Laboratory Identification of Blood Parasites

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Disclosures in the Past Year

- Disclosures Related to this Talk:
  - » NACMID (travel assistance)
- Other Professional Disclosures:
  - » American Society for Microbiology (Editorial Board, *Journal of Clinical Microbiology;* Editorial Board, *ASM Case Reports*; honoraria and travel support)
  - » Techcyte Inc. (collaborator/honoraria)
  - » College of American Pathologists (Microbiology Committee Member, 2024-present)
  - » ASCP – Virginia Chapter (honorarium)
  - » Illinois Society for Microbiology (sponsored travel; honorarium)
  - » University of Minnesota (sponsored travel)
  - » Association of Public Health Labs (sponsored travel)
  - » Mayo Clinic (sponsored travel)
  - » Southwestern Association for Clinical Microbiology (sponsored travel)
  - » Elsevier (publication honorarium)

NOTE: I will be mentioning a couple commercial products in this talk; this does not constitute a recommendation nor denouncement of the products.

Learning Objectives:

1. Describe common dogmas associated with blood parasite identification and how to avoid them
2. List the morphologic features separating *Plasmodium* and *Babesia*
3. List the non-microscopic diagnostic methods for Chagas disease and when each is appropriate
4. Describe key features used to identify microfilariae in blood

Malaria: Overview

Malaria: The Disease

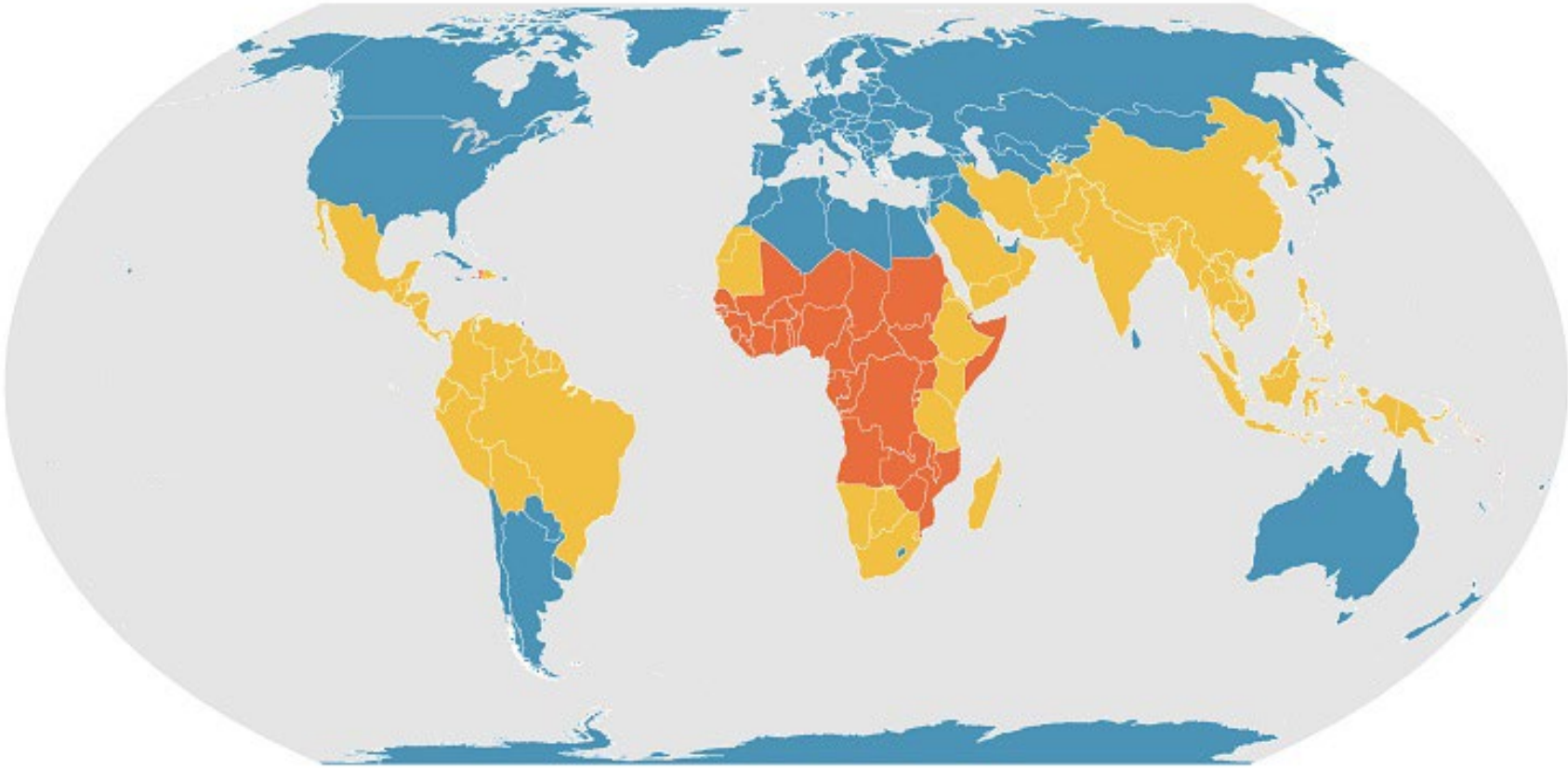
- Caused by apicomplexan parasites in the genus *Plasmodium* (upcoming slide).
- Transmitted by mosquitoes in the genus *Anopheles*.
- Half of the world's population lives in endemic areas.
- WHO reported 263 million cases in 2023, with ~94% being in the WHO African Region.
  - » Top 5 countries: Nigeria, DRC, Uganda, Ethiopia, Mozambique
- Roughly 2,000 cases diagnosed annually in the US in travelers and immigrants.
- Concerns related to diagnosis and identification
  - » Species-level ID can be important for patient management
- Immunologically-naïve patients may be symptomatic at a lower parasitemia.

Plasmodium species implicated in human disease

- 'Human' *Plasmodium* species
  - » *Plasmodium falciparum*
  - » *Plasmodium malariae*
  - » *Plasmodium vivax*
  - » *Plasmodium ovale*

- 'Zoonotic/Simian' *Plasmodium* species
  - » *Plasmodium coatneyi* (Malaysia)
  - » *Plasmodium cynomolgi* (SE Asia)
  - » *Plasmodium fieldi* (SE Asia)
  - » *Plasmodium inui* (SE Asia)
  - » *Plasmodium knowlesi* (SE Asia)
  - » *Plasmodium schwetzi* (West Africa)
  - » *Plasmodium simiovale* (Sri Lanka, Malaysia)
  - » *Plasmodium brasilianum* (South America) *
  - » *Plasmodium simium* (Brazil)*

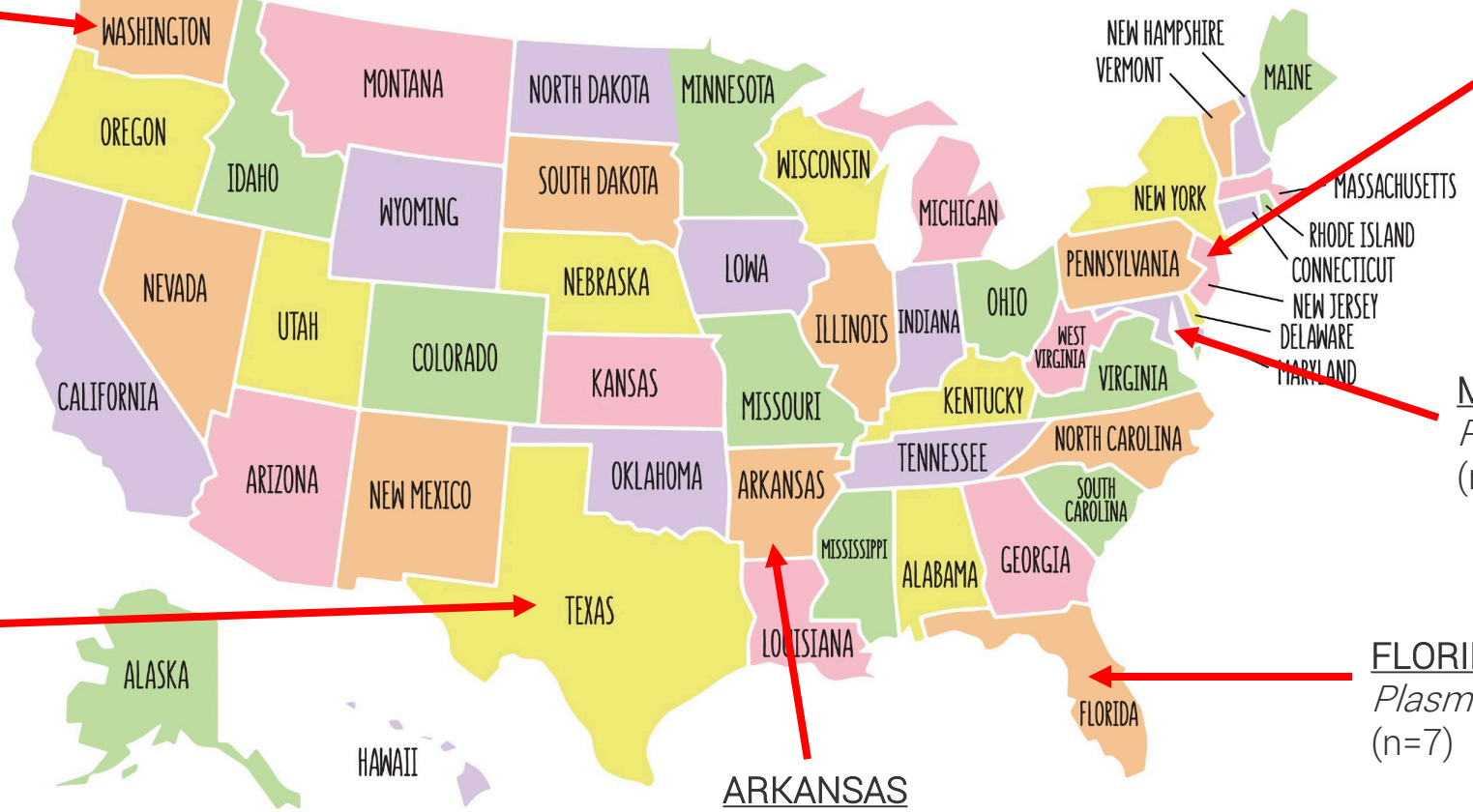
Malaria – Global Risk (CDC 2023)



Autochthonous Malaria, USA 2023 -present

WASHINGTON
Plasmodium sp., 2025
(n=1)

NEW JERSEY
Plasmodium sp., 2025
(n=1)



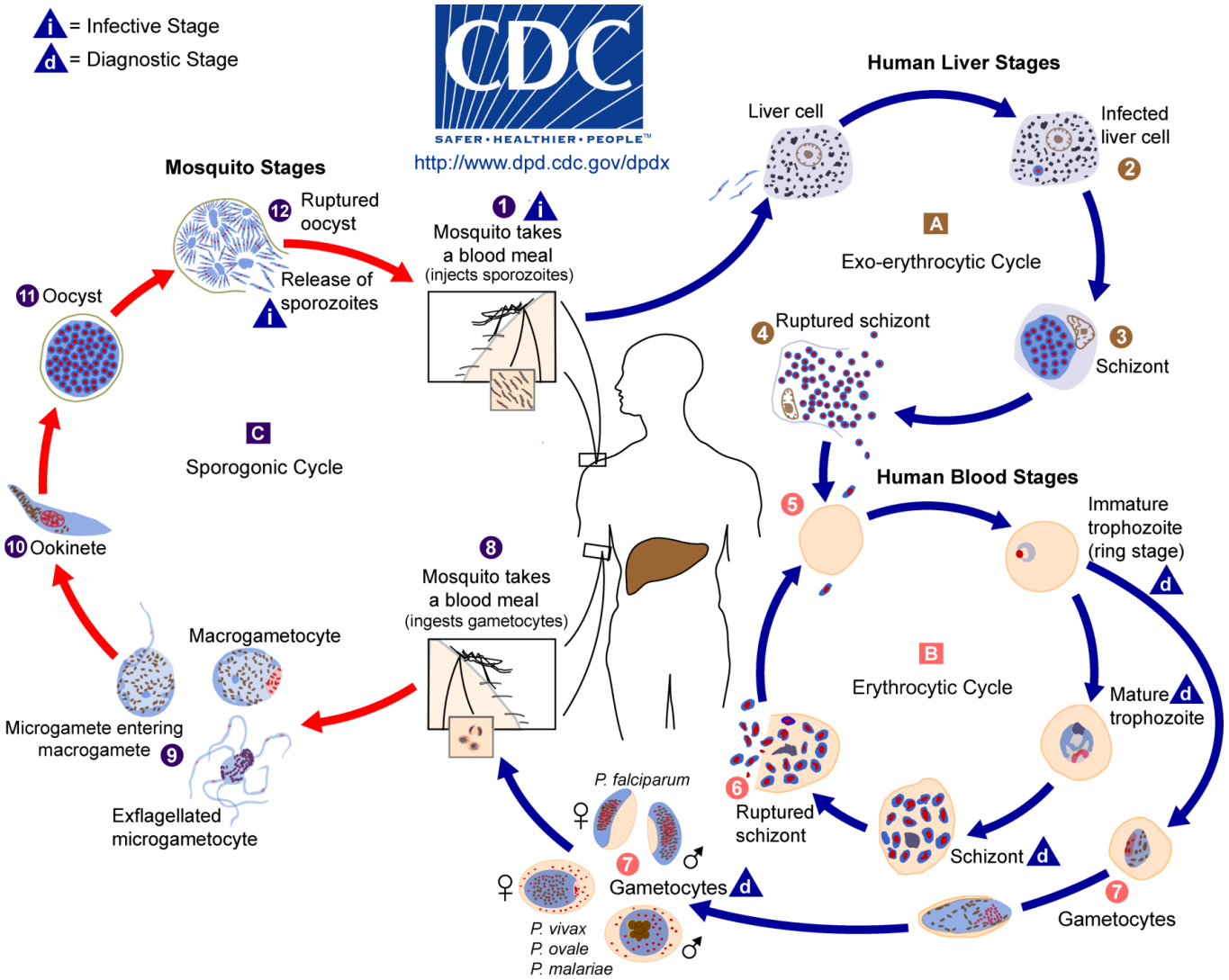
MARYLAND
Plasmodium falciparum, 2023
(n=1)

TEXAS
Plasmodium vivax, 2023
(n=1)

FLORIDA
Plasmodium vivax, 2023
(n=7)

ARKANSAS
Plasmodium vivax, 2023
(n=1)

Life Cycle of *Plasmodium* species



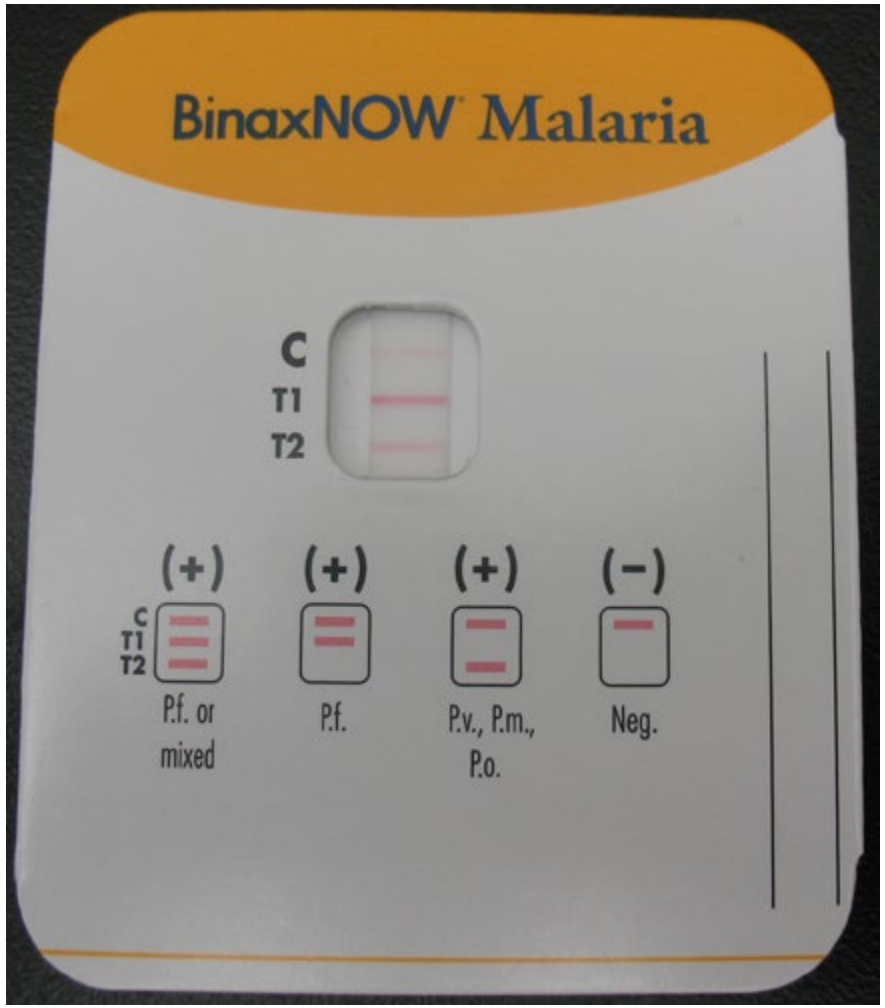
Malaria: Diagnostics

How is malaria diagnosed?

- Smear microscopy
  - » Still the 'Gold Standard'
- Molecular
  - » Becoming more popular, but still not as cost effective for screening and often performed only in large reference and public health labs (e.g., CDC)
- Antigen Detection
  - » Lots of options available worldwide; currently only BinaxNOW FDA-approved for use in the United States
- Antibody Detection
  - » Not generally recommended for clinical diagnosis; may be used for febrile patients returning from endemic areas who are repeatedly smear negative (esp. if immunologically naïve) and in cases of suspected tropical splenomegaly syndrome

Caveats with the BinaxNOW Malaria Test

- Less sensitive than microscopy
- Results, positive or negative, should be backed by microscopy
- Sensitivity and specificity does go down with non-*falciparum* species
- Emerging HRP-2-negative *P. falciparum* could affect interpretation
- Possible hook effect with too much antigen [false negative]



NOTE: Mentioning of this product does not constitute an endorsement nor denouncement of the product

BioFire® Global Fever Panel

- Developed for FilmArray® and FilmArray® Torch Systems
- Detects the following pathogens:
  - » Bacteria: *Leptospira* spp.
  - » Viruses: Chikungunya virus; Dengue virus (serotypes 1, 2, 3, 4)
  - » Protozoans: *Plasmodium* sp. (next)
- For malaria, separates as follows:
  - » *Plasmodium* species
  - » *Plasmodium falciparum*
  - » *Plasmodium vivax/ovale*
- Probably best for broad screening of non-specific fevers after travel to endemic areas rather than *Plasmodium*-specific testing

NOTE: Mentioning of this product does not constitute an endorsement nor denouncement of the product

BioFire® Global Fever Panel

Table 20. Observed Cross-Reactivity of BioFire Global Fever Panel Assays

Cross-Reactive Organism/Virus	Global Fever Panel Test Result
On-Panel	
Plasmodium knowlesi [1]	*Plasmodium vivax/ovale*
Plasmodium malariae [2]	
Off-Panel	
O'nyong-nyong virus	Chikungunya virus
Plasmodium berghei [3]	*Plasmodium* spp. and *Plasmodium vivax/ovale*
Plasmodium brasilianum [3]	
Plasmodium cynomolgi [3]	
Plasmodium fieldi [3]	
Plasmodium fragile [3]	
Plasmodium inui [3]	
Plasmodium simiovale [3]	

[1] Cross-reactive with the *Plasmodium vivax/ovale assay* at reduced sensitivity (~1,000×LoD).
[2] In silico analysis predicts potential cross-reactivity with the *Plasmodium vivax* assay at concentrations greater than 10^5 copies/mL.
[3] *Plasmodium* spp. that typically infect non-human primates and rodents, but are rarely found in humans.

NOTE: Mentioning of this product does not constitute an endorsement nor denouncement of the product

Assay Detection Outcomes			Result Banner	Result Summary*
Plasmodium spp.	*Plasmodium falciparum*	*Plasmodium vivax/ovale*		
Negative	Negative	Negative	N/A - No *Plasmodium* species detected in the sample (no results shown in Report).	*Plasmodium* spp. Not Detected *Plasmodium falciparum* Not Detected *Plasmodium vivax/ovale* Not Detected
Positive	Negative	Negative	***Plasmodium* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	***Plasmodium* spp. Detected** *Plasmodium falciparum* Not Detected *Plasmodium vivax/ovale* Not Detected
Positive	Positive	Negative	***Plasmodium falciparum* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	***Plasmodium* spp. Detected** ***Plasmodium falciparum* Detected** *Plasmodium vivax/ovale* Not Detected
Positive	Negative	Positive	***Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	***Plasmodium* spp. Detected** *Plasmodium falciparum* Not Detected ***Plasmodium vivax/ovale* Detected**
Positive	Positive	Positive	***Plasmodium falciparum* and *Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Note: The detection of 2 or more organisms is uncommon. Retest the Sample ONCE then Report the Results.	***Plasmodium* spp. Detected** ***Plasmodium falciparum* Detected** ***Plasmodium vivax/ovale* Detected**
Negative	Positive	Positive	***Plasmodium falciparum* and *Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Note: The detection of 2 or more organisms is uncommon. Retest the Sample ONCE then Report the Results.	*Plasmodium* spp. Not Detected ***Plasmodium falciparum* Detected** ***Plasmodium vivax/ovale* Detected**
Negative	Positive	Negative	***Plasmodium falciparum* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	*Plasmodium* spp. Not Detected ***Plasmodium falciparum* Detected** *Plasmodium vivax/ovale* Not Detected
Negative	Negative	Positive	***Plasmodium vivax/ovale* Detected** Note: See Instructions for Use for additional information on Plasmodium results. Report the Results.	*Plasmodium* spp. Not Detected *Plasmodium falciparum* Not Detected ***Plasmodium vivax/ovale* Detected**

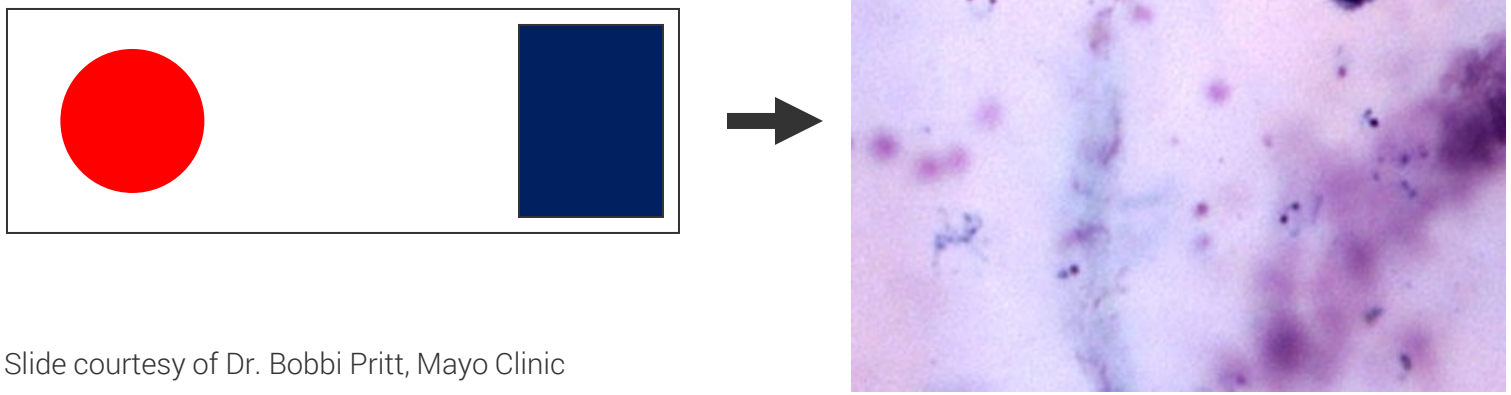
*Bolded results are displayed in the Detected column of the Result Summary. Non-bolded results are shown in the Not Detected column of the Result Summary.

Microscopy

- Thick and thin films
  - » Thick: detection
  - » Thin: identification
- Stains may include Giemsa, Wright, or Wright-Giemsa
  - » Features such as Schüffner's stippling and Maurer's clefts only usually visible with **true** Giemsa at a pH of 7.0

Thick Films

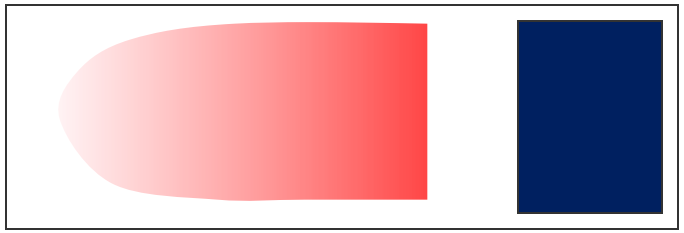
- Made by placing 2-3 drops of blood on a slide and allowed to air dry
  - » Scratching or etching the blood into the slide may improve adherence
- Blood is **NOT** fixed in methanol
  - » Blood may have to be mixed with water if the stain is alcohol-based
- The hypotonic nature of water-based stains lyses RBCs, releasing intracellular parasites.

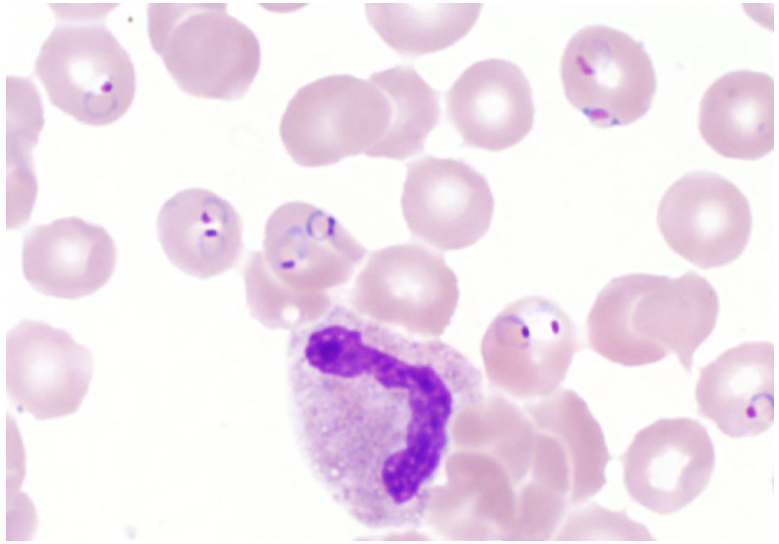


Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

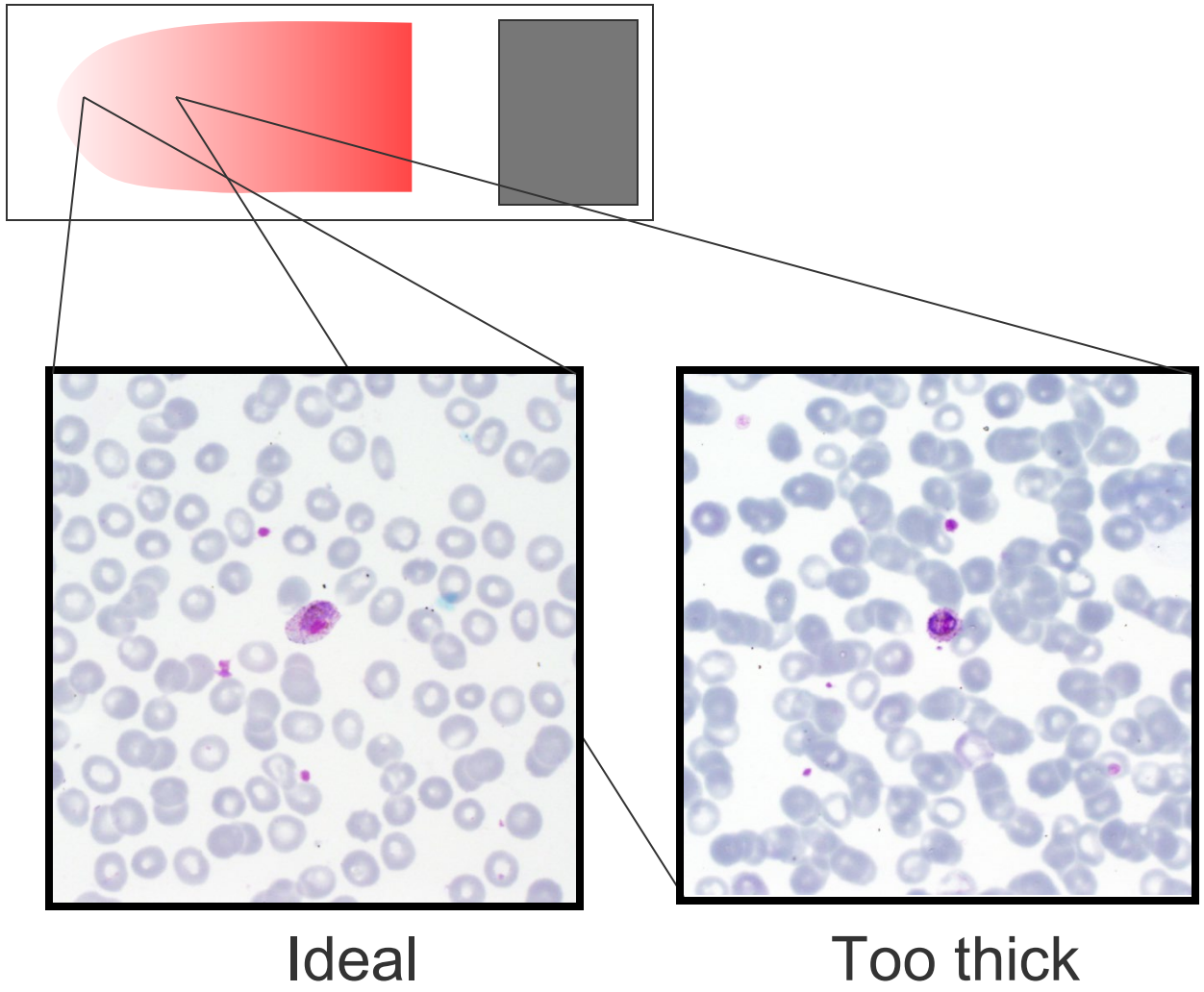
Thin Film

- Made in the same manner as a hematology smear
- FIXED in methanol prior to staining
- Morphology of RBCs remain intact, along with intracellular parasites



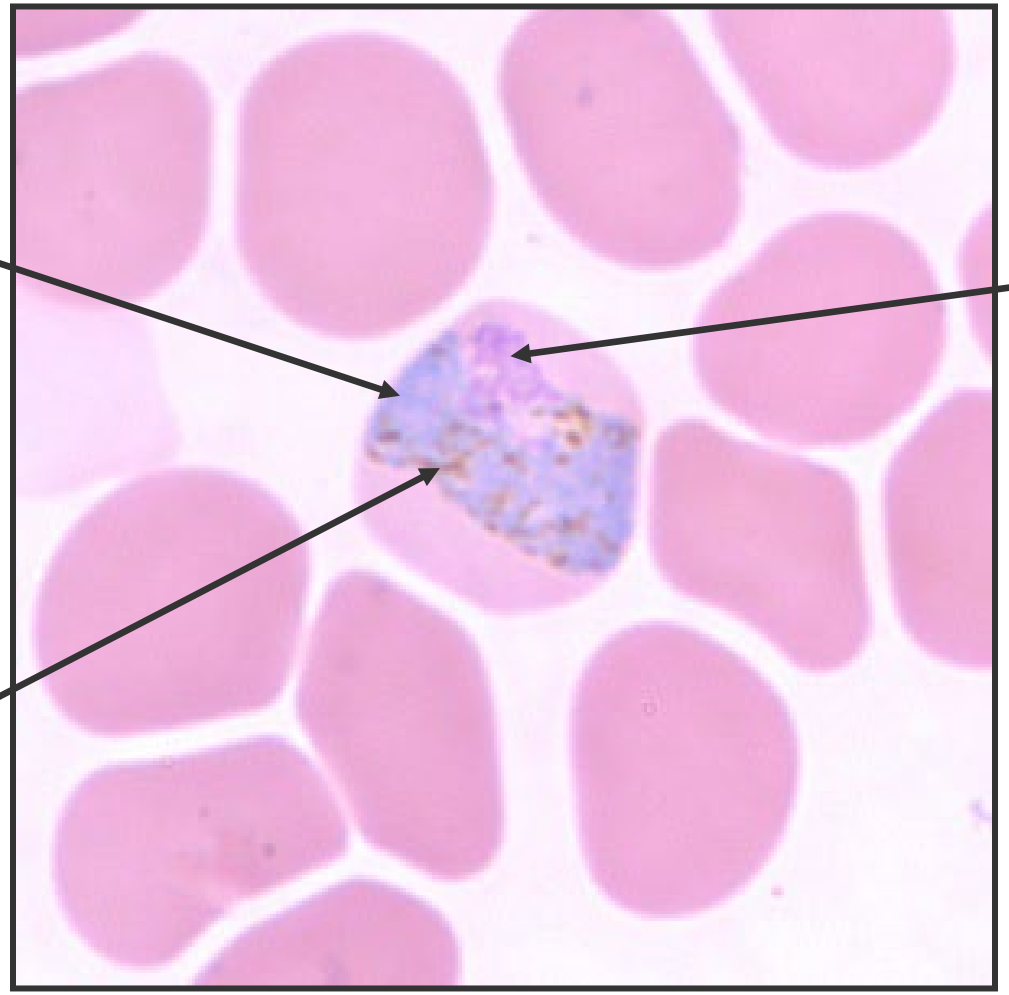


Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic



Slide courtesy of Dr. Bobbi Pritt, Mayo Clinic

Microscopy – what to look for

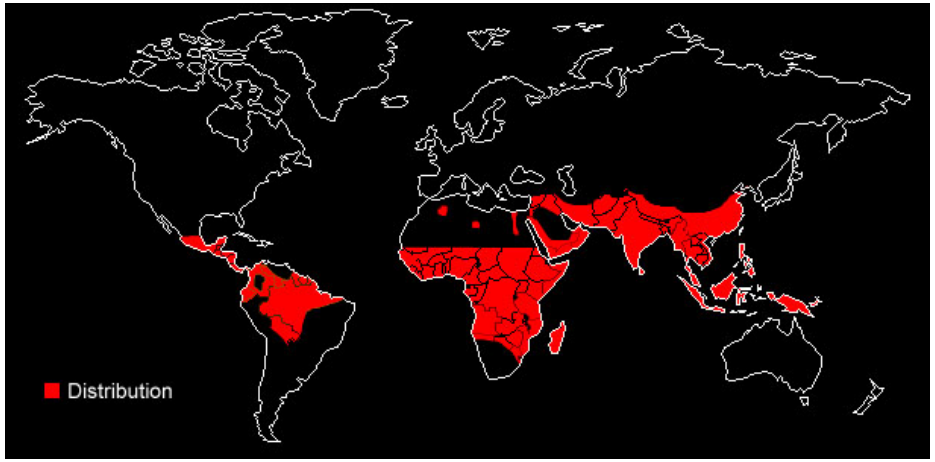


Calculating Parasitemia

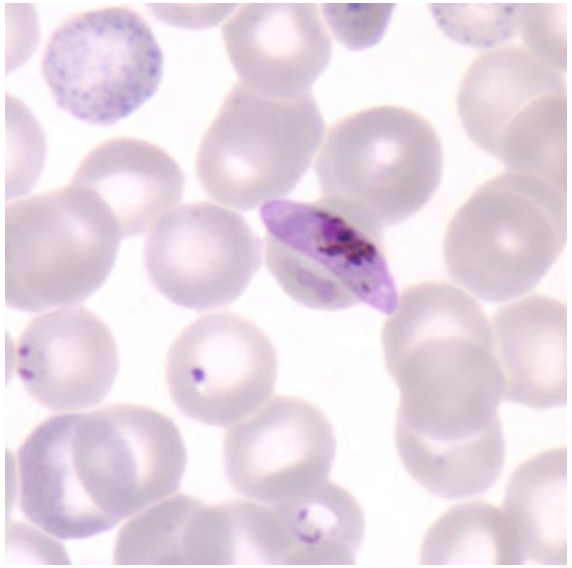
- Thin films (based on RBCs):
  - » count the parasitized RBCs among 1,000-10,000 RBCs on the thin smear and express the results as % parasitemia (% parasitemia = (parasitized RBCs/total RBCs) × 100.
  - » Count multiply-infected RBCs as '1'.
  - » Do not figure gametocytes into calculations
  - » Do not 'select' areas—pick random fields regardless of presence/absence of parasites. Pick areas with no overlapping RBCs.
- Thick films (based on WBCs):
  - » Tally the parasites against WBCs, until you have counted 500 parasites or 1,000 WBCs, whichever comes first. Express the results as parasites per microliter of blood, using the WBC count if known, or otherwise assuming 8,000 WBCs per microliter blood. Parasites/microliter blood=(parasites/WBCs) × WBC count per microliter<or 8,000>

Species of Human *Plasmodium*

Plasmodium falciparum

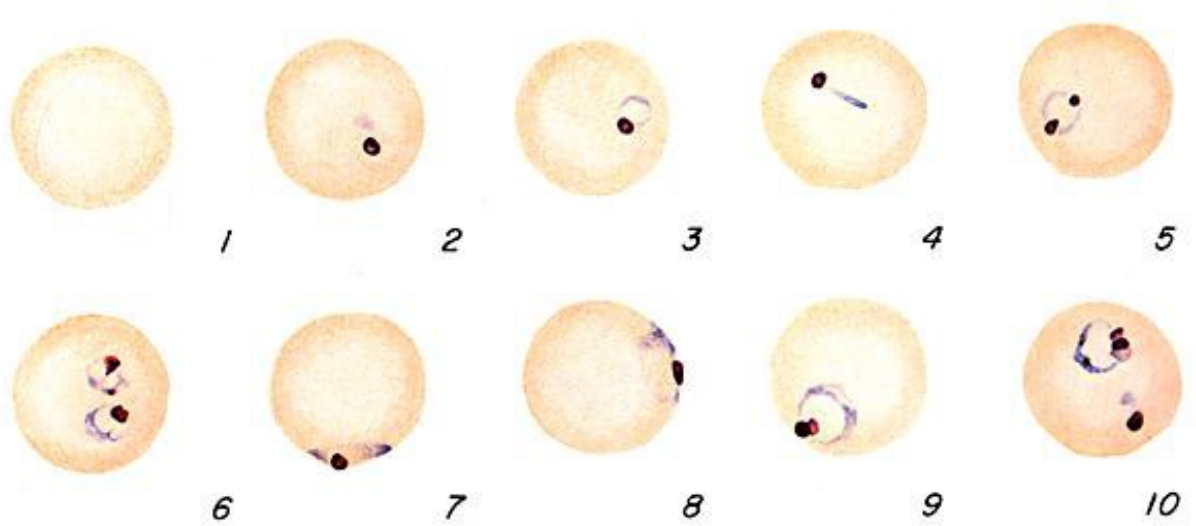


- Circumtropical distribution
- Infects all types of erythrocytes
  - » Allows for highest parasitemias
- Cause of cerebral malaria
- Resistance to chloroquine, mefloquine, and sulfadoxine-pyrimethamine in some areas
- Most commonly-encountered species in laboratories the United States
- Fever cycles every 24-48 hours
  - » Can be continuous at high parasitemias
- Can cause recrudescence in treatment failure

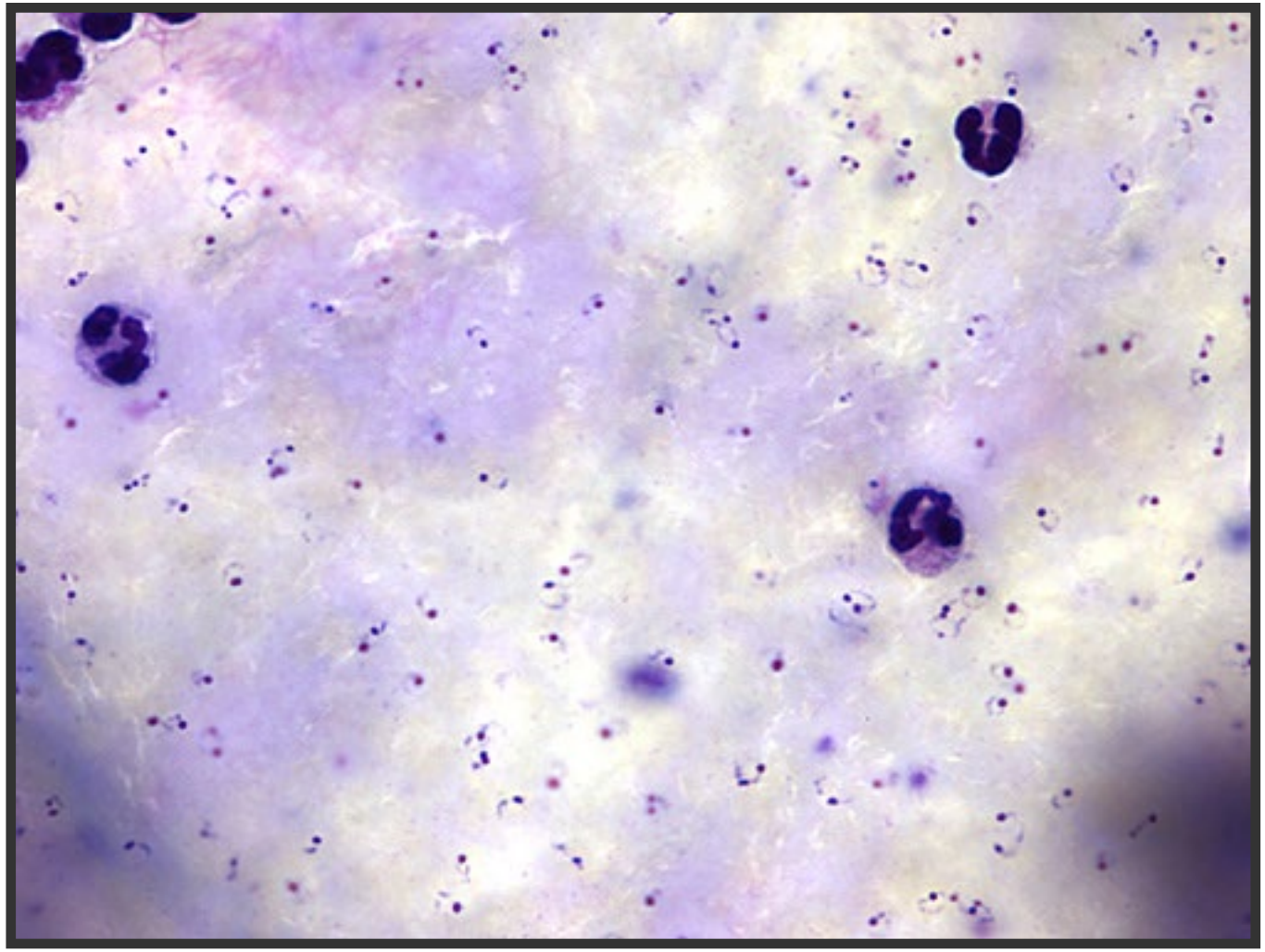


Plasmodium falciparum : Rings

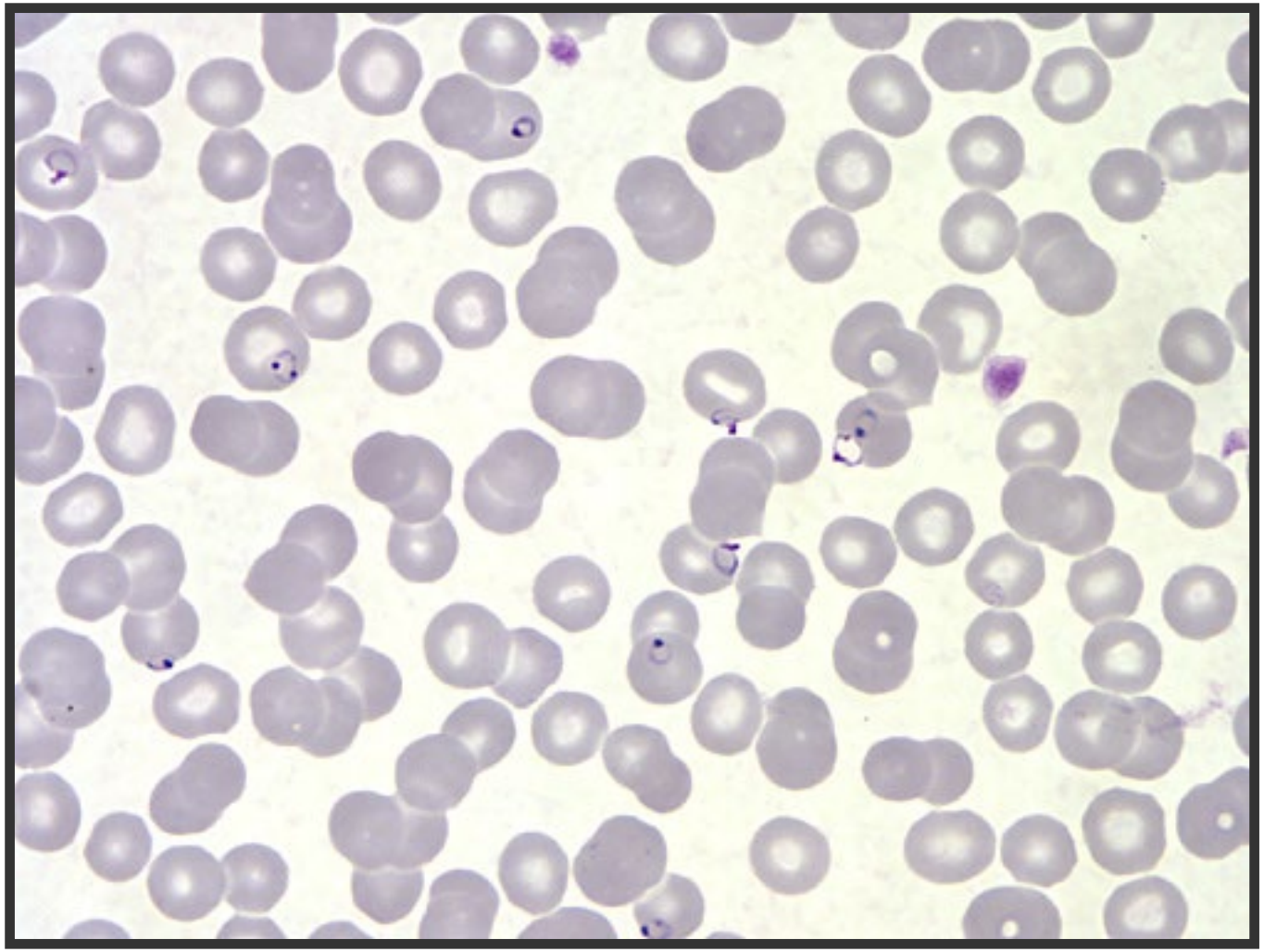
- The cytoplasm will appear thin and delicate.
- Multiple rings may be found inside individual RBC's.
- Double chromatin dots may be found.
- Appliqué forms may be found.



