Am. J. Trop. Med. Hyg., 35(2), 1986, pp. 255–258

DETECTION OF ANTIBODIES TO *PLASMODIUM VIVAX* BY INDIRECT IMMUNOFLUORESCENCE: INFLUENCE OF THE GEOGRAPHIC ORIGIN OF ANTIGENS AND SERUM SAMPLES

E. H. BENZERROUG,* P. DEMEDTS,** AND M. WÉRY**

**Bureau de Lutte Contre le Paludisme et les Maladies Parasitaires, Institut National de Santé Publique, Boulevard Abderrahmane Laala El Madania, Alger, Algerie, and*
***Laboratory of Protozoology, Institute of Tropical Medicine, Nationalestraat 155, 2000-Antwerpen, Belgium*

Abstract. The results of a double-blind serological study of 15 sera sampled in a residual focus of vivax malaria transmission in Algeria, and of 7 sera from patients with slide-proven *P. vivax* infections acquired in India, are analyzed. The reactivity of each of these serum samples was tested by indirect immunofluorescence using 6 different batches of antigen, including 3 batches of *P. vivax* antigen prepared with isolates from Zaire (Africa), India and the Solomon Islands, respectively.

The geometric mean of reciprocal titers (GMRT) calculated on the 7 sera from proven vivax infections fell from 289.8 using the homologous antigen from the same geographic origin (India) to 48.7 using a homologous (vivax) antigen originating from a different continent (Africa). Among the 15 samples from Algeria, the percentage of seropositives decreased from 100% using the homologous *P. vivax* antigen originating from the same continent (Africa) to 53.3% using a homologous antigen from India. Two aspects are included in the discussion: in seroepidemiological studies, sensitivity could be improved by the use of a homologous antigen from the same geographic origin; in detection of clinical cases of malaria and species identification based on serology, our results stress the need for caution in interpreting serological titers and for taking into account the geographic origin of the isolates used as antigen.

Recently, a seroepidemiological study was performed in a residual focus of vivax malaria transmission in Algeria.[1] The IFA test revealed a higher proportion of seropositive individuals using the heterologous antigen *P. falciparum* (prepared from in vitro cultures) than when the homologous *P. vivax* antigen (prepared from blood of a naturally infected person returning from India) was used. The difference observed was statistically significant ($P < 0.001$, χ^2 test).

Moreover, similar results were obtained during a comparative evaluation of 10 different batches of antigen, using a battery of 20 reference sera from patients with slide-proven infections.[2]

These observations apparently contrast with earlier reports, which indicate that the use of the homologous parasite species as antigen usually yields the highest titers.[3–5] However, our findings seemed to be in agreement with the results described by Mannweiler et al.[6]

This incited us to perform further investigations on the antigenicity of different strains of *P. vivax* according to their geographic origin. A double-blind experiment was set up in order to compare the reactivity of 15 sera from the above mentioned seroepidemiological study, and of 7 out of the 20 reference serum samples previously tested and selected on the basis of their geographic origin, towards 6 different batches of antigen, 3 of which were *P. vivax* from geographically distinct areas.

MATERIALS AND METHODS

Serum samples

Seven serum samples from patients with slide-proven *P. vivax* infections, all originating from India, included 5 sera from adopted children.

Accepted 9 November 1985.

TABLE 1

Summary of the serological results obtained in two consecutive seroepidemiological surveys in Khemis el Kechna, Algeria, 1984

Survey	Antigen (species and origin)	No. of serum samples	Results				%* seropositive	GMRT**
			Neg.	1/20	1/40	1/80		
1	*P. vivax* (India)	294	283	8	2	1	3.74	25.73
	P. falciparum (Kenya)	294	272	14	6	2	7.48	27.41
2	*P. vivax* (India)	172	150	16	5	1	12.79	24.94
	P. falciparum (Kenya)	172	139	18	13	2	19.18	28.58

* In both surveys, the percentage of seropositives differs significantly according to the antigens that are used ($P < 0.001$, χ^2 test).
** Geometric mean of reciprocal titers, calculated on the seropositives.

The malarial history of these children was unknown. Four of them were adopted at the age of 3 or 4 years, while the fifth had lived in India for 9 years. The attacks of vivax malaria occurred within a few months after their arrival in Belgium. Notwithstanding the predominance of *P. vivax* over *P. falciparum* in India, the possibility that these children had had *P. falciparum* as well as *P. vivax* can not be completely ruled out.

The 2 other patients had reported no previous malarial experience and consequently their serum samples contained antibodies resulting solely from *P. vivax* infection.

