

# Intestinal Protozoa

Amebae

Flagellate protozoa

Coccidia

and others

## Protozoa: numbers in millions

*Entamoeba histolytica*: 500; 48 severe; 0.07 deaths.

Malaria: 300-500; 2-3 deaths (mainly children).

*Giardia lamblia*: 500.

Mandell *et al.*, 2005 - WHO, 2001

# Eosinophilia > 10%

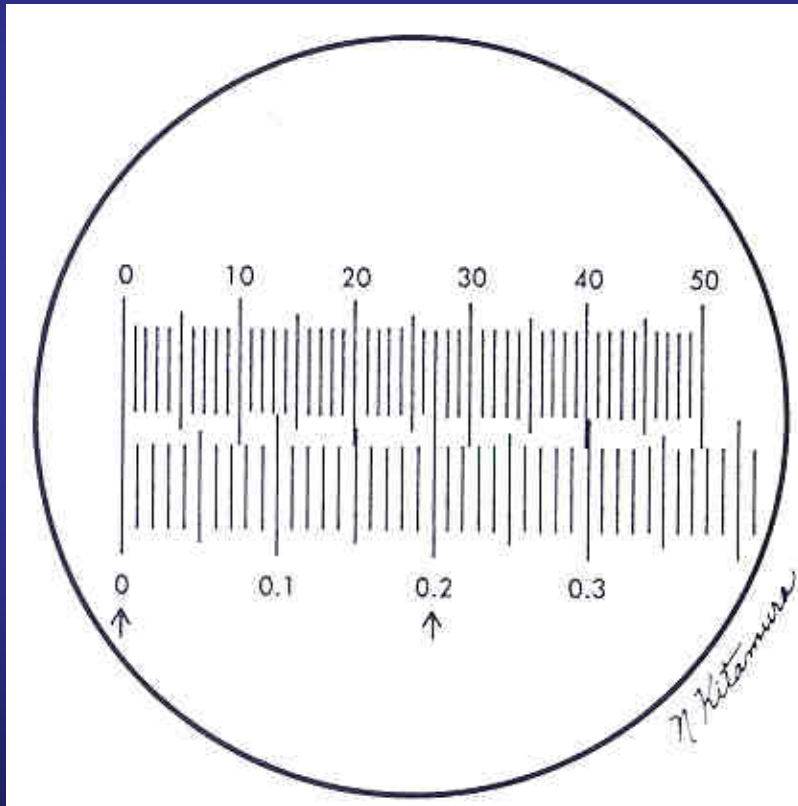
With helminths,  
insects (myiasis),

not with protozoa excepting  
*Isospora belli* and *Dientamoeba  
fragilis* (with pinworms?)

# Protozoa in faeces

- Cysts and trophozoites from amebae, flagellates, and coccidia.
- The size is essential for identification (to be measured with a calibrated micrometer).
- The aspect of the nucleus is also important for the identification.

# Ocular micrometer disk



- Each objective must be calibrated with reference material.
- Can be roughly checked with a counting chamber, with RBCs ...

# Direct examination in saline

- Standard-preparation, containing approximately 2 mg faeces.
- In fresh faeces it is possible to observe trophozoites (*Entamoeba histolytica*, ...).
- Cysts of protozoa are difficult to see, because they are colourless.

# Ritchie-enrichment and Lugol stain

- Screen the entire preparation with objective 10x.
- Suspect elements (cysts, eggs,...) are checked with higher enlargement (40x, 50x, 100x).
- Amebae and flagellates stain brown-yellow with Lugol.
- The identification of cysts from protozoa is based on the size and the aspect of the nucleus.

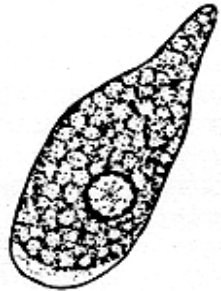

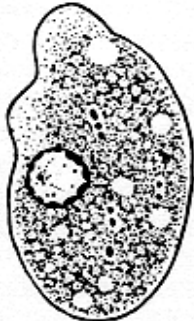
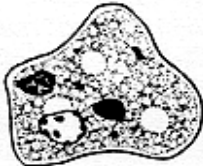
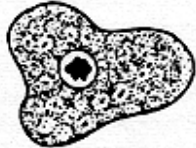
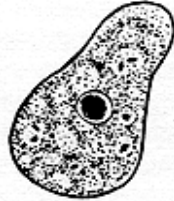





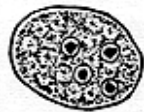

# Specific gravities

- S.G. of Zn-sulfate 33 %: 1.180
- S.G. of formol-solution 10 %: 1.019
- S.G. of ether: 0.714
- S.G. of parasites: *Ancylostoma* 1.055; *Giardia* 1.060; *Entamoeba histolytica (coli)* and *Endolimax nana* 1.065 - 1.070; *Ascaris* 1.110; *Trichuris* 1.150, *Chilomastix mesnili* 1.180; *Ascaris* (unfertilized) 1.200 (Bailenger, 1965).



