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Dengue infection as a potential trigger of an imported *Plasmodium ovale* malaria relapse or a long incubation period in a non-endemic malaria region

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SUMMARY

Objectives: To report that dengue fever (DF) could have triggered *Plasmodium ovale wallikeri* malaria.
Methods: A retrospective case report of *P. ovale* malaria and DF in a single patient in Rio de Janeiro, Brazil, who had lived in Angola, is presented.
Results: On the second week of illness, the patient was referred to our research service. As symptoms had persisted up to day 14, malaria was also considered, based on the patient's long-standing epidemiological history. On day 16 of illness, a thick blood smear was positive for *P. ovale* (3480 parasites/mm^3), PCR for malaria was positive for *P. ovale wallikeri*, and the kinetics of dengue virus (DENV) antibodies suggested a recent primary dengue infection.
Conclusions: Concurrent infections of DENV and malaria have rarely been reported; the actual impact of these sequential or simultaneous infections remains unknown. Therefore, DF must be considered as a potential co-morbidity for malaria, because of its influence on fluid electrolyte management. The case presented showed consistent temporal, clinical, and laboratory evidence that the relapse or the long incubation period of *P. ovale* malaria may have been triggered by a recent DF episode. To the authors' knowledge, this is the first report of DENV and *P. ovale* co-infection.

1. Introduction

Concurrent infections of dengue virus (DENV) and *Plasmodium vivax* or *Plasmodium falciparum* have rarely been reported. Thus, despite a wide geographical overlap of the two diseases in endemic areas, the actual impact of these sequential or co-infections is unknown. In fact, there are only a few reports of the simultaneous observation of dengue and malaria.[1–5] To the authors' knowledge, this is the first report of DENV and *Plasmodium ovale* co-infection.

The incidence of *P. ovale* malaria is low. Since its description in 1922, *P. ovale* malaria has typically remained restricted to tropical Africa and a few islands in Southeast Asia and the Middle East.[6] The real burden of *P. ovale* malaria is probably underestimated. Recent studies using molecular techniques have reported a prevalence of 15% in rural zones of Nigeria and Papua New Guinea and of 9.3% in children under 6 years of age in Equatorial Guinea.[7] In a recent Spanish report, *P. ovale* was diagnosed in approximately 7% of imported malaria cases, representing the second most frequently found species after *P. falciparum*, probably reflecting immigration from West Africa.[7,8] *P. ovale* malaria, usually clinically benign compared with malaria arising from other common species, has been well-studied because of its traditional use in the malaria therapy of neurosyphilis.

The existence of two species (or subspecies) of *P. ovale* occurring sympatrically in Asia and Africa – *P. ovale curtisi* and *P. ovale wallikeri* – is correlated with different patterns of relapse.[6]

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The prepatent period usually ranges from 12 to 20 days, with a median of 14.4 days. There are descriptions of incubation periods as long as 4 years, with maximum parasite counts registered around the ninth day of disease. The restriction of *P. ovale* to young erythrocytes could explain the low parasitemia usually found in infections (approximately 6000 parasites/μl). Relapses of *P. ovale* are less common than for *P. vivax*, with an estimated risk of 1:200, and relapses can arise as early as 17 days or as late as 4 years after effective therapy.[6,8] In Brazil, a total of 39 cases of *P. ovale* malaria have been recorded, showing a gradually increasing trend in recent years as a result of the migration of travelers from *P. ovale*-endemic regions.[9,10] The low prevalence of this parasite, combined with its morphological similarity to *P. vivax* and the low sensitivity of currently available rapid diagnostic tests, could increase the rates of *P. ovale* misdiagnosis.

Dengue is a mosquito-borne infection caused by four serotypes of the dengue *Flavivirus* (DENV1–DENV4) and is the most common arboviral disease worldwide.[11] Since the 1980s, dengue has become a major public health issue in Brazil, where all four serotypes have been isolated. Seven percent of dengue cases worldwide are reported from Brazil. In 2013, 921 716 dengue cases were registered by the Brazilian health surveillance system.[12,13] The incubation period ranges from 3 to 10 days, 30–50% of infections are asymptomatic, and 10% of cases may have a poor prognosis, with a 1–3% mortality rate even in the presence of ideal assistance care services.[14,15]

Even in overlapping regions, little is known about the reciprocal influence of malaria and dengue. A case of dengue in a patient from the city of Rio de Janeiro, Brazil, 2 years after leaving Luanda, Angola, is reported here. As there is no autochthonous *P. ovale* malaria in the Americas, the authors raise the possibility that dengue could have triggered a *P. ovale* malaria relapse or the expression of a latent infection not yet manifested.

