Case Report

*Plasmodium vivax* exflagellated microgametes in human peripheral blood: a potential diagnostic dilemma

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**Running title:** *Plasmodium vivax* exflagellated microgametes

**SUMMARY:** Both malaria and relapsing fever *Borrelia* are infectious diseases characterized by fever, headache, myalgia, hepatosplenomegaly and tendency to relapse. Exflagellation of microgametocyte in malarial parasites is seen only in the definitive host, i.e., mosquitoes. Here we report an unusual case of a 23-year-old man who presented *Plasmodium vivax* infection with multiple exflagellated microgametes in the peripheral blood smear.

**Keywords:** *Plasmodium vivax*, microgametocyte, microgametes, exflagellation, malaria, *Borrelia*

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INTRODUCTION
Malaria and relapsing fever *Borrelia* are two vector-borne diseases characterized by overlapping symptoms such as high fever, headache, myalgia, hepatosplenomegaly, as well as a marked tendency for relapses. Microscopic examination of peripheral blood smears is the most common test used to diagnose both diseases (Poulsen *et al.*, 1996, Colebunders *et al.*, 1993, Berger *et al.*, 2005). Here we report a case of *Plasmodium vivax* malaria with observed exflagellated microgametes in peripheral blood, which were initially mistaken for a co-infection of *P. vivax* malaria and *Borrelia* spp.

CASE REPORT
A 23-year-old male migrant from Eritrea arrived in Italy in March 2017, after traveling through Libya. He was admitted to San Luca Hospital in Lucca four days after his arrival because of high fever, headache, arthromyalgia, and general pruritus. Laboratory tests showed normocytic anemia (Hb 8.4 g/dl; RBC 2.59 x 10⁶/µl) and moderately high inflammatory parameters (PCT 1.11 ng/ml and CRP 4.22 mg/dl), while other blood parameters and renal and hepatic functions were normal. Some peripheral blood smears were set up about an hour after sample withdrawal. Examination of the blood smears, stained with May-Grunwald and Giemsa, showed the presence of trophozoites (Fig. 2) and gametocytes (Fig. 1) consistent with *P. vivax* diagnosis, with 2% of erythrocytes parasitized. In addition, several single forms of spirochete cell-like forms were also observed outside the erythrocytes (Fig. 2).

The presence of these thin and sinuous structures initially posed a diagnostic doubt, suggesting a combined infection of *P. vivax* and *Borrelia* spp. Laboratory testing for *Borrelia burgdorferi sensu lato* (anti-*Borrelia* IgG and IgM ELISA, Alegria, Orgentec) showed negative results. Therefore, the spirochete cell-like forms (Fig. 2) could have been due to the unusual presence of *P. vivax* microgametes. As required by the National Malaria Surveillance System in Italy (Ministry of Health, 2016), the blood smears were sent to the Italian National Institute of Health (ISS) to confirm *P. vivax* malaria diagnosis. As expected, ISS confirmed the presence of *P. vivax*. Moreover, the gene amplification assay (real-time PCR 16S rRNA for *Borrelia* spp.) (Parola *et al.*, 2011) confirmed the absence of *Borrelia* spp. Upon further microscopic examination, the *P. vivax* microgametes, about 12-15 µm in length, showed the presence of a central dark dot of a chromatin nucleus-like form. As shown in Figure 3, microgametocytes exflagellation was also observed. The definitive diagnosis was *P. vivax* mono-infection and the patient was treated with chloroquine (25 mg/kg) for three days with significant improvement. After his health returned to normal, he was discharged.
DISCUSSION
The detection of microgametes of malaria parasites in emoscopic preparations is a rare and unusual event, as these forms normally occur inside the mosquito following an infective blood meal. The occurrence of exflagellation in peripheral human blood has been well described in the literature for *P. falciparum* and *P. vivax* (Enger *et al.*, 2004, Tembhare *et al.*, 2009, Prasad *et al.*, 2011, Arnetha *et al.*, 2017; Bhar *et al.*, 2018). Microgametocytes collected in the test tube remain exposed to environmental conditions, thus triggering the maturation phase and starting mitotic division, with the subsequent release of microgametes. The exflagellation of microgametocytes is determined by several factors, mainly related to pH and temperature. Specifically, in a laboratory setting, temperature decreases and pH increases due to a shift in CO₂ levels. All these changes simulate the replication conditions in the mosquito's gut (Nijhout *et al.*, 1978, Hummert *et al.*, 1994, Tembhare *et al.*, 2009).

Morphological similarities between *P. vivax* exflagellated microgametes forms and *Borrelia spp.* pose a diagnostic problem on peripheral smears. However, the presence of chromatin material, which is absent in *Borrelia spp.*, could be a distinctive marker in order to carry out a differential diagnosis without molecular tests. Given that the presence of the nuclear-like form is not always marked and closely related to the staining procedure, the two infectious agents might be misdiagnosed. Figure 2 shows ovoid chromatin structures in the *P. vivax* exflagellated microgametes.

Our experience clearly demonstrates that to avoid diagnostic errors, clinics and laboratories should be aware that these and other unusual forms of malarial parasites can be observed under emoscopic examination (Enger *et al.*, 2004, Tembhare *et al.*, 2009, Prasad *et al.*, 2011, Arnetha *et al.*, 2017). In light of increased human migration, both clinicians and microbiologists may encounter patients from endemic areas with relapsing fever pathogens. Microscopic and molecular methods could thus be combined in order to eliminate misdiagnosis and thus ensure the correct therapeutic treatment.

**ACKNOWLEDGEMENTS:** This case report was presented at XLVI National Congress of the Italian Association Clinical Microbiologists, Rimini November 2017. Poster P143.
REFERENCES


**Figure 1**: Thin blood smear of the patient showing mature gametocytes of *Plasmodium vivax*. The blue arrow shows *Plasmodium vivax* gametocyte and merozoite, both in the same red blood cell (May-Grunwald and Giemsa stained; magnification 1000 X).
**Figure 2:** Thin blood smear of the patient: the red arrows indicates microgametes of *Plasmodium vivax* containing ovoid chromatin structures; the blue arrow indicates multiple infections of trophozoites inside the red blood cells (May-Grunwald and Giemsa stained; magnification 1000 X).
Figure 3: Thin blood smear of the patient showing exflagellating microgametocytes. Early flagella formation in panel A; exflagellation of numerous microgametes in panel B (May-Grunwald and Giemsa stained; magnification 1000 X).