Molecular Investigation into a Malaria Outbreak in Cusco, Peru: *Plasmodium falciparum* B\(_V1\) Lineage is Linked to a Second Outbreak in Recent Times

Sheila Akinyi Okoth,* Stella M. Chenet, Nancy Arrospide, Sonia Gutierrez, Cesar Cabezas, Jose Antonio Matta, and Venkatachalam Udhayakumar

Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; Atlanta Research and Education Foundation, Atlanta, Georgia; Instituto Nacional de Salud del Peru, Lima, Peru; Laboratorio de Referencia de La Convención, Cusco, Peru

Abstract. In November 2013, a *Plasmodium falciparum* malaria outbreak of 11 cases occurred in Cusco, southern Peru, where falciparum malaria had not been reported since 1946. Although initial microscopic diagnosis reported only *Plasmodium vivax* infection in each of the specimens, subsequent examination by the national reference laboratory confirmed *P. falciparum* infection in all samples. Molecular typing of four available isolates revealed identity as the B-variant (*B\_V1*) strain that was responsible for a malaria outbreak in Tumbes, northern Peru, between 2010 and 2012. The *P. falciparum* B\(_V1\) strain is multdrug resistant, can escape detection by PfHRP2-based rapid diagnostic tests, and has contributed to two malaria outbreaks in Peru. This investigation highlights the importance of accurate species diagnosis given the potential for *P. falciparum* to be reintroduced to regions where it may have been absent. Similar molecular epidemiological investigations can track the probable source(s) of outbreak parasite strains for malaria surveillance and control purposes.
four drug resistance–associated genes; \( Pfcrt \) (CQ resistance), \( Pfmdr1 \) (multidrug resistance), \( Pfdhfr \) (pyrimethamine resistance), and \( Pfdhps \) (sulfadoxine resistance) were polymerase chain reaction amplified and sequenced as described previously. Finally, we genotyped histidine-rich protein 2 (\( Pfhrp2 \)) and \( Pfhrp3 \) as reported by Akinyi and others. All four isolates had drug resistance profiles identical to the BV1 lineage, with an \( S72V73M74N75T76 \) haplotype in the \( Pfcrt \) gene, \( N86D144F184C1034D1042Y1246 \) in \( Pfmdr1 \), \( R50I51C59N108I164 \) in \( Pfdhfr \), and \( S436G437E540G581A613 \) in \( Pfdhps \) (bold font represents the mutant amino acid at each respective position).

All four isolates had drug resistance profiles identical to the BV1 lineage, with an \( S72V73M74N75T76 \) haplotype in the \( Pfcrt \) gene, \( N86D144F184C1034D1042Y1246 \) in \( Pfmdr1 \), \( R50I51C59N108I164 \) in \( Pfdhfr \), and \( S436G437E540G581A613 \) in \( Pfdhps \) (bold font represents the mutant amino acid at each respective position).

### TABLE 1

Characteristics of the 11 patients that presented with malaria during the 2013 outbreak in Palma Real, La Convención Province, Cusco Department, Peru

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Age</th>
<th>Sex</th>
<th>Parasitemia</th>
<th>Health center</th>
<th>Diagnosis date</th>
<th>Residence</th>
</tr>
</thead>
<tbody>
<tr>
<td>854*</td>
<td>26</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 14, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>1885*</td>
<td>32</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 14, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>1886*</td>
<td>30</td>
<td>Male</td>
<td>F§</td>
<td>Hospital Quillabamba</td>
<td>November 14, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>1930*</td>
<td>33</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 15, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>1913</td>
<td>17</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 15, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>1952</td>
<td>25</td>
<td>Female</td>
<td>F§</td>
<td>Palma real</td>
<td>November 16, 2013</td>
<td>Ivanqui</td>
</tr>
<tr>
<td>2033</td>
<td>32</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 17, 2013</td>
<td>Ivanqui</td>
</tr>
<tr>
<td>2109</td>
<td>52</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 18, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>2139</td>
<td>2</td>
<td>Female</td>
<td>F§</td>
<td>Palma real</td>
<td>November 18, 2013</td>
<td>Ivanqui</td>
</tr>
<tr>
<td>2303</td>
<td>24</td>
<td>Male</td>
<td>F§</td>
<td>Palma real</td>
<td>November 22, 2013</td>
<td>Rosalina</td>
</tr>
<tr>
<td>2443</td>
<td>28</td>
<td>Female</td>
<td>No slide available†</td>
<td>Palma real</td>
<td>November 25, 2013</td>
<td>Ivanqui</td>
</tr>
</tbody>
</table>

*Patient specimens available for molecular analysis.
†Plasmodium falciparum malaria was positively diagnosed (but undocumented) locally at the Palma Real Health Center. Blood film was not available to the Cusco Department of Health for confirmatory diagnosis.
‡Between 2 and 20 parasites per field in 100 fields.
§Between 21 and 200 parasites per field in 100 fields.
Overall, the genetic background of these isolates was identical to that of the BV1 lineage that caused the
*P. falciparum* outbreak in Tumbes in 2012 (Figure 1). This finding suggests that the BV1 lineage is the most likely source of the falciparum malaria outbreak in Cusco. This is the second malaria outbreak in Peru that involved the same *P. falciparum* lineage.

