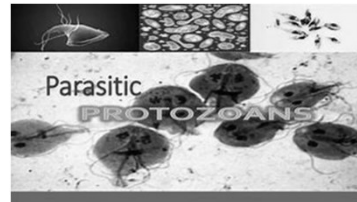


PARASITOLOGY CASE STUDIES

LYNDA BRITTON, PH.D., MLS(ASCP)^{CM}

OBJECTIVES

- Review parasitology pathogens laboratory diagnosis, epidemiology, and symptoms.
- Solve case studies.



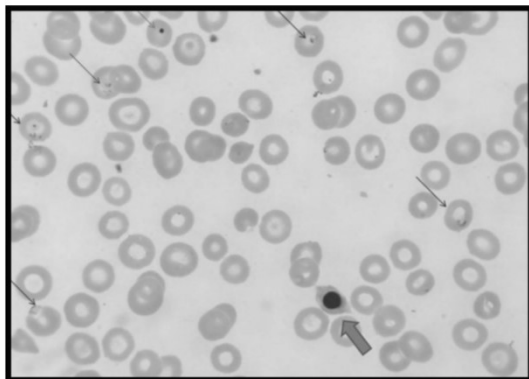
CASE 1: *BABESIA MICROTI*

- 79-year-old female farmer from Wisconsin
- Hemorrhagic shock secondary to spontaneous splenic rupture
- Transfused with 3 units of packed RBCs
- Underwent emergency splenectomy
- Re-admitted on postoperative day 10 for febrile hemolytic anemia
- Multiple tick bites 8 weeks prior to 1st admission

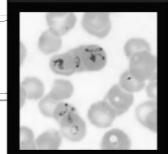
Am J Case Rep, 2018; 19: 335-341

CBC

Test	Result	Reference
WBC	14.9 K/mm ³ ↓	4.5-11 K/mm ³
RBC	2.25 ↓	4.2-5.4 M/mm ³
HGB	6.5 g/dL ↓	12.0-16.0 gm/L
HEMATOCRIT	19.6% ↓	37-47%
PLATELET	91 ↓	150-450 K/mm ³
NEUTROPHILS	50	37-62%
BAND	5	0-6%
LYMPHOCYTES	18 ↓	21-49%
MONOCYTE	23 ↑	2-10%
EOSINOPHIL	1	0-7%
BASOPHIL	3	0-2%



CASE 1



- Diagnosed with babesiosis and Lyme disease co-infections
- SR dreaded complication
- Can rapidly progress to hemorrhagic shock and death
- Not correlated with parasite burden or immune status of the affected host
- Parasitemia mild (1.3%)

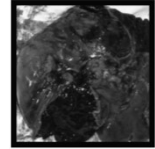
TICK-BORNE



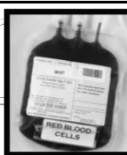
- Before rupture, asymptomatic
- Following splenectomy, more typical with febrile hemolytic anemia
- Developed anemia ~2 weeks following PRBC
- Incubation usually 6 mos. for TTB
- Patient had both IgM and IgG antibodies
- Probably not TTB

SPLENIC RUPTURE

- Most common cause blunt abdominal trauma
- Spontaneous classified as atraumatic-idiopathic 7%
- Atraumatic-pathological 93%
 - Neoplasm (30.3%)
 - Infections (27.3%)
 - Plasmodium, EBV and CMV
 - 9 cases of babesiosis associated with splenic rupture and 3 cases associated with splenic infarct
 - Inflammatory, non-infectious conditions (20%)



EPIDEMIOLOGY



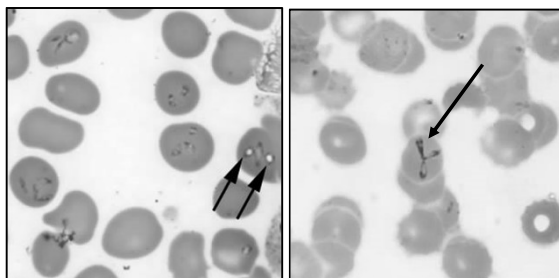
- Bite of an infected tick—during outdoor activities
- Transfusion from a blood donor who has a silent *Babesia* infection
 - Most common transfusion-transmitted infection in the U.S.-- mortality of up to 20%
- Rare congenital cases
- Report of 2 kidney transplant transmissions

BABESIA

- *B. microti*—most common in US NE, MW
- *B. divergens*—splenectomized Europe
- *B. duncani*—WA, CA
- Unnamed strain designated MO-1-- Missouri
- Sexual multiplication occurs in the tick

