Introduction to Parasitology

Robert W. Doms, M.D., Ph.D.
Chair, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia

Human Parasitic Diseases with Major Public Health Impact. Parasites remain major causes of morbidity and mortality. Significant parasitic diseases globally include:

Malaria - 300-500 million infections/yr, more than 1 million deaths per year
African trypanosomiasis (sleeping sickness) - 450,000 cases per year, 25 million total
Leishmaniasis - spread by the sandfly; 12 million total cases; 1.5 5o 2 million per year
Onchocerciasis (river blindness) - 18 million chronic infections
Shistosomiasis - 150 million infected; 20 million with severe symptoms
Toxoplasma - more than 1 billion infected
Various worms - more than 1 billion

Significant parasitic diseases in the U.S. that you may have heard of or will likely encounter include:
Cryptosporidiosis
Giardiasis
Toxoplasmosis
Trichomoniasis
Head lice
Entoamoeba histolytica

In general, parasites establish chronic infections that can last for years, even for life. Many if not most infections are asymptomatic. As a result, the number of infections are incredibly high. Note that to establish a chronic infection, a parasite must evade or

subvert the immune response - just like viruses or bacteria that establish chronic infections. Many mechanisms are used, including intracellular growth, varying surface antigens, camouflaging with 'host' molecules, producing proteins that interdict the immune response.

Covered in this lecture:
Malaria
Cryptosporidiosis
Giardiasis
Toxoplasmosis
Trichomoniasis
Entamoeba histolytica
Naegleria fowleri
Four examples of worms

Humans are typically not the normal hosts for parasites: we encounter the parasite in the environment or from a vector.

Parasites - a definition. A parasite is an organism that lives on or within another living organism, and obtains nourishment from it. Thus, all viruses are parasites, as are some bacteria and some fungi. Certain arthropods (biting insects) such as lice are also considered parasites, and further can serve as important vectors for other diseases. However, when we talk about parasites, we are generally referring to two types of eukaryotic organisms:

Protozoa - single cell organisms, such as amoeba and the flagellates. These tend to replicate in the host and have short life cycles. They can cause acute or chronic infections.
Helminths - worms, which are multicellular. These tend not to multiply in the host, but do lay eggs (tens of thousands per worm!) that are readily spread to new hosts. They have very long life cycles and cause chronic infections.

Parasite portals of entry. So, how do these things get into us? You should know the portal of entry for the 7 protozoa and 4 worms that will be covered in this lecture. The most common would be ingestion - intestinal parasites are among the most common, so ingestion via contaminated water is a common entry pathway. Some parasites, such as malaria, are spread by insect vectors (mostly mosquitoes, but also sandflies and other biting insects). T. gondii is known for causing congenital abnormalities as it can cross the placenta. Finally, some parasites directly penetrate our skin - this is a property of certain worms, with Schistosomes being a prime example (this results in swimmer's itch!).

Protozoa. Single-cell, eukaryotic organisms. They do not have cell walls, they do not photosynthesize. Most protozoa are motile, and they can move by various means:
Pseudopods - they basically stick out a foot, and drag themselves around
Cilia

Flagella

Protozoa typically reproduce by simple binary fission and obtain nutrients from the host by diffusion or pinocytosis. They very often produce cysts to survive under harsh conditions (especially outside of the host) and to aid in transmission, though some are spread by vectors and so do not need to do this.

Protozoa types.
The way in which protozoa move provides a way to classify them:
Amoeba. The example here is Entamoeba histolytica
Sporozoites (apicomplexan species). Malaria is the key example (Falciparum species)
Flagellates. Examples include Giardia, Trichomonas
Coccidia - these are non-motile. Cryptosporidium is a prime example.

The seven protozoa that will be covered - you should know something about their structure (flagellate, coccidia, etc), their epidemiology, how they are transmitted, clinical presentations, diagnosis and treatment.