# Protozoa in faeces

- Visible unstained: (mobile) trophozoites, *Blastocystis hominis*, *Cyclospora caeytanensis*.
- Visible with Lugol-stain: cysts of amebae and flagellates.
- With special stains: iron-hematoxylin (trophozoites and cysts); safranin stain (*Cryptosporidium parvum*).
- Sometimes the elements are already visible in the Gram stain: *Blastocystis hominis*, trophozoites and cysts of *Giardia lamblia*.

| AMEBAE      |  |  |  |  |  |  |   |
|-------------|--|--|--|--|--|--|---|
|             | <i>Entamoeba histolytica</i>   | <i>Entamoeba hartmanni</i>   | <i>Entamoeba coli</i>  | <i>Entamoeba polecki</i> <sup>1</sup>  | <i>Endolimax nana</i>  | <i>Iodamoeba bütschlii</i>   | <i>Dientamoeba fragilis</i> <sup>2</sup>  |
| Trophozoite |   |   |   |   |   |   |  |
| Cyst        |  |  |  |  |  |  | No cyst   |

<sup>1</sup>Rare, probably of animal origin

<sup>2</sup>Flagellate

Scale: | 0 5 10 μm

Courtesy ASM

FIGURE 1 Amebae and flagellate (*Dientamoeba fragilis*) found in human stool specimens. (From reference 4.)

