Research Article
High Prevalence of *Plasmodium falciparum* Infection in Asymptomatic Individuals from the Democratic Republic of the Congo

Dieudonné Makaba Mvumbi,1,2 Thierry Lengu Bobanga,3 Pierrette Melin,2 Patrick De Mol,2 Jean-Marie Ntumba Kayembe,4 Hippolyte Nani-Tuma Situakibanza,3,4 Georges Lelo Mvumbi,1 Célestin Ndosimao Nsibu,5 Solange Efundu Umesumbu,6 and Marie-Pierre Hayette2

1Biochemistry and Molecular Biology Unit, Department of Basic Sciences, School of Medicine, University of Kinshasa, P.O. Box Kin XI, Kinshasa, Democratic Republic of the Congo
2Department of Clinical Microbiology, University Hospital of Liege, 4000 Liege, Belgium
3Department of Parasitology and Tropical Medicine, School of Medicine, University of Kinshasa, P.O. Box Kin XI, Kinshasa, Democratic Republic of the Congo
4Department of Internal Medicine, School of Medicine, University of Kinshasa, P.O. Box Kin XI, Kinshasa, Democratic Republic of the Congo
5Department of Pediatrics, School of Medicine, University of Kinshasa, P.O. Box Kin XI, Kinshasa, Democratic Republic of the Congo
6National Malaria Control Program, P.O. Box Kin XI, Kinshasa, Democratic Republic of the Congo

Correspondence should be addressed to Dieudonné Makaba Mvumbi; didimvumbi@gmail.com

Received 23 September 2015; Revised 20 November 2015; Accepted 29 December 2015

Academic Editor: Sasithon Pukrittayakamee

Copyright © 2016 Dieudonné Makaba Mvumbi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Malaria remains a major public health problem in the Democratic Republic of Congo (DRC) with 14 million cases reported by the WHO Malaria Report in 2014. Asymptomatic malaria cases are known to be prevalent in endemic areas and are generally untreated, resulting in a significant source of gametocytes that may serve as reservoir of disease transmission. Considering that microscopy certainly underestimates the prevalence of *Plasmodium* infections within asymptomatic carriers and that PCR assays are currently recognized as the most sensitive methods for *Plasmodium* identification, this study was conducted to weigh the asymptomatic carriage in DRC by a molecular method. Six provinces were randomly selected for blood collection in which 80 to 100 individuals were included in the study. Five hundred and eighty blood samples were collected and molecular diagnosis was performed. Globally, almost half of the samples collected from asymptomatic individuals (280/580; 48.2%) had *Plasmodium* infections and the most species identified was *P. falciparum* alone in combination with *P. malariae*. The high prevalence reported here should interpellate the bodies involved in malaria control in DR Congo to take into account asymptomatic carriers in actions taken and consider asymptomatic malaria as a major hurdle for malaria elimination.

1. Introduction

Malaria is a parasitic disease caused by *Plasmodium* species transmitted to man by a mosquito bite [1]. Four *Plasmodium* species infect human but *Plasmodium falciparum* is responsible for the major morbimortality. Infection with *P. falciparum* can result in a simply asymptomatic carriage, uncomplicated or severe malaria. In fact, there is an exposure-related immunity in malaria that could explain these different expressions [2].

In high transmission settings, symptomatic malaria is often concerning children below five years as they have
2. Malaria Research and Treatment
not been for long time exposed to the parasite and asymptomatic infections generally concern adults that acquired an antiseed and/or an antiparasite immunity during their exposure time [3–6]. Asymptomatic malaria cases are known to be prevalent in endemic areas [7–9] and are generally untreated, resulting in a significant source of gametocytes that may serve as reservoir of disease transmission [10].

The Democratic Republic of the Congo (DRC) is the second largest country in Africa and has a population that is estimated to be 75.5 million people. It is estimated that 97% of the population live in zones with stable transmission. In the WHO malaria report for 2014, 14 million of malaria cases were reported in DRC [11]. Some studies conducted in DRC revealed significant prevalence of asymptomatic Plasmodium carriers. Matangila et al. reported a prevalence of 21.6%, 27.4%, and 29.5% of asymptomatic P. falciparum infection in pregnant women in Kinshasa, respectively, determined by microscopy and RDT [14]. But Tiono et al. showed in their work that RDTs have some limits in detecting asymptomatic carriers of P. falciparum [15].