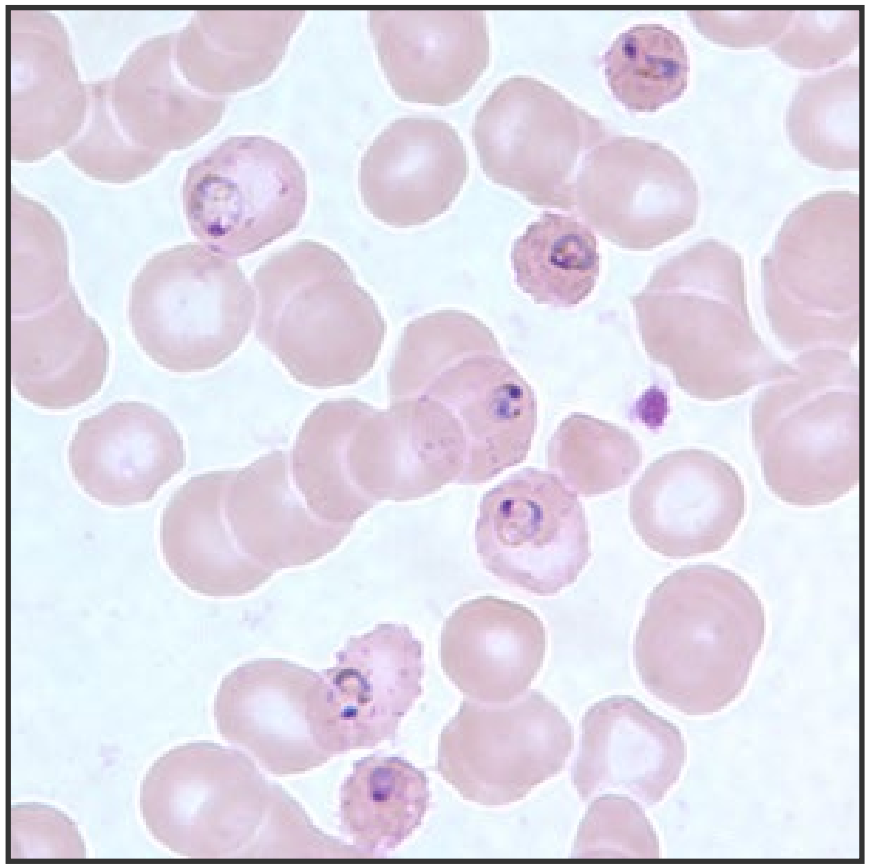
Plasmodium falciparum : thick film

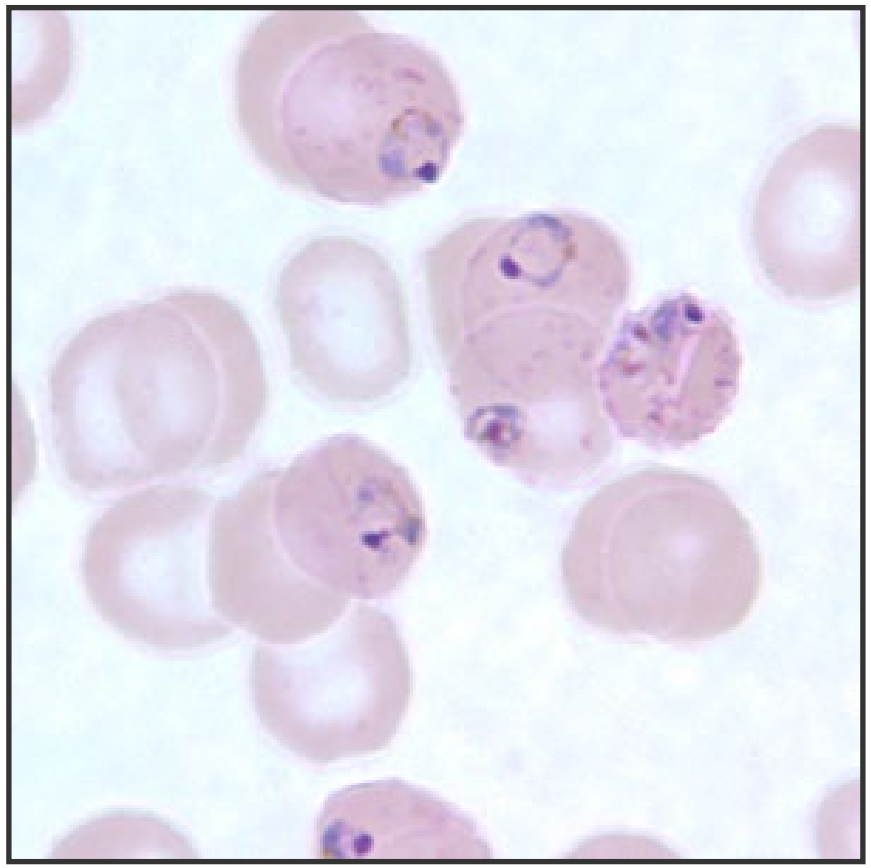


Plasmodium falciparum : thin film



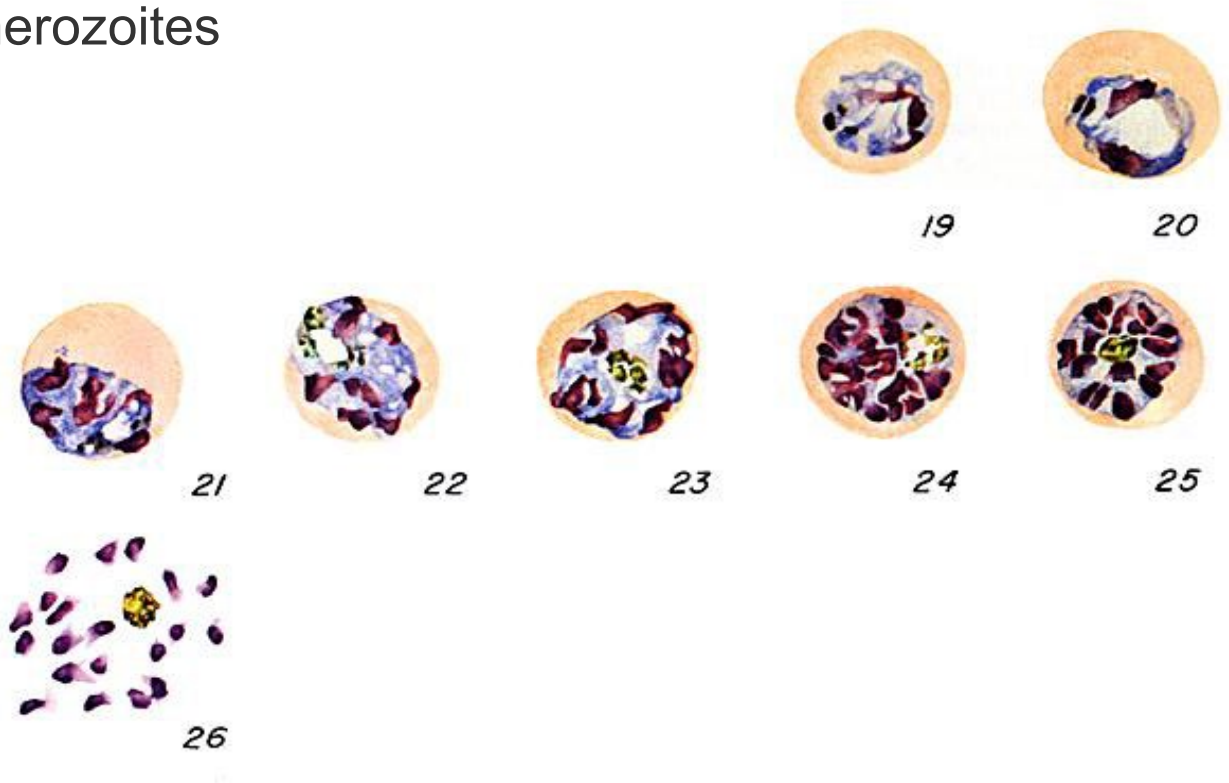
Plasmodium falciparum : Maurer's clefts



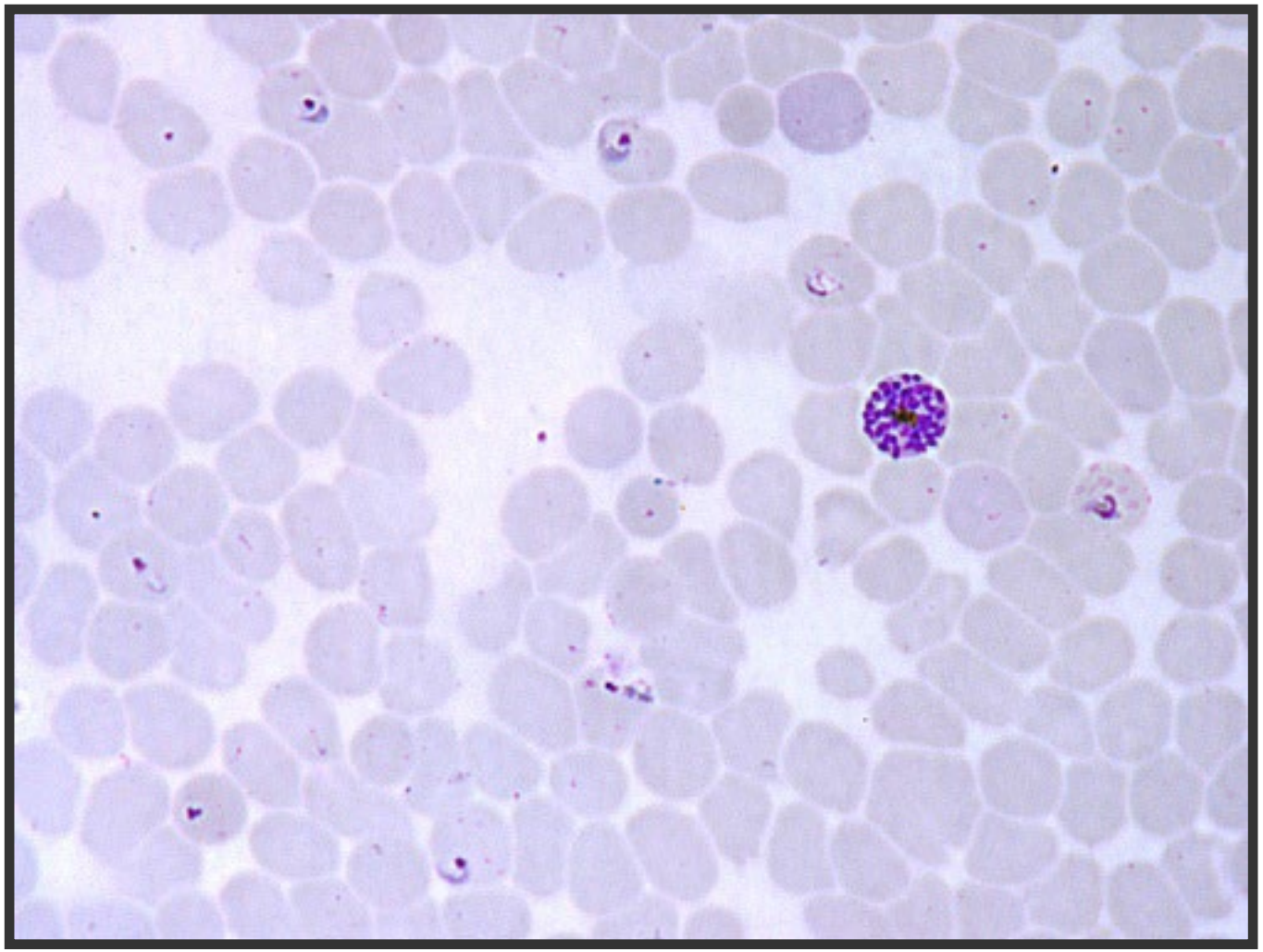


Plasmodium falciparum : schizont

- Rarely seen on stained films of peripheral blood
- 8 to 24 merozoites

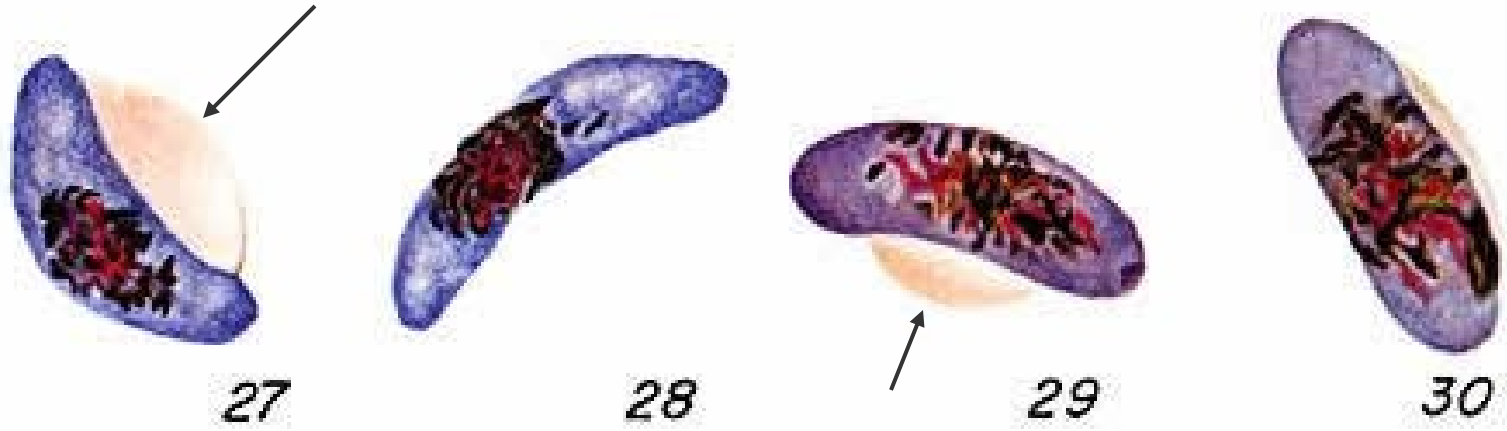


Plasmodium falciparum : Schizont

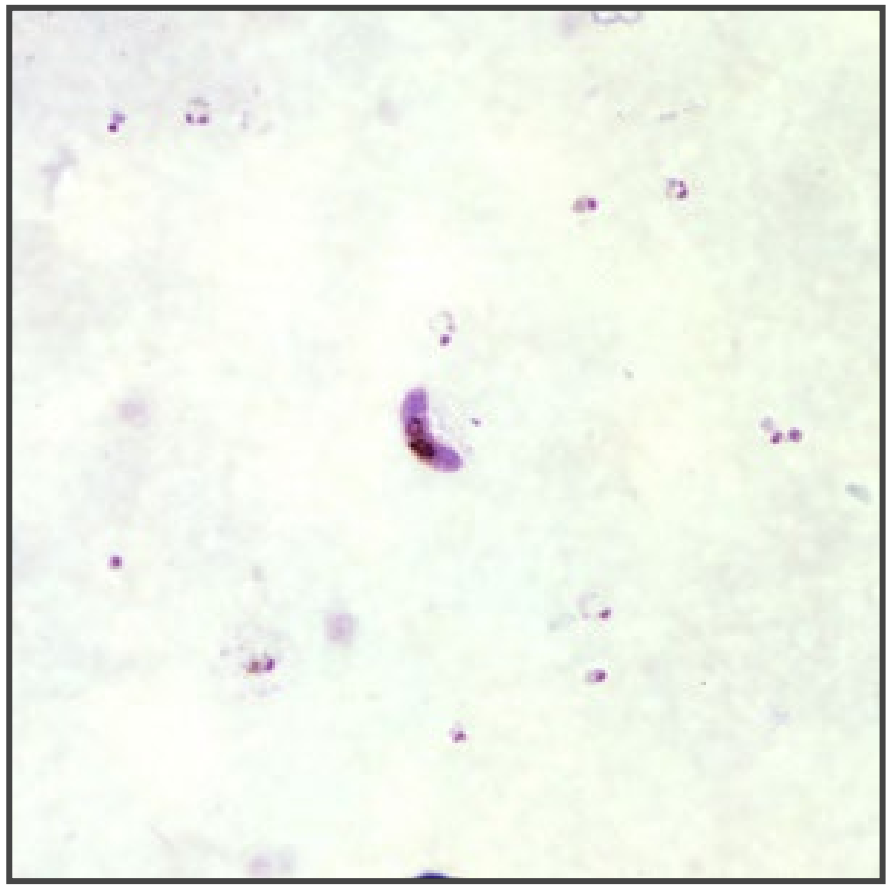


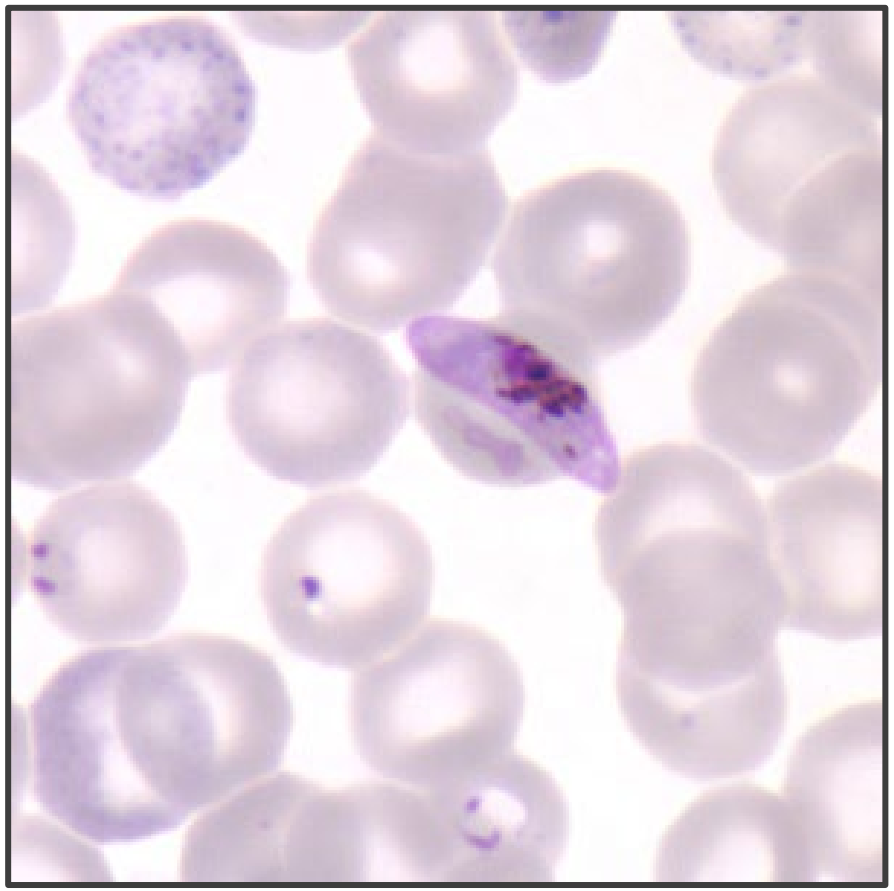
Plasmodium falciparum : Gametocytes

- Typically crescent or banana shaped with pigment
- Remnants of host RBC may be seen in a structure known as Laveran's bib (arrows).

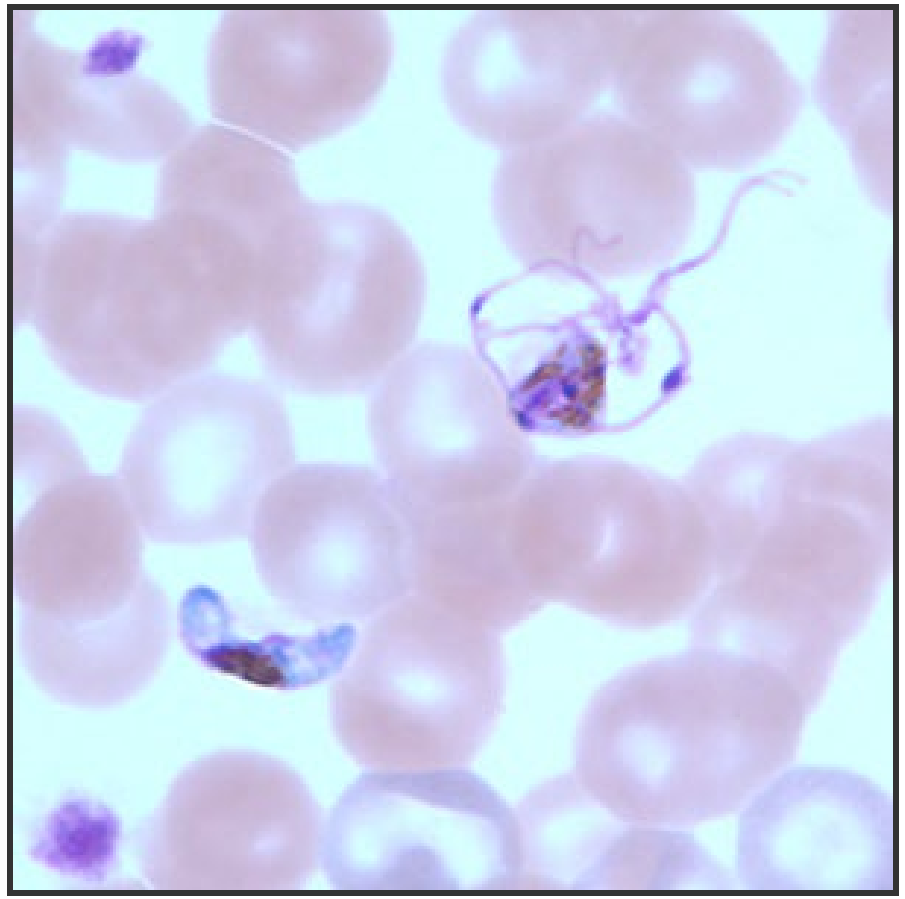


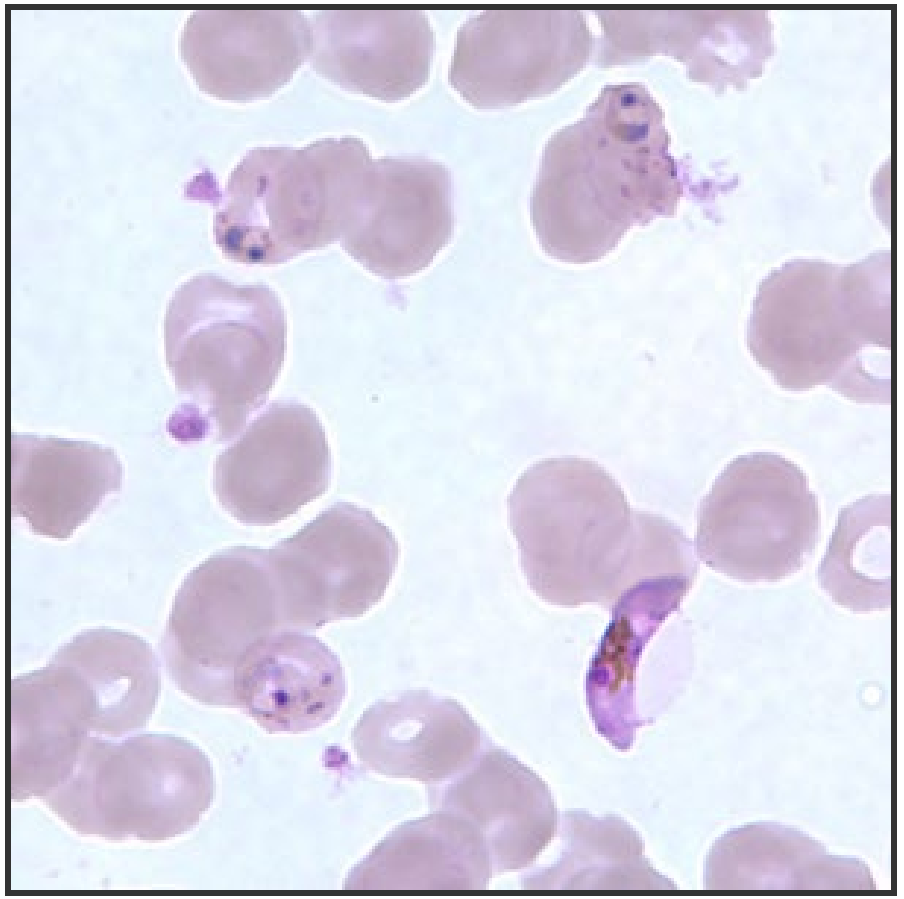
Plasmodium falciparum : gametocytes





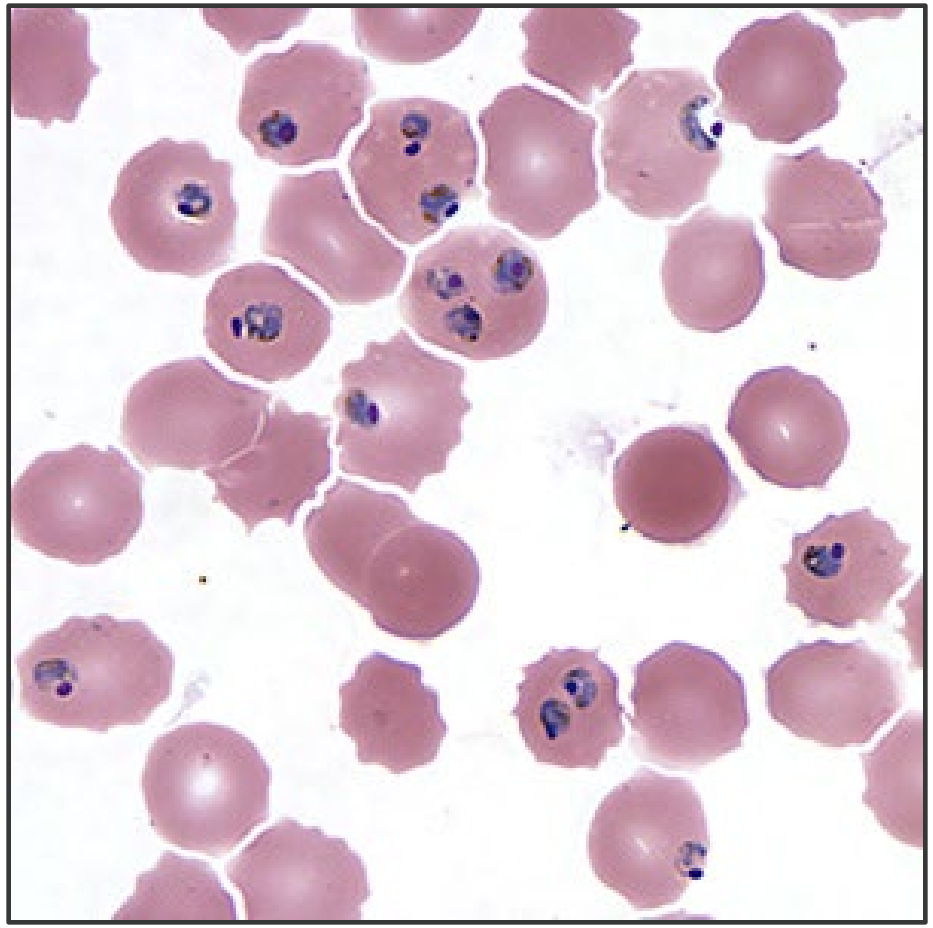
Plasmodium falciparum : gametocytes





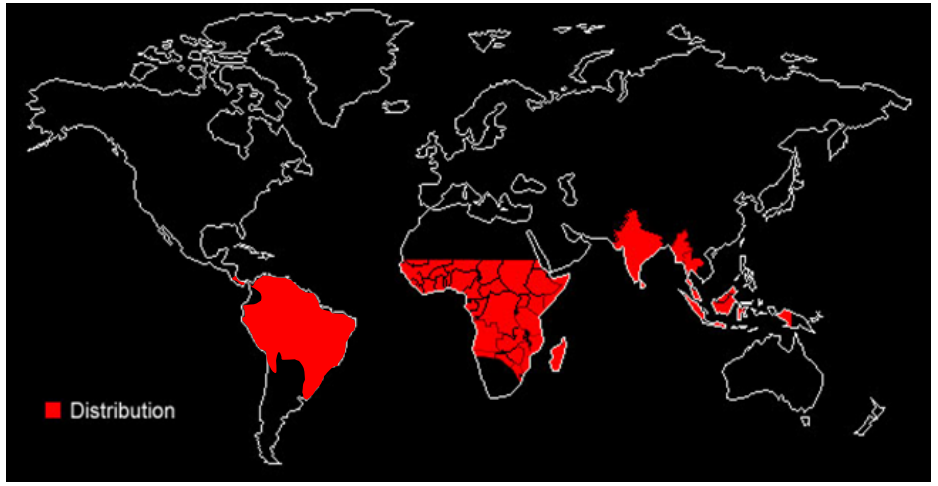
Atypical Presentation of *P. falciparum* Trophozoites

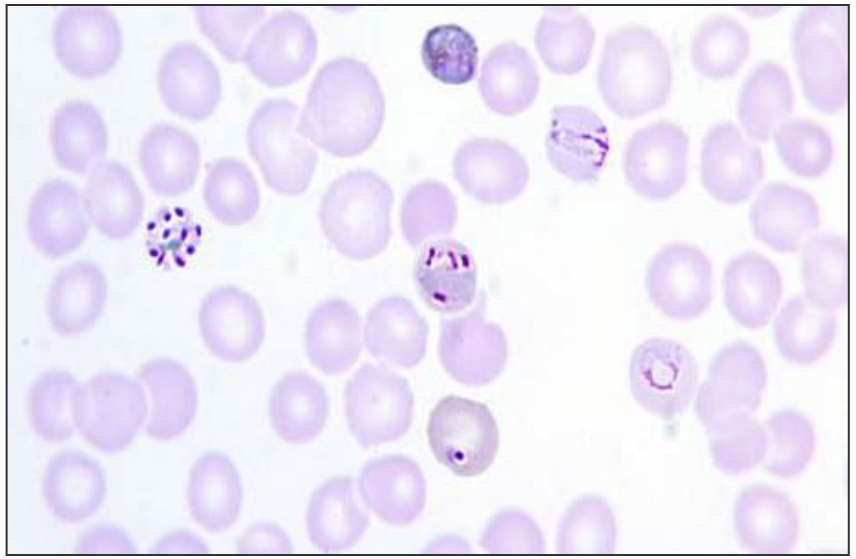
- May occur when blood smears are not made in a timely manner from blood that has been held at room temperature.
- Typically complicates an accurate diagnosis



Plasmodium malariae

- Circumtropical (but more focal)
  - » *Plasmodium brasilianum* is probably *P. malariae* that made a host switch from humans to monkeys after introduction to New World
- Prefers older erythrocytes
  - » Low parasitemia due to restricted host cells availability
- Often asymptomatic
- Fever cycles every ~72 hours
- Fourth most reported species in the US

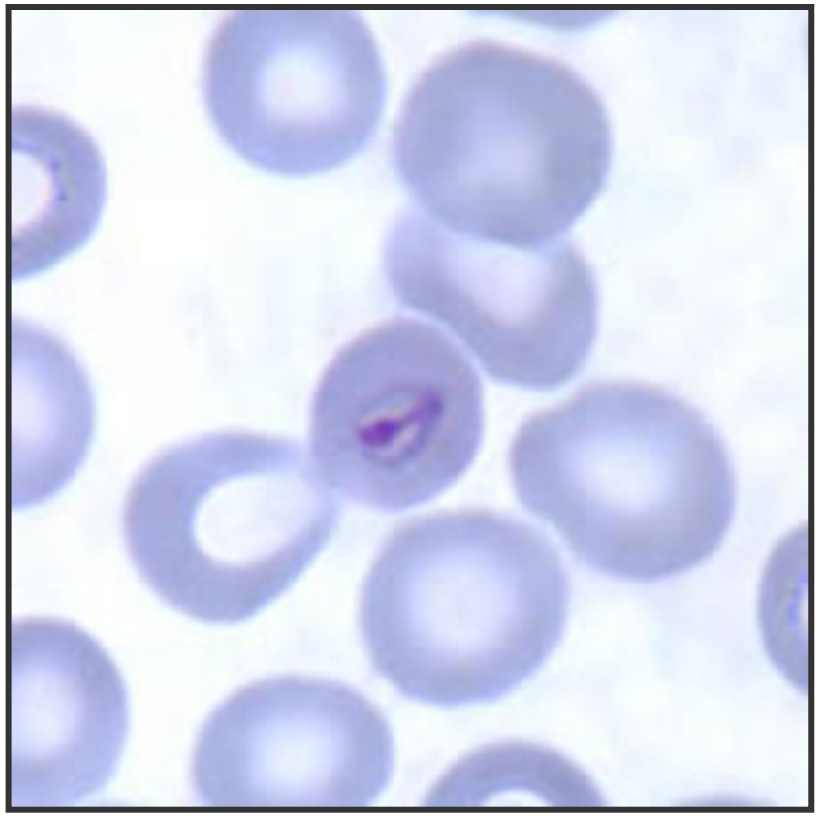


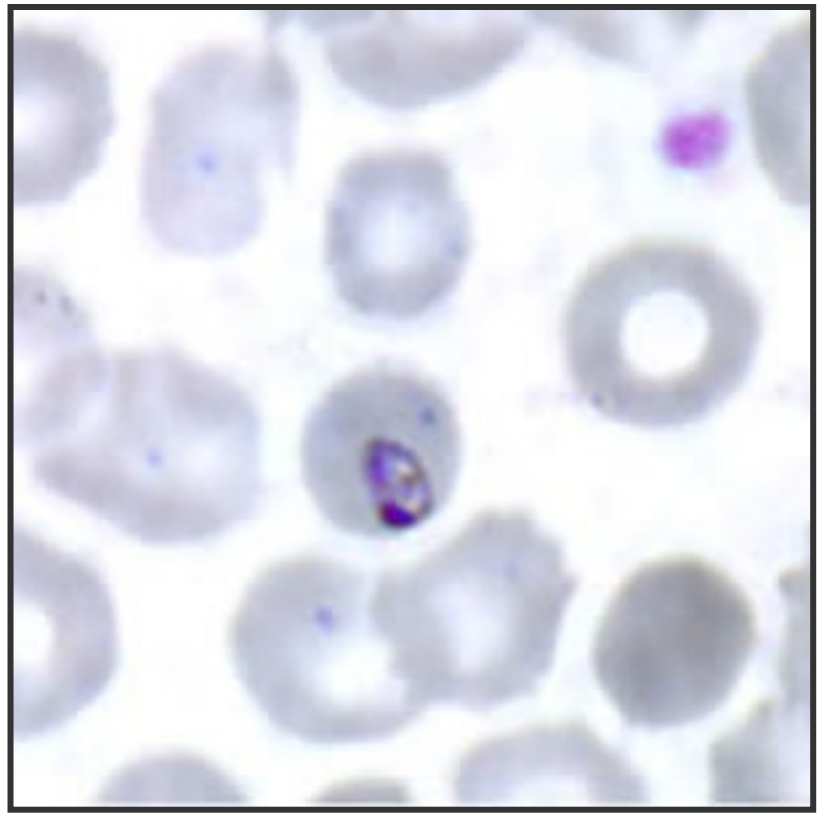


Plasmodium malariae : rings

- Rings usually small.
- Often one, sometimes two, chromatin dots.
- May see ‘bird’s eye’ forms.

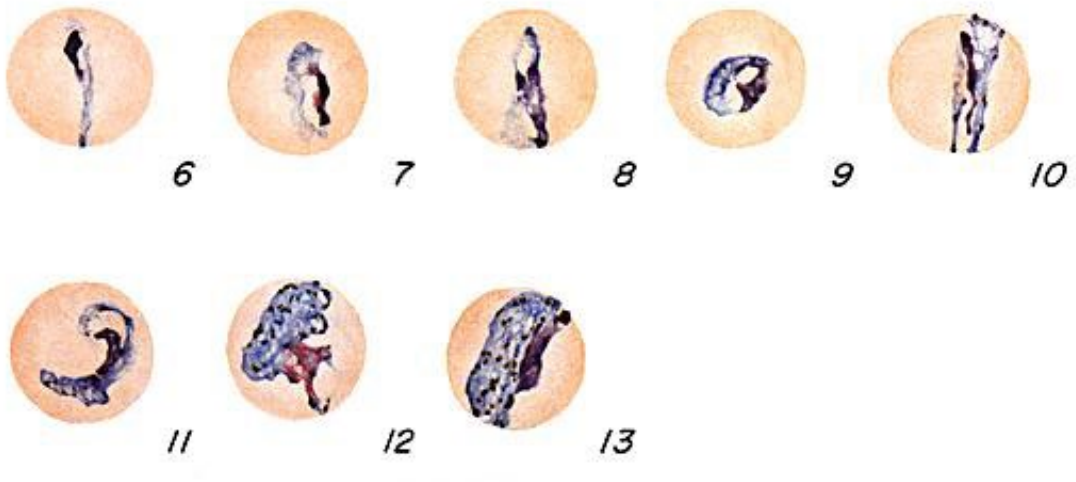
Plasmodium malariae : rings, thin film



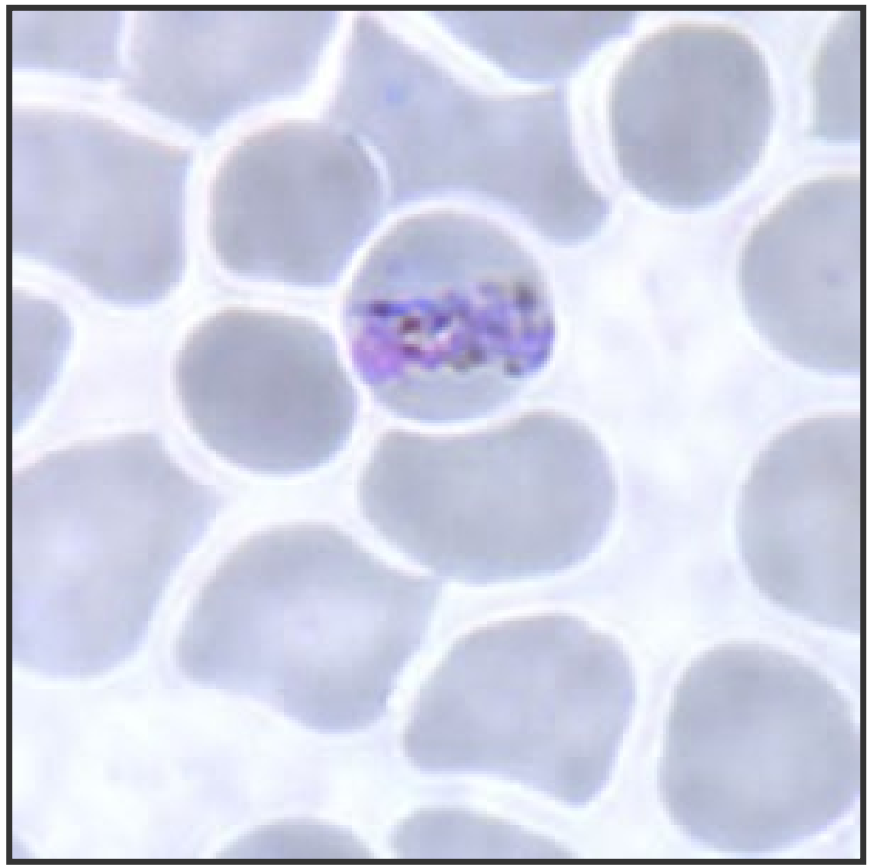


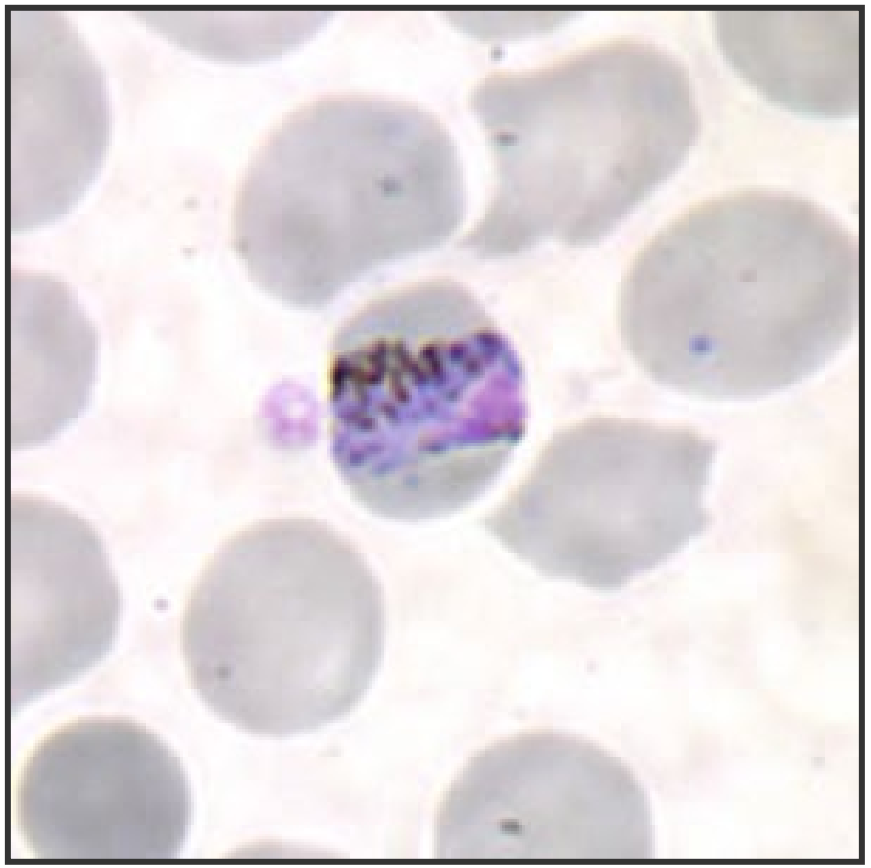
Plasmodium malariae : trophozoites

- Elongate as they develop.
- May see ‘basket’ or ‘band’ forms.

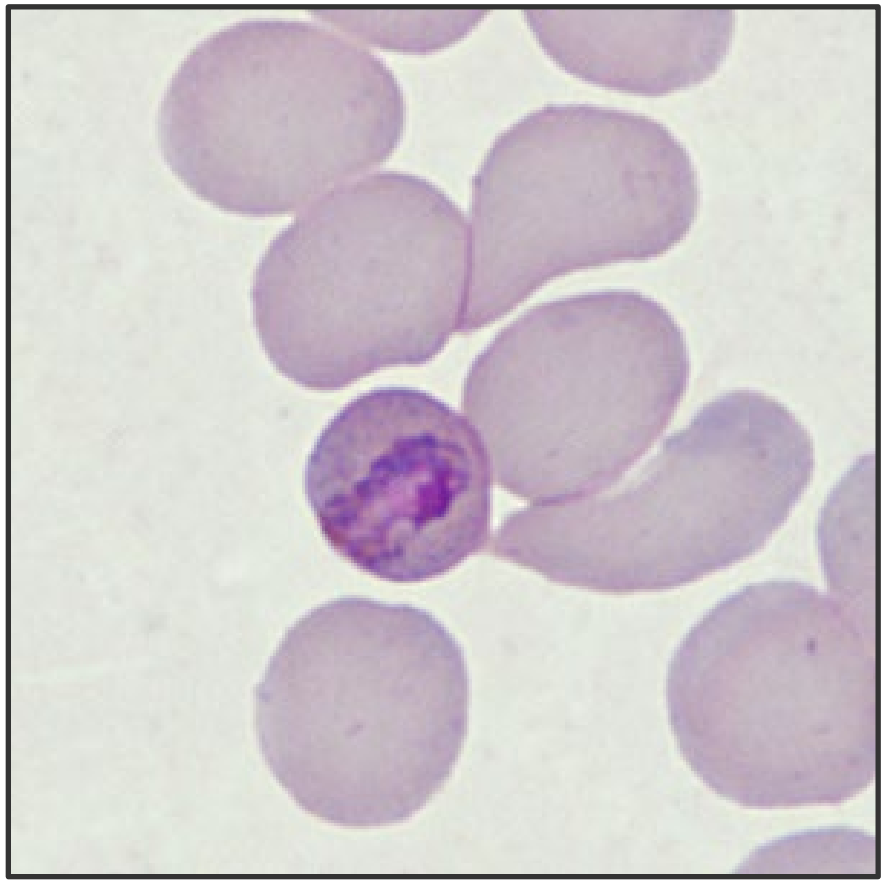


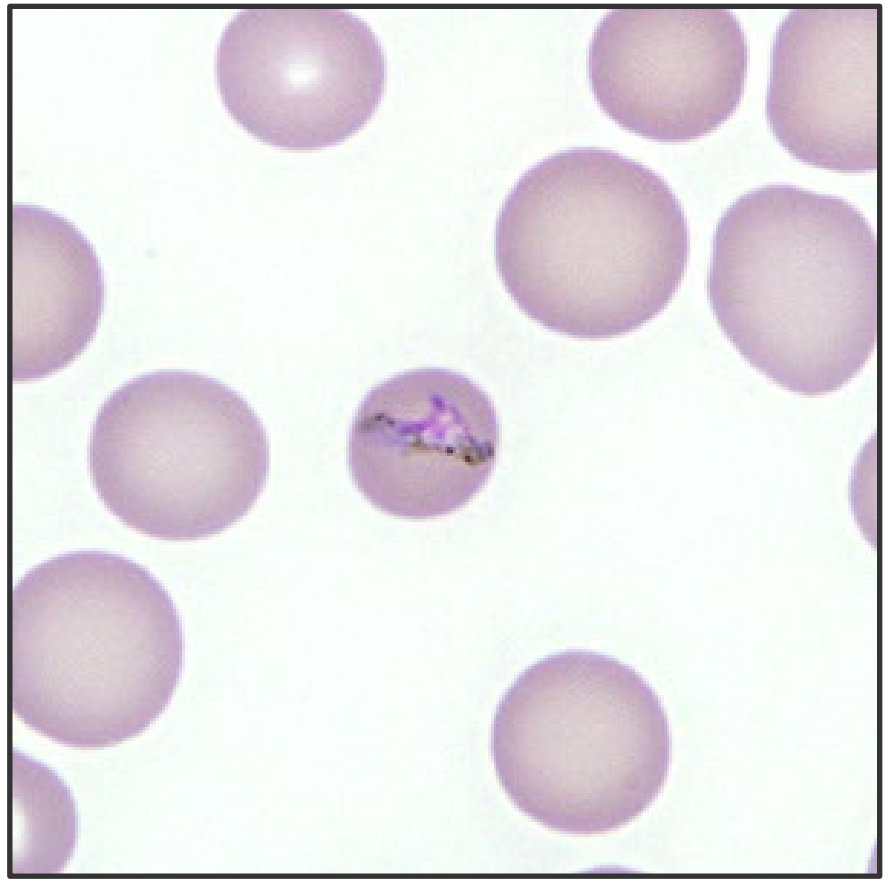
Plasmodium malariae : band -form trophs



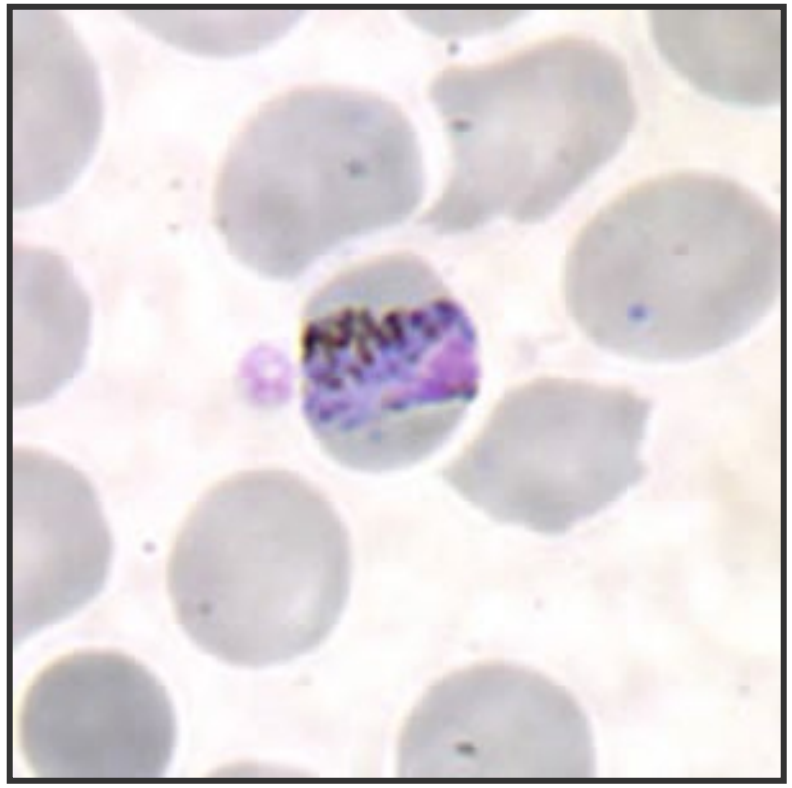


Plasmodium malariae : band -form trophs

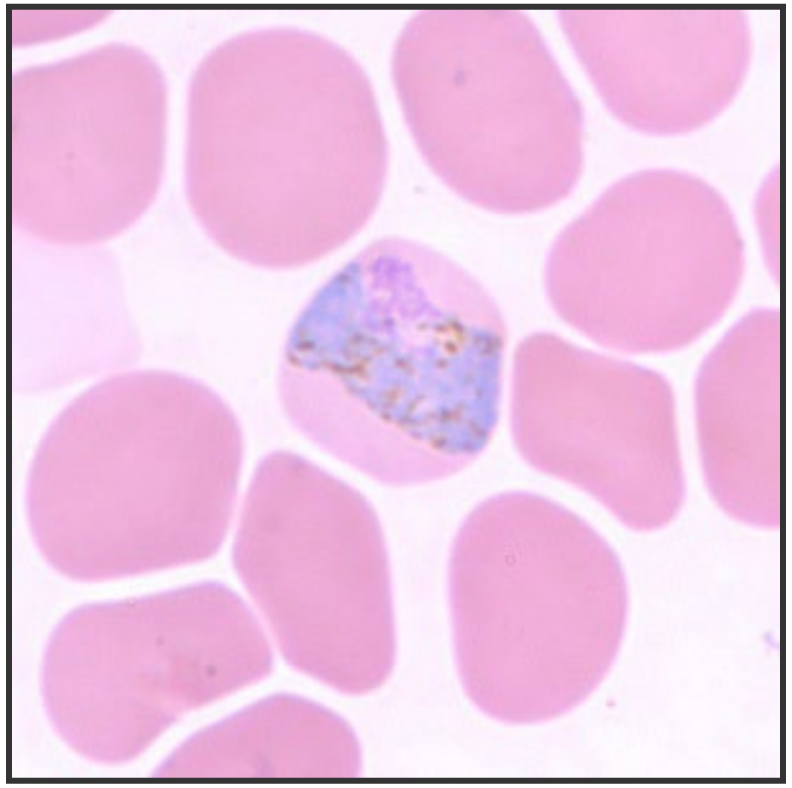




Band Forms: *P. malariae* vs. *P. vivax*

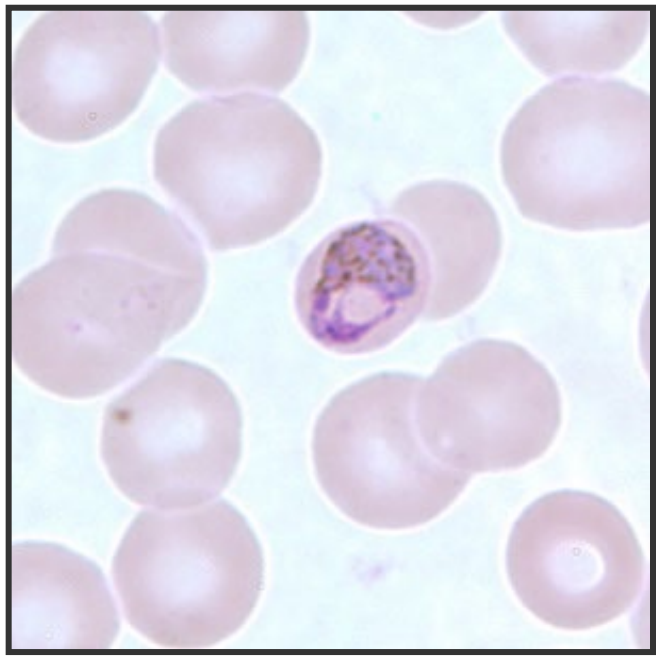


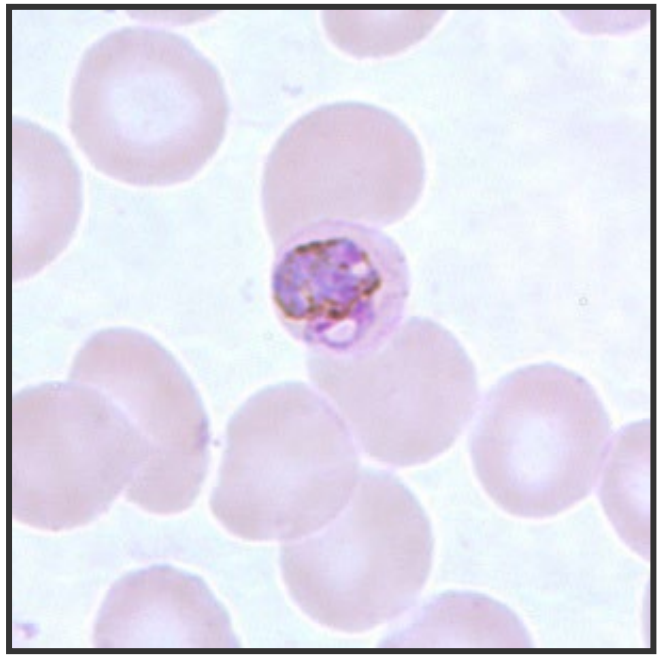
P. malariae

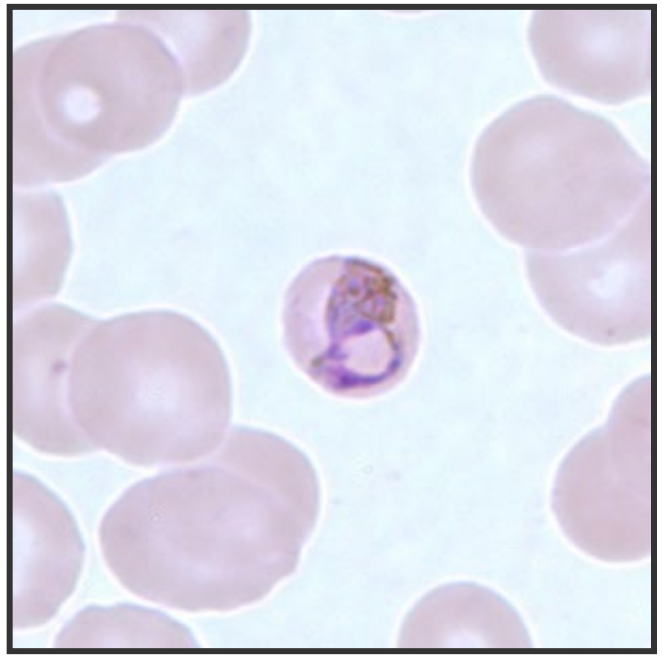


P. vivax

Plasmodium malariae : basket -form trophs

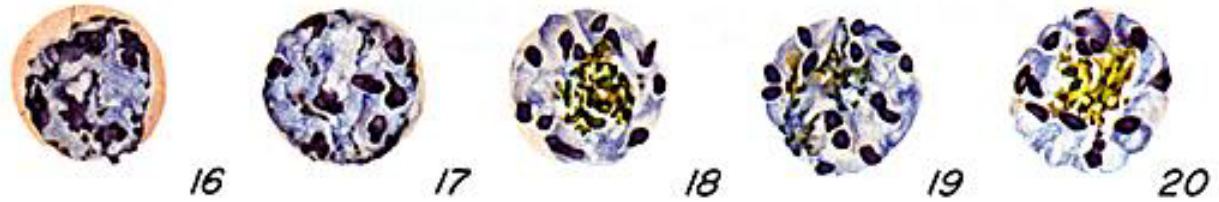


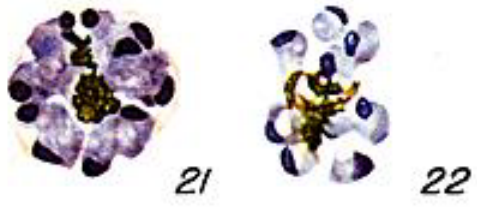




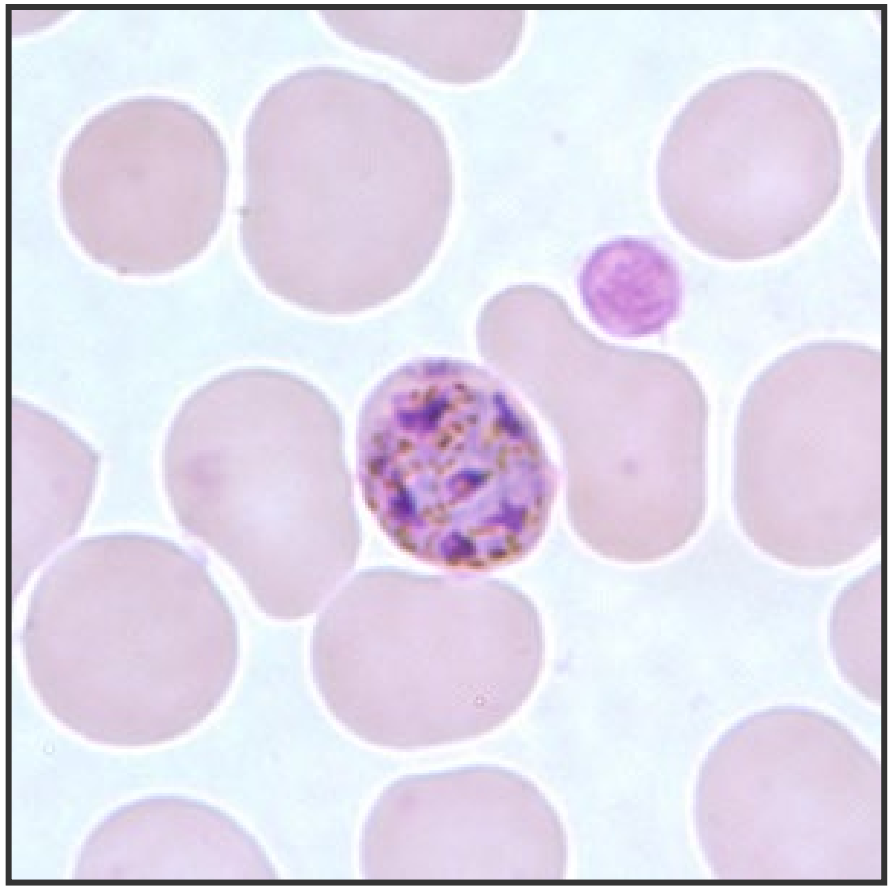
Plasmodium malariae : schizonts

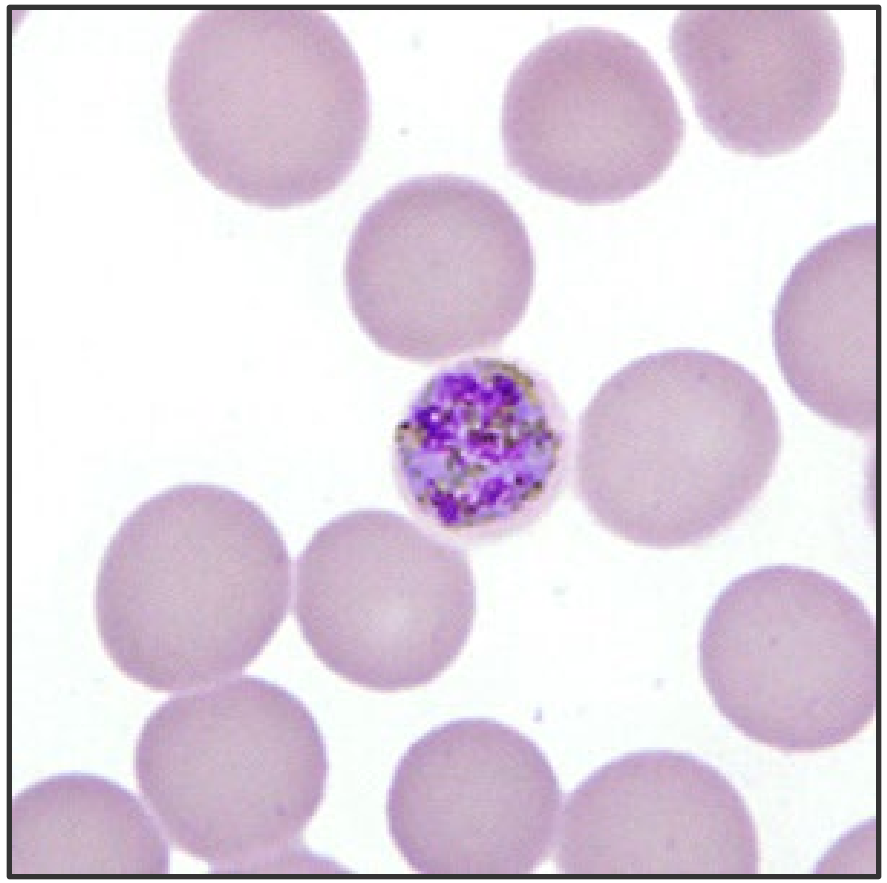
- Mature schizonts have 6-12 merozoites.
- Often in rosette-shaped patterns around coalesced and centrally-located pigment.
- May be smaller than host RBC.



