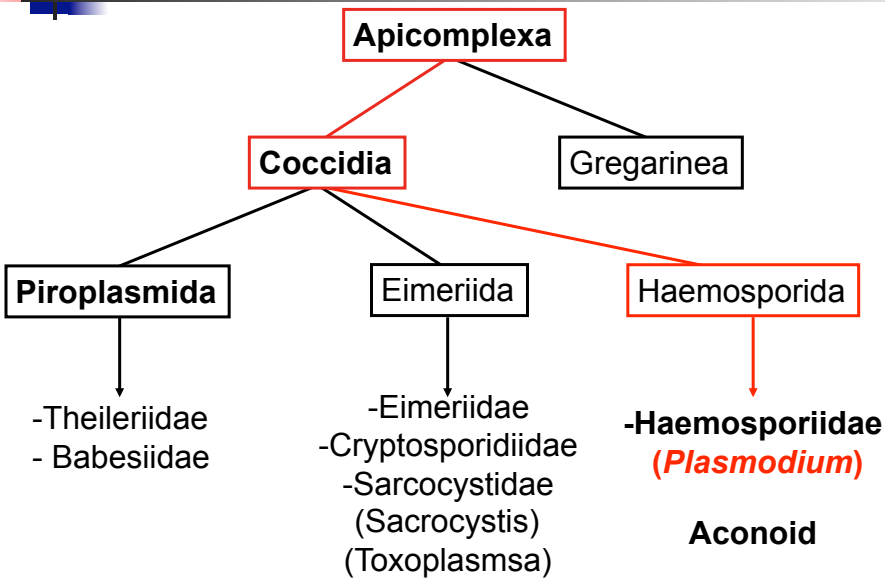
Extra-intestinal coccidians



Plasmodium species

- Causitive agent of Malaria
- ~155 species named
  - Infect birds, reptiles, rodents, primates, humans
- Species is specific for host and vector
- 4 species cause human disease
- No zoonoses or animal reservoirs
- Transmission by *Anopheles* mosquito

- ***P. falciparum***
- ***P. vivax***
- ***P. malariae***
- ***P. ovale***

New human species – *P. knowlesi* has been recently documented to cause human infections in many countries of Southeast Asia.

Distribution of Malarial Parasites

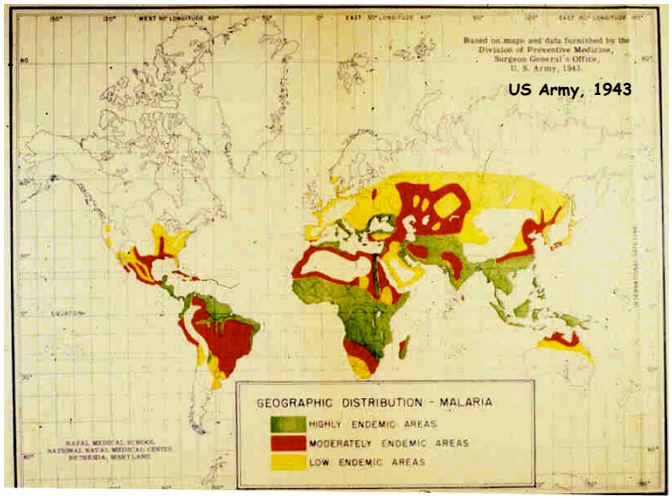
P. vivax
most widespread, found in most endemic areas including some temperate zones

P. falciparum
primarily tropics and subtropics

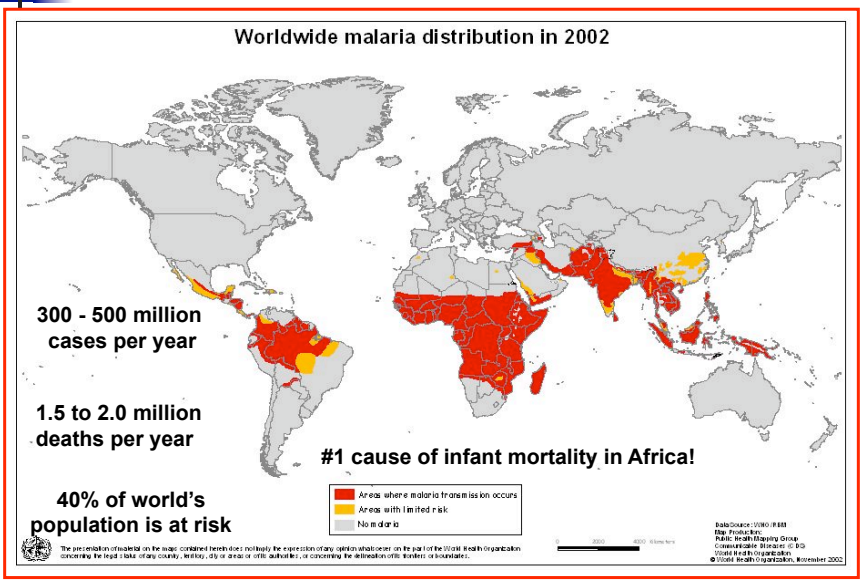
P. malariae
similar range as *P. falciparum*, but less common and patchy distribution

