

PARASITE PUZZLES

*Gut wrenching stories, Wisdom from the
Bench*

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November 17th, 2011

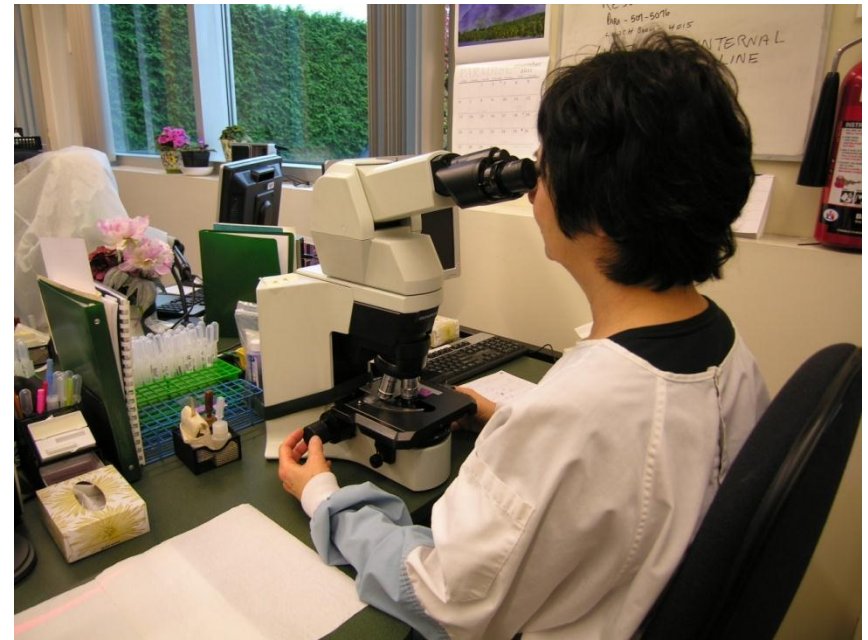
Some Basics

Routine Ova and Parasite
examination for intestinal parasites
(stool)

Complete Ova and Parasite examination for intestinal parasites

- Preserved stool specimen (SAF) - essential in an outpatient setting or whenever processing cannot occur immediately.
- Complete exam requires both a concentrated preparation and a permanent stained smear

- Microscopic examination of each slide takes about 10 minutes by an experienced technologist.
- More time needs to be available for:
 - difficult identifications
 - multiple parasites present
 - or if the technologist is inexperienced

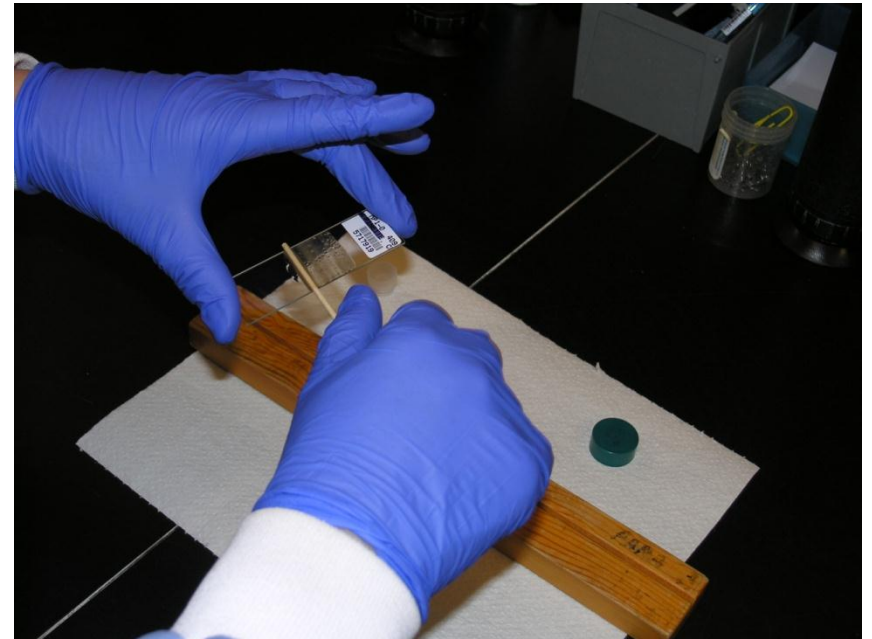


Permanent stained smear

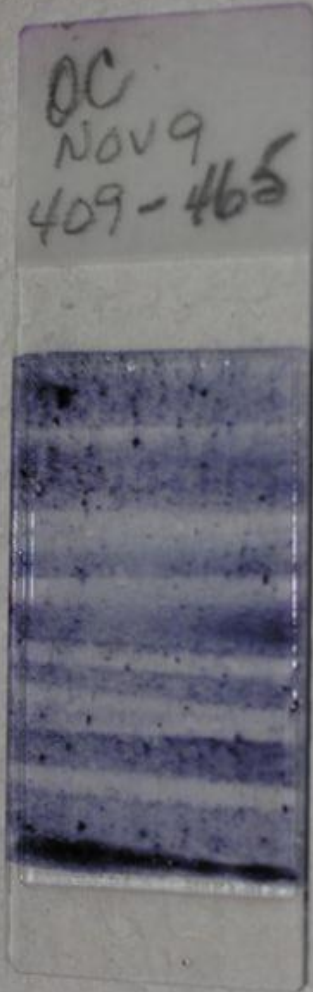
- Permanent stained smear allows identification of protozoan trophozoites, cysts, oocysts, and flagellates.
- We use the combination stain – Kinyoun's carbol fuchsin with iron haematoxylin which allows screening for acid-fast organisms at the same time as examination for other organisms.

Permanent Stained Smear

- Permanent stained smear is made after the stool has been “washed with saline and spun to remove formalin.



Stained Smear



- Rolling motion in application of stool to slide creates thick and thin bands.
- Rare organisms may show up in the thick bands
- Organisms are easier to identify in the thinner bands.

- Acid-fast staining confirms oocysts of coccidian parasites –
 - Cryptosporidium*
 - Cyclospora*
 - Cystoisospora belli* (*Isospora belli*)
- Morphology of trophozoites is clearer in the stained smear
- Staining may distort helminth ova

Concentrated stool

- The concentrated preparation is where helminth ova and larvae, and protozoan cysts and oocysts will be found.
- Concentration techniques separate the parasites from fecal debris by either centrifugation (sedimentation) or differences in specific gravity (flotation techniques)

Concentrates cont.

- Sedimentation techniques are commonly used.
- Washed stool specimen is concentrated with ethyl acetate to remove fecal fats and float off other debris making a cleaner preparation
- Final sediment is in 10% formalin



Concentrates cont.

- Trophozoites may be found but may be distorted or destroyed by ethyl acetate making them difficult to identify.
- Use of ethyl acetate may result in a loss of *Giardia* cysts in the concentrate.
- Identification of *Entamoeba histolytica/dispar* trophozoites requires a stained smear.
- Morphology of coccidian parasites is often better in the concentrate than the stained smear.

