

# A Difficult Diagnosis of Plasmodium ovale Malaria

## Um Diagnóstico Difícil de Malária por Plasmodium ovale



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### ABSTRACT

Malaria is a major cause of suffering, disease, and death worldwide and is considered the most important of all human parasitic diseases. Malaria is still endemic in most tropical and sub-tropical areas and globalization has contributed to an increase of imported cases around the world. We report a *Plasmodium ovale* infection in a traveler with recent return from a long land trip across West Africa. He declared adherence to mefloquine chemoprophylaxis only at the start of the trip. Initially, he was seen at two different hospitals and in both he was screened for malaria by microscopy and rapid diagnostic test, but his diagnosis was not confirmed. The traveler was then diagnosed at our hospital with a malaria infection by *Plasmodium ovale*. Complete blood count showed mild anemia, but leukocytes and platelets were already normal. Symptoms resolved in 24 hours after treatment started. Microscopy of stained blood films remains the gold standard for malaria diagnosis, which is critically dependent on trained eyes. In non-endemic regions with few cases during the year, training programs in malaria microscopy are crucial. The aim is to prevent the reintroduction of malaria in Europe, reduce individual morbidity and suffering, and thus contribute towards reduction in deaths caused by this disease.

**Keywords:** Europe; Malaria/diagnosis; Malaria/therapy; Plasmodium ovale; Portugal; Travel

### RESUMO

A malária é uma das principais causas de sofrimento, doença e morte no mundo e é considerada a mais importante doença parasitária em humanos. A malária ainda é endêmica na maioria das áreas tropicais e sub-tropicais e a globalização tem contribuído para o aumento de casos em todo o mundo. Relatamos um caso de infeção por *Plasmodium ovale* num viajante com regresso recente de uma viagem por terra por vários países de África Ocidental. O viajante aderiu à profilaxia com mefloquina apenas no início da viagem. Já em Portugal, sentindo-se doente, febril, dirigiu-se a dois hospitais, onde realizou despiste para malária por microscopia e teste rápido, que foram descritos como negativos. Posteriormente, o viajante dirigiu-se ao nosso serviço onde foi diagnosticada uma infeção de malária por *Plasmodium ovale*. Os achados analíticos revelaram uma contagem de leucócitos normal, anemia moderada e, uma contagem de plaquetas já normal. Os sintomas terminaram com apenas 24 horas de tratamento. O método de referência do diagnóstico de malária continua a ser a microscopia, que é altamente dependente da experiência do pessoal do laboratório. Em regiões não endémicas com poucos casos durante o ano, é essencial promover e manter programas de capacitação em microscopia para prevenir a reintrodução da malária na Europa, diminuir a morbilidade e o sofrimento, e contribuir assim, para a redução do número de mortes pela doença.

**Palavras-chave:** Europa; Malaria/diagnóstico; Malaria/tratamento; Plasmodium ovale; Portugal; Viagem

### INTRODUCTION

Malaria is a major cause of suffering, disease and death worldwide and it is considered the most important of all human parasitic diseases. Malaria is still endemic in most tropical and sub-tropical areas and globalization has contributed to an increase of imported cases around the world. Malaria was successfully eradicated from Portugal, with the last endemic case in 1959, and that was acknowledged by the World Health Organization in 1973.<sup>1</sup> Since then, all malaria cases were imported, mainly from the former Portuguese colonies in Africa. By far, *Plasmodium falciparum* malaria is the most common, but non-*falciparum* malaria is diagnosed in about 25% of the cases, both in Portugal and in Europe.<sup>2</sup>

Human malaria is caused by any one of five *Plasmodium* species, *P. falciparum* (*P.f.*), *P. malariae* (*P.m.*), *P. vivax* (*P.v.*), *P. ovale* ssp. (*P.o.*) and *P. knowlesi* (*P.k.*).<sup>3</sup> *Plasmodium ovale* malaria has two sympatric species, i.e. two sub-population/variant types of the same parasite species that share the same epidemiology, morphology, are

non-recombining species but their discrimination is possible through molecular methods), namely *Plasmodium ovale curtisi* (classic type) and *Plasmodium ovale wallikeri* (variant type).<sup>4</sup> *P. ovale* is endemic in all of Africa South of the Sahara desert, South Asia and islands of the Western Pacific.<sup>5-6</sup> It is relatively unusual outside of Africa, and severe illness or death are most uncommon.<sup>7-8</sup> Malaria can be avoided by travelers using two approaches: chemoprophylaxis and protection against mosquito bites. The protection against mosquito bites includes the use of clothes that cover most of the body, use of insect repellent in the exposed skin, and use of mosquito nets (with long-lasting insecticide). The hallmark of malaria symptoms is rigors, fever and profuse sweats characterize the paroxysm (malaria attack), comprising three successive stages, including the cold stage, hot stage and sweating stage. However, clinical manifestations might be non-specific and confused with a flu-like illness.