# Nuclei of intestinal protozoa



Courtesy ASM

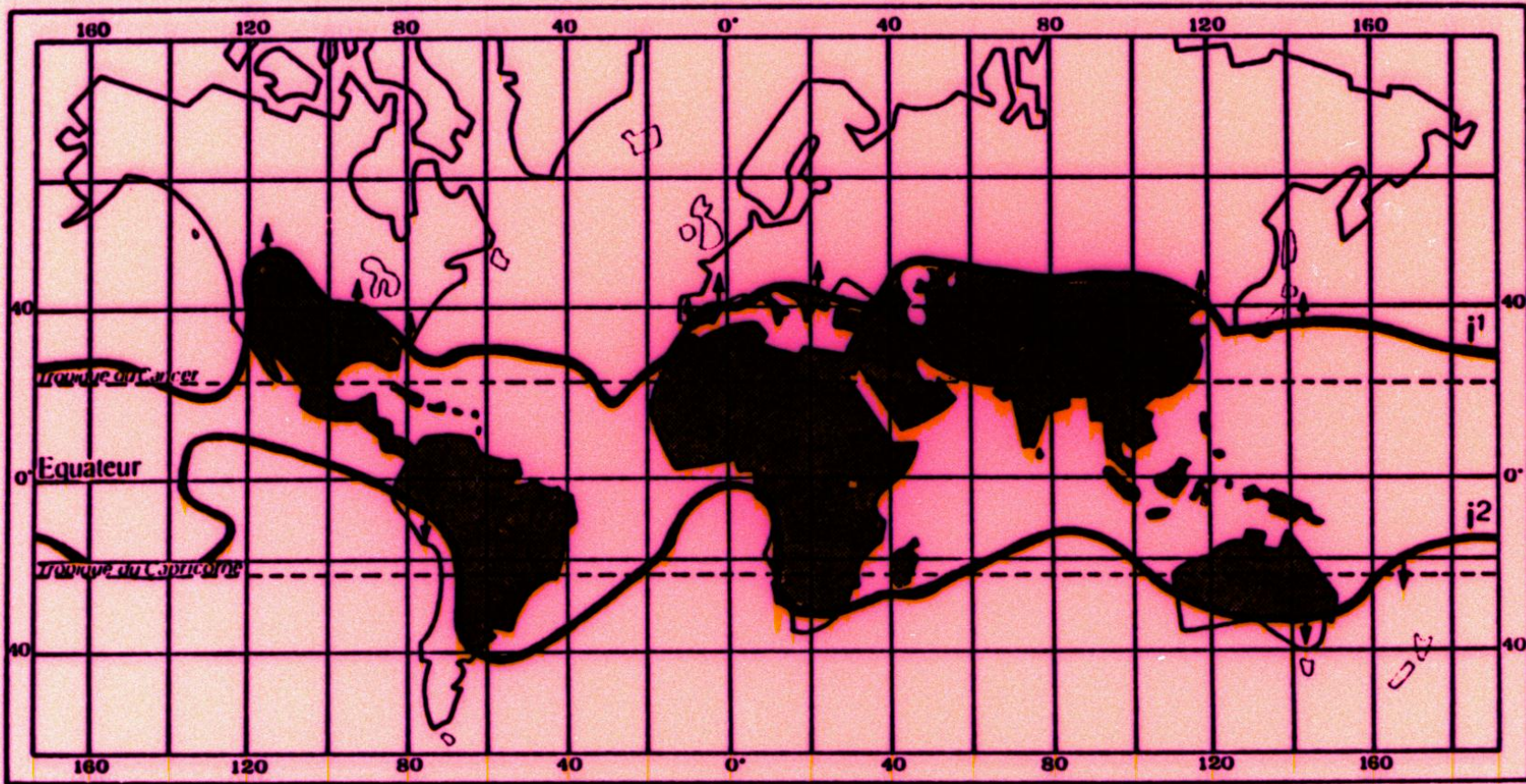
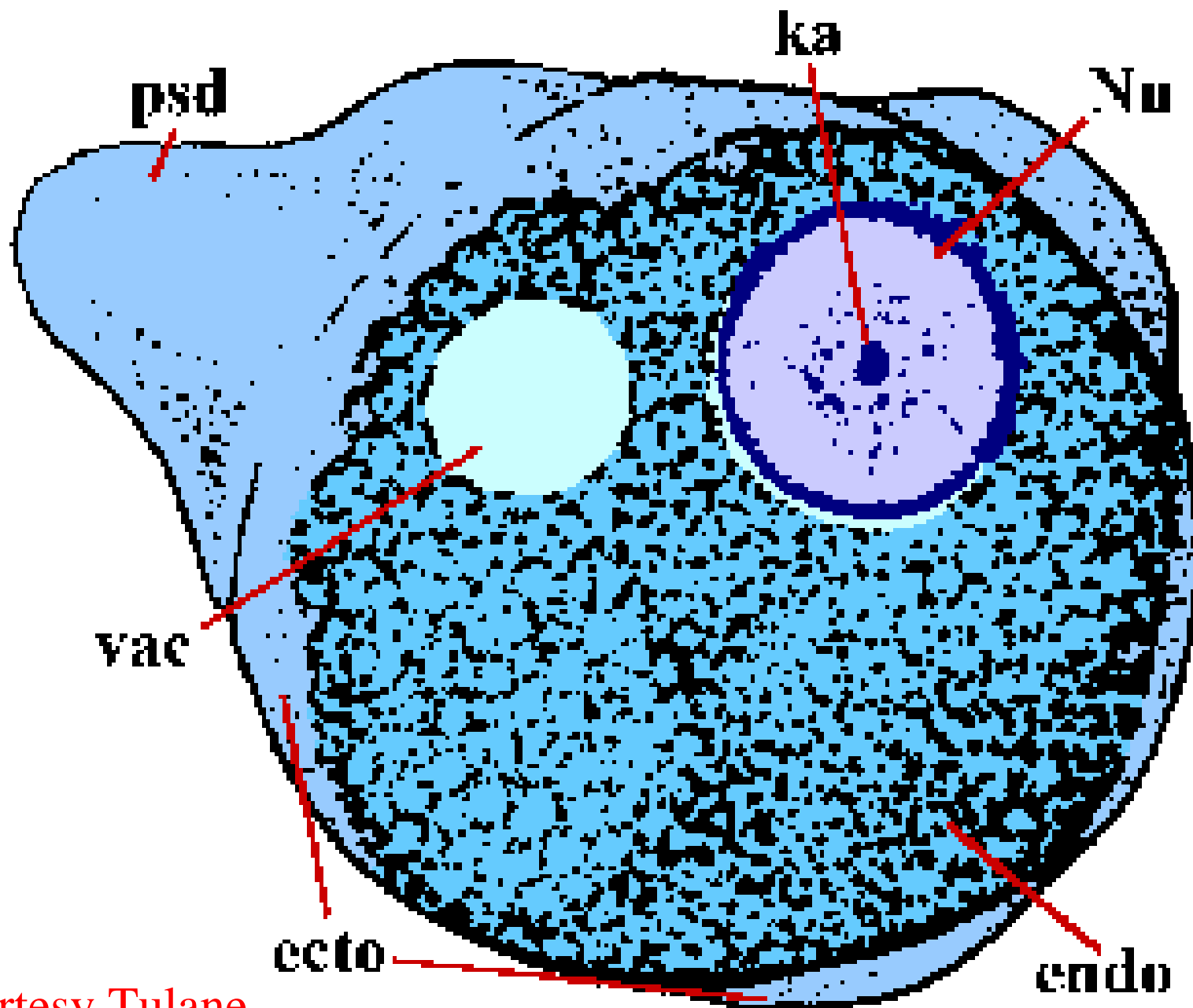