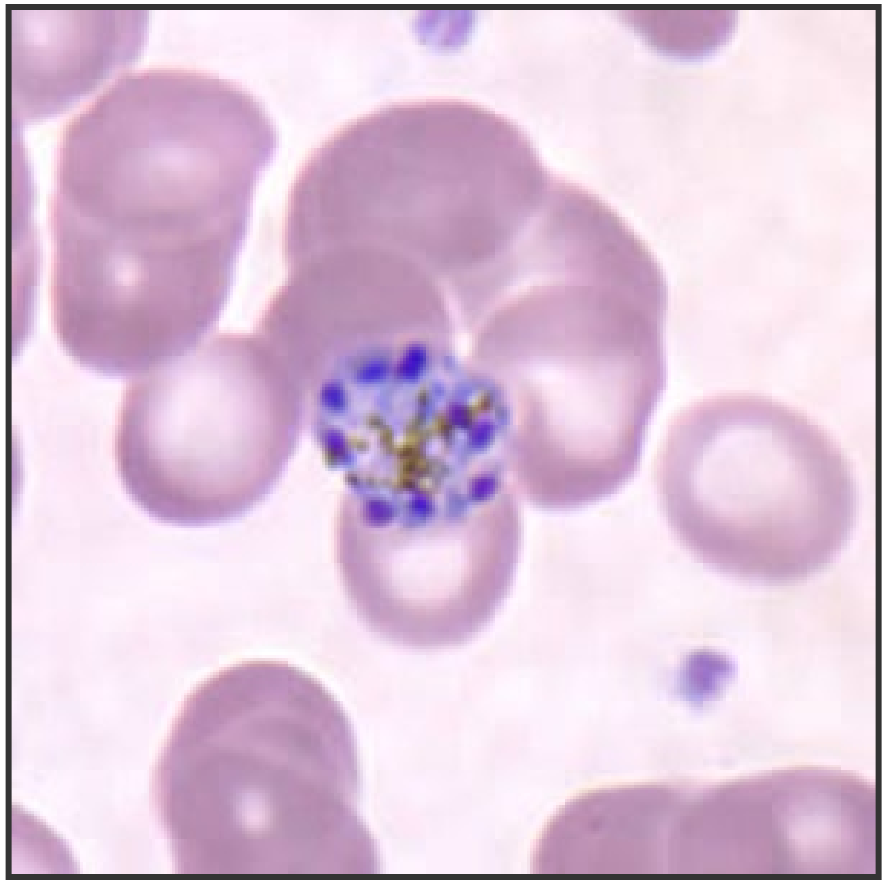


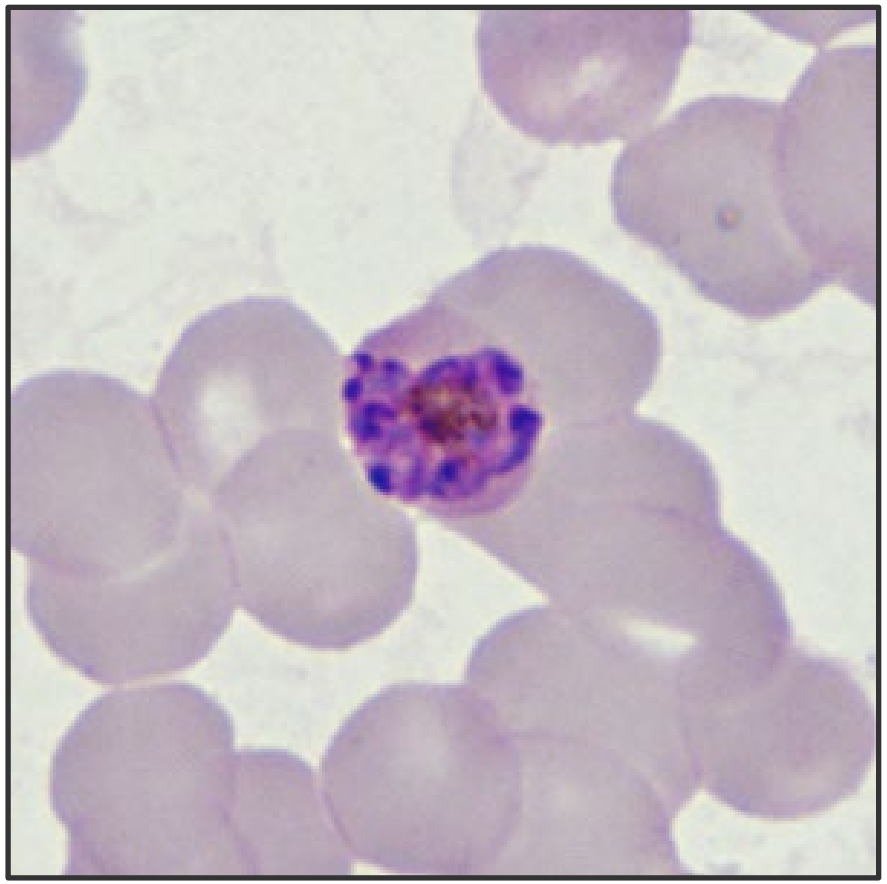
Plasmodium malariae : immature schizonts





Plasmodium malariae : mature schizonts

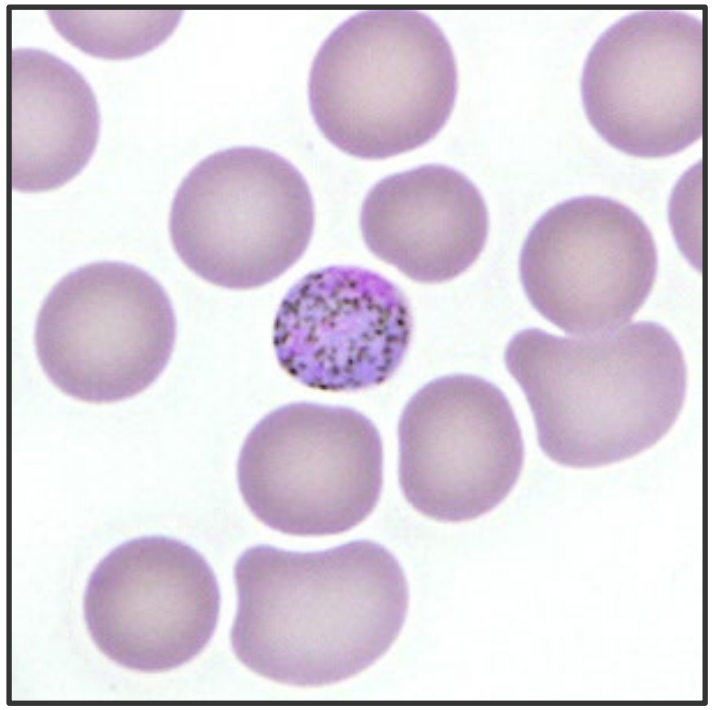


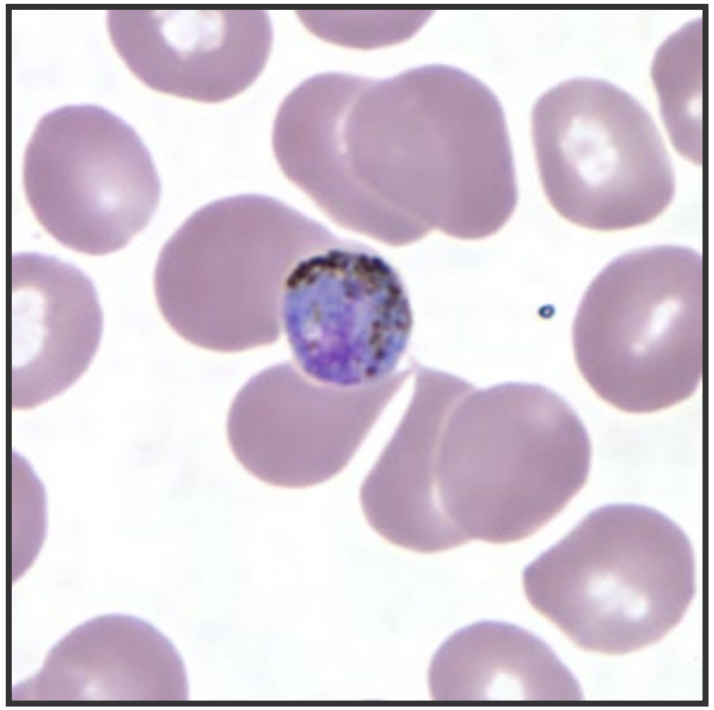


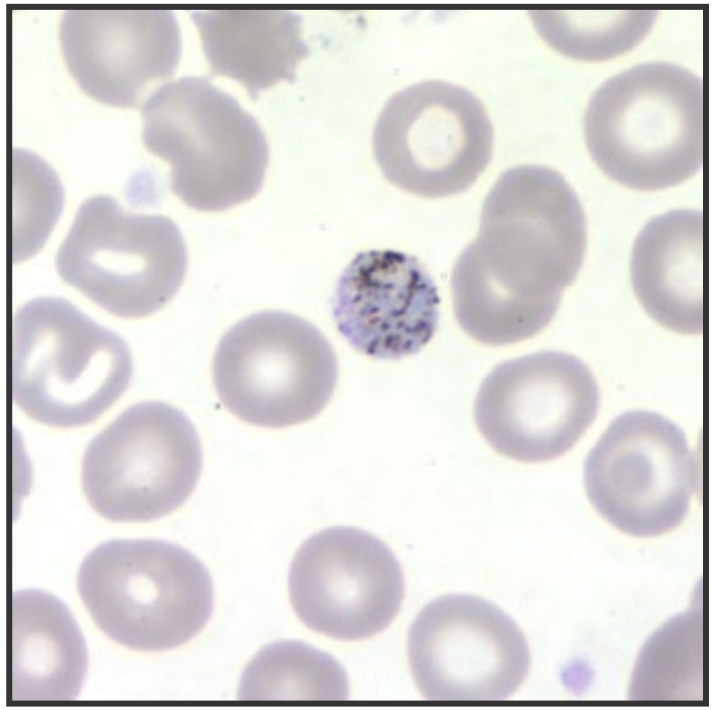
Plasmodium malariae : gametocytes

- Round in shape.
- May be smaller than normal RBC.
- Pigment usually coarse.

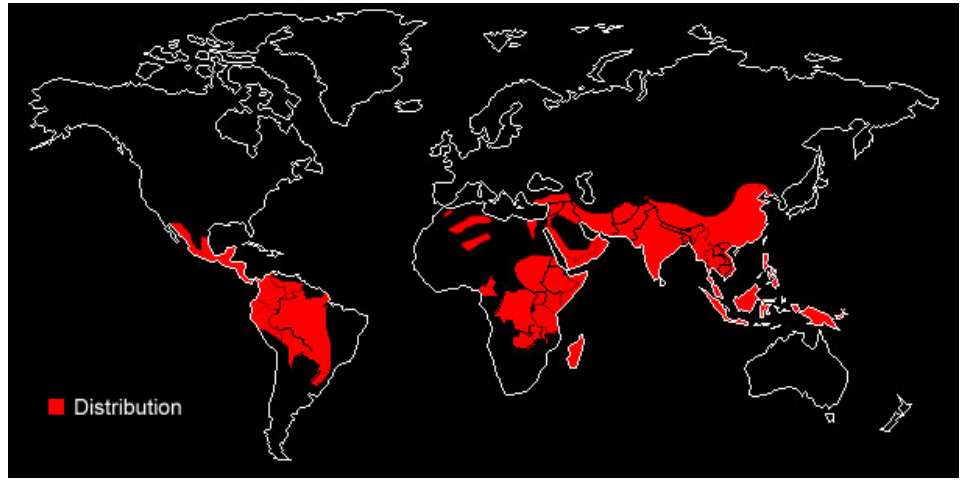
Plasmodium malariae : gametocytes



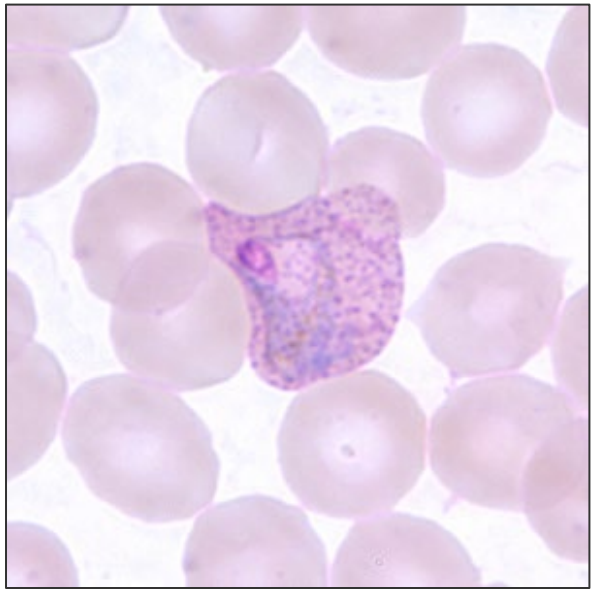




Plasmodium vivax

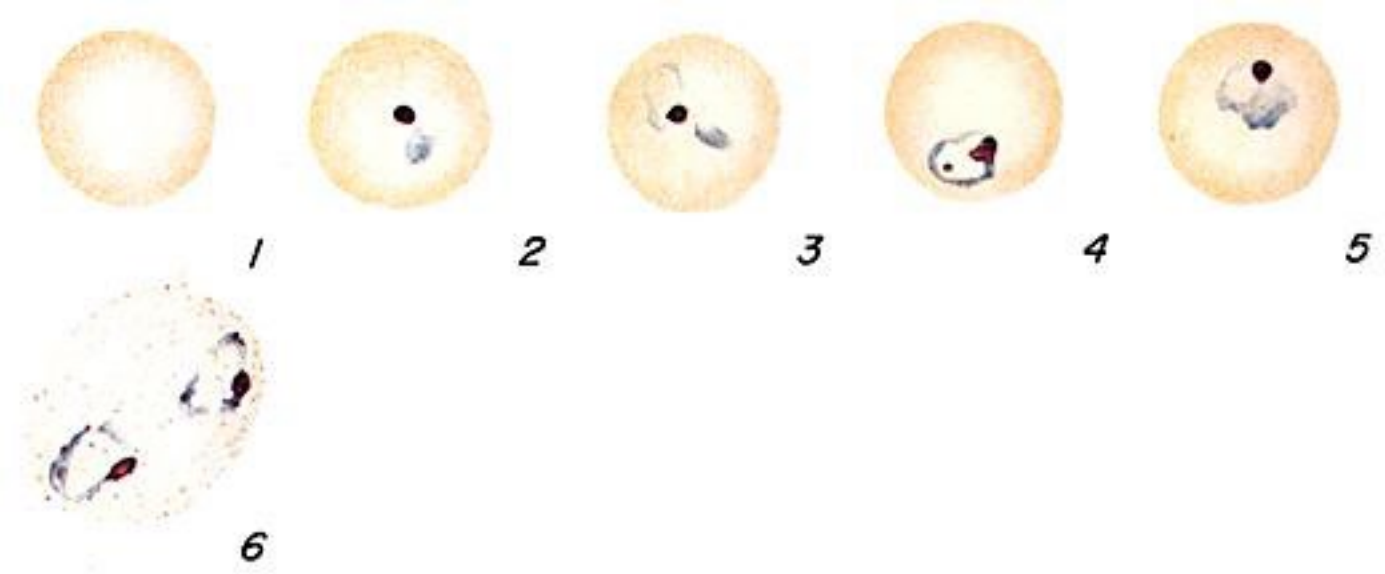


- Distribution: East Africa, Central to Southeast Asia, Central and South America
  - » *Plasmodium simium* is probably *P. vivax* that made a host switch from humans to monkeys after introduction to South America.
- Prefers immature erythrocytes (reticulocytes)
  - » Lower parasitemia due to restricted host cell availability
- Second most common species reported in the US healthcare
- Fevers cycle every ~48 hours
- Liver phase (hypnozoites) can reactivate months or years after infection
- Chloroquine resistance, esp. in Indonesia and Papua New Guinea

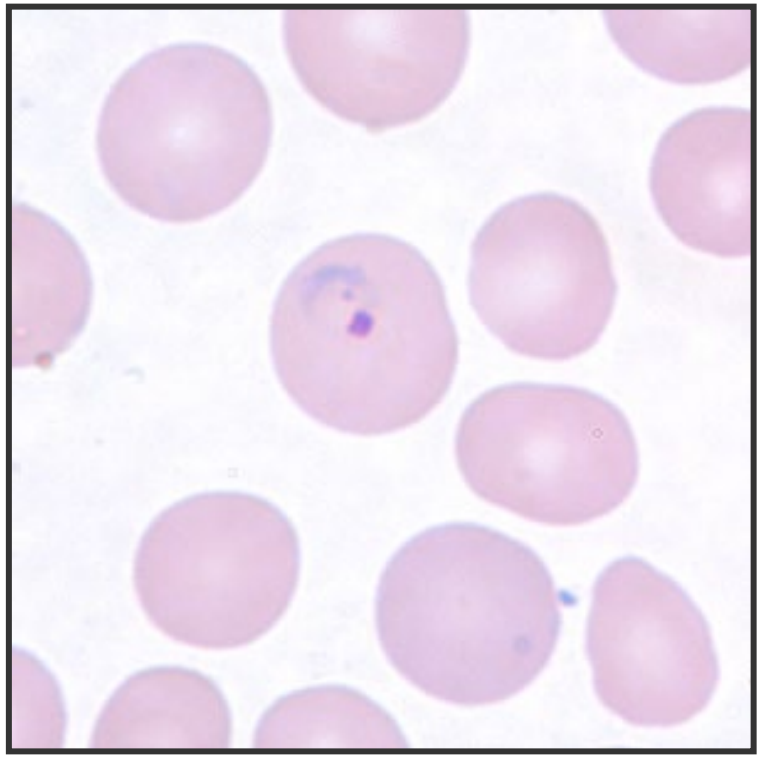


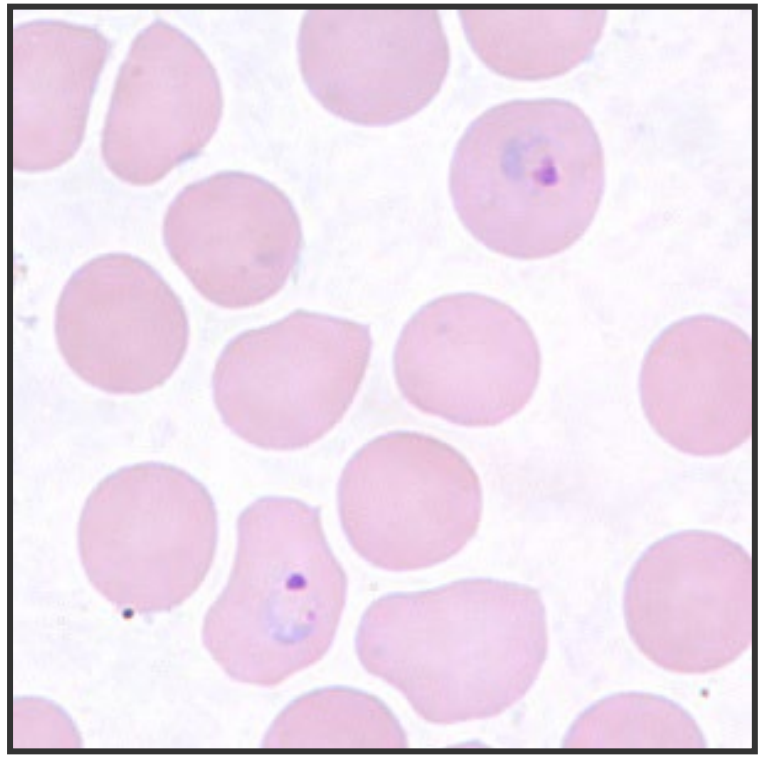
Plasmodium vivax : rings

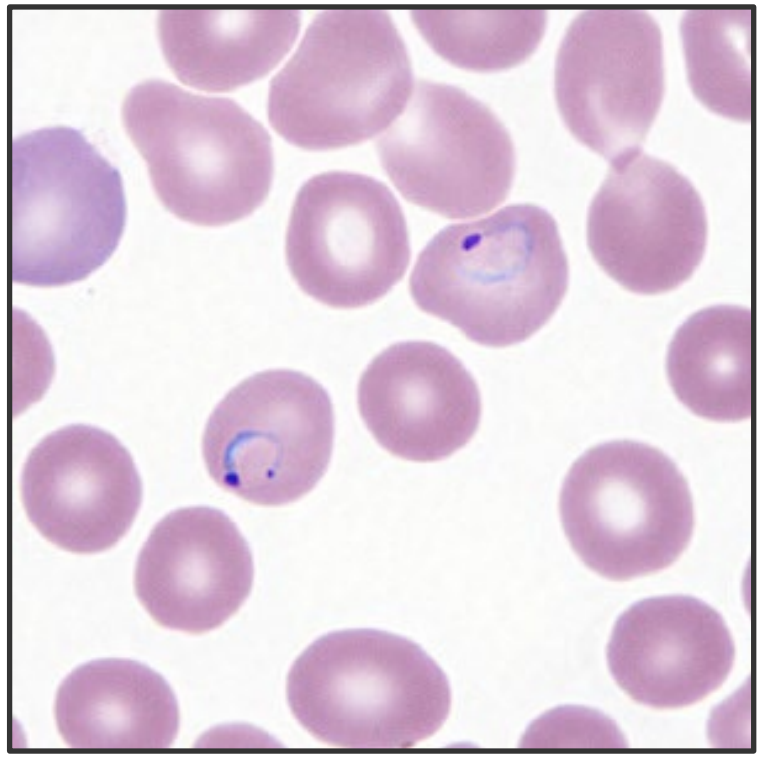
- Rings usually thicker, with a single larger chromatin dot.
- Often one per host RBC, but multiply-infected cells can be seen.



Plasmodium vivax : rings

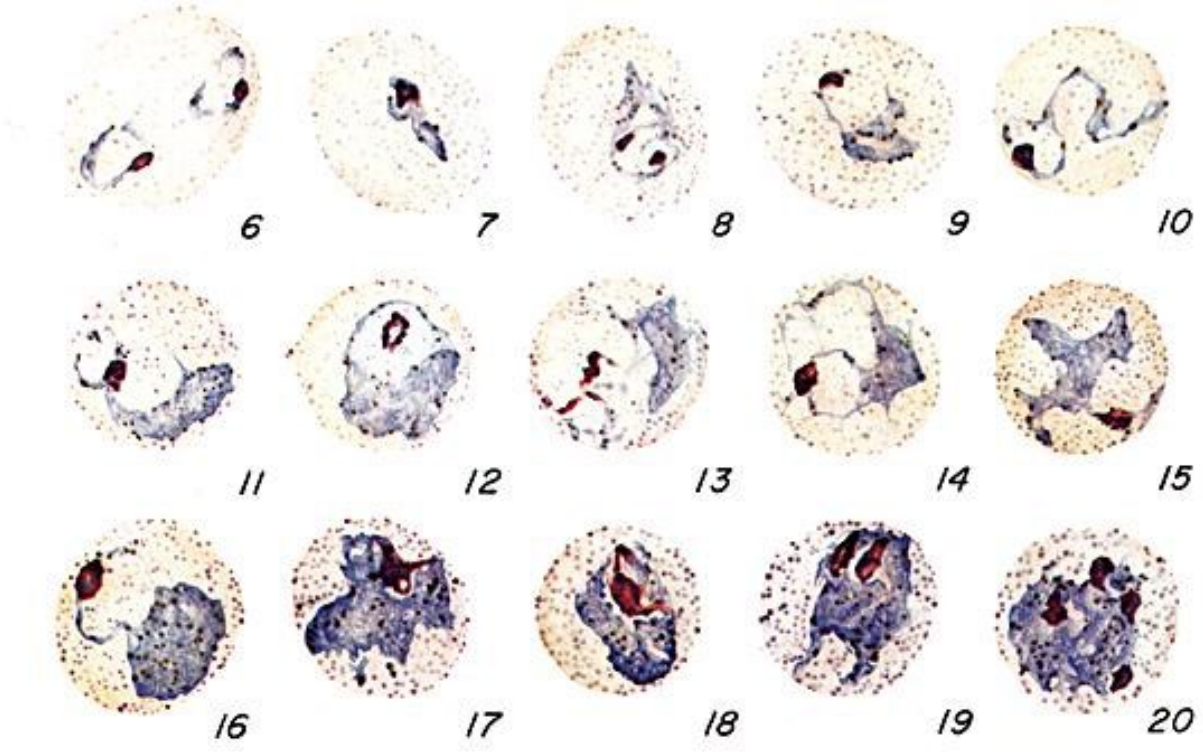




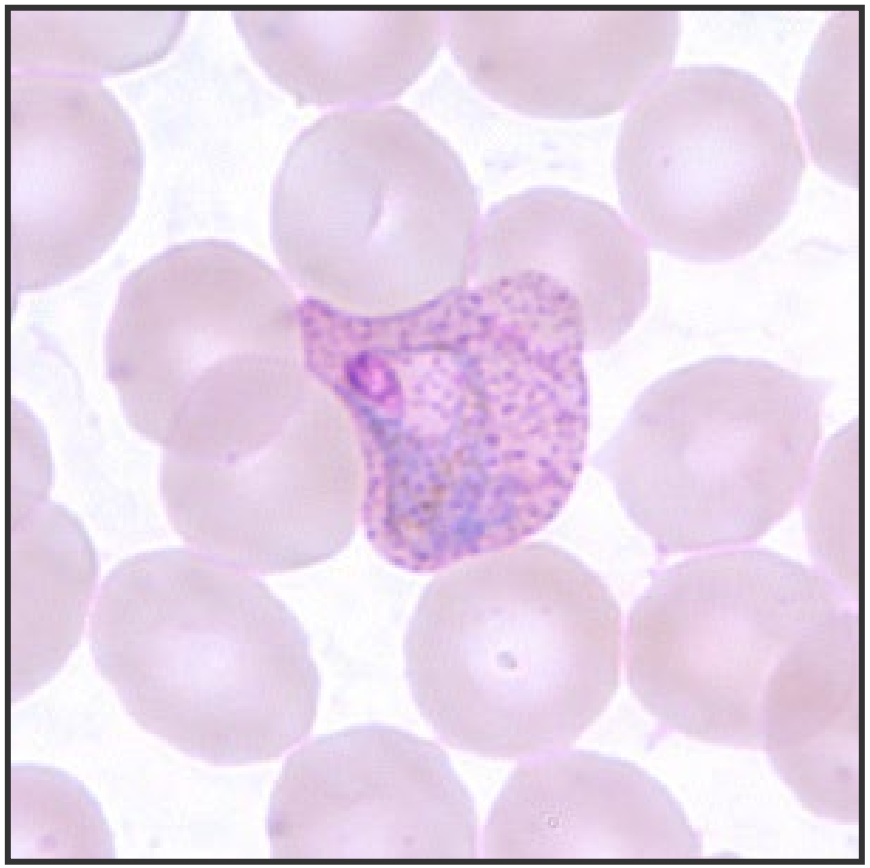


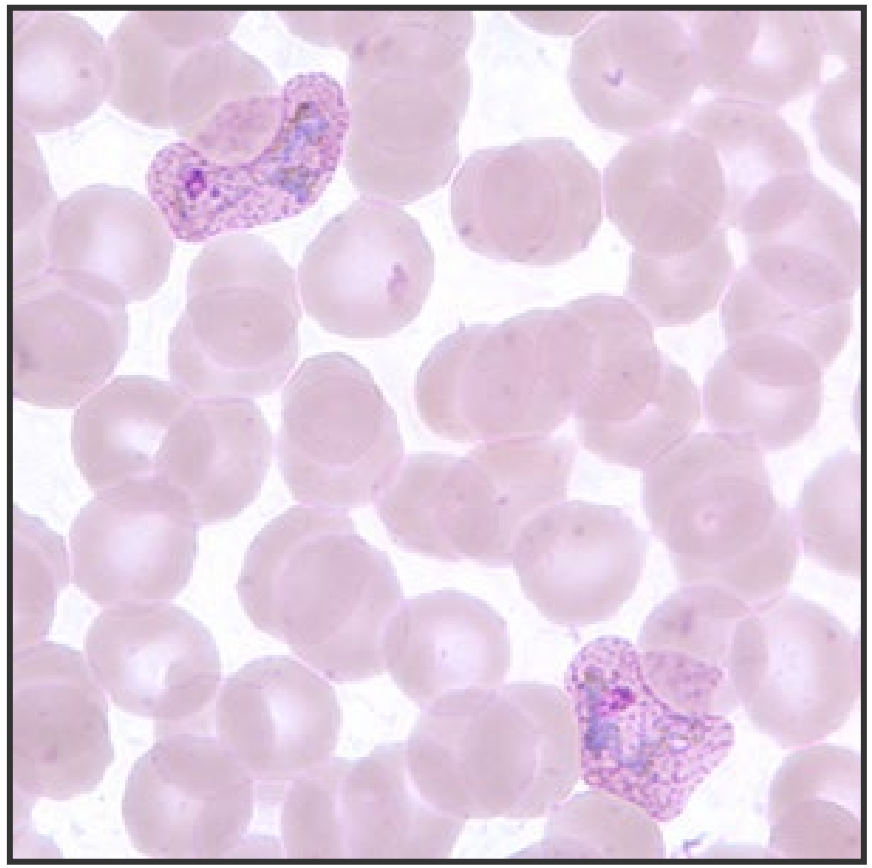
Plasmodium vivax : mature trophozoites

- Developing trophozoites usually amoeboid in appearance.
- Infected RBCs may be enlarged (1.5x normal RBC).
- Schüffner's dots may be present.

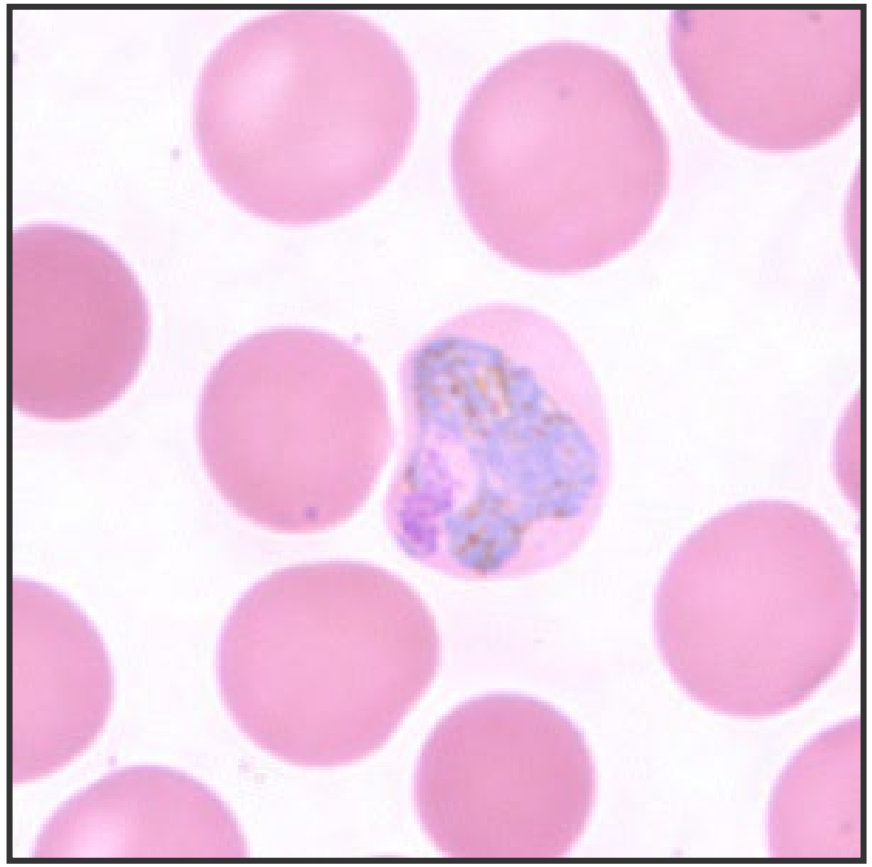


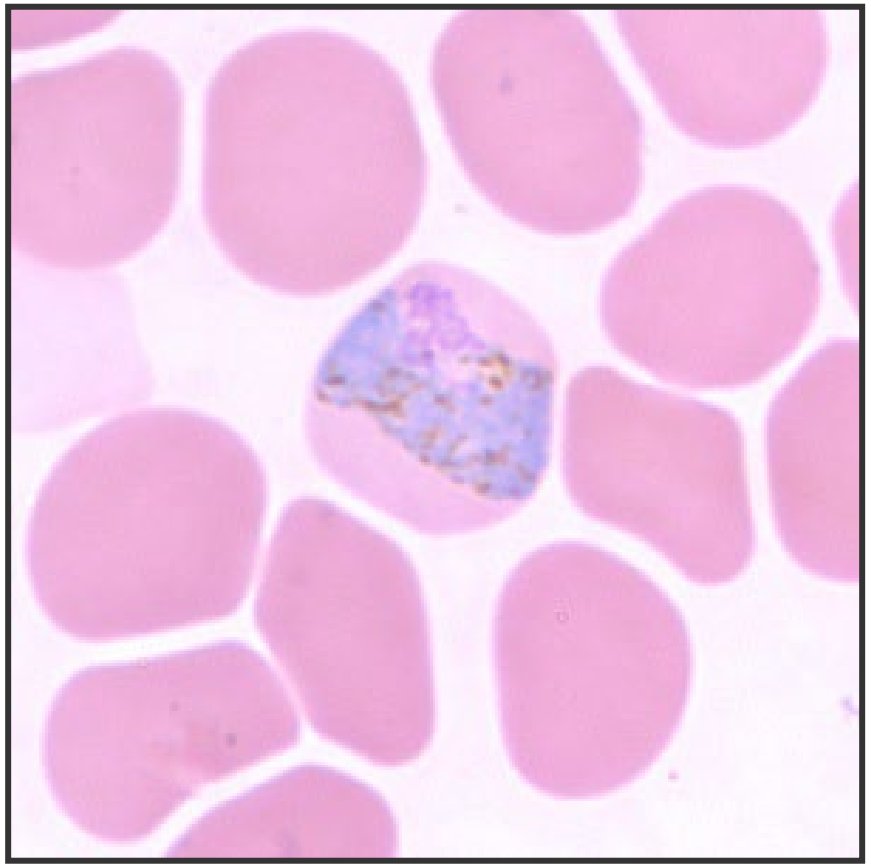
Plasmodium vivax : mature trophozoites





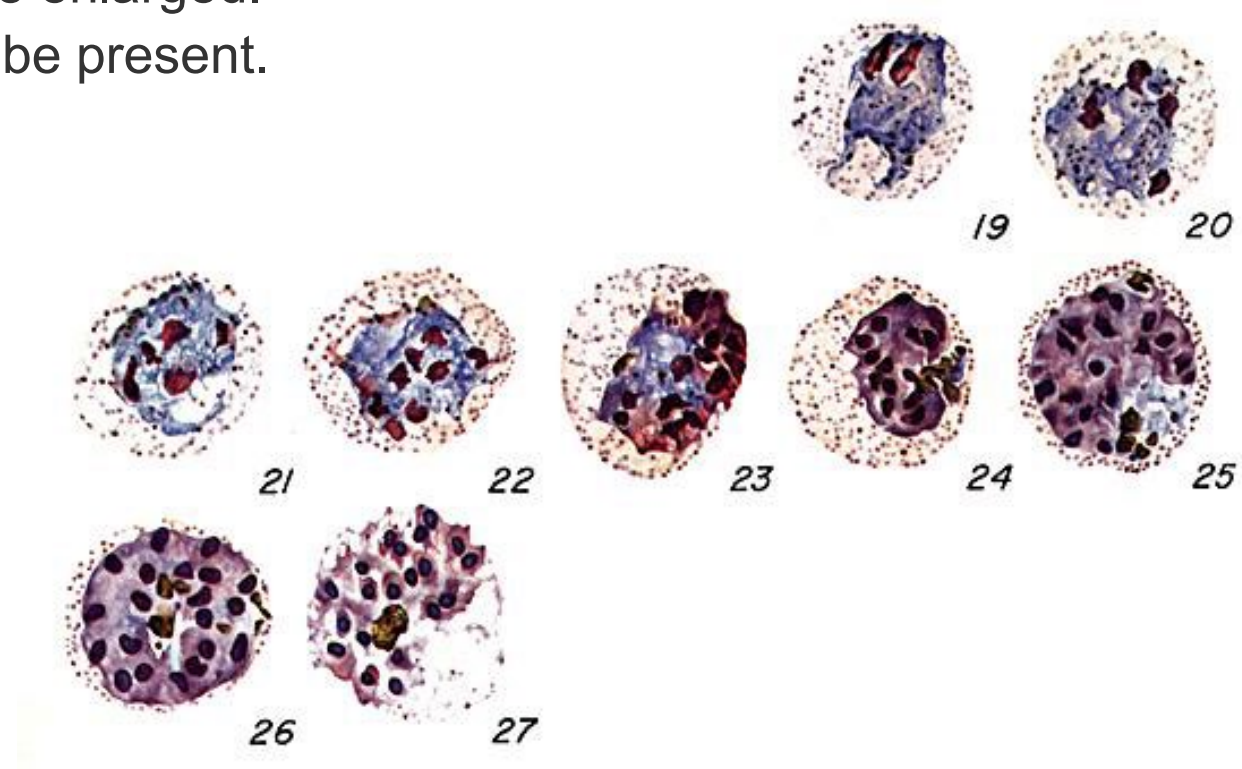
Plasmodium vivax : mature trophs, Wright stain



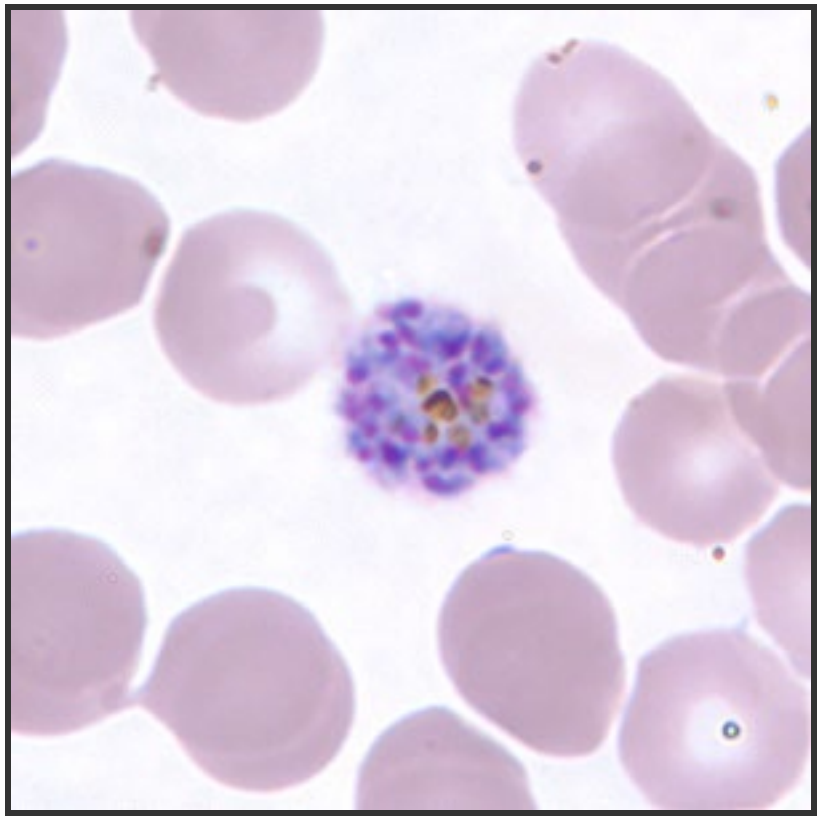


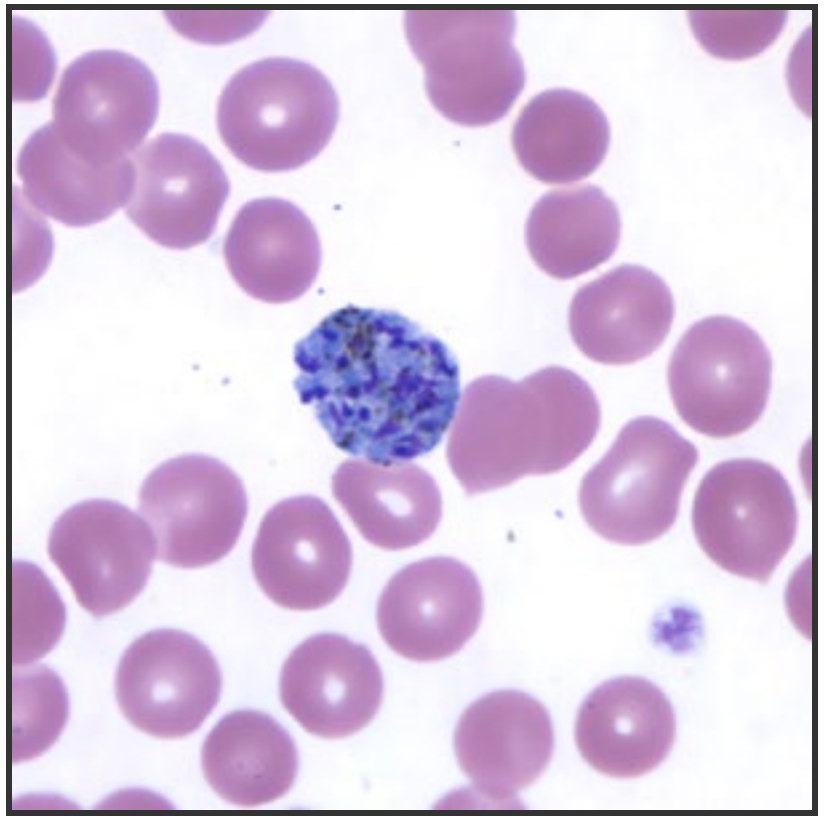
Plasmodium vivax : schizonts

- Mature schizonts usually contain >13 (16-24) merozoites.
- Mature schizonts have coalesced pigment.
- Infected RBCs may be enlarged.
- Schüffner's dots may be present.



