

## RELAPSING *PLASMODIUM VIVAX* MALARIA WITH ATYPICAL PARASITE FORMS AND PHAGOCYTOSIS BY PERIPHERAL NEUTROPHILS

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### Summary :

A case of atypical *Plasmodium vivax* malaria is presented. The clinical follow-up has allowed to characterize three consecutive malaria clinical episodes within one year. At the first attack, 39 % of the infected red blood cells were parasitized by gametocytes. Furthermore, rare crisis forms, exceptional « pseudo-parthenogenesis » forms, a few equatorial trophozoites, malaria pigment-containing leucocytes and phagocytized parasites were also found in the thin blood smears. At the second malaria episode, morphological aspects were quite similar, but the gametocyte percentage decreased and that of the equatorial trophozoite forms increased. Only at the third attack, was the morphology typical of *P. vivax*. The *Plasmodium* species and the absence of mixed infection were unequivocally confirmed using polymerase chain reaction. Atypical strains of *P. vivax* are relatively frequent. Nevertheless, to our knowledge, neither so high a gametocyte percentage, nor extensive *P. vivax* peripheral phagocytosis were previously reported.

**KEY WORDS :** *P. vivax*, atypical morphology, peripheral phagocytosis.

### Résumé : RECHUTES DE PALUDISME À *PLASMODIUM VIVAX* AVEC FORMES PARASITAIRES ATYPIQUES ET PHAGOCYTOSE PAR LES NEUTROPHILES PÉRIPHÉRIQUES

Nous présentons un cas d'infection palustre à *Plasmodium vivax* atypique. Le patient a contracté 3 accès palustres en 1 an. Lors du premier accès, les gamétocytes représentaient 39 % des formes parasitaires intra-érythrocytaires. Parallèlement on observait de rares formes de pseudo-parthénogénèse, quelques trophozoïtes en plaque équatoriale et des leucocytes renfermant soit du pigment malarique, soit des formes parasitaires. Lors du second accès, les aspects morphologiques parasitaires étaient identiques à ceux décrits précédemment. Cependant le pourcentage de gamétocytes était diminué tandis que celui des trophozoïtes en plaque équatoriale était augmenté. Lors du dernier et troisième accès palustre, la morphologie parasitaire était enfin typique de *P. vivax*. Le diagnostic d'espèce de *Plasmodium* et l'absence d'infection multiple ont été confirmés par PCR. Les souches de *P. vivax* atypiques sont relativement fréquentes. Cependant à notre connaissance, c'est la première fois qu'un pourcentage aussi élevé de gamétocytes est rapporté lors d'une infection à *P. vivax* et qu'une phagocytose périphérique des différentes formes parasitaires intra-érythrocytaires est notée pour cette espèce de *Plasmodium*.

**MOTS CLÉS :** *P. vivax*, morphologie atypique, phagocytose périphérique.

## INTRODUCTION

**A**mong human malaria species, *Plasmodium falciparum* is the most fearsome pathogen, whereas *P. vivax* has the most widespread

geographical distribution. Indeed, *P. vivax* can develop in female *Anopheles* from a temperature of 16 °C, instead of 20 °C for *P. falciparum* (Brumpt, 1949). So *P. vivax* is in some countries the most common malaria pathogen (Prasad *et al.*, 1990). The first case of chloroquine resistance by *P. vivax* from Papua-New Guinea was described in 1989 (Rieckmann *et al.*, 1989), and similar cases have been described from other parts of the world (Baird *et al.*, 1995). Only one case of resistance to mefloquine has been reported (Amor *et al.*, 1992).

In this article, we present an unusual case of *P. vivax* studied on three successive clinical malaria episodes which developed within one year, after return to France from an endemic country. Atypical parasite forms and numerous peripheral phagocytized parasites were observed in the thin blood smears. The microscopic identification was confirmed using polymerase chain reaction (PCR).