P. ovale
occurs primarily in tropical west Africa

Distribution of Malaria



MALARIA



Malaria Atlas Map Project

- **http://www.map.ox.ac.uk/**

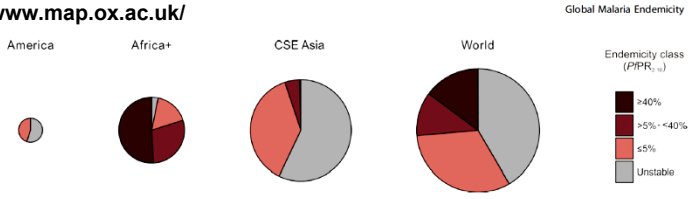


Figure 7. Pie Charts Showing the PAR of *P. falciparum* Malaria in 2007

The charts show the proportion of the population living in each predicted $PfPR_{2-10}$ endemicity classes for the America, Africa+, CSE Asia regions, and worldwide. The charts are scaled proportionally to the total population at risk in each region and the segments are coloured to match the endemicity classes shown in Figure 4.

doi:10.1371/journal.pmed.1000048.g007

Malaria in the United States

- Malaria was quite prevalent in the rural South
- It was eradicated after world war II in an aggressive campaign using, treatment, vector control and exposure control (along with overall improvement of living conditions)
- This was the CDCs initial misssion

Time magazine - 1947

Was a widely available, cheap insecticide

DDT resistance
Half-life in mammals - 8 years!
US banned use of DDT in 1973

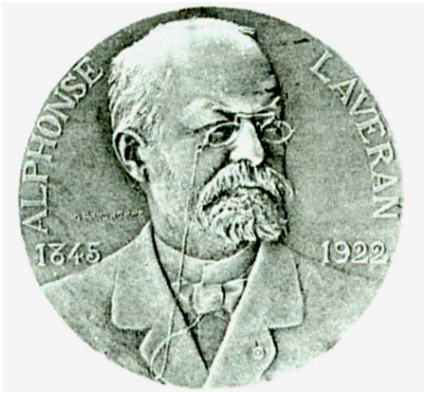
History of Malaria

- Considered to be the most important infectious disease on a worldwide scale
- Mal aria - “bad air”
- Swamp disease
- Periodic fever episodes
- Egyptian records
- Roman empire
- World War II

http://www.malariasite.com/MALARIA/History.htm

History of Malaria

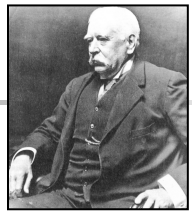
- Based on epidemiological considerations Alphonse Laveran concluded that “Swamp fevers are due to a germ”
- He discovered parasite life cycle stage (gametes) in the blood of patients with fever which were absent in samples from healthy individuals



