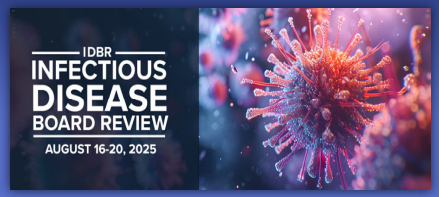


Protozoa That Could Be Tested

Edward Mitre, MD
Rockville, MD

8/4/2025

1

Disclosures of Financial Relationships with Relevant Commercial Interests

- None

Disclaimer: Dr. Mitre is giving this presentation in a personal capacity. The views expressed in this presentation are the sole responsibility of the presenter and do not necessarily reflect the views, opinions, or policies of the Uniformed Services University of the Health Sciences, the Department of Defense, or the United States Government.

2

Protozoa

Protozoa - Extraintestinal

Apicomplexa
- Plasmodium
- (Babesia)
- (Toxoplasma)

Flagellates
- Leishmania
- Trypanosomes
- (Trichomonas)

Amoebae
- Naegleria
- Acanthamoeba
- Balamuthia

Protozoa - Intestinal

Apicomplexa
- Cryptosporidium
- Cyclospora
- Cystoisospora

Flagellates
- Giardia
- Dientamoeba

Amoebae
- Entamoeba

Ciliates
- Balantidium

Maybe Not Protozoa **Fungi Kingdom:** Microsporidiosis agents
SAR supergroup: Blastocystis

3

Protozoa

Protozoa - Extraintestinal

Apicomplexa
- Plasmodium
- (Babesia)
- (Toxoplasma)

Flagellates
- Leishmania
- Trypanosomes
- (Trichomonas)

Amoebae
- Naegleria
- Acanthamoeba
- Balamuthia

Protozoa - Intestinal

Apicomplexa
- Cryptosporidium
- Cyclospora
- Cystoisospora

Flagellates
- Giardia
- Dientamoeba

Amoebae
- Entamoeba

Ciliates
- Balantidium

Maybe Not Protozoa **Fungi Kingdom:** Microsporidiosis agents
SAR supergroup: Blastocystis

4

Question #1

PREVIEW QUESTION

IDBR INFECTIOUS DISEASE BOARD REVIEW 2025

A 54-year-old woman presents with fever, chills, and oliguria one week after travel to Malaysia.

Vitals: **39.0°C**, HR 96/min, RR 24/min, **BP 86/50**
Labs: Hct 31%, platelets14,000/µl, Cr of 3.2 mg/dL.

Peripheral blood smear has intraerythrocytic forms that are morphologically consistent with *Plasmodium malariae*.

What is the most likely infectious agent causing the patient's illness?

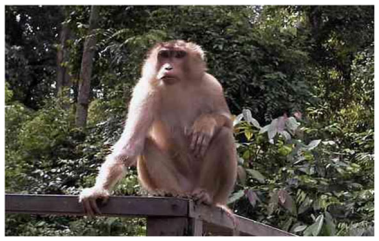
A. Plasmodium malariae
B. Plasmodium knowlesi
C. Plasmodium vivax
D. Plasmodium falciparum
E. Babesia microti

5

P. knowlesi

Morphologically similar to *P. malariae*

Usually a parasite of long-tailed macaques



Increasingly recognized in Myanmar, Phillipines, Indonesia, and Thailand

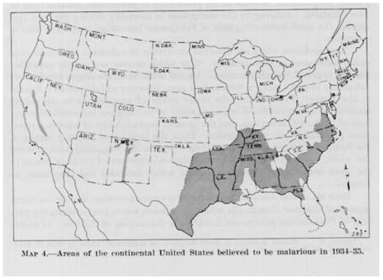
Causes high parasitemia

Highly morbid and can be lethal

6

Malaria

One of the most important pathogens in the history of the world



MAP 4.—Areas of the continental United States believed to be malarious in 1934-35.

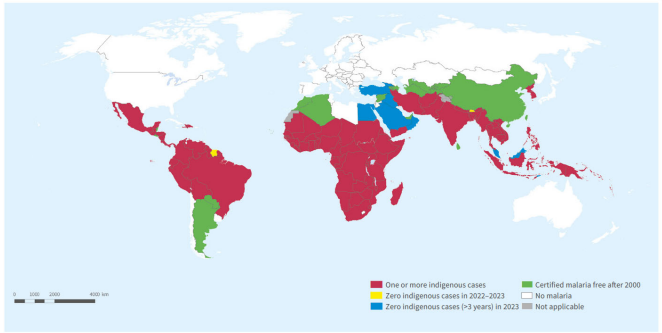
National Malaria Elimination Program: 1947- 1951
→ DDT spraying, drainage of wetlands
→ Atlanta was chosen for the Office of Malaria Control in War Areas (the predecessor agency of the CDC) in part because of its location in a malaria-endemic region

In 1775 the Continental Congress purchased quinine for George Washington's troops

7

Disease Burden – WHO 2024 World Malaria Report

263 million cases worldwide in 2023
597,000 deaths
U.S. ~ 2000 cases reported each year

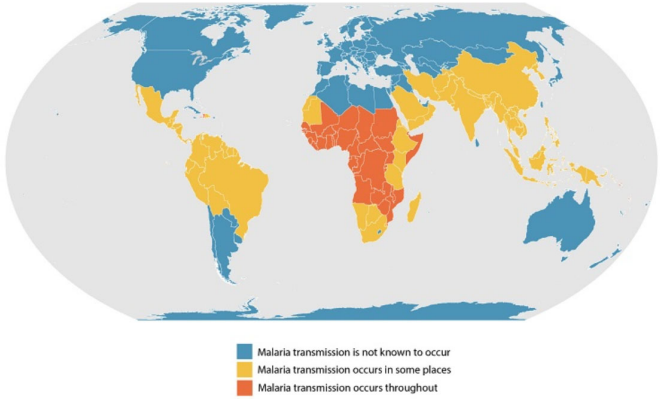


WHO: World Health Organization.
[a] Malaysia has a significant number of indigenous malaria cases caused by *Plasmodium knowlesi* infection.
[b] Countries and areas with zero indigenous cases for at least 3 consecutive years are considered to have eliminated malaria. In 2023, Malaysia reported zero indigenous cases caused by human *Plasmodium* species[a] for the sixth consecutive year, and Saudi Arabia and Timor-Leste reported zero indigenous cases for the third consecutive year, ending the malaria epidemic. Cabo Verde and Belize were certified malaria free in 2023, following 4 years of zero malaria cases. Egypt has since been certified malaria free in 2024.