GI Protozoa:
Entamoeba histolytica
Giardia lamblia
Cryptosporidium

Genitourinary protozoa:
Trichomonas vaginalis

Blood and Tissue Protozoa:
Toxoplasma gondii
Falciparum (malaria)
Naegleria fowleri (primary amoebic meningoencephalitis – the 'brain eating' amoeba)

Intestinal Protozoa - Entamoeba histolytica. This is the only amoeba that I will cover. When it is growing, it is termed a trophozoite, and it also makes a cyst that is responsible for dissemination. It is spread by the fecal-oral route after ingestion of a cyst (the cysts have multiple nuclei, which helps in diagnosis); low pH in the stomach activates the cyst, and trophozoites emerge. These attach to and invade the colonic epithelium - lysis of cells provides nutrients for the parasite. A very characteristic pattern associated with E. histolytic are **flask-shaped ulcers.** Both cysts and trophozoites are excreted, but the trophozoites die quickly - it is the cysts that spread the infection. A cyst is shown in the upper right corner of the slide.

Resected colon, E. histolytica infection. You are looking at the mucosal surface of the colon - the little bumps/craters are the lesions; the inset shows the flask-shaped ulcers in cross-section.

E. histolytica - epidemiology.

Found all over the world; infections associated with poor sanitation. In such areas, attack rates can be very high - 10% to 50% of the population. In the U.S., 1% to 2% may be infected, but this might be an over-estimate due to contamination with other, nonpathogenic Entamoebae species. Many patients are asymptomatic, and the infection is cleared - whether you get symptoms or not depends in part on the infective dose. Millions of cysts are shed in the stool per day.

E. histolytica - clinical

There are three possible outcomes - the most common is an asymptomatic carrier state, which can last for some time. At the population level, asymptomatic carriers clear their infection at the rate of about 3% a month, with a median of more than 15 months. The Second outcome is intestinal amebiasis, and this is related to destruction of colonic epithelial cells. Patients can have cramping, diarrhea (they are not absorbing water well), pain, and bloody stools. This needs to be treated. The third outcome includes extraintestinal manifestations, including fever and rigors. The liver is the primary target, as it is for many other GI pathogens that enter the portal circulation; this can lead to abscess formation in the liver. Immunosuppressed individuals are at greater risk for this.

E. histolytica - diagnosis and treatment.

• Detection of trophozoites and cysts (multiple nuclei) in stool
• Serology can be useful, but not in endemic areas since most people will be seropositive
• Metronidazole followed by iodoquinol. Metronidazole was covered in the ABx lectures - its mechanism of action is somewhat hazy, but it seems to result in fragmentation of DNA. It is the drug of choice for a number of protozoal infections.

Intestinal Protozoa - Giardia lamblia - a flagellate

• Flagellate with trophozoite and cyst forms, just like E. histolytica. Has a very distinctive appearance - sort of looks like a smiley face
• 10 – 25 cysts are infective dose
• Acid in stomach releases trophozoites from cyst
• Attaches to villi and divides in small intestine; interfere with absorption
• No ulcerations, unlike E. histolytica
• Very rarely spreads to other organs
• Cysts and trophozoites released – trophozoites non-infectious

Giardia lamblia - epidemiology and clinical

•World-wide distribution
• Lives in streams, lakes. In this country, typically associated with camping, when water is not purified properly - resistant to chlorine, so need to filter or boil water
• Giardia has animal reservoirs, and as it is water borne mammals such as muskrats and beaver are typical hosts.
• 50% symptomatic
• Chronic inflammation leads to villus blunting, leads to mild diarrhea to severe malabsorption
•Incubation period about 10 days

•Sudden onset, foul-smelling, watery diarrhea, cramps, lots of gas, steatorrhea (fatty stool)
• Blood/pus rare, consistent with non-ulceration - this is different from E. histolytica

Giardia lamblia - diagnosis and treatment
•Detection of trophozoites and cysts in stool
• Should test multiple stool samples - this is true for any GI parasite - it can be hard to detect cysts/parasites, and sometimes they are released in 'waves'
• Rapid antigen test available
• Don't drink stream water when camping, especially in areas with beaver – need to boil
• Metronidazole is the drug of choice, just like E. histolytica
- Don't keep beavers as pets!

