

The views of the authors do not purport to reflect the position of the Department of the Army of the Department of Defense of the United States.

Human use: the investigators adhered to the guidelines of a human use protocol approved by the University of Brasília and the US Army Surgeon General's Committee for the use of humans as subjects for research conducted in this report.

⁺Department of Entomology, Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100, U. S. A.

^{**}Instituto de Biologia do Exército, Rua Francisco Manuel, 102 20911-270 Rio de Janeiro, RJ, Brasil

^{***}Laboratório Central de Entomologia, Divisão de Malária, FNS, Ministério da Saúde, 70058-900 Brasília, DF, Brasil.

Received 20 August 1991.

Accepted 30 March 1992.

Klein et al., 1991b; Lourenço-de-Oliveira et al., 1989).

MATERIALS AND METHODS

Study site – The study was conducted at the malaria clinic in Costa Marques, a small frontier town situated on the Guaporé River which represents the border between Brazil and Bolivia. Both *P. falciparum* and *P. vivax* are endemic. The prevalence of malaria (1984 through 1986) ranged from 8-9% in residents of the town and 14-26% in pioneers living along primary and secondary roads (McGreevy et al., 1989).

Volunteers – Thick and thin blood films from patients with signs and symptoms of malaria seen between 1987-1990 were stained with Giemsa stain and examined for *Plasmodium* parasites (1,000X). Males that were > 21 years old and had a single infection of *P. vivax* with circulating asexual and sexual parasites were considered for study under a human use protocol. These patients were given a medical examination by a physician and excluded if they had complications (anemia, vomiting and high fever). The remaining patients were admitted to the study with informed consent.

Treatment – Five patients were treated orally with 600 mg chloroquine diphosphate (manufactured by Brazilian pharmaceutical companies for the Central de Medicamentos for distribution by the Superintendency of Public Health Campaigns – SUCAM) (0 h) followed with 300 mg at 8, 24 and 48 h. Patients were treated concurrently with 15 mg primaquine phosphate every 24 h, beginning at 0 h, for 14 days to eliminate exoerythrocytic parasites. All patients responded to chloroquine treatment and asexual parasites were not present beyond the third day of treatment.

Mosquito rearing – Anopheline mosquitoes were collected from human-bait near the town of Costa Marques (Klein & Lima, 1990). Mosquitoes were provided bloodmeals on human volunteers on mefloquine prophylaxis, identified to species according to Faran & Linthicum (1981) and Lane (1953), and reared as described by Klein et al. (1990). The insectary was maintained at $26 \pm 2^\circ\text{C}$ and $65 \pm 20\%$ RH. The light cycle, a combination of natural and fluorescent lighting, was maintained at 12 ± 2 h daylight.

Mosquito feeds – Twenty five to 50, laboratory reared, 3-6 day old, F_1 *An. darlingi* mosquitoes were placed in screen topped 215 cm³ (pint) cartons, starved for 4-6 h, and then exposed for 20 min on a vivax malaria patient with circulating gametocytes. Mosquitoes were exposed to patients prior to treatment (-0.5 h) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60 and 72 h after the first dose of chloroquine and primaquine. Unfed and partially fed mosquitoes were discarded. Engorged females were provided a 10% sucrose solution daily and maintained in the insectary for the duration of the study.

A portion of the mosquitoes (> 10) from each of the hourly feeds were dissected for oocysts on day 8 after feeding. However, all mosquitoes in a group were dissected on day 8 when the total number of surviving mosquitoes was < 20 or the frequency of infection was < 25%. Wet mounts of mosquito midguts were examined under a compound microscope (400X) to count the number of oocysts. Midguts with > 200 oocysts were counted as 200 when calculating the mean number of oocysts. Salivary glands from the surviving mosquitoes were dissected on day 15 after feeding and examined by light microscopy (400X) to detect salivary gland sporozoites. The salivary gland infections were graded as negative, 1-10 sporozoites, 11-100 sporozoites, 101-1,000 sporozoites and > 1,000 sporozoites.

RESULTS

The mean number of oocysts and the mosquito infection rate are shown for five patients treated concurrently with chloroquine plus primaquine (Figs 1-5). The mean number of oocysts per mosquito ranged from 0.1 to 183.0 for groups fed on different patients before treatment. Vivax malaria patient infectivity decreased rapidly and, except for one patient who vomited 15 min after the initial dose (Fig. 1), patients did not infect mosquitoes beyond 10 h after the first dose of chloroquine plus primaquine (Table).

