



Gastrointestinal parasites

Revisiting and rebooting our knowledge of organisms, detection methods, and practical laboratory issues.

Marc Roger Couturier Ph.D., D(ABMM)

Director of Microbiology and Molecular Microbiology, NorDx

Professor of Internal Medicine (adj.), Tufts University

Objectives

- 1 Review the life cycle and identifying features of common gastrointestinal parasites
- 2 Recognize the pathogenic potential of gastrointestinal parasites
- 3 Discuss non-microscopy methods for parasite detection versus conventional microscopy
- 4 Recognize the dogmas and conventions that exist in parasitology that need revisiting
- 5 Understand patient presentation/risk factors and appropriate test utilization to guide physicians

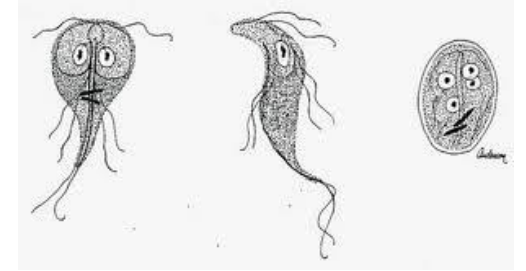


Refresher on Common Test Methods

Ova and parasite exam

Wet mount on fresh stool

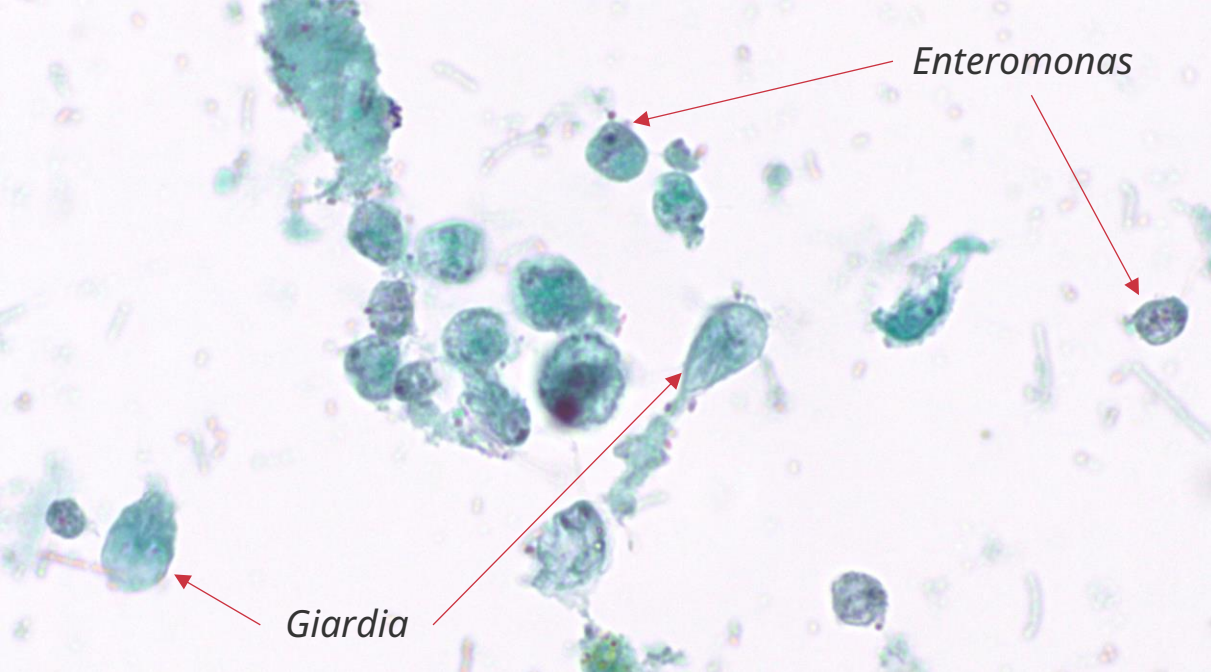
- Look for motility (circa 1800s)
 - Difficult to identify/perform (<30 mins)
 - Organisms stop moving shortly after expulsion



Fixed stool (formalin & polyvinyl alcohol)

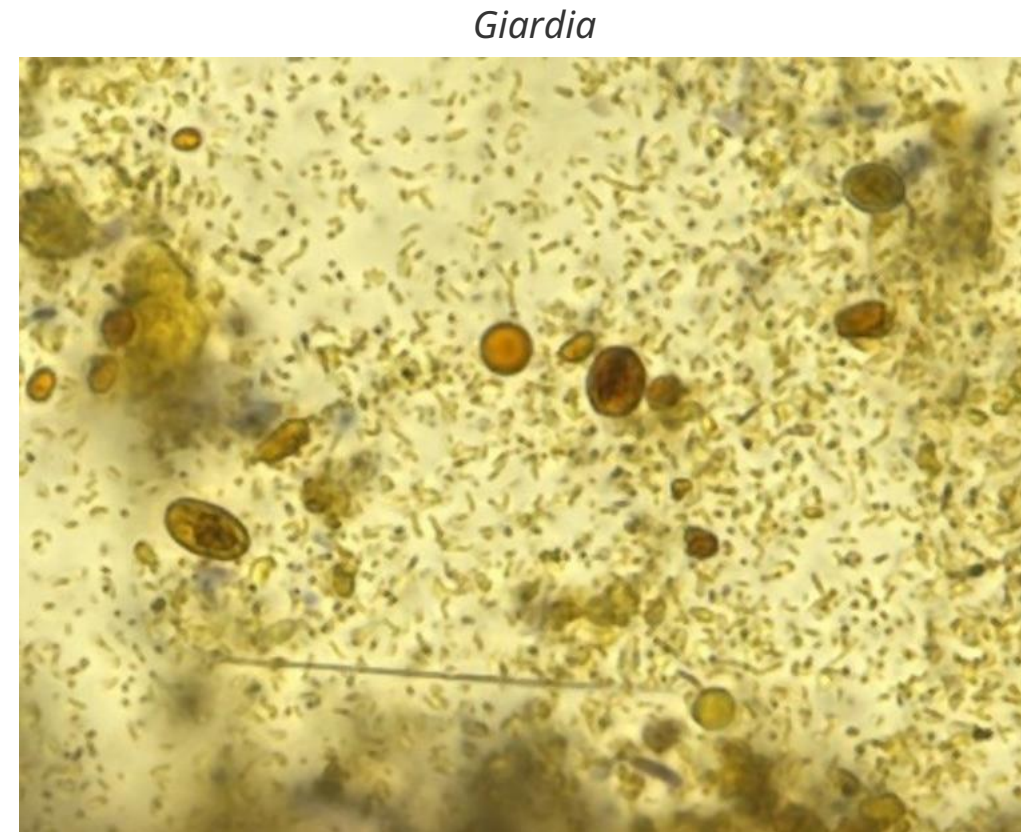
- Most used method
- Specimen is concentrated (↑ sensitivity)
- **Trichrome** stain: specimen smeared on slide & stained
- **Wet mount (+/- Iodine)**: specimen added to slide, mixed with iodine and visualized





Trichrome stain

Iodine stain (wet mount)



O&P considerations

Recommend 3 specimens on separate days

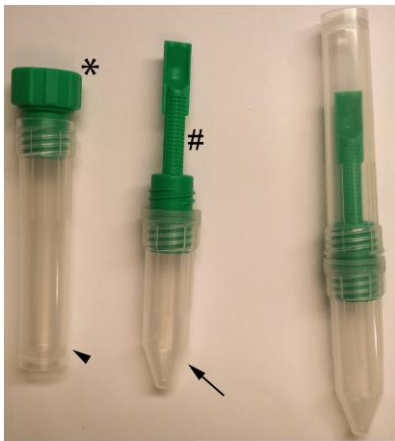
- Span 5-7 days)
- 5+ collections for helminths

Only for patients with high pre-test probability

- Immunocompromised patients
- Pertinent exposure history (immigrants, hikers, splash parks)
- Pertinent travel history
- Having eaten at Olive Garden or Longhorn...

Stool concentration techniques

- Formalin-ethyl acetate or ether sedimentation
- Zinc sulfate flotation
- Vertical filtration/centrifugation



Stool concentration techniques

Concentration	Pros	Cons
Formalin-Ethyl acetate sedimentation	Provides good concentration	Toxic Required formalin disposal Cumbersome workflow
Ether sedimentation	Provides good concentration	Toxic Explosive Cumbersome workflow
Zn flotation	Excellent concentration for worms and helminth eggs	Cumbersome workflow Less sensitive for protozoa
Vertical filtration (Separate filter device)	Good concentration Available commercially as a kit Better workflow than above methods Can be used with any submission fixative	Requires some additional manipulation and time delays before centrifugation Not ideal for high test volumes
Vertical filtration (Integrated filter device)	Good concentration Commercially available Best workflow (fastest processing time) Best high test volume application	Tube system is provided to patient, may be incorrectly assembled Concentrates entire specimen*

Stool collection

- Choose your poison
 - Formalin containing
 - 10% formalin (PVA second vial)
 - Sodium acetate-acetic acid-formalin
 - Alcohol-based
 - AlcorFix (Apacor)
 - EcoFix (Meridian)
 - TOTAL-FIX (MCC)



Stool collection comparison

*Assay specific

Fixative	Composition	Indication for use	Pros	Cons
PVA	Polyvinyl alcohol with Cu, Zn, or Hg	Permanent stained trichrome slides	Hg-PVA provides excellent fixation & morphology	Highly toxic (if Hg) Requires formalin vial
Formalin	10% formalin in buffered saline	Wet-mount examination Coccidia stains Microsporidia stains	Excellent fixation properties. Multiple uses	Hazardous Not compatible with trichrome stains
AlcorFix	EtOH, ZnSO ₄ , acetic acid, glycerin, MeOH, iPrOH, small % PVA	Trichrome, wet-mount Coccidia stains Microsporidia stains	Good morphology Less toxic Good fixation Can be sold in Parasep tube Single vial	Looks best with EcoStain If used in Parasep, requires change in workflow
EcoFix	EtOH, ZnSO ₄ , acetic acid, glycerin, MeOH, iPrOH, small % PVA	Trichrome, wet-mount Coccidia stains Microsporidia stains NAAT tests*	Good morphology Less toxic Good fixation Single vial	Looks best with EcoStain
SAF	Sodium acetate formalin	Trichrome, wet-mount Coccidia stains Microsporidia stains	Good fixation Great for wet-mounts Single vial	Highly toxic Trichrome is monochrome and difficult to interpret
TOTAL-FIX	EtOH, ZnSO ₄ , acetic acid, iPrOH, MeOH	Trichrome, wet-mount Coccidia stains Microsporidia stains NAAT tests* Antigen tests*	Less toxic Good fixation Single vial	Morphology is acceptable, not always optimal Looks best with EcoStain

ARUP Fixative blind comparison

- Negative stool spiked with live *Giardia* and *Cryptosporidium*
- Specimens submitted through the lab with 8 technologists: trichrome, wet-mount, modified acid-fast (MAF)
- Blinded to fixative used
- Qualitative scoring and comments recorded

Fixative	Average weighted score	Comments/observations
PVA/Formalin	+++	Trichrome: Morphology is good, color contrast is good. Wet-mounts: A bit fuzzy, Ok. MAF: Good
Alcorfix	++++	Trichrome: Morphology is great, color contrast is ok*. Wet-mounts: Look great. MAF: Good
EcoFix	++++	Trichrome: Morphology is great, color contrast is ok*. Wet-mounts: Look great. MAF: Good
SAF	+	Trichrome: Morphology is horrible, color contrast is awful. Wet-mounts: Looks amazing, easy to find and identify. MAF: Poor contrast
TOTAL-FIX	++	Trichrome: Morphology is not optimal, color contrast is ok*. Wet-mounts: Ok, not optimal. MAF: Good

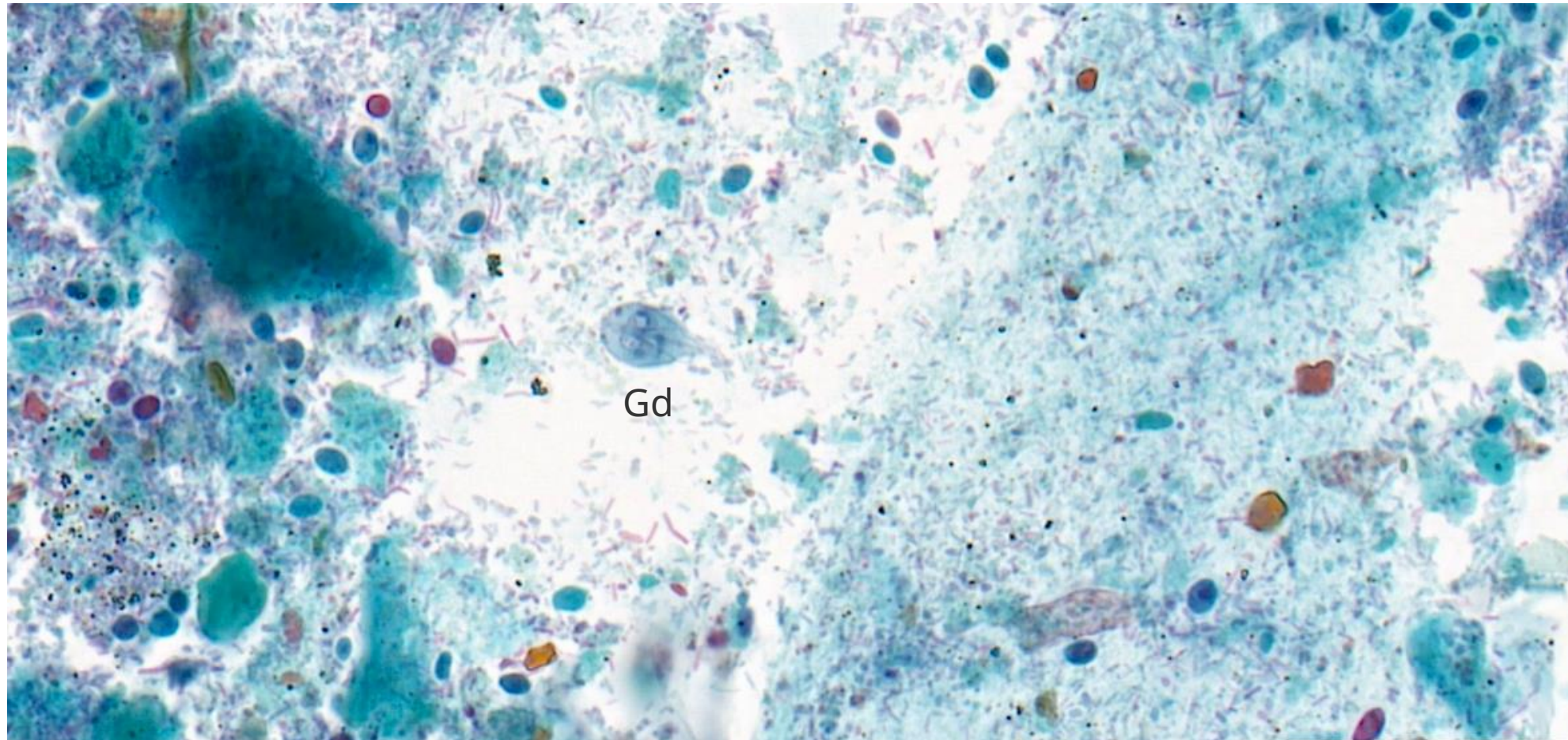
* Study performed with Wheatley's trichrome. Color contrast with EcoStain later found superior

ARUP Fixative blind comparison

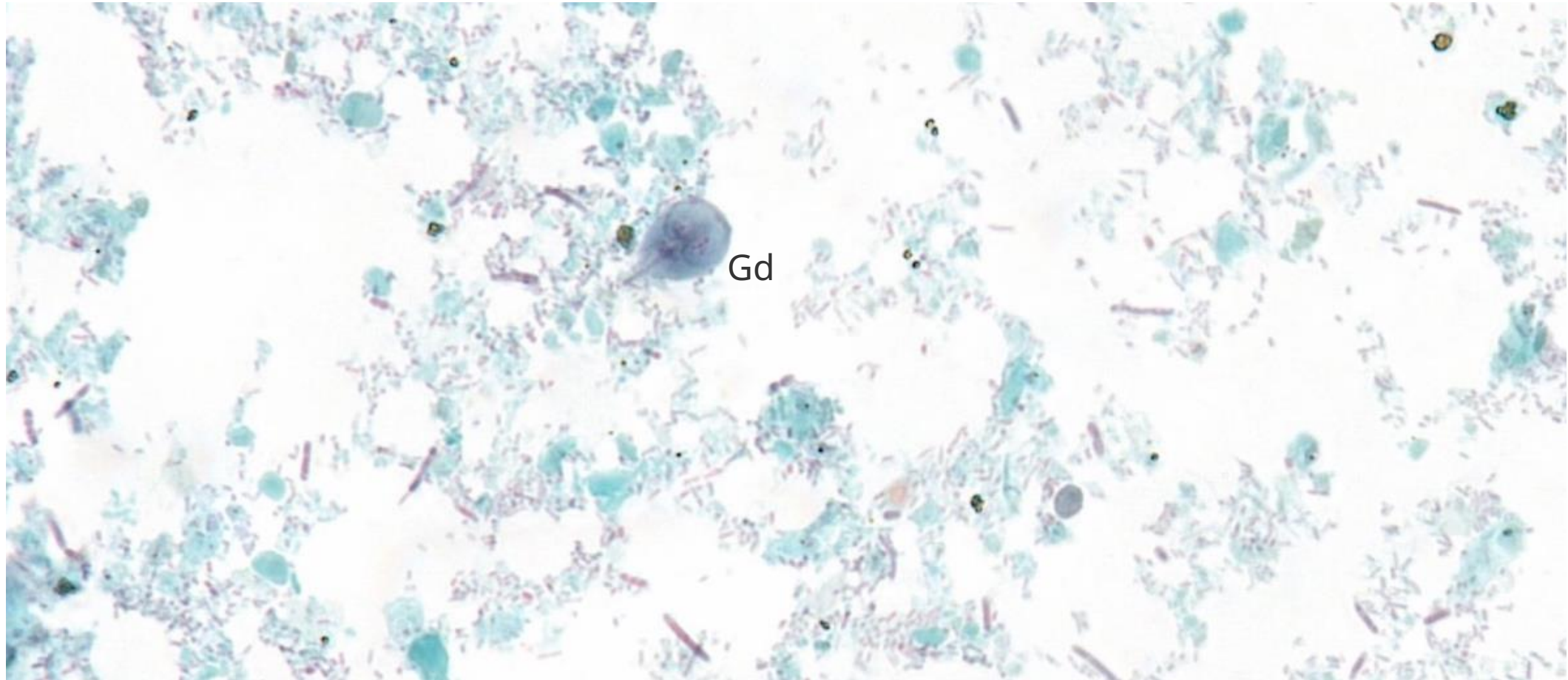
- Bottom line...

Fixative	Does it work
PVA/Formalin	Yes
EcoFix	Yes, best with EcoStain
Alcorfix	Yes, best with EcoStain
TOTAL-FIX	Yes, best with EcoStain
SAF	Marginally...for trichromes

