2,2'-azino-di(3-ethyl-benzthiazoline sulphonate) peroxidase substrate solution (ABTS, Kirkegaard & Perry Laboratory) for 30 min, at room temperature. The reaction was stopped with 10% aqueous sodium dodecyl sulphate (SDS), and the optical density (OD) read at 414 nm in an ELISA reader. All sera were tested in duplicate. Positive and negative controls were included in each assay.

Forty sera from a blood bank in São Paulo, the donors of which had never been to a malarious area and had no history of malaria episodes, were used to determine the cut-off OD. The donors were parasitologically negative by Giemsa-stained thick blood film examination at the time of bleeding and seronegative by indirect fluorescent antibody test using asexual forms of *P. vivax*. The mean OD value of these sera was 0.149 and the SD was 0.069. The cut-off value for determining positive reactions was taken as 0.287 (mean + 2 SD).

**Capture and handling of mosquitoes**

Extracts of the whole body of 1375 anophelines, 1207 *A. oswaldoi* and 168 *A. deaneorum*, prepared during the 1990–1991 survey (BRANQUINHO et al., 1993), were stored, after the addition of protease inhibitors, at −70°C.

These anophelines had been collected using the local residents as human ‘bait’ (with their informed consent) inside and around their houses and in Shannon traps in the peri-domestic areas; in a few very instances, collections were made in open country. Mosquitoes were identified according to FARAN (1980).

'Sandwich' enzyme-linked immunosorbent assay of anopheline extracts

Extracts of anophelines were examined by a 'sandwich' ELISA using the mab Pam 172 (UDHARYAKUMAR et al., 1994) directed against the repetitive region of the *P. vivax*-like/*P. simiovale* CSP (APGANQEGGAA)₃ as described by BRANQUINHO et al. (1993).

Wells of flexible polyvinyl chloride microtitre plates (Dynatech Laboratoriest) were coated overnight with Pam 172 (60 μL/well of PBS containing 1 μg of mab protein per mL). After 5 washings with PBS-T and blocking with PBS plus 5% bovine serum albumin, 30 μL of mosquito extract were added per well. Incubation for 1 h at 37°C was followed by 5 washes with PBS-T, addition of 50 μL of the biotinylated mab solution, and an additional incubation for 2 h at 37°C. Further washings were followed by 30 min incubation with 50 μL of a streptavidin–biotin–peroxidase complex (Calbiochem–Behring Diagnostics). After 3 washings with PBS-T and 3 with distilled water, 60 μL/well of ABTS were added. The reaction was stopped after 30 min by addition of 50 μL/well of 20% SDS. The OD was read at 414 nm. All positive samples were retested, and mean OD was taken as the final value. Positive and negative controls were included in each assay.

One hundred uninfected laboratory-raised anophelines were examined similarly; the mean OD value was 0.156 (SD=0.021), and the cut-off value for determining positive reactions was taken as 0.287 (mean + 3 SD).

**Results**

The prevalence of antibodies against the *P. vivax*-like/*P. simiovale* CSP in the sera tested by ELISA was 15% (18/120). The mean optical density of the positive sera was 0.426 (median 0.368) (Fig. 1).

Twelve of 1207 *A. oswaldoi* (1-0%) and 2 of 168 *A. deaneorum* (1-2%) had *P. vivax/P. simiovale* infection detected by the ‘sandwich’ ELISA (Fig. 2). The positive OD values ranged from 0.295 to 0.667, i.e., from 6 SD up to 21 SD.

**Discussion**

The prevalence of anti-*P. vivax*-like antibodies was similar to that previously found in Brazil, both in an endemic malaria area (QARI et al., 1993a) and an area of low endemicity (CURADO et al., 1997). Seven of the positive samples had OD values close to the cut-off value. If these sera are excluded, the antibody prevalence would be 9-2% (11/120), rather than 15%.

At the same time that sera were collected, 294 anophelines were captured and examined only for the presence of sporozoites in their salivary glands. Among these were 34 *A. oswaldoi* and the only infected salivary gland was found in one of them (BRANQUINHO et al., 1996).