Malaria Case Studies

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Disclosure of Potential Conflict of Interest

- Financial Disclosures
  - Research / Grant support – Public Health Agency of Canada; Public Health Ontario
Clinical Case

- 60 year-old previously well, Canadian-born man, working in rural Ghana x 3 months 1 year ago
- Intermittently adherent to mefloquine ppx
- Presents with a 3-week history of fever, fatigue, and 25-lb weight loss

- On exam, febrile (T 40), tachycardic, mildly tachypneic, looks unwell
The most likely explanation for the malaria microscopy and RDT pattern is:

- Expired RDT kit
- Infection with Plasmodium ovale
- Infection with Plasmodium malariae
- Absence of malaria infection
Malaria due to *P. ovale*

- Increasing importation of *P. ovale* to Ontario in the past 2 years from West Africa
- *P. ovale* may have a prolonged incubation and present many months after exposure
- RDT assays have poor sensitivity for non-falciparum malaria, but *P. ovale* in particular
- On microscopy, *P. ovale* will classically demonstrate Schuffner’s dots, elongation and enlargement of RBCs, and comet-shaped RBCs

### Table 2. Characteristics of Imported Cases of Malaria among 2822 U.S. Travelers from 1992 through 1998.

<table>
<thead>
<tr>
<th>Plasmodium Species</th>
<th>No. of Cases</th>
<th>Early Onset (≤2 mo after Return)</th>
<th>Late Onset (&gt;2 mo after Return)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Use of Effective Prophylaxis</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>number (percent)</td>
<td>number (percent)</td>
<td>number (percent)</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>1290</td>
<td>1231 (95.4)</td>
<td>167 (13.6)</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>1321</td>
<td>510 (38.6)</td>
<td>148 (29.0)</td>
</tr>
<tr>
<td><em>P. ovale</em></td>
<td>87</td>
<td>21 (24.1)</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td><em>P. malariae</em></td>
<td>124</td>
<td>73 (58.9)</td>
<td>23 (31.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2822</strong></td>
<td><strong>1835 (65.0)</strong></td>
<td><strong>347 (18.9)</strong></td>
</tr>
</tbody>
</table>

## RDT Assays

<table>
<thead>
<tr>
<th>Species</th>
<th>Overall Sensitivity of RDT Assays</th>
<th>Sensitivity of Binax at PHOL (vs real time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>77.4—98.1%</td>
<td>93.4%</td>
</tr>
<tr>
<td>P. vivax</td>
<td>68.9%</td>
<td>78.3%</td>
</tr>
<tr>
<td>P. malariae</td>
<td>21.4—45.2%</td>
<td>79%</td>
</tr>
<tr>
<td>P. ovale</td>
<td>5.5—86.7%</td>
<td>31%</td>
</tr>
</tbody>
</table>

P. knowlesi – in travelers, 1/6 tests positive
Treatment of P. ovale

- Blood Schizonticide
  - Chloroquine
  - Atovaquone-Proguanil
- Tissue Schizonticide (radical cure)
  - Primaquine
### G6PD Deficiency

<table>
<thead>
<tr>
<th>Class of Deficiency</th>
<th>Enzyme Activity</th>
<th>Ethnicities</th>
<th>Primaquine Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Severe deficiency; &lt;1% G6PD activity; chronic HA</td>
<td>Sporadic mutation Hx neonatal jaundice</td>
<td>Contraindicated</td>
</tr>
<tr>
<td>II G6PD&lt;sub&gt;Med&lt;/sub&gt;</td>
<td>Severe deficiency; 1-10% G6PD activity; acute HA with RBC stress</td>
<td>Asian males Mediterranean descent</td>
<td>Contraindicated</td>
</tr>
<tr>
<td>III G6PD&lt;sub&gt;A&lt;/sub&gt;-</td>
<td>Mild-to-Mod deficiency 10-60% activity Intermittent HA</td>
<td>Asians African Americans Hispanics</td>
<td>Cautious use as weekly PART; Mild hemolysis by d4</td>
</tr>
<tr>
<td>IV</td>
<td>Normal to mild deficiency; &gt;60% activity; no hemolysis</td>
<td>Women</td>
<td>Regarded as safe</td>
</tr>
</tbody>
</table>

