Non-falciparum malaria in Dakar: a confirmed case of *Plasmodium ovale wallikeri* infection

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**Abstract**

**Background:** *Plasmodium ovale* is rarely described in Senegal. A case of clinical malaria due to *P. ovale wallikeri* in West Central of Senegal is reported.

**Case:** A 34-year-old male baker in Dakar, with no significant previous medical history, was admitted to a health clinic with fever and vomiting. Fever had been lasting for 4 days with peaks every 48 h. As monospecific *Plasmodium falciparum* HRP-2 RDT was negative, he was treated with antibiotics. However, owing to persisting symptoms, he was referred to the emergency unit of the Youssou Mbargane Diop Hospital, Dakar, Senegal. Clinical examination found impaired general condition. All other physical examinations were normal. Laboratory tests showed anaemia (haemoglobin 11.4 g/dl), severe thrombocytopenia (platelets 30 $\times$ 10$^9$/mm$^3$), leukopenia (3650/mm$^3$), lymphocytopenia (650/mm$^3$). Renal function was normal as indicated by creatininaemia and uraemia (11 mg/l and 0.25 g/l, respectively) and liver enzymes were slightly elevated (aspartate aminotransferase 77 UI/l and alanine aminotransferase 82 UI/l). Blood smear evaluations in Parasitology Laboratory of Aristide Le Dantec Hospital showed malaria parasites of the species *P. ovale* with a 0.08 % parasitaemia. Molecular confirmation was done by real time PCR targeting the 18S rRNA gene. The *P. ovale* infection was further analysed to species level targeting the *potra* gene and was identified as *P. ovale wallikeri*. According to the hospital’s malaria treatment guidelines for severe malaria, treatment consisted of intravenous quinine at hour 0 (start of treatment) and 24 h after initial treatment, followed by artemether–lumefantrine 24 h later. A negative microscopy was noted on day 3 post-treatment and the patient reported no further symptoms.

**Conclusion:** Malaria due to non-falciparum species is probably underestimated in Senegal. RDTs specific to non-falciparum species and/or pan specific RDTs should be included as tools of diagnosis to fight against malaria in Senegal. In addition, a field-deployable molecular tool such as the loop-mediated isothermal amplification can be considered as an additional useful tool to detect low malaria parasite infections and for speciation. In addition, national malaria control policies should consider other non-falciparum species in treatment guidelines, including the provision of primaquine for the treatment of relapsing parasites.

**Keywords:** *Plasmodium ovale*, Malaria, Fever, RDT, Microscopy, Diagnostic, Treatment, Primaquine, Dakar
Background

Despite the fact that *Plasmodium ovale* is endemic in tropical Africa, malaria due to *P. ovale* is rarely described in Senegal [1]. The most common pathogen of malaria in Africa is *Plasmodium falciparum* [2]. In the 1990s, *P. ovale* infections were described in the south of Senegal in the context of the longitudinal study at Dielmo village located 280 km South East of Dakar [1]. During the first year of the project in 1990, the prevalence of *P. ovale* was determined, among symptomatic and asymptomatic villagers of all groups age, to be 5.5 % either as a mono-infection or as mixed infection with *P. falciparum* [3]. Concerted efforts to control malaria in Senegal contributed to decrease in the overall prevalence of malaria and by 2010 *P. ovale* infections were rarely reported [1]. Recently, a molecular survey found *P. ovale* in Kedougou only as mixed infections with *P. falciparum* at 11.5 % of prevalence [4].

*Plasmodium ovale* consists of two distinct species: *P. ovale curtisi* (classic type) and *P. ovale wallikeri* (variant type) [5]. The two species are indistinguishable by microscopy but seem to differ in their duration of latency [6]. The recent description of dimorphism in the gene encoding *P. ovale* tryptophan-rich antigen (*potra*) allows for the distinction between the two *P. ovale* species. Sequence and size variations were noted between the *tryptophan-rich antigen* genes from *P. ovale curtisi* (*poctra*) and *P. ovale wallikeri* (*powtra*) [5]. This was exploited in a nested PCR detection assay [7], where the species are discriminated by the size of the amplified fragments (299 or 317 bp for *poctra*; 245 bp for *powtra*). In addition, Tanomsing et al. developed a semi-nested PCR protocol which efficiently discriminate *P. ovale wallikeri* and *P. ovale curtisi* and found that the 299 bp fragment was overlapping between the two subspecies [8] and the 245 bp, represented *P. ovale wallikeri*.