Plasmodium vivax : mature schizonts

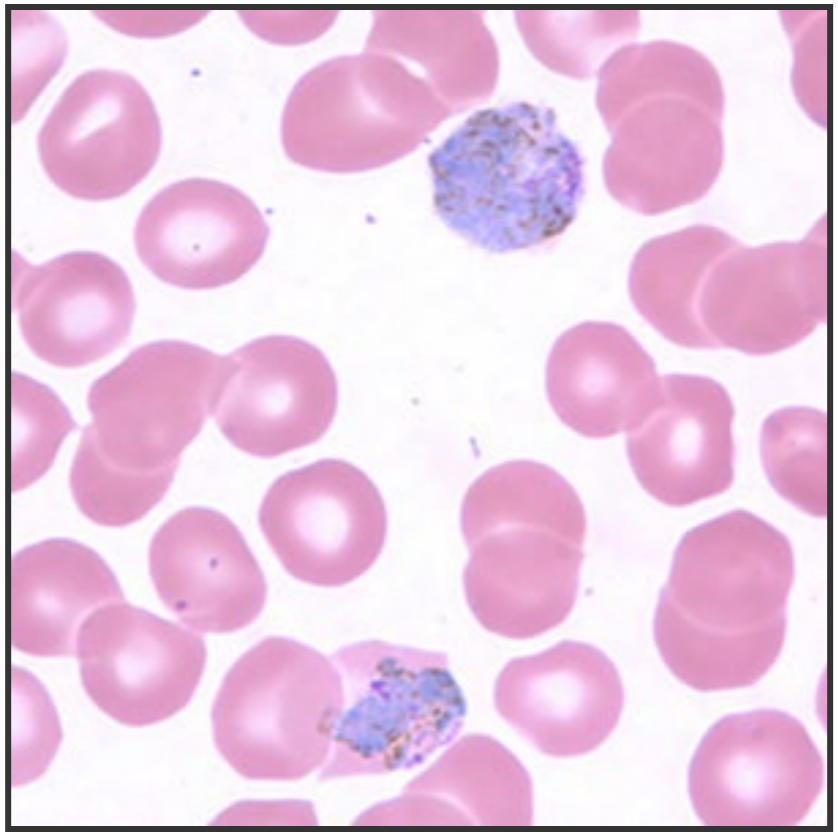


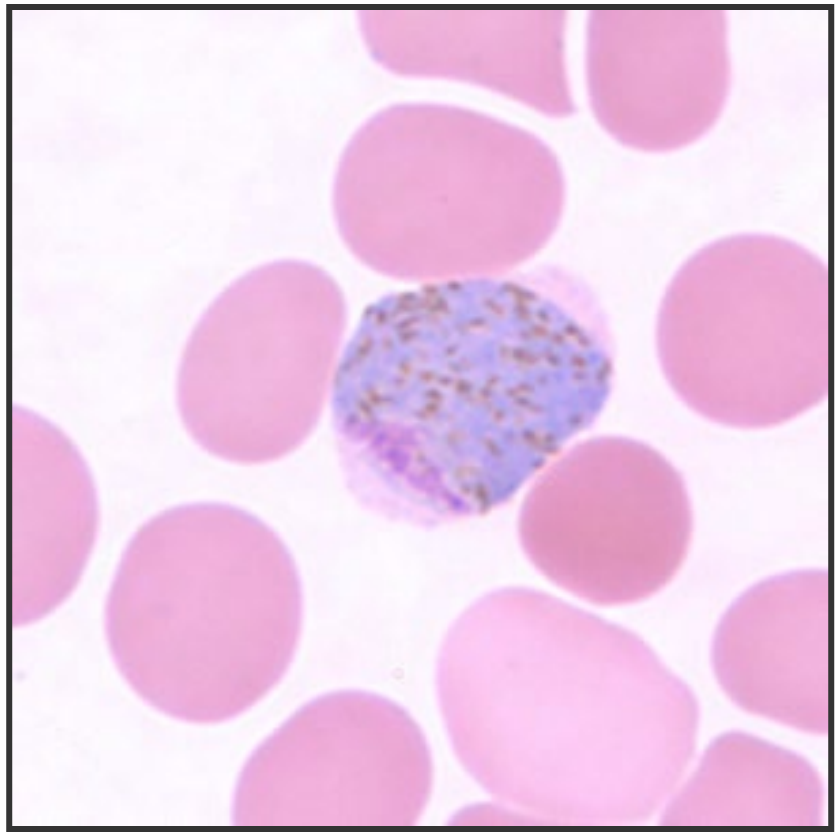


Plasmodium vivax : gametocytes

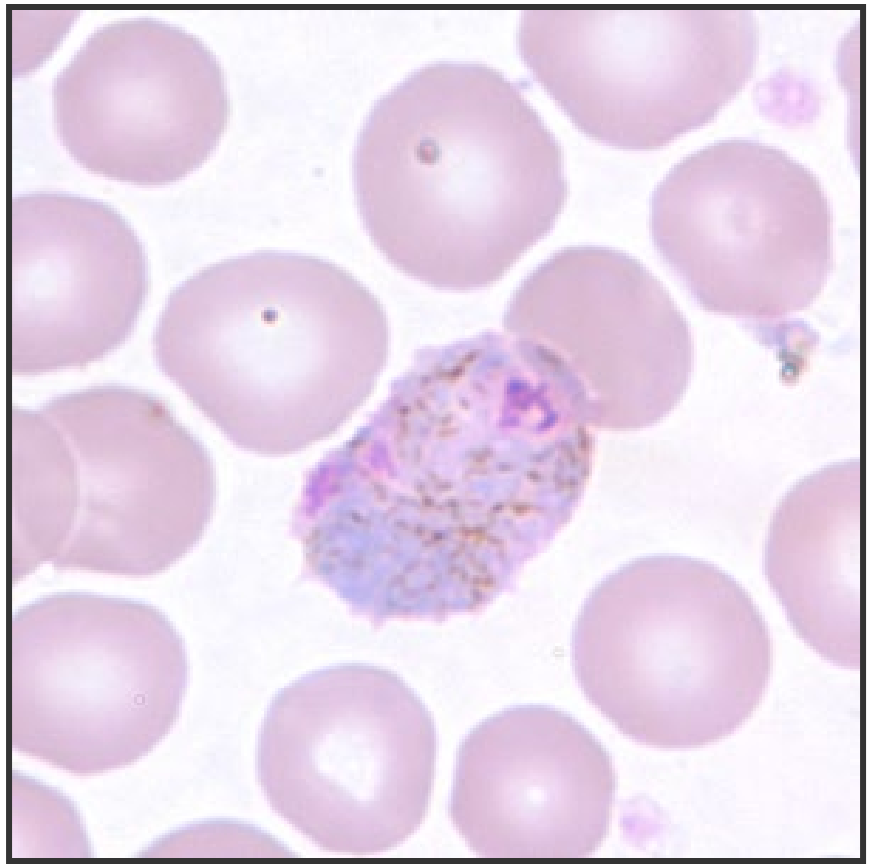
- Mature gametocytes enlarged to 2x normal RBC.
- Round to oval and usually fill host RBC.
- Pigment is usually fine and evenly dispersed.
- Schüffner's dots may be seen.

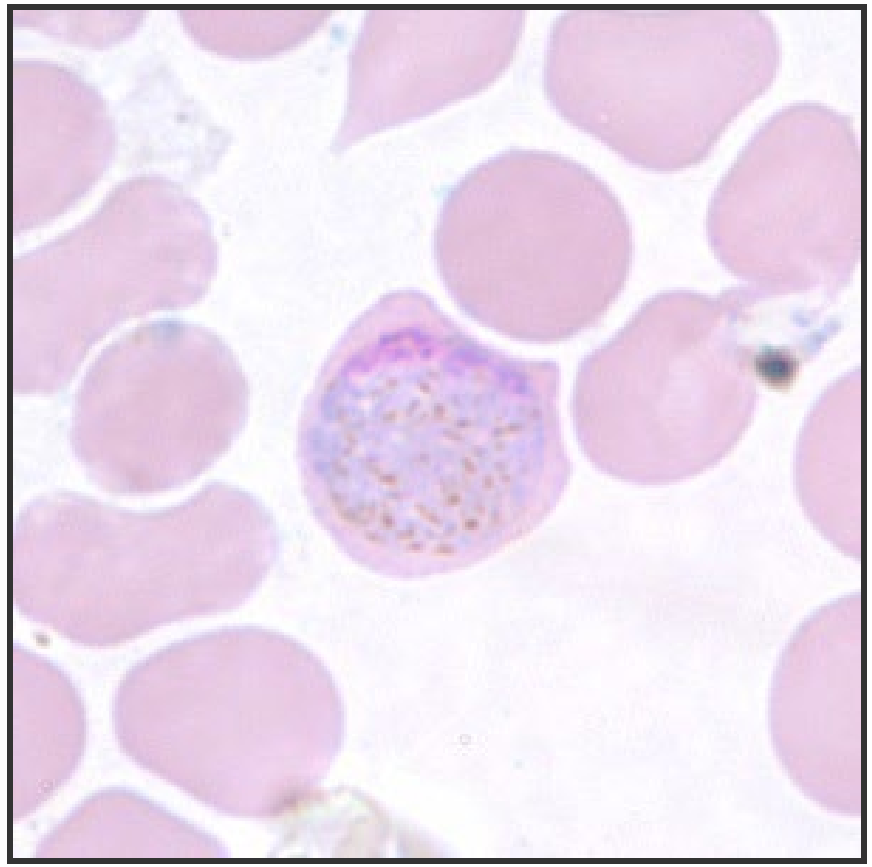
Plasmodium vivax : gametocytes



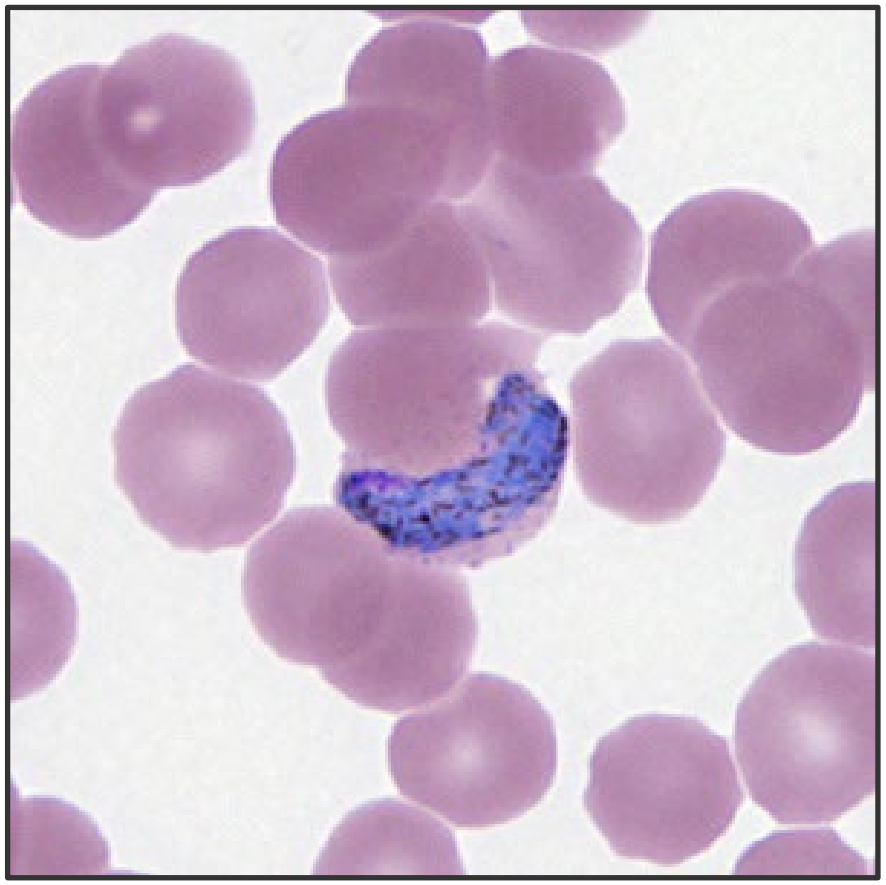


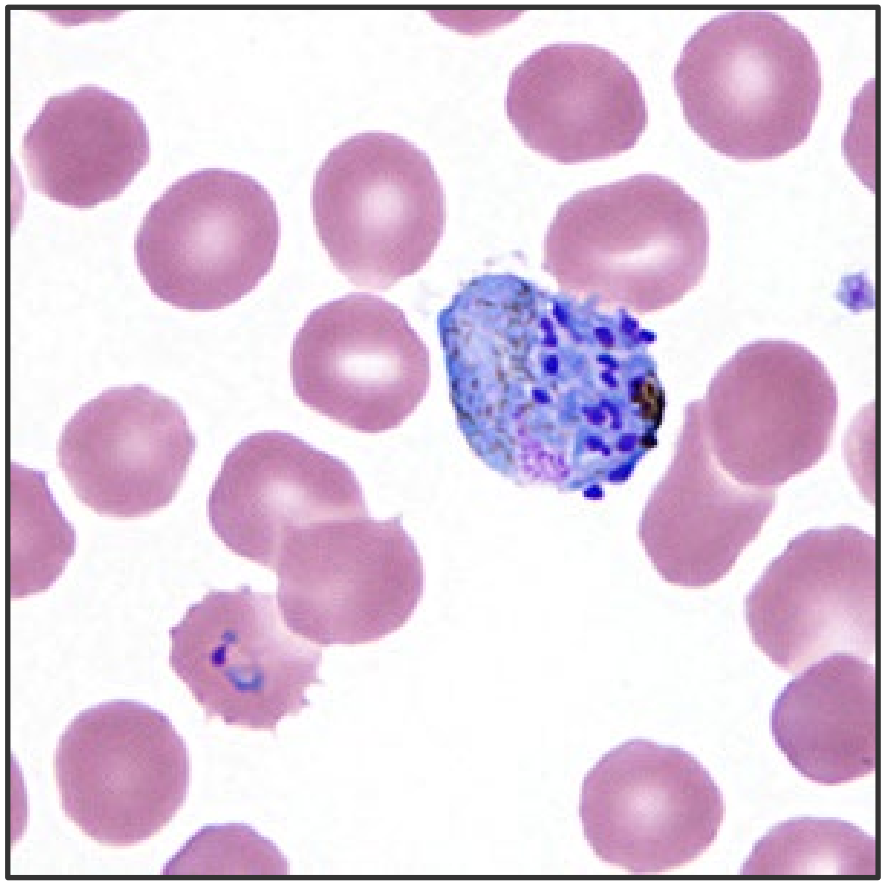
Plasmodium vivax : gametocytes



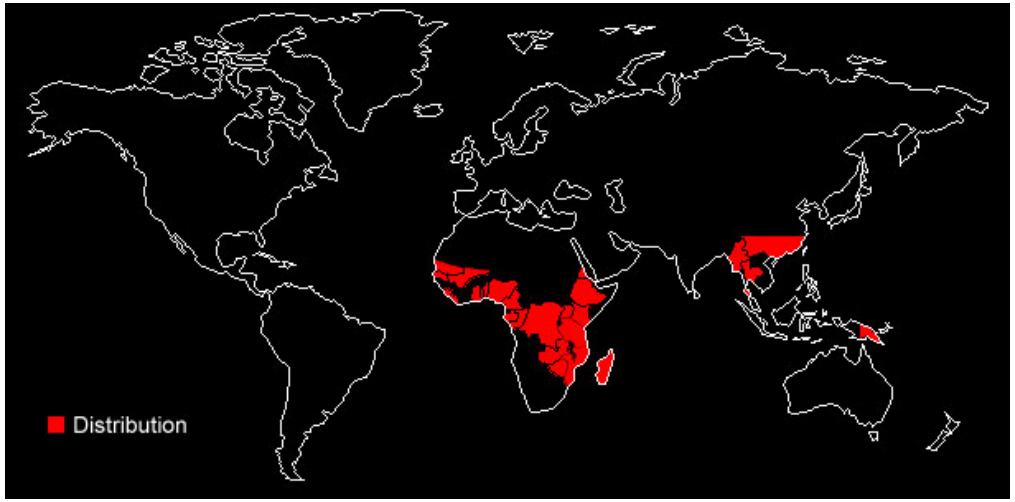


Plasmodium vivax : odd presentations

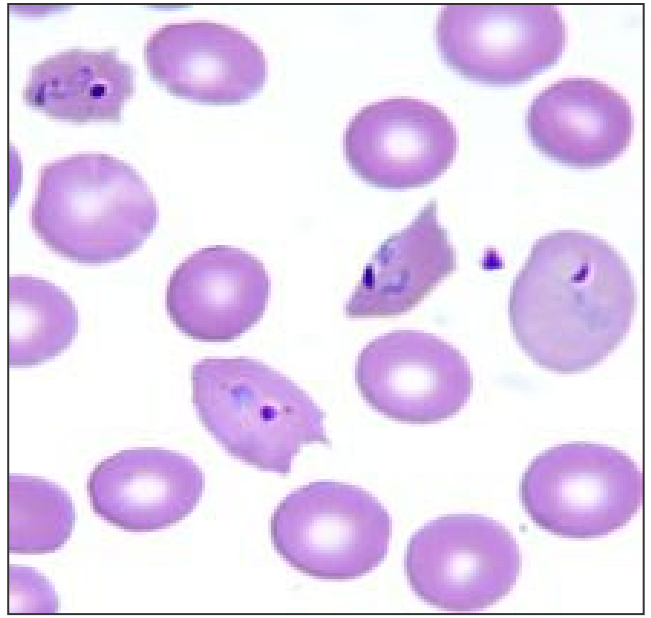




Plasmodium ovale



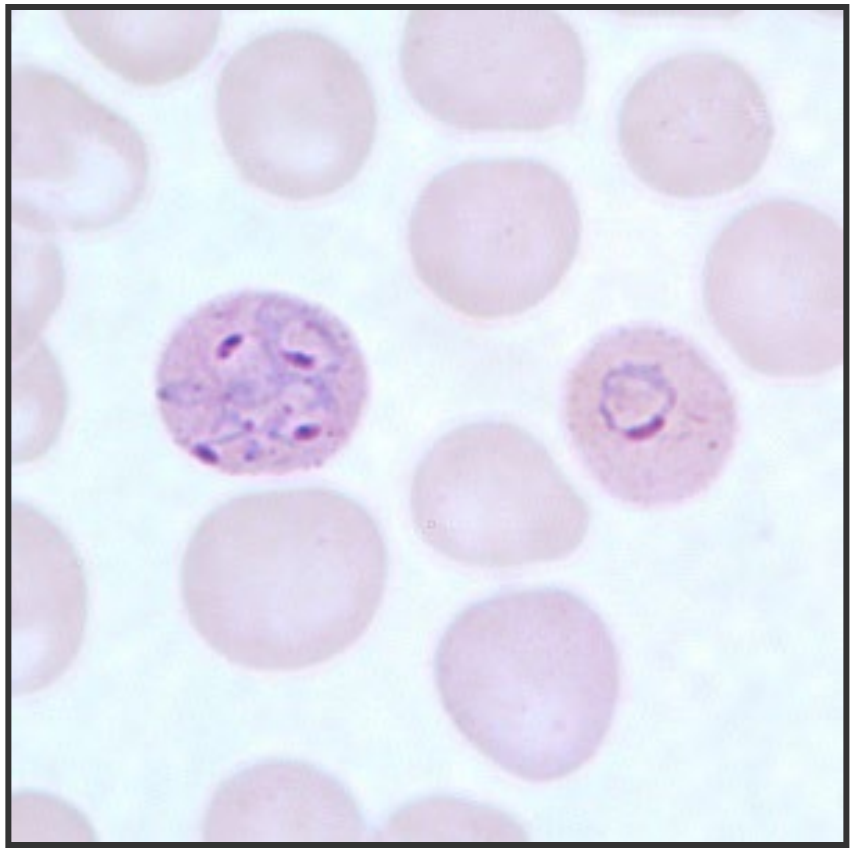
- Distribution: West, tropical Africa; Southeast Asia
  - » Only species **not** known to have been introduced to the New World
  - » Recently broken into two 'groups' (*wallikeri*, *curtisi*), but nomenclatural issues being resolved
- Third most common species reported in the US healthcare system
  - » Most geographically constrained
- Fever cycles every ~48 hours
- Liver phase (hypnozoites) can reactivate months or years after infection

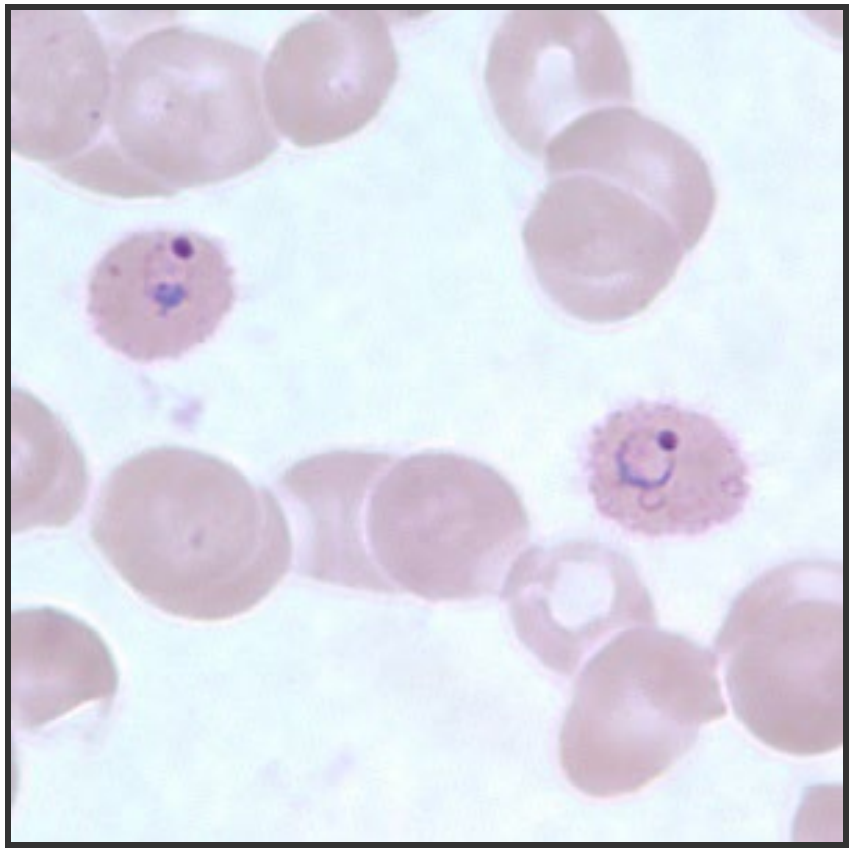


Plasmodium ovale : rings

- Usually thicker with a single chromatin dot (may be double).
- Often one parasite per infected RBC, but multiply-infected RBCs not uncommon.
- Schüffner's dots may be present.

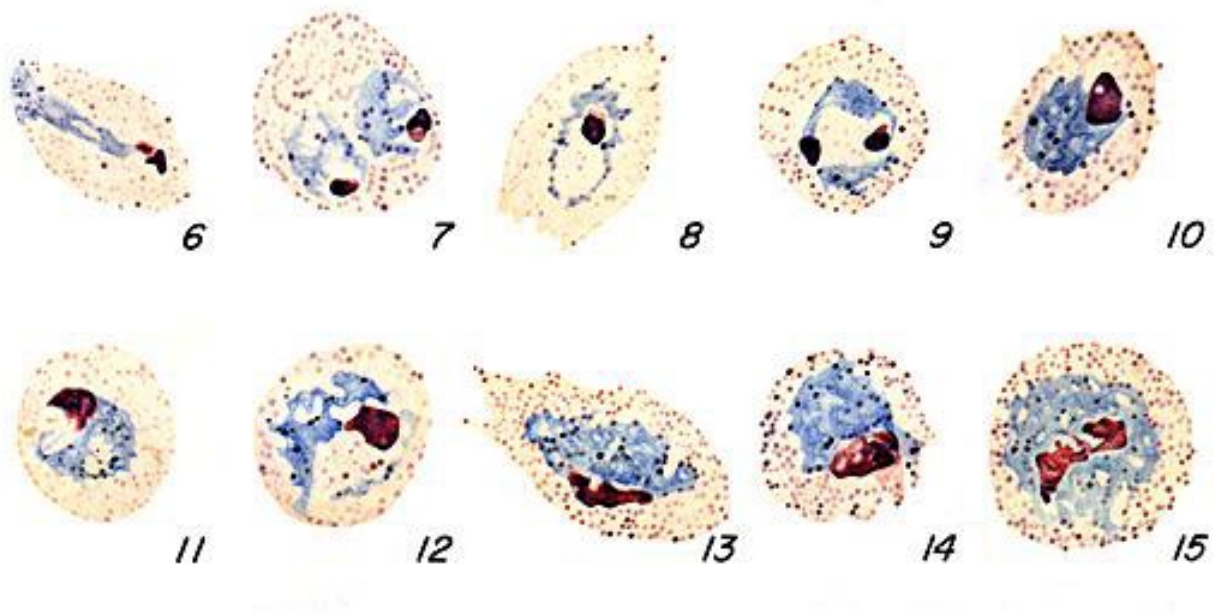
Plasmodium ovale : rings



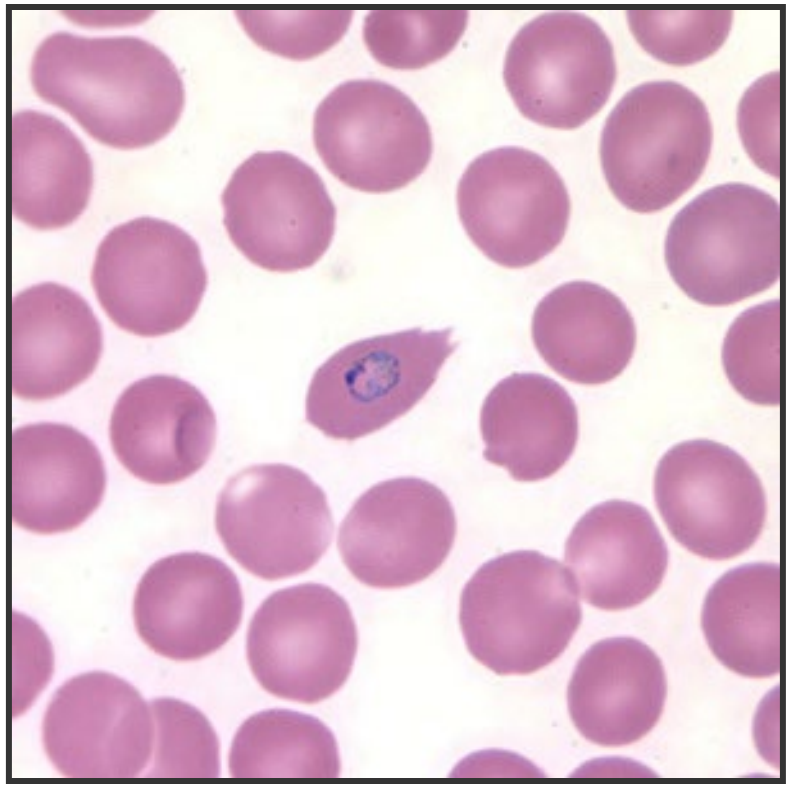


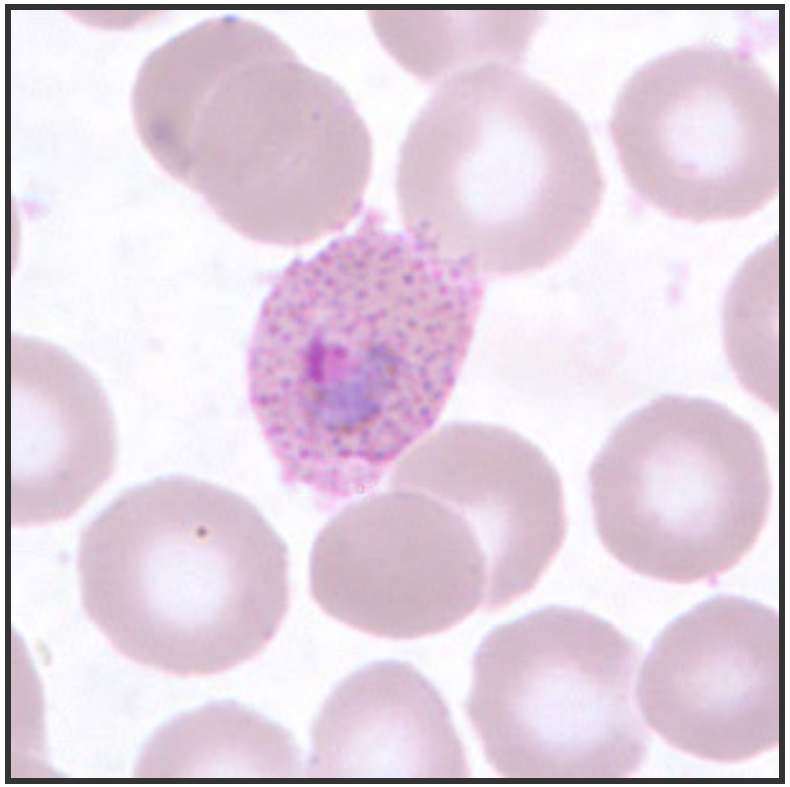
Plasmodium ovale : mature trophozoites

- Trophozoites may start to become amoeboid, but not to the degree as *P. vivax*.
- Schüffner's dots may be seen.
- Enlarged, but usually not as much as with *P. vivax*.
- Elongation and fimbriation common (note difference from crenation).

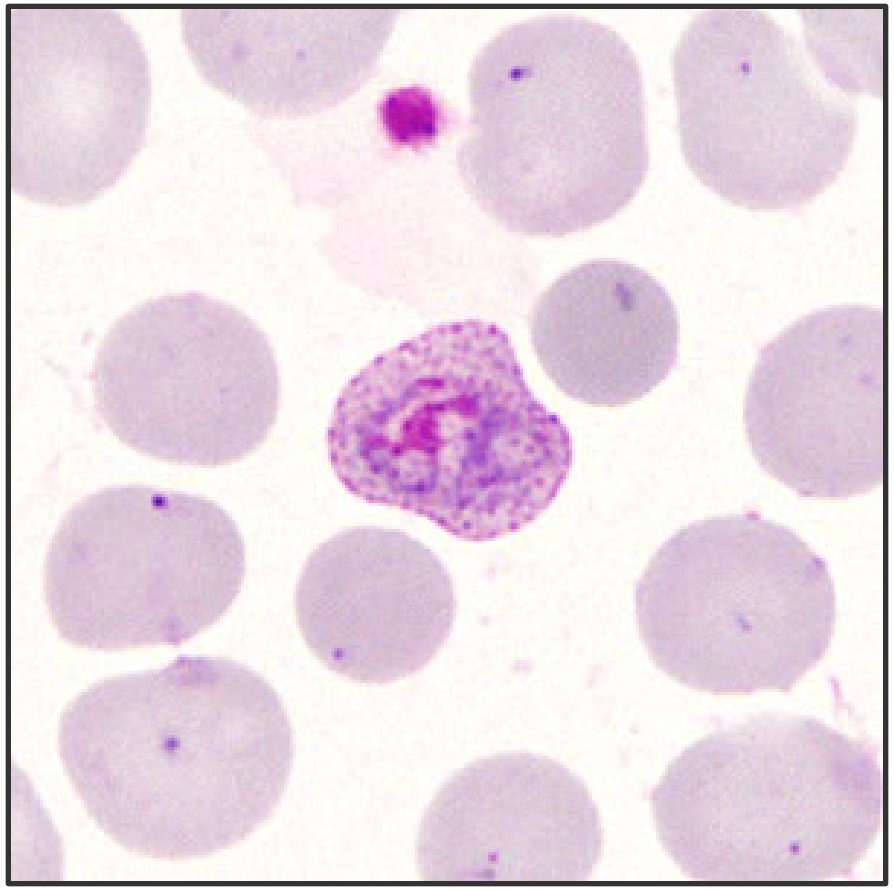


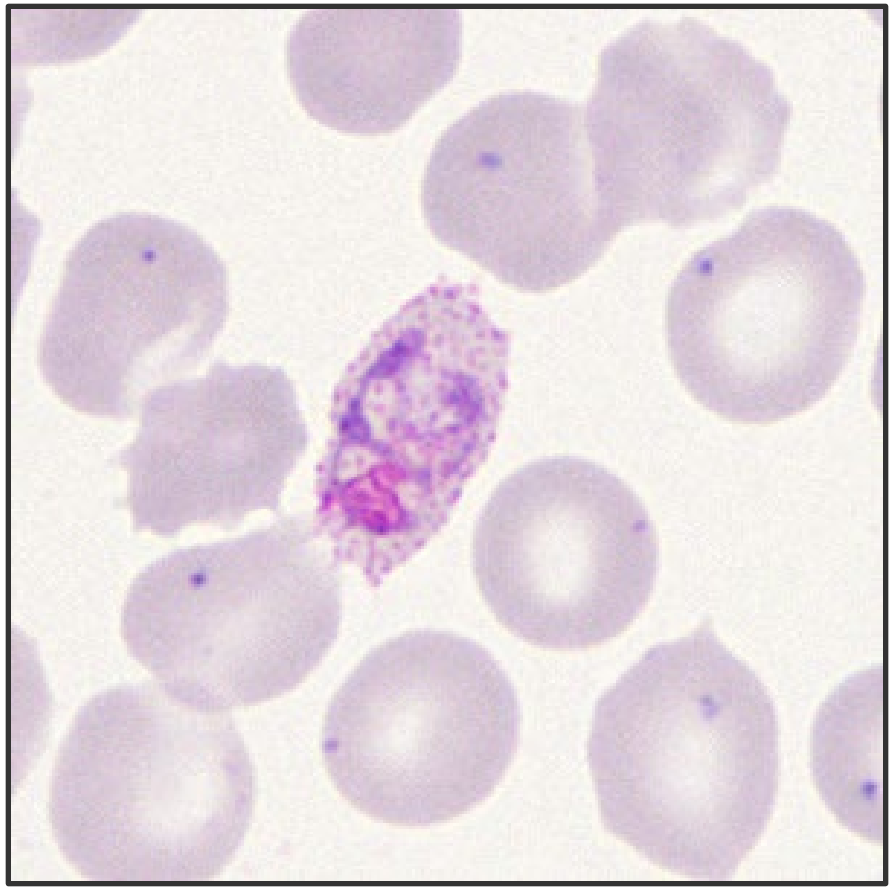
Plasmodium ovale : developing trophozoites





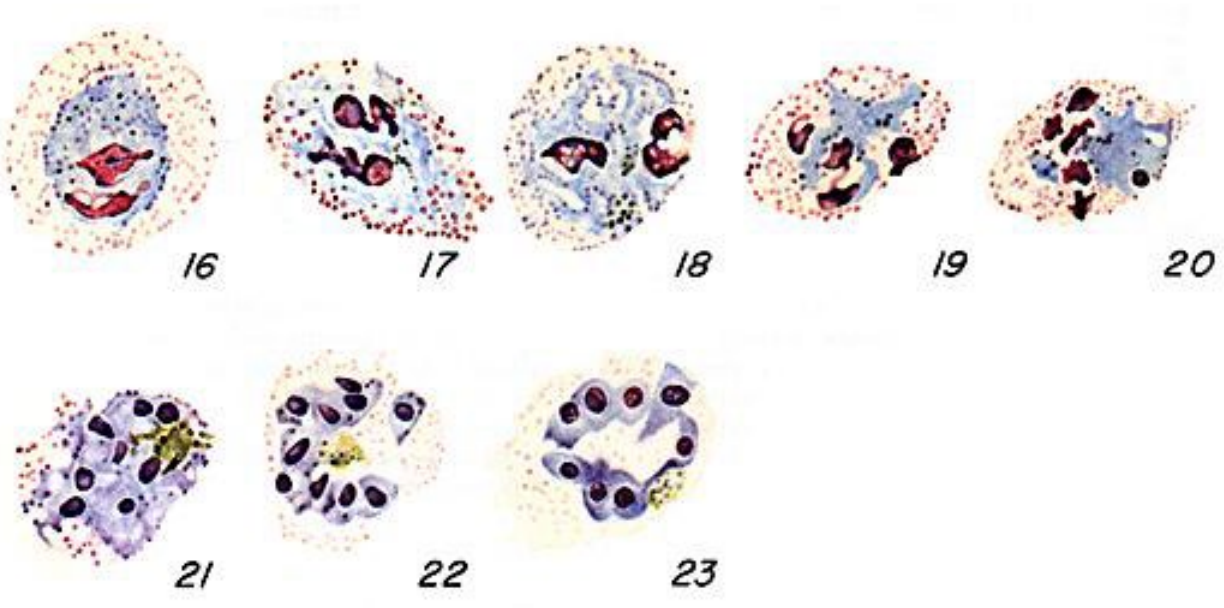
Plasmodium ovale : mature trophozoites



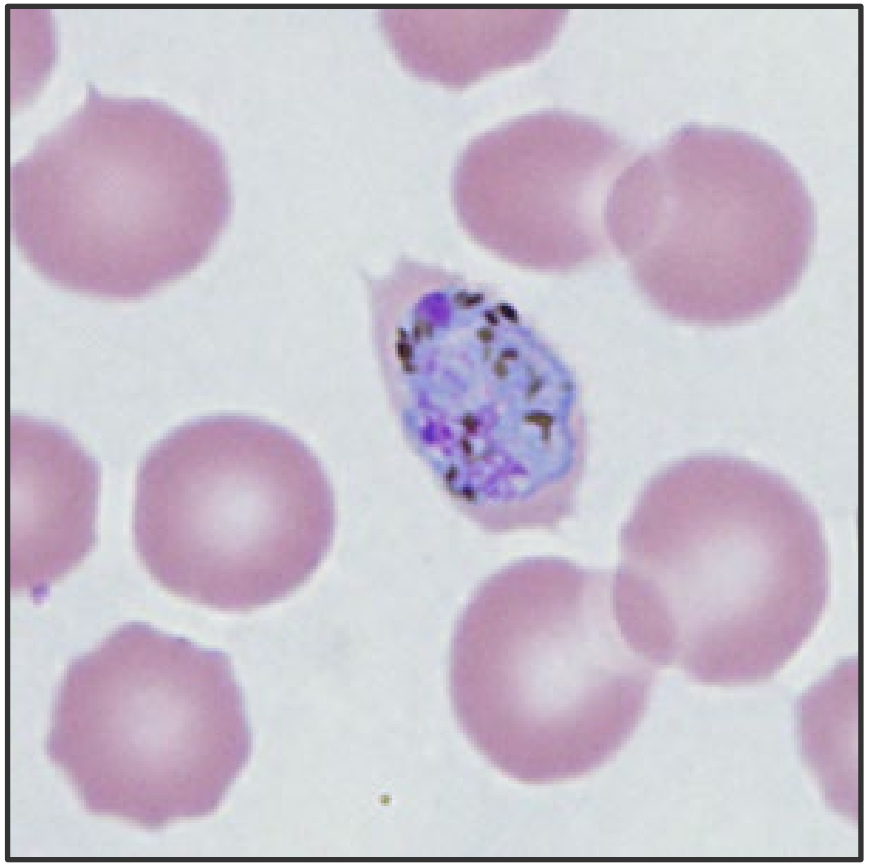


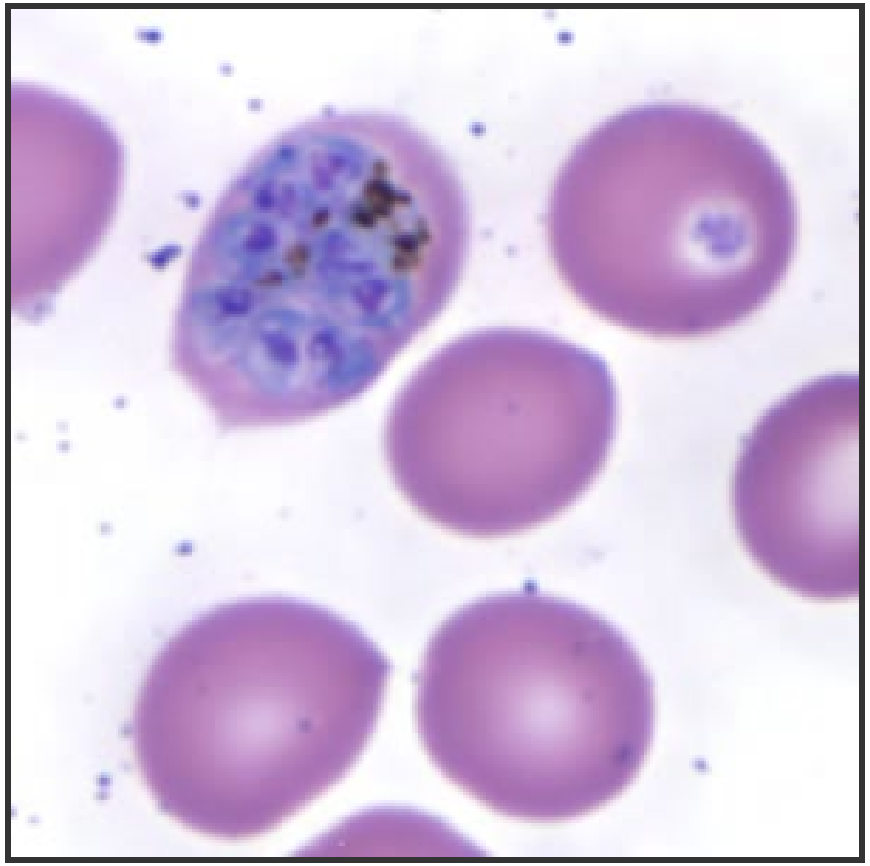
Plasmodium ovale : schizonts

- Mature schizonts usually have <13 merozoites (6-16).
- Schüffner's dots may be seen.
- Enlarged, but usually not as much as with *P. vivax*.
- Elongation and fimbriation can occur (note difference from crenation) but may also be rounded.



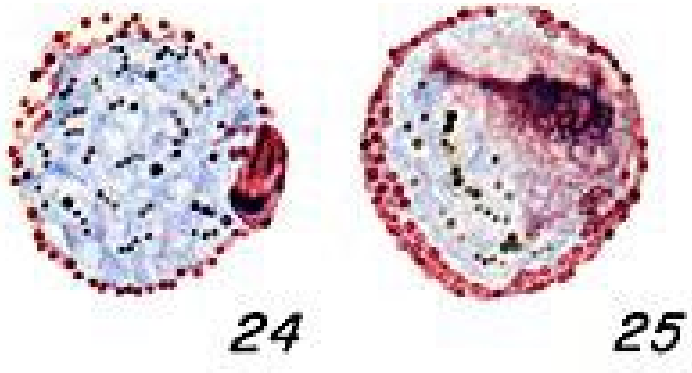
Plasmodium ovale : schizonts



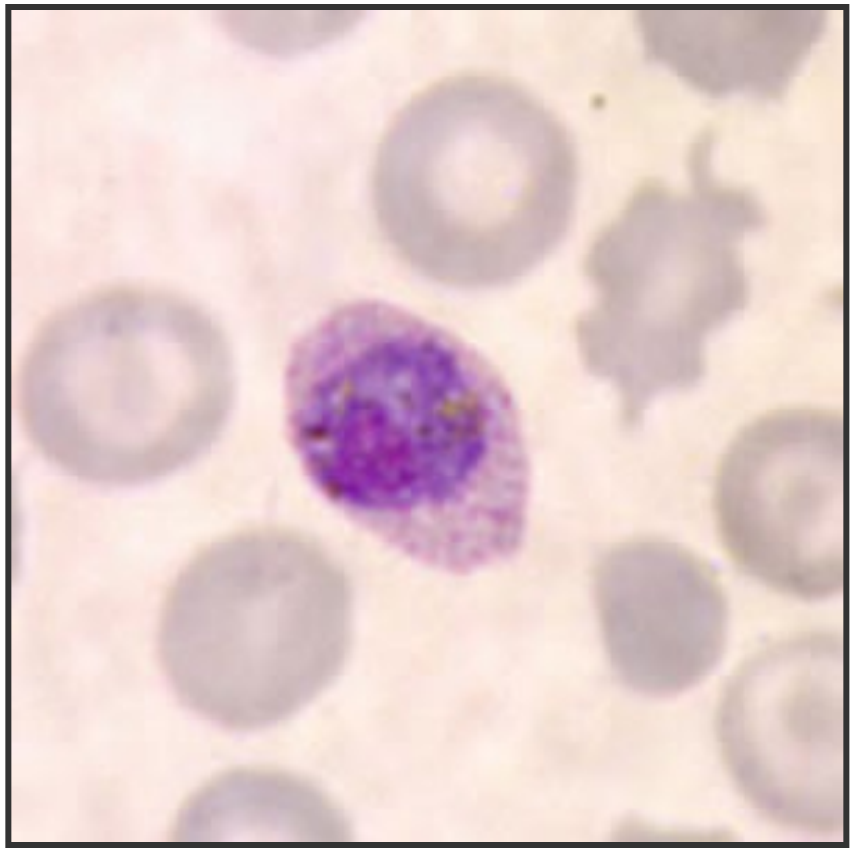


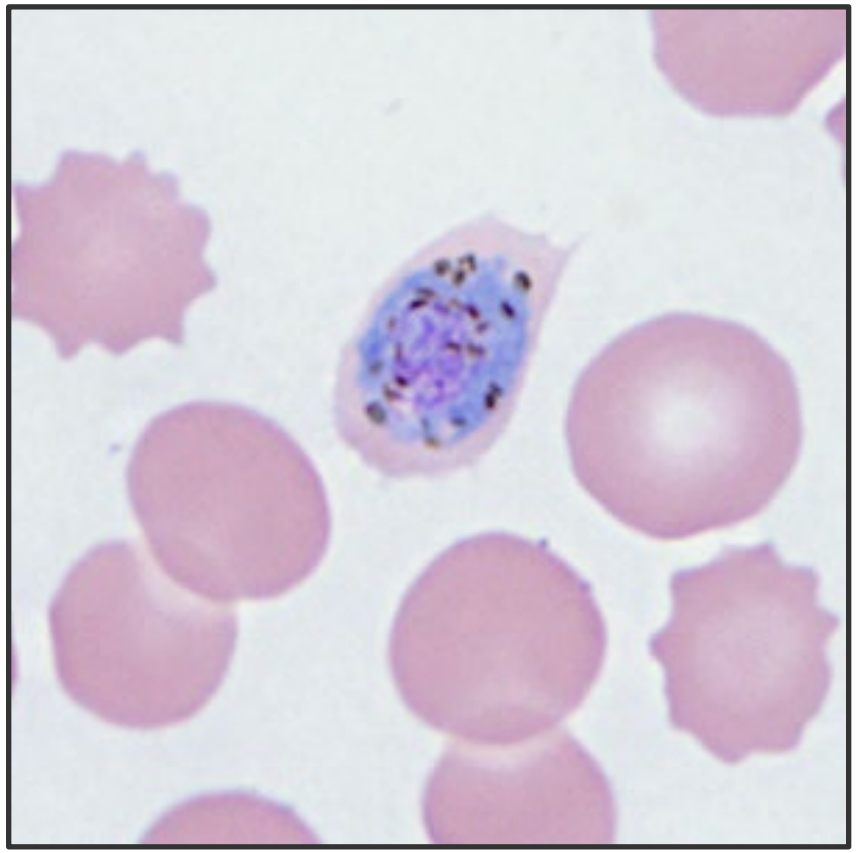
Plasmodium ovale : gametocytes

- Enlarged, but not usually as big as *P. vivax* (1-1.25x normal RBC).
- Pigment usually more coarse than *P. vivax*.
- Schüffner's dots may be seen.
- Usually rounded (may be elongated and fimbriated, especially in immature gametocytes).

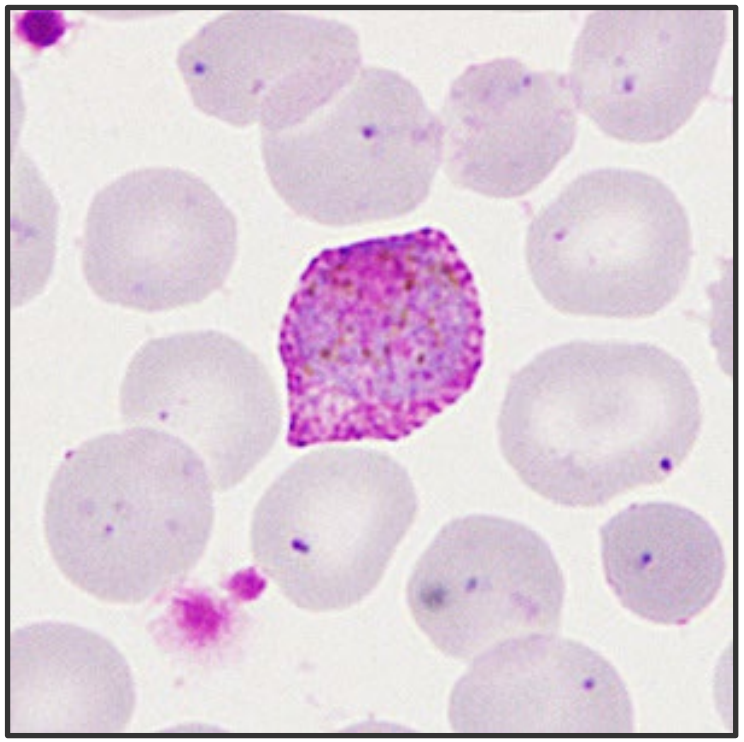


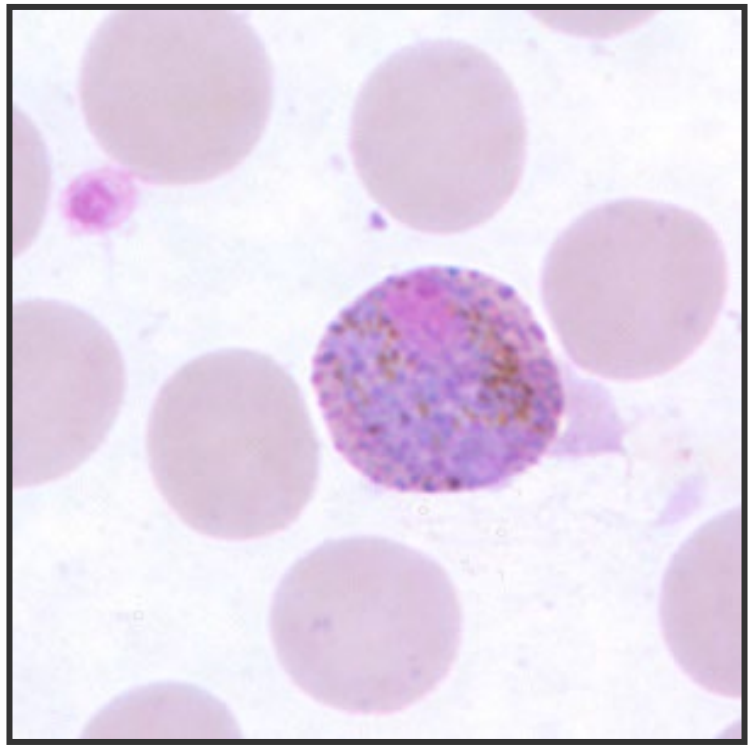
Plasmodium ovale : developing gametocytes

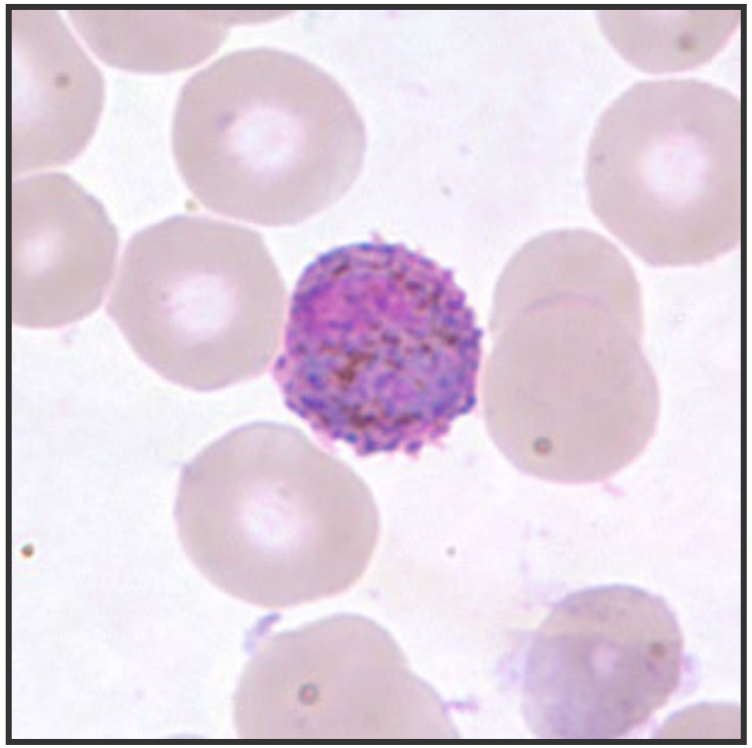




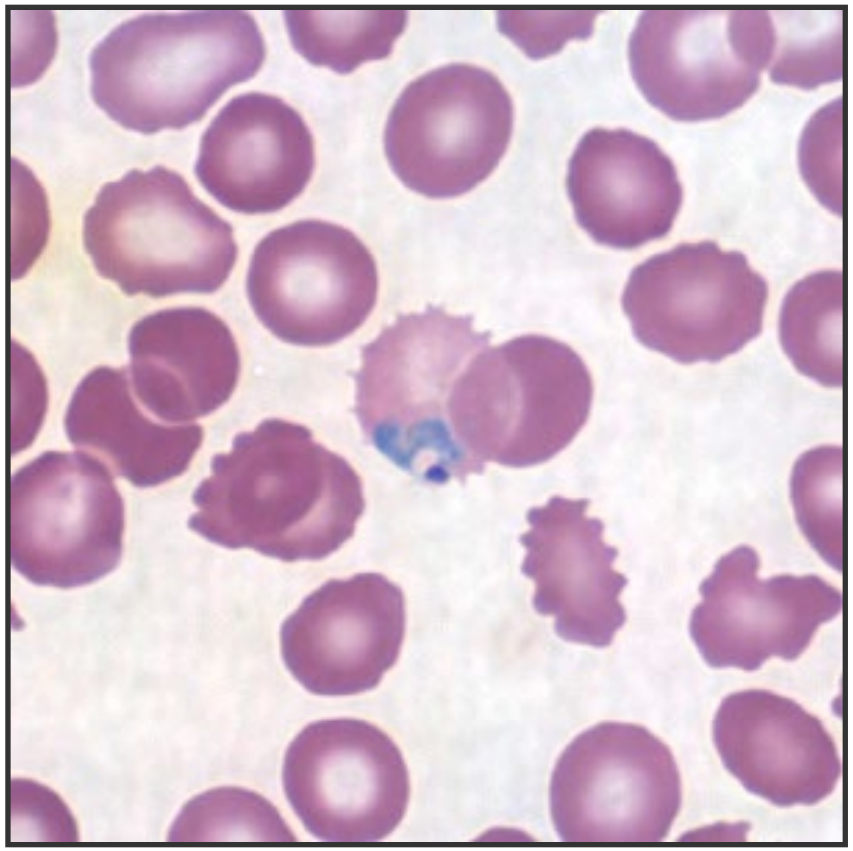
Plasmodium ovale : mature gametocytes

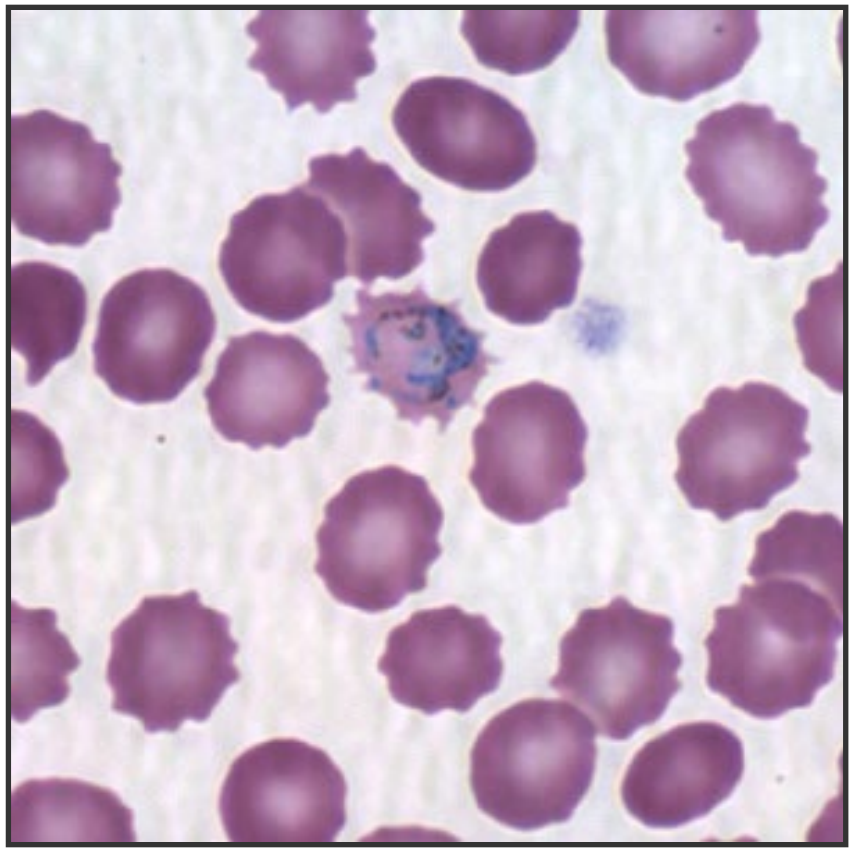






Crenation (not fimbriation)





Babesiosis

The primary morphologic differential for malaria

Babesiosis

- Caused by apicomplexan parasites in the genus *Babesia*.
- Transmitted by ticks in the genera *Ixodes* and (possibly) *Dermacentor*
- Several species endemic to North America (*B. microti*, NE; *B. duncani*, West, PNW; *Babesia* MO-1, PNW, Missouri River Valley).
- Most at-risk groups include immunocompromised, elderly, asplenic patients.
- Species cannot be separated morphologically. PCR or serologic testing needed for species-level ID (epidemiologic data can be helpful).