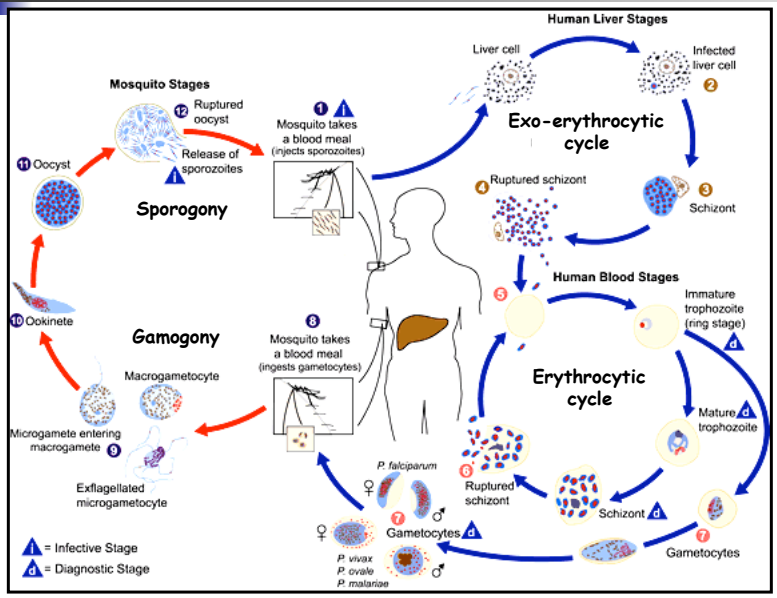
History of Malaria

- Patrick Manson (discovered mosquito transmission of *Wucheria*) proposes same transmission pathway for malaria
- With Manson’s encouragement Ross discovers the insect part of the lifecycle in 1897 while a military doctor in India using a bird model
- Ross reported to the world that Malaria is transmitted by mosquitoes - however he never directly demonstrated the transmission to humans
- William Trager (Rockefeller) - 1976 was the first to culture *Plasmodium falciparum* - did not require an animal model. Greatly changed research capabilities for studying Malaria.

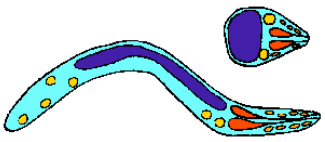
“you can't study something that you can't grow”



Plasmodium Life Cycle

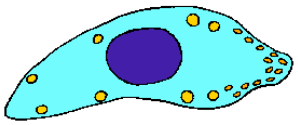


Parasite forms



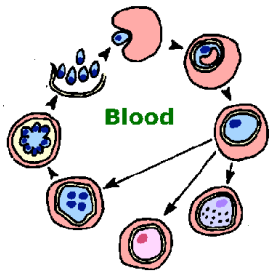
Merozoite: invades erythrocytes

Sporozoite invades mosquito salivary glands and liver cells

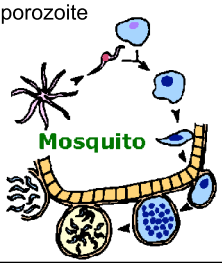


Ookinete invades mosquito gut epithelial cells

Mosquito forms: gametocytes, Oocyst, ookinete, sporozoite



Blood forms:
merozoites, rings, trophozoite, schizonts



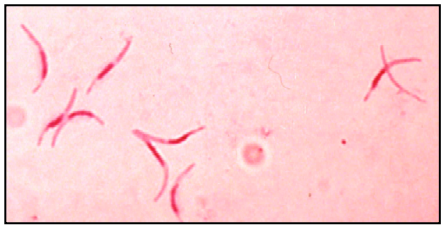
Transmission

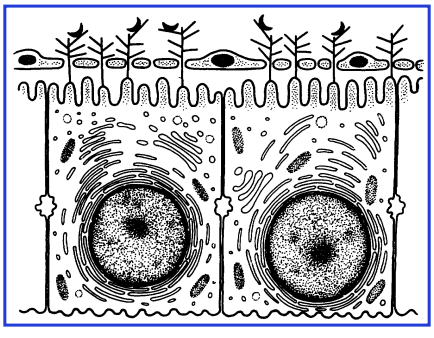
- Injection of sporozoites
- Average of 15 sporozoites are injected per bloodmeal
- <10 will initiate an infection
- Sporozoites will rapidly invade hepatocytes
  - Invasion <1 hr (minutes?)
  - Initiates the exoerythrocytic cycle
  - Adhesion/motility mediated by TRAP
- Sporozoites 1 of 2 motile stages

Female *Anopheles* mosquito

Invasion of Hepatocytes

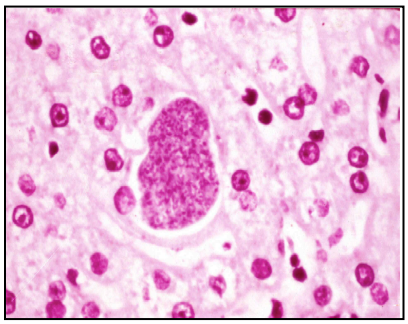
- Sporozoite invasion is specific for liver cells
- 2 sporozoite surface proteins contain hepatocyte adhesive domains
  - CSP - circumsporozoite protein
  - TRAP - thrombospondin related anonymous protein
- Both proteins bind to glycosoaminoglycans on the surface of hepatocytes
- Initiates the exoerythrocytic cycle