A case of malaria due to *P. ovale wallikeri* is described in a patient admitted to the emergency unit service of the Youssou Mbargane Diop Hospital of Rufisque, a department of the peripheral region of Dakar in Senegal.

Case presentation

A 34-year-old male baker living in Diamniadio (Dakar region) sought a consultation at a health centre near his home for fever, headache, vomiting and diarrhoea. Fever had lasted for 4 days with peaks every 48 h. The patient was suspected of malaria and was tested using an HRP-2 based RDT (detecting only *P. falciparum*). The test was negative for *P. falciparum* malaria and the patient was treated by three intravenous (IV) injection of cefotaxim (third generation of cephalosporin) at 100 mg per kg per day to cover potential bacterial infection. Owing to persisting symptoms 4 days later, he was referred to the emergency unit of the Youssou Mbargane Diop Hospital, Dakar, Senegal.

The patient had no particular previous medical history. He was born in Thies and moved to Touba at the age 12 and finally at age 23 to Diamniadio, where he has lived since. The patient claimed that he had never been out of these three areas, which are all in West Central of Senegal. Physical examination found impaired general condition but the patient was conscious. Abdomen was tender. All other physical examinations were normal. Another HRP-2 based RDT was performed and was also negative. Other laboratory tests showed anaemia (haemoglobin 11.4 g/dl), thrombocytopenia (platelets 30 × 10^9/mm^3), leucopenia (3650/mm^3) and lymphocytopenia (650/mm^3). Kidney function was normal as indicated by creatininemia and uraemia (11 mg/l and 0.13 g/l, respectively). Transaminases were slightly elevated.

Blood smear evaluations in the Parasitology Laboratory of Aristide Le Dantec University Hospital showed malaria parasites in normal and enlarged red blood cells (RBCs). Some infected RBCs were oval shaped and some were fimbriated. However, Schüffner’s dots were not observed likely due to staining defect (Fig. 1). All malaria parasite stages (rings, trophozoites, schizonts and gametocytes) were observed. These malaria parasites were identified as *P. ovale* and a 0.08 % parasitaemia was calculated. Parasite density was measured by determining the ratio of parasites/leucocytes on the basis of 8000 leucocytes/μl after counting 500 leucocytes. That ratio was found to be 1.1 giving a parasite density of 8800 parasites/μl of blood.

An 18S rRNA gene real-time PCR molecular assay was undertaken as previously described [9] to confirm the *P. ovale*, infection (Fig. 2). No others species of *Plasmodium* was detected. The parasite was then subtyped using the *potra* gene as target as previously described [7]. This genotyping identified the presence of *P. ovale wallikeri* (Fig. 3).

Based on clinical signs and biological findings, the patient was treated as severe malaria accordingly to the hospital’s treatment guideline. Intravenous quinine was given at admission and 24 h later. 48 h after initial treatment, oral artemether–lumefantrine was given every 6 h at six doses. On day 3 after treatment, microscopy was negative and the patient reported no further symptoms. Primaquine was not given (see “Discussion” section).

Discussion

*Plasmodium ovale* is a malaria parasite that is endemic in sub-Saharan Africa, although not very commonly described [2]. In Senegal, little attention has been paid to *P. ovale* malaria in the recent years. Here, a case of *P. ovale* infection that appears to have been acquired in Diamniadio, near Dakar, in West Central of Senegal is reported.
This case highlights the need to consider non-falciparum malaria infections in this region for case management. *Plasmodium ovale* malaria is often mild in clinical presentation [10] and severe forms of the disease are relatively rare [11–13]. The parasite densities are often less than 50 parasites/μl and a parasitaemia higher than 5000 parasites/μl is rarely reported [14]. This is believed to be due to the fact that *P. ovale* prefers younger RBCs, which are rare in peripheral blood [2]. The patient in this report presented a relatively high density parasitaemia of 8800 parasites/μl and severe thrombocytopaenia. Equally high parasitaemia levels were found in Spanish travelers infected with *P. ovale wallikeri* imported from different countries in Africa and the same study observed that severe thrombocytopaenia was more commonly associated with *P. ovale wallikeri* infection than with *P. ovale curtisi* infections [15]. While the mechanisms that produce thrombocytopaenia in malaria is still not clear it seems likely to be linked to a greater severity of the illness [16].

