Detection of *Plasmodium* Parasites in Blood Donors and Strategies to Identify and Defer Malaria-Risk Donors

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IPFA/PEI Workshop on Surveillance and Screening of Blood Borne Pathogens  
Zagreb, Croatia,  
May 26, 2010
Malaria Life Cycle, Biology and Pathogenesis and Implications for the Risk of TTM

- *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale*
- Biology and pathogenesis of malaria parasites depend on *Plasmodium* species and intensity of transmission.
  - Length of liver stage cycle varies for each species.
  - Some species can establish life-long infections.
  - Individuals from endemic areas may have chronic, low-grade parasitemia.
Prepatent period is the time between sporozoite infection to first appearance of blood form parasites (generally 9-30 days). However, this period varies among species: *P. vivax* and *P. ovale* have dormant liver form stages causing relapse infections (months to a year or more).

*P. malariae* blood form parasites can persist as chronic infections for up to 40 years or possibly longer.

Individuals born in endemic countries or expatriates with prolonged residence in such areas can become asymptomatic carriers of malaria.

Parasite burdens in asymptomatic carriers are not known.

The infectious dose of intraerythrocytic parasites is very low.
Malaria Mortality and Morbidity

- Transmitted in more than 100 countries
- More than 250 million clinical cases annually
- Approximately 800,000 deaths each year.
Malaria Incidence in India

Source: Tom Wellems, NIH
Malaria in the United States

- Natural mosquito-borne malaria infections are rare in the USA.
- Approximately 1,500 clinical cases each year are acquired outside the USA.
- Malaria can be transmitted by transfusion of blood from infected donors.
How Malaria Infections Reach the United States

- An average of 34 million US residents arrive annually in countries where malaria is transmitted.
- Approximately 18 million US residents arrive in Mexico annually, mostly entering non-endemic areas.
- Most malaria in the USA is introduced by travelers to and immigrants from endemic countries.
- Rates of malaria transmission vary greatly among different geographical areas, so the risk of malaria exposure depends directly on the area of travel or residence.

Kiszewski et al. 2004
The number of clinical cases and the percentage of each *Plasmodium* species implicated as causative agent has remained stable for the last several years.

Relatively complete information on geographical regions where infections were acquired and whether reported clinical cases had a prior history of malaria exposure is available.

These data allows to identify the donor populations who present the highest risk of transmitting malaria by blood transfusions.
Malaria cases in the United States 2007

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falciparum</td>
<td>654</td>
<td>(43.4)</td>
</tr>
<tr>
<td>Vivax</td>
<td>305</td>
<td>(20.3)</td>
</tr>
<tr>
<td>Malariae</td>
<td>30</td>
<td>(2.0)</td>
</tr>
<tr>
<td>Ovale</td>
<td>53</td>
<td>(3.5)</td>
</tr>
<tr>
<td>Mixed Sp</td>
<td>9</td>
<td>(0.6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>454</td>
<td>(30.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1505</td>
<td></td>
</tr>
</tbody>
</table>
Imported Malaria Cases Among US and Foreign Residents, by Region Of Acquisition – United States, 2007

Modified from MMWR 2009
• No FDA-approved laboratory test to screen blood donors for malaria risk.
• In U.S., prospective blood donors who present the risk of transmitting malaria infections through blood transfusion are identified by donor questionnaire based on history of travel or residence in malaria endemic areas.
FDA Recommendations for Donor Deferral for Malaria Risk

• **Three-year deferral**
  – History of clinical malaria
  – Prior residents of endemic country

• **One-year deferral**
  – Visit to malaria-endemic area by residents of nonendemic countries

TTM in United States between 1963 through 2009

Data from NEJM 2001, MMWR and recent cases of TTM
Changing Pattern of TTM in USA

Data from NEJM 2001, MMWR and recent cases of TTM
Characteristics of Donors Implicated in Cases of TTM in the USA 1963 through 2009

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Former resident of malarious area</td>
<td>4</td>
<td>5</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Former resident of malarious area who visit the country of origin</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>US civilian traveler</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>US military personnel</td>
<td>6</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*All five donors were immigrants from sub-Saharan Africa.

NEJM 2001, MMWR and unpublished
TTM and Donor Availability

• Approximately 150,000 blood donors lost annually due to perceived risk of malaria exposure.

• Of these, ~61,000 (41%) are deferred because of travel to endemic areas in Mexico.

• Unknown number of self deferrals—probably substantial.