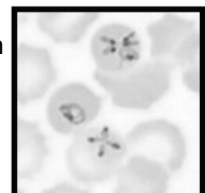


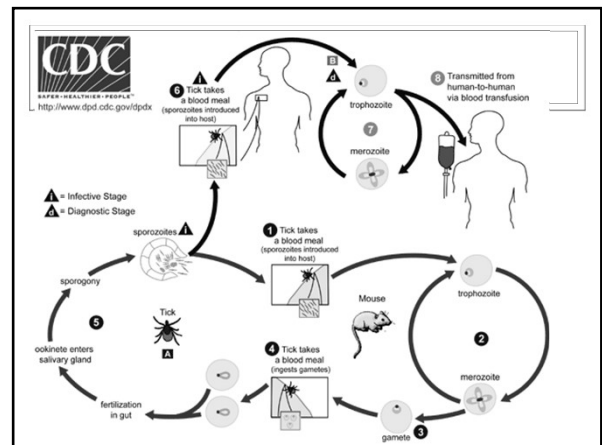
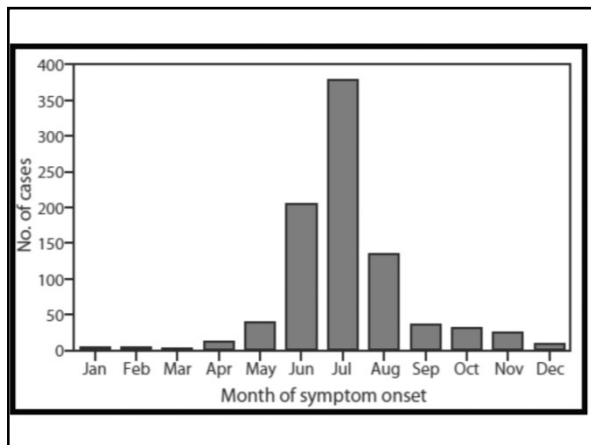
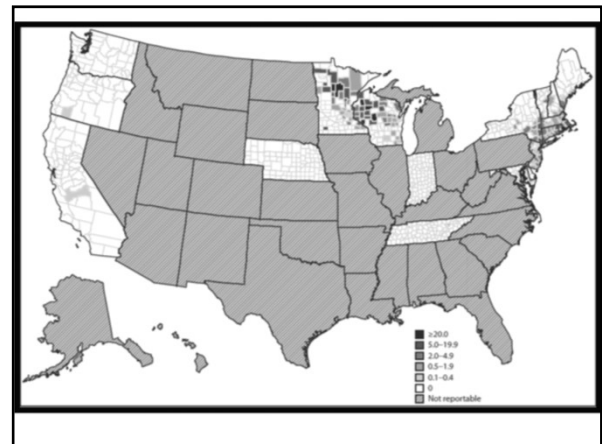
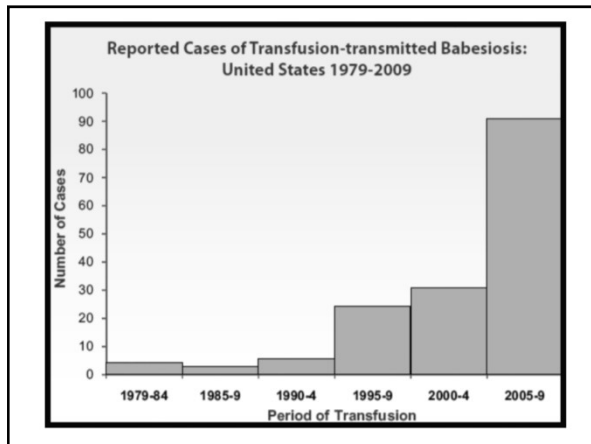
THIN BLOOD SMEAR



BABESIA MICROTI

- Organisms infect red blood cells
- Pleomorphic ring-like structures resemble *Plasmodium falciparum*
- Occasionally a tetrad formation: "Maltese Cross"
- Malarial pigment never seen






CLINICAL FEATURES

- Most infections asymptomatic
- Fever, chills, sweating, myalgias, fatigue, hepatosplenomegaly, and hemolytic anemia
- Incubation period 1-4 weeks
- Can last several weeks
- More severe in patients who are immunosuppressed, splenectomized, and/or elderly

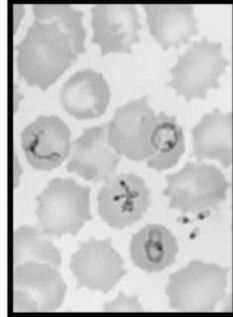
CONFIRMATORY DIAGNOSIS

- Light microscopy: Giemsa, Wright, or Wright-Giemsa–stained blood smear
- *Babesia microti* DNA by PCR
- Isolation of *Babesia* organisms from a whole blood specimen by animal inoculation



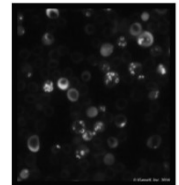
BABESIA MICROTI

- Multiple rings per RBC
- Smaller and more pleomorphic
- May form tetrad formations
- No other stages



SUPPORTIVE DIAGNOSIS

- *Babesia microti* IFA, total Ig or IgG $\geq 1:256$ (or $\geq 1:64$ in epidemiologically linked donors or recipients)
- Positive immunoblot IgG
- *Babesia divergens* IFA $\geq 1:256$
- *Babesia duncani* IFA $\geq 1:512$



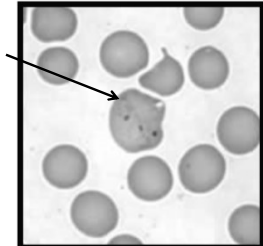
PREVENTION

- Avoid overgrown grass or brush
- Wear long pants, long sleeved shirt—tuck pant legs into socks
- Remove ticks as soon as possible
- Apply repellents
- Shower soon after



CASE 2: PLASMODIUM FALCIPARUM

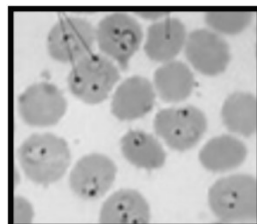
- 15-year-old male sickle cell disease from NY
- Acute febrile illness
- No travel outside U.S.
- Chest pain
- Malaise
- Receiving monthly erythrocytapheresis
- Last transfused 24 days prior



Transfusion. 2018 September ; 58(9): 2115-2121.

CASE 2

- 4 days after discharge, fever 100°F & back pain
- CBC
 - 118,000 platelets
 - 22,000 WBCs
- 0.5% paracitemia
- PCR confirmed *P. falciparum*




TRANSFUSION ASSOCIATED

- <1 in million units
- 2000-2017 11 cases--8 *P. falciparum*
- 228 million cases of malaria worldwide and 405,000 deaths
- Increases in immigration from, and travel to, endemic areas
- 99% symptomatic within 1 year of travel
- Small # congenital, transfusion-related, needle stick associated, or undetermined




TRANSFUSION ASSOCIATED

- Blood centers rely on screening questionnaires
- FDA: 3 year deferral for donors who are former residents of malaria endemic countries and for donors who have ever had malaria
- 1 year deferral for residents of U.S. who travelled to malaria endemic countries
- 150,000 deferrals/year of 6.8 million donors
- 70% TTM due to errors in donor questionnaires
- Most asymptomatic with low parasitemia




