Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Microscopic Identification of Microbes in Primary Blood Smears

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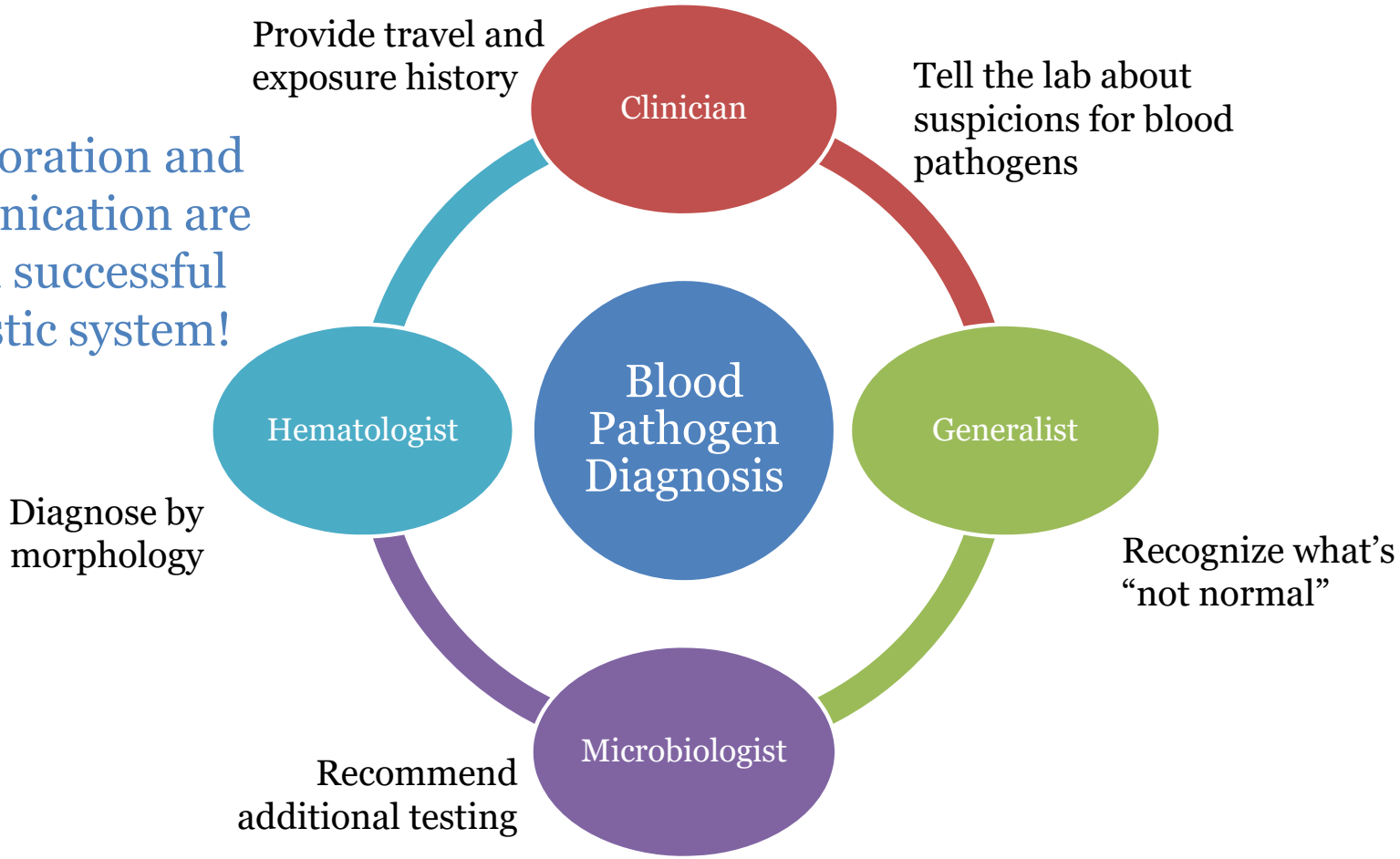
Contents

Wisconsin focused discussion on microbes you can find in primary blood smears for a laboratory audience

- Morphology (pictures!)
- Supporting clinical data
- Additional testing

*We will not cover treatment or blood cultures

*Collaboration and communication are key to a successful diagnostic system!

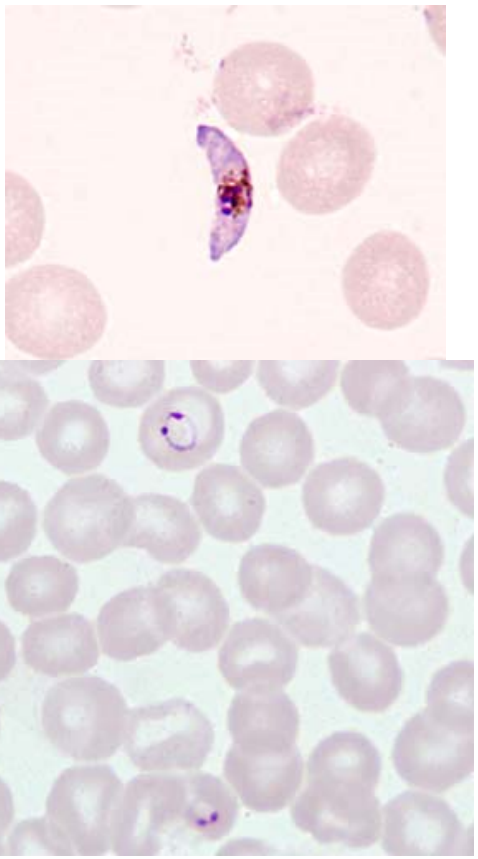


Blood Smear Methods

- Most of this talk is based on detection using a Giemsa stain.
- Wright and Giemsa-Wright stains can also be used to detect blood pathogens but they may not be preferred for certain characteristics like stippling on red cells from Malaria or observing the sheath of a microfilaria.
- Pathogens can also be observed in gram stains and wet mounts from primary blood so anyone viewing these slides should be aware of the possible pathogens they could encounter.

Malaria

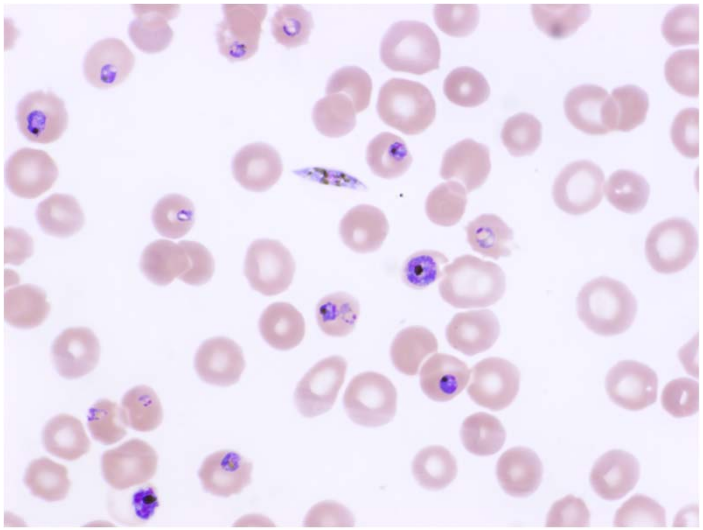
- Malaria can be immediately life threatening.
- Testing should be available on a 24-hour STAT basis
- Requests for testing should be accompanied by information related to clinical signs and symptoms, travel history, and receipt of malaria chemoprophylaxis or therapeutic antimalarial agents.



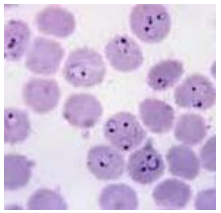
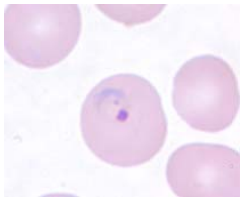
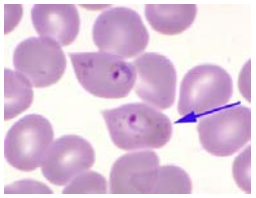
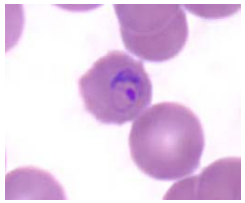
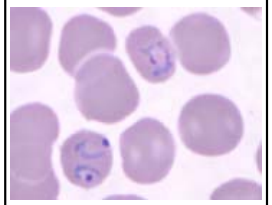
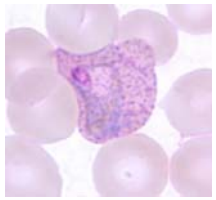
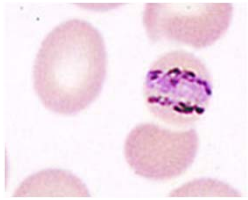
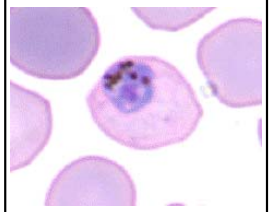
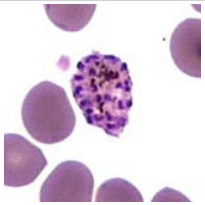
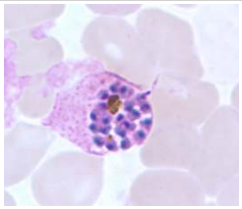
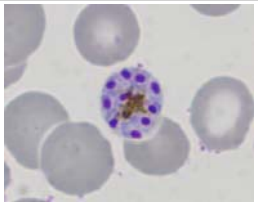
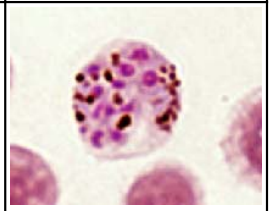
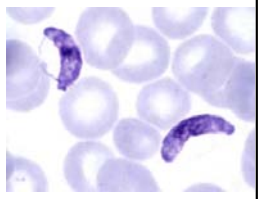
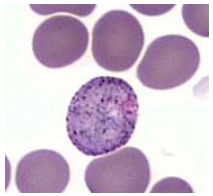
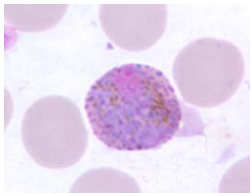
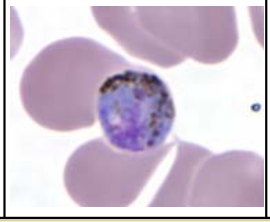
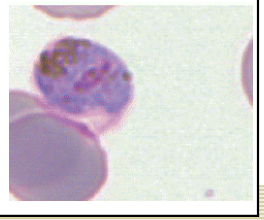
Additional Resource

WCLN Malaria Diagnostics Webinar

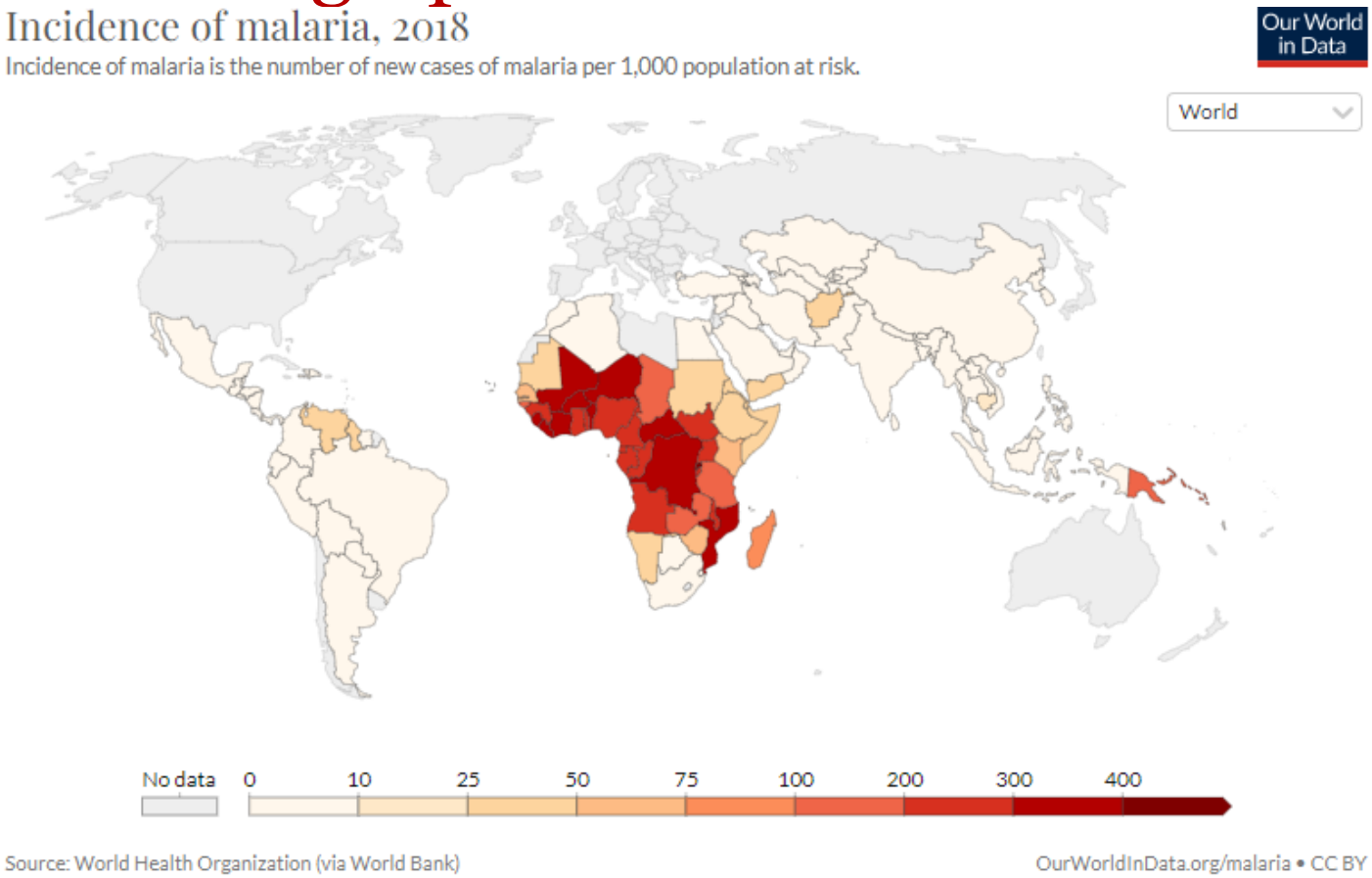
http://www.slh.wisc.edu/event/wcln-webinar-malaria-diagnostics/



Plasmodium morphological features in peripheral blood

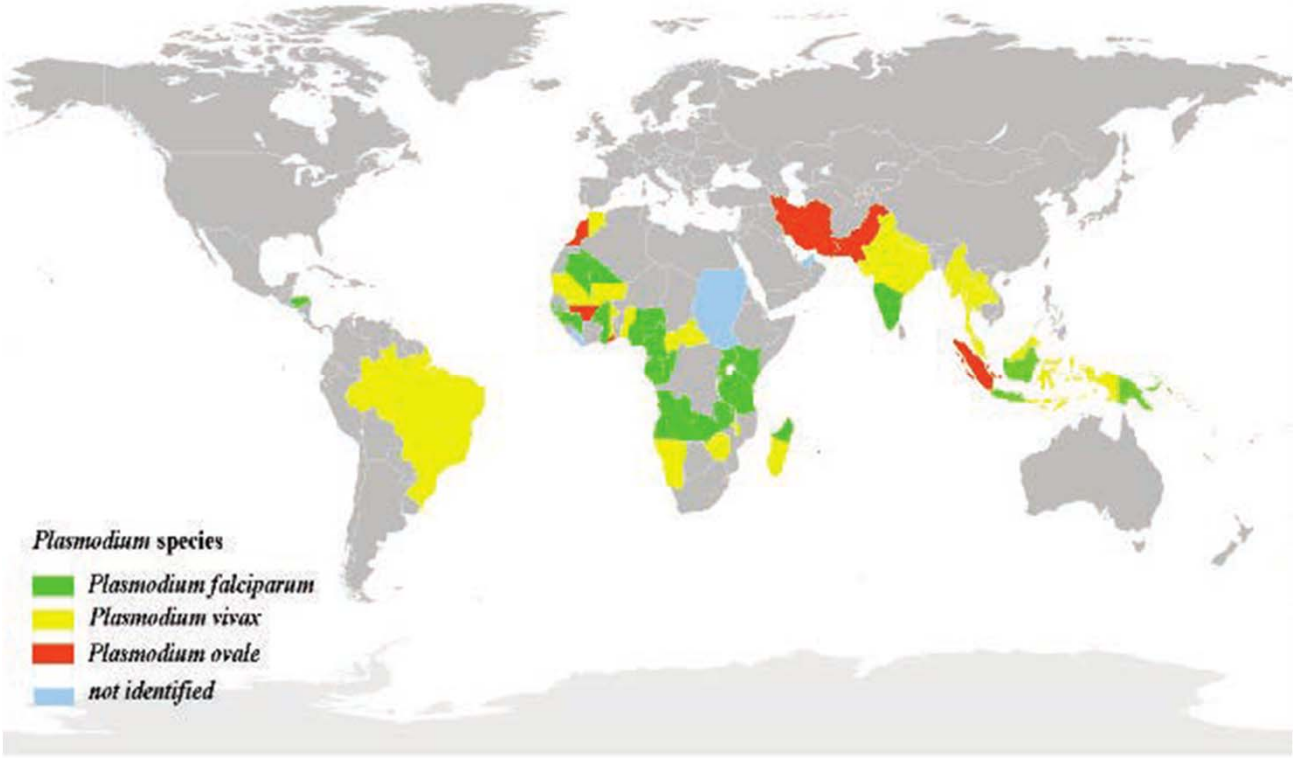
	falciparum	*vivax*	*ovale*	*malariae*	*knowlesi*
Immature Trophozoite (ring form)					
Mature Trophozoite (amoeboid form)	Very rarely seen				
Schizont	Very rarely seen				
Gametocyte					

Geographic Distribution



https://ourworldindata.org/malaria

Distribution of the Species



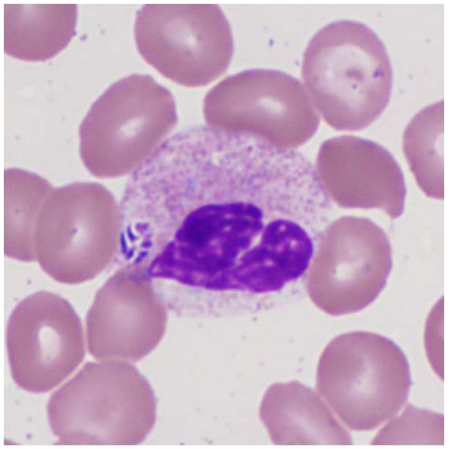
Testing methods

- Microscopic examination of blood films
  - Fast, cheap, can ID to the species, and provide % parasitemia
- Molecular analysis
  - Less subjective, more accurate, and more sensitive.
  - No parasitemia, slower and expensive
- Antigen detection
  - Inexpensive, fast
  - May not detect all species, no parasitemia
- Susceptibility testing at CDC
- Please send all positives to WSLH

Case

- A 68 year old man underwent elective knee surgery. Due to complications from the procedure he received multiple blood products.
- Seven days after the surgery he developed sepsis and multisystem failure.
  - Blood cultures were negative.
- 22 days after admission a peripheral blood smear identified inclusions in neutrophils.
- Retrospective review of blood smears right after his surgery did not find these inclusions.

