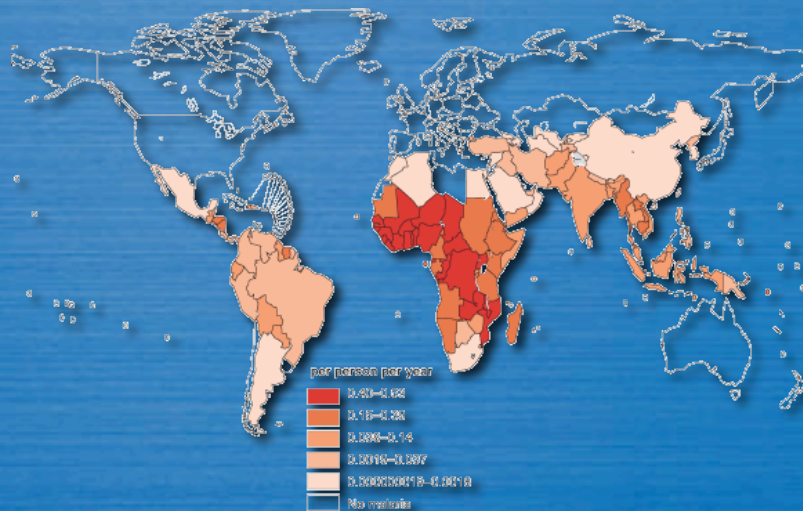




Malaria and the Promise of Microbial Genomics

By Ben Robinson

Malaria Incidence



- Malaria is one of the most widespread infectious diseases
- 41% of the world's population live in areas where malaria is transmitted
- An estimated 500 million infections of malaria occur each year resulting in nearly 3 million fatalities
- Malaria is the 4th leading cause of death in children of developing countries after perinatal conditions, pneumonias, and diarrheal diseases
- Malaria causes 10.7% of all children's deaths in developing countries

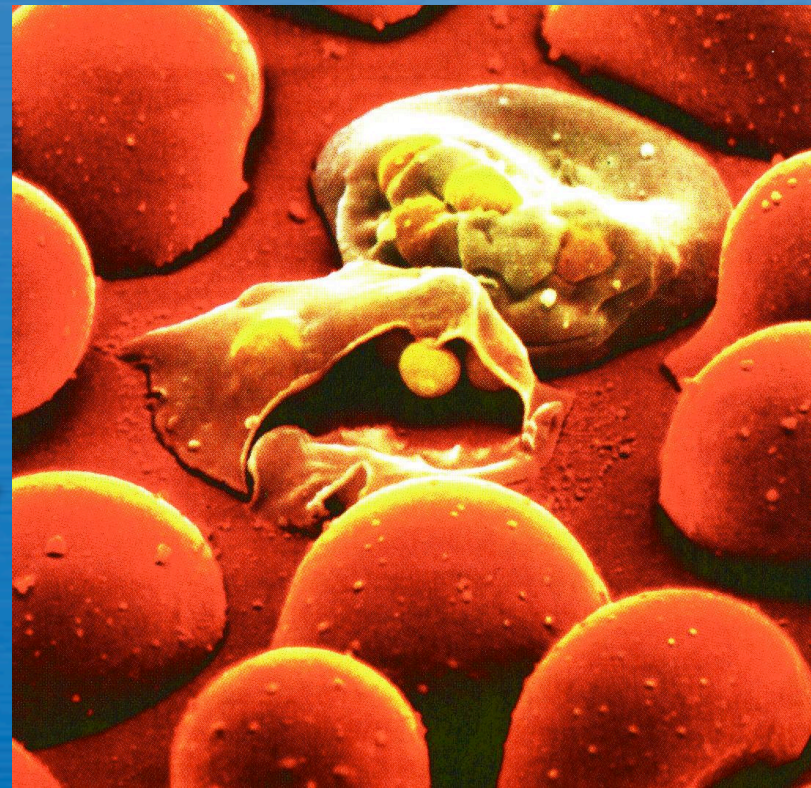
Burden of Malaria

- Estimates during the past 35 years indicate that the yearly gross domestic product has risen up to 2% less in countries where malaria remains highly endemic than in countries with an otherwise similar background where malaria does not occur
- The African continent shows the greatest effects of this disease, accounting for more than 90% of the burden, followed by Southeast Asia with almost 9% of the burden
- The disease costs Africa about \$12 billion each year