8

Malaria Epidemiology

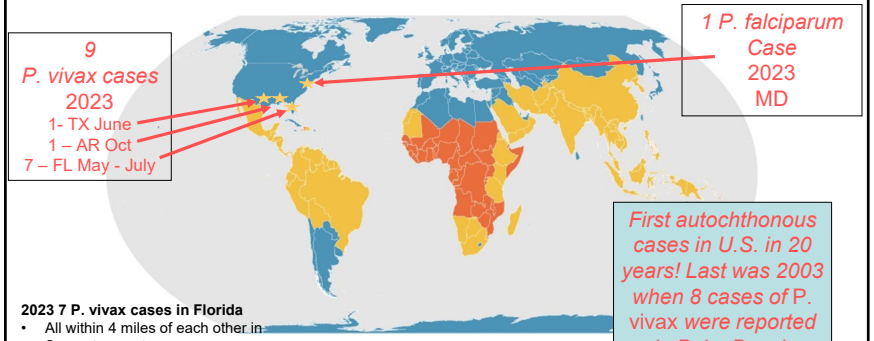
CDC map from a few years ago…what's missing?



https://www.cdc.gov/malaria/about/distribution.html

9

2023 U.S. Malaria Cases



2023 7 P. vivax cases in Florida

- All within 4 miles of each other in Sarasota county
- All with fever and low platelets
- 3 individuals were homeless
- April 20th there had been an imported P. vivax case
- CDC testing of 407 Anopheles mosquitoes → 3 A. crucians were PCR+

https://www.cdc.gov/mmwr/volumes/72/wr/mm7236a1.htm

https://www.cdc.gov/malaria/about/distribution.html

10

In non-immune patients, falciparum malaria is a medical emergency!!

One of the most common causes of fever in a returned traveler

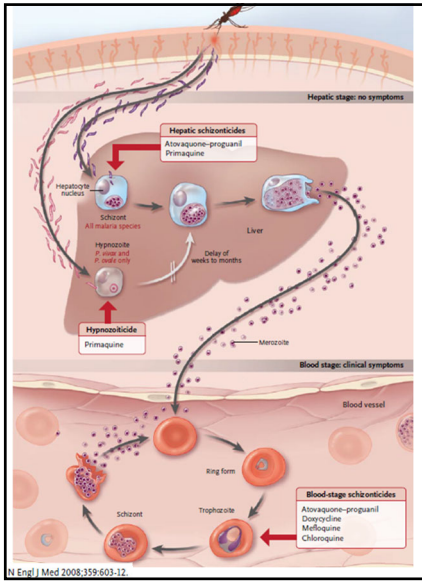
Infected individuals can rapidly progress from appearing well to being critically ill

11

Some Helpful Heuristics

If patient has	Make sure patient doesn't have
Fever and freshwater contact---------->	Malaria
Fever and unpasteurized milk---------->	Malaria
Fever and undercooked meat---------->	Malaria
Fever and raw vegetables---------->	Malaria
Fever and untreated water---------->	Malaria
Fever and wild dog bite---------->	Malaria
Fever and abdominal pain---------->	Malaria
Fever and headache---------->	Malaria
Fever and diarrhea---------->	Malaria
Fever and cough---------->	Malaria
Fever and dysuria---------->	Malaria

12



Sporozoites

- **Infective stage**
- Come from mosquito

Liver schizont

- **Asymptomatic replicative stage**
- Become 10,000 to 30,000 merozoites

Hypnozoite

- Dormant liver stage in **vivax and ovale**
- Release merozoites weeks to months after primary infection

Merozoites

- Infect RBCs and develop into ring-stage trophozoites
- Mature into schizonts, which release merozoites which infect more RBCs

Gametocytes

- Infective stage for mosquitoes

13

Characteristics of Human Malaria Species

	P. falciparum	P. knowlesi	P. vivax	P. ovale	P. malariae
incubation	8 - 25 d	prob 8-25 d	~ 2 wks	~ 2 wks	~ 3-4 wks
hypnozoite	no	no	yes	yes	no
RBC age	any	any	young	young	old
parasitemia	high	high	< 2%	< 2%	< 1%
morbidity	high	high	high	moderate	low
mortality	high	moderate	low	low	low

14

Possible Evolutionary Defenses Against Malaria

Duffy antigen negative

(*P. vivax* uses Duffy Ag to enter RBCs)

Sickle cell trait

(increases survival during *P. falciparum* infection, perhaps by selective sickling of infected RBCs)

Glucose-6-phosphate dehydrogenase deficiency

(malaria parasites grow poorly in G6PD deficient RBCs, perhaps b/c this results in an overall increase in reactive oxygen species in RBCs)

15

Uncomplicated (Mild) Malaria

Symptoms:

- Fevers, chills, headache, fatigue
  *NOTE: abdominal pain presenting symptom in 20%

→ *Periodicity of fevers not common when patients seen acutely*

Labs:

- Thrombocytopenia in 50%
- Mild anemia in 30%
- Typically, no leukocytosis
- May see evidence of hemolysis with mild increase T bili and LDH

16

Complicated (Severe) Malaria

- Cerebral malaria (altered mental status, seizures)
- Respiratory distress/pulmonary edema
- Severe anemia (hct <15% in children, <20% in adults)

} Often seen in children of endemic countries. Adults more often get multiorgan failure.

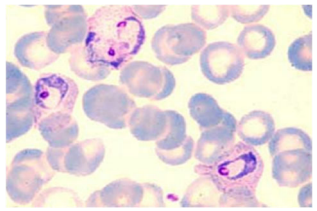
- Renal failure
- Hypoglycemia
- Shock (SBP < 80 mm Hg or capillary refill > 3 seconds)
- Acidosis (often lactic acidosis)
- Jaundice (total bilirubin > 3 mg/dL)
- Bleeding disorder (spontaneous bleeding or evidence of DIC)

These complications primarily occur with *Plasmodium falciparum,* usually when parasitemia ≥ 2%.

NOTE: in the absence of end organ damage, parasitemia ≥ 5% is often used as the cut-off to treat for severe malaria in the U.S.

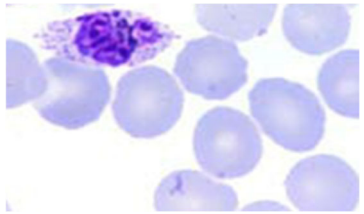
17

P. vivax or ovale



- Intracellular schüffner's dots
- Enlarged infected cells

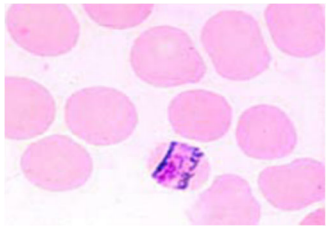
P. ovale



- Elongated or oval
- 6-12 merozoites (vs 12-24 for vivax)

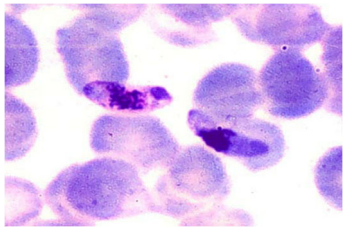
18

P. malariae



- Band form
  (also seen in *P. Knowlesi*)

P. falciparum

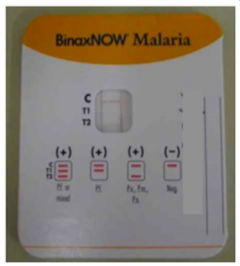


- Banana shaped gametocyte

19

Malaria: Diagnosis

Rapid diagnostic antigen tests
→ sensitivity > 90% for P. falciparum
(about 85% for P. vivax, lower for P. knowlesi and P. ovale)



Binax Now® ICT assay for the detection of *Plasmodium falciparum* malaria according to the level of parasitemia

Parasitemia (no. of parasites/μL of whole blood)	Microscopy (no. positive)	NOW ICT (no. positive)	Sensitivity (%)
1–100	4	3	75.0
101–1,000	26	25	96.2
1,001–10,000	37	36	97.3
>10,000	34	33	97.1

Am. J. Trop. Med. Hyg., 69(6), 2003, pp. 589–592

For P. falciparum (T1) → tests for histidine-rich protein 2
For all species (T2) → tests for aldolase

*Most false-negative antigen tests are due to low parasite burden
→ Retest suspected patients that initially test negative

*Increasing false negative cases occurring worldwide due to mutations in HRP2

20

Question #2

PREVIEW QUESTION

A 33-year-old woman is traveling to Uganda to do field studies in anthropology. She is two months pregnant.

