A focus of hyperendemic *Plasmodium malariae – P. vivax* with no *P. falciparum* in a primitive population in the Peruvian Amazon jungle

Studies by means of immunofluorescence and blood smear

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Findings in a sample population in southeastern Peru with a very high rate of malaria infection, due to Plasmodium malariae *and* P. vivax *with apparently no* P. falciparum, *are described. The proportion of persons with* P. malariae *in this sample population, as determined by slide examination, appears to be the greatest ever reported for any area before the introduction of control measures. Although very few* P. vivax *were found on stained slides, results of the indirect immunofluorescence test indicated that this species was probably as prevalent as* P. malariae *; the absence of* P. falciparum *was supported by results of serologic tests. Possible reasons for this focus of malaria with no* P. falciparum *are discussed.*

The irregular distribution of *Plasmodium malariae*, as compared to *P. falciparum* and *P. vivax*, the other two most common malaria parasites of man, has been attributed to a number of factors (1, 2). Reasons given include the need for a long-lived vector to ensure the development of infective stages, the need for very specialized vector species, and the presence of an animal reservoir in endemic areas in the recent past. Whatever the cause, *P. malariae* is usually sporadic, accounting at best for only a small percentage of the total cases of malaria in areas where malaria control has not been instituted. As control measures become effective and the total number of cases is reduced, the residuum is often due mostly to persistent *P. malariae* infection.

Knowles & White (3) gave the proportions of each malaria species in the area bounded by the 70°F summer isotherm as: *P. vivax*, 43%; *P. malariae*, 8%; and *P. falciparum*, 49%. In their rather exhaustive study, carried out a few years before large-scale malaria control and eradication was attempted, they found that, with very few exceptions, *P. malariae* was responsible for fewer cases in any area than either of the other two species. The reason for this remains unclear. To resolve this question, malariologists should find areas relatively undisturbed by malaria control measures, where there is a very high rate of endemic malaria due predominately to infection with *P. malariae*. In such populations, seroepidemiological methods could be applied to determine the extent to which serological reactions agree or disagree with findings obtained by more classical methods for determining the prevalence of malarial species.

We report here two samplings of a population of primitive people from an isolated area in southeastern Peru, of whom 63% had malarial parasites in blood specimens taken at the first sampling and 85% had parasites in specimens taken 3 months later. On the basis of blood slide examination the most common species by far was *P. malariae*, occasionally mixed with *P. vivax*; there was no evidence of *P. falciparum* infection. Patterns of malarial antibody responses in the immunofluorescence test confirmed the absence of *P. falciparum* but indicated that *P. vivax* was present in many more individuals than the slide

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examination indicated. Geographic and climatic conditions are believed to have contributed to this apparently isolated focus of *P. malariae–P. vivax*.

MATERIALS AND METHODS

The centre for our collection of specimens was the Franciscan Mission Cutiverini, located in southeastern Peru at the confluence of the Rio Ene with the Rio Cutiverini. The Rio Ene is a tributary of the Rio Tambo, which empties into the Rio Ucayali; this river in turn joins the Amazon below Iquitos.

The population at the mission is composed of a culturally primitive group of Amerindians belonging to the group called " Campas " in Peru. Culturally, these people are nomadic hunter-gatherers, dependent on large areas of jungle for their livelihood (4). Since their area is being converted to agricultural use (5), they are being forced into a more settled life. The group sampled had lived at the mission for about 3 years.

The first samples were collected in June 1973, when thick and thin smears were prepared from blood specimens from 123 persons. The slides were sent to the National Repository for Malaria Slides, Parasitology Division, Center for Disease Control (CDC), Atlanta, Georgia, USA, where they were Giemsa stained and carefully examined for the presence of malarial parasites and identification of the species. Since the possibility of zoonotic malaria had to be considered, a representative sample of the slides containing *P. malariae* parasites were submitted to the Primate Malaria Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Chamblee, Georgia, USA. There they were examined for differentiation of *P. malariae* and *P. brasilianum*.

The population was sampled again in September 1973, about 1 month after the rainy season had begun. Because a high prevalence of malaria was found in the first sampling, all mission personnel were given 500 mg of chloroquine on a 2-day basis 2 weeks before the second sampling. At the second sampling, serum samples for antibody determination as well as blood for films were obtained from 127 persons. These blood films were also examined at the CDC. The sera were tested in the Immunfluorescence Laboratory, Parasitology Division, CDC, according to the method of Sulzer et al. (6).