Fig. 5-3. – Répartition géographique de l'amibiase (d'après R. Deschiens). I<sup>1</sup> : isotherme 25 °C de juillet; I<sup>2</sup> : isotherme 25 °C de janvier. En grisé, les zones d'endémie de l'amibiase; les flèches indiquent les zones de débordement les plus fréquentes de l'amibiase en zone tempérée.

Courtesy Gentilini M. & Duflo B.



Courtesy Tulane

# Trophozoite

# *Entamoeba histolytica*

Trophozoite (*magna* variety) in faeces. Diameter approximately 30  $\mu\text{m}$ . Nucleus with typical fine chromatin picture (Iron-hematoxylin stain).



# *Entamoeba histolytica*

Trophozoite (*magna* variety) in faeces. Diameter approximately 30  $\mu\text{m}$ . Nucleus with typical fine chromatin picture and central karyosome (Lugol stain).



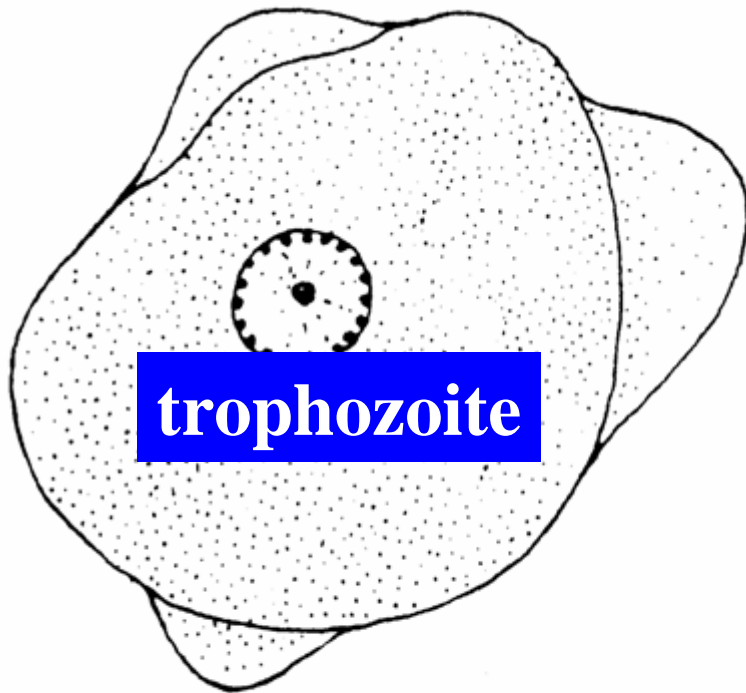
# *Entamoeba histolytica*

Trophozoite (*minuta* variety) in faeces. Diameter approximately 15  $\mu\text{m}$ . Nucleus with typical fine chromatin picture (Iron-hematoxylin stain).



