Introduction to Protozoa Kingdom
DNA data suggests actually several kingdoms.

Some Key, Relevant Features
Unicellular eukaryotes - so which domain?
No tissues/organs, but do show development
Metabolism: chemicals for energy
Most reproduce sexually or asexually
Major Phyla - Motility-Driven (sort of)
Old Flagellates: no organelles, maybe later - GI
New Flagellates: Leishmania, Trypanosoma
Amoebae: maybe later - GI
Ciliates: maybe later - GI
Sporozoa (non-motile): Plasmodium
New Flagellates

Genus 1 - Leishmania
About 30 genera, 20 pathogenic
Carried by tropical sandflies - on dogs, rats…
In insect: promastigote - flagella, extracellular
In human: amastigote - non-motile, intracellular
4 species, 3 distinct diseases or regions
Leishmaniasis
(Leishmania spp.)

Sandfly Stages

1. Sandfly takes a blood meal (injects promastigote stage into the skin)
2. Promastigotes are phagocytized by macrophages
3. Promastigotes transform into amastigotes inside macrophages
4. Amastigotes multiply in cells (including macrophages) of various tissues
5. Sandfly takes a blood meal (ingests macrophages infected with amastigotes)
6. Ingestion of parasitized cell
7. Amastigotes transform into promastigote stage in midgut
8. Divide in midgut and migrate to proboscis

Human Stages

i = Infective Stage
d = Diagnostic Stage
Leishmaniasis 1: Cutaneous
Insect bite injects agent just below skin surface
Infect white blood cells - lesions, scars, resolves
New World *mexicana* - Latin America
Old World *tropica* - Asia, Africa, Middle East
Leishmaniasis 2: Mucocutaneous
More invasive *braziliensis* - Latin America
Like cutaneous but deeper, melts nose, mouth
Secondary infections, fever, more scarring
Leishmaniasis 3: Visceral or Kala Azar
Most invasive *donovani* - tropics/subtropics
Skin to white blood cells - no symptoms for years
Then, fever, gray skin, hepatosplenomegaly
75-90% lethal if untreated; 30% lethal if treated
While multi-million dollar awareness campaigns educate the masses on the deadly effects of HIV/AIDS, another auto-immune disease is quietly spreading like wildfire: Kala-Azar, or the "black fever."

Photography:
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Leishmaniasis - Epidemiology, Treatment
350 million worldwide; 2 million new cases/year
Military problem - Iraq, Afghanistan, Columbia
Unique outer membrane molecule - LPG
Antimony drugs target LPG - toxic side effects
Genus 2: Trypanosoma
2 major species, insect vectors, diseases
In insect: epimastigote - small sail flagella,
In human: trypomastigote - full sail flagella
Some evidence of amastigotes - chronic cases
American Trypanosomiasis/Chagas

*T. cruzi* discovered 1909, taken seriously 1960s
Assassin/kissing bug (chinches) carries in feces
Animal and dwelling-associated - thatch roof…
Feces/agent enters bites on face - “el barbeiro”
Also via blood/organ donors, mother-infant

*Both Chagas, Cruzi = Brazilian epidemiologists in early 1900s - major public health heroes.*
Trypanosomiasis, American (Chagas disease)

**(Trypanosoma cruzi)**

**Triatomine Bug Stages**

1. Triatomine bug takes a blood meal (passes metacyclic trypanomastigotes in feces, trypanomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)

2. Metacyclic trypanomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.

3. Amastigotes multiply by binary fission in cells of infected tissues.

4. Intracellular amastigotes transform into trypanomastigotes, then burst out of the cell and enter the bloodstream.

5. Triatomine bug takes a blood meal (trypanomastigotes ingested)

6. Epimastigotes in midgut

7. Multiply in midgut

8. Metacyclic trypanomastigotes in hindgut

**Human Stages**

1. Infective Stage
2. Diagonal Stage
Adult *Rhodnius prolixus*, a kissing bug.
WHO/TDR/Stammers
Chagas - The Disease

Through bite, agent invades to blood slowly…
2 Stages - acute (months), chronic (10 years)