Anaplasma phagocytophilum



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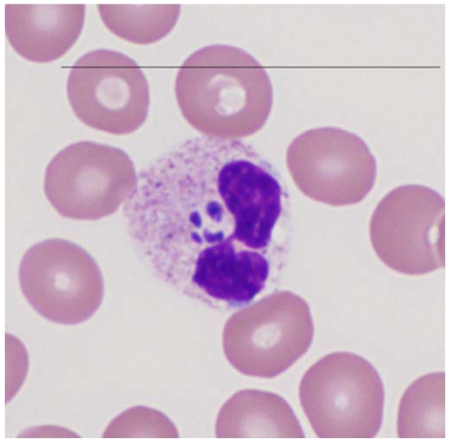
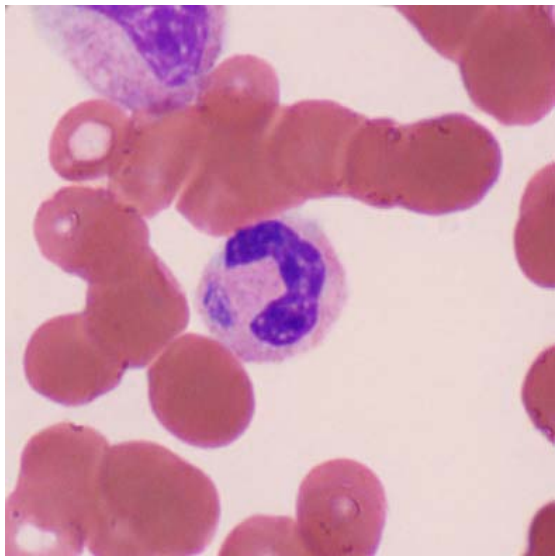
Anaplasma phagocytophilum

- Spread by tick bite
  - Ixodes and lone star ticks
- Less commonly, spread through blood transfusion and organ transplant
  - Can survive for a week in refrigerated blood
  - Blood products are not routinely screened for *A. phagocytophilum*

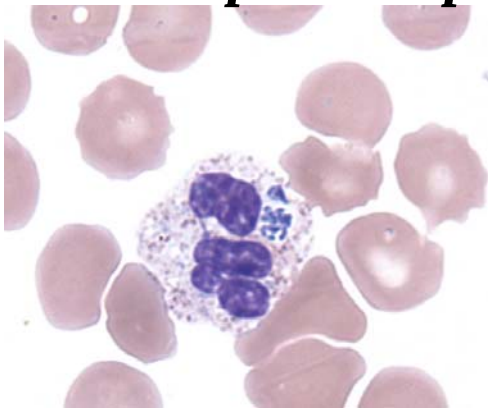


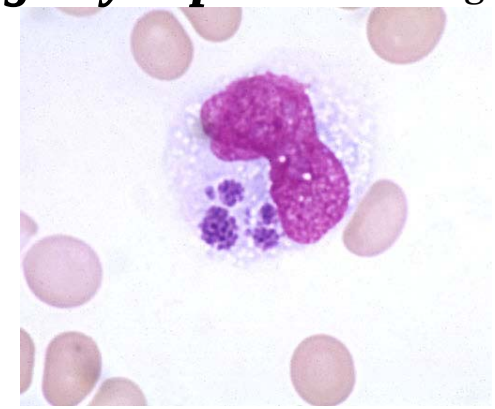
Signs and Symptoms

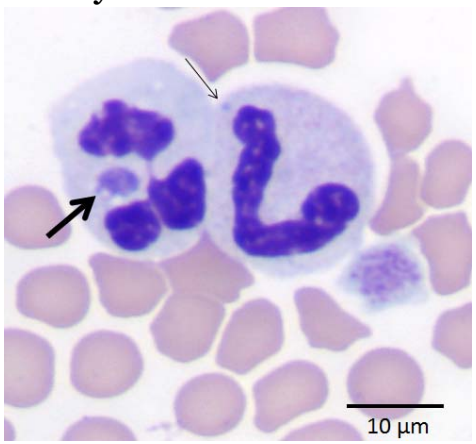
- Fever, chills
- Severe headache
- Muscle aches
- Nausea, vomiting, diarrhea, loss of appetite
- Respiratory failure
- Bleeding problems
- Organ failure
- Death



Anaplasma phagocytophilum- in granulocytes

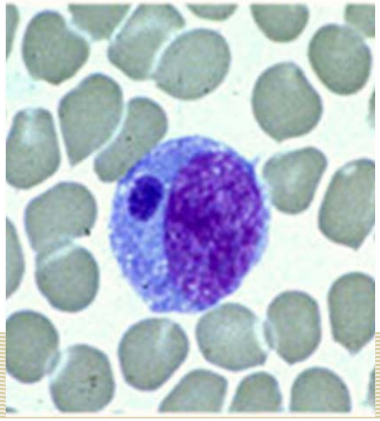


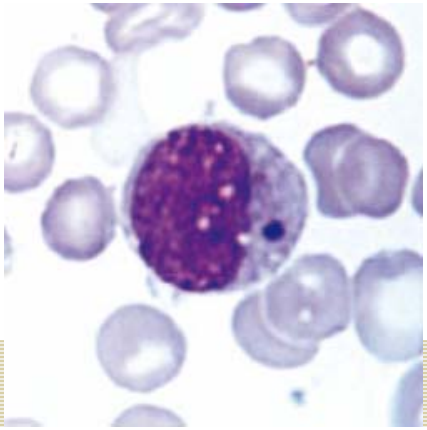


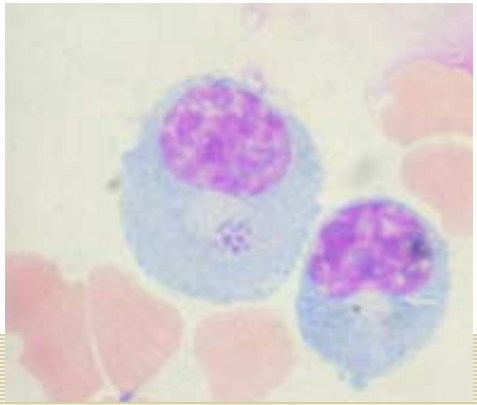


*The observance of morulae in a particular cell type cannot conclusively differentiate among *Ehrlichia* species or between *Ehrlichia* and *Anaplasma*

***Ehrlichia* species-** Usually in monocytes, can be in granulocytes

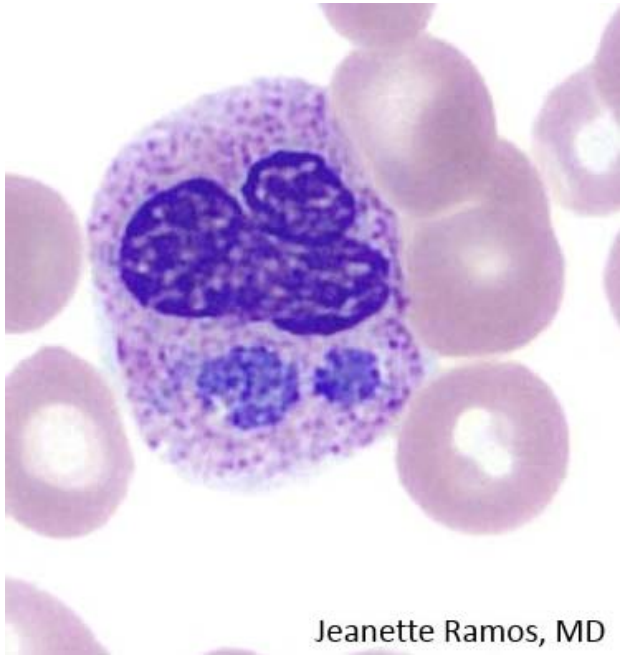






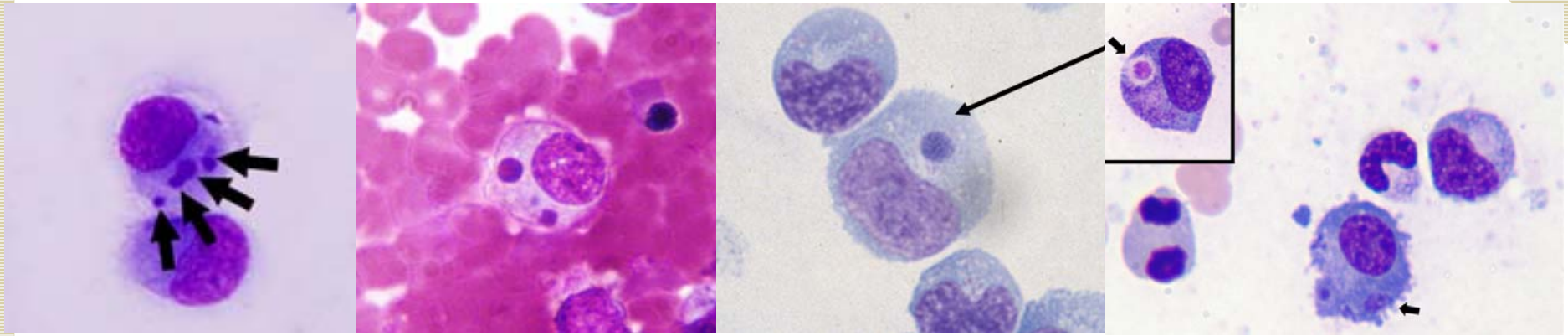
Ehrlichia Species

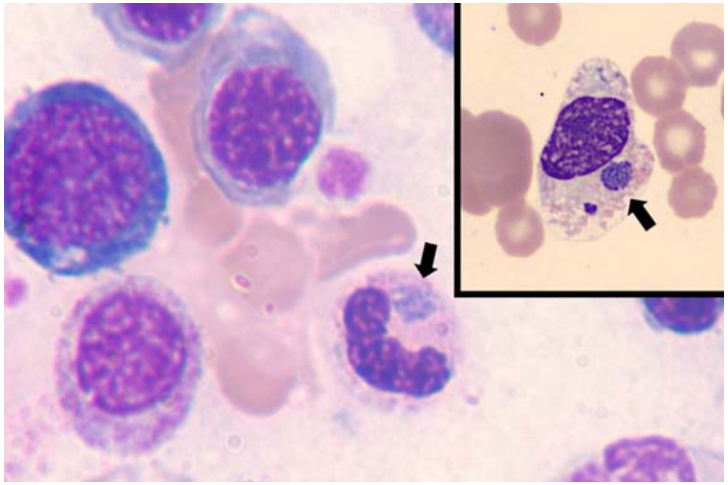
- Spread by tick bite
  - Ixodes and lone star ticks
- Multiple species can cause disease
  - *E. chaffeensis* most commonly infects monocytes.
  - *E. ewingii* more commonly infects granulocytes.
  - No target cell has been identified for *E. muris eauclairensis*.
- Blood smear examination is relatively insensitive and should not be relied upon solely for diagnoses
- In rare cases, *Ehrlichia* species have been spread through blood transfusion and organ transplant.

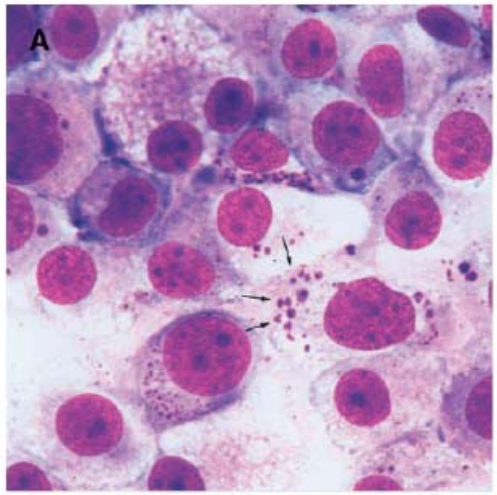


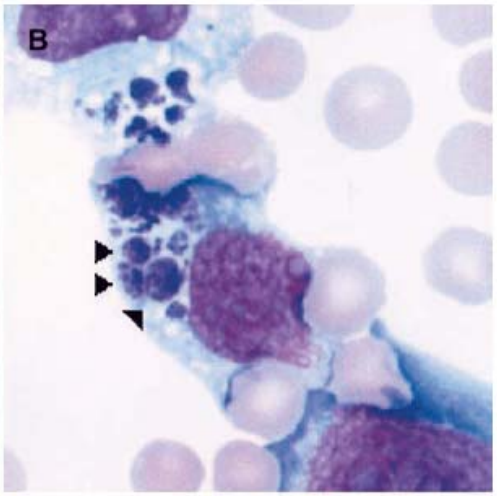
Jeanette Ramos, MD

Ehrlichia



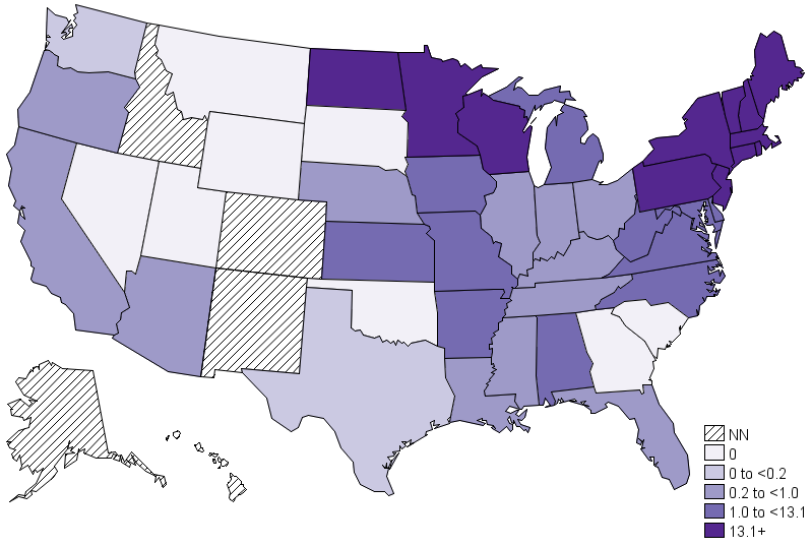






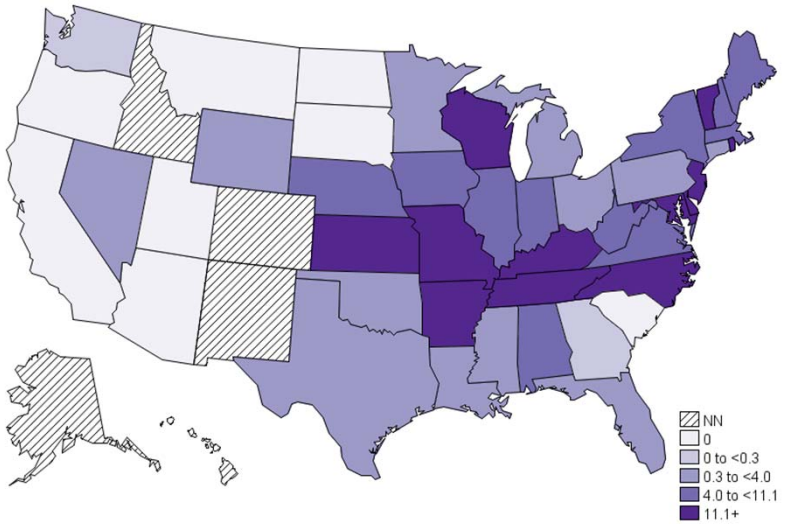
Widespread in the US

Anaplasma phagocytophilum



https://www.cdc.gov/anaplasmosis/stats/index.html

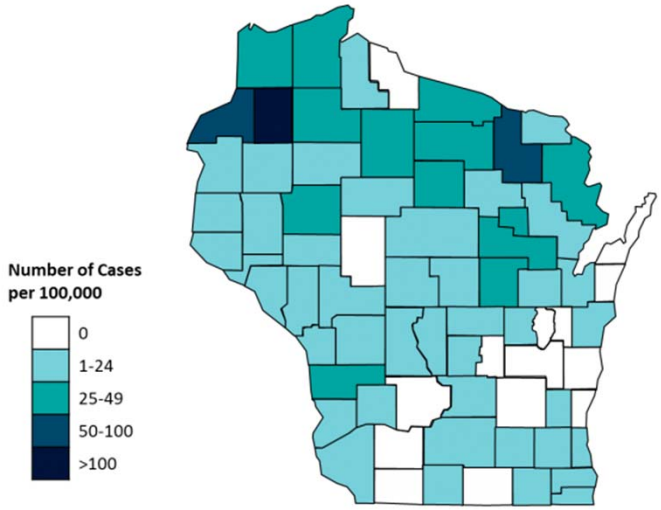
Ehrlichia spp.