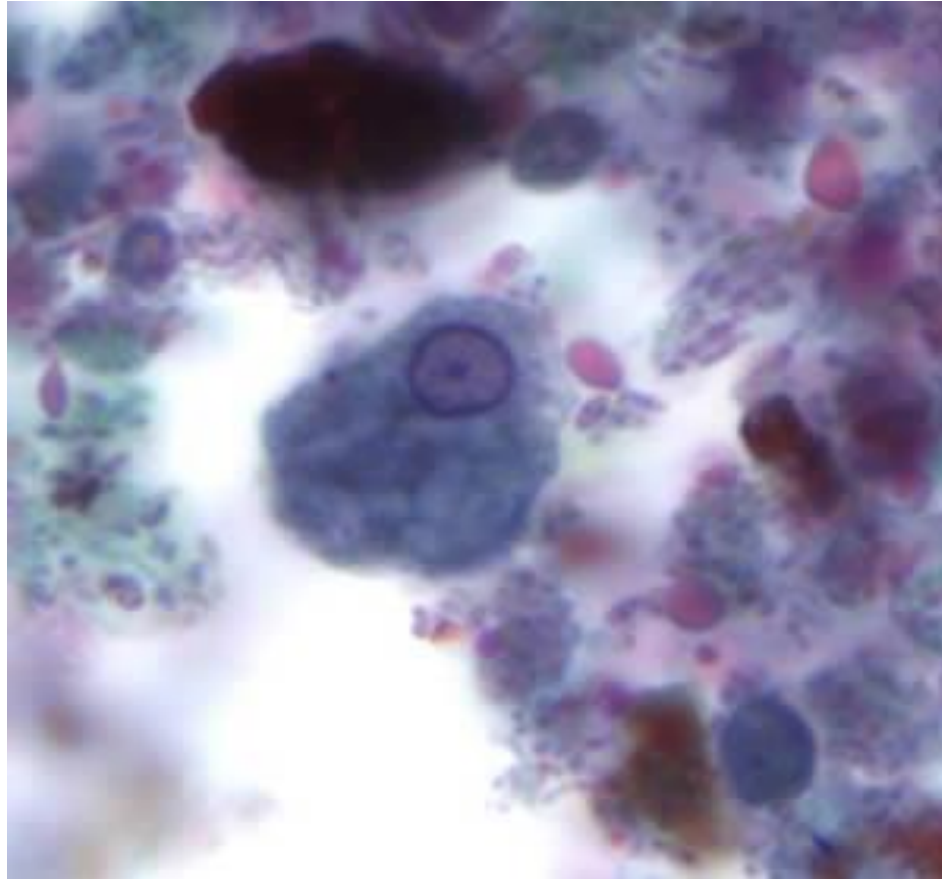
32 year old traveler

- 32 year old man
- Returned 1 week prior to presentation
- 3 month backpacking trip through Central America
- 2 weeks before return developed diarrhea,
- Increasing mucous, some blood
- Increasing lower abdominal pain
- No fever

Investigations

- Hgb 135
- WBC $7.4 \times 10^9/L$ Neuts $5.3 \times 10^9/L$
- Stool C & S
No *Salmonella*, *Shigella*, *Campylobacter*,
Yersinia, or
E.coli O1:57
- Stool O&P exam

Stool O & P



Amoebic protozoa

- Pathogen:

Entamoeba histolytica

- Non-pathogens:

Entamoeba coli

Entamoeba hartmanii

Entamoeba polecki

Iodamoeba butschlii

- Non-pathogens: [morphologically indential to *E. histolytica*

Entamoeba dispar

Entamoeba moshkovskii

Standard O&P method

- At least three specimens recommended.
- Must differentiate *E. histolytica/dispar* from *E. coli*, *E. hartmanni* and *E. polecki*.
- Trophozoites seen in a concentrate must be confirmed in the stained smear for the best differentiation of nuclear structures.

Stool – Ova and Parasite exam

E. histolytica/dispar

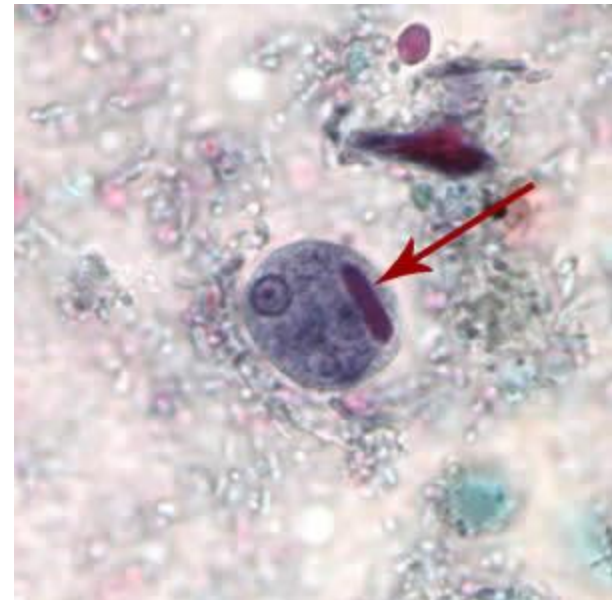
Cyst in concentrate:

- Rounded chromatoidal bars
- 4 nuclei in mature cyst



Cyst in stained smear:

- 10 to 20 microns in size (allow for shrinkage)

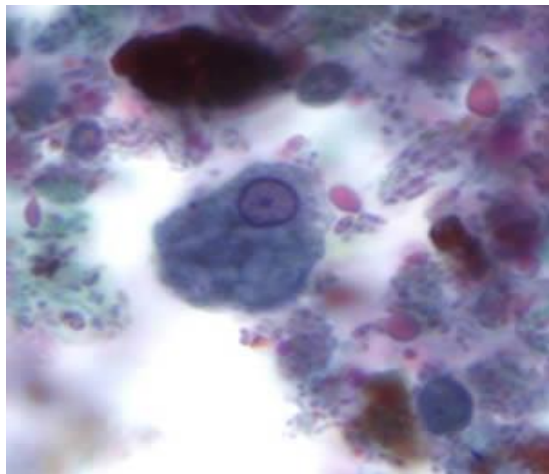


Laboratory identification cont.