Chloroquine plus primaquine, while rapidly decreasing the average number of oocysts, did not appear to affect the sporogonic development of the oocysts. The proportion of oocyst infected mosquitoes with sporozoites were similar whether they fed on the patients before treatment or during the first day of chloroquine

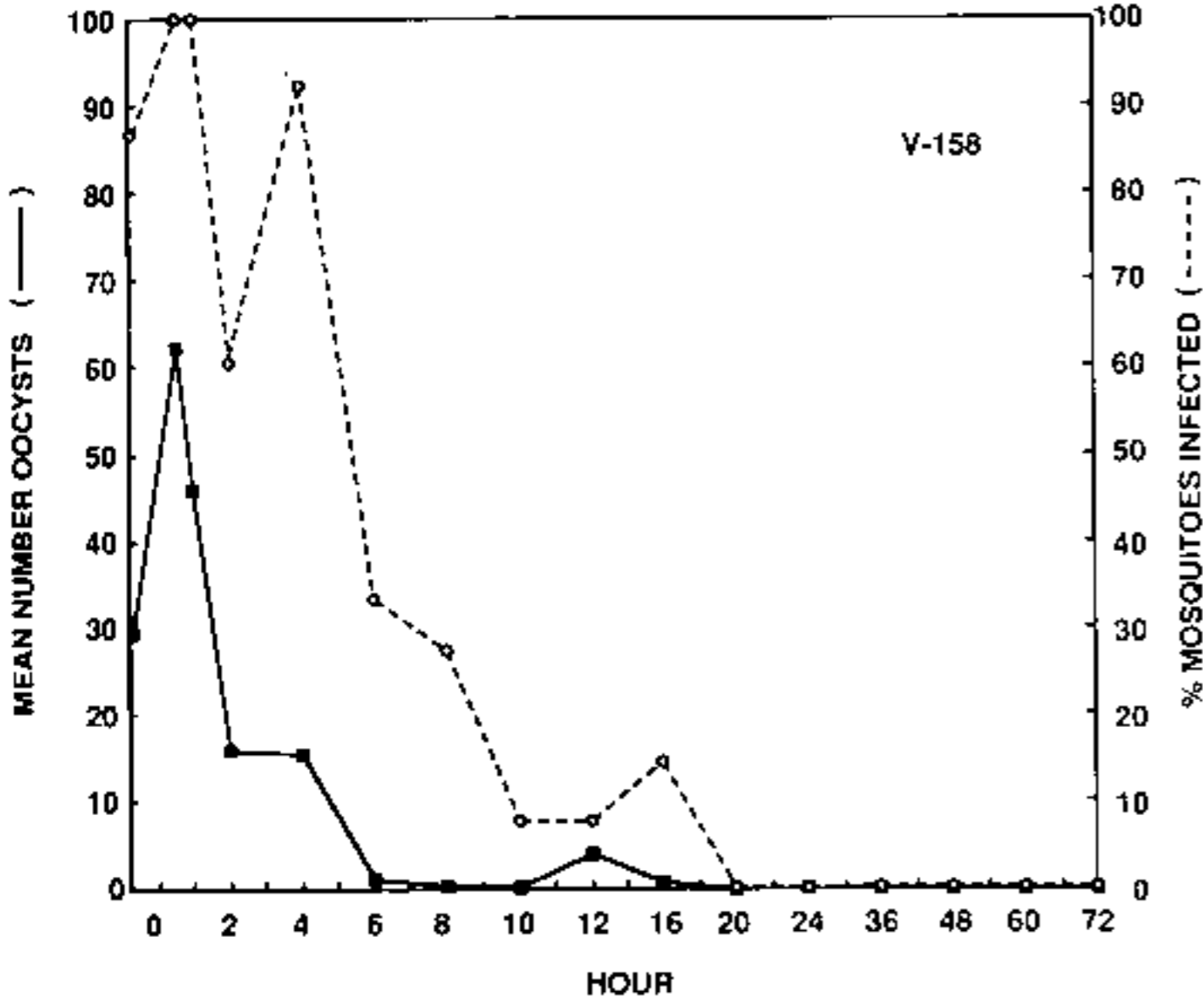


Fig. 1: mean number of oocysts (number oocysts/number mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient (V-158) before and during treatment with chloroquine diphosphate plus primaquine. Patient vomited 15 min after the first dose of chloroquine plus primaquine.