https://www.cdc.gov/ehrlichiosis/stats/index.html

Cases in Wisconsin

Cases of Anaplasmosis per 100,000 Wisconsin Residents, 2018

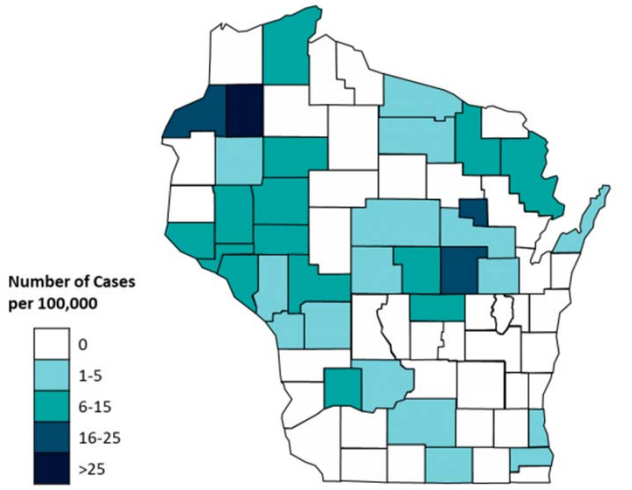


Data Source: Wisconsin Department of Health Services

https://www.dhs.wisconsin.gov/tick/anaplasmosis-about.htm

Cases of Ehrlichiosis per 100,000 Wisconsin Residents, 2018

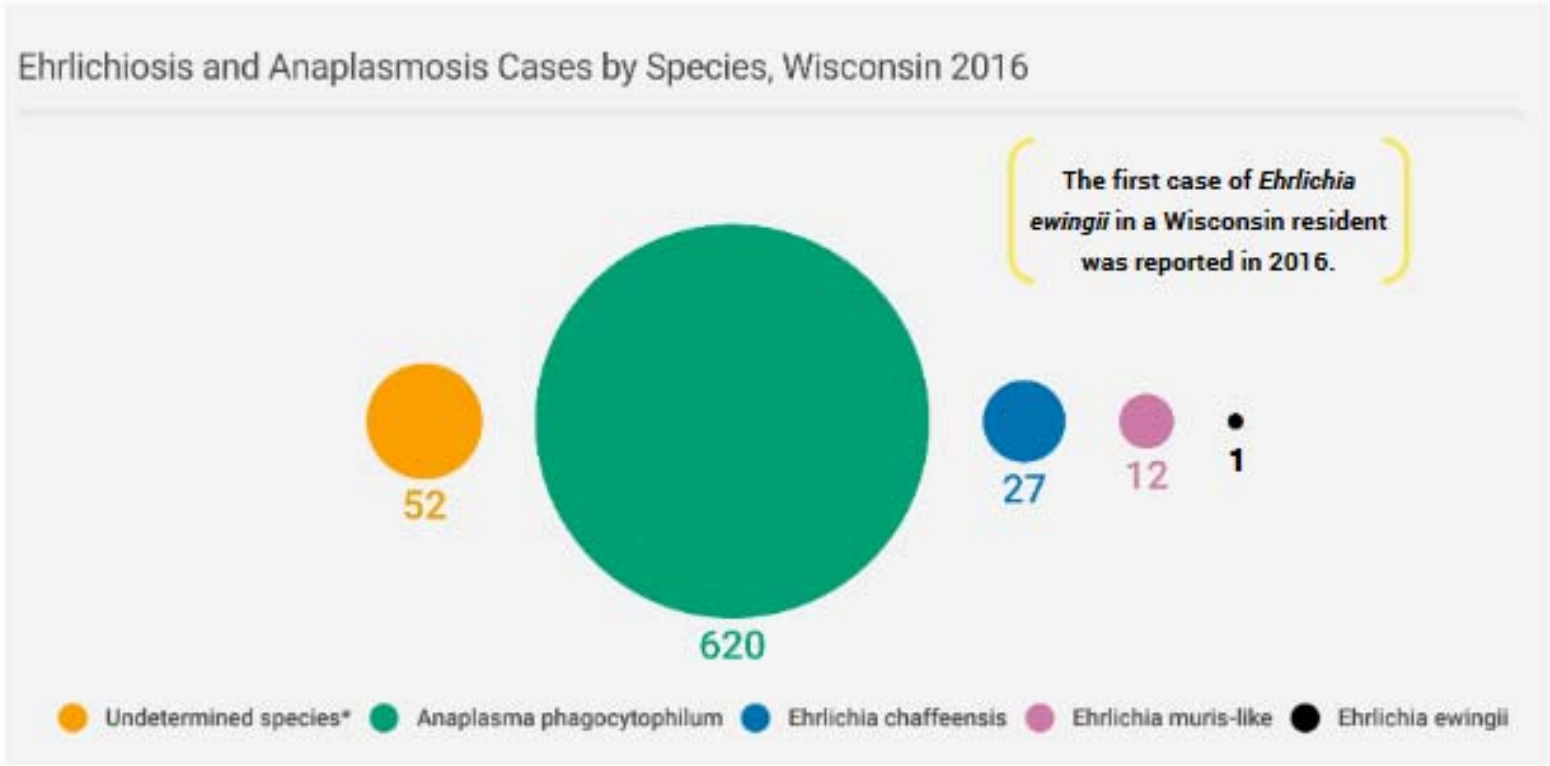
This map includes cases of *E. chaffeensis, E. muris eauclairensis, and E. ewingii.*



Data Source: Wisconsin Department of Health Services

https://www.dhs.wisconsin.gov/tick/ehrlichiosis-data.htm

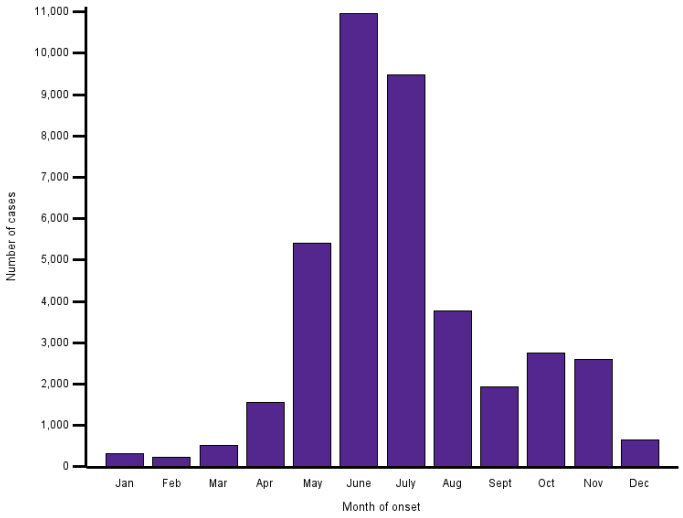
Anaplasma more common in WI



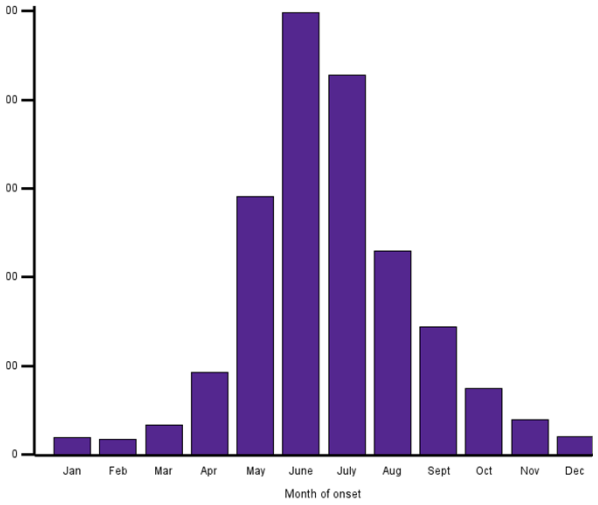
*Anaplasma and Ehrlichia species are reported as "undetermined" in cases of dual infection, cross reactivity, and when the infection is identified by blood smear only.

Seasonality Overlaps with Tick Season

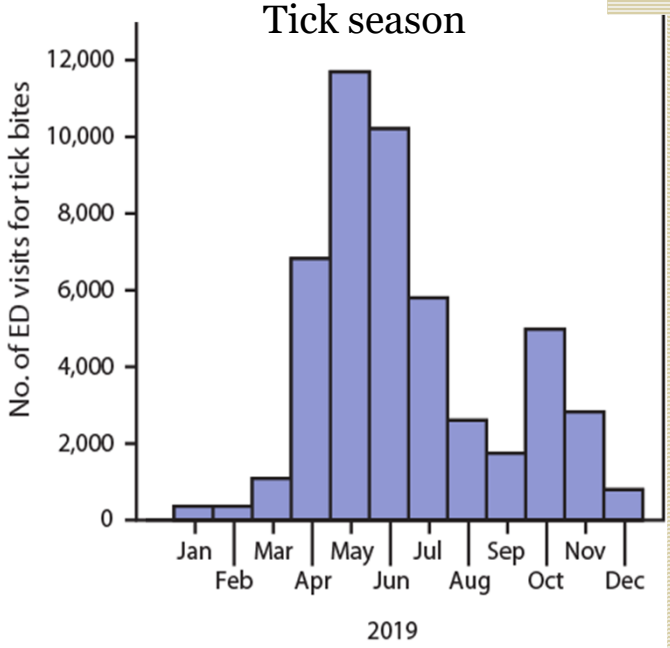
Anaplasmosis



Ehrlichiosis

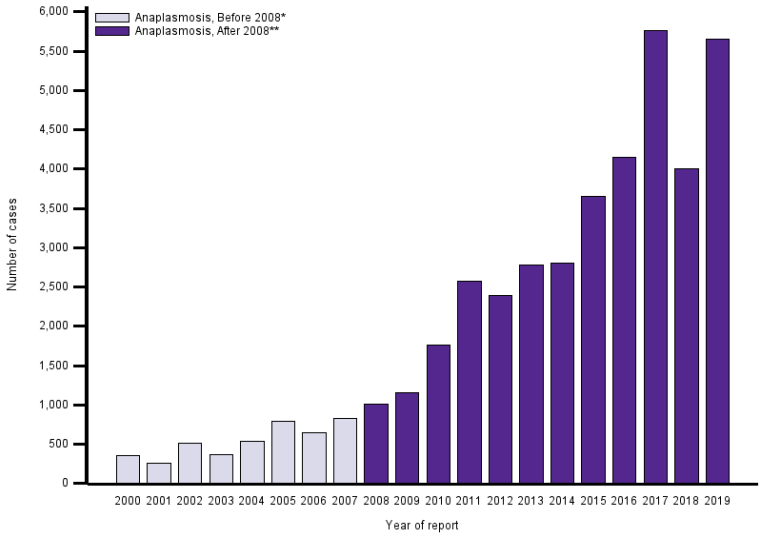


Tick season



Cases increasing

Anaplasmosis



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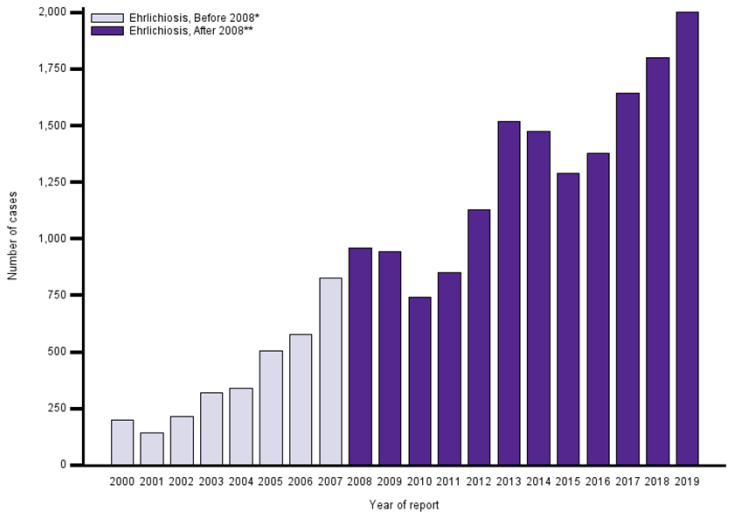
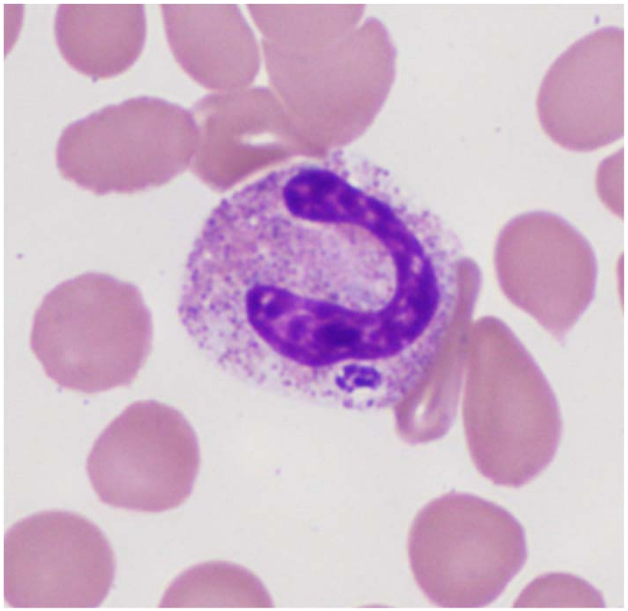
Ehrlichiosis



https://www.cdc.gov/ehrlichiosis/stats/index.html

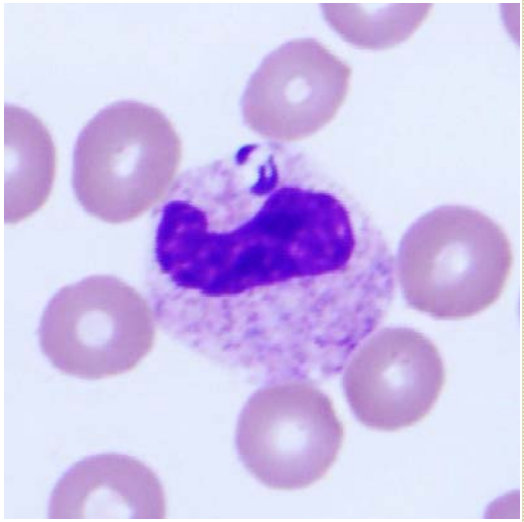
Risk Factors

- Highest among males and people over 40 years of age.
- People with weakened immune systems
- People who live near or spend time in known tick habitats



Additional Testing

- PCR from whole blood
  - Most sensitive in the first week of illness
  - Can distinguish between species
- Serology- acute and convalescent serum
  - IgM antibodies can persist for many months after disease has resolved.
  - Cross reactivity between *Anaplasma* and *Ehrlichia*
- Co-infection with other tickborne diseases should be considered.



Case Conclusion

- After appropriate therapy the patient recovered and was discharged home 2 weeks later
- Trace back found that one of the blood donors was positive for *A. phagocytophilum* by PCR and IFA.
- The donor did not recall being bitten by a tick, but had spent time in wooded areas of northeast Minnesota the month before her donation.
- She reported no history of fever during the month before or after her donation. No other patients received blood components from her donation.