BEST PRACTICES



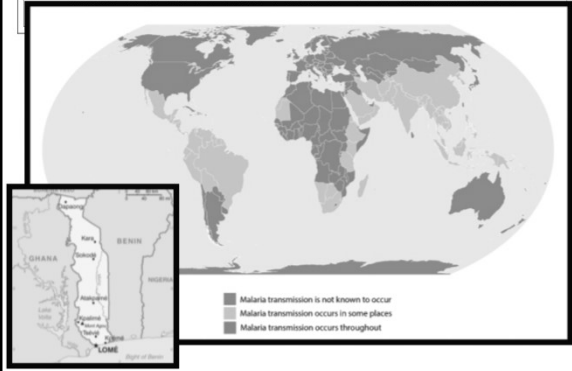
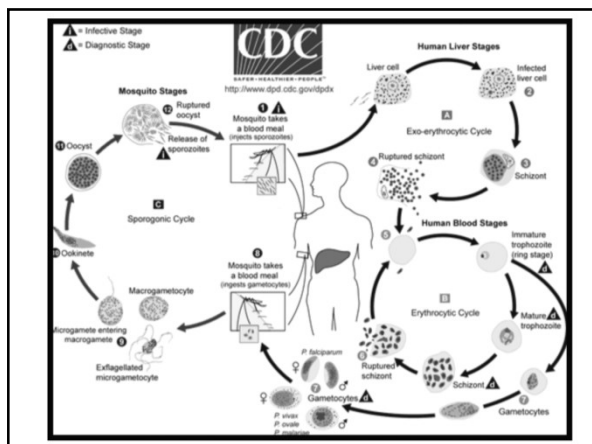
- Prompt tracing of donors
- Using sensitive serologic methods to identify donors with a history of exposure
- RT-PCR
- PCR testing to directly identify parasites in donor blood
- Microsatellite analysis to match parasites from donor
- Use epidemiologic data and re-interview

THIS CASE

- Applied to all samples available among all donors
- Donor from Togo but longer than 3 years
- Partially immune low paracitemia



MALARIA ENDEMIC COUNTRIES

CLINICAL MANIFESTATIONS

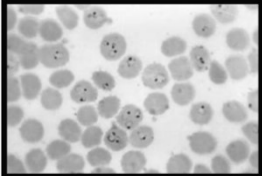
- Uncomplicated malaria can be mild and missed
- Untreated malaria can progress to severe forms that may be rapidly (<24 hours) fatal
- Fever and chills, headache, myalgias, arthralgias, weakness, vomiting, and diarrhea
- Splenomegaly, anemia, thrombocytopenia, hypoglycemia, pulmonary or renal dysfunction, and neurologic changes

CLINICAL MANIFESTATIONS

- Varies depending on the infecting species, the level of parasitemia, and the immune status of the patient
 - P. falciparum* infections can progress to severe, potentially fatal forms with CNS involvement (cerebral malaria), acute renal failure, severe anemia, or adult respiratory distress syndrome
 - P. falciparum* most deadly
 - P. vivax* malaria complications: splenomegaly
 - P. malariae* complications: nephrotic syndrome


CLASSIC PAROXYSM

- Cold stage -- 1-2 hours
- Fever -- high fever spikes, skin hot and dry
- Sweats -- marked sweating and drop in body temperature



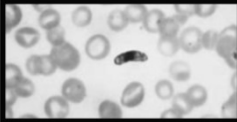
MALARIA IN PREGNANCY

- Pregnant women especially vulnerable to malaria
- Important cause of stillbirths, infant mortality and low birth weight
- Twice as attractive to malaria-carrying mosquitoes as non-pregnant women
- Due to a greater volume of exhaled air (21%) and a warmer (0.7° C) skin surface




DIAGNOSIS OF MALARIA




- 18S-rRNA-based, real-time polymerase chain reaction
- Indonesian study found sub-microscopic parasitemia in 80%--7.8 times more infections than microscopy
- Anemia strongly correlated with prevalence & load



DIAGNOSIS

- Rapid diagnostic tests
 - Plasmodium specific lactate dehydrogenase
 - Histidine rich protein II of *P. falciparum* and pan-malarial antigen aldolase
 - May not detect *P. knowlesi*

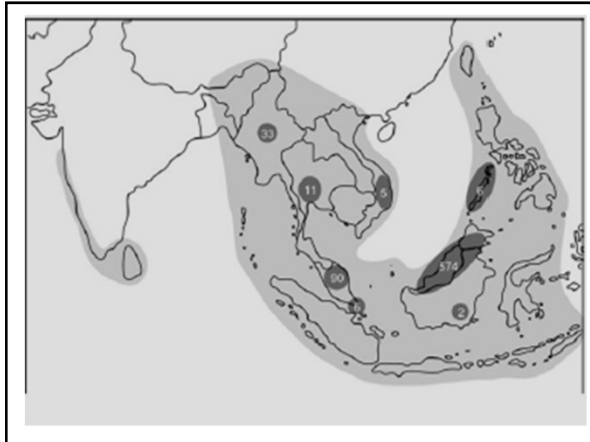


 T1 POSITIVE – Positive result for *P. falciparum* (P.f.)
 T2 POSITIVE – Positive result for *P. vivax* (P.v.), *P. malariae* (P.m.), or *P. ovale* (P.o.). In some cases the appearance of only the T2 line may indicate a mixed infection with two or more of P.v., P.m., and P.o.
 T1 + T2 POSITIVE – Positive result for *P. falciparum* (P.f.). In some cases the appearance of both the T1 and T2 lines may indicate a mixed infection with another species.

P. KNOWLESI

- 6 deaths in Sabah, Malaysia 2015–2017
- Median age was 40 (range, 23–58)
- 4 cases (67%) in males
- 3 (50%) had significant cardiovascular comorbidities
- 1 was pregnant
- Delays in administering appropriate therapy contributed to 3 (50%) deaths

Risk Factors for Fatal *P. knowlesi* Malaria • cid 2019:69 (15 November) •



SYSTEMATIC REVIEW

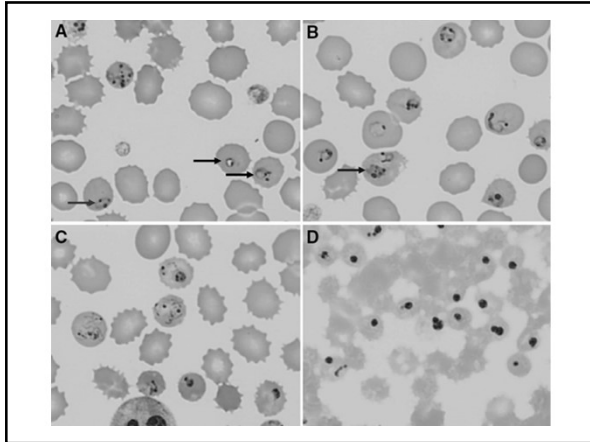
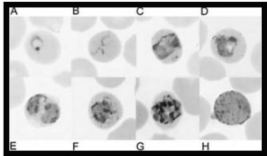
- Additional 26 fatal cases
- Cardiovascular-metabolic disease--34%
- 90% microscopic misdiagnosis
- Delay in treatment in 36%
- 2010–2017 death rate 2.5/1000: 6.0/1000 for women and 1.7/1000 for men ($p = .01$)
- Independent risk factors for death included female sex ($p = .04$), and age ≥ 45 years ($p < .01$)