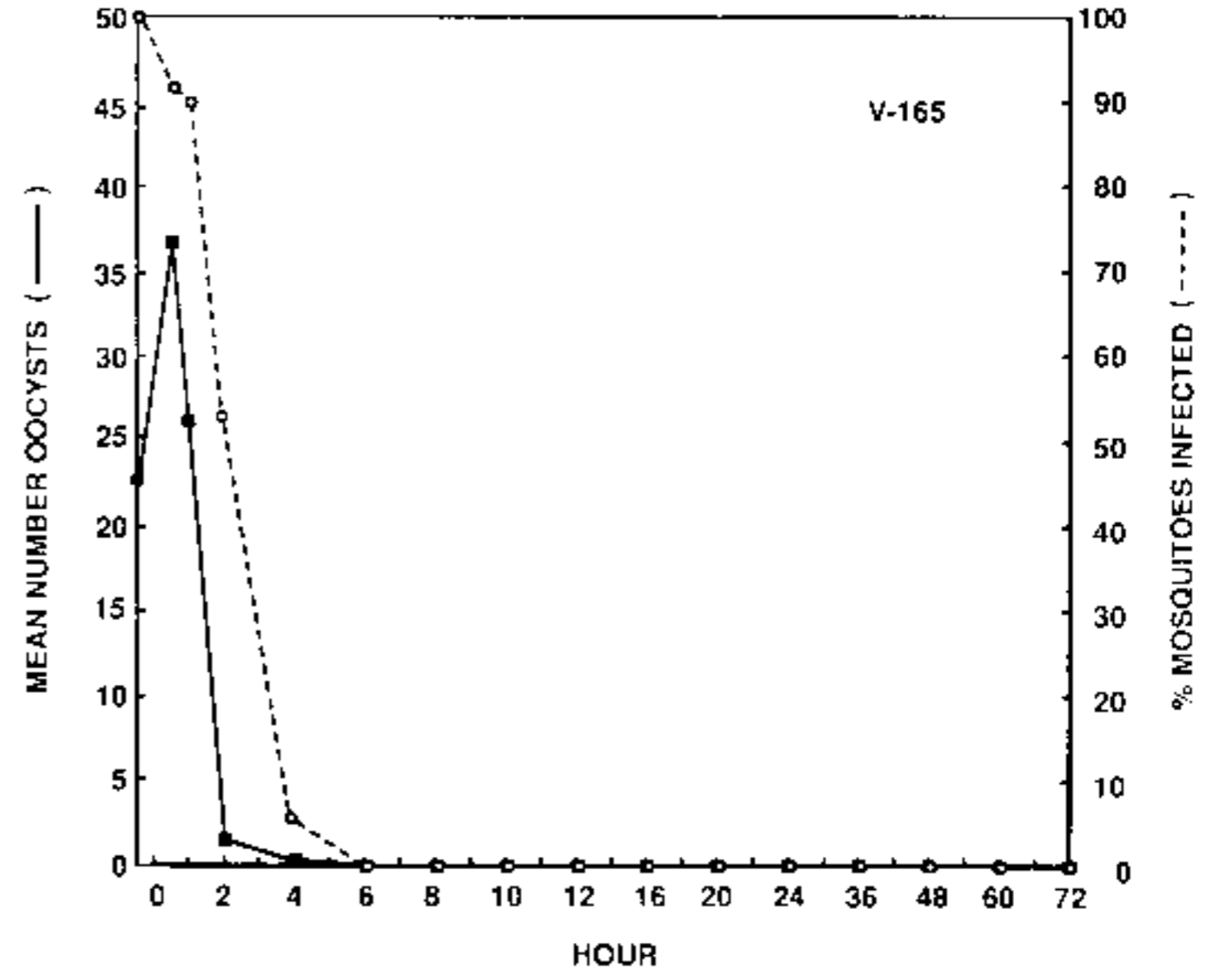


Fig. 3: mean number of oocysts (number oocysts/number mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient (V-165) before and during treatment with chloroquine diphosphate plus primaquine.