Intestinal Protozoa - Cryptosporidium - a Coccidia (and so is non-motile)
• Fecal-oral transmission
• Ingest oocyst
• Carried by many farm animals, so is in the water table
• Oocyst incredibly stable, and small
• Identified only in 1976; on CDC emerging ID list
• Causes large water-borne outbreaks

Cryptosporidium parvum
Can cause really impressive outbreaks due to contaminated drinking water. Most famous case was in Milwaukee, where one of two water treatment plants became contaminated. About half of the population got water from this plant (about 800,000), and half of these came down with watery diarrhea. There were about 200 deaths, virtually all immunocompromised patients (oncology patients, status-post bone marrow transplant, AIDS). This led to the introduction of smaller filters (4 to 5 microns) in municipal water treatment plants. Other outbreaks have been associated with pools, water parks.

Cryptosporidium parvum
•Can be asymptomatic
• Usually mild, self-limiting enterocolitis – watery diarrhea without blood.
• Remission after a 10 day course
• More severe in the immunocompromised – massive fluid loss, lasts for months to years – life threatening
No specific treatment!!!

Urogenital protozoa - Trichomonas vaginalis - a flagellate – covered in detail in the STD lecture
Most common STD in the world!!!
• ONLY a trophozoite – no cysts
• Four flagella and undulating membrane
• Sexually transmitted
• Found in urethras and vagina of women; urethras and prostate glands of men

Trichomonas vaginalis - a flagellate

• Most common STD in the world – 180 million cases/yr worldwide; 7 million in the U.S.
• Prevalence in women in developed countries 5% - 20% in women, 2% - 10% in men
•Most infected women asymptomatic, or with scant, watery discharge
•Vaginitis upon occasion
•Men almost always asymptomatic
•Microscopic diagnosis; serologic test also available
•Metronidazole drug of choice

Blood and tissue protozoa: Toxoplasma gondii - a Coccidia (and so is non-motile)

• Intracellular organism, found in numerous animals
• Cats important source of infection for humans

This is why:

• Your mom told you to wash your hands after handling uncooked meat.
• Don't eat undercooked meat
• Pregnant women: don't clean the litterbox!

Toxoplasma gondii

• Cats excrete the cysts of the parasite in their stool and then almost any warm-blooded animal can become infected by ingestion or inhalation of the cysts
• By adulthood, 50% of the US population is infected with this organism
• People become infected by:
–Handling cat feces
–Eating or handling raw or undercooked meat
–Transplacentally, if mother's infection is her first, primary infection

Toxoplasma gondii

Cycle in Humans:

• Released from ingested cyst, engulfed by and macrophages and transported to all organ systems in the body, **especially CNS**
• Parasite grows and replicates, causes macrophages to rupture, leading to spread
• Immune response destroys many of the parasites or causes them to encyst
• Immune response is responsible for latency – cysts can persist for life
• Can be reactivated if immune response wanes (as in immunodeficiency).

Toxoplasma gondii - Disease

•Most infections benign/asymptomatic
•Can cause acute disease in normal host when parasite moves to tissues, associated with cell destruction.
–Sxs nonspecific: fevers, headaches, chills, fatigue
–sometimes mimics infectious mononucleosis: fever, sore throat, rash, and lymphocytosis for 2-3 days
•Disease in Immunocompromised Host
–this form is a serious, often fatal disease
–if it's acute disease: widespread dissemination to every organ
–if it's reactivation disease: **usually presents as encephalitis -- common in HIV/AIDS**

Toxoplasma gondii - Disease

•Congenital disease

–Acute maternal infection in 1st trimester leads to abortion or very severe disease

–If infection is after first trimester, can have numerous different outcomes:

- •Epilepsy
- •Microcephaly
- •Blindness
- •Mental retardation
- •Anemia
- •Jaundice

–Infant may be asymptomatic at birth, develop symptoms later – most often chorioretinitis with or without blindness and other neurologic symptoms

Toxoplasma gondii - diagnosis/treatment

•Laboratory Diagnosis:

1) Serology – look for IgM or rising antibody titers to detect acute infection - so many people infected (and thus antibody positive) that you are looking for changes in serology - just finding that someone is seropositive is not helpful. A rising titer shows active infection.