E. histolytica/dispar

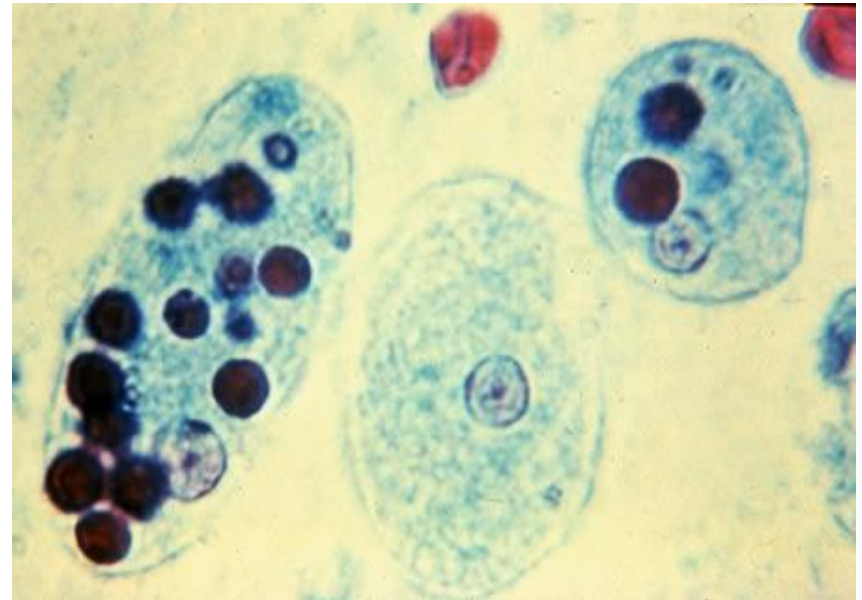
Trophozoites

- 12 to 60 microns
- Smooth peripheral chromatin and dense compact central karyosome



Trophozoites with ingested RBC's

- Diagnostic for *E. histolytica*



Size comparisons

- Size of trophozoites:

E. hartmanni 5-12 microns

E. histolytica/dispar 12 to 60 invasive forms >20

E. coli 15 to 50

- Size of cysts:

E.hartmanni 5-10 microns

E.histolytica/dispar 10-20

E. coli 10-35

- Shrinkage of the organism may occur on stained smear. Be sure to include the “halo” of unstained area around the item being measured.

Tips from the Bench

- Proper preservation is crucial for good staining.
- If numbers of organisms are quite low, make multiple stains for examination.
- Refer out if necessary to a number of reference labs. Good learning opportunity if you have having difficulties.

More on size – nuclei

- Trophozoites:
 - *E. histolytica/dispar* – 3 to 6 microns
 - *E. coli* – 4 to 7 microns
- (T. Scholten, Ontario ministry of Health)

Nuclear sizes – distinguishing between *E. hartmanni* and *E. histolytica/dispar*

- When the size of the trophozoite or cyst isn't helping with identification because it measures at the extreme of the size range, look at the nuclei
- *E. hartmanni* nuclei scale down in size
- ?“small race *E. histolytica*” distinct from *E. hartmanni* ?
- Source: Diagnosis of Intestinal Parasites Price, Donald L. CRC Press 1994

E. histolytica/ dispar

- Morphologically the same
- Techlab ELISA kit
- PCR

Immunodetection in stool specimens

- Specific and sensitive
- ELISA [TechLab]
- Differentiate between *E. histolytica* and *E. dispar*
- Need unpreserved stool
- Best results with fresh stool within 24 hours.
Stools maybe frozen, but freezing and thawing especially multiple times may result in a loss of sensitivity.
- Expensive

32 year old traveler

- Treated with metronidazole
- Followed by paromomycin
- Good recovery

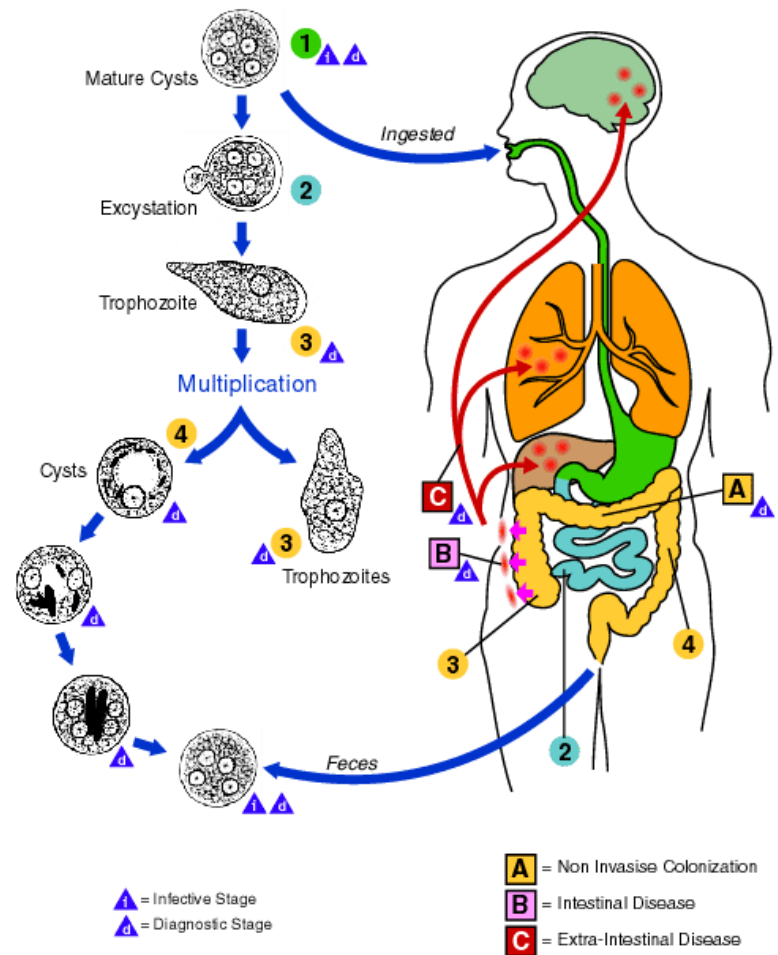
- Should his partner be tested?

32 year old traveler

How did he get infected ?

1. eating
2. drinking unsafe water
3. swimming
4. sexual contact

- Faecal excretion of cysts
- Oral ingestion of contaminated food or water
- Oral anal sexual contact
- Transmission rare in developed countries



Life cycle notes

- Trophozoites may remain in the intestinal lumen (non-invasive infection)
- In invasive disease, the trophozoites invade the intestinal mucosa, or through the bloodstream invade extraintestinal sites such as the liver, brain and lungs.
- Usual infective form is amoebic cysts
- Transmission through sexual contact (exposure to fecal matter) occurs.

.

Mr LA

- 42 year old man
- Immigrated 12 years ago from Columbia
- 3 wk history of fever, chills, abdominal and back pain
- diarrhea at beginning of illness.

Mr LA

- Recurrent travel to El Salvador for work, last there 1 week before onset of illness
- Lives with wife and son, no pets
- No drugs, no alcohol, no smoking

Mr LA

On admission

WBC 12.7×10^9 /L

afebrile

CT of abdomen showed solitary 9.5 cm thick rimmed non-enhancing cystic mass in right lobe of liver



Mr LA

What tests would be helpful in making a diagnosis?

1. imaging
2. smear and culture of abscess fluid
3. stool O&P
4. serology
5. PCR of abscess fluid

Mr LA

- Patient started on cipro and flagyl
- Drain placed into abscess, culture negative
- Blood cultures negative
- Stool for O&P sent
- Serology sent

Mr LA

- O&P negative
- Serology positive for *E. histolytica*

Serological methods

- Serology is more likely to be positive in extraintestinal disease such as liver abscess.
- Positive serology results together with the clinical picture make a diagnosis highly probable.

