Experimental infections

Venous blood samples were obtained, with informed consent, from individuals presenting high parasitaemia of *P. vivax* parasites. The blood was collected in heparinized tubes and then distributed to pre-warmed (37°C) membrane feeders (RUTLEDGE *et al.*, 1964). Most of these blood donors had a history of past malaria episodes.

The F1 progenies of field-captured *An. oswaldoi s.s.* and *An. konderi* were fed for 15 min on the *P. vivax*-infected blood. *An. darlingi* were fed simultaneously and used as control of each infection because of their high susceptibility to malaria parasites.

Fully fed mosquitoes were maintained under the same environmental conditions, with permanent access to a 10% sucrose solution without further blood meals, in the insectary at Porto Velho, Rondônia, for 10–12 days. After that the midguts and the salivary glands of fed mosquitoes were removed into a drop of saline solution and examined under a coverslip by light microscopy for the presence of oocysts and sporozoites, at a magnification of ×400.

Results

We examined the male genitalia of anophelines from 47 families originating from mosquitoes captured in Rondônia and 48 families from Acre. According to the morphological characteristics of the male genitalia, all families from Rondônia were *An. konderi*, while 41 anopheline families (85·0%) from Acre corresponded to *An. oswaldoi s.s.* and 7 to *An. konderi*. The number of female mosquitoes raised from the 7 families of *An. konderi* from Acre was very low. Therefore, experimental infections with *An. konderi* were conducted with specimens only from Rondônia.

The dissection of both *An. oswaldoi s.s.* and *An. konderi*, fed on *P. vivax*-infected blood, showed that these 2 species developed oocysts in the midguts. The percentage of oocyst-positive mosquitoes for *An. oswaldoi* (13·8%) ($n = 29$) was higher than for *An. konderi* (3·3%) ($n = 30$) (Fig. 1).

Comparing the infections of the salivary glands, sporozoites were found in only 2 (6·9%) of 29 *An. oswaldoi s.s.* We did not find sporozoites in the salivary glands of any dissected *An. konderi* (Fig. 2).

Infection rates in *An. darlingi* ranged from 22·5% to 30·0% for both oocysts and sporozoites (Figs 1 and 2).

Discussion

Circumsporozoite proteins (CSPs) of human malaria parasites have been used to identify infection by *Plasmodium* species in anophelines by RIA and ELISA (ZAVALA *et al.*, 1982; COLLINS *et al.*, 1984; ARRUDA *et al.*, 1986; WIRTZ *et al.*, 1987; BRANQUINHO *et al.*, 1993). Using these techniques other anophelines, besides *An. darlingi*, have been incriminated as vectors of malaria parasites in the Amazon Region.

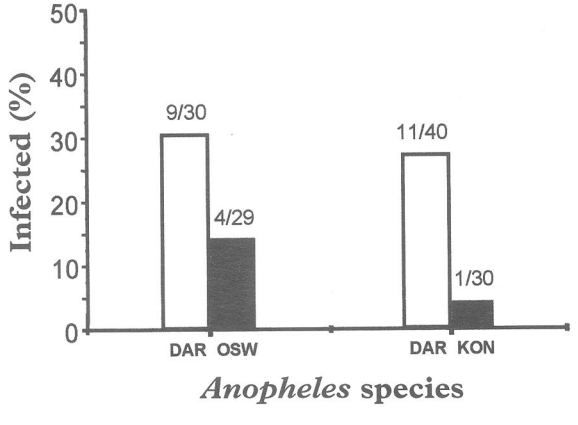

Fig. 1. Percentage of *Anopheles oswaldoi s.s.* and *An. konderi* infected with *Plasmodium vivax* oocysts in the midgut compared to percentage for *An. darlingi*. OSW, *An. oswaldoi s.s.*; KON, *An. konderi*; DAR, *An. darlingi*. Numbers above bars: positive/total of examined mosquitoes.

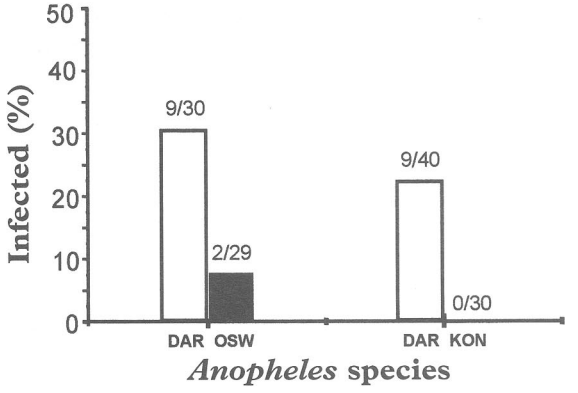

Fig. 2. Percentage of *Anopheles oswaldoi s.s.* and *An. konderi* infected with *Plasmodium vivax* sporozoites in the salivary glands compared to percentage for *An. darlingi*. OSW, *An. oswaldoi s.s.*; KON, *An. konderi*; DAR, *An. darlingi*. Numbers above bars: positive/total of examined mosquitoes.

Very little is known about anopheline infection rates and prevalence of anthropophilic mosquito species in the State of Acre. In a previous study by our group in the State of Acre, Brazil, 3056 anophelines were captured and extracts of whole mosquitoes were tested by 'sandwich' ELISA with monoclonal antibodies against CSPs of *P. falciparum, P. malariae, P. vivax* VK210 and the variant VK247. Of the specimens collected, 85·3% were identified as *An. oswaldoi s.l.*, and only 0·8% were *An. darlingi* (NATAL *et al.*, 1992). Among the *An. oswaldoi s.l.* 7·8% were positive for *Plasmodium*. The only other positive species was *An. deaneorum*, with 4·3% positivity (BRANQUINHO *et al.*, 1993).

Because CSP can be detected prior to the release of sporozoites from the oocysts, positive results from tests on whole mosquitoes do not indicate that the salivary glands are infected with sporozoites (WIRTZ *et al.*, 1987). Thus, immunological evidence for the presence of CSP may indicate that a mosquito is a potential malaria vector, but it is not proof that the sporozoites are located in the salivary glands and can be transmitted to a vertebrate host by mosquito bite.

For these reasons, BRANQUINHO *et al.* (1996), continuing their work at the same area in the State of Acre, dissected 294 wild-caught anophelines to determine the oocyst and sporozoite rates. Only 1 mosquito, identified as *An. oswaldoi s.l.*, was found infected with oocysts and sporozoites, and mosquitoes belonging to other anopheline species were negative. On that occasion, 11·5% of the anophelines were *An. oswaldoi s.l.* Despite this low positivity rate, these findings again suggested *An. oswaldoi* as a malaria vector in that region. Further data supporting the involvement of this species in malaria transmission were provided in another study that associated the presence of CSP antigen of *P. vivax*-like parasites in *An. oswaldoi s.l.* with antibodies against this parasite in the human population of Acre (MARRELLI *et al.*, 1998).

KLEIN *et al.* (1991a, b), working in Costa Marques, Rondônia, reported a very low infection rate in the salivary glands of *An. oswaldoi s.l.*, as compared to that of *An. darlingi*, when fed on patients infected with *P. vivax* or *P. falciparum*. Since *An. oswaldoi* is a highly exophilic and zoophilic species, associated more with the forest than with anthropic environments, it has been