Disease Overview

- Malaria is an infectious disease caused by parasites of the *Plasmodium* genus
- 4 species of this genus represent nearly all cases of malaria: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*
- *P. falciparum* causes most of the severe disease and deaths attributable to malaria and is prevalent in Sub-Saharan Africa and in parts of South- East Asia and the Western Pacific
- *P. vivax* and *P. ovale* have dormant liver stage parasites which can reactivate and cause malaria several months or years after the infecting mosquito bite
- *P. malariae* produces long-lasting infections and if left untreated can persist asymptotically in the human host for years, even a lifetime

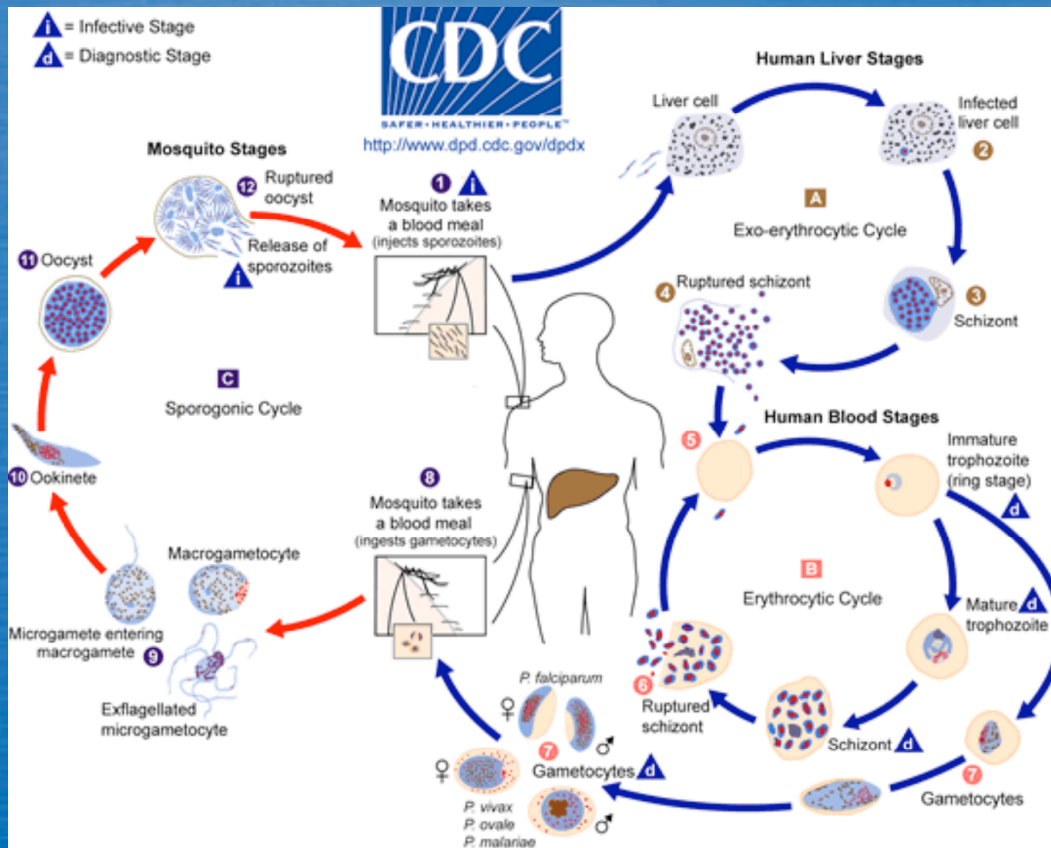


Vector



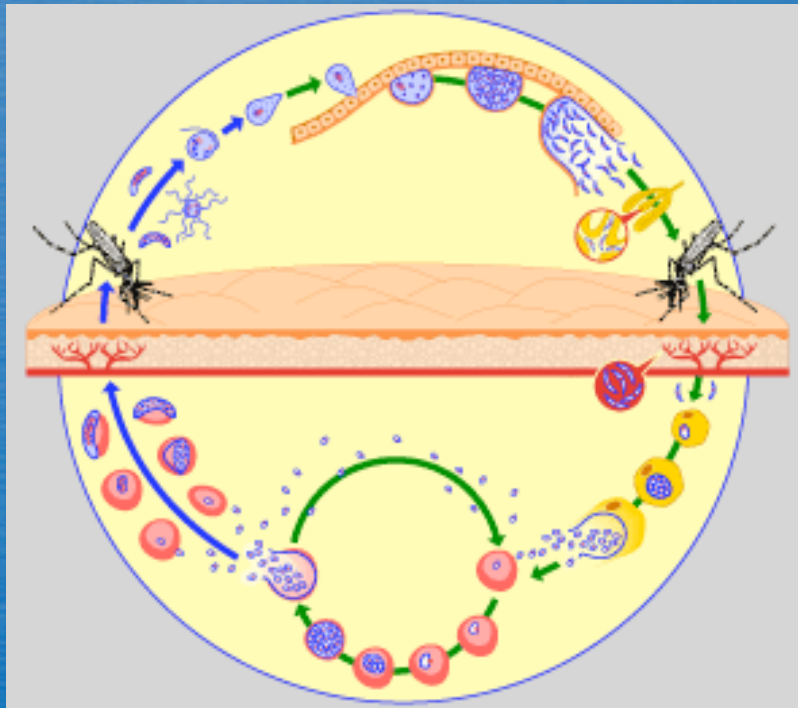
- Malaria is transmitted by mosquitoes of the *Anopheles* genus
- Female mosquitoes take blood meals from humans to carry out egg production
- In doing so, they insert plasmodium parasites into the bloodstream through their saliva
- Of the approximately 430 known species of *Anopheles*, only 30-50 transmit malaria in nature

Malaria Lifecycle



- Upon the bite of an infected mosquito, *Plasmodium* parasites in the sporozoite stage quickly lodge themselves in human liver cells
- These sporozoites divide rapidly over the next week, one parasite giving rise to around 30,000 daughter parasites (merozoites)
- After this time, these swollen liver cells lyse, releasing thousands of merozoites into the blood stream
- These parasites then infect red blood cells, where, safe from the immune system, they again grow and divide, forming up to 32 daughter merozoites