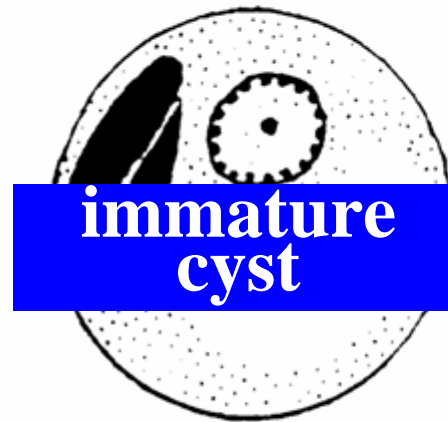


# *Entamoeba histolytica*



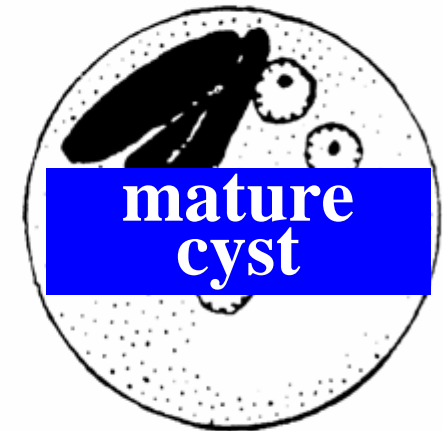
**trophozoite**

trophozoite



**immature  
cyst**

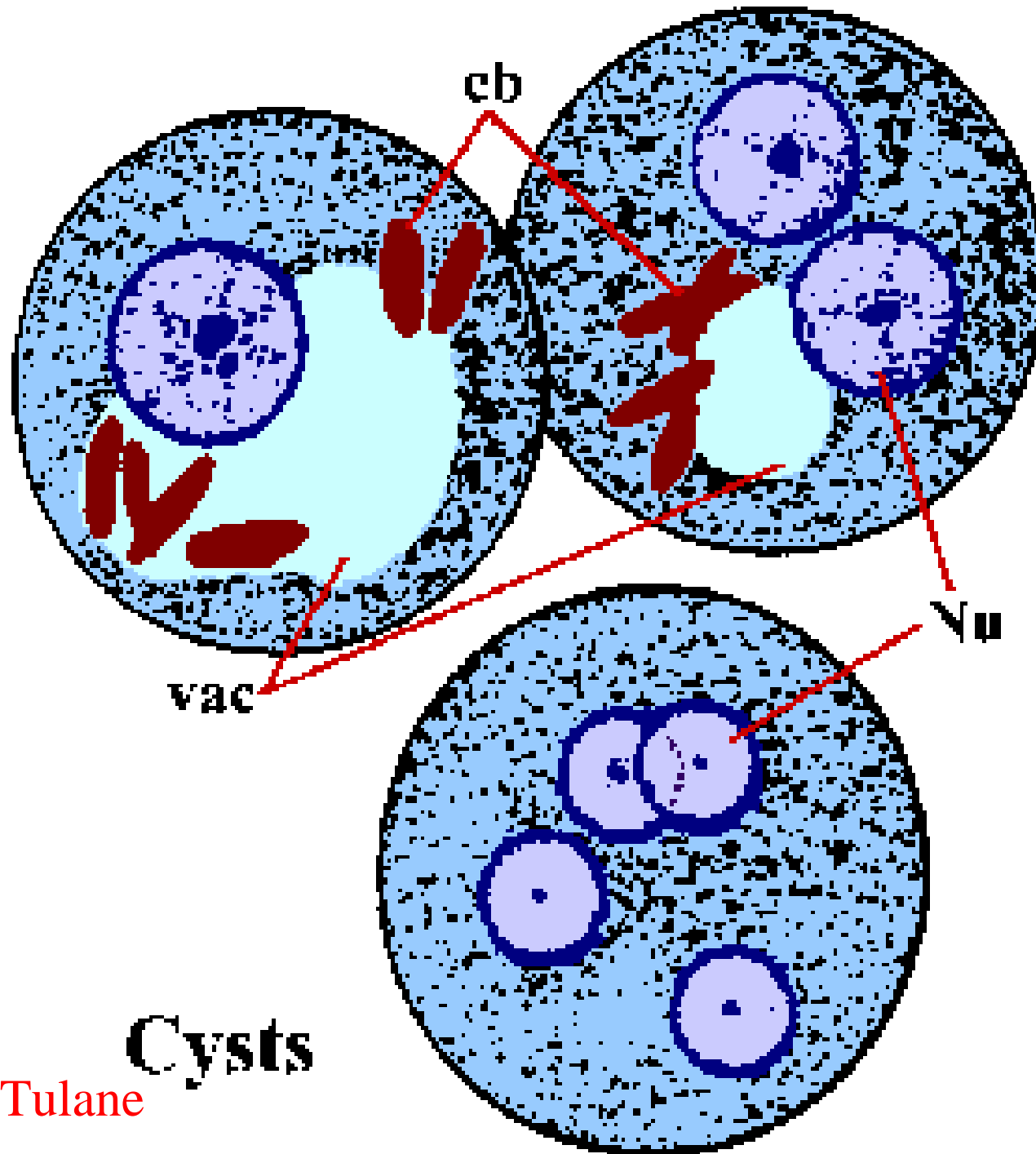
immature cyst



**mature  
cyst**

mature cyst

Courtesy Tulane



**Cysts**

Courtesy Tulane

# *Entamoeba histolytica*

Cyst in faeces with three visible nuclei. In the nucleus at the right we clearly see the central karyosome (Lugol stain).