RESULTS

Results of slide examination of the specimens collected in the two surveys are shown in Table 1. In the first sampling 79 (64%) of 123 persons sampled had malaria parasites in the peripheral blood; *P. malariae* alone was identified in 66 of these. The proportion of *P. malaria* infections to patent parasitaemias was, therefore, 83.5%.

In the second sampling, made about 3 months after the first and 2 weeks after chloroquine had been administered, 109 (86%) of 127 persons were found to have patent parasitaemia. Of the 109 persons with parasitaemia, 106 (97.2%) showed *P. malariae* infection. Between the first and second sampling, the percentage of parasitized individuals (as deter-

Table 1. Number of persons with malaria parasites in blood smears by malaria species and proportion of each species to total positives. Two samplings of a population of primitive people at the Mission Cutiverini, southeastern Peru, 1973

Species found	First sample (June 1973)		Second sample (September 1973)	
	number	proportion of total positives	number	proportion of total positives
Negative	44 (35.8 %)		18 (14.2 %)	
P. malariae	66 (53.7 %)	66/79 (83.5 %)	106 (83.5 %)	106/109 (97.2 %)
P. vivax	8 (6.5 %)	8/79 (10.1 %)		
P. malariae + *P. vivax*	4 (3.3 %)	4/79 (5.1 %)	1 (0.8 %)	1/109 (0.9 %)
Plasmodium spp.	1 (0.8 %)	1/79 (1.3 %)	2 (1.6 %)	2/109 (1.8 %)
Total	123 (100 %)		127 (100 %)	
Total positive	79 (64.3 %)		109 (85.8 %)	

Table 2. Titres on 127 sera in the immunofluorescence test for malaria with antigens of *P. vivax* and *P. brasilianum* compared with *P. falciparum* antigen

P. vivax— *P. brasilianum* [a]	*P. falciparum*							
	<1:4	1:4	1:16	1:64	1:256	1:1024	1:4096	total
<1:4	1							1
1:4								
1:16								
1:64		1						1
1:256	2	3	1	4				10
1:1024	1	1	2	19	17	3		43
1:4096		2	3	22	21	17	1	66
1:16 384			1		4	1	1	7
Total	4	7	7	45	42	21	2	128

[a] The titre indicated is the highest titre obtained with either one or both of these species. The two antigen species were tested separately.

mined by positive blood smears) rose from 64 to 86 and the proportion of *P. malariae* cases increased from 83% to 97%.

No evidence of *P. falciparum* was found on any of the slides. Because of the propinquity of wild primates to the human habitations and because of the well-known similarity of *P. malariae* to *P. brasilianum*, the possibility of the latter species infecting man was considered. A representative sample of the slides found to contain *P. malariae* were examined for the presence of *P. brasilianum*. The organisms were confirmed as *P. malariae*; no *P. brasilianum* were found.

Results of immunofluorescence tests for malaria antibody are shown in Tables 2 and 3. Table 2 shows that in only 3 of 127 sera did antibodies against *P. falciparum* equal antibodies against one or both of the other two species; one serum did not react with any of the three antigens. Antibody titres with either *P. vivax* or *P. malariae* or both were $\geqslant 1:256$ with 125 of the 127 sera in the sample.

When reactions with *P. vivax* antigen were compared with those with *P. malariae* antigen (Table 3), the titres were found to be almost equal. The titres were identical in 62 cases; in 34 the titre with *P. vivax* was greater than that with *P. malariae* and

Table 3. Titres of 127 sera in the immunofluorescence test for malaria using antigens of *P. vivax* and *P. brasilianum* (= *P. malariae*)

P. brasilianum (= *P. malariae*)	*P. vivax*								
	<1:4	1:4	1:16	1:64	1:256	1:1024	1:4096	1:16 384	total
<1:4	1				3	2	2		8
1:4						1			1
1:16				1		1			2
1:64					1				1
1:256	1				5	8	4		18
1:1024				1	4	26	9		40
1:4096						23	28	3	54
1:16 384							4		4
Total	2			2	13	61	47	3	128

in 31 the titres were 4-fold greater with *P. malariae*. The titres were, however, 16-fold greater with *P. vivax* in 13 instances and with *P. malariae* in only two. The general equality of titres is also reflected in geometric mean titres with the two antigens: these were 1 : 959 with *P. malariae* and only slightly greater, 1 : 1383, with *P. vivax*. These values include results on sera that failed to react.

