**CASE REPORT**

**Plasmodium malariae** infections as a cause of febrile disease in an area of high *Plasmodium falciparum* transmission intensity in Eastern Uganda

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**Abstract**

**Background:** *Plasmodium falciparum* is responsible for the vast majority of (severe) clinical malaria cases in most African settings. Other *Plasmodium* species often go undiagnosed but may still have clinical consequences.

**Case presentation:** Here, five cases of *Plasmodium malariae* infections from Eastern Uganda (aged 2–39 years) are presented. These infections were all initially mistaken for *P. falciparum*, but *Plasmodium* schizonts (up to 2080/µL) were identified by microscopy. Clinical signs included history of fever and mild anaemia.

**Conclusion:** These findings highlight the importance of considering non-falciparum species as the cause of clinical malaria. In areas of intense *P. falciparum* transmission, where rapid diagnostic tests that detect only *P. falciparum* antigens are commonly used, non-falciparum malaria cases may be missed.

**Keyword:** *Plasmodium malariae*, *P. falciparum*, Diagnosis, Misdiagnosis, Schizonts, Microscopy

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surveys in African countries, where prevalence of up to 12% has been reported [11, 12]. Rapid diagnostic tests (RDTs), which are simple to use and require minimal training, are increasingly being used to diagnose malaria. In Africa, most RDTs detect histidine-rich protein 2 (HRP2), an antigen that is specific for \( P. \) falciparum; use of these HRP2 RDTs is likely to contribute further to under-diagnosis of \( P. \) malariae infections.

Here, five cases are presented of symptomatic, uncomplicated \( P. \) malariae. These cases were coming from an area of intense \( P. \) falciparum transmission in Eastern Uganda and were initially misdiagnosed as \( P. \) falciparum by microscopy.

**Case presentation**

Patients described in this case report presented at either Masafu General Hospital or Nagongera Health Centre IV. Masafu General Hospital is a district referral facility in Busia District, an area in south-eastern Uganda that borders Kenya and Lake Victoria, where transmission of \( P. \) falciparum is perennial and intense [13]. Nagongera Health Centre IV is located in Nagongera sub-county in Tororo district. Nagongera sub-county is predominantly a rural setting, with traditionally very high malaria transmission [14]. Between 2019 and 2020 the total Outpatient Department treatment (OPD) attendance in Masafu General Hospital was 40,471, whilst in Nagongera Health Centre IV 18,624 [15].

In both settings, hospital and health centre, malaria diagnosis is routinely performed by HRP-2 based rapid diagnostic test (Standard Q Malaria Pf Ag, SD Biosensor, South Korea). However, as part of a research study on \( P. \) falciparum, clinic technicians all received formal microscopy training and thick and thin blood films were used for malaria diagnosis, instead of RDTs. Haemoglobin measurements were performed by Hemocue™ (haemoglobinometer, Angelholm, Sweden).

**Case-patient 1**

On January 30th 2020, a 15-year-old male presented to the OPD with history of fever, headache and joint pain for 3 days with no evidence of severe malaria or danger signs. At initial presentation, he had an axillary temperature of 36.8 °C and his physical examination was within normal limits. Malaria parasites were reported on the thick and thin blood film. By thick blood smear, total parasite count was 896 parasites/μL; malaria schizonts were observed at 176 parasites/μL (and included in the total parasite density estimate). Haemoglobin concentration was 12.3 g/dL. The patient was diagnosed with \( P. \) falciparum malaria.

**Case-patient 2**

On February 19th 2020, a 20-year-old female presented to the OPD with history of fever for 3 days and headache, weakness and joint pain for 2 days. Her temperature on admission was 37.2 °C and her physical examination did not show abnormalities. After reading the thick blood film, malaria infection was diagnosed with a total of 2288 parasites/μL (2080 schizonts/μL). Mild anaemia was detected with haemoglobin of 10.8 g/dL. The patient was diagnosed with \( P. \) falciparum malaria.

**Case-patient 3**

On February 27th 2020, a 39-year-old female presented to the OPD with history of fever and joint pain for 2 days and headache, weakness and low back pain for 4 days. Her axillary temperature on admission was 37.0 °C and she was diagnosed with uncomplicated \( P. \) falciparum malaria based on a thick blood film with 336 parasites/μL (272 schizonts/μL). Mild anaemia was detected with haemoglobin of 9.5 g/dL.

**Case-patient 4**

On March 3rd 2020, a 2-year-old boy presented to the outpatient department with persistent vomiting, anorexia and headache. He had an axillary temperature of 38.3 °C. Microscopy slides were interpreted as \( P. \) falciparum/\( P. \) malariae mixed infection based on presence of 32 ring-stage parasites/μL and 520 schizonts/μL for \( P. \) falciparum and one trophozoite “band form” for \( P. \) malariae. Haemoglobin was 7.8 g/dL. The patient was diagnosed with uncomplicated mixed species malaria infection.

**Case-patient 5**

On April 29th 2020, a 14-year-old male with mild symptoms of fever, headache, joint pain and anorexia presented to Nagongera Health Center IV, Tororo district. He had an axillary temperature of 37.4 °C. Malaria was diagnosed by microscopy with 4016 parasites/μL (96 schizonts/μL). Haemoglobin was 11.1 g/dL. The patient was diagnosed with uncomplicated \( P. \) falciparum malaria.