Life Cycle of *Babesia microti*

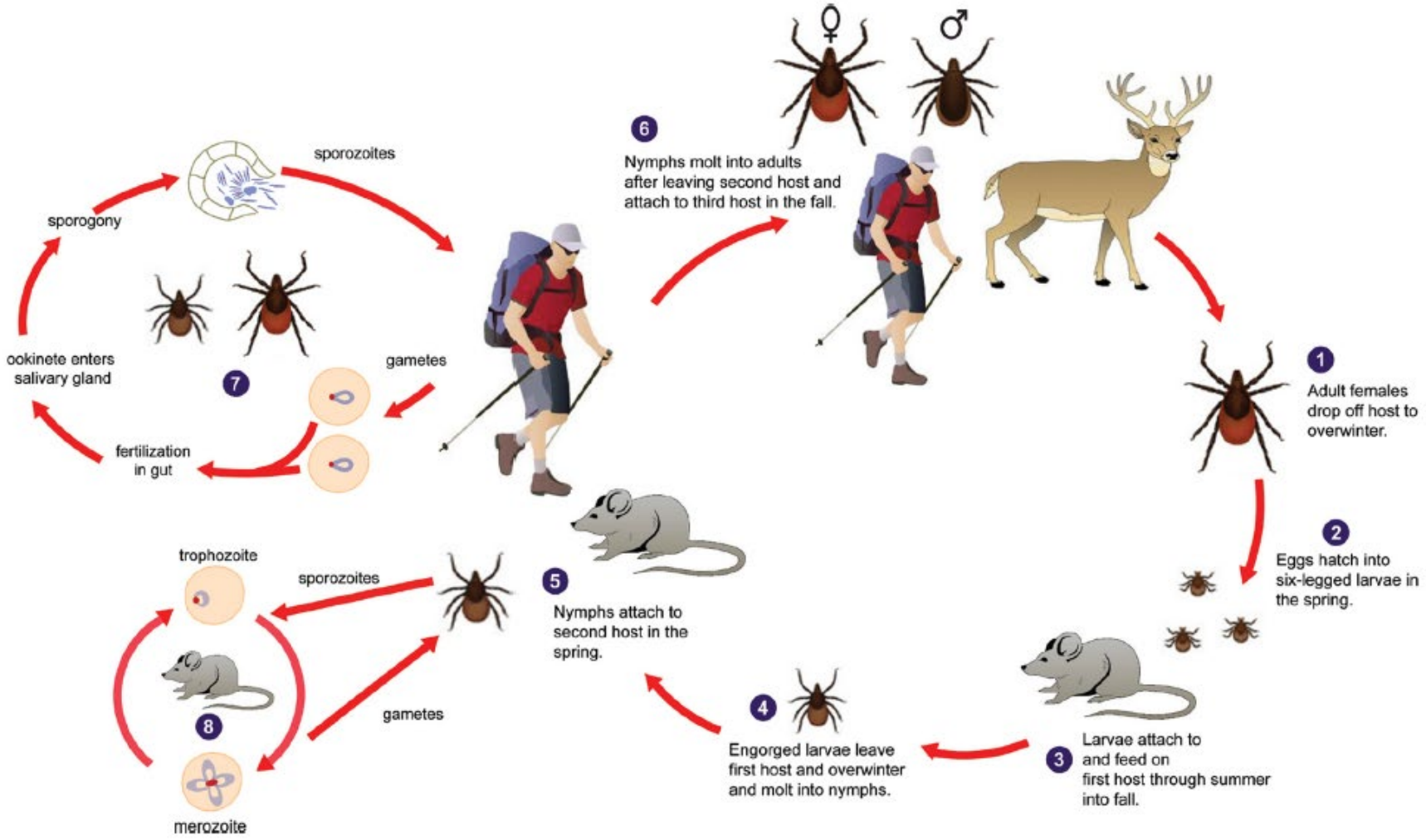
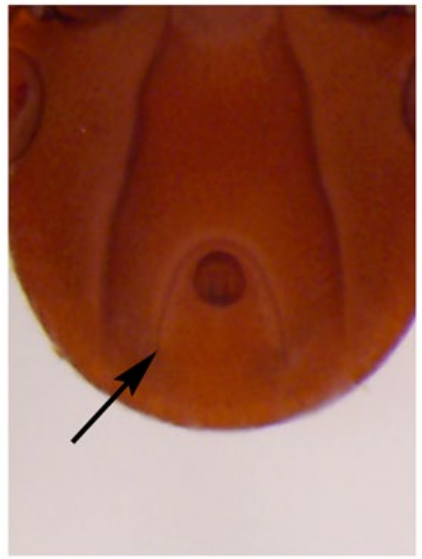


Image from: Westblade et al., J Clin Microbiol. 2017. PMID 28747374

North American Medical Important of *Ixodes*

- *Borrelia burgdorferi*, *B. mayonii* (Lyme disease)
- *Anaplasma phagocytophilum* (HGA)
- *Babesia* spp. (babesiosis)
- *Borrelia miyamotoi*
- *Ehrlichia muris eauclairensis* (EML)
- Powassan Virus II (Deer Tick Virus)

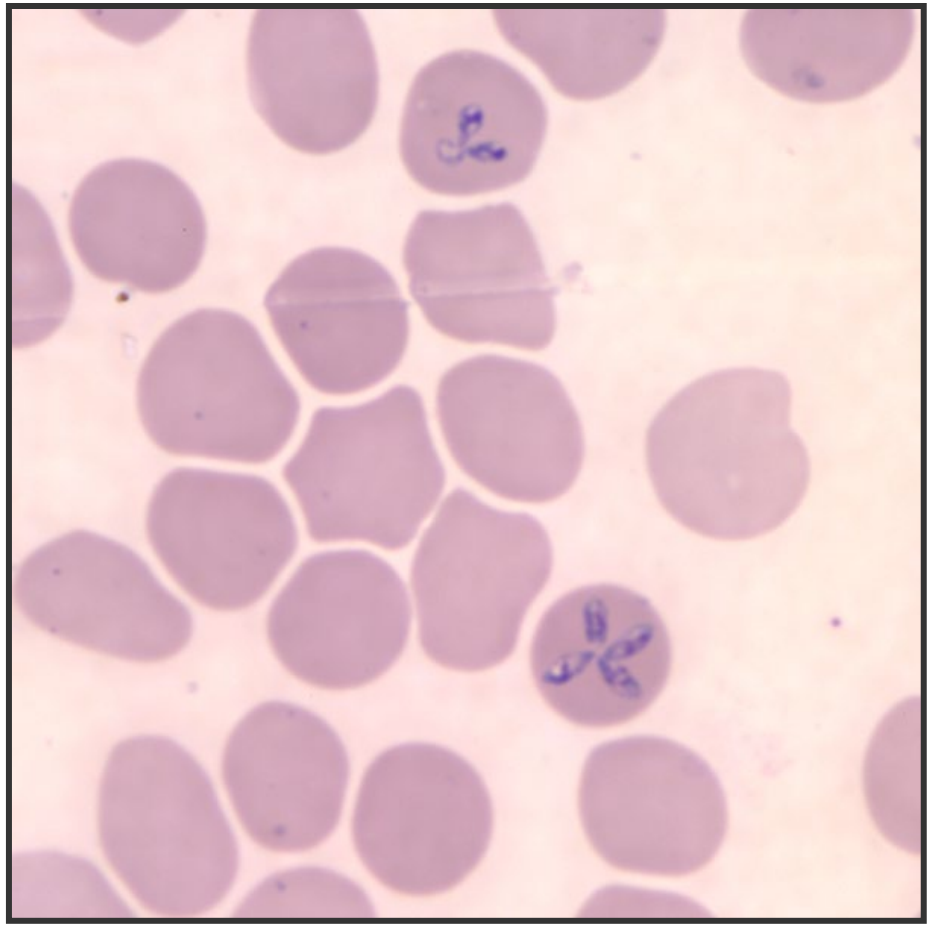


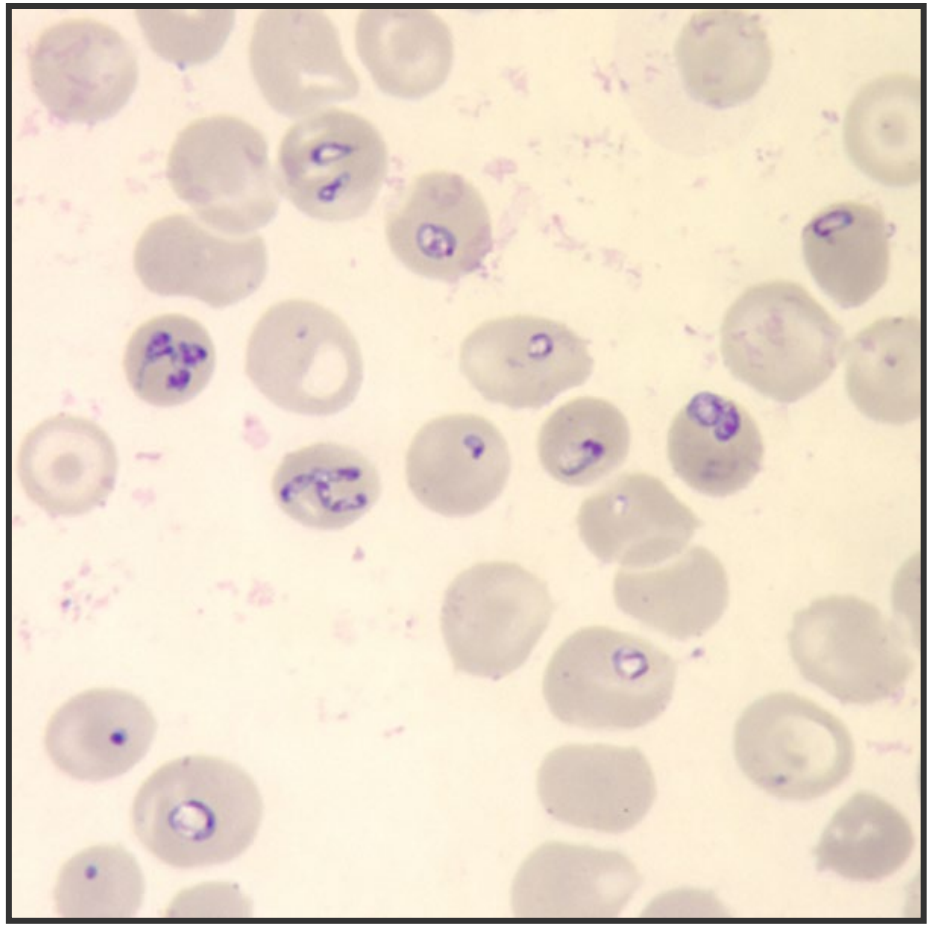
Images from Mathison BA, Pritt BS 2015, CAP

Babesia spp. – Morphologic Identification

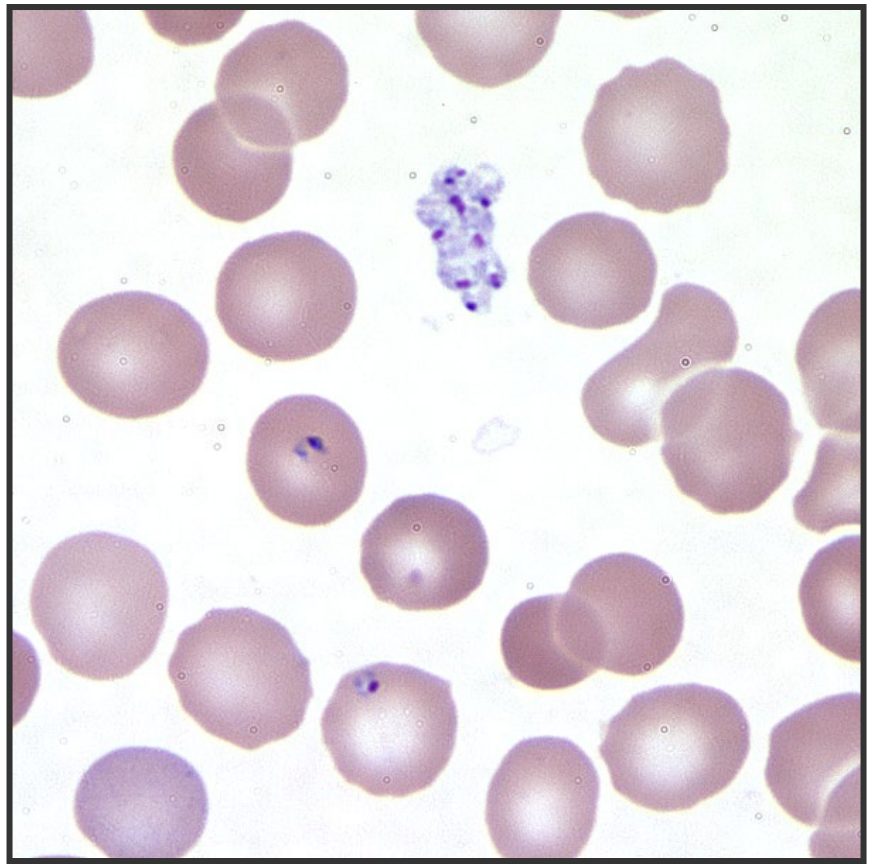
- Extraerythrocytic and intraerythrocytic forms may be found.
- Morphologically resemble ring forms of *Plasmodium* spp.
- Erythrocytic forms may be pyriform, oval, or round, and often are amoeboid or vacuolated.
- More than one parasite can be found in a single RBC.
- Pigment is not present in the organisms.
- There is no enlargement of the RBC.
- No gametocytes or schizonts detected in blood films.
- In some species, tetrads are formed as a result of fission ('Maltese Cross').

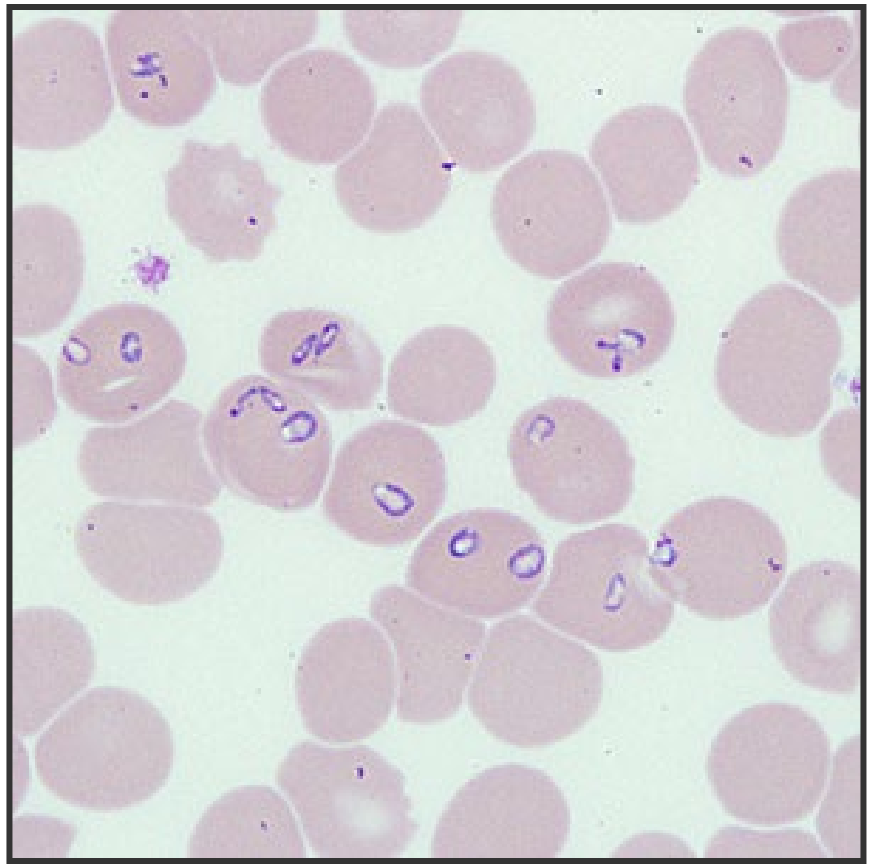
Babesia spp. – thin films





Babesia spp. – thin smears





Extraerythrocytic forms

Babesia vs. *Plasmodium*

Feature	*Plasmodium*	*Babesia*
Ring morphology	Generally consistent within a given specimen	Highly variable in size, shape, form
Advanced stages morphologically-defined	Yes (e.g., schizonts, gametocytes)	No
Enlargement of infected RBC	Sometimes	Never
Pigment production	Yes	Never
Extracellular forms	No[1]	Yes

[1]Individual merozoites of recently-ruptured schizonts may be observed

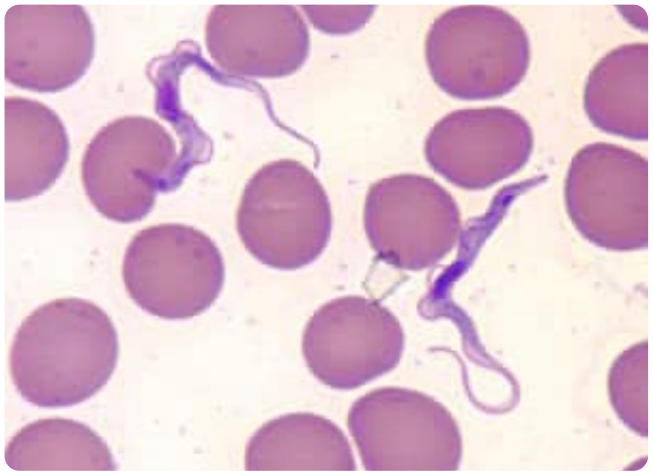
Take-home and other important messages in *Plasmodium* and *Babesia* identification

- Always have a 'big picture' approach
- Make sure you examine enough examples (if the parasitemia is high enough) to appreciate the full extent of morphologic variation in the specimen
- When using size as a criterion, do it where there is a good monolayer with no overlapping or compressing of RBCs (feathered edge)
- Be careful in interpreting classic textbook definitions
  - » Double-chromatin dots, multiply-infected rings, band- and basked-form trophozoites
- When counting merozoites in a schizont, make sure the schizont is mature (look for coalesced pigment)
- Presence of pigment rules-out *Babesia*
- Recommend having a backread policy and a way of handling discrepant results
- Archive all positive specimens for training new staff and periodic competency assessment.

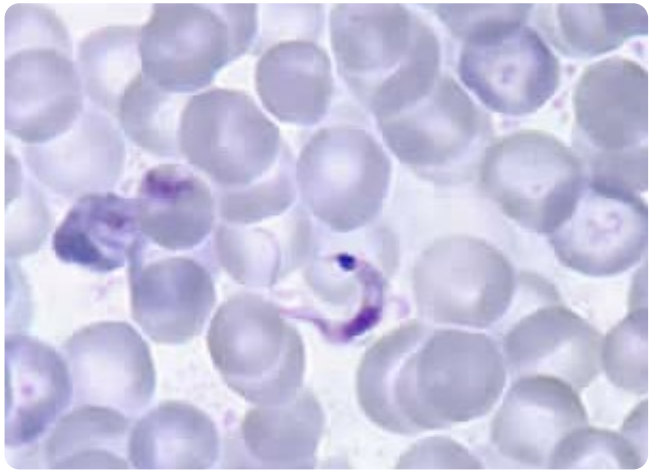
Trypanosomiasis

Trypanosomiasis

- Kinetoplastid protozoans in the genus *Trypanosoma*
- Two species responsible for most human infections:
  - *T. brucei* (African trypanosomiasis; African sleeping sickness)
  - *T. cruzi* (American trypanosomiasis; Chagas disease)

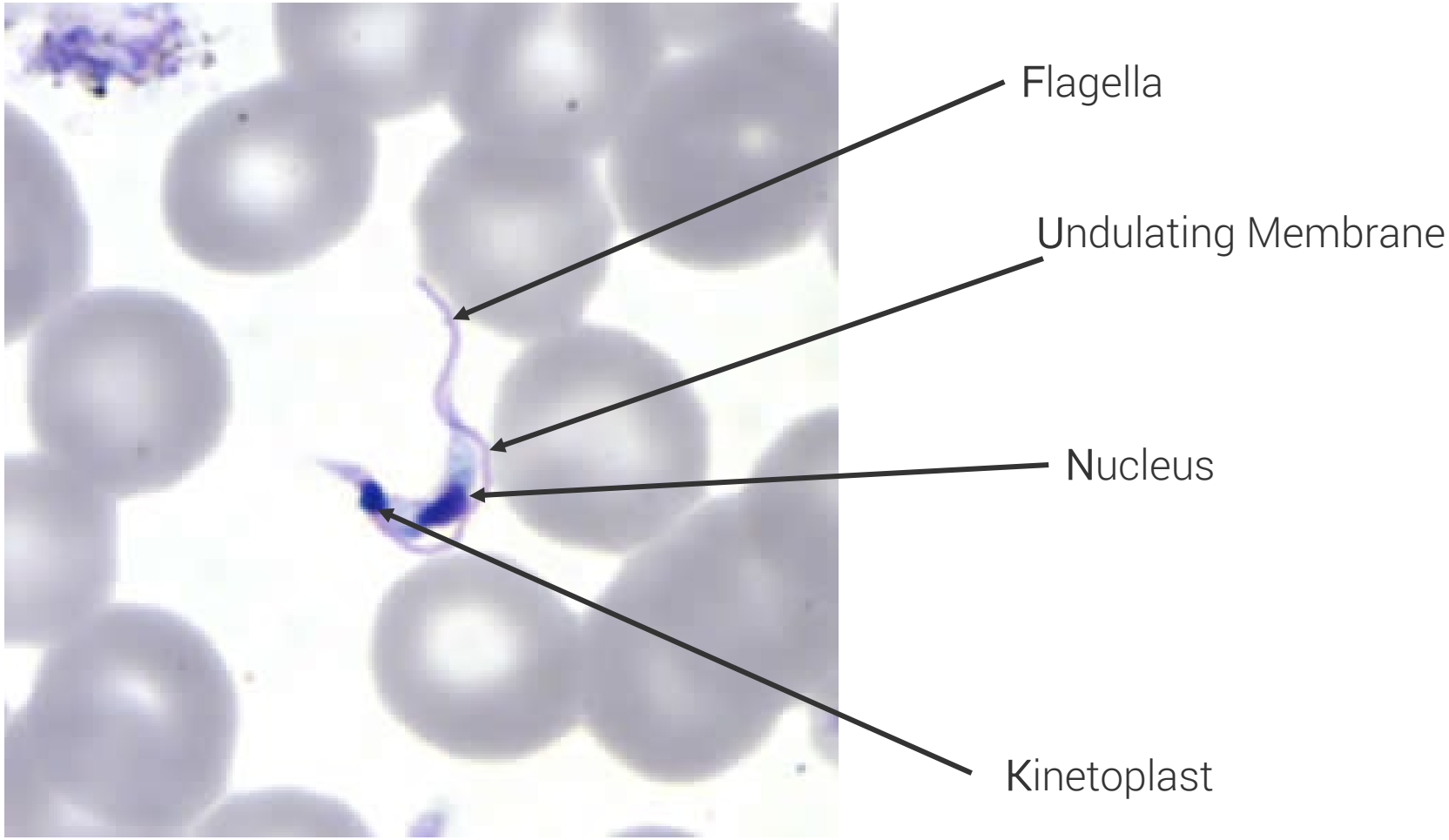


- Other, rare zoonotic species occasionally responsible for human disease:
  - *T. lewisi* (rats; worldwide)
  - *T. rangeli* (mammals; South America)
  - *T. evansi* (ungulates, equids; tropical Africa, Asia, Latin America)



Images courtesy of CDC-DPDx

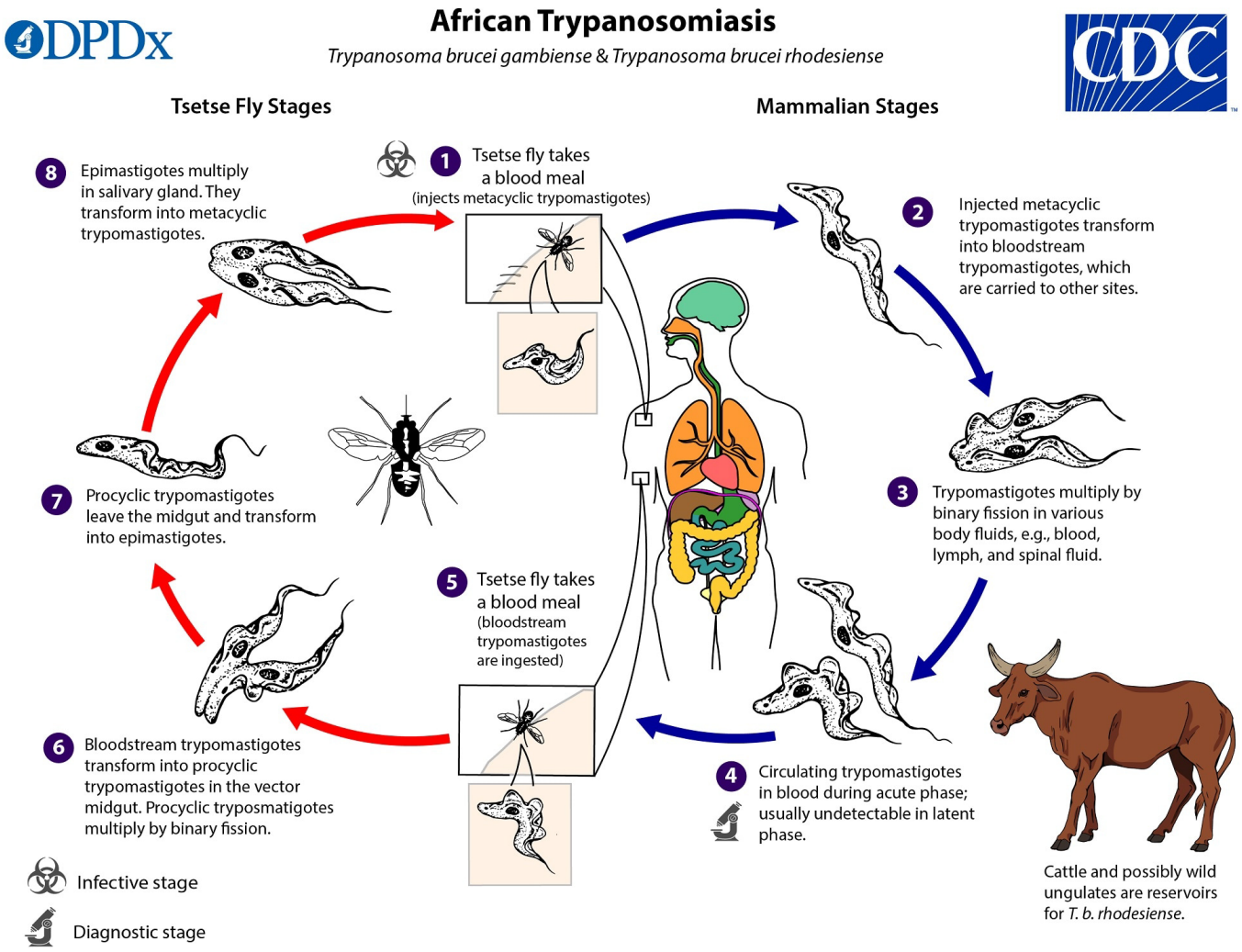
Tryps got da F.U.N.K.!



African Trypanosomiasis

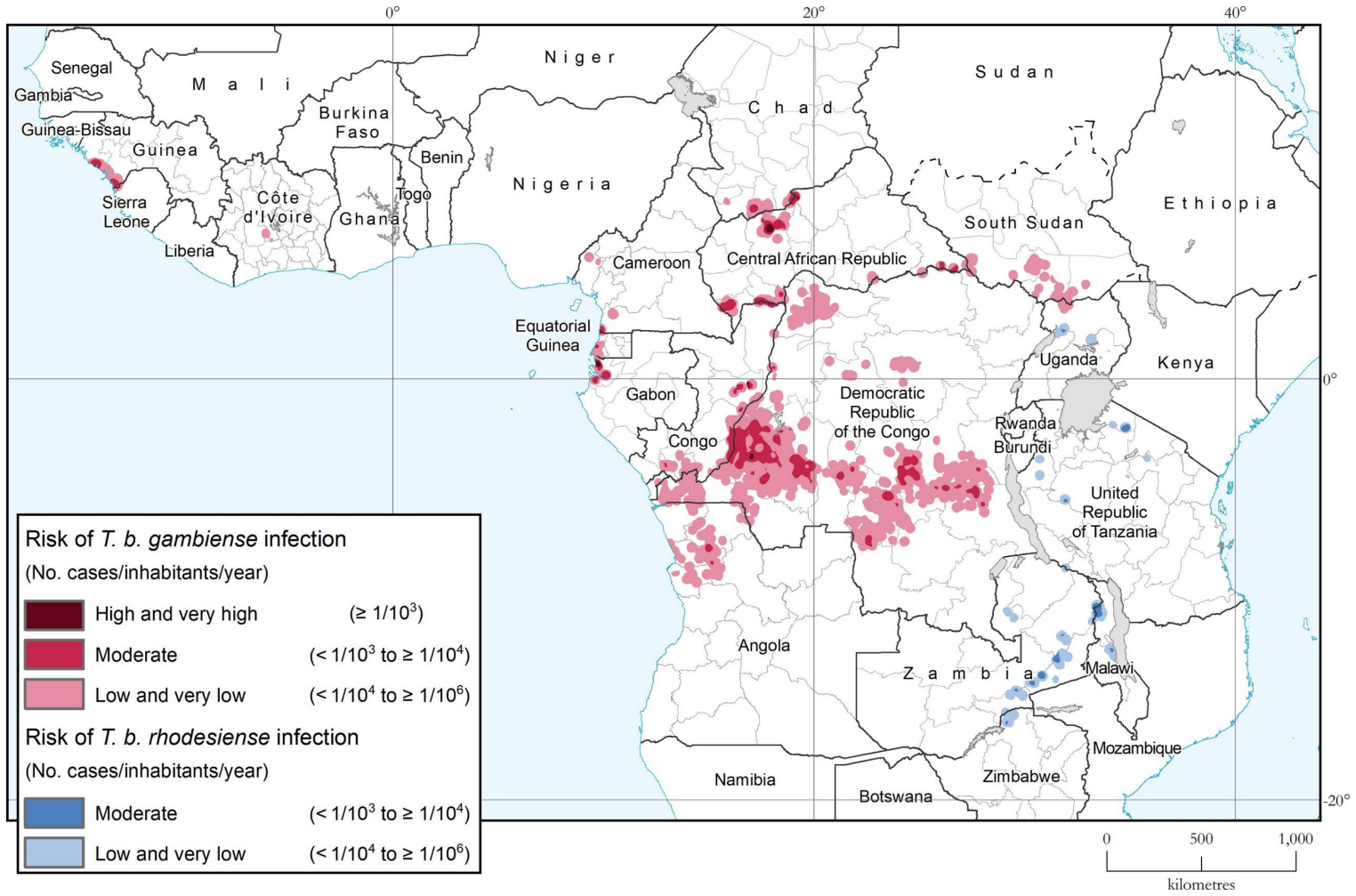
- Caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*
  - » The third, nominate subspecies, *T. b. brucei* infects non-human mammals
- Two subspecies divided geographically:
  - » Western HAT (*T. b. gambiense*) – more chronic, less severe disease
  - » Eastern HAT (*T. b. rhodesiense*) – more acute, more severe disease
- Occurs focally in sub-Saharan Africa (upcoming slide)
- Transmitted by **tsetse flies** in the genus *Glossina*.
- Diagnosis is primarily by the finding of trypomastigotes in blood and other bodily fluids; quantification buffy coat method may be useful for increasing sensitivity.

Life Cycle of *Trypanosoma brucei*



Life cycle courtesy of the CDC-DPDx

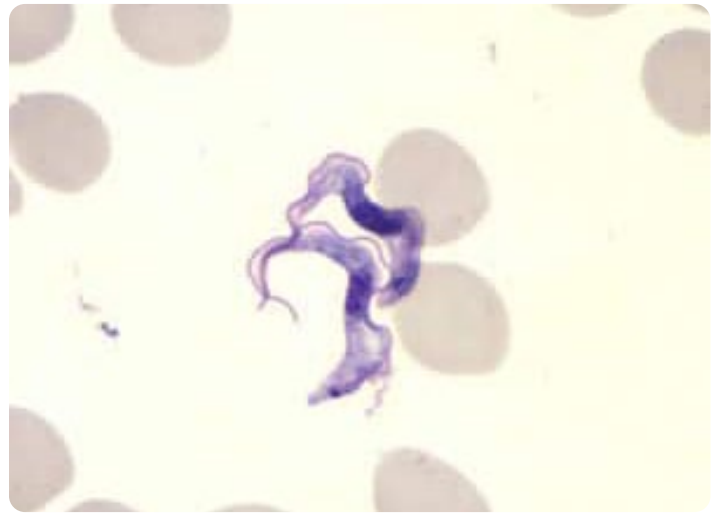
Geographic Distribution of African Trypanosomiasis

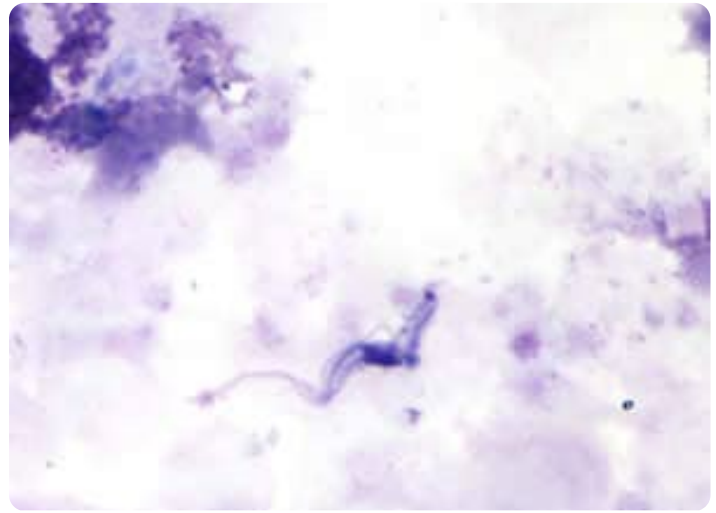


Map from Franco et al. 2022 (PMID 35041668). Image reprinted under license Creative Commons BY 4.0. © 2022 Franco et al.

Morphologic Features of *T. brucei*

- The two subspecies cannot be morphologically separated
- Trypomastigotes slender, measuring 14-33 µm long
  - » Shorter ‘stumpy’ forms may be observed
- Prominent undulating membrane
- **Tiny kinetoplast**
- Dividing forms may be seen in clinical specimens (e.g., blood)





Dividing forms of *T. brucei*

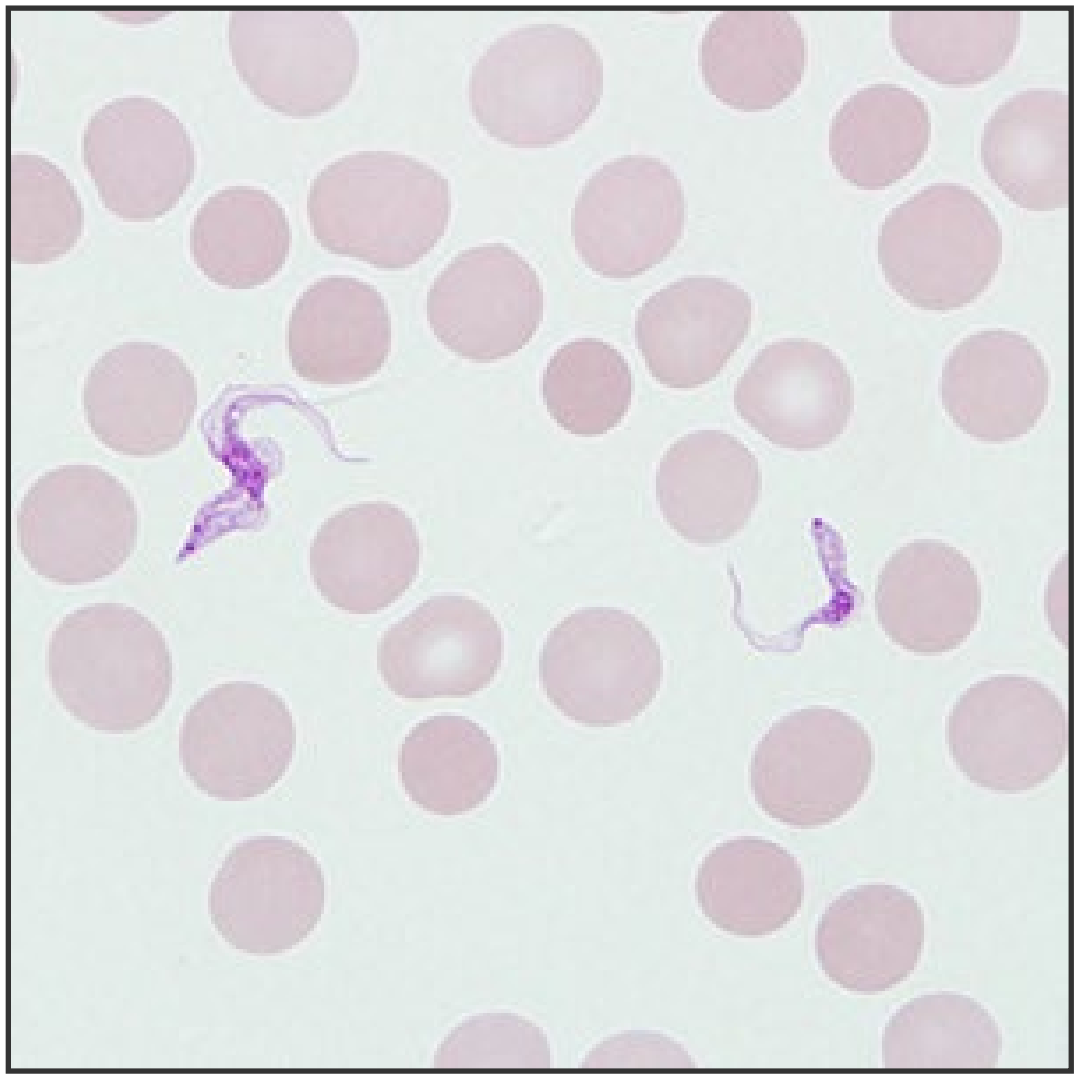
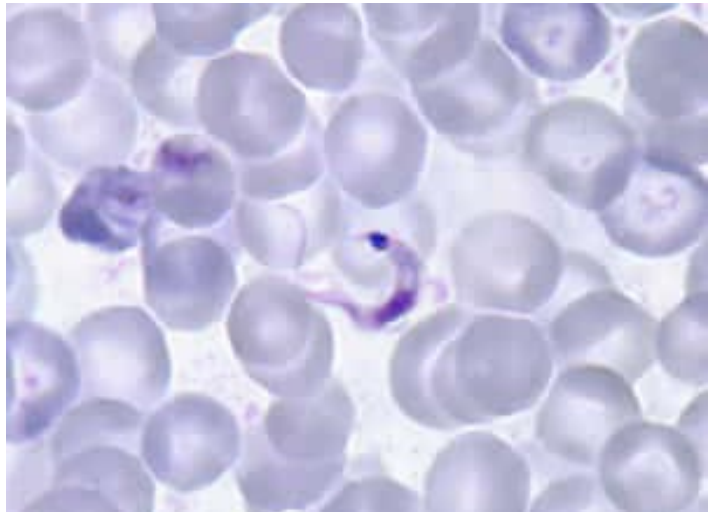
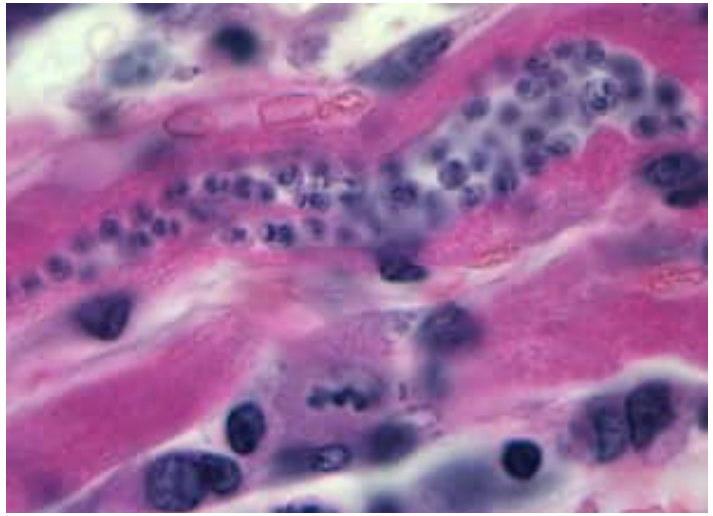


Image courtesy of CDC-DPDx

American Trypanosomiasis (Chagas Disease)

- Occurs from southern North America to South America
- Multiple roads to infection:
  - » Vector-borne (**triatomine bugs**)
  - » Blood transfusion
  - » Solid organ transplantation (e.g., heart)
  - » Congenital
  - » Ingestion (foodborne)
  - » Laboratory accidents (needle sticks)
- Diagnosis is very challenging and varies based on stage of infection:
  - » Acute: blood film microscopy, PCR
  - » Chronic: serology





Images courtesy of CDC-DPDx

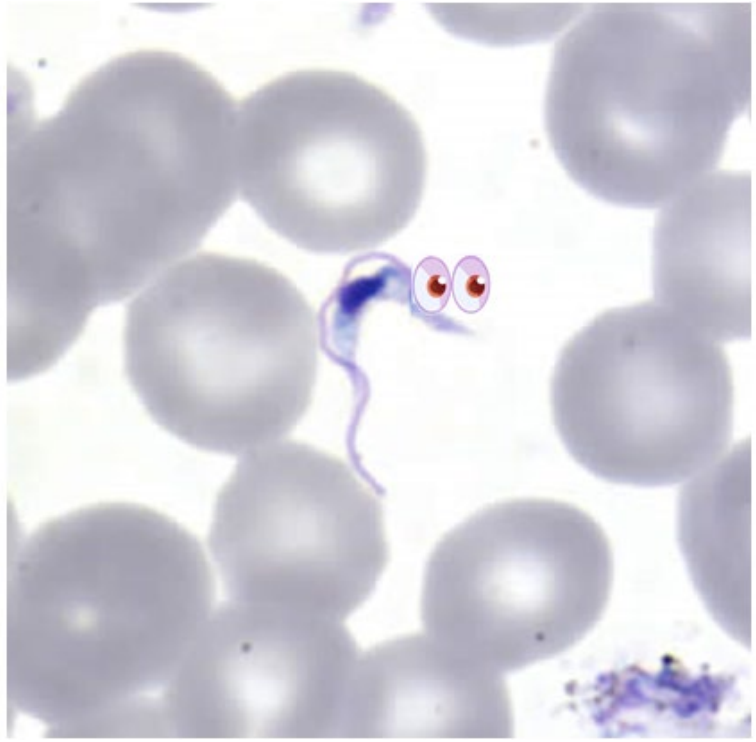
Serologic Testing for Chagas Disease

- The following clinical scenarios:
  - » Confirmation of seropositive donors by the Red Cross and other blood collection agencies
  - » Asymptomatic potential chronic infections
  - » Screening potential organ donors
  - » Initial transplant recipients (especially if a high pretest probability for disease)
  - » Following potential congenital cases (note: newborns may have maternal antibodies)
  - » Confirm positive results from commercial/clinical/reference labs.

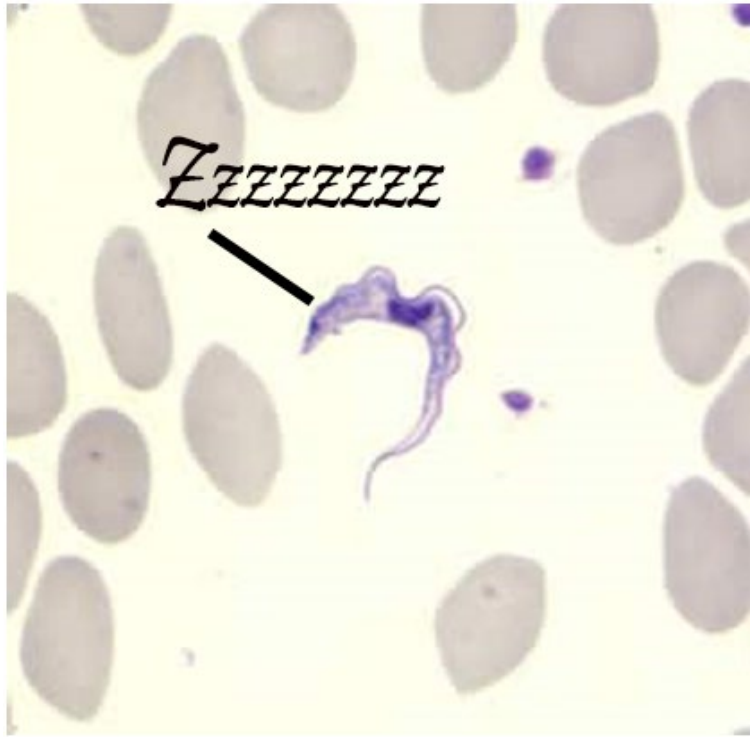
Molecular testing for *T. cruzi*

- Clinical scenarios:
  - » Monitoring of organ transplant recipients previously confirmed with chronic *T. cruzi* infection
  - » Patient with triatomine bite/exposure in an endemic area within two months of exposure
  - » Accidental inoculation (e.g. lab accidents)
  - » Suspect congenital cases
  - » Blood transfusion recipients if the donor had confirmed infections (rare in the US)

Review: Morphologic separation of trypanosomes



Trypanosoma cruzi:
Large kinetoplast!
Its eyes are open as it's 'cruz'ing around looking for trouble!



Trypanosoma brucei:
Tiny kinetoplast!
Its eyes are closed as it's sleeping…

Filariasis

What is Filariasis?