DISCUSSION

The malaria in this population was brought to our attention when the padre in charge of the mission became ill and was brought to the hospital in Lima, where his illness was diagnosed as malaria. He reported that the population at his mission had similar symptoms, including the typical chills and fever of malaria. The project described was therefore planned and carried out to determine the extent of the problem.

The situation at Cutiverini was well suited to the use of both stained blood smears and seroepidemiology for determining the extent of malaria infection. Our results are an excellent illustration of the way in which the two methods can complement each other, especially when the species present can be determined by serology.

Slide examination revealed at least four interesting points:

Firstly, it was immediately apparent that this population is a focus of highly endemic malaria. Because of the limitations of time we collected specimens for slide examination at both samplings on a single day. If we had collected the specimens over several consecutive days we would probably have observed an increase in the percentage of persons with parasitaemia in the two samplings. The 106 cases of *P. malariae* infection found in the second sampling is more than three times the number of *P. malariae* cases reported for the whole of Peru in 1972.[a]

Secondly, the malaria situation at Cutiverini seems to be unique in that *P. malariae* accounted for 83% of the malaria cases detected and *P. falciparum* was not present on any slides examined. The proportion of *P. malariae* cases compared to the total number of cases found in the first sampling is one of the greatest, if not the greatest, ever reported for a population before the introduction of malaria control measures. Knowles & White (3), in a report published before the widespread attempts to control malaria, cited only four areas where *P. malariae* accounted for more than 50% of the detected cases. The area with the greatest proportion was a locality in Java, with 72% of the cases caused by *P. malariae*. *P. falciparum* was not totally absent from the slide specimens from any of these areas. Reports since that time indicate that the situation has not changed (7).

Thirdly, after chloroquine was administered to the residents of Cutiverini, the percentage of individuals with parasitaemia actually increased to about 86% (Table 1). Furthermore, since 106 of the 109 slide-positive individuals detected in the second sample had *P. malariae* only, the proportion of this species increased to 97%. The increase in the percentage of the population with parasitaemia in the face of drug administration might be due to the advent of the rainy season in August and the probable consequent increase in transmission. The almost complete disappearance of *P. vivax* and the much lighter parasitaemias in the second sampling as compared to the first was, however, striking. This might have been due to the introduction of chloroquine, to an immune response in the population, or to the interaction of these two factors. The relatively few cases of *P. vivax* infection found before the drug was administered could also be attributed to the immune response being more effective against this species than against *P. malariae*.

Fourthly, individual experience with malaria may be reflected by results of such serological procedures as the indirect immunofluorescence test for malaria antibodies. This test reflects better than stained blood slides the history of malaria in an area (8). In places such as Cutiverini, where control measures are lacking or have just been initiated, the results of the indirect immunofluorescence test probably reflect better than stained blood slides the present status of malaria. In people subject to exposure to malaria for their entire lives, very high antibody titres may be encountered, even in the absence of patent parasitaemia (9, 10, 11). In tests of sera from persons with their first attack of malaria or with short exposure periods to malaria, the greatest titre was found to be with the species found on the slide (12). We believe, therefore, that if a serum reacts at a greater titre with one antigen species than with the others tested, the subject is probably infected with the species giving the greatest reaction. Applying this view to the results shown in Table 2,

[a] Status of malaria eradication in the Americas. Twenty-first report to the XXII meeting of the Directing Council, Pan American Health Organization/XXV meeting of the Regional Committee of the World Health Organization, 1973. Unpublished document CD22/4.

it is probable that in our sample as many persons were infected with *P. vivax* as were infected with *P. malariae*, since 34 sera reacted at four-fold or greater dilutions with *P. vivax* than with *P. malariae*, and 31 specimens reacted at greater levels with *P. malariae* than with *P. vivax*. Of the remainder, 61 sera reacted equally with the two antigens, indicating the possibility of infection with both species; one serum sample failed to react with either antigen. The possibility that the observed differences in reactivity might be an artifact due to a more reactive *P. vivax* antigen, as compared to *P. malariae* antigen, is ruled out by continual checks of the two antigens with sera from known infections. In these trials the validity of speciation with the antigens used was confirmed.