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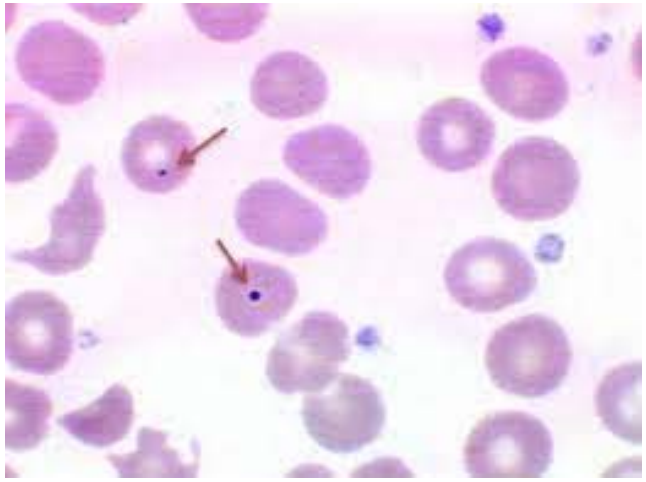
Case Presentation

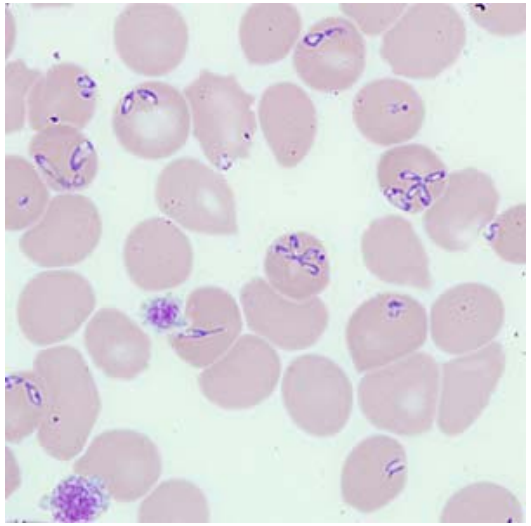
- A 76 year old man presents after a fall in his kitchen.
- On admission he has a fever and seems confused. His wife reports that he hasn't been feeling well for a few days with off and on fevers but he didn't want to go to the doctor.
- The patient had not travelled outside of his northern Wisconsin town for several years. He spends most of his time gardening.
- His spleen was removed 5 years ago due to a car accident

Under the Microscope

- The patient's blood was flagged as abnormal by the automated instrument during cell counts
- The Hematologist observed the following:



Howell-Jolly bodies



Parasitemia 16%

Babesiosis

25% of cases are asymptomatic or mild

Flu-like illness Fever, chills, aches, sweats, nausea, fatigue

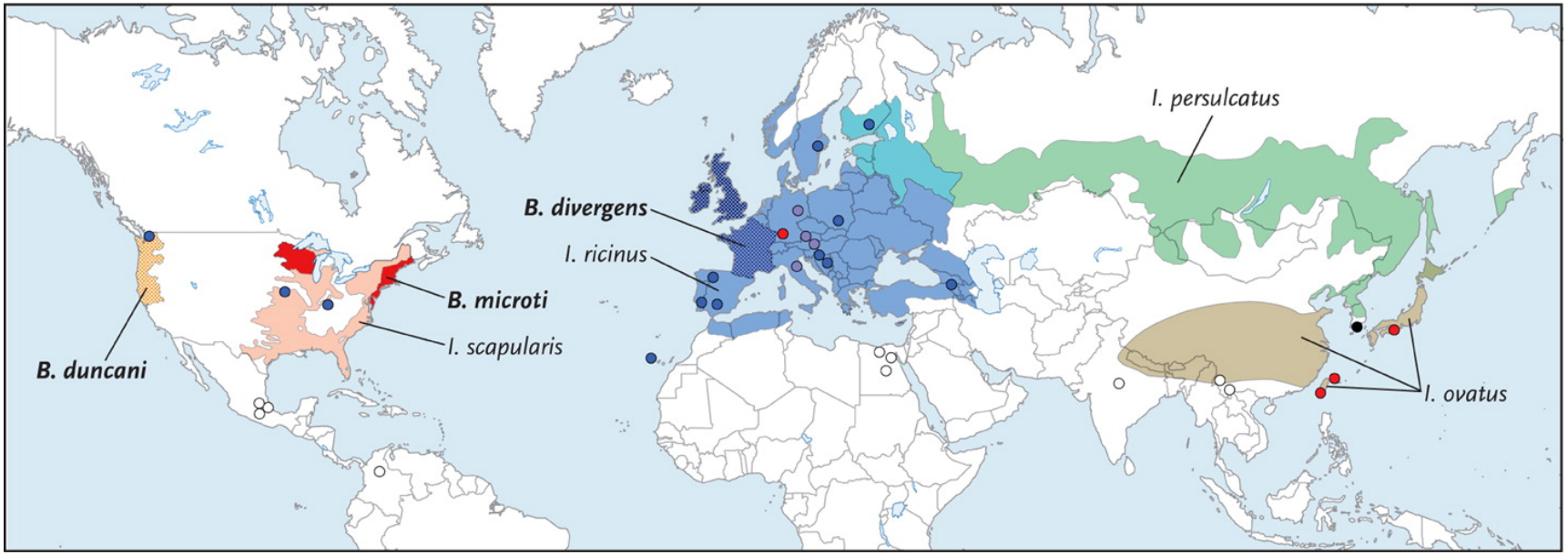
- Usually lasts 1-2 weeks, no periodicity
- Can become an asymptomatic carrier

Less common but more severe symptoms

- low and unstable blood pressure
- severe hemolytic anemia (hemolysis)
- Jaundice
- very low platelet count (thrombocytopenia)
- disseminated intravascular coagulation (DIC)
  - can lead to blood clots and bleeding
- malfunction of vital organs (such as the kidneys, spleen, and heart)
- death.

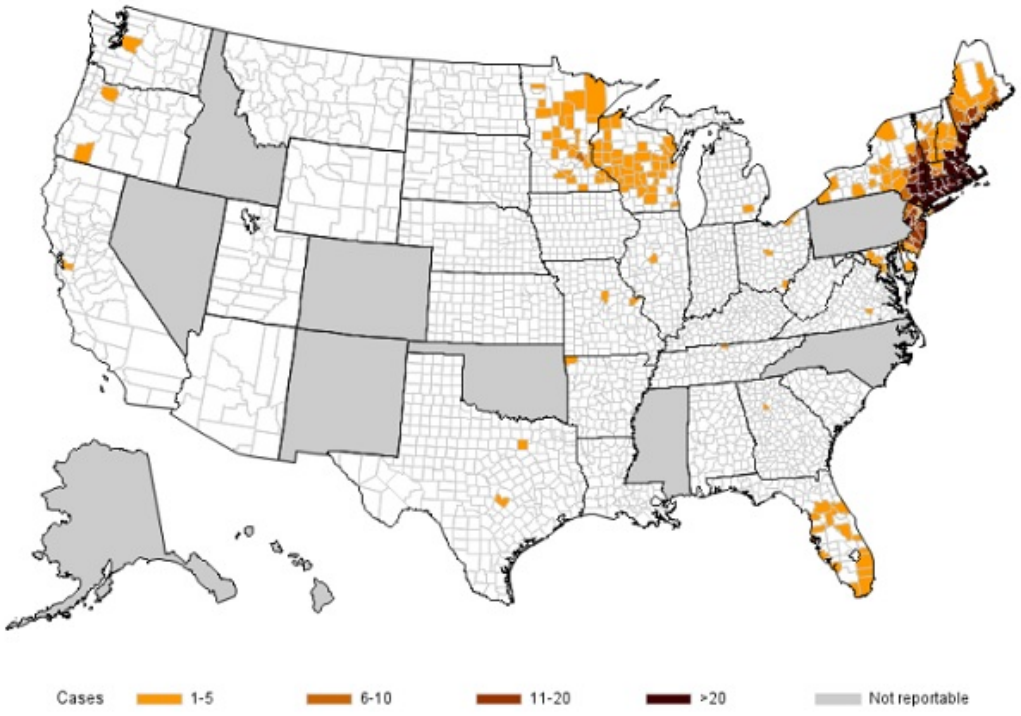
Species

Over 100 species, only a few cause human disease

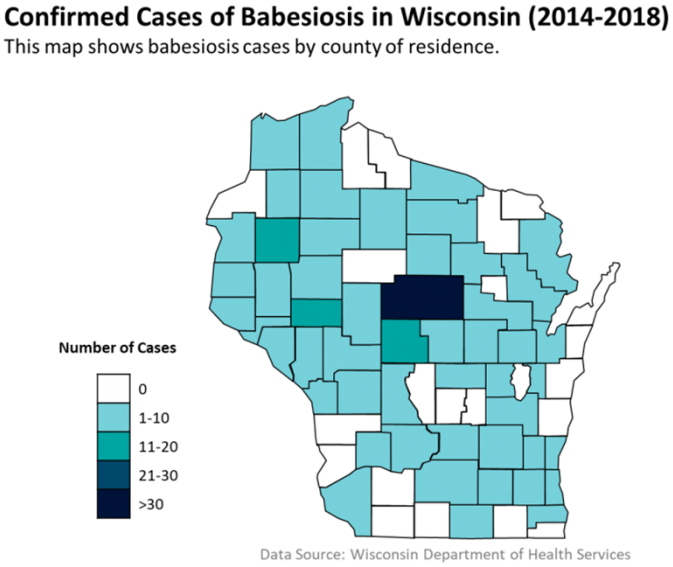


*Hard to tell apart and doesn't impact treatment.- Most labs report as Babesia Spp.

Geographic distribution



https://www.cdc.gov/parasites/babesiosis/data-statistics/maps/maps.html

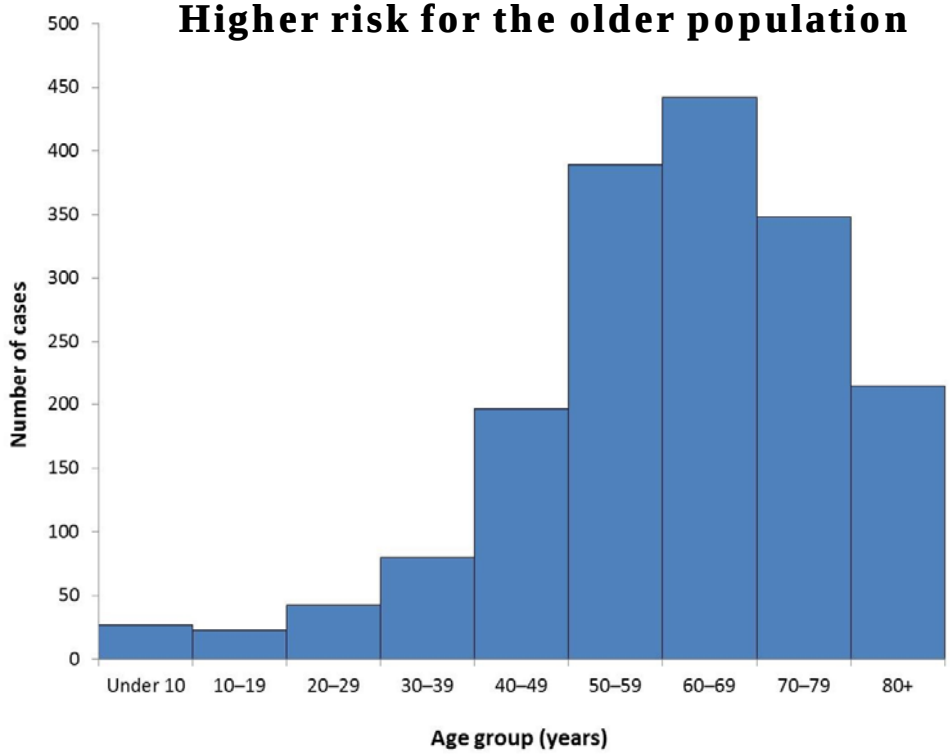


https://www.dhs.wisconsin.gov/tick/babesiosis-data.htm

Exposure History

- No Travel (for Wisconsin residents) or travel to endemic regions
- Spends time outdoors in areas with lush vegetation, often in the woods, particularly in northern Wisconsin
  - Hiking, camping, gardening, walking the dog
- History of recent tick bite- especially *Ixodes scapularis*

Risk factors for severe disease

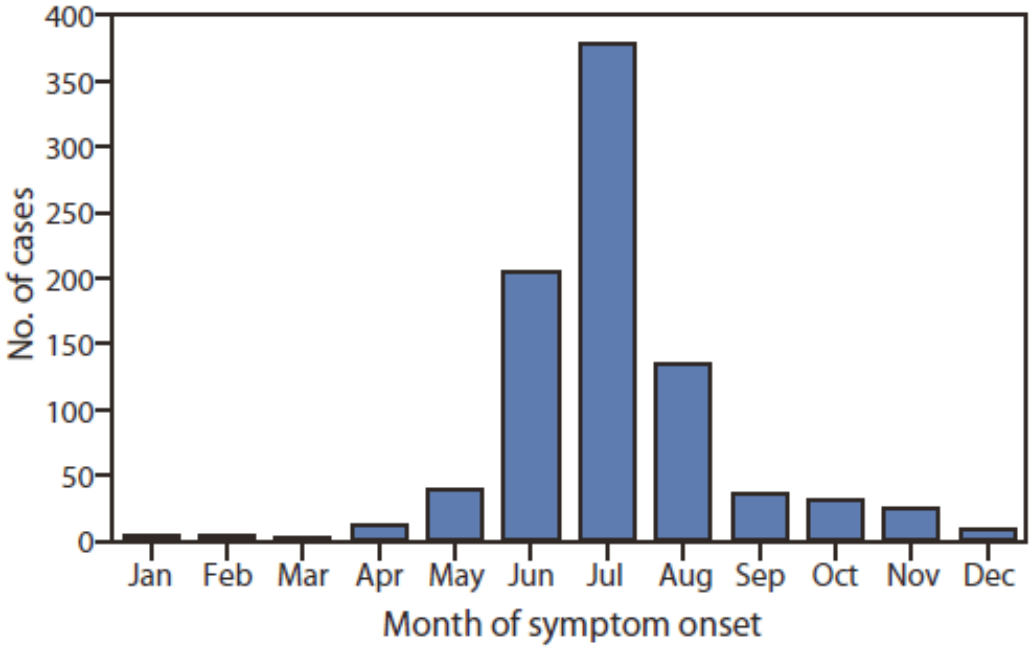


- Advanced age
- Poor spleen function or loss of the organ (helps clear disease)
- Immune compromise
- Other serious health conditions (liver and Kidney disease)

5-20% mortality

Babesiosis coincides with tick exposures

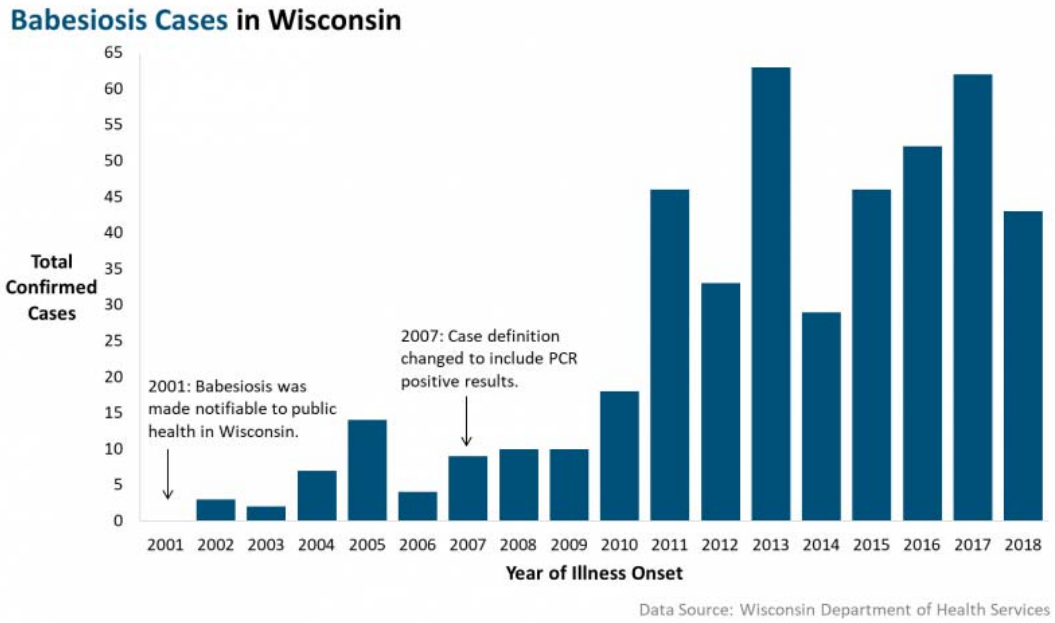
Number of reported cases by month



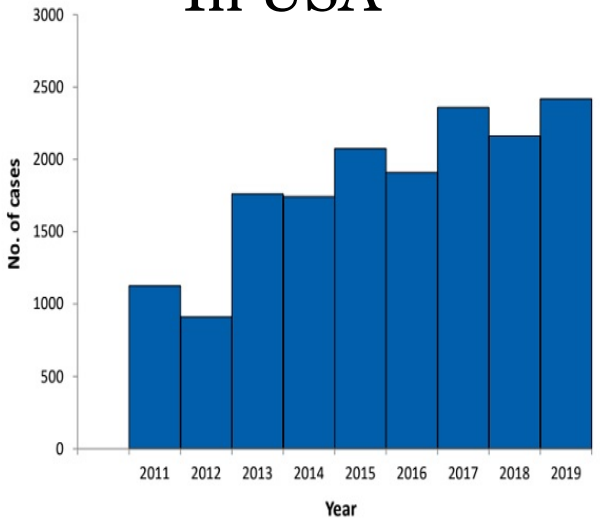
- Symptoms arise 1-8 weeks after a tick bite
- Can also be transmitted by blood transfusion, organ transplant, or congenitally

