CASE REPORT

AN AUTOCHTHONOUS CASE OF MALARIA IN PETROPOLIS, RIO DE JANEIRO, BRAZIL

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ABSTRACT

In Brazil, about 99% of malaria cases occur in Brazil’s Legal Amazon. In all other states malaria has been noted but with imported cases. Here we describe an autochthonous case of malaria in Petropolis city, Rio de Janeiro. The clinical symptoms and epidemiological aspects were compatible with malaria and the presence of Plasmodium was confirmed through molecular diagnostic testing performed on a blood sample from a local resident after an ecological hike in the Atlantic Forest on the outskirts of town. After treatment with chloroquine and primaquine, the symptoms ceased. This city was regarded malaria free for many years although it still presents the malaria vectors. Considering that Plasmodium sp. is an important cause of morbidity and mortality worldwide, this clinical case serves as an epidemiological alert.

KEY WORDS: Malaria; clinical case; Plasmodium vivax

INTRODUCTION

Malaria is transmitted by the Anopheles species of mosquito found mainly in forests. The disease is promoted by a protozoan of, at least, six different species namely: Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi. According to WHO World Malaria Report, in 2015, 212 million new cases were reported with 429,000 deaths (WHO, 2016). Plasmodium can cause a number of clinical complications, such as fever, nausea, severe anemia and multiple organ failure, and can lead to death if the infection is caused by P. falciparum, P. knowlesi and P. vivax (Arends et al., 2013), therefore early laboratory diagnosis and monitoring of patients is desirable to avoid further difficulties (Ding et al.,...
2017). Most cases of malaria in humans are promoted by *P. falciparum* and *P. vivax*, with an incubation period that varies from 12-14 days to 12 months (Arends et al., 2013; Kim et al., 2013). *P. vivax* parasite can engender relapses varying from weeks to months due to the activation of dormant liver-stage parasites (hypnozoites) which cause the spread of other infections (Howes et al., 2016; Benavente et al., 2017).

Petrópolis is a municipality in Rio de Janeiro state; it is about 68 kilometers from the state capital Rio de Janeiro, location: 22°30′18″S, 43°10′44″W. According to the Instituto Brasileiro de Geografia e Estatística (IBGE, 2019) Petrópolis covers an area of 795,798 km² and has a population of 305,917 inhabitants. The city was reported to be malaria-free for many years until the first quarter of 2017 when 5 cases were reported by the Petrópolis Health Center. The malaria vector can be found in parks and in the forest surrounding the city. Considering that *Plasmodium* sp. is an important cause of morbidity and mortality worldwide, this clinical case serves as an epidemiological alert.

**CASE REPORT**

A 29-year-old woman, local resident of Petrópolis, presented prostration on March 17th, 2017 with no other symptoms. The prostration began 13 days after an afternoon visit to São Vicente Park in Petrópolis (location: 22°32′27″S, 43°13′68″W). On March 25th, as well as the prostration, the patient began having headaches, myalgia and night fever (40°C). These symptoms persisted for 3 days, after which they appeared every other day. The patient only sought medical attention at Santa Teresa Hospital on April 7, 2 weeks after the symptoms first appeared. An hemogram and chest x-ray were performed but neither presented any abnormality, leading the physician to consider the diagnosis inconclusive and prescribe medication for pain and fever, advising the patient to make an appointment with a clinician the following week.

On April 8th, the patient returned to Santa Teresa Hospital and was informed of five malaria cases reported in town with the same symptoms she was presenting but, once again, the diagnosis was inconclusive and the case was forwarded to the Infectious and Parasitic Disease Service (DIP).

On April 10th, the patient was seen at the DIP and sent to Petrópolis Health Center where a blood smear test was performed and then repeated on April 12th, when the malaria diagnosis was confirmed. A blood sample was sent to the Laboratório de Diagnóstico Molecular e Hematologia at the Universidade Federal do Rio de Janeiro for molecular investigation.

Treatment was started on the same day with primaquine, 2 tablets a day for 7 days, and chloroquine, 4 tablets on the first day, 3 on the second and third days. By the second day of treatment no more symptoms were noted.

On May 5, a new hemogram was performed and no alterations were detected.
Two hundred microliters of whole blood were used to extract DNA by Illustra blood genomicPrep Mini Spin kit (GE Healthcare®) according to the manufacturer’s instructions. Nested PCR amplification was performed using two different protocols (Snounou et al., 1993; Isozumi et al., 2014), the products were visualized by electrophoresis and ethidium bromide impregnation under ultraviolet light. The PCR products were purified and sent to the Genomic Plataform - DNA Sequencing, PDTIS/FIOCRUZ in order to undergo sequencing by Sanger’s method. Also, real time PCR was performed by SYBR Green and subsequent RFLP analysis (Xu et al., 2015) to determine the species involved. The Snounou et al. (1993) protocol presented amplification of a product around 120 bp with specific *P. vivax* primers but due to the small size of the fragment, adequate sequencing was impaired. Through the Isozumi et al. (2014) protocol, there was amplification of a fragment around 430 bp with specific genus primers. Sequencing showed 98.67% similarity with *P. vivax*. Real time PCR showed Ct = 16.41 to *P. falciparum*, Ct = 19.50 to *P. vivax* as positive controls and Ct = 25.37 to the blood sample. Digestion with the restriction enzymes Alul and FspBI showed the same profile to *P. vivax*.

**DISCUSSION**

Although in recent years Brazil has achieved the best levels in malaria control by reducing case incidence to 75%, so far most of the malaria cases in Central and South America occurs in this country. An estimated 99% of the cases in Brazil come from the Legal Amazonian Region, which comprises nine states: Acre, Pará, Maranhão, Amazonas, Rondônia, Roraima, Amapá, Mato Grosso and Tocantins.

The other federated units in Brazil are in the Extra-Amazonian Region and account for less than 1% of the cases. Autochthonous cases of malaria in the extra-amazonian region were confirmed in almost all the states according to SINAN (National Notification System for Medical Complaints). The last epidemiological bulletin in Rio de Janeiro state reported 4 cases in 2012, 8 cases in 2013, 2 cases in 2016 and 34 cases in 2017 (SINAN, 2019). This shows a significant increase in less than a year, serving as a prevention alert. It is noteworthy that the sequenced region corresponds to mitochondrial cytochrome C as described by Isozumi et al. (2014), identified *P. vivax* and by the digestion profile after real time PCR also identified *P. vivax* as the ethiological agent in this infection. Of the autochthonous cases that occurred in Rio de Janeiro State in 2017, 28 cases underwent mitochondrial DNA sequencing proving *Plasmodium simium* to be strongly linked to the Atlantic Forest (Brasil et al., 2017). According to the authors, *P. simium* differs from the most closely related *P. vivax* in two single nucleotide polymorphisms (SNPs) in the mitochondrial genome, therefore it is impossible to confirm the species identified in the patient.
Almost all cases report ecotourism activities as the case described here. In addition, in this case the patient reported visiting the forest to enjoy the sunset, exactly when vector activity begins. Preventive action should include the use of mosquito nets, warning the vulnerable population and training health professionals for more precise clinical and laboratory diagnosis.

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