Case Report

Plasmodium ovale malaria in Brazil: report of an imported case with a prolonged incubation period

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Abstract

We report the first case of imported *Plasmodium ovale* in Brazil, confirmed using both conventional microscopy and PCR-based protocols. The patient was a 36-year-old Brazilian male who had been working as a miner in Cabinda Province, Angola. Based on his travel history, the parasite was dormant for at least two years. The relatively long period of incubation of *P. ovale* may obscure the link between exposure and disease. The recent increase in the number of people travelling to regions where *P. ovale* is endemic, suggests that a PCR-based protocol should be included as a complementary tool for malaria reference laboratories.

Key words: Malaria*; Plasmodium ovale;* long incubation period

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Introduction

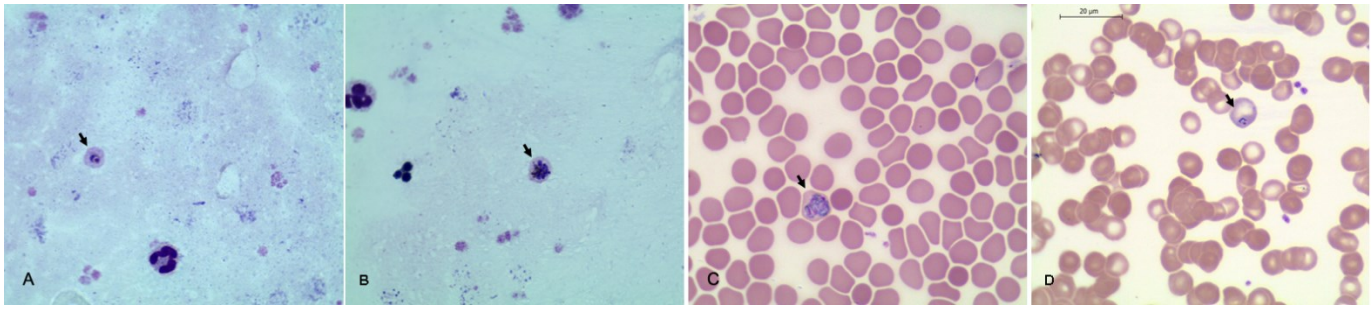
Five parasite species cause human malaria. *Plasmodium vivax* and *Plasmodium falciparum* are the most prevalent and are frequently associated with morbidity and fatalities. The geographic occurrence of *Plasmodium malariae* infection is reported as being sporadically distributed, but it has been observed in all major malaria-endemic regions. The simian parasite *Plasmodium knowlesi*, recently described in human infections, has been responsible for a large number of malaria hospitalizations in Asia [1]. *Plasmodium ovale* was thought to have a more limited distribution, with endemic transmission traditionally described as limited to tropical Africa, Middle East, Papua New Guinea, Burma, West Papua, and Indonesia [2]. Imported *P. ovale* malaria cases have been sporadically described in other areas. The low prevalence of this parasite, combined with its morphological similarity to *P. vivax*, has led to this type of malaria being overlooked. Consequently, ovale malaria is often misdiagnosed [3]. In patients returning from ovale-endemic countries, additional challenges include: (i) potentially long period of incubation (median of 70 days, but there are some reports of several years of incubation), (ii) low parasite densities, and (iii) low sensitivity of all rapid diagnostic tests (RDTs) currently available [4, 5].

In Brazil, 38 cases of *P. ovale* malaria have been recorded, showing a gradually increasing trend in recent years [6]. None of the reported cases was confirmed by molecular methods. Here, a case of *P. ovale* infection with a prolonged incubation period confirmed by parasitological and molecular diagnosis is reported.