Clinically suspicious cases of malaria should be

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confirmed by diagnostic laboratory tests: microscopy, rapid diagnostic test and/or polymerase chain reaction (PCR). Microscopy remains the gold standard to detect, identify, quantify parasite density, and characterize parasite morphology, allowing to differentiate parasite species through a blood films.<sup>9</sup> However, it has important disadvantages, namely being time consuming and requiring trained eyes, especially at low parasite density.<sup>10</sup> Despite these disadvantages, microscopy remains the best method for malaria diagnosis.

We report a clinical case of *Plasmodium ovale*.

## CASE REPORT

A healthy 39-year-old Portuguese man with high fever was seen at the outpatient clinic of the Infectious and Tropical Medicine department of Egas Moniz Hospital. He reported having just arrived from a two month long land trip across West Africa (Mauritania, Senegal, Gambia, Guinea-Bissau, Guinea-Conakry, Ivory Coast and Mali). He had already been seen at the emergency department of two other Portuguese hospitals, complaining that his symptoms were suggestive of malaria and was discharged from both with

laboratory results reported as negative. He said that during his trip he slept in his car or in a camping tent. He had started mefloquine chemoprophylaxis but stopped after a neuropsychiatric adverse reaction. He had never been diagnosed with malaria, even after short stays in Africa, South America and Southeast Asia.

The general laboratory results had only showed moderate anaemia and leukopenia (at the three hospitals). Rapid diagnostic tests were always negative. The microscopy examination showed one trophozoite, with morphology compatible with *Plasmodium ovale* ssp., and a schizont and a gametocyte were also seen. Parasite density was estimated to be 7 parasites/ $\mu$ L of *Plasmodium ovale* ssp. (Fig. 1). Molecular testing by nested PCR and Sanger sequencing was performed to confirm the diagnosis to mono-infection from *Plasmodium ovale wallikeri* (Fig. 2).<sup>11,12</sup>

The patient was treated with dihydroartemisinin-piperaquine for three consecutive days, with apyrexia in 24 hours. Later on, the patient was treated with primaquine 30 mg daily for 14 days, to avoid future relapses, and he had no further febrile episodes, until now, after 23 months.