2. Methods

The study was performed at the Laboratório de Doenças Febris Agudas at the Instituto Nacional de Infectologia Evandro Chagas (INI-FIOCRUZ), a regional dengue center that integrates the Center for Malaria Research and Training (CPD-Mal) of FIOCRUZ, Rio de Janeiro, Brazil. CPD-Mal covered 25% to 30% of attendant care of all confirmed malaria cases registered in the state of Rio de Janeiro from 2005 to 2014 (unpublished data). During the period December 2010 to June 2014, 24 patients were diagnosed with dengue and malaria co-infection (unpublished data). All of these patients had a history of recent travel to a malaria endemic area. The patients had never used chemoprophylaxis for malaria and all had been immunized for yellow fever.

The diagnosis of malaria cases at CPD-Mal is usually made on the basis of direct microscopic identification of the hematozoa on thick and thin blood smears stained with Giemsa, rapid diagnostic tests (RDT), and a single conventional PCR for Plasmodium[16] and *P. vivax*[17] and nested PCRs for *P. falciparum*[18] and *Plasmodium malariae*.[19] In the case of *P. ovale* diagnosis, a first reaction with the genus-specific primer set rPLU1–rPLU5 is used, followed by the second reaction using two pairs of primers: rOVA1–rOVA2 for *P. ovale curtisi* amplification[19] and rOVA1v–rOVA2 v for *P. ovale wallikeri* detection.[20]

At CPD-Mal, a dengue diagnosis is considered routinely in the protocol for the investigation of fever, as well as in suspected cases of malaria, and dengue is screened for in serum samples obtained at admission. A dengue diagnosis is confirmed by standard serological tests, IgM-Panbio in paired samples, conventional PCR, and NS1 antigen test (NS-1 Ag) within the first 5 days of symptom onset. In the case presented here, the IgG-CDC titled assay was also performed to confirm the recent dengue infection,[21–23] since the patient was referred to our service only after day 14 of fever. The previous samples collected in the basic health service units were not recovered, because during the epidemic season, the storage period is limited to 1 week, with the exception of samples from critical patients and those who have died.

3. Case report

In April 2013, a 52-year-old man was referred to CPD-Mal, FIOCRUZ, with a history of chills, fever, headache, arthralgia, myalgia, choluria, discrete jaundice, anorexia, diffuse abdominal pain, nausea, and one episode of vomiting. The illness had begun 16 days before, and the patient had already received medical care at a basic health unit, where he had been monitored every 2 days in accordance with the Brazilian protocol as a suspected dengue patient. IgM ELISA confirmed the infection on day 7 of disease, and laboratory examinations revealed leukopenia and thrombocytopenia. The patient had no criteria for severe dengue disease during the first week. He received supportive treatment, but as his fever persisted at day 14, his epidemiological history of travel was reviewed. Malaria was considered, as well the possibility of hepatitis, typhoid fever, and unusual forms of dengue, after confirmation of a long stay in Luanda, Angola.

The patient had high blood pressure and reported previous episodes of *P. falciparum* malaria in 2009 and 2011 in Angola, both treated successfully with only therapeutic schemes effective

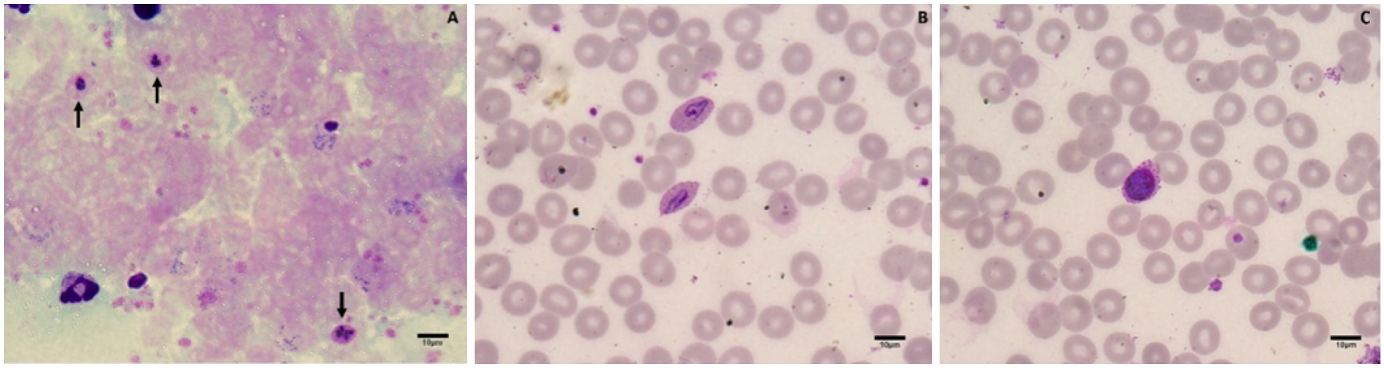

Figure 1. Giemsa-stained thick and thin blood smears. (A) *Plasmodium ovale* trophozoites (arrows) in thick smear. (B) *Plasmodium ovale* trophozoites in thin smear; see slightly enlarged oval-shaped infected erythrocytes. (C) *Plasmodium ovale* gametocyte in thin smear; note fimbriated borders of red blood cells showing dark Schüffner's granules.