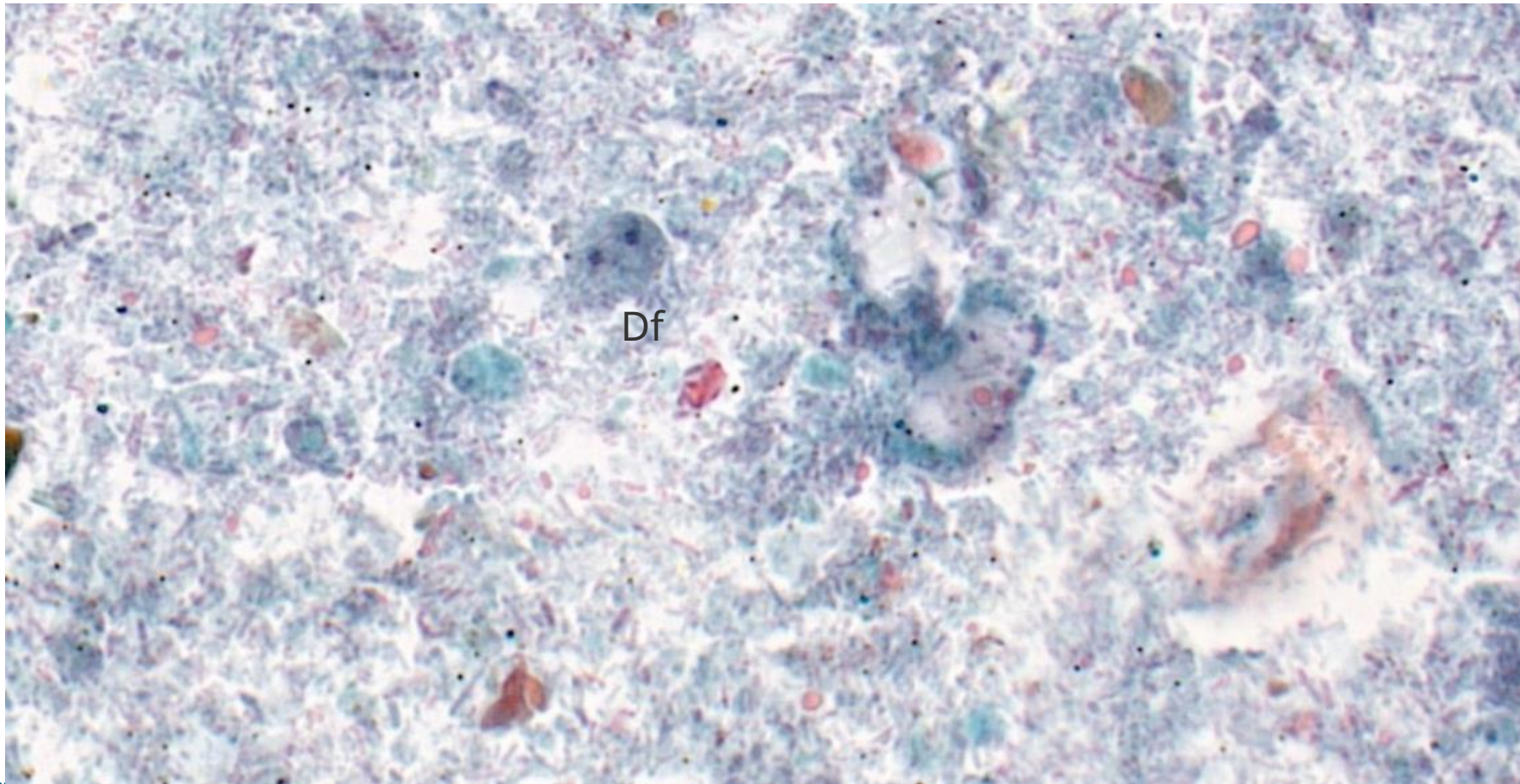
Alcorfix with EcoStain



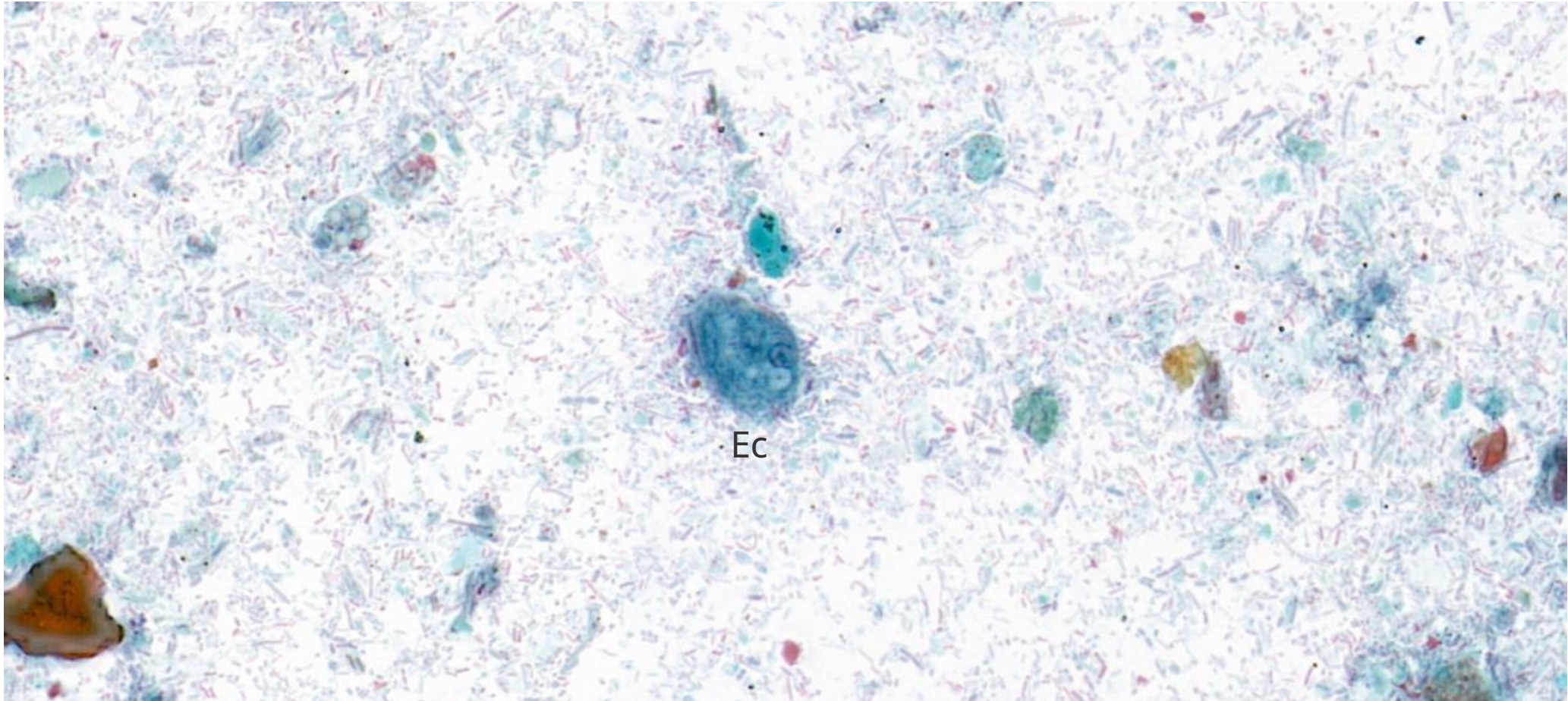
Alcorfix with EcoStain



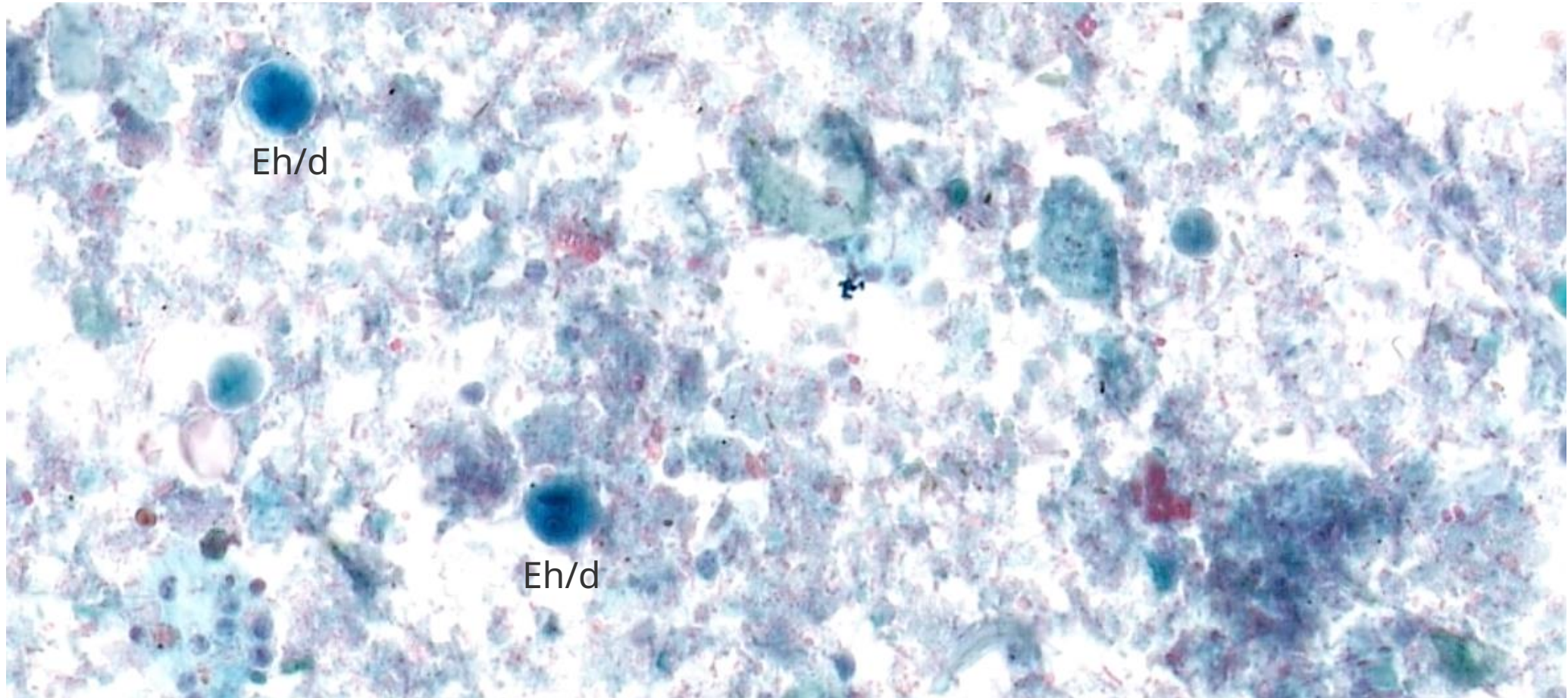
Ecofix with Ecostain



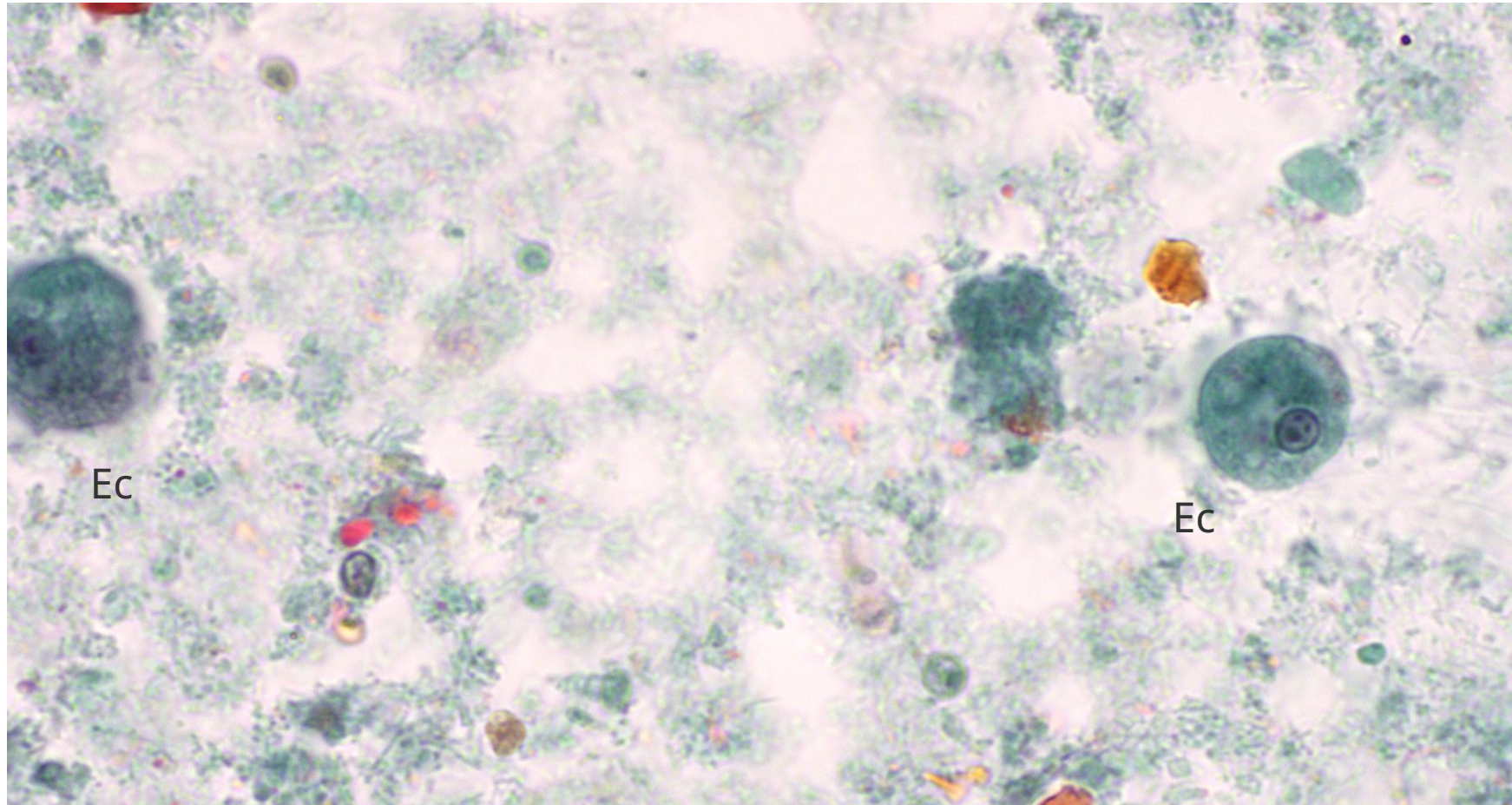
Ecofix with Ecostain



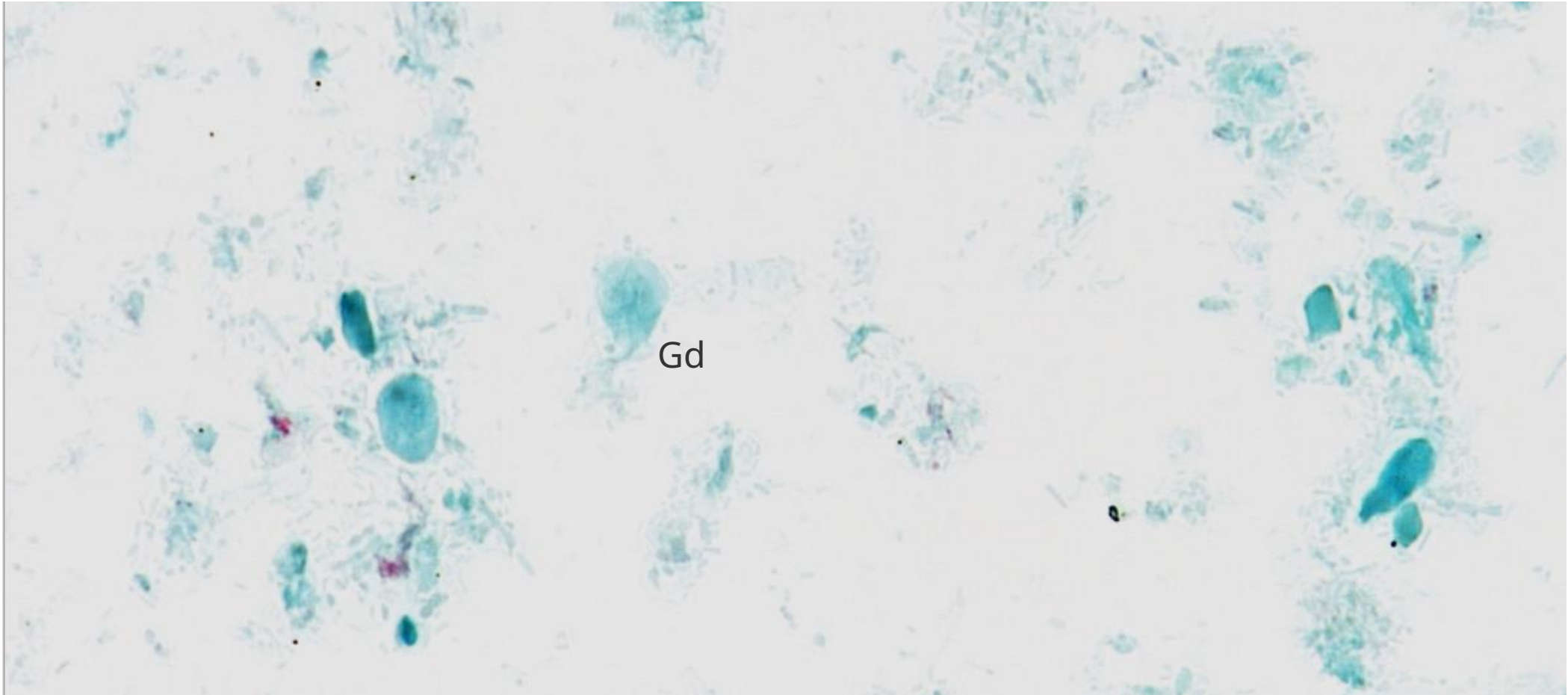
TOTAL-FIX with Ecostain



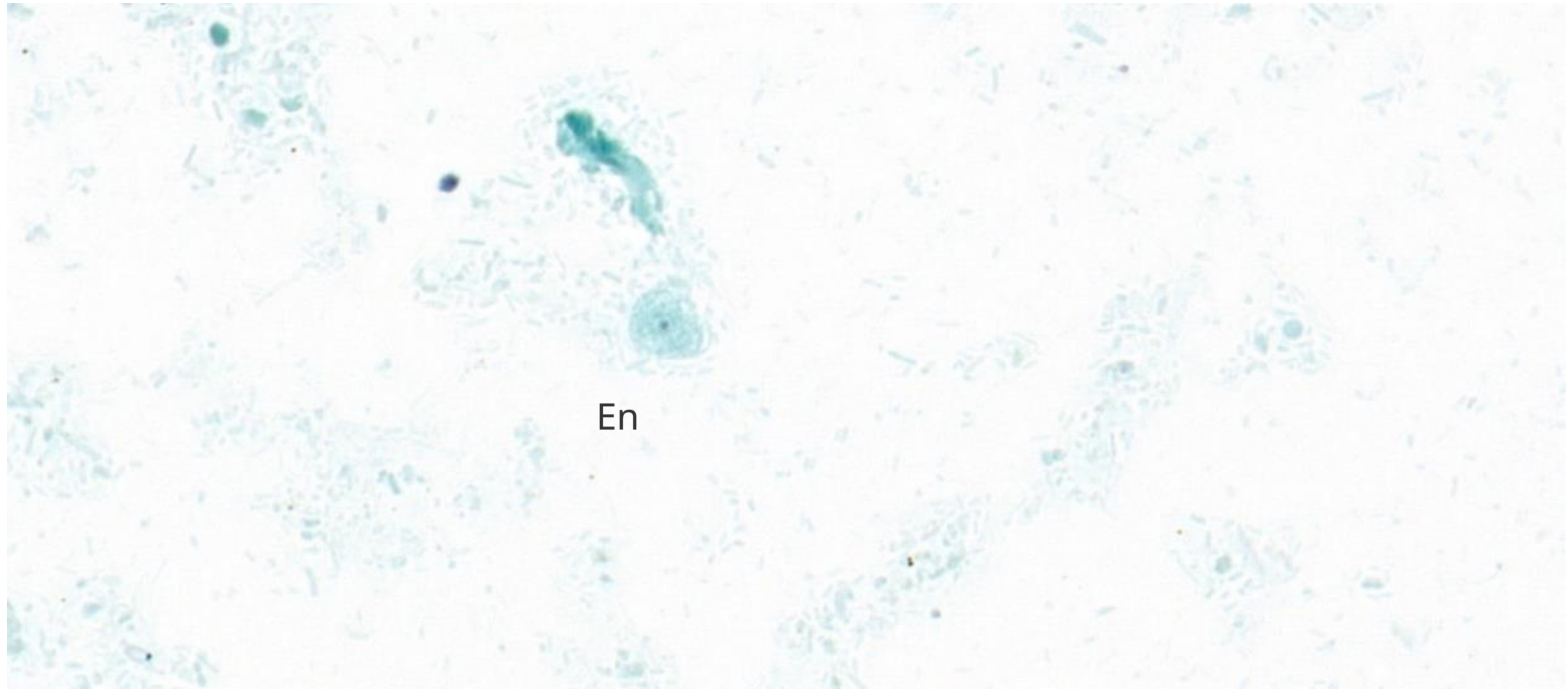
PVA with Wheatley's Trichrome



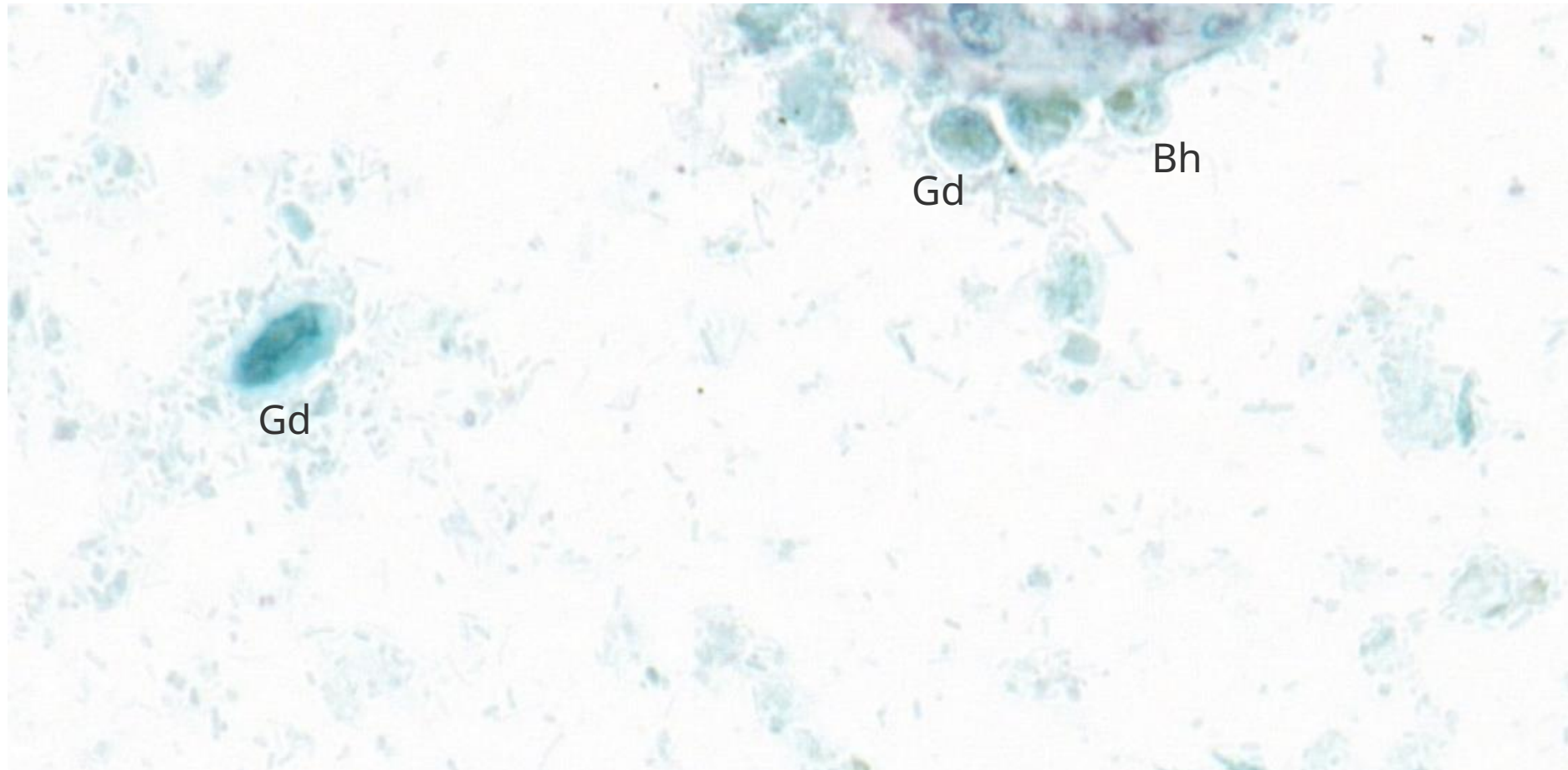
SAF with Wheatley's Trichrome



SAF with Wheatley's Trichrome



SAF with Wheatley's Trichrome



What about others?

- MIF – Merthiolate-iodine-formaldehyde – toxic, rarely used. Single vial. Very good morphology (less with thimerosal removed)
- Unifix – Not commonly used, previous reports do not recommend for use due to poor morphology. Formalin free, single vial.
- Proto-fix – Not widely used, few detailed evaluations published. Similar to EcoFix, AlcorFix. Formalin-free, single vial. No PVA.
- Para-Pak SVT – (Newer) No performance studies published, composition proprietary. Formalin-free, single vial.

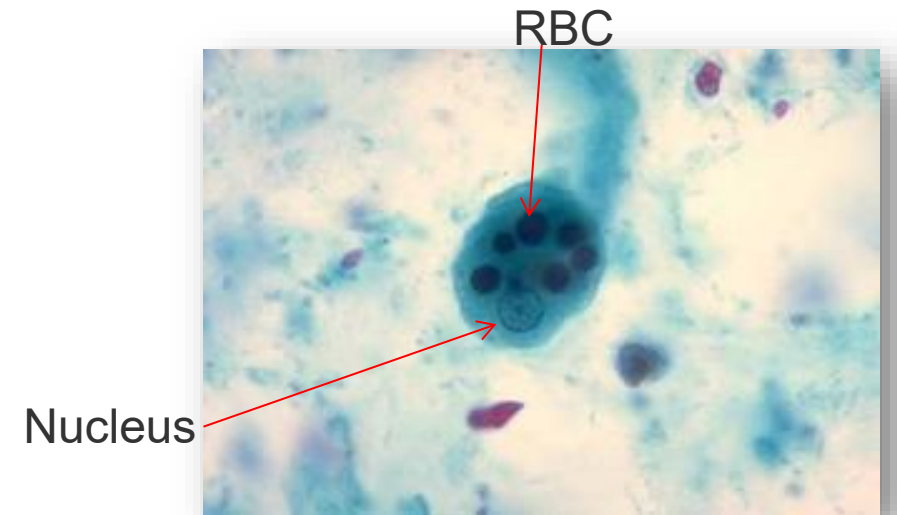
O&P Trichrome Limitations

Cannot detect

- *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, Microsporidia

Does not readily identify

- *Entamoeba histolytica* from non-pathogenic *E. dispar*

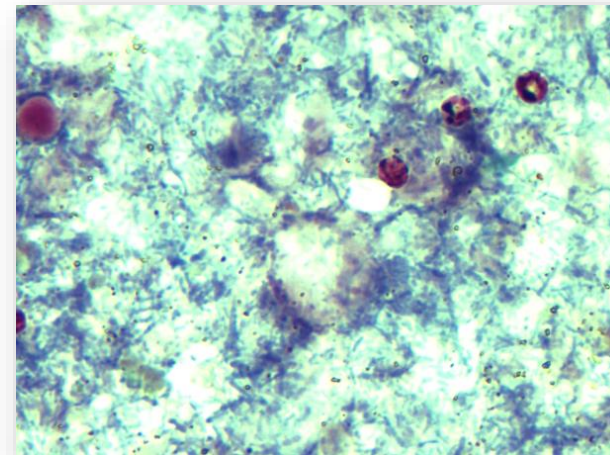


Modified acid fast/safranin stain

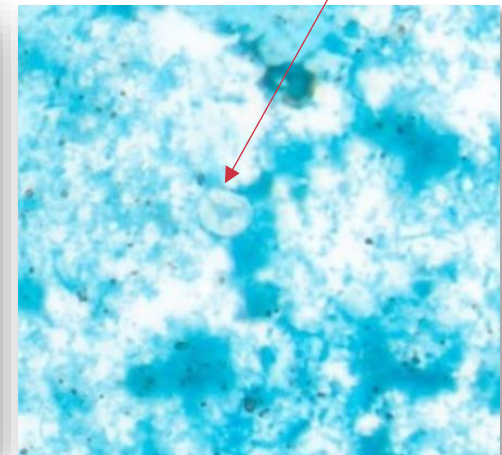
Cryptosporidium, *Cyclospora*, *Cystoisospora* do not retain trichrome stain

- Sensitivity improved with modified acid fast stain or modified safranin
- Can be difficult to interpret
- “Ghosts”

Often not ordered when indicated



Cryptosporidium

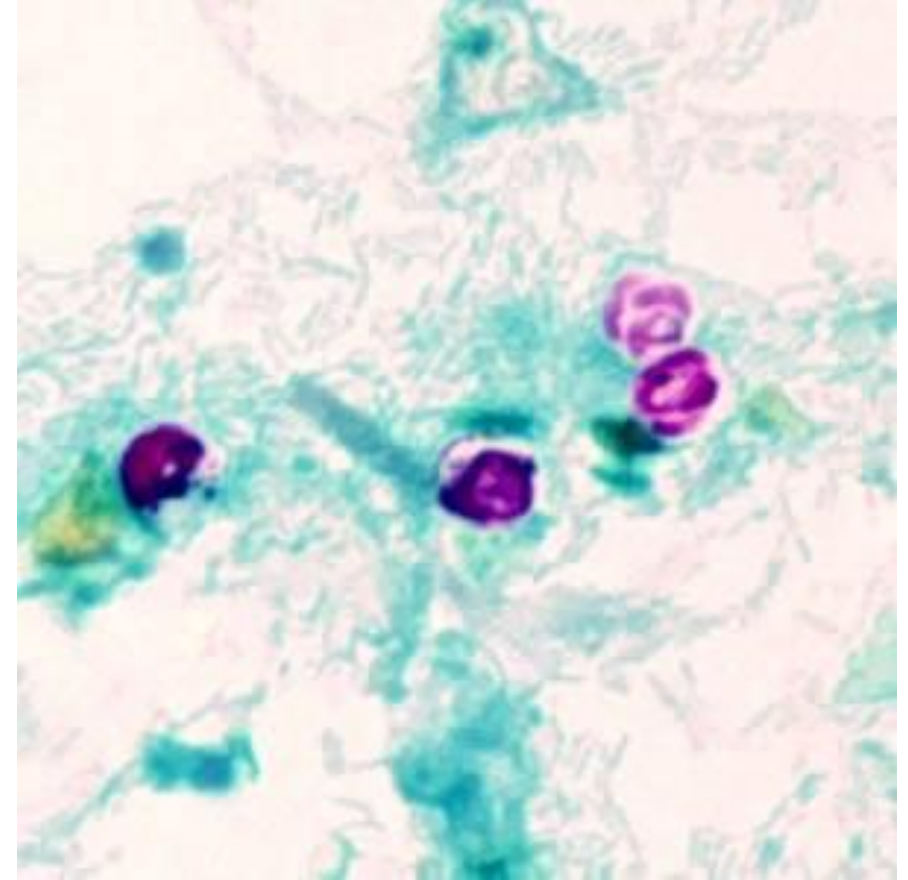
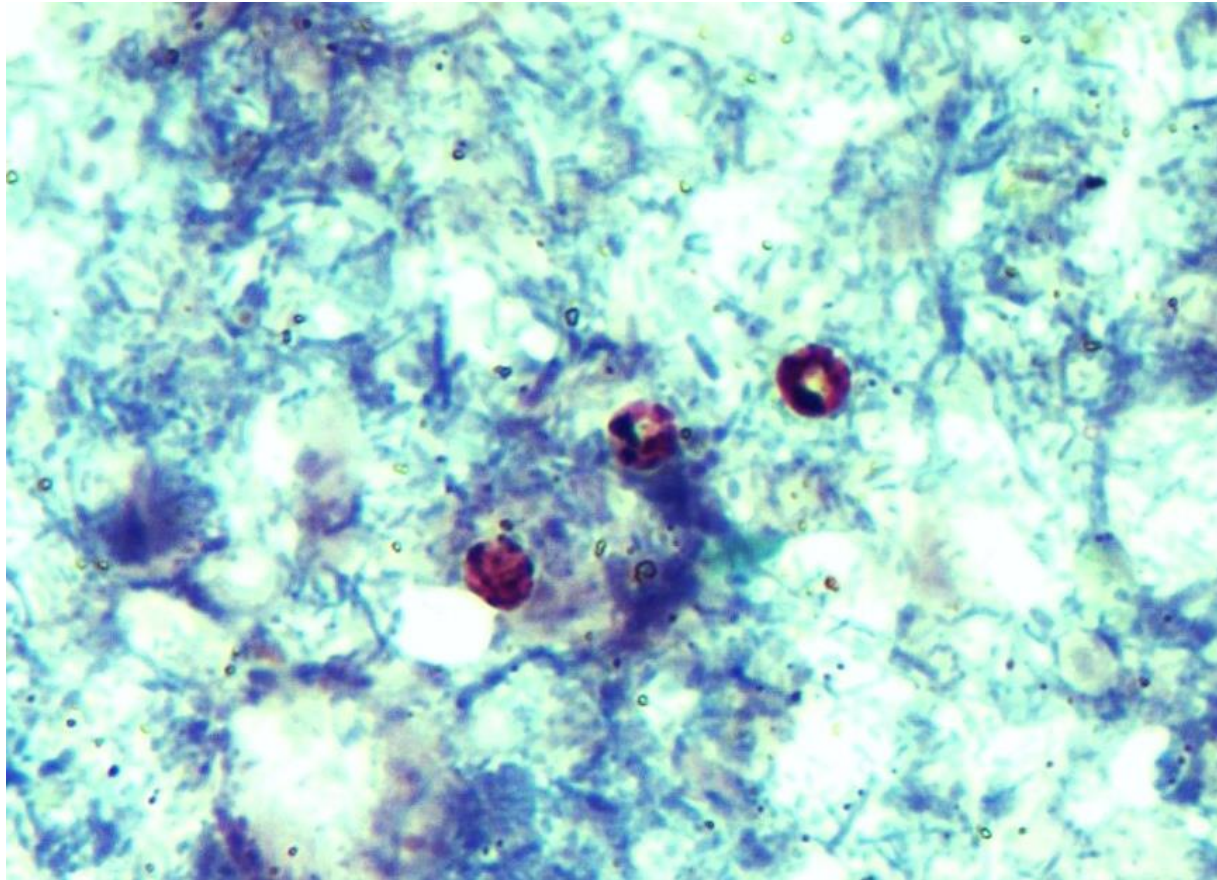


Cyclospora

Other stains – coccidia & *Cryptosporidium*

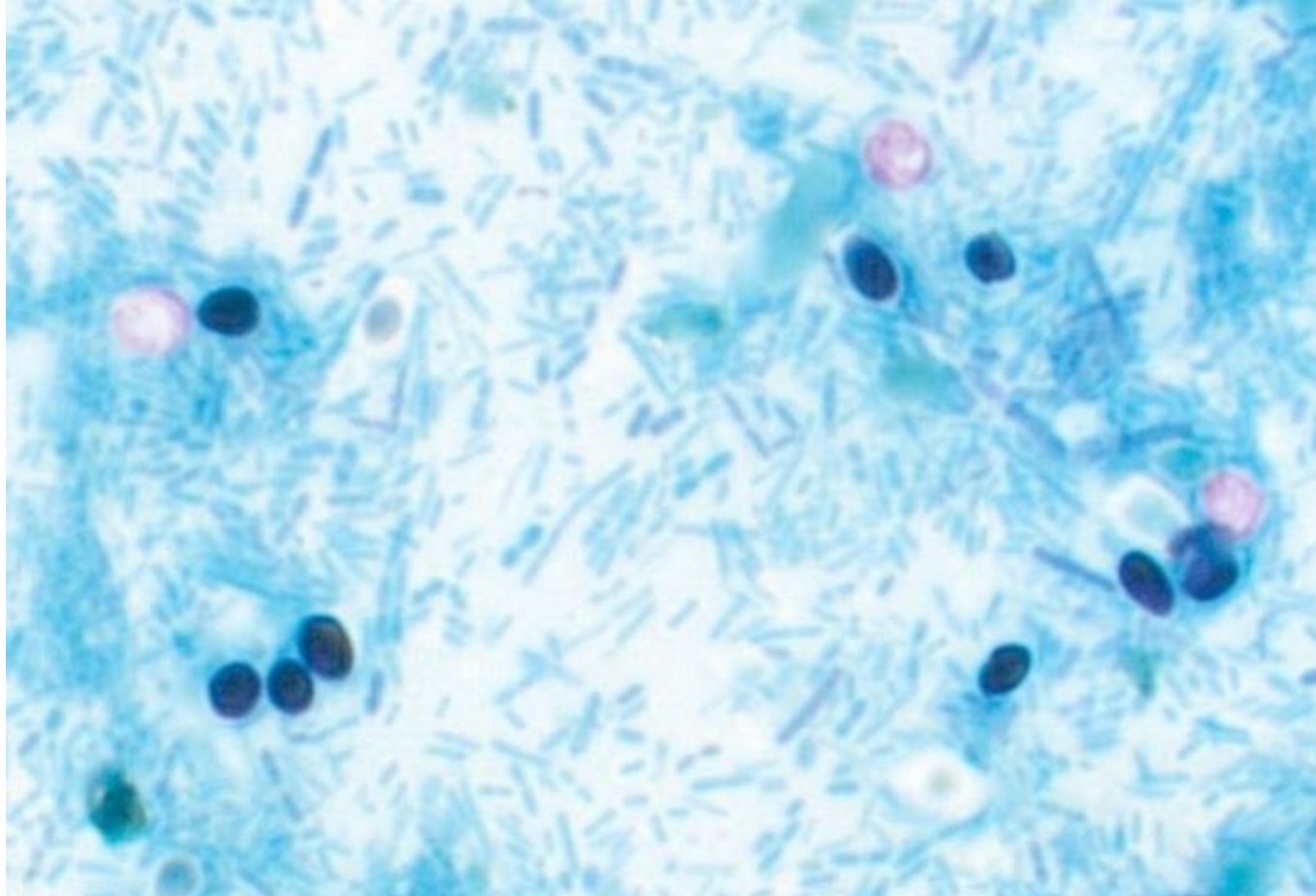
Stain	Pros	Cons
Modified Kinyoun – malachite green	Great contrast of organism vs background (pink vs green)	<i>Cyclospora</i> and <i>Cystoisospora</i> often occlude stain (“ghost”)
Modified Kinyoun – methylene blue	Good contrast of organism vs background (pink versus blue/purple)	<i>Cyclospora</i> and <i>Cystoisospora</i> often occlude stain (“ghost”) Yeast can pick up counterstain and pink (e.g. artifacts)
Modified Ziehl-Nielson	Good contrast of organism vs background (pink vs blue)	<i>Cyclospora</i> and <i>Cystoisospora</i> often occlude stain (“ghost”) Yeast can pick up counterstain and pink (e.g. artifacts)
Modified safranin	Excellent stain retention for all organisms Easy to distinguish organism from background (pink vs pale green)	Messy; requires heating Difficult to scale up for high volume.

Modified Kinyoun – malachite green [*Cryptosporidium*]

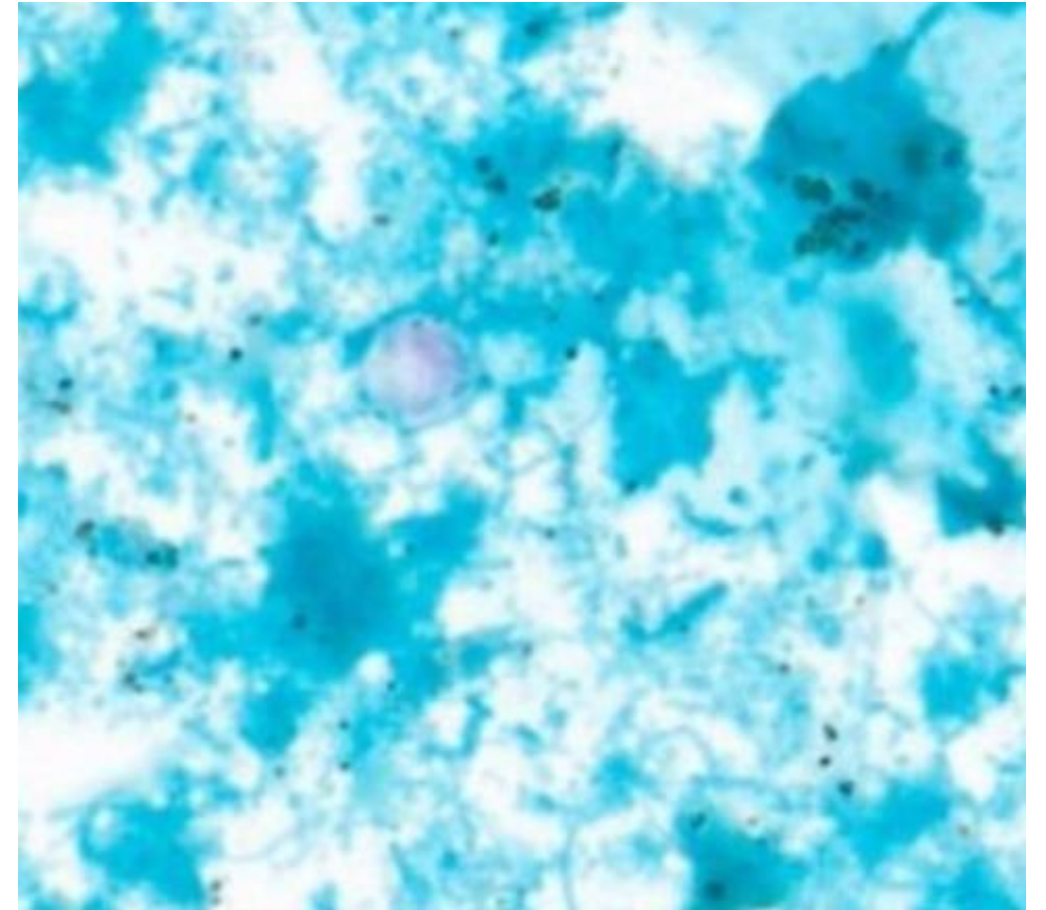
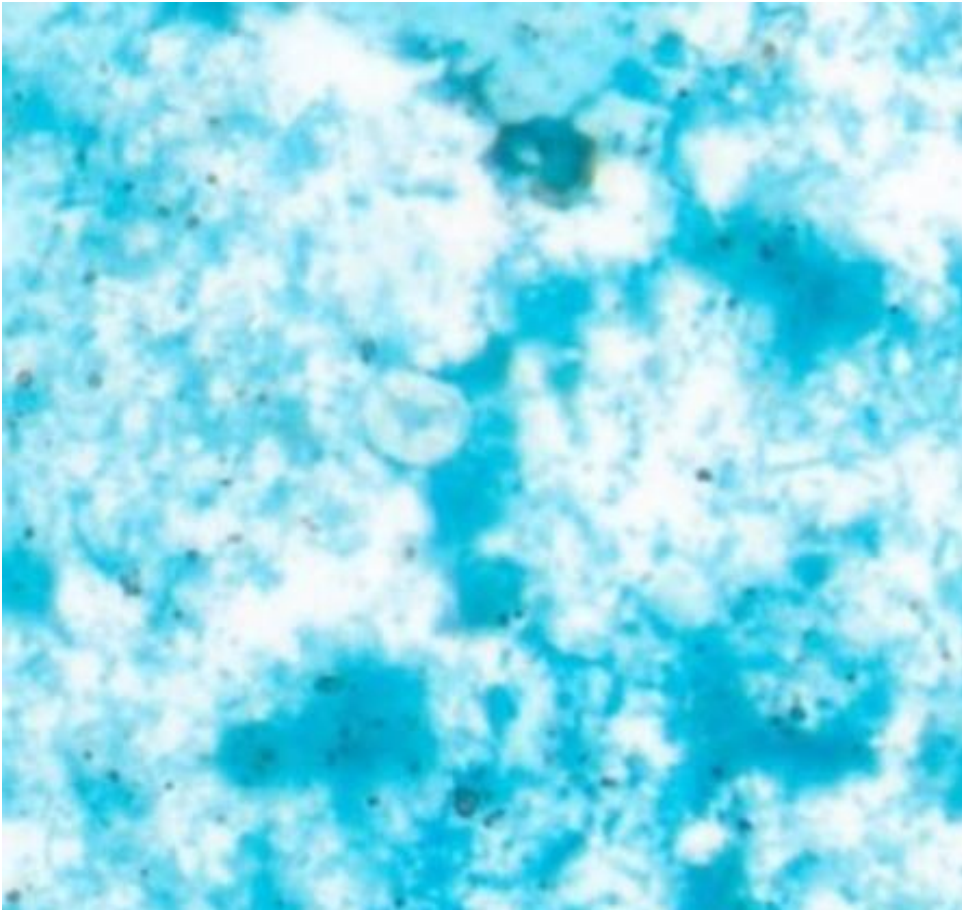


<https://www.cdc.gov/dpdx/cryptosporidiosis/index.html>

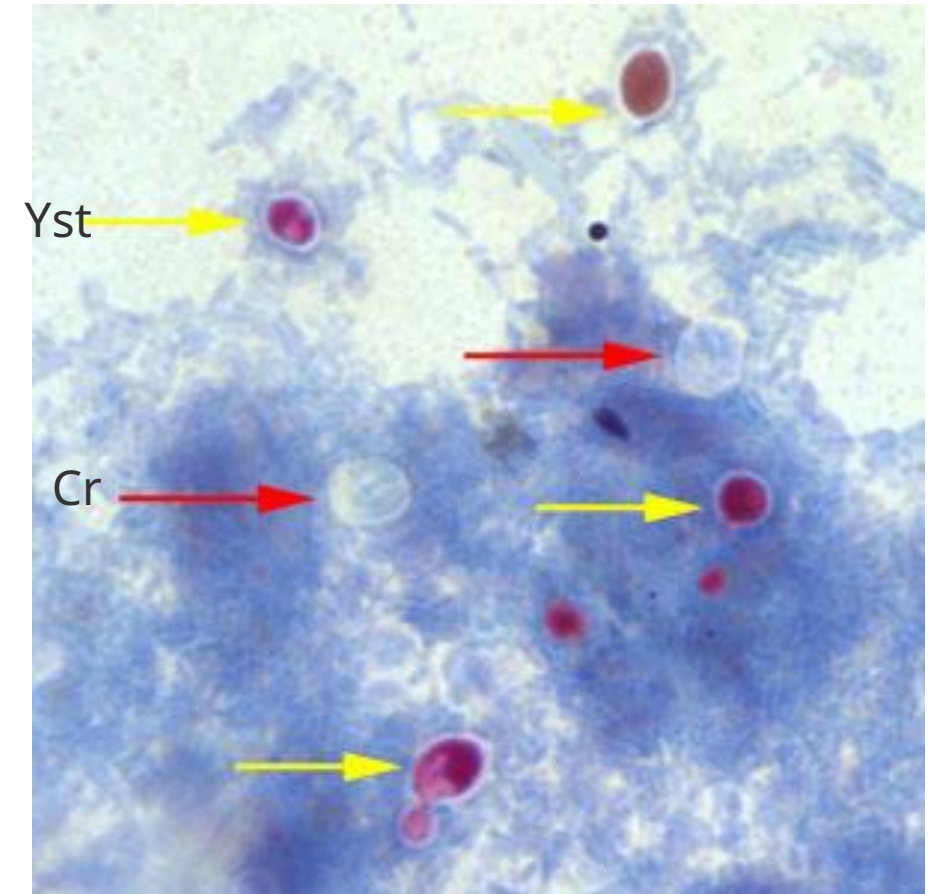
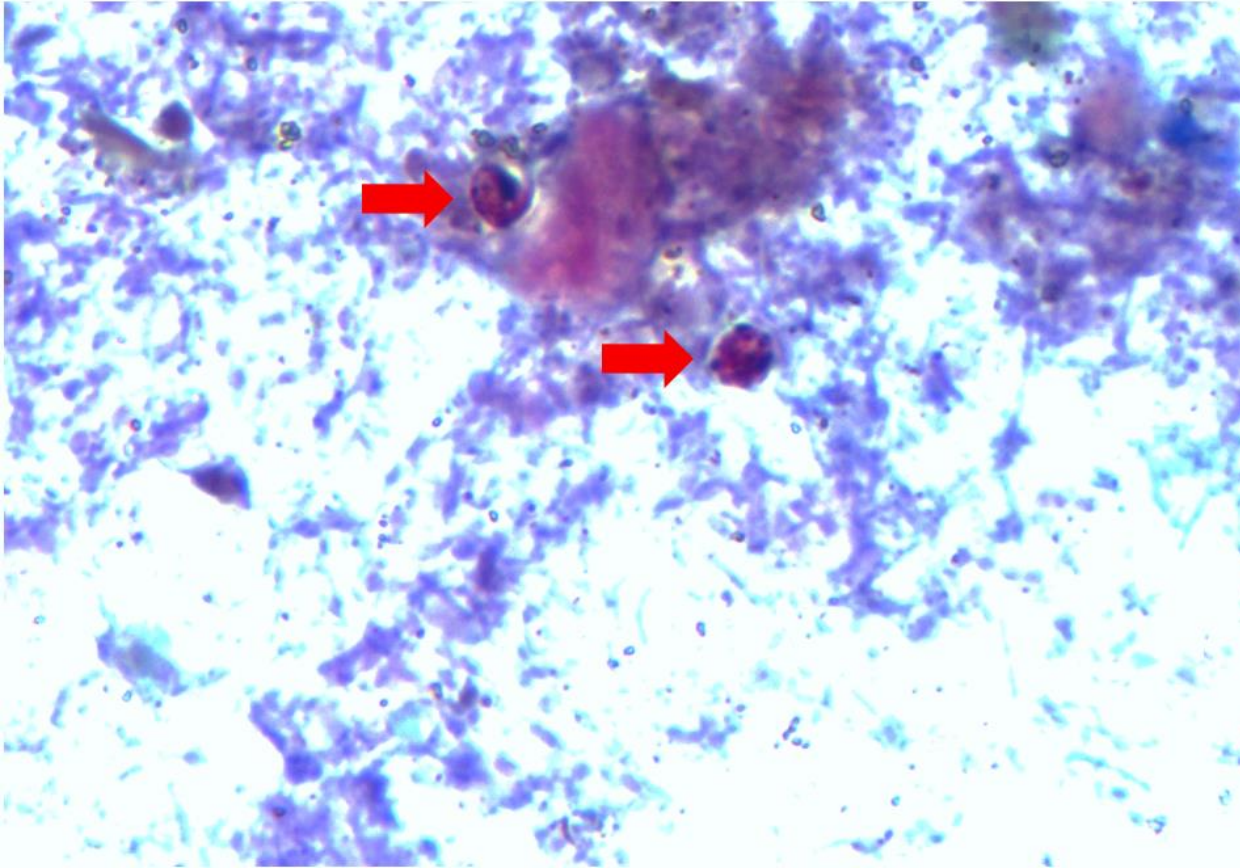
Modified Kinyoun – malachite green [*Cryptosporidium*]



Modified Kinyoun – malachite green [*Cyclospora*]

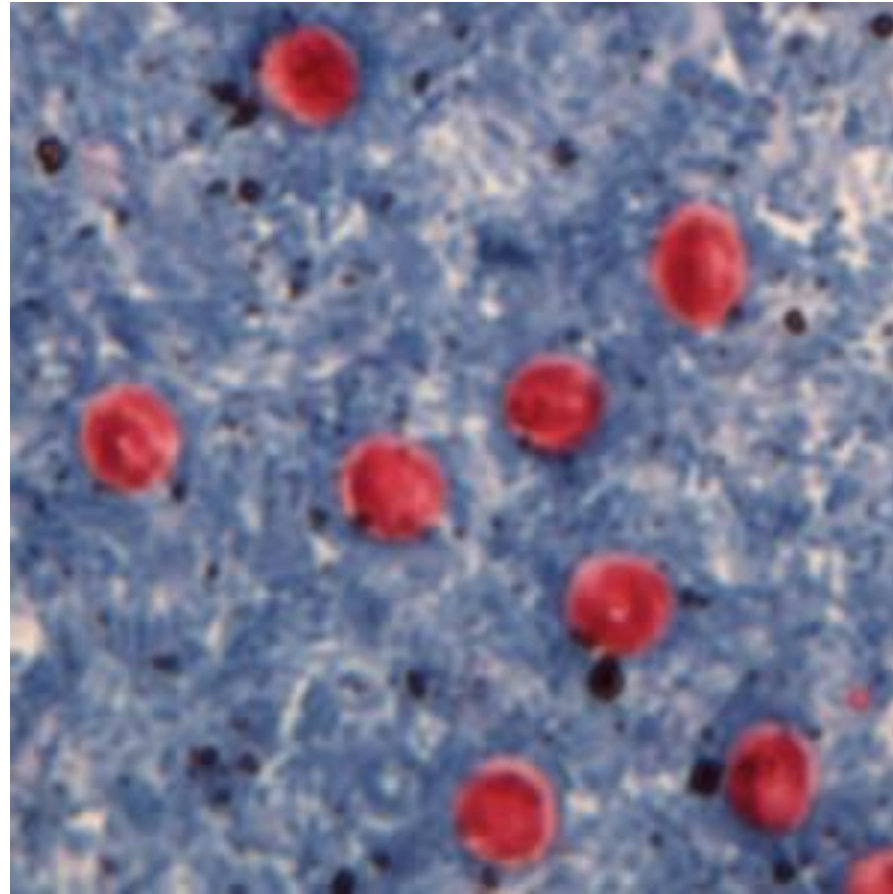


Modified Kinyoun – methylene blue [*Cryptosporidium*]