It is important to point out that the BV1 lineage derived from the original “B clonal lineage” found in Peru since 1999. The highly clonal dynamic observed in this *P. falciparum* population is the result of decreased outcrossing and genetic drift primarily resulting from inbreeding and asexual replication.

In general, the low *P. falciparum* genetic diversity found in South America relates to the low transmission intensity in this region. With fewer parasite types, there are limited opportunities for outcrossing during meiosis in the mosquito, which results in highly related or even clonal parasites. Moreover, since the *P. falciparum* population underwent strong drug selection, expansions of few drug-resistant parasite lineages is expected.

Epidemiological data suggest that the Cusco *P. falciparum* outbreak was the result of human migration from Loreto Department when a road construction company set up a camp in Rosalina to build a road to Kiteni, Echarate District. In September 2013, prior to the outbreak, two road workers who were originally from Iquitos were diagnosed with severe malaria and, because of their critical condition, were subsequently transferred to another city for medical examination. It is suspected that these may have been the index cases to the ensuing outbreak. Unfortunately, specimens from these suspected index cases were not available for molecular analysis to confirm the proposed link. Nevertheless, even with the limited number of samples analyzed, this molecular investigation corroborated the epidemiological data suggesting that the

*P. falciparum* BV1 lineage from Loreto is the most likely source of the Cusco outbreak.

In addition to Peru, BV1 lineage parasites have been detected in isolates collected in Amazonas Department, Colombia, from as far back as 2005 (Figure 2). *Anopheles darlingi* and *Anopheles oswaldoi* are the main malaria species in Amazonas Department. The discovery of BV1 lineage parasites in other parts of the Amazon region of South America presents a major challenge for regional malaria control and elimination due to the persistence and wide distribution of this *P. falciparum* lineage, which seems highly adaptive to different transmission settings and can escape detection by PfHRP2-based rapid diagnostic tests because of Pfhrp2/3 deletions.

Finally, this study highlights the need for local health posts to perform high-quality malaria diagnosis to detect *P. falciparum* introductions, particularly in areas where *P. vivax* transmission is known or thought to occur exclusively, because *P. falciparum* lineages could possibly establish themselves in the long term. In addition, although there have been some reports on the use and interpretation of *Plasmodium* molecular genetics tools for outbreak investigations, this methodology should be standardized for routine use to identify not only the *Plasmodium* species but the source(s) of a malaria outbreak and the prevalence of long-lasting drug-resistant *P. falciparum* strains.

Received June 16, 2015. Accepted for publication August 29, 2015.
Published online October 19, 2015.

Acknowledgments: We are grateful to the personnel at the Ministry of Health in Cusco and Lima as well as the Reference Laboratory in La Convención, Cusco, Peru, who were involved in the response to the outbreak, assisted with patient sample collection, and provided specimens to the Malaria Laboratory at the CDC.
Financial support: Sheila Akinyi Okoth was supported by the Atlanta Research and Education Foundation. Stella M. Chenet was supported by the American Society of Microbiology/CDC Postdoctoral Fellowship Program. We acknowledge the support of the CDC Antimicrobial Resistance Working Group and the Atlanta Research and Education Foundation for the investigation.

Authors’ addresses: Sheila Akinyi Okoth and Stella M. Chenet, Division of Parasitic Diseases and Malaria, CDC, Atlanta, GA, E-mails: jyo3@cdc.gov and ynw0@cdc.gov. Nancy Arrospide, Sonia Gutierrez, and Cesar Cabezas, Instituto Nacional de Salud de Peru, Lima, Peru, E-mail: narrospide@hotmail.com, scgg68@hotmail.com, and salljaruna@yahoo.com. Jose Antonio Matta, Laboratorio de Referencia de La Convención, Cusco, Peru, E-mail: antonio_matta@hotmail.com. Venkatachalam Udhayakumar, Division of Parasitic Diseases and Malaria, Atlanta, GA, E-mail: vxu0@cdc.gov.

REFERENCES