Case Report

In June 2011, a 36-year-old male was admitted to hospital in Uberlândia, in Minas Gerais state, Brazil, with a fever of eight days duration. He was a Brazilian resident in the city of Patrocínio, Minas Gerais, who had worked as a miner in Cacongo and lived in Buco Zau, two villages situated in Cabinda Province, Angola, on the African continent. He had contracted malaria in Angola in December 2008 and was treated with anti-malarial drugs, but he could not remember the type of malaria nor the therapeutic treatment

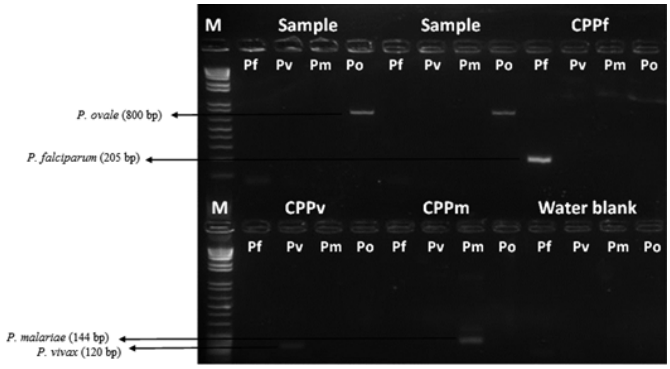
Figure 1. Giemsa-stained thick blood smear (x 1000): **(A)** Immature trophozoite with thick, compact cytoplasm and prominent circular nucleus (arrow), **(B)** Schizont of *P. ovale* with a few merozoites (arrow). Wright-Giemsa-stained thin blood smear (x 1000): **(C)** Early schizont of *P. ovale* (arrow). **(D)** *P. ovale* ring with sturdy cytoplasm and large chromatin dot (arrow).



applied. The patient was not administered antimalarial chemoprophylaxis during the time he remained in Africa. He returned to Brazil in January 2009, but went back to Cabinda in April 2009, where he remained until June 2009, before establishing permanently in Brazil.

On admission to hospital, his body temperature was 39°C. Results of clinical examinations were within normal parameters. A routine hemogram detected low grade anemia (hemoglobin 12.7 g/dl) without thrombocytopenia (182,000/mm^3). Other hematologic values were in the normal range. Values of the enzymes alkaline phosphatase (79.9 U/L), gamma-glutamyl transferase (25.9 U/L), g-oxaloacetic transaminase (34.7 U/L), and g-pyruvic transaminase (21.0 U/L) were also in the normal range. Thick and thin blood smears were prepared according to WHO recommendations [7]. Using conventional microscopy, rings and schizonts highly suggestive of *P. ovale* (Figure 1) were observed. The parasite density was 425 parasites/µl in the thick blood smear. The patient was treated with chloroquine (1.5 g for three days) and primaquine (30 mg daily for 7 days) to prevent relapse. No *Plasmodium* stages were detected on the follow-up of 60 days of the peripheral blood smear.

Figure 2. *Plasmodium* species-specific nested PCR with samples from positive controls for *P. falciparum* (CPPf), *P. vivax* (CPPv), and *P. malariae* (CPPm), with the patient sample in duplicate (sample). The black arrows indicate the band of 800 bp, which confirms *P. ovale* infection.



To confirm the type of *Plasmodium* and evaluate the possibility of a mixed infection, two molecular biological protocols, nested polymerase chain reaction (PCR) and real-time polymerase chain reaction were performed. Before treatment, DNA was extracted from 1 mL of whole blood using a genomic DNA purification kit (Puregene, Gentra Systems, Minneapolis, USA), according to the manufacturer's protocol. Parasite species identification was conducted via amplification of the small subunit ribosomal RNA gene (18S ssuRNA), as described for nested-PCR [8] in Figure 2 and real-time PCR [9]. Both PCR-based protocols confirmed a single infection by *P. ovale* in the patient's peripheral blood.