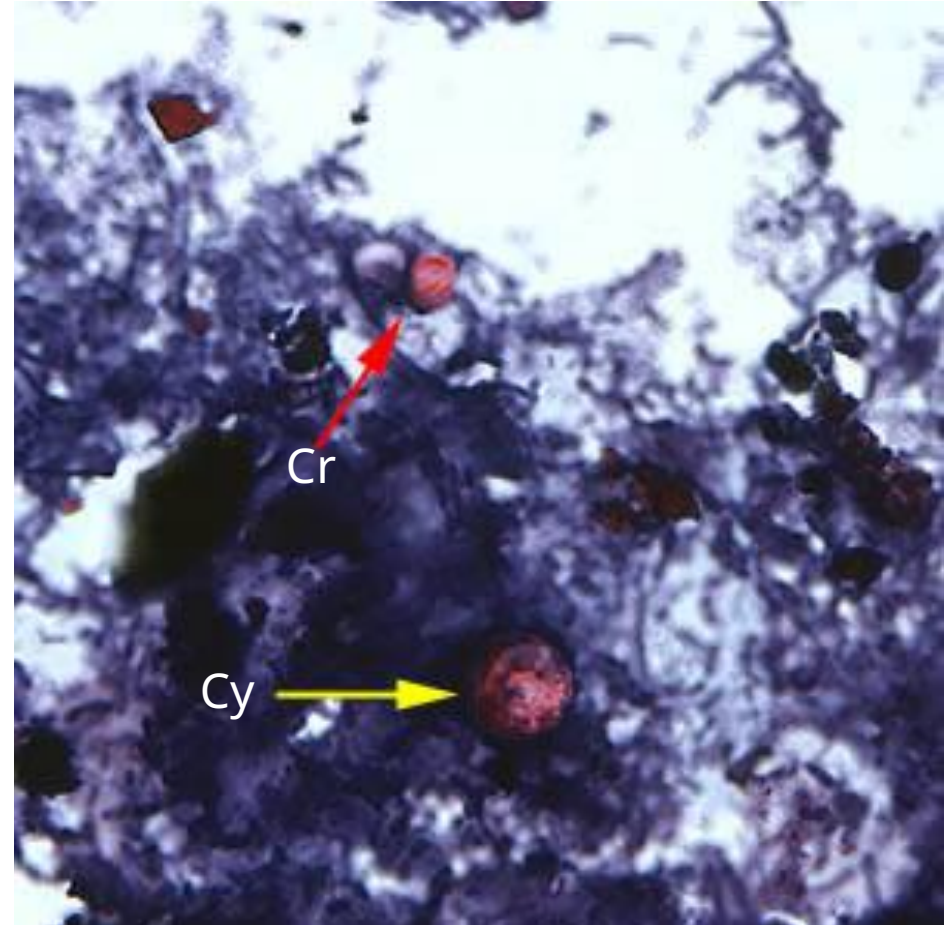
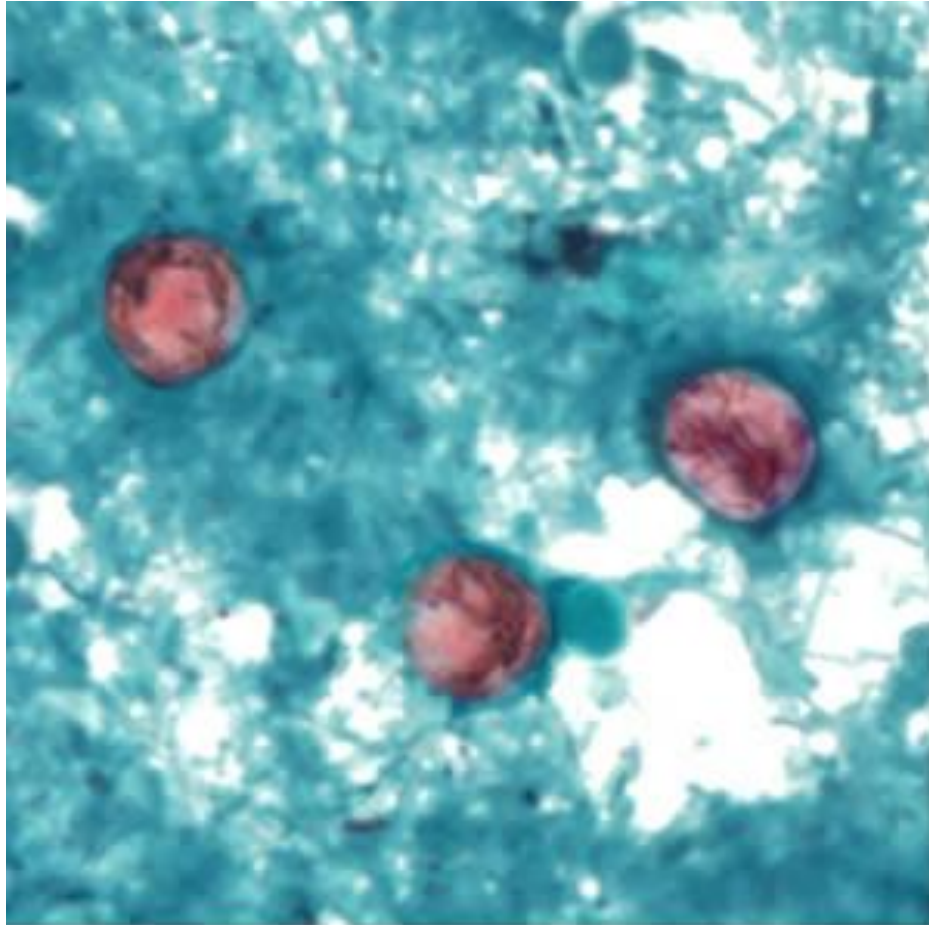
<https://www.cdc.gov/dpdx/cryptosporidiosis/index.html>

Modified Ziehl-Neilson [*Cryptosporidium*]



Oregon State Public Health Laboratory <https://www.cdc.gov/dpdx/cryptosporidiosis/index.html>

Modified safranin [*Cyclospora* & *Cryptosporidium*]

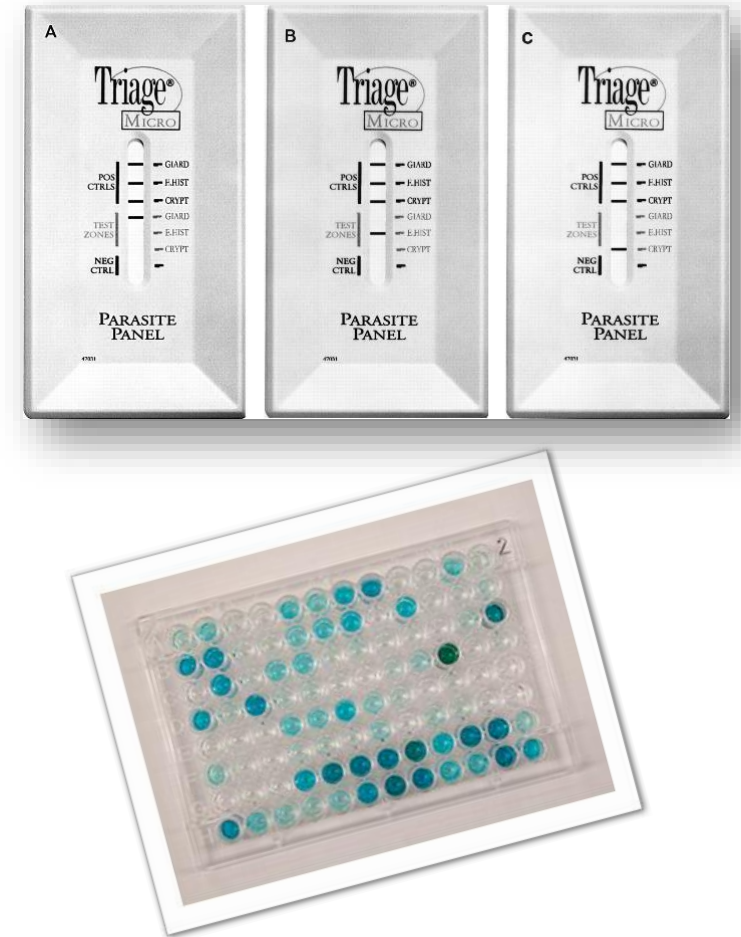


<https://www.cdc.gov/dpdx/cyclosporiasis/index.html>

Fecal antigen EIA (Enzyme Immunoassay)/LFA (lateral flow assay)

Detect antigen shed through stool

- Decreased TAT versus O&P
- High sensitivity, variable specificity
- Detect live & dead organisms
- **Cannot** be used as test of cure



Fecal antigen EIA/LFA

Common screening tests for *Giardia*, *Cryptosporidium*, and *Entamoeba histolytica*

- Especially for domestic diarrheal illness
- Species-specific EIA is best method to identify *E. histolytica* in stool
 - Less sensitive than *E. histolytica/dispar* generic assays

Antigen screening algorithms will miss other pathogenic protozoa

Fecal antigen EIA/LFA

Pros

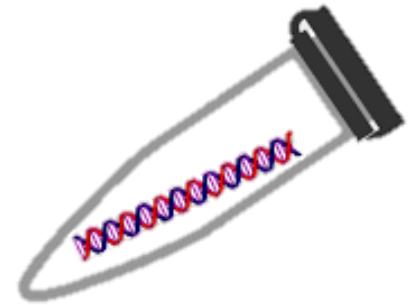
- Inexpensive
- Rapid
- High specificity (mostly)
- More sensitive than microscopy
- Detect common endemic protozoa
- Primary screen for smaller settings w/out parasitology lab

Cons

- Limited spectrum of targets
- No test for *Cyclospora*
- Variable sensitivity
- Unclear “true” performance vs NAAT

Nucleic Acid Amplification testing (NAAT)

- More recent technology (~13 years)
- Common practice in CE-IVD market
- Less embraced in FDA market
- Standalone (few) protozoa (+/- microsporidia)
or
multiplex with bacteria & viruses



NAAT for parasites

Pros

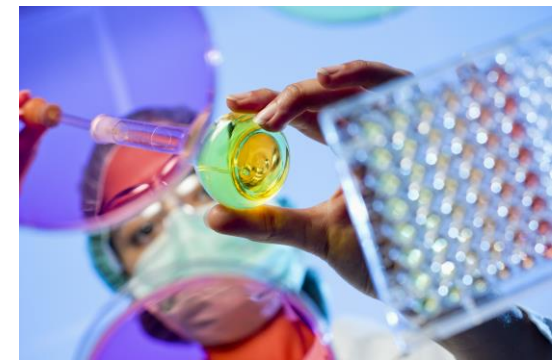
- Rapid
- Very high specificity (mostly)
- More sensitive than microscopy...antigen(?)
- Detect common endemic protozoa
- Primary screen for smaller settings w/out parasitology lab



NAAT for parasites

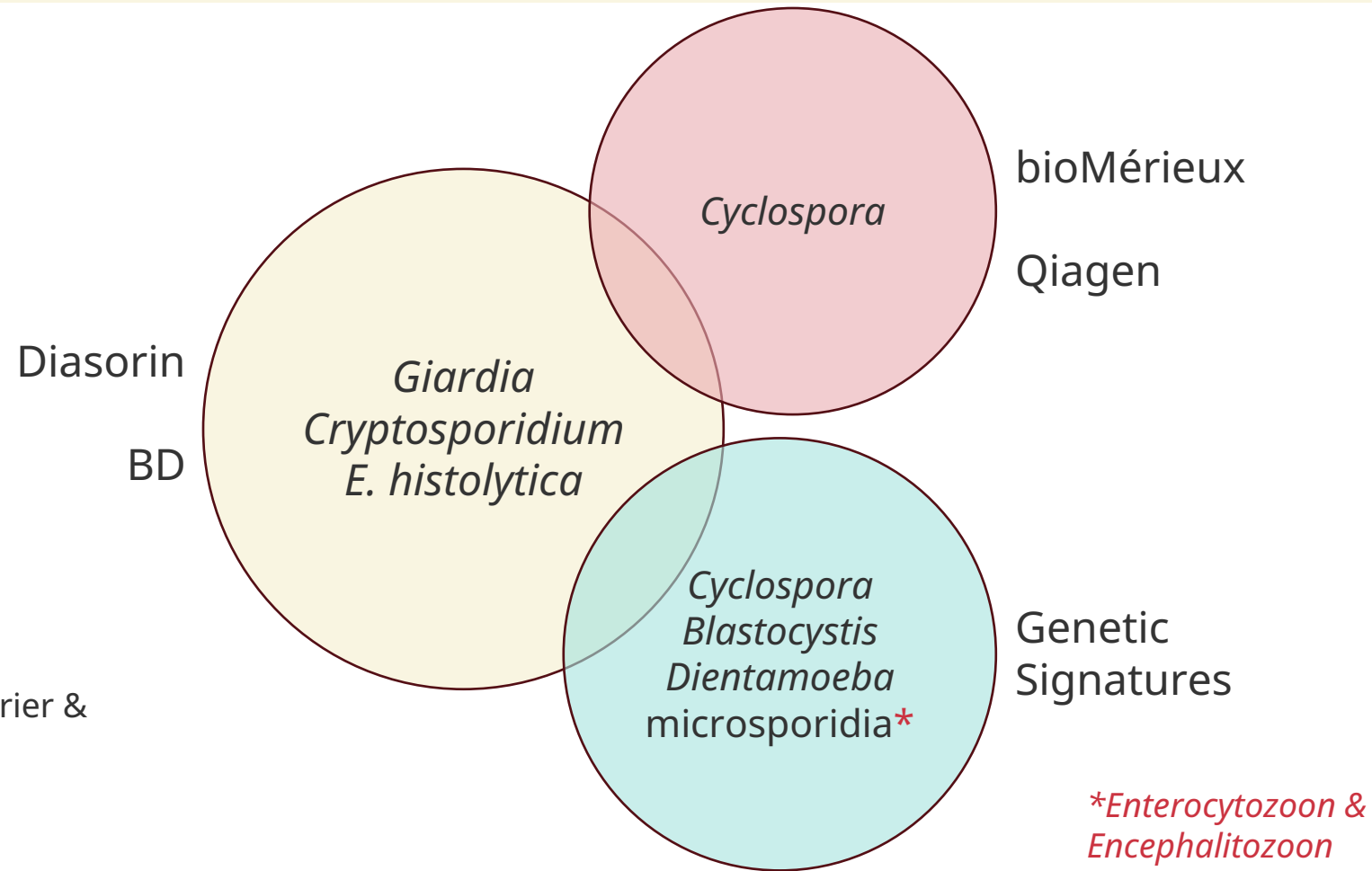
Cons

- Expensive
- May require advanced molecular expertise &/or specialized equipment
- Limited spectrum of targets (most assays)
- Reimbursement issues in USA
- Limited outcomes data
- Challenges with extraction chemistry



Commercially available NAATs

- 5 FDA cleared
- Multiple CE-IVD labeled
- See Manual of Clinical Microbiology, Chapter 143 *Gastrointestinal and Urogenital Protozoa* by Couturier & Mathison for full list of products, targets, and manufacturer/platforms



Digital Slide Scanning and Artificial Intelligence for GI parasites

- Very recent (~6 years)
 - No FDA cleared options
 - Require slide scanner
 - Solutions for trichrome, modified acid-fast, and wet-mount
- (See session tomorrow for full details)

Digital Slide Scanning and Artificial Intelligence for GI parasites



Trichrome stain model

techcyte

Q

Search

DM

Dr. Marc Couturier

Marc Couturier Demo

RUO

EcDfEhartBh

Barcode EcDfEhartBh 2019 Accession New Customer Demo Patient Legacy demo sets - 2019, 2020, 2021

DOB 3/17/2019

μm

Sizes

Confidence

count

total

present

Fecal Smear

0

350

Blastocystis sp.

59

Giardia duodenalis

0

10

Giardia Cyst

8

Giardia Troph

2

Dientamoeba fragilis

141

Endolimax/Iodamoeba Troph

8

Entamoeba hartmanni

0

9

Entamoeba hartmanni Troph

9

Entamoeba sp.

0

87

Dientamoeba fragilis

10.2 μm

11.3 μm

9.4 μm

14.3 μm

11.5 μm

13.5 μm

10.9 μm

9.8 μm

12.2 μm

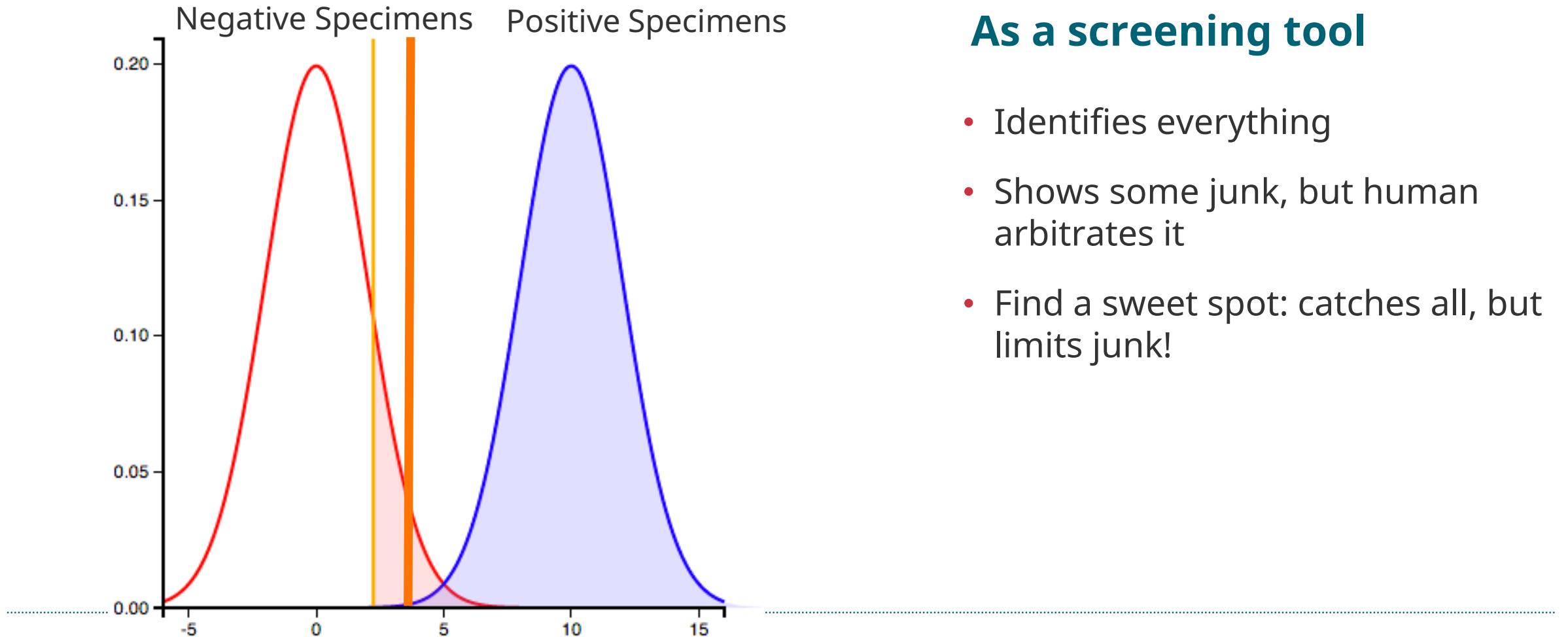
12.5 μm

9.9 μm

10.6 μm

Assessment (8 of 46)

Perfect Sensitivity, Lower Specificity...

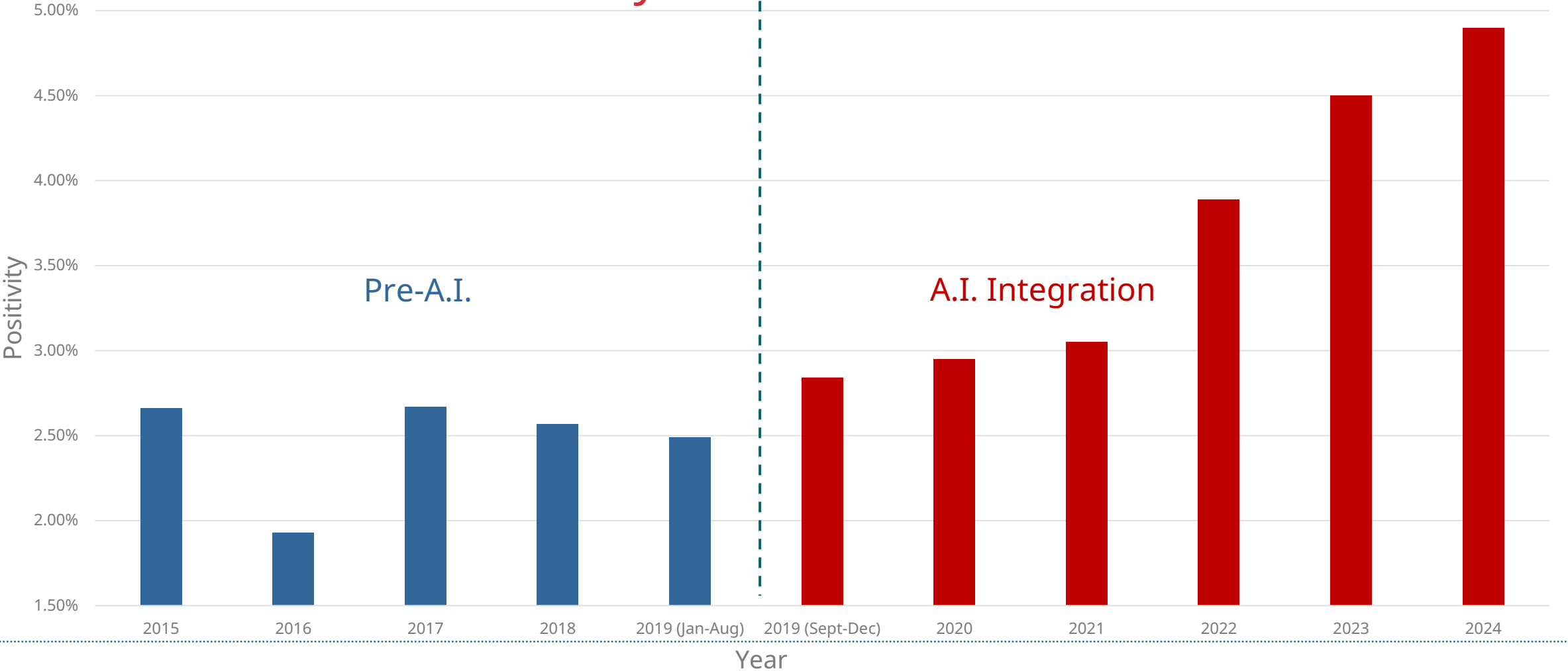


As a screening tool

- Identifies everything
- Shows some junk, but human arbitrates it
- Find a sweet spot: catches all, but limits junk!

(Using Trichrome AI only)

Positivity Rate of O&P Exams



Wet-Mount

Accuracy: Initial

Organism	Positive Accuracy	Negative Accuracy
<i>Dientamoeba fragilis</i>	9/10 (90%)	99/100 (99%)
<i>Giardia duodenalis</i>	10/10 (100%)	100/100 (100%)
<i>Endolimax nana</i>	10/10 (100%)	99/100 (99%)
<i>Cyclospora</i> species	10/10 (100%)	100/100 (100%)
<i>Entamoeba hartmanni</i>	10/10 (100%)	99/100 (99%)
<i>Blastocystis</i> species	15/15 (100%)	100/100 (100%)
<i>Chilomastix mesnili</i>	6/10 (60%)	99/100 (99%)
<i>Entamoeba</i> species	10/10 (100%)	99/100 (99%)
<i>Iodamoeba buetschlii</i>	10/10 (100%)	99/100 (100%)
<i>Cystoisospora belli</i>	10/10 (100%)	100/100 (100%)
<i>Balantiodides coli</i>	10/10 (100%)	100/100 (100%)
<i>Ascaris lumbricoides</i>	9/10 (90%)	100/100 (100%)
<i>Trichuris trichiura</i>	8/10 (80%)	100/100 (100%)
Hookworm/ <i>Trichostrongylus</i> sp.	10/10 (100%)	100/100 (100%)
Fish Tapeworm	9/10 (90%)	100/100 (100%)
<i>Taenia</i> species	10/10 (100%)	100/100 (100%)
<i>Enterobius vermicularis</i>	10/10 (100%)	100/100 (100%)
<i>Strongyloides</i> species	9/10 (100%)	100/100 (100%)
<i>Rodentolepis nana</i>	10/10 (100%)	100/100 (100%)
<i>Hymenolepis diminuta</i>	10/10 (100%)	100/100 (100%)
<i>Schistosoma mansoni</i>	10/10 (100%)	100/100 (100%)
<i>Paracapillaria philippinensis</i>	10/10 (100%)	100/100 (100%)
<i>Paragonimus</i> species	10/10 (100%)	100/100 (100%)
<i>Fasciola</i> sp./ <i>Fasciolopsis buski</i>	10/10 (100%)	100/100 (100%)
<i>Clonorchis</i> / <i>Opisthorchis</i> spp.	10/10 (100%)	100/100 (100%)
<i>Schistosoma japonicum</i>	5/10 (50%)	100/100 (100%)
Total Agreement	250/265 (94.3%)	94/100 (94%)

Mathison et. al.
Accepted, JCM.

Wet Mount

Accuracy: Resolved

66% increased diagnostic yield

Mathison et. al.
Accepted, JCM.

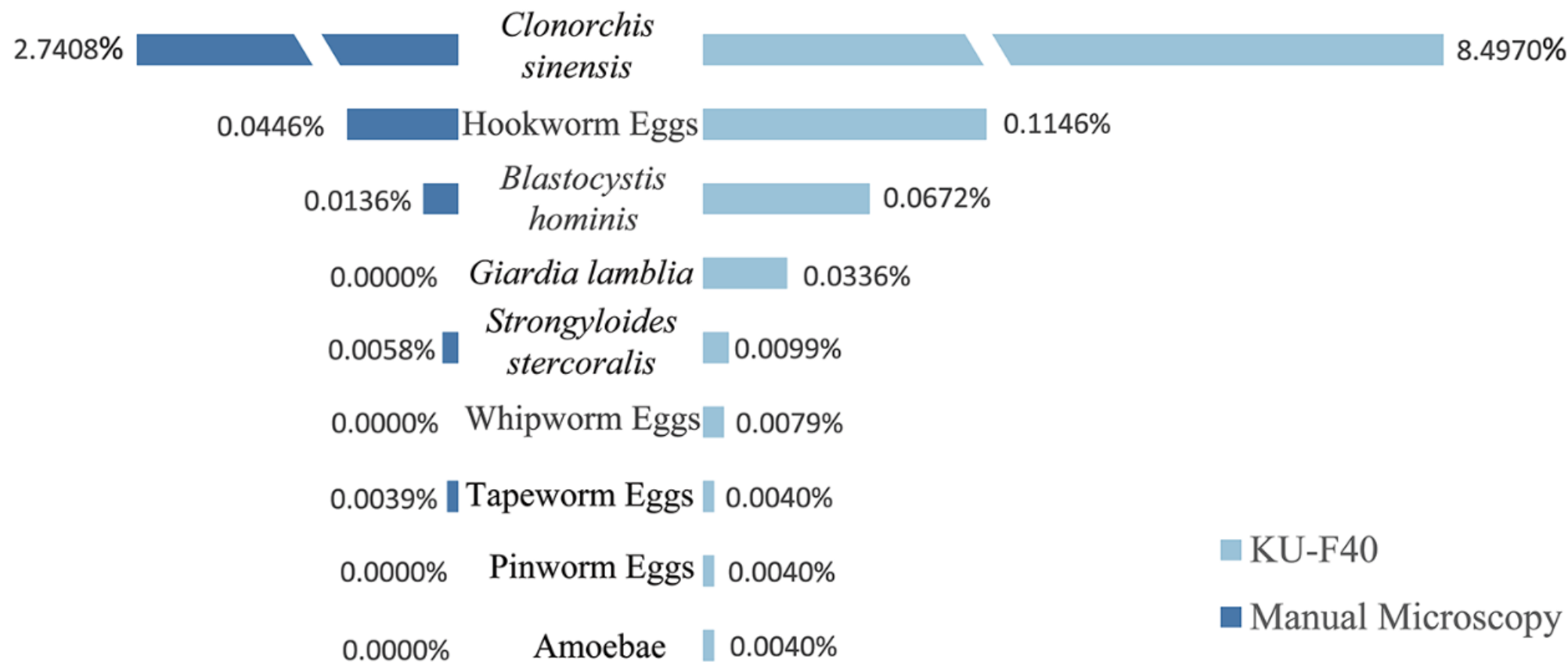
Organism	Positive	Negative
<i>Dientamoeba fragilis</i>	11/11 (100%)	102/103 (99%)
<i>Giardia duodenalis</i>	26/26 (100%)	103/104 (99%)
<i>Endolimax nana</i>	21/21 (100%)	102/103 (99%)
<i>Cyclospora</i> species	11/11 (100%)	103/104 (99%)
<i>Entamoeba hartmanni</i>	12/12 (100%)	102/103 (99%)
<i>Blastocystis</i> species	61/61 (100%)	103/110 (94%)
<i>Chilomastix mesnili</i>	13/16 (81%)	102/104 (98%)
<i>Entamoeba</i> species	49/49 (100%)	102/112 (91%)
<i>Iodamoeba buetschlii</i>	20/20 (100%)	102/104 (98%)
<i>Cystoisospora belli</i>	10/10 (100%)	103/103 (100%)
<i>Balantioides coli</i>	13/13 (100%)	103/103 (100%)
<i>Ascaris lumbricoides</i>	17/18 (95%)	103/104 (99%)
<i>Trichuris trichiura</i>	30/30 (97%)	103/103 (100%)
Hookworm/ <i>Trichostrongylus</i> sp.	26/26 (100%)	103/103 (100%)
Fish Tapeworm	10/10 (100%)	103/103 (100%)
<i>Taenia</i> species	10/10 (100%)	103/103 (100%)
<i>Enterobius vermicularis</i>	11/11 (100%)	103/103 (100%)
<i>Strongyloides</i> species	17/18 (94%)	103/104 (99%)
<i>Rodentolepis nana</i>	13/13 (100%)	103/103 (100%)
<i>Hymenolepis diminuta</i>	10/10 (100%)	103/103 (100%)
<i>Schistosoma mansoni</i>	10/10 (100%)	103/103 (100%)
<i>Paracapillaria philippinensis</i>	10/10 (100%)	103/103 (100%)
<i>Paragonimus</i> species	10/10 (100%)	103/103 (100%)
<i>Fasciola</i> sp./ <i>Fasciolopsis buski</i>	10/10 (100%)	103/103 (100%)
<i>Clonorchis</i> / <i>Opisthorchis</i> spp.	10/10 (100%)	103/103 (100%)
<i>Schistosoma japonicum</i>	9/9 ² (100%)	103/103 (100%)
Misc. Small Protozoans ¹	22/22 (100%)	102/111 (92%)
Total Agreement	472/477 (98.9%)	Variable by target*

KU-F40 Fully Automated Feces Analyzer

- Wet-mount only
- High throughput
- Multiple focal depth imaging
- Flotation and sedimentation
 - +/- iodine
- Currently O-US



KU-F40 System retrospective study



Tornado diagram for parasite (egg) species detection levels by manual microscopy and KU-F40. Note: Due to the significant difference in detection levels between *Clonorchis sinensis* eggs and other parasites (eggs), an open section is used to represent the omitted portion of the actual detection level



Protozoa

Which of the following is a pathogen?

- *Balantioides coli*
- *Entamoeba histolytica*
- *Entamoeba hartmannii*
- *Entamoeba coli*
- *Entamoeba dispar*
- *Dientamoeba fragilis*
- *Chilomastix mesnili*
- *Giardia duodenalis*
- *Iodamoeba butschlii*
- *Cryptosporidium parvum*
- *Endolimax nana*
- *Cystoisospora belli*
- *Cyclospora* spp.
- *Blastocystis*

Which of the following is a pathogen?

- *Balantioides coli*
- *Entamoeba histolytica*
- *Entamoeba hartmannii*
- *Entamoeba coli*
- *Entamoeba dispar*
- *Dientamoeba fragilis*
- *Chilomastix mesnili*
- *Giardia duodenalis*
- *Iodamoeba butschlii*
- *Cryptosporidium parvum*
- *Endolimax nana*
- *Cystoisospora belli*
- *Cyclospora* spp.
- *Blastocystis*

Pathogen
Questionable
Avirulent

Case

A weekend in the woods

- 22 yo male, returned from hiking Mt. Katahdin
- Ate dehydrated meals prepared w/ boiled H₂O
- Drank filtered water
- Swam in Chimney Pond
- Slept in a lean-to with open walls
 - No tick bites, multiple mosquito bites
 - No animal exposures
- No new sexual partners (heterosexual/monogamous)



Case 1

A weekend in the woods

- Developed acute onset cramping 5 days later
- Diarrhea 5-6 times a day
- Foul smelling stool and belching
- Resolves for a day or two then starts again

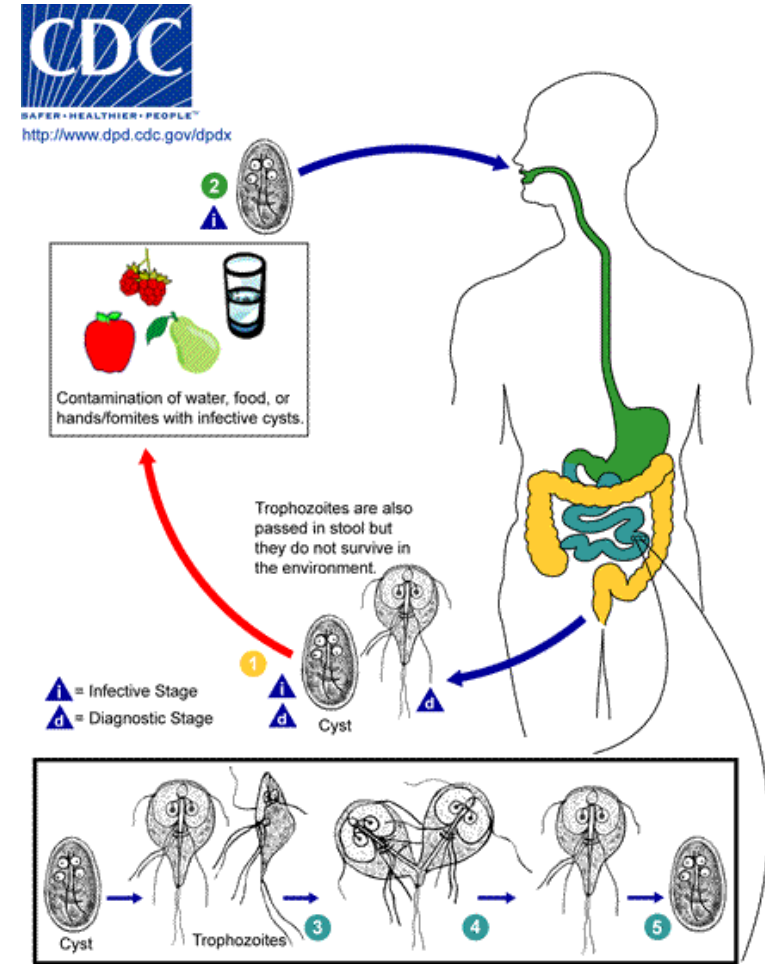
What are you thinking?