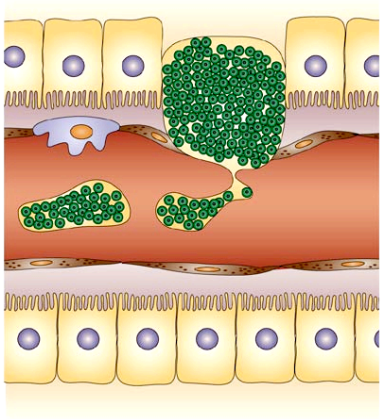
Exoerythrocytic Cycle

- Develop into tissue schizonts
- 5-7 days in liver
- Formation of >10,000 merozoites - schizogony
- Upon rupture of hepatocytes, released merozoites will invade RBC's
- Hypnozoites (sleeping animalcules)
  - Dormant sporozoites
  - Causes relapsing malaria - months or years later
  - *P. vivax* and *P. ovale*



Schizont in hepatocyte

Exoerythrocytic Cycle



Nature Reviews Microbiology (2006): 4-849

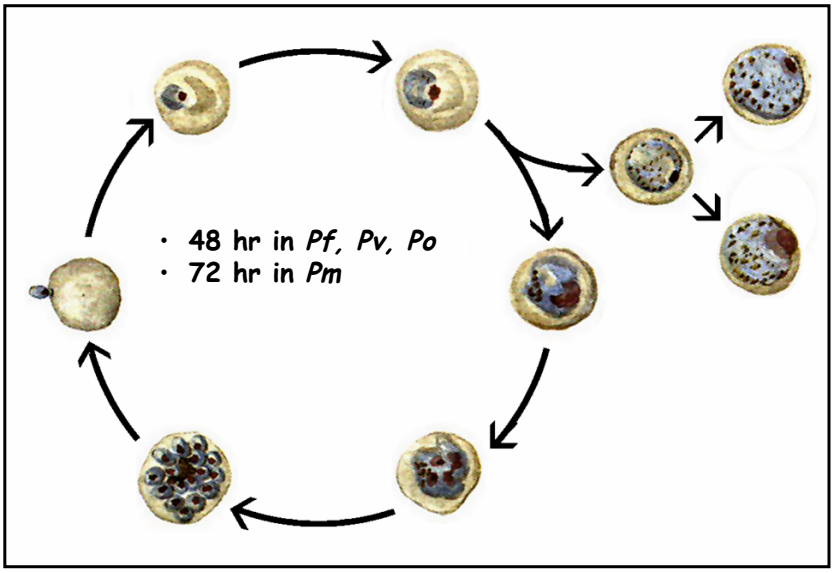
- Schizonts take 5-7 days to develop
- formation of >10,000 merozoites
- upon rupture (??) of merosome, released **merozoites** will invade rbc's

The final step involves the release of merozoites (green) into the bloodstream.

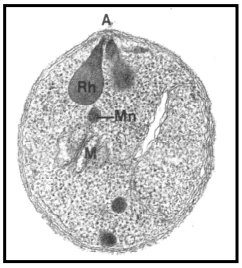
The signal(s) that trigger the release remain unknown.

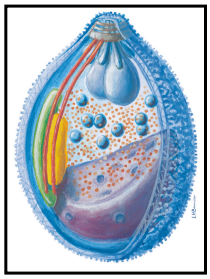
Plasmodium merozoites are released by the formation of merozoite-filled vesicles (merosomes), which bud off from the infected hepatocytes into the sinusoidal lumen.

