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Diagnosis and Clinical Complications

David Sullivan, MD

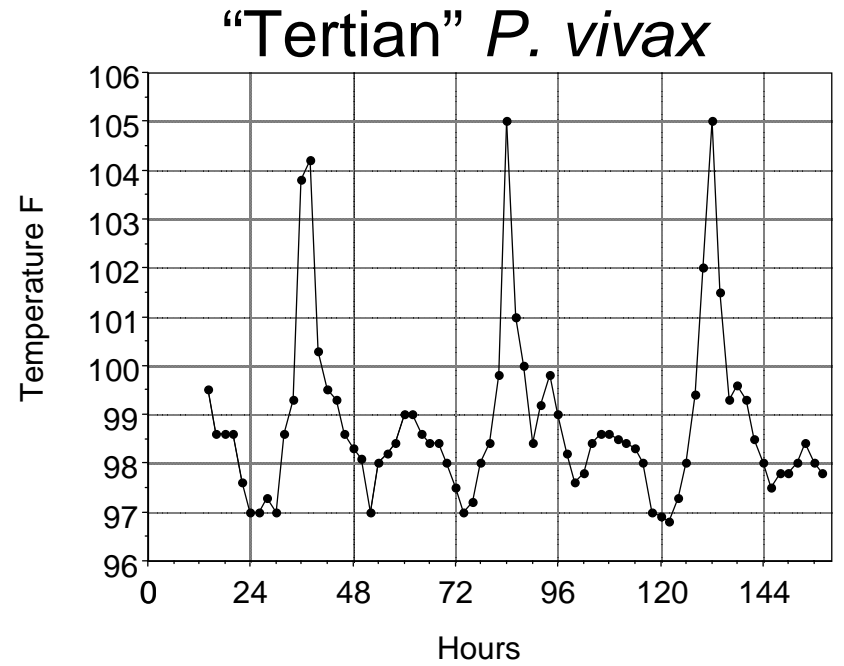
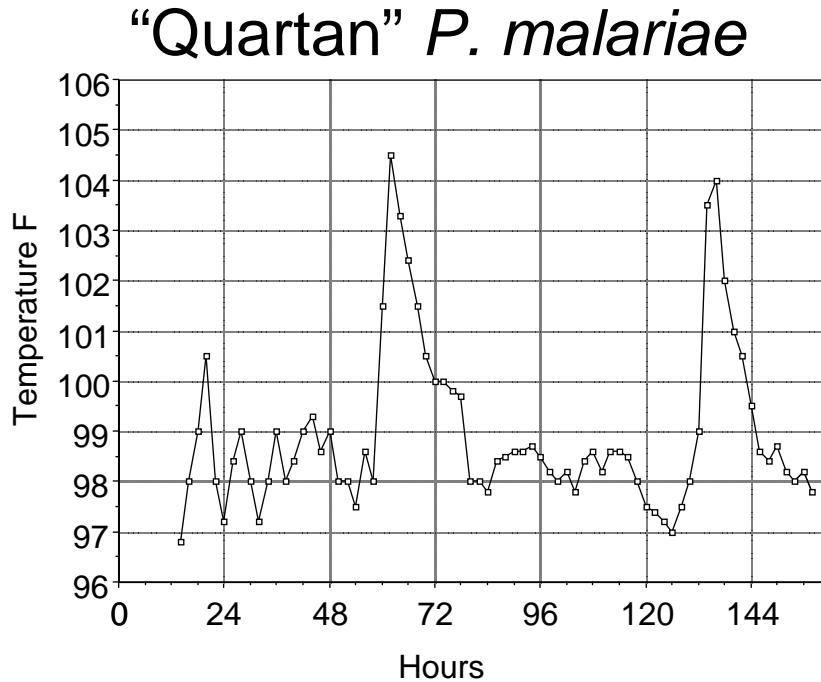
Classical Malaria

- Fever
- Splenomegaly
- Anemia

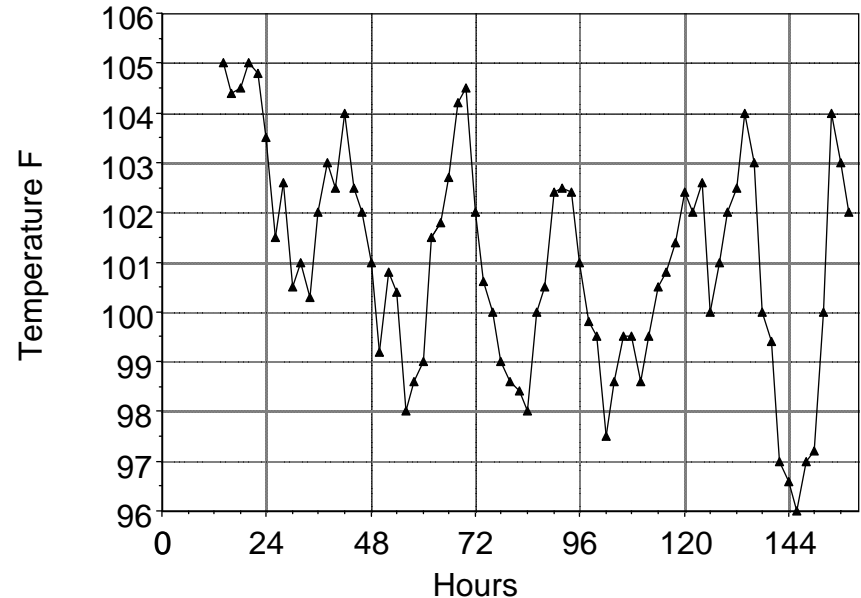
Hippocrates, 5th Century BC

Comparison of Malaria Fever Curves

Adapted from Thayer and Hewetson
Johns Hopkins Hosp Reports V 1895 p. 3-224



“Aestivo-autumnal” “Quotidian” *P. falciparum*



Diagnosis Based on Clinical Features

Advantages

Cheap

Fast



Disadvantages

Lack of precision

Over-treatment

Axial temperature is not a good indicator of malaria infection in children under holoendemic conditions, as often less than 10% of infections are associated with fever.

Inaccuracies of Clinical Diagnosis

- Malaria is difficult to diagnose clinically
- In studies $> 70\%$ of +ve diagnoses are non-parasitemic
- Beware statistics based on clinical reports

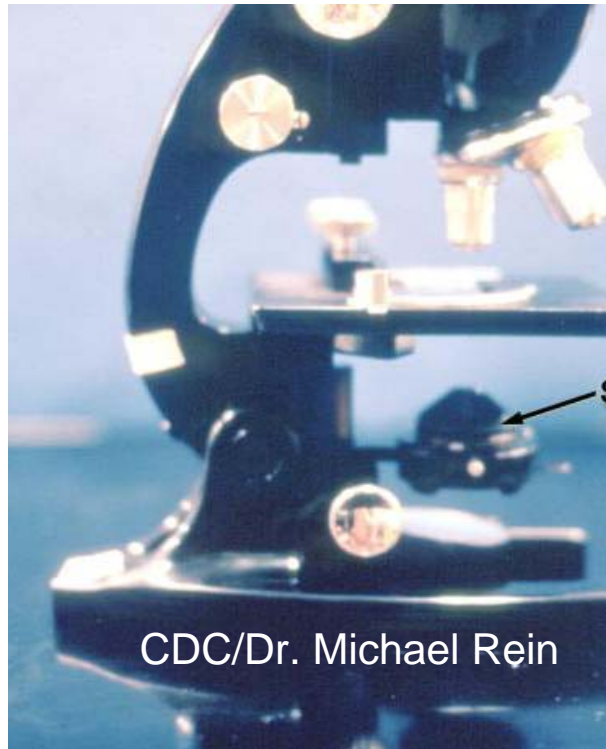
Diagnosis Based on Microscopy

Advantages

Gold standard

Quantitative

Useful for other diseases



Disadvantages

Time consuming

Relies upon good microscopes, reagents, and trained technicians

Useful Web Sites for Training in Blood Film Analysis

This site is presented by the Division of Laboratory Medicine at Royal Perth Hospital.

<http://www.rph.wa.gov.au/malaria.html>

- Dr. Richard Davis AM PhD MSc FAACB FIBMS MASM
- Mr. Graham Icke MSc CBiol FIBiol FIBMS Grad Dip Bus

CDC site

http://www.dpd.cdc.gov/dpdx/HTML/Malaria.asp?body=Frames/M-R/Malaria/body_Malariadiagfind2.htm

