Dispelling dogmas in clinical parasitology

A TRIPLE ATTACK BY BLAINE MATHISON, HENRY BISHOP, & MARC COUTURIER

Dispelling Dogmas in Stool Testing

1. *Dientamoeba fragilis* always has two nuclei
2. Size measurements can separate *Entamoeba coli* vs *E. histolytica*
3. If you concentrate stool for trichrome stain, you lose sensitivity
4. Alcohol-based fixatives are universally compatible with NAAT

Dientamoeba fragilis always has two nuclei

SURVEY SAYS?

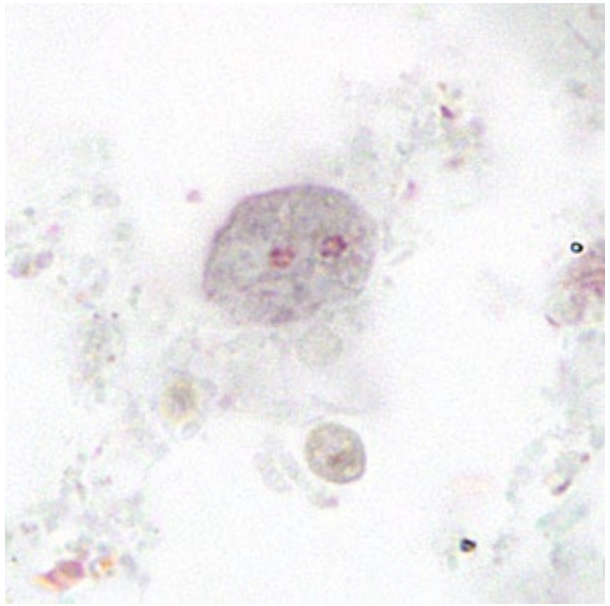
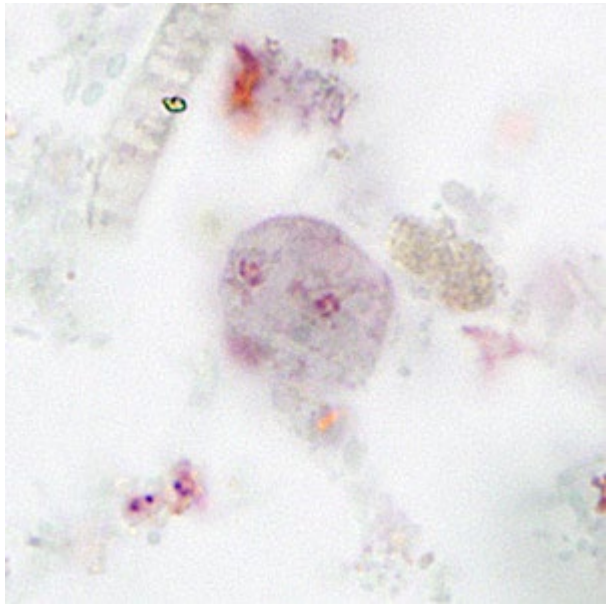
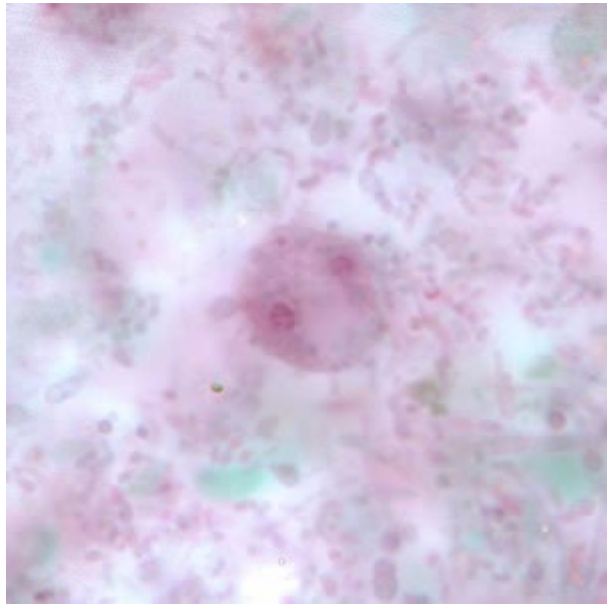
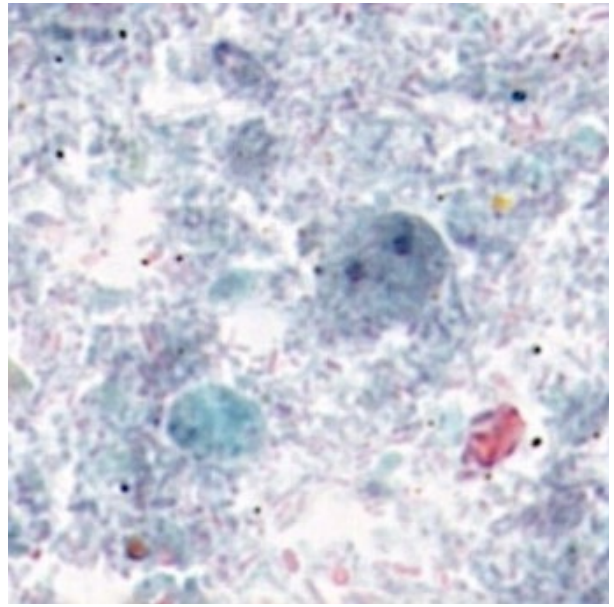
Dientamoeba fragilis always has two nuclei

- False…

- *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

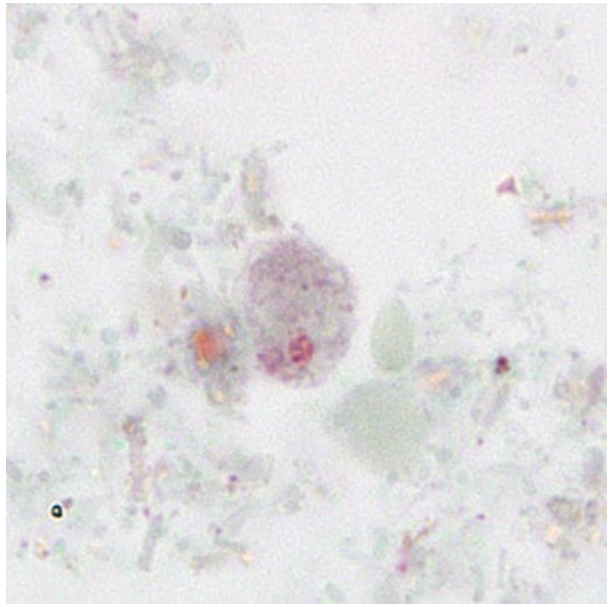
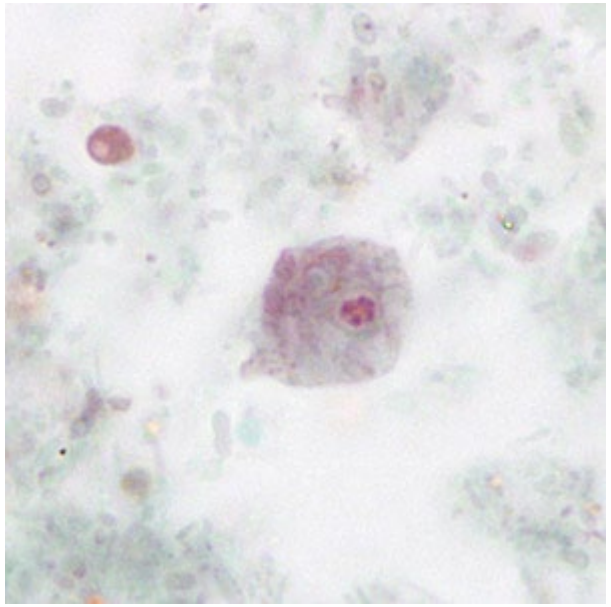
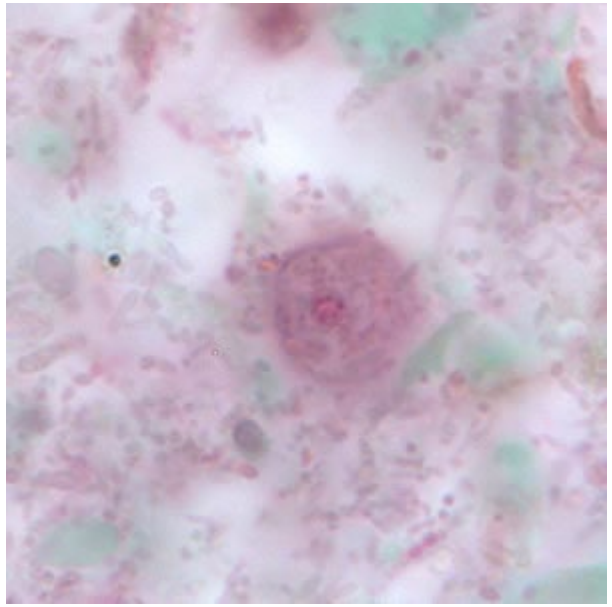
- Two nuclei, tetrad forms

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

Dientamoeba fragilis always has two nuclei

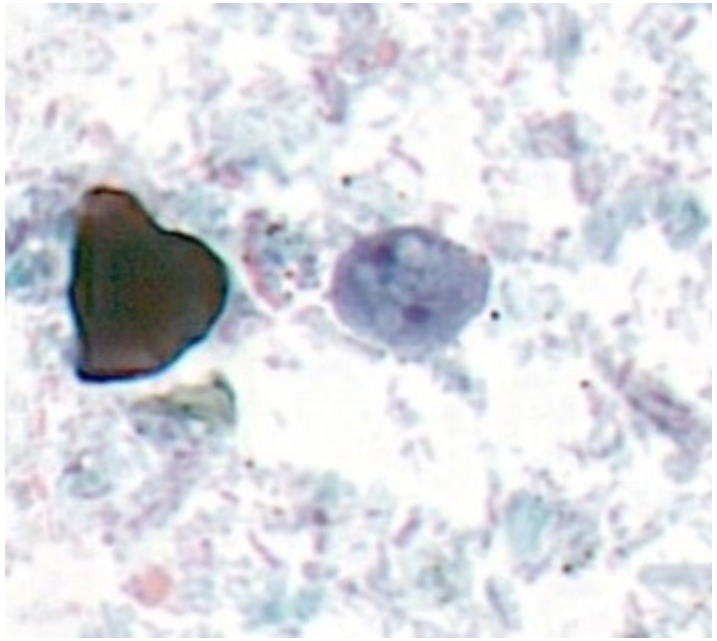
- Uninucleate, tetrad forms

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

Dientamoeba fragilis always has two nuclei

- Non-tetrad forms



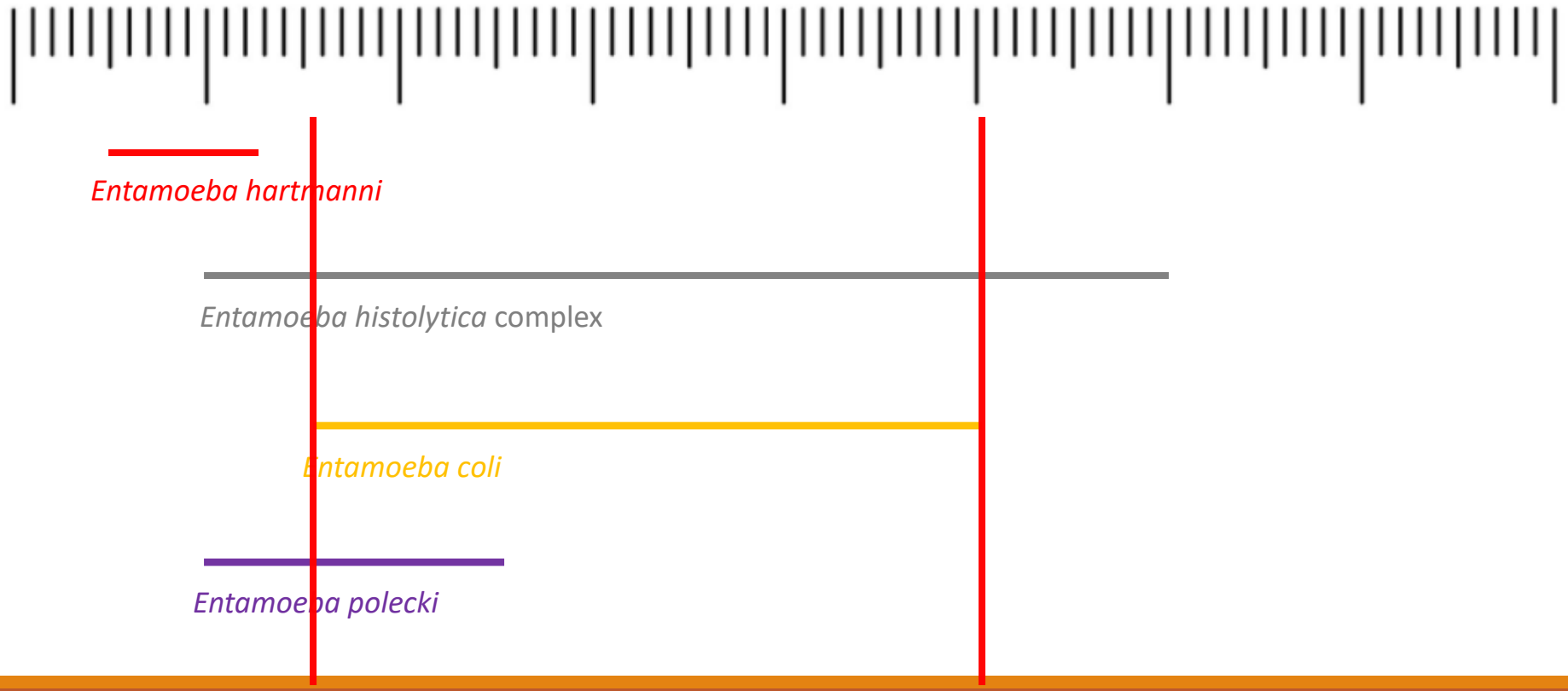
Size measurements can separate *Entamoeba coli* vs *E. histolytica*

SURVEY SAYS?

SLIDES MODIFIED COURTESY OF BLAINE MATHISON

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

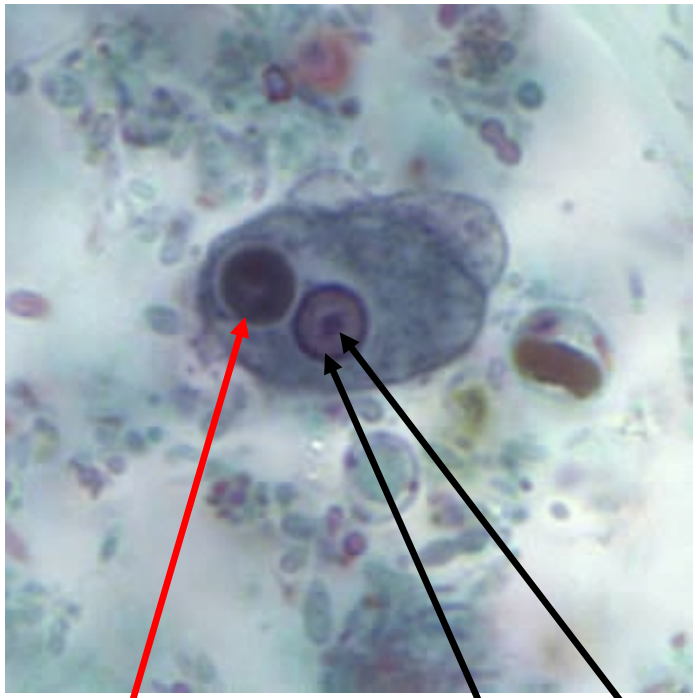
Entamoeba spp. identification

- 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

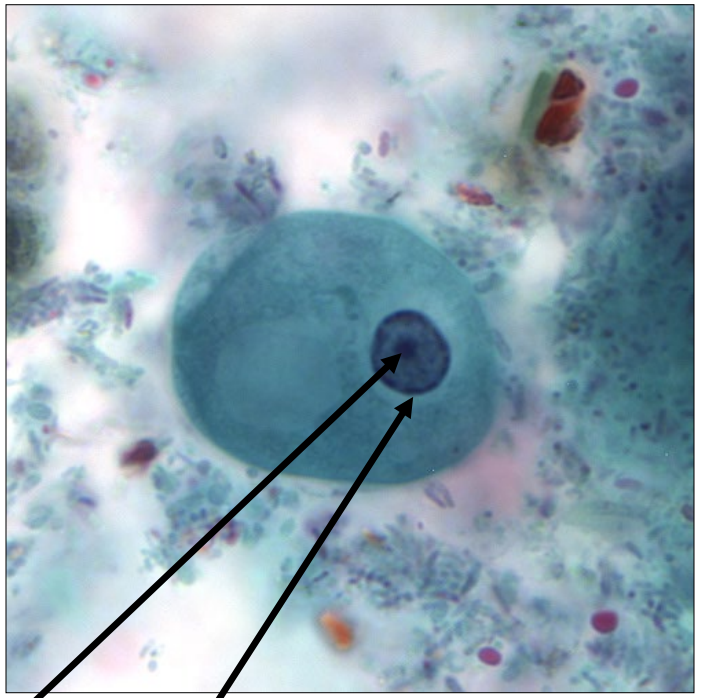


Ingested erythrocyte!