Giardia duodenalis

Symptoms & Epi

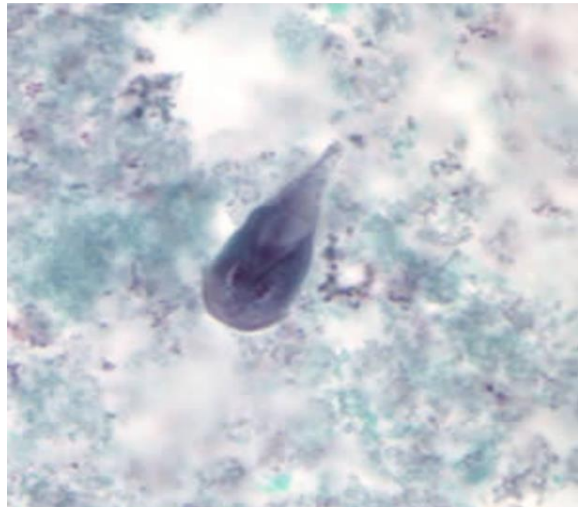
- Asymptomatic → Mild → Severe symptoms
- Diarrhea
Malabsorption
Abdominal pain/Bloating (Sulphurous belches)
Nausea/Vomiting
 - 1-3 weeks
- Can become chronic
- Worldwide distribution



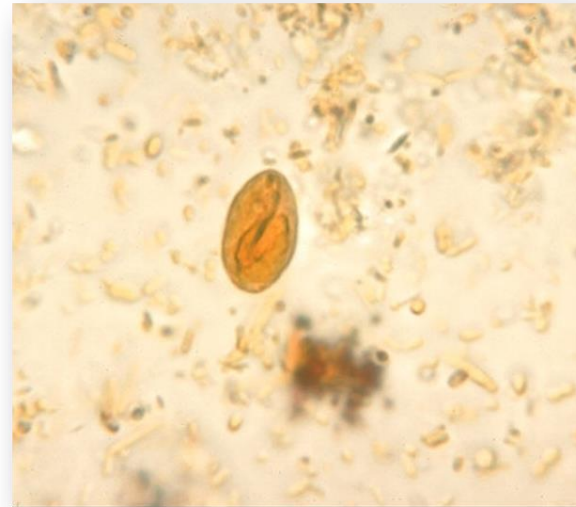
Giardia duodenalis

Diagnostic features & testing

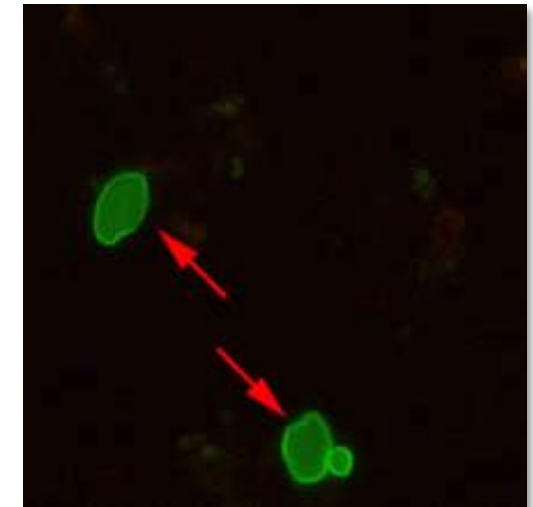
- O&P
- DFA
- Antigen
- NAAT
- Histopath



Trophozoite (10-20 μ m)



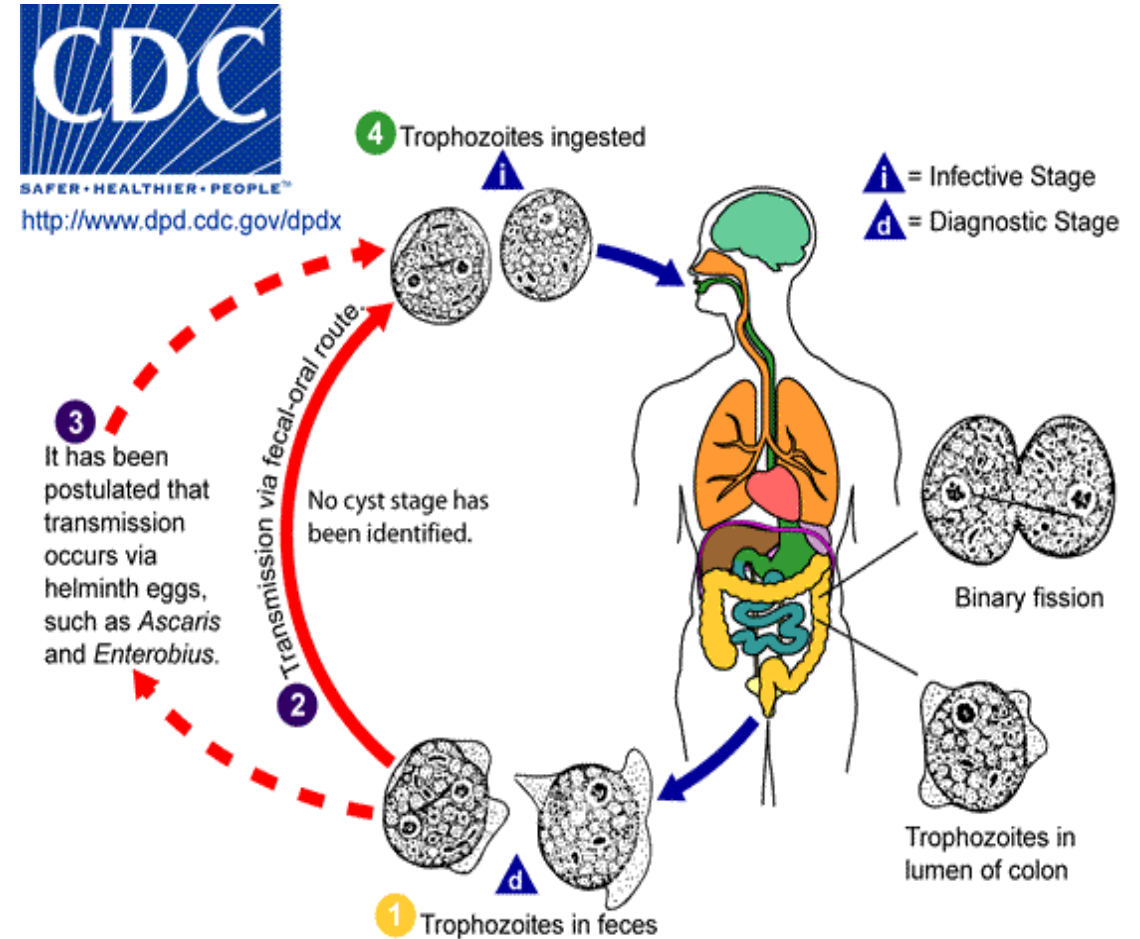
Cyst (10-14 μ m)



Dientamoeba fragilis

Symptoms & Epi

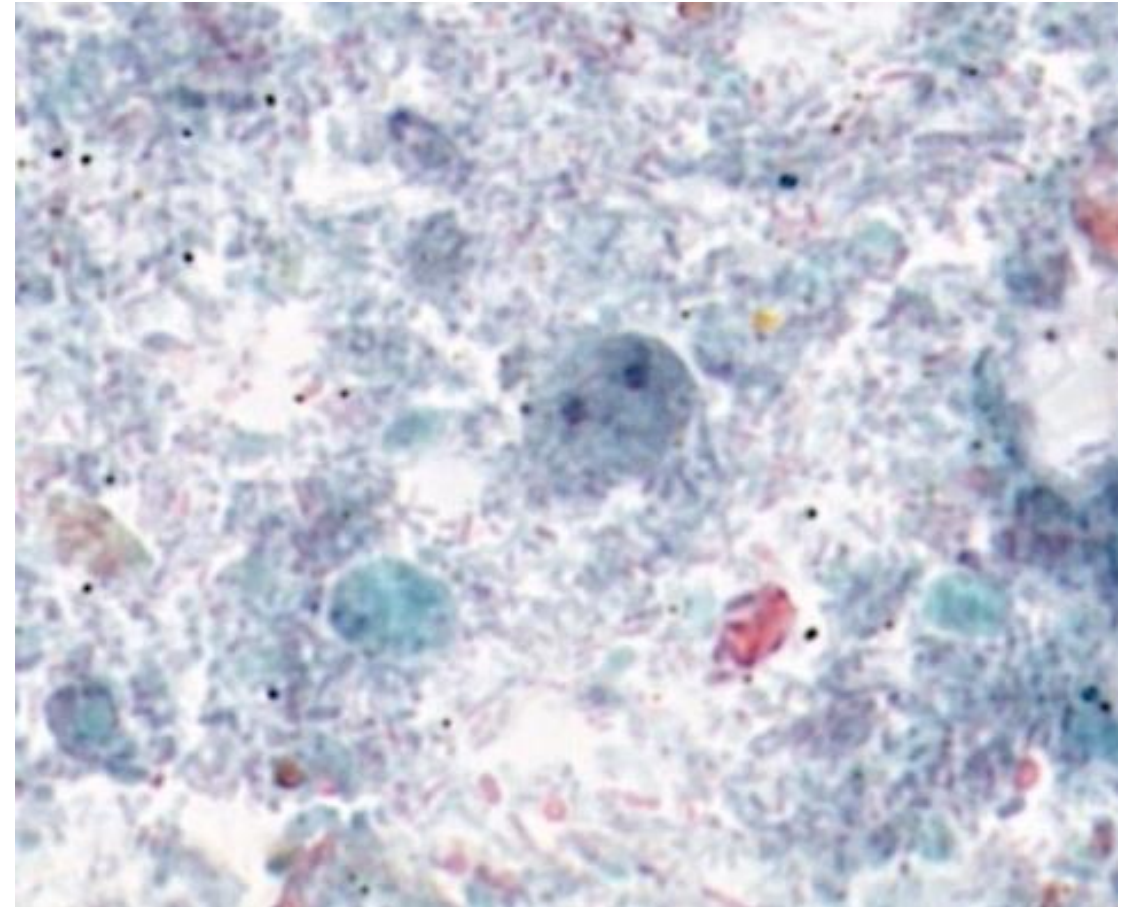
- Pediatric skew in symptomatic patients
- Intermittent diarrhea
Abdominal pain
Nausea
Fatigue/malaise
Anorexia/poor weight gain
OR asymptomatic
- Worldwide distribution



Dientamoeba fragilis

Diagnostic features & testing

- Mono- or binucleated, flagellate (not visible)
 - Fragmented nuclei (tetrad)
- Commonly identified protozoal pathogen
 - True for developed countries
- Detect by O&P or NAAT
(only Genetic Signatures)



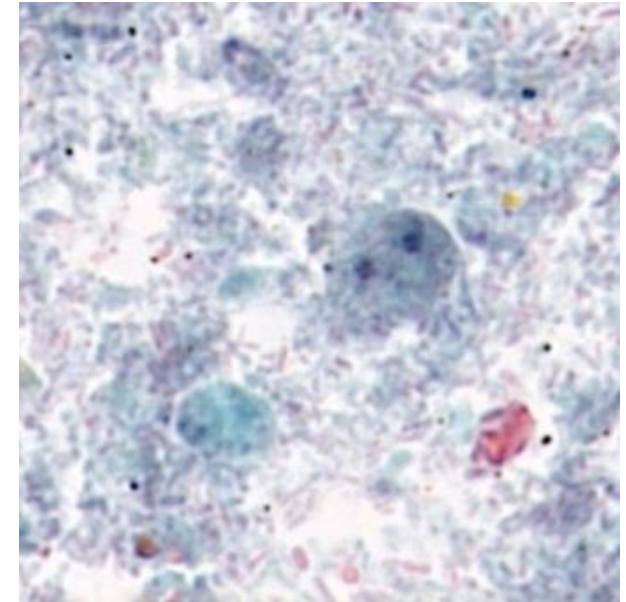
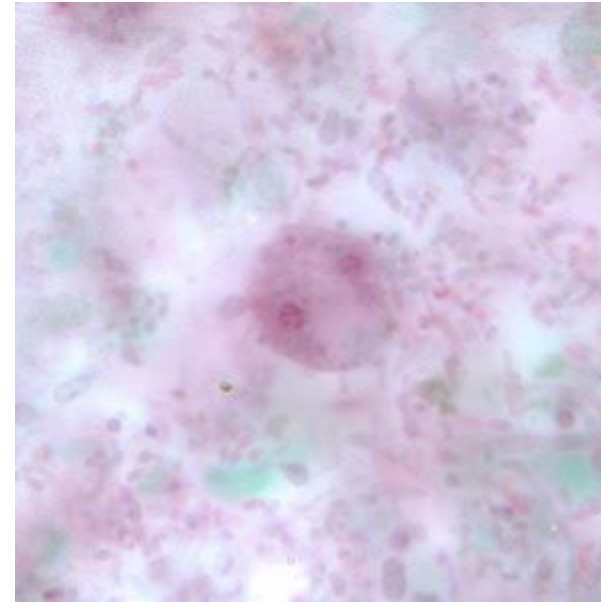
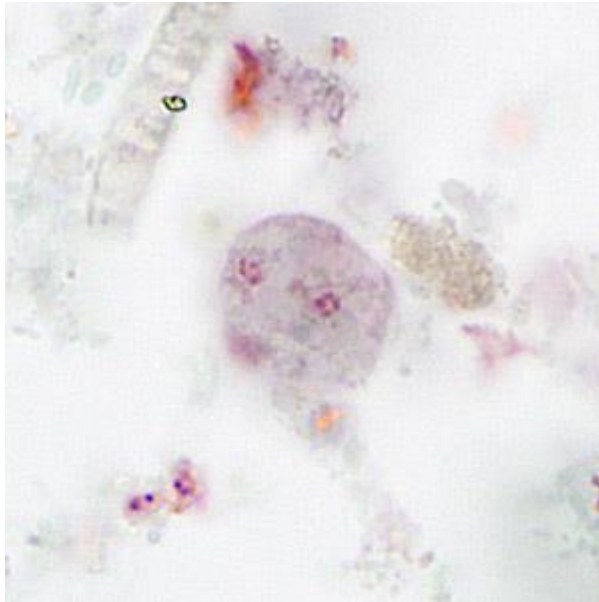
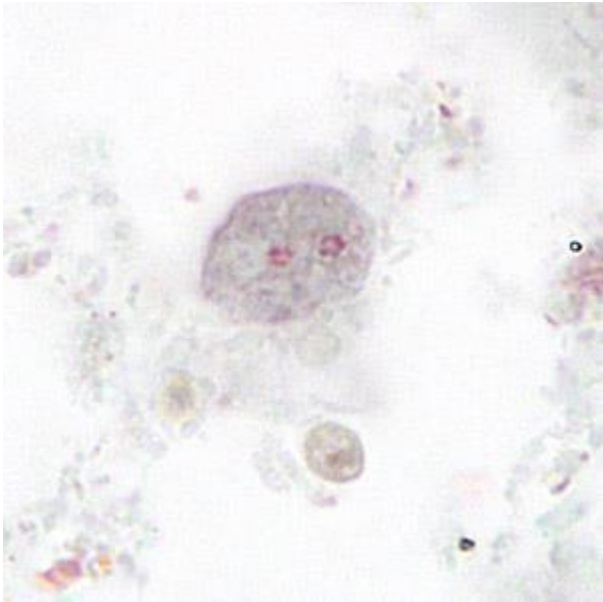
DOGMA:

Dientamoeba fragilis always has two nuclei and no cyst

- False...and maybe?
- *D. frag* typically has two nuclei...but not always
- Nuclei are “tetrad”/fragmented...but not always
- Cytoplasm is fuzzy, often with many ingested bacteria

Dientamoeba fragilis always has two nuclei

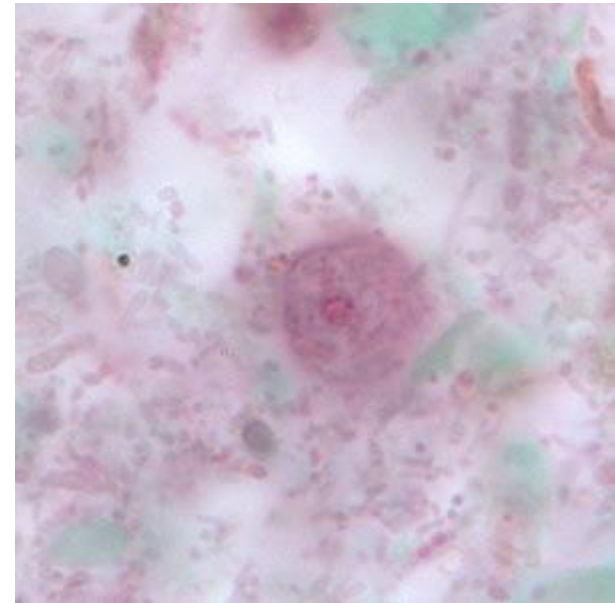
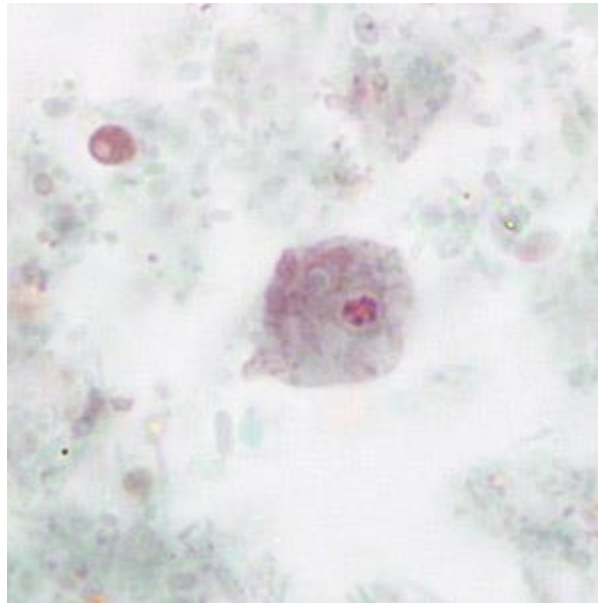
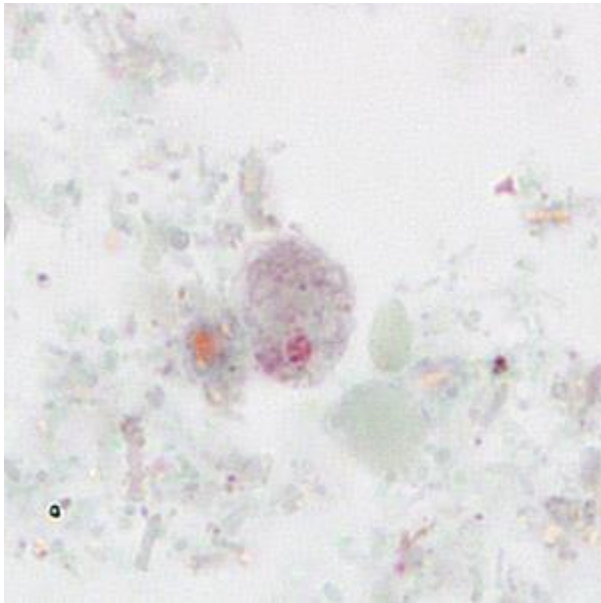
- Two nuclei, tetrad forms



<https://www.cdc.gov/dpdx/dientamoeba/index.html>

Dientamoeba fragilis always has two nuclei

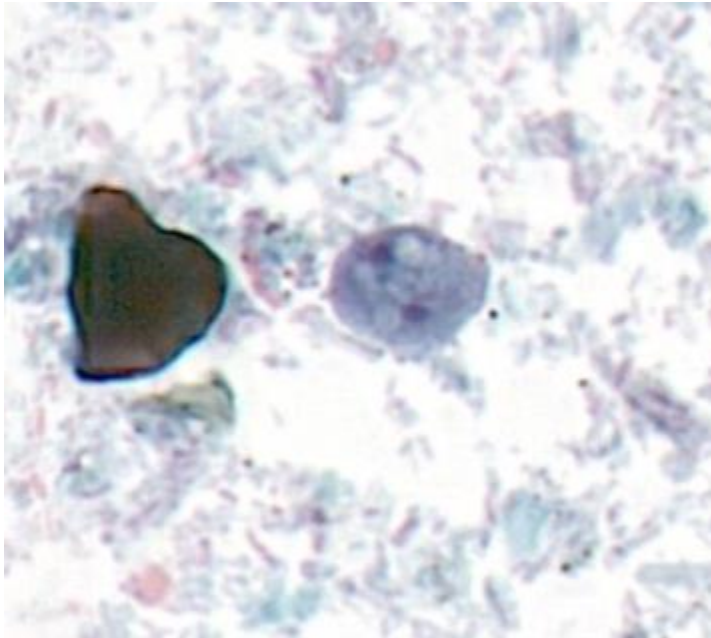
- Uninucleate, tetrad forms



<https://www.cdc.gov/dpdx/dientamoeba/index.html>

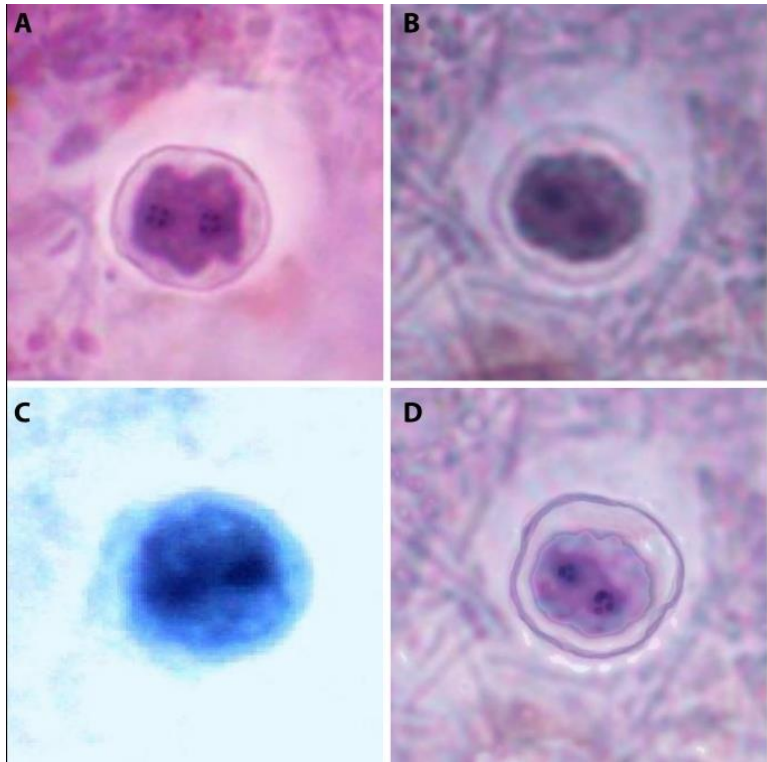
Dientamoeba fragilis always has two nuclei

- Non-tetrad forms

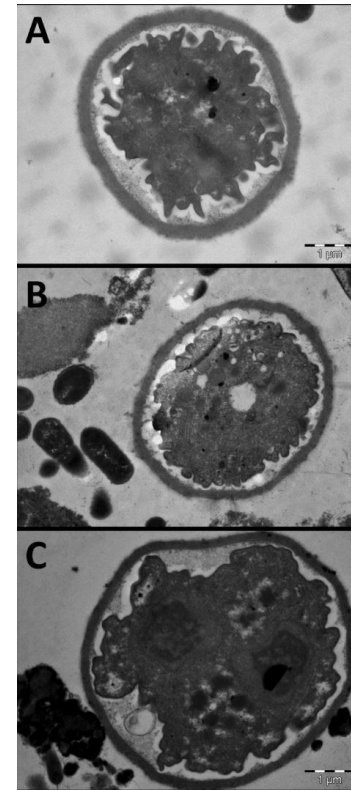


Dientamoeba fragilis has no cyst

Evidence for cysts



From human stool



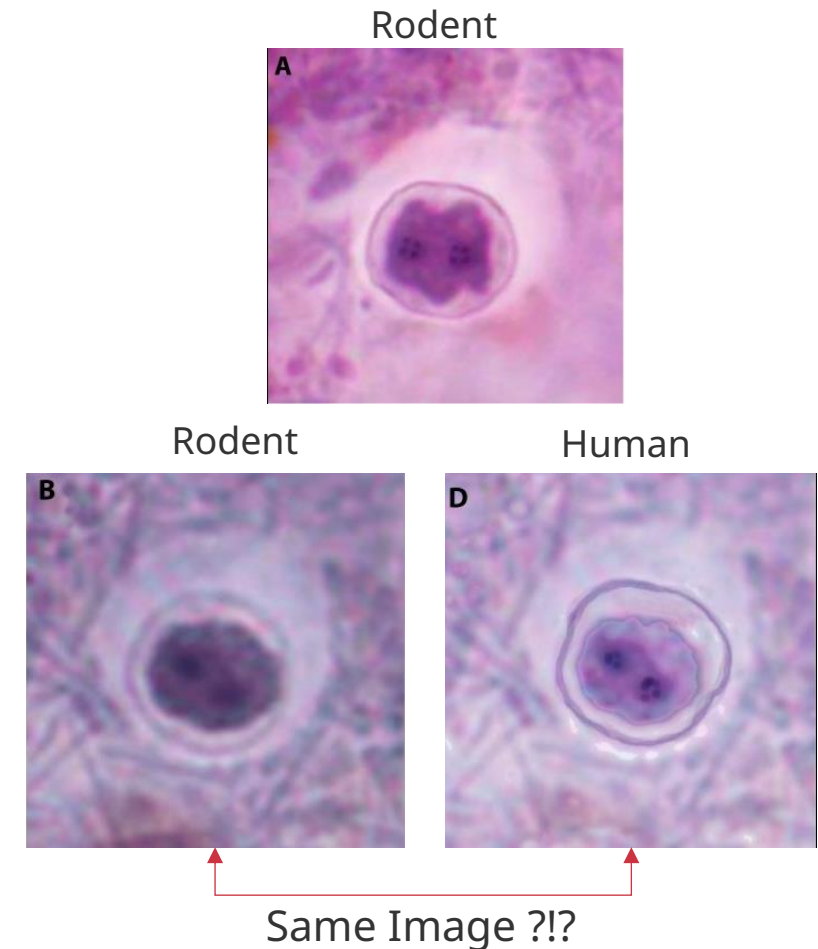
From lab mice

Stark D et al. Clin Microbiol Rev. 2016 Jul;29(3):553-80.

Dientamoeba fragilis has no cyst

Evidence against cysts

- Order Trichomonadida do not form cysts
 - *D. fragilis* would be the only evolutionary exception?
- *D. frag* = Common in +O&Ps in dev. world
 - Why don't we see these then?
 - 0/~200+
 - AI has not captured
 - Photo manipulation (?)
 - Anyone else??



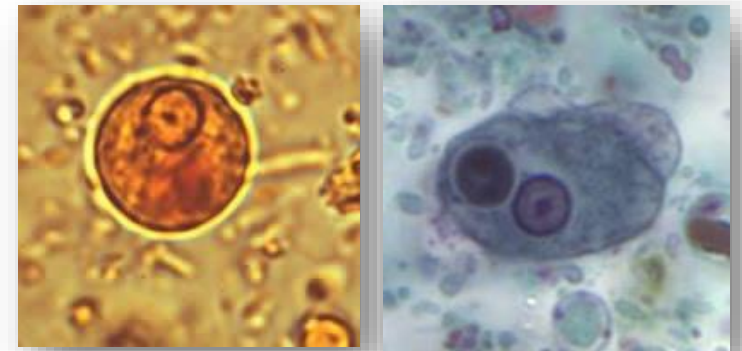
Stark D et al. Clin Microbiol Rev. 2016 Jul;29(3):553-80.

Case 2

An unexpected journey

- 21 yo male, recently started college school year
- Presented to clinic with diarrhea, abdominal pain, fever, and possible blood in stool
- No travel history
- No antibiotics
- Regular diet, nothing exotic
- Drank only tap water
- Single, multiple new sexual partners
- No animal exposures

What are you thinking?



Case 2

An unexpected journey

- Recently became fraternity pledge
- Attended a party and admitted to having sex with a female sex worker from Central America
- Patient admitted to performing cunilingus, anilingus, vaginal, and anal sex with the woman
 - Wore barrier prophylaxis during insertive sex

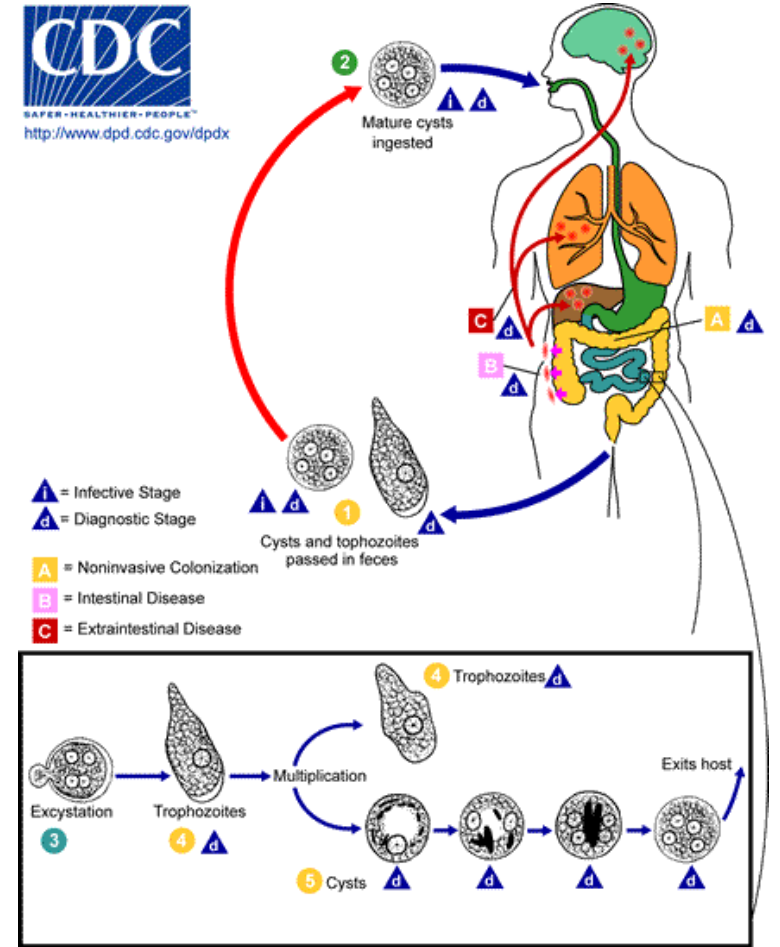


What are you thinking?

Entamoeba histolytica

Symptoms & Epi

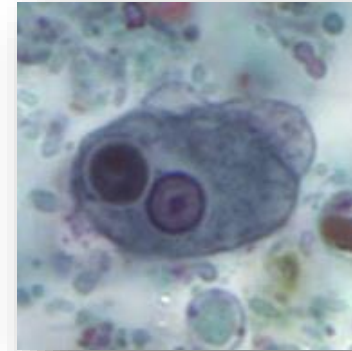
- Amoebiasis
 - Diarrhea in most cases with cramping
 - Asymptomatic
 - Amoebic dysentery
 - Fever
 - Bloody stool
 - Severe stomach pain
 - Amoebic liver abscess
 - Brain, lung
 - Worldwide, more common with poor sanitation
- Can be fatal



Entamoeba histolytica

Diagnostic features

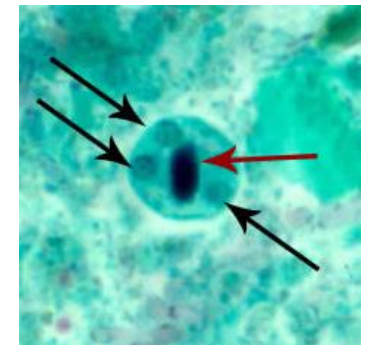
- Cannot separate from *E. dispar* unless RBCs ingested
- Trophozoites
 - Nuclei features
 - “Central” chromatin dot
 - Smooth peripheral chromatin
 - Cytoplasm “clean”, granular
- Cysts
 - 4 or less nuclei
 - Bluntly rounded, few chromatoid bodies



Troph 15-20 μm
(range 10-60 μm)



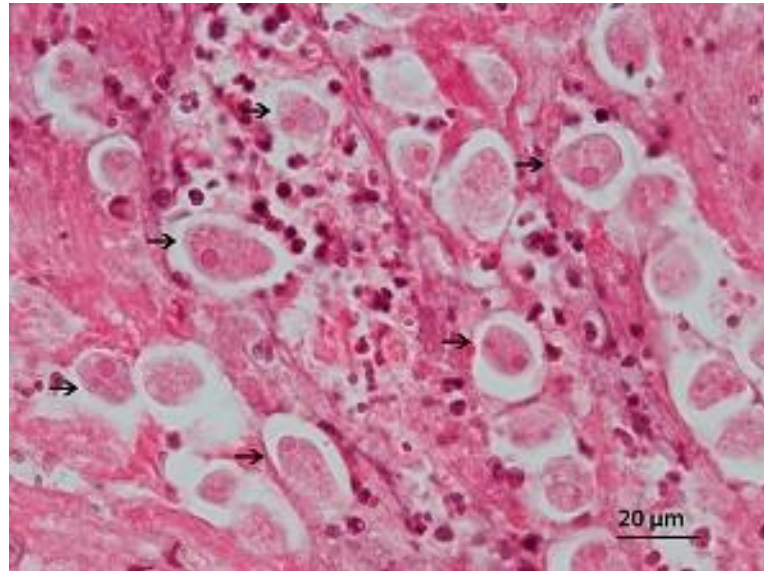
Cyst 12-15 μm



Entamoeba histolytica

Testing

- Amoebiasis
 - O&P
 - Antigen
 - NAAT
 - Histopathology

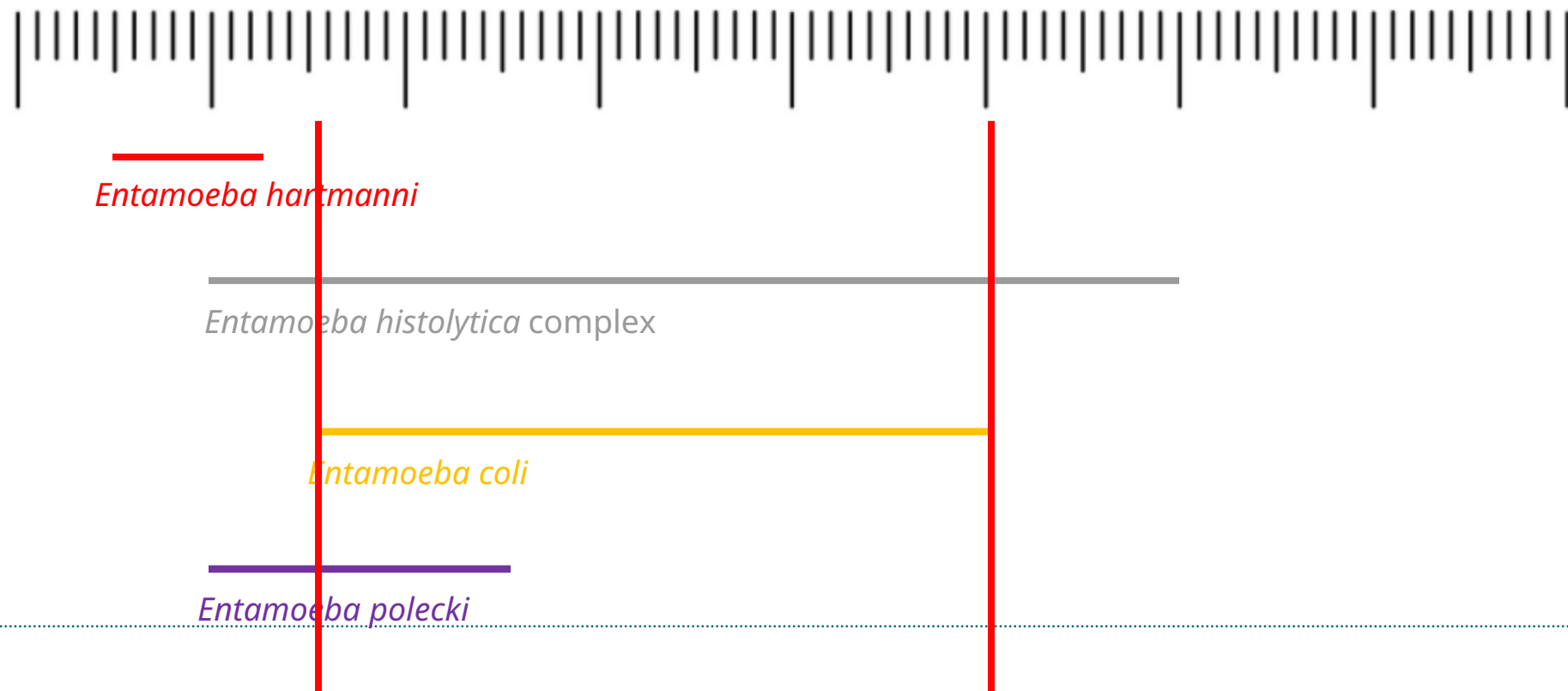


<https://www.cdc.gov/dpdx/amebiasis/index.html>

- Amoebic liver abscess
 - IgG antibody
 - Imaging
 - NAAT (abscess fluid)
 - Histopathology

DOGMA: Size measurements can separate *Entamoeba coli* vs *E. histolytica*

- False...beware overlap



Entamoeba spp. identification

- Trophozoites are pleomorphic & variable size/shape
- Overlap in morphologic features among different species
 - Size
 - Nuclear structure
- Delay in processing affects all features
- Variability in morphology due to the fixative and/or stain used

Entamoeba spp. identification

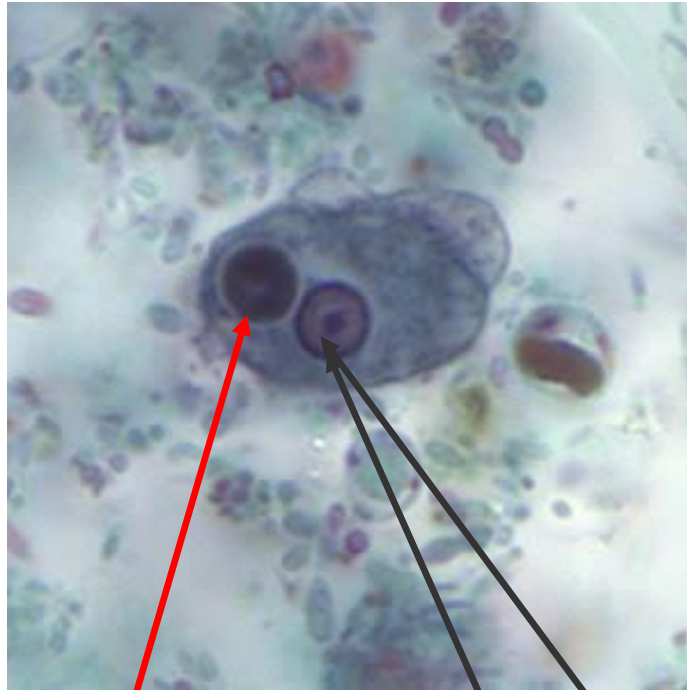
- Size
 - Consistently less than 10 μm = *E. hartmanni*
- Nuclear structure
 - Peripheral chromatin
 - Karyosome
- Cytoplasm consistency
 - Be cautious of vacuolation due to a delay in processing!
- Mature cysts
 - Nucleus number, form of chromatoid bodies



Madison Sant and Blaine Mathison

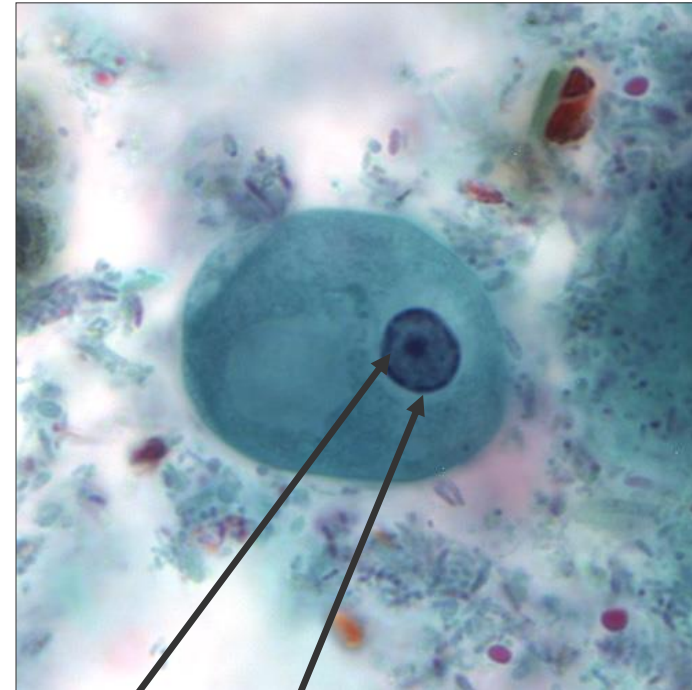
Entamoeba species – nuclear structure

Entamoeba histolytica complex



Ingested erythrocyte!