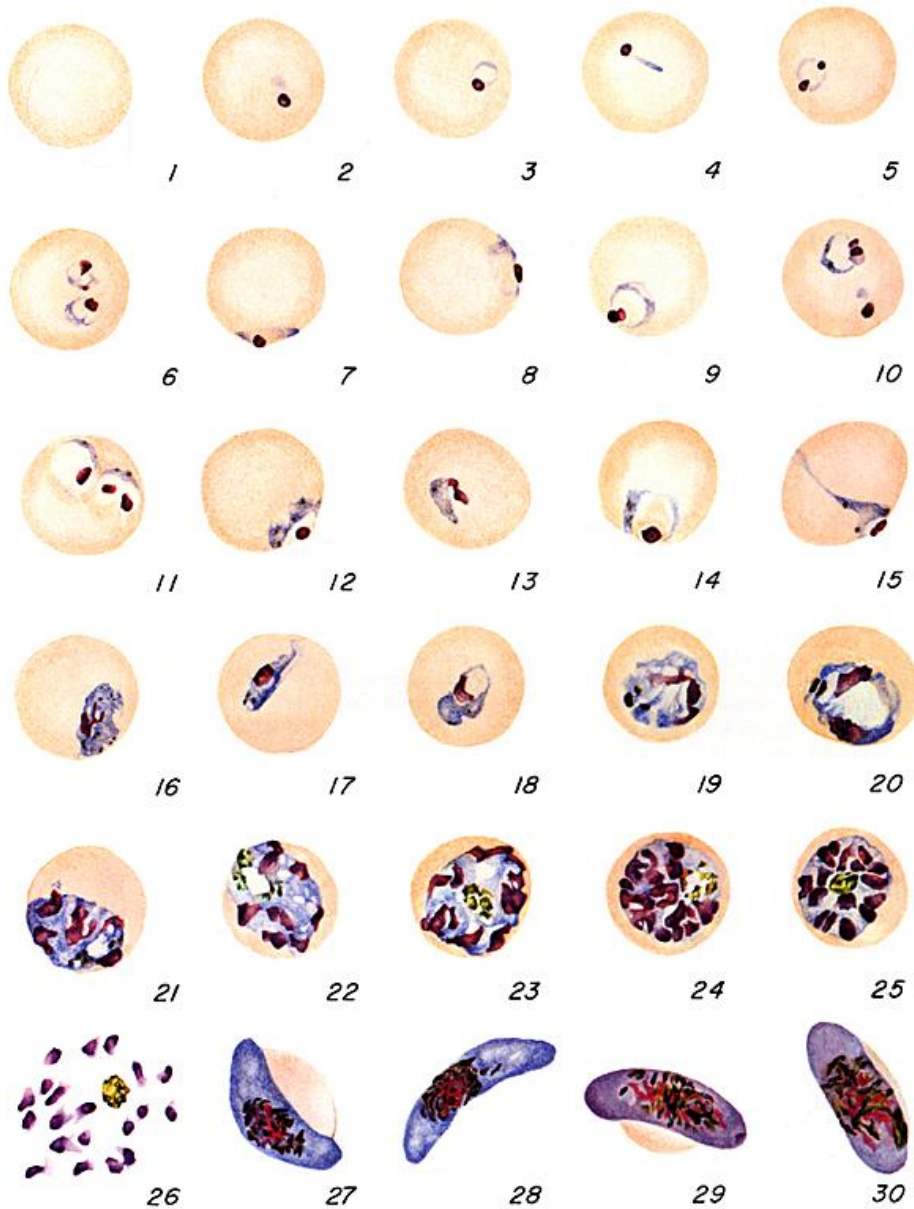
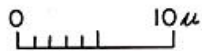


Fig. 1: Normal red cell
Figs. 2-18: Trophozoites (among these, **Figs. 2-10** correspond to ring-stage trophozoites)
Figs. 19-26: Schizonts (**Fig. 26** is a ruptured schizont)
Figs. 27 & 28: Mature macrogametocytes
Figs. 29 & 30: Mature microgametocytes (male).

Illustrations from: Coatney GR, Collins WE, Warren M, Contacos PG The Primate Malaria. U.S. Department of Health, Education and Welfare, Bethesda, 1971.



PLASMODIUM FALCIPARUM

J. H. Nicholson

P. falciparum Thick film

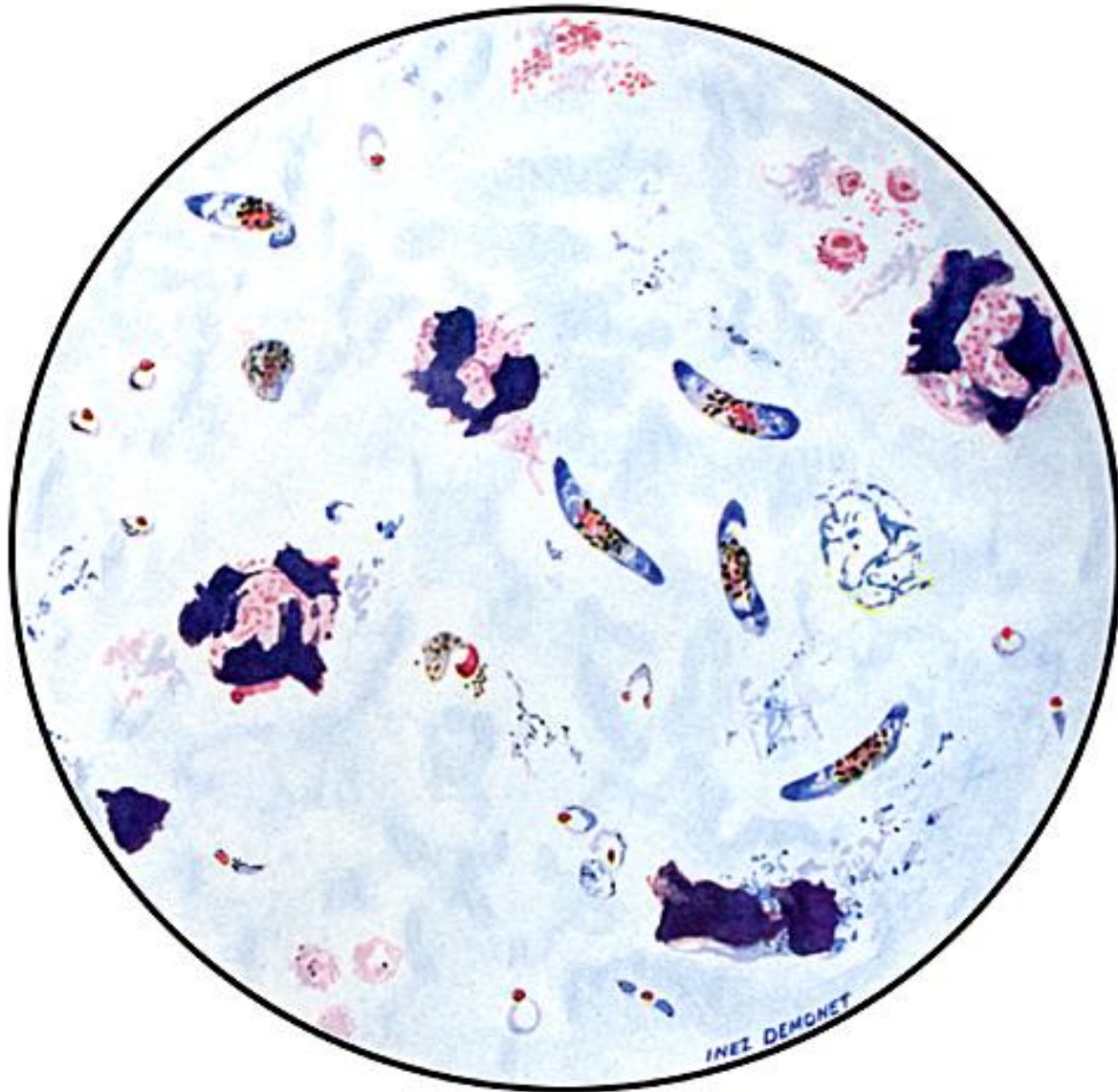


Illustration from: Wilcox A. Manual for the Microscopical Diagnosis of Malaria in Man. U.S. Department of Health, Education and Welfare, Washington, 1960.

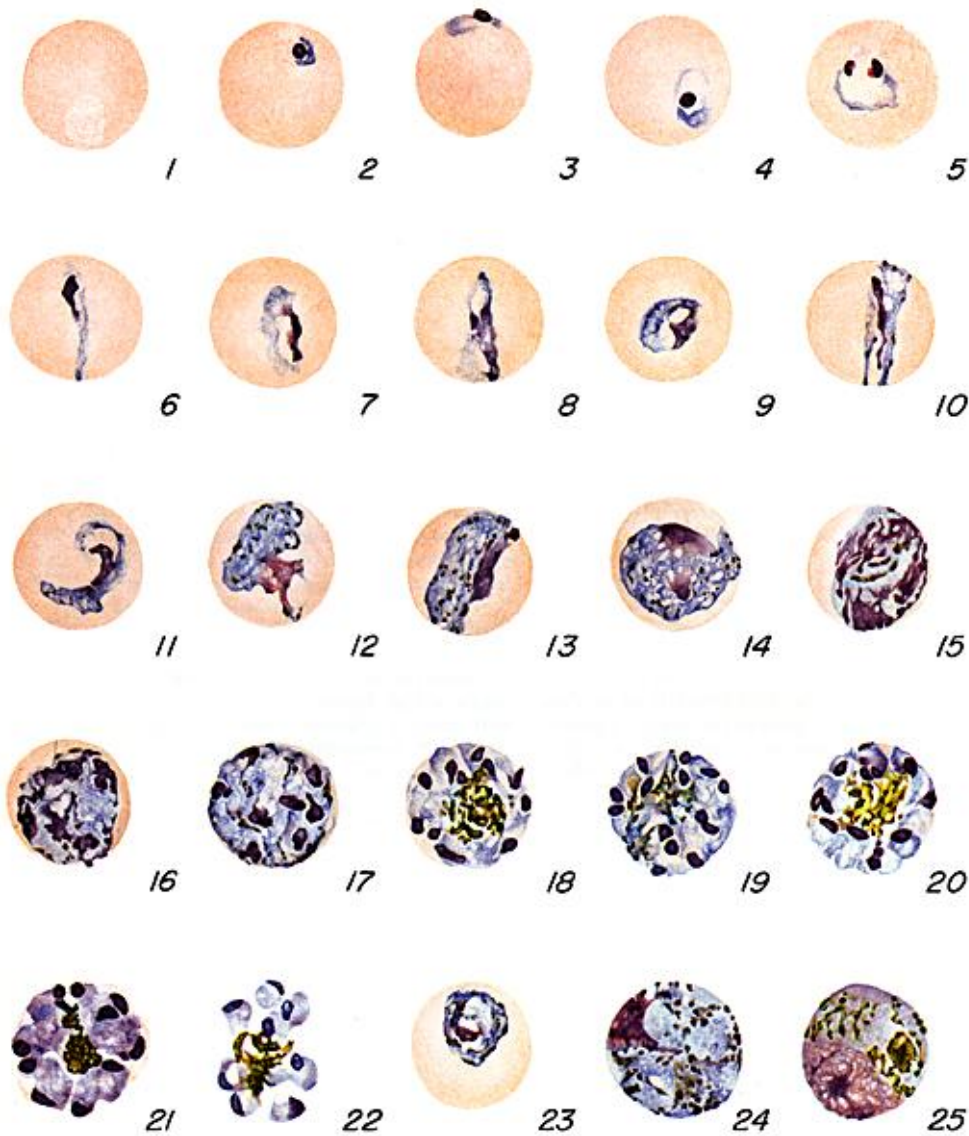


Fig. 1: Normal red cell
Figs. 2-5: Young trophozoites (rings)
Figs. 6-13: Trophozoites
Figs. 14-22: Schizonts
Fig. 23: Developing gametocyte
Fig. 24: Macrogametocyte (female)
Fig. 25: Microgametocyte (male)

Illustration from: Coatney GR,
 Collins WE, Warren M, Contacos PG.
 ^ The Primate Malariae. U.S.
 Department of Health, Education and
 Welfare, Bethesda, 1971.



