Victims, Vectors and Vaccines: Battling Malaria in the 21st Century

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Plasmodium falciparum: merozoite stage (Photo:WHO/TDR)
Alexander the Great may have died from malaria in 323BC.

Children of Lugnano in Imperial Rome died of malaria in 450AD.
“Malaria disease leaves uncultivated two million hectares of land in Italy .... it poisons every year about two million inhabitants and kills 15,000 of them .... There is no other health problem so deeply linked to the prosperity of our country.”

Giustino Fortunato and Leopoldo Franchetti
Members of the Italian Parliament
July 14, 1898
“Malaria is the single largest disease in Africa and a primary cause of poverty. Every day 3,000 children die from malaria. Every year there are 500 million cases among children and adults.”

Dr. Gro Harlem Brundtland
Director General
World Health Organization
May 13, 1998
“... the world has yet to commit the resources needed to control this preventable and treatable disease.

Today is Africa Malaria Day, but malaria is a global problem, and the responsibility for stopping it extends to the entire international community.”

Dr. Regina Rabinovich
Director
Infectious Diseases Program
Bill & Melinda Gates Foundation
April 25, 2003
What is malaria?

Why is it still a problem?

What is being done to fight it?

What are the prospects for the future?
What is malaria?

Italian, from *mala aria* “bad air”: 1740

Infectious disease

Intracellular protozoan parasite

Vector-borne (mosquitoes)

High degree of host specificity
Etiological agents in humans:

*Plasmodium falciparum*

*Plasmodium vivax*

*Plasmodium ovale*

*Plasmodium malariae*
Transmitted to humans by mosquitoes in the genus *Anopheles*

442 described species

68 associated with malaria transmission

40 as main vectors

Humans are the only natural reservoirs of the four species that cause disease

No free-living forms
Malaria parasites have a complex life cycle

Developmental stages in both hosts
Epidemiology

Malaria is endemic to the poorest countries
Epidemiology

300 to 500 million clinical cases

Greater than one million deaths each year
(>2,700 deaths/day; ~2 persons every minute)

More than 90% of malaria deaths occur in Sub-Saharan Africa
Age-related prevalence

Despommier, Gwadz, Hotze, and Knirsch 2000
Clinical aspects:

Children and pregnant women are affected most severely

Symptoms of uncomplicated malaria:

Fever, malaise, fatigue, anemia, headache, myalgias

Clinical aspects (cont.):

Severe and complicated malaria (often associated with *P. falciparum*) characterized by:

- Cerebral malaria (seizures, coma, convulsions)
- Respiratory distress
- Pulmonary edema
- Acute renal failure
- Metabolic acidosis

*Newton et al., 1998 Am J Trop Med Hyg 58: 673-683*
Economic impact:

Sub-Saharan Africa:

- Slows economic growth by as much as 1.3%/year
- GDP/person average is 3X higher in malaria-free countries

WHO/Harvard University/London School of Hygiene and Tropical Medicine Report
Economic impact:

One healthy year of life is gained for every $1 to $8 spent on effectively treating malaria cases.

This makes malaria treatment as cost-effective a public health investment as measles vaccinations.
STANDARD ECONOMIC MODEL

ECONOMIC GROWTH → HEALTH

NEW ECONOMIC MODEL

ECONOMIC GROWTH ↔ HEALTH
Why is malaria still a problem?

Anti-vector measures

insecticide resistance
Prophylactic/therapeutic drugs

Chloroquine, mefloquine, fansidar, doxycycline, etc.

Drug resistance
RESISTANCE TO ANTIMALARIAL DRUGS

- Chloroquine: 16 years
- Fansidar: 6 years
- Mefloquine: 4 years
- Atovaquone: 6 months
Other contributing factors

Little private sector (commercial) interest

Decaying healthcare infrastructure

Political turmoil
What is being done to fight it?