Which of the following do you prescribe for malaria prophylaxis?

A. Doxycycline
B. Chloroquine
C. Mefloquine
D. Atovaquone/progruanil
E. No prophylaxis

21

Malaria Chemoprophylaxis (note: no malaria vaccine approved for U.S. travelers)

SOME REGIONS IN CENTRAL AMERICA and the MIDDLE EAST

	Pre-Exposure	During	Post-Travel
Chloroquine 500mg tabs	1 tab/wk x 2 wks	1 tab/wk	4 weeks
EVERYWHERE			
Atovaquone/proguanil 250/100mg	1 tab daily x 2 d	1 daily	7 days
Doxycycline 100mg tabs	none	1 daily	4 weeks
Tafenoquine* 100mg tabs	2 tab daily x 3 d	2 tab/wk	2 tab after 1 wk
Mefloquine (not SE Asia)** 250mg tabs	1tab/wk x 2-3 wks	1 tab/wk	4 weeks

** **Tafenoquine can precipitate severe hemolytic anemia in individuals that are G6PD deficient***

*** **FDA black box warning mefloquine can cause neurologic symptoms, hallucinations, and feelings of anxiety, mistrust, and depression. Can also cause QT prolongation. Thus, many U.S. practitioners now reserve mefloquine for pregnant travelers to areas with chloroquine resistance***

22

P. falciparum Treatment

Excellent review → 2022 JAMA, 328(5):460-47, PMID: 3591684

Uncomplicated P. falciparum malaria (no organ dysfunction, low parasitemia, able take po)

If chloroquine sensitive area → Chloroquine or hydroxychloroquine

If not chloroquine sensitive area (most cases) → **Artemether/lumefantrine (Coartem)**
ACTs are treatment of choice, WHO 2022 guidelines
Alternatives if artemether/lumefrantrine not available:
Atovaquone/proguanil (Malarone), quinine + doxycycline, mefloquine

Severe Malaria

→ **IV artesunate** (CDC malaria hotline: 770-488-7788)

NOTES

1. Treatment failures can occur with artemether/lumefrantrine, especially when > 65 kg
   Sonden K. et al, *Clinical Infectious Diseases* 2017 PMID: 27986683
2. Artemisinin resistance reported in SE Asia (Cambodia, Laos, Myanmar, Thailand, Vietnam), parts of Africa (Uganda, Rwanda), and in S. America (Guyuna)
3. Delayed-onset anemia in 2.7% of U.S. patients after treatment with artesunate
   Abanyie F. et al, *Clinical Infectious Diseases* 2022 PMID: 36052468
4. Hypoglycemia and ARDS are complications that can occur during treatment of malaria.
   ARDS sometimes develops even as pt improving from malaria.

23

P. vivax/P. ovale Treatment

Chloroquine x 3 days, or ACT (artemether/lumefrantrine in U.S.)
Note: PNG, Indonesia, Oceania have CLQ R P. vivax → use ACT

Then ANTIRELAPSE THERAPY with primaquine or tafenoquine

→ Need to check G6PD status before administering primaquine OR tafenoquine
(as both can cause severe hemolysis in patients with G6PD deficiency)

→ Both primaquine and tafenoquine contraindicated during pregnancy

- **Primaquine – weight-based dosing and duration as determined by G6PD activity**

  ALWAYS LOOK UP DOSING BEFORE ADMINISTERING

  -> Usually 30 mg primaquine base per day x 14 days if normal G6PD activity

  -> Do not exceed 30 mg primaquine base per day

  -> If over 70 kg, can calculate total dose 6 mg/kg and then extend duration of 30 mg daily doses until total goal met

  -> if G6PD deficient consider weekly chloroquine x 1 year

- **Tafenoquine (two 150 mg tabs once, given on 1st or 2nd day of chloroquine therapy)**
  (Tafenoquine was approved for radical cure of *P. vivax* in 2018, *P. ovale* treatment is off-label)

2020: Company (GSK) reported some failures when tafenoquine was used after ACT treatment of P. vivax.
NEW FDA LABELING: **Tafenoquine now only approved and recommended after chloroquine treatment**

https://www.cdc.gov/malaria/hcp/clinical-guidance/treatment-uncomplicated.html

24

Suggestions for All ID Practitioners

1. Make sure the facility where one works has the means to rapidly test for malaria
2. Ensure that hospital pharmacy has access to appropriate medications for treatment of malaria

25

Babesia

Transmission

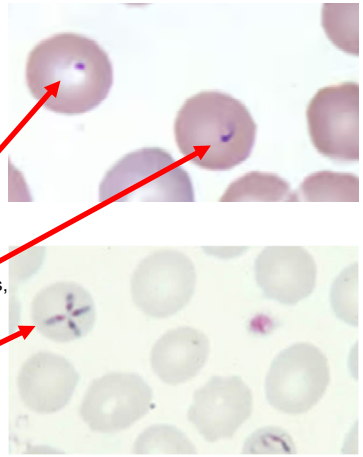
- Ixodes ticks in Northeast and upper midwest
  → co-infection with Lyme and Anaplasma
- Transfusion
  (Ab screening tests approved by FDA in 2018)

Symptoms: fever, headache, chills, myalgias
less common: nausea, dry cough, neck stiffness, vomiting, diarrhea, arthralgias
→ severe disease: in HIV, asplenia

Labs: anemia, thrombocytopenia, mild increase LFTs, normal/low/high WBC

Diagnosis: small ring forms in RBCs, PCR, Ab
merozoites can make tetrad ("Maltese cross")

Treatment: azithromycin + atovaquone
(clindamycin + quinine is alternative)
→ Exchange transfusion for severe disease



CDC DpDx

26

Protozoa

Protozoa - Extraintestinal

Apicomplexa
Plasmodium
(Babesia)
(Toxoplasma)

Flagellates
Leishmania
Trypanosomes
(Trichomonas)

Amoebae
Naegleria
Acanthamoeba
Balamuthia

Protozoa - Intestinal

Apicomplexa
Cryptosporidium
Cyclospora
Cystoisospora

Flagellates
Giardia
Dientamoeba

Amoebae
Entamoeba

Ciliates
Balantidium

Maybe Not Protozoa **Fungi Kingdom:** Microsporidiosis agents
SAR supergroup: Blastocystis

27

Leishmaniasis

→ **Obligate intracellular protozoan infection**
→ **Transmitted by sand flies** **(noiseless, active in evenings)**

Lutzomyia
New world leishmaniasis

Phlebotomus
Old world leishmaniasis

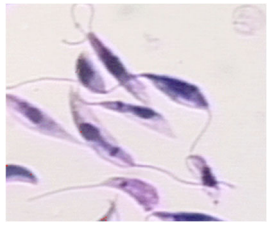
28

13 Protozoa That Could Be Tested
Speaker: Edward Mitre, MD

Leishmaniasis Life Cycle – Two Stages

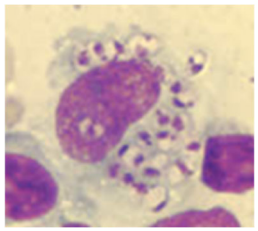
Promastigote

Extracellular, in sand fly
2 µm wide x 20 µm long
- Flagella
- Large central nucleus
- Band shaped kinetoplast



Amastigote

Intracellular (macrophages)
Round or oval
Wright-Giemsa:
- Dark-purple nucleus
- Small rod shaped kinetoplast



CDC DpDx

29

Question #3

A 42-year-old man from Bolivia presents with nasal stuffiness and is found to have nasal septal perforation. Biopsy demonstrates intracellular amastigotes consistent with Leishmania.