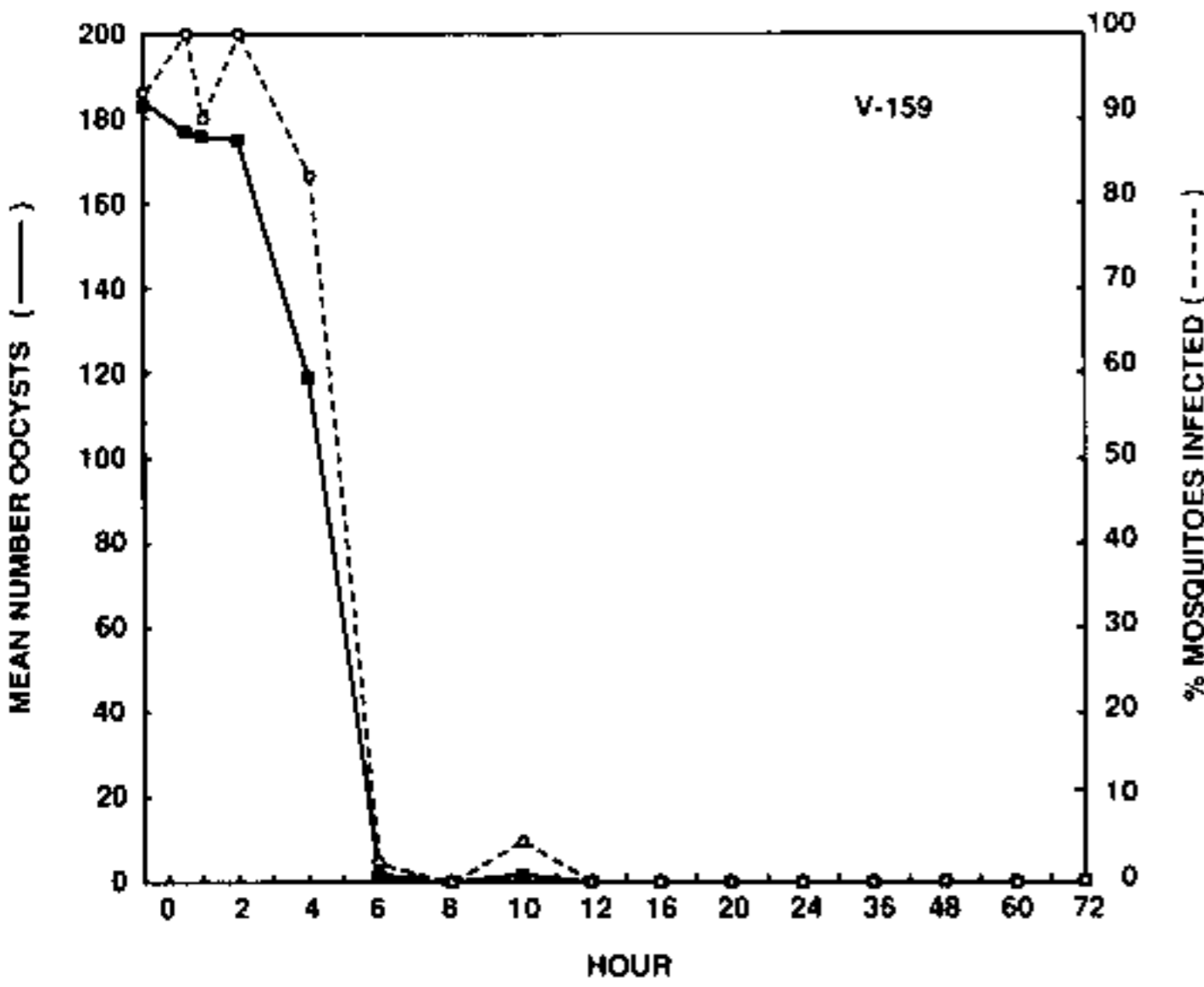


Fig. 2: mean number of oocysts (number oocysts/number mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient (V-159) before and during treatment with chloroquine diphosphate plus primaquine.