P. KNOWLESII

- Now most common cause of malaria in Malaysia and parts of western Indonesia
- 6%–9% of symptomatic adults severe disease
- 20 (69%) diagnosed as *P. malariae*, 4 (14%) as *P. falciparum*, and 2 (7%) as *Plasmodium vivax*
- Thrombocytopenia, increased creatine, hyponatrimia

P. KNOWLESII

- Inability of routine microscopy to reliably distinguish *P. knowlesi* from other species
- Poor sensitivity and specificity of available rapid diagnostic tests
- Some patients low parasitemia
- Rare in children

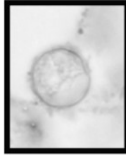


PREVENTION



CASE 3: CYCLOSPORIASIS

- 2 multi-state outbreaks linked to prepackaged vegetable trays and fast food salads
- Sickened more than 700 people 2018
- Advanced Molecular Detection (AMD) technologies
- Complex genome and difficult to extract DNA



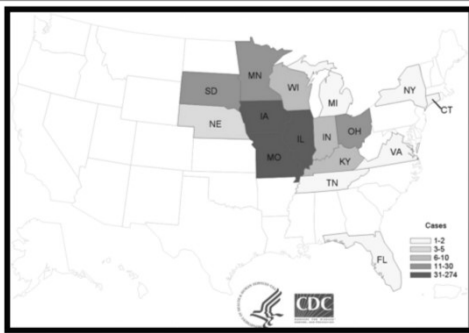
Microorganisms 2019, 7, 317; doi:10.3390/microorganisms7090317

2018 MCDONALD'S OUTBREAK

- Romaine lettuce and carrot mix distributed by Fresh Express in Streamwood, IL.
- 511 laboratory-confirmed cases in 15 states and New York City
- Consumed variety of salads from McDonald's restaurants in the Midwest
- 24 hospitalizations, 0 deaths

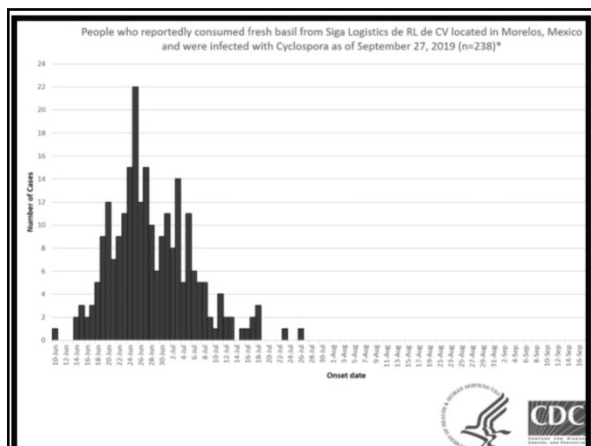
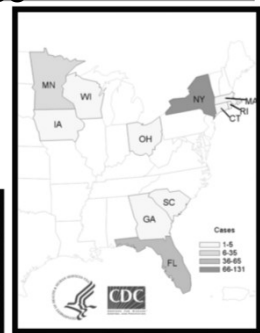


LOCATIONS OF INFECTED



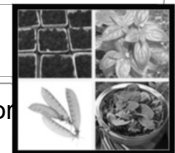
2019 FRESH BASIL FROM SIGA LOGISTICS DE RL DE CV OF MORELOS, MEXICO

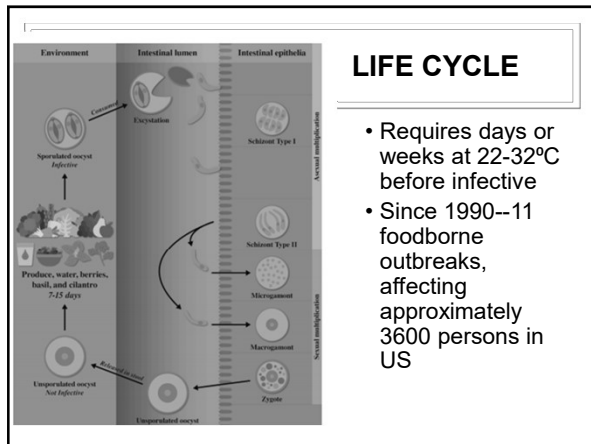
- Laboratory-confirmed cases: 241
- States: 11; exposures occurred in 5 states
- Hospitalizations: 6
- Deaths: 0



EPIDEMIOLOGY

- Spread in contaminated food or water
- Not person-to-person--requires 10-14 days to become infective
- Tropical and subtropical regions
- Imported fresh produce--no commercially frozen or canned
- Travel to endemic areas



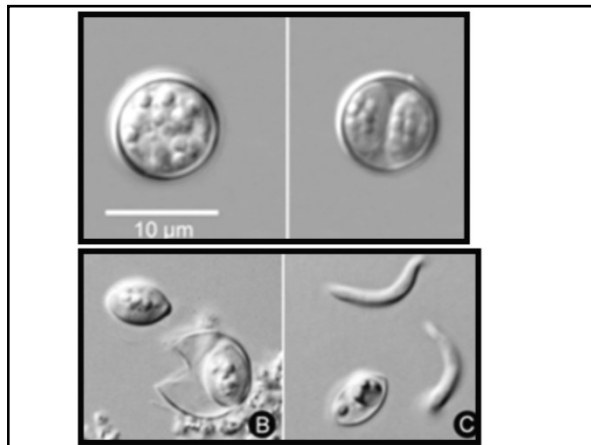


LIFE CYCLE

- Requires days or weeks at 22-32°C before infective
- Since 1990--11 foodborne outbreaks, affecting approximately 3600 persons in US

DISEASE

- Watery diarrhea (most common)
- Loss of appetite
- Weight loss
- Cramping
- Bloating
- Increased gas
- Nausea
- Fatigue
- Lasts weeks to month