karyosome

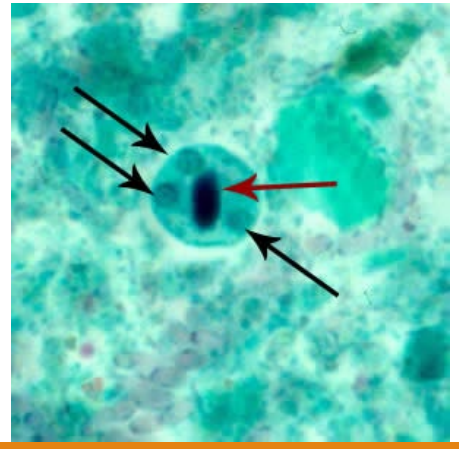
peripheral chromatin

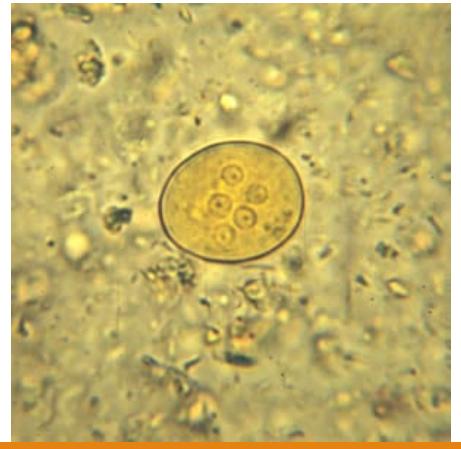
Entamoeba coli

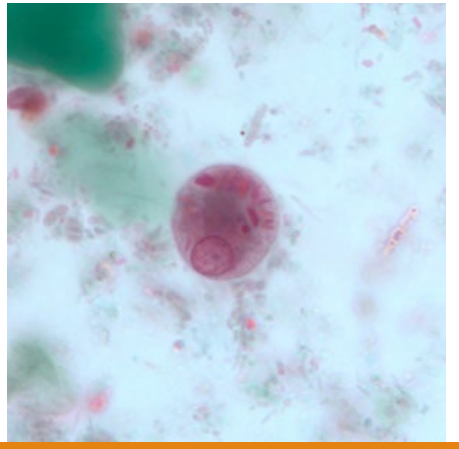


Entamoeba spp. – mature cysts

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







If you concentrate stool for trichrome stain, you lose sensitivity

SURVEY SAYS?

If you concentrate stool for trichrome stain, you lose sensitivity

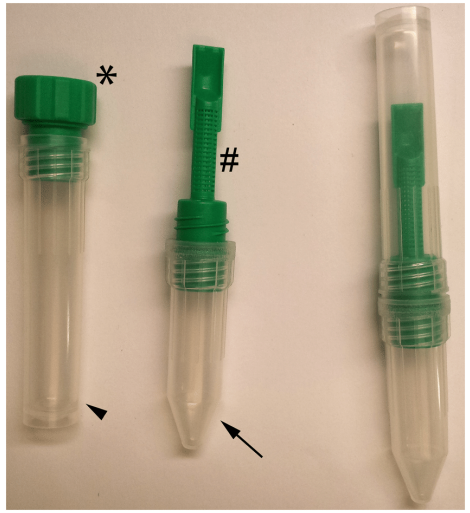
- False

- Where does this come from??
  - No published comparator study to prove this
  - Expert opinion and personal experience (?)

- Ok…well, I like data.

If you concentrate stool for trichrome stain, you lose sensitivity

- Parasep tubes allow for semiautomation of processing
- Single tube & alcohol-based fixative streamlines prep
- Vertical filtration achieved inside of tube/spork device



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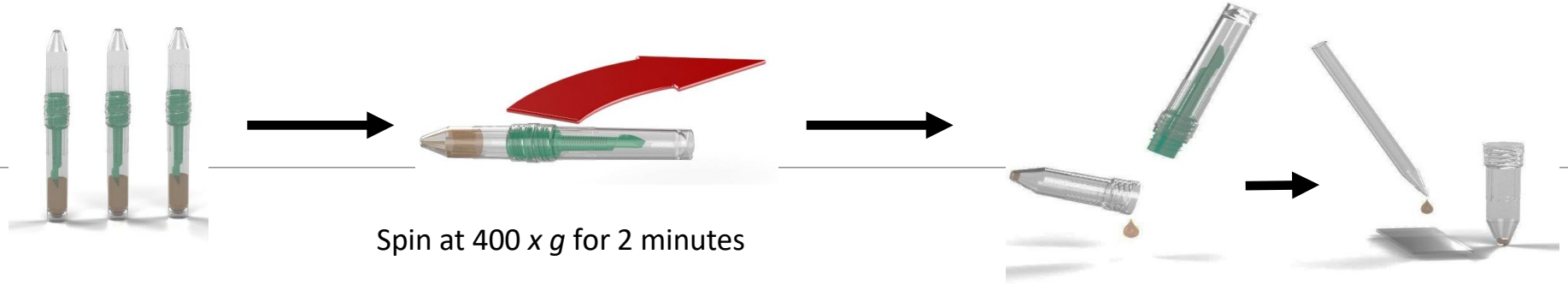
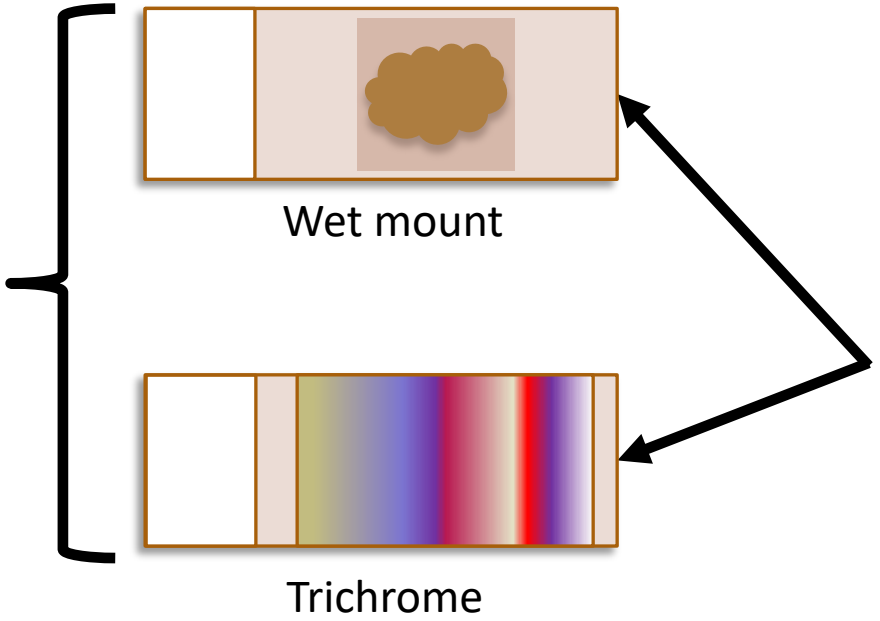
Single preparation workflow



Spin at 400 *x g* for 2 minutes

Decant

Diagnose



If you concentrate stool for trichrome stain, you lose sensitivity

- 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 (× *g*)	Trichrome stain result
1	Unconcentrated	Uninterpretable
	200	Uninterpretable
	400	Uninterpretable
2	Unconcentrated	1+ *Chilomastix*
	200	2+ *Chilomastix*
	400	3+ *Chilomastix*
3	Unconcentrated	1+ *B. hominis*
	200	1+ *B. hominis*
	400	1+ *B. hominis*
4	Unconcentrated	Negative
	200	Negative
	400	Negative
5	Unconcentrated	1+ *B. hominis*
	200	1+ *B. hominis*
	400	1+ *B. hominis*
6	Unconcentrated	Rare *E. histolytica/Entamoeba dispar*
	200	1+ *E. histolytica/E. dispar*
	400	1+ *E. histolytica/dispar*
7	Unconcentrated	Rare *B. hominis*
	200	Rare *B. hominis*
	400	1+ *B. hominis*
8	Unconcentrated	1+ *B. hominis*, Rare *E. coli*
	200	1+ *B. hominis*, 1+ *E. coli*
	400	1+ *B. hominis*, 1+ *E. coli*
9	Unconcentrated	1+ *B. hominis*, 1+ *E. coli*
	200	1+ *B. hominis*, 1+ *E. coli*
	400	1+ *B. hominis*, 1+ *E. coli*
10	Unconcentrated	Rare *Giardia* cysts
	200	1+ *Giardia* cysts
	400	1+ *Giardia* cysts

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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 

Alcohol-based fixatives are universally compatible with NAAT

SURVEY SAYS?

Alcohol-based fixatives are universally compatible with NAAT

- Kinda…but not really

Alcohol-based fixatives and NAAT tests

- NAAT tests can be performed on most alcohol-based fixatives
  - *e.g.* TOTAL-FIX, EcoFix, AlcoFix, PVA
- Formalin or formalin derivatives is BAD
  - *e.g.* 10% formalin, SAF, MIF
- Compatibility with some FDA cleared & CE-IVD labeled stool protozoa panels
  - See package insert for intended use statements & acceptable specimen types

Alcohol-based fixatives and NAAT tests

- CLIA/CAP require full LDT validation studies if fixative is used for testing that IS NOT labeled in the NAAT IFU
  - Do not tempt your inspector with a citation

- If a fixative lists “compatible with NAAT”, that is a claim of their product only
  - Does not mean it is “***ACCEPTABLE***” with every commercial NAAT
  - *i.e.* regulatory clearance of assay trumps fixative compatibility statement

Examples from the marketplace

- biomérieux FilmArray GI Panel (FDA-IVD)
  - Intended use: Cary-Blair preserved stool
  - TOTAL-FIX, EcoFix, AlcorFix, PVA etc. would be off-label and require full validation

- Genetic Signatures EasyScreen Gastrointestinal Parasite Detection Kit (CE-IVD)
  - Intended use: TOTAL-FIX, EcoFix
  - Alcorfix, PVA, etc. would require full validation

- Novodiag (Hologic) Stool Parasites (CE-IVD)
  - Intended use: eSwab with liquid Amies
  - TOTAL-FIX, Ecofix, Alcorfix, PVA, etc. would require full validation

Dispelling dogmas in clinical parasitology

A TRIPLE ATTACK BY BLAINE MATHISON, HENRY BISHOP, & MARC COUTURIER

Question 1:

I see double chromatin dots! This must be *Plasmodium falciparum*!

Answer: FALSE!

Plasmodium falciparum – ring morphology

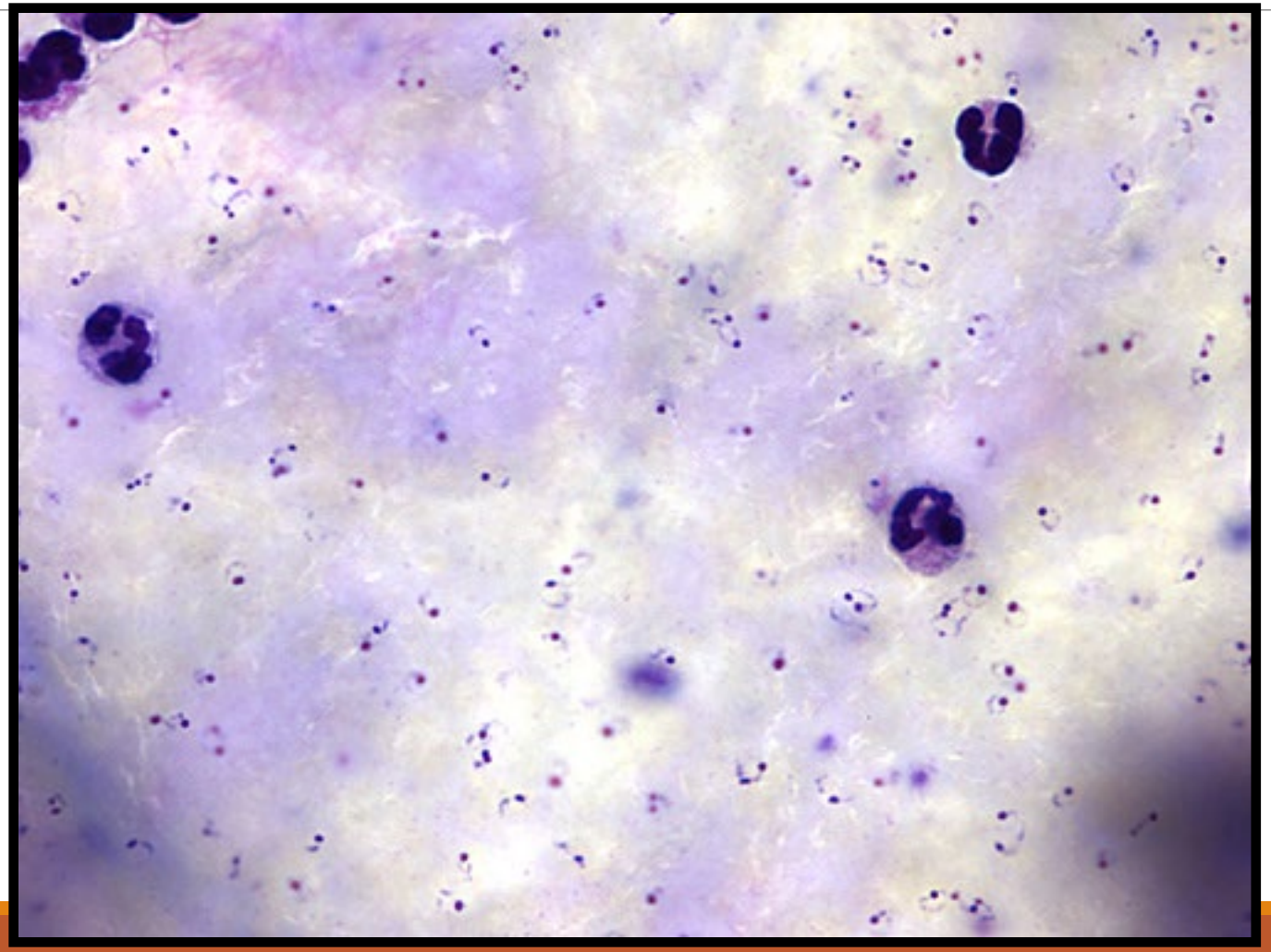
Four textbook definitions of *Plasmodium falciparum* ring morphology:

- Rings with thin, delicate cytoplasm
- Double chromatin dots
- Applique (or accolé) forms – on the periphery of the RBC
- Multiply infected erythrocytes

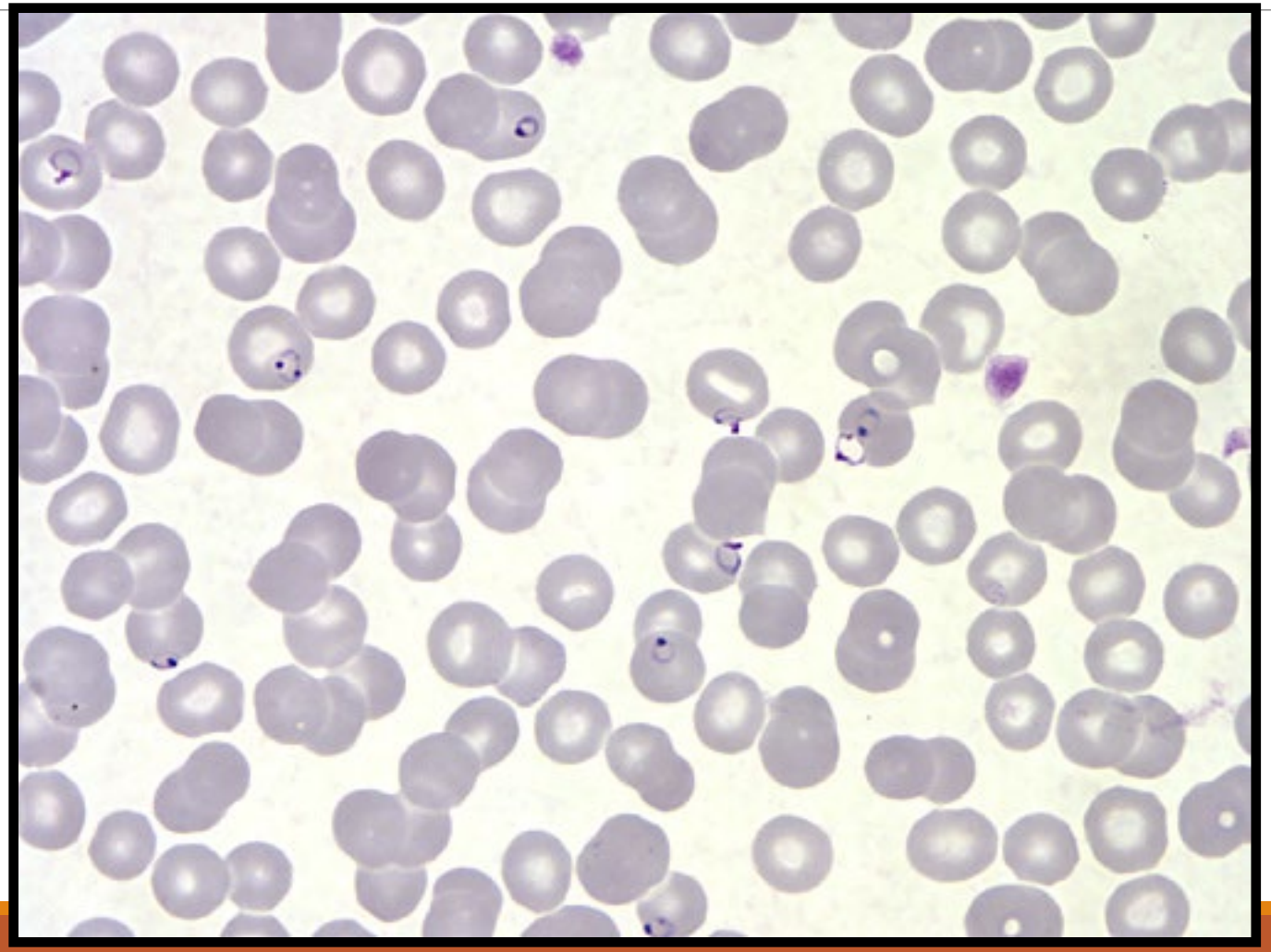
When these four structures are seen together, especially in the absence of other stages, this is highly supportive of *P. falciparum*. BUT…

It is important to understand these four features can be seen among all four human*Plasmodium* species!