Liver abscess McGill

❑ Stat serology

Echinococcus negative

E. histolytica positive

❑ 10 ml of gray viscous material

❑ No amoebae were seen in the material aspirated from the edge

❑ *E. histolytica* PCR on this material was positive (the *E. dispar* PCR was negative)

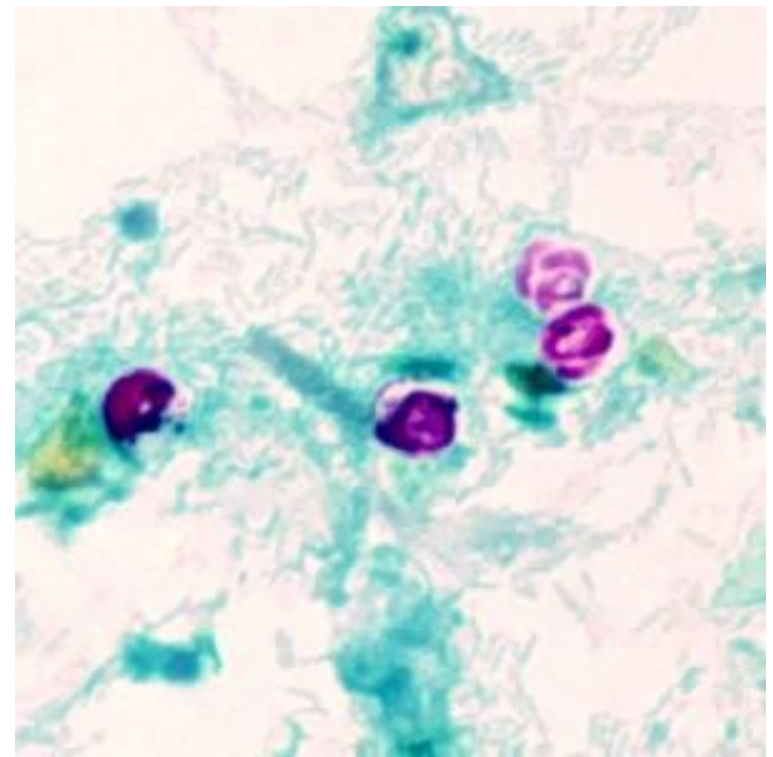
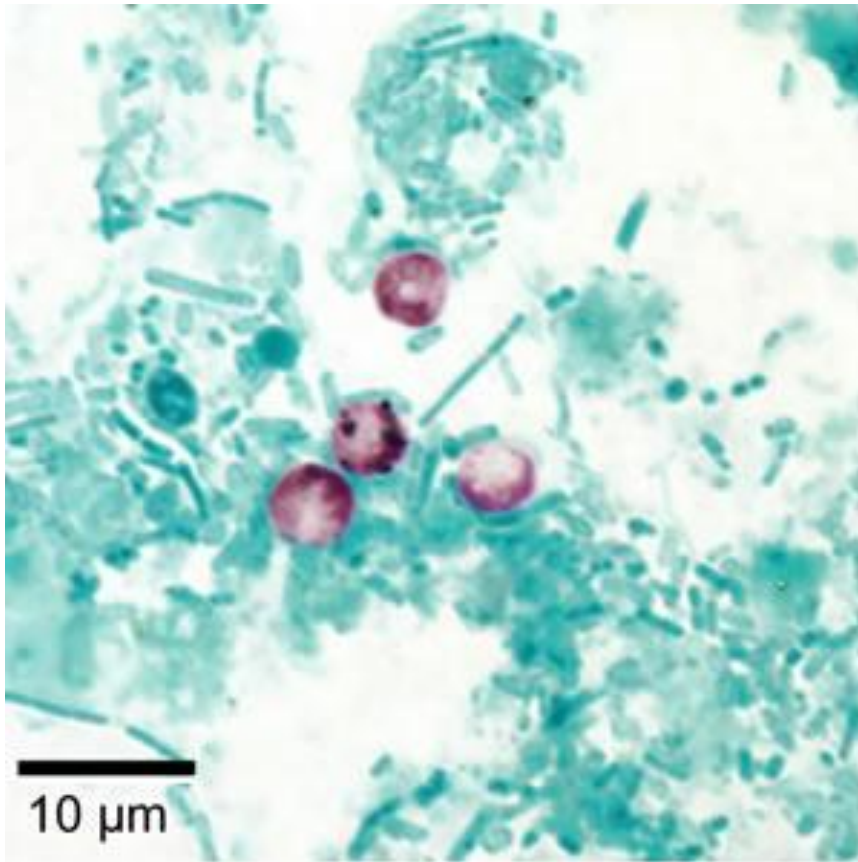
3 year old girl

- end of August
- 3 year old previously healthy girl
- 6 days of profuse watery diarrhea,
- no blood,
- some vomiting,
- fever 37.5 - 38⁰ C.
- In swimming lessons,
- Had been to a petting zoo.
- No travel

3 year old girl

- Stool C&S negative
- Verotoxin negative
- Stool O&P

Acid-fast stain



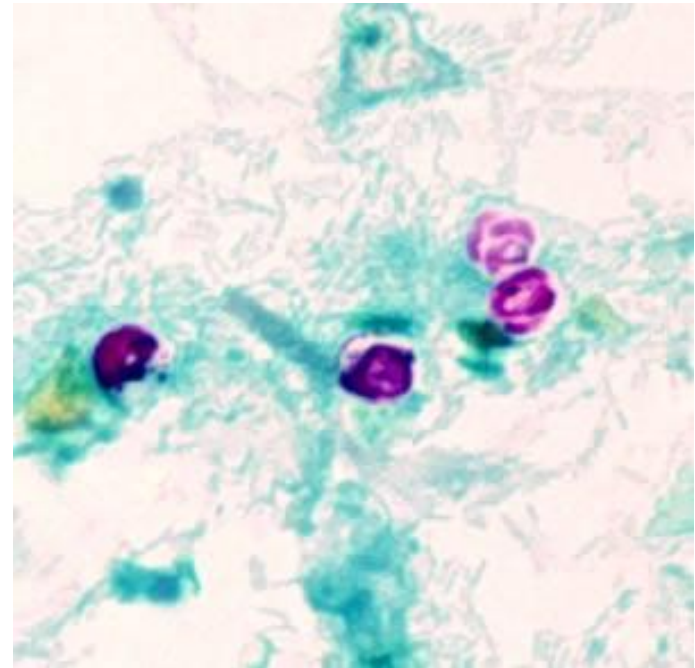
Morphology *Cryptosporidium* in concentrated preparation

- 4 to 6 microns
- “pac-man” shaped
- Does not stain with iodine
- Yeast similar in size but stain with iodine.
- Distinguish from *Cyclospora* oocysts which are 8 to 10 microns (about size of *E. hartmanni* cysts)



Morphology in stained smear

- Acid-fast
- Stain somewhat variably from pale to deep and “ghosts” or unstained oocysts are possible.
- Watch for acid-fast yeast – morphology is important
- Distinguish from Cyclospora – size, more variability in the staining.