Table 1
Clinical and laboratory data, according to the patient's follow-up

Febrile syndrome	Laboratory examinations			Malaria examinations			Dengue examinations		
Day of disease	Symptoms	Hb (g/dl)/ Hct (%)	WBC ($\times 10^9$/l)	Platelets ($\times 10^9$/l)	Thick blood smear, parasites/mm^3	Malaria Plasmodium PCR/ RDT	IgM-Panbio/ NS-1 Ag	IgG-CDC	Dengue PCR
1	Fever, chills, intense myalgia, anorexia, headache, abdominal pain, nausea and vomiting	13.0/38.0	5.80	114	NP	NP	NP	NP	NP
5	Same	11.9/36.0	5.90	92	NP	NP		NP	NP
7	Same	NA/35.5	8.30	95	NP	NP	Reactive[a] /NP	NP	NP
8	Same	11.6/35.7	9.60	137	NP	NP	NP	NP	NP
Patient begins follow-up at FIOCRUZ									
16	Fever, chills, anorexia, myalgia, nausea, abdominal pain	10.0/32.4	8.98	145	3480[a]	Positive[a]/Negative	Reactive[a] / Negative[a]	1/2560[a]	Negative
19	Anorexia and nausea	10.7/32.1	9.99	299	Negative	Positive[a]/Negative	Reactive /NP	1/2560[a]	NP
21	Nausea	10.0/30.0	10.98	421	Negative	Negative/Negative	NP	NP	NP
35	None	11.0/32.1	6.48	254	Negative	NP/Negative	NP	1/10 240[a]	NP
60	None	13.1/38.6	4.69	200	Negative	NP/Negative	Reactive /NP	1/10 240[a]	NP
90	None	NP	NP	NP	NP	NP	Not Reactive /NP	1/10 240[a]	NP

Hb, hemoglobin; Hct, hematocrit; WBC, white blood cell count; RDT, rapid diagnostic test; Ag, antigen; CDC, US Centers for Disease Control and Prevention; NP, not performed; NA, not available.
[a] Results that support the hypothesis of the study.

for *P. falciparum* malaria, without the use of primaquine. Epidemiologically important facts were his residence in Angola between 2007 and 2011, with short visits to a non-endemic malarial area in Brazil every 3 months. In December 2011, he moved back to Brazil and did not return to Angola or visit another malaria endemic area.

On admission to the outpatient service of FIOCRUZ on day 16 of disease, the patient presented moderate anemia, fever, mild jaundice, and dehydration, without signs of circulatory collapse. There was no tachycardia, no respiratory distress, and no coagulation disturbance. A thick blood smear was positive for *P. ovale* and the parasitemia was 3480 parasites/mm^3, with the presence of gametocytes, schizonts, and trophozoites (Figure 1). The PCR for malaria was positive for Plasmodium and negative for *P. vivax*, *P. falciparum*, and *P. malariae*. An RDT was negative. In view of these results, a blood sample was submitted to *P. ovale* PCR using primers specifically designed for the diagnosis of *P. ovale curtisi*[19] and *P. ovale wallikeri*[20]; *P. ovale wallikeri* was identified.

The patient had no glucose 6-phosphate enzyme deficiency. The results of a complete blood count were as follows: hemoglobin 10.0 g/dl, hematocrit 32.4%, platelets 145 $\times 10^9$/l , and white blood cell count 8.98 $\times 10^9$/l (with differential 0/0/0/1/15/53/21/10). Other values included alkaline phosphatase of 130.0 U/l, gamma-glutamyl transferase of 382.0 U/l, aspartate aminotransferase of 80.0 U/l, alanine aminotransferase of 36.0 U/l, C-reactive protein of 10.5 mg/dl, total bilirubin of 1.96 mg/dl, and creatinine of 1.67 mg/dl. Table 1, shows a summary of the most relevant laboratory results and clinical signs for the complete patient follow-up.