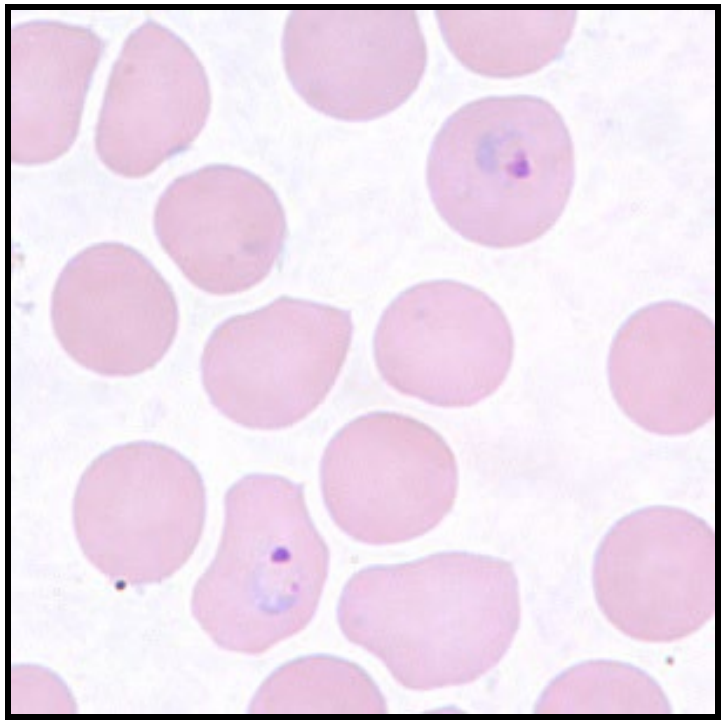
Plasmodium falciparum: Thick film

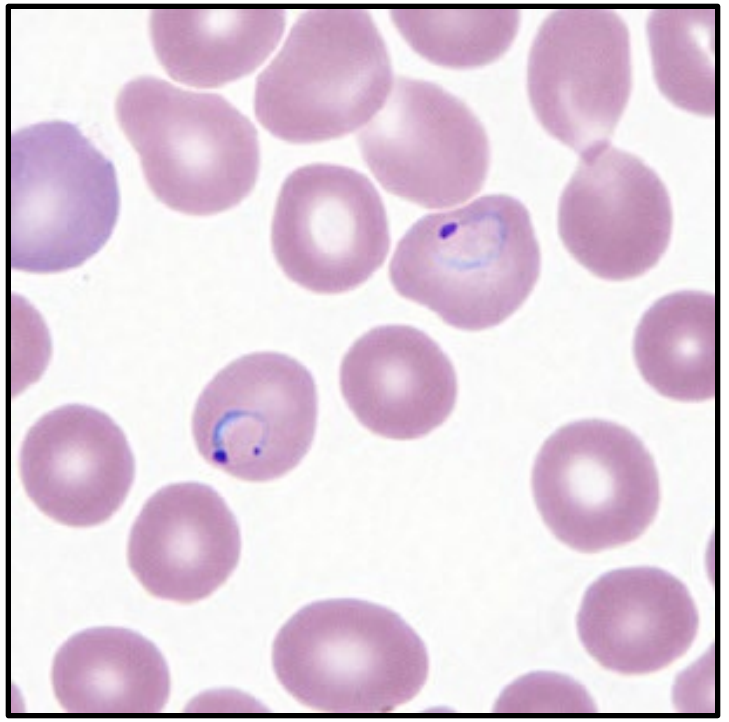


Plasmodium falciparum: Thin film



Plasmodium vivax: Rings in thin films



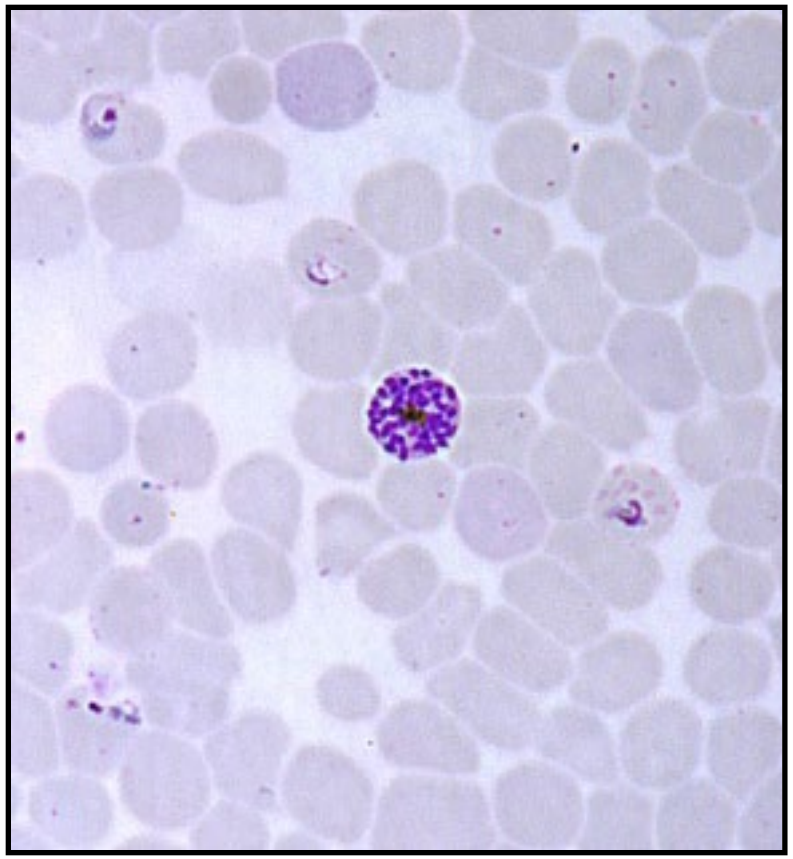


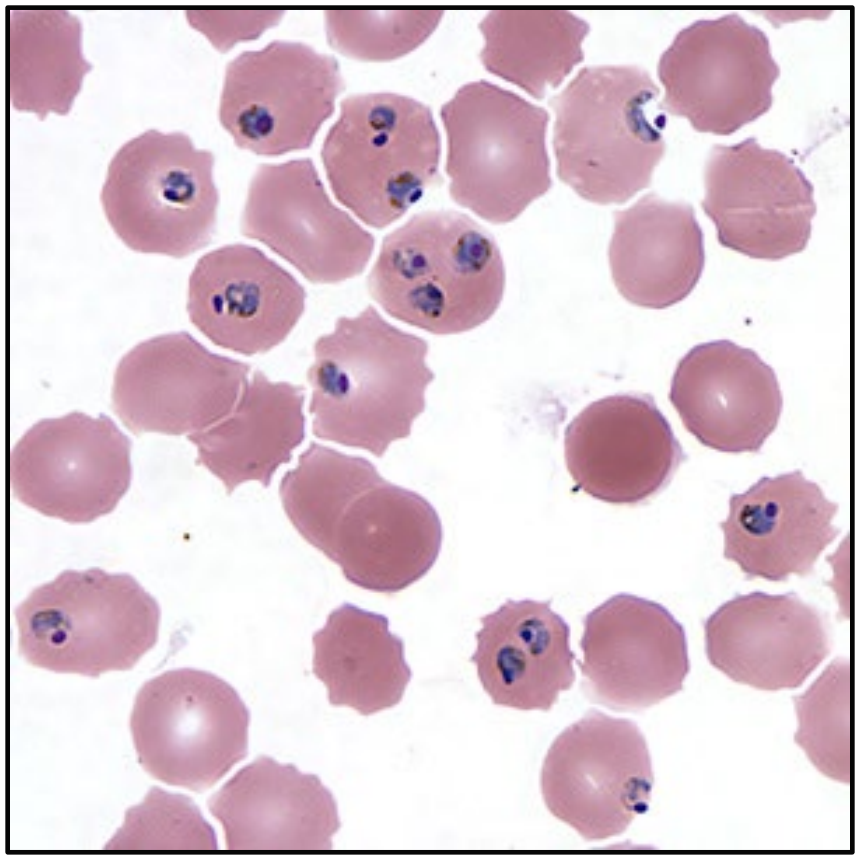
Question 2:

You never see late trophozoites or schizonts of *P. falciparum* in blood films!

Answer: False

Plasmodium falciparum



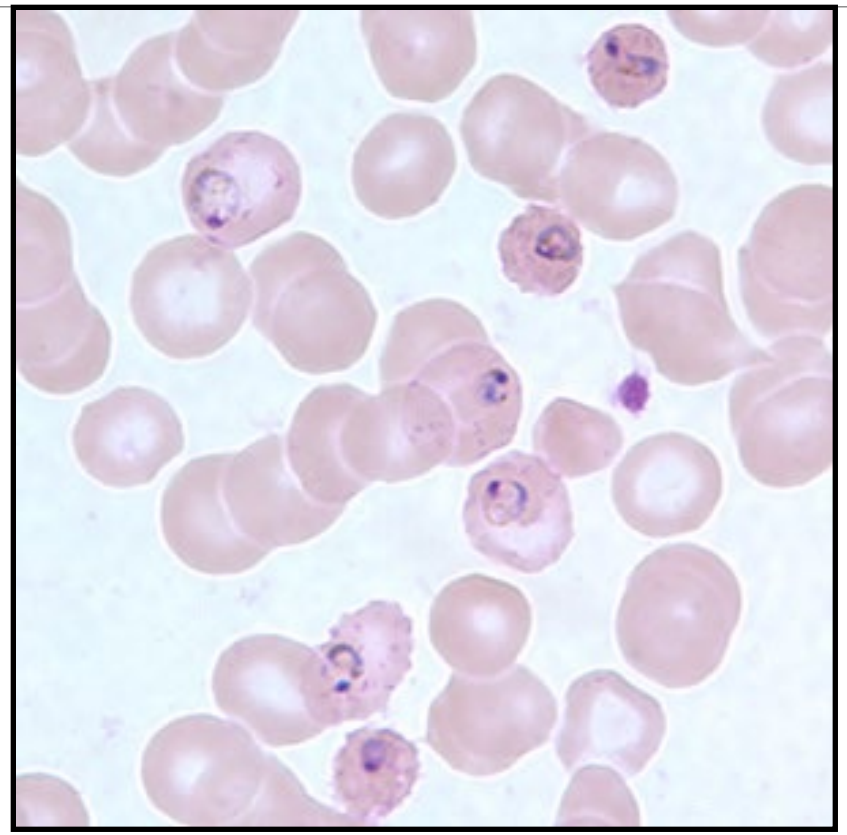


Question 3:

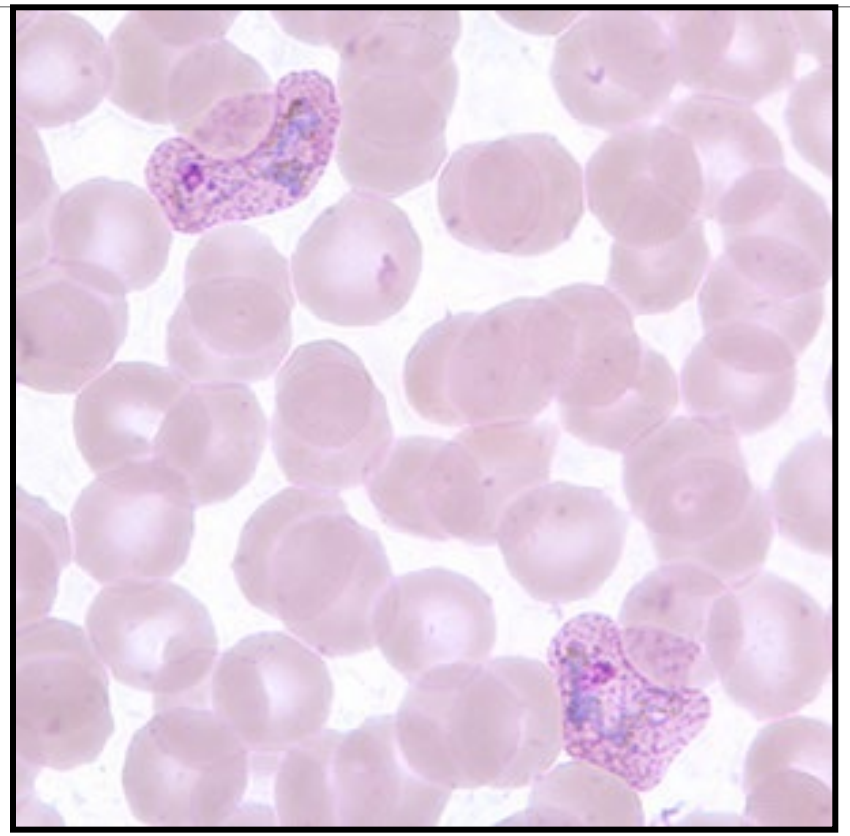
I see inclusions in the cytoplasm of the erythrocytes. This represents Schüffner's stippling and we are looking at *P. vivax* or *P. ovale*

Answer: Well…you could be right…

Cytoplasmic Inclusions in Erythrocytes



Maurer's Clefts: *P. falciparum*



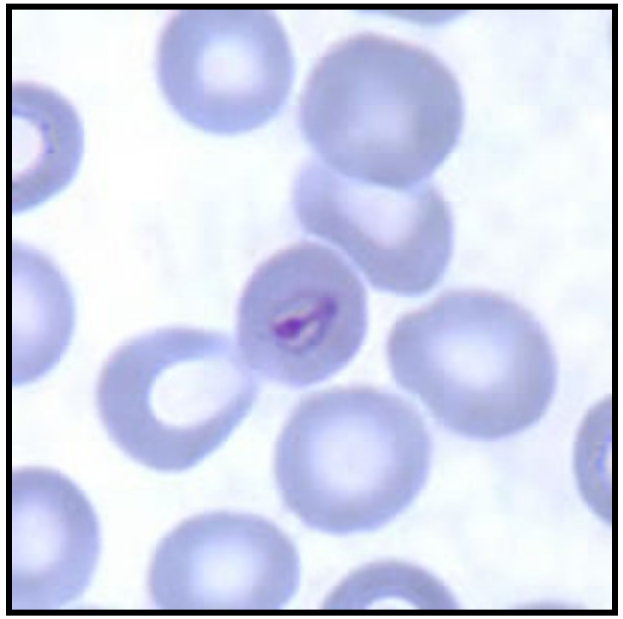
Schüffner's stippling: *P. vivax*/*P. ovale*

Question 4:

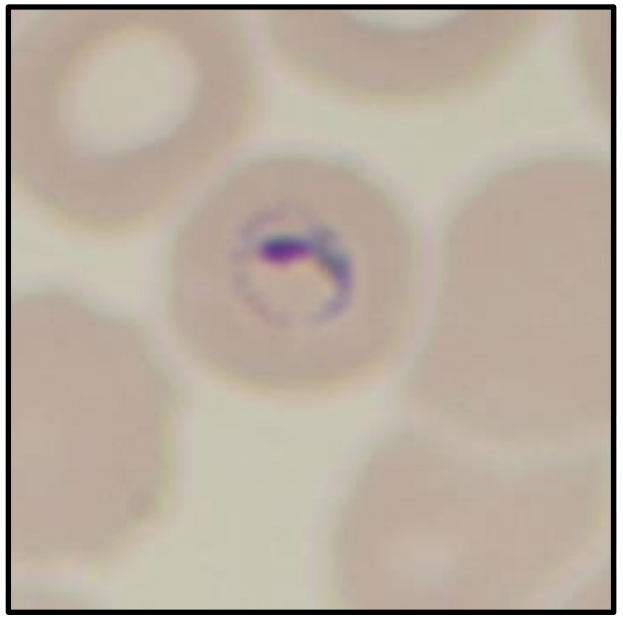
Birds-eye ring forms are always indicative of *P. malariae*

Answer: False!