Currently, Polymerase Chain Reaction (PCR) assays are the most sensitive manner to detect Plasmodium DNA [16,17] and it detects only viable Plasmodium [18]. Some PCR assays can detect less than 1 parasite/μL as the technique validated by Cnops et al. [19].

Discrepancies have been often found between microscopy and PCR results for the determination of asymptomatic Plasmodium carriage. For example, Baliraine et al. reported 20.7% difference (12.6% by microscopy and 33.3% by PCR) [20], Dal-Bianco et al. found 25% difference (27% by microscopy and 52% by PCR) [21], and May et al. showed 19% difference (48.9% by microscopy and 67.9% by PCR) [22].

We conducted this study to assess the weight of asymptomatic carriage of P. falciparum in the DRC, based on a highly sensitive RT-PCR assay for malaria parasite identification.

2. Materials and Methods

2.1. Study Sites and Participants. We randomly identified health areas within 6 provinces of the DRC with different malaria transmission dynamics: Bolenge in Equateur, Luvizila in Kinshasa, Mweca in Kasai-Occidental, Butembo in Nord-Kivu, Punia in Maniema, and Kapolowe in Katanga Province. This household survey recruited one hundred individuals per province, except for Maniema where only 80 individuals could be included. Every age and sex was taken into account after being given an informed consent by adults and parents/guardians of children. People with fever or other malaria related symptoms were not included in this study. Samples were collected during the period from March to November 2014.

2.2. Blood Collection and DNA Extraction. Five hundred and eighty blood samples were obtained from finger prick and dropped on filter paper (Whatman 3MM®) that were dried and stored into individual plastic bags with desiccant. Plasmodium DNA has been extracted using the QIAamp DNA Mini Kit® (Qiagen Benelux, Venlo, Netherlands) according to manufacturer’s recommendation. Briefly, 3 circles of approximately 3 mm diameter were punched out from a blood spot and placed into a 1.5 mL microcentrifuge tube in which 180 μL of buffer ATL was added. The final elution volume was 150 μL. Each dried blood spot was treated individually into a sterile petri dish to avoid contamination. One negative control (sterile water) was included for each dozen of blood spots treated. DNA was stored at −20°C till further analysis.

2.3. Parasite Identification by Real-Time PCR. A real-time PCR having a sensitivity varying from 0.02 to 0.006 parasites/μL for human Plasmodium species identification was run, as previously described by Cnops et al. [19]. PCR tests were run on a light cycler 480 instrument (Roche®) and in the presence of positive controls (provided by the Parasitology Unit, Institute of Tropical Medicine, Antwerp, and the Laboratory of Clinical Microbiology, University Hospital of Li`ege). PCR conditions were as follows: 2 min at 95°C, followed by 50 cycles of 15 s at 95°C and 60 s at 60°C.

2.4. Ethical Considerations. This study has received the ethical approbation of the Ministry of Public Health of the DRC and of the Institutional Committee of the Faculty of Medicine, University of Kinshasa.

3. Results and Discussion

P. falciparum was correctly identified in 280 samples (48.2%) among which 6 infections were mixed (P. falciparum + P. malariae). Prevalence of positive samples by age groups is represented in Table 1. The other human Plasmodium species P. ovale and P. vivax were not found. The real-time PCR cycles thresholds (Ct) vary from 21.72 to 39.23.

Prevalence of P. falciparum infection by province was 51% for Equateur, 62% for Kinshasa, 31% for Kasai-Occidental, 22% for Nord-Kivu, 63.7% for Maniema, and 63% for Katanga, as shown in Figure 1.

4. Conclusion

Efforts to control or to eradicate malaria should take into account asymptomatic Plasmodium carriers because elimination of parasites in only symptomatic patients will not be enough as long as the pool of asymptomatic carriers will continue to act as a parasite reservoir [23]. Some researchers have suggested to screen and to treat asymptomatic carriers with an Artemisinin-based combination as part of a surveillance strategy towards malaria elimination [24, 25].

The high prevalence of Plasmodium infections in asymptomatic carriers found in this study stressed the importance
Figure 1: Prevalence of \textit{P. falciparum} infection by collection sites based on a RT-PCR assay.