Cases are Increasing

Reported cases by year



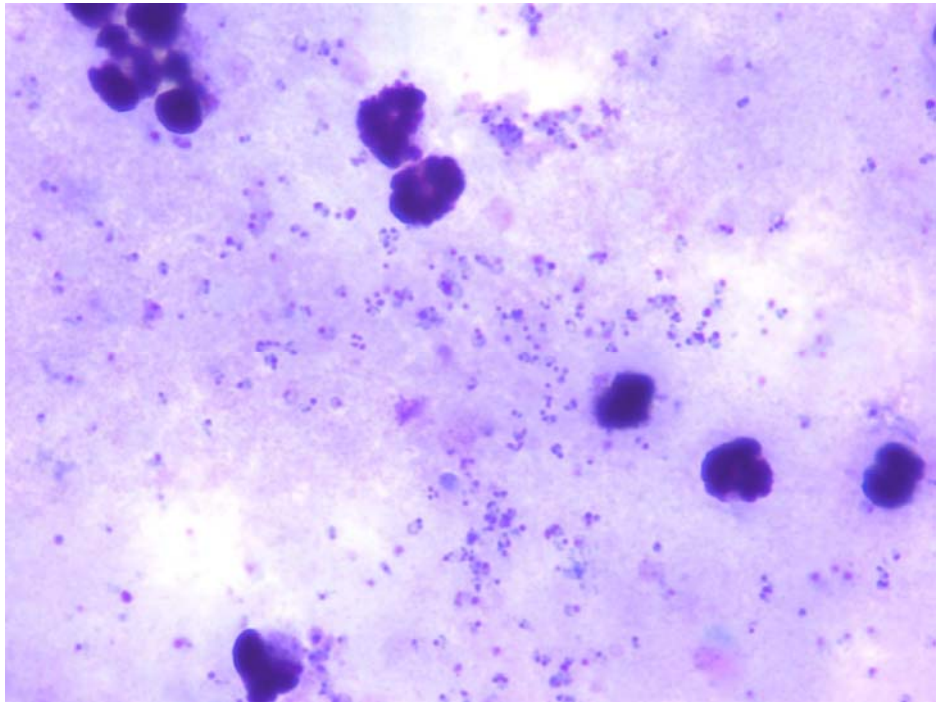
In USA

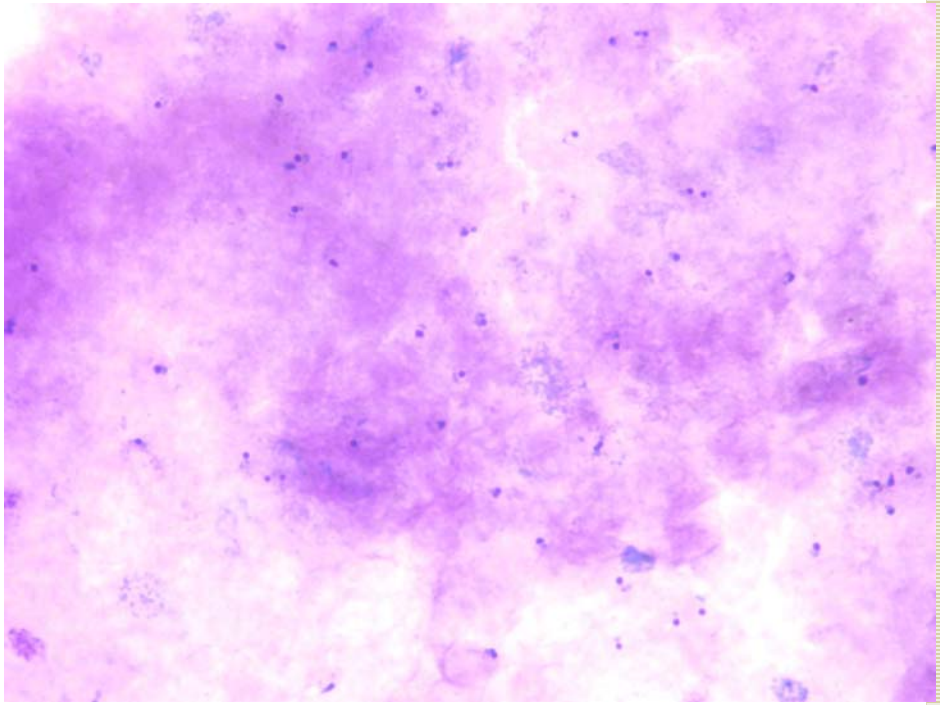


https://www.cdc.gov/parasites/babesiosis/data-statistics/graphs/graphs.html

CDC, MMWR, *Weekly* / July 7, 2017 / 66(26);687–691

Babesia vs Malaria on Thick Smears



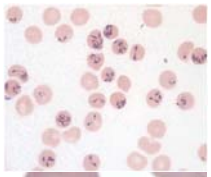


Babesia vs Malaria

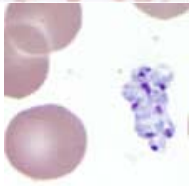
Babesia

Tear drop shaped ring forms

Size variability and clear vacuole



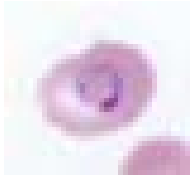
Higher parasitemia (1-10%, can be 80%)



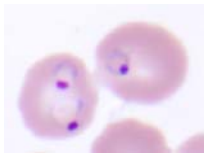
Extracellular forms

Maltese cross

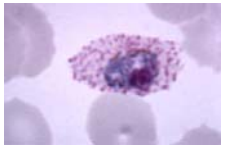
Malaria



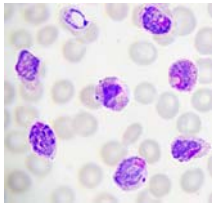
Round signet ring forms



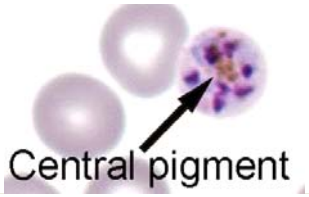
Fewer parasite per red cell



Schuffner's dots

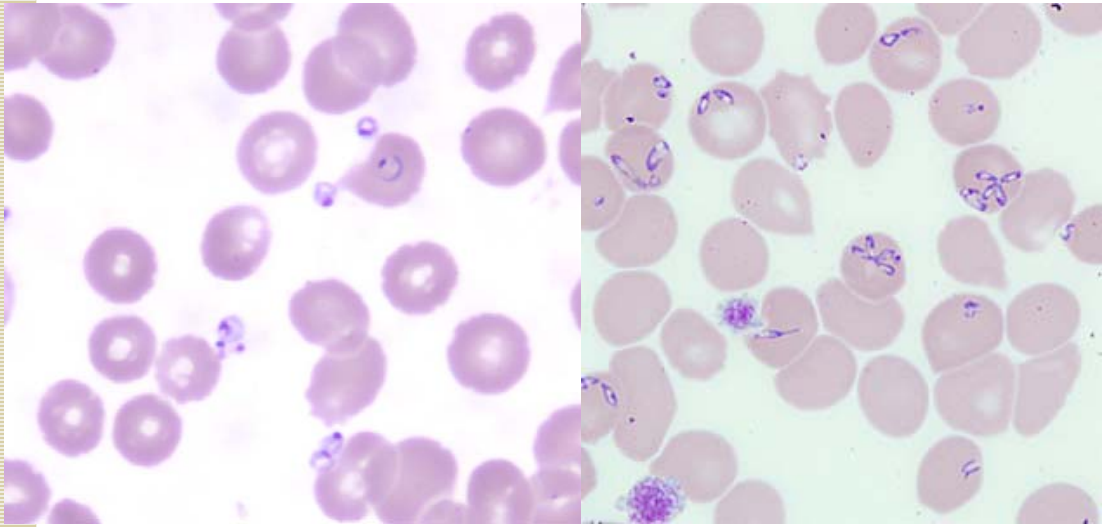


Advanced forms



Hemazoin

Reporting Parasitemia



- Reporting parasitemia is not required but is often requested by clinicians.
- Higher risk for severe babesiosis when parasitemia ≥4%. (can happen with less)
- High parasitemia may predict prolonged or relapsing disease.
- Persistent parasitemia can inform on extending therapy

Additional Steps

- Results can be confirmed by serology or PCR tests
  - Antibody detectable 2 weeks after infection
  - PCR at WSLH can confirm ID as *B. microti*

- All positives should be reported to the Division of Health

Case Conclusion

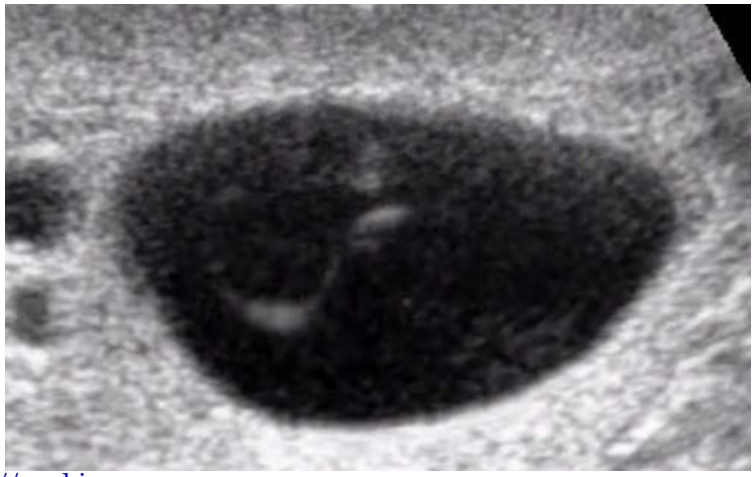
- His disease was severe and prolonged, likely related to his advanced age and asplenia. The parasitemia was checked every day near the start of treatment and then every few days until it was <0.5% and he was substantially recovered.

- After extensive therapy our patient eventually recovered. He had some prolonged hearing loss associated with the therapy.

Case

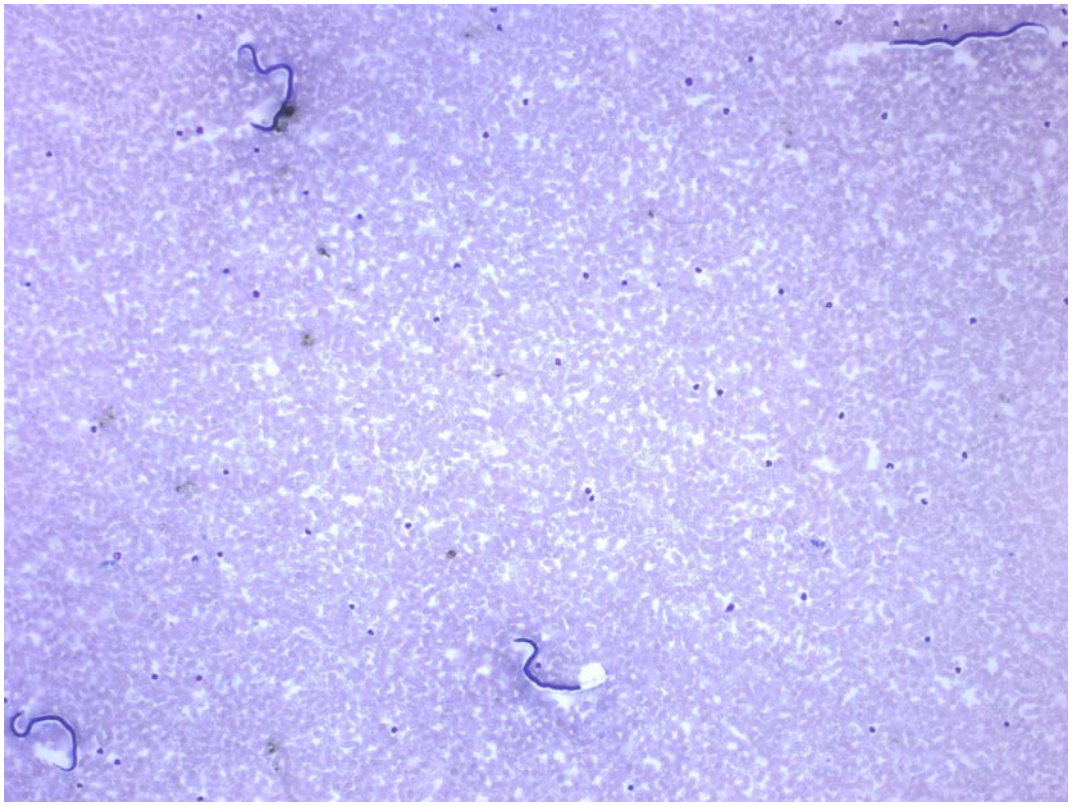
- A 32 year old woman presents to her doctor with ongoing breast tenderness.
- She recently moved to the US from central Africa
- Blood smears ordered for 2 am and 2 pm for 3 days

Ultrasound



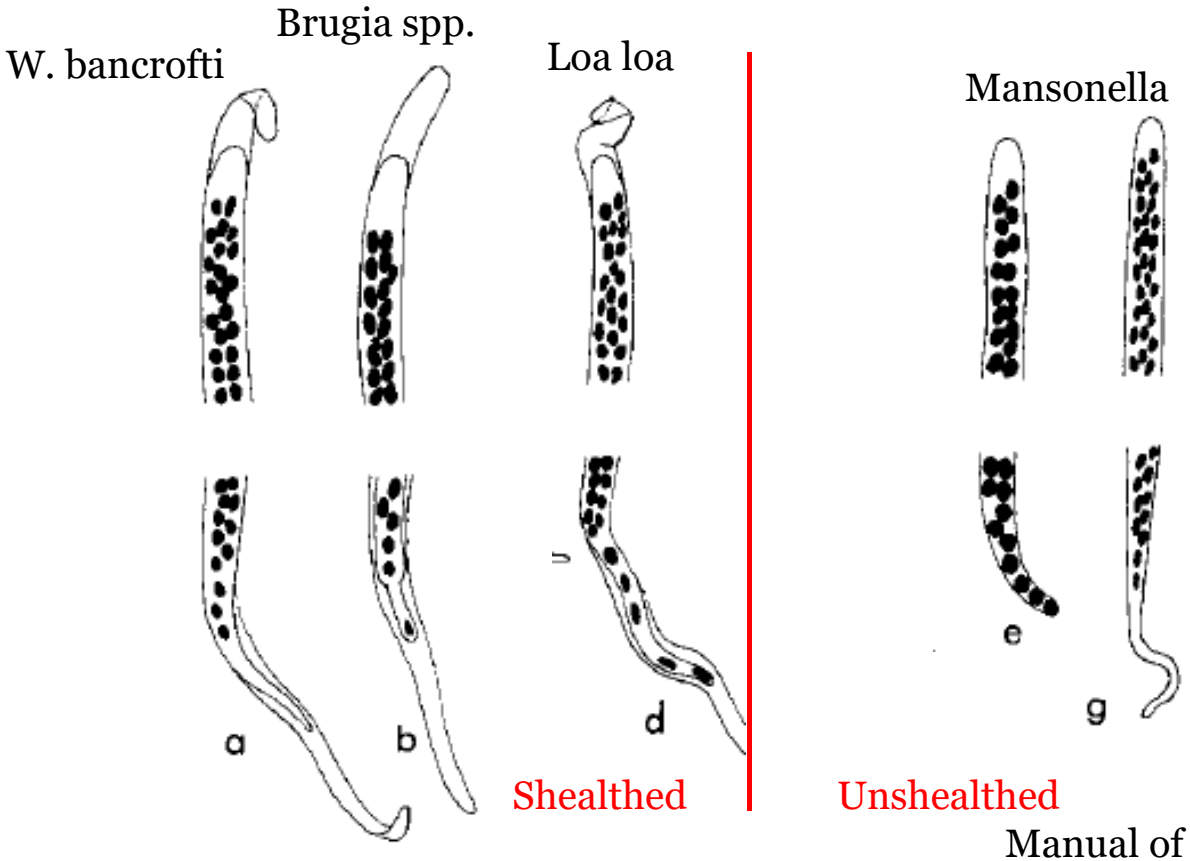
https://prod-images-static.radiopaedia.org/images/41322804/96fc0136ded3be3ebe0b10c5c313f2.mp4