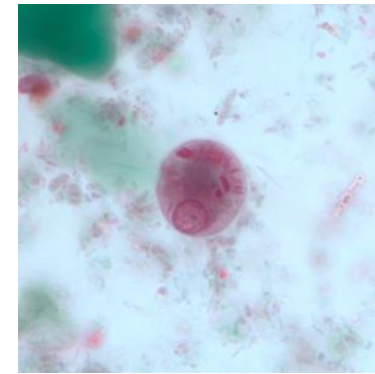
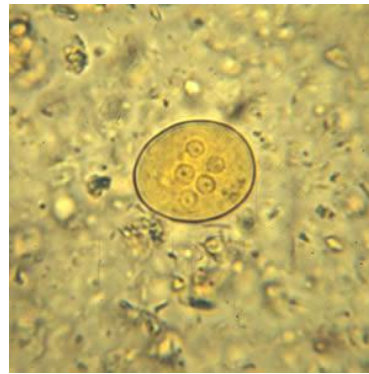
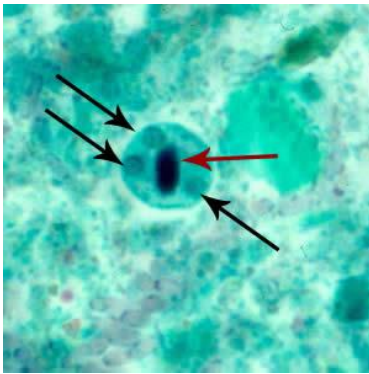
Entamoeba coli



karyosome
peripheral chromatin

Entamoeba spp. – mature cysts

Species	Number of Nuclei	Chromatoid Bodies
<i>Entamoeba hartmanni</i>	4	Bluntly rounded, few
<i>Entamoeba histolytica</i> – complex	4	Bluntly rounded, few
<i>Entamoeba coli</i>	≥ 8	Fragmented ends, few to several
<i>Entamoeba polecki</i>	1 (rarely 2)	Highly variable in size and form, many present

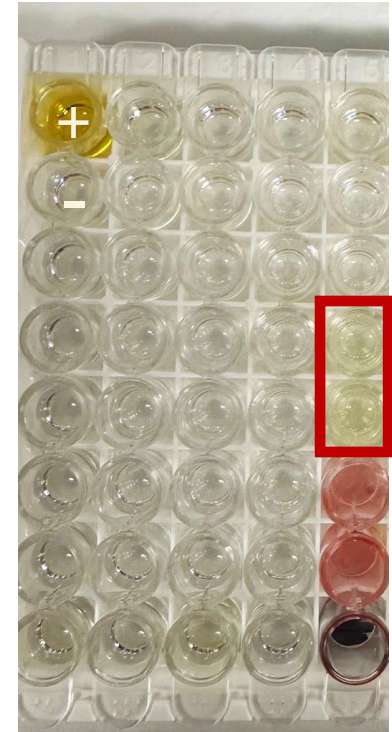


Case 3

Microscopy for the win

- 25 yo male w/PMH HIV (uncontrolled)
 - No prior HAART
 - CD4 ↓, viral load ↑
- Recent immigrant from Mexico
- 2 weeks watery, severe diarrhea
- Multiplex protozoa PCR performed
 - Negative: *Giardia*, *Cryptosporidium*, *E. histolytica*
- Antigen for *Cryptosporidium*, negative...

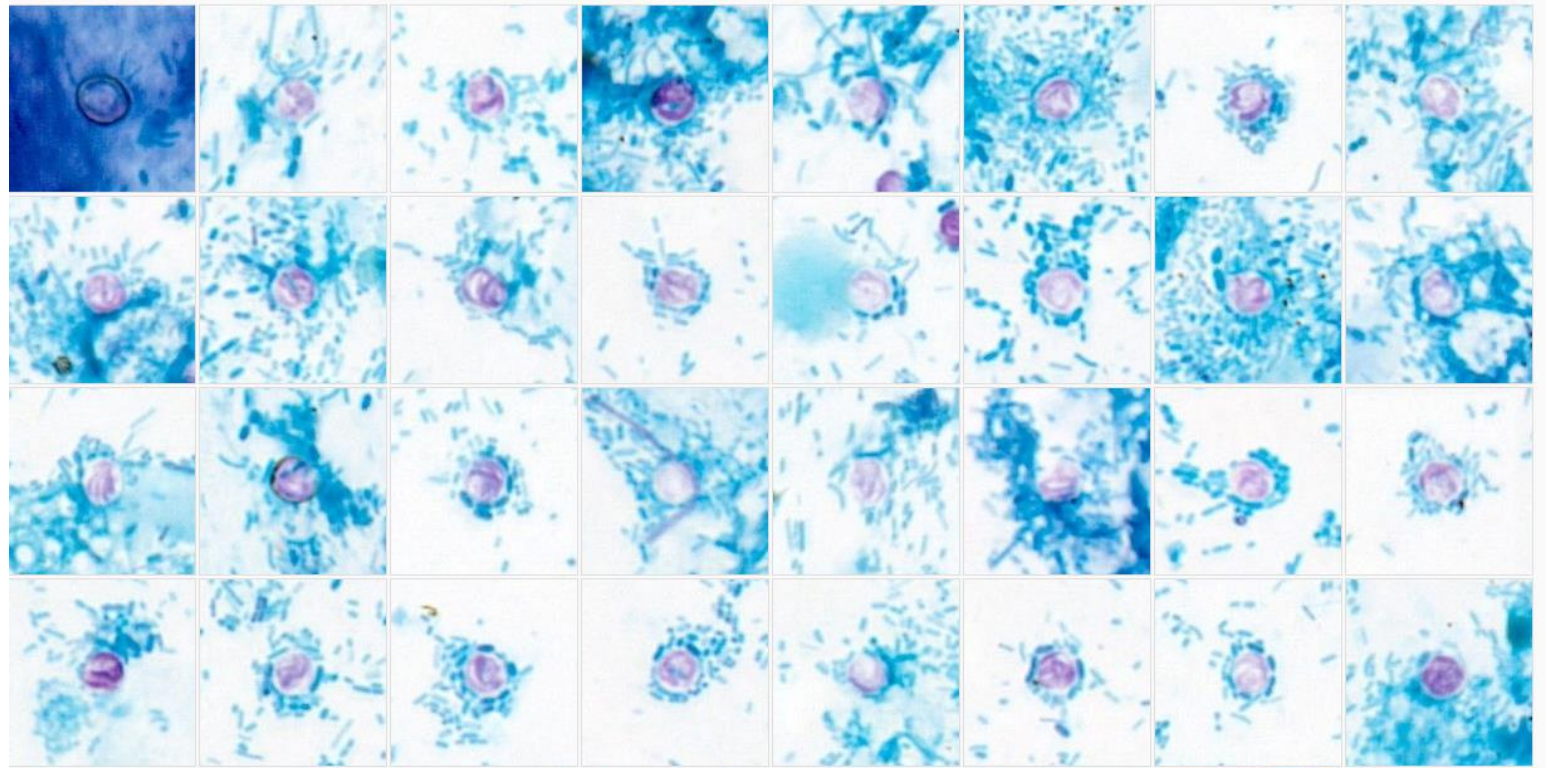
What are you thinking?



Case 3

Is it *Cyclospora*?

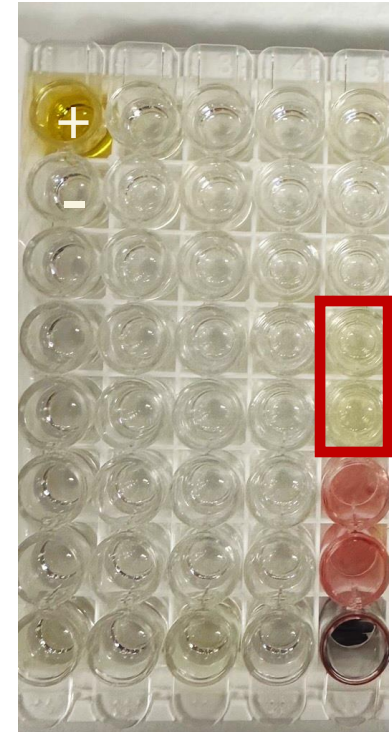
- Patient has risk factors
- Epi is possible
- Symptoms possible
- Modified acid fast by AI
 - 4-6 μm organisms
- What is this?



Cryptosporidium spp.

How?

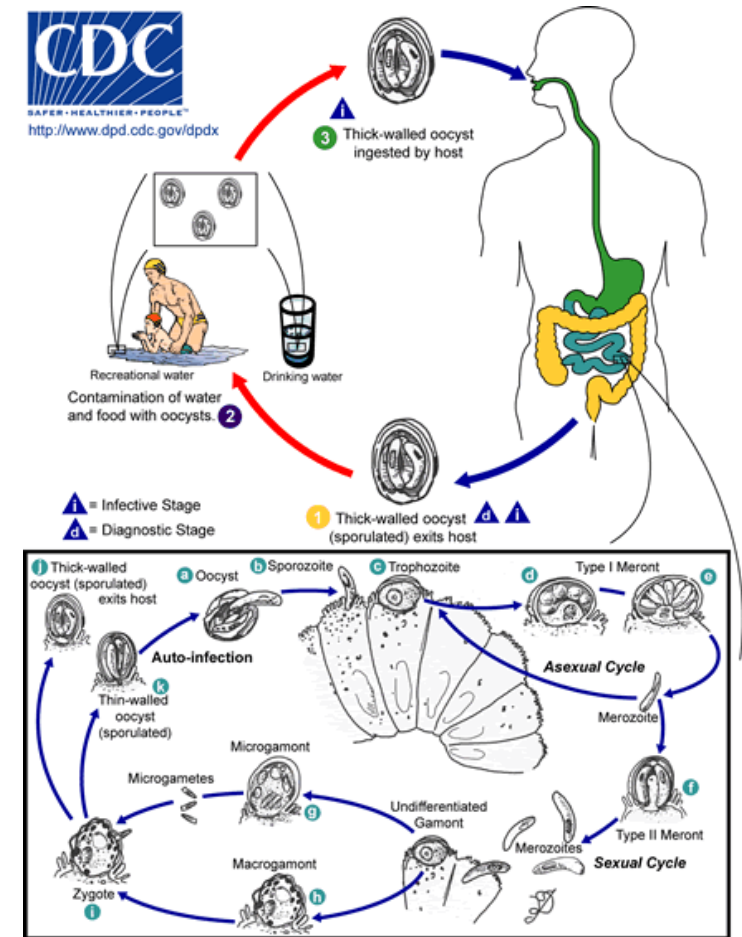
- Commercial PCR only detects *C. parvum/hominis*
- Antigen test most sensitive for *C. parvum/hominis*
 - Some reactivity just below cutoff
 - Crossreactivity?
- Likely zoonotic *Cryptosporidium* sp.
 - E.g. *C. canis*, *C. felis*, *C. meleagridis*, *C. chipmunk* genotype 1



Cryptosporidium spp.

Symptoms & Epi

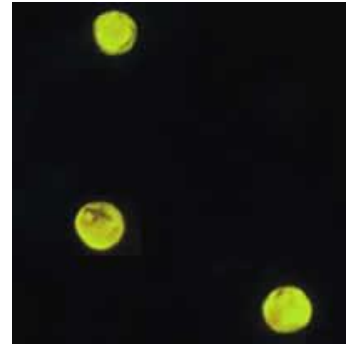
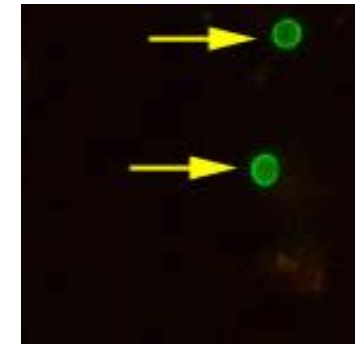
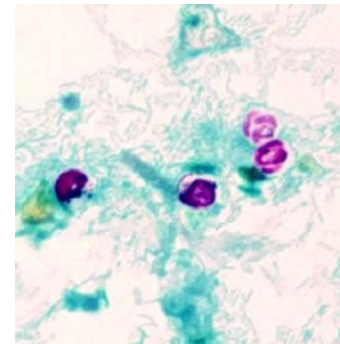
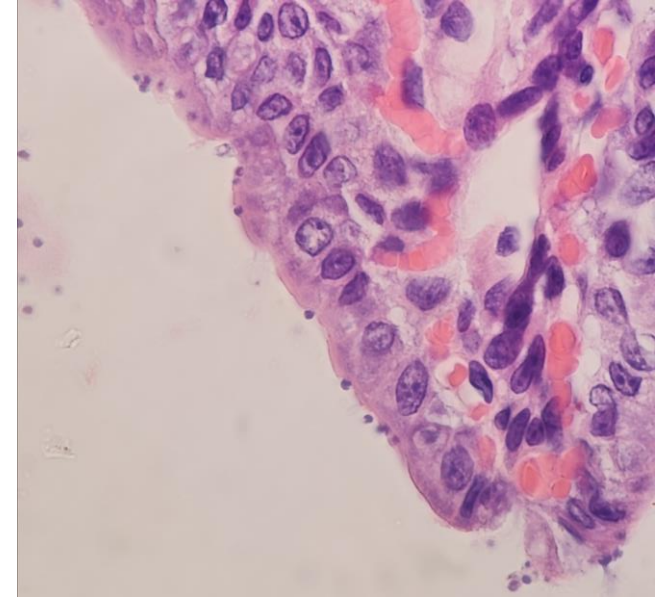
- Watery diarrhea (1-2 weeks); shed 2 weeks
- Nausea/cramps
Dehydration/weight loss
Vomiting
Asymptomatic
- Immunocompromised can shed for > month
 - Can be deadly
- Oocysts immediately infective when shed
- Worldwide distribution



Cryptosporidium spp.

Diagnostic features & testing

- Light Microscopy – Modified AF, safranin
 - Oocysts (4-6 μm) may see up to 4 sporozoites
- Fluorescent microscopy
 - Auramine-rhodamine
 - DFA
- Antigen
- NAAT
- Histopath



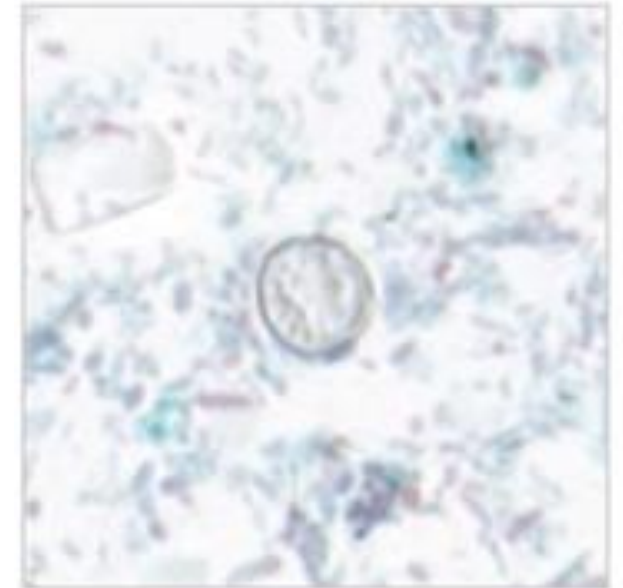
<https://www.cdc.gov/dpdx/cryptosporidiosis/index.html>

Case 4

Salad and suffering

- 50 yo female phm colon cancer, lives in Park City, UT
 - No tx for 6 years
- No recent travel, no Abx
- 30 days prior, rapid onset diarrhea
 - Voluminous, watery, 5-6X per day
 - Cramping, pain, bloating
 - No blood, mucous, or fever
- Noted recent diet started of only salads ~45 days prior
- O&P x1 ordered

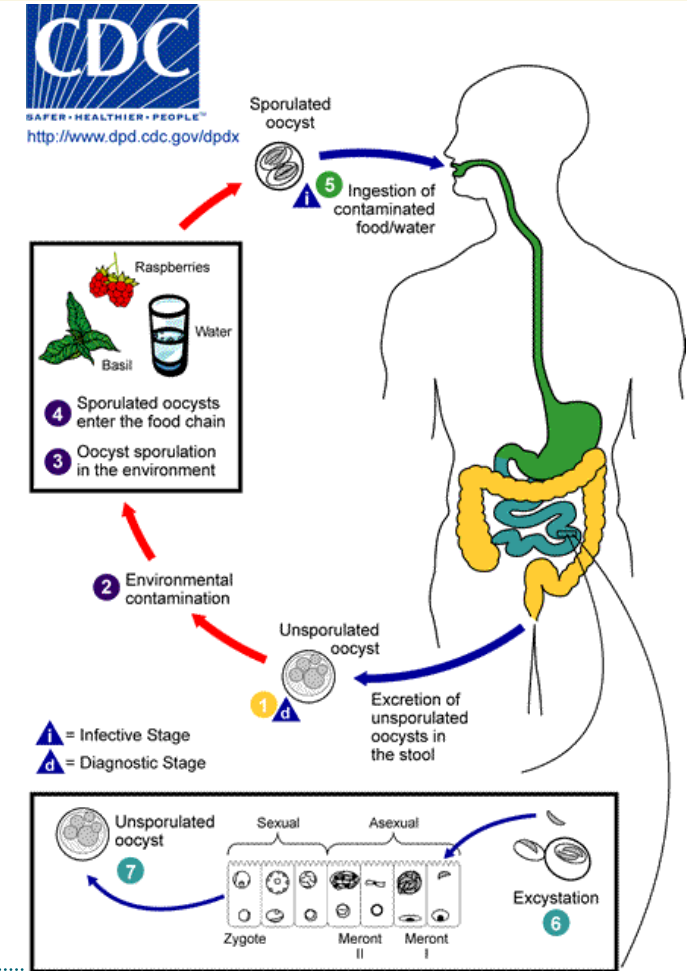
What are you thinking?



Cyclospora spp.

Symptoms & Epi

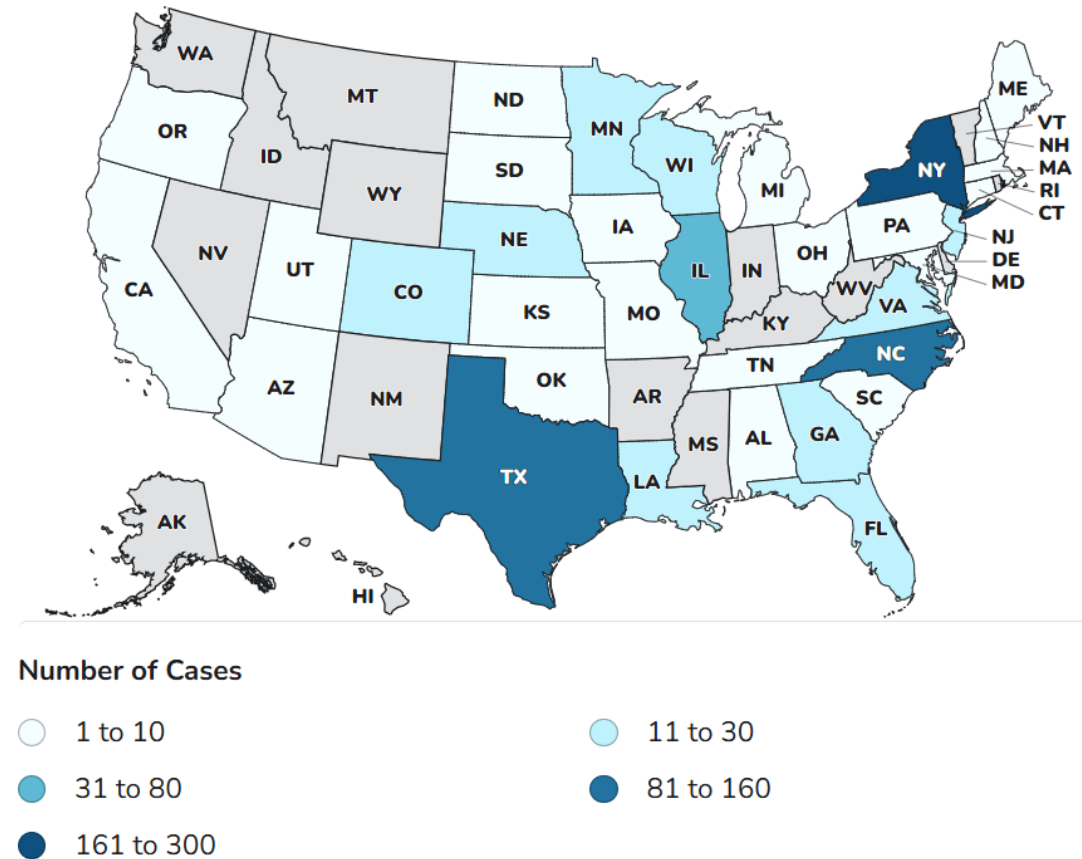
- Watery diarrhea
(can last months if untreated regardless of immune status))
- Cramping/nausea
Weight loss/loss of appetite
Gas/bloating
Fatigue
Asymptomatic
- Vomiting & low fever (rarely)
- Oocysts not infective when shed
- Tropical/subtropical



Cyclospora spp.

Outbreaks and seasonal illness

- 2025 CDC data
- 752 reported & confirmed domestic cases
- Annual outbreaks reported since 2013
 - Sources often not identified
 - Previous sources include:
 - Cilantro
 - Raspberries
 - Basil
 - Lettuce

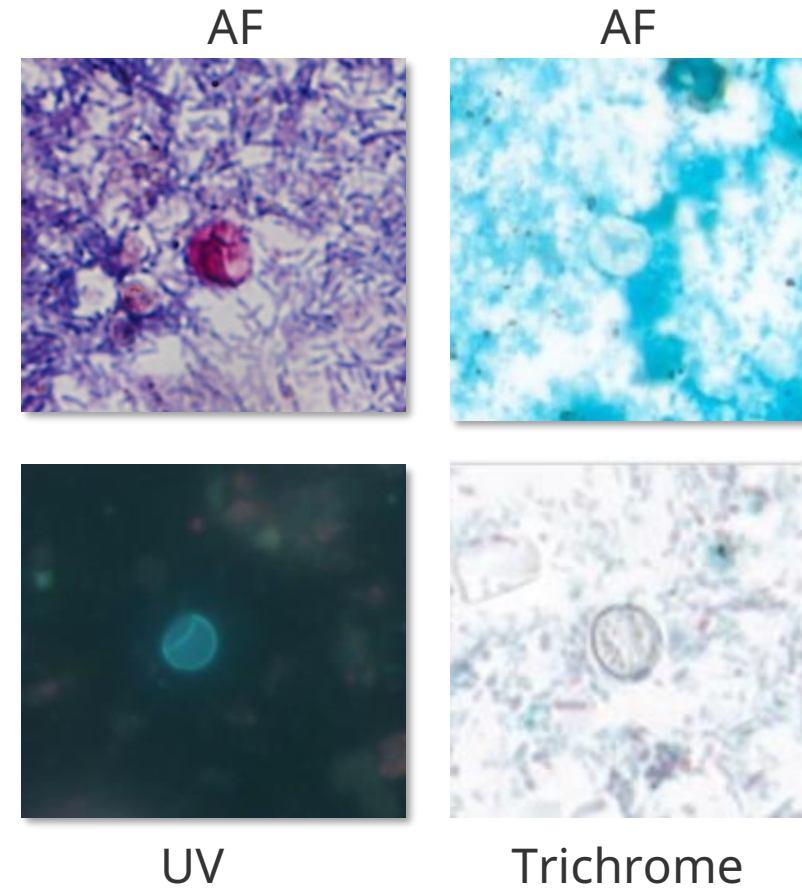


https://www.cdc.gov/cyclosporiasis/php/surveillance/index.html#cdc_generic_section_2-surveillance

Cyclospora spp.

Diagnostic features & testing

- Microscopy
 - 8-10 μm oocysts, “wrinkled plastic”, thick cell wall
 - Modified AF & safranin
 - AI can detect in trichrome
- NAAT
- Histopathology
- UV autofluorescent microscopy

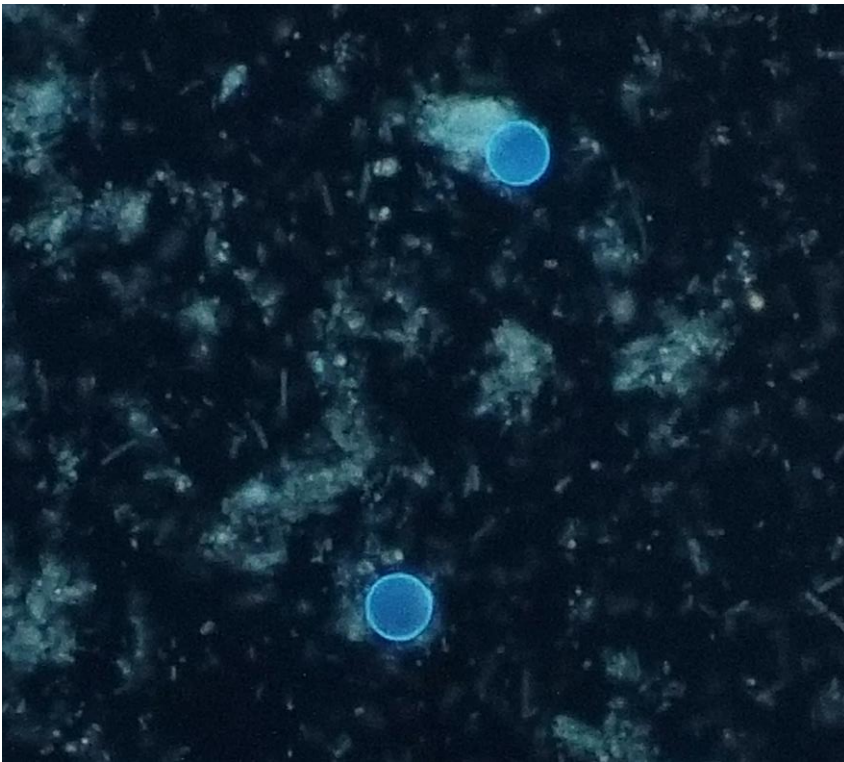


UV autofluorescence

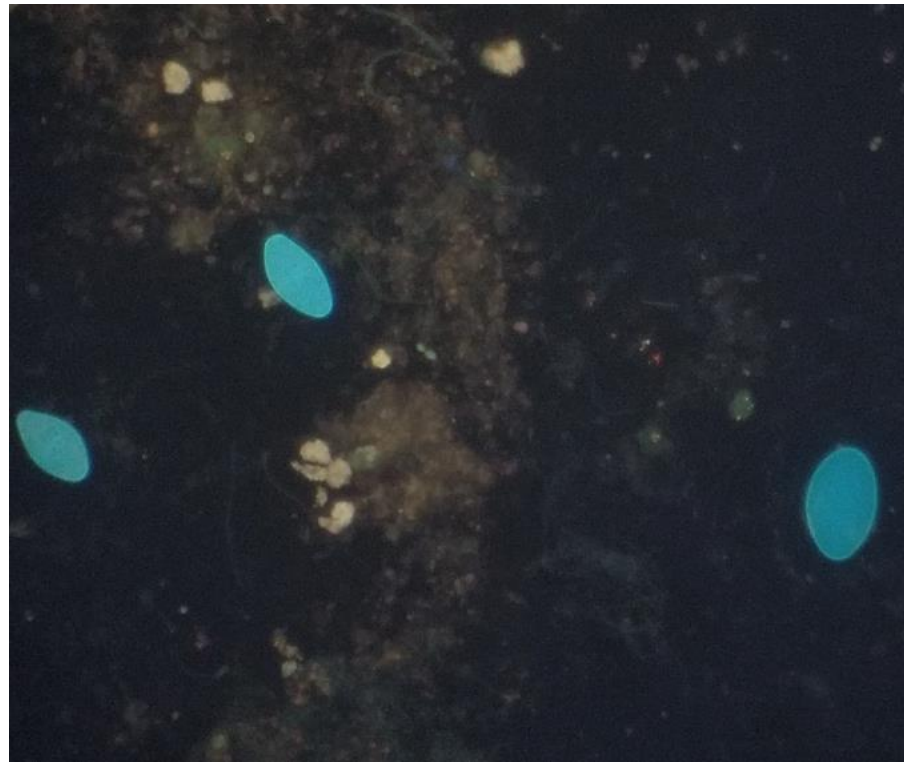
Does your lab perform this method?

- Screen wet-mount at 400X magnification
 - Blue = UV excitation filter 330-365 nm
 - Pale green = 450-490 nm
- Can detect *Cyclospora*, *Cystoisospora*, *Sarcocystis*
- Bonus! - Helmith eggs

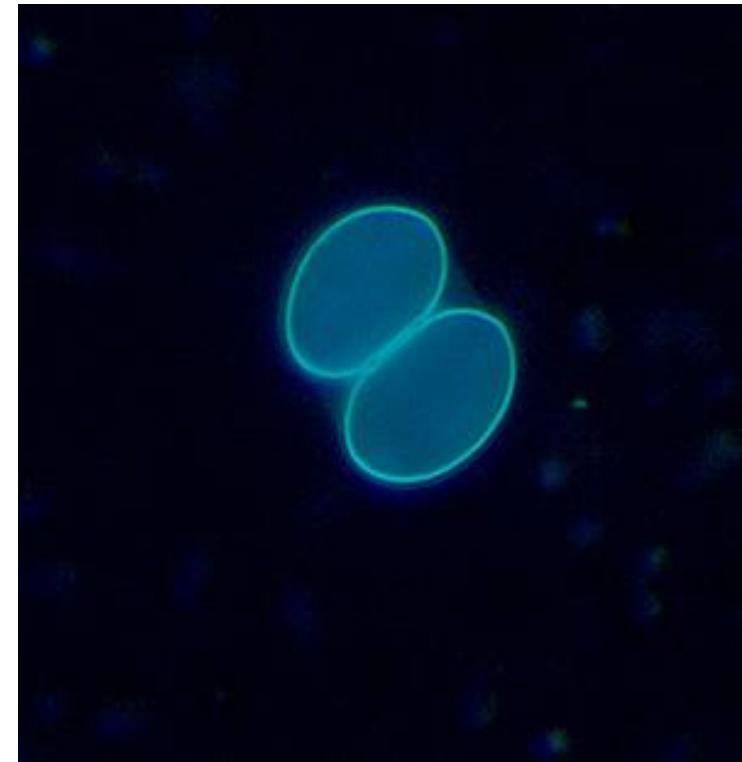
UV autofluorescence



Cyclospora spp.



Cystoisospora spp.



Sarcocystis spp.