2) demonstrate trophozoite – not the cyst - in tissue

•Treatment

–No need to treat normal, acute infections

–Chronic or CNS infections: pyrimethamine plus sulfadiazine – both target folic acid pathway

Naegleria fowleri. I added this to the lecture this year, because there were two high-profile cases of this 'brain-eating amoeba' in the news. N. fowleri is a heat loving amoeba that inhabits fresh bodies of water, mostly in the southern US (also elsewhere in the world).

N. fowleri has 3 stages in its life cycle. The trophozoite form of the amoeba is the stage that is infective for humans. If you are exposed to contaminated water (typically swimming in a lake) and it enters your nose, N. fowleri penetrates the nasal tissue and migrates to the CNS via the olfactory nerve. Symptoms start several days after swimming, and include severe headache, fever, nausea and vomiting. Symptoms of meningitis (stiff neck) and encephalitis (seizures, altered mental status) ensue.

There are only a handful of cases each year. Over the past 30 years, there have been 130 documented cases, with 127 deaths. Of the two cases in the US this past summer, the little boy died, while the girl survived. She obtained an investigational breast cancer drug that the CDC now keeps a stock of. Whether she survived because of this is not known. You do NOT get this parasite from drinking contaminated water – it has to enter your nose. SO, when swimming in warm fresh water lakes, hold your nose closed!

Blood and tissue protozoa: Plasmodium species - cause malaria

One of the major infectious diseases in the world – 40% of the world's population lives in endemic areas; there are at least 300 million cases a year, 2 million deaths (now more than deaths from HIV). It is an increasing problem due to drug resistance and spread of mosquitos. Malaria is caused by Plasmodium, a protozoa, and is transmitted by Anopholes mosquitos.

Four species infect humans.
P. falciparum: this accounts for 95% of all deaths, about 65% of cases
P. vivax: this strain uses the Duffy antigen (a protein) on the surface of red blood cells to enter and replicate in these cells. People living in West Africa are frequently Duffy antigen-negative, making them resistant to P. vivax, but not other Plasmodium strains.
P. ovale
P. malariae

Step 1 of the life cycle: an infected mosquito bites a human; sporozoites are injected via the mosquito's saliva. Sporozoites enter capillaries and make their way to the liver, where they enter hepatocytes and begin to replicate. This takes between 1 and 2 weeks, and this phase is asymptomatic. Vaccines are being tested that target the sporozoite stage in an attempt to prevent them from infecting hepatocytes.

Step 2 of the life cycle: Each infected hepatocyte produces >10,000 merozoites that are released upon cell rupture. Merozoites invade red blood cells, replicate and produce more merozoites that then infect other RBCs.

Note: P. ovale and P. vivax have dormant liver stages – infected hepatocytes can be activated weeks to years after initial infection, resulting in relapsing malaria. P. falciparum does not do this.

Step 3 of the life cycle: Sometimes an infected RBC produces gametocytes (male and female). These are infectious for the mosquito. If a mosquito takes up both a male and female gametocyte from a blood meal, sexual reproduction occurs in the mosquito, resulting in the production of sporozoites that are ready to infect a new human host.

Erythrocytic Phase: Clinically, malaria is largely a disease of the blood. Upon entering a RBC, the parasite enters a trophic (growth) phase. If you do a blood smear, it is easy to see infected RBCs (this is how the diagnosis is often made). An early stage trophozoite is termed the 'ring stage', since the infected RBC looks like it has a ring inside of it. As the trophozoite grows and divides, it produces schizonts, which look like little blue dots – you get perhaps 40 of these per RBC. The RBC bursts, releasing the schizonts, which are then called merozoites, and these then infect RBCs (not liver cells).