All five cases were treated for uncomplicated \( P. \) falciparum infection according to national Ugandan treatment guidelines with artemether lumefantrine 20 mg/120 mg twice per day for 3 days and discharged home [16]. Although there are some reports of persisting \( P. \) malariae parasites after artemether-lumefantrine [5, 17], suggestive of reduced treatment efficacy for this species, most available data support the use of artemether-lumefantrine for \( P. \) malariae [18, 19]. Thick and thin smears were re-read by expert microscopists and representative images taken (Fig. 1). Parasite DNA was extracted from fixed Giemsa-stained thick and thin smears using a
modified protocol for isolating genomic DNA from dried blood spots (QIAamp® DNA Micro kit, cat numb. 56304) [20]. *Plasmodium* speciation was performed using modified methods described by Snounou et al. [21]. *Plasmodium malariae* parasite DNA was detected in material from all slides; *P. falciparum* was also detected for case 4 (Table 1).

**Discussion and conclusions**

In these five symptomatic malaria cases, *P. malariae* was the most likely cause of the clinical symptoms. All cases were classified as uncomplicated malaria, although they presented with anaemia, a condition that is often associated with chronic infection with *P. malariae* [3, 4]. In four cases, *P. falciparum* infection was initially diagnosed. Only after an expert microscopist reviewed the blood slides was *P. malariae* identified as the infecting species. The presence of symptomatic *P. malariae* mono-infections has implications for the use of HRP-2 RDTs. In both of the study settings, HRP-2 RDTs are routinely used to diagnose malaria. Since non-falciparum *Plasmodium* species do not express HRP-2 antigen, at least four of these cases would presumably have been missed in routine practice and would not have received malaria treatment.

The cases presented here all reported to the health facilities within a relatively short 13-week period. No effort is made to systematically investigate for non-falciparum malaria among individuals presenting with suspected malaria under standard care. This suggests that a considerable number of patients may present with uncomplicated non-falciparum malaria in this setting. The *P. malariae* cases reported here were older than typical *P. falciparum* cases in the area [22], which could be the consequence of a lower force of infection of non-falciparum malaria, older age at first infection, and higher average age at clinical presentation. However, other studies found *P. malariae* infections predominantly in children [23, 24], further highlighting the need for research into the clinical burden of non-falciparum malaria in African settings.

![Fig. 1 Features of Plasmodium malariae parasites in thin and thick blood smears. A typical band form mature trophozoite. × 10 ocular, × 100 magnification, thin smear. B young trophozoite. Pictures A, B taken with a smart phone camera. C, D “daisy shape” schizonts. × 10 ocular, × 100 magnification, thin film. E, F schizonts. × 10 ocular, × 100 magnification, thick smear. C-F pictures taken with Axio Cam MRc-5, Zeiss, Germany. Blood smears were stained with 10% Giemsa solution](#)

**Table 1** Patient characteristics at presentation

| Case | Age  | Gender | Temperature (°C) | Symptoms                                                                 | Haemoglobin (g/dL) | Trophozoite (rings) count | Schizont count* | Diagnosis by microscopy | Diagnosis by 18S qPCR |
|------|------|--------|------------------|--------------------------------------------------------------------------|--------------------|--------------------------|-----------------|------------------------|-----------------------|
| 1    | 15   | Male   | 36.8             | Headache 3d, joint pain 2d, fever                                        | 12.3               | 720                      | 176             | Pf                     | Pm                    |
| 2    | 20   | Female | 37.2             | Headache 2d, general body weakness and joint pain 3d, fever 3d           | 10.8               | 208                      | 2080            | Pf                     | Pm                    |
| 3    | 39   | Female | 37.0             | Fever and joint pain 2d, general body weakness, headache, backache 4d    | 9.5                | 64                       | 272             | Pf                     | Pm                    |
| 4    | 2    | Male   | 38.3             | Persistent vomiting, anorexia, fever, headache                           | 7.8                | 32                       | 520             | Pf+Pm                  | Pm/Pf                 |
| 5    | 14   | Male   | 37.4             | Headache, fever 3d, joint pain, anorexia                                | 11.1               | 3920                     | 96              | Pf                     | Pm                    |

* Density per microlitre of blood
Identifying the species of *Plasmodium* infections using molecular diagnostic techniques is needed to quantify the burden of malaria in areas where multiple species may be present.

**Abbreviations**

HRP2: Histidine-rich protein 2; OPD: Outpatient department treatment; PCR: Polymerase chain reaction; RDTs: Rapid diagnostic tests.

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**Authors’ contributions**

DA, BO, JO read the blood smears and counted the parasites; OM, DA, CA wrote the draft of the manuscript; SGS and TB conceived and prepared the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

Not applicable. All data are included in the manuscript.

**Declarations**

**Ethics approval and consent to participate**

Data was collected under a protocol that was approved by the School of Biomedical Sciences Research and Ethics Committee Makerere University College of Health Sciences (SOM-REC IRB; Protocol # SBS-363), UNCEST (Number H5-2110), the Human Research Protection Program Institutional Review Board of the University of California-San Francisco (IRB # 16-2050), the London School of Hygiene & Tropical Medicine and the Liverpool School of Tropical Medicine (15-025).

**Consent for publication**

All authors have given their consent for this publication.

**Competing interests**

The authors have declared that no competing interests exist.

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