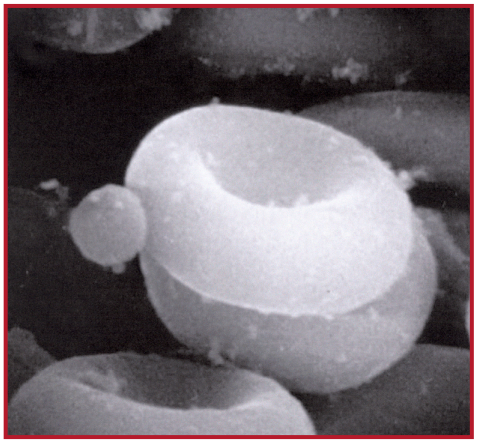
Erythrocytic Schizogony



Merozoite invasion



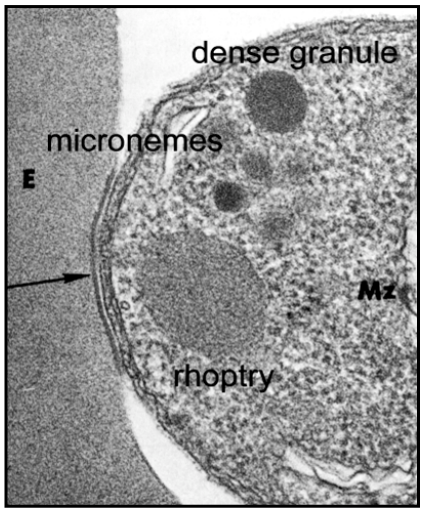




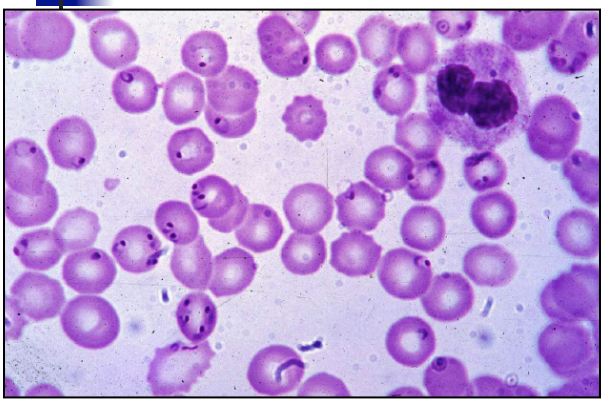
The merozoite is briefly present in the blood between leaving one red blood cell and entering the next one.
Lemon shape, Aprox 1 by 1.6 μm micron.

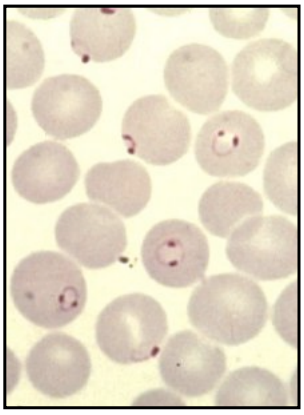
Invasion of Red Blood Cells

Reorientation on RBC surface



Ring Stage

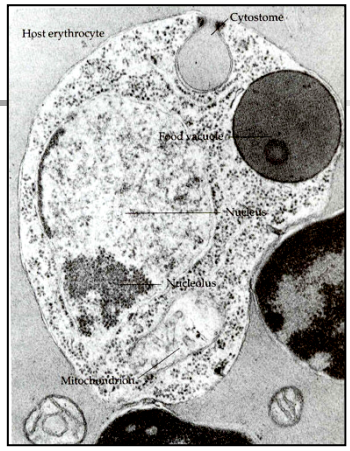


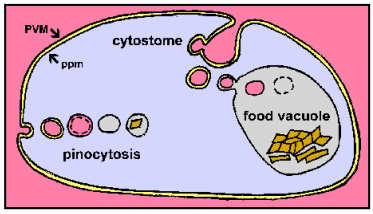


- 1st 14-16 hours spent as ring stage, or young trophozoite
- little to no Hb degradation
- Usually only see this stage in RBC for *P. falciparum*

Trophozoite stage

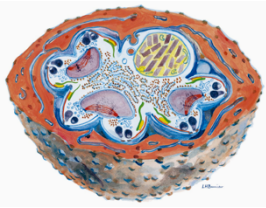
- Actively digesting RBC cytoplasm (Hb)
- Trophozoites take in RBC cytoplasm by endocytosis through the cytostome
- digestive vacuole is site of Hb digestion (50-100% digested in < 10 hrs)
- several proteases required (plasmepsins and cysteine proteases) endpoint of digestion is a toxic heme product ferriprotoporphyrin (FP)
- FP is polymerized to form hemozoin, the "malaria pigment" which is thought to detoxify heme for the parasite