Erythrocyte changes: Infected red cells become deformed; they develop knobs on their surface and are not flexible – these misshapen RBCs are recognized as malformed and degraded by the reticuloendothelial system (macrophages), particularly the spleen. Thus, splenomegaly is a common physical finding in patients with malaria. In addition, the RBCs become sticky and so adhere to the endothelium, forming clots with these leading

not just to RBC loss, but also to end-organ damage due to hypoperfusion. Finally, lysis of infected RBCs also results in RBC loss. If parasitemia is high (and it often is with primary infection with P. falciparum), then it is not surprising that patients often have some degree of anemia and jaundice.

Clinical features: Early symptoms are non specific and can be mistaken for a viral infection. But, with time, the classic symptoms appear: high fever, chills, rigors, and anemia. The release of parasites from RBCs can be relatively synchronous (especially with P. ovale and P. vivax). When this happen, the patient has spiking fevers followed by chills with regular periodicity (every 2 to 3 days depending on the strain). But, especially with P. Falciparum, the fever is simply irregular in nature, spiking at various times and alternating with chills. With P. falciparum, especially in children with no immunity, you can get cerebral malaria with convulsions, change in mental status, and death. If a patient survives, they can still get re-infected, but they usually have some degree of immunity and so their symptoms are ameliorated to a degree.

Treatment: Qunine was the first treatment, followed by derivatives such as chloroquine. However, in many areas of the world malarial parasites are resistant to these drugs, necessitating the use of others. Options include malarone, doxycycline, mefloquine, and primaquine. If you travel to a country that is endemic for malaria, check in with international medicine so that you can discuss taking appropriate prophylaxis.

Helminths

•Multicellular, eukaryotes, elongated and bilaterally symmetrical; typically macroscopic
• May have a protective cuticle or tegument
Three major types:
•Nemotodes: round worms; digestive system, male and female.

•Trematodes: flukes – leaf-shaped bodies, snails are intermediate hosts and other aquatic animals/plants are intermediate hosts. No digestive system. Hermaphrodites.

•Cestodes: tapeworms. Ribbons, segmented, no digestive system. Hermaphrodites.

Example of a nematode (fluke): Enterobius vermicularis - the pinworm

•Tiny worm, 10 mm in length
•Ingest or inhale ova
•Rarely causes serious disease
•Common pediatric infection
•Female crawls out and deposits eggs in perianal area – not stool!
•Anal itching (puritis), esp. at night
–may get 2^{o} bacterial infections 2° itching
•Scotch tape or swab anal region
•Treat entire family with albendazole or mebendazole

Example of a trematode (fluke) - Schistosoma mansoni

- All trematodes need two species to complete life cycle:
    - snail or mollusks
    - some other host
- Many species; S. mansoni most important; >150 M infected
- Obligate intravascular parasite in man
- Snail release free-swimming cercaria into the water column
- Attracted to sunlight; warmth
- Bind to and penetrate skin
- Attach with vessels in portal system
- Develop into worms; mate; produce eggs

Schistosoma mansoni

Clinical Symptoms:

Dermatitis at site of skin penetration

Swimmer's itch: penetration of skin by an Schistosoma sp. (not mansoni) that is not adapted for humans; dies immediately, but elicits an immune response

Flukes inhabit mesenteric vessels; their blockade can lead to hepatosplenomegaly

Fever, malaise, abdominal pain

Thickening of bowel wall due to granulomatous inflammation (chronic)

Ascitic fluid; portal vein can become fibrotic due to reaction to eggs

Treatment:

Praziquantel

Example of a BIG nematode: *Ascaris* – the giant roundworm.

Perhaps $1/6^{th}$ of all the people in the world are infected with this GI worm (fecal oral route – ingesting eggs). Grow to about 1.5 feet in length and lay 200K eggs per day, every day. Often asymptomatic, but a heavy parasite burden can lead to abdominal discomfort, distension and blockage. Usually, treatment clears the infection without complications.

Example of a cestode (tapeworm) - Taenia solium - the 'pork tapeworm'

Associated with eating under-cooked pork

We ingest the larval worm

The scolex (hooks) attaches to our intestines

Grows and lays eggs - if disseminates, can have eggs in any solid organ, leading to abscess formation. Abdominal pain and weight loss are typical.