**Acute**: no symptoms, or skin/eye swelling…
fever, hepatosplenomegaly, myocarditis

**Chronic**: permanent damage to heart (30%)…
GI (15%), nervous system (<10%)

20% acute resolve on own - seldom young/old.
Chagas - Epidemiology, Treatment

Latin America: 16-18 million affected annually
Up to 50,000 die each year - fatal if untreated
10% rural population show heart defects
Nifurtimox reduces acute cases - NOT chronic
Target is DNA in dividing cells - BIG side effects
Most programs - homes, insecticides, education

US blood not screened, not notifiable, about
50,000 immigrants infected.
SE BUSCA
VIVA O MUERTA

RECOMPENSA
EVITAR LA ENFERMEDAD DE CHAGAS
¡Cúñdate de las CHINCHES y así evitarás tener la ENFERMEDAD DE CHAGAS!

Esta enfermedad es producida por un parásito llamado Tripanosoma cruzi y transmitida por unos insectos conocidos como "CHINCHES PICUDAS"

MANIFESTACIONES DE LA ENFERMEDAD

- Fiebre, escalofríos, malestar, agrandamiento del hígado y el bazo, y en la mayoría de los casos no se presentan síntomas
- Cuando el parásito penetra a través de los ojos, se inflaman los párpados durante un periodo de 4 a 6 semanas, que es lo conocido como Signo de Romaña
- 10-20 años después de que la persona se ha infectado, puede presentar dolor al corazón y provocar la muerte

¿COMO SE TRANSMITE?

- A través de las CHINCHES PICUDAS infectadas con el parásito
- Por transfusión de sangre infectada
- De las mujeres embarazadas infectadas al niño por nacer

¿DONDE SE ENCUENTRAN ESTAS CHINCHES?

- En techos de material vegetal
- En gallineras construidas con material vegetal
- En materiales de construcción acumulados
- En paredes de bahareque, de adobe agrietado o revueltas flojas
- Debajo o detrás de muebles
- En marcos de puertas o ventanas

MEDIDAS DE PREVENCIÓN

- Mantener la casa limpia y ordenada
- Cambiar los techos de material vegetal por tejados o tejas
- Cambiar pisos de tierra por ladrillo o cemento
- Evitar dormir con los animales domésticos dentro de la vivienda
- Evitar acumular desperdicios de materiales de construcción o madera
- Revocar o repeler las paredes de las viviendas
- Alejar las carrozas de la pared para que las chinchas no suban fácilmente
- Si encuentra chinchas, no las toque directamente con las manos
African Trypanosomiasis/Sleeping Sickness

*T. brucei* - 2 strains: gambiense, rhodesiense

Carried by Tse Tse fly/*Glossina* salivary glands

TBG - “riverine” flies, humans main host

TBR - savannah, antelopes main host

Also via blood/organ donors, mother-infant

*Although recognized and dealt with early (1910)
- serious surveillance lapses… re-emerging!!!*
African Sleeping Sickness (Trypanosoma brucei)
Sleeping Sickness, African (African trypanosomiasis)
(Trypanosoma brucei gambiense)
(Trypanosoma brucei rhodesiense)

Tsetse fly Stages

1. Tsetse fly takes a blood meal (injects metacyclic trypomastigotes)
   - Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.

2. Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.

3. Trypomastigotes in blood

4. Trypomastigotes in blood

5. Tsetse fly takes a blood meal (bloodstream trypomastigotes are ingested)
   - Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly’s midgut. Procyclic trypomastigotes multiply by binary fission.

6. Procyclic trypomastigotes leave the midgut and transform into epimastigotes.

7. Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.

8. Tsetse fly Stages

i = Infective Stage
d = Diagnostic Stage
Sleeping Sickness - The Disease
Following bite/lesion, depends on strain…
**TBG**: chronic, asymptomatic/fever 6-9 months
**TBR**: acute, asymptomatic 3-6 weeks
Major Symptoms - organ failure, crosses BBB…
Convulsions, sleeping, insomnia, speech loss
Sleeping Sickness - Epidemiology, Treatment

0.5 million infected/year - TBG/West; TBR/East
Up to 40,000 die/year, 20,000 acquire/year
Ongoing epidemics - Uganda, Congo Basin
5-10 tourists/20,000 acquire/year - most TBR
Suramin pre-brain; Arsobal post-brain symptoms

*Suramin targets unique protozoa metabolic reactions (safer); arsobal = toxic arsenic*
Sporozoa - Also Apicomplexa