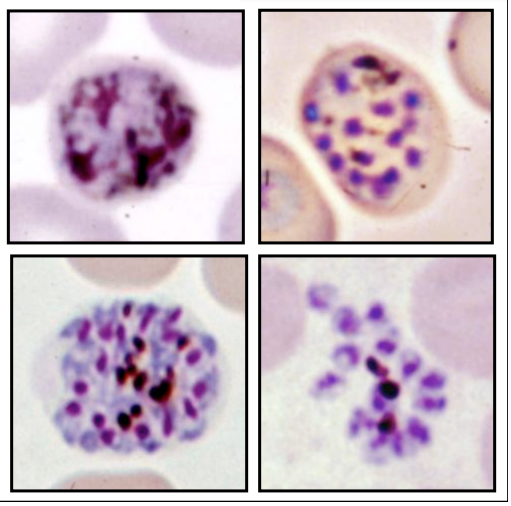




Erythrocytic Schizogony

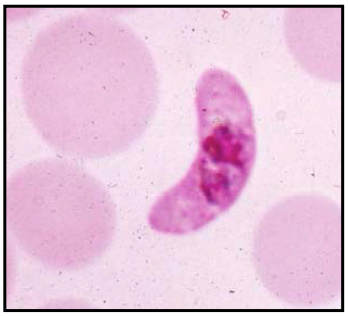
- Schizogony: form of asexual reproduction in which multiple mitoses take place, followed by cytokinesis resulting in multiple daughter cells
- multiple mitoses produce 20-24 nuclei
- once nuclei & organelles replicated cytokinesis occurs





Gamogony

- Formation of gametocytes
  - Occurs in mammalian host
  - Different forms for Plasmodium species
- Induction factors are unknown - in vitro studies suggests stress is a factor
  - Drug treatment can ↑ #'s
- Sexual dimorphism
- No pathology
- Infective stage for mosquito
- Further development in mosquito - fertilization



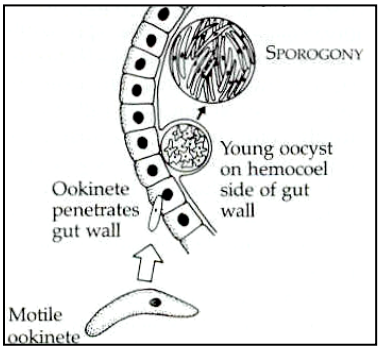
P. falciparum has the classic banana shape

Gamogony in Mosquito

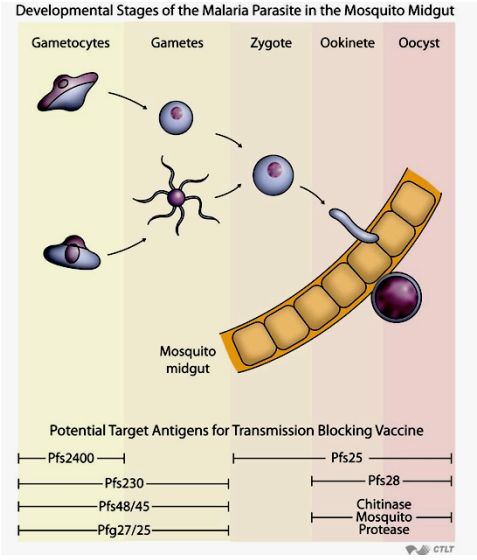
- Occurs in gut
  - Xanthurenic acid in gut
    - Gametocyte activating factor
    - High conc. in insect vs. human
  - ↑ pCO_2
  - ↓ pH (8-8.3)
  - ↓ temperature
- Microgametocytes undergo astonishing change
  - Rapidly undergo multiple nuclear division - form 8 microgametes
  - Exflagellation - free to swim and fertilize macrogamete
  - Ross actually observed this!
- Resulting ookinete

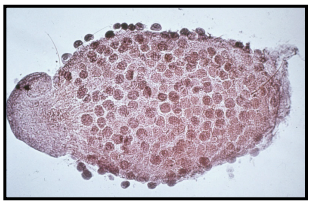
Development in Mosquito

- Sporogony in mosquito gut
- Zygote = Ookinete
  - penetrates intestinal cell
  - Hemocoel side
- Transformation into oocyst
- Formation of thousands of sporozoites
  - Sporogony 10-21 days
- Infection is correlated with increased mosquito feeding
- Experimentally infected mosquitoes can harbor huge numbers of oocysts
- Field isolates rarely have more than one oocyst per gut



