Short Report: A Case of Quadruple Malaria Infection Imported from Mozambique to Japan

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Abstract. A 35-year-old Japanese man had an intermittent fever and mild headache for eight weeks after he returned to Japan from working in Mozambique. He had taken antimalarial prophylaxis (doxycycline) for 25 weeks, and stopped taking this drug two weeks after his return. Microscopic examination of a peripheral blood smear showed a mixed infection with Plasmodium vivax, P. falciparum, and P. ovale. In addition, a nested polymerase chain reaction and subsequent sequencing detected specific DNA sequences of four species of Plasmodium, including P. malariae. The patient was successfully treated with artemether-lumefantrine and primaquine phosphate. The present case is a rare instance of a mixed infection with four species of Plasmodium. Nonimmune persons in malaria-endemic areas may have a risk of mixed infection. All four species must be identified by using sensitive and specific tests, such as a nested polymerase chain reaction, in addition to morphologic identification.

Although many cases of mixed malaria infection have been reported in malaria-endemic countries, coincidental infection with more than one species of Plasmodium spp. is rare in non-endemic countries. In Japan, only approximately 50–80 cases of malaria are reported each year, and are all imported malaria. In this report, we describe a Japanese man who was given a diagnosis of a mixed infection with four species of Plasmodium (P. vivax, P. falciparum, P. ovale wallikeri, and P. malariae) by using a nested polymerase chain reaction (PCR) after he returned from Mozambique.

CASE REPORT

A 35-year-old Japanese man had intermittent fever and mild headaches for eight weeks after returning from Mozambique. He returned to Japan at the end of June after working as an instructor in operating construction machinery during for two years (June 2010–June 2012). He had taken antimalarial prophylaxis (doxycycline) for 25 weeks from January through the second week of July in 2012. He had no symptoms suggestive of malaria while in Mozambique, but a slight fever developed on August 25, 2012, in Japan. A high fever developed on August 25, 2012, in Japan. Physical examination at admission showed a body temperature of 37.9°C, a respiratory rate of 27 beats/minute, a heart rate of 100 beats/minute, a blood pressure of 108/68 mm Hg, anemia in the palpebral conjunctiva, hepatomegaly, and splenomegaly. The hemoglobin level was 11.3 g/dL, the mean corpuscular volume was 88.7 fl, and the platelet count was 54 × 10^9/L. Biochemical tests showed increased serum levels of aspartate aminotransferase (32 U/L, reference value < 30 U/L), lactate dehydrogenase (379 U/L, reference range 110–219 U/L) and C-reactive protein (5.57 mg/dL, reference value < 0.3 mg/dL). A coagulation test result for the prothrombin time–international normalized ratio was slightly prolonged (1.18, reference range = 0.80–1.10). An ultrasonographic examination showed hepatomegaly (140 mm thickness along the mammary line), splenomegaly (160 × 75 mm), and reactive swelling of the portal hepatic lymph nodes.

A rapid diagnostic test (OptiMAL-IT; DiaMed, Cressier, Switzerland) for malaria with blood of the patient showed three positive bands for the control, Plasmodium spp., and P. falciparum. A thin blood smear stained with Giemsa showed P. vivax, P. falciparum, and P. ovale (Figure 1). Trophozoites of P. vivax were dominant in the microscopic examination, and the total parasitemia including other species was 1.7%.

The patient was treated with Coartem (artemether 20 mg/ lumezantrine 120 mg) in a six-dose regimen for three days (total = 24 tablets), followed by primaquine, 15 mg/day for 14 days. The patient soon became afebrile and subsequently left the hospital on the seventh day of hospitalization. Since that time, Plasmodium has not been detected, and his symptoms did not recur.

To identify the exact Plasmodium species, Plasmodium DNA was extracted from the patient's blood by using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) and was subjected to a nested PCR specific for the 18S ribosomal RNA gene. Plasmodium genus–specific primers and four pairs of species-specific primers described in a previous study were used for the nested PCR. Results showed that the patient was infected with four species of Plasmodium, including P. malariae, which was not identified in the blood smear by microscopy (Figure 2). Amplified fragments were cloned into a vector and sequenced. The fragment amplified with P. falciparum-specific nested primers showed 100% identity with the P. falciparum 18S ribosomal RNA gene (GenBank accession no. JQ627152.1, expected = 3 × 10^-35). Fragments amplified with P. vivax-specific, P. ovale-specific, and P. malariae-specific nested primers showed a single nucleotide substitution in the P. vivax 18S ribosomal RNA gene (GenBank accession no. JQ627158, expected = 1 × 10^-39), the P. ovale 18S ribosomal RNA gene (GenBank accession no. AB182490.1, expected = 9 × 10^-35), and the P. malariae 18S ribosomal RNA gene (GenBank accession no. M548971.1, expected = 9 × 10^-34), respectively.