<table>
<thead>
<tr>
<th>Study sites</th>
<th>Age groups</th>
<th>0–5</th>
<th>6–15</th>
<th>16–59</th>
<th>&gt;60</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N) (%)</td>
<td>(N) (%)</td>
<td>(N) (%)</td>
<td>(N) (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equateur</td>
<td>15 (29.4)</td>
<td>15 (29.4)</td>
<td>21 (41.1)</td>
<td>0 (0)</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Kinshasa</td>
<td>7 (11.3)</td>
<td>10 (16.1)</td>
<td>45 (72.5)</td>
<td>0 (0)</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>K-occ</td>
<td>3 (9.6)</td>
<td>6 (19.3)</td>
<td>20 (39.2)</td>
<td>2 (6.4)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Kivu</td>
<td>2 (9.1)</td>
<td>5 (22.7)</td>
<td>15 (68.1)</td>
<td>0 (0)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Punia</td>
<td>4 (7.8)</td>
<td>6 (11.7)</td>
<td>37 (72.5)</td>
<td>4 (7.8)</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Katanga</td>
<td>13 (20.6)</td>
<td>26 (41.2)</td>
<td>21 (33.3)</td>
<td>3 (4.7)</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>44 (15.7)</strong></td>
<td><strong>68 (24.2)</strong></td>
<td><strong>159 (56.7)</strong></td>
<td><strong>9 (3.2)</strong></td>
<td><strong>280</strong></td>
<td></td>
</tr>
</tbody>
</table>

K-occ = Kasai-occidental.

of including this item in malaria control programs by the DRC Ministry of Health.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


pregnancy and can be more cost-effectively detected by rapid
diagnostic test than by microscopy in Kinshasa, Democratic

between *Plasmodium* infection, anaemia and nutritional status
in asymptomatic children aged under five years living in
stable transmission zones in Kinshasa, Democratic Republic of

[14] Demographic and Health Survey, Democratic Republic of
FR300.pdf.

learned from the use of HRP-2 based rapid diagnostic test
in community-wide screening and treatment of asymptomatic
carriers of *Plasmodium falciparum* in Burkina Faso,” *Malaria
Journal*, vol. 13, no. 1, article 30, 2014.

[16] G. Snounou, S. Viriyakosol, Xin Ping Zhu et al., “High sen-
sitivity of detection of human malaria parasites by the use of
nested polymerase chain reaction,” *Molecular and Biochemical
Parasitology*, vol. 61, no. 2, pp. 315–320, 1993.

[17] N. Steenkeste, S. Incardona, S. Cly et al., “Towards high-
throughput molecular detection of *Plasmodium*: new
approaches and molecular markers,” *Malaria Journal*, vol.
8, no. 1, article 86, 2009.

[18] W. Jarra and G. Snounou, “Only viable parasites are detected by
PCR following clearance of rodent malarial infections by drug
treatment or immune responses,” *Infection and Immunity*, vol.

[19] L. Cnops, J. Jacobs, and M. Van Esbroeck, “Validation of a four-
primer real-time PCR as a diagnostic tool for single and mixed
*Plasmodium* infections,” *Clinical Microbiology and Infection*, vol.

prevalence of asymptomatic *Plasmodium falciparum* Infections
in a highland area of western Kenya: a cohort study,” *Journal of

prevalence of asymptomatic *Plasmodium falciparum* infection
in Gabonese adults,” *American Journal of Tropical Medicine and

mixed and subpatent malarial infections in southwest Nigeria,”
*The American Journal of Tropical Medicine and Hygiene*, vol. 61,

of active case detection to target reservoirs of asymptomatic
malaria and gametocyte carriers in a rural area in Southern
Province, Zambia,” *Malaria Journal*, vol. 9, no. 1, article 265,
2010.

tomatic carriers with artemether-lumefantrine: an opportunity
to reduce the burden of malaria?” *Malaria Journal*, vol. 9, no. 1,
article 30, 2010.

screening and treatment of asymptomatic carriers of *Plasmod-
iuim falciparum* with artemether-lumefantrine to reduce malaria
disease burden: a modelling and simulation analysis,” *Malaria
Journal*, vol. 10, article 210, 2011.
Submit your manuscripts at http://www.hindawi.com