Development in Mosquito





Malaria the disease

WHO-TDR

In Africa, malaria kills one child in 20 before the age of five (STI

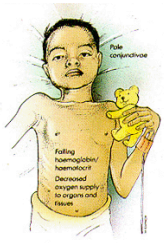
Malaria the disease

3 Severe manifestations

Cerebral malaria

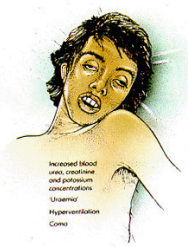
Irritability, loss of reflexes, neurological symptoms similar to menigitis, coma 20% fatality

Severe anemia



Progressive severe drop of hematocrit, poor oxygen Supply for organs and tissues

Renal failure

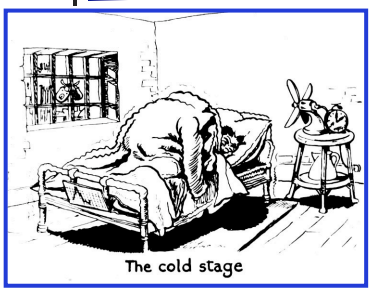


Dwindling urine, high urea Level in serum, hyperventilation Coma, poor prognosis

Others

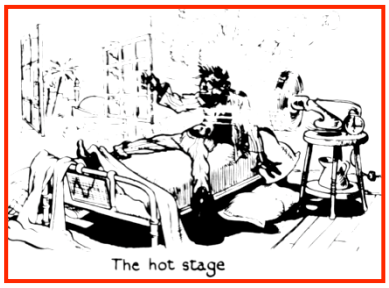
- Hepatic dysfunction
- Hypoglycaemia
- Shock ('algid' malaria)
- Pulmonary oedema
- Fluid/electrolyte imbalance
- Vomiting and diarrhoea
- Secondary bacterial infection
- Bleeding and clotting disturbances
- Haemoglobinuria and blackwater fever

The Disease



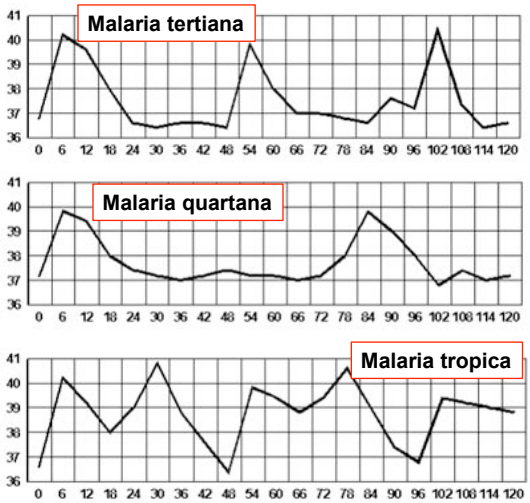
cold stage
- feeling of intense cold
- vigorous shivering, rigor
- lasts 15-60 min

hot stage
- intense heat
- dry burning skin
- throbbing headache
- lasts 2-6 hours



sweating stage
- profuse sweating
- declining temperature
- exhausted, weak → sleep
- lasts 2-4 hours

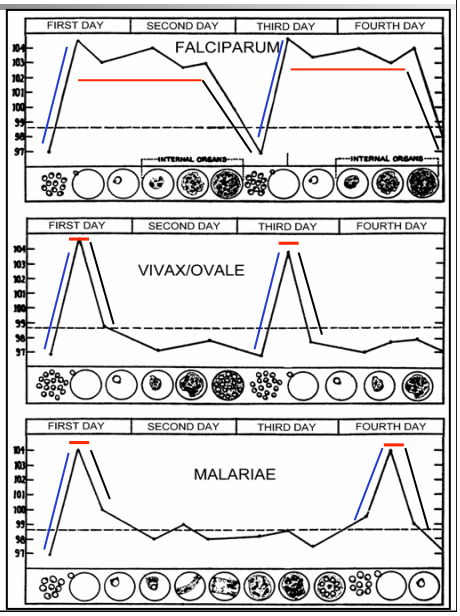
Malaria Paroxysm



- Malaria tertiana: 48h between fevers (P. vivax and ovale)
- Malaria quartana: 72h between fevers (P. malariae)
- Malaria tropica: irregular high fever (P. falciparum)