Because two types of P. ovale strains (classic and variant types) are prevalent in Africa and Asia, and it has been reported that the two types are distinct species, we generated specific nested-primers specific for the P. ovale cytochrome b
Some triple or quadruple mixed malaria infections have been reported among semi-immune residents in malaria-endemic areas of Asia and Africa. However, triple or quadruple mixed malaria infections have been rare among non-residents of such areas or among nonimmune persons. The present case is the first imported case of quadruple mixed malarial infections among nonimmune persons.

**DISCUSSION**

Some triple or quadruple mixed malaria infections have been reported among semi-immune residents in malaria-endemic areas of Asia and Africa. However, triple or quadruple mixed malaria infections have been rare among non-residents of such areas or among nonimmune persons. The present case is the first imported case of quadruple malaria in a nonimmune Japanese patient.

In mixed malaria infections, the level of parasitemia is different for each *Plasmodium* spp. In Japan, some mixed infections have been confirmed by combined use of morphologic detection and other alternative methods, including immunoassays and the molecular analyses. The findings in this report, as well as in other reports, indicate that molecular detection methods, such as nested PCR and real-time PCR, have superior sensitivity and specificity, which enables identification of low levels of infection and differentiation of species.

For antimalarial chemoprophylaxis, mefloquine is recommended in Japan, but it cannot be used in areas with mefloquine-resistant malaria. Doxycycline and atovaquone/proguanil can be used in all malaria-endemic areas, and these drugs are used in Japan. These drugs are effective against erythrocytic forms and also inhibit development of the normal liver stages, but they do not affect hypnozoites. Therefore, development of clinical malaria from hypnozoites of *P. vivax* or *P. ovale* has been reported more than two months after returning among travelers who continued chemoprophylaxis based on standard regimens.

Daily use of doxycycline is recommended during travel to malaria-endemic areas and for four weeks after returning to a area where malaria is not endemic. However, the patient in this study stopped taking doxycycline after two weeks. Similar prophylactic failures for doxycycline against *P. falciparum* or *P. vivax* are associated with use of inadequately low doses.

Moreover, the prevalence of *P. falciparum* isolates with reduced susceptibility to mefloquine and doxycycline has been reported in Africa. In nonimmune persons taking chemoprophylaxis, efficacy differs for each *Plasmodium* spp. Furthermore, different levels of drug resistance to each strain and the dose of drugs used influence the clinical manifestations in cases with chemoprophylactic failure. *P. falciparum* might dominate over *P. vivax* by inducing a primary infection, as occasionally observed in patients simultaneously infected with the two species. In the present case, however, *P. vivax* predominated over *P. falciparum*. A partial effect of doxycycline was conceivably obtained because the parasitemia with *P. falciparum* and *P. malariae* was low. Detection of *P. malariae* was difficult by
microscopy and was confirmed by using the nested PCR. A variant strain of *P. ovale* could not be detected by PCR for the classic strain, and its infection was confirmed by the more specific protocol of PCR used for the present case.

Although the Duffy-negative phenotype *FY*<sup>BE</sup><sup>ES</sup>, which is not susceptible to infection with *P. vivax*, is dominant in sub-Saharan Africa,21 Duffy-negative persons infected with *P. vivax* have been reported and suggested as a possible reservoir of *P. vivax* to Duffy-positive persons, such as those seen in Japanese persons.22 As more nonimmune persons visit malaria-endemic countries in Africa, there are likely to be more cases of imported mixed infections of *Plasmodium* species, including *P. vivax*. Nonimmune persons who stay in malaria-endemic areas for long periods face an increasing risk of mixed infection, and clinical features are modified by chemoprophylaxis. When nonimmune persons show atypical clinical features for malaria or when chemoprophylaxis failure is suspected, all four species of human malaria parasites should be considered as causative agents, and the patients should be examined by using sensitive and specific tests, such as the PCR, in addition to conventional morphologic analyses.

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