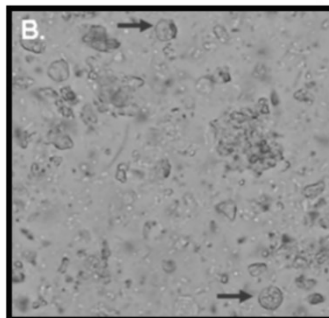


DIAGNOSIS

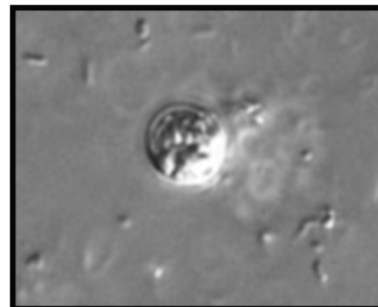
- May be excreted intermittently and in small numbers (~1-2 logs lower than *Cryptosporidium*)
- Multiple stools specimens (3) on different days
- Formalin-ethyl acetate
- Centrifuge 10 minutes at 500 x g
- Stain sediment
- Performed on request unless outbreak
- Add note to report that a routine O&P will not detect *Cyclospora*, *Cryptosporidium* or *Cystoisospora*

MICROSCOPY

- Wet mount: Refractile spheres 8-10 µm
- Distinct oocyst wall
- Not stained by iodine

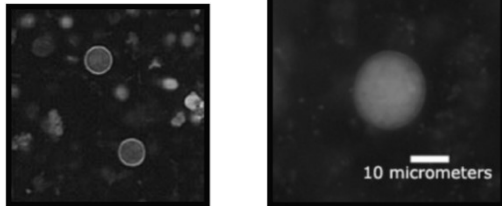


DIFFERENTIAL INTERFERENCE CONTRAST (DIC)



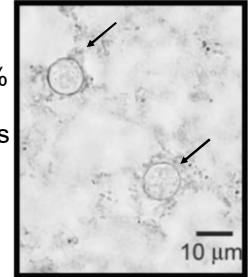
AUTOFLUORESCENCE

- Green with 450-490 DM filter
- Blue with 330-365 DM filter preferred
- 1+-3+



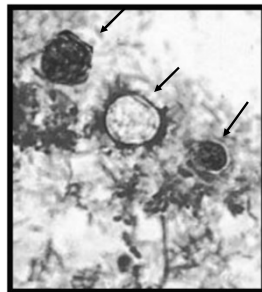
STAINED

- Trichrome: does not stain
- Modified acid-fast (1-3% variable: unstained are glassy, wrinkled spheres)
- Stained oocysts deep red to light pink, with granules or bubbles
- No sporozoites visible

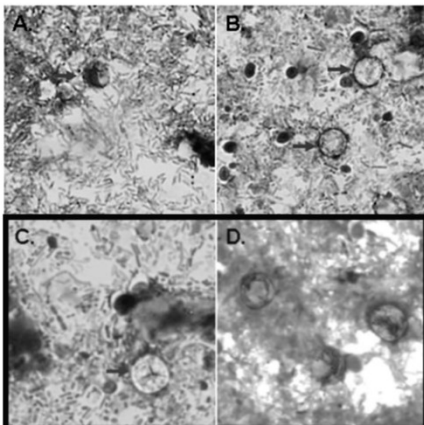
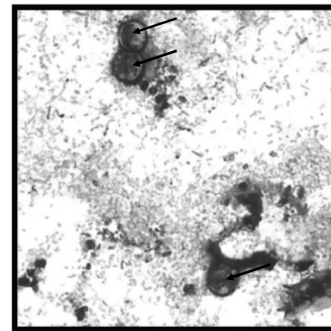


MODIFIED ACID FAST

- 8-10 μm *Cyclospora*, stained and unstained
- 4-6 μm *Cryptosporidium*
- Small artifact

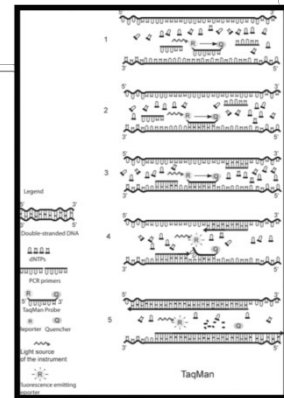


HOT SAFRANIN STAIN



MOLECULAR

- Multiplex PCR detects real time:
 - *Cyclospora*
 - *Cystoisospora*
 - *Encephalitozoon bienersi*



PREVENTION & TREATMENT

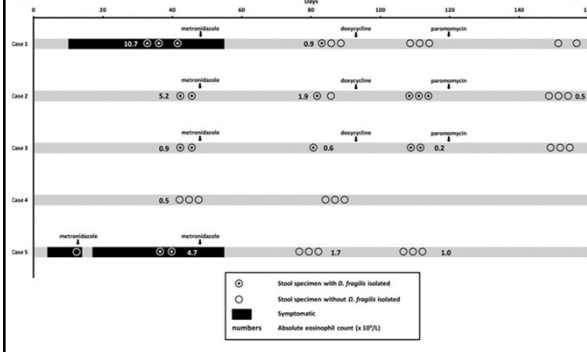
- Avoid food and water contaminated with feces
- Chlorine or iodine does not kill
- Precise way food and water contaminated is not fully understood
- CDC monitors foodborne outbreaks
- Trimethoprim/sulfamethoxazole only drug

CASE 4: FAMILY OUTBREAK OF *DIENTAMOEBIA FRAGILIS*

- 45-year-old male, wife, 2 daughters, sister-in-law
- Frequently ate sashimi
- Absolute eosinophilia
- Sister-in-law symptomatic and treated with metronidazole
- Others treated with paromomycin and tetracycline

Clinical Infectious Diseases, Volume 57, Issue 6, 15 September 2013, Pages 845–848

TEMPORAL RELATIONSHIP BETWEEN 5 CASES

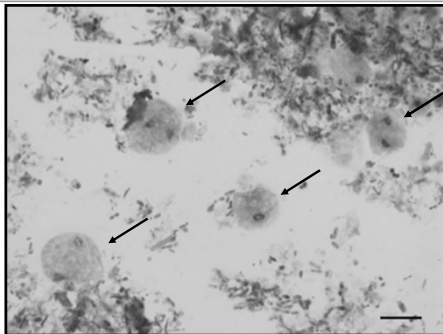


CASE 4

- Heavy burden of *D. fragilis* in stool thought to cause eosinophilia
- No other pathogens seen or found in serology
- After treatment, eosinophilia returned to normal
- No definitive source identified



