OCCULT PLASMODIUM VIVAX INFECTION DIAGNOSED BY A POLYMERASE CHAIN REACTION–BASED DETECTION SYSTEM: A CASE REPORT

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Abstract. After a trip to Zambia, a previously healthy adult traveler presented with a prolonged illness characterized by low-grade fevers and fatigue. Although malaria smears and antibody tests results for Plasmodium species were negative, a diagnosis of malaria was ultimately determined by polymerase chain reaction (PCR) amplification and species-specific nucleic acid hybridization techniques. The patient was successfully treated and cured. Clinical use of PCR technology may facilitate the identification of cases of smear-negative malaria, which up to the present time, have been difficult to diagnose.

INTRODUCTION

We report the case of a previously healthy returning adult traveler who presented with a prolonged illness characterized by low-grade fevers and fatigue. Malaria smears and antibody test results for Plasmodium species were negative. A diagnosis of malaria was made by polymerase chain reaction (PCR) amplification and species-specific nucleic acid hybridization techniques.

CASE REPORT

The patient was a 23-year-old woman with no significant prior medical history. She presented with daily low-grade fevers, malaise, and fatigue for three weeks after returning from Zambia. She had spent the month of March 2002 in Zambia volunteering at an acquired immunodeficiency syndrome (AIDS) orphanage. Prior to travel to Zambia, she had been seen in the Travel Clinic at University Hospitals of Cleveland. She had received appropriate vaccinations. She used malaria prophylaxis that she had acquired locally in Zambia but was not able to recall its name. She reported no illnesses in Zambia. One year previously she had spent a month in Mozambique where she also volunteered at an AIDS orphanage. She reported having no illnesses from that trip. Her current symptoms were assessed at this initial visit with a thick and thin malaria smear, which showed no organisms. Results of all other tests including a complete blood count (CBC), chemistry panel, and liver function tests were unremarkable.

Two weeks later, she returned to Travel Clinic with persistent fatigue, low-grade fevers to 38.1°C, and occasional nausea that did not significantly limit her food and liquid intake. She had a repeat malaria smear that was unremarkable. Her thyroid stimulating hormone level was normal.

She was followed up one month later at which time the symptoms had returned. She was seen again in the clinic, where test results for antibody levels to all four human Plasmodium species were negative. Her blood was then sent in EDTA-coated Vacutainer® (Becton Dickinson, Franklin Lakes, NJ) tubes to a research laboratory for PCR amplification of malarial parasites and species-specific nucleic acid hybridization analysis as previously described.1 The results of these studies were positive for Plasmodium vivax (Figures 1 and 2). She was retreated for malaria with chloroquine (a loading dose of 600 mg base orally, followed by 300 mg base given six hours after the first dose and again on days 2 and 3) and given a course of primaquine (15 mg base per day orally for 14 days) after the results of a test for glucose-6-phosphate dehydrogenase were normal.

After receiving definitive treatment for P. vivax infection, she has remained afebrile and her energy level has returned to baseline. The PCR testing was repeated on three blood samples collected in EDTA-coated tubes at follow-up and the results were negative for Plasmodium species.

DISCUSSION

Malaria is always a primary consideration when travelers complain of fevers after returning home from abroad. The disease has been eradicated from most temperate zone countries, but there are still > 100 million cases of malaria worldwide per year. Although most of these cases occur in the disease-endemic regions of the tropics, there are still a small number that are recognized in returning travelers from areas

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As PCR technology improves, it may allow detection of malaria parasites. DNA was extracted from whole blood using the QIAamp DNA blood mini-kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. *Plasmodium* genus diagnosis was made by a small subunit ribosomal DNA-based nested PCR. Lane A, DNA amplification ladder; lane S, sample; lane p, *P. falciparum*; lane p, *P. vivax*; lane p, *P. malariae*; lane p, *P. ovale*. P = genus *Plasmodium*. Lane S demonstrates that the sample belongs to the genus *Plasmodium*

not endemic for malaria, such as North America. For example, there were 1,167 cases of malaria diagnosed in the United States in 1995.2

This case of *P. vivax* malaria was difficult to diagnose because the results of the standard techniques normally used to evaluate a returning traveler for malaria were unrevealing. In most situations, the diagnosis of malaria in a non-immune traveler can be made with thick and thin blood smears. When malaria is suspected in a febrile returning traveler, the standard approach is to check Giemsa-stained thick blood smears every 12 hours until 3 are negative.3 The rationale for this protocol is based on the periodicity of the *Plasmodium* life cycle and the concept that the smear is more likely to be positive during the schizont rupture phase. However, White, noting that the first smear is positive in 95% of malaria cases, questioned the collection of additional smears.4 Although this patient did have three smears prepared during her two months of close monitoring, they did not aid in the diagnosis. In fact, these negative test results argued against the presence of malaria, and the disease remained a consideration only because of her persistent symptoms and a lack of evidence to suggest an alternative diagnosis.

The pivotal event in her clinical course was the improvement of her symptoms with a trial of atovaquone/proguanil. Atovaquone selectively inhibits parasite mitochondrial electron transport and is paired with proguanil because of demonstrated synergism and because resistance develops rapidly when the drug is used alone. This medication is well-tolerated and has been shown to be effective treatment for uncomplicated *P. falciparum* and *P. vivax* malaria in patients who do not take it for prophylaxis.5 However, similar to mefloquine and chloroquine, this drug combination does not eliminate the hypnozoite phase of *P. vivax* in the liver. It is therefore not surprising that although the patient began to feel better after taking the medication, her symptoms returned soon after discontinuing treatment.

Although an empirical trial of atovaquone/proguanil is not a prerequisite for further evaluation for malaria, the brief response to anti-malarial medication in this case suggested that additional tests were warranted. The diagnosis ultimately required the use of a PCR-based detection system. The detection of *Plasmodium* nucleic acids by PCR may soon become the standard for suspected cases of malaria that are not confirmed by thick and thin blood smears. It offers excellent sensitivity and specificity, approaching 100%, with few false-negative or false-positive results.6,7 It is especially useful in patients with low-grade parasitemia, detecting levels as low as 5 parasites/µL.6,7 The disadvantages are that the technique is expensive, labor-intensive, requires technical expertise, and cannot distinguish between viable and non-viable organisms. In addition, the time lag between sample collection and acquisition of results limits the usefulness of the PCR in clinical practice.8 At this time, efforts are being made to address these limitations and develop a reliable PCR method with a fast turnaround.9,10 As PCR technology improves, it may allow increased recognition of the syndrome of smear-negative malaria that up to this time has been difficult to diagnose. Currently, 30,000 travelers from industrialized countries are found to have malaria each year.11 Other returning travelers with persistent fevers and undiagnosed *Plasmodium* infections may benefit from this technology.

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**Figure 1.** Polymerase chain reaction (PCR) detection of malaria parasites. DNA was extracted from whole blood using the QIAamp DNA blood mini-kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. *Plasmodium* genus diagnosis was made by a small subunit ribosomal DNA-based nested PCR. Lane A, DNA amplification ladder; lane S, sample; lane p, *P. falciparum*; lane p, *P. vivax*; lane p, *P. malariae*; lane p, *P. ovale*. P = genus *Plasmodium*. Lane S demonstrates that the sample belongs to the genus *Plasmodium*.

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