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MALARIA SEROLOGY AS AN ALTERNATIVE TO PARASITE DETECTION IN A HIGHLY ENDEMIC AMAZONIAN COMMUNITY

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Abstract: The Indian colony under study is located in relatively inaccessible part of the Amazonian jungle, restricting frequent visits. Malaria survey in such a community requires methods which would yield reliable results even from a single visit. Malaria was regarded as a minor disease by the inhabitants who appeared to be free from malaria. The reported incidence was very low, and in fact, no parasitemia was detected in the blood smears of a group of inhabitants obtained through our short visit. But on the contrary, an avidin biotin peroxidase complexed enzyme-linked immunosorbent assay (ABC-ELISA) revealed a 100% prevalence of malaria antibodies in the population. Therefore, serological studies which can obtain the *period prevalence* are useful for the assessment of malaria situation in the highly endemic community which is not readily accessible, or whose populations seem to have acquired a certain degree of immunity depressing parasitemia to submicroscopic levels.

INTRODUCTION

Immunological methods are regarded as the most useful for determining malaria experience of populations especially in the advanced stages toward the eradication (Collins *et al.*, 1968; WHO, 1972). Ordinary slide observation cannot detect latent malaria foci in the controlled area because of the lowered possibility of encountering patent parasitemias (Pampana, 1969). However, we present another circumstance in which serological investigation in retrospective diagnosis of malaria is of high practical value.

We had an opportunity to visit an Amazonian Indian colony in the state of Pará, which per se had been isolated culturally from the surroundings. We could just stay there for a very short time, during which time we obtained the blood samples and detailed malarial histories through a questionnaire. Parasites were not detected in any individuals examined, and the declared malaria histories were rather incidental. However, the ABC-ELISA revealed a high antibody prevalence in the community. The serological profile was considered to give a truer picture of the endemicity of malaria at the place.

MATERIALS AND METHODS

Study area

The colony was located along the Xingu, about 700km to the south-west of Belém, the capital of the state of Pará (Figure 1). The inhabitants were of the Amazonian Indian tribe of Kaiapós with a population of 912 people then (Photo 1). They were basically a subsistent community, isolated from the rest of the people economically and culturally. However, the Brazilian Government started to preserve their way of life, helping the promotion of community health. A nurse was being sent who stayed with them and took care of their health and needs. The malaria situation of the state of Pará was one of the worst in Brazil. Annual report of 1988 of Fundação Nacional de Saúde (National Foundation of Health) described 125,628 positive cases out of a total population of 4,857,199 in Pará (Ministério da Saúde, 1988). In the nearest city

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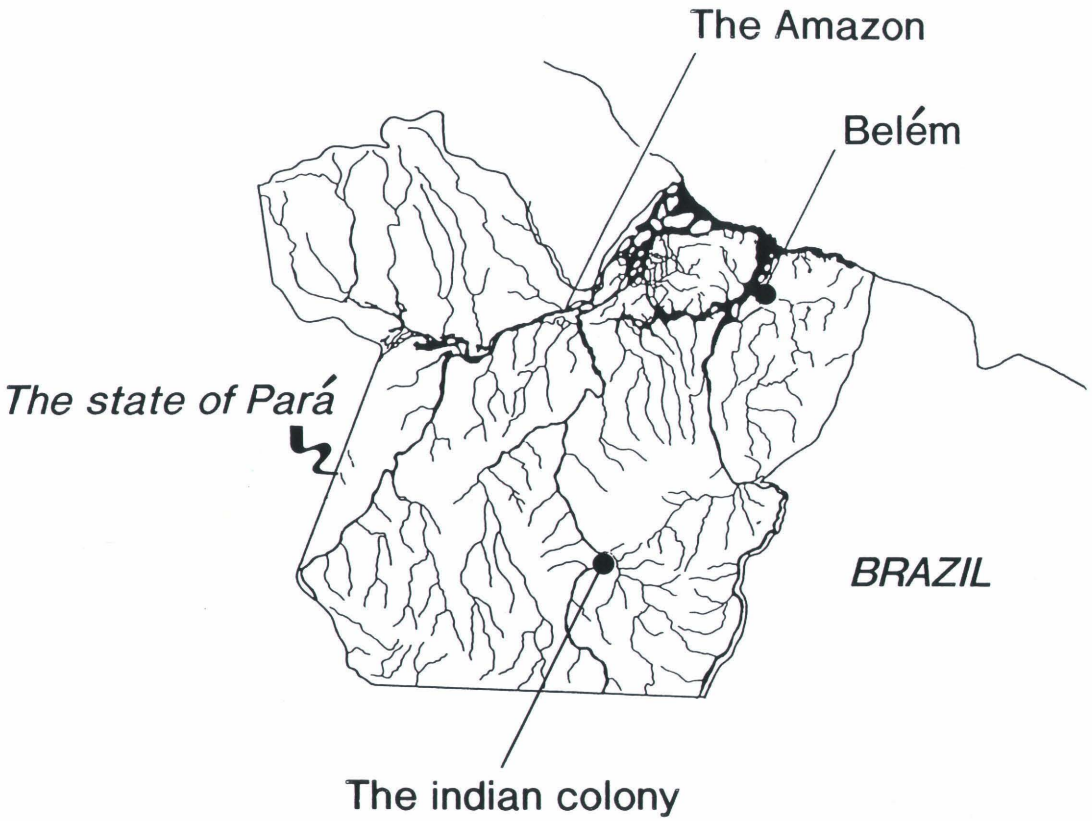