CDC

Laboratory notes

- Characteristic morphology is easiest to see in the concentrated preparation.
- Numbers of organisms will be higher in concentrate than on stained smear.
- Staining the concentrate with acid-fast stains gives the best sensitivity.

Reporting

- Several morphologically identical species infecting people – most commonly *C. hominis* and *C. parvum*
- Report as *Cryptosporidium* spp.

3 year old girl

- 3 year old recovered after 10 days
- 5 year old brother now has mild diarrhea

Cryptosporidium spp.

5 problem characteristics

- low infectious dose: median 130 oocysts
- Small size: 3 to 6 microns: Can pass through standard filters
- Highly chlorine resistant
- Infectious at time of excretion
- *C. hominis* responsible for many outbreaks in Canada/USA
- *C. parvum* and other species: zoonotic

Cryptosporidium

4 settings

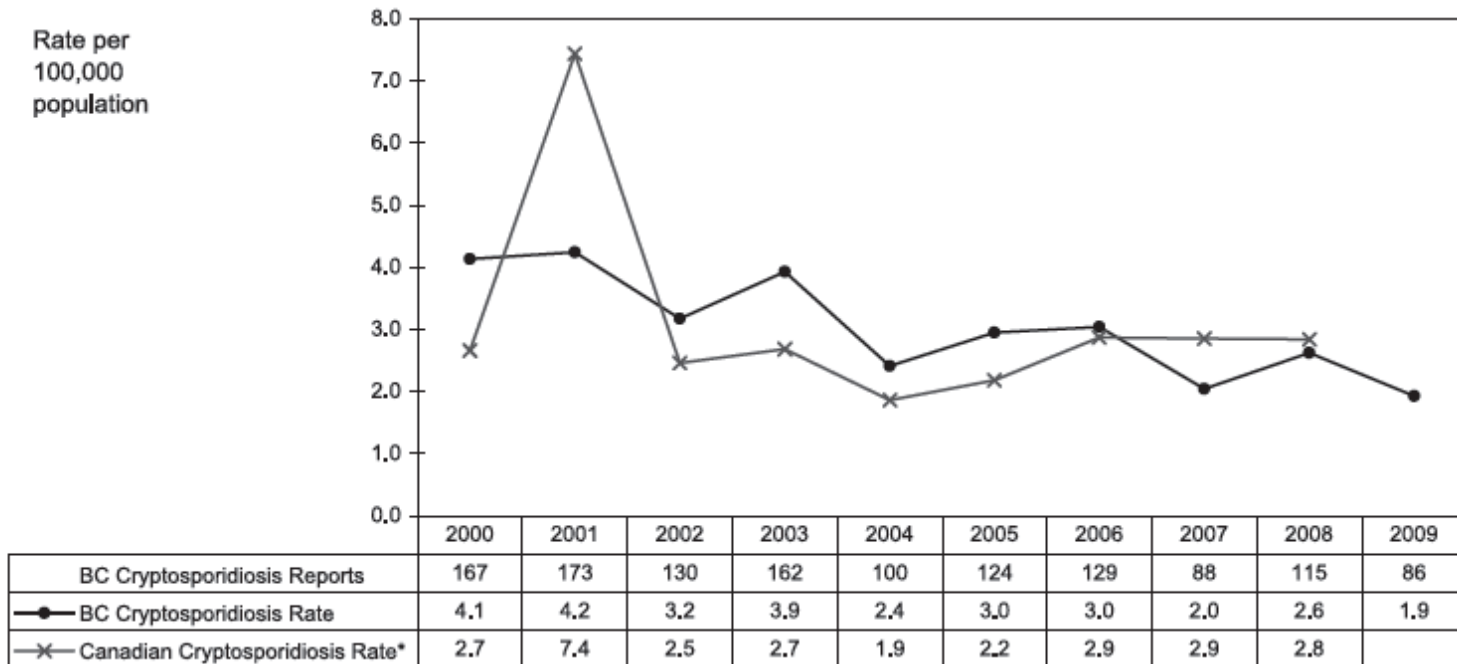
- Endemic childhood diarrhea in developing areas
- Traveler's diarrhea in visitors to endemic areas
- Waterborne disease outbreaks in developed countries
- Protracted diarrhea in immunocompromised

Extraintestinal cryptosporidiosis

Immunocompromised

- Disseminated infection may occur in immunocompromised patients
- Sputum specimens should be fixed and processed the same way stool specimens are.

22.1 Cryptosporidiosis Rates by Year, 2000-2009



Note: Cryptosporidiosis became nationally notifiable in January 2000

*Please see Sources and Explanatory Remarks, Section 11 on Page 123 in regard to the national rate

Decreased water borne parasitic disease

- Disinfection practices improved
- Monitoring of disinfection levels
- Automated warning systems
- Cross-connection control programs
- New water treatment plants
- Education of operators
- Quality assurance programs

-- Fall 2003, in Surrey BC

9 people in 2 months have *Cryptosporidium*

[Zero to 2 positive samples in those 2 months
in the previous 4 years]

-- All people positive have swum or have family members associated with same pool.

Worms

[Helminths]

- Nematodes: round worms
ascaris, trichuris, hookworm, strongyloides
- Cestodes: tapeworms
- Trematodes: flukes

Italian couple

- Journal of Travel Medicine 2011:18: 138-140

O&P specimen from husband [1 out of 5]
O&P specimen from wife -negative

340 x 30 microns



This worm is ?



- *Strongyloides stercoralis* larva
- hookworm larva
- spurious infection –not significant
- *Ascaris lumbricoides*

O&P Findings

- Diagnostic stage is rhabditoid (noninfective) *Strongyloides* larvae.
- Recovered from concentrated stool.
- The larvae are 180 to 380 microns long by 30-34 microns wide.
- “Short and sexy” - a short buccal cavity and a prominent sexual primordium

Strongyloides morphology

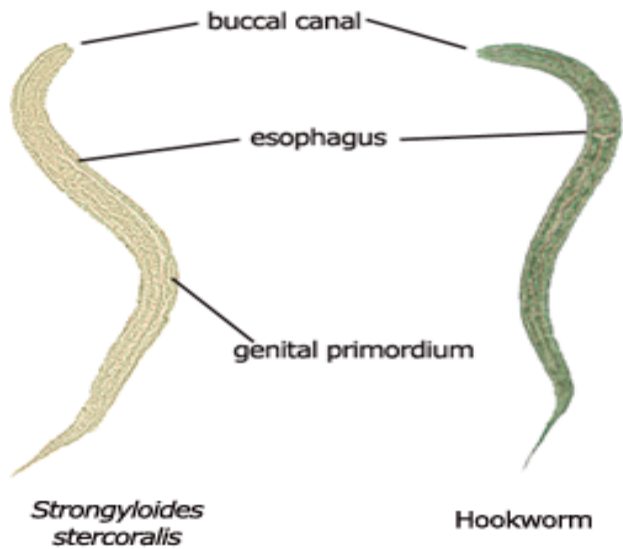
- Short buccal cavity, prominent sexual primordium



O&P Findings

- Ova may be recovered when there is severe diarrhea or from duodenal aspirates. They are very similar to hookworm ova (slightly smaller and rounder). This is extremely rare.
- It is important to be able to differentiate *Strongyloides* larvae from hookworm.