Plasmodium
Several geographically distinct species
Carried by tropical Anopheles mosquito saliva
Extremely complex life cycle - will not cover all
In insect: various extracellular forms
In host: various intracellular forms - liver, blood
Malaria Transmission Cycle

1st Vector

Initial human host

Liver infection

Blood infection

2nd Vector

Next human host

In utero transmission

Plasmodium sporozoites
The plasmodium cycle involves:

**Mosquito Stages**
- **11. Oocyst**
- **12. Ruptured oocyst**
- **Release of sporozoites**

**Sporogonic Cycle**
- **10. Ookinetes**
- **Macrogametocyte**
- **Microgamete entering macrogamete**
- **Exflagellated microgametocyte**

**Human Liver Stages**
- **A. Liver cell**
- **Infected liver cell**

**Exo-erythrocytic Cycle**
- **B. Ruptured schizont**
- **Schizont**
- **Schizont**

**Human Blood Stages**
- **C. Mosquito takes a blood meal (injects sporozoites)**
- **D. Mosquito takes a blood meal (ingests gametocytes)**
- **E. Immature trophozoite (ring stage)**
- **F. Mature trophozoite**

**Erythrocytic Cycle**
- **G. Ruptured schizont**
- **Schizont**
- **Schizont**
- **Schizont**

**Plasmodium Species**
- **P. falciparum**
- **P. vivax**
- **P. ovale**
- **P. malariae**

**Infective Stage**

**Diagnostic Stage**
Malaria - The Disease

2 weeks following bite/lesion, most strains…
Infection sites = red blood cells (RBC), liver
Periodic Fever - 48-72 hours (strain-dependent)
Anemia - RBC killing, dark urine/blackwater fever
Circulation - vasodilation, pressure differences
Organ Complications - liver, kidney, spleen

While many resolve, others - chronic liver or fatal brain infections.
Malaria - Epidemiology

#5 Killer, 1-3 million die annually, 80-90% Africa

300-500 million cases annually worldwide

* vivax*: tropics and subtropics, most widespread

* falciparum*: only equatorial tropics

* ovale*: primarily Africa, some Asia
Malaria Endemic Countries, 2000

Note: This map shows countries with endemic malaria. In most of these countries, the malaria risk is limited to certain areas.
Malaria - Sickle Cell Disease Connection
Genetic disease that protects against malaria
Defective hemoglobin halts RBC infection
Each person get 2 gene copies - H/good, h/SC
SO - 3 Kinds of People: HH, hh, Hh (carriers)
In malaria areas, carriers (Hh) survive best
25% tropical race maintain sickle cell gene

Meanwhile, HH more susceptible to malaria, hh
die from full-blown sickle cell.
Normal hemoglobin:

- Primary structure: Val, His, Leu, Thr, Pro, Glu, Glu...
- Secondary structure: α subunit
- Tertiary structure: α and β chains
- Quaternary structure: Two α2 and two β2 chains
- Function: Molecules do not associate with one another; each carries oxygen.
- Red blood cell shape: Normal cells are full of individual hemoglobin molecules, each carrying oxygen.

Sickle-cell hemoglobin:

- Primary structure: Val, His, Leu, Thr, Pro, Val, Glu...
- Secondary structure: Exposed hydrophobic region
- Tertiary structure: β subunit
- Quaternary structure: Two α2 and two β2 chains
- Function: Molecules interact with one another to crystallize into a fiber; capacity to carry oxygen is greatly reduced.
- Red blood cell shape: Fibers of abnormal hemoglobin deform cell into sickle shape.
Distribution of malaria caused by *Plasmodium falciparum* (a protozoan)

Frequencies of the sickle-cell allele
- 0–2.5%
- 2.5–5.0%
- 5.0–7.5%
- 7.5–10.0%
- 10.0–12.5%
- >12.5%
Malaria - Treatment
Most drugs based on plant-derived compounds
S. American Cinchoa bark, Chinese Artemisia
Chloroquine - target rapidly dividing agent DNA
Artemisinin - break down agent membranes
Mounting resistance, not unique to malaria

Some drugs for treatment post-infection; others
for prophylaxis to avoid infection.
Cinchona officinalis

Quinine