- After 48 hours, the host red blood cell bursts, releasing merozoites into the blood to repeat the cycle

Lifecycle Continued



- Some merozoites also divide sexually to form male and female gametocytes, which are ingested by the mosquito
- In the mosquito's stomach the gametocytes fuse to produce sporozites, which migrate into the salivary glands, continuing the cycle
- Some mature merozoites insert molecules into the red blood cell surface that hook onto host receptor molecules found on the lining of small blood vessels, sequestering the infected cells
- It is presumed that this provides some advantage to the parasite, either by protecting it from passing through the spleen, where infected red blood cells might be recognized and removed, or by providing optimum conditions for the parasite to grow

Symptoms

- The symptoms of malaria are due to three main events: the destruction of red blood cells, the release of cytokines in response to merozoites in the blood, and the sequestration of mature merozoites
- The symptoms of uncomplicated malaria, fever, headache, rigors, muscle pains, lassitude, and cough result mainly from the immune response mediated by cytokines
- The features of severe malaria, caused by complications of *p. falciparum* infection, include: impairment of consciousness, seizures, coma, severe anemia, hemoglobinuria, metabolic acidosis, respiratory distress, and cardiovascular collapse and shock
- The mass destruction of red blood cells and the obstruction of blood vessels causes reduced oxygen delivery, explaining the majority of these symptoms