PLASMODIUM MALARIAE

Dr. H. Nicholson

P. malariae Thick film

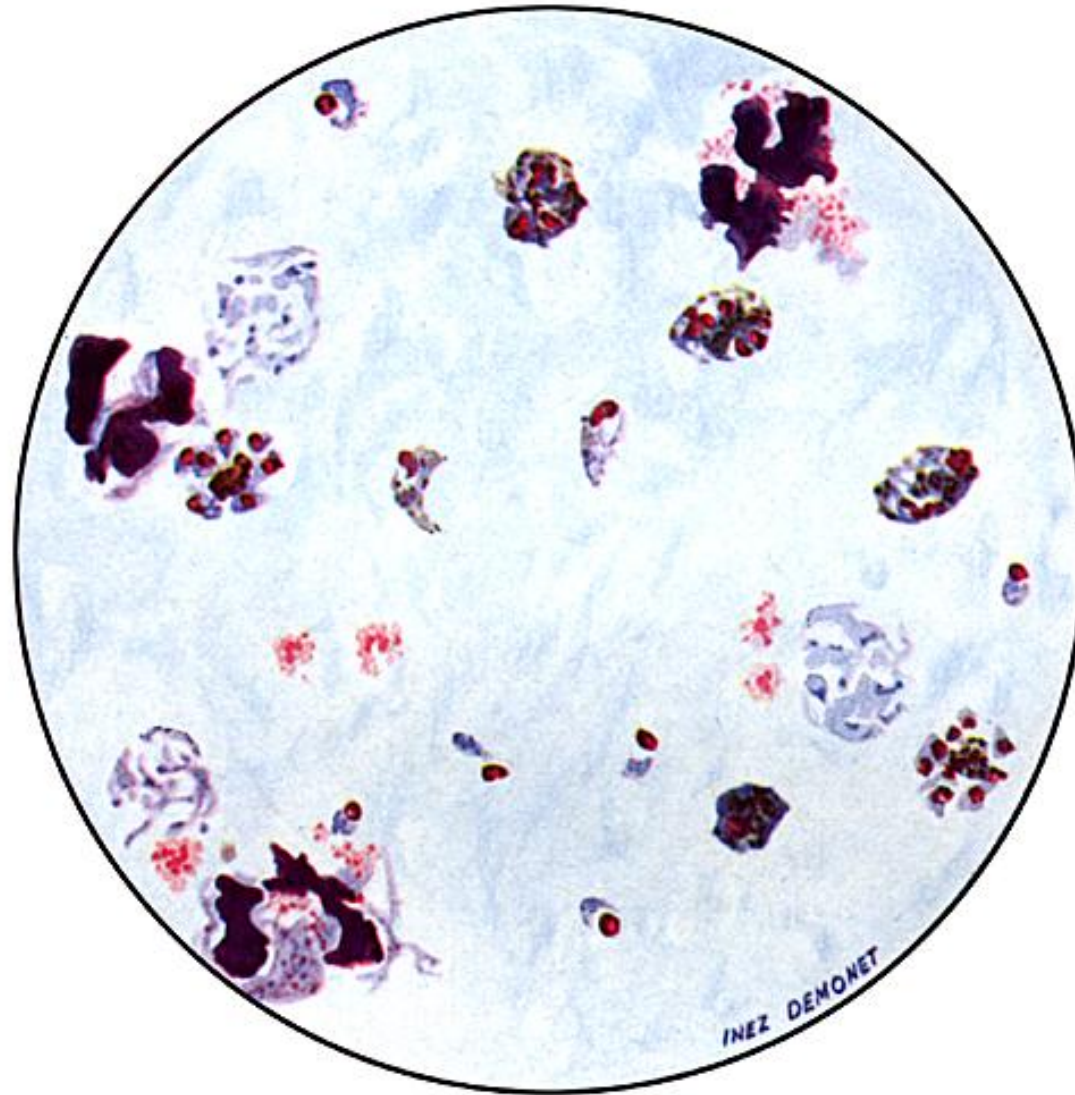


Illustration from: Wilcox A. Manual for the Microscopical Diagnosis of Malaria in Man. U.S. Department of Health, Education and Welfare, Washington, 1960.

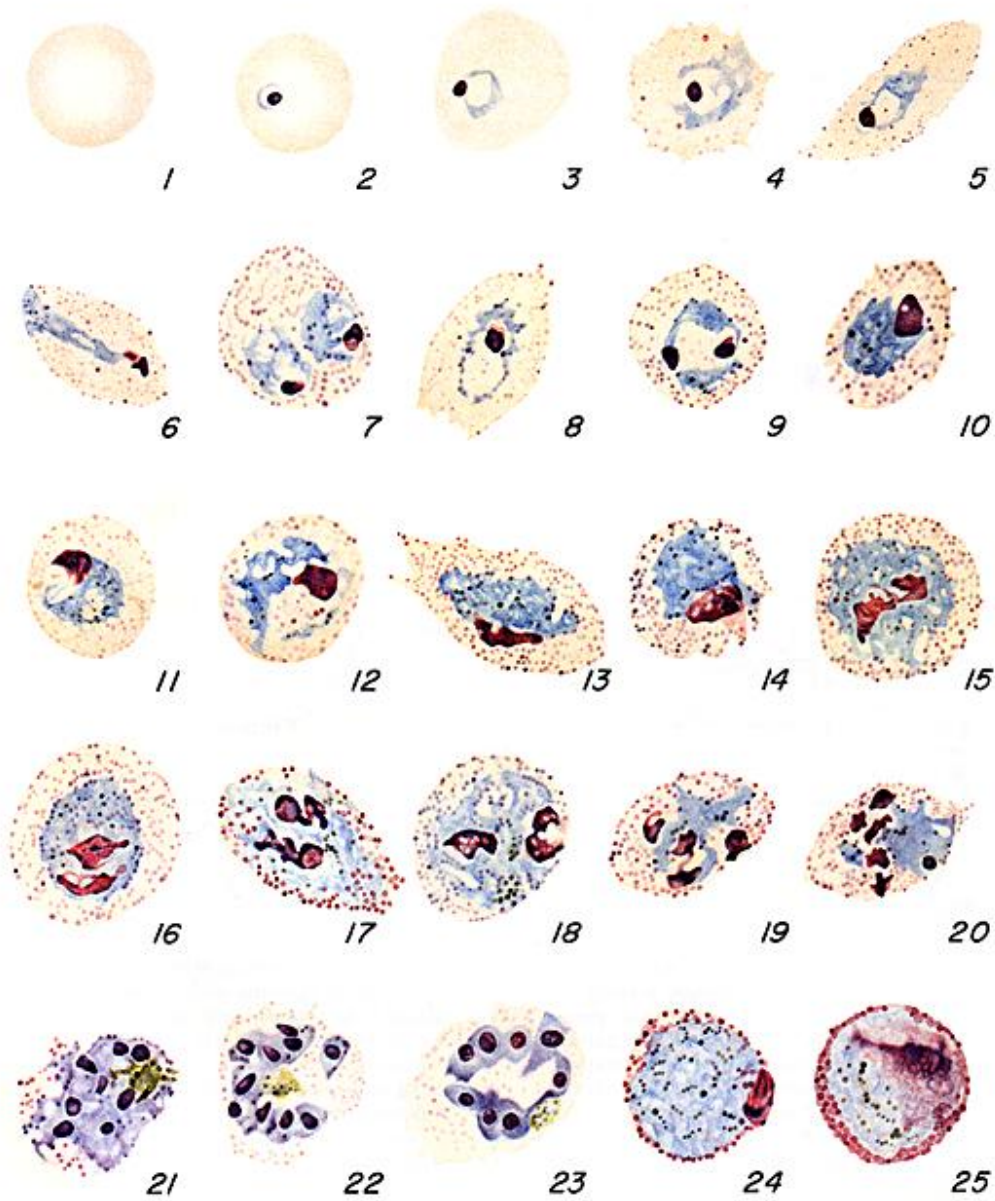


Fig. 1: Normal red cell
Figs. 2-5: Young trophozoites (Rings)
Figs. 6-15: Trophozoites
Figs. 16-23: Schizonts
Fig. 24: Macrogametocytes (female)
Fig. 25: Microgametocyte (male)