Under the Microscope



Under the Microscope

Microfilariae



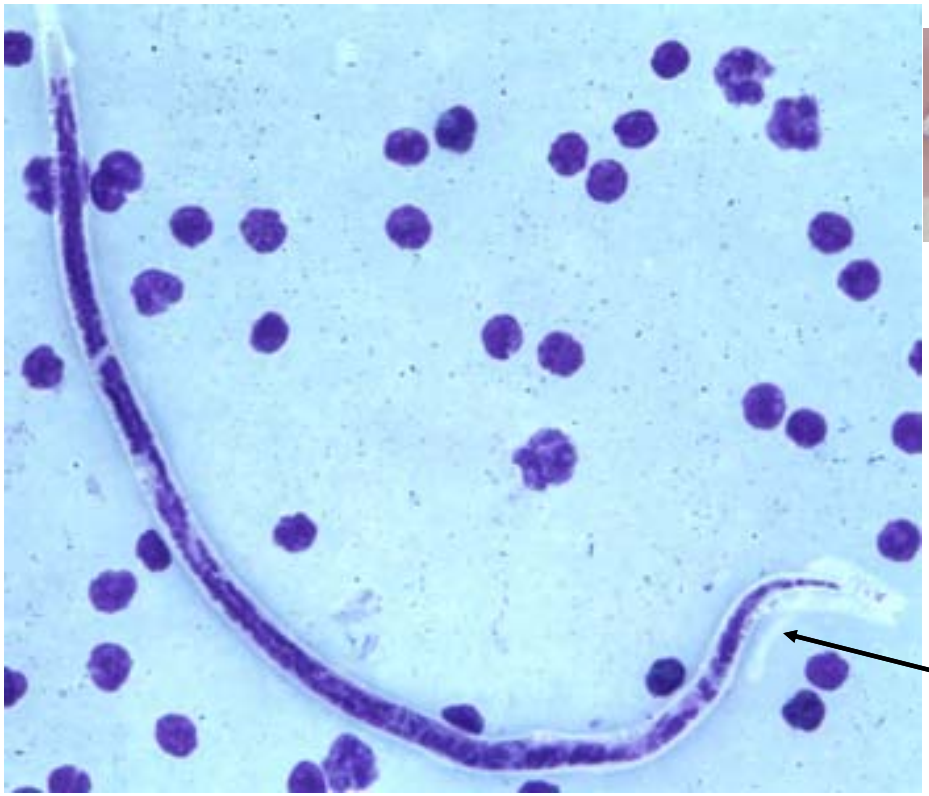
Manual of Clinical Microbiology

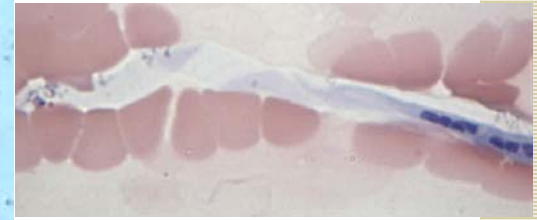
TABLE 1 Filarial parasites of humans

Species	Distribution	Vector	Primary pathology	Location(s)	Periodicity	Size (μm)	Tail	Sheath	
								Presence	Staining properties
Wuchereria bancrofti	Tropics	Mosquito	Lymphatic, pulmonary	Blood, hydrocele fluid	Nocturnal, subperiodic	298 by 7.5–10	Pointed tail devoid of nuclei	+	Does not stain
Brugia timori	Indonesia	Mosquito	Lymphatic	Blood	Nocturnal	300 by 5–6	Nuclei in tail	+	Tends not to stain
Brugia malayi	Southeast Asia	Mosquito	Lymphatic, pulmonary	Blood	Nocturnal, subperiodic	270 by 5–6	Nuclei in tail	+	Bright pink with Giemsa
Loa loa	Africa	Deer fly	Allergic	Blood	Diurnal	Up to 300	Irregularly arranged nuclei extend to end of tail	+	Does not stain
Mansonella perstans	Africa, South America	Midge	Probably allergic	Blood	None	203 by 4–5	Blunt tail contains nuclei	–	
Mansonella ozzardi	Central and South America	Midge	?	Blood	None	224 by 4–5	Long tail with no nuclei in it	–	

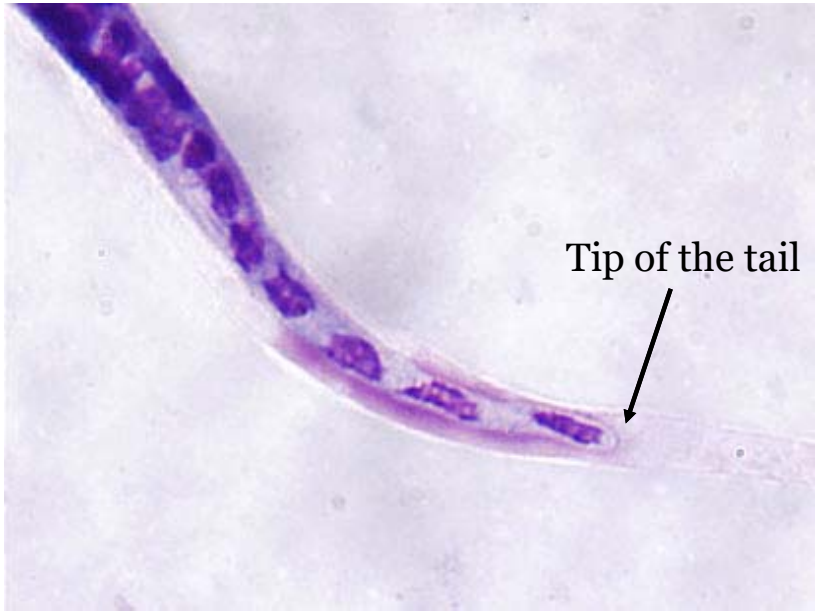
MCM v 11

Sheath Ghosts



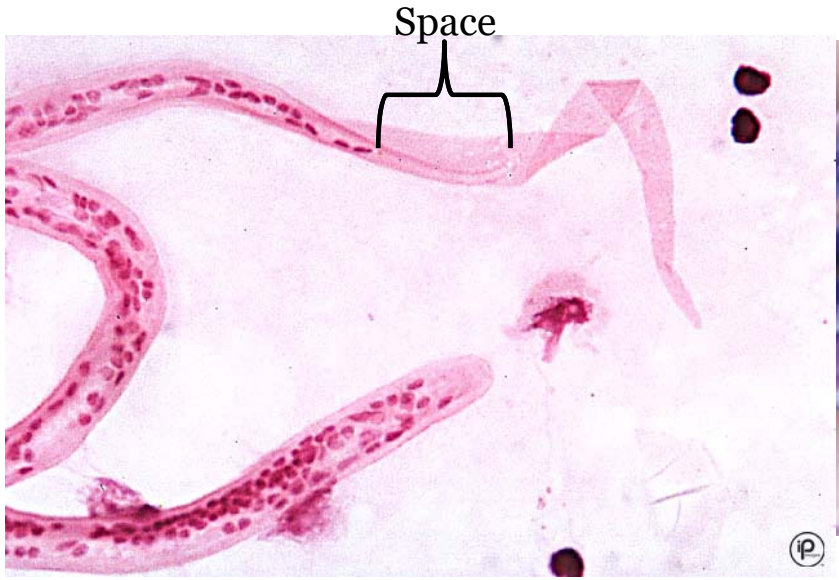


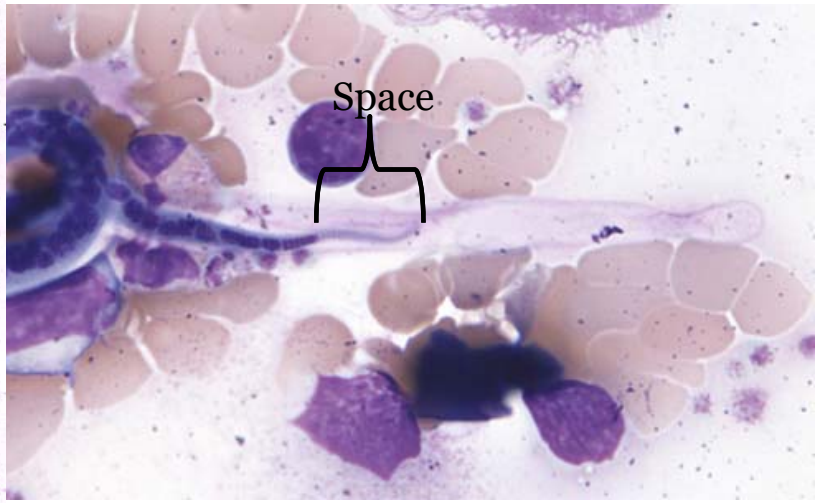
Loa Loa



*Nuclei go the tip

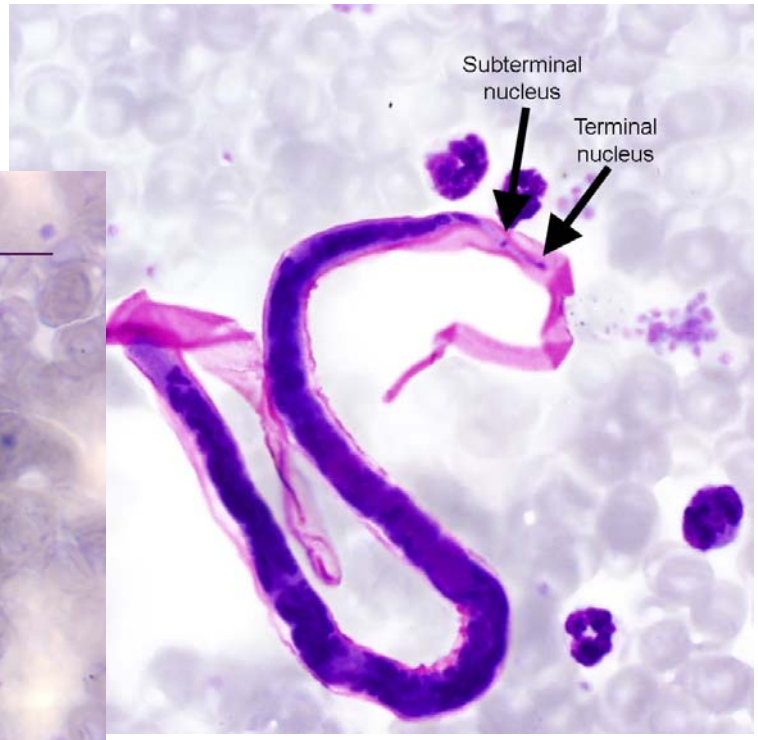
W. bancrofti





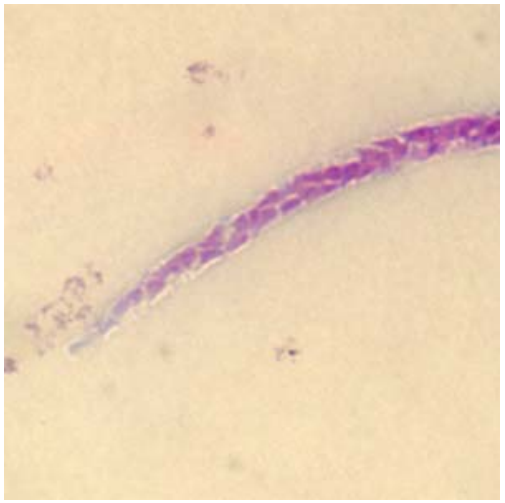
*Nuclei do not go the tip

Brugia spp



*space between terminal nuclei

Mansonella



Microfilaria

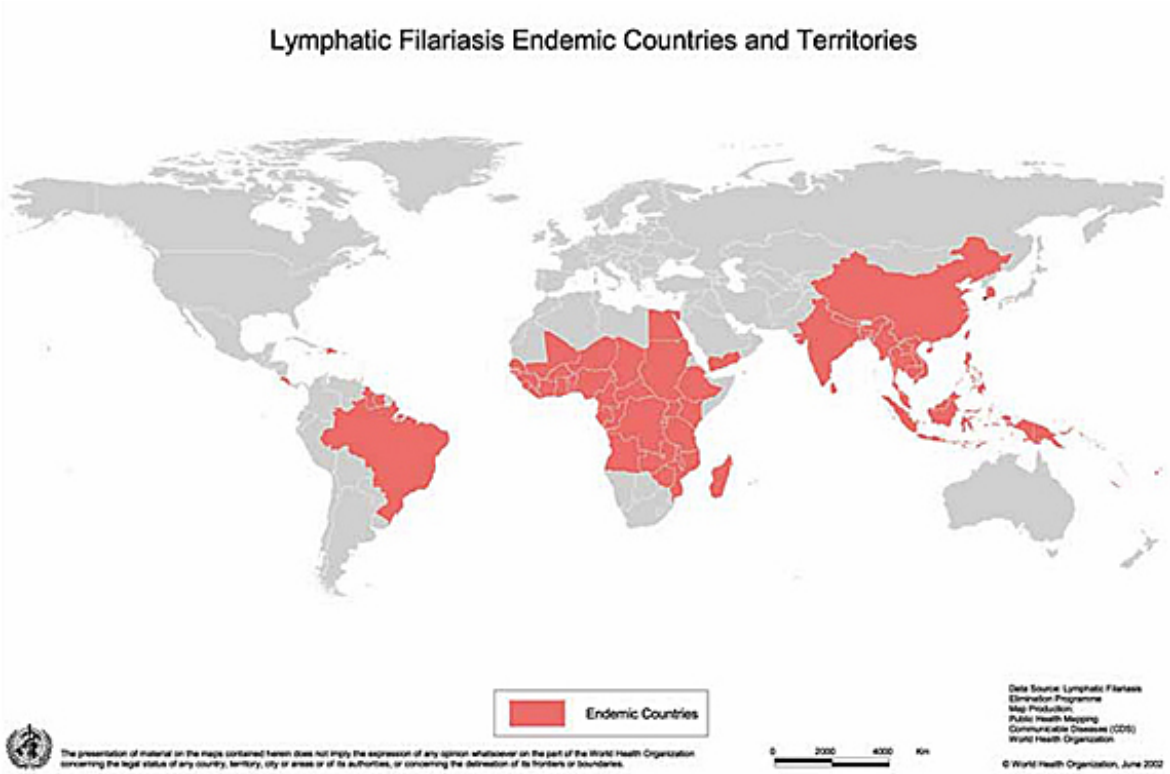
- The adult lives in the lymphatics and offspring are present in the blood at certain times of day
- Larva take several months to years to develop into adults
- Adults live 3 months to 3 years
- Wide range of vectors, all blood sucking arthropods

Symptoms

- Elephantiasis
- Majority of infections are asymptomatic
- Tenderness in lymphatic tissue
- Tropical pulmonary eosinophilia syndrome includes cough, shortness of breath, and wheezing

https://www.cdc.gov/parasites/lymphaticfilariasis/disease.html

Travel History



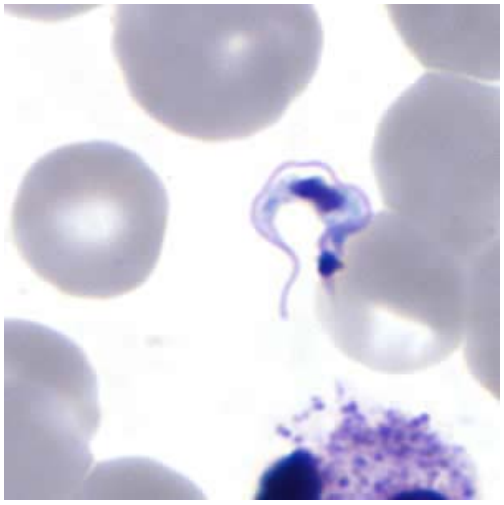
Additional testing

- Ultrasound
- Antigen detection
- PCR
- Antibody testing- extensive cross reactivity