# *Entamoeba histolytica*

Cyst in faeces with two visible nuclei. In the nucleus at the left we clearly see the central karyosome (Lugol stain).



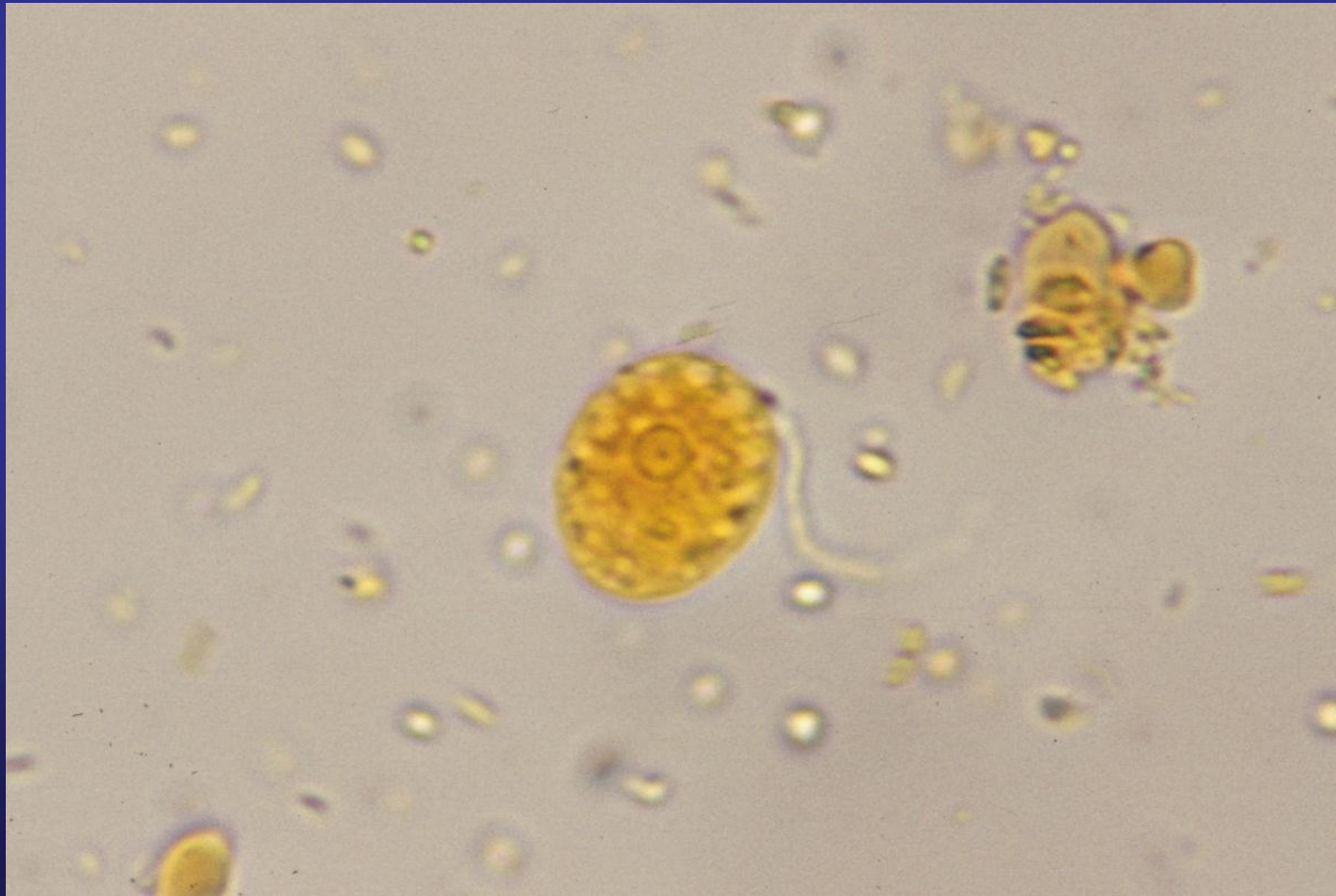
# *Entamoeba histolytica*

Cyst in faeces with two visible nuclei. We clearly see the central karyosomes (Lugol stain).