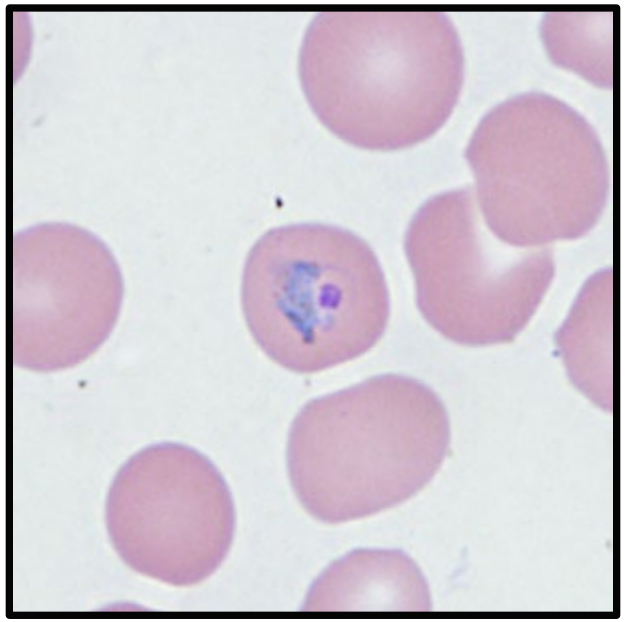
Birds-eye Ring Forms



Plasmodium malariae



Plasmodium falciparum



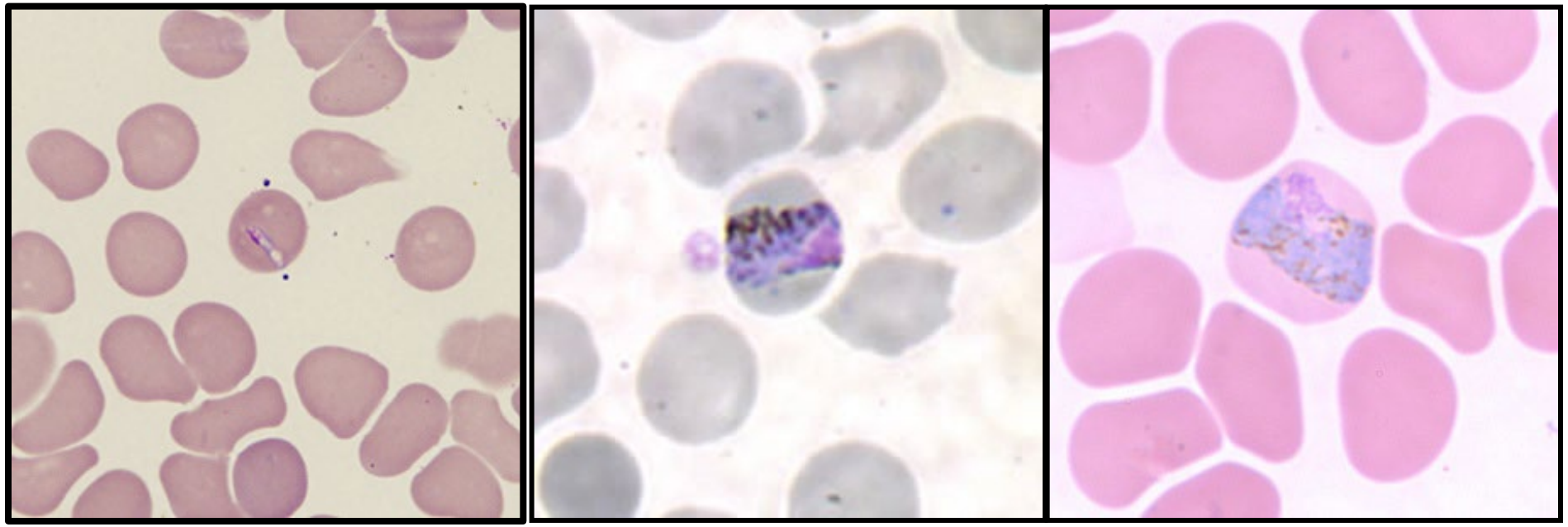
Plasmodium ovale

Question 5:

Band-from Trophozoites are always indicative of *P. malariae*

Answer: False!

Band Forms



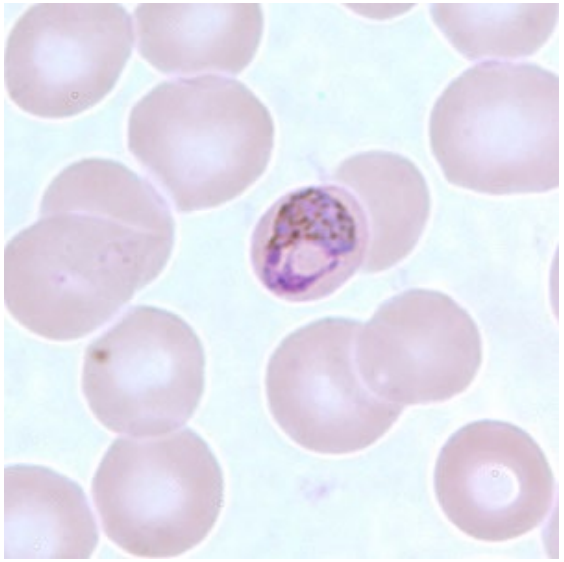
Babesia *P. malariae* *P. vivax*

Question 6:

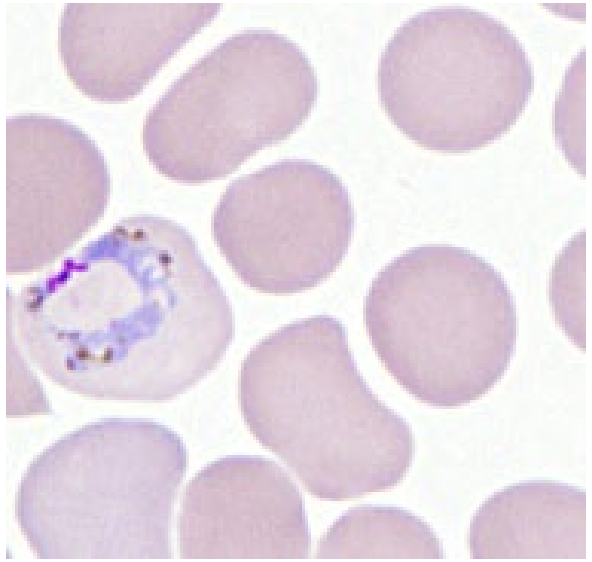
Basket-form trophozoites are always indicative of *P. malariae*

Answer: False!

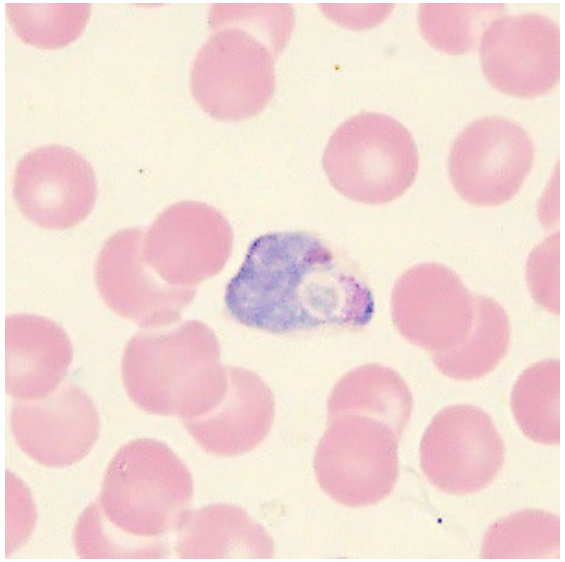
Basket Forms



Plasmodium malariae



Plasmodium vivax



Plasmodium vivax

Question 7:

I see Schüffner's stippling in an erythrocyte that contains a schizont. The schizont has only 8 merozoites, so it must be *P. ovale*.

Answer:

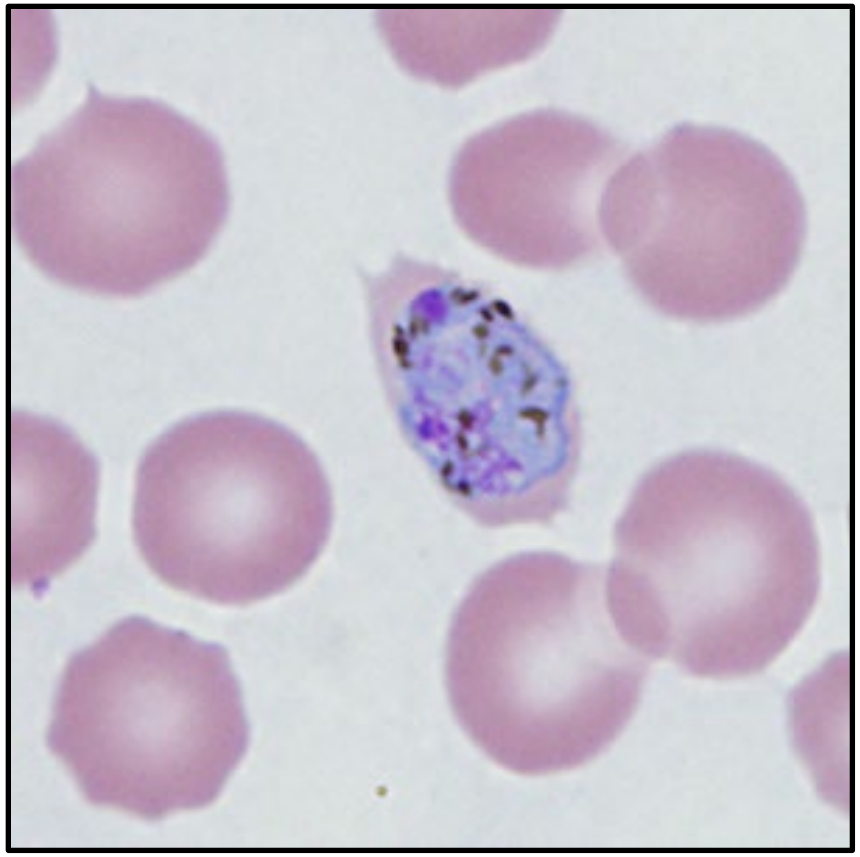
You might be right, but…

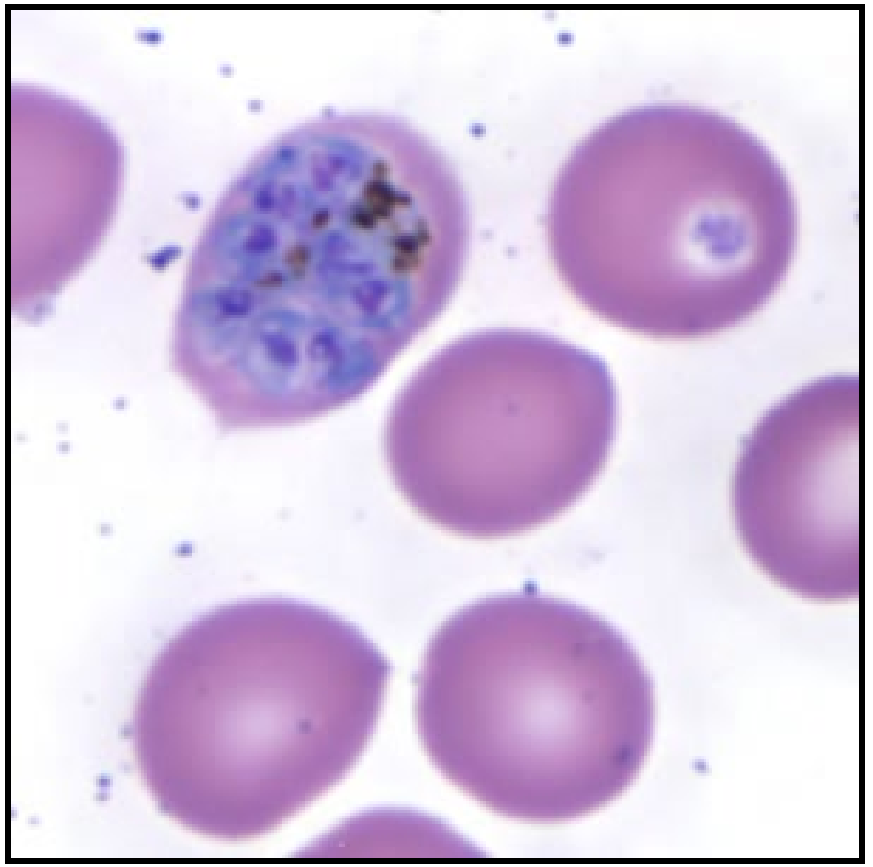
Answer:

You might be right, but…

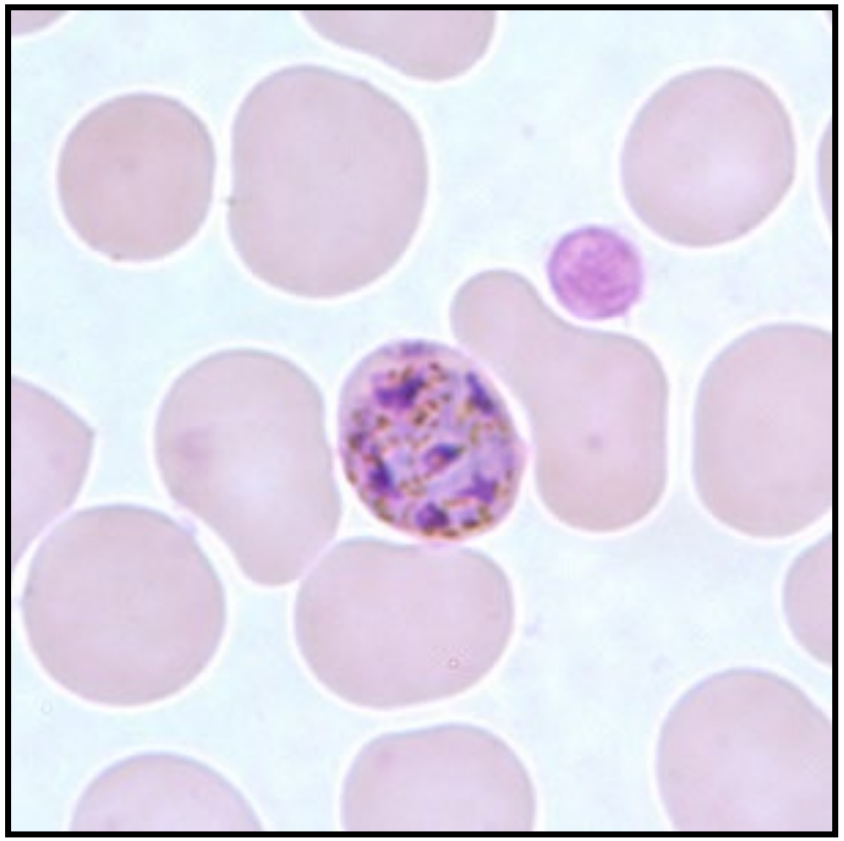
do you know when to count merozoites?