*Patient recovered with appropriate therapy

Case

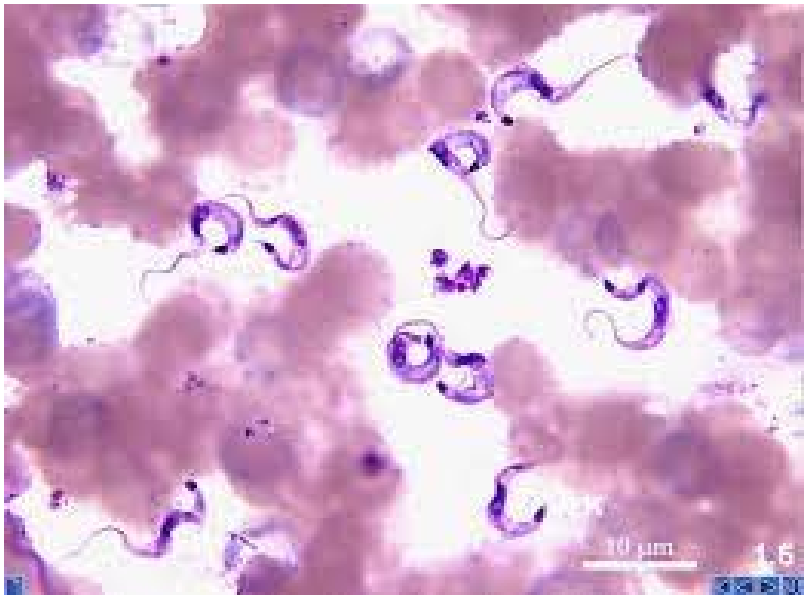
- A 64 year old man presented with anorexia, fever, and diarrhea of 2 weeks' duration.
- Immunosuppressed to prevent organ rejection from a heart transplant 2 month prior
- Surprise find in the peripheral blood smear

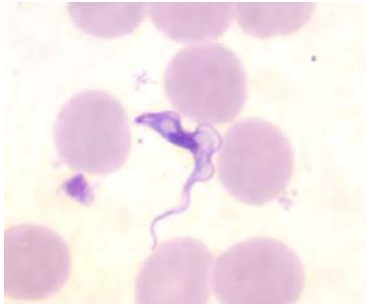


https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5529a3.html
https://www.cdc.gov/dpdx/trypanosomiasisamerican/index.html

Trypanosomes

- The form in blood is the Tryptomastogote
- Long flagellum running the length of the body
- Flagella is powered by a large kinetoplast at the posterior end





Chagas

- Acquired through rubbing the feces of a triatomine bug into a wound or mucus membrane.
- Referred to as American Trypanosomiasis or Chagas Disease
- Caused by *Trypanosoma cruzi*

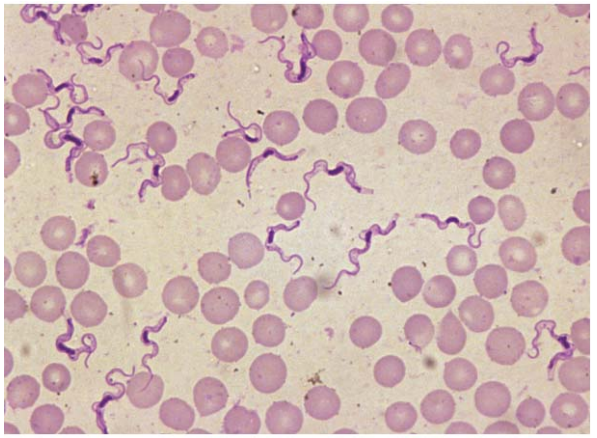
Triatomine- reduvid

Romaña's sign

Trypanosomiasis

- Acquired through the bite of a Tsetse fly
- Referred to as African sleeping sickness
- Caused by *Trypanosoma brucei* (distinct geography)
  - "East" *T.b. rhodesiense*-
    - human and cattle
    - higher parasitemia, more acute disease
  - "West" *T.b. gambiense*
    - mostly humans
    - rarely detected in blood
  - *T.b. brucei*- mostly in cattle

*morphologically indistinguishable



Symptoms

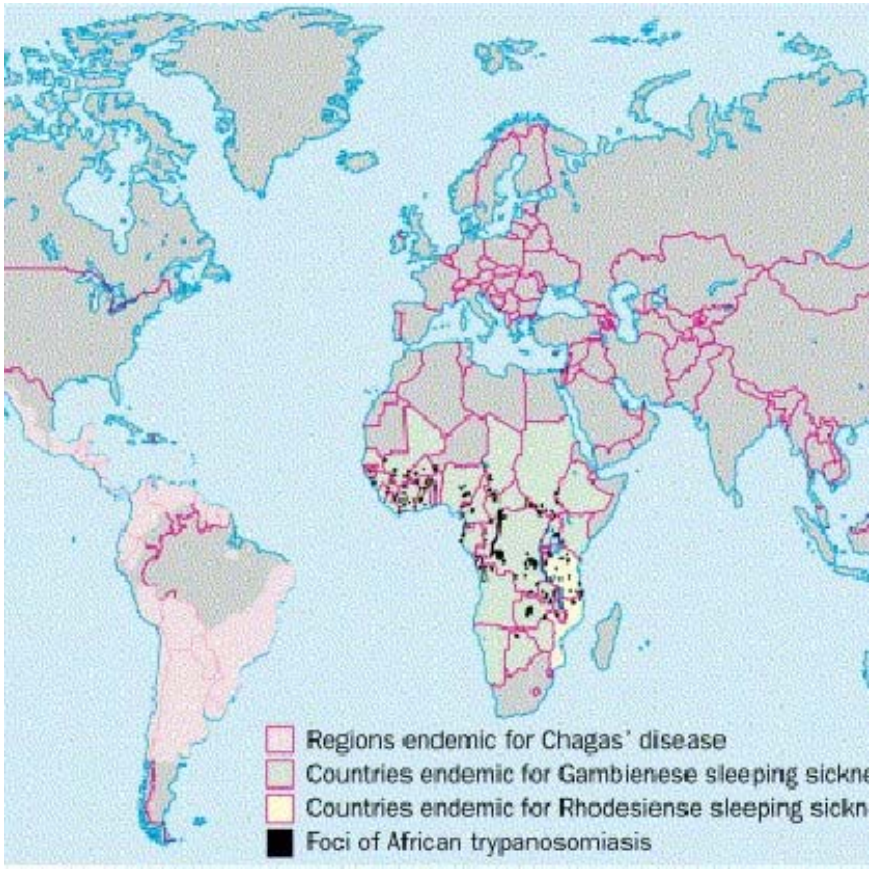
T. cruzi

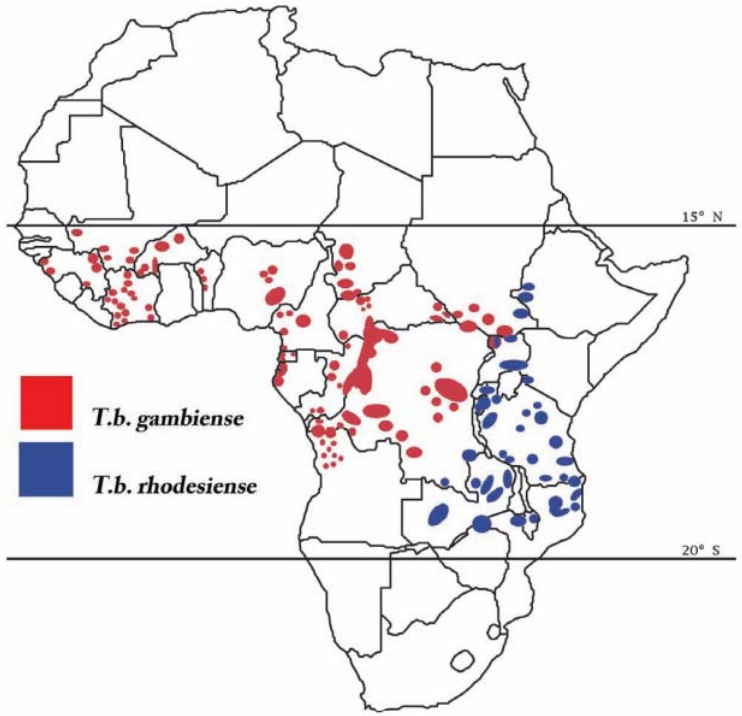
- Romaña's sign (swelling of the eyelid), fever, fatigue, body aches, headache, rash, loss of appetite, diarrhea, vomiting, lymphadenopathy, hepato-splenomegaly, myocarditis, or meningoencephalitis

T. brucei

- Chancre at bite site, generalized rash, headache, cyclical fever, cervical lymphadenopathy, hepato-splenomegaly, facial edema, sleep dysregulation, poor coordination, emaciation

Geographic Distribution





Yahya M, Hamid U, Zuraina M. Bioinformatics. Chapter 2. p.31-54

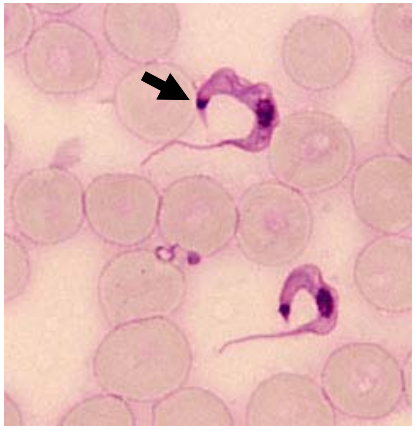
Mary Klassen-Fischer, Wayen Meyers, Ronald Neafie. African Trypanosomiasis. Ch 3.

Comparison

T. brucei

- Larger (14 to 33 µm long)
- More often an “S” shape
- Smaller, more faint kinetoplast

T. cruzi



- Smaller
- Consistent “C” shape
- Large, dark, kinetoplast

Additional Testing

- Direct examination if the primary diagnostic tool
- Some referral labs have PCR, not a routine method for diagnosis
- Antibody testing: IFA, ELISA, agglutination assays

T. cruzi

- *T. cruzi* culture in NNN media
- Tissue biopsy for amastigotes
- Ab tests can cross react with Leishmania and Toxoplasma
- Xenodiagnosis using the triatomid bugs

T. brucei

- *T. brucei* does not grow in vitro
- No other forms in humans
- Ab test can cross react with Malaria
- CNS testing for meningoencephalitis

Case Continued

- Blood cultures were positive for *T. cruzi* and endomyocardial biopsy specimens contained amastigotes.
- Seronegative for *T. cruzi* antibodies but positive for *T. cruzi* DNA by polymerase chain reaction (PCR).
- Parasitemia rapidly cleared with treatment but he still died 2 months later due to acute organ rejection.

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5529a3.htm

Case Conclusion

- No identifiable risk factors for *T. cruzi* infection (e.g., travel to a country endemic for Chagas disease)
- Trace back found that the donor was seropositive.
- The donor was UW born but had traveled to *T. cruzi* endemic Mexico.
- Three additional patients received liver and kidneys from the same donor.
- All three remain seronegative and continue to be monitored.

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5529a3.htm

Next Case

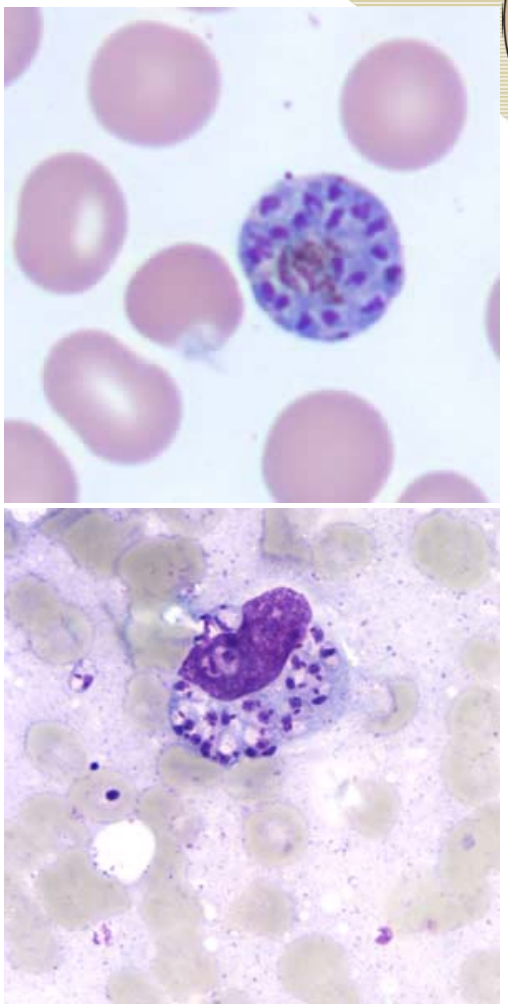
- 5 year old child from rural Nepal
- Presents with an enlarged spleen and liver, high fever, abdominal pain, and constipation for 3 months
- Pancytopenia
  - hemoglobin – 7.2 g/dl,
  - RBC 2.94 × 10^{12}/L
  - WBC 2.90 × 10^{9}/L
  - Platelet count- 63 × 10^{9}/L.

Under the Microscope

- *P. vivax* schizont seen in blood film

Visceral leishmaniasis and malaria co-infection!

- *Leishmania donovani* seen in monocyte

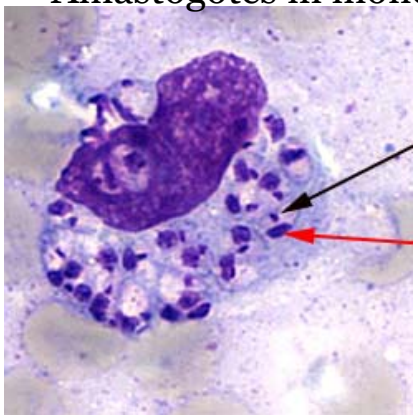


https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-019-4478-1

Leishmaniasis

- Transmitted by the sandfly
- Most often seen in tissue biopsies, skin scrapings, and touch preps
- Visceral Leishmaniasis can be detected in the blood

Amastogotes in monocytes



https://www.cdc.gov/dpdx/leishmaniasis/index.html

Additional testing

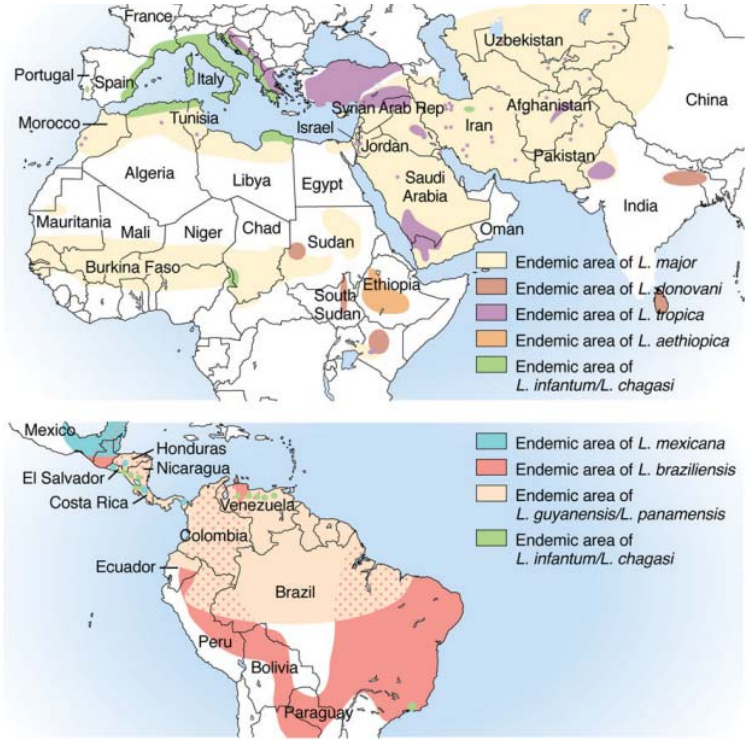
Detection and species determination

- Culture- Isoenzyme analysis (e.g. NNN medium)
- Antibody – DAT, rK39 (can cross react with T. cruzi)
- PCR- conventional and Real time
- Cellulose acetate electrophoresis
- Sequencing- rRNA internal transcribed spacer 2 (ITS2)
- Xenodiagnosis (mice and hamsters)

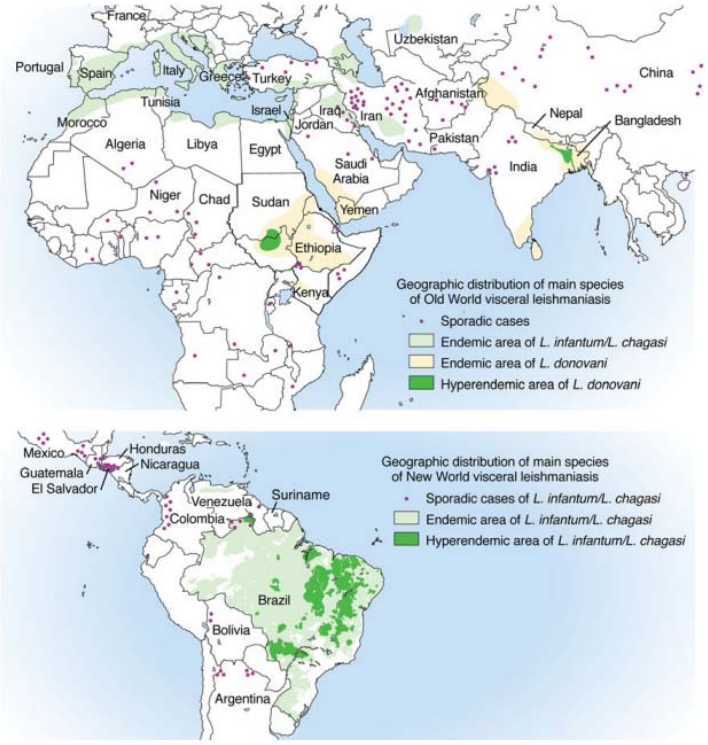
Aronson, N, et al. Diagnosis and treatment of Leishmaniasis: Clinical Practice Guidelines by the IDSA and ASTMH. *Clin. Infec. Dis.* 2016

Where in the World?