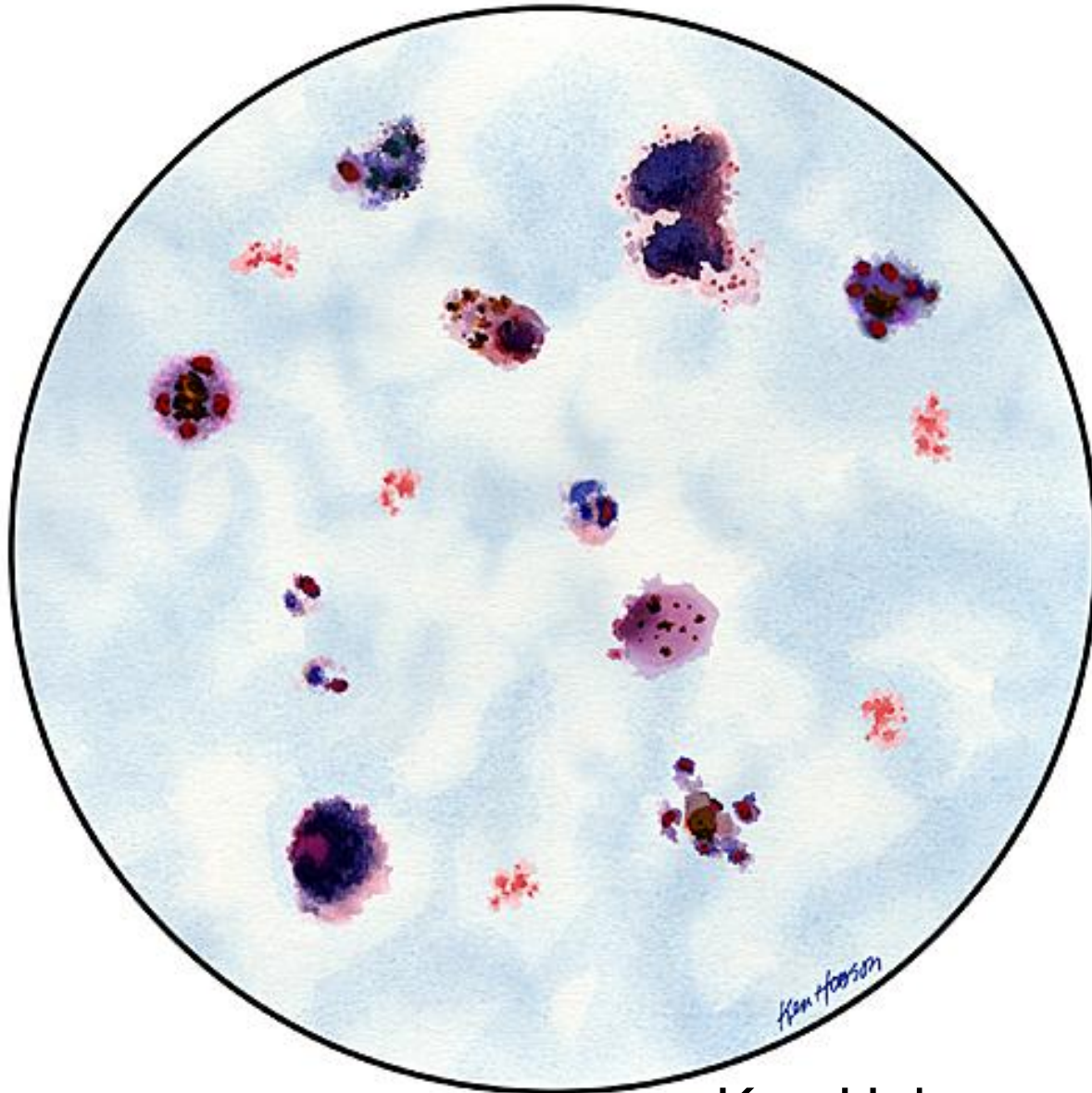
Illustration from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malarias. U.S. Department of Health, Education and Welfare, Bethesda, 1971.



PLASMODIUM OVALE

B. H. Nicholson

P. ovale Thick film



Ken Hobson

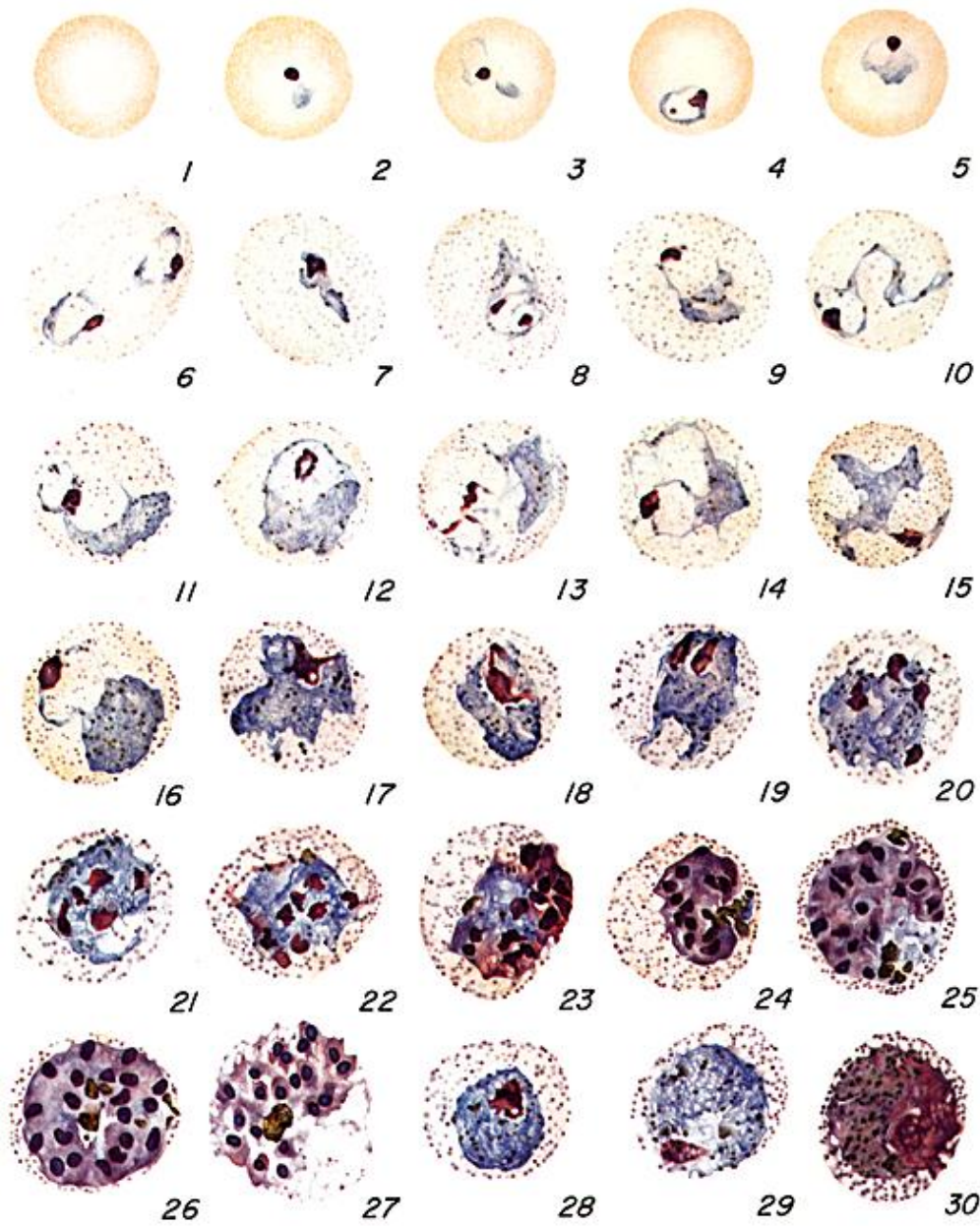


Fig. 1: Normal red cell
Figs. 2-6: Young trophozoites (ring stage parasites)
Figs. 7-18: Trophozoites
Figs. 19-27: Schizonts
Figs. 28 and 29: Macrogametocytes (female)
Fig. 30: Microgametocyte (male)

Illustration from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malaria. U.S. Department of Health, Education and Welfare, Bethesda, 1971.