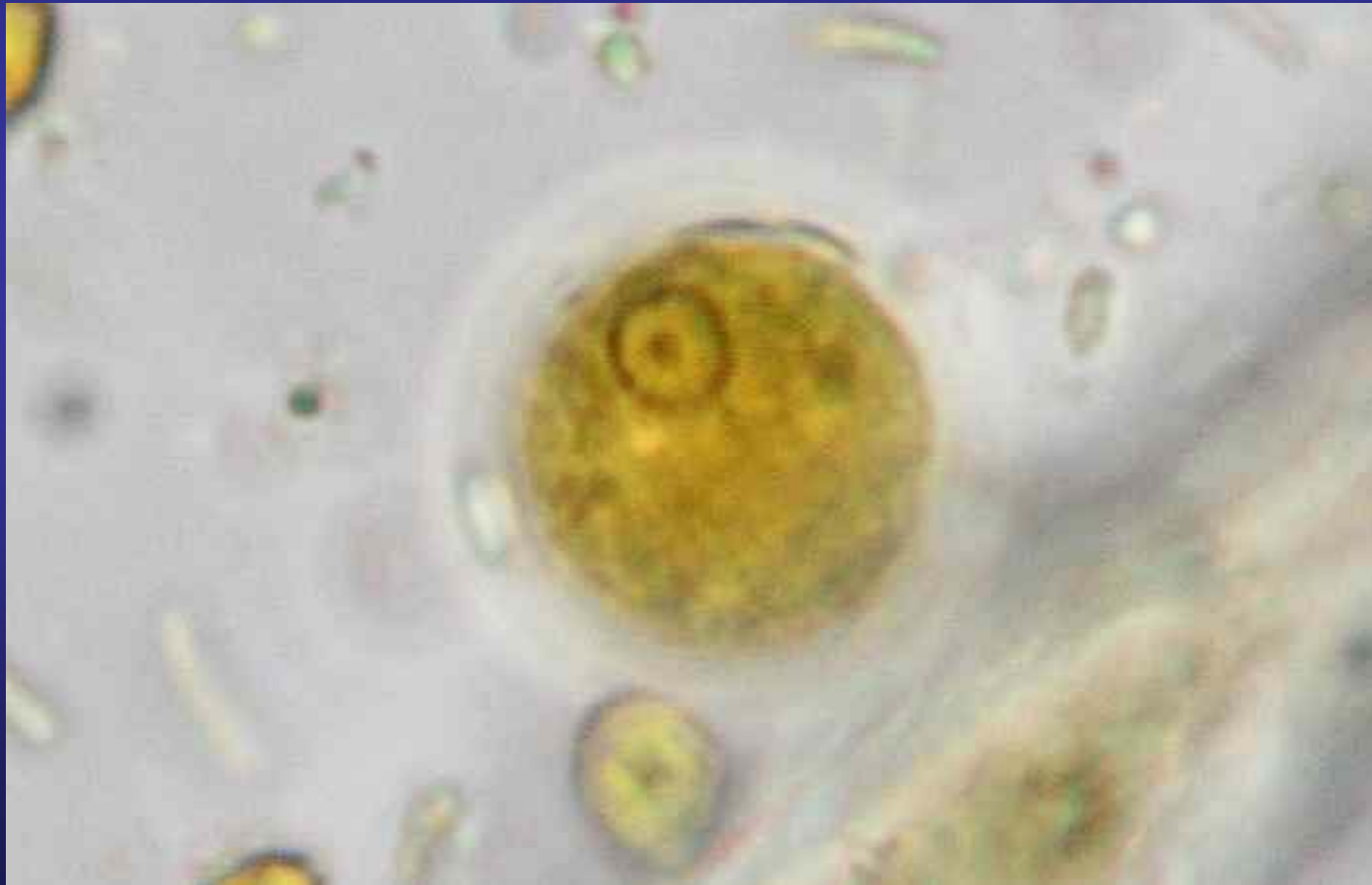
# *Entamoeba histolytica*

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).



# *Entamoeba histolytica*

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).



# *Entamoeba histolytica*

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).