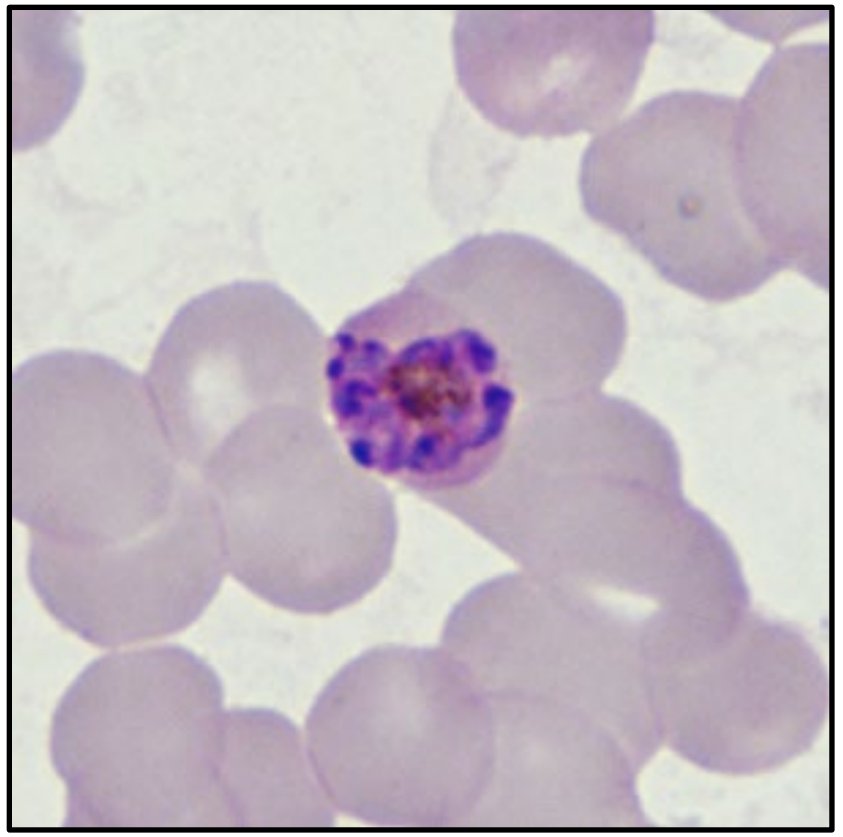
Plasmodium ovale: Schizonts, thin smears



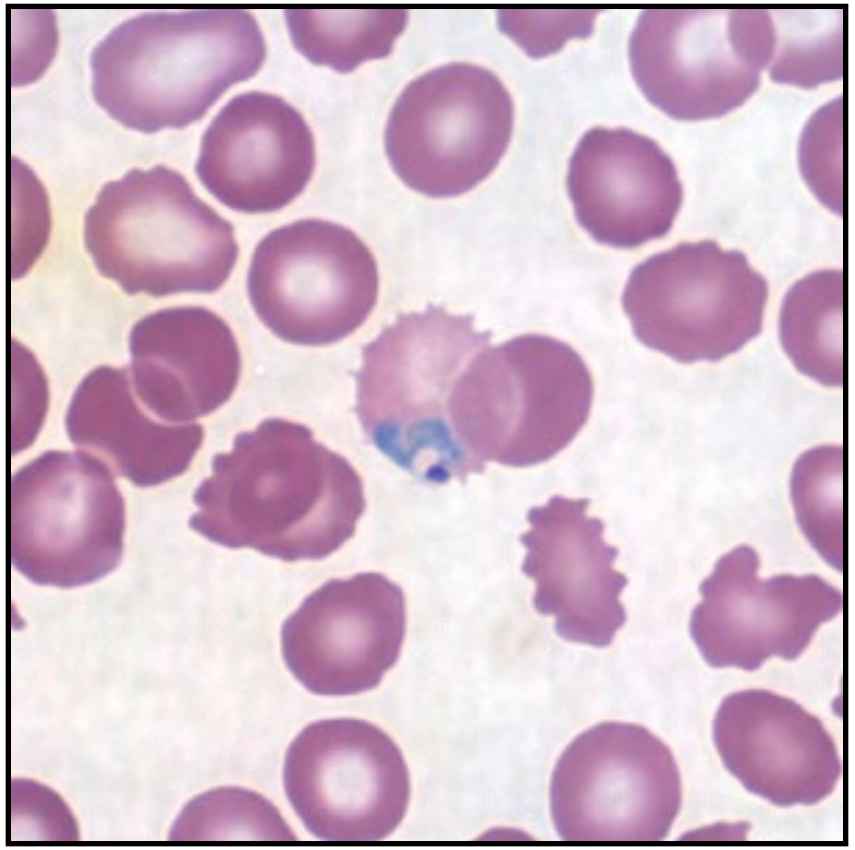


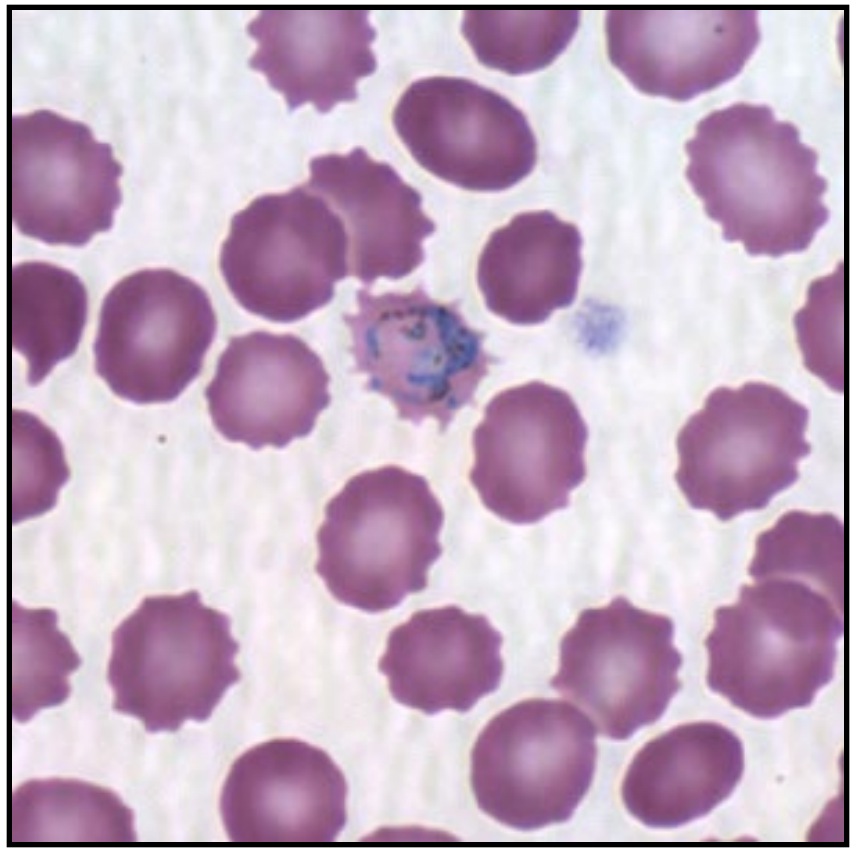
Schizonts of *P. malariae,* thin film





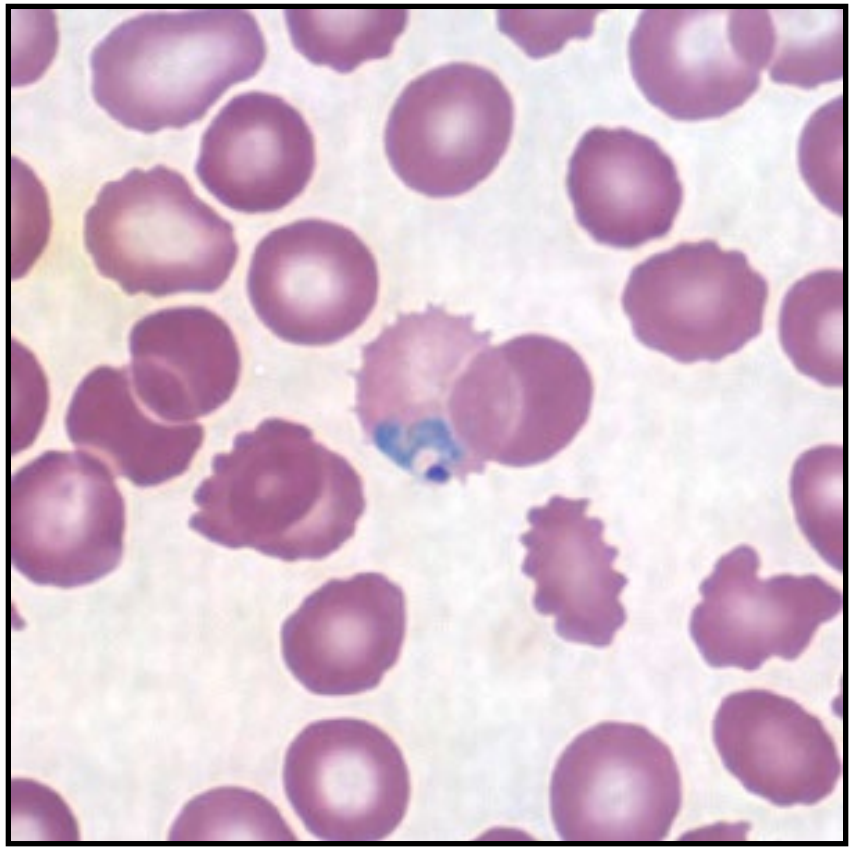
Question 8: The fimbriation in the following images supports a diagnosis of *P. ovale*

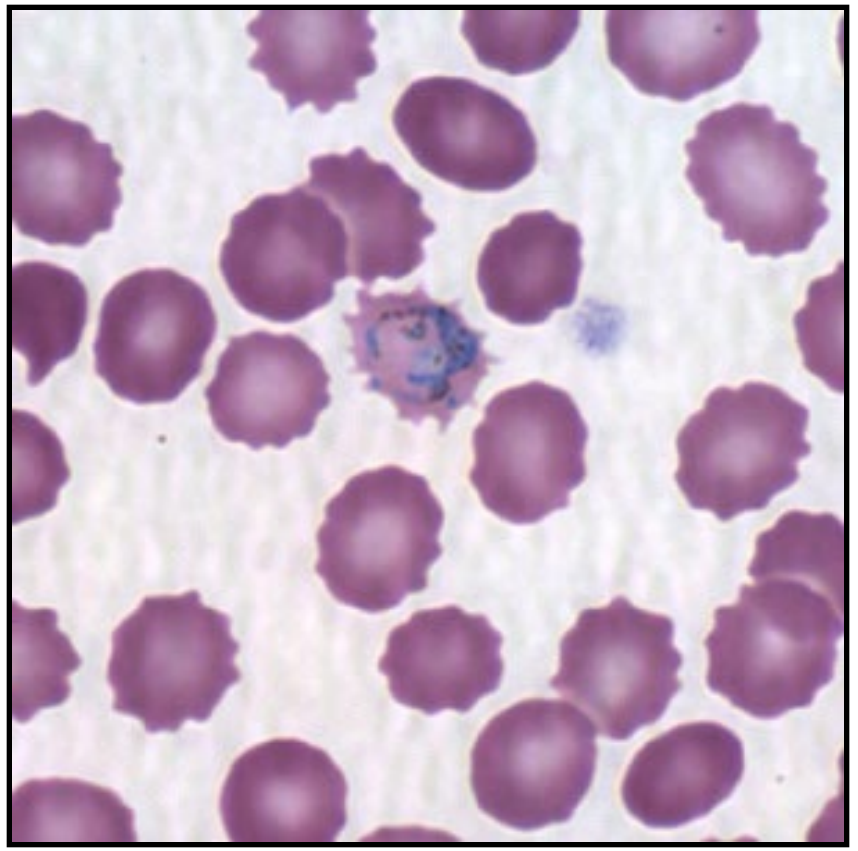




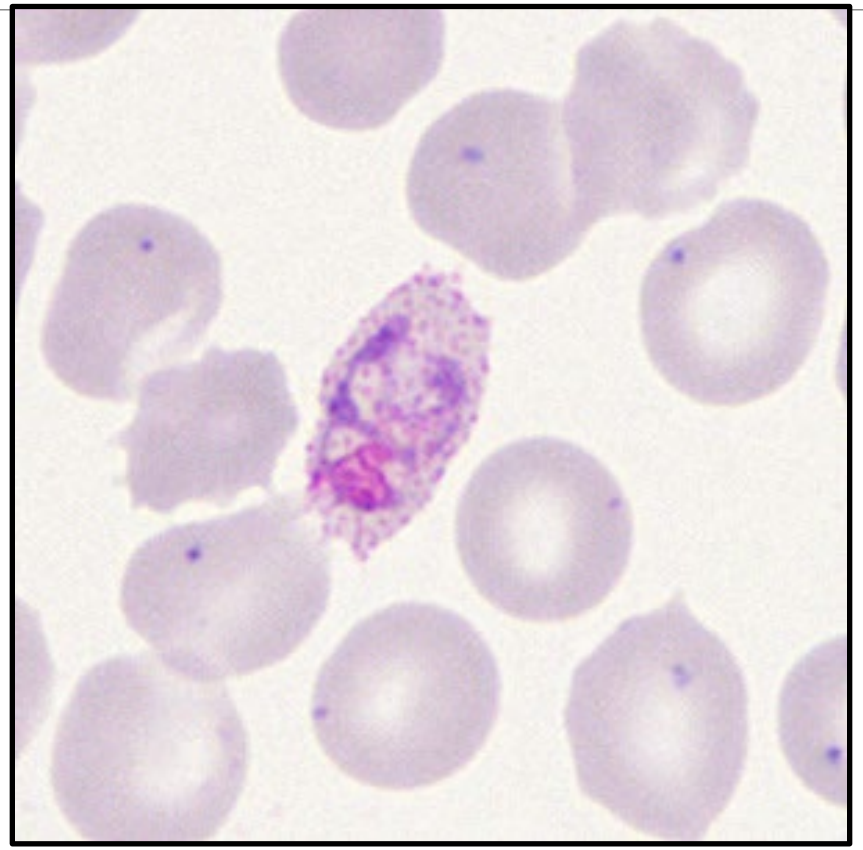
Answer: False!

Crenation

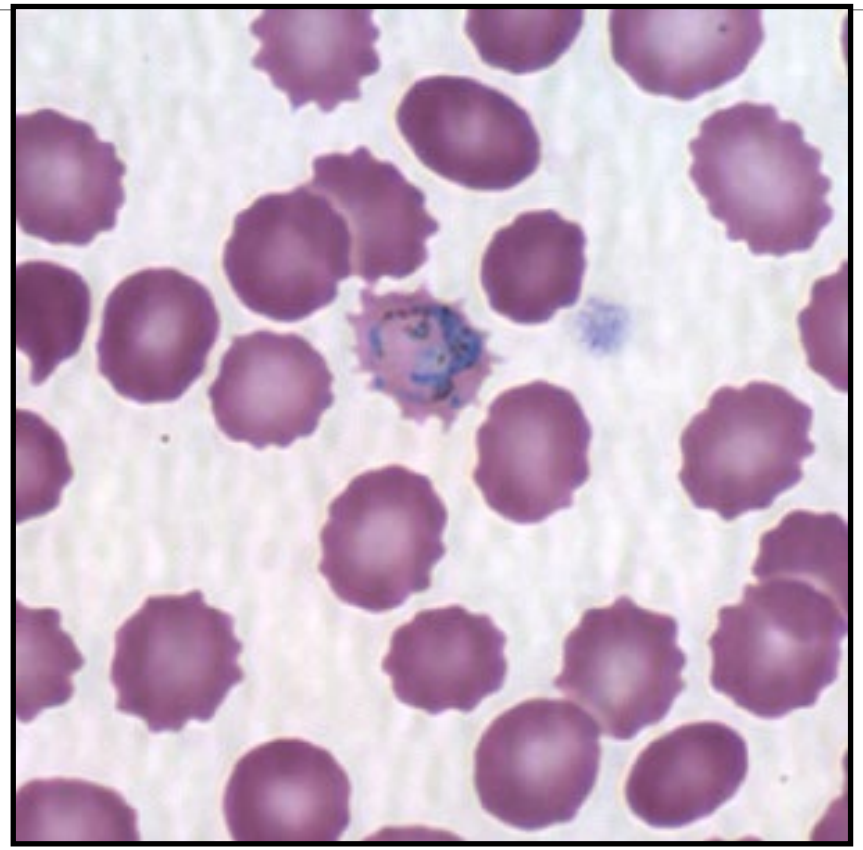




Crenation vs Fimbriation



fimbriation



crenation

Take-home and other important messages in *Plasmodium* identification

Always have a ‘big picture’ approach

Make sure you examine enough examples (if the parasitemia is high enough) to appreciate the full extent of morphologic variation in the specimen

When using size as a criterion, do it where there is a good monolayer with no overlapping or compressing of RBCs (feathered edge)

Be careful in interpreting classic textbook definitions

- Double-chromatin dots, multiply-infected rings, band- and basked-form trophozoites

When counting merozoites in a schizont, make sure the schizont is mature (look for coalesced pigment)