PLASMODIUM VIVAX

H. H. Nicholson

P. vivax Thick Film

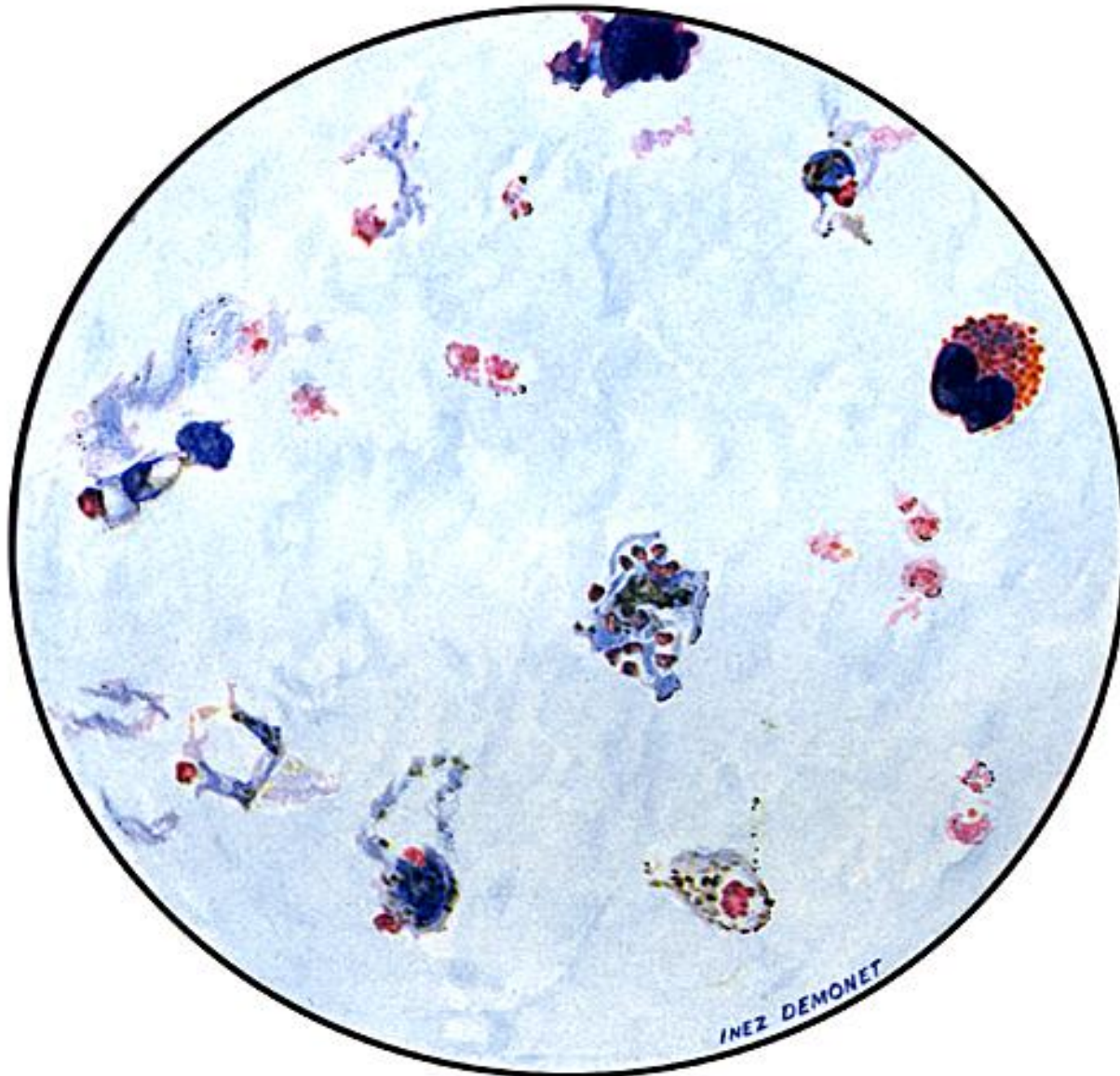
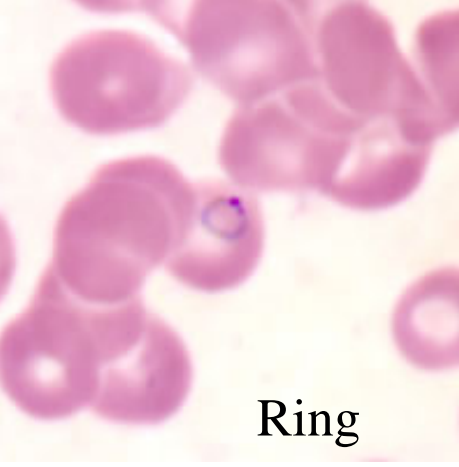


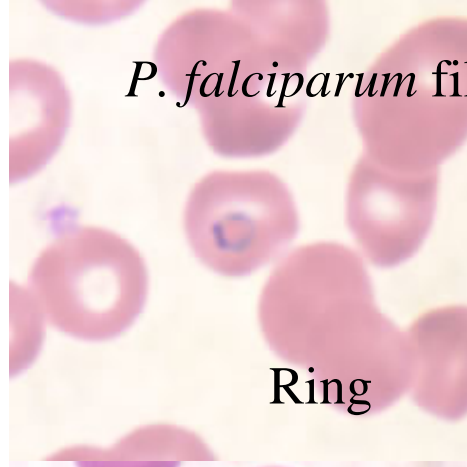
Illustration from: Wilcox A. Manual for the Microscopical Diagnosis of Malaria in Man. U.S. Department of Health, Education and Welfare, Washington, 1960.

Distinguishing Blood Film Characteristics

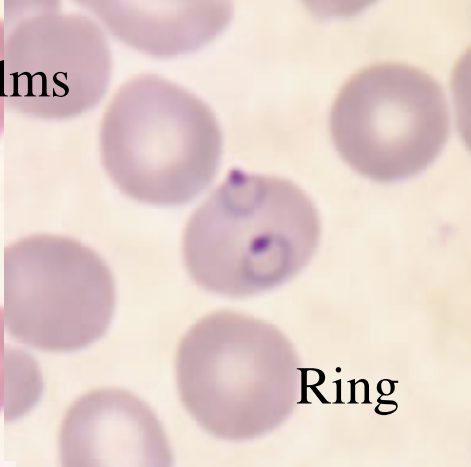
| Feature | <i>P. falciparum</i> | <i>P. vivax</i> | <i>P. ovale</i> | <i>P. malariae</i> |
|------------------------|---|-----------------------|--------------------------------|-------------------------------|
| Red cell size | Normal | Large | Large | Normal |
| Merozoites in schizont | Up to 32 | Up to 16 | Up to 8 | Up to 8 |
| Rings | Fine, delicate double chromatin dots and applique forms | Large, irregular | Large, irregular | Square or band appearance |
| RBC cytoplasm | Maurer's dots | Schuffner's dots | Schuffner's dots | |
| Gametocytes | Sickle or banana shape | Round | Round | Round |
| Special | Trophozoite and schizonts rare | Amoeboid trophozoites | Comet/oval rbc. Only in Africa | Band form and daisy schizonts |