**Case Resolution**
Case Resolution

- Patient treated with Malarone
- G6PD testing revealed normal enzyme levels
- Radical cure was initiated with a 2-week course of primaquine, which the patient tolerated well
- He recovered uneventfully

Clinical Case

- 77 year-old Indian-born resident of India visiting Canada x 2 weeks
- Presents with a 16-day history of fever, anorexia, headache, myalgia, and fatigue
- Co-morbidities include diabetes and hypertension
- Febrile on examination in the ER
- CBC – Hb 97 g/L, WBC 7.3 bil/L, Platelets 81 bil/L
Malaria Diagnostics Workflow

1. EDTA Blood
   - Microscopy
     - Mixed Infection or Unable to Speciate
   - Immuno-Chromatography Test
     - Neg or Pf, Pv, Pm, Po
       - Neg or Pf or non-Pf
         - Discrepant Results
         - Resolve by Real Time PCR
           - Pf, Pv, Pm, Po, Pk
The most likely explanation for the malaria microscopy, RDT, and PCR pattern is:

- Expired RDT kit
- Infection with Plasmodium ovale
- Infection with Plasmodium malariae
- Absence of malaria infection

Further History

- For 6 weeks prior to arrival in Canada, he had been staying with other family in Massachusetts near the New Hampshire border
- Wooded area in the backyard, and patient endorsed finding ticks on his person
- Daughter removed a small dark foreign-body from the patient with tweezers upon arrival in Canada
### Plasmodium vs Babesia

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Babesia</th>
<th>Plasmodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>Rings</td>
<td>Rings</td>
</tr>
<tr>
<td></td>
<td>Tetrads (Maltese Cross)</td>
<td>Schizonts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gametocytes</td>
</tr>
<tr>
<td>Malaria Rapid Diagnostic Test</td>
<td>Negative</td>
<td>Positive for Pf</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less sensitive for non-Pf</td>
</tr>
<tr>
<td>PCR</td>
<td>Cross-reactive with standard 18S genus level assays</td>
<td>Logarithmic curve with lower ct value at parasitemia &gt;1%</td>
</tr>
</tbody>
</table>

### Clinical Case

- 28 year-old previously well, Ghanian-born woman, traveled home to VFR in urban Ghana x 1 month
- Presents 5 days after return home with a day of fever and chills
- Pre-travel advice obtained, Malarone prescribed and filled in Canada
- Patient took Malarone each day 1-hr before breakfast, and began her prophylaxis 1 day prior to departure from Canada
The most likely explanation for the malaria microscopy and RDT pattern is:

- Infection with P. falciparum malaria
- Inadequate absorption of atovaquone-proguanil
- Drug resistant P. falciparum infection
- All of the above

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- Infection with *P. falciparum* malaria
- Inadequate absorption of atovaquone-proguanil
- Drug resistant *P. falciparum* infection
- All of the above
Atovaquone is highly lipophilic with low aqueous solubility and is therefore poorly absorbed unless consumed with a fatty meal.

Co-administration of atovaquone and a fatty meal leads to a 5-fold increase in maximum plasma concentration (Cmax) over fasting.

Serum Drug Concentrations

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Parasitemia (by thin film microscopy)</th>
<th>Expected plasma drug concentration</th>
<th>Plasma drug concentration(^a), atovaquone</th>
<th>Plasma drug concentration(^a), proguanil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 of illness</td>
<td>3%</td>
<td>Atovaquone: 11.5 µg/mL; Proguanil: 0.509 µg/mL</td>
<td>2 ng/mL (0.002 µg/mL)</td>
<td>1.3 ng/mL (0.0013 µg/mL)</td>
</tr>
<tr>
<td>Day 3 of illness</td>
<td>&lt;0.1%</td>
<td>Atovaquone(^b): 0.93 µg/mL; Proguanil(^b): 0.102 µg/mL (102 ng/mL)</td>
<td>1.3 ng/mL (0.0013 µg/mL)</td>
<td>0.7 ng/mL (0.0007 µg/mL)</td>
</tr>
</tbody>
</table>

\(^a\) By LC-MS/MS; limit of detection for UV-HPLC is 100 ng/mL.