DIENTAMOEBIA FRAGILIS



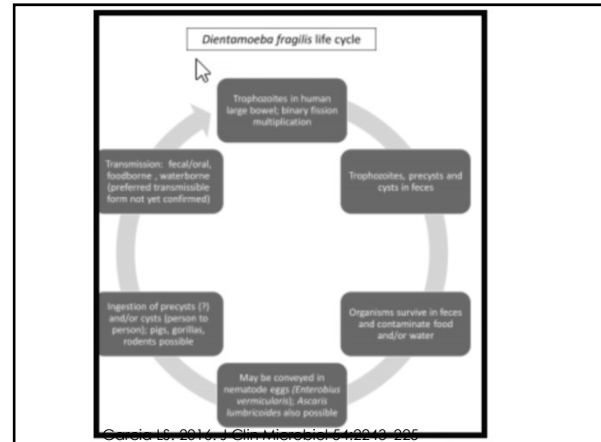
EPIDEMIOLOGY

- Worldwide
- True incidence considerably higher than reported
- Fecal/oral route
- Human to human transmission
- Pigs are natural hosts and harbor genotypes found in humans—potential zoonotic transmission

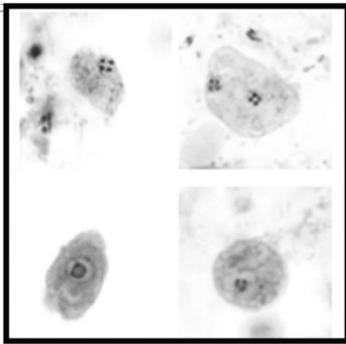


SYMPTOMS

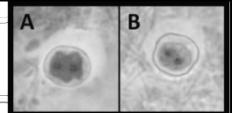
- From asymptomatic to intermittent diarrhea
- Abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain, and unexplained eosinophilia
- ~ half of the patients have eosinophilia
- Most common: intermittent diarrhea, abdominal pain, and fatigue



D. FRAGILIS TROPHOZOITES

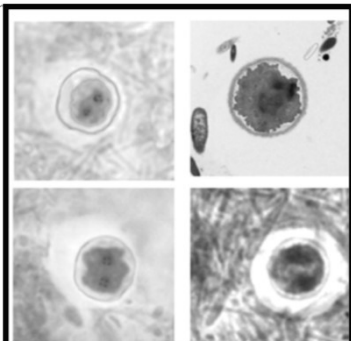


CYSTS



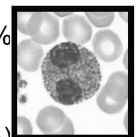
- Once thought not to exist
- Precysts and cysts extremely difficult to identify & tend to be quite rare (5%)
- Distinct cyst wall (~5 μm) with a clear zone around the cyst
- Nuclear structure morphologically identical to that found in trophozoites
- Contain two nuclei, with each nucleus containing a large central karyosome with a delicate nuclear membrane
- Fragmented into distinct granules of chromatin
- Rarely encountered in clinical samples

D. FRAGILIS CYSTS



SPANISH STUDY

- 108 patients
- 63% immigrants, 30% native & 7% travelers
- 45% presented symptoms, and eosinophilia observed in 26 (24%)
- 55% asymptomatic
- 21 asymptomatic with eosinophilia



Am. J. Trop. Med. Hyg., 99(5), 2018, pp. 1170-1173

2016 Jan 1;25(1)

CASE 5: TRYPANOSOMA BRUCEI

- A 53-year-old veterinarian developed fever on the 8th day of a holiday trip in Uganda
- Had adequate mefloquine chemoprophylaxis
- Headache, confusion, dyspnea and painful red lesion on left leg
- Returned home where physician prescribed doxycycline for presumed African tick typhus
- Next day-- fever (38.7 °C), headache and cough
- Acutely ill with tachycardia, hypertension, tachypnea and diffuse wheezing

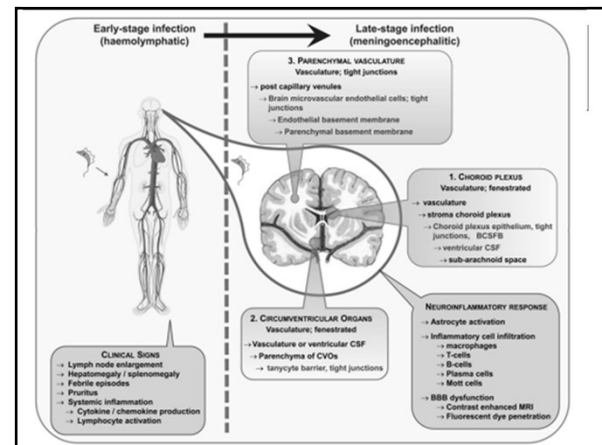
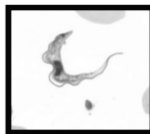
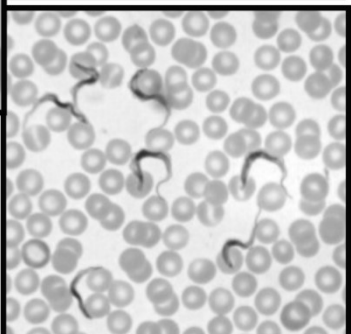
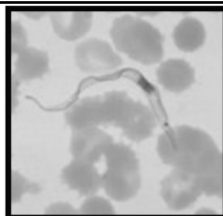
Frontiers in Immunology (2019) 10: 39

CASE 5

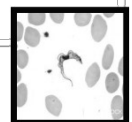
- Oxygen saturation 97%
- Neurological exam normal
- Facial and limb edema
- Generalized erythematous, macular exanthema and a large, painful chancre with necrotic center measuring 5 cm in diameter at the suspected bite area on the left calf
- Hepatosplenomegaly seen on ultrasound