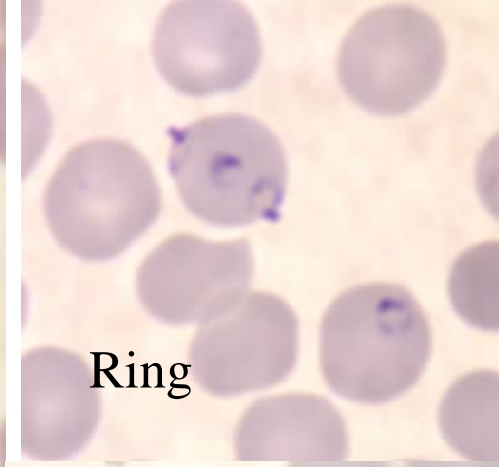
Ring



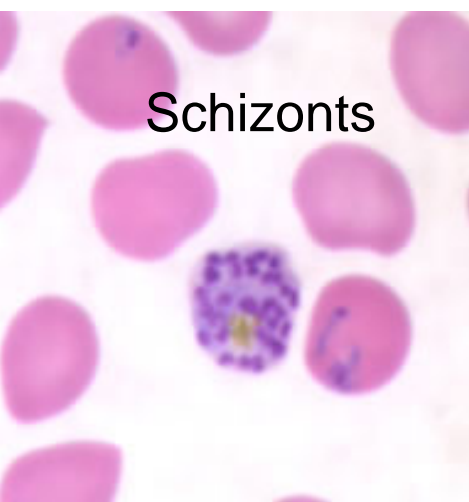
Ring



Ring



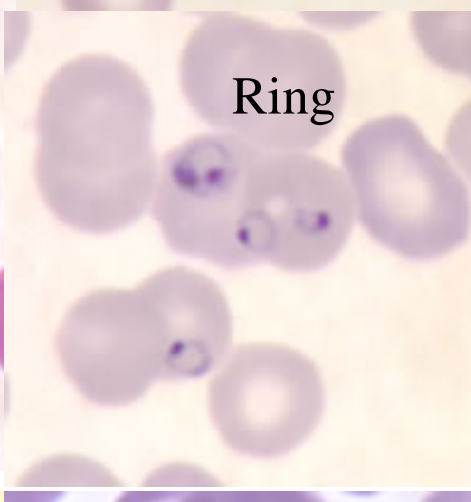
Ring



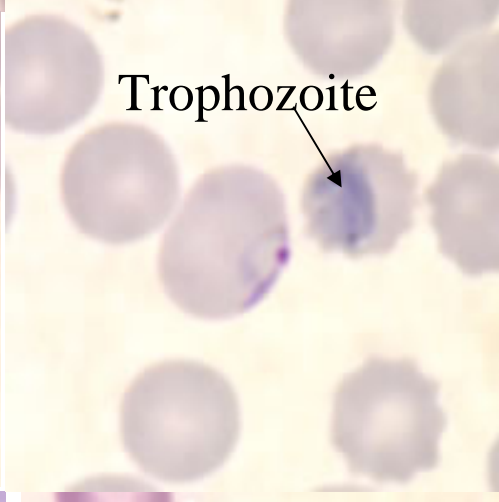
Schizonts



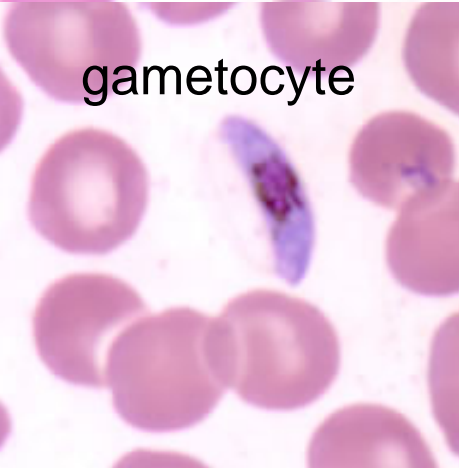
Schizonts



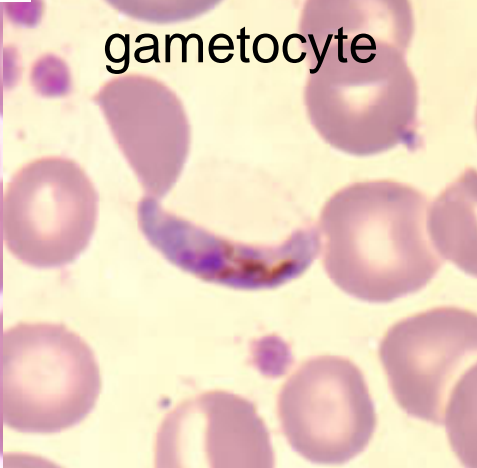
Ring



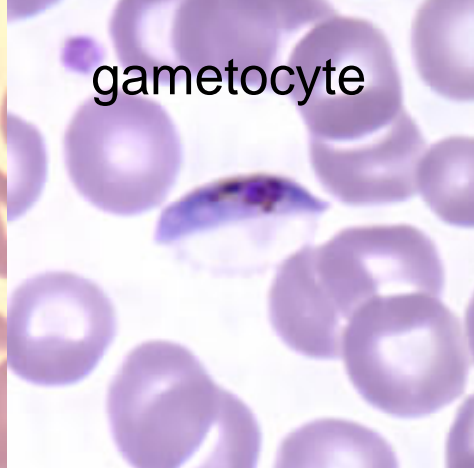
Trophozoite



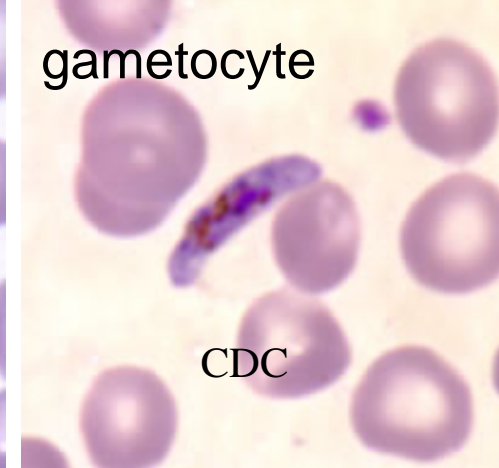
gametocyte



gametocyte



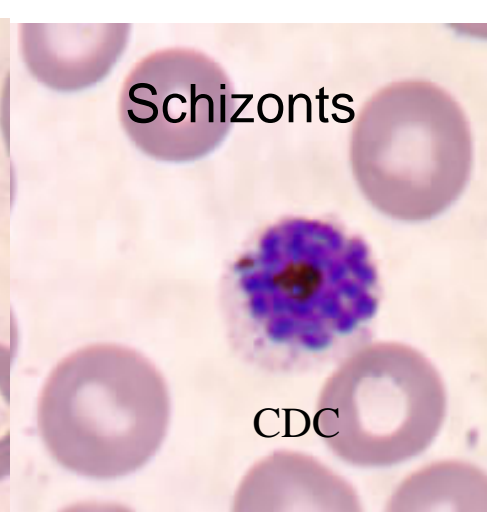
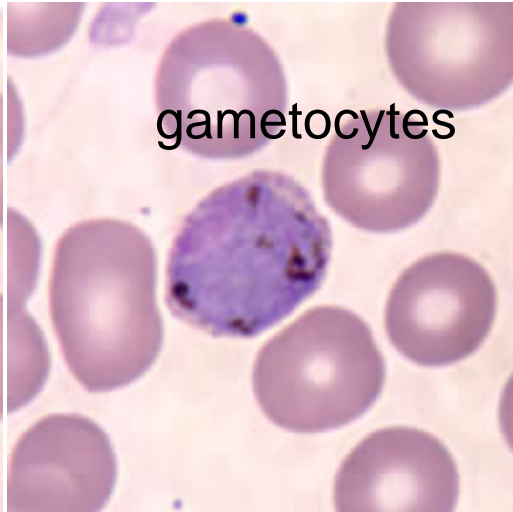
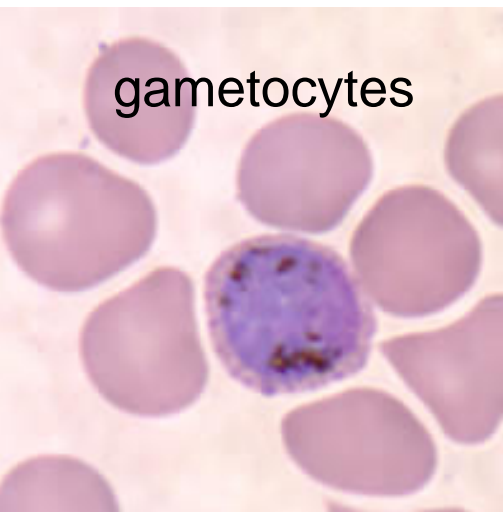
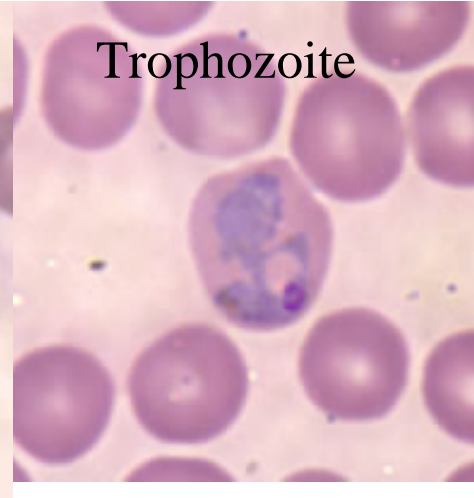
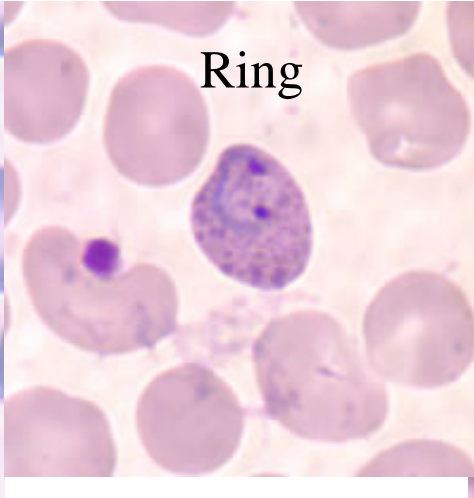
gametocyte



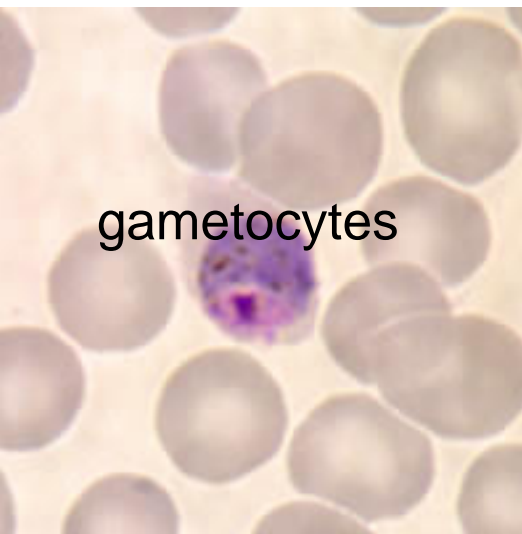
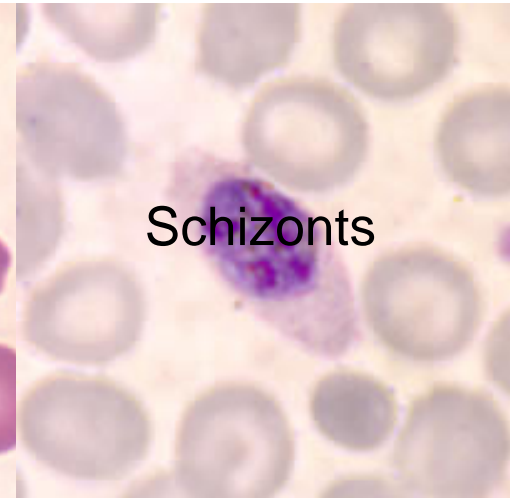
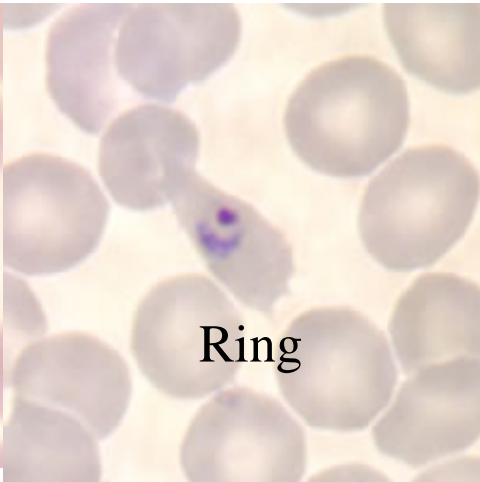
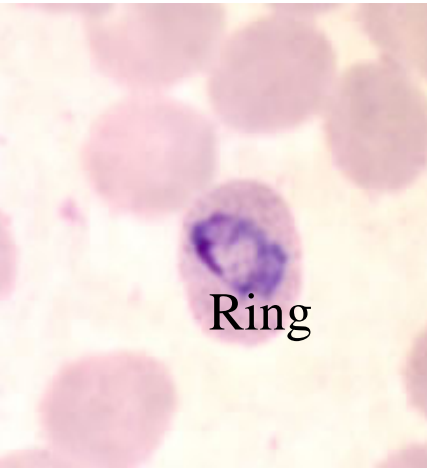
gametocyte