\(^b\) Half-life of atovaquone is 59 h, and that of proguanil is 14.5 h [11].
The most likely explanation for the malaria microscopy and RDT pattern is:

- Infection with *P. falciparum* malaria
- Inadequate absorption of atovaquone-proguanil
- Drug resistant *P. falciparum* infection
- All of the above
Resistance to atovaquone results from a single point mutation in parasite cytochrome b, which leads to reduced binding affinity for atovaquone.

Resistance to proguanil involves the stepwise development of point mutations in the dhfr gene.
Anti-Malarial Resistance Marker Analysis

pfmdr1 gene. A→T nucleotide change may confer chloroquine resistance

Clinical Sample A
100% A – Wild Type

Clinical Sample B, mixed pop
58% T – Mutant
42% A – Wild Type

Sequencing results of *Plasmodium falciparum* isolate

- Cytochrome b Y268N/S/C = Y (wild type)
- DHFR C50R = C (wild type)
- DHFR N51I = I (mutant)
- DHFR C59R = R (mutant)
- DHFR S108N = N (mutant)

- Patient did not absorb atovaquone and then was left with proguanil monophylaxis in the setting of a triple-mutant *P. falciparum* infection
Clinical Case

- 16-year-old male volunteered in Ghana x 3 weeks in June and July, without malaria prophylaxis
- 10 days after return, presented to ER with 4-day hx fever, chills, HA, nausea, vomiting
- Past travel history – Kenya 1 year prior
- Physical Exam / Labs:
  - Hypotension
  - Platelets 19 bil/L
  - ALT 134, AST 106
Case Continued

- Thick and thin blood film positive for P. falciparum malaria, parasitemia 2.5%
- Malaria RDT positive for HRP-2 antigenemia
- Treated with a 3-day course of IV artesunate + po Malarone
- Clinically improved on day 5 of admission, and discharged home with negative blood smears, though HRP-2 remained detectable
- Follow-up 1 week post-discharge: asymptomatic

Case Continued

- One month later, patient returned to ER with 2-day hx of recurrent HA, nausea, and vomiting without fever
- Laboratory investigations benign
- Repeat malaria testing:
  - Thick and thin blood films positive, parasitemia <0.1%
  - RDT positive for isolated HRP-2 antigenemia
What could be going on here?
<table>
<thead>
<tr>
<th>Date</th>
<th>Parasite</th>
<th>Stages</th>
<th>Parasitemia (%)</th>
<th>ICT kit</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 28</td>
<td>Pf</td>
<td>Rings</td>
<td>2.5</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>July 28</td>
<td>Pf</td>
<td>Rings, Young trophs</td>
<td>1.2</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>July 29</td>
<td>Pf</td>
<td>Rings</td>
<td>2.0</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>July 29</td>
<td>Pf</td>
<td>Rings</td>
<td>0.1</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>July 30</td>
<td>Pf (based on ICT kit)</td>
<td>No parasites found</td>
<td>N/A</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>Aug 1</td>
<td>Pf (based on ICT kit)</td>
<td>No parasites found</td>
<td>N/A</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>Aug 3</td>
<td>Pf (based on ICT kit)</td>
<td>No parasites found</td>
<td>N/A</td>
<td>T1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>Sep 6</td>
<td>Po only Pf not seen</td>
<td>Growing and Mature Trophs of Po</td>
<td>&lt; 0.1</td>
<td>T1 1+ T2 Neg</td>
<td>Both Pf and Po detected</td>
</tr>
<tr>
<td>Sep 7</td>
<td>Po only Pf not seen</td>
<td>Growing and Mature Trophs of Po</td>
<td>&lt; 0.1</td>
<td>T1 1+ T2 Neg</td>
<td>Not done</td>
</tr>
<tr>
<td>Sep 10</td>
<td>Po only Pf not seen</td>
<td>Growing and Mature Trophs of Po</td>
<td>&lt; 0.1</td>
<td>T1 1+ T2 Neg</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Malaria J 2015;14:350.
Case Continued