• Non-returning of deferred donors.
Strategies to Minimize the Risk of TTM and Improve Donor Availability

- Donor testing
- Exploiting *Plasmodium* biology
- Exposure risk based donor deferral: malaria risk in all endemic areas is not equal
- Testing of donors who had visited the areas where malaria risk is low but travels to those areas result in significant donor loss.
*P. falciparum* in infected human blood in Giemsa stained thin smears at different hours of culture after storage at 4°C for different time

<table>
<thead>
<tr>
<th>Days of storage at 4°C</th>
<th>Hours in culture after taken out of refrigerator (4°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Day 1</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 7</td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 14</td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 21</td>
<td><img src="image22.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 28</td>
<td><img src="image29.png" alt="Image" /></td>
</tr>
</tbody>
</table>

[★ Positive in thick smear only]
Donor Screening in the U.S.

Recommended Tests
• HIV: EIA and NAT
• HCV: EIA and NAT
• HBV: HBsAg and Anti-core
• WNV: NAT

Test Performed but not recommended
• HBV: NAT
• CMV: EIA (Only Targeted testing)
New Test Implementation and Declining Risk of Viral Infections from Transfusion

Busch M. et al.
Methods to Detect Malaria Parasites

• Direct parasite demonstration
  - Microscopy
    - Thick blood film
    - QBC method

• Antigen detection
  - HRP, LDH etc. based dip sticks
  - Nucleic acid based methods
    - PCR test, TaqMan assay, Real-time PCR and Microarray

• Indirect demonstration of parasite exposure
  - Antibody based methods: IFAT, ELISA
AN IMPROVED METHOD FOR THE MICROSCOPICAL DIAGNOSIS OF INTERMITTENT FEVER.

BY RONALD ROSS, C.B., F.R.S., F.R.C.S. ENG., D.P.H.,
LECTURER ON TROPICAL MEDICINE AT UNIVERSITY COLLEGE, LIVERPOOL;
WALTER MYERS LECTURER AT THE LIVERPOOL SCHOOL OF
TROPICAL MEDICINE; LATE MAJOR, I.M.S.
Nucleic Acid based Tests for Donor Screening for Malaria

- Nucleic Acid Based tests
  - Difficult to ascertain the desired sensitivity of NAT for donors screening for malaria.
- Infectious dose of *P. vivax* malaria is 10 infected red cells or less.
- Minimum parasite burden in asymptomatic malaria carriers is not known.
Microarray Chip Based Detection of human Plasmodium by Targeting the Species-Specific 18s rRNA Sequences

\[ Pf \]

\[ Pv \]

\[ Pm \]

\[ Po \]
Sensitivity in PCR-based Detection by two-step Amplification of 18s rRNA

Direct Boiling Method

QiaAmp Blood Kit

Thick film sensitivity: 20,000 parasites/ml (0.0005% parasitemia)

Estimates were made assuming RBC count as $4 \times 10^6$ cells/µl
Sample Preparation: Failure of Magnetic Beads Coated with Immune Sera to Bind *P. falciparum* Schizonts Spiked in Normal Human Blood

- Blood spiked with 5% Parasites
- Normal Blood

Beads

Flow Through
Detection of *P. falciparum* in discarded blood clot

- Blood was drawn directly in tubes spiked with 20,000 or 2000 parasites/ml (without anticoagulant). Samples were allowed to clot for a few hours.

- Mechanical disruption of the clot by passing through 70μm sieve followed by saponin lysis. Samples were washed to remove the hemoglobin.

- DNA preparation by boiling the pellet in 200 μL of water.
**P. falciparum Detection in Blood Clot:**
PCR amplification of 18S rRNA

<table>
<thead>
<tr>
<th>Lane</th>
<th>P. falciparum parasites/ml</th>
<th>Vol. of blood used (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In Blood Clot</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>2000</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>20,000</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>20,000</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>+ Control</td>
<td>NA</td>
</tr>
<tr>
<td><strong>In Serum Sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>2000</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>2000</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>20,000</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>20,000</td>
<td>10</td>
</tr>
</tbody>
</table>

*Detection limit of first PCR is from 200 to 2000 parasites/ml that equates to 0.000005% and 0.00005% parasitemia.*
Antibody based Tests for Donor Screening for Malaria

- Must be highly sensitive and specific
- Ideally should detect antibodies against all four *Plasmodium* species
- All sero-positive donors will not be parasitemic
- May be useful in the reentry of antibody negative donors who had visited endemic areas.
Antibody based Tests for Donor Screening for Malaria (contd.)