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## CASE REPORT

### CLINICAL COURSE

Mr JC, a 26-year-old French man, travelled in French Guyana from March to June 1991, and served in the army in Djibouti for two years (from October 1992 to the 9th of October 1994). During his stays in French Guyana and in Djibouti, he took malaria chemoprophylaxis (chloroquine = Nivaquine<sup>®</sup>, 1 tablet of 100 mg per day), daily and for 40 days after his return to France.

On the 8th of January 1995, he developed a fever attack of 40 °C and was admitted to the general hospital of Valenciennes. The clinical examination revealed an enlarged spleen. Blood counts showed thrombocytopenia ( $40,000 \times 10^6$  platelets/L). Blood smears showed a malaria infection. The patient was treated with mefloquine (Lariam<sup>®</sup>: 6 tablets of 250 mg, in 3 doses on the same day), which brought about the resolution of the fever and an increase of platelets count.

Four and half months later, Mr JC had several fever peaks and was again admitted to the hospital on May 27, 1995. Blood counts were normal except thrombocytopenia ( $44,000 \times 10^6$  platelets/L), and blood smears showed again a malaria infection. The clinical episode was managed with chloroquine treatment (900 mg the first day, 300 mg the second and third days). The parasitaemia became undetectable by microscopy 3 days after initiation of treatment.

Seven months later, he had a fever attack (38.3 °C) which remained high for 36 hours. On the 10th of January 1996, blood counts were normal and thin blood smears showed another malaria relapse. Cure was obtained by mefloquine treatment, as above.

### MICROSCOPIC OBSERVATIONS

Blood samples were examined the January 8, 1995, the May 27, 1995 and the January 10, 1996. Five ml of whole blood were obtained by venepuncture into one tube containing EDTA as anticoagulant. Thin blood smears were stained with 10 % Giemsa for 20 min.

In the thin blood smears of January 8, 1995, parasitaemia was very low ( $5,922 \times 10^6$  infected corpuscles/L = 0.14 %). All parasite stages were observed: numerous trophozoites and gametocytes, and rare schizonts. Moreover, multiply-infected red blood cells (RBC), malaria pigment-containing leucocytes and some phagocytized parasites were observed (Fig. 1). Indeed fifty-five point six percent of infected RBC were invaded by trophozoites and measured between 6.5 and 9 µm (mean 7.5 µm). Fifty-five point six percent of infected RBC were invaded by one trophozoite,

1.5 % by two trophozoites, or rarely by three trophozoites. Trophozoites were polymorphous (Fig. 1): *i*) their size ranged between 1.5 and 4 µm (mean 2.5 µm), *ii*) few of them showed an equatorial plate morphology, *iii*) others were binuclear trophozoites. Schüffner's dots were not observed in most of the RBC parasitized with trophozoites (Fig. 1). A few schizonts were observed in these smears: they were ruptured mature forms, containing five to sixteen merozoites with malaria pigment (Fig. 1). Thirty-nine percent of the infected RBC were invaded by gametocytes. Their size ranged between 7 and 12 µm (mean 9 µm) and gametocytes measured between 6 and 10 µm (mean 7.5 µm). Rare « pseudo-parthenogenesis » forms were also observed (Fig. 1); these forms correspond to simultaneous infections of a same RBC with an asexual form and a sexual one (Poirriez *et al.*, 1991, 1996). Furthermore, numerous malarial pigment-containing neutrophils and monocytes were detected by microscopy, especially under polarized light (Lawrence & Olson, 1986) (Fig. 1). Phagocytized parasites were also observed (Fig. 1). Eight percent of the neutrophils (83 % of total leucocytes) contained haemozoin, parasites (trophozoites, schizonts, gametocytes or merozoites) (4 %), whereas only 3 % of the monocytes (6 % of total leucocytes) contained parasite material.