Which is the most likely species?

A. L. mexicana
B. L. braziliensis
C. L. peruviana
D. L. infantum chagasi
E. L. major

30

Leishmania Taxonomy and Disease Simplified

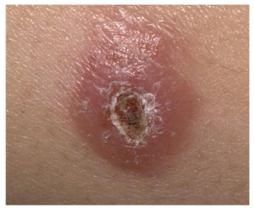
	Cutaneous	Mucosal	Visceral
NEW WORLD			
L. mexicana complex	X		
L. braziliensis	X	X	
L. infantum chagasi			X
OLD WORLD			
L. tropica	X		
L. major	X		
L. donovani			X
L. infantum chagasi			X

*Note: *L. braziliensis* is in the Viannia subgenus. *L. V. guyanensis* and *L. V. panamensis* also cause mucosal disease. *L. peruviana* DOES NOT

31

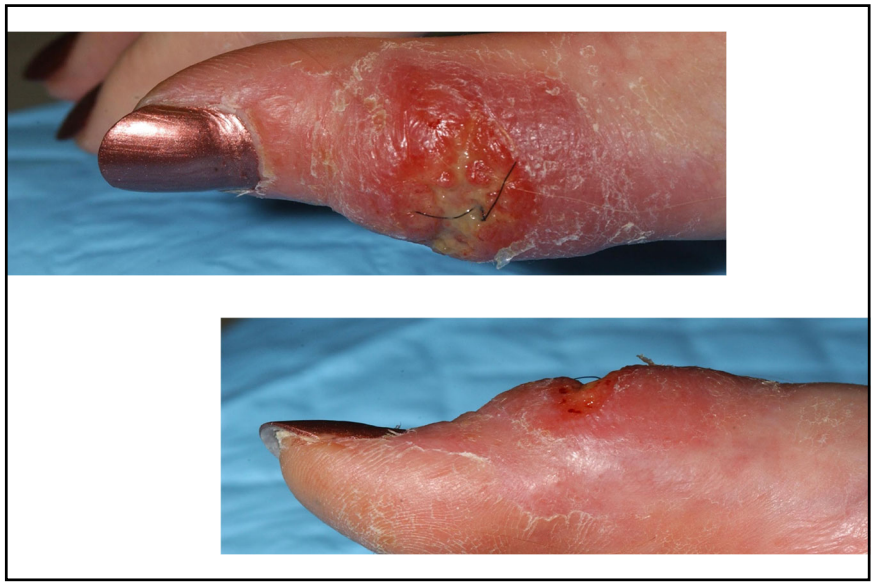
Cutaneous Leishmaniasis: Clinical Presentation

- **Papule → nodule → ulcerative lesion → atrophic scar**
  - **Ulcerative lesion may have:**
    - **Induration**
    - **Scaliness**
    - **Central depression**
    - **Raised border**

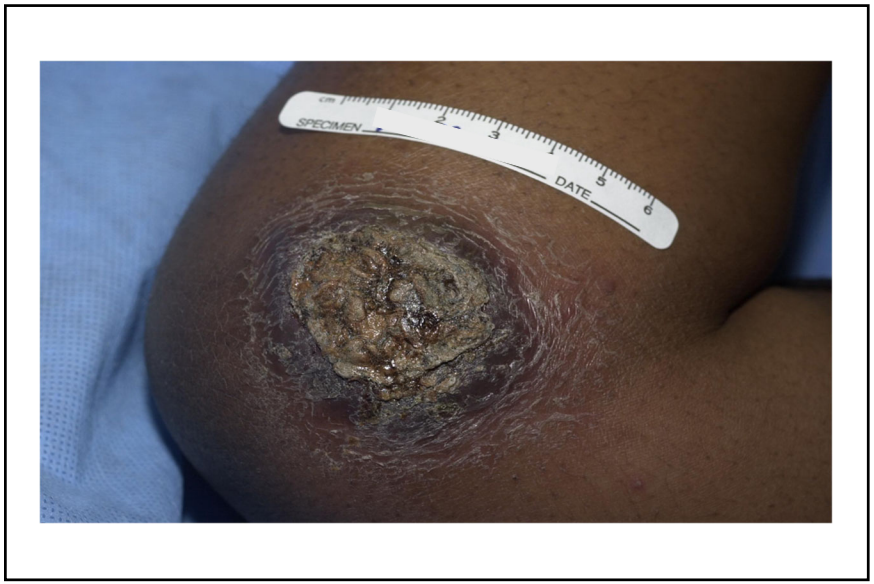


- **Takes weeks to months to develop**
- **Usually painless, unless superinfected**
- **Most lesions will eventually resolve on their own**