Discussion

For travel-associated malaria, *P. falciparum* is the main target for anti-malarial prevention due to its wide endemic distribution and clinical severity. Since imported non-falciparum malaria is globally less prevalent and usually has a benign course, information is limited, especially for *P. ovale* [4]. Currently, available data on the global prevalence and distribution of ovale malaria are based on conventional microscopy. However, routine microscopy is not highly effective in distinguishing *P. vivax* from *P. ovale*, because the morphology of the infected red blood cells cannot be evaluated after hemolysis the parasite shape is affected. Therefore, WHO recommends an examination of thin smear in addition to the thick smear. In a study conducted in the United Kingdom, diagnoses made by several laboratories were compared to those of two reference laboratories and the morphological identification of *P. ovale* was correct only in 29.4% of cases [3]. In addition, the number of parasites is usually low compared to that in

patients infected by *P. falciparum* or *P. vivax*, reflecting the restriction of *P. ovale* to development in younger erythrocytes [10]. Parasitemia under 500/µl (0.01% of red blood cells) are a common occurrence in *P. ovale* infections [4]. For this reason, experienced microscopists are required to achieve high levels of accuracy in parasite diagnosis. Higher density parasitemia usually occurs after an average of nine days into the erythrocytic cycle [10]. The parasitemia level of 425/µl found in the current study probably reflects an early stage of the cycle.

Most of the time, there are no pathognomonic differences in clinical presentation of *P. vivax* and *P. ovale* infection, and in both chills and fever are observed in 48 hours cycles. Nevertheless, the distinction between the two species is important for clinical, epidemiological, and therapeutic reasons. Resistance to chloroquine, for instance, occurs in *P. vivax* but not in *P. ovale* [11]. Moreover, cases of severe malaria caused by *P. vivax* have been reported, while cases associated with *P. ovale* are, in most circumstances, benign [10, 12].

Cases of malaria with long incubation periods are reported in many parts of the world, a phenomenon mainly attributed to the variation in parasite lineages and the use of chemoprophylaxis [13, 14]. Since anti-malarial drugs used in chemoprophylaxis only affect the erythrocytic stages of the parasite, the symptoms of the initial infection are masked, but *P. vivax* and *P. ovale* latent forms can remain viable, and the appearance of symptoms may occur only after months or years. In the current report, the patient reported no use of chemoprophylaxis. In addition, since the patient did not recall the therapeutic treatment used, failure to administered primaquine during the primary episode might have resulted in hypnozoite persistence.

For *P. vivax*, the prolonged relapse periods have been reported for strains from the northern hemisphere, in comparison to those from the southern hemisphere. Similarly, prolonged relapse periods in infections of *P. ovale* have been reported [4, 15, 16], but the frequency of occurrence, length, and incidence of relapse in these cases are probably underestimated. The recent report of the existence of two species of *P. ovale* occurring sympatrically in Asia and Africa: *P. ovale curtisi* and *P. ovale wallikeri* [17, 18], enhances the understanding on these species. The authors highlight the need to determine differences in biological, epidemiological, and clinical characteristics of these two types of malaria, since those differences may have implications for control of the species involved. The incubation period and relapse periodicity, for instance, may differ between these species.

Molecular studies show that the prevalence of *P. ovale* in endemic areas, as well as the number of imported cases in other areas, has been underestimated [19]. Since most diagnostic centers have no staff experienced in malaria diagnosis, the likelihood of correct microscopic identification of *P. ovale* is limited. The molecular detection of *P. ovale* using PCR-based protocols may be a powerful complementary tool for malaria reference laboratories in cases of uncertain diagnosis, including unexplained recurrent fever in travellers returning from malaria endemic areas. There are no reports of autochthonous cases of *P. ovale* in the Americas. However, some species of Anopheles vectors have been shown to be experimentally infected with *P. ovale* (*Anopheles (Nyssorhynchus) albimanus*, *An. (Ano.) quadrimaculatus* and *An. (Ano.) freeborni*). They are reported in several American countries, but not in Brazil [10, 20]. We reported an imported case of infection by *P. ovale* with a lengthy incubation period most probably as a result of a relapse. This case emphasizes the need to consider malaria in the differential diagnosis of fever, regardless of time after exposure in areas of disease transmission.

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