Rhabditiform larvae



- *Strongyloides* – short buccal cavity and prominent genital primordium (short and sexy). This is the most likely larvae you will find in stool O&P.
- Hookworm – long buccal cavity and indistinct genital primordium

Tips from the bench

- If strongyloidiasis is suspected, examine the entire concentrate. Often very low numbers of larvae present.
- Ensure the concentrate is not too thick – larvae can hide in the faecal debris
- Watch for artifacts – plant hairs (peach fuzz)
Blunt ends, lack of well defined esophagus and intestine will indicate an artifact.

Agar plate culture for larvae

- Handle very, very carefully if you try this – infective filariform larvae may be present
- Agar plate culture is considered the most sensitive of various culture techniques. Can be used for stool or sputum. Considered more sensitive than concentrated stool.
- Small amount of unfixed stool is placed in the centre of a plate of brain heart infusion/ meat extract agar and the plate is incubated at 37 degrees C.
- Observe daily for up to six consecutive days for tracts
- Larvae may be recovered from the plate using a wash of 10% formalin, spinning and examining the sediment.



Serology

- Enzyme immunoassay EIA has a sensitivity of 84 to 92%
- Cannot differentiate between past and present infection
- Cross reactions with filariasis and other nematode infections may occur.
- Useful to monitor treatment – antibody levels should decrease markedly within 6 months of successful treatment

How did this couple get infected?

1. eating sushi
2. swimming in infected water
3. walking barefoot
4. mosquito bite
5. sexually transmitted disease

Life cycle

- Parasitic and free-living cycles
- In the intestinal tract, ova hatch releasing rhabditiform larvae which are passed in the stool.
- In the soil, these larvae molt to become filariform larvae.
- Filariform larvae have two options 1) find a host and continue the parasitic cycle or 2) continue to develop and go the free-living route.

Parasitic Cycle

- Infective filariform larvae penetrate intact skin.
- Larvae enter the circulation and reach the intestines through a migration involving the lungs, and trachiobronchial tree where they are swallowed.
- Larvae may bypass this entirely and migrate to the intestine by any available route.
- Swallowed larvae reach the intestine, molt twice to become adult female worms

Autoinfective cycle

- Rhabditiform larvae may become infective filariform larvae within the lumen then penetrate the host through the intestinal mucosa or the skin of the perianal area.
- May result in a heavy worm burden which can be maintained for years.
- May result in sudden fatal disseminated disease in immunocompromised patients (although not a problem in AIDS)
- Disseminated Strongyloidiasis and hyperinfection syndrome may present as bacteremia and/or meningitis as they carry enteric flora with them throughout the body.

Italian couple continued

- Treated with ivermectin for 2 days, repeated after 1 month
- All clinical signs disappeared.
- Serology positive after 1 month but negative at 3 and 6 months after treatment.

Strongyloides

- ❑ Acute strongyloides:
 - bare skin exposure to humid soil –walking barefoot in the soil around the bungalow
 - larval migration syndrome
- ❑ Chronic strongyloides: Dyspepsia in a person from the tropics
- ❑ Hyperinfection syndrome: immunosuppression – transplants and corticosteroids

60 year old man

- 60 year old male from the Caribbean
- Admitted with nasopharyngeal cancer with metastases to the spine
- Received corticosteroids and morphine
- After a month long admission developed unexplained anemia and severe constipation with intermittent ileus, thought due to pain meds and spinal lesions

60 year old man



- He then developed a cough with purulent sputum
- The sputum culture plate looked like this

Soil transmitted helminths

Asia, Africa, Americas

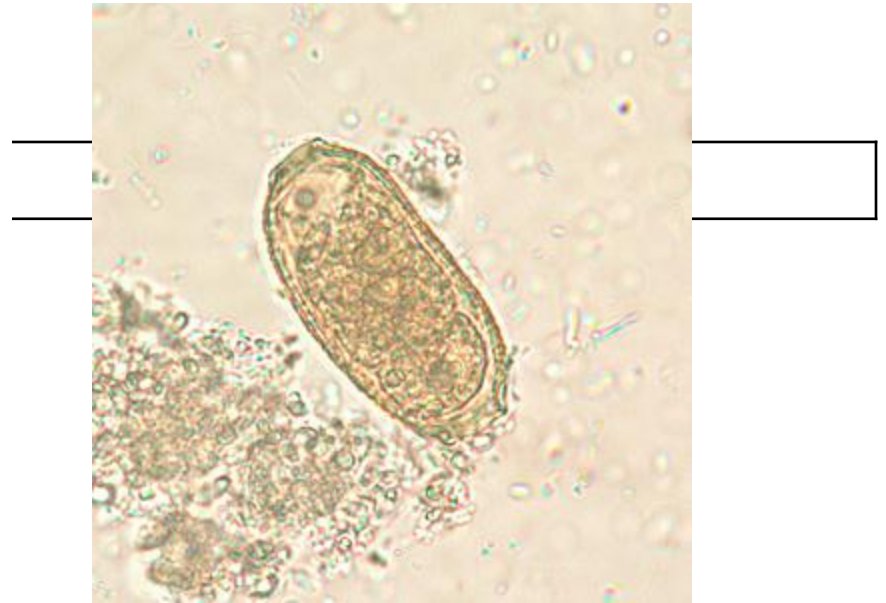
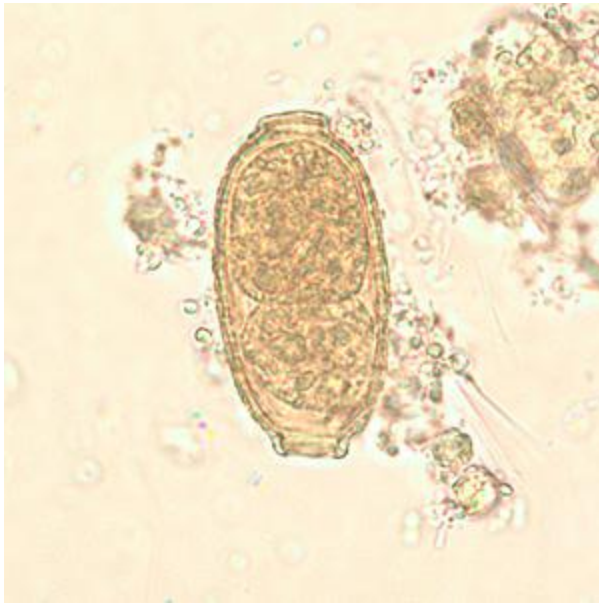
	No of cases worldwide	Major disease	Major disease
<i>Ascaris</i>	807 million	retarded growth and development	Intestinal obstruction
<i>Trichuris</i>	604 million	retarded growth and development	Colitis, dysentery
Hookworm	576 million	retarded growth and development	Iron deficiency anemia
<i>Strongyloides</i>	50 million		

72 year old man

- New England Journal of Medicine
359: 1 p.75

Final O&P exam positive for this ova

42 x 20 microns



This ova is?