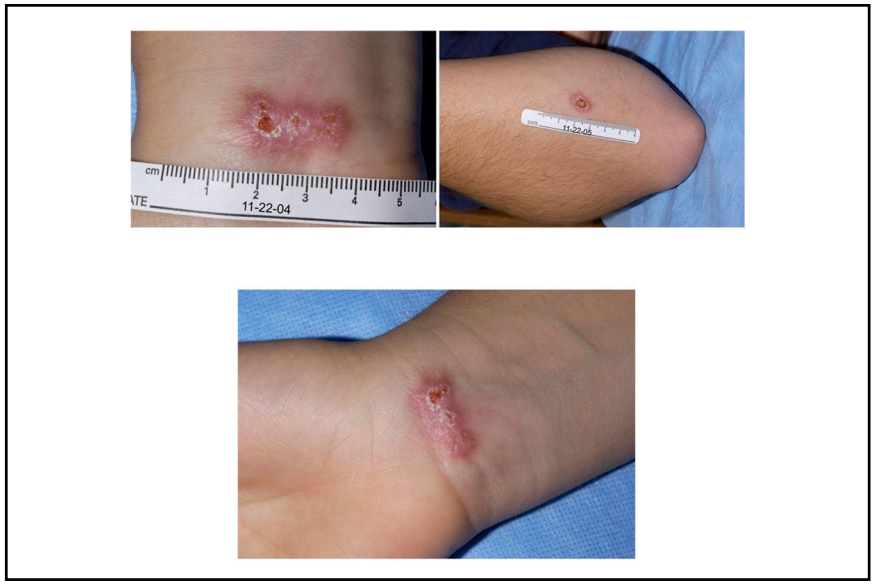
32



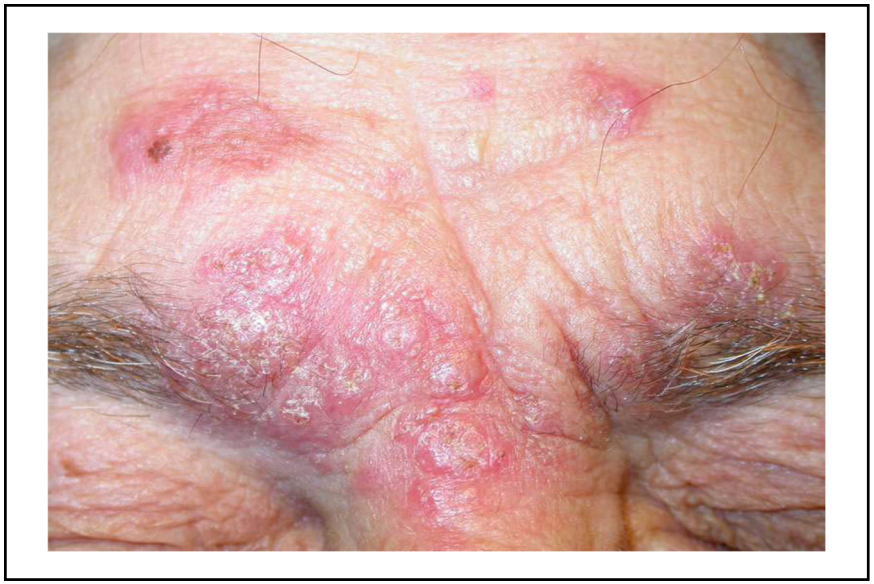
33



34



35



36

13 Protozoa That Could Be Tested

Speaker: Edward Mitre, MD

Cutaneous Leishmaniasis: Diagnosis

Definitive diagnosis is very helpful because:

1. Allows you to rule out other possibilities
2. May help in deciding whether and how to treat

Diagnostic Tools:

Edge of ulcer skin: scraping, aspirate, punch

- Touch prep with examination under oil looking for amastigotes
- Culture on triple N media (may take weeks to grow)
  - (Nicolle's modification of Novy and MacNeal's medium – biphasic)
- Histology
- PCR

37

Cutaneous Leishmaniasis: Treatment Recommendations

→ **Treat systemically if *L. (V.) braziliensis, guyanensis, panamensis***

→ **If not, ok to observe if there are:**

- **Few lesions, they are < 5 cm, not on face/fingers/toes/genitals, normal host, no subcutaneous nodules**

Treatment Options

- **Local: Heat with radiotherapy (FDA approved), cryotherapy, intralesional therapy systemic**
- **Oral: Miltefosine for certain species, especially New World CL species**
  Ketoconazole, fluconazole (off-label)
- **IV: Liposomal amphotericin B (off-label)**
  pentavalent antimony *(meglumine antimoniate, ASTMH website has instructions for obtaining on IND from Sanofi)*

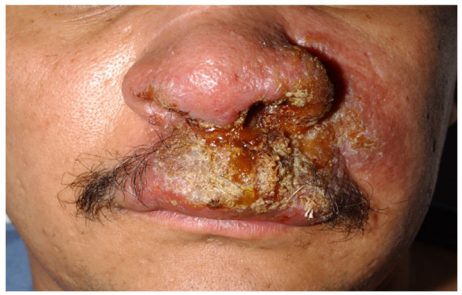
*****2016 IDSA GUIDELINES FOR TREATMENT OF LEISHMANIA*****
http://www.idsociety.org/Guidelines/Patient_Care/IDSA_Practice_Guidelines/Infections_by_Organism/Parasites/Leishmaniasis/

38

Mucosal Leishmaniasis

Leishmania (Viannia) braziliensis, guyanensis, panemensis

- Dissemination to nasal mucosa
- Slow, progressive, destructive
- Can occur months or years after cutaneous ulcer



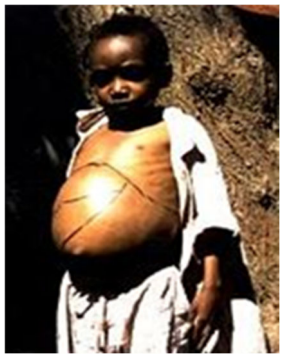
Treatment:
- Oral miltefosine (FDA approved for *L. braziliensis*)
- IV lip. amphotericin (off-label)
- IV antimony (no longer commercially available)

Miltefosine notes
Side effects: nausea, vomiting, diarrhea, increased AST/ALT
Contraindicated in pregnancy, use contraception for 5 months after treatment ($t_{1/2}$ = 30 d)

39

Visceral Leishmaniasis

- ***L. donovani*** *(South Asia, East Africa)*
- ***L. infantum chagasi*** *(Middle East, Central Asia, Mediterranean, Central and S. America)*
- Amastigotes in macrophages go to local LNs then hematogenously to liver, spleen, bone marrow



A persistent disease that can reactivate
TNF blockade, HIV CD4 < 200

- Wks/months of fevers, chills, hepatosplenomegaly
- Pancytopenia & hypergammaglobulinemia

Diagnosis: PCR, culture, or histopathology for intracellular amastigotes
→ bone marrow aspirate preferred, can also check LN, spleen, or buffy coat
Antibody to rK39 recombinant Ag (dipstick test)

Treatment: Liposomal ampho B (FDA approved)
Miltefosine (oral) FDA approved for *L. donovani*
(Combination treatment for L. donovani in people living with HIV in SE Asia)

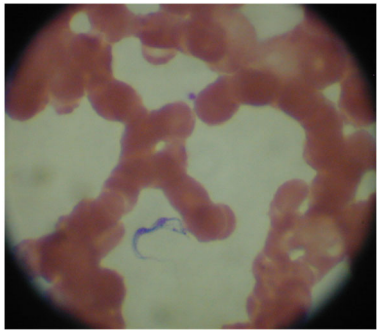
40

Question #4

A 41-year-old woman presented to a local emergency department with a one-day history of fever associated with swelling and redness in her groin four days after returning from safari in Tanzania. Peripheral blood smear is obtained.

What is the most likely diagnosis?

A. Leishmania donovani
B. Plasmodium vivax
C. Trypanosoma brucei
D. Wuchereria bancrofti
E. Leptospira interrogans



41

African Trypanosomiasis

(Sleeping Sickness)

Vector = Tsetse fly (Glossina sp)

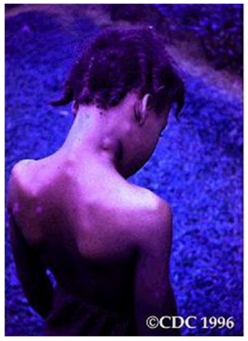
Trypanosoma brucei gambiense (W. Africa)
- Humans as reservoirs
- Progression over many months

Trypanosoma brucei rhodesiense (E. Africa)
- Cattle and game park animals as reservoirs
- Progression over weeks

Disease
Within 5 days: Chancre at Tse Tse fly bite
Regional lymphadenopathy

For weeks: Fever, hepatosplenomegaly, lymphadenopathy, faint rash, headache

Late: Mental status changes, terminal somnolent state



W.H.O.

42

African Trypanosomiasis: Lab Findings

Non-specific Lab Findings
- Anemia
- Thrombocytopenia
- Elevated IgM
- Hypergammaglobulinemia

Diagnostic Lab Findings
- Detection of parasite in lymph node, circulating blood, or CSF
  - Do FNA of lymph node while massaging node, then push out the aspirate onto a slide and immediately inspect under 400x power. Trypanosomes can be seen moving for 15-20minutes, usually at edge of the coverslip
- A card agglutination test that detects T.b. gambiense sp. Antibodies
  - V. sensitive (94-98%), but poor specificity
  - Can get false +s in pts with Schisto, filaria, toxo, malaria

43

African Trypanosomiasis: Life Cycle

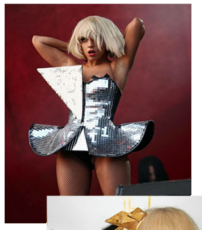
Question

Why are Trypanosoma brucei infections associated with persistently elevated IgM levels?

