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BRIEF REPORT

Missed *Plasmodium ovale* Infections Among Symptomatic Persons in Angola, Mozambique, and Ethiopia

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The majority of symptomatic malaria in sub-Saharan Africa is caused by *Plasmodium falciparum*. Infection with *Plasmodium ovale* is often not recorded and not considered clinically relevant. Here, we describe 8 cases of *P ovale* infection from 3 African countries—all of which were misdiagnosed at the presenting health facility.

Keywords. diagnosis; malaria; *Plasmodium ovale*; rapid diagnostic tests.

Malaria is a major public health concern in many global settings with approximately 229 million cases in 2019 alone [1]. Malaria infection in sub-Saharan Africa is most often due to the *Plasmodium falciparum* parasite, which causes the most severe forms of the disease [1]. As such, *Plasmodium ovale* and *Plasmodium malariae* are not of focus for clinical diagnosis or national malaria programs. Endemic transmission of *P ovale* has been reported in areas of sub-Saharan Africa, Papua New Guinea, the eastern parts of Indonesia, and the Philippines [2]. Even in areas with known *P ovale* transmission, the proportion of malaria cases due to *P ovale* seldom exceeds 5% [2]. It is generally considered to cause mild infection and is seldom accounted for in epidemiological estimates [3]. There may be several reasons for the paucity of data regarding *P ovale* epidemiology, including challenging diagnosis by microscopy due to generally low parasite densities and short duration of symptomatic infection [3]. In the case of coinfection with *P falciparum* and *P ovale*, *P ovale* may be difficult to distinguish from the higher-density *P falciparum* parasite, so the case may be classified as *P falciparum* only [4]. *Plasmodium ovale* is morphologically similar to *Plasmodium vivax*, making it difficult to distinguish by light microscopy [3, 5]. In addition, malaria rapid diagnostic tests (RDTs) have poor sensitivity for detecting *P ovale* infection, with the only current antigen target able to detect presence of *P ovale* being the pan-*Plasmodium* lactate dehydrogenase [6]. In *P falciparum*–dominant areas, many RDTs only utilize detection of histidine-rich protein 2 (HRP2) for malaria diagnosis, which would leave *P ovale* cases undiagnosed [1].

In malaria-endemic settings, when *P ovale* elicits a symptomatic infection, it is generally short-lived and relatively mild [7]. Additionally, *P ovale* can cause relapsing infection due to latent parasites (hypnozoites) that remain in the liver for weeks to years after treatment with antimalarial drugs, and hypnozoite clearance would require a regimen with an 8-aminoquinoline drug (eg, primaquine or tafenoquine) [2].

Here, we describe a series of symptomatic *P ovale* cases. Eight symptomatic *P ovale* cases were identified from a post hoc analysis of dried blood spot (DBS) samples collected during health facility surveys in Angola (2016) and Mozambique (2018), and a therapeutic efficacy study (TES) in Ethiopia (2017). All of these cases were either misdiagnosed as infection with other malaria species, or not diagnosed as malaria.

METHODS

The Angola [8] and Mozambique [9] health facility surveys have been described previously. In brief, any person attending the health facility, regardless of symptoms, was eligible to participate. Enrolled participants were tested with a *P falciparum* or *P falciparum/P vivax* RDT and any antimalarial drugs were administered based on RDT results and national treatment guidelines. For the TES in Ethiopia, persons presenting to the health facility with symptoms of malaria and diagnosed with *P falciparum* or *P vivax* monoinfection by microscopy were eligible for the study.

For all studies, finger-prick blood was dried on filter paper (Whatman 903 cards, GE Healthcare) to create a DBS for further laboratory analysis by a bead-based assay for detection of *Plasmodium* antigens [10], and detection of *Plasmodium* DNA by photo-induced electron transfer polymerase chain reaction (PCR) [11]. Samples showing presence of pan-*Plasmodium* antigens and low or absent HRP2 through multiplex assay screening had DNA extracted for PCR assays with primer sets specific for each of the 4 human malaria parasites.