- Some European countries, Australia and New Zealand allow testing of otherwise deferred at-risk donors for the presence of anti-malarial antibodies. In those countries, an otherwise deferred at-risk donor found negative for antibodies to *P. falciparum* and *P. vivax* parasites is allowed to reenter the donor pool after a shortened deferral period (4 – 6 months).
Cross-species recognition of recombinant *Plasmodium* antigens with sera from *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* infected patients as determined by western blot analysis

**Pooled P. falciparum sera**

1: rPfCSP, 2: rAMA-1, 3: rMSP142, 4: rPvCSP, 5: rPmCSP

**Pooled P. vivax sera**

**Pooled P. malariae sera**

**Pooled P. ovale sera**
### Annual number of donor deferrals in the US in 2006

Source: REDS data, Bryan Spencer et al. Transfusion 2009

<table>
<thead>
<tr>
<th>Region</th>
<th>Total (%) REDS-II</th>
<th>Projected annual number of donors deferred nationally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>78 (3.7)</td>
<td>5,500</td>
</tr>
<tr>
<td>Asia</td>
<td>323 (15.3)</td>
<td>24,524</td>
</tr>
<tr>
<td>Caribbean</td>
<td>280 (13.3)</td>
<td>18,859</td>
</tr>
<tr>
<td>Central America</td>
<td>462 (21.9)</td>
<td>33,494</td>
</tr>
<tr>
<td>Middle East</td>
<td>37 (1.8)</td>
<td>2,533</td>
</tr>
<tr>
<td>North America * (Mexico)</td>
<td>870 (41.3%)</td>
<td>61,494</td>
</tr>
<tr>
<td>Oceania</td>
<td>0</td>
<td>123</td>
</tr>
<tr>
<td>South America</td>
<td>58 (2.7)</td>
<td>4,009</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,108</strong></td>
<td><strong>150, 537</strong></td>
</tr>
</tbody>
</table>
Malaria Morbidity in Mexico
by Plasmodium species 1998-2009

Malaria-Endemic and Non-Endemic States of Mexico in 2009

Malaria transmission in Mexico is of low intensity, stable and uneven (2007-2009).

<table>
<thead>
<tr>
<th>Mexican States</th>
<th>Number of Malaria Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007</td>
</tr>
<tr>
<td>Chiapas</td>
<td>1483</td>
</tr>
<tr>
<td>Oaxaca</td>
<td>369</td>
</tr>
<tr>
<td>Chihuahua</td>
<td>142</td>
</tr>
<tr>
<td>Durango</td>
<td>46</td>
</tr>
<tr>
<td>Sinaloa</td>
<td>108</td>
</tr>
<tr>
<td>Tabasco</td>
<td>86</td>
</tr>
<tr>
<td>Nayarit</td>
<td>35</td>
</tr>
<tr>
<td>Quintana Roo</td>
<td>14</td>
</tr>
<tr>
<td>Sonora</td>
<td>12</td>
</tr>
<tr>
<td>Jalisco</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2297</strong></td>
</tr>
</tbody>
</table>

Data from Secretariat of Health Mexico
FDA Risk Assessment for Antibody Testing in Visitors to Mexico

• Approximately 150,000 donors are lost annually due to a perceived risk of malaria exposure; 61,000 donors (approximately 40%) are deferred for visits to malaria endemic regions in Mexico alone.

• An FDA risk-benefit assessment model was constructed to estimate effects of antibody testing on donor availability and risk of TTM in the following model donor testing scenarios
  - all presenting blood donors (universal testing)
  - all malaria-at-risk donors only
  - travelers who visited malaria-endemic areas in Mexico only.

The model assumed a four-month deferral after donors left malaria-endemic areas and before antibody testing.
Model predicts that testing for malarial antibodies in travelers to Mexico (61,000 annually) would allow to reenter approximately 37,000 donors annually with a reduced deferral period.

A significant but unknown number of travelers to Mexico who self defer for malaria risk are also anticipated to present for testing, and thus increase the donor pool.
The contribution of different Mexican states to the number of donor deferrals for malaria exposure among U.S. travelers is highly uneven.

According to two independent surveys by blood centers (REDS and BSRI), approximately 69.5% of donors were deferred for malaria exposure because they had traveled to Quintana Roo, a state with very low malaria transmission.
FDA’s probabilistic risk model suggested a point estimate of 0.088 malaria-infected blood collections per year resulting from visits to endemic areas in Mexico, and a combined point estimate of 0.016 infected blood collections per year due to visits to Quintana Roo alone which could result in 1 additional case in 61 years.
Conclusions

- TTM remains a risk to blood safety in the U.S.
- Approximately 1% of all blood donors are deferred for malaria-risk.
- Donor screening tests are needed to identify, defer and reenter malaria-risk donors.
- In the absence of testing, novel strategies based on the stratification of exposure risk—factors such as level of transmission in areas of travel or prior residence—may be given consideration to protect TTM.
Acknowledgements

OBRR/FDA
Hira Nakhasi, Hong Zheng, Babita Mahajan, Paul Mied, David Asher, Jay Epstein

OBE/FDA
Mark Walderhaug, Hong Yang, Richard Forshee, Steven Anderson

CDC
Paul Arguin

ARC
Bryan Spencer

BSRI
Brian Custer