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DIAGNOSIS OF MALARIA IN A TRAVELER 9 MONTHS AFTER RETURNING FROM WEST AFRICA BY *ILLUMIGENE*® LAMP ASSAY: A CASE REPORT

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Loop-mediated isothermal amplification (LAMP) is a rapid molecular technique that has been introduced into malaria diagnosis. The test is easy to perform and offers high sensitivity. We report a 53-year-old male patient who was hospitalized with fever attacks, chills, and headache caused 9 months after returning from Africa. During his stay in Africa, he used malaria chemoprophylaxis. Microscopy of thin and thick blood films and rapid diagnostic antigen testing remained negative for three times. The EDTA blood samples were tested using the Meridian *illumigene*® malaria LAMP assay that gave a positive result for *Plasmodium* spp. Diagnosis of malaria was subsequently specified as *P. ovale* infection by real-time PCR. Ovale malaria often manifests with delay and low parasitemia. The patient was treated with atovaquone-proguanil, followed by primaquine for prophylaxis of relapse. This case illustrates the usefulness of the *illumigene*® malaria LAMP assay for initial screening of malaria parasites.

Keywords: *Plasmodium ovale*, malaria, LAMP, screening

Introduction

Malaria is diagnosed in travelers returning from endemic regions. The appropriate management of patients depends on early microbiological diagnosis

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after the onset of symptoms and initiation of the specific therapy to prevent the progression to severe disease or persistent infection [1]. Standard diagnosis is based on microscopy of thin and thick blood smears. Sensitivity and specificity of microscopy is influenced by the practical experience of the laboratory staff [2, 3]. In addition, rapid diagnostic antigen tests (RDTs) are recommended by current guidelines [1, 4]. RDTs are lateral flow immunochromatographic assays that offer an easy handling and results within a few minutes, but they are less sensitive than microscopy and it is qualified only to identification of *Plasmodium falciparum* infections, whereas the sensitivities for other species are too low [3]. Although less common, the prevalence of *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*, and *Plasmodium knowlesi* is probably underestimated due to often low parasitemia [5]. In non-endemic settings, molecular tests are new promising tools that may offer a rapid and accurate screening for exclusion or identification of malaria parasites in travelers [6]. Polymerase chain reaction (PCR) has been shown to be highly sensitive and specific but most assays are laborious and take several hours before results are available, making them less suitable for an initial screening of patients [7]. An alternative amplification technique that is simple to perform is provided by loop-mediated isothermal amplification (LAMP). LAMP offers high-speed amplification under isothermal conditions without the need of DNA purification because of using a *Bst* DNA polymerase with strand-displacement activity [8–10]. The commercially available *illumigene*® malaria LAMP assay (Meridian Bioscience/Bioline, Luckenwalde, Germany) is a rapid molecular test for the detection of *Plasmodium* spp. in venous EDTA whole blood specimens, reaching a sensitivity that is comparable with PCR [11]. In this report, we describe a malaria case caused by *P. ovale* that would not have been diagnosed without using a molecular screening assay.

Case Presentation

A 53-year-old male patient was referred to our hospital with chills, fever attacks up to 39.5 °C for 5 days, and headache. Apart from a type-1 diabetes mellitus, the medical history of the patient was unremarkable. Nine months before admission, he was on a backpacking tour in Ghana, Togo, and Benin and he had traveled to Greece recently. During his visit to Africa, he used malaria prophylaxis as recommended (atovaquone-proguanil; Aliud Pharma, Laichingen, Germany). He was presented at the hospital admission with elevated levels of C-reactive protein (CRP; 119.2 mg/L), procalcitonin (0.90 ng/ml), lactate dehydrogenase (489.6 U/L), aspartate aminotransferase (36 U/L), bilirubin (53 μmol/L), and a decreased platelet count of 61 Gpt/L. White blood cells were within the normal

range (4.7 Gpt/L). The initial blood pressure was 120/70 mmHg, the heart rate was 88/min, and the respiratory rate was within the normal range. Abdominal ultrasound revealed splenomegaly (18 cm) and a chest X-ray showed pulmonary infiltrations. Blood cultures were taken three times but remained sterile. An antibiotic treatment with ampicillin/sulbactam was initiated but did not lead to an improvement of the elevated CRP level (121.4 mg/L on Day 4) or of clinical symptoms. Procalcitonin increased to 4.74 ng/ml and antibiotic therapy was escalated to piperacillin/tazobactam. Laboratory evaluation showed increasing bilirubin (65 μmol/L), aspartate aminotransferase (336 U/L), and alanine aminotransferase (294 U/L). As haptoglobin was below lower limit of detection and hemoglobin was decreased to 5.5 mmol/L, a diagnosis of hemolysis could be made in combination with elevated levels of bilirubin and lactate dehydrogenase. A computed tomography evaluation of thorax and abdomen was performed, but despite a hepatosplenomegaly, no infectious focus could be identified. Dengue fever RDT was negative. The patient had received vaccination against yellow fever before traveling. We could detect IgG antibodies with an 1:160 titer without IgM antibodies. PCR for *Leishmania* spp. from an EDTA blood sample remained negative. Because of the travel history, malaria testing was performed. Repeated microscopy of thick and thin blood films on three consecutive days and RDT (BinaxNow® Malaria; Alere Technologies, Cologne, Germany) were negative. The antigen targets of the RDT included the *P. falciparum*-specific histidine-rich protein 2 and the *Plasmodium* genus-specific aldolase [12]. All microscopic slides were examined by a specialist in malaria diagnosis.