- Because they keep changing their outer surface protein
  - T. brucei contains as many as 1000 genes encoding different VSGs (VSG = variant surface glycoprotein)
  - Each trypanosome expresses one, and only one, VSG at a time
  - Individual parasites can spontaneously switch the VSG they express

44

African Trypanosomiasis: The Lady Gaga of the Microbial World





45

African Trypanosomiasis: Treatment

July 16, 2021: Oral fexinidazole FDA approved for T. gambiense

West African (T. gambiense)

If < 6 yo or < 20 kg: lumbar puncture
- CSF < 5 WBC/ul → iv pentamidine
- CSF > 5 WBC/ul → iv eflornithine + nifurtimox

If > 6 yo and > 20kg: confusion, ataxia, anxiety, abnl speech, motor weakness, abnl gait?
- No suspicion of late disease → oral fexinidazole
- If suspicion of CNS disease →obtain lumbar puncture
  - CSF < 100 cells/ul (non-severe 2nd stage) →oral fexinidazole
  - CSF > 100 cells/ul → iv eflornithine+ nifurtimox

East African (T. rhodesiense)

If < 6 yo or < 20 kg: lumbar puncture
- CSF < 5 WBC/ul → suramin
- CSF > 5 WBC/ul → melarsoprol

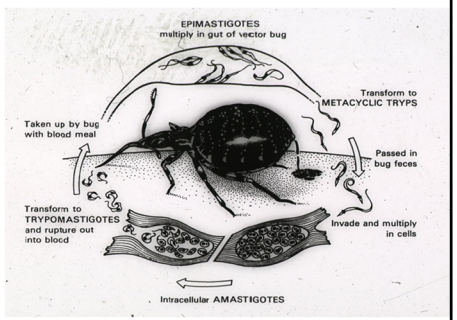
If > 6 yo and > 20kg → oral fexinidazole (IV melarsoprol or suramin depending on CSF counts if unable to take po)

Notes: 1. Melarsoprol associated with ~5% death rate due to reactive encephalopathy
2. This is reduced by co-administration of corticosteroids

46

Chagas Disease

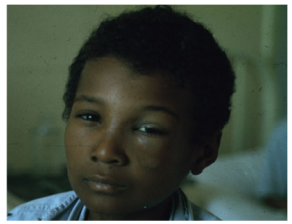
- Caused by *Trypanosoma cruzi* (Also blood transfusion and congenitally)
- Vector: Reduviid (triatomine) bugs
- Reservoirs: Opossums, rats, armadillos, raccoons, dogs, cats
- Autochthonus cases in the U.S.:
  - Texas
  - Louisiana
  - Mississippi
  - Missouri
  - California



- Oral ingestion of food and drinks contaminated with reduviid bugs or the feces of those bugs is a major route of infection (acai and sugar cane juice)

47

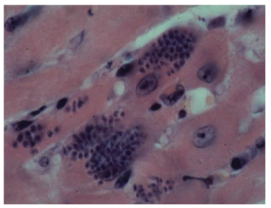
Chagas: Clinical Disease

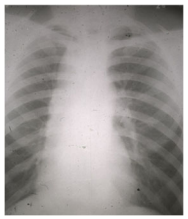


Acute (starts 1 week after infection, can persist for 8 weeks)
- Fever
- Local lymphadenopathy
- Unilateral, painless periorbital edema

Indeterminate stage
- Serology positive, no evidence of disease

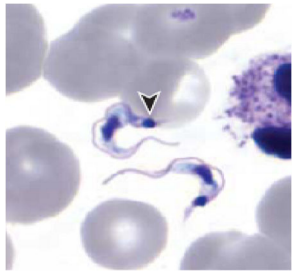
Chronic


Dilated cardiomyopathy, R>L (CHF, syncope, arrythmia)


Megaesophagus

48

Chagas: Diagnosis & Rx



Acute disease
- Identification of parasites in blood

Chronic disease
- *T. cruzi* specific IgG antibodies in serum

→ **Two antibody tests using different antigens and different techniques recommended for dx**

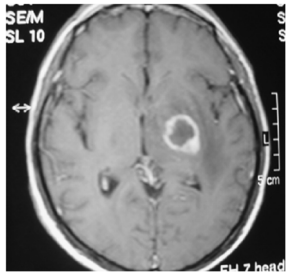
(research: xenodiagnosis, hemoculture, PCR)

NOTE: U.S. blood supply screened for 1st time donors

Treatment
- Benznidazole for 30 – 60 d, alternative: Nifurtimox (both FDA approved)
- **Benznidazole AEs:** peripheral neuropathy, granulocytopenia, rash
- **Nifurtimox AEs**: abdominal pain/vomiting, tremors, peripheral neuropathy
- **Always offer:** acute infection, congenital, < 18 yo, reactivation disease
- **Usually offer:** 19-50 years old and no advanced cardiac disease
- **Individual decision:** > 50 years old and no advanced cardiac disease

49

Chagas in Immunosuppressed Patients



2008 Int J Infectious Diseases

T. cruzi and AIDS

Primarily reactivation neurologic disease
- Acute, diffuse, necrotic meningoencephalitis
- Focal CNS lesions (similar to Toxo)**

T. cruzi and solid organ transplant
- Recipient of infected organ: fevers, hepatosplenomegaly, myocarditis
- Disease often does not occur until months after transplant

ALSO…reactivation myocarditis occurs in ~40% of patients that receive heart transplant because of Chagas cardiomyopathy

50

Protozoa

Protozoa - Extraintestinal

Apicomplexa
- Plasmodium
- (Babesia)
- (Toxoplasma)

Flagellates
- Leishmania
- Trypanosomes
- (Trichomonas)

Amoebae
- Naegleria
- Acanthamoeba
- Balamuthia

Protozoa - Intestinal

Apicomplexa
- Cryptosporidium
- Cyclospora
- Cystoisospora

Flagellates
- Giardia
- Dientamoeba

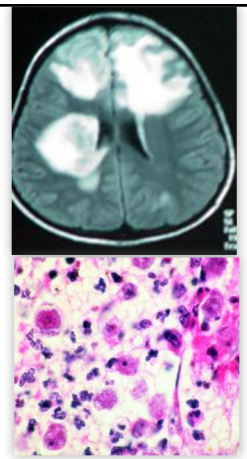
Amoebae
- Entamoeba

Ciliates
- Balantidium

Maybe Not Protozoa **Fungi Kingdom:** Microsporidiosis agents
SAR supergroup: Blastocystis

51

Free-living Amoebae



Naegleria fowleri
- Warm freshwater exposure
- Enters through olfactory neuroepithelium
- Fulminant meningoencephalitis
- Immunocompetent children/young adults

Acanthamoeba
- Found in soil and water
- Enter through lower respiratory tract or broken skin
- Subacute granulomatous encephalitis
- Immunocompromised hosts
- Chronic granulomatous keratitis (contact lens, LASIK)

Balamuthia mandrillaris
- Likely enters through lower respiratory tract or broken skin
- Transmission by solid organ transplantion has been reported
- Subacute granulomatous encephalitis
- Normal and immunocompromised hosts