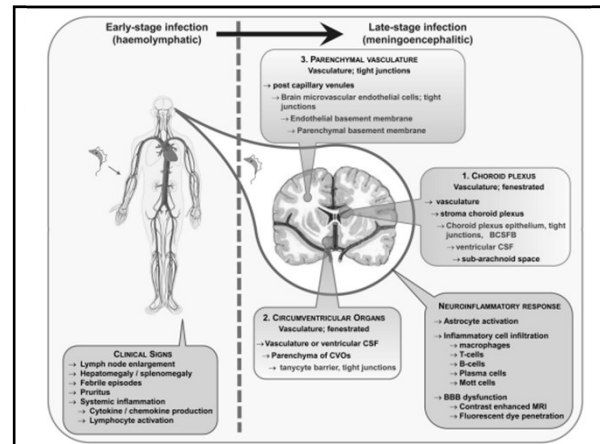
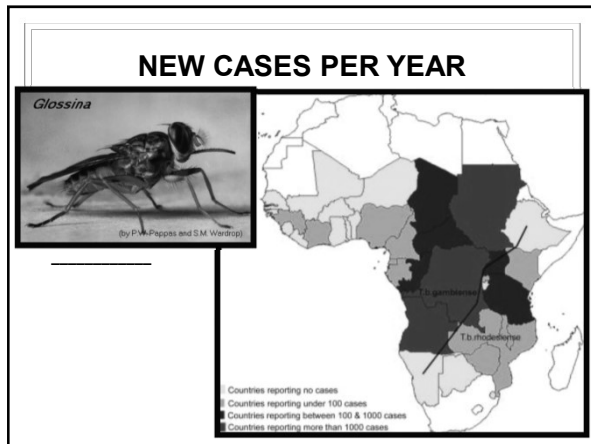
CASE 5

- Blood count showed mild leukopenia (3200 cells/ μ l) with 83% neutrophils
- Platelet count normal (161,000/ μ l)
- C-reactive protein 265 mg/l
- Liver enzymes 5–10 times normal
- Glomerular filtration rate normal
- Lactate \uparrow 5.8 mmol/l
- Recalled tsetse flies in her car on the 4th day of travel, but did not notice any bites

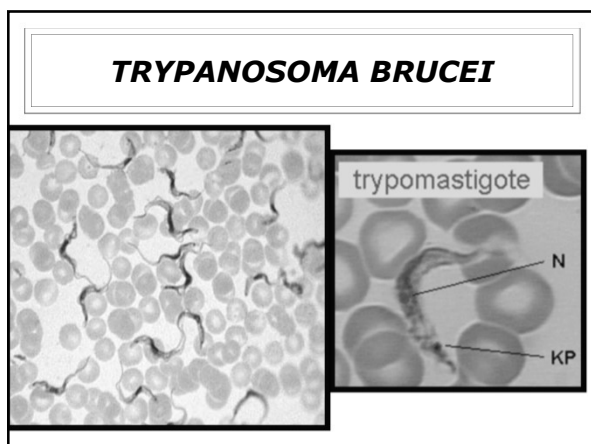
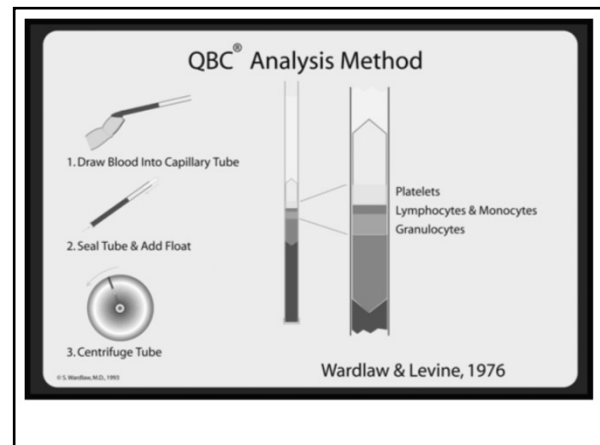
**BLOOD SMEAR****TRYPANOSOMA BRUCEI**

- Two subspecies
- Morphologically indistinguishable
- Cause distinct disease patterns in humans:
 - *T. b. gambiense*
 - *T. b. rhodesiense*
- Uganda only country that harbors both subspecies
- PCR confirmed *Trypanosoma b. rhodesiense*





- ### LABORATORY DIAGNOSIS
- Microscopic examination of chancre fluid, lymph node aspirates, blood, bone marrow, cerebrospinal fluid
 - Wet preparation for motile trypanosomes
 - Giemsa stained slide
 - Concentration techniques--examination of the buffy coat, mini anion-exchange/centrifugation and Quantitative Buffy Coat (QBC)



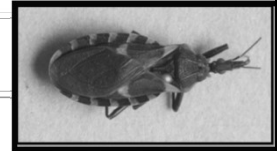
- ### CEREBRAL CRITERIA
- Observation of trypanosomes in CSF
 - CSF white cell count of ≥ 6
 - Elevated protein
 - Increase in nonspecific IgM in CSF
-

CASE 6: CHAGAS DISEASE

- A 26-year-old, previously healthy, professional male
- 6-week history of being bitten throughout his body
- Lived with his parents in a well-manicured suburban home in San Francisco Bay area
- No pets and had not traveled

Am. J. Trop. Med. Hyg., 95(5), 2016, pp. 1115–1117

CASE 6



- Kept getting bitten
- Engaged exterminator
- Found 8 adult reduviid bugs, 9 nymphs, and 2 eggs in his bed sheets
- Contacted CDC to test bugs
- Found *Trypanosoma cruzi* in one

CASE 6 BEDROOM

- Served as home office
- Contained several cardboard boxes from domestic companies
- Otherwise spotless
- After removal of boxes, no further bugs seen

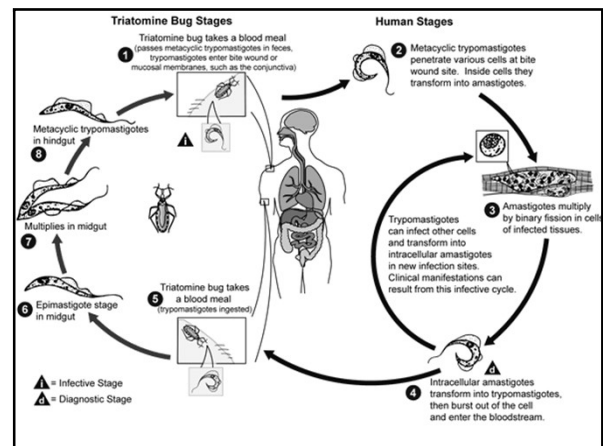
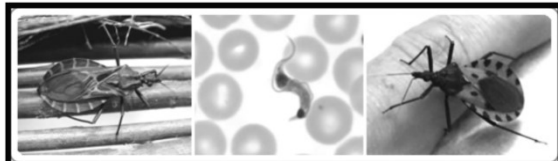