In addition, molecular diagnostic was performed using the *illumigene*® malaria LAMP assay that targets a sequence of *Plasmodium* spp. mitochondrial DNA non-coding region conserved across *P. falciparum, P. vivax, P. ovale, P. malaria*, and *P. knowlesi*. An amount of 50 μl of EDTA blood was added to a ready-to-use tube containing lysis buffer I and incubated at room temperature for 2 min. An amount of 50 μl of the lysate was transferred into the sample preparation tube and filtered into a clean Eppendorf tube. Each 50 μl of the filtrate was pipetted into both the test and internal control chamber of the test device containing lyophilized master mixes. The test was run in an *illumipro*-10 machine (Meridian Bioscience) for 40 min. The EDTA whole blood sample of the patient was tested positive for *Plasmodium* spp. For species identification, an aliquot was sent to the German National Reference Center for Tropical Pathogens at the Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany). *Plasmodium* infection was confirmed by real-time PCR (RealStar Malaria PCR Kit 1.0; altona Diagnostics, Hamburg, Germany) and *P. ovale* was identified as the causative species using species-specific in-house multiplex PCR [6]. Therapy with atovaquone-proguanil was initiated, followed by a 14-day course of primaquine for prophylaxis of

relapse. With this treatment, fever disappeared, the general condition improved, and CRP levels and platelet counts normalized. At the time of discharge from hospital, CRP was 2.6 mg/L and platelet count was 286 Gpt/L. Liver enzymes were decreasing and gave a negative result at a control 4 weeks after discharge. Anemia disappeared and haptoglobin, bilirubin, and lactate dehydrogenase were normalized as well.

Discussion

Current US and European guidelines recommend microscopy of thick and thin blood films combined with RDT as standard procedures for the diagnosis of malaria [13, 14]. To rule out infection, febrile patients returning from malaria-endemic regions have to be tested three times because microscopy cannot reliably identify parasite numbers <50/µl [1, 2]. RDTs detect ≥100–200 parasites/µl and have a low sensitivity for identification of non-*P. falciparum* infections, especially for *P. malariae* and *P. ovale* as observed in this case [4, 12, 15, 16]. Limitations to identify infections with low parasitemia can be overcome by PCR that can detect <5 parasites/µl [8, 9, 17, 18]. LAMP also has an adequate analytical sensitivity to detect low parasite-level parasitemia, compared to PCR [7, 10, 11].

The *illumigene*® malaria test is a simple and rapid molecular diagnostic test allowing its integration into a 24/7 routine workflow. Because of a simplified sample preparation step and ready-to-use reagents, no technical expertise in molecular biology is needed and the results can be obtained within 1 h. In a recent study, both sensitivity as well as specificity of LAMP was found to be 100% versus microscopy when discrepant results were resolved using PCR [11]. Therefore, the *illumigene*® malaria test represents a useful and cost-effective tool for emergency diagnostics [6, 7]. Because of its high negative predictive value, it has been suggested that the current test algorithm of three microscopic tests to rule out an infection can be replaced by a single LAMP test, thereby reducing the inconvenience for the patient and saving costs [11].

In this case report, we presented a patient who developed unspecific symptoms 9 months after a travel to West Africa. *P. ovale* infection would not have been identified without a sensitive molecular test. *P. ovale*, which is now separated into the two sympatric species *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*, is generally linked to rather mild disease but severe complications can develop [19]. The delay in the manifestation of symptomatic disease is characteristic even when atovaquone/proguanil chemoprophylaxis has been used. Liver hypnozoites of *P. ovale* probably arrest early enough during the developmental cycle and may not be eliminated by anti-malaria drugs [20].

Because a late onset of disease after *P. ovale* or *P. vivax* infection is observed in a major part of the patients, it is recommended to consider malaria within 2 years after people return from endemic areas. This case illustrates that the integration of the *illumigene*® malaria LAMP assay into the diagnostic algorithm for malaria infections is beneficial in patients with a low density of parasites in peripheral blood.

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Conflict of Interest

The authors state that there is no conflict of interest.

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