Diagnostics

Therapeutics

Prevention
Three milestone efforts:


Diagnostics

Malaria symptoms overlap other febrile diseases

Clinical diagnosis can be unreliable

Laboratory confirmation of clinical diagnosis is desirable (laboratory diagnosis can be confounded by the prevalence of circulating blood parasites in individuals without symptoms)

Microscopic confirmation difficult in peripheral health facilities and in the community
Diagnostics

Development of Rapid Diagnostic Tests (RDTs)

RDTs do not:

- measure severity of infection
- discriminate among all species
- discriminate between sexual and asexual parasite stages
- Discriminate between malaria antigens that persist in the blood following treatment

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>% Sensitivity/Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ParaSight-F</td>
<td>Becton Dickinson, U.S.A.</td>
<td>93/92.4</td>
</tr>
<tr>
<td>ICT Pf/Pv</td>
<td>-do-</td>
<td>96/93.1 (Pf)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.5/99 (Pv)</td>
</tr>
<tr>
<td>Rapid Test Malaria</td>
<td>Quoram Diagnostics, Canada</td>
<td>100/98.3</td>
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<tr>
<td>Pf Check-1</td>
<td>Veda Lab., France</td>
<td>87.7/98.9</td>
</tr>
<tr>
<td>Determine™ Malaria Pf</td>
<td>Dainabot Co., Japan</td>
<td>96.5/87.2</td>
</tr>
<tr>
<td>ACCU Stat Malaria</td>
<td>Millennium Bio-Technology Inc., USA</td>
<td>86.9/90.3</td>
</tr>
<tr>
<td>Paracheck</td>
<td>Orchid Biomed. Systems (Goa)</td>
<td>95.8/85.7</td>
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<tr>
<td>OptiMAL</td>
<td>DiaMed, Switzerland</td>
<td>92.2/99.3 (Pf)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94.5/98.2 (Pv)</td>
</tr>
</tbody>
</table>

“dipstick test”  Gene amplification tests
Therapeutics

It is estimated that a new drug must be available every five years. 

Dr. Robert Ridley, 
Chief Scientific Officer 
Medicines for Malaria Venture/WHO

Genomics has led to the discovery of hundreds of new possible targets


POWER OF PREVENTION: IMPACT OF VACCINES IN THE U.S.

- Poliomyelitis
- Diphtheria
- Measles
- Rubella (German Measles)
- Mumps
- Pertussis (Whooping Cough)

(Adapted from data presented by the Boston Consulting Group)
Targets for vaccine development

1) Infection-blocking (sterile)
5) Anti-disease
6) Transmission-blocking
Insecticide-treated bed nets

Can reduce transmission of malaria by 17%, and severe malaria by as much as 35-50% when used properly

Research Program at UCI

Genetic Control Of Vector-Borne Diseases

Vectors:
- Aedes aegypti
- Anopheles gambiae
- Anopheles stephensi
- Anopheles darlingi

Malaria parasites:
- Plasmodium gallinaceum
- Plasmodium falciparum

Dengue Viruses

Identification of Malaria Antigens for Vaccine Development

Surface antigens on the vertebrate-infectious forms of the parasites, the sporozoites
1) Gametocytes are ingested and are carried in bloodmeal to midgut
2) Gametes fuse to form zygote
3) Zygote transforms into ookinete and penetrates midgut epithelium
4) Ookinete develops into oocyst on basal surface of midgut epithelium wall
5) Sporozoites develop in oocyst
6) Sporozoites burst through oocyst wall and migrate through hemolymph to salivary glands
7) Sporozoites invade salivary glands in preparation for transmission to a new vertebrate host
Hypothesis

The introduction into a population of vector insects of a gene that confers resistance to a pathogen should lead to a decrease in transmission of that pathogen.

Implicit in this hypothesis is that less transmission will result in less morbidity and mortality.
Major Areas of Research

Molecular genetic manipulation of mosquitoes (laboratory)
Introduction of genes into populations (laboratory and field)
Population characteristics of target species (field)
Molecular genetic manipulation of mosquitoes

Develop transgenesis technology

Identify promoters for expressing antiparasite genes

Develop antiparasite effector molecules
**TRANSGENESIS TECHNOLOGY**

![Diagram of transposase action](diagram.png)

### Mosquito | Element | First Citation
---|---|---
*Anopheles gambiae* | *piggyBac* | Grossman *et al.*, 2001
*An. stephensi* | *Minos piggyBac* | Catteruccia *et al.*, 2000 Nolan *et al.*, 2002
*An. albimanus* | *piggyBac* | Perera *et al.*, 2002
*Culex quinquefasciatus* | *Hermes* | Allen *et al.*, 2001
# PROMOTERS EXPRESSING ANTIPATHOGEN GENES