Figure 1 The study area.

Photo. 1 Beautifully painted and dressed indian children in the colony.
The photograph was taken with their permission.

(50km from the colony), Saõ Felix do Xingu, which was the center of deforestation and gold mining, 24,145 parasite positive cases out of 69,570 people were reported (Ministério da Saúde, 1993).

Subjects

Blood smears and sera were collected with informed consent from 20 donors, 8-75 years of age (September 5, 1990). They were asked their age, sex, and malaria episodes (Table 1). The samples were brought back to Gunma University School of Medicine and subjected to microscopical observation and the ABC -ELISA. The results were made known to them after the tests were completed.

ABC-ELISA

The measurement of antibodies by the ABC-ELISA was carried out according to the method described by Sato *et al.* Briefly, the antigen was obtained from an *in vitro* cultivation (Trager and Jensen, 1976; Miyagami and Waki, 1985) of *Plasmodium falciparum* (*P.f.*) strain (SGE1 strain; donated by Dr. Ambroise-Thomas in 1979). Twenty microliter of the antigen solution at a protein concentration of 250 μg/ml was placed in each well of a 96-well U-bottom microtiter plate (Greiner, Germany). The plate was left overnight allowing the antigen to dry and coat the bottom of the wells. Sera at 2-fold dilutions, 1:32-1:1024, were applied. Enzyme reaction was performed by the VECTASTAIN™ ABC Kit, which consisted of biotinylated, affinity-purified anti-human IgG made in goat, avidin DH and biotinylated horseradish peroxidase H (VECTOR Laboratories Inc., CA). Addition of the substrate, 4-chloro-1-naphtol, generated a blue color in the well with a positive serum, clearly readable with the naked eye. This test can be carried out without using major electrical equipment. The final dilution of a serum sample which gave this color was considered as the antibody titer.

RESULTS

Malarial incidence

No parasite was detected from microscopical observation of Giemsa-stained thick blood smears which were obtained from the 20 donors. Examination of local malarial records kept by Fundação Nacional de Saúde revealed 5 parasite-positives out of 222 feverish cases in the colony for the 2-week period prior to our visit. Inhabitants' response to the questionnaire indicated that malaria was considered as a minor disease and that no prophylactic measures were taken against the contraction of malaria.

The ABC-ELISA titers and declared malaria episodes

Table 1 is the individual records on age, sex, the ABC-ELISA titers and declared malaria episodes. Examinees numbered 3 and 9 declared the last malaria episode 5 days before the survey and showed consider-