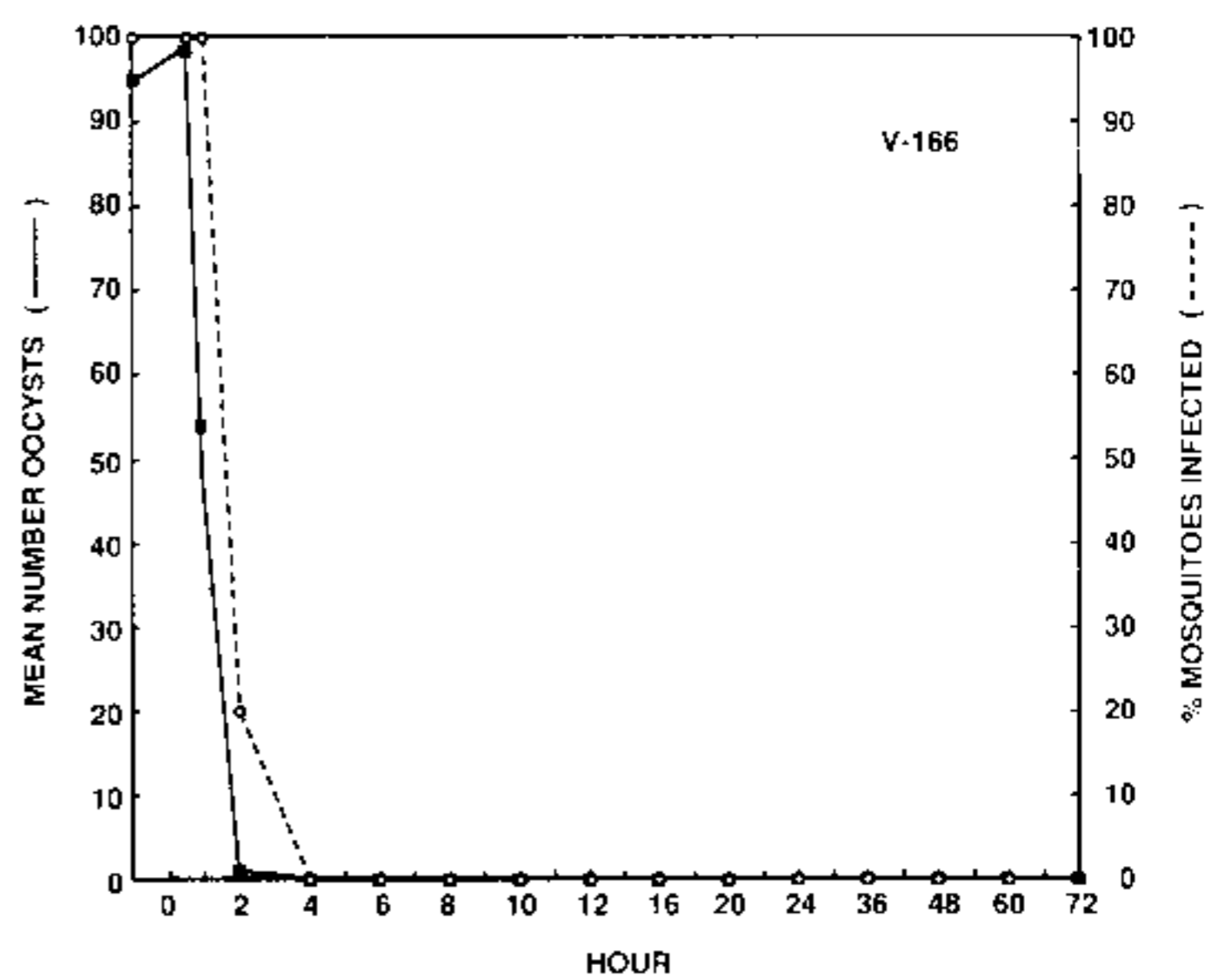


Fig. 4: mean number of oocysts (number oocysts/number mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient (V-166) before and during treatment with chloroquine diphosphate plus primaquine.

plus primaquine treatment. Indeed, where we only considered mosquitoes with oocysts on day 15 after feeding, 76% of those which fed on patients before treatment had sporozoites in the salivary glands while 77% of those that fed during the first 6 h of treatment had sporozoites in the salivary glands (Fig. 6).

DISCUSSION AND CONCLUSIONS

Malaria control programs require that the number of vivax malaria patients with infective gametocytes be reduced. *Plasmodium vivax*

gametocytes mature in about 2 days and can be detected in the peripheral circulation 4-5 days after the first erythrocytic infection (Mackerras & Ercole, 1949; Coatney et al., 1971; Klein et al., 1991b). In Costa Marques, about 20% of the vivax malaria patients seeking treatment at the malaria clinic were reported by SUCAM to have circulating *P. vivax* gametocytes (Klein et al., 1991a). This is probably an underestimate since, at SUCAM, there is no requirement for the microscopists to identify patients with *P. vivax* gametocytes and only a limited number of fields are examined.