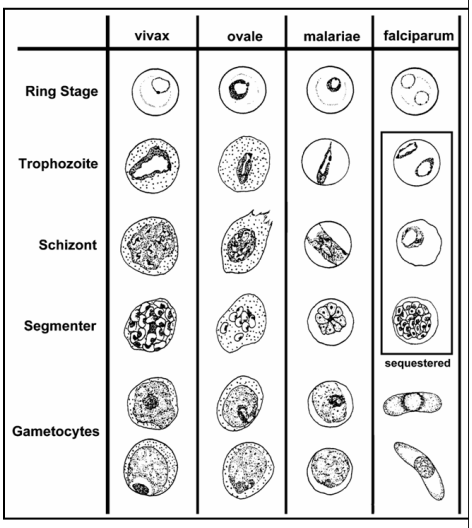
Malaria Paroxysm

- Paroxysms are associated with the synchrony of merozoite release - rupture of RBCs - release of toxins
- Rapid climb in body temp.
  - Mild delirium
- Between paroxysms patient feels well with normal temps
- *P. falciparum* may not exhibit classic paroxysms
  - Continuous fever
  - Less synchronous
- Concurrent infections with more than 1 species is common



Diagnostics

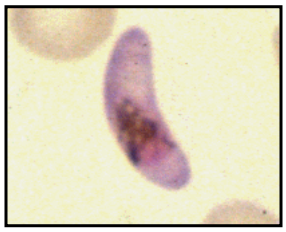
- **blood-stage morphology**
  - **Blood smears**
- **minor life cycle variations**
  - **hypnozoite stage (*vivax, ovale*)**
  - **sequestration (*falciparum*)**
- **host erythrocyte preference**
  - **immature erythrocytes (*vivax, ovale*)**
  - **senescent (*malariae*)**
  - **no preference (*falciparum*)**
- **disease and clinical manifestations**

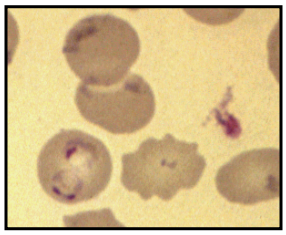


Many more color images available on the web!!

Plasmodium falciparum

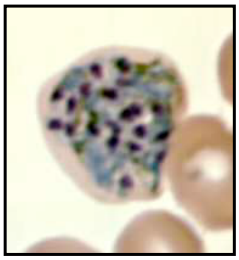
- Most pathogenic of the 4 species
  - More than 50% of Malaria cases
  - High parasitemia - large number of merozoites are produced
- Causes irregular fevers
  - Malignant malaria
  - Subtertian malaria
- Crecent shaped gamonts
- Often multiple rings in RBCs
- Only rings and young trophozoites in peripheral blood
- Other forms are sequestered

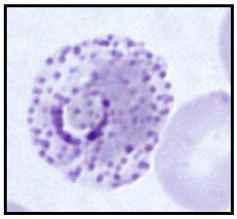




Plasmodium vivax

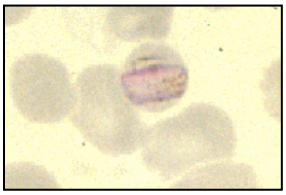
- Most widespread, only parasite reported in temperate climates
- Causes tertian Malaria
  - Benign malaria
- Significant cause of Morbidity
- Merozoites only invade young erythrocytes - reticulocytes
- Bind Duffy blood group antigen Fy^a and Fy^b
  - Fy/Fy individual are refractory to infection - no antigen present
- Schizonts give rise to 12-14 merozoites





Plasmodium malariae

- Represents only about 7% of reported cases
  - Low parasitemia
  - Invades only aging RBCs that are quickly cleared from the circulation
- Causes fever every 72 hours
  - Quartan Malaria
- Characteristic band form of the trophozoite - so to develop, 48 hr
- Schizonts often show very regular flower or rosette pattern of nuclei



Plasmodium ovale

- Only 1% of the cases!
  - Quite rare
- Restricted to West Africa
- Cause of malaria tertiana
- Infected cells are often ovoid

Disease Severity

	Pv	*Po*	*Pm*	*Pf*
Average (per mm^3)	20,000	9,000	6,000	50,000-500,000
Maximum (per mm^3)	50,000	30,000	20,000	2,500,000
Paroxysm Severity	moderate to severe	mild	mild to moderate	severe
Duration				
Disease	3-8 w	2-3 w	3-24 w	2-3 w
Infection	5-8 y	12-20 m	>20 y	6-17 m
Anemia	++	+	++	++++
Complications			renal	cerebral