The failure to find *P. falciparum* on any of the slides and the conclusion that this species was absent in the population is also supported by serological results (Table 3). In only three cases was the titre with *P. falciparum* not surpassed with either *P. vivax*, or *P. malariae*, or both. In most cases titres with the other two antigens were at least 16-fold higher than that with *P. falciparum*. Since in three cases the titre with *P. falciparum* equalled that of the other two antigens, there is a slight possibility that *P. falciparum* was present; we believe, however, that if it had been present, this highly malignant species would have appeared on at least a few of the slides examined. This species seems to be absent from this population but, unless malaria control measures are taken, it may be introduced with disastrous results.

The finding of this highly endemic focus of malaria in an area reported to be malaria free in 1972 [a] emphasizes the problems posed in malaria eradication and control in isolated groups of Amerindians in Amazonia. Gabaldon (13) discussed this problem very ably and at length. He recognized that the cooperation of such populations was both essential and difficult to obtain. It was only with the help of the mission padre, the Rev. William Brown, whom the population at Cutiverini respected and trusted, that we were able to make this survey. It is only through such individuals, who have convinced the local populations of their goodwill and sympathy, that such surveys are likely to succeed. If this cooperation is to continue during follow-up surveys, the persons who do the work must also demonstrate their willingness to work to the advantage of the local people.

The reasons for the high proportion of *P. malariae* in this focus are not clear. As noted, the hosts' immune responses may have successfully suppressed the *P. vivax* infections in most cases below patent levels. Three of the major conditions postulated by Garnham (1) and Coatney et al. (14) for a focus of *P. malariae* are met at Cutiverini. These are: a primitive culture; a possible simian reservoir; and an environment favourable to the vector. The culture is certainly primitive, with people living under conditions of constant exposure to vectors. Dwellings are little more than bamboo platforms with thatched roofs under which sleeping hammocks are hung; the whole life of the individual is spent essentially out of doors. The animal reservoir, although a possible one is present in the local monkeys, is probably not important. The number of patent cases was so great that man-to-man transmission can keep the cycle going. Furthermore, examination of blood films revealed no resemblance of the parasites to *P. brasilianum*. It might be interesting, however, to examine some of the local simians to determine whether they might have been infected from man, since South American monkeys have been found to be susceptible to both *P. malariae* and *P. vivax*. The vector is certainly favoured by the local climate. Even in the dry season, water in the streams and reservoirs in the surrounding jungle is sufficient to ensure their continued breeding. The location, about 15 degrees south of the equator and at an elevation of less than 1000 m, ensures that the temperature never drops to levels inhibitory to the mosquitoes. Exposure to mosquitos is enhanced by activities in the shady fields of bananas and in the surrounding jungle. Clearing of some of the jungle may displace mosquito species normally found in the canopy and introduce them into new habitats in the houses and cultivated fields.

Determining whether other such isolated populations with a high proportion of *P. malariae* and *P. vivax* infections exist in South America would increase our understanding of the epidemiology of malaria. These populations should exist if, as some believe (15), these two species were present in South America before the 15th century. This would account for the absence of *P. falciparum* in the population at Cutiverini. Because of the isolation of this group there might not have been sufficient time and opportunity for *P. falciparum* to have diffused from

[a] Status of malaria eradication in the Americas. Twenty-first report to the XXII meeting of the Directing Council, Pan American Health Organization/XXV meeting of the Regional Committee of the World Health Organization, 1973. Unpublished document CD22/4.

imported European or African cases to such isolated groups. A study of similar isolated populations to determine if other foci of *P. vivax–P. malariae* exist in Amazonia is desirable. If such populations are common, and if *P. falciparum* is absent, a study of the related epidemiology might provide new information on the disease.

The unusual situation at Cutiverini seems to merit further study, including determination of the vector species present and the parasites in the non-human primates. Further studies on the human population are in progress, including the collection of more blood for slides and of more sera for immunological studies. The population is now being treated for malaria and control with antimalarial drugs and insecticides is being instituted.