<https://www.cdc.gov/dpdx/index.html>

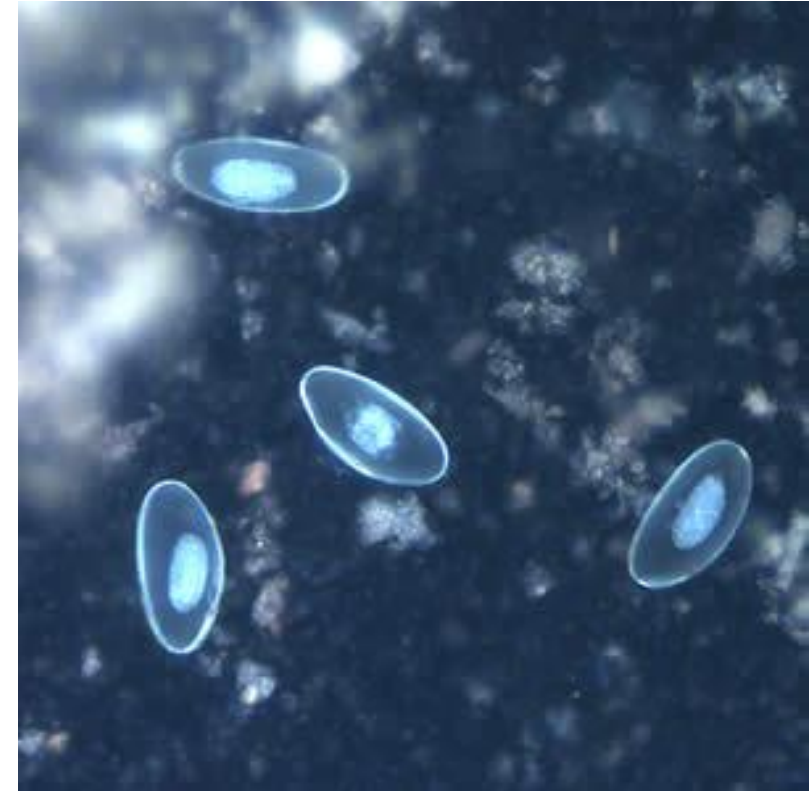
UV autofluorescence



Hookworm



Trichuris

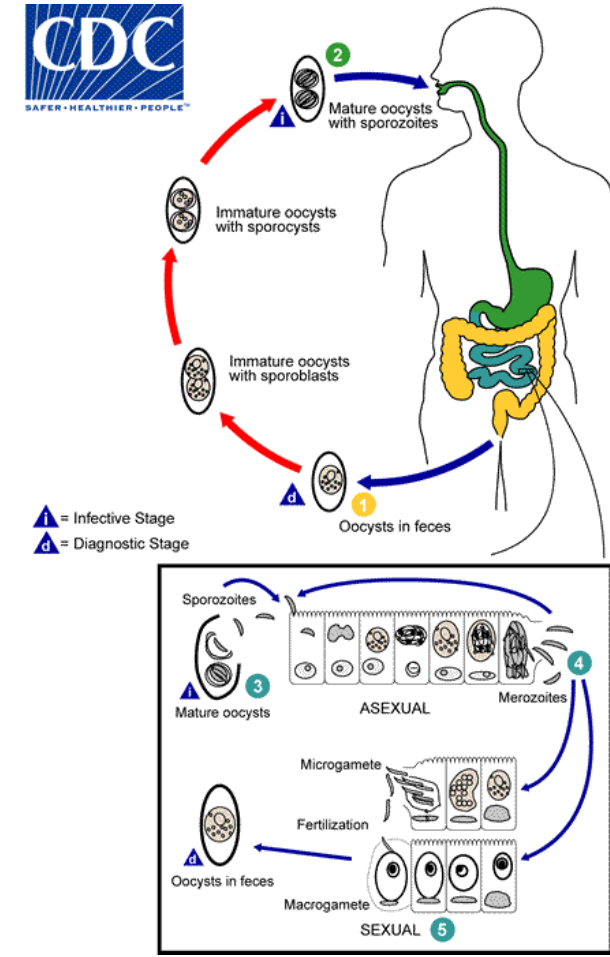


Enterobius

Cystoisospora belli

Symptoms & Epi

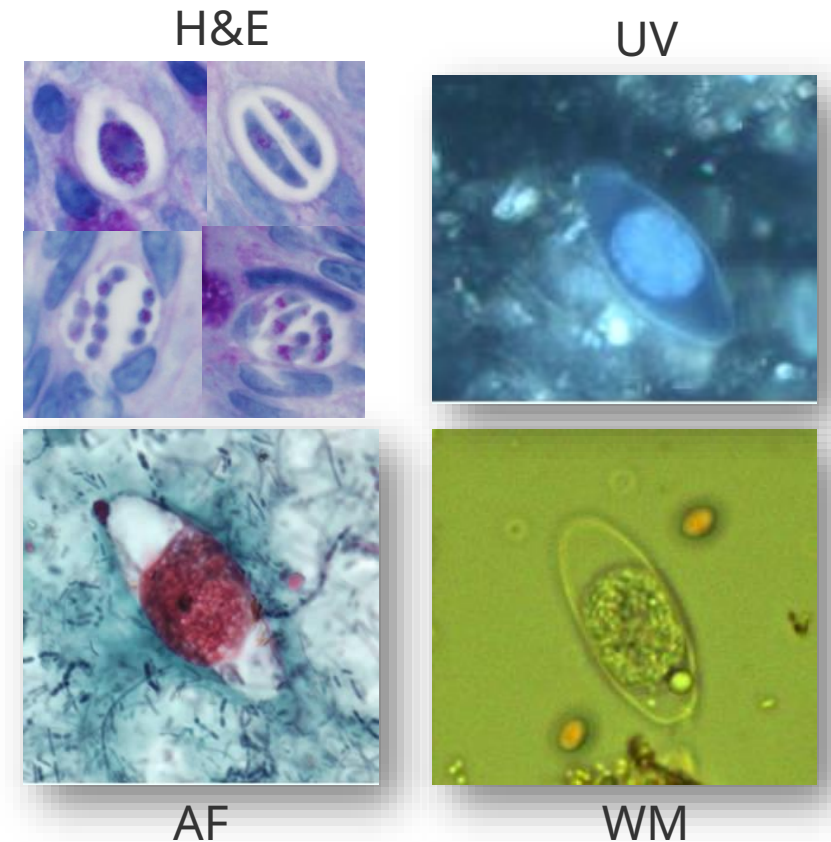
- Non-bloody diarrhea
- Abdominal cramps (wks. to mos.)
Malabsorption
Weight loss
- **Eosinophilia!**
- Can be deadly w/severe immunosuppression
- Oocysts not immediately infective
 - Must develop sporozoites
- Worldwide, but more prevalent in tropic/subtropic



Cystoisospora belli

Diagnostic features & symptoms

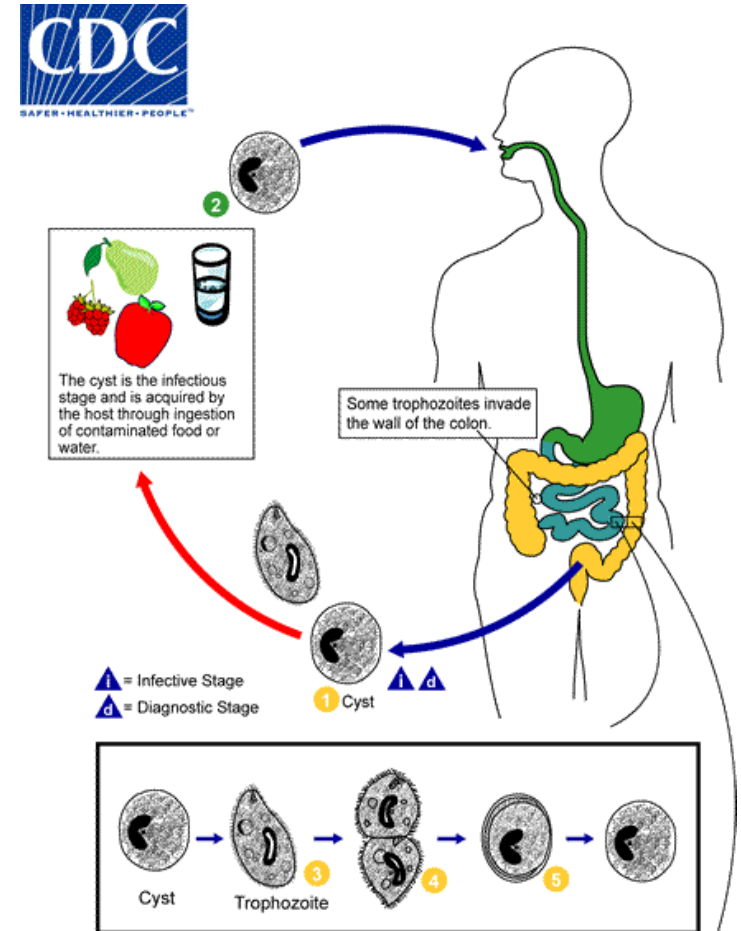
- 25-25 μm oblong, football-shaped oocysts
 - Contain one or two sporoblasts
- Microscopy
 - Modified AF
 - Wet mount
 - UV autofluorescence
- Histopath



Balantidioides coli

Symptoms & Epi

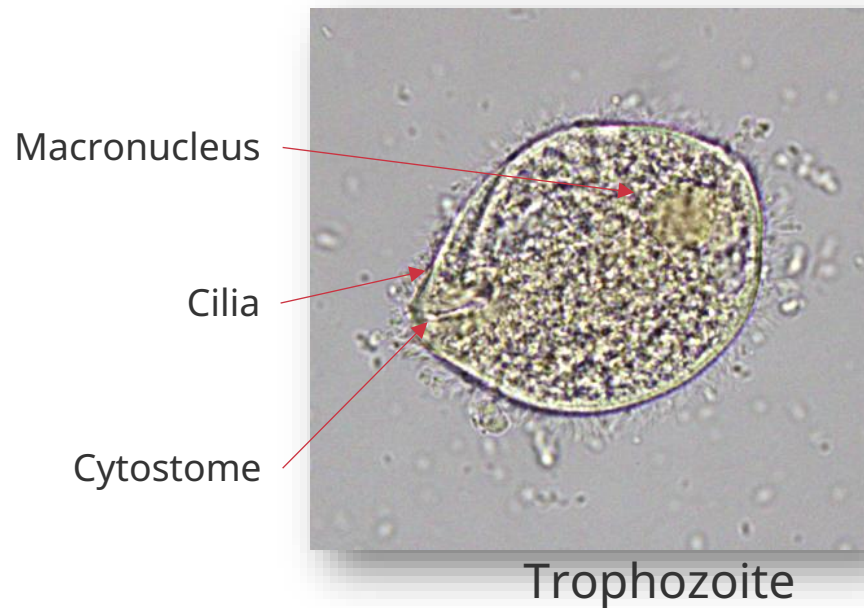
- Mostly asymptomatic
- Persistent diarrhea
Rare dysentery (invasion)
Abdominal pain
Weight loss
- Severe symptoms in compromised hosts
- Worldwide, esp. where pigs raised



Balantioides coli

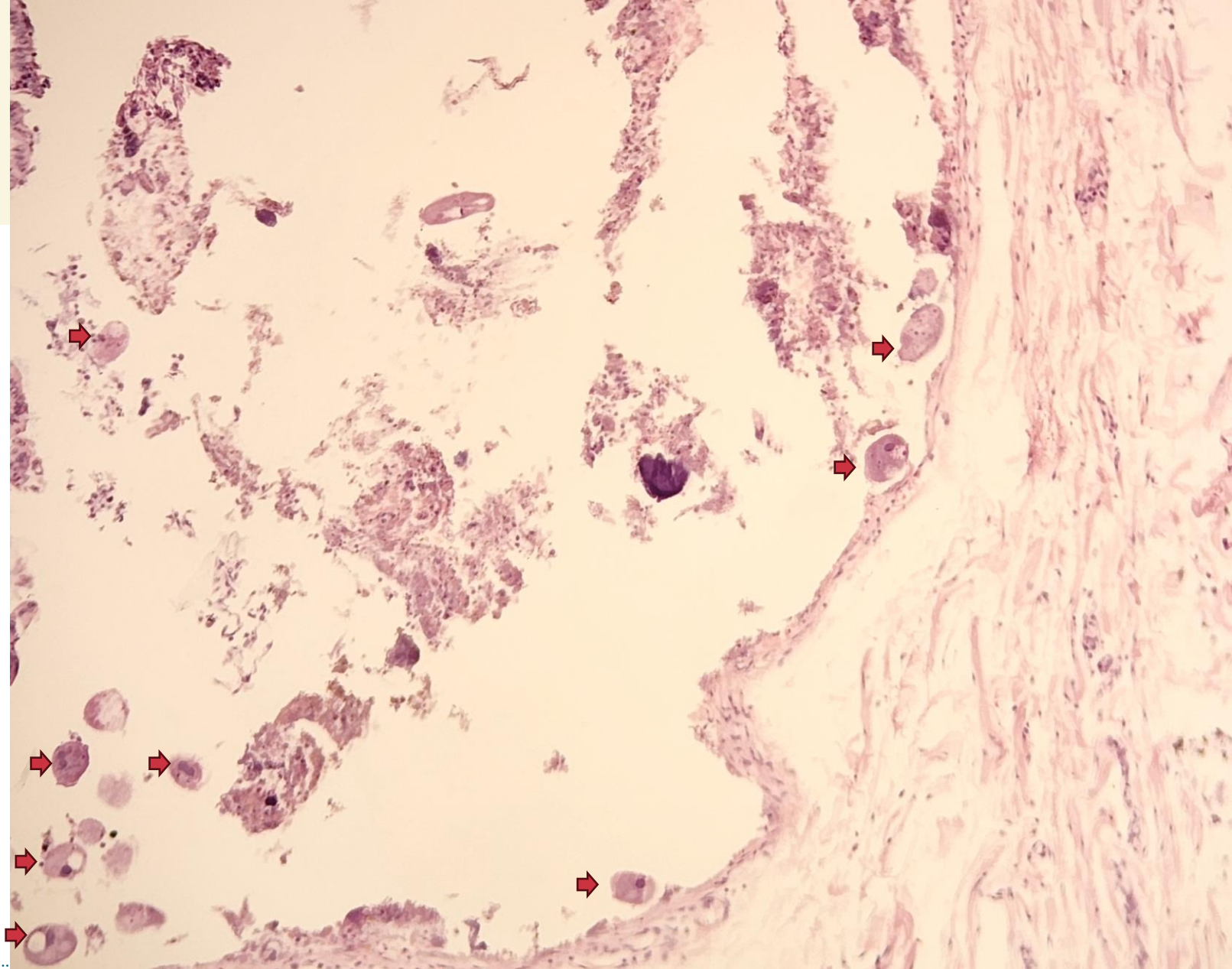
Diagnostic features & testing

- Microscopy
 - O&P
 - Large 50-70 μm
 - Peripheral cilia
 - Macronucleus
 - Micronucleus
 - Cytostome



Balantioides coli

Histopath



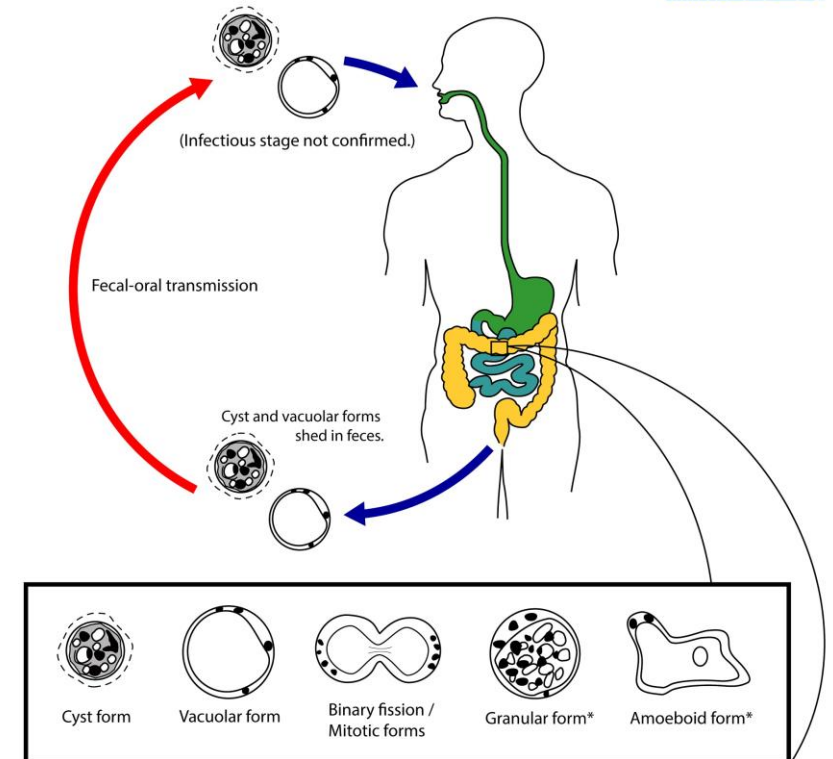
Blastocystis sp.

Symptoms & Epi

- Many symptomatic and asymptomatic
 - Diarrhea
 - Abdominal discomfort
- Not a definitive pathogen
 - 17 subtypes, 10 in humans, 1-4 most common, 1&3 common with IBD, 2&3 more severe symptoms
 - Very common in O&Ps
- Distributed worldwide
- Reported with semi-quantification

4DPDx

Blastocystis sp.



*Various forms that may occasionally be seen in human stool samples and in culture. Their biological significance is not well understood.

Blastocystis sp.

Why do we quantitate?

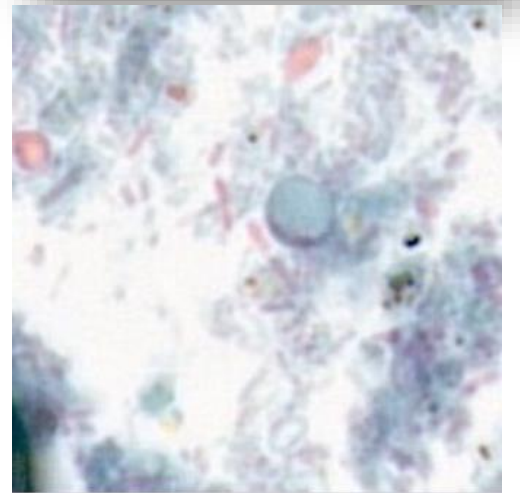
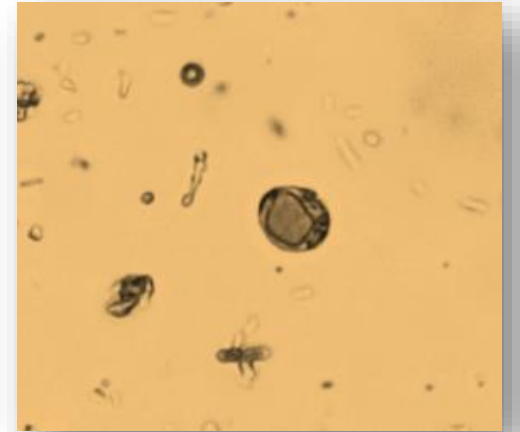
- Historically we just didn't know...so...give as much info as we can.
- Does quantity predict disease?
 - Not really
 - Chronic 1+ *Blasto* story
- Is 1+ of ST3 more concerning than 4+ ST6?



Blastocystis sp.

Diagnostic features & testing

- Microscopy – O&P
 - 6-40 μm
 - Vacuolar forms most common
 - Central body
 - Peripheral nuclei <6
- NAAT
 - Currently only 1 FDA cleared (Genetic Signatures)

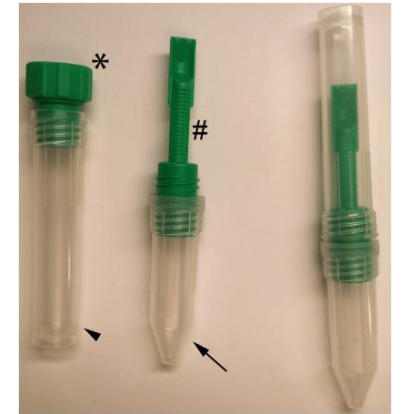


DOGMA: If you concentrate stool for trichrome stain, you lose sensitivity for *Blastocystis et al.*

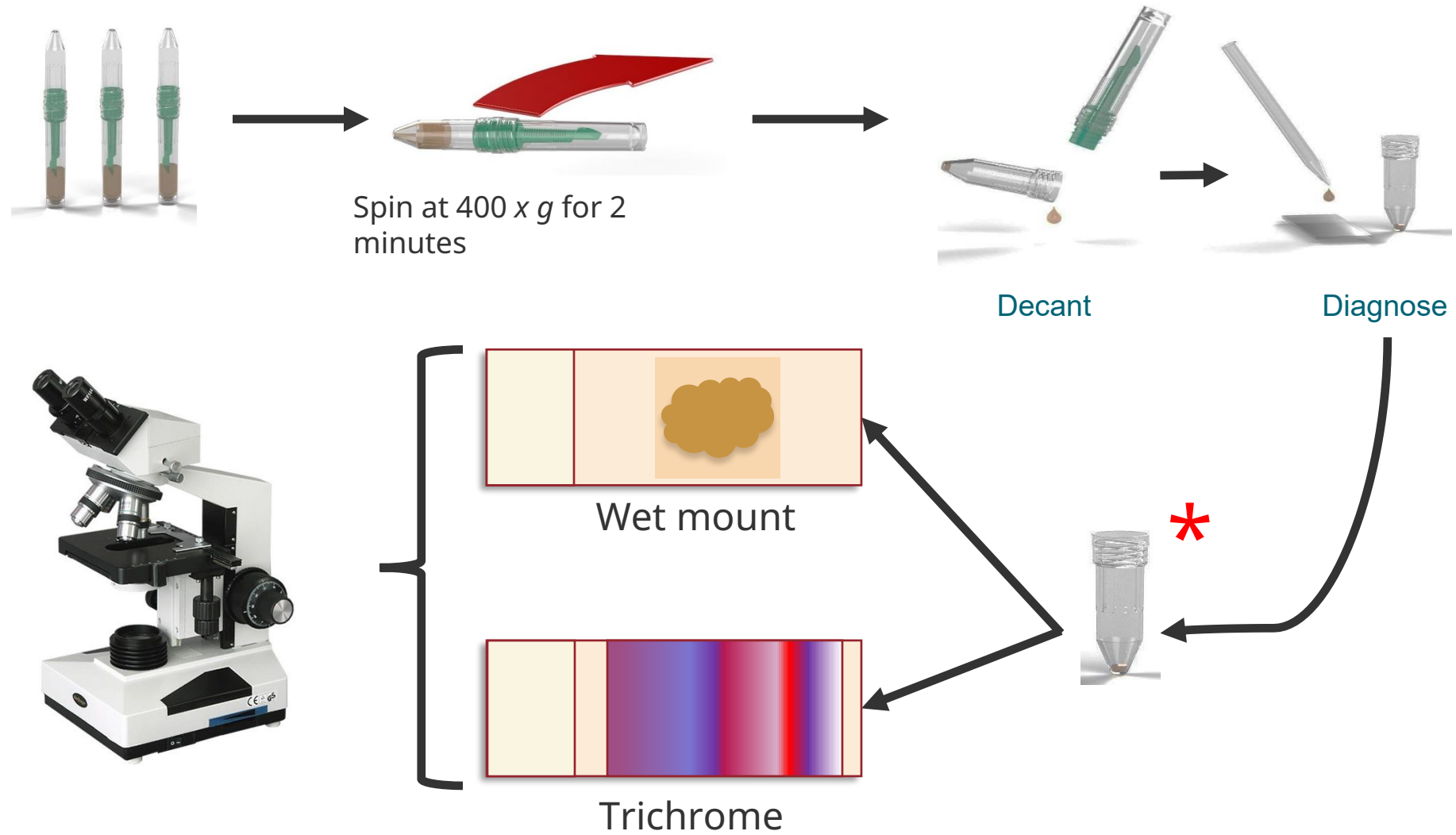
- False
- Where does this come from??
 - No published comparator study to prove this
 - Expert opinion and personal experience (?)
 - Most countries do not even do trichrome stains!
- ...well, I like data.

If you concentrate stool for trichrome stain, you lose sensitivity for *Blastocystis et al.*

- Parasep and Paradevice tubes allow for semiautomation of processing
- Single tube & alcohol-based fixative streamlines prep
- Vertical filtration achieved inside of tube/spork device
- BUT...All specimen is concentrated



Single preparation workflow



If you concentrate stool for trichrome stain, you lose sensitivity for *Blastocystis et al.*

- Compared 10 well characterized stools collected in PVA
 - Previously identified as positive for protozoa
- Targeted common organisms, enriched for *Blastocystis*
- Compared direct smear to 2 centrifugal speeds
 - Concentrated trichrome preps vs direct smear dogma

Concentrated stool is equally sensitive for trichrome

Sample no.	Centrifugation speed ($\times g$)	Trichrome stain result			
1	Unconcentrated	Uninterpretable	6	Unconcentrated	Rare <i>E. histolytica</i> /Entamoeba dispar
	200	Uninterpretable		200	1+ <i>E. histolytica</i> /E. dispar
	400	Uninterpretable		400	1+ <i>E. histolytica</i> /dispar
2	Unconcentrated	1+ <i>Chilomastix</i>	7	Unconcentrated	Rare <i>B. hominis</i>
	200	2+ <i>Chilomastix</i>		200	Rare <i>B. hominis</i>
	400	3+ <i>Chilomastix</i>		400	1+ <i>B. hominis</i>
3	Unconcentrated	1+ <i>B. hominis</i>	8	Unconcentrated	1+ <i>B. hominis</i> , Rare <i>E. coli</i>
	200	1+ <i>B. hominis</i>		200	1+ <i>B. hominis</i> , 1+ <i>E. coli</i>
	400	1+ <i>B. hominis</i>		400	1+ <i>B. hominis</i> , 1+ <i>E. coli</i>
4	Unconcentrated	Negative	9	Unconcentrated	1+ <i>B. hominis</i> , 1+ <i>E. coli</i>
	200	Negative		200	1+ <i>B. hominis</i> , 1+ <i>E. coli</i>
	400	Negative		400	1+ <i>B. hominis</i> , 1+ <i>E. coli</i>
5	Unconcentrated	1+ <i>B. hominis</i>	10	Unconcentrated	Rare <i>Giardia</i> cysts
	200	1+ <i>B. hominis</i>		200	1+ <i>Giardia</i> cysts
	400	1+ <i>B. hominis</i>		400	1+ <i>Giardia</i> cysts

Bottom line

- This procedure has been used for years
 - ARUP & internationally
- No loss in sensitivity shown
- Positivity rate is consistent; increased with process change
 - Increased more with AI integration subsequently
- You will be ok...concentrate that 

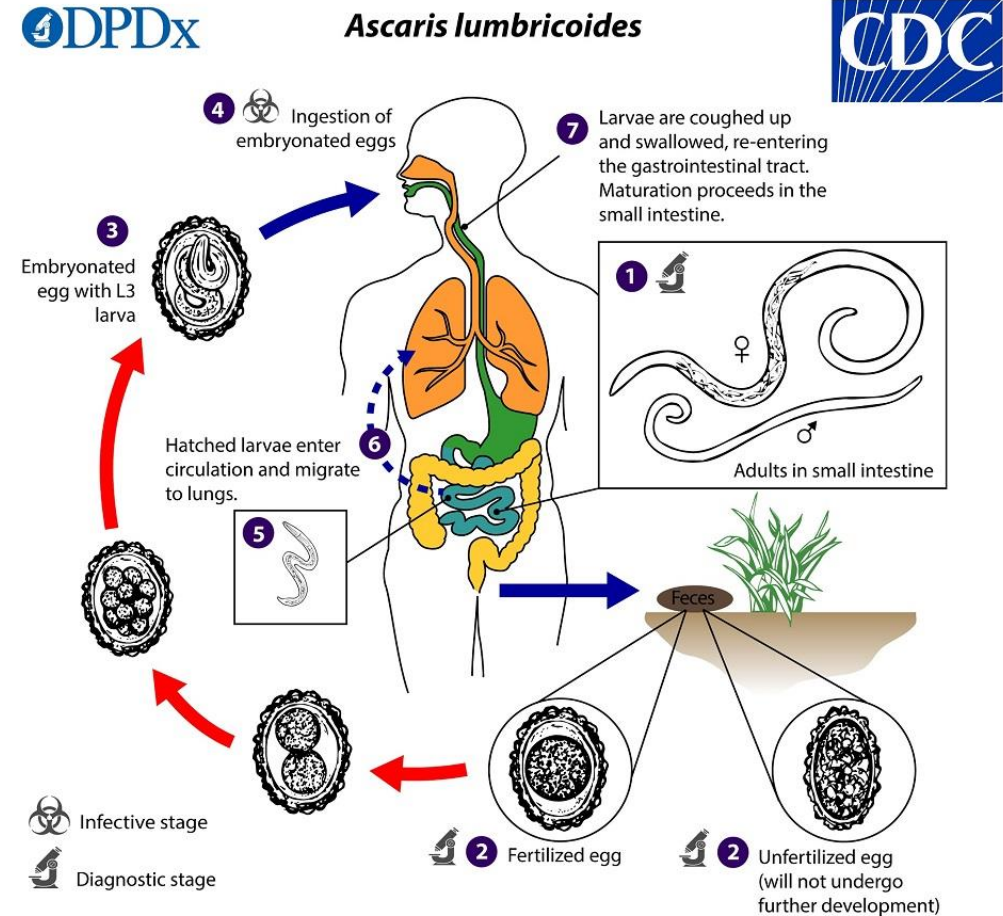


Nematodes

Ascaris lumbricoides

Symptoms & Epi

- Most asymptomatic
- Peds stunted growth
- ↑ Worm burdens
 - Abd. pain
 - Intestinal obstruction
 - Perforation
- Worldwide, most sig. tropic/subtropic
 - Dev'd. Countries – poor sanitation



Ascaris lumbricoides

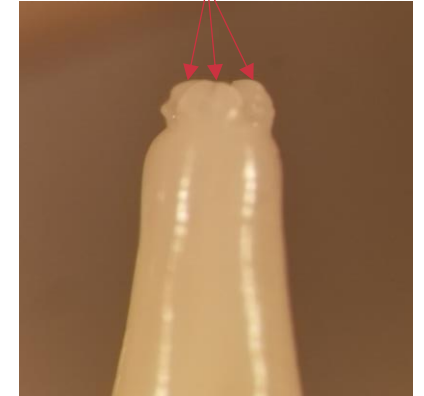
Diagnostic features & testing

- Macroscopic worm examination - Adult worms
 - Large 15-31 cm w/curved tail
 - 3 anterior “lips”
 - Annulations on tegument
- O&P exam - Eggs
 - Fertile (45-75 μ m) vs Unfertilized (<90 μ m)
 - Mamillated vs decorticated

Curved tail



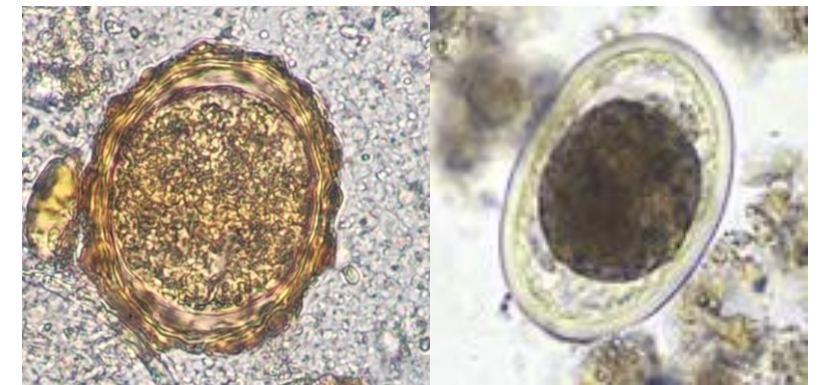
Three “lips”



Unfertilized



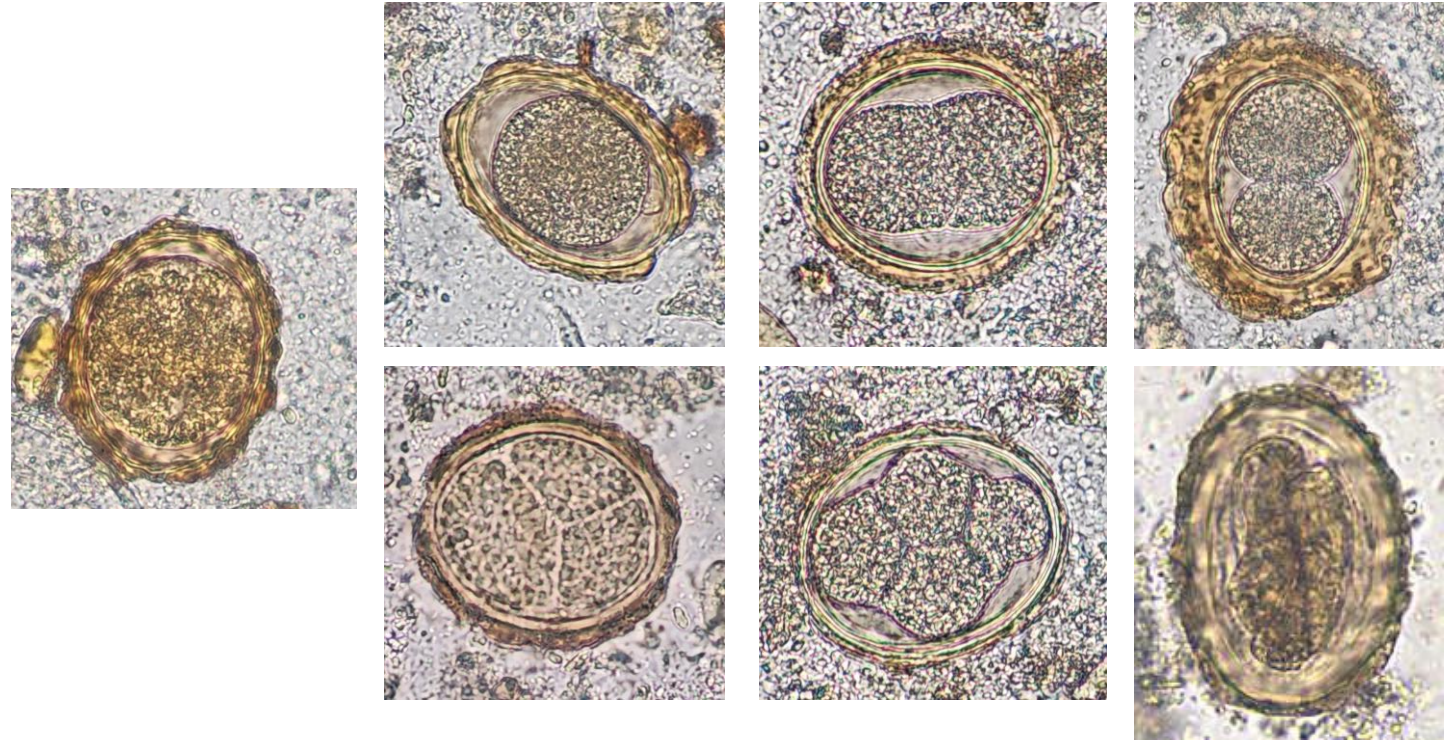
Fertilized



<https://www.cdc.gov/dpdx/ascariasis/index.html>

Ascaris lumbricoides

Eggs can continue to develop in fixative



Case 5

Coughed up by a patient with severe sudden onset upper abdominal pain

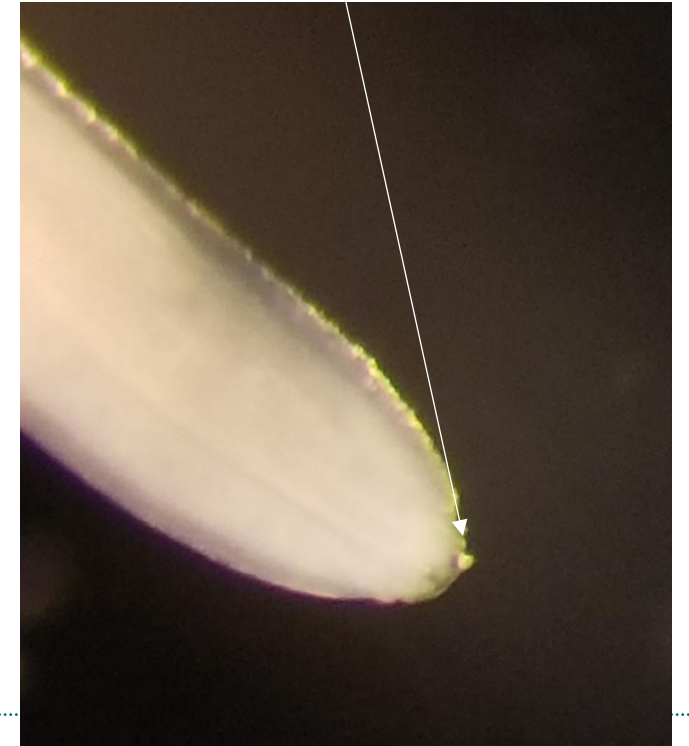
Lips



2.5 cm



Mucron



Case 5

Anisakid L3 larva (*Anisakis*, *Pseudoterranova*, *Contracaecum*)