Outcome → often fatal (amphotericin B, azoles, pentamidine, others tried)

52

Protozoa

Protozoa - Extraintestinal

Apicomplexa
- Plasmodium
- (Babesia)
- (Toxoplasma)

Flagellates
- Leishmania
- Trypanosomes
- (Trichomonas)

Amoebae
- Naegleria
- Acanthamoeba
- Balamuthia

Protozoa - Intestinal

Apicomplexa
- Cryptosporidium
- Cyclospora
- Cystoisospora

Flagellates
- Giardia
- Dientamoeba

Amoebae
- Entamoeba

Ciliates
- Balantidium

Maybe Not Protozoa **Fungi Kingdom:** Microsporidiosis agents
SAR supergroup: Blastocystis

53

When to Suspect an Intestinal Protozoan Infection

Patient has: Protracted watery diarrhea (weeks to months)

AND/OR:
- History of travel [domestic (esp. camping) or foreign]
- Recreational water activities
- Altered immunity (HIV infection)
- Exposure to group care (daycare)

Note: Discussion will focus on intestinal protozoa as they occur in patients seen in the U.S. These are leading causes of diarrhea, morbidity, and mortality worldwide, especially in young children.

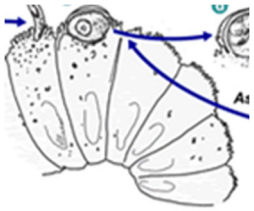
54

Intestinal Apicomplexa Parasites

Cryptosporidium
- *C. parvum:* Cows
- *C. hominis:* Humans

Cyclospora cayetanensis

Cystoisospora belli



Cryptosporidium in enterocyte. CDC DpDx

- All have worldwide distribution
- All transmitted by water or food contaminated with oocysts
- Organisms invade enterocytes
- All cause watery diarrhea that can be prolonged & severe in immunocompromised

55

Intestinal Apicomplexa: Clinical Clues

Cryptosporidium
- Watery diarrhea of several weeks
- Cattle workers and daycare outbreaks
- Cysts are resistant to chlorine (water supply outbreaks)
  - ➢ #1 cause of water park/swimming pool outbreaks

Cyclospora cayetanensis
Self-limited immunocompetent BUT can last up to 10 weeks!

- Abrupt onset with nausea, vomiting, and fever early
- Anorexia, weight loss, fatigue late in course
- Food associated outbreaks: raspberries, lettuce, herbs
- Esp. Nepal, Peru, Guatemala

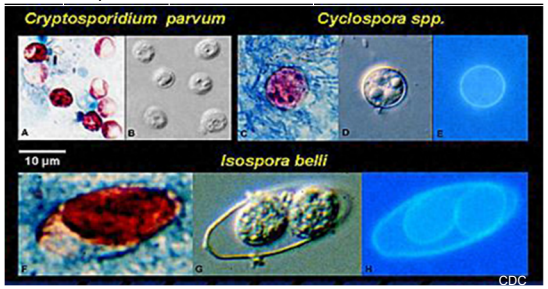
Cystoisospora belli
- No animal reservoirs known
- Watery diarrhea
- May be associated with a peripheral eosinophlia!
  (the ONLY intestinal protozoa that does this)

56

13 Protozoa That Could Be Tested
Speaker: Edward Mitre, MD

Intestinal Apicomplexa Characteristics

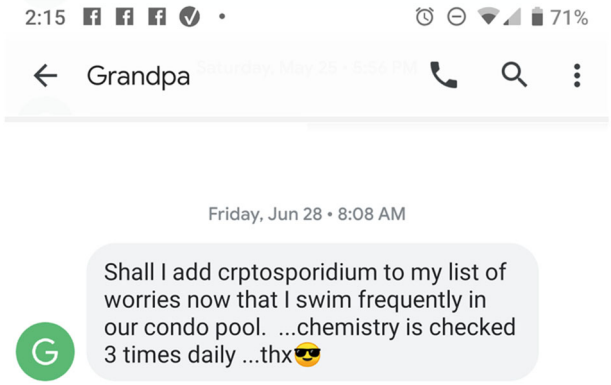
Pathogen	Size	Stain	Treatment
Cryptosporidium	4 µm	m acid-fast	(None) Nitazoxanide or Paromomycin
Cyclospora	10 µm	m acid-fast	TMP/SMX
Cystoisospora	20 µm	m acid-fast	TMP/SMX



Molecular tests

- ***Most stool multiplex PCR assays detect cryptosporidium AND Cyclospora but NOT Cystoisospora***
- *Stool Ag tests commercially available for cryptosporidium*

57



58

Morbidity and Mortality Weekly Report

Cryptosporidiosis Outbreaks — United States, 2009–2017

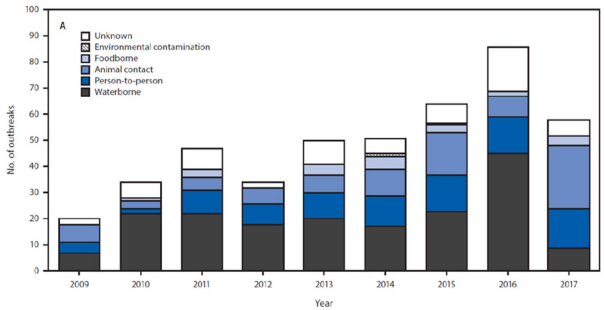
MMWR / June 28, 2019 / Vol. 68 / No. 25

59

Morbidity and Mortality Weekly Report

Cryptosporidiosis Outbreaks — United States, 2009–2017

MMWR / June 28, 2019 / Vol. 68 / No. 25



"The number of reported outbreaks has increased an average of approximately 13% per year."

60

Question #5

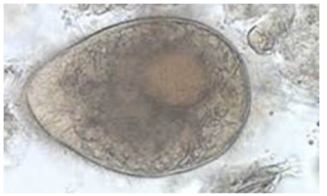
A 28-year-old woman returns after studying mosquito breeding habits in Honduras for one year. She reports intermittent abdominal pain and diarrhea for several months. Stool ova and parasite exam is positive for the presence of a ciliated single cell organism.

What is the most likely diagnosis?

A. *Balantidium coli*
B. *Entamoeba histolytica*
C. *Giardia lamblia*
D. *Dientamoeba fragilis*
E. *Endolimax nana*

61

Balantidium coli



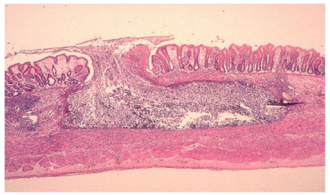
CDC DpDx

- The only ciliated pathogen of humans!
- Largest protozoan pathogen of humans! (about 70 µm wide and up to 200 µm long)
- Found worldwide, especially Central and S. America, S.E. Asia, and Papua New Guinea
- Associated with eating food/water contaminated with pig feces
- Symptoms: Most people asymptomatic
  Can cause colitis with abdominal pain, weight loss, +/- diarrhea
  (Especially in malnourished and immunocompromised)
- Treatment: Tetracycline (!) or metronidazole

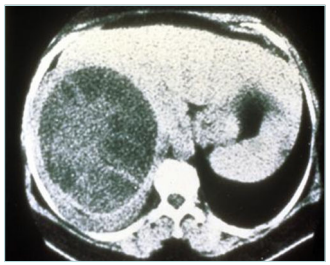
62

Entamoeba histolytica

- Strictly human pathogen
- Fecal/oral (contaminated food/water)
- Cysts = infective stage
- Trophozoites = active form, tissue-destructive