<table>
<thead>
<tr>
<th>Mosquito Tissue-Specific Promoter</th>
<th>Antipathogen Effector Gene</th>
<th>3'UTR</th>
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</thead>
<tbody>
<tr>
<td>Midgut</td>
<td>Tissue receptors</td>
<td></td>
</tr>
<tr>
<td>Fat body</td>
<td>Parasite surface ligands</td>
<td></td>
</tr>
<tr>
<td>Salivary gland</td>
<td>Insect immune response</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parasite gene expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antiparasite toxins</td>
<td></td>
</tr>
</tbody>
</table>

Taken from B. Jobling, Anatomical drawings of biting flies, British Museum.

**MICROARRAY OF A. GAMBIAE GENES AFTER MULTIPLE BLOOD MEALS**

- 24 HPBM 1st meal
- 24 HPBM 2nd meal
EFFECTOR MOLECULES

**Malaria:**

- scFv

**Dengue viruses:**

- 3’UTR
- 2prMA
- FitB

-Jasinskiene and James, unpublished

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Capurro et al., 2000

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Effector Molecule Strategies

- Interfere with mosquito tissue-recognition molecules (receptors)
- Interfere with parasite surface ligands
- Induce antiparasite insect immune response
- Interfere with parasite gene expression
- Secretion of antiparasite toxins
Blocking strategy:

prevent sporozoites from invading salivary glands
Plasmodium gallinaceum Circumsporozoite proteins

- Surface protein on immature and mature sporozoites
- 64-76 kiloDalton polypeptide
- Single-copy gene
- Contains highly-immunogenic tandem repeat amino acid sequences

Image courtesy of Nirmala Xavier
The mouse monoclonal antibody, N2H6D5, binds the circumsporozoite protein of the avian parasite, *P. gallinaceum*, and blocks sporozoite invasion of salivary glands.
Single-chain antibodies as anti-Plasmodium effector molecules

- F(ab’)2
- Fv
- IgG
- Fv
- Linker
- 16 aa linker
- scFv
- 4 aa linker
- Dibody
Sindbis virus vectors for transient expression of antipathogen scFvs in blocking experiments

Control virus

Sindbis Promoter

EGFP

Control single-chain virus

Sindbis Promoter

Mal-I

4G2scFV

E-tag

Experimental single-chain virus

Sindbis Promoter

Mal-I

N2scFV

E-tag

Capurro et al., 2000
Sindbis-expressed N2scFv binds sporozoites \textit{in vivo}

Sporozoites isolated from the hemolymph of virus-infected mosquitoes and reacted with anti-E-tag antibodies

Capurro \textit{et al.}, 2000
Mosquitoes with salivary gland mean intensities of infection as low as 10 sporozoites were able to infect chickens.

The target for salivary gland sporozoite intensities is zero.

Jasinskie and James, unpublished
“Eleven patients requiring malaria therapy were inoculated intradermally with … Plasmodium vivax. Estimated doses of 10 sporozoites were given to four patients, of 100 to three patients, of 1,000 to two patients, and of 10,000 to two patients. Parasitaemia was detected in all patients 12 to 17 days after inoculation; fever began on the 14th to 19th days.”

Future directions

Move from animal models to human pathogens

Develop mechanisms for driving genes through populations

Study the transmission dynamics of malaria in the field

Define target vector populations
“Roll Back Malaria” (RBM) Goals to cut by half malaria deaths in Africa by 2010

Insecticide treated bed nets must be made available to more people more quickly (only 4% use them now). Taxes and tariffs on them must be removed.

Pregnant women must get bed nets and anti-malarial medicines as a priority.

Next-generation drugs must be made available to treat chloroquine-resistant malaria. Even at $1-3 per treatment these drugs cost 100X more than chloroquine.

Malaria Early Warning Systems, using weather forecasting and data on malaria hotspots, must be created to help authorities mitigate outbreaks.
“Big-picture” challenges:

Consider development of a live vaccine

Educate scientists and public on genetically-engineered vectors

Design malaria control approaches with eradication as the ultimate goal

Secure appropriate funding