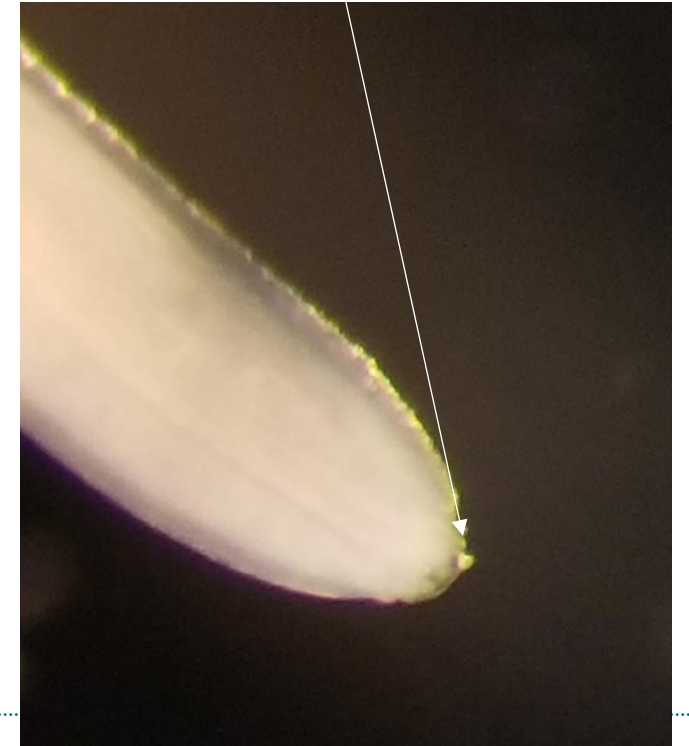
Lips



2.5 cm



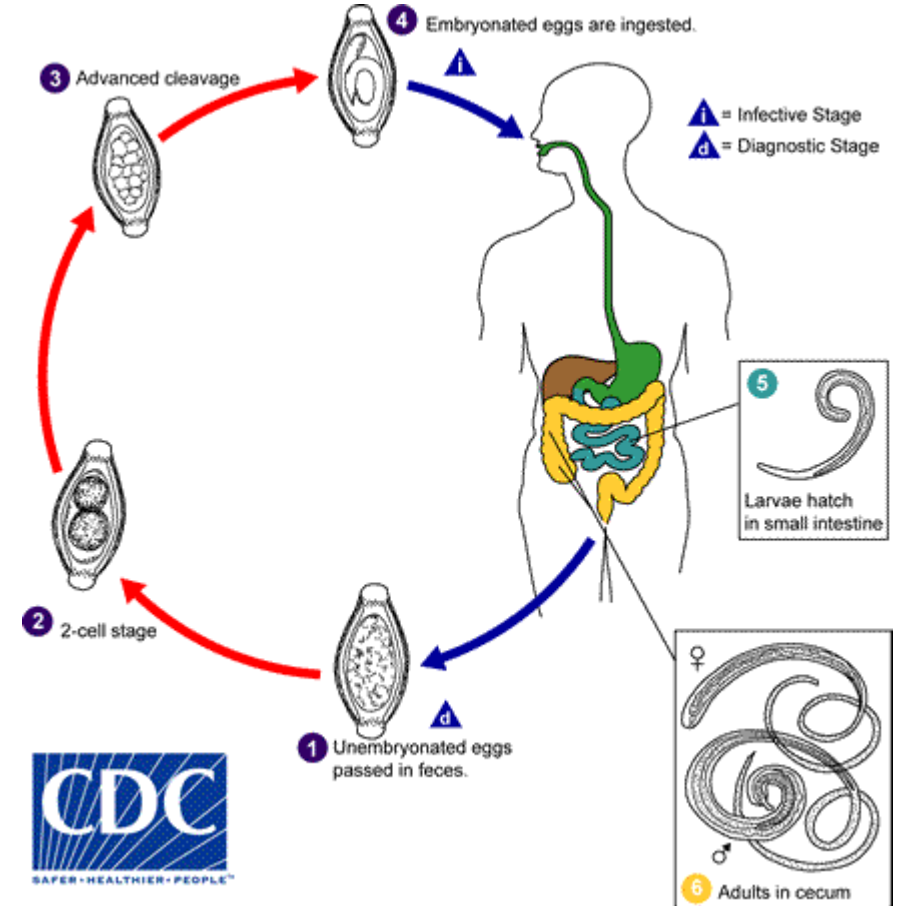
Mucron



Trichuris trichiura

Symptoms & Epi

- Most asymptomatic
 - ↑ Worm burden in peds = GI symptoms & growth retardation
 - Extreme = rectal prolapse
- Worldwide, most sig. tropic/subtropic
 - Developing countries - peds
 - Dev'd. Countries – poor sanitation



Trichuris trichiura

Diagnostic features & testing

- Macroscopic examination
 - 30-45mm
 - Often post endoscopy
- O&P
 - Eggs – 50-55 μm ,
 - Bipolar plugs
 - Unembryonated
 - Refractile
 - Autofluorescent



Anterior



Trichuris trichiura

Variable egg morphology



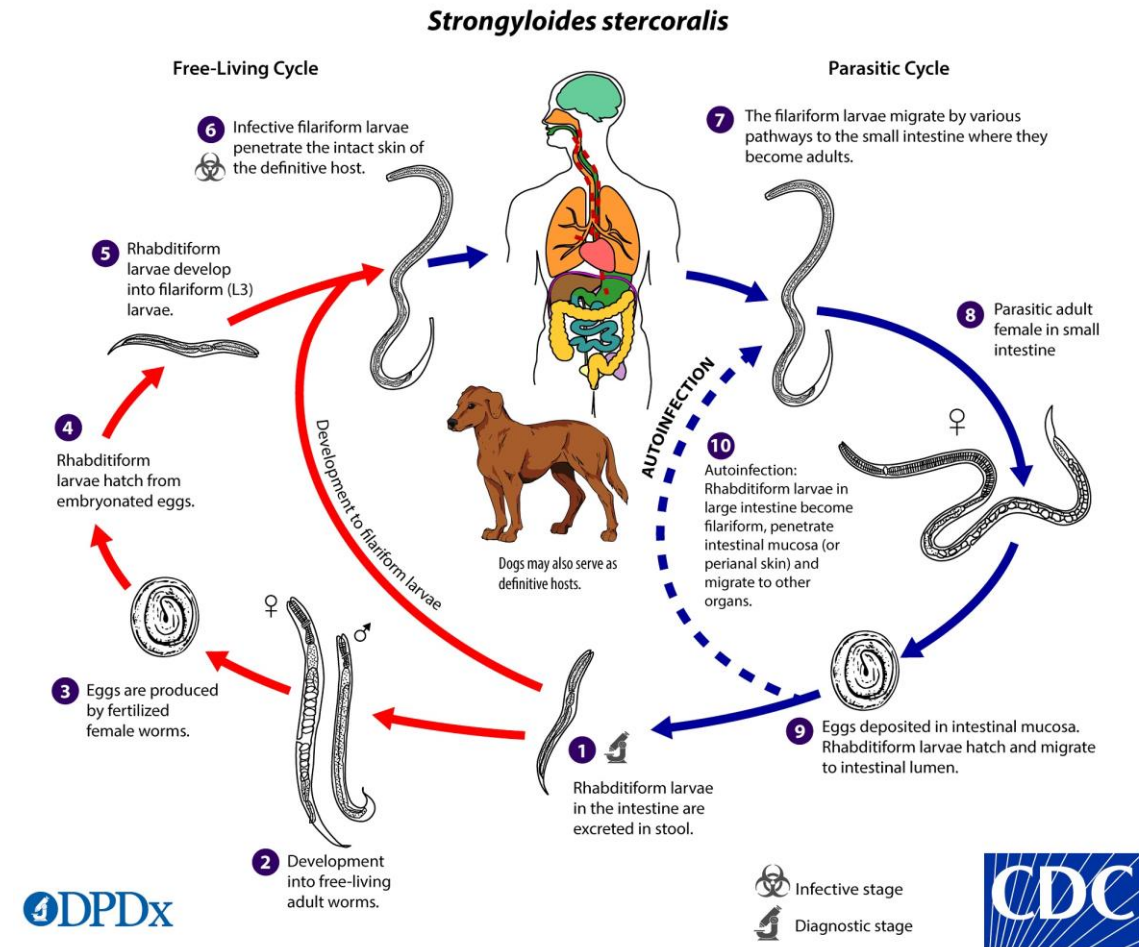
35-45 μm

Paracapillaria phillipinensis

Strongyloides stercoralis

Symptoms & Epi

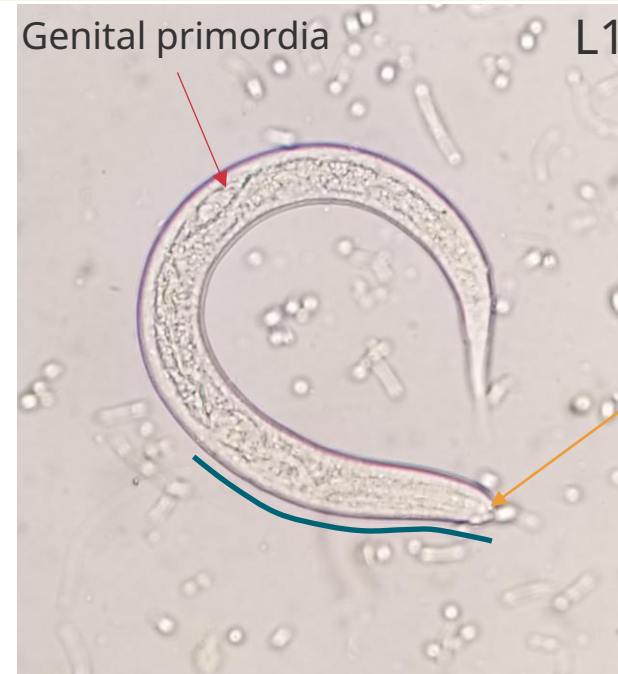
- Acute:
 - Rash @ penetration site
 - Tracheal irritation dry cough
 - Diarrhea, abdominal pain
- Chronic:
 - Often asymptomatic unless immunosuppressed
 - Hyperinfection syndrome can be fatal
- Tropic/subtropic
 - Temperate areas – poor sanitation, rural, remote
 - e.g. S. Appalachia, AL, MS, LA



Strongyloides stercoralis

Diagnostic features & testing

- O&P
 - Rhabditiform larvae (L1) – 180-380 μm (stool) vs filariform larvae (L3) - <600 μm (resp., other)
 - Short **buccal cavity** (L1&L3)
 - Prominent **genital primordia** (L1)
 - Tail: L1 pointed, L3 forked
 - **Esophagus**: Intestine = L1, 1:3 L3, 1:1
- Serology IgG
 - 70-85 % sens/spec method dependent
- Histopathology (incidental)



Buccal cavity



Strongyloides stercoralis

Additional best practice

- Agar plate culture
 - Most sensitive method
 - Labor intensive
 - Biosafety considerations



30°C



10% formalin
(if +ve)

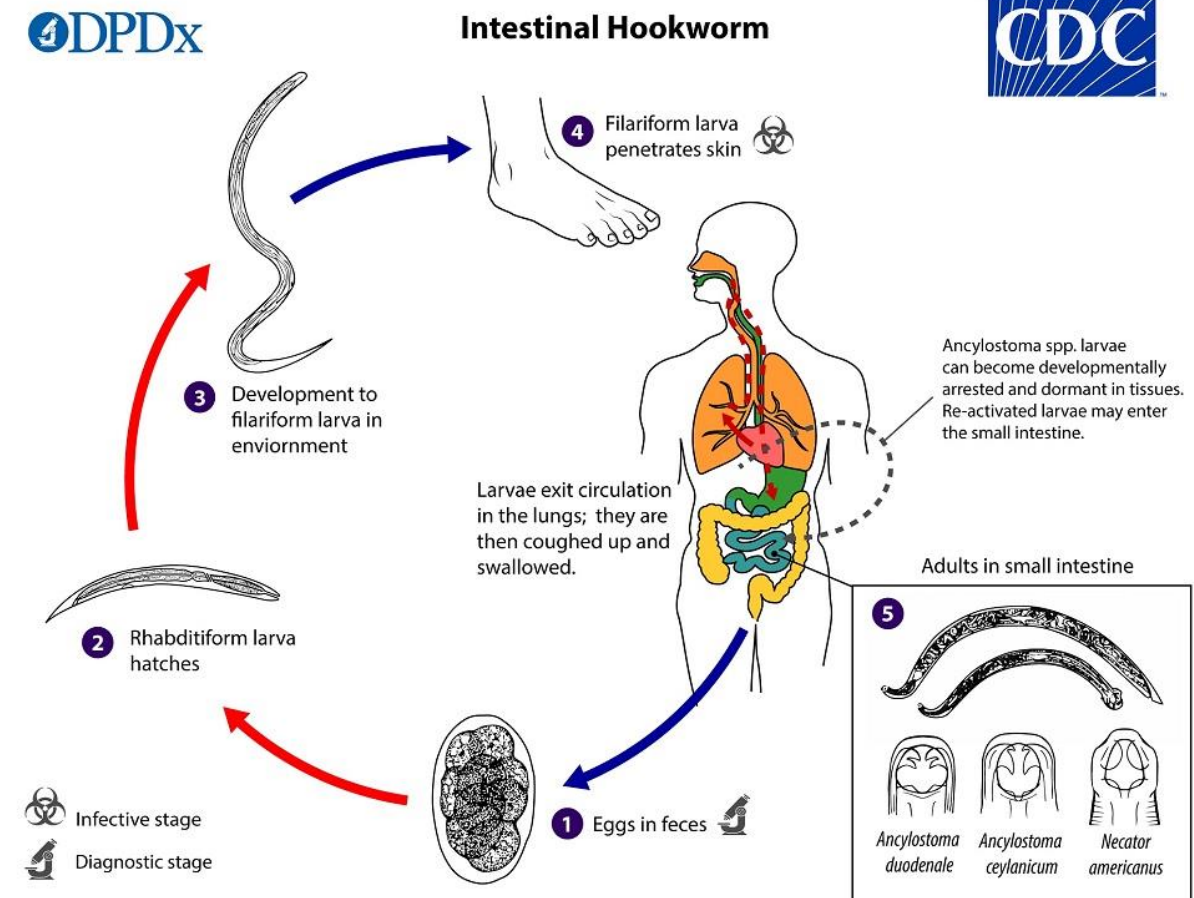


Wet-mount to confirm

Hookworm – *Ancylostoma* spp. and *Necator*

Symptoms & Epi

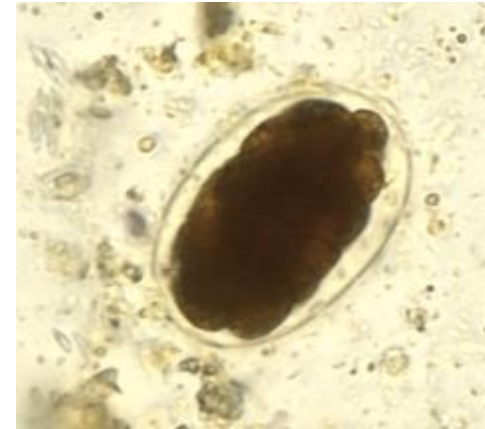
- Mostly asymptomatic
- Possible Fe deficiency anemia with ↑ loads
- Rash after L3 penetration (uncommon)
- Worldwide with warm, moist climates & soil



Hookworm – *Ancylostoma* spp. and *Necator*

Diagnostic features & testing

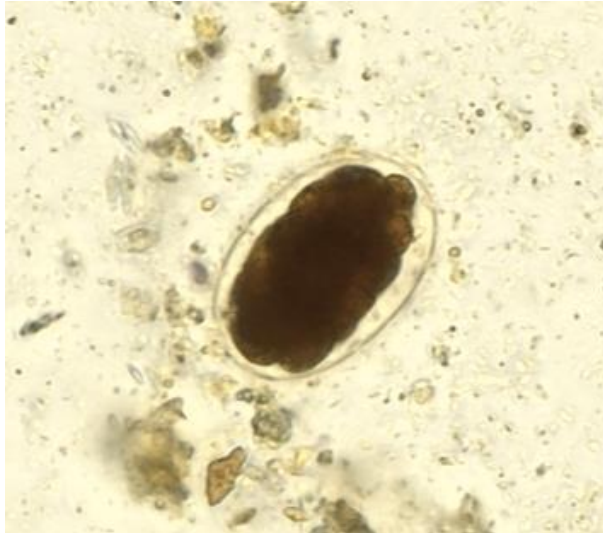
- O&P:
 - Eggs 60-75 μm
 - Thin shell
 - Larvae only seen if delay in fixation*
 - Long buccal cavity
- Macroscopic adult worm (rare)
 - Endoscopy collect (?)
 - Anc. = fangs Nec. = plates



Hookworm – *Ancylostoma* spp. and *Necator*

Land of confusion

60-75 μm



Hookworm

50-60 μm



Strongyloides

50-60 μm



Enterobius

~100 μm



Mite (?)

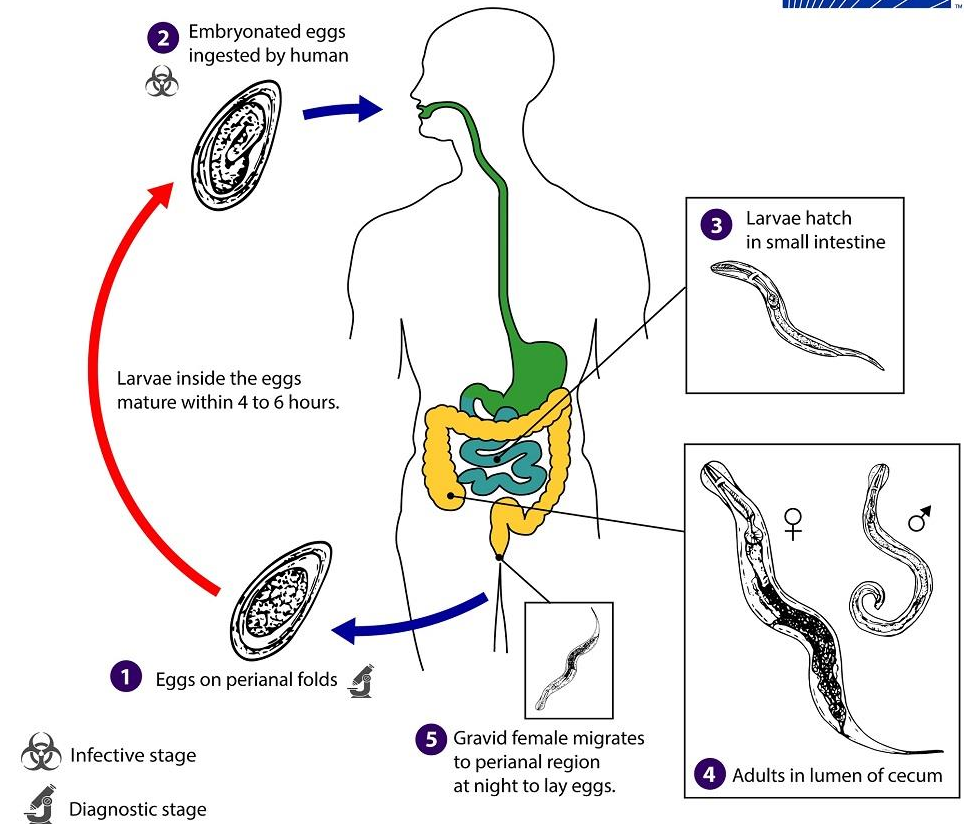
Enterobius vermicularis

Symptoms & Epi

- Perianal itching
- Vulvovaginitis
- Appendicitis (?)
- Worldwide - cosmopolitan



Enterobius vermicularis



Enterobius vermicularis

Diagnostic features & testing

- Cellophane tape prep or adhesive paddle
 - Collect early AM
 - Eggs & female may be detected
 - Eggs – 50-60 μm , flat on one side
 - Females 8-13 mm
- O&P – not indicated...
 - Can detect (esp. w/AI)



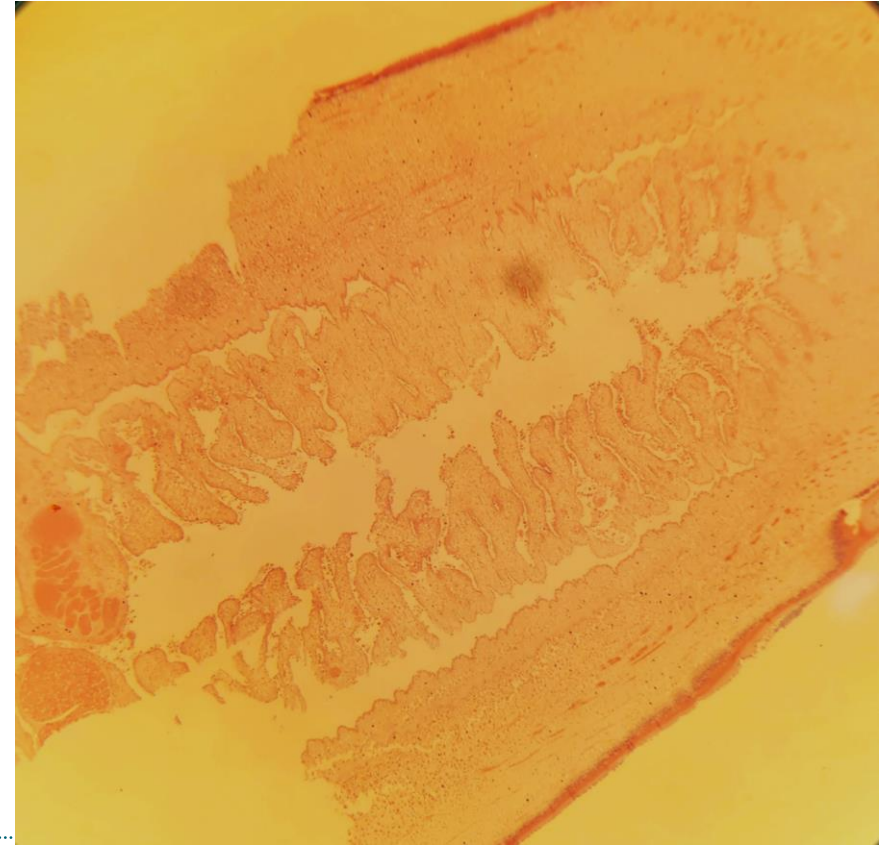


Cestodes

Case 6

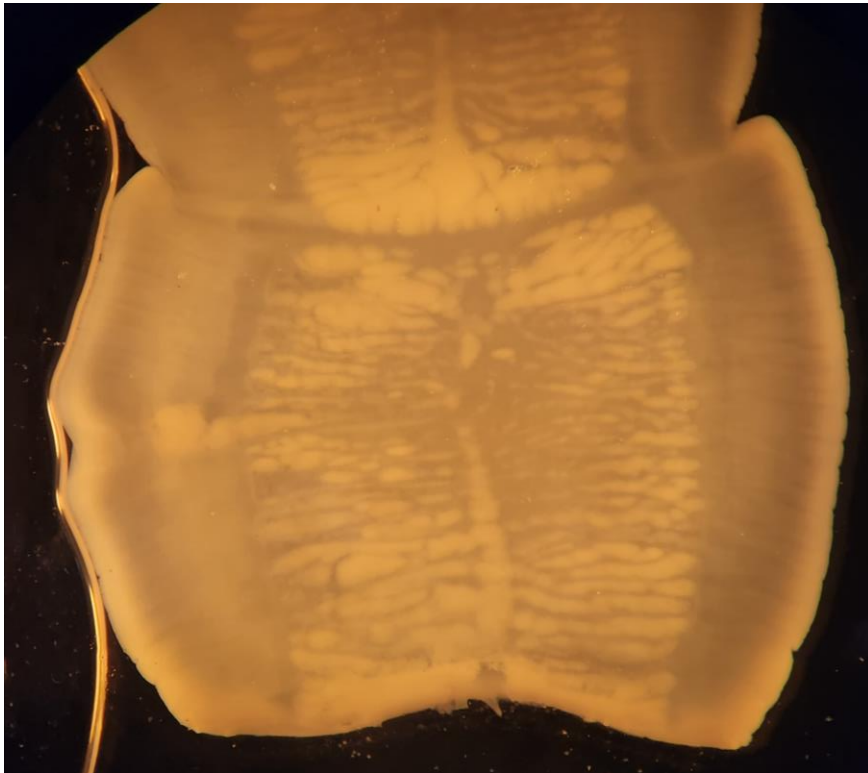
Is this *Taenia*? Is it *T. solium* or *saginata*?
...and Why do we care?

- Proglottids submitted in EtOH
- Patient asymptomatic

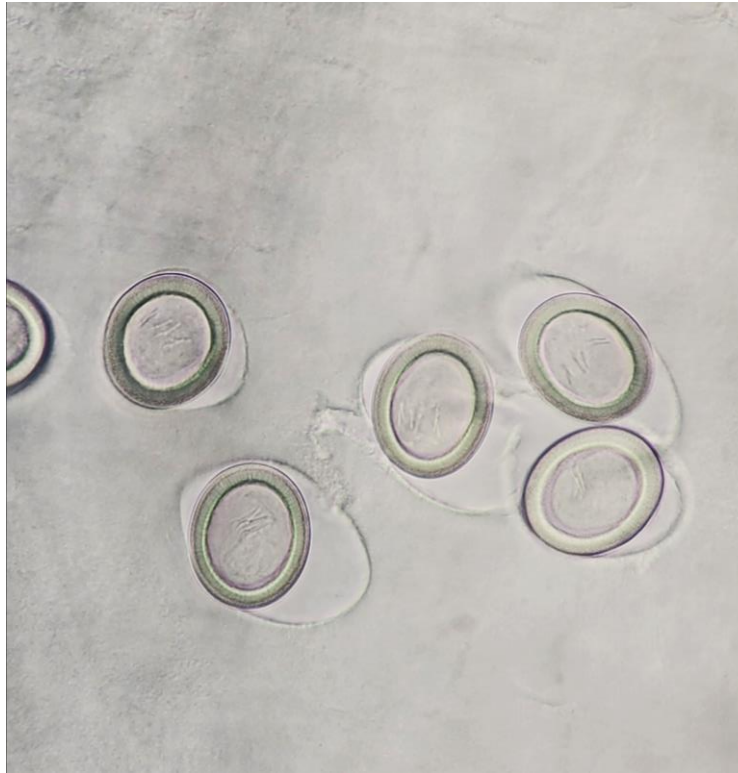


Case 6

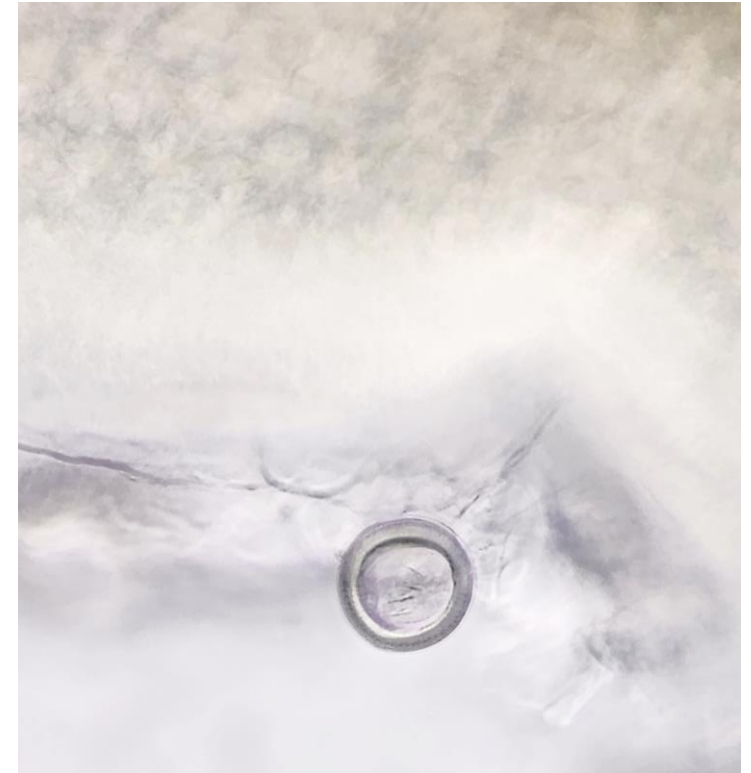
Who am I?



Lactophenol cleared



In utero eggs

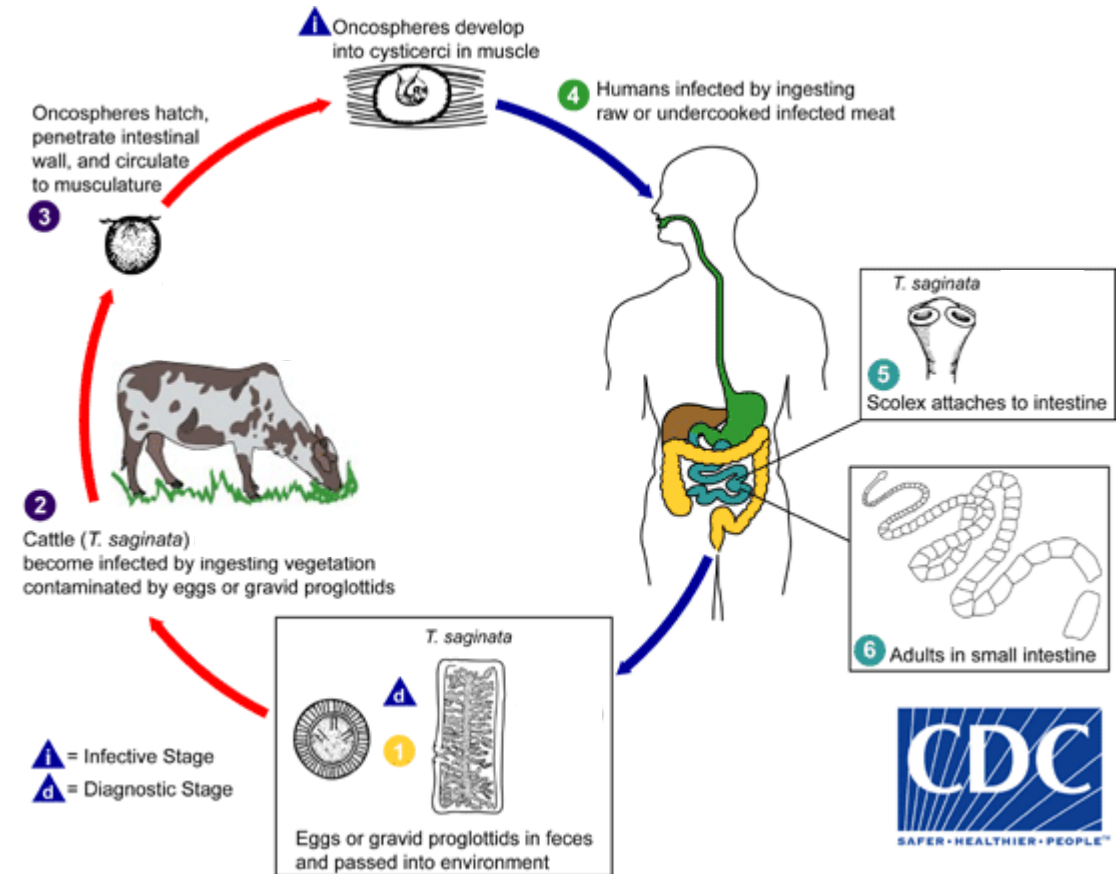


Liberated egg

Taenia saginata

Symptoms & Epi

- Mostly asymptomatic
- Rare bowel obstruction for severe infection
- Passing motile proglottids
- Worldwide where cattle are raised





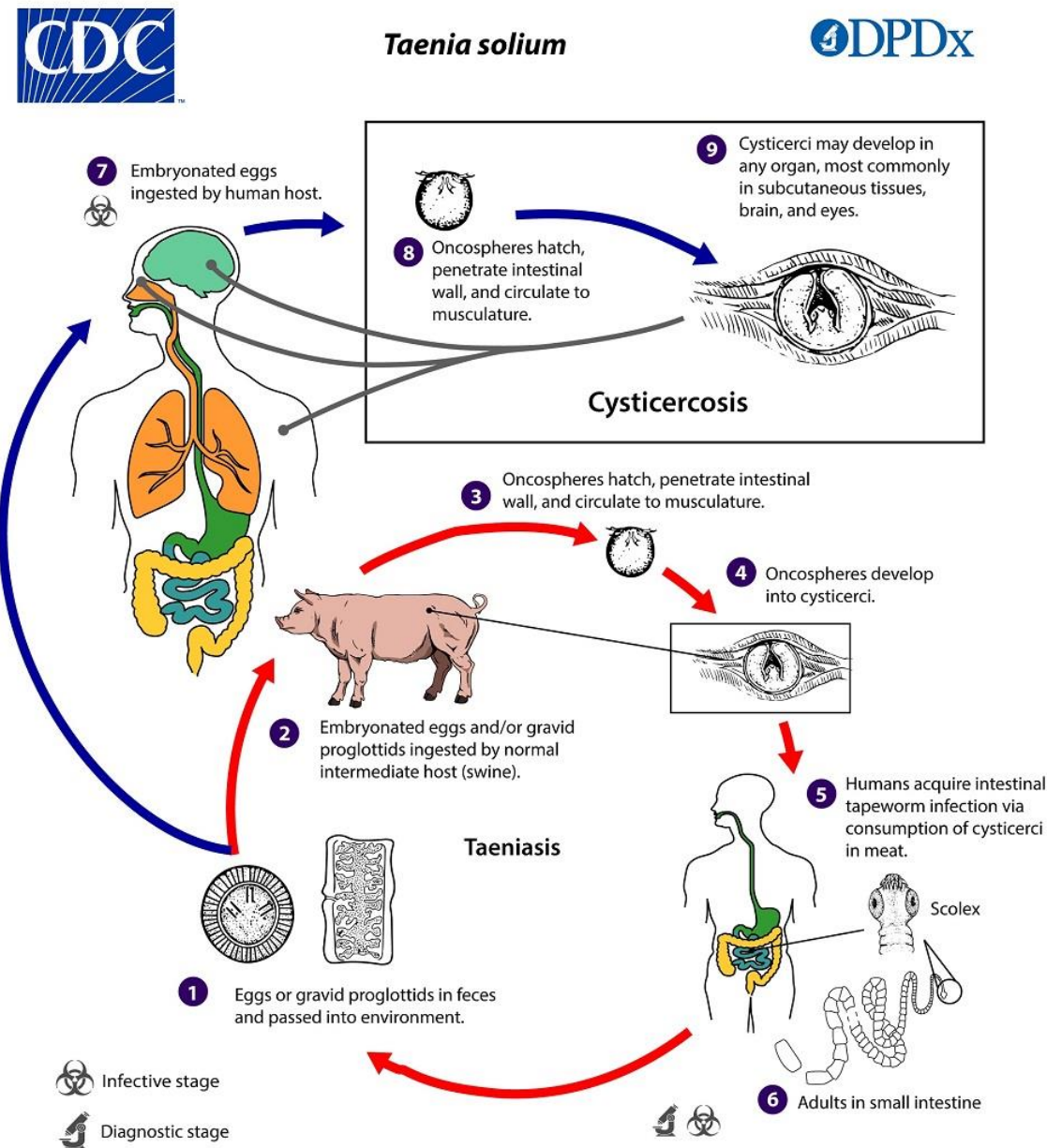
How do you get
neurocysticercosis?



Taenia solium

Symptoms & Epi

- Mostly asymptomatic (intestinal)
- Neurocysticercosis
 - Cysts in brain
 - Seizures
 - Neurological deficit
 - Or asymptomatic
- Worldwide where cattle & pigs are raised
 - And humans eat human 🦌



Taenia saginata & *T. solium*

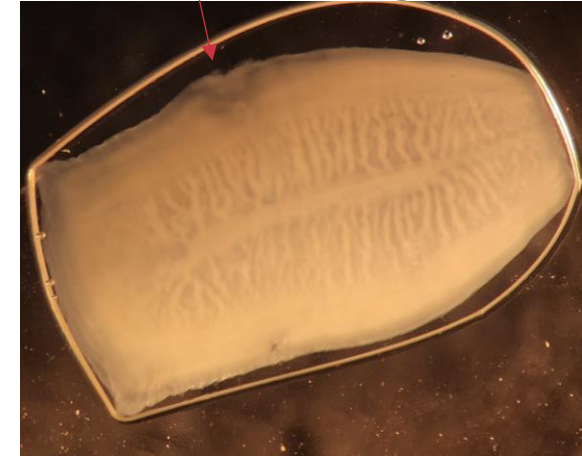
Diagnostic features & testing

- O&P – Eggs (30-35 μ m)
 - Round, thick striated shell
 - Indistinguishable
- Macroscopic examination
 - Lateral genital pore
 - Clearing in lactophenol
 - +/- injecting with India ink (better with fresh proglottids)
 - Uterine branches
T.sol = 7-13 T. sag = 12-30



Genital pore

T. sag



Lactophenol & transmitted light

India Ink



T. sag



T. sol

<https://www.cdc.gov/dpdx/taeniasis/index.html>

Taenia saginata & *T. solium*

SCOLEX for the win!