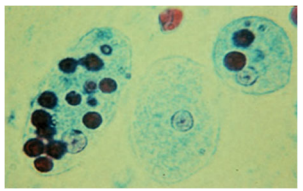


Clinical Presentations
- Asymptomatic
- Traveler's diarrhea
- Colitis
  Sharp abdominal pain
  Bloody diarrhea
  Fever
  Flask-shaped ulcerations
  ➢Onset can occur weeks to months after travel
- Ameboma
- Liver and brain abscesses, esp. in young men, usually 2-5 months after travel



63

Entamoeba histolytica



E. histolytica trophozoites with ingested RBCs.

Diagnosis

Stool PCR (multiplex or single)
- **Close to 100% sensitivity and specificity**

Stool O/P
- Only 50% sensitive for colitis and abscess
- Poor specificity b/c unable to differentiate *E.histolytica* from non-pathogenic *E. dispar* and the diarrhea-only causing *E. moshkovskii*
  (note: ingested RBCs suggestive of Eh, but not 100%)

Stool antigen testing > 85% sensitive for intestinal disease

Serology 95% sensitive for liver abscess, 85% sensitive for intestinal infection

Treatment

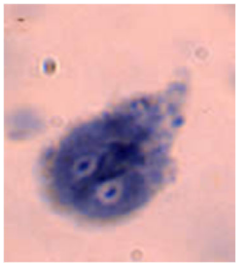
Asymptomatic: Luminal agents such as paromomycin
Symptomatic: Tissue agents such as metronidazole or tinidazole THEN luminal agent
Liver abscess: Medical therapy (tissue agent then luminal agent) usually sufficient!
Drainage if no response to medical therapy or dx unclear or v large abscess

64

Giardia duodenalis → described by Antony van Leeuwenhoek

Flagellated protozoan

- Fecal/oral via ingestion of cyst form in food/water
- Cyst is chlorine resistant
- Cysts from humans (beavers, muskrats)



Disease in U.S.

- Most common parasitic infection in the U.S
  (20k cases reported/year, likely 2M)
  → U.S-acquired cases peak in the late summer/early fall
  → A leading cause of traveler's diarrhea

Symptoms

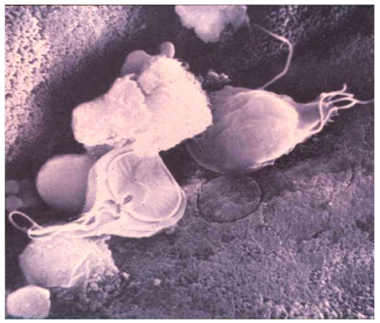
- Intermittent watery diarrhea weeks to months
- Foul smelling stools, flatulence, "sulfur burps"

65

Giardia

At risk populations

- International travelers
- Swimming in lakes/streams, outdoor survival/camping
- Infants in daycare
- Child-care workers
- Immunoglobulin deficiencies (esp CVID)
- HIV when CD4 < 100



Diagnosis

- Stool antigen test
- Stool multiplex PCR

Treatment

- Tinidazole (FDA approved)
- Metronidazole (off-label), nitazoxanide (FDA-approved), and albendazole (off label)

66

Other Intestinal Protozoa

Non-pathogens

Amoebae

Entamoeba dispar
Entamoeba hartmanni
Entamoeba coli
Endolimax nana
Iodamoeba bütschlii

Flagellates

Chilomastix mesnili
Trichomonas hominis

Treat if symptomatic: *Dientamoeba fragilis* (implicated in IBS)

67

Protozoa

Protozoa - Extraintestinal

Apicomplexa
Plasmodium
(Babesia)
(Toxoplasma)

Flagellates
Leishmania
Trypanosomes
(Trichomonas)

Amoebae
Naegleria
Acanthamoeba
Balamuthia

Protozoa - Intestinal

Apicomplexa
Cryptosporidium
Cyclospora
Cystoisospora

Flagellates
Giardia
Dientamoeba

Amoebae
Entamoeba

Ciliates
Balantidium

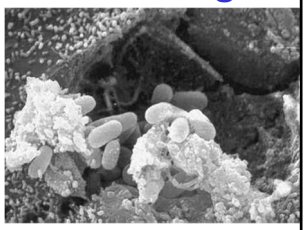
Maybe Not Protozoa

Fungi Kingdom: Microsporidiosis agents
SAR supergroup: Blastocystis

68

Microsporidia – Obligate Intracellular Fungi!

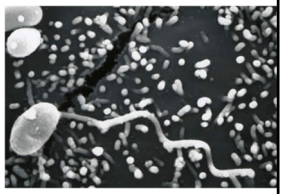
→ Produce extracellular, 1-2 micron, infective spores
→ Spores have a coiled organelle called a polar tubule
→ After ingestion, the spore germinates, and the polar tubule is used to inject sporoplasm into a host cell



Spores of *E. hellem* bursting out of a cell (CDC DpDx)

Enterocytozoon bieneusi
- Watery diarrhea
- Biliary disease (cholangitis, acalculous cholecystitis)

Encephalitozoon intestinalis
- Watery diarrhea
- Biliary disease
- Disseminated disease (liver, kidney, lung, sinuses)



Polar tubule inserted into a eukaryotic cell (CDC DpDx)

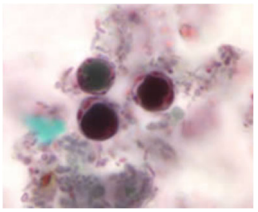
Encephalitozoon cuniculi, hellem
- Can cause disseminated disease of multiple organs, plus eye

Many species (including *Vittaforma corneae*)
- Punctate keratoconjunctivitis
  (contact lens use, after eye surgery, bathing in hot springs)

DIAGNOSIS: Modified trichrome stain, Calcofluor white, IFA to detect spores in stool
TREATMENT: Albendazole (not effective for *E. bieneusi*)

69

Blastocystis



Blastocystis cyst-like forms , trichrome (CDC DpDx)

What is it?
- Currently classified as a protozoa
- Forms are 5-40 microns wide
- Anaerobic
- Eukaryotic
  → Cystic, ameboid, granular, and vacuolar forms

Often the most common eukaryotic organism found in human stool samples

Does it cause disease?
- Maybe
- Associated with watery diarrhea, abdominal discomfort, nausea, and flatulence

Diagnosis
- Light microscopy of stool samples

Treatment
- Metronidazole, tinidazole, TMP/SMX, or nitazoxanide (none FDA-approved)

70

Protozoan Infections that can Reactivate in the Severely Immunocompromised

- Toxoplasmosis
  - Encephalitis with mass lesions
  - Pneumonitis
  - Retinitis
- Leishmania
  - Reactivation of visceral and cutaneous reported
  - Visceral with fever, hepatosplenomegaly, pancytopenia
- Chagas
  - Encephalitis with mass lesions
  - Hepatosplenomegaly and fevers
  - Myocarditis in 40% that receive heart transplant b/c Chagas disease
- Malaria

Some other protozoa that can case severe disease in immunocompromised
- Cryptosporidium
- Giardia
- Microsporidia
- Babesia
- Acanthamoeba

71

Good luck on the exam!

Edward Mitre, M.D.
edwardmitre@gmail.com

72