CDC

P. vivax Films

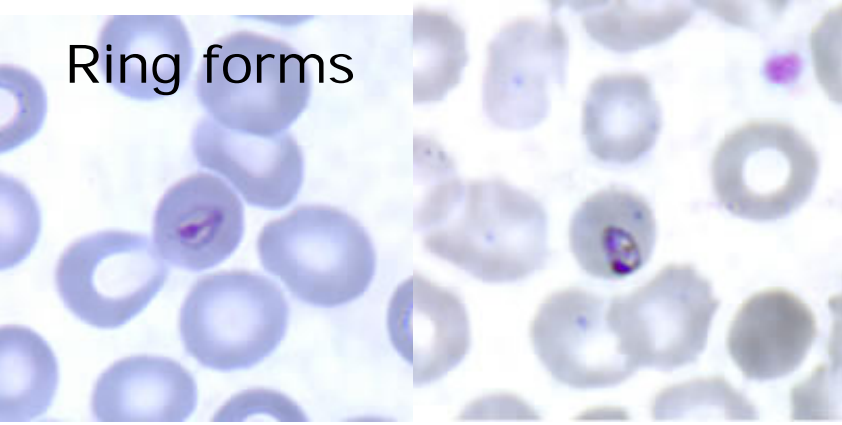


P. ovale Films



P. malariae Films

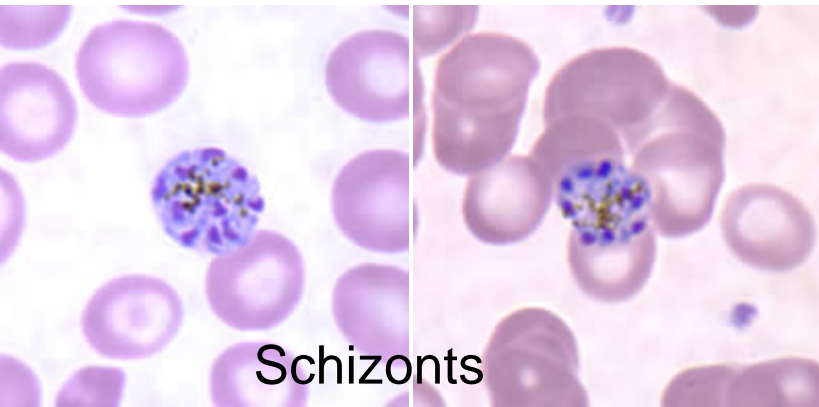
Ring forms



Mature trophozoites
(band forms)



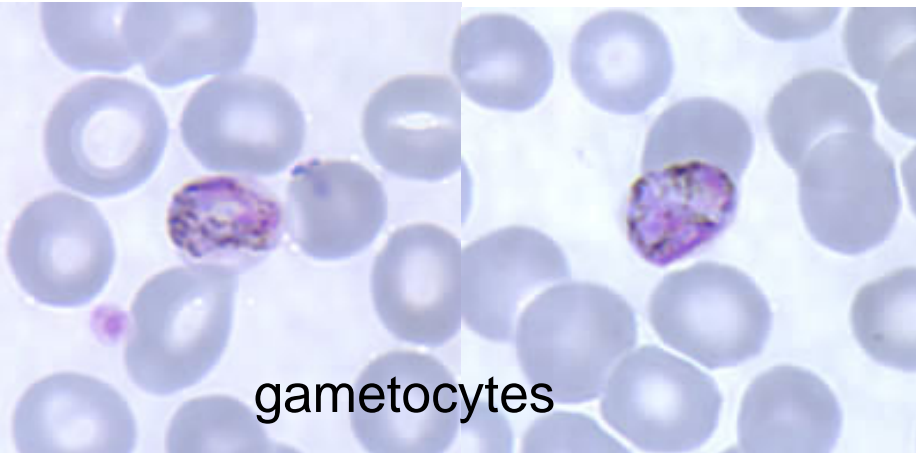
Schizonts



Basket trophozoite



gametocytes



Rapid Diagnostic Tests

Advantages

- Sensitive
- Fast
- Simple to perform
- No need for special equipment or electricity

Disadvantages

- HRPII:
 - Not suitable for non Pf species
 - Remains positive for 2 weeks after treatment
- Not quantitative
- Expensive (US\$0.60-2.50 per test)

Why Target HRP II for Detection?

- Multiple His-Ala repeating regions for antibody epitopes
- Present in infected RBC cytoplasm and parasite digestive vacuole
- Secreted in plasma
- Directly related to parasitemia, parasite biomass, and parasite developmental stage

PfHRP II

MVSFSKNKVL SAAVFASV
 LLLDNNNSAFNNNLCSKNA
 KGLNLNKRL LHETQAHVDD
 AHHAHHVADAHHAHHAAD
 AHHAHHAADAHHAHHAAD
 AHHAHHAADAHHAHHAAY
 AHHAHHAADAHHAHHASD
AHHAADAHHAAY
 AHHAHHAADAHHAHHASD
 AHHAADAHHAAY
 AHHAHHAAD
 AHHAADAHHATD
 AHHAHHAAD
 ARHATDAHHAADAHHATD
 AHHAADAHHAADAHHATD
 AHHAADAHHATDAHHAAD
 AHHAADAHHATD
 AHHAHHAADAHHAAAHHATD
 AHHATDAHHAAAHHEAATHCLRH

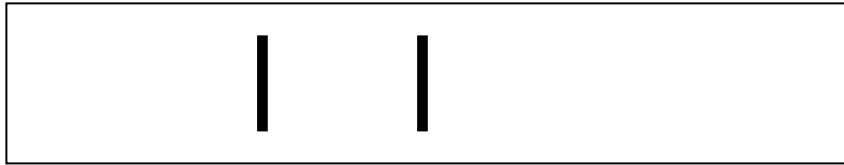
Secretary
 leader

PfHRP III

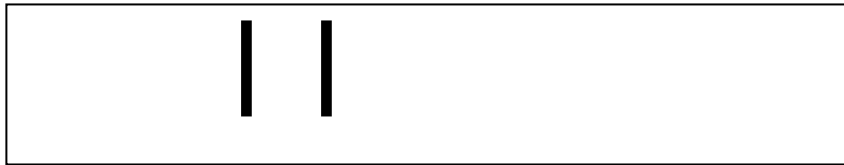
MVSFSKNKILSAAVFASVLLLDN
 NNSEFNNNLF SKNAKGLNSNKRL
 LHESQAHAGD
 AHHAHHVADAHHAHHAAN
 AHHAANAHHAANAHHAANAHHAA
 NAHHAANAHHAANAHHAANAHHA
 ANAHHAANAHHAANAHHAANAHH
 AANAHHAANAHHAANAHHAADAN
 HGFHFNLDNNSHTLHHAKANAC
 FDDSHHDD
 AHHDGAHHDDAHHDGAHHDDAHH
 DGAHHDDAHHDGAHHDDAHH
 DGAHHDGAHHDGAHHNATTHHLH
 H

- Aldolase and lactate dehydrogenase enzymes
- Abundant production by parasites
- Aldolase has over 90% identity at amino acid level
- LDH has less and enables species specific monoclonal antibodies
- LDH is basis for Optimal test
- Aldolase is in ICT test as non HRP II band
- Both aldolase and LDH have short half life and go away within 1-2 days of treatment
- HRP II can linger for more than a week

Result



Other than *P. falciparum*



P. falciparum



P. falciparum/mixed



negative

Control

aldolase

HRP II

RDTs in Africa?

Current situation

Problems

- Asymptomatic parasitaemia
- Expense

Special situations

- Complex emergencies
- Malaria epidemics
- Low transmission settings
- Military
- Travellers

RDTs in Africa

Future options

- Changing cost-benefit
 - Rising drug costs
- Possible uses
 - Confirmation of treatment failure (pLDH)
 - Severe disease in peripheral settings
- BUT...
 - Will RDT diagnosis change clinical practice?
- Need for operational studies

Malaria Rapid Diagnostic Tests

WHO site: <http://www.wpro.who.int/sites/rdt>

Contains

- Explanation of RDT
- Use of RDT
- Guidelines on purchasing an RDT including an important table that compares good manufacturing practice on known suppliers
- Collections of published reviews and trials
- Collection of publications and committee documents
- Useful links pertaining to malaria diagnosis

<http://www.wpro.who.int/sites/rdt/links.htm>

Clinical Complications of Malaria

P. Falciparum

- Cerebral coma
- Anemia
- Pulmonary edema
- Shock
- Lactic acidosis
- Hypoglycemia
- Tropical splenomegaly
- Pregnancy
 - Maternal Death
 - Stillbirth
 - Low birth weight
 - Anemia