On May 27, 1995 (first relapse), parasitaemia was low ( $14,620 \times 10^6$  infected corpuscles/L = 0.34). Ninety point two percent of infected RBC were invaded by trophozoites, they measured between 6 and 9 µm (mean 8 µm). Eighty-four percent, 5.5 and 0.7 % of the infected RBC were invaded by one, two or three trophozoites respectively. The trophozoites measured between 2 and 4 µm (mean 3.5 µm). They were also polymorphous and a high proportion of equatorial forms (10 % of trophozoites) was observed (Fig. 1). The rare mature schizonts contained five to eight merozoites. Nine point six percent of infected RBC were invaded by gametocytes. Their diameter ranged between 6 and 10 µm (mean 8.5 µm). Gametocytes measured between 5 and 8 µm (mean 7 µm) (Fig. 1). As in the first clinical malaria episode, few malaria pigment-containing leucocytes and phagocytized parasites were also present. No « pseudo-parthenogenesis » forms were observed.

On January 10, 1996 (second relapse), parasitaemia was the lowest ( $0.8 \times 10^6$  infected corpuscles/L = 0.017 %). In this third episode, 87.5 and 12.5 % of infected RBC were invaded respectively by trophozoites and gametocytes. No schizonts were observed in these blood smears. A typical *P. vivax* morphology was found: enlarged and pale parasitized RBC, amoeboid shape of trophozoites, relative late development of Schüffner's dots. Only rare RBC were invaded by two trophozoites or binuclear trophozoites.