- Patient treated with 3-day course of Malarone and resolved quickly.
- Species identification results then obtained, and patient treated with 4 doses of oral chloroquine and 14-day course of primaquine following G6PD testing.
- Follow-up 2 weeks post-treatment: asymptomatic, negative blood smears.

Clinical Case

- Healthy 11-month-old Canadian-born baby developed high fever 10-days following a 5-week VFR trip to Cameroon.
- 3-days into illness, presented to ED:
  - Looked generally well
  - Temp 38.9 C, vitals otherwise normal
  - CBC – Hb 62 g/L, WBC 11.6 bil/L, Platelets 134 bil/L
  - Bilirubin 55 umol/L
- Thin blood film revealed.......
Audience Question: Which of the following statements is True?

- The patient has mild disease therefore likely has non-falciparum malaria
- The patient has mild disease and so should be treated with oral therapy
- Given the high parasitemia, the patient should be admitted to the ICU, urgently dialysed, exchange transfused, and ventilated
- VFR is the most common travel reason associated with imported malaria in Canada
Clinical Case

- Started on iv artesunate and transfused 1 unit of PRBCs
- Defervesced by 48-hours with clearance of parasitemia
- Stepped down to po atovaquone-proguanil to complete a 7-day course
- 1-month post-discharge remained well and blood films negative

Clinical Case

- 1-month prior to child’s illness, mother developed *P. falciparum* malaria while in Cameroon and responded to a 3-day course of iv therapy overseas
- Fantastic parasitemia in setting of clinically mild illness?
- Child was breastfed from birth and throughout mother’s and own illness
Inhibitory Factors in Breastmilk, Maternal and Infant Sera Against *in vitro* Growth of *Plasmodium falciparum* Malaria Parasite

- 144 Nigerian maternal milk samples with paired mother and infant sera
- Significant antibody titres to all stages of *P. falciparum* in breastmilk
- Significant in *vitro* growth inhibition of *P. falciparum* by whole breastmilk and breastmilk constituents such as lactoferrin and sIgA

Therefore suggest a protective *in vivo* role for breastmilk in the possible modulation of malaria frequency, severity and complications.


<table>
<thead>
<tr>
<th>Reason for travel</th>
<th>Total no. of cases</th>
<th><em>P. falciparum</em></th>
<th>Severe or cerebral malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 18,870)</td>
<td>456</td>
<td>282</td>
<td>26</td>
</tr>
<tr>
<td>Tourism (n = 8136)</td>
<td>54</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>Immigration (n = 4967)</td>
<td>62</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>VFR (n = 1966)</td>
<td>174</td>
<td>117</td>
<td>5</td>
</tr>
<tr>
<td>Missionary, volunteer, research, aid (n = 1656)</td>
<td>73</td>
<td>42</td>
<td>7</td>
</tr>
<tr>
<td>Business (n = 1643)</td>
<td>77</td>
<td>51</td>
<td>7</td>
</tr>
</tbody>
</table>
5 Key Points – Malaria

- Microscopy remains the gold standard diagnostic tool and is the only technique that can reliably distinguish asexual from sexual parasitemia
- RDTs are designed to detect *P. falciparum* with >95% sensitivity at parasitemia of 0.004% (200 parasites/uL)
- Sensitivity of RDTs for non-falciparum malaria can be as low as 15-30% and is reduced for all species with very low parasitemias
- Babesiosis can produce false positive results on microscopy and standard genus-level *Plasmodium* PCR assays
- Thick and thin smears ± RDT should be performed on all febrile returned travelers from risk areas even in the setting of seemingly appropriate prophylaxis

Contact Information

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