- 100% diagnostic...but rarely recovered
- *T. solium*
 - Armed with hooklets
 - Remember...NASTY, pathogen
- *T. saginata*
 - Unarmed
 - Remember...innocent bystander

T. solium



T. saginata



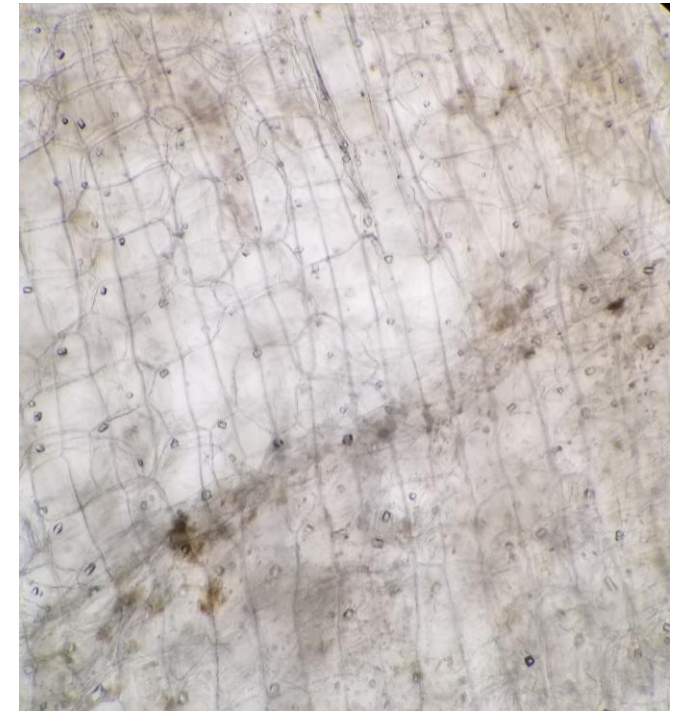
<https://www.cdc.gov/dpdx/taeniasis/index.html>

Case 7

Chain of proglottids passed in the stool

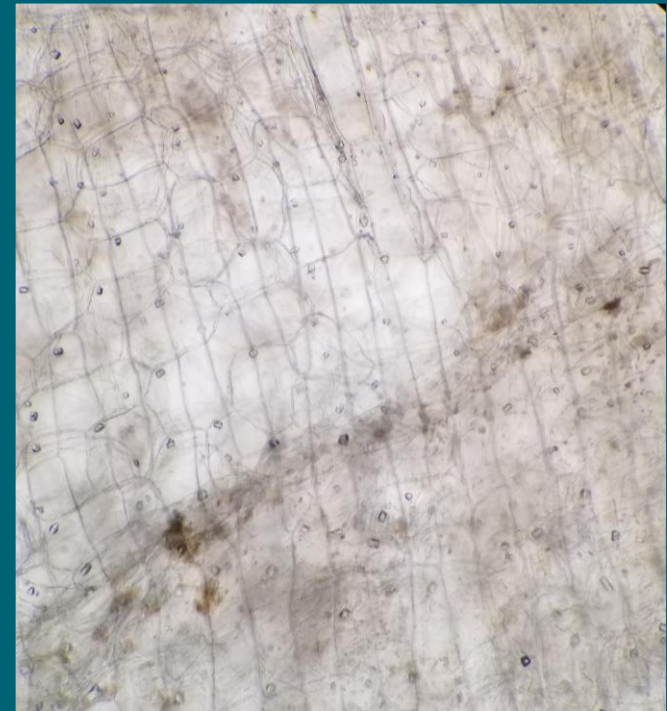


Lactophenol





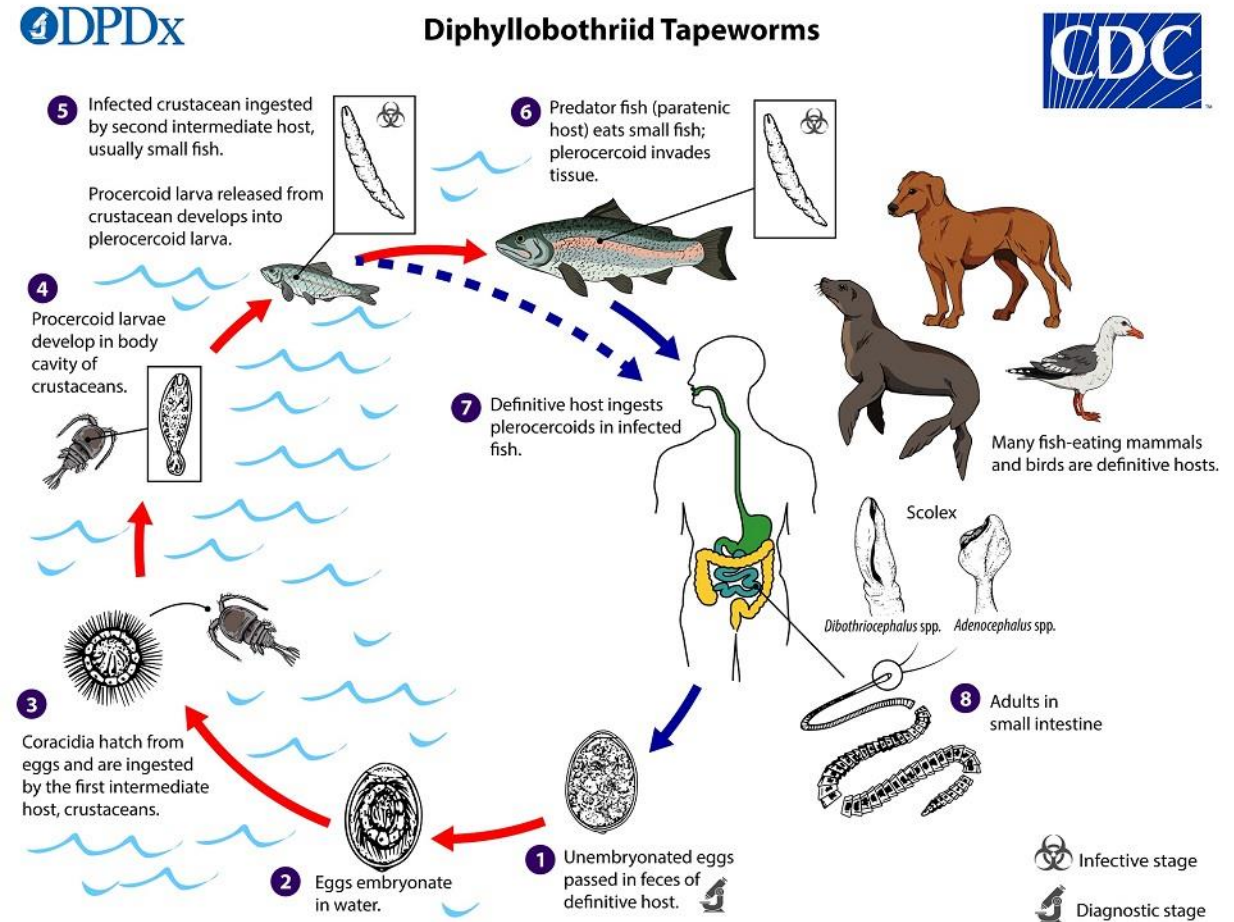
Who am I?



Fish tapeworms (*Diphyllobothrium*, *Adenocephalus*, *Dibothriocephalus*)

Symptoms & Epi

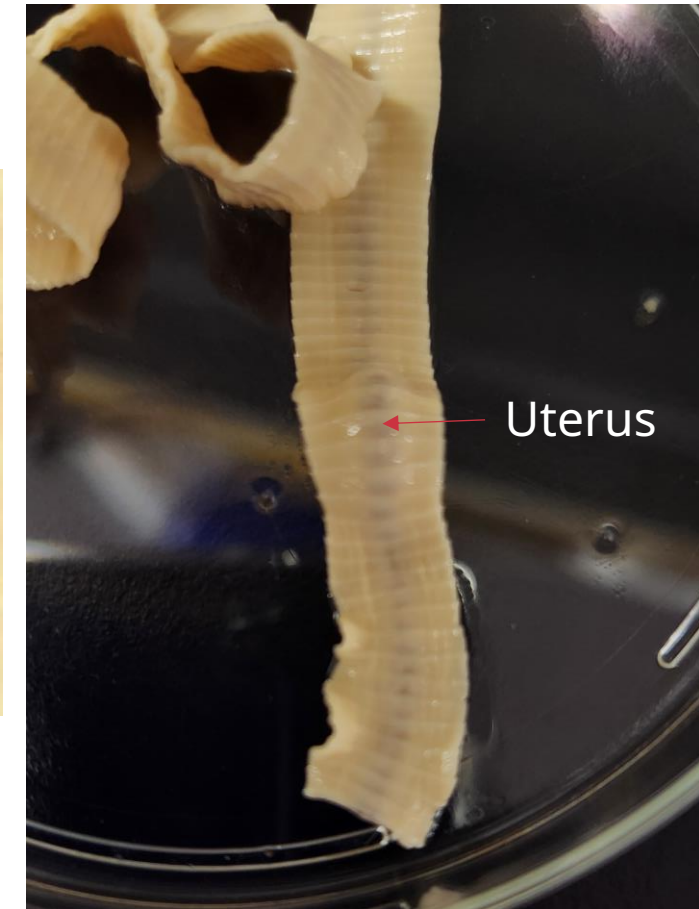
- Mostly asymptomatic
- Infections can last decades
- Vitamin B12 deficiency occasionally
- Circumpolar distribution
 - Where fish are eaten uncooked
 - Salted, cold smoked



Fish tapeworms (*Diphyllobothrium*, *Adenocephalus*, *Dibothriocephalus*)

Diagnostic features & testing

- O&P – Eggs resemble trematodes
 - 55-75 μm
 - Operculum
 - Abopercular knob
- Macroscopic examination of proglottids
 - Ventral genital pore (inconspicuous)
 - “Rosette” uterus
 - Broader than tall
 - Sections > meters long

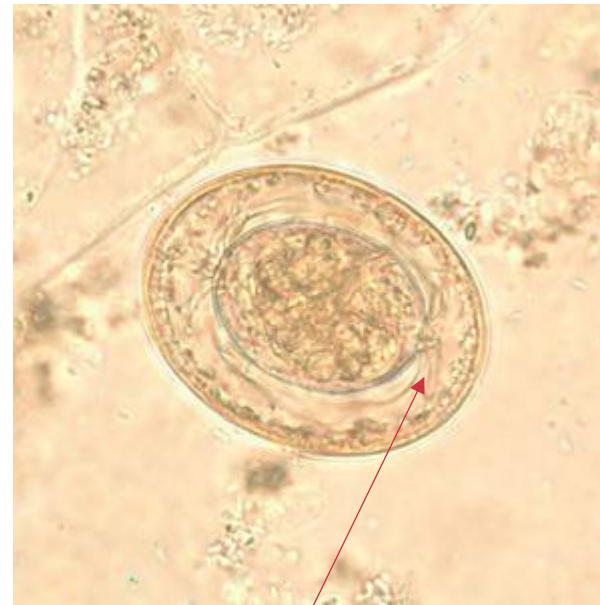


Rodentolepis nana and *Hymenolepis diminuta*

Symptoms, Epi, ID

- Mostly asymptomatic
- Worldwide (common)
- Identify primarily by eggs in O&P
- Adult worms rare encountered
 - Likely degrade

30-50 μm



Polar filaments

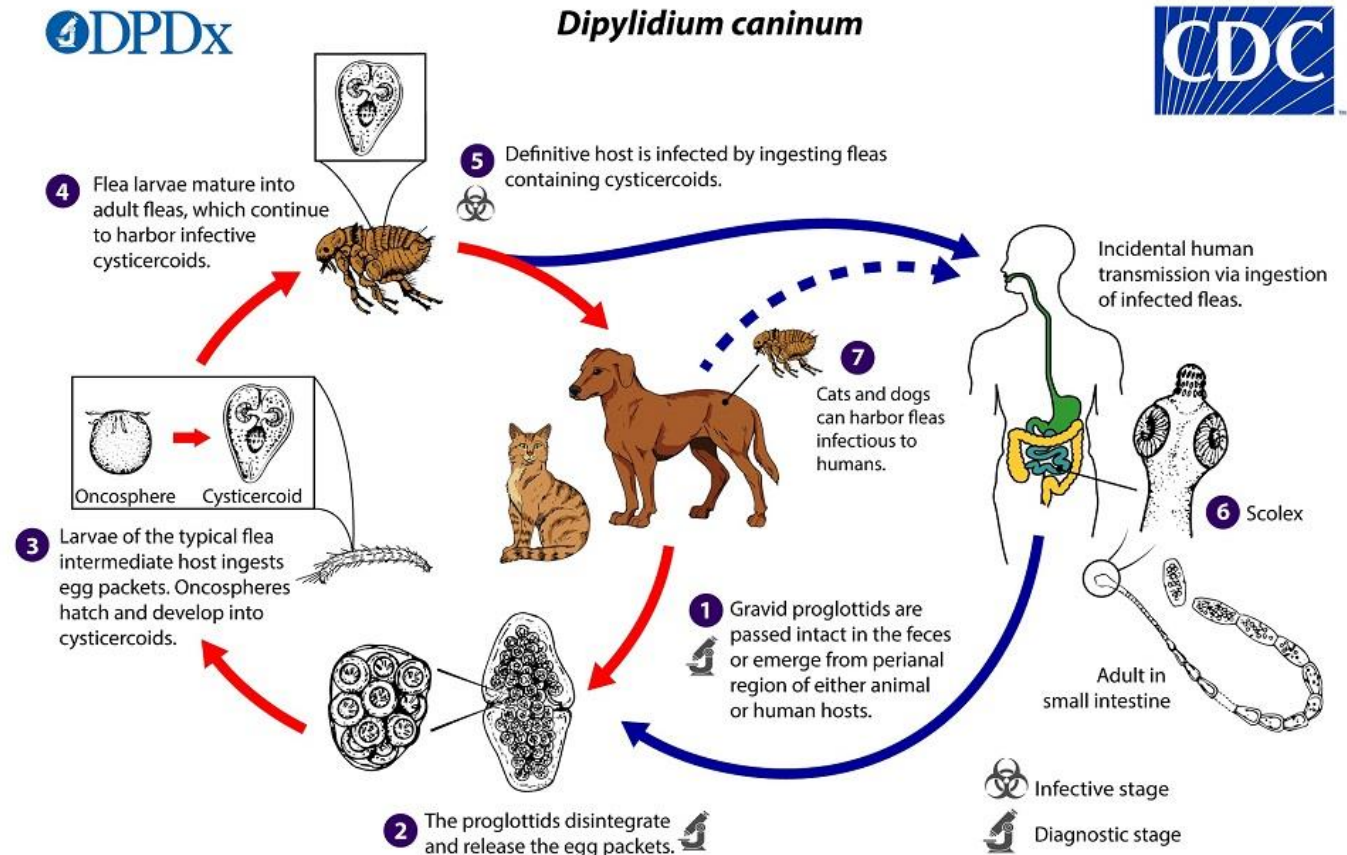
70-85 μm



Dipylidium caninum

Symptoms & Epi

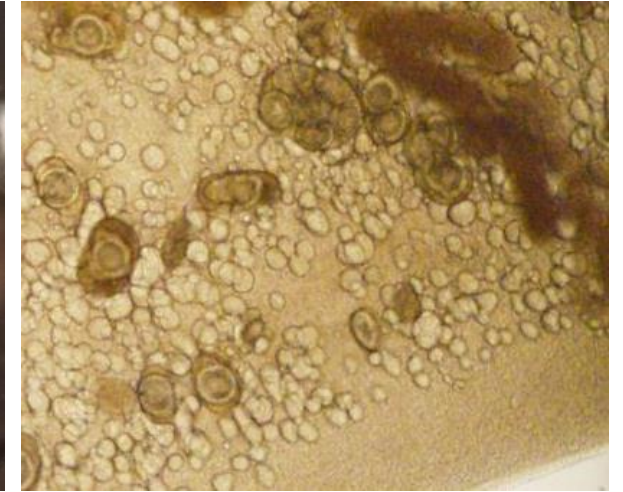
- Mostly asymptomatic
- Shed motile proglottids
- Worldwide: Cat and dog owners



Dipylidium caninum

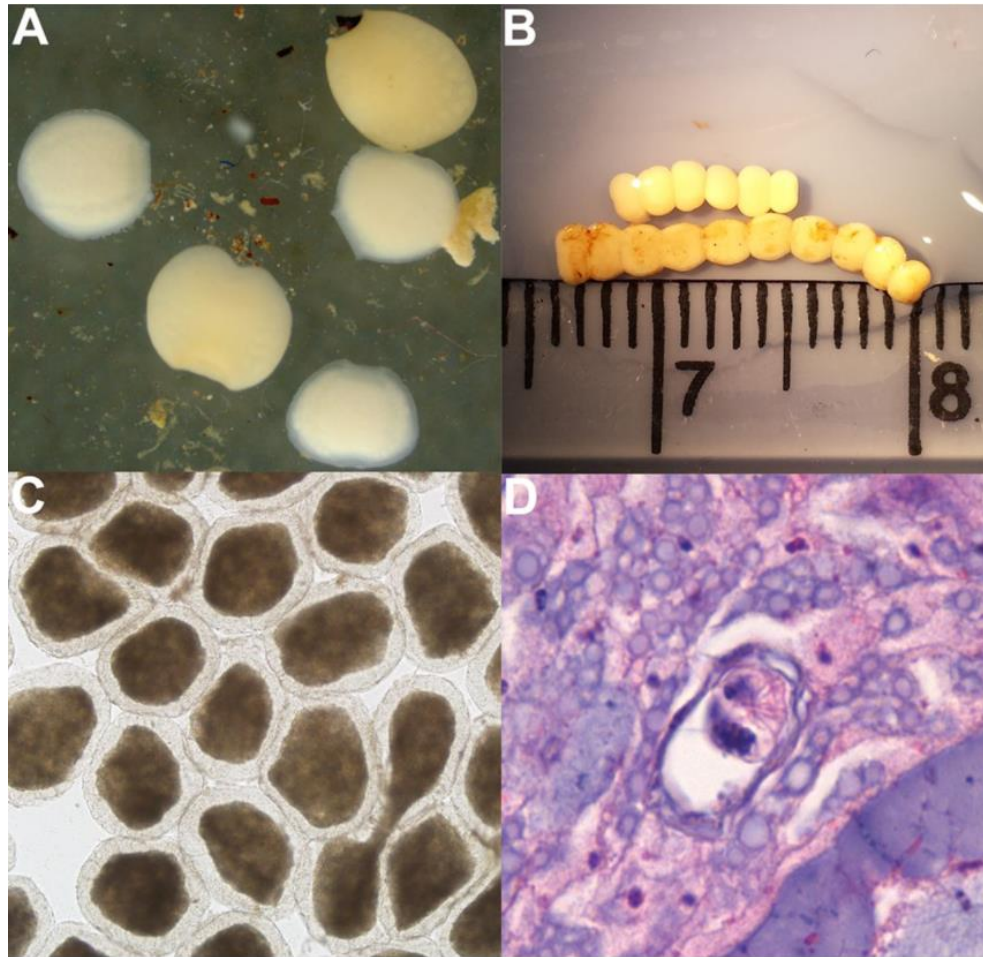
Diagnostic features & testing

- O&P
 - Egg packets rarely seen (degrade)
 - Contain 5-15 eggs
- Macroscopic examination of proglottids
 - Bilateral genital pores
 - Small “rice like”
 - Long, narrow



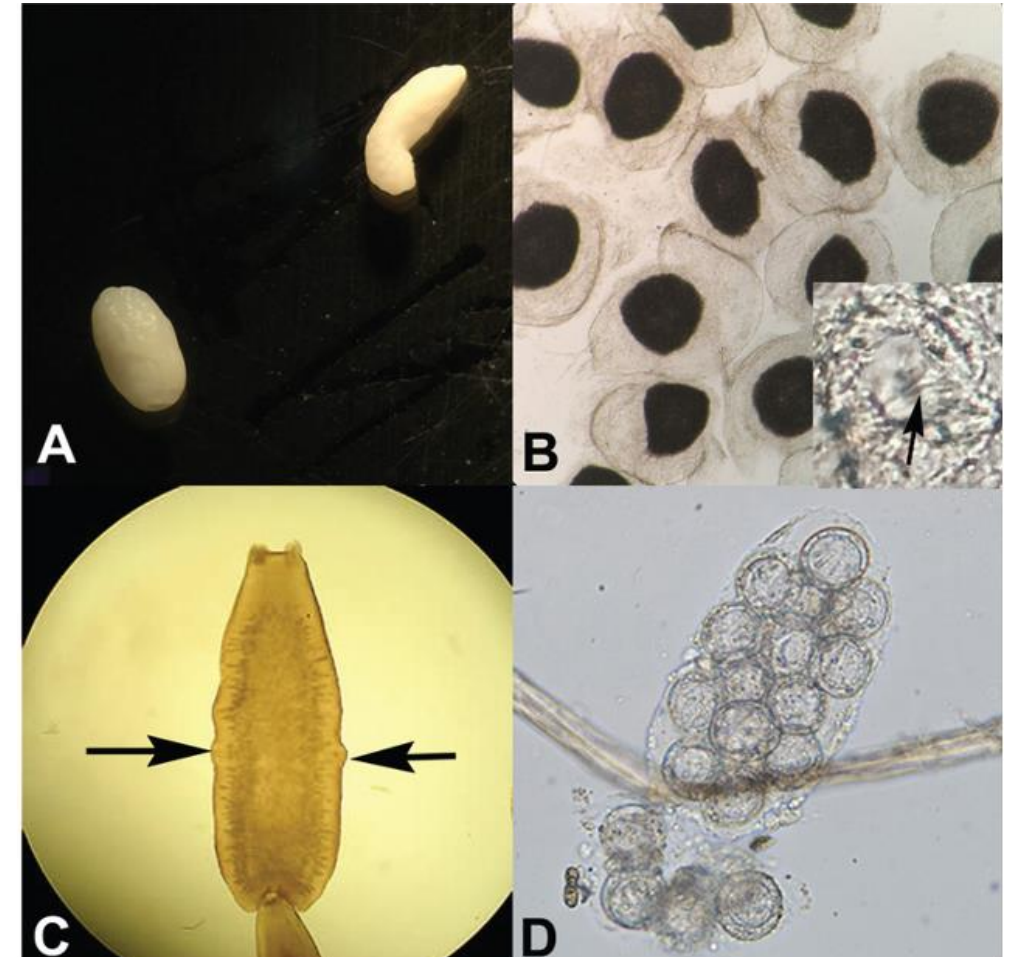
<https://www.cdc.gov/dpdx/dipylidium/index.html>

Look-a-likes – *Raillietina* & *Inermicapsifer*



Raillietina

Raillietina



Dipylidium

Kendall BA...Couturier MR. J Clin Microbiol. 2017 Aug;55(8):2293

Davis RE, Mathison BA, Couturier MR. Clin Infect Dis. 2019 Aug 30;69(6):1053-1055.



Trematodes

Case 8

Newly immigrated patient to Maine

- Asymptomatic
- Had stool exam performed as part of immigration/refugee screening procedures
- From Somalia
- Object measures ~150 μm

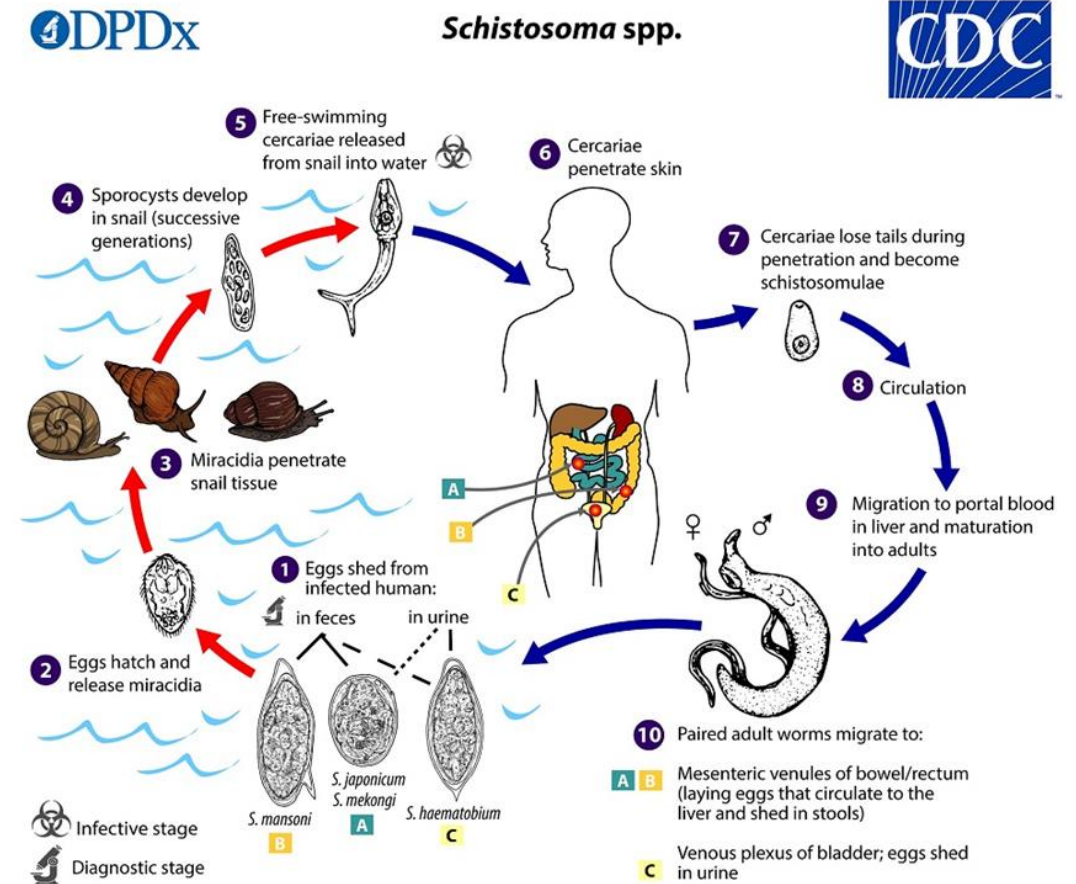
Schistosoma mansoni
+ *Entamoeba coli*



Schistosoma spp.

Symptoms & Epi

- Most asymptomatic (Sx mostly immune-mediated)
 - Acute: Hypersensitivity rxn - Katayama fever
 - Fever, cough, abd. Pain, diarrhea, eos.↑
 - Chronic: ectopic egg deposition & inflammation
 - *S. haem* – urinary, haematuria, squamous cell carcinoma
 - *S. man./jap.* – liver, brain, spinal cord



Schistosoma spp.

Diagnostic features & testing

- O&P –
 - Stool – *S. man*/*S. jap*
 - Occasionally *S. haem*.
 - Urine – *S. haem*
- IgG – late acute or chronic
 - Crossreactivity
 - Sensitivity varies
 - Species
 - Stage

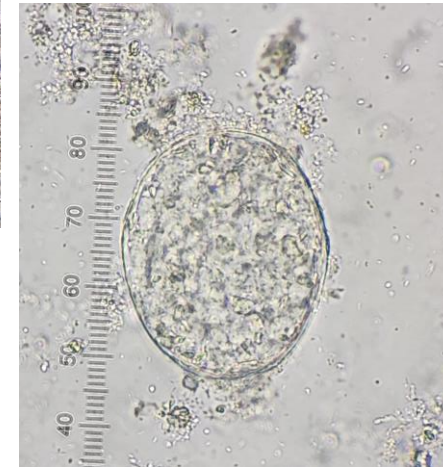


S. haematobium
110-170 μm



S. mansoni
114-180 μm

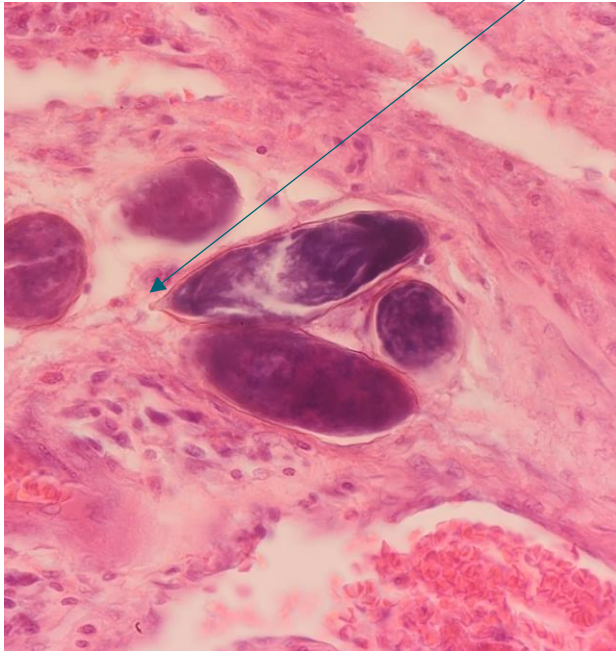
S. japonicum
70-100 μm



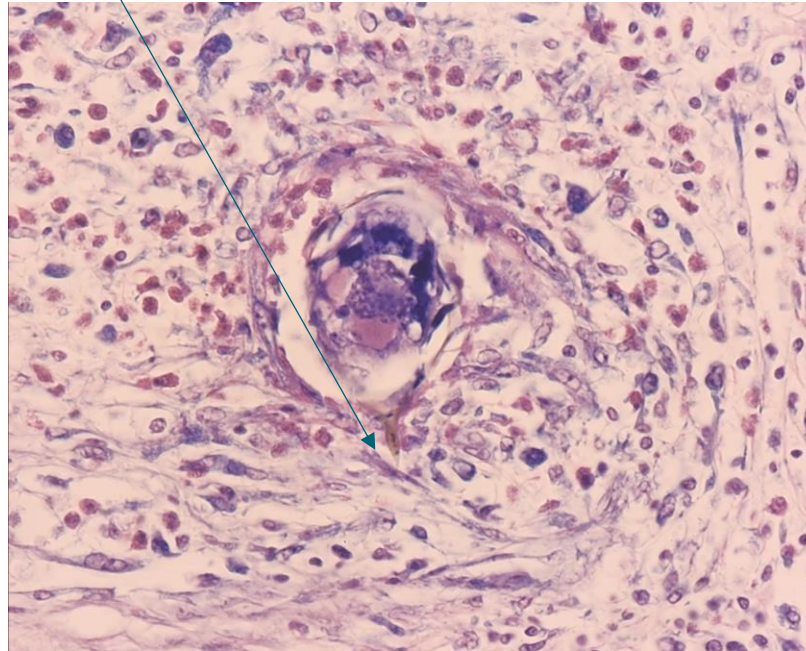
Schistosoma spp.

Histology

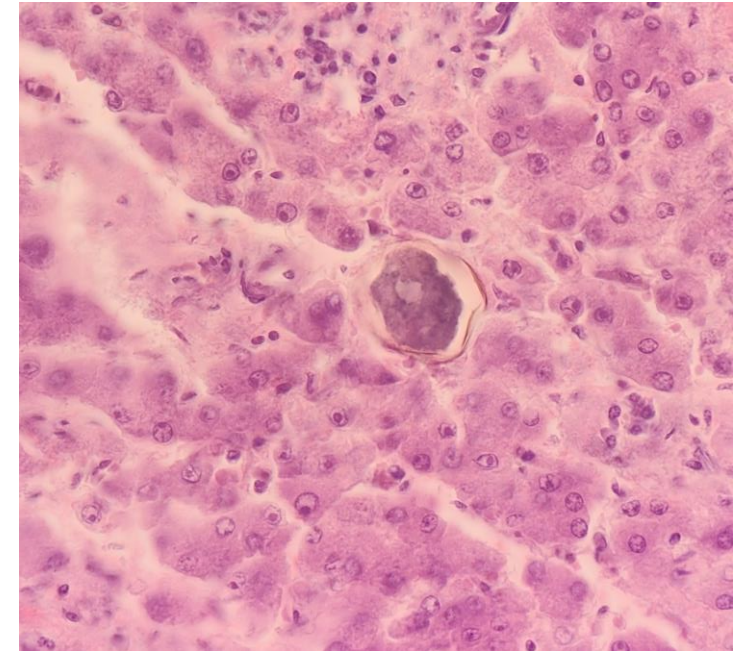
spine



S. Haematobium
Bladder



S. mansoni
Liver



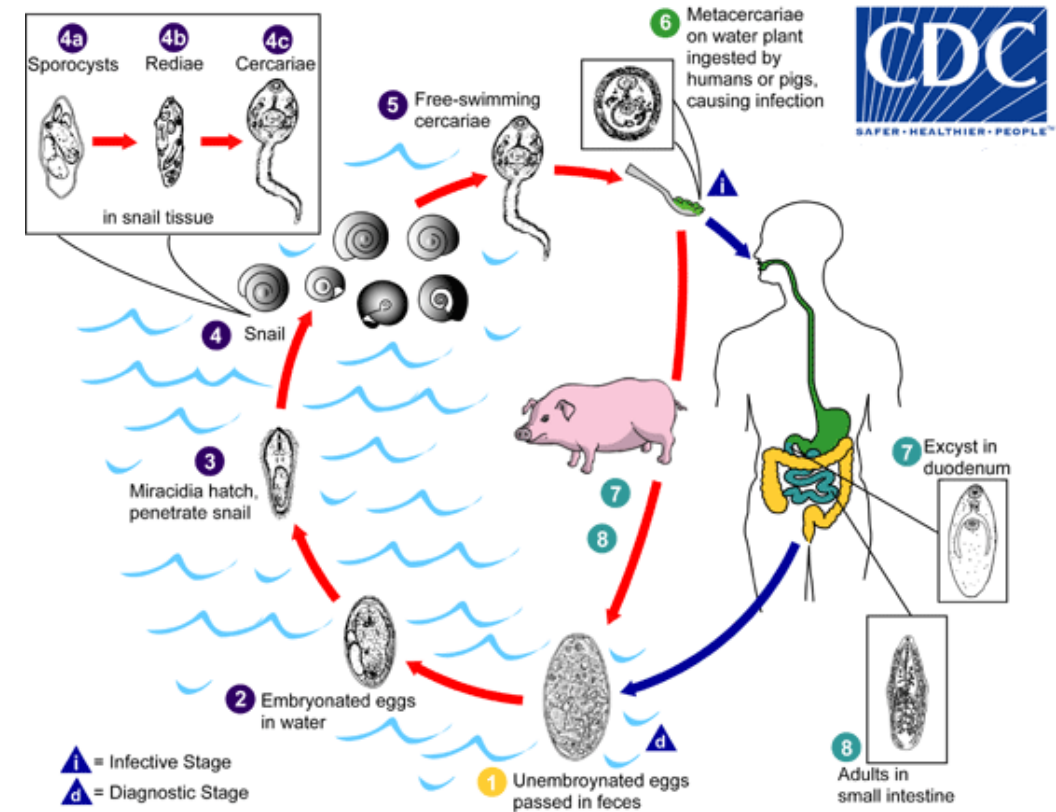
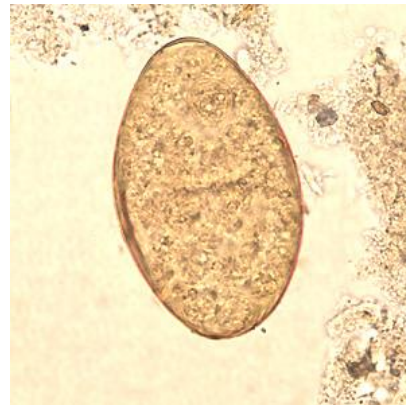
S. japonicum
Liver

Fasciolopsis buski

Symptoms, Epi, Testing

- Mostly asymptomatic
 - Less common, diarrhea, abdominal pain
- Asia/Indian subcontinent where pigs are raised and freshwater plants are eaten
- O&P exam

130-150 μm





Questions?

Marc.couturier@mainehealth.org