- Trichuris
- Capillaria
- Ascaris

Laboratory findings

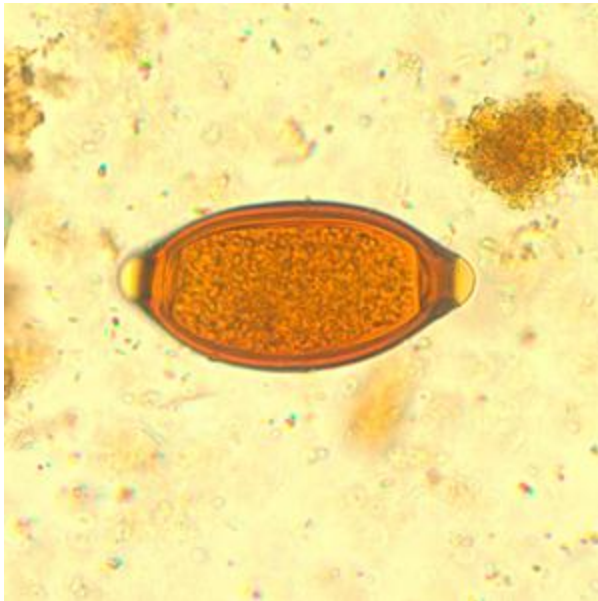
- ***Capillaria philippinensis* ova**
 - 36 to 45 microns long
 - 20 to 25 microns wide
 - thick shelled
 - striated
 - peanut shaped



Egg comparisons

Trichuris trichiura

- 50-55 microns long by 22-24 microns wide



Capillaria philippinensis

- 36 to 45 microns long by 20 to 25 microns wide



72 yr old man

- How did he get this?
- 1. peanut butter
- 2. raw fish
- 3. salad
- 4. drinking water
- 5. sand fleas

Life Cycle *C. philippinensis*

- Nematode parasite of fish eating water birds and is considered a zoonosis in humans
- Infection is from eating whole fish raw
- Adults reside in the mucosa and lumen of the small intestine. Females produce ova and larvae.
- Larvae and ova are passed in stool.
- In water, ova mature in 10 to 14 days

Life Cycle cont.

- Ova are ingested by fish
- Ova hatch in fish intestine, invade mucosa, mature to infectious state in 3 weeks
- Raw, uncleaned fish are eaten transferring the infective larvae to the intestinal tract of humans/birds.
- Like Strongyloides, autoinfection may occur resulting in a large worm burden

- *Capillaria philippinensis*

very rare but possible fatal disease

distinguish from

Trichuris and

Capillaria hepatica

Laboratory findings

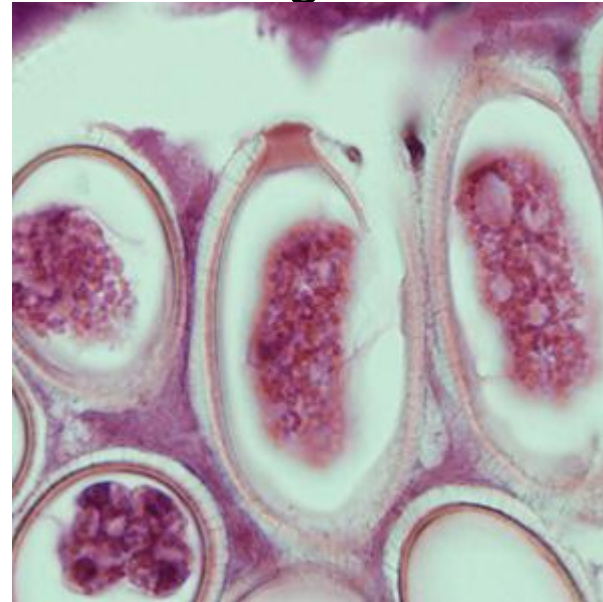
Capillaria philippinensis ova

- 36 to 45 microns long, 21 wide. Thick shelled, striated, peanut shaped, polar plugs



Capillaria hepatica ova

- 50 to 70 microns, long, 35 wide, thick shelled, striated, This image from liver tissue:



New Zealand traveler

- J of Travel Medicine 2011 Vol 18 p 59-60

Is this ?

1. Hookworm

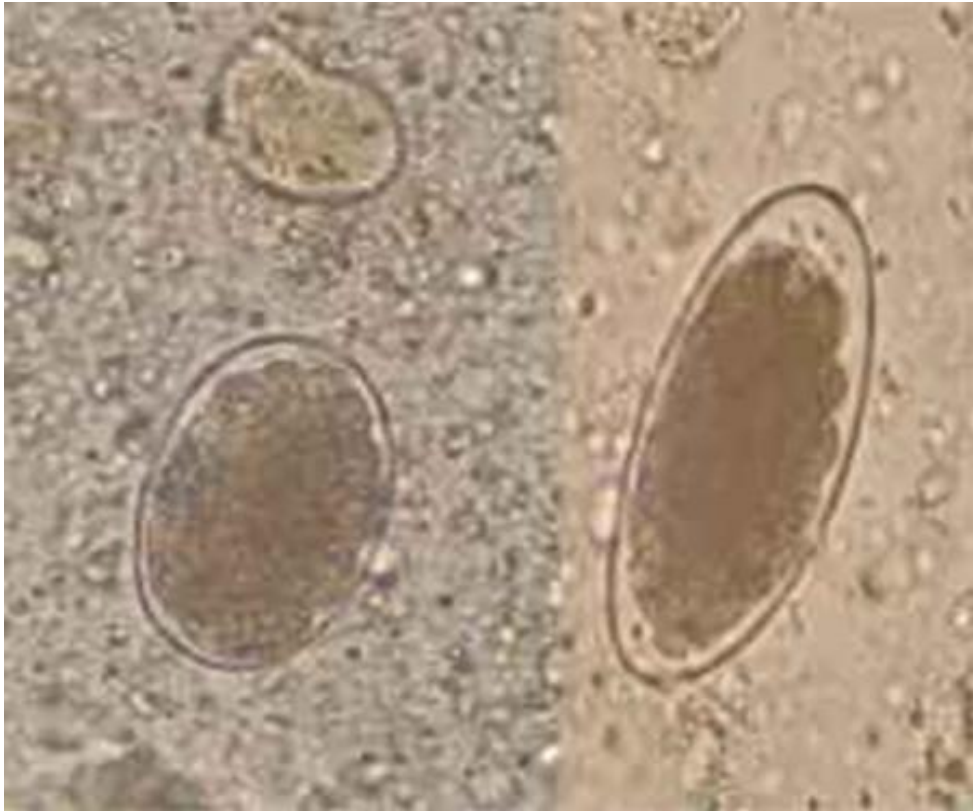
2. *Trichostrongylus*

3. *Diphyllobothrium*



CDC

Ova – Hookworm, Strongyloides and Trichostrongylus



- Hookworm ova measure 55 to 75 microns and are oval in shape.
- *Strongyloides* ova are similar but are slightly smaller and rounder – very rarely seen.
- *Trichostrongylus* ova measure 75 to 95 microns and distinctive tapered end.