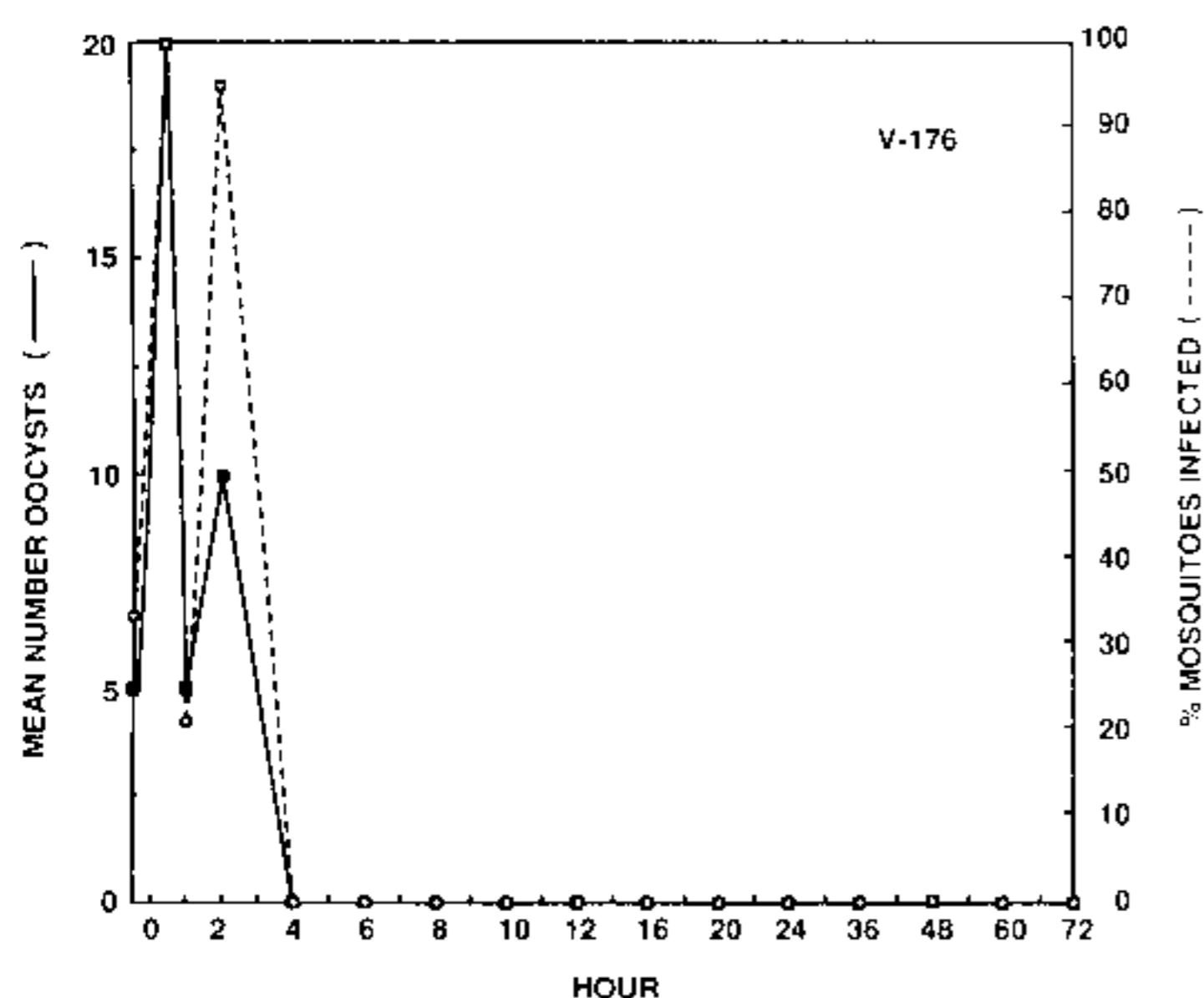


Fig. 5: mean number of oocysts (number oocysts/number mosquitoes dissected) and percent of *Anopheles darlingi* infected that fed on a vivax malaria patient (V-176) before and during treatment with chloroquine diphosphate plus primaquine