Diagnosis

- **Clinical Diagnosis** - based on identification of symptoms (fever, chills, sweats, headaches, muscle pains, nausea and vomiting). Difficult because they are common to flu and viral infections
- **Microscopic Diagnosis** - based on recognition of parasites in blood samples. maintenance of the microscopes and the quality of microscopy in rural clinics is difficult
- **Antigen Detection** - based on the detection of various antigens. It is very rapid, achieving results in 2-10 minutes. In highly malaria-endemic areas, however, many healthy individuals have parasitaemia, so a positive test does not prove that malaria is the cause of illness. High cost is another limiting factor of rapid diagnostic tests.
- **Molecular Diagnosis** - based on the amplification through PCR of parasitic nucleic acids. Very accurate but limited to areas with specialized labs or equipment

Treatment



- Treatments for malaria were initially derived from the *cinchona* plant, found in the Amazon
- The plant's active ingredient, quinine, was isolated in 1820
- Since then derivatives with fewer side effects, such as chloroquine and mefloquine, have been developed
- Discovered more recently, artemisinin, which is derived from the *artemisia* or *qinghao* plant of China, is also effective in treating malaria
- Due to increasing resistance to presently available treatments, especially chloroquine, Artemisinin Combination Therapies (ACTs) are being looked to

Control and Eradication

- The Global Malaria Eradication Campaign was adopted by the World Health Assembly in 1955, dictating the widespread use of DDT against mosquitoes and of antimalarial drugs to treat malaria and eliminate the parasite in humans
- While successful in eradicating endemic malaria by 1967 in all developed countries and parts of tropical Asia and Latin America, the approach employed in the campaign was both unsustainable and not feasible for global implementation
- The recent strategy, adopted in 1993, is based largely upon the primary health care approach and requires flexible, cost-effective, sustainable, and decentralized programs based upon disease rather than parasite control



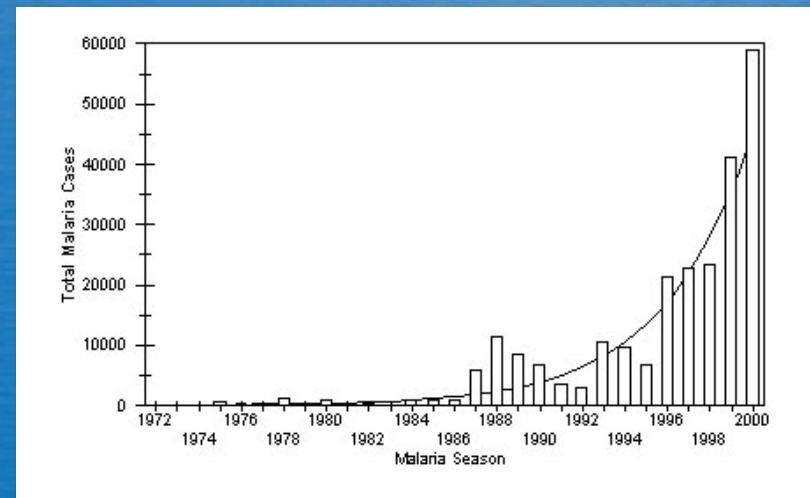
Methods of Control



- Malaria control is now primarily based on accurate diagnosis and prompt treatment, which involves the development of better primary healthcare in affected areas
- Certain vector control measures, however, are still in place in certain areas of high transmission risk
- Insecticide Treated Nets (ITN's) are the primary strategy adopted for vector control by most countries in Africa
- Issues with ITN's are cost and need for retreatment
- Indoor Residual Spraying (IRS) is useful for achieving a rapid reduction in transmission during epidemics and other emergency situations, but it involves the usage of large amounts of insecticides including DDT, and it requires precise timing

Recent Reemergence

- In the 1980's and 1990's, despite measures implemented to eradicate malaria, there was an increase in the disease's burden in terms of proportions of population at risk, the severity of infections and the number of deaths
- Malaria re-appeared in several countries in Central Asia and Transcaucasia where it had previously been eradicated
- In rural sub-Saharan Africa, child mortality caused by malaria is estimated to have increased by up to 100% during the 1980's and the early 1990's, while mortality resulting from other causes decreased over the same period