P. vivax (P. ovale)

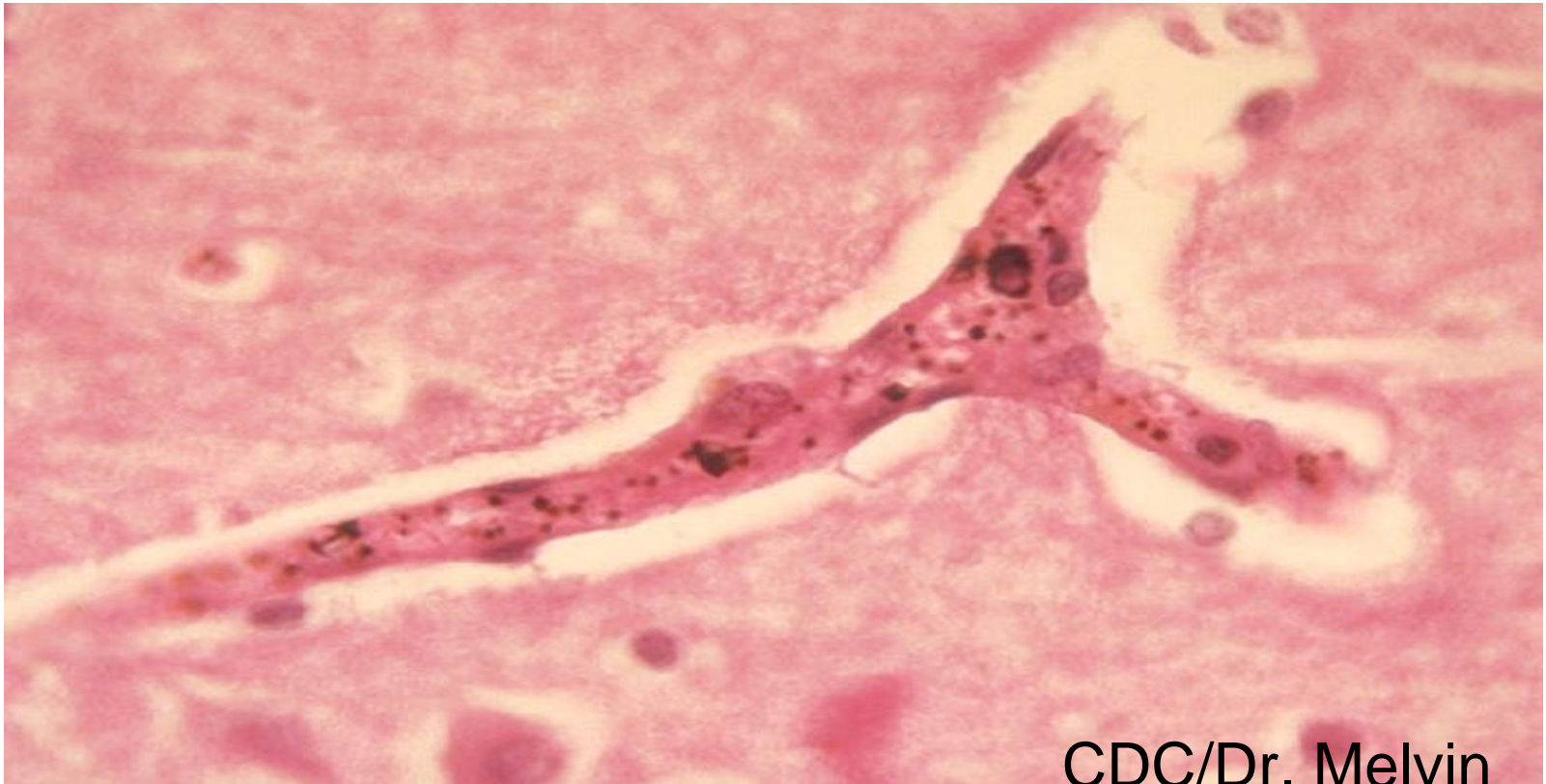
- Splenic rupture
- Anemia (mild)
- Debilitating fevers
- Higher TNF-alpha per parasite

P.malariae

- Immune complex
- Glomerulonephritis leading to nephrotic syndrome

Cerebral Malaria

Adherent parasites release cytokines. In one study 94% of persons with cerebral malaria had adherent parasites compared with 13% of those without change in mental status. Steroids have no effect on mortality, no increase in vascular permeability is observed, anaerobic glycolysis in brain tissue predominates.



CDC/Dr. Melvin

Cerebral Malaria: Signs and Symptoms

- About 90% become comatose before dying
- Gradual impairment or coma following seizure
- Extensor posturing
- Immobile or tossing about

Neurologic Sequelae

- Uncommon in adults or non immunes
- Common in African children
 - Psychosis
 - Extraparamidal tremor
 - Cranial nerve lesions
 - Polyneuropathy
 - Mononeiritis multiplex
 - Guillain-Barre syndrome
 - Focal epilepsy

Modified Glasgow Scale

Best Verbal Response

- Oriented: 5
- Confused: 4
- Inappropriate Words: 3
- Incomprehensible sounds: 2
- None: 1

Best Motor Response

- Obeys commands: 6
- Localizes pain: 5
- Flexion to pain:
 - Withdrawal: 4
 - Abnormal: 3
- Extension to pain: 2
- None: 1

Blantyre Scale

Eye Movements

- Directed: 1
- Not Directed: 0

Verbal Response

- Appropriate cry: 2
- Moan or inappropriate cry: 1
- None

Best Motor Response

- Localizes painful stimulus: 2
- Withdraws limb from pain: 1
- Non-specific or absent response: 0

Total = 0-5

Unrousable coma <2

Severe Anemia

- Not only red blood cell destruction, but also decreased production
- Due to iron deficiency and ineffective erythropoiesis, Rouleaux formation of uninfected erythrocytes increases spleen destruction
- Peak incidence in African children from holoendemic areas between ages of 6 months and 2 years
- Associations with secondary bacterial infections
- Transfusion is life saving

Pathogenesis of Severe Anemia

- Degree of anemia corresponds to duration and severity of parasitemia.
- Parasitemia does not predict risk of death in severe anemia and in Kenya over half of children with severe anemia had less than 10,000 parasites per ul.
- Treated uncomplicated *P. falciparum* malaria will decrease the hematocrit by one seventh.
- Severe anemia kills as hemoglobin falls below 5g/dl
- Mortality rises with tissue hypoxia and metabolic acidosis.
- Another infection can tip to catabolic metabolism.

Placental Malaria

Unstable epidemiology

Maternal death, abortion,
stillbirth, premature delivery,
low birthweight

Stable (Holoendemic)
epidemiology

Clinical symptoms and
parasitemia is higher in
primigravida

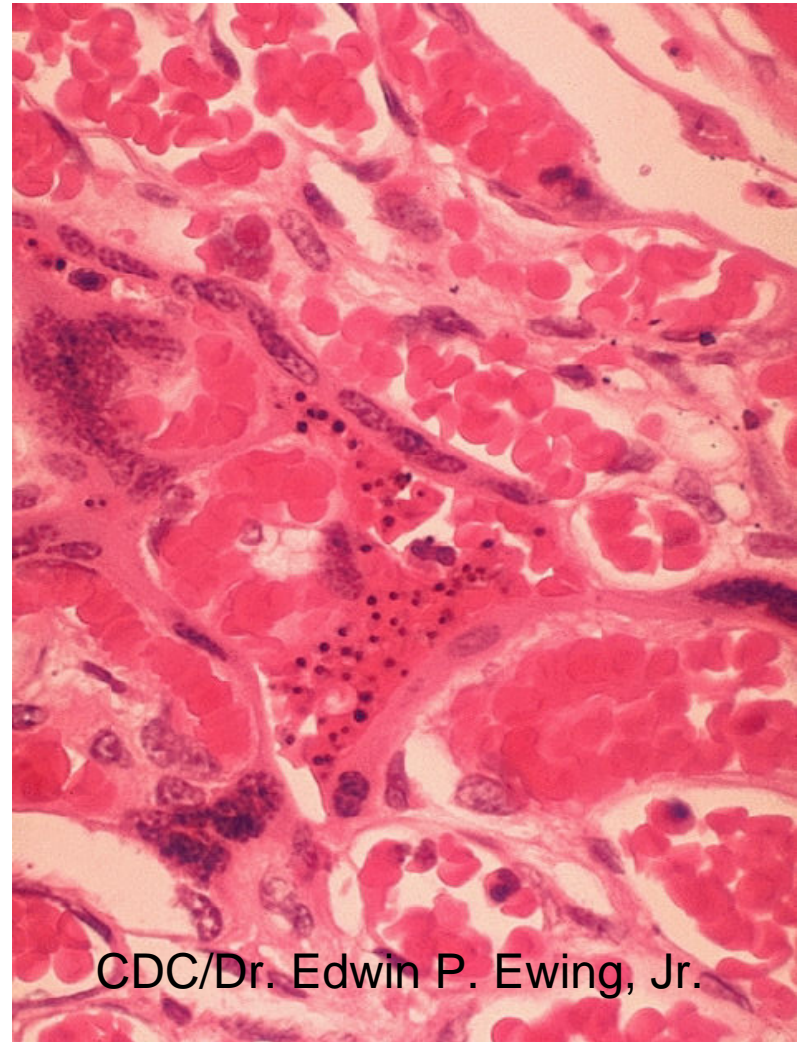
Low birthweight

Non-immunes

Higher mortality

Progressive anemia

Quinine induced
hypoglycemia



CDC/Dr. Edwin P. Ewing, Jr.

Primigravida women expose chondroitin sulfate A on placenta endothelial cells to which a new population of *P. falciparum* parasites predominates and causes microvascular sequestration in the placenta, disrupting its function.

Vertical transmission

Congenital

Parasitemic neonate within 7 days of birth

Blood transfusion

P. malariae

Pulmonary edema

May develop at any stage of disease

Iatrogenic (presents as patient recovering)

Increased RR, dyspnea, crepitations are first clinical signs

ARDS with normal right heart pressures

CXR

Bronchopneumonia

Metabolic acidosis

ARDS

Tropical Splenomegaly Syndrome

- Also known as Hyperreactive malarial splenomegaly
- Progressive, massive, splenic enlargement
- 80% of some areas of PNG
- Past medical history of repeated attacks of fever or malaria

Tropical Splenomegaly Syndrome

- Abdominal distention, vague dragging sensation, sharp abdominal pains
- Peritonism suggesting perisplenitis
- Cachexia
- Lower leg ulcerations
- NC/NC Anemia with hemolytic episodes
- Very low or undetectable parasitemia

Tropical Splenomegaly Syndrome

- Untreated mortality rate is high
- Death due to overwhelming pulmonary or skin infections
- Definition:
 - Gross splenomegaly
 - Elevated IgM (polyclonal)
 - Clinical and immunological response to anti-malarial prophylaxis

Why Is *P. falciparum* So Dangerous?

- Ability to infect all age of RBCs
- Higher multiplication capacity
- Sequestration (cytoadherence and rosetting)
- Capillary leak syndromes
- End organ failure

The Numbers

- 70 kg person has @ 5 liters of blood = 5×10^3 ml = 5×10^6 μ L times 5×10^6 RBCs per μ L of blood = 2.5×10^{13} RBCs
- 1% parasitemia = 1 in 100 iRBCs = 2.5×10^{11} parasites = 250 billion parasites
- *P. vivax* invades predominately reticulocytes and so has a built-in ceiling, but *P. falciparum* can invade all ages of RBCs.
- Pyrogenic density *P. falciparum* 10,000/uL nonimmune; 100,000/uL immune; *P. vivax* 100/uL