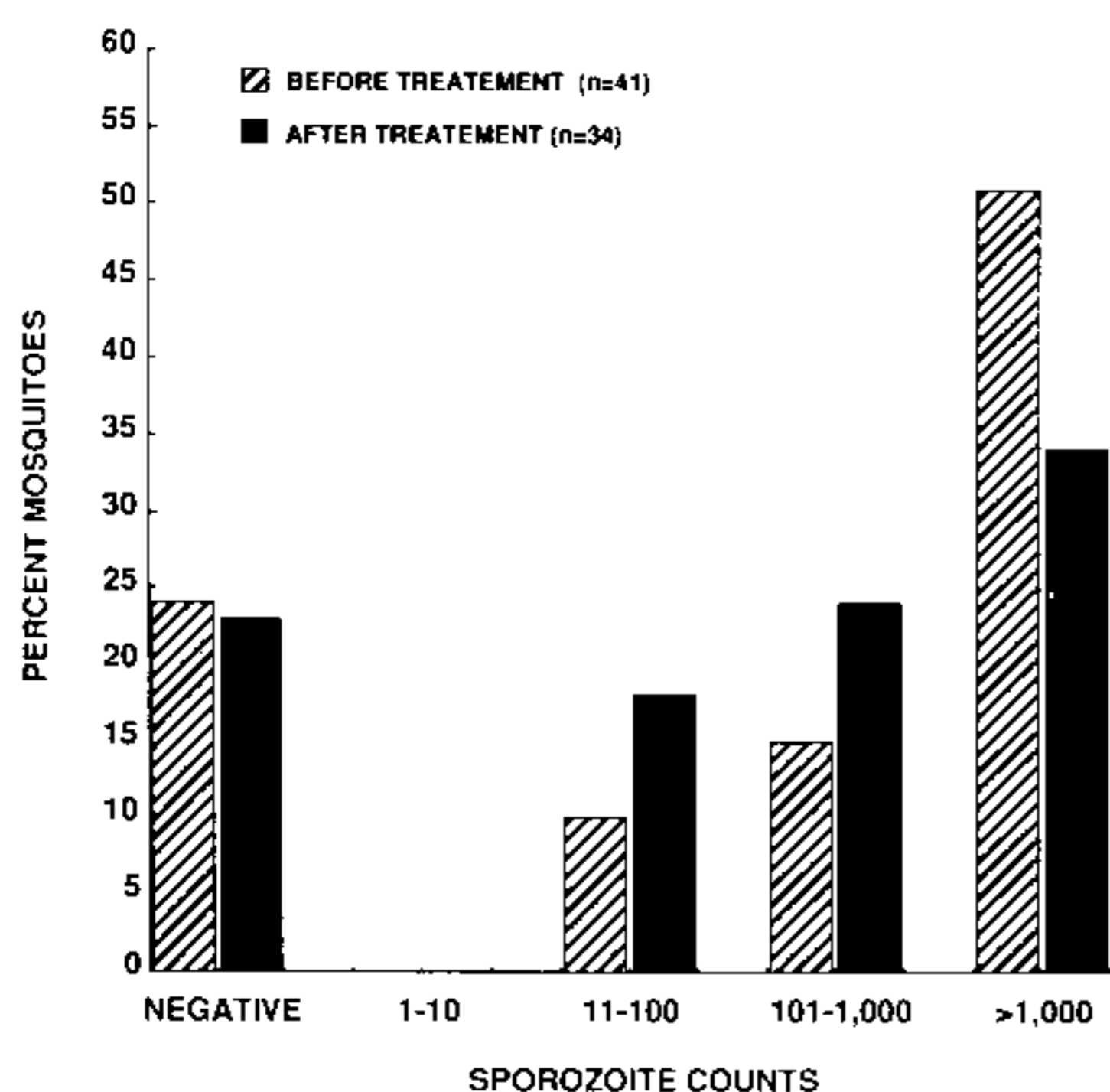


Fig. 6: comparison of numbers of sporozoites in *Anopheles darlingi* mosquitoes with oocysts that fed on *Plasmodium vivax* infected volunteer patients before or during the first 6 h after treatment with chloroquine plus primaquine.

Indeed, in Thailand, about 50% of all *P. vivax* infected patients (males, > 21 years) seeking treatment were infective to *Anopheles* mosquitoes (Andre et al., 1982). Laboratory studies conducted on the susceptibility of *Anopheles* in Costa Marques showed that 97% of the *P. vivax* infected patients with gametocytes in thick blood films were infective to *An. darlingi* (Klein et al., 1991b). In the population of rural pioneers living near Costa Marques, the mean time from the onset of symptoms to arrival at the malaria clinic and diagnosis was 3-4 days.

TABLE

Changes in mean numbers of oocysts and percent of mosquitoes fed before (0 h) and during (0.5 - 72 h) treatment with chloroquine plus primaquine

Hour	Grand mean oocysts ^a	Percent with oocysts	Percent reduction of mosquitoes with oocysts
-0.5	66.1	77.4	0.0
0.5	75.4	82.3	increase
1	60.3	76.8	0.8
2	38.8	50.3	35.0
4	27.0	36.1	41.3
6	0.2	7.7	90.1
8	<0.1	5.5	92.9
10	1.0	2.5	96.8
12	0.8	1.5	98.1
16	0.1	2.9	96.3
20-72	0	0	100.0

^a: determined as the sum of the mean number oocysts from mosquitoes fed on each volunteer/5 (no. volunteers) since unequal numbers of mosquitoes were dissected for different patients.

Many of these patients are probably infective to mosquitoes one or more nights preceding diagnosis and treatment. This delay in diagnosis, chemotherapy treatment and subsequent sterilization of *P. vivax* gametocytes presumably increases the potential for malaria transmission from man to mosquito.

Our results on patient infectivity to mosquitoes fed before treatment and 24, 48 and 72 h after the first dose are similar to those of other researchers and clearly indicate that chloroquine plus primaquine administered concurrently reduces the potential for malaria transmission from man to mosquito. Primaquine exhibits rapid sporontocidal activity against *P. vivax* while chloroquine is a slower acting gametocytocidal drug (Shute & Maryon, 1954; Young & Burgess, 1957; Wernsdorfer, 1980; Desjardins et al., 1988). Patients only treated with chloroquine are able to infect mosquitoes for up to 36 h (Klein et al., 1991a) or up to 72 h after the first dose (Andre et al., 1982). In the present study, when patients were treated with chloroquine and primaquine concurrently, patient infectivity was reduced to < 10 h, except for one patient who vomited 15 min after the first dose. However, in a study conducted in Thailand, one patient treated with chloroquine plus primaquine remained infective to mosquitoes 24 h after treatment (Andre et al., 1982). The period of patient infectivity after treat-

ment may be related to the initial patient infectivity since our results indicated that the patient producing the highest initial oocyst infections (0 h), infected mosquitoes for the longest period of time (Fig. 2). Although the combination of the rapid acting gametocytocidal drug primaquine given concurrently with chloroquine may reduce vivax malaria transmission by interrupting the man-mosquito cycle, vivax malaria patients should be protected from bites of malaria vectors for at least 24 h after the initial dose (Shute & Maryon, 1954; Klein et al., 1991a).

New antimalarial drugs are being investigated for their human safety and efficacy to eliminate asexual parasites. Additionally, the investigators also should determine the gametocytocidal and sporontocidal activity of these new antimalarial drugs which may impact on the epidemiology of malaria.