The DBS samples from all 3 studies were shipped to the US Centers for Disease Control and Prevention's (CDC) Malaria Branch laboratory in Atlanta for post hoc testing. CDC

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researchers did not have access to identifying information. All 3 studies were reviewed by the CDC (2016-003, 2017-517, and 6892.0) and local ethics review boards, and were conducted consistent with applicable federal law and CDC policy.

RESULTS

In total, 1267 samples were screened from the Angola survey, 1861 samples from the Mozambique survey, and 147 samples from the Ethiopia TES. Based on the selection criteria outlined in the Methods, 35 samples underwent PCR assays to determine infecting *Plasmodium* species: 8 from Angola, 6 from Mozambique, and 21 from Ethiopia. Through PCR analysis, 8 samples among these 3 studies were positive for *P ovale* DNA. One of the Mozambique participants had a mixed *P falciparum*/*P ovale* infection. The 8 samples from *P ovale* infections were all obtained from persons experiencing at least 1 clinical symptom consistent with malaria, including fever, chills, headache, or joint pain. The majority of the *P ovale* infections were in persons 15 years or younger (5/8 [62.5%]) (Table 1). The most common symptom was fever (87.5%) and headache (87.5%), followed by chills/weakness (37.5%) and joint pain (37.5%).

For 7 of the 8 patients, no malaria diagnosis was provided during the routine health facility visit. All 7 were from the Mozambique (4) or Angola surveys (3) where the only malaria diagnostic test was a *P falciparum* or *P falciparum*/*P vivax* RDT—all returned a negative test result and no antimalarial treatment was provided. All 7 cases were prescribed an antipyretic to treat their symptoms, and 5 were prescribed antibiotics for treatment of their ailments. For the *P ovale* case from Ethiopia, though the individual was negative by *P falciparum*/*P vivax* RDT, a *P vivax* diagnosis was given based on microscopic examination of the parasite, and the patient was erroneously enrolled in the *P vivax* arm of the clinical trial and provided chloroquine treatment followed by a 14-day primaquine course.

DISCUSSION

Although often considered a clinically mild parasite of minor importance in sub-Saharan Africa, *P ovale* can cause symptomatic infection leading to treatment-seeking behavior. Through screening samples for malaria parasite antigen and DNA, we found 8 *P ovale* cases that caused symptomatic disease in patients from 3 African countries. Seven of the 8 cases received no malaria diagnosis at presentation to the health facility and therefore did not receive any treatment for malaria. The misdiagnosed case in Ethiopia (*P ovale* diagnosed as *P vivax*) received treatment with chloroquine and 14-day primaquine—the same treatment for *P ovale*. While this switched diagnosis was not an issue clinically, widespread misdiagnosis of *P ovale* as *P vivax* by microscopy could affect the estimation of *Plasmodium* species

Table 1. Characteristics of Persons With *Plasmodium ovale* Infection

Country (Province)	Survey Type, Year	Age	Sex	Fever	Symptoms	Original Diagnosis	RDT Result	Test Type Used	PCR Result	Treatment Received or Prescribed
Angola (Uige)	HF survey, 2016	15 mo	Female	Yes	Stomachache, weakness, headache	None	Negative	Pf/Pv RDT	*Plasmodium ovale*	Antipyretic
Angola (Uige)	HF survey, 2016	4 y	Male	Yes	Headache, stomachache, chills	Enteric disease	Negative	Pf/Pv RDT	*P ovale*	Antipyretic and an antibiotic
Angola (Uige)	HF survey, 2016	60 y	Female	Yes	Joint pain, headache, lack of appetite	None	Negative	Pf/Pv RDT	*P ovale*	Antipyretic and an antibiotic
Mozambique (Maptuo)	HF survey, 2018	23 y	Female	No	Chills, weakness, joint pain, headache	None	Negative	Pf RDT	*P ovale*	Antipyretic and albendazole
Mozambique (Zambezia)	HF survey, 2018	4 y	Female	Yes	Headache, vomiting	None	Negative	Pf RDT	*P ovale*	Antipyretic and an antibiotic
Mozambique (Zambezia)	HF survey, 2018	45 y	Female	Yes	Headache, joint pain, backache	None	Negative	Pf RDT	*P ovale*	Antipyretic and an antibiotic
Mozambique (Zambezia)	HF survey, 2018	15 y	Male	Yes	Headache	None	Negative	Pf RDT	*P ovale* + *Plasmodium falciparum*	Antipyretic and an antibiotic
Ethiopia (Amhara)	TES, 2017	15 y	Male	Yes	Unknown	*Plasmodium vivax* malaria	Negative	Microscopy, Pf/Pv RDT	*P ovale*	Chloroquine and 14-d primaquine