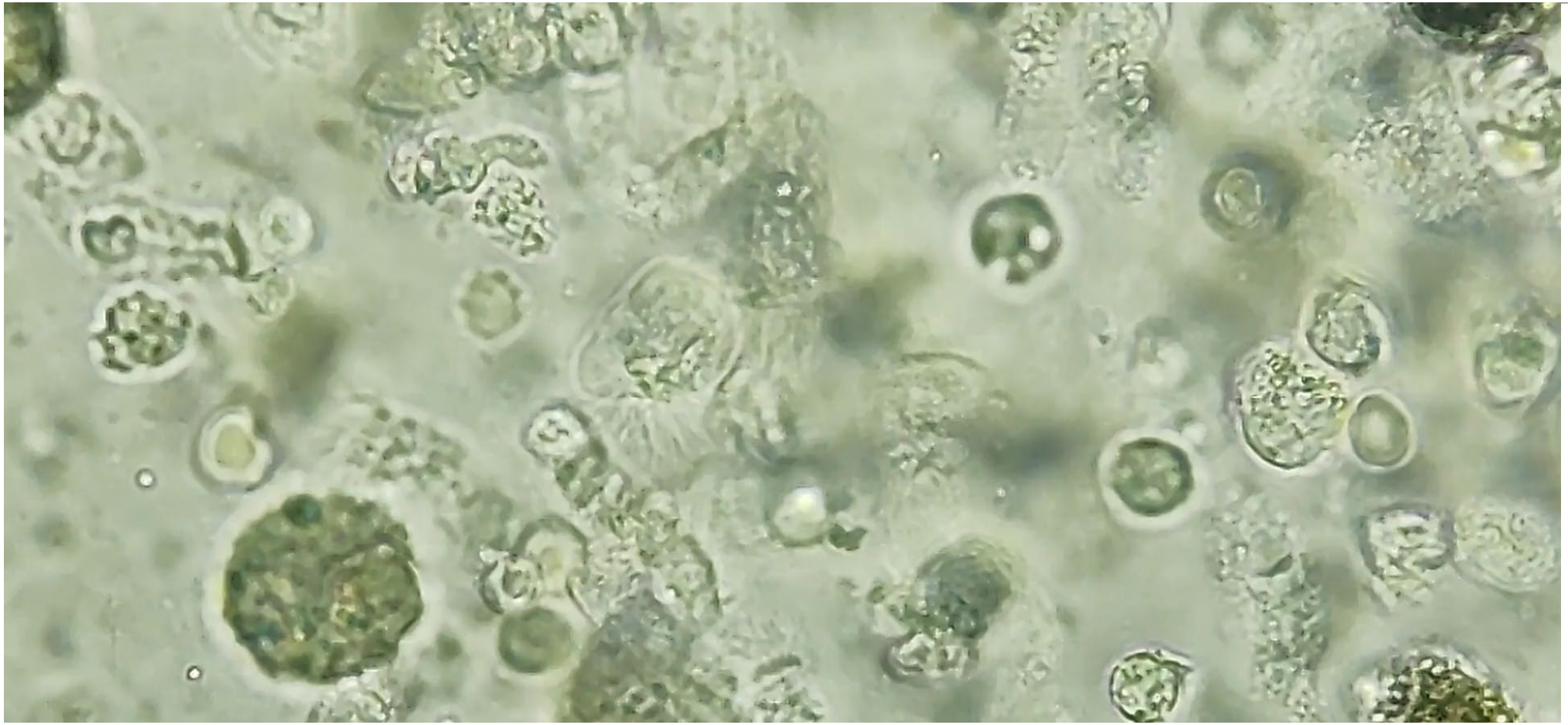
Presence of pigment rules-out *Babesia*

Dispelling dogmas in clinical parasitology

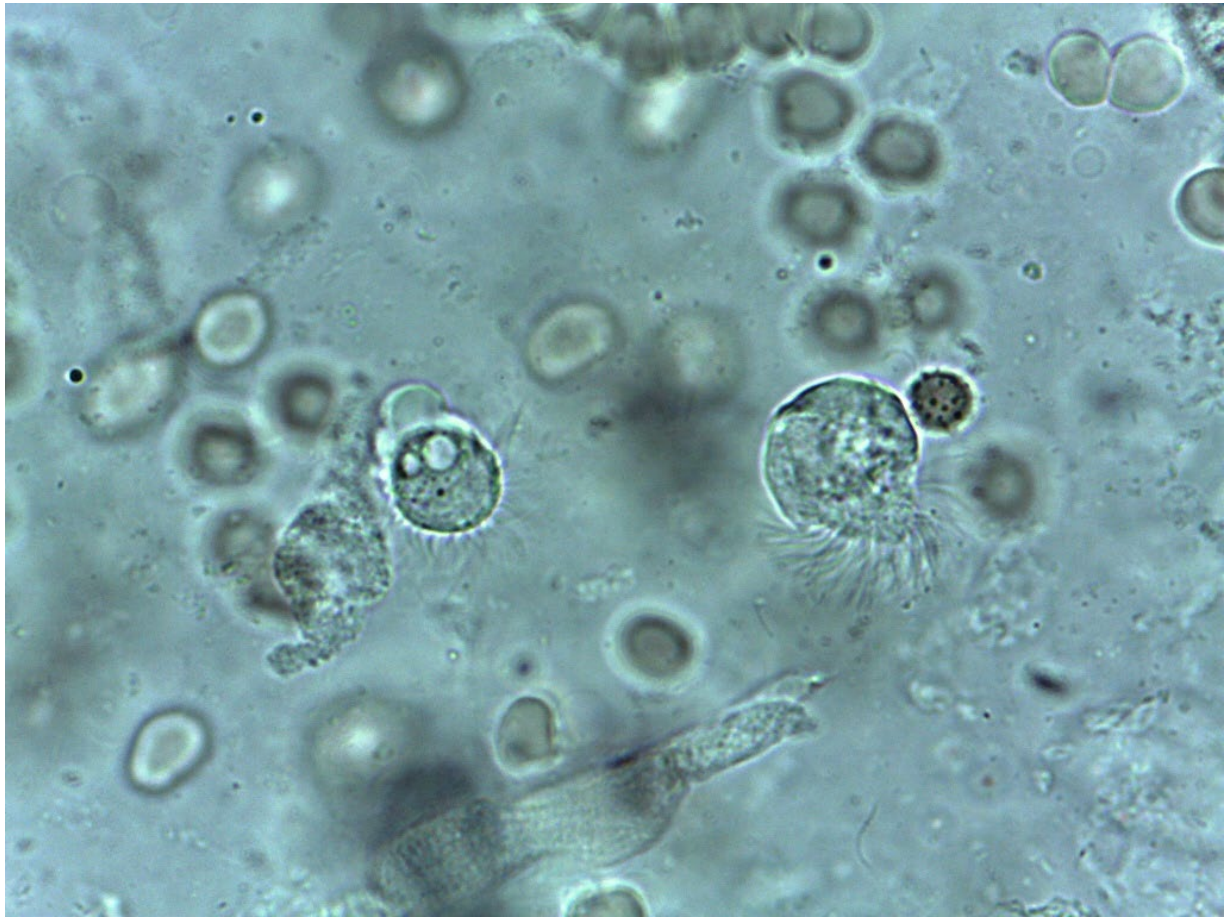
A TRIPLE ATTACK BY BLAINE MATHISON, HENRY BISHOP, & MARC COUTURIER

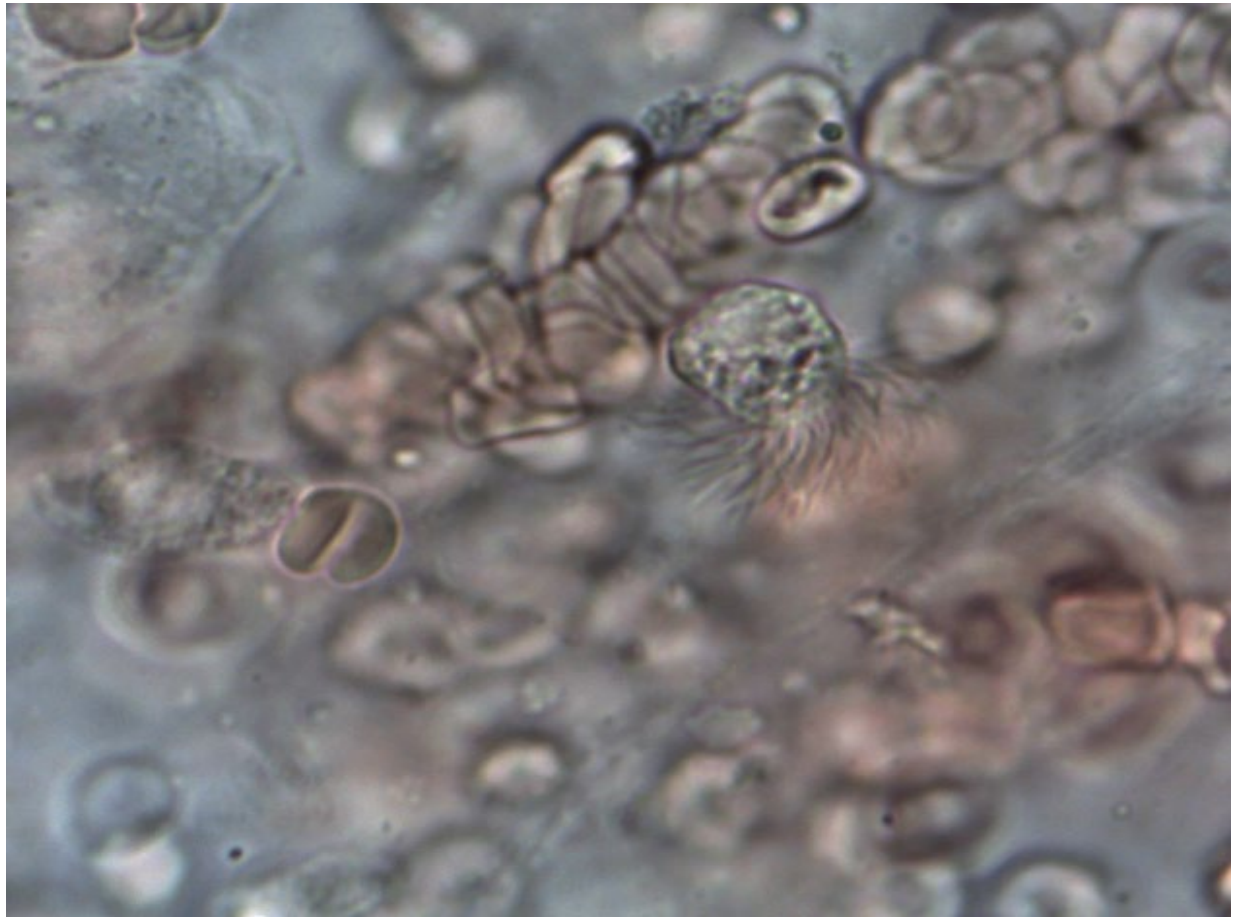
I see parasites….

Something is observed moving in a BAL (bronchoaveolar lavage) specimen so it **must** be a parasite