ACKNOWLEDGEMENTS

To Luis Gil, Fatima Barros and Jose Vargas, USAMRU-B, and Valdecir Ortis and Valdevino Ortis, SUCAM, Costa Marques for their technical support. To Lyman Roberts and Russell Coleman, Walter Reed Army Institute of Research; Maria A. F. Costa, U. S. Army Medical Research Unit-Brasília; Ilineu P. A. Silva and Daniel N. da Silva, Instituto de Biologia do Exército, Rio de Janeiro; Orlando Ramirez, SUCAM, Rondônia; Agostinho Marques and Dilermando Fazito de Rezende, Ministério da Saúde, SUCAM, Brasília, DF; and Aluzio Prata and Vanize Macedo, University of Brasília, Brasília, DF, for their support.

REFERENCES

- ANDRE, R. G.; DOBERSTYN, E. B. & TEERAKIARTKAMJORN, C., 1982. Sporontocidal effect of antimalarials on *Plasmodium vivax* malaria in *Anopheles dirus* and *Anopheles maculatus*. *Ann. Meet. Entomol. Soc. Amer. Canada, Toronto, Canada*.
- COATNEY, G. R.; COLLINS, W. E.; WARREN, M. C. W. & CONTAGOS, P. G., 1971. *The Primate Malariae*. U.S. Department of Health Education and Welfare, Washington, DC.
- DESJARDINS, R. E.; DOBERSTYN, E. B. & WERNSDORFER, W. H., 1988. The treatment and prophylaxis of malaria. p. 827-864 in W. H. Wernsdorfer & I. McGregor (eds), *Malaria, Principles and Practice of Malariology*. Vol. 1, Churchill and Livingstone, New York.
- FARAN, M. E. & LINTHICUM, K. J., 1981. A handbook of the Amazon species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Mosq. Systematics*, 13: 1-81.
- JEFFERY, G. M., 1958. Infectivity to mosquitoes of *Plasmodium vivax* following treatment with chloroquine and other antimalarials. *Am. J. Trop. Med. Hyg.*, 7: 207-211.
- KLEIN, T. A. & LIMA J. B. P., 1990. Seasonal distribution and biting pattern of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. *J. Am. Mosq. Control Assoc.*, 6: 700-707.
- KLEIN, T. A.; LIMA, J. B. P. & TODA TANG, A., 1990. Colonization and maintenance of *Anopheles deaneorum* Rosa-Freitas (Diptera: Culicidae) in Brazil. *J. Am. Mosq. Control Assoc.*, 6: 510-513.
- KLEIN, T. A.; TADA, M. S.; LIMA, J. B. P. & KATSURAGAWA, T. H., 1991a. Infection of *Anopheles darlingi* fed on patients infected with *Plasmodium vivax* before and during treatment with chloroquine in Costa Marques, Rondônia, Brazil. *Am. J. Trop. Med. Hyg.*, 45: 471-478.
- KLEIN, T. A.; LIMA, J. B. P.; TADA, M. S. & MILLER, R., 1991b. Comparative susceptibility of potential vectors of *Plasmodium vivax* in Rondônia, Brazil. *Am. J. Trop. Med. Hyg.*, 45: 463-470.
- LANE, J. 1953. *Neotropical Culicidae*. Univ. São Paulo. São Paulo. 2 vol. 1112p.
- LOURENÇO-DE-OLIVEIRA, R.; GUIMARÃES, A. E.; ARLE, M.; FERNANDES-DA-SILVA, T.; GONÇALVES CASTRO, M.; ALBUQUERQUE MOTTA, M. & DEANE, L. M., 1989. Anopheline species some of their habits and relation to malaria, in endemic areas of Rondônia state, Amazon Region of Brazil. *Mem. Inst. Oswaldo Cruz*, 84: 501-504.
- MACKERRAS, M. J. & ERCOLE, Q. N., 1949. Some observations on the action of quinine, atebirin and plasmoquine on *Plasmodium vivax*. *Trans. R. Soc. Trop. Med. Hyg.*, 42: 443-454.
- MCGREEVY, P. B.; DIETZE, R.; PRATA, A. & HEMBREE, S. C., 1989. Effects of immigration on the prevalence of malaria in rural areas of the Amazon basin of Brazil. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, 84: 485-491.
- SHUTE, P. G. & MARYON, M., 1954. The effect of pyrimethamine (Daraprim) on the gametocytes and oocysts of *Plasmodium falciparum* and *Plasmodium vivax*. *Trans. R. Soc. Trop. Med. Hyg.*, 48: 50-63.
- WERNSDORFER, W. H., 1980. The importance of malaria in the world. p. 1-93 in J. P. Kreier, *Malaria*. Vol. 1, Academic Press, New York.
- YOUNG, M. D. & BURGESS, R. W., 1957. Effect of 25 milligrams of pyrimethamine on the infectivity of *Plasmodium vivax*, St. Elizabeth strain, to *Anopheles quadrimaculatus*. *Am. J. Trop. med. Hyg.*, 6: 805-807.