Fifteen serum samples with low serological titers were from individuals living in a residual focus of vivax malaria in Algeria.[1] It is unlikely that these serum samples contained specific antibodies to *P. falciparum*, since, in the past, the prevalence of *P. vivax* in Algeria has been higher than 90%, while the last 3 cases of autochthonous falciparum malaria had been observed in the western part of the country in 1974.[7]

Antigens

P. falciparum antigen was prepared from a strain originating from Kenya, isolated in late May of 1983 and adapted to continuous culture. The cultures were *not* synchronized in view of the preparation of the IFAT-antigen and consequently the antigen consisted mainly of ring forms and trophozoites.

The *P. vivax* antigens were prepared from the blood of 3 individuals who acquired vivax malaria during stays in India, the Solomon Islands and Zaire, respectively.

The *P. ovale* antigens were prepared from blood of 2 individuals with slide-proven infections: one fell ill after a stay in Zaire and Cameroon, while the second was infected in Cameroon.

Parasitemia was high enough in all these isolates to permit comfortable reading of the immunofluorescence.[8] The stage of parasite development was comparable for all batches of *P. vivax* and *P. ovale* antigen, containing mainly ring forms and young trophozoites and only a minor proportion of schizonts and gametocytes.

Experimental scheme

All serum samples and antigens were coded in order to force blind testing.

Reading of the tests was performed on a Leitz Ortholux II microscope, equipped with a Ploem vertical illuminator and a mercury HBO 50 lamp. The following tests were applied for statistical analysis of the serological results: χ^2 test for the comparison of the percentage of seropositives, and Snedecor and Fisher's F, and the Student's *t*-test for the comparison of mean titers.

In the IFA test, the endpoint titer was defined as the highest serum dilution producing a bright fluorescence with the *majority* of the parasites (trophozoites). The observed differences in titers therefore reflect immunological differences between the various malarial antigens, rather than differences in reactivity related to the stages of development of the parasites.

RESULTS

The results obtained during 2 consecutive seroepidemiological surveys (on 294 and 172 serum samples) in Khemis el Kechna (Algeria) are shown roughly in Table 1 and illustrate that in a vivax focus, the *P. falciparum* antigen detected more positive individuals than the homologous antigen. The percentage of seropositive individuals during the first survey was 3.74 using the homologous antigen (*P. vivax* isolate from India)

TABLE 2

Reactivity to different antigens of 7 serum samples from clinical cases of vivax malaria acquired in India

Antigen (species and origin)	No. of serum samples	Results							% seropositive	GMRT*
		Neg.	1/20	1/40	1/80	1/160	1/320	1/640		
P. vivax (India)	7	0	—	—	1	—	5	1	100	289.8
P. vivax (Solomon Islands)	7	0	—	—	2	3	—	2	100	195.0
P. vivax (Zaire)	7	1	2	—	2	2	—	—	85.7	48.7
P. falciparum (Kenya)	7	1	2	—	4	—	—	—	85.7	40.0
P. ovale (Zaire/Cameroon)	7	0	2	—	2	3	—	—	100	72.4
P. ovale (Cameroon)	7	0	2	—	2	2	1	—	100	80.0

* Geometric means of reciprocal titers, calculated on the entire group of serum samples, are significantly different ($P < 0.001$, Snedecor's F test).

and 7.48 using the heterologous *P. falciparum* antigen. During the second survey, these percentages were 12.79 and 19.18, respectively. In both cases, the difference was statistically significant ($P < 0.001$, χ^2 test).

The results obtained with 7 serum samples from proven vivax infections acquired in India are listed in Table 2. The GMRT was as high as 289.8 using the homologous antigen from the same geographic origin, while it was only 48.7 using a homologous (vivax) antigen originating from Africa. The difference is statistically significant ($P < 0.001$, t-test). On the other hand the GMRT values obtained with the homologous antigen from India compared with the same antigen species from the Solomon Islands were not significantly different.

The results obtained with 15 sera, collected in a residual focus of vivax malaria transmission in Algeria are summarized in Table 3. Since in general the observed titers were on the low side, the percentage of seropositive individuals detected is most relevant for the comparison. Indeed, the percentage of seropositives decreased from 100% using the homologous antigen originating from the same continent (Africa) to 86.6% using the homologous antigen from the Solomon Islands and even further to 53.3% using the homologous antigen from India. Moreover, the proportion of seropositives detected using the homologous *P. vivax* antigen from India was inferior to the percentage detected by the use of the heterologous antigens *P. falciparum* and *P. ovale* (from Zaire/Cameroon). On the whole, GMRT values differed significantly ($P < 0.001$). This difference is mainly attributable to the results obtained with the Indian *P. vivax* antigen in contrast to those obtained using the *P. vivax* antigen from Zaire ($P < 0.001$, $t = 3.46$).

It is also worthwhile mentioning that among the heterologous *P. ovale* antigens important differences in cross-reactivity were observed. Only the 3 serum samples that showed the highest reactivity with the *P. vivax* antigens were reactive to the *P. ovale* antigen from Cameroon and this only at the lowest significant dilution of 1/20.