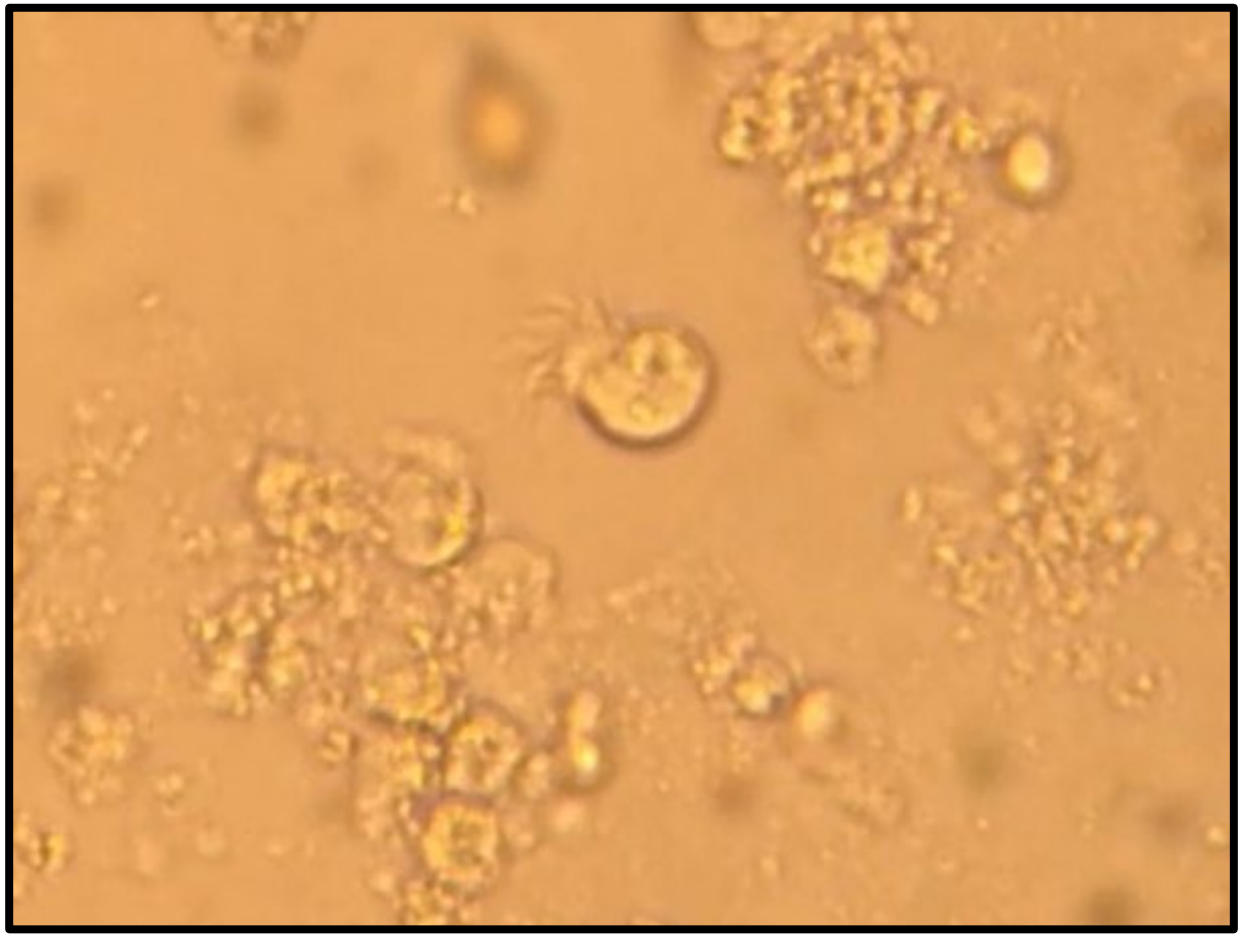


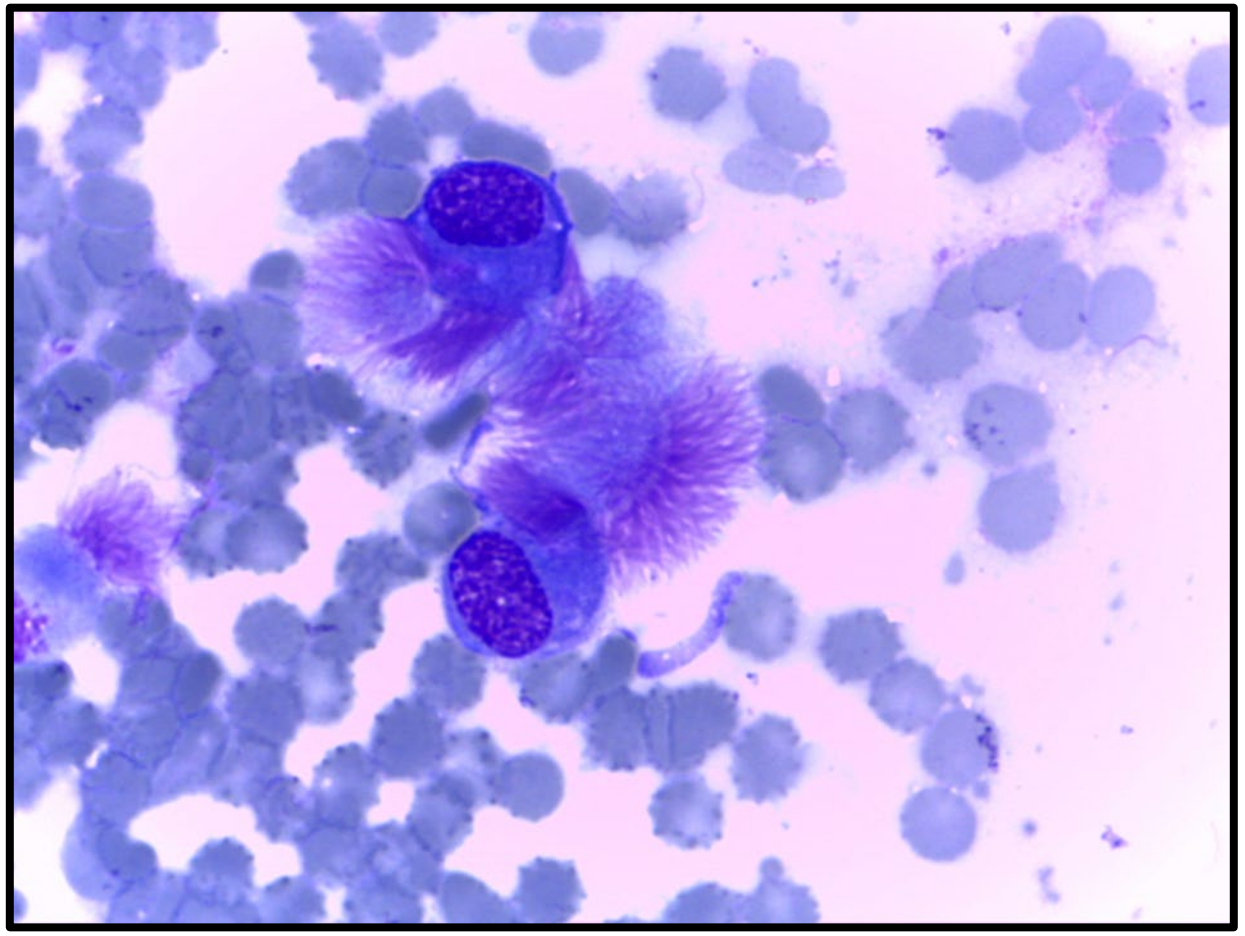
Wet mount static images





Wet mount static image and Giemsa stained prep

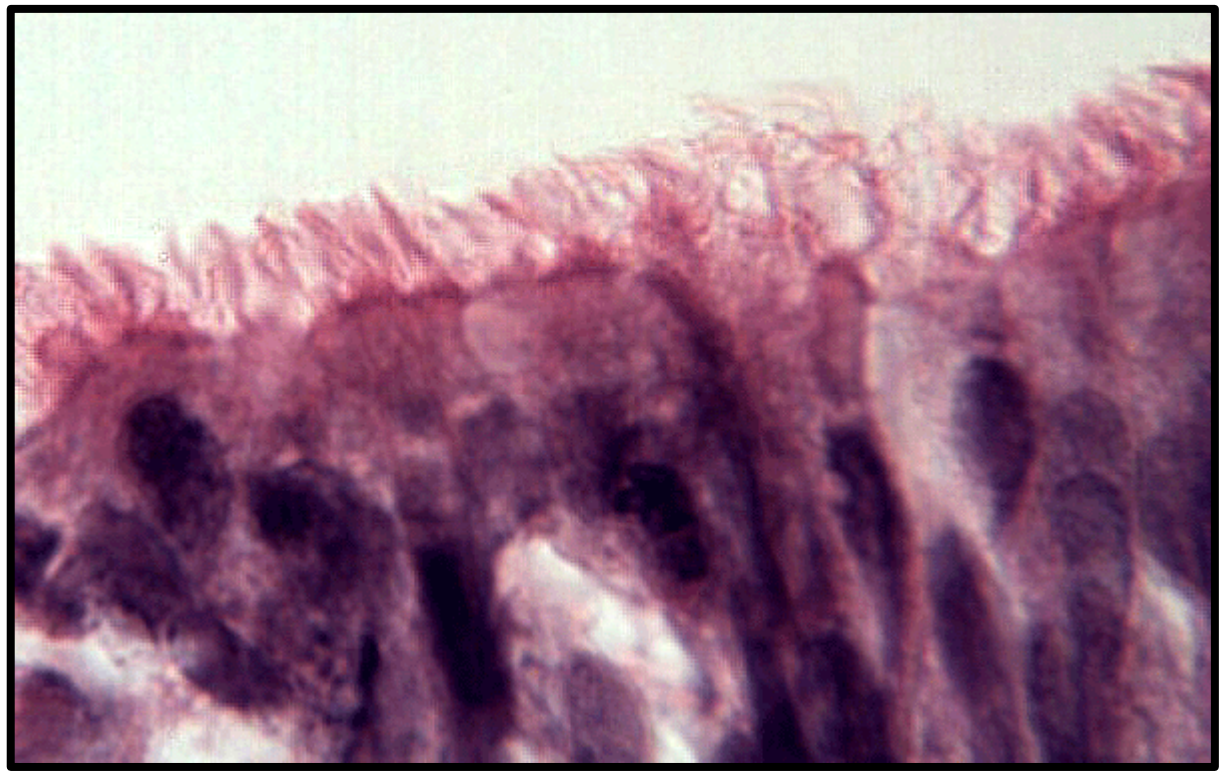




Parasite or something else?

Ciliocytophthoria

Detached ciliary tufts (remnants of ciliated epithelium) seen in a variety of body fluids, especially peritoneal, amnionic, and respiratory specimens; they can be observed being motile and thus confused with ciliated or flagellated protozoa.



Differential Characteristics:

Ciliated epithelial cells:

- Cells with normal physiological functions – not parasites
- Upper respiratory tract, fallopian tube, epididymis
- Basal nucleus far from the cilia
- Terminal bar from which the cilia arise

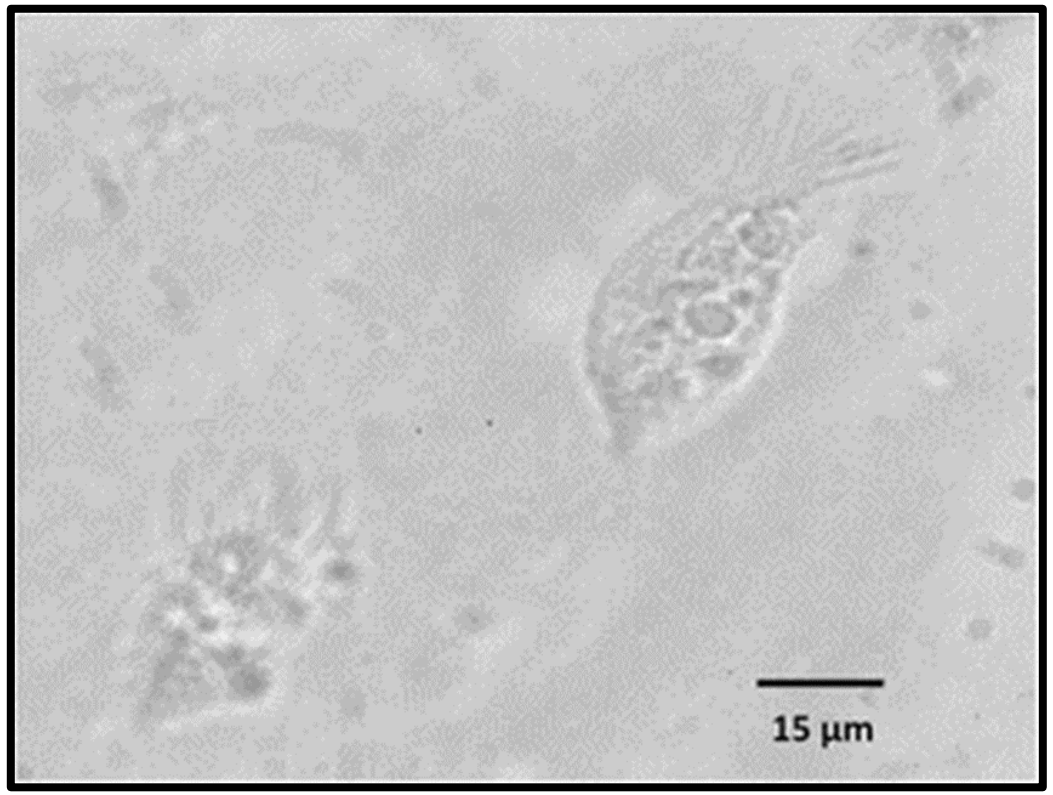
Lophomonas blattarum (Stein, 1860):

- Parasite of digestive tracts of various arthropods
- Flagellated, arising from anterior end
- Anterior vesicular nucleus
- Perinuclear tubules
- Axial filament

Cilia	Flagella
Short, hair like appendages.	Long, threadlike appendages.
Short	Longer than cilia, can vary
Motor-like, rotational, very fast	Wave-like, undulating, sinusoidal, slow
Many (hundreds) per cell	Few (less than 10) per cell

Illustration from *Protozoology*, RR Kudo, 1939. (p. 280)

Lophomonas blattarum



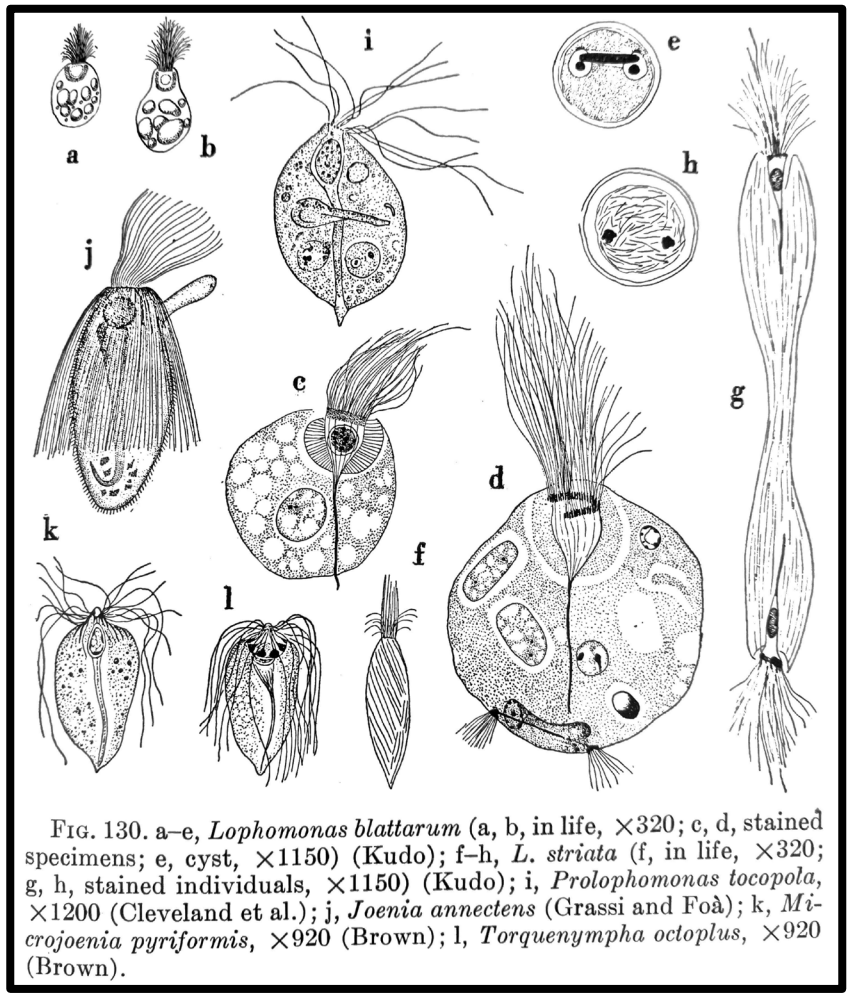
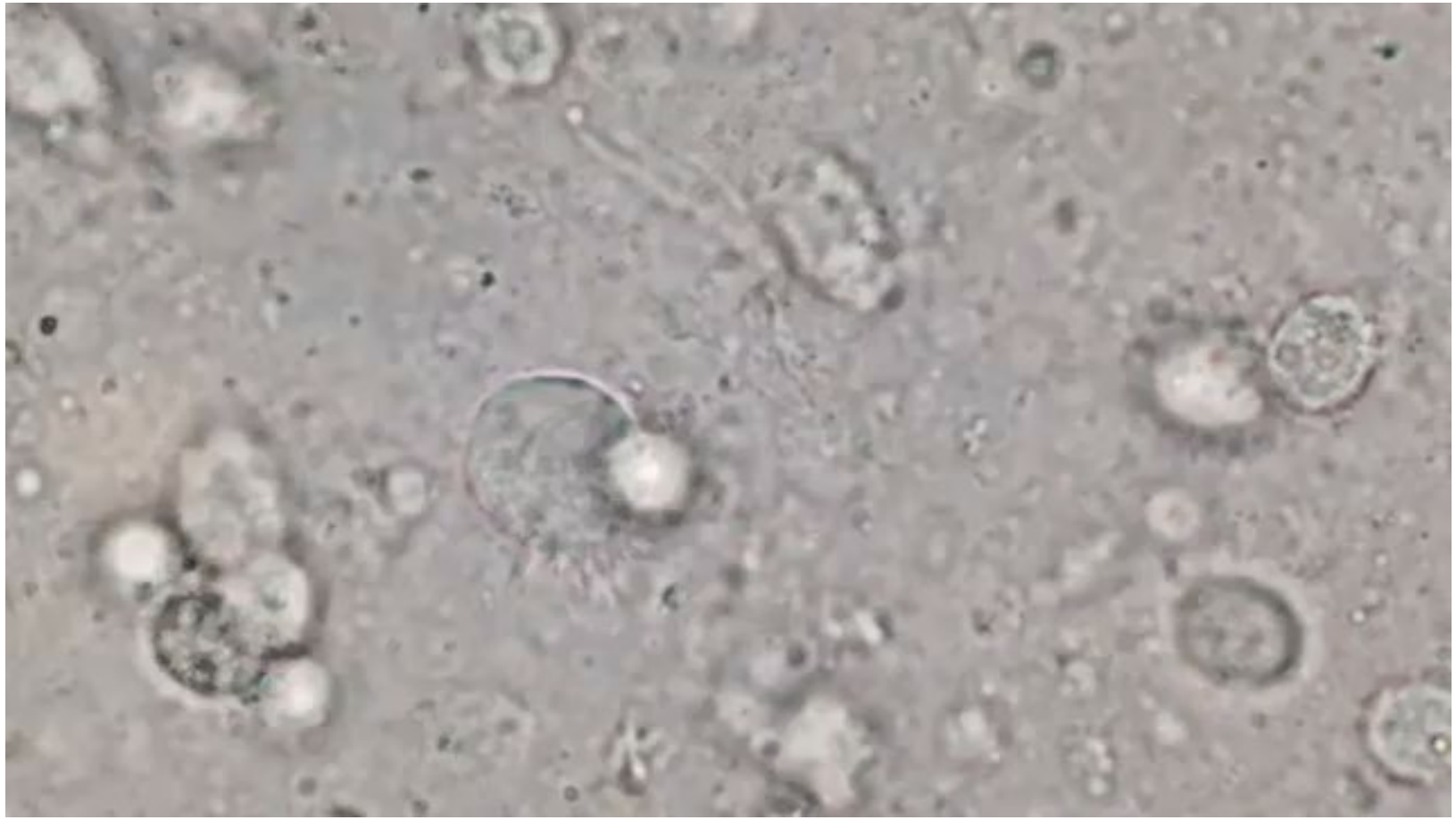


FIG. 130. a–e, *Lophomonas blattarum* (a, b, in life, ×320; c, d, stained specimens; e, cyst, ×1150) (Kudo); f–h, *L. striata* (f, in life, ×320; g, h, stained individuals, ×1150) (Kudo); i, *Prolophomonas tocopola*, ×1200 (Cleveland et al.); j, *Joenia annectens* (Grassi and Foà); k, *Microjoenia pyriformis*, ×920 (Brown); l, *Torquenympha octoplus*, ×920 (Brown).



It’s not **moving** but sure looks like a worm

- A worm was coughed up by a toddler and submitted for identification.
- Toddlers are notorious for exploring their world by taste (this can include earthworms, maggots, etc).
- Since the worm was coughed up, the skeptic in you may suspect case is not a true parasitic infection but you **must examine** the worm (or object). **DO NOT SEND OUT TO PATHOLOGY FOR HISTOLOGIC PROCESSING** before attempting to at least make a preliminary identification.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Examine the “worm” using a dissecting microscope to look for diagnostic features such as:

- Is it flat or round
- What is the size, both length and diameter
- Is it segmented
- Can you make out “heads” or “tails” (literally!)
- Can you see spines or other protuberances

Keep in mind that once you slice, dice, and color it in, these basic features can be lost and a definitive ID may be more difficult or no longer even feasible.