Cases of Malaria in South Africa



Causes of Reemergence

- **Climate instability:** droughts and floods can increase malaria transmission in different epidemiological circumstances
- **Global warming:** global warming can increase transmission in some highland areas, but is unlikely to lead to a wide geographical spread of malaria
- **Civil disturbances:** civil unrest results in the collapse of malaria treatment programs and crowding of refugees, some of whom might come from non-endemic areas, enhancing malaria transmission
- **Travel:** increasing travel within endemic areas as well as by travelers from non-endemic to endemic areas puts many non-immune individuals at risk
- **HIV:** HIV increases susceptibility to malaria, raises the burden on the health services, and reduces the number of clinical staff available to treat malaria
- **Drug resistance:** parasite resistance to various drugs is probably the major cause of the deteriorating malaria situation in Africa
- **Insecticide resistance:** resistance to pyrethroids (used to treat bed-nets) has emerged in *Anopheles gambiae* in west Africa and in *A. funestus* in southern Africa.

Genomics of Malaria



- Efforts begun in 1996 by the Sanger Institute, TIGR, and Stanford University, to sequence the genome of *Plasmodium falciparum* culminated in October 2002 with the publishing of the complete genome
- The genome of the *Anopheles gambiae* mosquito was also published in the same issue of *Nature*, as was that of *Plasmodium yoelii*, the infectious agent in rodent malaria, providing the scientific community with both a human pathogen and its animal model simultaneously
- The information in each of these genomes in addition to that of the human provides novel approaches to the problems previously mentioned with the control of malaria



Parasite Genome

- The *P. falciparum* genome consists of 14 chromosomes, encoding about 5,300 genes
- This genome provides a complete map of its metabolic pathways, the genes encoding metabolic enzymes being recognized by comparison with other organisms
- Each step in the metabolic pathway could represent a novel drug target, one example being a protein involved in the synthesis of type II fatty acids
- Scientists have also identified a family of 59 genes responsible for the parasite's ability to change the proteins on the erythrocyte surface, thereby evading the host's immune system
- This along with the identification of numerous other potential antigens will be useful in designing a malaria vaccine, a challenge that has not yet been met
- Finally, with the sequences of all the genes known, DNA microarrays can be designed to look at how the expression of the genes changes in the presence of a given drug
- Such experiments will help identify the genes involved in drug resistance



Mosquito Genome

- The *An. gambiae* genome includes 79 odorant receptor genes, all potentially useful in the development of mosquito repellants or traps
- It also contains about 200 genes that encode glutathione-S-transferases, cytochrome P450s, and carboxylesterases. These and possibly other genes probably play a critical role in detoxification of insecticides, and could be exploited for the development of new insecticides
- The ability to introduce foreign genes into *Anopheles* vectors is an exciting advance that might facilitate the development of transgenic mosquitoes immune to malaria parasites
- However, the implementation of this control strategy must take into consideration concerns about the environmental impact of releasing genetically altered mosquitoes

Human Genome



- The human genome also provides insights useful for the control of malaria
- Currently there is a poor understanding of the molecular basis of naturally acquired immunity
- A few clues have emerged, including studies of HLA-B – a protein that presents molecules from invading organisms to the immune system, variants of which are present at an unusually high frequency in West Africans and are associated with protection from severe malaria
- Since HLA-B is expressed on liver cells and not on red blood cells, this suggests that immune mechanisms targeted against the liver stage of malaria infection are a significant factor in protection against severe malaria, bolstering efforts to develop a liver-stage vaccine
- A more complete understanding of natural immunity will aid exponentially in the quest for a malaria vaccine



Sources

- <http://www.cdc.gov/malaria>
- <http://www.nature.com/nature/malaria/index.html>
- <http://www.rbm.who.int/cgi-bin/rbm/rbmportal/custom/rbm/home.do>
- <http://www.wellcome.ac.uk/en/malaria/home.html>
- <http://www.sciencemag.org/feature/data/mosquito/index.shtml>