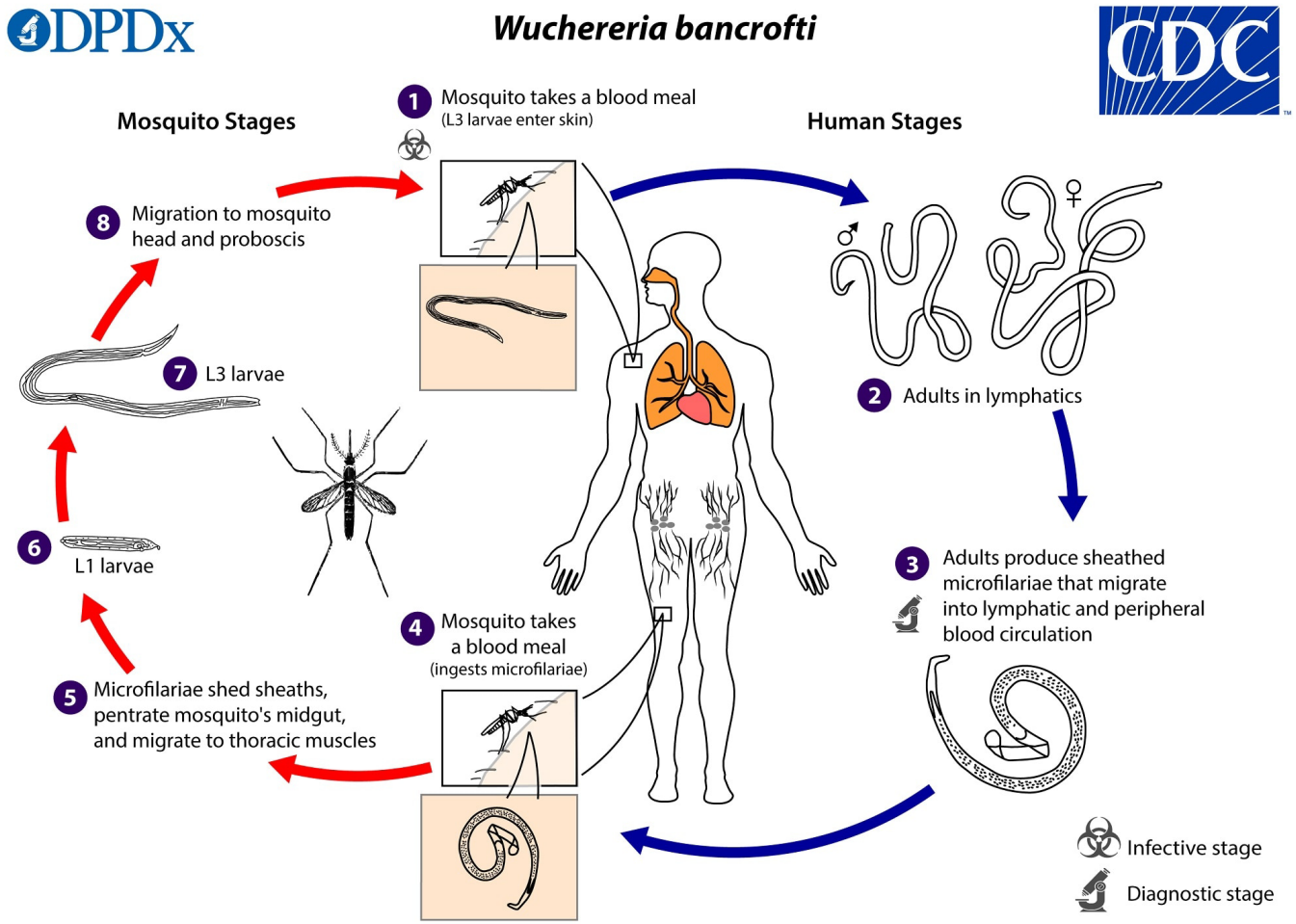
- Infection with filarial nematodes
- A specific clade of nematodes (Filarioidea: Onchocercidae) characterized by an early larval stage known as a **microfilaria**.
- Two host life cycles; human infecting species cycle between the human definitive host and a blood-feeding **insect vector** and intermediate host.
- Responsible for some of the most debilitating diseases worldwide, especially in tropical and subtropical resource-poor regions.
- Diagnosis is usually made by the identification of microfilariae in blood or skin snips
    - » Adults may be identified in biopsy specimens or extracted from skin or the eyes
    - » Diagnosis may be enhanced by concentration techniques (e.g. Knott's concentration)
    - » Serology available in the US for lymphatic filariasis
    - » Screening assays may be available in endemic areas
- Identification is important, as different species may be treated differently, and in areas where multiple species occur, all potential agents need to be ruled out and in the case of mixed infections, a complex treatment algorithm may need to be applied

NOTE: treatment discussions here are in relation to identification and do not constitute medical advice

Primary Clinical Presentations of Filariasis

Species	Clinical Presentation
BLOOD	
Wuchereria bancrofti	Lymphatic Filariasis
Brugia malayi, *B. timori*	Lymphatic Filariasis
Loa loa	Loiasis; **Calabar swellings**, ectopic ocular infection
Mansonella perstans, *M. ozzardi*	Generally asymptomatic
SKIN	
Onchocerca volvulus, *Onchocerca* spp.	Onchocerciasis; skin nodules, **River Blindness**
Dirofilaria spp. (non-*immitis*)	Cutaneous Dirofilariasis; skin nodules, ocular infection
Mansonella streptocerca	Cutaneous infection
PULMONARY	
Dirofilaria immitis	Pulmonary infarcts; **coin lesions**

Life Cycle of a Filarial Nematode



Life cycle courtesy of CDC-DPDx

Diagnostic Criteria for Filarial Nematodes

- Specimen type (blood or skin)
- Size (approximate or measured length)
- Geographic distribution
- Periodicity
- Sheath – presence or absence, and if presence, color when stained with Giemsa
- Arrangement of nuclear column

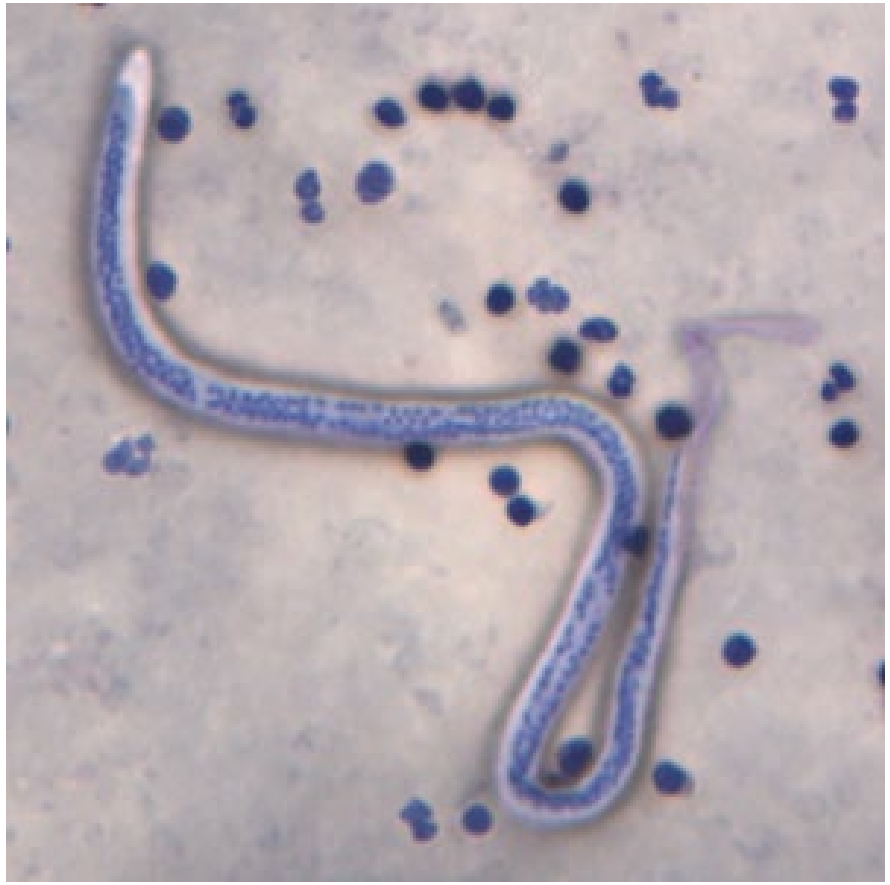
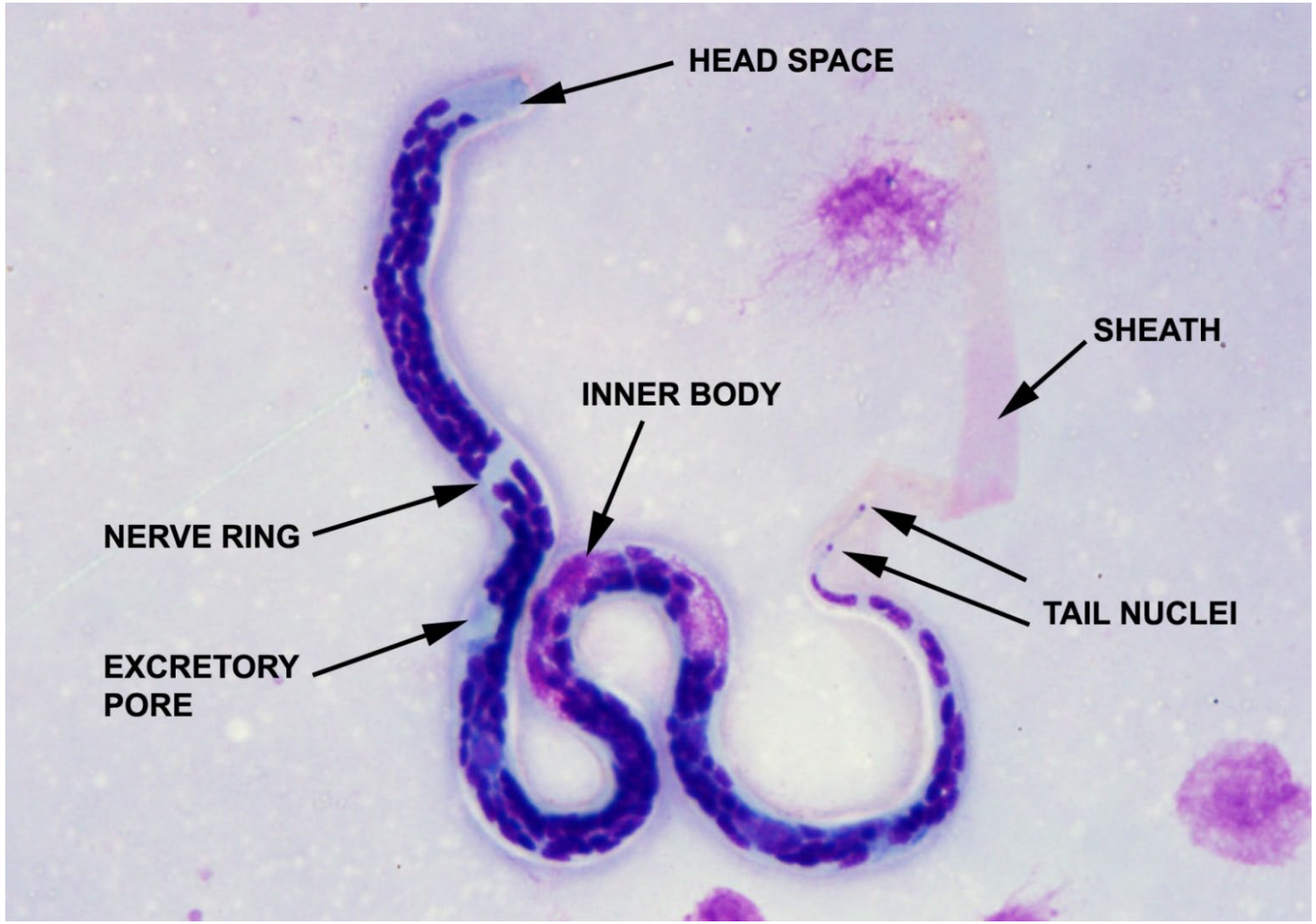


Image courtesy of CDC-DPDx

Anatomy of a Microfilaria



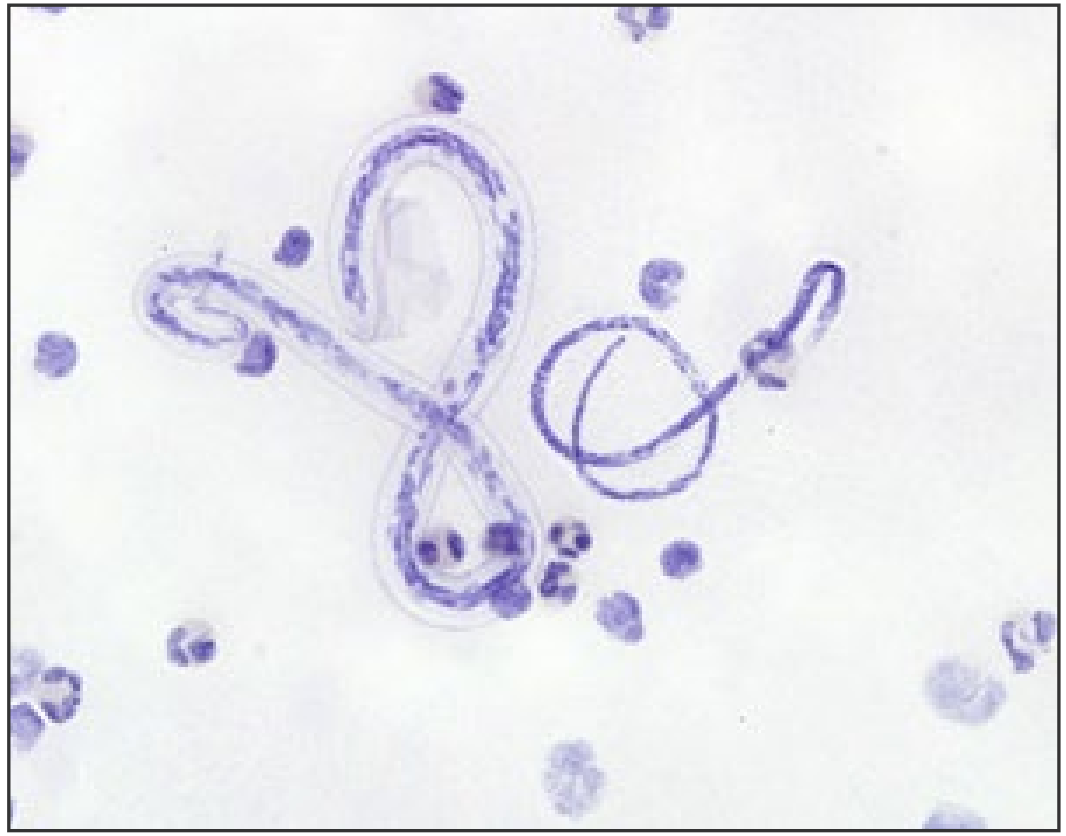
Diagnostic Criteria: Size

BIG ONES!

Loa loa

Wuchereria bancrofti

Brugia spp.



little ones

Mansonella spp.

Image courtesy of CDC-DPDx

Diagnostic Criteria: Distribution

Diagnostic Criteria: Periodicity

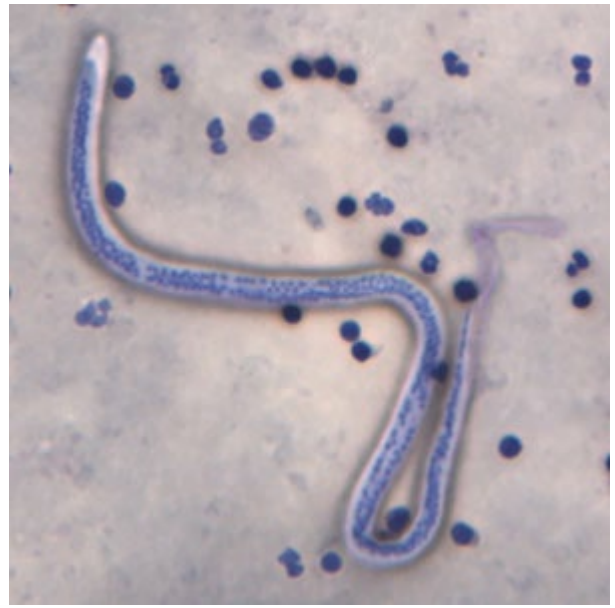
- Periodicity is the habit of the microfilariae of certain species to be in peripheral blood to match the optimal feeding times of the intermediate host/vector.
- Diurnal periodicity: microfilariae circulate during the day (*Loa*)
  - » Collect between 10AM-2PM
- Nocturnal periodicity: microfilariae circulate at night (*Wuchereria**, *Brugia*)
  - » Collect between 10PM-2AM
- Aperiodic: no periodicity (*Mansonella*)

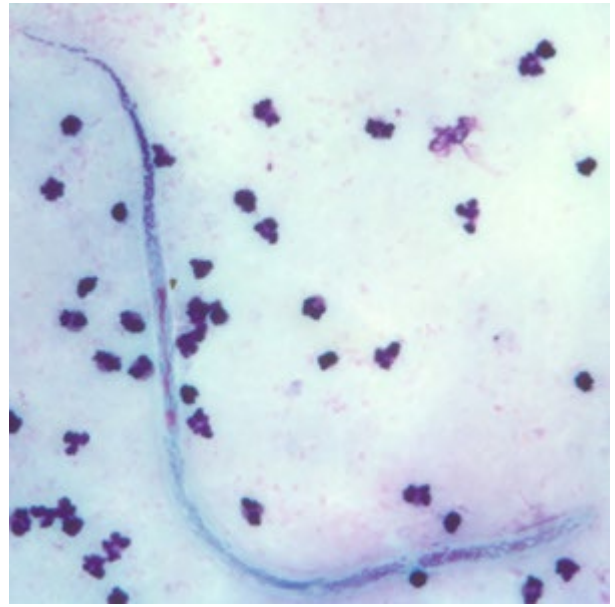
- *note: populations of *W. bancrofti* in South Pacific circulate primarily between noon and 6PM.

Diagnostic Criteria: the pesky sheath!

The sheath is a membrane surrounding the microfilariae of some filarial nematodes.

- » May be present (*Loa*, *Wuchereria*, *Brugia*) or absent (*Mansonella*, *Onchocerca*)
- » Absence of sheath in clinical specimen does not mean it is not an unsheathed species!!!!
- » May be colorless to hot pink; color is pH-dependent and not strictly a biological phenomenon.



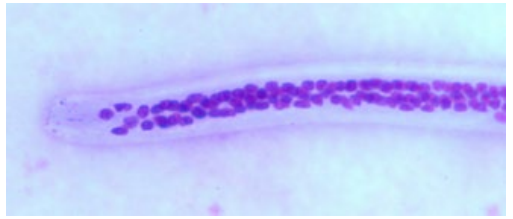


Images courtesy of CDC-DPDx

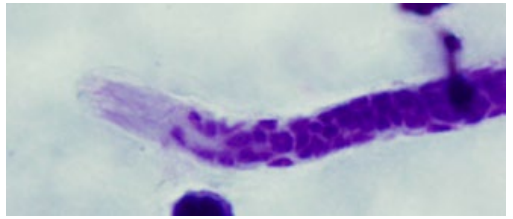
Diagnostic Criteria: Heads or Tails?

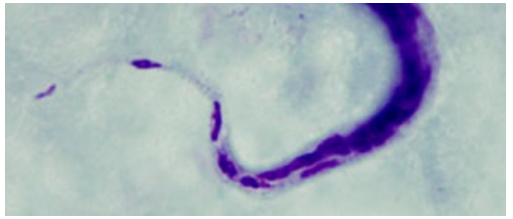
Head space and tail nuclei

Wuchereria bancrofti

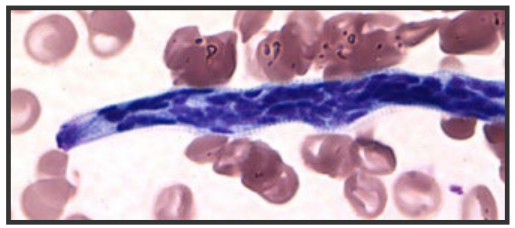


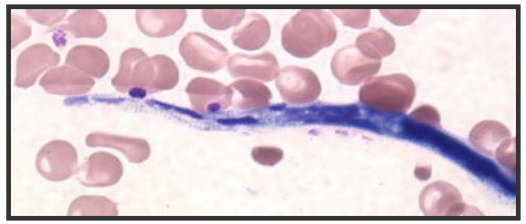
Brugia malayi
B. timori



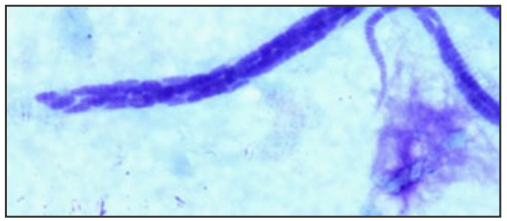


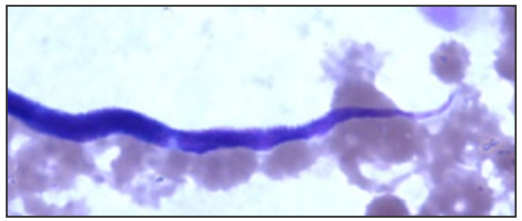
Loa loa



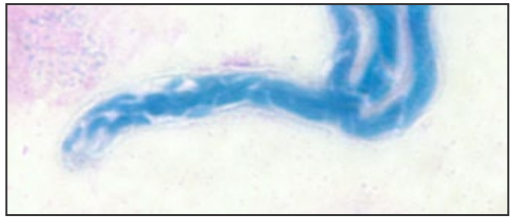


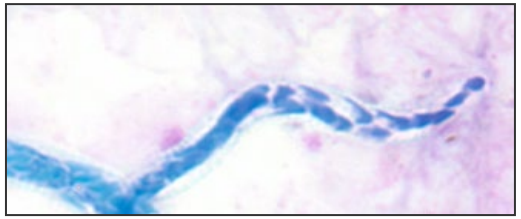
Mansonella ozzardi





Mansonella perstans





Lymphatic Filariasis

- Caused by *Wuchereria bancrofti*, *Brugia malayi*, *B. timori*
- Geographic distribution varies by species:
  - *W. bancrofti*: circumtropical
  - *B. malayi*: Central and Southeast Asia, Pacific Islands
  - *B. timori*: Lesser Sunda Archipelago
- Transmitted by various genera and species of **mosquitoes**
- Adults reside in **lymphatic tissue** (lymph vessels, lymph nodes, testes)
- Microfilaria circulate in the blood during the night (**nocturnal periodicity**)
- Treatment is diethylcarbamazine (DEC); might be contraindicated in areas with loiasis and onchocerciasis

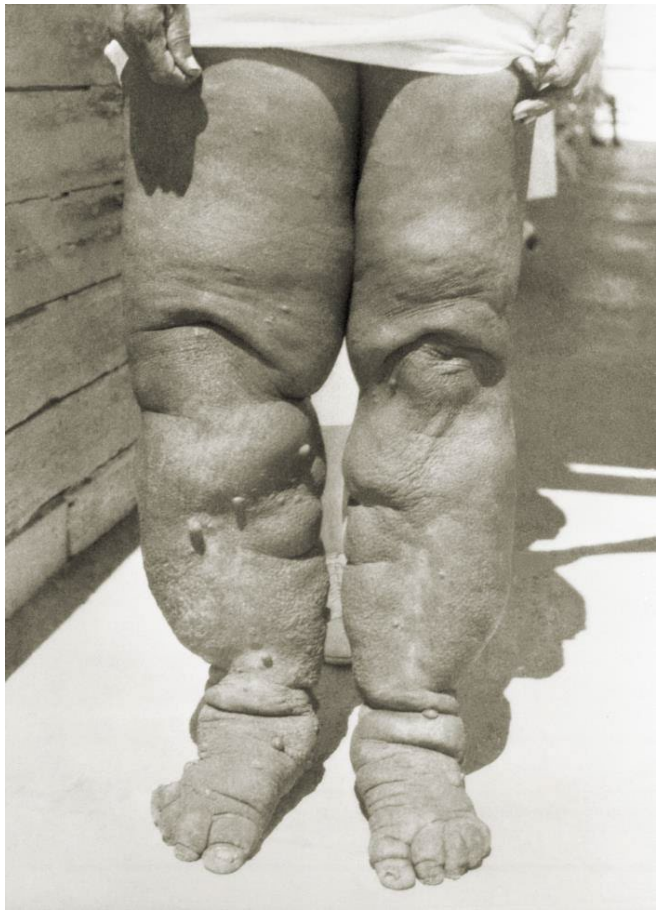
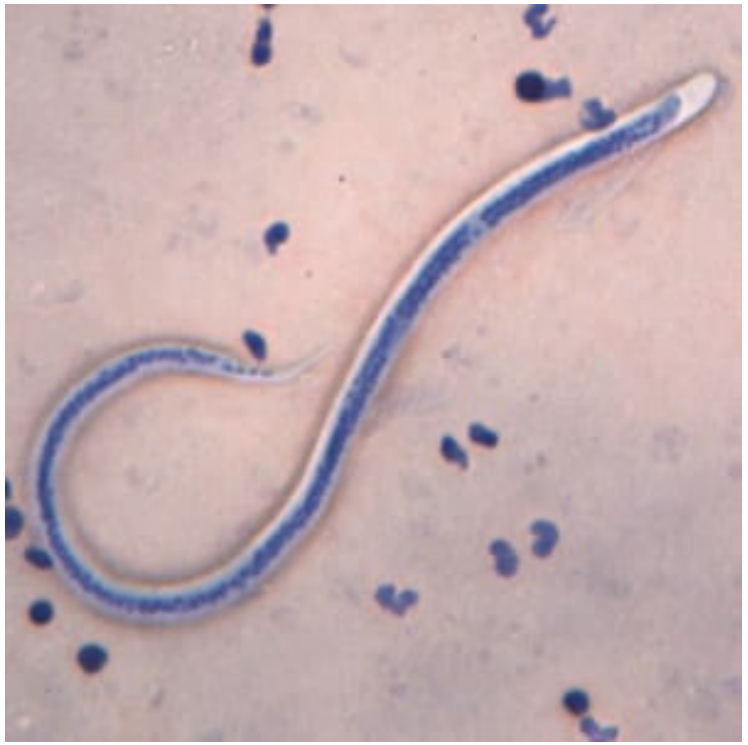


Image courtesy of CDC-PHIL

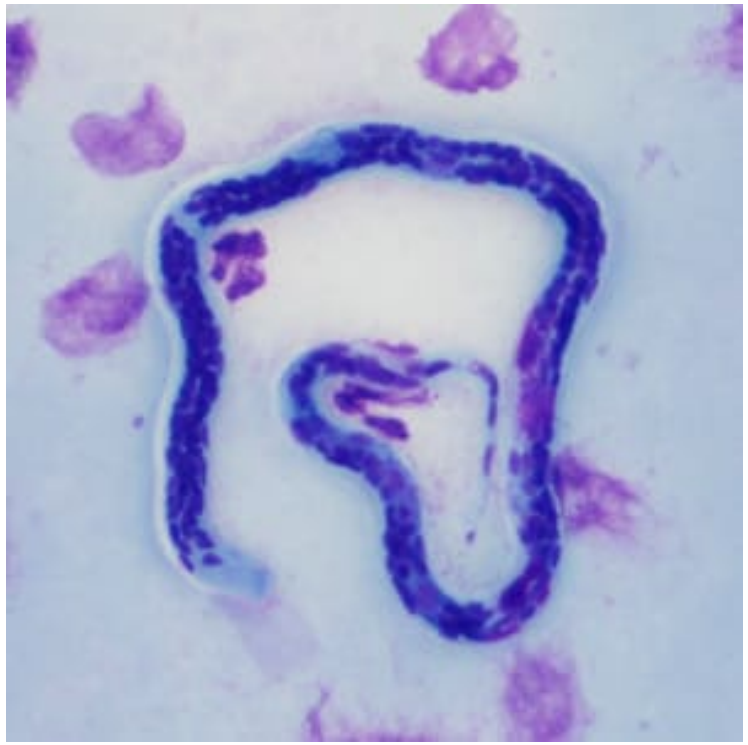
Lyphatic Filariasis: Morphology

- Microfilariae large:
  - » *W. bancrofti* 244-296 µm
  - » *B. malayi* 177-230 µm
  - » *B. timori* 310 µm
- Normally possess a sheath; often pink for *B. malayi* and colorless for other two
- Head space: short for *W. bancrofti*; long for *Brugia* spp.
- Tail nuclei:
  - *W. bancrofti*: **anucleate**
  - *Brugia* spp.: **terminal and subterminal nuclei separated by gaps**

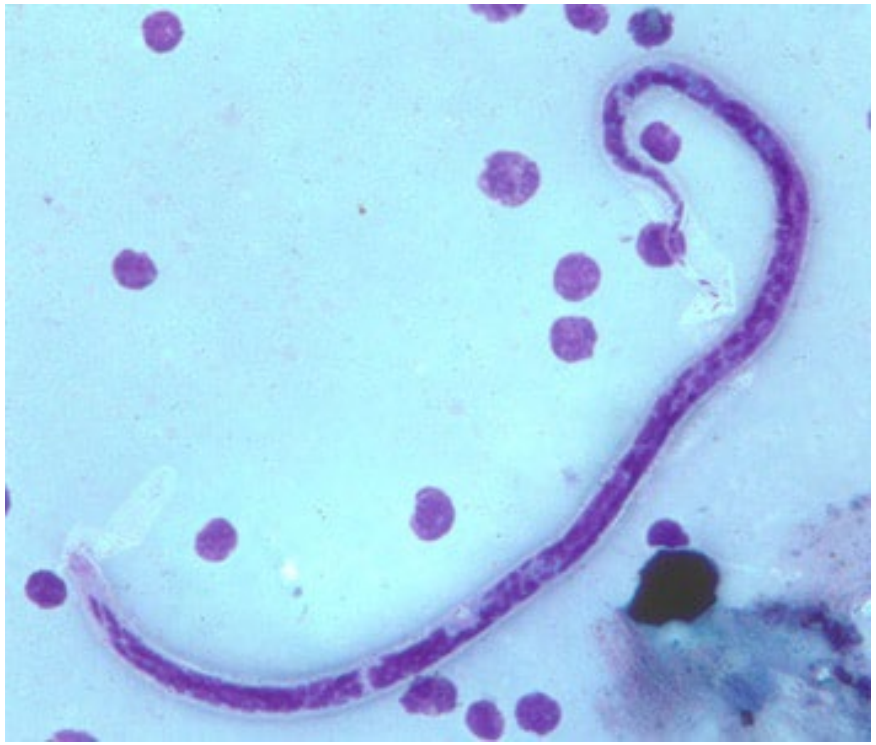
Lymphatic Filariasis



Wuchereria bancrofti, hematoxylin



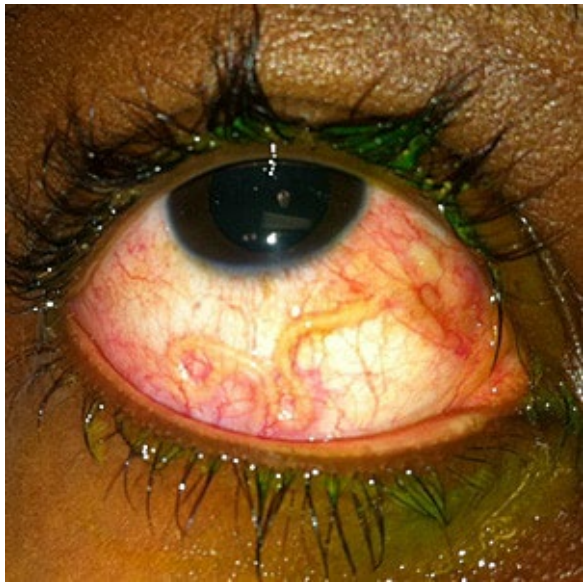
Brugia malayi, Giemsa



Brugia timori, Giemsa

Images courtesy of CDC-DPDx

Loiasis



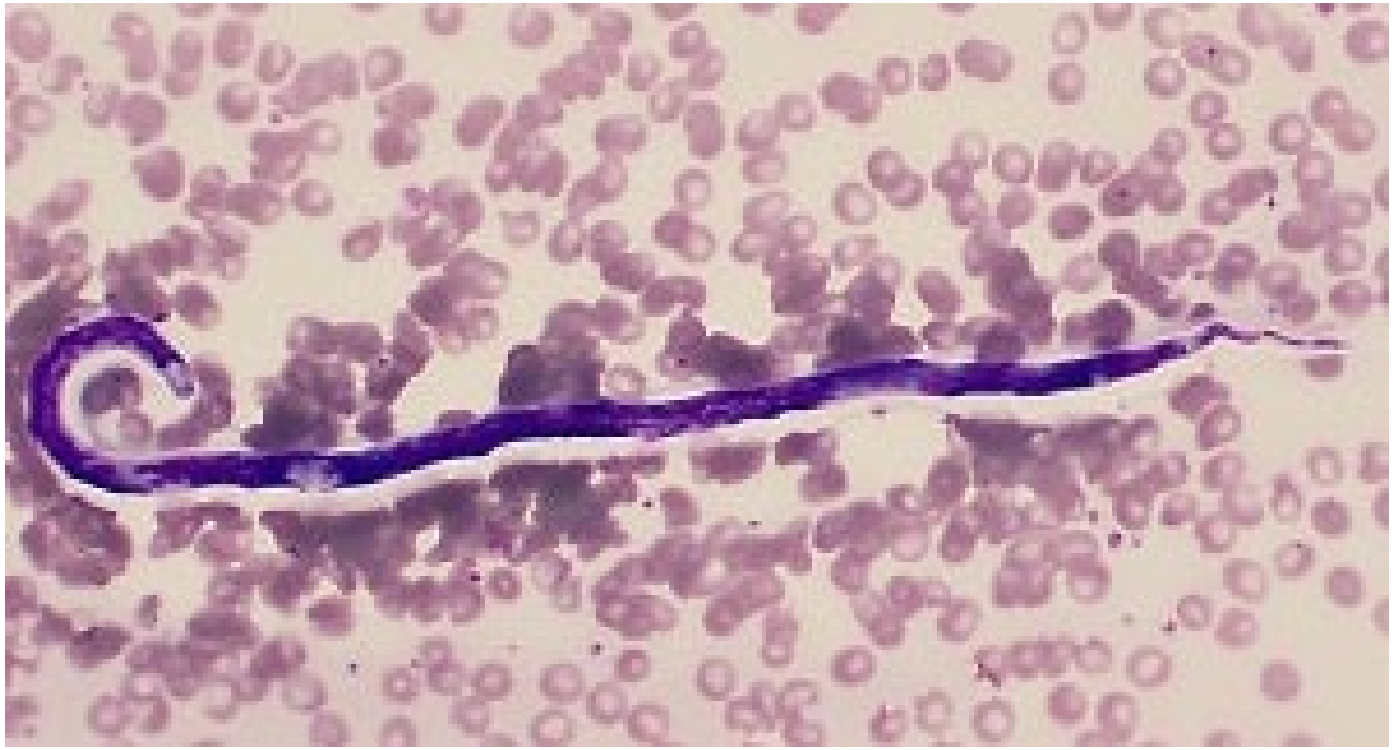
- Caused by *Loa loa* the 'African eye worm'
- Endemic to West-Central Africa (esp. Cameroon, DRC)
- Transmitted by **deer flies** in the genus *Chrysops*
- Adults typically occur in the subcutaneous tissues (**Calabar swellings**)
  - » ectopic migration to the eye
- Microfilaria circulate during the day (**diurnal periodicity**)
- Treatment is DEC; however, in cases of heavy microfilaremia (>8000 mf/mL), treat first with albendazole to reduce mf burden **or** apheresis followed by DEC

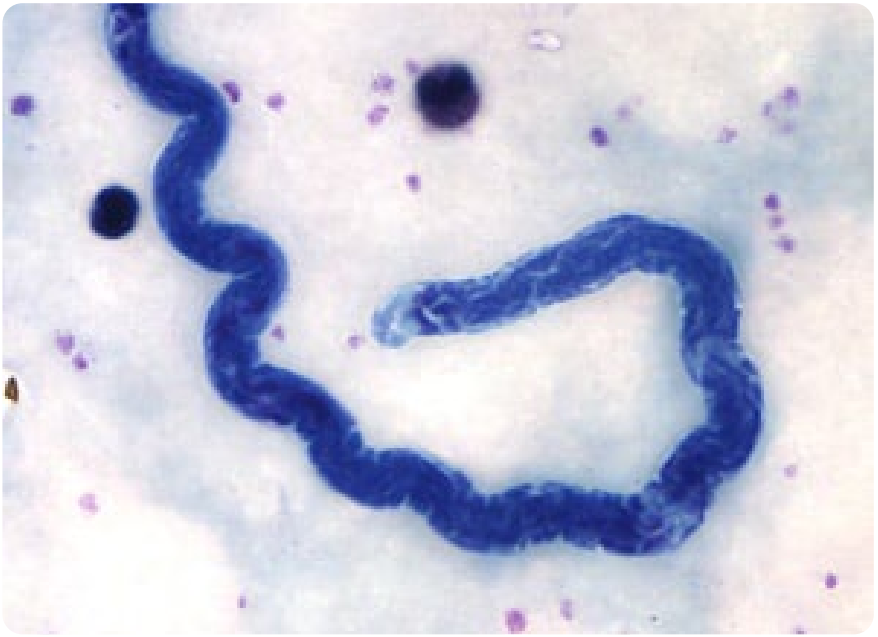
Image courtesy of CDC-DPDx

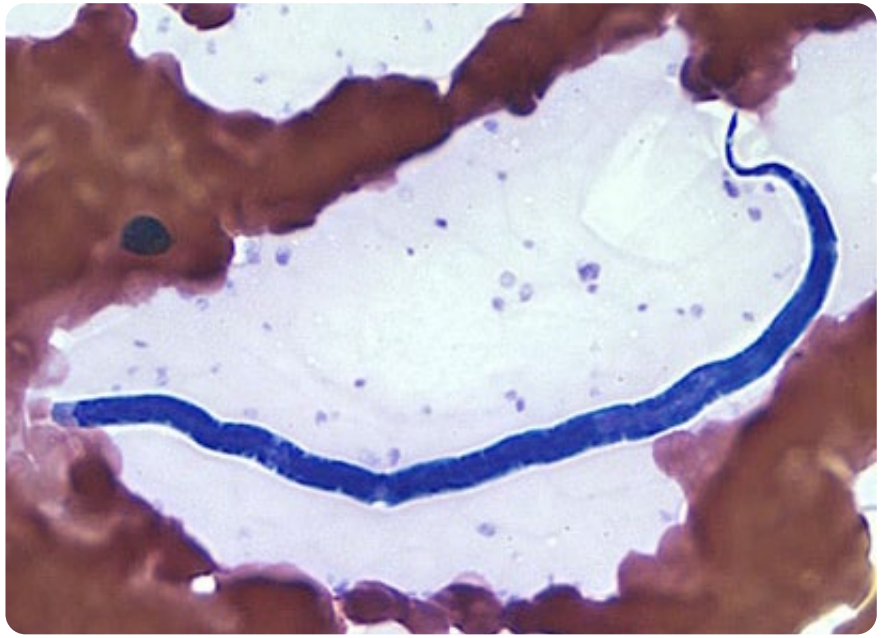
Loa loa: microfilarial morphology

- Microfilariae large, 231-250 µm long
- Sheath usually present (prone to loss)
- Short head space
- Tail with nuclei randomly spaced to the tip of the tail

Loa loa





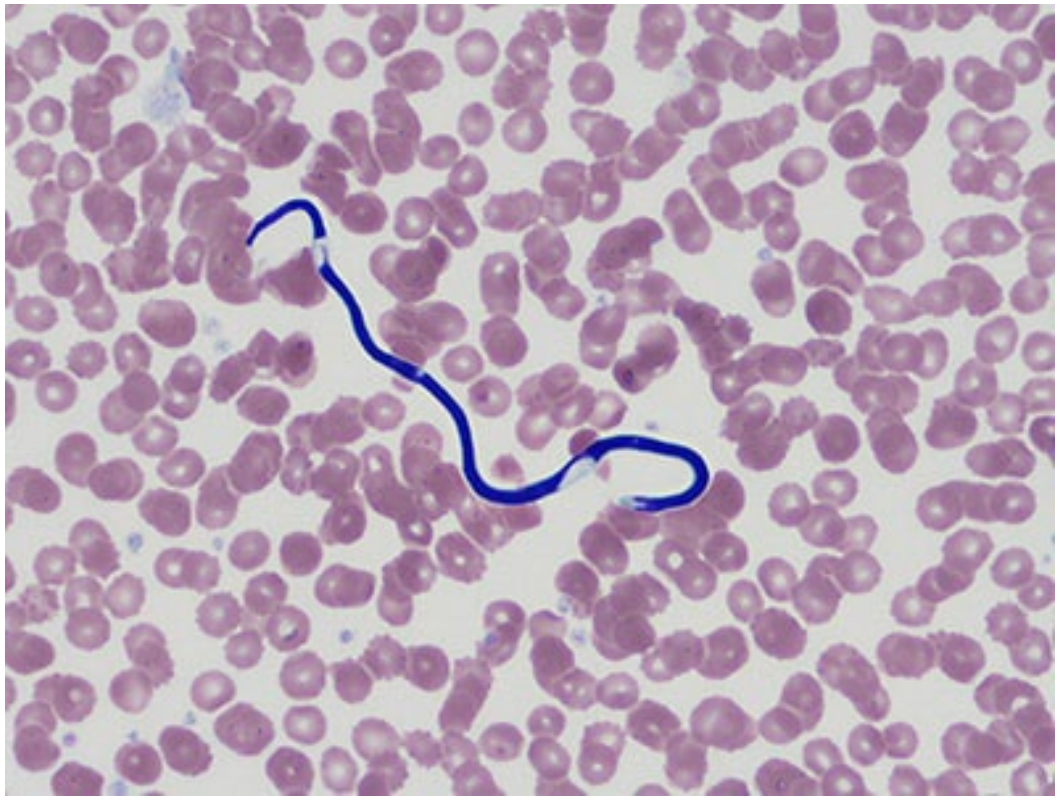


Images courtesy of CDC-DPDx

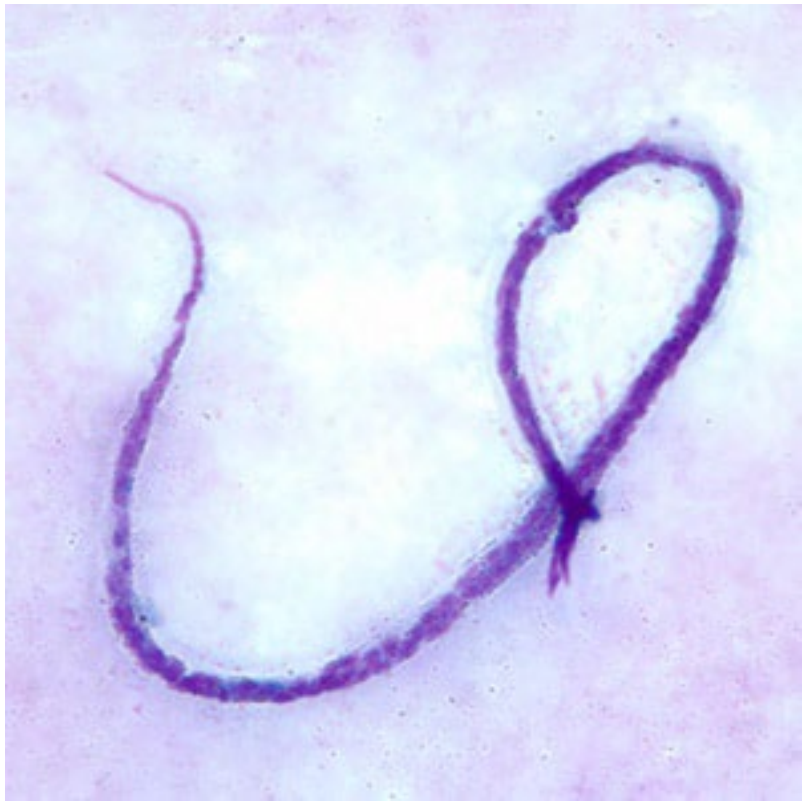
Mansonellosis

- Two primary species with microfilariae that circulate in blood:
  - » *Mansonella perstans* - native to Africa, introduced to Latin America
  - » *Mansonella ozzardi* – native to Latin America
- Transmitted by biting midges (*M. ozzardi* can also be transmitted by black flies)
- Location in definitive host:
  - » *Mansonella perstans*: mesenteries, connective tissues, visceral organs
  - » *Mansonella ozzardi:* skin
- Aperiodic
- Morphology:
  - » Small species; generally under 300 microns
  - » Never possess a sheath
  - » Tail nuclei:
    - *M. ozzardi*: blunt tail; nuclei to tip
    - *M. ozzardi*: anucleate tail (difficult to appreciate) that tapers to point

Mansonella species



Mansonella perstans, Giemsa



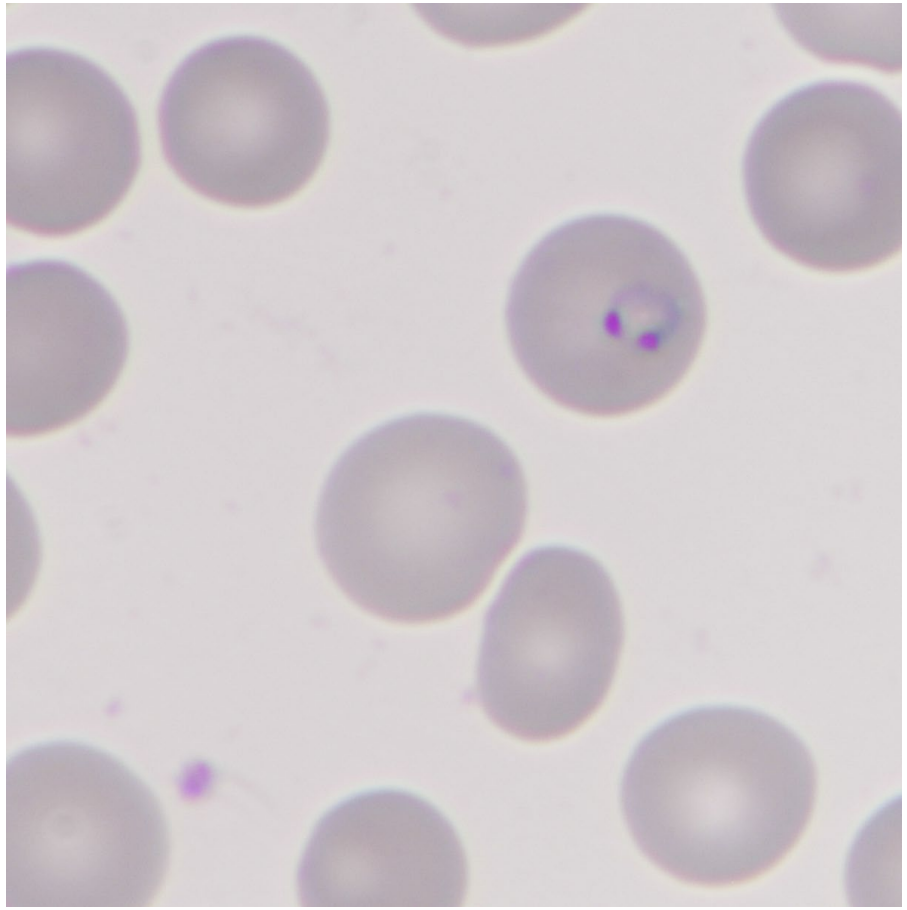
Mansonella ozzardi, Giemsa

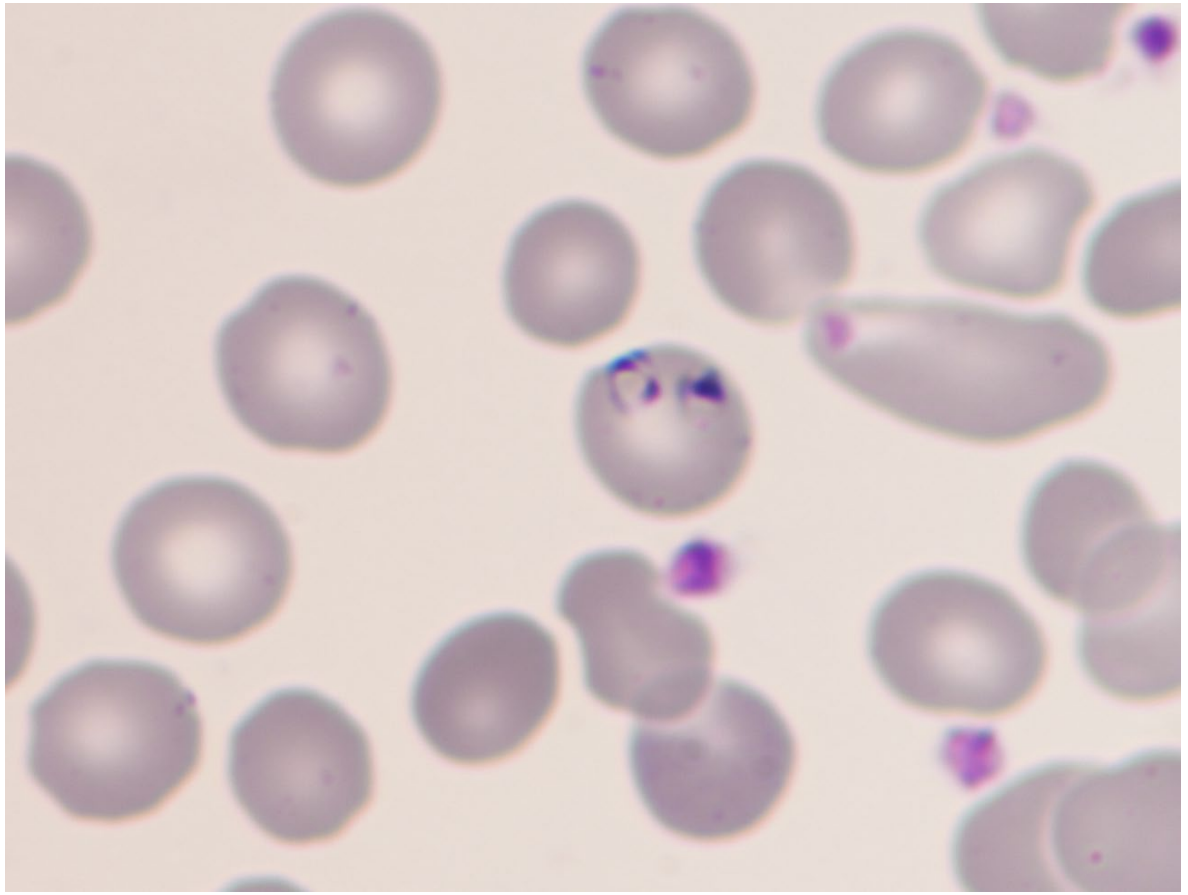
Images courtesy of CDC-DPDx

Cases: What have we learned?