In Senegal, *P. ovale* subtyping is not documented. Determining the infecting *P. ovale* species may be important because recent observations suggest that the two species might differ in their relapsing patterns [6] and secondly, *Plasmodium ovale wallikeri* seems to be slightly more pathogenic [15]. The previously described dimorphisms observed in gene encoding *P. ovale* tryptophan-rich antigen (*potra*) [5] were used to identify the infecting *P. ovale* species as *P. ovale wallikeri*. This is most likely the first time *P. ovale* species determination have been undertaken when describing *P. ovale* infections in Dakar, Senegal.

The patient in this study denied having travelled outside Diamniadio suggesting that the *P. ovale* infection was likely acquired locally. Since Diamniadio is a crossroads town located at the town exit of Dakar where many different human populations from neighbouring regions and countries intersect there are two possible likely sources of the *P. ovale* parasites: (1) from an existing *P. ovale* reservoir in or near Diamniadio, or (2) that the source of the *P. ovale* infection came from another region or neighbouring countries. In the 1990s, in a study in Dielmo, exposure to *P. ovale* was reported across all ages and perennial transmission of *P. falciparum* was also noted [17], therefore, it is possible that *P.
falciparum cross immunity could protect against non-falciparum species [2]. However, in the West Central of Senegal like Diamniadio where malaria transmission is very low, this cross immunity might be low or none existing, making it easy to acquire malaria, including non-falciparum infections, as in this case report.

This present findings highlight the need to consider non-falciparum malaria infections in clinical settings both for the appropriate treatment of the infection and in order to further understand the epidemiology of malaria in the country. Plasmodium ovale infections present unique challenges because of the reported long latency period and the fact that two distinct species are known to exist [8]. In fact, the commonly used diagnostic tools in many endemic malaria regions, microscopy and RDT, are less sensitive when parasitaemia is very low and need expert microscopists or molecular assays to recognize uncommon species. In Senegal, P. falciparum is the most prevalent parasite detected when patients are suspected of malaria. Because of this, the national malaria control programme provides all health clinics of the country with HRP-2 based RDT, which are specific to P. falciparum. Therefore, it is possible that many cases of non-falciparum malaria infections are just not detected.

This case report highlights the urgent need to develop standardized protocols capable of detecting all Plasmodium parasites in Senegal in order to achieve the malaria elimination agenda. In addition, although the existence of P. ovale hypnozoite remain controversial [18], they pose an important challenge in that they can cause relapse malaria which has great implications for treatment and transmission. Therefore, more species specific RDTs and field-usable molecular tools, such as the loop-mediated isothermal amplification (LAMP) are needed to assess the actual prevalence of P. ovale and other non-falciparum malaria parasites in Senegal and in other parts of Africa as previously suggested [19].

The current World Health Organization treatment guidelines recommend intravenous (IV) artesunate for the treatment of severe malaria due to all Plasmodium species [20]. In Senegal, since the national malaria control policies targets especially P. falciparum, ACT and quinine are the recommended and registered anti-malarials. The patient discussed in this case report was treated using IV quinine
This case also highlights the fact that *P. ovale* malaria is not as benign as previously thought and that it is important to consider other non-falciparum malaria infections for appropriate patient care and for the success of the malaria control programme.

**Abbreviations**
- ACT: artemisinin-based combination therapy
- HRP: histidine rich protein
- LAMP: loop mediated isothermal amplification
- PCR: polymerase chain reaction
- *potra*: *P. ovale* tryptophan-rich antigen
- RDT: rapid diagnostic test
- rRNA: ribosomal ribonucleic acid

**Authors’ contributions**
MAD, ASB and KD performed the microscopy examination. MAD collected clinical and biological data and drafted the manuscript; NWL performed the Rougemont real time PCR. MAD, AD, TN, LKS performed the *potra* genotyping experiment; MIG, AG, MSY participated in the laboratory work. ASB, KD, NWL, VU and DN critically commented on the manuscript. All authors read and approved the final manuscript.

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**Competing interests**
The authors declare that they have no competing interests.

**Availability of data and materials**
The datasets supporting the conclusions of this article are included within the article.

**Consent for publication**
Written informed consent was obtained from the patient for publication of this case report.

**Ethics approval**
The study received ethical clearance from the Ethic Committee of the Senegalese Ministry of Health. Informed consent was obtained from the patient.

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