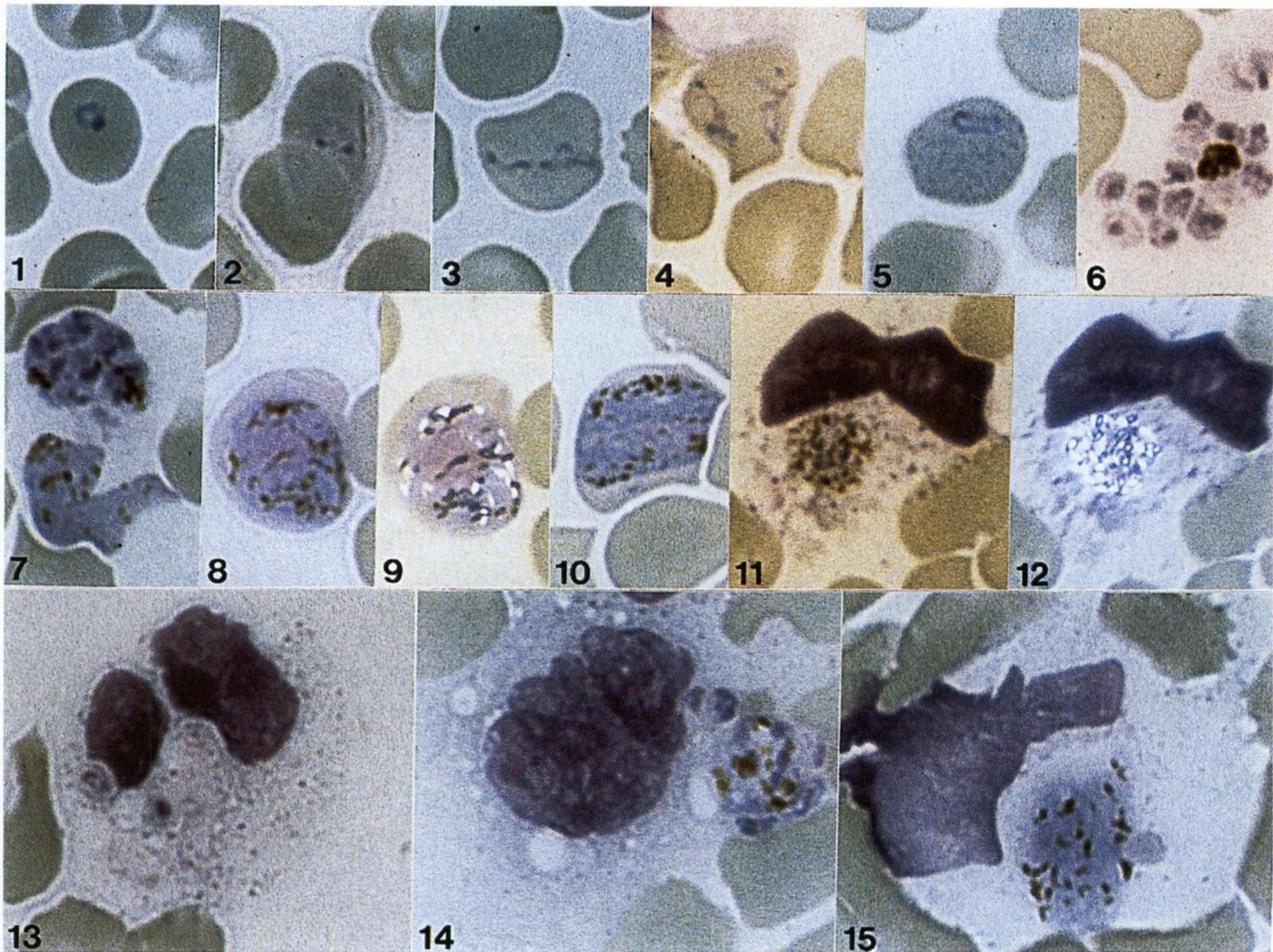


Fig. 1. Atypical forms of *P. vivax*, and peripheral phagocytosis. Admission thin blood smears at the first clinical malaria episode and at the first relapse.

1-5: ring and trophozoite forms; 1: *P. falciparum*-like ring; 2: *P. falciparum*-like ring with two chromatin dots; 3: equatorial plate trophozoite (« band form »); 4: two amoeboid trophozoites infecting one red blood cell; 5: trophozoite with Schüffner's dots; 6: ruptured schizont releasing 15 merozoites and malarial pigment; 7: simultaneous gametocyte-schizont infection of a red blood cell, so-called « pseudo-parthenogenesis »; 8: a macrogametocyte observed with normal light; 9: the same macrogametocyte observed under polarized light; 10: equatorial plate microgametocyte; 11: malaria pigment-containing neutrophil, observed with normal light; 12: the same malaria pigment-containing neutrophil, observed with polarized light; 13: phagocytized merozoites; 14: phagocytized schizont; 15: phagocytized gametocyte (Original magnification  $\times 1,000$ ).

### PLASMODIUM DNA AMPLIFICATION

A whole blood sample harvested during the first and the third clinical malaria episodes was taken to perform a nested-PCR on the small subunit ribosomal DNA sequences, as previously described (Snounou *et al.*, 1993). Only one amplified product of 120 bp, specific of *P. vivax*, was detected in the two samples.

### DISCUSSION

We present here a case of *P. vivax* malaria with three clinical episodes occurring over a period of one year. In the first two clinical episodes, parasite morphology was atypical, and

phagocytosis of whole *P. vivax* parasites by neutrophils was described for the first time.

At the first malaria clinical episode, the microscopic diagnosis of the *Plasmodium* species was somewhat difficult because of the atypical morphology of the parasites. Indeed, a mixed infection could not be eliminated. Multiply-infected RBC, small and/or binuclear rings (Fig. 1) were observed, suggesting the presence of *P. falciparum* parasites mixed with *P. vivax*, and leading to mefloquine treatment. A concurrent infection with *P. malariae* was also suspected because of the presence of trophozoites in equatorial plate (« band forms ») and of small schizonts containing five to eight merozoites. The definitive identification of the *Plasmodium* species was achieved through the use of a highly sensitive and specific nested PCR, able to

detect less than ten parasites, to identify the four human *Plasmodium* species and therefore to detect mixed malaria infections (Snounou *et al.*, 1993). The only detected *Plasmodium* species was *P. vivax*. The PCR assay has clearly allowed to identify the parasite species and to exclude unequivocally a mixed infection diagnosis, which was suspected by routine microscopy.

At the second malaria episode (first relapse), the parasites found in the thin blood films were morphologically quite similar to those seen during the first attack. Unfortunately, a blood sample had not been taken for PCR assay. Although *P. falciparum*-like and *P. malariae*-like blood forms were observed, the diagnosis of *P. falciparum* and *P. malariae* was considered most unlikely: this second attack occurred eight months after the return of the patient to France, about five months after the first attack which was adequately treated with mefloquine and in which the PCR assay detected only *P. vivax*. The presence of *P. ovale* was also excluded because the morphological features of this species were absent (Poirriez *et al.*, 1991).