Still don’t know what to make of it? Try to clear it with **lacto-phenol**

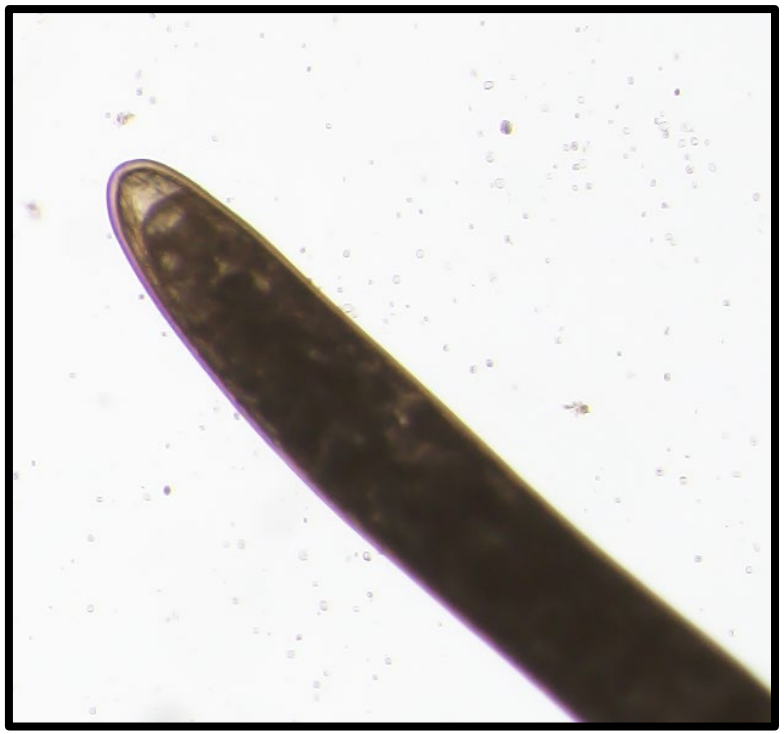
- Solution can made in-house or purchased commercially (minus cotton blue)
- You may be able to make out internal organs or eggs (may need to squeeze out a few)
- If you believe you have a cestode (tapeworm), you may be able to count uterine branches if a taeniid;
- Examination of the cuticle may be enhanced

Still can’t figure it out? **Now** you can consider to send to pathology but it is critical that you have some cross-sections (esp. for nematodes) to facilitate identification of morphologic features

Diagnostic Features

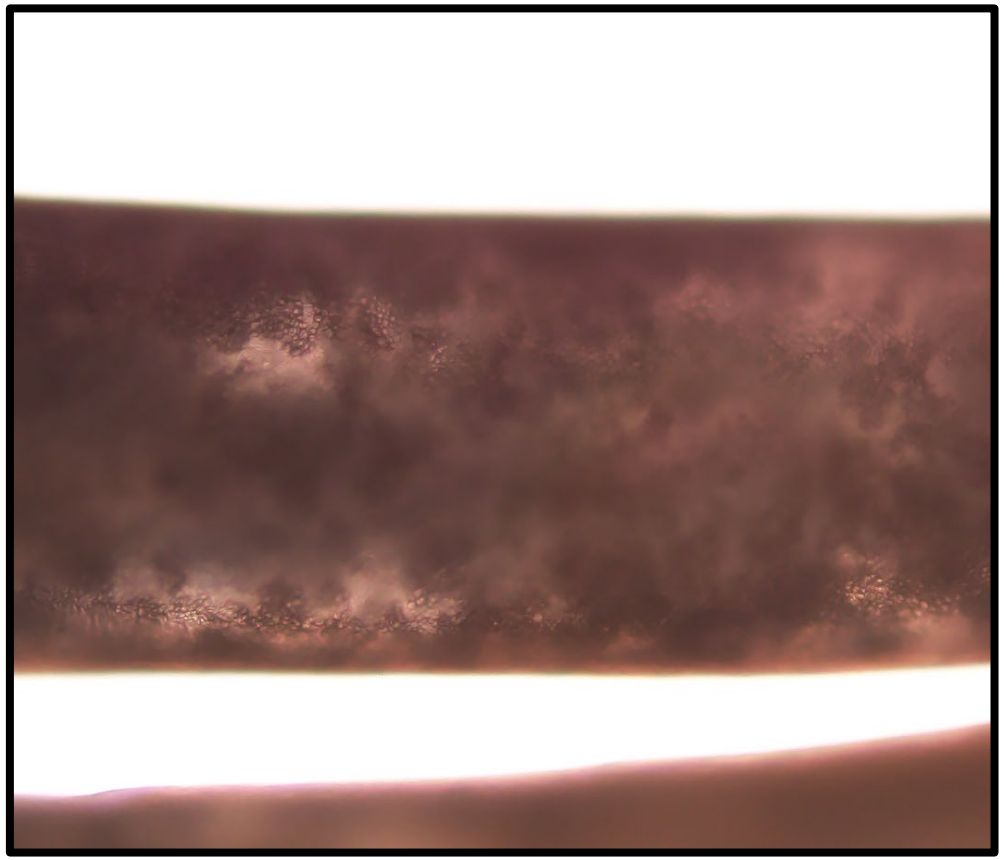
SHARPLY NARROWED ANTERIOR

DISTINCTLY ROUNDED POSTERIOR

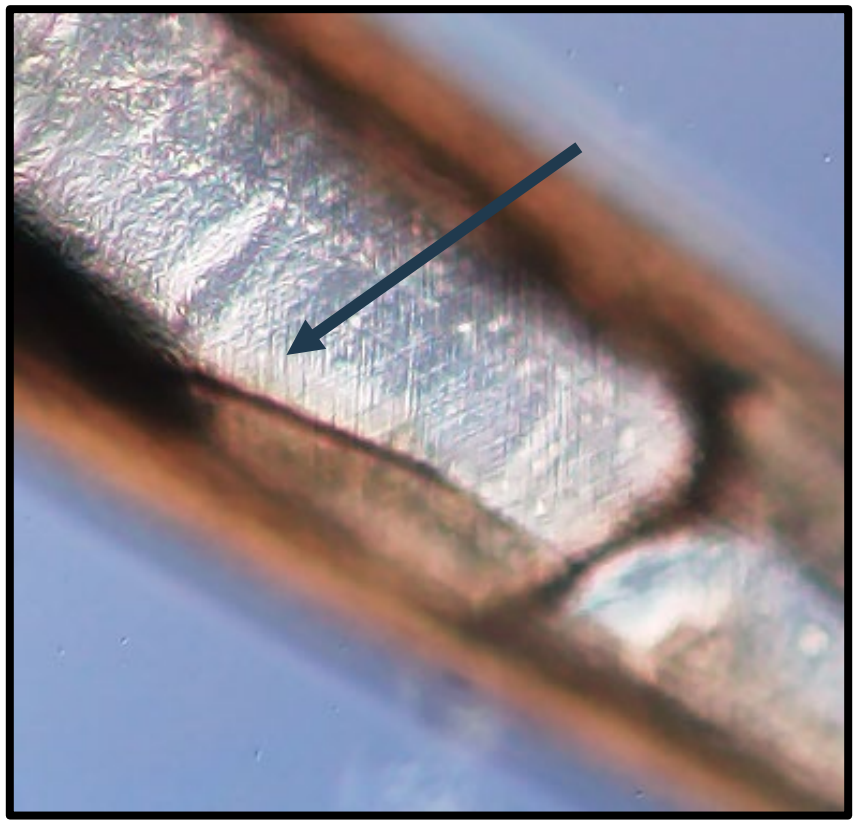


Diagnostic Features

EGG CLUSTERS



CUTICLE COMPOSED OF DIAGONAL CROSS-FIBERS

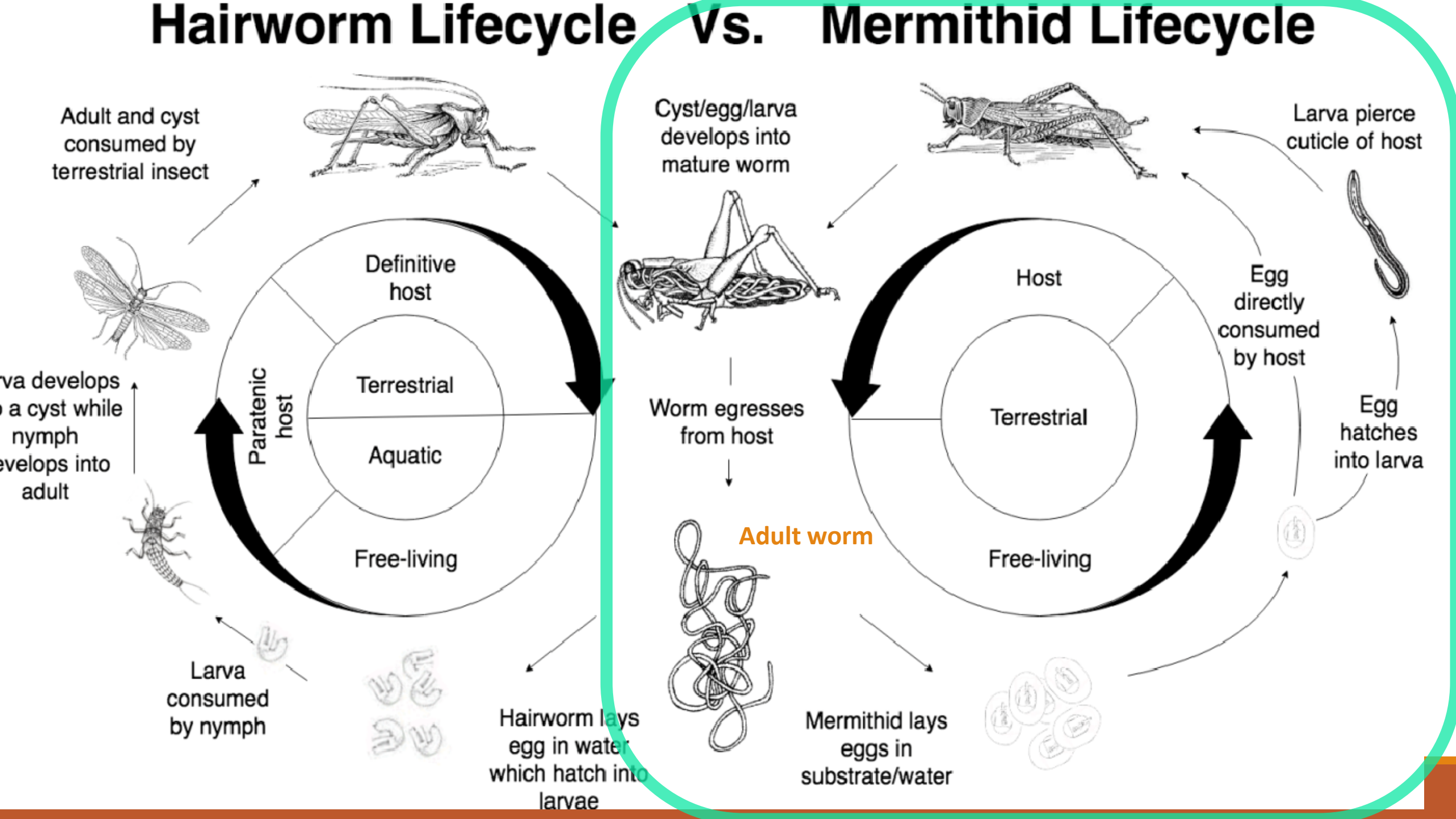


Does the kid have worms?

“No Parasites Found”

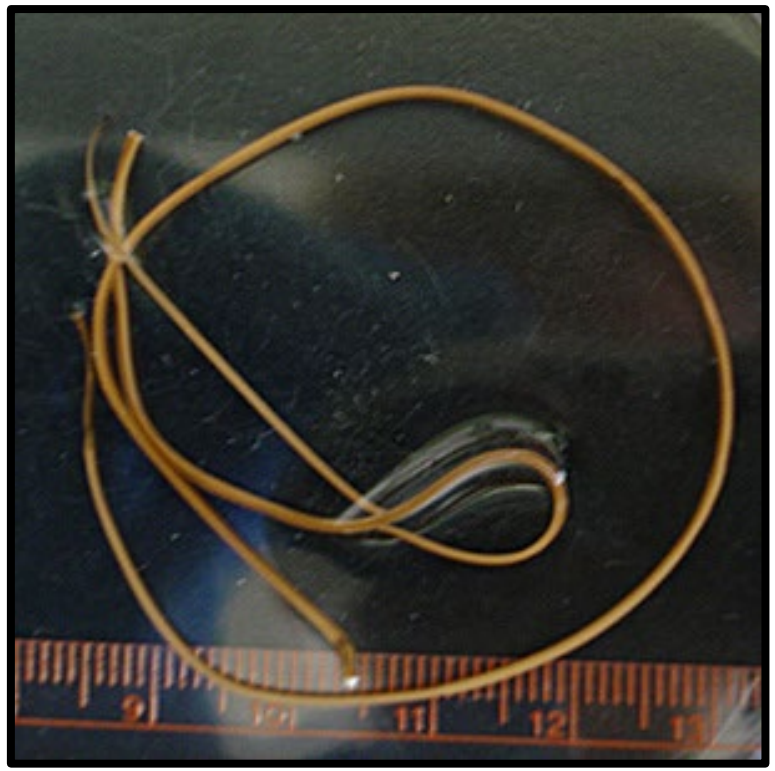
- Likely **Mermis sp.** (phylum Nematode)
- Parasite of arthropods as larvae, with a free-living adult stage
- Spurious passage following ingestion of infected insect, causing worm to emerge and be vomited/coughed up
- Similar life cycle to “**horsehair/Gordiid worm**” (phylum Nematomorpha); completely unrelated convergent evolution

Hairworm Lifecycle Vs. Mermithid Lifecycle

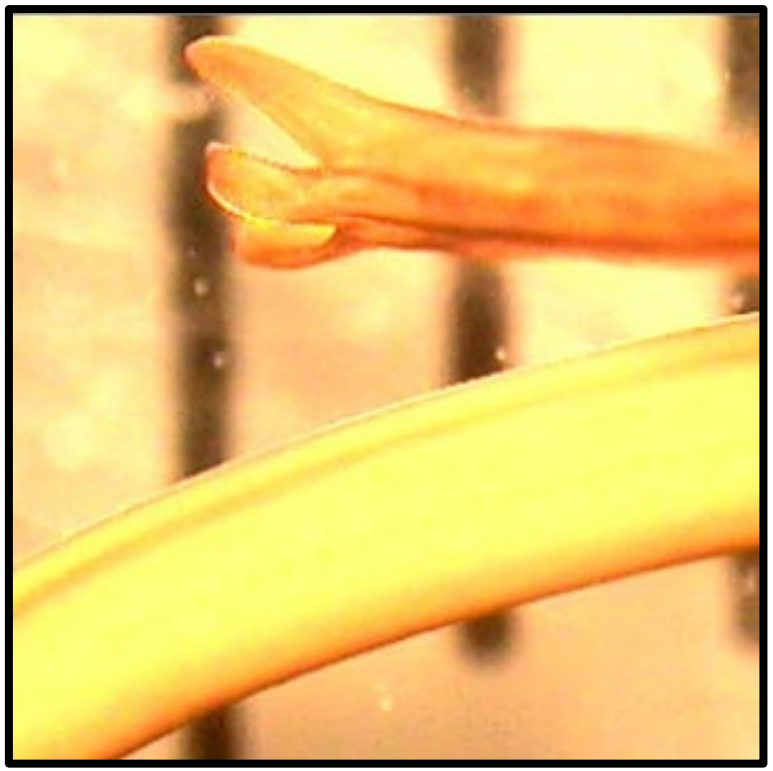


Diagnostic Features (*Gordius* sp)

POSTERIOR NOT TAPERED



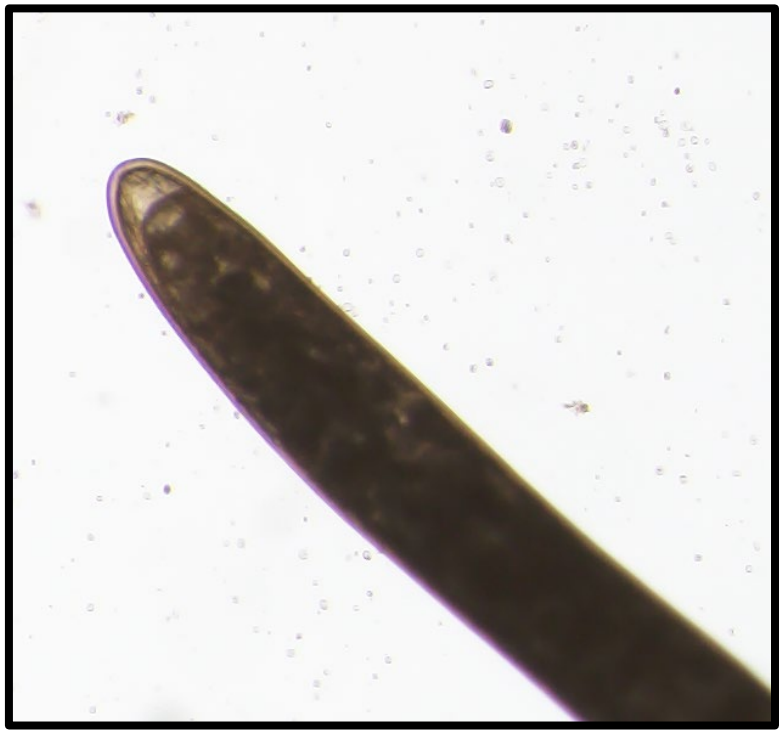
LOBED POSTERIOR; SMOOTH CUTICLE



Diagnostic Features

SHARPLY NARROWED ANTERIOR WITH NON-FUNCTIONAL ESOPHAGUS

DISTINCTLY ROUNDED POSTERIOR



In Summary:

Just because it looks like a parasite, be it worm or other organism, doesn't necessarily mean it is.

Be as thorough as possible to try to make an accurate identification

Use all reference material at your disposal

You are like a detective – morphologic features are the clues you use to arrive at the diagnosis