Cutaneous Leishmaniasis



Visceral Leishmaniasis



90% of the world's cases of visceral leishmaniasis are in India, Bangladesh, Nepal, Sudan, and Brazil.

Aronson, N, et al. Diagnosis and treatment of Leishmaniasis: Clinical Practice Guidelines by the IDSA and ASTMH. *Clin. Infec. Dis.* 2016

Case conclusion

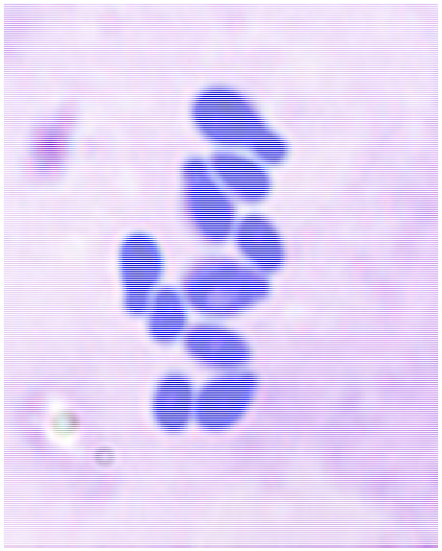
With appropriate therapy, the young patient made a full recovery!

Case

- 42 year old Male
- Hospitalized, immune compromised cancer patient on parenteral nutrition
- New onset fever, not responding to antibiotics
- Blood sent to the lab for CBC and culture

In the Lab

- An astute generalist noted unusual shapes in the primary blood smear “that’s not normal!”
- They recognized it as a microbe but couldn’t identify it on their own. So, they consulted with Microbiology.
- Based on the morphology, yeast were suspected.
- They called the clinician to recommend ordering a special yeast culture with oil from concentrated primary blood

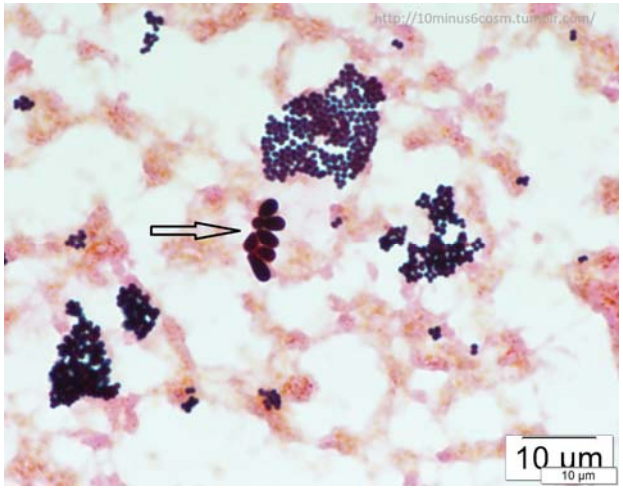


https://link.springer.com/chapter/10.1007/978-3-642-03616-3_8

More in the Lab

- The next day *Malassezia furfer* was identified from a yeast culture with oil.

- The blood culture bottle also flagged that day and the same yeast forms were seen again.



Sabouraud Agar overlaid with edible oils in the locality.

Edible oils	Growth rate
Groundnut oil	++++
Coconut oil	+++
Olive oil	+++
Margarine	+++
Castor oil	+++
Palm oil	+
Palm Kernel oil	+

++++ = Excellent Growth, +++ = Good Growth, ++ = Fair Growth, + = Poor Growth.

Ofonime Ogba. European Journal of Pharmaceutical and medical Research. 2016. 3 (9):71-76

What Happened?

- The line was infected!
- The parenteral nutrition provided a rich fatty diet for this oil loving fungus.
- With antifungals and a line change the patient cleared their infection.

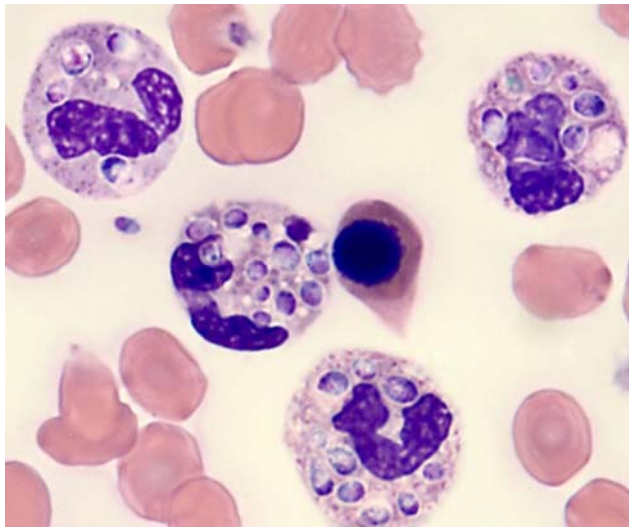
Summary

- Cross team collaboration allowed for a much more rapid diagnosis than routine culture alone.

- Familiarity of the morphology of this fungus helped to start the specialized culture needed to grow this organism producing a diagnosis 3 or more days faster than would have occurred otherwise.

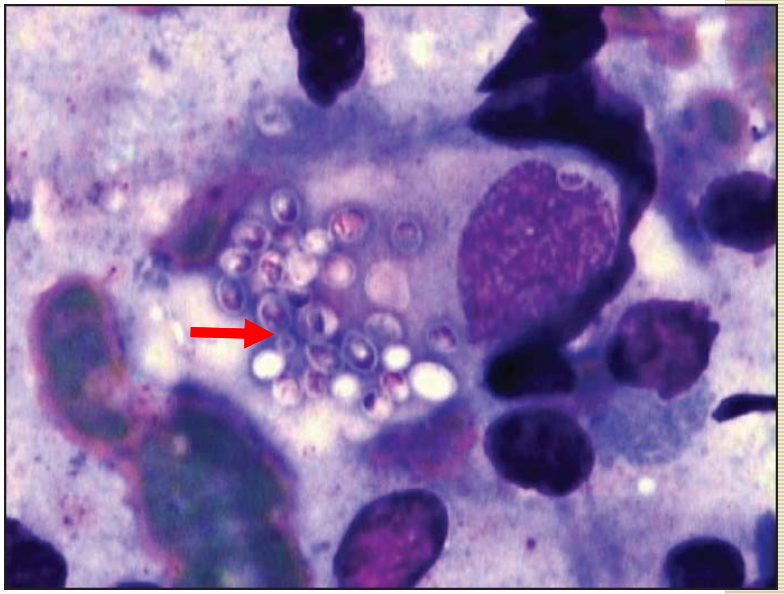
Case

- A 76 year old man presented to the ER with a 10 day fever.
- He had a procalcitonin of 4.4 ng/mL (1.00-0.05 normal), suggestive of severe sepsis.
- The patient deteriorated rapidly and died of septic shock within 24 hours of admission and before proper cultures could be obtained.
- A peripheral blood smear identified the cause of disease



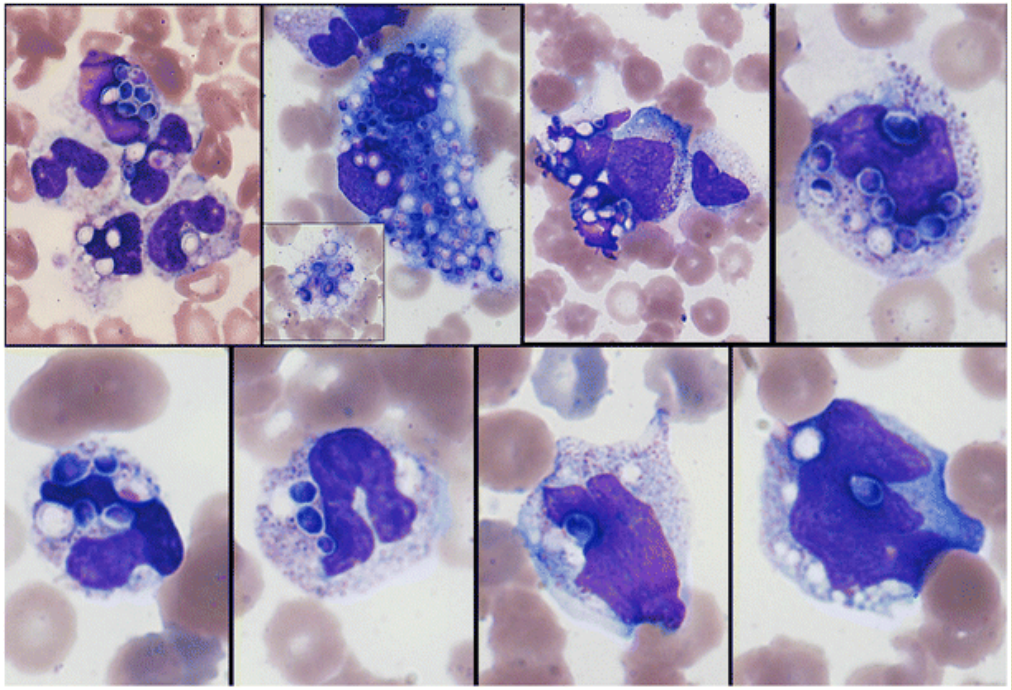
Under the Microscope

- The peripheral blood smear showed neutrophilic leukocytosis with left shift and toxic change.
- Many monocytes and neutrophils showed numerous inclusions.
- Inclusions were 1-4 uM and were surrounded by a pseudocapsule.
- Rarely, extracellular organisms and budding were seen



Histoplasma capsulatum

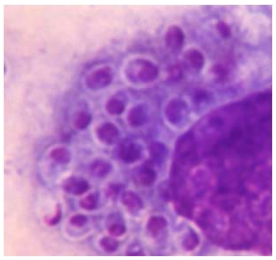
- Acquired through inhalation but can disseminated throughout the body within leukocytes.
- Rare to find in the blood
- Usually in severely immune compromised patients



Bhawna Jha and Smeeta Gajendra. Annals of Hematology. 2017. 96, 709-710

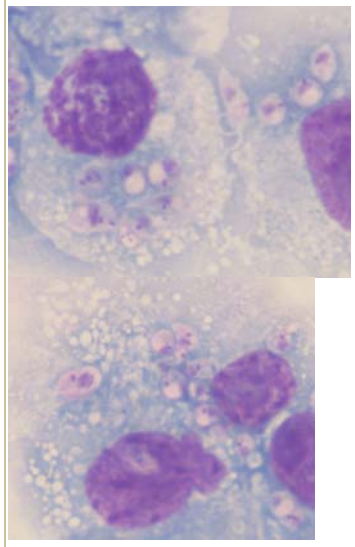
Look Alikes

Histoplasma capsulatum



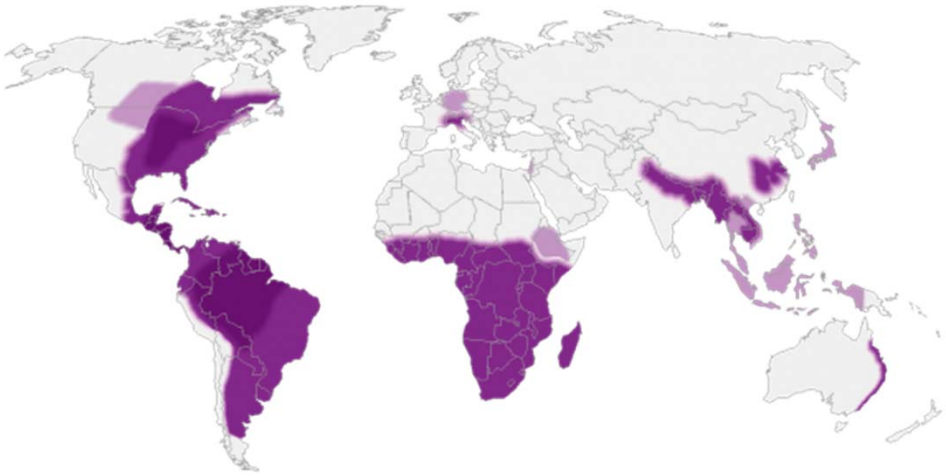
- Ovoid
- Budding
- Thin pseudocapsule
- One large nucleus

Leishmania



- Irregular shape
- No budding
- Clearing, but no capsule/cell wall
- Second nuclear dot (kinetoplast)

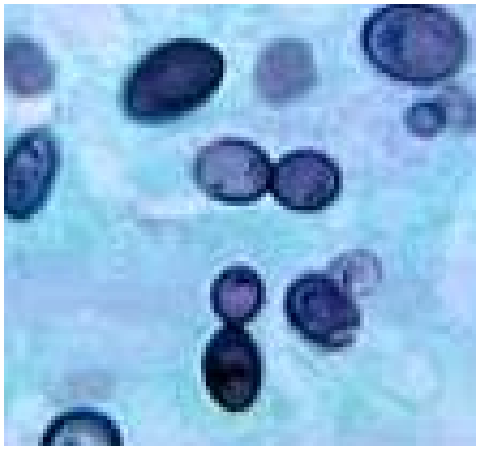
Geographic distribution



https://www.cdc.gov/fungal/diseases/histoplasmosis/maps.html

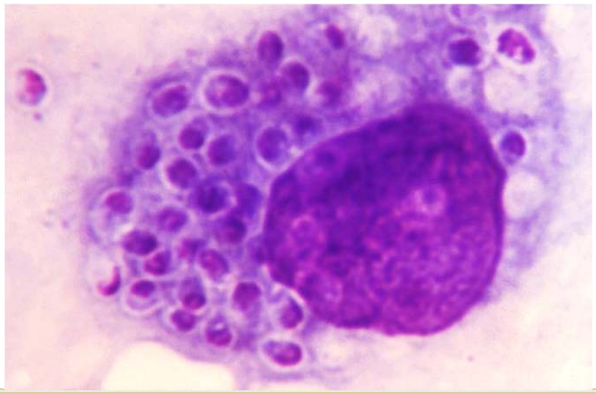
Common Symptoms

- Shortness of breath, fever, fatigue, anorexia, and weight loss
- White pallor, hepatosplenomegaly, lymphadenopathy, petechiae, and in some patients, skin or mucous membrane lesions as ulcers, or nodules
- It may involve the bone marrow and then usually presents with anemia, leukopenia, and thrombocytopenia



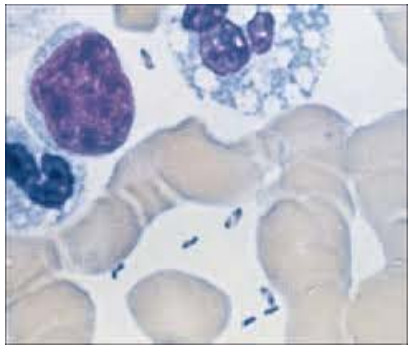
Additional Tests

- Silver staining – Grocott's Methenamine Silver (GMS) can help confirm it is yeast
- Fungal culture for isolation and identification
- Histoplamsa urine and serum antigen tests
- Chest X-ray or other imaging for dissemination to other organs



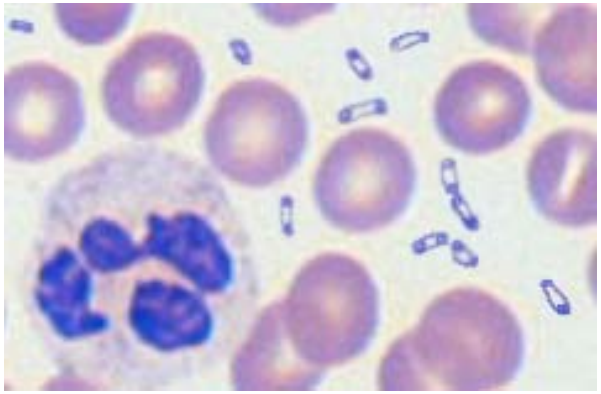
Case

- A 14 year old male presents to the ER with fever, and severe sepsis
- Many bacteria were seen in the peripheral blood smear
- Blood cultures were drawn that later identified Yersinia pestis bacteria
- The patient died within 24 hours.
- They had been hiking in an endemic region and had been poking dead animals with a stick



Take Away Point

- Obvious, free bacteria in a primary (uncultured) blood smear is immediately life threatening and should be called to the clinician
- Very few bacteria are able to grow to this level in the blood before the patient dies from toxic shock.



What You Can Find in Primary Blood

- Parasites
  - Babesia
  - Plasmodium
  - Microfilaria
  - Trypanosomes
  - Leishmania
- Bacteria
  - Anaplasma
  - Ehrlichia
  - Yersinia pestis
- Fungi
  - Histoplasma
  - Malassezia

*Anything else unusual is worth asking about

Summary

- Strong communication with clinicians and between laboratorians can reduce the time diagnosis and improve patient care.
- Even if you aren't sure what it is, knowing what's not normal can be enough to start an investigation.
- When it come to pathogens seen in primary blood, time is of the essence, don't wait for a culture, start communicating!