Abbreviations: HF, health facility; Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*; RDT, rapid diagnostic test; TES, therapeutic efficacy study.

distribution in a country. These findings from 3 separate settings highlight the potential of *P ovale* to cause clinical disease in diverse parts of sub-Saharan Africa.

In sub-Saharan Africa, *P falciparum* is the predominant malaria species causing morbidity and mortality and, as a result, is the primary target for malaria diagnosis and reporting in many settings [1]. It follows that *P ovale* infections are likely underdiagnosed in sub-Saharan Africa. Many studies throughout *P ovale*–endemic settings have found a very low (<1.0%) or complete absence of this species by microscopy, but PCR assays on blood samples from the same populations found prevalence an order of magnitude higher [3]. In Nampula Province, Mozambique, a high seroprevalence (43%) for the *P ovale* MSP-1_{19} antigen has been reported [12]. In Ethiopia, *P ovale* seroprevalence was estimated to be 3.1% in 2015 [13]. The prevalence of *P ovale* infection in eastern Tanzania, which neighbors Mozambique, was estimated to be 3.6% by PCR and infected persons were not found to have greater odds of symptoms vs uninfected persons [14]. This and other PCR studies have shown that *P ovale* transmission does not necessarily decline as the prevalence of *P falciparum* declines in a region [14, 15]. This suggests that despite improved efforts for vector control and parasite reduction, if *P ovale* is not accounted for in surveillance and clinical practices, it may remain undiagnosed and the true magnitude of transmission unknown. One study in Cameroon found 5 symptomatic *P ovale* cases that were RDT negative/microscopy positive for malaria [16]. Although there are few reports of clinical *P ovale* infections in Africa, there have been several reports of symptomatic *P ovale* infection among travelers returning from Africa [17–21]. While the majority of *P ovale* cases are uncomplicated malaria, a systematic review found that 3% of patients infected with *P ovale* developed severe malaria [22].

For this current study, the suspicion of non-*falciparum* malaria infection was initially generated though the post hoc multiplex screening of these sample sets for *Plasmodium* antigens. Those samples showing presence of pan-*Plasmodium* antigens (and low or absent HRP2) had DNA extracted and were speciated by PCR. For that reason, this study may have underestimated true *P ovale* infection from these samples as we could have missed *P falciparum*/*P ovale* mixed infections if abundant HRP2 antigen was present in the blood sample.

Although reports of *P ovale* infection are rare, when considering the diagnostic difficulties due to low parasitemia, HRP2-only RDTs, and distinguishing between *P ovale* and *P falciparum* and/or *P vivax* using microscopy, cases of *P ovale* may be underreported. The majority of malaria diagnostic tests used in sub-Saharan Africa are not set up to identify *P ovale* species. Next-generation diagnostic tools that accurately identify all 4 species of human malaria with high sensitivity would allow for timely detection and treatment in Africa and elsewhere. In the meantime, patients with persistent fever following a negative RDT result may be referred to a hospital for secondary microscopic diagnosis as is currently practiced in multiple countries in sub-Saharan Africa. Future studies investigating the burden of *P ovale* in sub-Saharan Africa will help inform the epidemiological significance of these results.

Notes

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Patient consent. Written patient consent was obtained for all studies. For the Angola health facility survey, review and approval were given by the Angolan Ministry of Health. The Mozambique health facility survey was reviewed and approved by the Mozambique National Health Bioethics Committee. The Ethiopia therapeutic efficacy study protocol was approved by the Ethiopian Public Health Institute, the National Ethical Committee, and the Food, Medicine and Health Care Administration and Control Authority in Ethiopia. All 3 studies were reviewed and approved by the Centers for Disease Control and Prevention (CDC) (2016-003, 2017-517, and 6892.0).

Disclaimer. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the CDC.

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