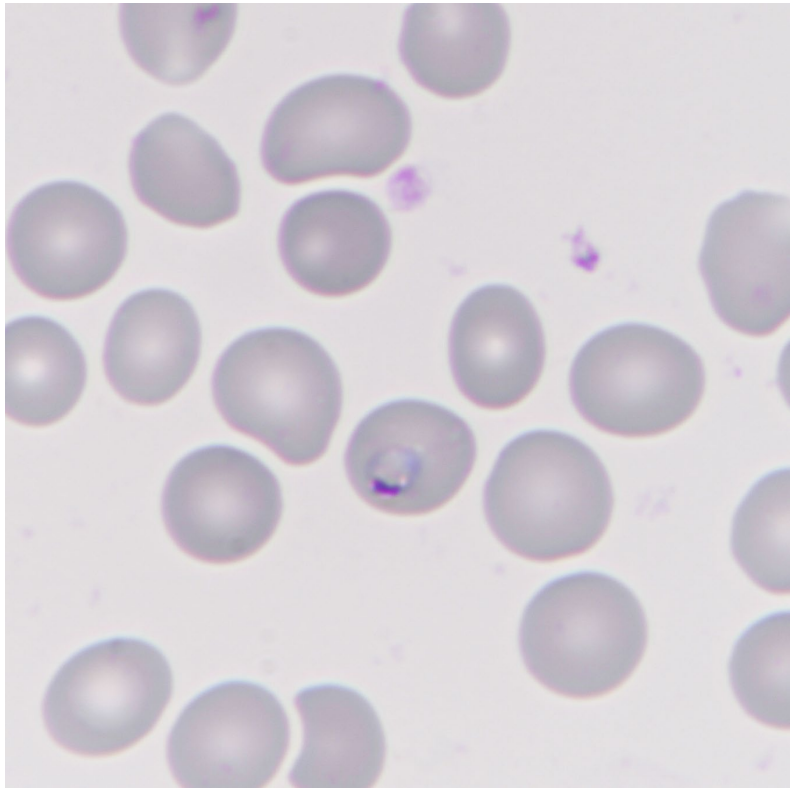
Case 1

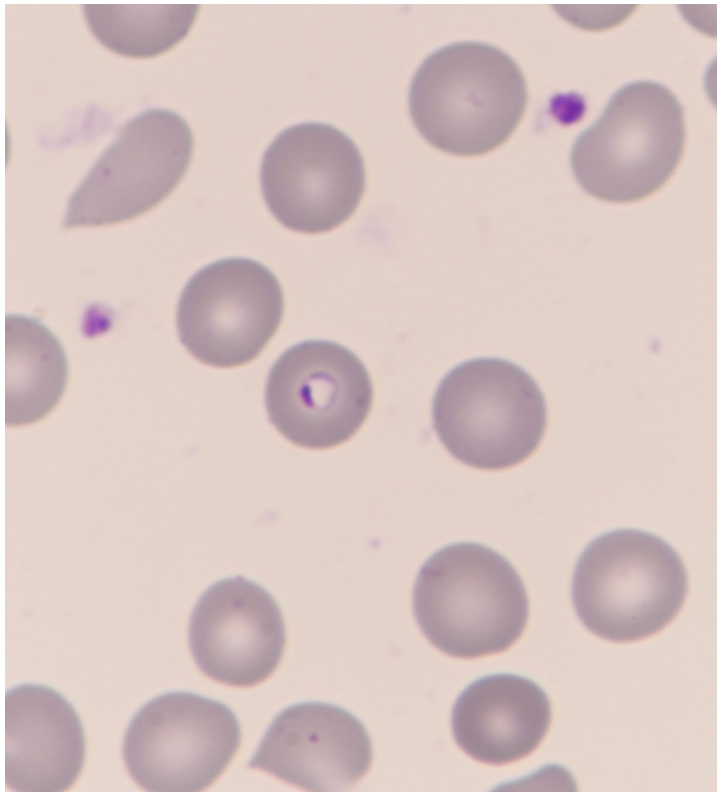
- The following objects were seen on thin blood films in a patient with travel to Africa.

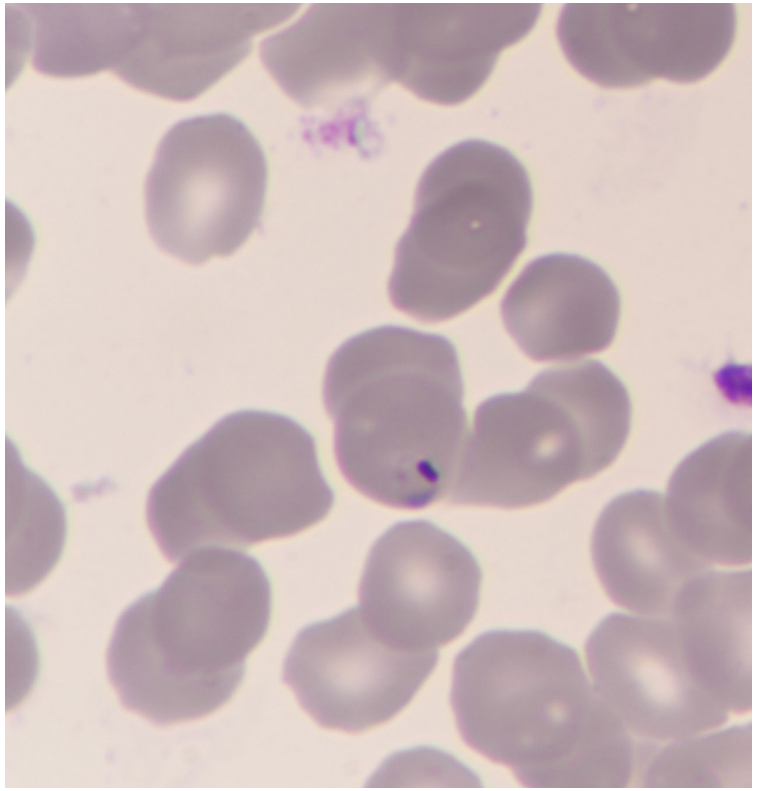




Case 1 – More images



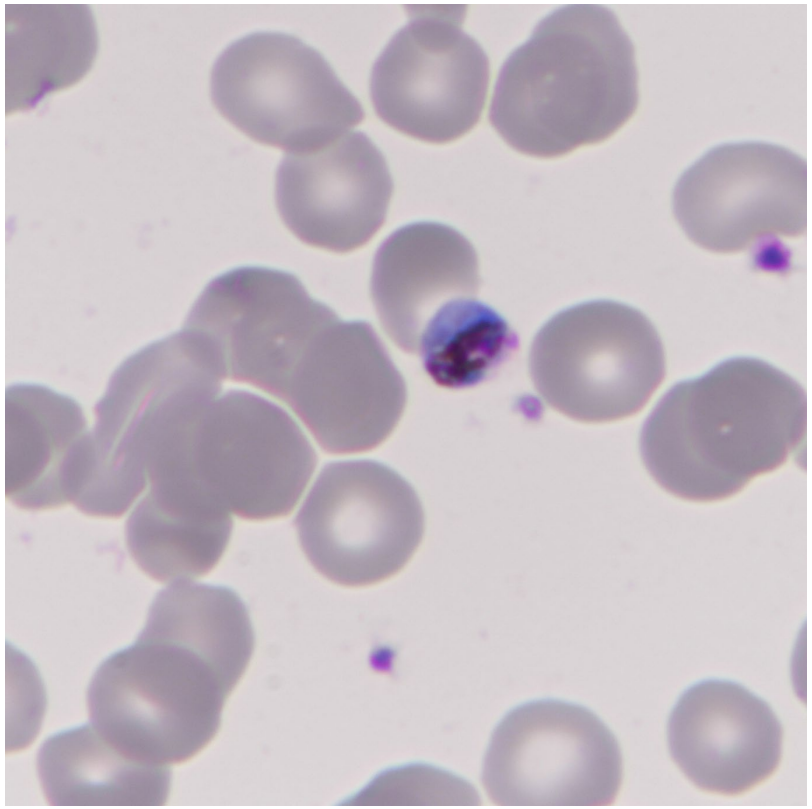


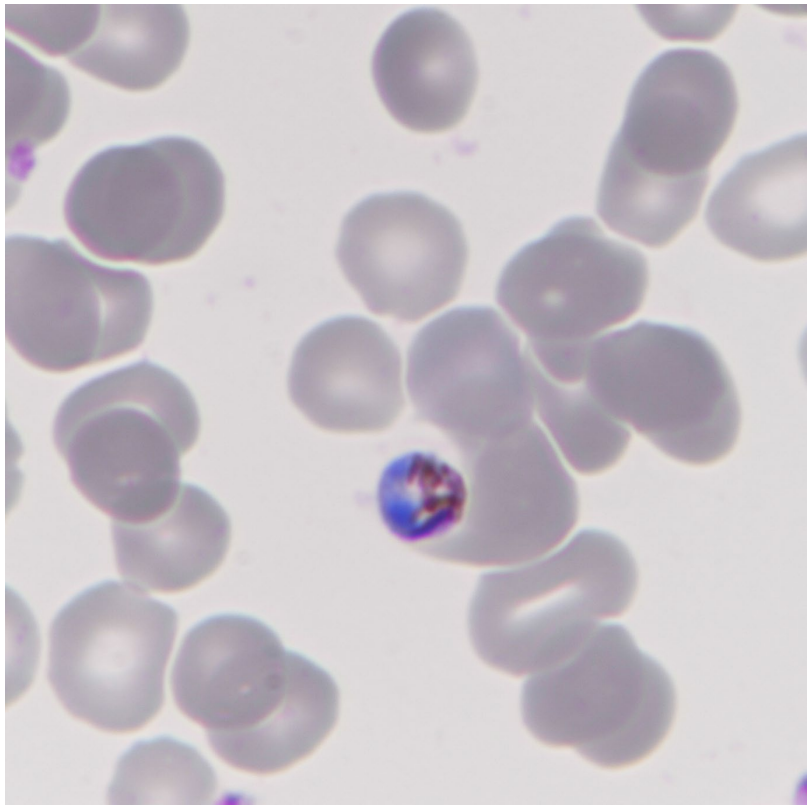


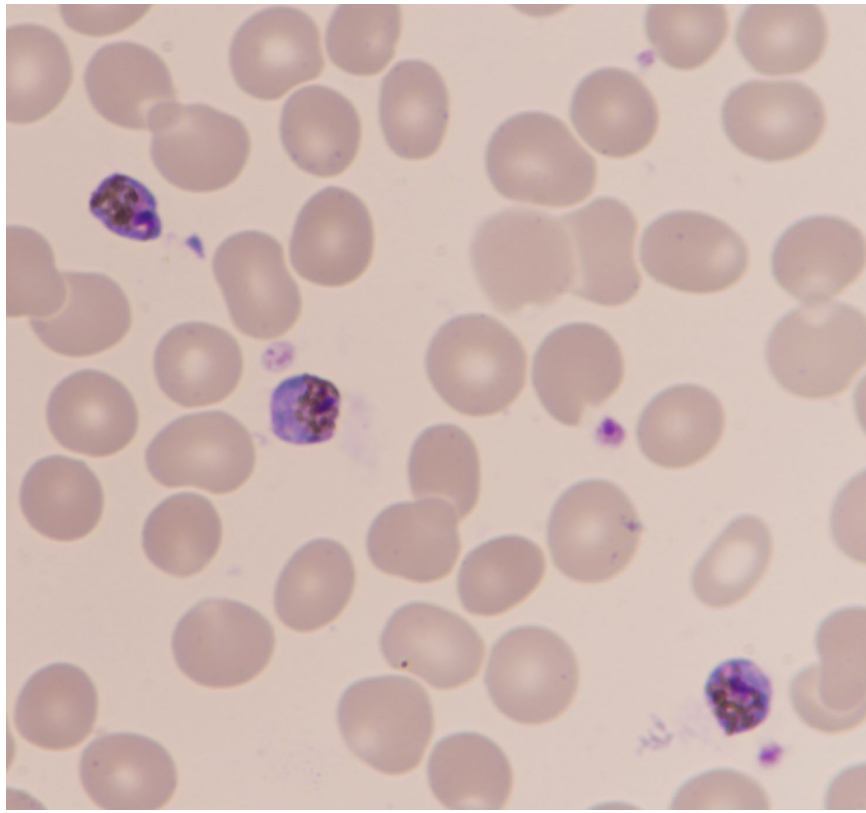
Case 1

- What are we seeing in these images?
- What are some initial thoughts?
- Differentials?

Case 1: These were also seen on the thin films



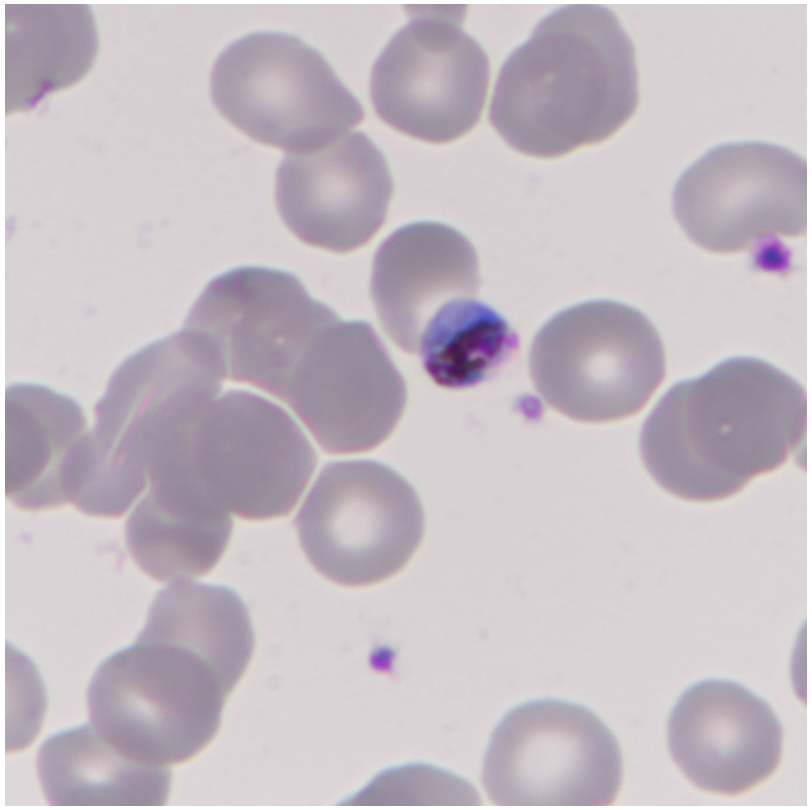


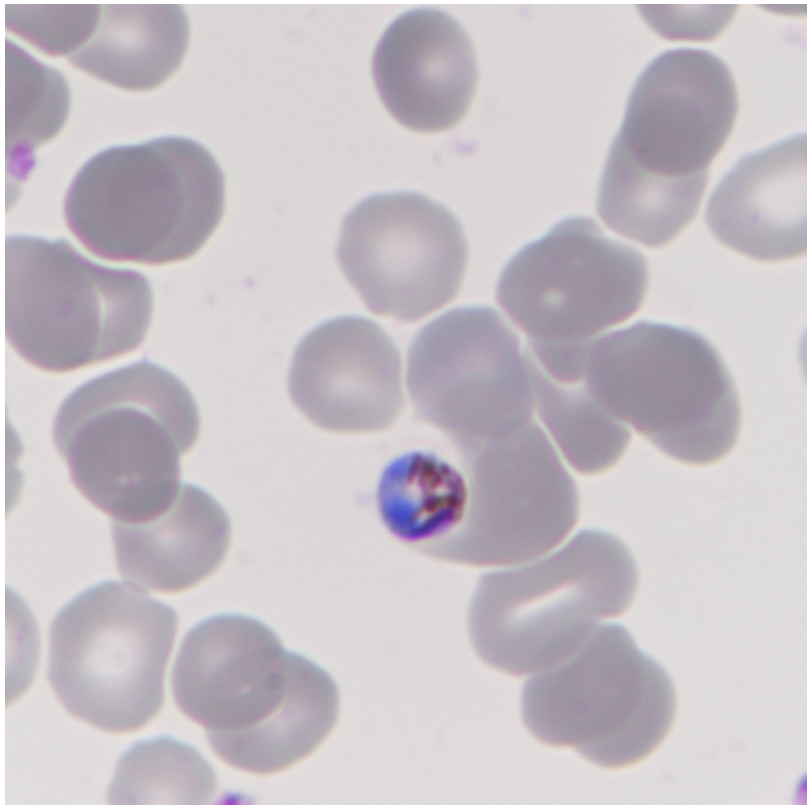


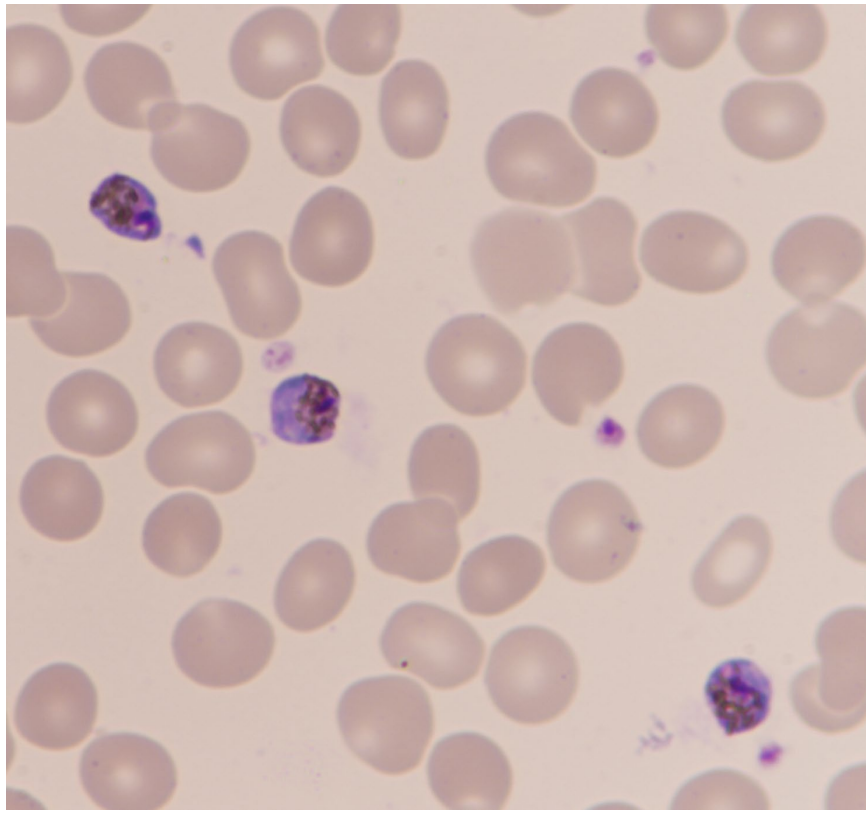
Case 1, continued

- Thoughts?
- Differentials?
- Does this change the identification?

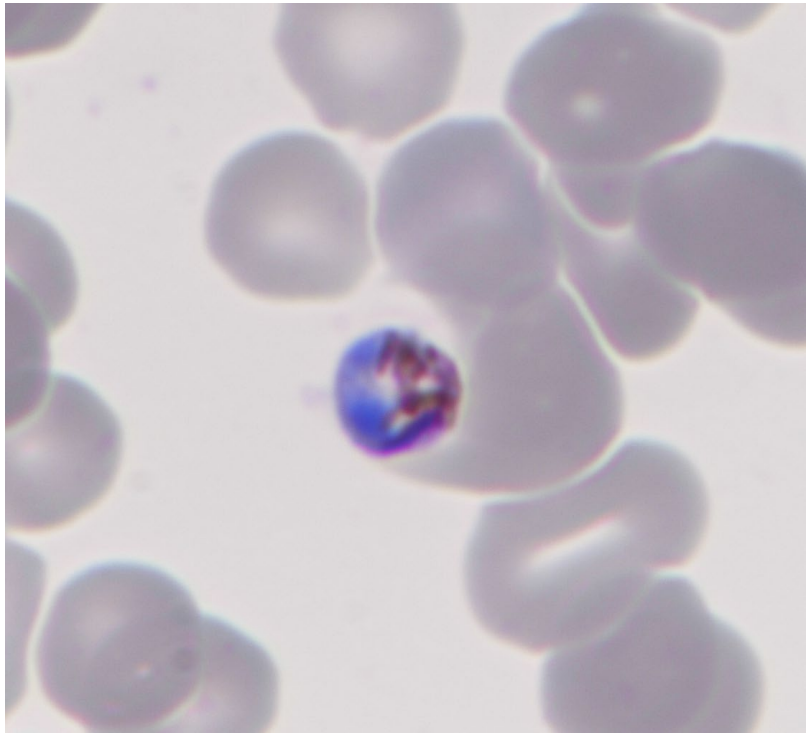
Case 1: Rounded gametocytes of *P. falciparum*



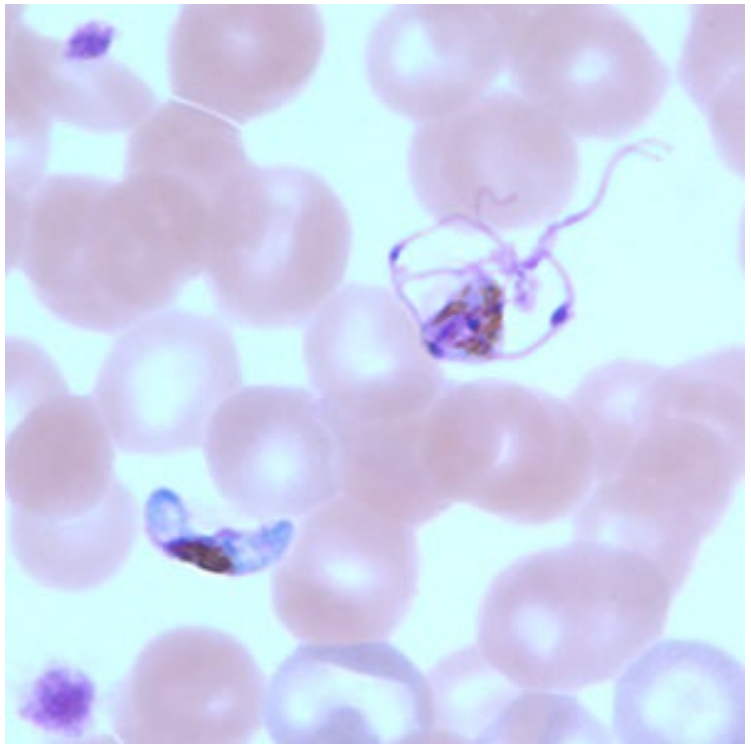




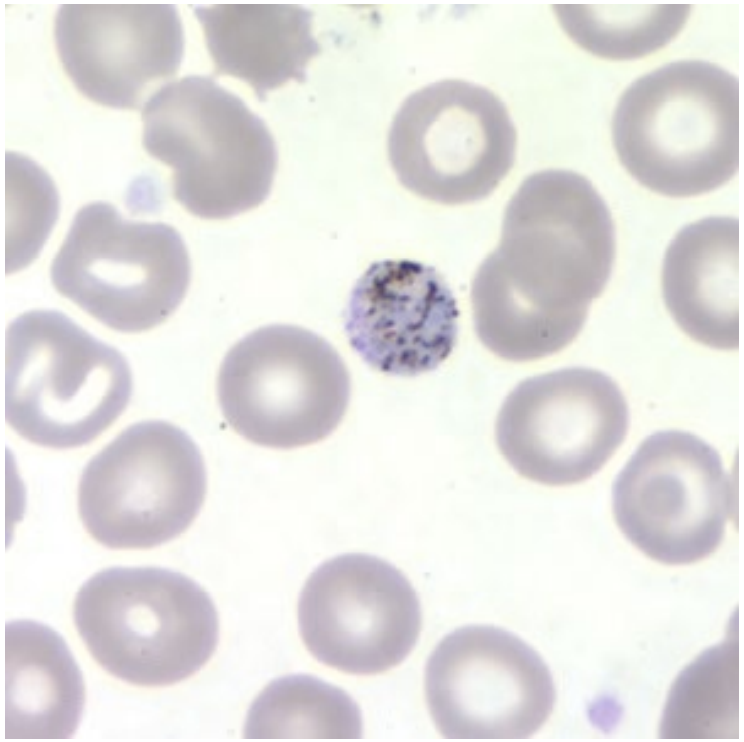
Rounded and Exflagellating Gametocytes of *P. falciparum*



Rounded gametocyte



exflagellating gametocyte

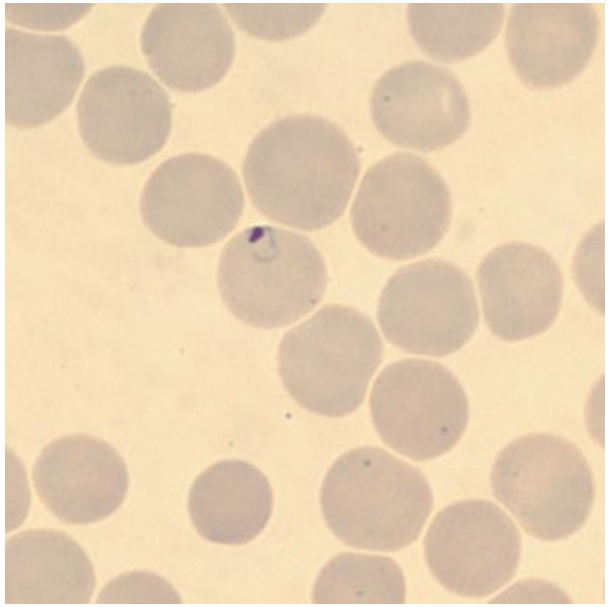


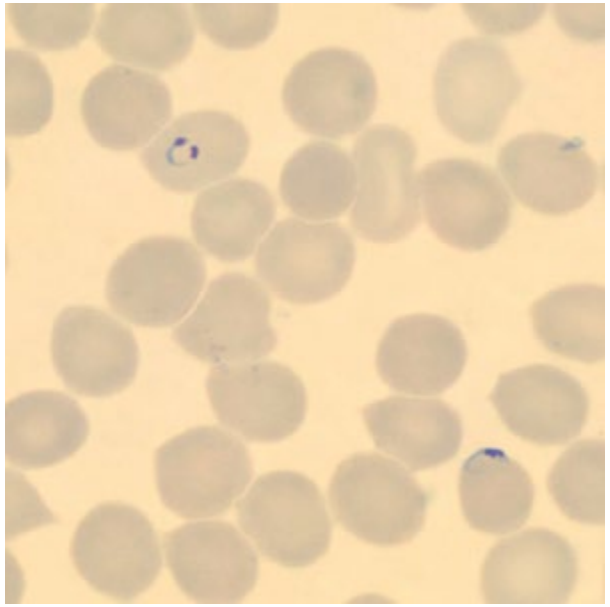
P. malariae gametocyte

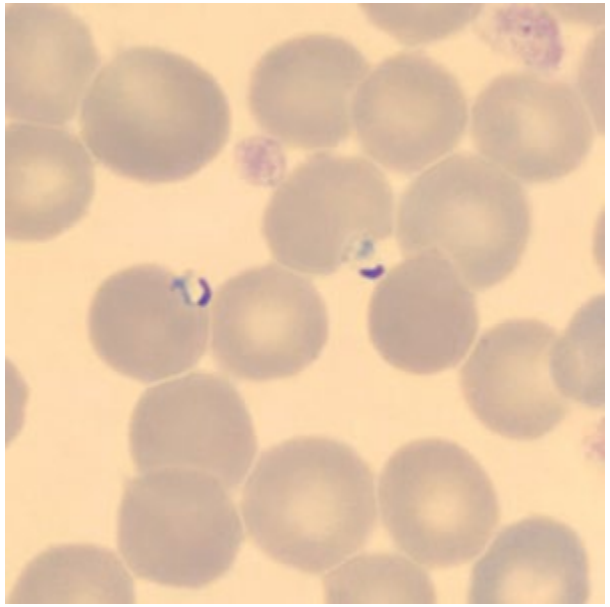
Case 2

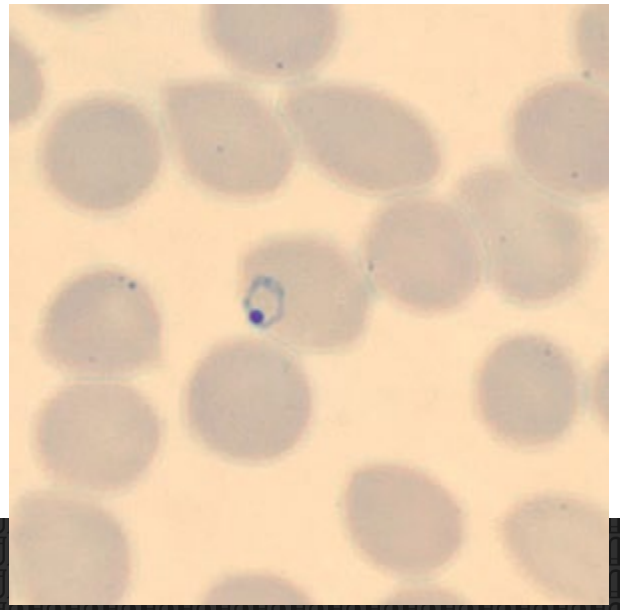
- The following case and images are courtesy of the California Dept. of Health and the CDC-DPDx
- The following blood images were taken from blood films on a patient with recent travel to Cambodia.

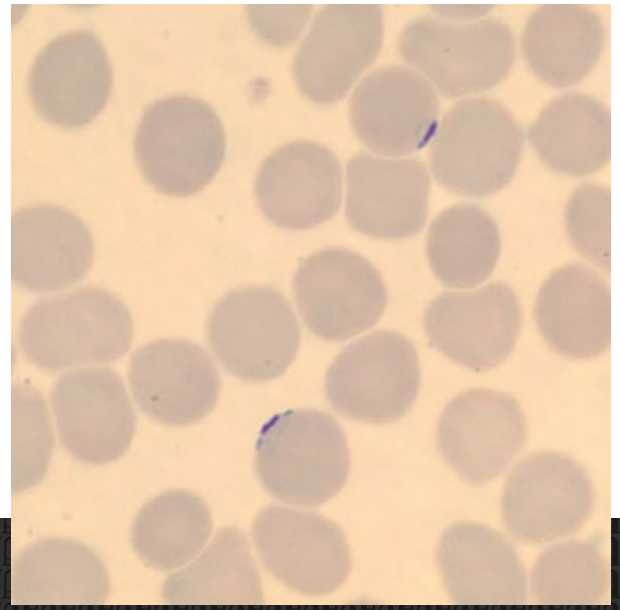
Case 2, Images











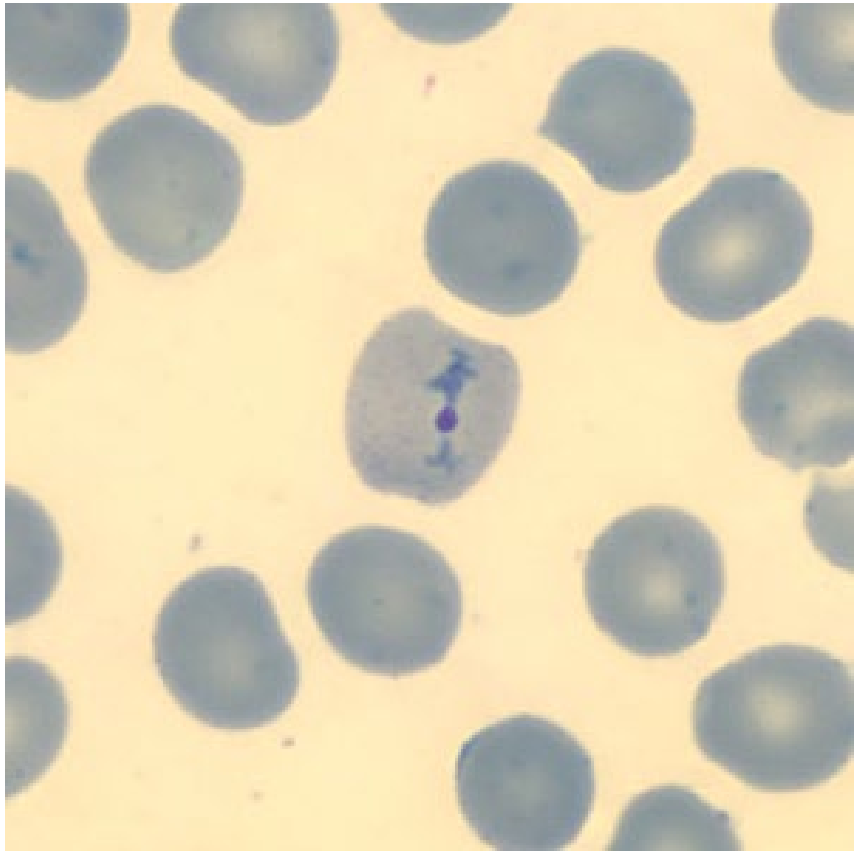
Case 2

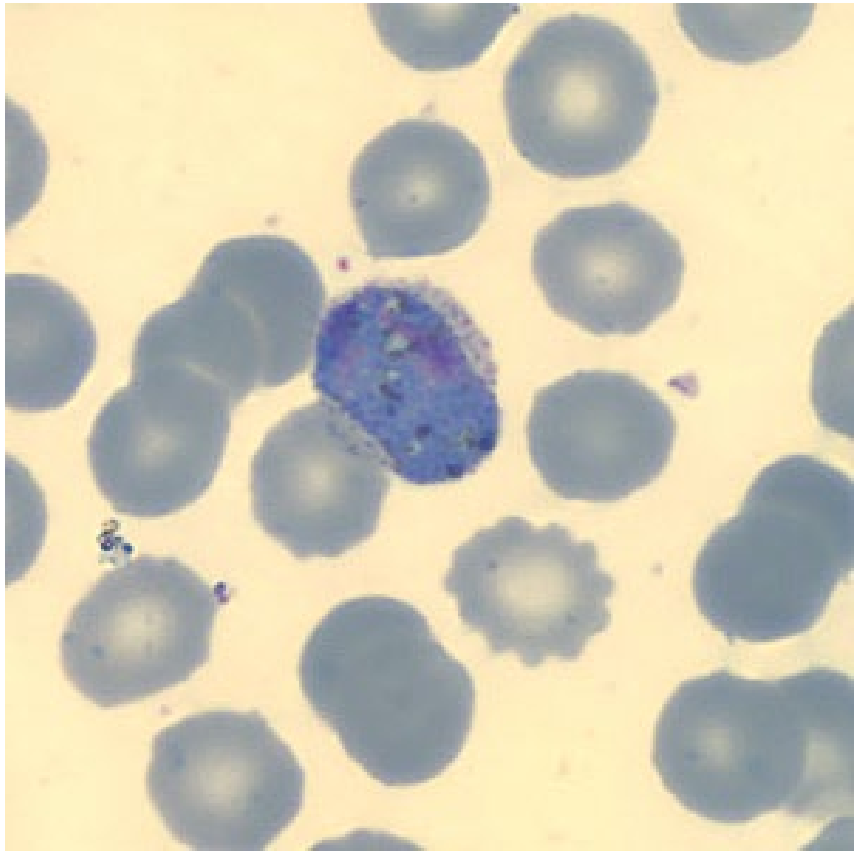
- What are we seeing in these images?
- What are some initial thoughts?
- Differentials?

Case 2, continued

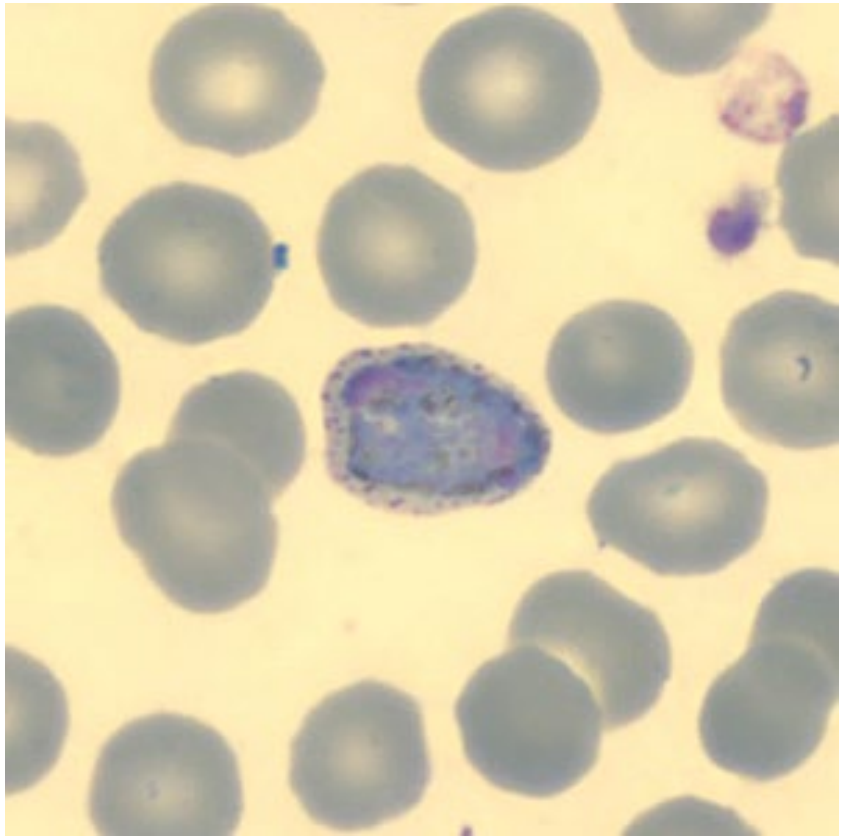
- The specimen was reported as *Plasmodium falciparum* and the patient was treated accordingly.
- One month later, the patient returned with recurrent fevers.
- There had been no documented international travel during that month.
- More blood films were collected and…

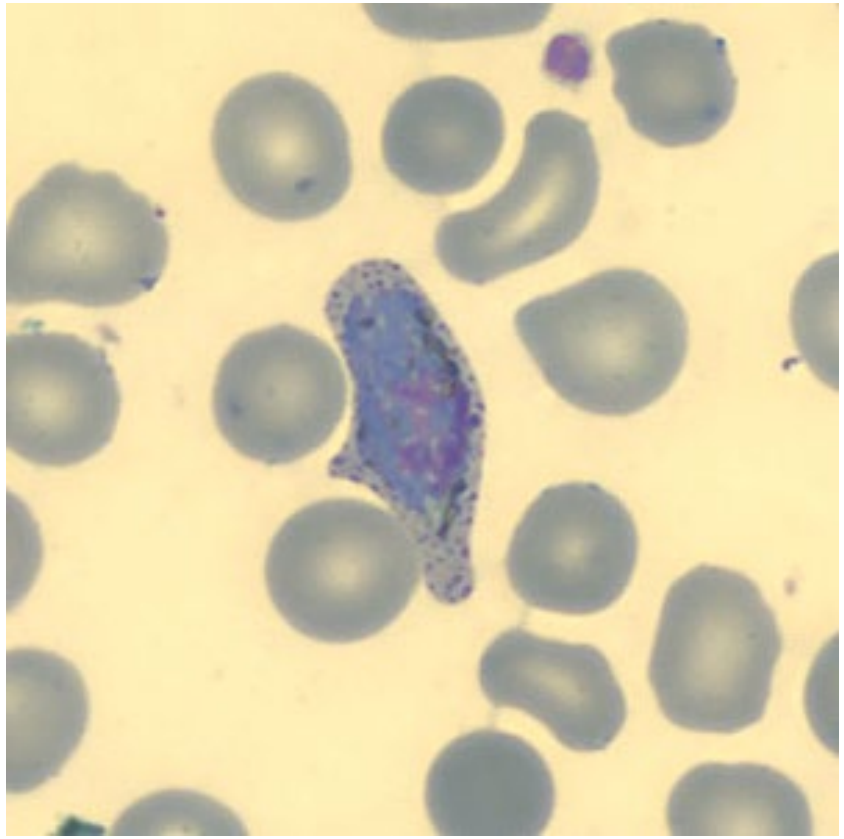
Case 2, images from one month later





Case 2, images from one month later





Case 2, continued

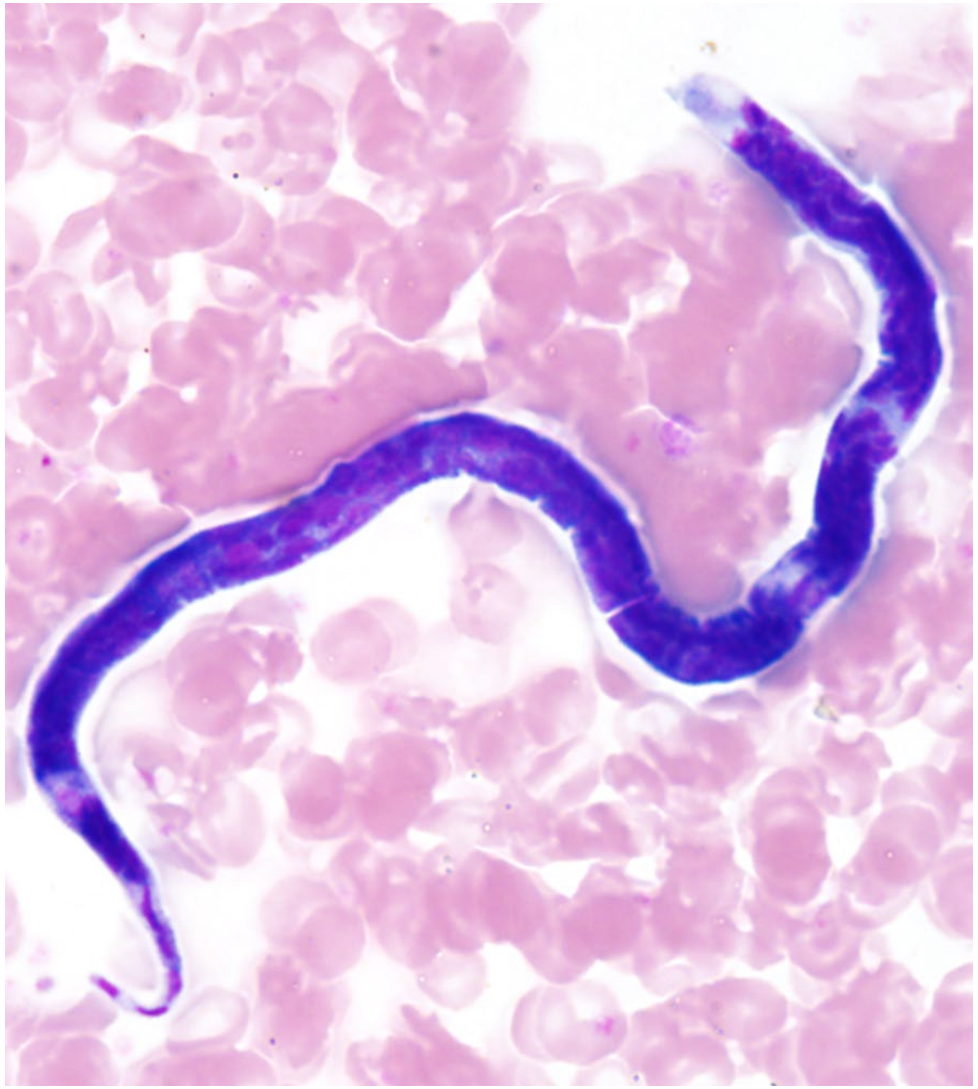
- What is seen in these newer images?
- How does it affect the initial diagnosis?
- Thoughts?

Case 2, conclusion

- PCR was performed on the initial and follow-up blood specimens
  - » Initial draw: Positive for *P. falciparum* and *P. vivax*
  - » Second draw: Positive for *P. vivax* (only)
- The patient initially had a mixed infection of *P. falciparum* and *P. vivax*.
  - » The patient was successfully treated for *P. falciparum* initially.
  - » The *P. vivax* was probably below the microscopic detection level
  - » The *P. vivax* later relapsed from its hypnozoite stage in the liver!

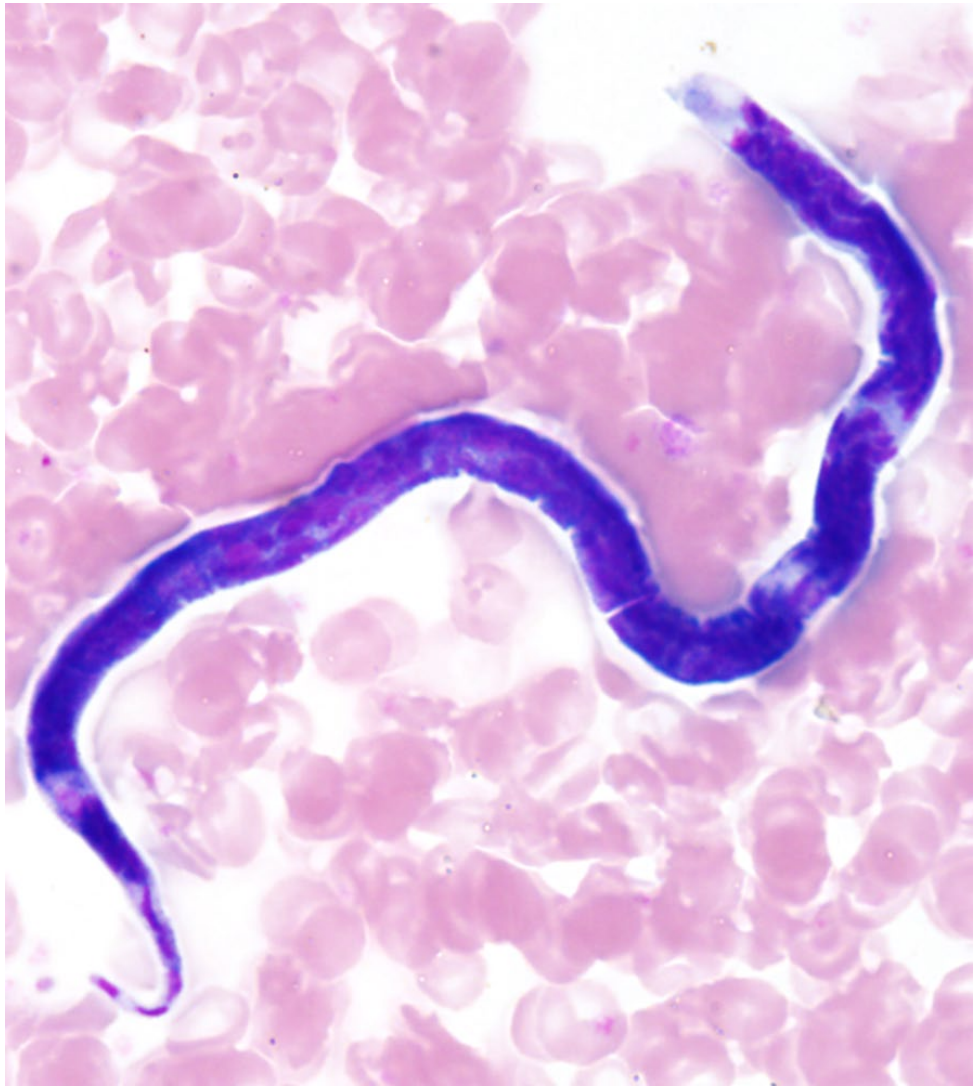
Case 3

- The following was seen in a thin blood film in a patient with travel to Cameroon.
- Object measured 245 µm
- Thoughts?



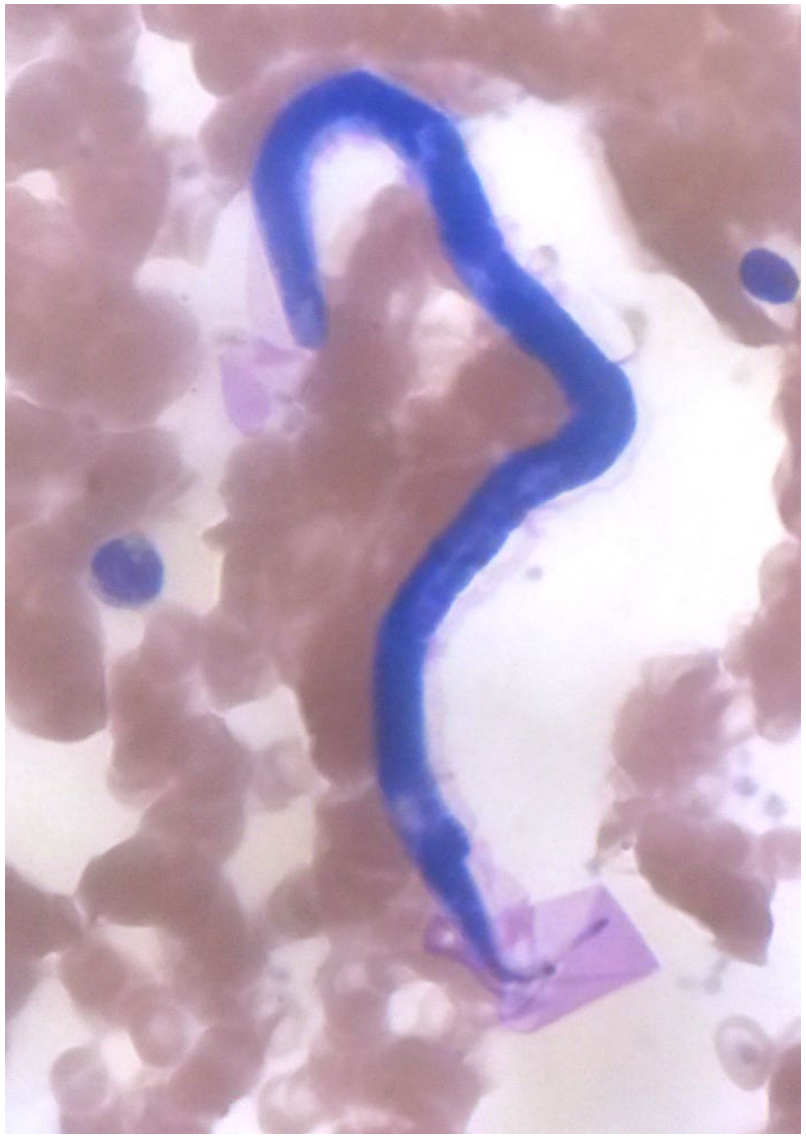
Case 3

- Identification: *Loa loa* (that lost its sheath)



Case 4

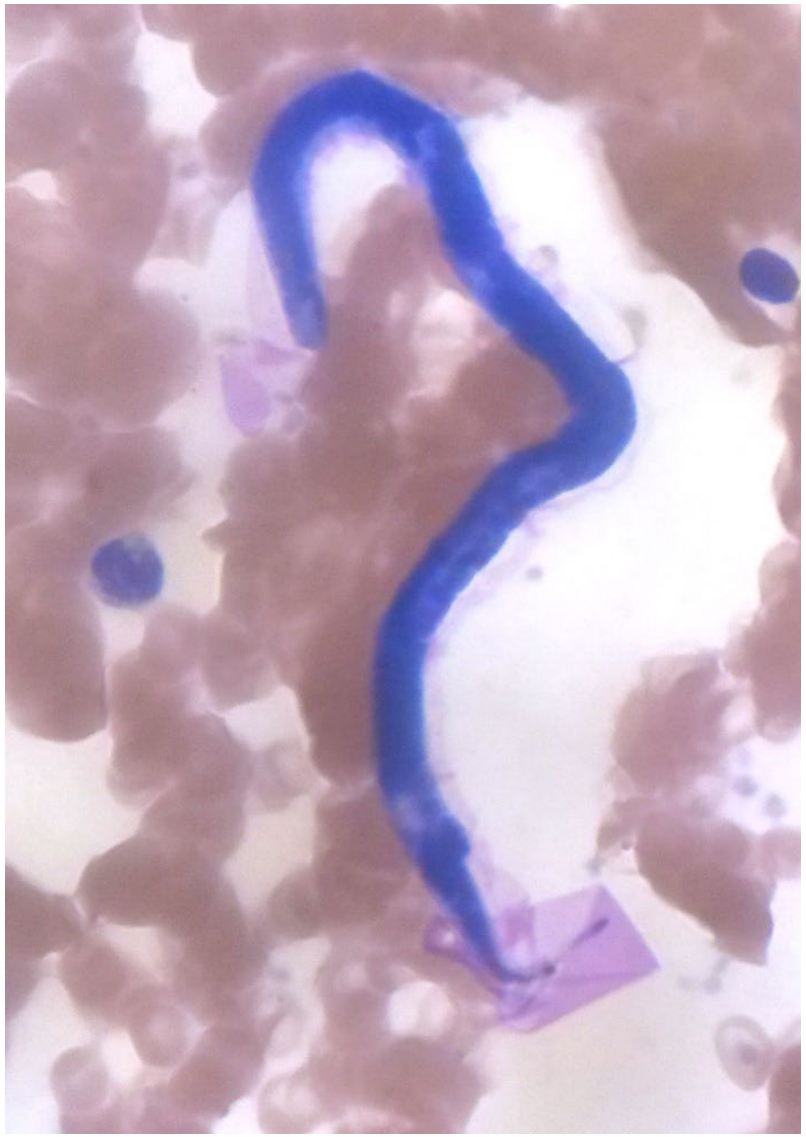
- Case courtesy of the South Carolina State PHL
- Thin blood film in a patient with travel to Gabon, Nigeria, and Mozambique
- Object measured 230 µm
- Thoughts?



image/case courtesy of the South Carolina PHL

Case 4

- Diagnosis: *Loa loa* with a pink sheath!!!