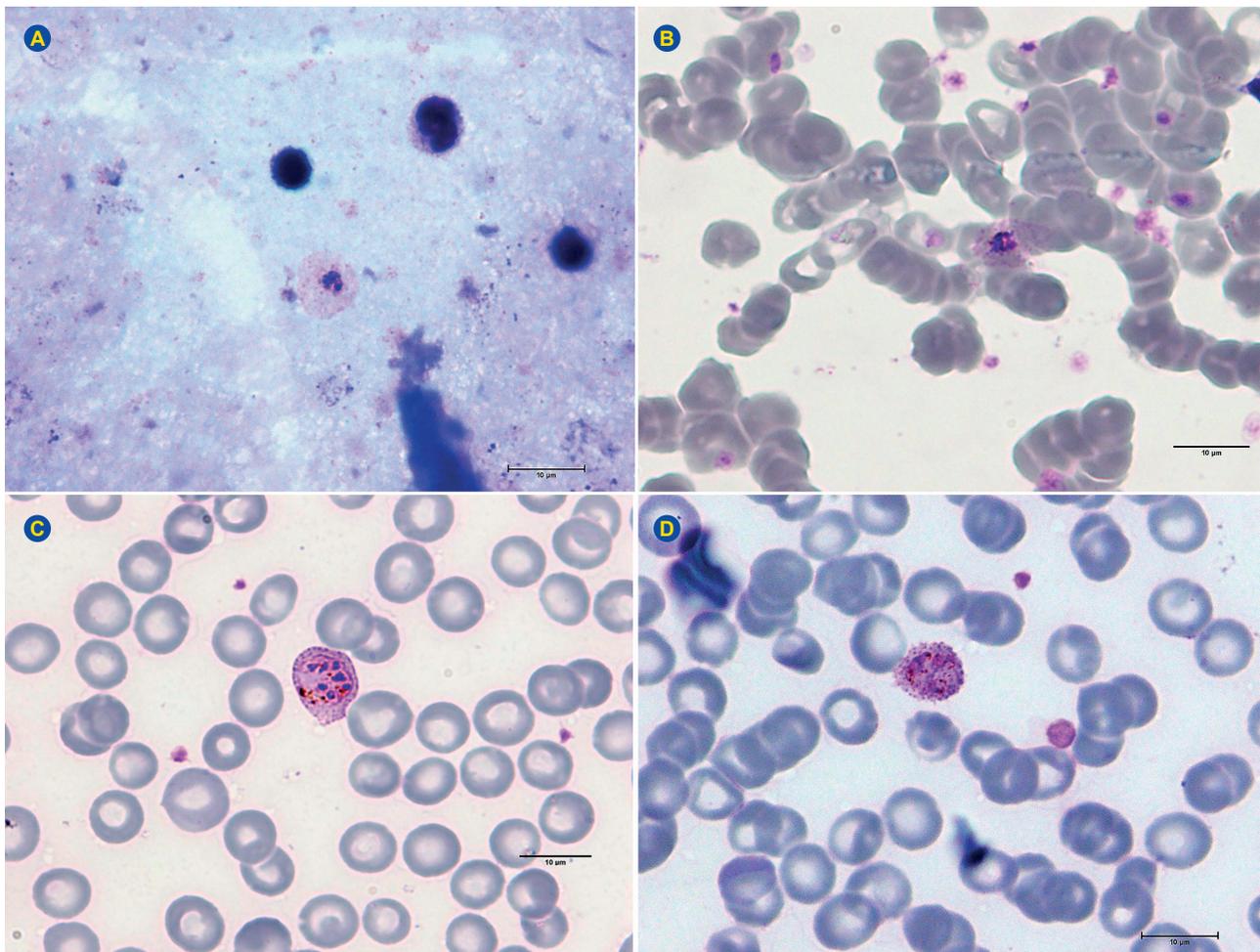
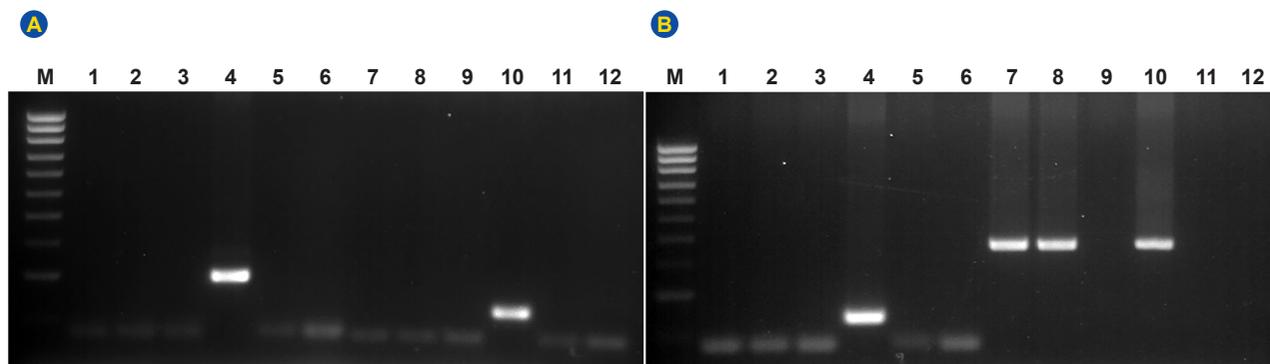


Figure 1 – *Plasmodium ovale* ssp. parasites Giemsa-stained blood films of case report (magnification 1.000 X).

(A) Thick blood with ring trophozoite, (B) Thin blood, compact trophozoite with RBC fimbriated edges and Schüffner stipplings, (C) Thin blood, schizont with hemozoin, Schüffner stipplings and seven distinctive merozoites inside. (D) Thin blood, gametocyte in infected red blood cell show some fimbriation and pigment is scattered throughout the cytoplasm.



**Figure 2** – PCR products (second reaction with species-specific primers) of the SSU rRNA gene of *Plasmodium* spp. were visualized on 2% agarose gel.