RÉSUMÉ

UN FOYER D'INFECTION HYPERENDÉMIQUE À *PLASMODIUM MALARIAE* ET *P. VIVAX*, SANS *P. FALCIPARUM*, DANS UNE POPULATION PRIMITIVE DE LA JUNGLE AMAZONIENNE DU PÉROU. ÉTUDES PAR IMMUNOFLUORESCENCE ET EXAMEN D'ÉTALEMENTS DE SANG

Un foyer d'infection hyperendémique à *Plasmodium malariae* et *P. vivax* a été découvert en 1973 sur les hauteurs du bassin de l'Amazone, dans la partie sud-est du Pérou. La population de cette région, naguère encore isolée dans un environnement de jungle, est une population primitive qui sort à peine du stade où l'homme vit de chasse et de cueillette. L'enquête s'est faite par examen d'étalements de sang colorés et par épreuves d'immunofluorescence. Sur 127 sujets examinés, 109 étaient porteurs de parasites du paludisme. Pour 106 d'entre eux, l'infection était à *P. malariae*, pour un d'entre eux il s'agissait d'une infection mixte à *P. malariae* et *P. vivax* et chez les deux autres, le parasite a été identifié comme étant *Plasmodium* sp. Aucun étalement ne contenait de parasites de l'espèce *P. falciparum*. Les épreuves d'immunofluorescence ont révélé que chez 126 sujets les titres d'anticorps dirigés contre *P. malariae* ou *P. vivax* ou les deux étaient égaux ou supérieurs à 1: 256; les réactions à l'antigène de *P. falciparum* se situaient à un niveau beaucoup plus as. L'absence de *P. falciparum*, révélée à la fois par l'examen des étalements de sang et par les épreuves sérologiques, est un phénomène sans précédent dans un foyer de paludisme hyperendémique. Le tableau du paludisme dans cette population (présence de *P. malariae* et *P. vivax* et absence de *P. falciparum*) est celui que l'on suppose avoir existé dans le nouveau monde avant l'arrivée de Christophe Colomb. Cette constatation corrobore donc l'hypothèse que le paludisme existait en Amérique du Sud avant le quinzième siècle. La découverte de ce foyer de paludisme hyperendémique dans une région où l'on pensait que la maladie avait été jugulée souligne les difficultés que présentent les enquêtes paludologiques dans des groupes primitifs isolés. L'enquête n'a été possible que parce que ce groupe s'était récemment installé auprès d'une mission. Il paraît indiqué d'entreprendre dans la même région des études plus poussées sur cette population et sur d'autres, ainsi que sur les vecteurs et les réservoirs animaux possibles, afin de pouvoir déterminer l'étendue de ce foyer d'infection paludéenne et son origine.

REFERENCES

1. GARNHAM, P. C. C. Malaria parasites and other haemosporidia. Oxford, Blackwell, 1966.
2. COATNEY, R. G. ET AL. *American journal of tropical medicine and hygiene*, **20**: 795-803 (1971).
3. KNOWLES, R. & WHITE, R. S. *Indian medical research memoirs*, No. 18 (1930).
4. GUTIERREZ MERE, L. Metodologia de equipamiento para la Communidades Campas del Rio Ene. Tesis de Bachiller de Arquitectura, Universidad Particular Ricardo Palma, Miraflores, Lima, Peru, 1972.
5. PONCE DE LEON, J. ET AL. Peru, challenge and response. Lima, Publicaciones Continente, 1972.
6. SULZER, A. J. ET AL. *American journal of tropical medicine and hygiene*, **18**: 199-205 (1969).
7. ONORI, E. *Bulletin of the World Health Organization*, **37**: 330-331 (1967).
8. BRUCE-CHWATT, L. ET AL. *Lancet*, **2**: 547-551 (1973).
9. VOLLER, A. ET AL. *Journal of tropical medicine and hygiene*, **74**: 45-52 (1971).
10. COLLINS, W. E. ET AL. *American journal of tropical medicine and hygiene*, **20**: 199-205 (1971).
11. SULZER, A. J. & WILSON, M. *Bulletin of the World Health Organization*, **45**: 375-379 (1971).
12. GLEASON, N. N. ET AL. *American journal of tropical medicine and hygiene*, **20**: 10-13 (1971).
13. GABALDON, A. *American journal of tropical medicine and hygiene*, **21**: 634-639 (1972).
14. COATNEY, R. G. ET AL. The primate malarias. Bethesda, Maryland, US Department of Health Education and Welfare, 1971.
15. BRUCE-CHWATT, L. J. *Bulletin of the World Health Organization*, **32**: 363-387 (1965).