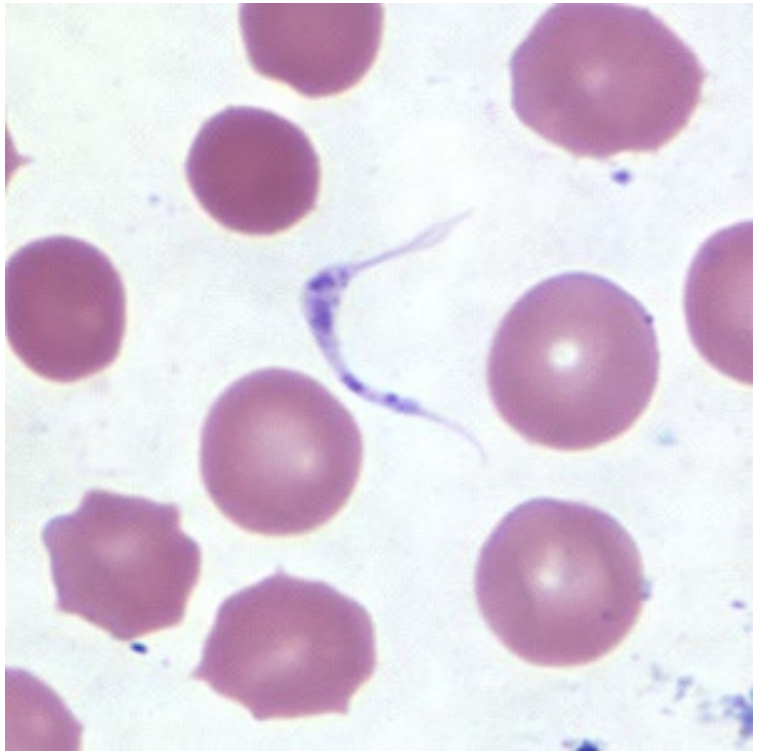


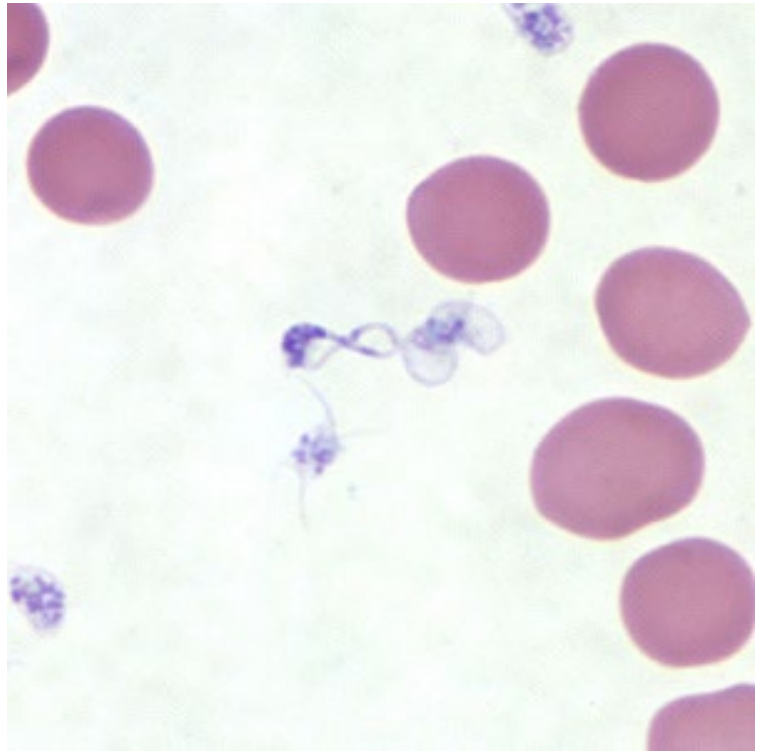
image/case courtesy of the South Carolina PHL

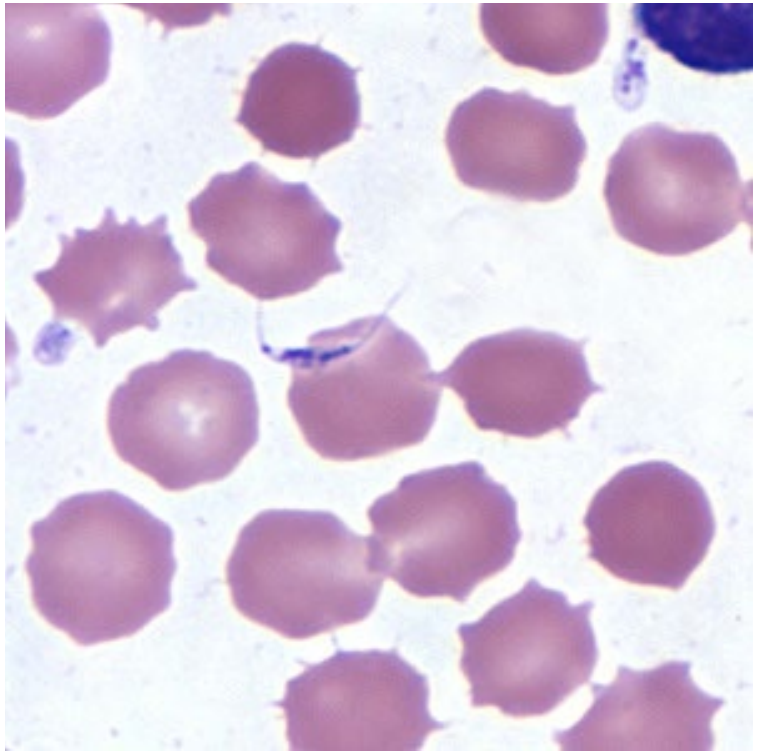
Case 5

- The following images were seen from a patient with two month travel to Senegal and The Gambia
- Upon return, reported to his health care provider with fatigue, headache, and fever
- Patient noted being bitten by insects while traveling

Case 5, images





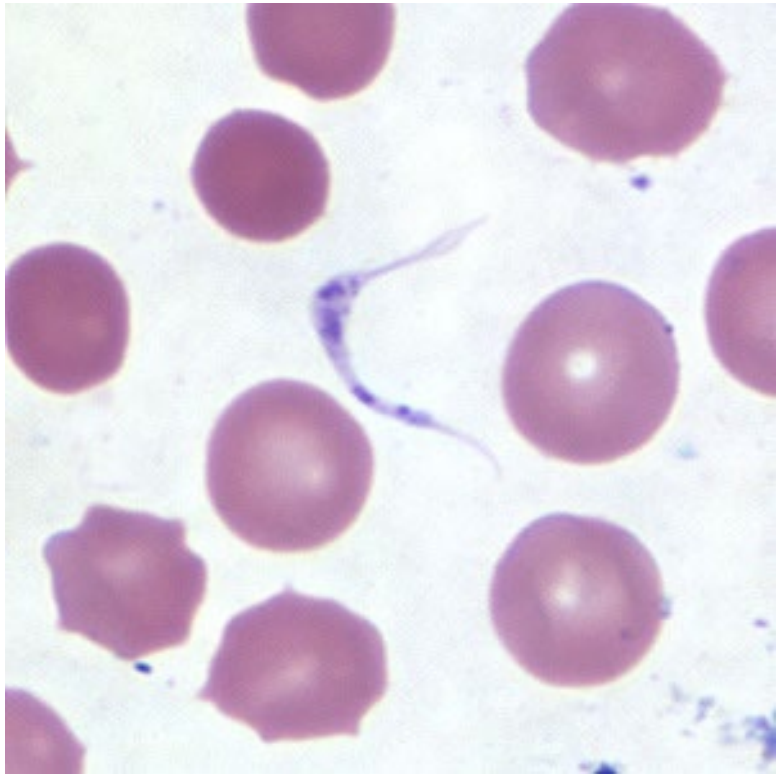


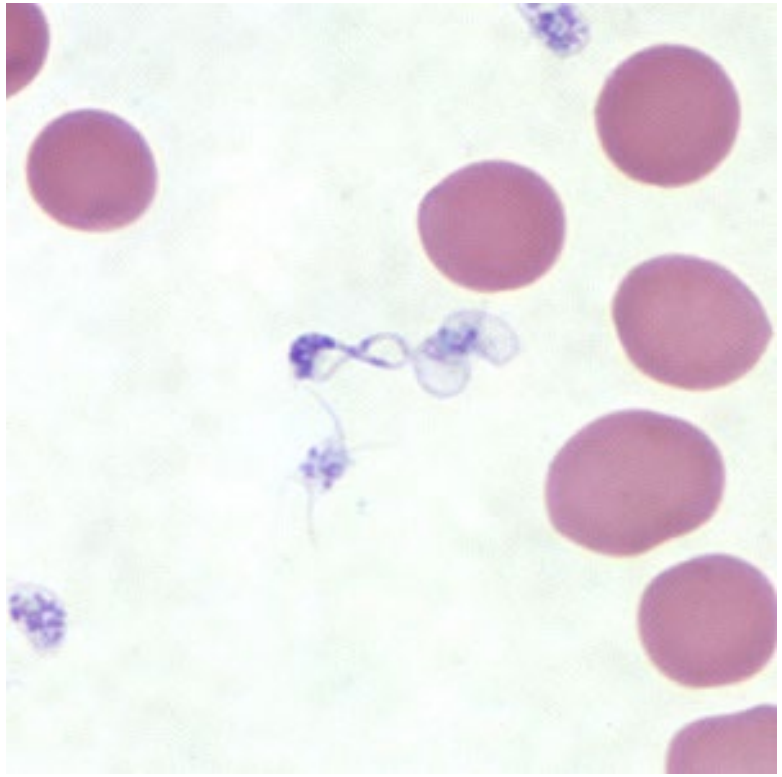
Case 5

- What are we seeing in these images?
- What are some initial thoughts?
- Differentials?

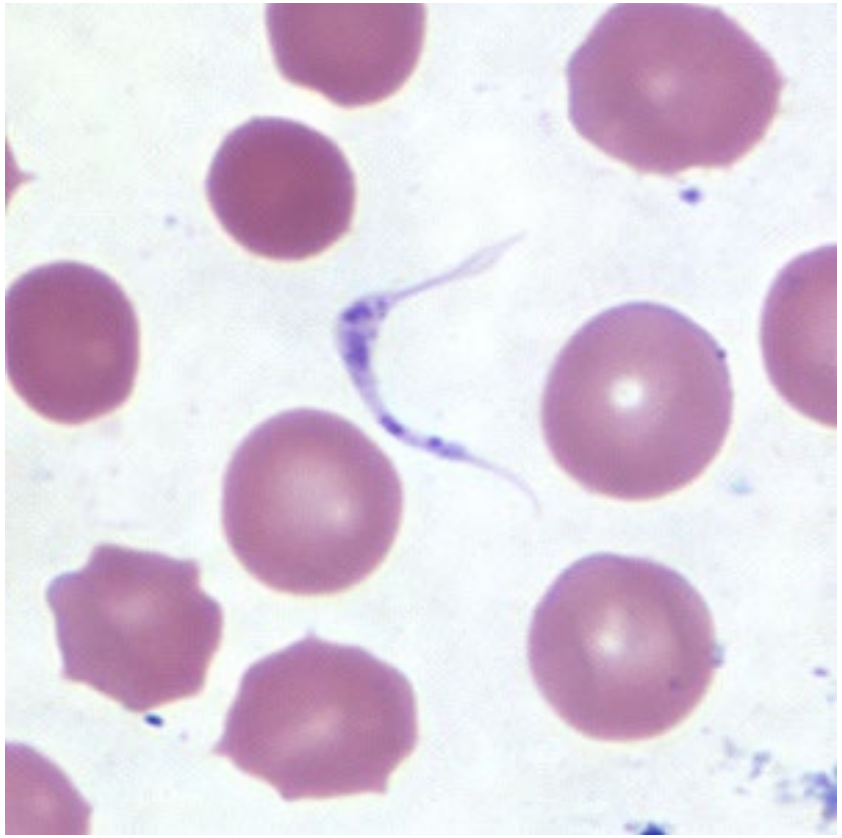
Case 5: answer

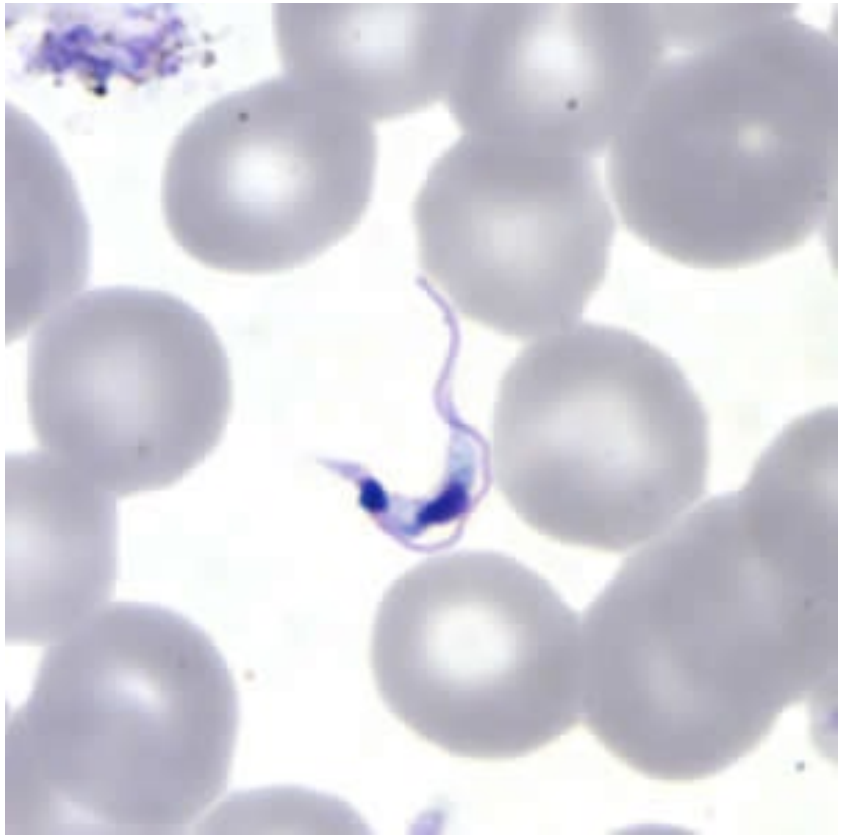
- Degrading platelets





Case 5: answer

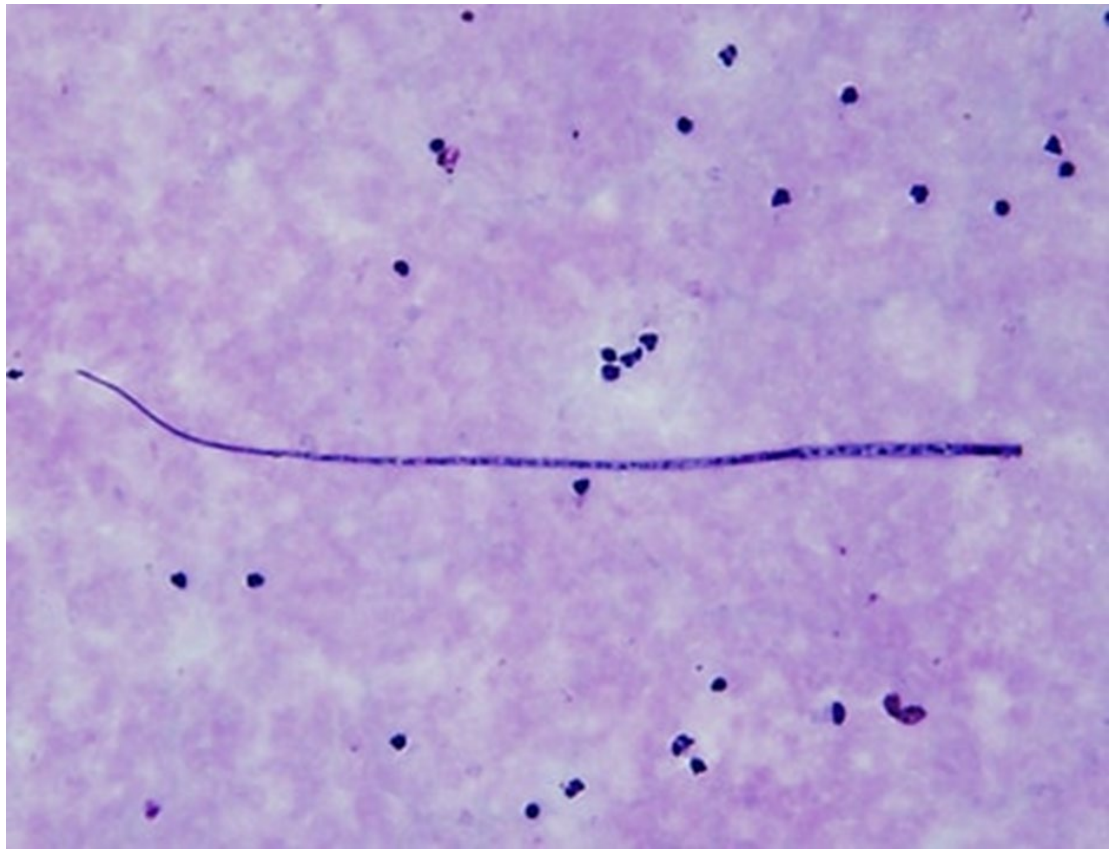




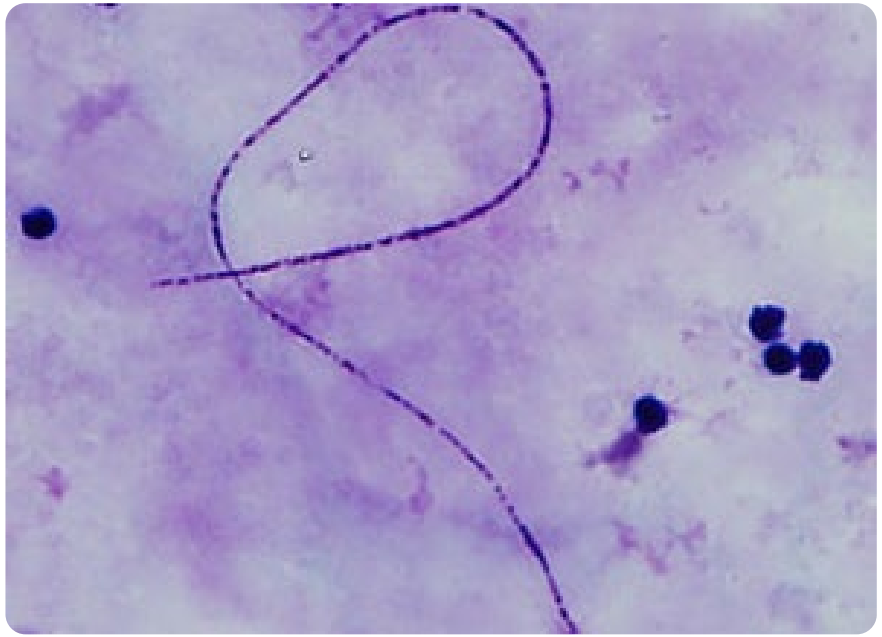
Case 6

- The CDC conducted a survey with the Ministry of Health in Uganda to evaluate the prevalence of filariasis among the local population.
- Skin snips and blood specimens were collected and sent to a regional laboratory for preparation
- Thick blood films were made and stained with Giemsa and then forwarded to the CDC in Atlanta for evaluation.
- The following were observed on thick blood films stained with Giemsa

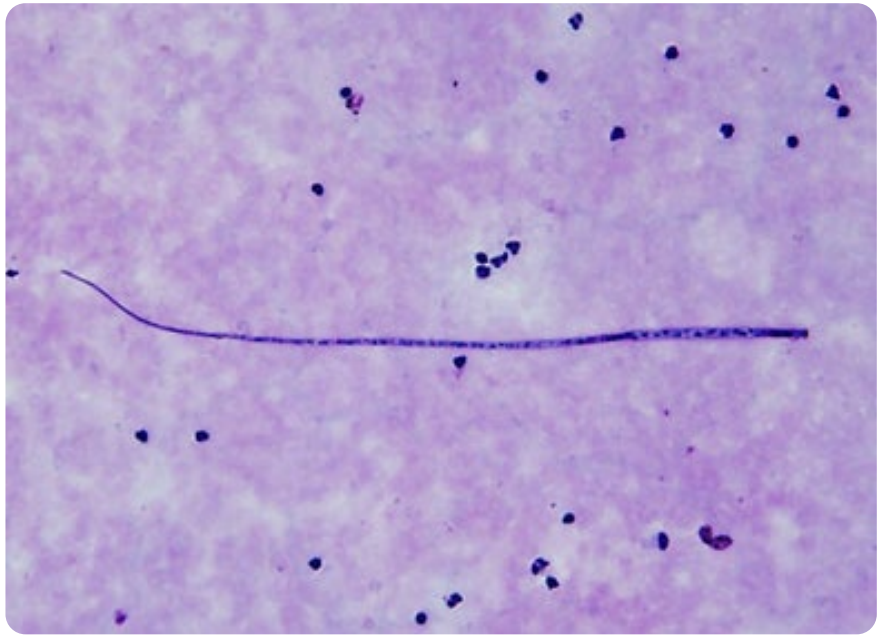
Case 6, images



Length:
225 µm



Length:
325 µm

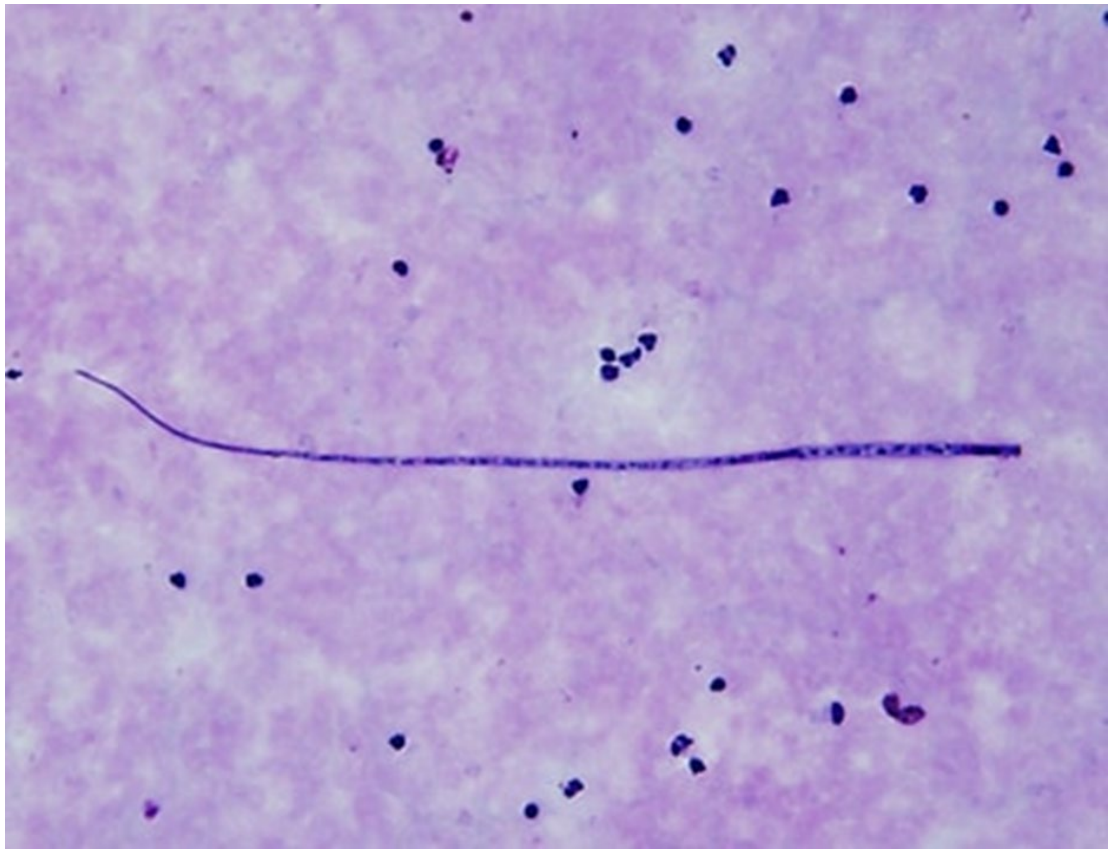


Case 6, continued

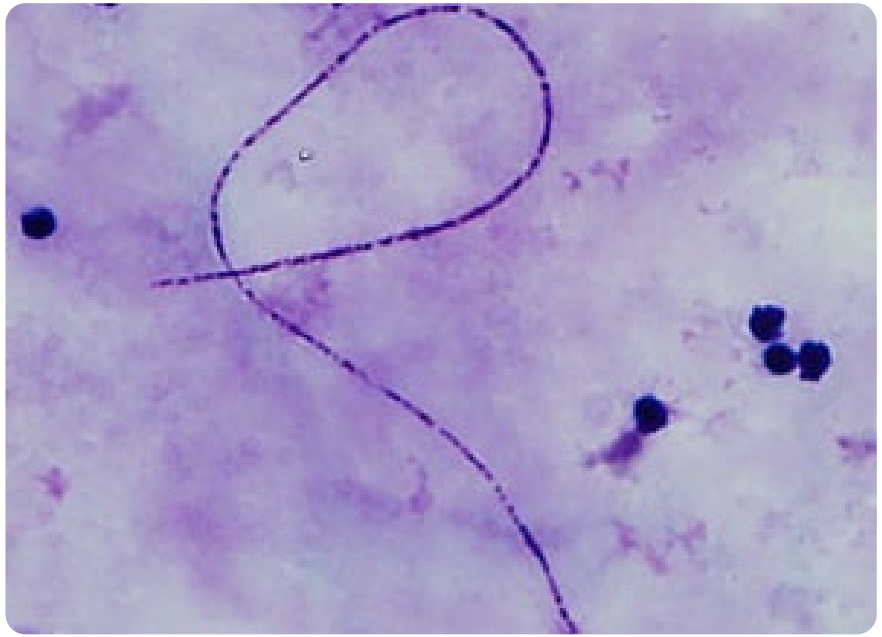
- What are we seeing in these images?
- What are some initial thoughts?
- Differentials?

Case 6, answer

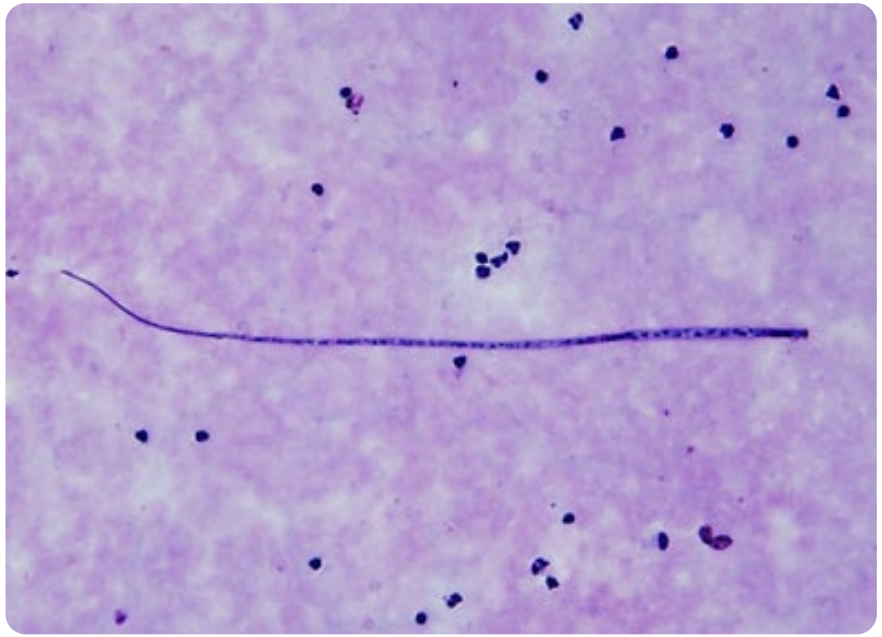
Diagnosis: fungal elements!



Length:
225 µm



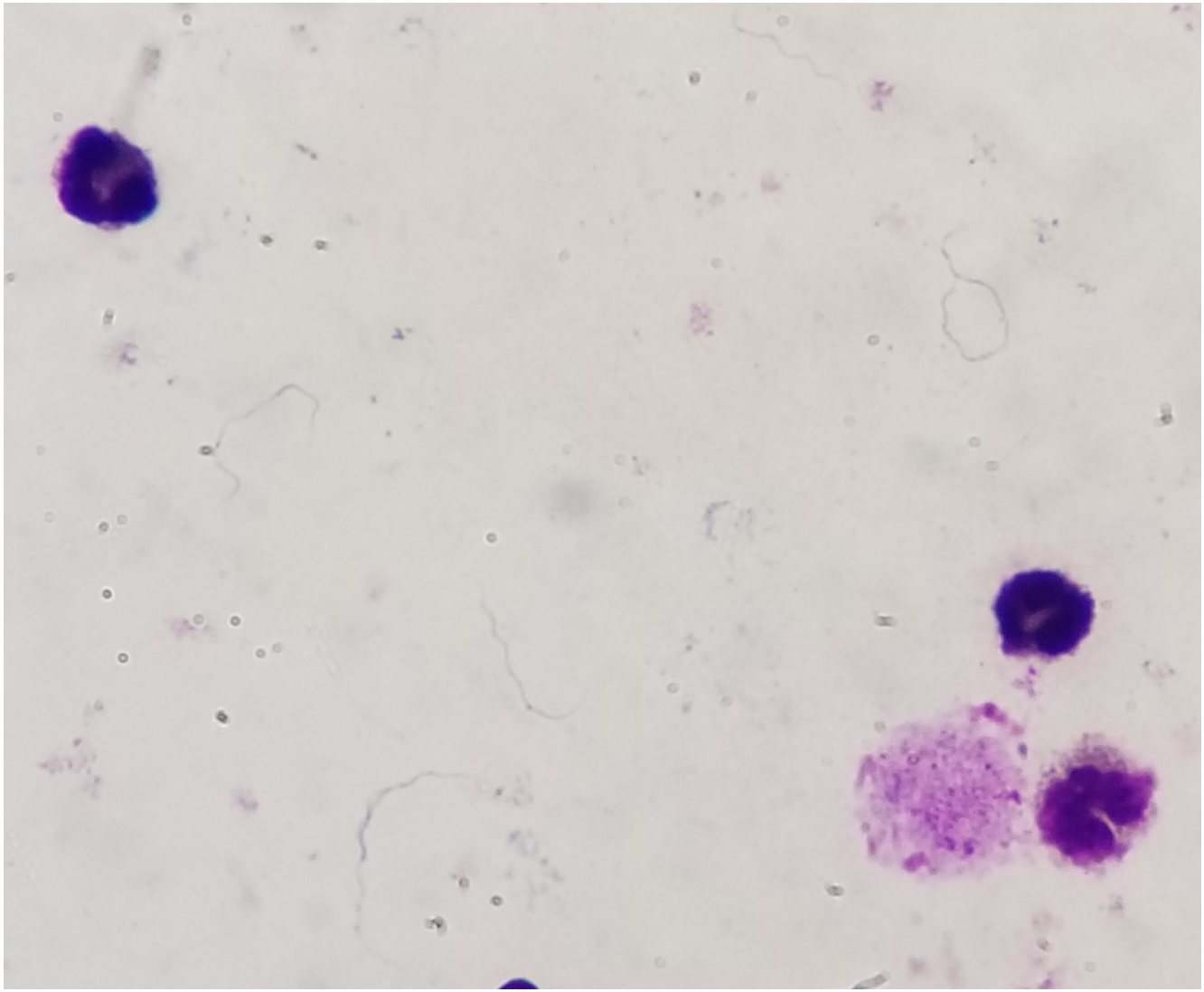
Length:
325 µm

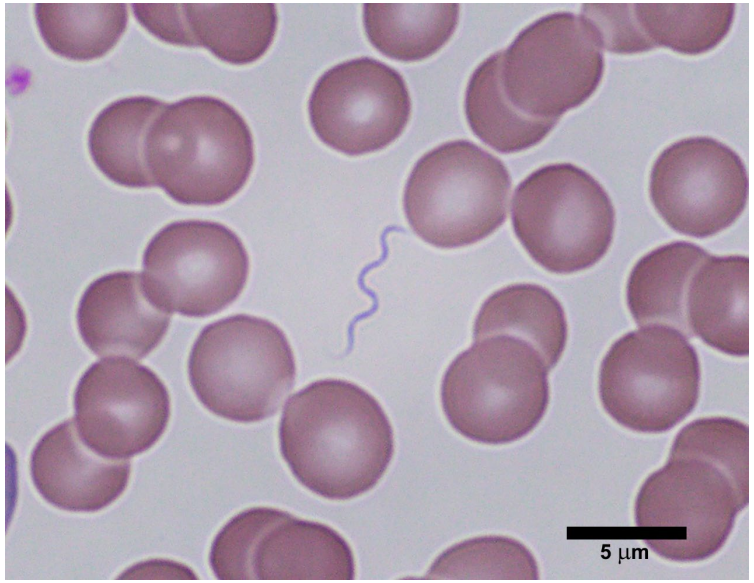


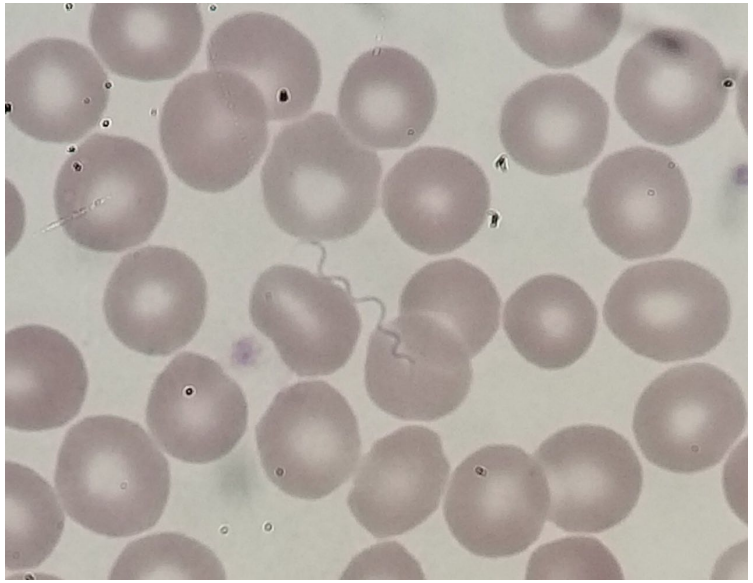
Case 7

- The following objects were seen on blood films in a patient in Utah with no documented international travel
- The patient is an avid outdoorsman and likes to fish, hunt, and camp.
- He owns a rustic cabin in the Wasatch Mountains outside Salt Lake City.
- He has two pet dogs but no other known animal contacts.

Case 7, Images







Images courtesy of Dr. Marc R. Couturier

Case 5

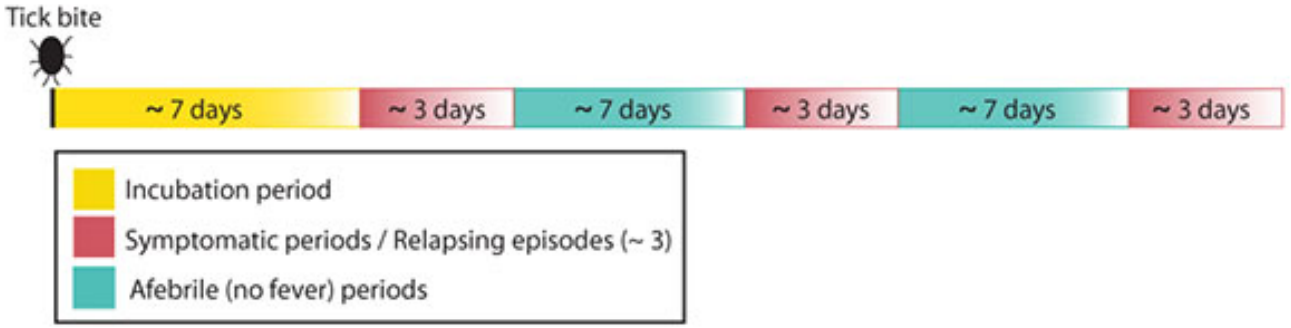
- What are we seeing in these images?
- What are some initial thoughts?
- Differentials?

Case 7, Answer

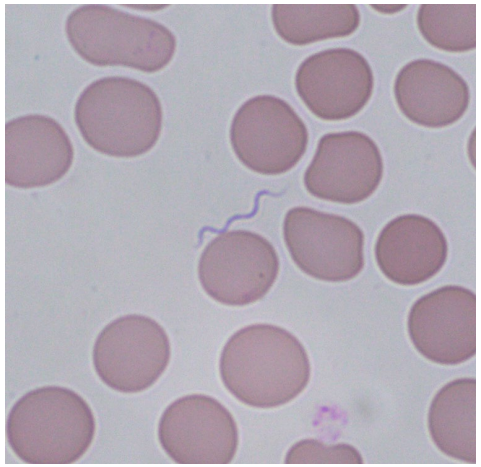
- Relapsing fever spirochete (*Borrelia* sp.)

Relapsing Fever *Borreliosis*

- Detected in blood smears (intentionally or accidental)
- NAATs (most sensitive)
- Serology (retrospective)



- Recurring febrile episodes ~3 days separated by afebrile period ~7 days
  - » 75%: headache, myalgia, chills, nausea
  - » 50%: arthralgia, vomiting
  - » 25%: abdominal pain, dry cough, eye pain, diarrhea, photophobia, neck pain

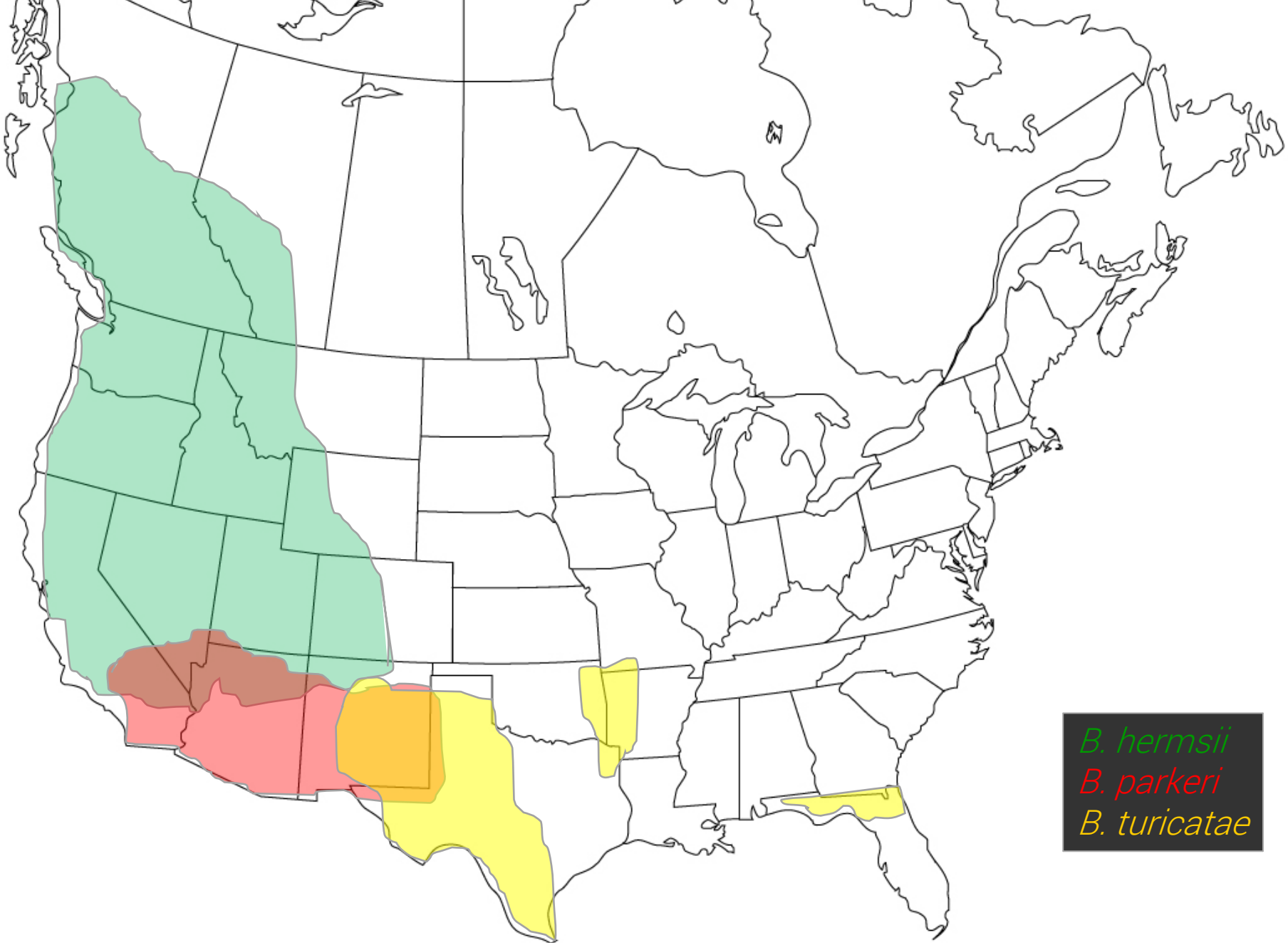


Slide courtesy of Dr. Marc Roger Couturier

Soft Ticks and Relapsing Fever *Borrelia*

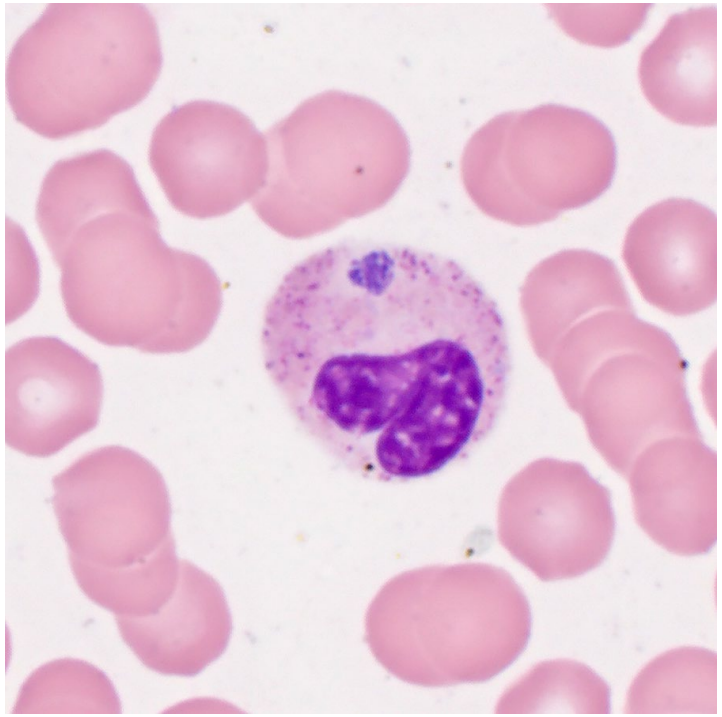
Relapsing Fever Borrelia	Tick Vector(s)	Geographic Distribution
Borrelia baltazardii	Unknown	Iran
Borrelia braziliensis	*Ornithodoros braziliensis*	Brazil
Borelia caucasica	*Ornithodoros verrucosus*	Western Asia
Borrelia crocidurae	*Ornithodoros sonrai*	West Africa
Borrelia duttonii	*Ornithodoros moubata*	Central and East Africa
Borrelia graingeri	*Ornithodoros graingeri*	Kenya
Borrelia hermsii	***Ornithodoros hermsi***	**Western North America**
Borrelia hispanica	*Ornithodoros marocanus*, *O. occidentalis*, *O. kairouanensis*	Iberian Peninsula, North Africa
'*Candidatus* Borrelia kalaharica'	*Ornithodoros savingnyi*	Southern Africa
Borrelia latyschewii	*Ornithodoros tartakowskyi*	Central Asia, Middle East
Borrelia mazzottii	*Ornithodoros talaje*	Central America
Borrelia parkeri	***Ornithodoros parkeri***	**Western United States**
Borrelia persica	*Ornithodoros tholozani*	North Africa, Middle East, Central Asia
Borrelia turicatae	***Ornithodoros turicata***	**Southern United States, Mexico**
Borrelia venezuelensis	*Ornithodoros rudis*	Central and South America

Ornithodoros turicata

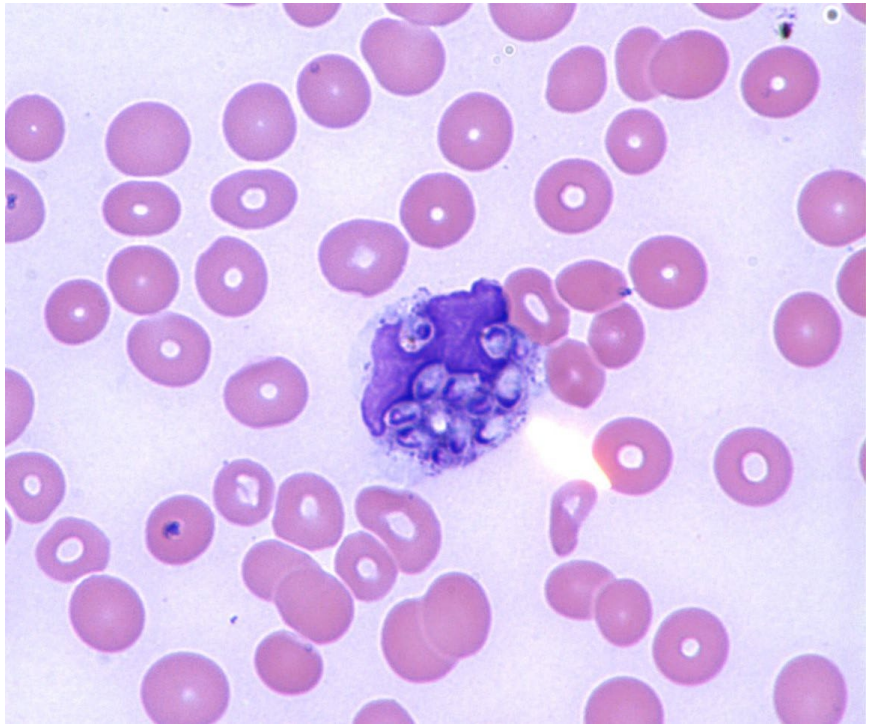


Map courtesy of Dr. Marc Roger Couturier

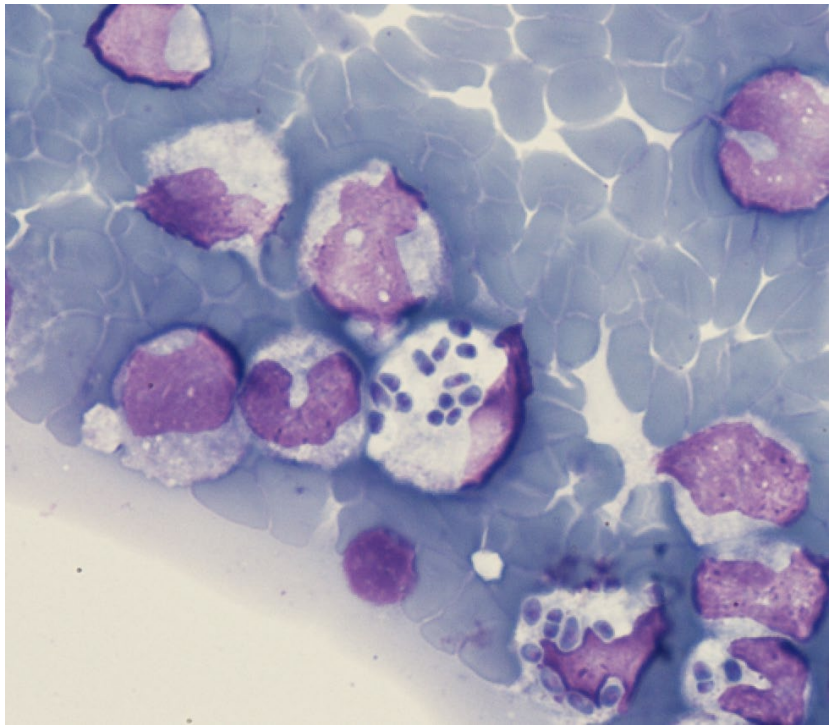
What else non-parasitic etiologic agents can be seen on blood films?



Anaplasma phagocytolyticum



Histoplasma capsulatum



Talaromyces marneffei

Images courtesy of Dr. Bobbi S. Pritt

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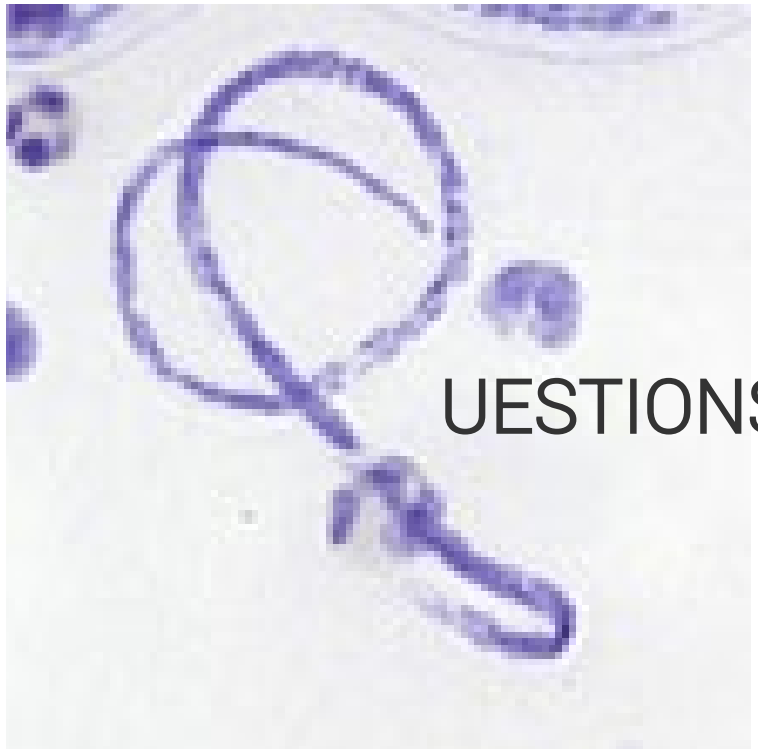
- CDC-DPDx (images and life cycles)
- Numerous collaborators who provided images and cases



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