The serology for dengue panel was repeated on day 16, and the serum was reactive for IgM and IgG. NS-1 Ag and dengue PCR results were negative, as expected. This step was performed in order to differentiate between sequential and simultaneous infections. There was a significant dengue IgG-CDC titer increase (seroconversion), and dengue IgM-Panbio was still reactive on day 60 and negative on day 90 of follow-up, suggesting a recent primary dengue infection, although a previous DENV infection could not be ruled out completely (Table 1). Acute viral hepatitis and typhoid fever were ruled out by serological tests and cultures.

Malaria treatment was initiated immediately using chloroquine and primaquine (total cumulative dose 3.2 mg/kg), after which the patient became apyretic. The parasitemia decreased rapidly and became negative on day 3 of treatment. Follow-up was performed weekly until day 90, and the patient was considered cured, with complete clinical recovery after this period.

4. Discussion

This is the first report of concurrent or sequential dengue and ovale malaria. Considering that it would be difficult to discriminate a malaria relapse or a delayed incubation period from a new *P. ovale* infection in endemic regions and that there is no transmission of this species in the Americas, it was considered that this case deserved to be reported. Unfortunately, as a result of the lack of initial samples, the demonstration of recent DF had to be investigated with only the specific antibody kinetics. Nevertheless, the profile and chronology of the clinical presentation of the disease clearly points to the temporal precedence of dengue in relation to the *P. ovale wallikeri* malaria episode. This raises the possibility that the dengue may have triggered the emergence of a latent previous *P. ovale* infection or the relapse of a very old ovale malaria. The intense inflammatory reaction, classically recognized in symptomatic dengue and well-described in severe cases,[24] could have caused a stress response resulting in the re-emergence of *P. ovale* in the circulation.

Because malaria is classically recognized to be a disease caused by an agent endowed with important mitogenic and polyclonal

B-lymphocyte activation (PBA) properties,[25,26] one could ask whether the dengue serological evidence could not have arisen from the marked malaria-associated PBA.[27,28] However, some evidence indicates that this was not the case in the present patient: (1) clinically, there were two clear disease patterns; (2) false-positive serological results related to the malaria-associated PBA usually present low titers and decrease early after treatment,[28] unlike the situation described here in which there were significant antibody dengue titers at up to day 90 of the disease; and (3) although the anti-DENV IgM test was negative on day 90, the specific IgG titers continued to rise after malaria treatment on day 16, despite parasite clearance (confirmed by negative PCR on day 19) and it was still high on day 90.

The outcome of the concurrence of a non-severe dengue with *P. ovale* malaria reported here was positive, possibly as a result of a co-infection of the DENV with a relatively benign Plasmodium. However, this outcome should not always be expected, as both diseases may present severe forms and co-infection could enhance their respective morbidities. This has already been reported in DENV and *P. falciparum* and in DENV and *P. vivax* co-infections.[5,29–38]

Some authors have recently reported that usually benign forms of *P. vivax* malaria are becoming more severe in some regions of South America and Asia. Because dengue and malaria overlap in some of these regions,[29,37–39] the data presented here emphasize the need for the accurate recognition of dengue as a co-morbidity of malaria to avoid the false diagnosis of severe disease caused by a relatively benign Plasmodium such as *P. vivax*.

The clinical case presented here showed temporal, clinical, and laboratory evidence consistent with a relapse or a long incubation period of a *P. ovale wallikeri* malaria being triggered by a dengue episode. Clinicians must therefore keep this possibility in mind when facing cases with a clinical evolution similar to that described here. The results also highlight the importance of adequate training for malaria diagnosis, allowing the detection of imported cases caused by species of Plasmodium that are not prevalent in the country, such as *P. ovale* in Brazil.

Finally, this is the first description of *P. ovale wallikeri* in Angola. Further studies should be done using *P. ovale*-specific PCR to assess the prevalence of *P. ovale curtisi* and *P. ovale wallikeri* in endemic areas of Angola.

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Ethics statement: Written consent was obtained from the patient under a research project approved by the Ethic Committees of IPEC/FIOCRUZ: "Detecção de formas não usuais de dengue a partir da vigilância de síndrome febris agudas", CAAE 0026.0. 009.000-07.

Conflict of interest: The authors have no competing interests to declare.

Author contributions: OL and FR liaised with the clinic regarding the management of the patient, collected the clinical information, and drafted the manuscript; SS and GMZ established the parasitological diagnosis of *P. ovale* malaria; AL performed the malaria molecular diagnosis; RMRN performed the antigenic research and the serological and molecular tests for the diagnosis of dengue; MFFC supervised the molecular malaria diagnosis and reviewed the manuscript; CTDR and PB discussed and reviewed the manuscript. All authors read and approved the final manuscript.

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