(A) M- Molecular marker 100 bp ladder (upper 1000 bp; lower 100 bp); Lane 1 to 6 PCR products with specific primers of *P. falciparum*: 1, 2 are product of amplification from duplicated sample; 3 is negative control of extraction, 4 is DNA positive control of *P. f.* (205 bp), 5 and 6 are negative control of *P. f.*, in the first and second PCR reaction respectively; lane 7 to 12 PCR products with specific primers of *P. vivax*: 7 and 8 are product of amplification from duplicated sample; 9 is negative control of extraction, 10 is DNA positive control of *P. v.* (120 bp), 11 and 12 are negative control of *P. v.*, in the first and second PCR reaction respectively; (B) M- Molecular marker 100 bp ladder (upper 1000 bp; lower 100 bp), Lane 1 to 6 PCR products with specific primers of *P. malariae*: 1, 2 are product of amplification from duplicated sample; 3 is negative control of extraction, 4 is DNA positive control of *P. m.* (144 bp), 5, 6 are negative control of *P. m.* in the first and second PCR reaction respectively; lane 7 to 12 PCR products with specific primers of *P. ovale* ssp.: 7, 8 are product of amplification from duplicated sample; 9 is negative control of extraction, 10 is DNA positive control of *P. o.* (375 bp), 11 and 12 are negative control of *P. o.* in the first and second PCR reaction, respectively.

## DISCUSSION

Our case illustrates the difficulty in diagnosing non-*falciparum* malaria in non-endemic countries, both for clinicians and for laboratory staff.<sup>1</sup> *P. ovale* is believed to be the most difficult of malaria species to identify because of usual low parasite density and some morphologic similarities with *P. vivax* and *P. malariae*. Microscopy is time consuming and requires technical expertise, and it is easy to miss the small number of parasites present. Furthermore, most RDTs are optimized to *P. falciparum* malaria and are usually negative for non-*falciparum* species as *P. ovale*, due to low sensitivity for this parasite.<sup>6,13</sup> Clinical suspicion for malaria increased since the patient was a healthy heavily exposed person (sleeping in the car or under the stars), with partial chemoprophylaxis, fever episodes, plus suggestive complete blood count (CBC) results. Even if not specific, useful clues for a suspected diagnosis of malaria may be apparent on CBC: leucopenia, anaemia or thrombocytopenia.<sup>6,13-15</sup> Thrombocytopenia is usually present in early acute malaria, and the platelet count can then rebound at the same time as parasite density starts to decline. In this kind of situation, reexamination of the blood's films should be done, trying to exclude missed malaria parasites. If clinical suspicion persists, blood film repetition at 12h and 24h after the first microscopy observation is recommended to rule out malaria.<sup>16</sup> It is important to improve the quality of malaria diagnosis, because misdiagnosis in non-*falciparum* malaria presents both an individual and collective risk. Misdiagnosis and/or incomplete treatment could promote routes of transmission by transfusion, post-transplant, and congenital malaria.<sup>17,18</sup> The access to travel advice can improve the knowledge about the exposure risk and adherence to chemoprophylaxis in order to ensure great protection for the health of travelers. *Anopheles atroparvus* is the dominant malaria

vector across Europe and it is a competent vector of some strains of *Plasmodium* sp. with potential local transmission. Local malaria transmission has been reported in several countries in Europe caused mainly by *P. vivax*, and a few cases by *P. falciparum*. Ecological changes in vectors due to rising temperature, low adherence to chemoprophylaxis, and malaria gametocyte carriers are essential ingredients to re-establish the vector, parasite and human host cycle.

Globalization, global warming and mass air travel conspire to introduce new challenges in healthcare services in non-endemic malaria regions. Maintaining expertise in malaria diagnosis by microscopy is crucial. It is the only method able to cover all *Plasmodium falciparum* and non-*falciparum* malaria cases quickly in emergency settings. The creation of a Portuguese and/or European network to share case reports and threats to malaria diagnosis would promote a better training/expertise in this diagnosis. Excellent communication between physicians and laboratory staff is key to encourage exhaustive observation of blood films. Furthermore, repeat of blood collection is recommended to guarantee confidence in results, in all suspected malaria cases and even in cases with unsuspecting initial fever. Both rigorous implementation of training in malaria microscopy and routine microscopy quality control are the only way to avoid misdiagnosis and/or delays in malaria diagnosis, resulting in adverse clinical outcomes. In addition, they are important to reduce the danger of reintroduction of malaria in Europe.

## AUTHORS CONTRIBUTION

ASR, JN: Both authors had the same intellectual contribution for the conception and design of the article; data analysis and interpretation; draft of the manuscript; critical review, approval of the final version.

## PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

## DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patient's data publication.

## PATIENT CONSENT

Informed consent for publication of his Case Report was given by the patient.

## COMPETING INTERESTS

The authors have declared that no competing interests exist.

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