DISCUSSION

Our intention was to demonstrate the immunological differences existing between various strains of *P. vivax* and to indicate that these differences may be so important that titers obtained

TABLE 3

Reactivity to different antigens of 15 serum samples from individuals residing in a residual focus of vivax malaria in Algeria

Antigen (species and origin)	No. of serum samples	Results				% seropositive	GMRT*
		Neg.	1/20	1/40	1/80		
P. vivax (India)	15	7	5	1	2	53.3	18.2
P. vivax (Solomon Islands)	15	2	8	2	3	86.6	26.3
P. vivax (Zaire)	15	0	6	3	6	100	40.0
P. falciparum (Kenya)	15	2	7	4	2	86.6	26.3
P. ovale (Zaire/Cameroon)	15	2	8	3	2	86.6	25.1
P. ovale (Cameroon)	15	12	3	—	—	20	11.4

* Geometric means of reciprocal titers, calculated on the entire group of serum samples, are significantly different ($P < 0.001$, Snedecor's F test).

with a given homologous antigen may become equal or inferior to those obtained with a heterologous antigen. These differences are best demonstrated (and emphasized) by the use of homogeneous groups of homologous antisera.

From Tables 2 and 3 it may appear that African *P. vivax* and *P. falciparum* are more similar immunologically than 2 strains of *P. vivax,* isolated from Africa and India. However, one should take into account that only homogeneous groups of sera were studied. When a group of antisera to *P. falciparum* (mainly of African origin) was tested using this series of antigens, distinct higher titers were obtained with the homologous antigen (*P. falciparum* from Kenya) than with any of the *P. vivax* antigens, while differences in titers among the various vivax antigens were negligible (P. Demedts, personal communication).

The finding of important differences in the GMRT values and the proportion of seropositive individuals detected according to the geographic origin of the strain of *P. vivax* used as antigen raises 2 types of problems:

In seroepidemiological studies, which are very useful for delimiting residual foci of malaria in control and eradication programs, the geographic origin of the *P. vivax* strains used as antigen has rarely been mentioned. Most probably the sensitivity of these serological surveys could be improved by the use of a local homologous antigen.

In the diagnosis of malaria, especially in the absence of parasitological evidence, important strain-related differences among antigens may hamper species identification based on serology. The present results also stress the need for integration of the geographic origin of the antigens into the interpretation of serological titers.

These observations contribute to the characterization of strains of malaria parasites, as defined in a recent WHO memorandum.[9] However, additional comparative evaluations of *P. vivax* antigens should be made to determine in more detail the variations within and between geographic areas.

REFERENCES

1. Benzerroug, E. H., and Wéry, M., 1985. Séro-épidémiologique du paludisme au niveau d'un foyer residuel en Algerie, commune de Khemis el Kechna. *Rev. Epidém. et Santé Publ.* (In press.)
2. Demedts, P., and Wéry, M., 1985. Indirect immunofluorescence test using differentiating antigens in the detection of imported malaria. *Ann. Soc. Belge Med. Trop., 65, Suppl. 2:* 89–96.
3. Diggs, C. L., and Sadun, E. H., 1965. Serological cross-reactivity between *Plasmodium vivax* and *Plasmodium falciparum* as determined by a modified fluorescent antibody test. *Exp. Parasit., 16:* 217–223.
4. Ungureanu, E. M., Marinov, R., Stratulat, C., Teodorescu, C., Mihai, M., Bornuz, M., and Tudose, M., 1980. The value and limits of homologous and heterologous antigens of human plasmodium species for evaluating the immune response in malaria by the indirect fluorescent antibody test. *WHO Document* WHO/MAL/80.920.
5. Ambroise-Thomas, P., 1981. Diagnostic immunologique du paludisme en pratique individuelle et de masse. *Méd. et Mal. Infect., 11:* 382–387.
6. Mannweiler, E., Zum Felde, I., Mohr, W., and Muhlpfordt, H., 1977. Strain related differences in the immunodiagnosis of malaria. *Tropenmed. Parasit., 28:* 23–25.
7. Benzerroug, E. H., and Gassabi, R., 1982. Le programme national d'éradication du paludisme. Critères d'evaluation. Resultats. *Rapport annuel sur la campagne antipaludique. Document BCEP/INSP/ministère de la santé/Algerie.*
8. Sulzer, A. J., Wilson, M., and Hall, E. C., 1969. Indirect fluorescent antibody tests for parasitic diseases: V. An evaluation of a thick-smear antigen in the IFA test for malaria antibodies. *Am. J. Trop. Med. Hyg., 18:* 199–205.
9. World Health Organization, 1981. Malaria parasite strain characterization, cryopreservation and banking of isolates: A WHO memorandum. *Bull. W.H.O., 59:* 537–548.