How did the patient get this infection?

1. walking barefoot
2. swimming
3. insect bite
4. eating

How did the patient get this infection?

1. walking barefoot
2. swimming
3. insect bite
4. eating



Life cycle

- Once passed in feces, the *Trichostrongylus* ova quickly hatch to rhabditiform larvae.
- Molt to filariform larvae and are ingested by herbivores or humans on contaminated vegetation.
- Worldwide distribution, common where livestock are raised.

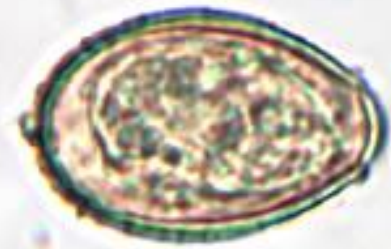
- Do not use uncomposted animal manure as a crop fertilizer.
- Separation of grazing land from cultivation of raw foods.

62 year old man -Korea

- New England Journal of Medicine
358;16 April 17, 2008

This egg found in stool is ??

30 microns x ---14 microns



CDC

1. *Metorchis conjunctis*
2. *Trichuris*
3. *Clonorchis sinensis*
4. *Fasciola*
5. *Ascaris*

O&P exam - Ova found in stool

Clonorchis sinensis



- 27 to 35 microns in length
- Prominent shoulders
- seated operculum
- Knob or “boss” opposite the operculum
- “cracked mud” shell markings (focus carefully with oil immersion)
- Miracidium asymmetrical
- Ova may be quite rare

Other small trematode ova – get out a good atlas for these!

Metorchis spp. – **liver fluke** found in North America – absence of “shoulders” shell markings resembling “brain”. Miracidium asymmetrical

• **Small intestinal flukes:**

Heterophyes heterophyes, *Metagonimus yokogawai* – absence of shell markings, external shoulders absent but *H. heterophyes* may have internal shoulders. Miracidium is symmetrical

62 year old man

- He ate:
- 1.Unwashed vegetables
- 2.Uncooked fish
- 3.Pork
- 4.Bear meat

Life Cycle

- Embryonated ova are passed in feces.
- Two intermediate hosts ,
a snail and then fresh water fish
- Metacercariae encysted in fresh water fish are the infective form for humans through ingestion of undercooked, salted, pickled or smoked freshwater fish.

62 year old man

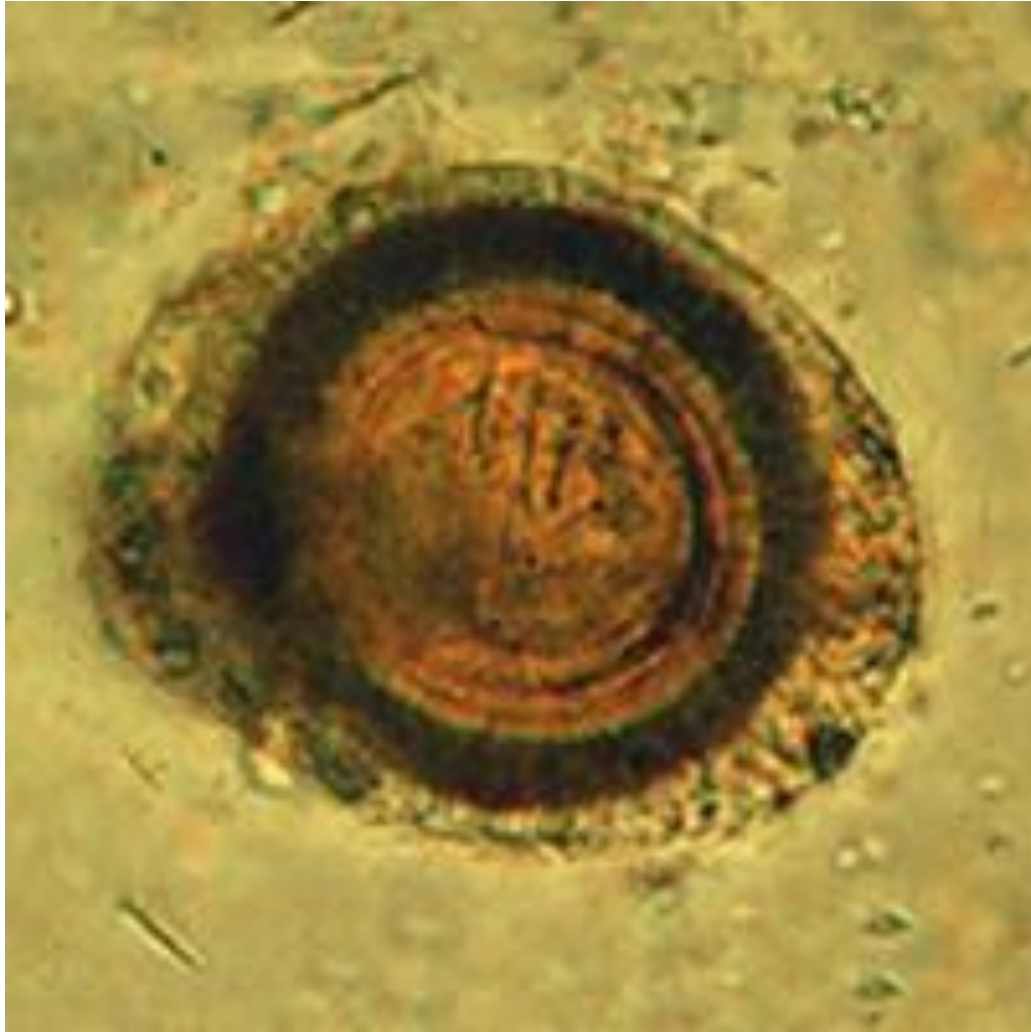
Rx praziquantel

- *Clonorchis* persists for decades
- Can cause diarrhea and/or
- Acute cholangitis and pancreatitis

- Associated with cholangiocarcinoma

51 year old man with seizure

- Burneo et al
CMAJ Mar 17,2009



WHO

Taenia eggs

50 different species Taenia

All look the same

Proglottids can be sent to BCCDC for speciation
but they must be unfixed

Not in SAF

Cysticercosis

- Most common preventable cause of epilepsy
- Most common cause of epilepsy in Latin America

Taenia solium/ cysticercosis

- If you eat infected raw pork, you get the tapeworm
you are the definitive host.
- If you ingest eggs from the person with the tapeworm you get cysticercosis
you are the intermediate host

Taenia solium cysticercosis

- Endemic in pig raising/pork consuming areas
- Associated with poverty
inadequate sanitation
lack of meat inspection and control
- Spread by
migration
overseas domestic workers
international travel
marketing and transport of pigs

References

- Images from CDC Atlanta website
www.dpd.cdc.gov/DPDx/default.htm
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- Diagnostic Medical Parasitology, 5th Edition, Garcia, Lynn Shore ASM Press 2007
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