At the third episode (second relapse), the diagnosis of *P. vivax* infection was firmly established by microscopy on the basis of the typical blood parasite morphology, and confirmed by PCR.

A high degree of polymorphism has long been known in *P. vivax* (as multiply-infected RBC, crisis forms, « pseudo-parthenogenesis », or scarce Schüffner's dots) and has been reviewed recently (Prasad *et al.*, 1990; Poirriez *et al.*, 1991, 1995). In the present case, *P. vivax* was considered atypical partly because of the high gametocyte percentage detected at the first clinical malaria episode. Indeed, to our knowledge, so high a gametocyte percentage (39 %) has never been reported in *P. vivax*. It is interesting to note that the typical *P. vivax* morphology was only observed at the second relapse, one year after the first clinical malaria episode. Usually, atypical strains became typical throughout the course of a clinical attack or during a relapse (Poirriez *et al.*, 1991).

Malaria chemoprophylaxis prevents neither malaria infection, nor a relapse of *P. vivax* or *P. ovale*; it is only aimed at preventing the first clinical manifestations. After the prophylaxis ceases, relapses can be observed at varying times depending on the parasite strain. In the present case, the *P. vivax* strain was chloroquine sensitive and could be considered as a II-III intermediate type, in regard to the WHO classification (Garnham *et al.*, 1975).

At the first clinical malaria episode, less so during the second attack, numerous leucocytes contained malaria pigment (haemozoin), which is produced by *Plasmodium* sp erythrocytic parasites as an end product of haemoglobin digestion. In addition, numerous neu-

trophils and some monocytes were also found to have phagocytized various stage parasites (trophozoites, schizontes, gametocytes and merozoites). Pigment-containing leucocytes are more frequently observed in the peripheral blood of patients with severe malaria, where high percentage of neutrophils containing malarial pigment could reflect acute hyperparasitaemia (Metzger *et al.*, 1995). In severe *P. falciparum* malaria, a count of peripheral neutrophils containing visible pigment > 5 % would predict a fatal outcome with higher sensitivity and specificity than parasitaemia (Hoan Phu *et al.*, 1995). To our knowledge, the significance of the level of peripheral pigment-containing neutrophils has not been investigated in *P. vivax* infections. In our case, the high percentage of these cells recorded, especially at the first clinical episode, was not correlated with a bad prognosis.

One century ago, Golgi called attention to circulating leucocytes containing not only pigment, but also malarial organisms, either well preserved or in various stages of desintegration (Taliaferro & Mulligan, 1937). Nevertheless, he noticed that the extent of phagocytosis in the peripheral blood was incomparably less than that occurring in the spleen, the liver and the bone marrow. Several years later, Taliaferro et Mulligan (1937) reported that phagocytic neutrophils are rarely encountered in human malaria either in the peripheral blood or in the internal organs, except occasionally in cases of extremely acute pernicious malaria. More recently, a peripheral phagocytosis by only monocytes of whole parasites in *P. falciparum* malaria, was reported in three patients living in Gabon (Vernes *et al.*, 1980). Our unusual observation indicates that leucocytes, especially neutrophils, are able to engulf not only *P. falciparum* parasites in cases of pernicious malaria but also whole *P. vivax* parasites without predicting a bad clinical evolution.

In conclusion, atypical strains of *P. vivax* are relatively frequent (Prasad *et al.*, 1990; Poirriez *et al.*, 1991, 1995). The most atypical features of the *P. vivax* strain described here are the following: *i*) a high gametocyte percentage detected at the first clinical malaria episode, *ii*) an important percentage of leucocytes containing malarial pigment at the first malaria episode, *iii*) a *P. vivax* marked peripheral phagocytosis at the first and the second malaria episodes.

## ACKNOWLEDGEMENTS

We thank Richard Torka for his valuable technical assistance, and Gérard Vinchent for the excellent photography reproduction.

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Reçu le 27 mars 1997  
 Accepté le 12 juin 1997