CARDBOARD BOXES

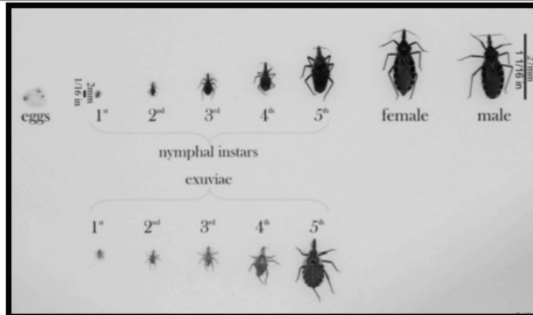


TRYPANOSOMA CRUZI

- Causes Chagas disease
- Zoonotic disease transmitted to humans by blood-sucking triatomine bugs: reduviid bug



REDUVIID (TRIATOMINE) BUGS



REDUVIID BUG DEFECATION



- Nocturnal
- Feed on blood of mammals
- Rarely infest plaster/dry wall houses

TRANSMISSION

- Bite wounds, intact mucus membranes or conjunctiva
- Vertical or congenital transmission
- Blood transfusions, bone marrow or organ transplants
- Certain foods and drinks
- Accidental laboratory exposure



TRYPANOSOMA CRUZI

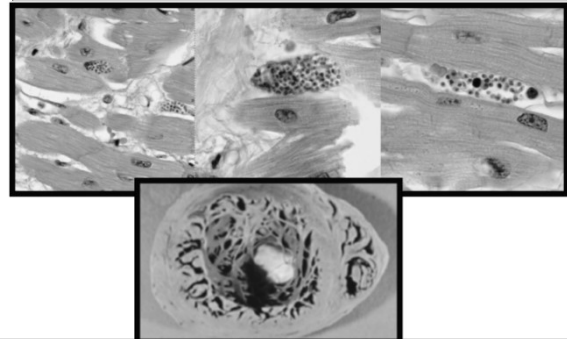
- Worldwide 10 to 12 million people infected
- ~50,000 people die each year
- 8–10 million people in Mexico, Central America, and South America infected
- >300,000 people in US infected
- Acquired in endemic countries



CHAGAS DISEASE

- Infants and children often die from swelling of brain
- Parasite may lie dormant for years
- Emerge and lodge in heart muscle, causing serious and irreversible damage to the heart
- Leading cause of heart disease in South America

AMASTIGOTE IN HEART MUSCLE



11 SPECIES OF TRIATOMINE BUGS



BLOOD TRANSFUSION



- AABB recommends testing
- Began in 2007
- Performed in major labs that test 65% of blood
- Recently approved by FDA
- 1800 confirmed cases in donors
- 0.2% of blood donors in the San Francisco Bay Area tested positive for antibodies

**TRYPANOSOMA CRUZI
GEOGRAPHIC DISTRIBUTION**

- Americas from the southern US to southern Argentina
- Mostly in poor, rural areas of Central and South America
- Mud huts with thatched roofs
- Blood transfusion in the United States



CLINICAL FEATURES

- A local lesion (chagoma, palpebral edema) at the site of inoculation
- Acute phase is usually asymptomatic:
 - Can present with fever, anorexia, lymphadenopathy, mild hepatosplenomegaly, and myocarditis
 - Most resolve over 2 to 3 months into an asymptomatic chronic stage

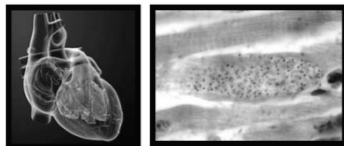


Romaña's sign



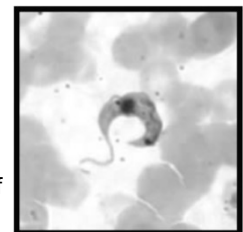
**TRYPANOSOMA CRUZI CLINICAL
FEATURES**

- Symptomatic chronic stage may not occur for years or even decades
 - Cardiomegaly (the most serious manifestation)
 - Megaesophagus and megacolon
 - Weight loss
 - Can be fatal



**TRYPANOSOMA CRUZI LABORATORY
DIAGNOSIS**

- Microscopic examination
 - Fresh anticoagulated blood or buffy coat for motile parasites
 - Thin and thick blood smears stained with Giemsa, for visualization of parasites

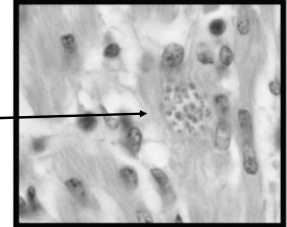


TRYPANOSOMA CRUZI LABORATORY DIAGNOSIS

- Isolation of the agent by:
 - Inoculation into mice
 - Culture in specialized media
 - Xenodiagnosis where uninfected reduviid bugs are fed on the patient's blood, and their gut contents examined for parasites 4 weeks later
- Indirect fluorescent antibody (IFA), enzyme immunoassay (EIA) or (ELISA)
- PCR

TRYPANOSOMA CRUZI

- Amastigote
- Indistinguishable from *Leishmania*
- 2-6 μm
- Large nucleus and rod-shaped kinetoplast
- Found in heart muscle, not macrophages



ANOTHER CHAGAS CASE

- 2017 Missouri 53-year-old woman
- Blood donor
- Tested positive to antibody test and confirmatory test
- Referred to physician
- No bug bites

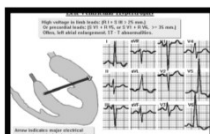
AUTOCHTHONOUS CASE

- Lived in Missouri entire life
- Travel history: trip to California ~28 years earlier--crossed the Mexican border for a few hours to go shopping
- Traveled to Florida and Alabama for vacation



CHAGAS CASE

- ECG: arrhythmias and left ventricular hypertrophy
- Consistent with chronic Chagas
- 60-day course of benznidazole
- Rare: only 28 autochthonous infections documented from 1955 to 2015
- First documented autochthonous case in Missouri



PREVENTION

- In endemic areas
 - Improved housing
 - Spraying insecticide inside housing
 - Screening of blood donations
 - Early detection and treatment of new cases, including mother-to-baby
- In US and in other regions where Chagas disease is now found but is not endemic
 - Control focused on preventing transmission from blood transfusion, organ transplantation, and mother-to-baby



QUESTIONS