Table 1 Individual records of a batch of 20 donors

No.	Declared age	Sex	ABC-ELISA titer	Declared malaria episode	
				No. of times	Last episode prior to survey
1	38	f	64	1	several years
2	26	f	128	2	7 months
3	32	f	1024	2	5 days
4	40	f	256	None	
5	73	m	512	1	1 year
6	44	f	64	1	several years
7	74	m	512	1	1 month
8	28	f	32	1	several years
9	8	m	256	3	5 days
10	73	f	128	1	several years
11	30	m	64	1	4 months
12	32	f	64	1	1 year
13	56	m	512	None	
14	28	f	64	None	
15	75	m	512	None	
16	25	f	64	2	5 months
17	47	f	512	None	
18	47	f	512	None	
19	24	f	64	1	1 year
20	50	f	128	1	1 month

able high ABC-ELISA titers at 1:1024 and 1:256 respectively. Six individuals declared that they had never contracted malaria, however, one of them showed the titer at 1:64, another at 1:256 and the rest 4 at 1:512. This discrepancy will be explained granting that these 6 people had acquired a certain degree of immunity against malaria. In fact, there is a correlation between the ABC-ELISA titers and ages of the donors (excluding No. 3 and 9) with a correlation coefficient at 0.73, which suggests that one is likely to manifest higher titer after the cumulative infection as one grows older in the community.

The frequency distribution curve of antibody titers

The frequency distribution curve of the ABC-ELISA titers is shown in Figure 2. A bimodal distribution of titers was obtained: the first peak which consists of the individuals showing the titer at 1:32 to 1:128 is suggestive of past malaria episodes from months to years before the survey, and the second one at 1:256 to 1:1024 may be reflecting the recent malaria episodes or cumulative malaria infection. This pattern of frequency distribution curve suggests that malaria transmission was actively taking place in the colony (Rivera, 1993).

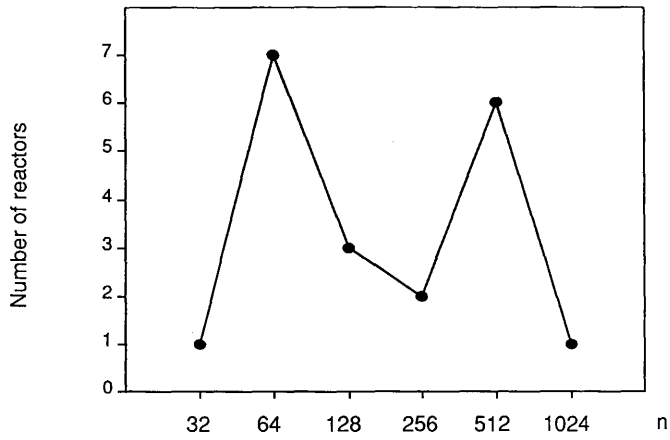

Figure 2 The frequency distribution curve of the ABC-ELISA titers.

DISCUSSION

Seroepidemiology provides more informative *period* prevalence data than can be obtained through microscopy (Collins and Skinner, 1972; Draper, 1972). It is particularly useful if, like in our present survey, the area where malaria epidemiological studies are to be conducted is not easily accessible, or one cannot stay there long enough to get standard malariometric indices of the population. In our present survey, *point* prevalence data obtained by microscopical observation of the blood smears showed no evidence of malaria transmission. But the serological method revealed a variety of positive antibody titers. Indeed, the frequency distribution curve of the ABC-ELISA titers with a peak of high antibody prevalence suggested potential risks of malaria epidemics in the population, or that active transmission of the disease were actually occurring in the area (Rivera, 1993). Thus, we could get a truer picture of the endemicity of malaria in the village by the serological method even though our visit to the place was short and temporary.

Generally, the absence of patent parasitemia can be misleading, since patency is influenced by the use of antimalaria drugs and often occurs only intermittently during malaria infections (Kagan, 1972). However this big discrepancy between parasite and antibody rates in the present study may probably be due to a certain degree of acquired immunity among the indians which depresses parasitemia down to submicroscopic levels (Draper, 1972). Repeated infection with malaria parasites maintaining continuous antigenic stimulation may have produced adequate antibody levels of the inhabitants which play, in part, a protective role against malaria (Kuvin and Voller, 1963).

Serological assessment has considerable value in the advanced stages of a malaria control program when the endemicity of malaria has become very low in the target area (Kagan, 1972; Voller and Draper, 1982). Despite this, we stress in this manuscript the importance of serological surveys of malaria for populations in a highly endemic area. The value and ease of application of a new serological method, the ABC-ELISA, which requires no major electrical equipment will also facilitate seroepidemiological studies in remote rural communities.

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