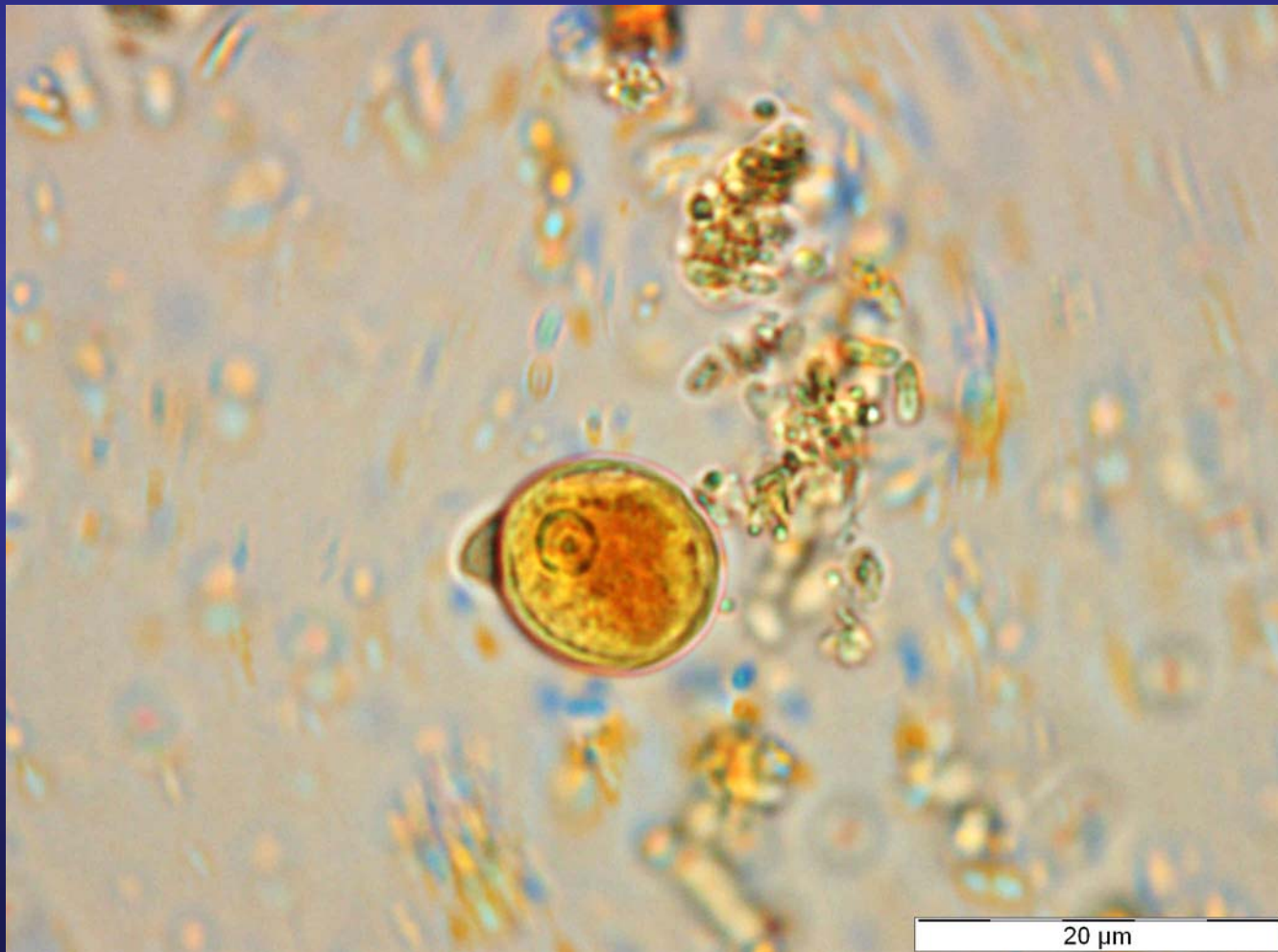
# *Entamoeba histolytica*

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).



# *Entamoeba histolytica*

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).



# *Entamoeba histolytica*

Cyst with one visible nucleus and one cylindrical chromatoidal body (Lugol stain).



# *Entamoeba histolytica*

Cyst with two visible nuclei and one cylindrical chromatoidal body (Lugol stain).



# *Entamoeba histolytica*

Cyst with one visible nucleus and one cylindrical chromatoidal body (Lugol stain).



# *Entamoeba histolytica - dispar*

- *Entamoeba polecki*  
*Entamoeba hartmanni* (small race *E. histolytica*)
- *Entamoeba histolytica* Laredo strain  
*Entamoeba dispar*  
non pathogenic strains grow between 20 and 37°C, pathogenic only at 37°C  
isoenzyme analysis: only 9 zymodemes are pathogenic

# *Entamoeba histolytica - dispar*

- PCR, isoenzyme analysis, and antigen detection (JCM, 1998, 449).
- Monoclonal antibodies (JCM, 2001, 716).
- ITM-Antwerp: PCR on fecal material.



# *Entamoeba hartmanni*

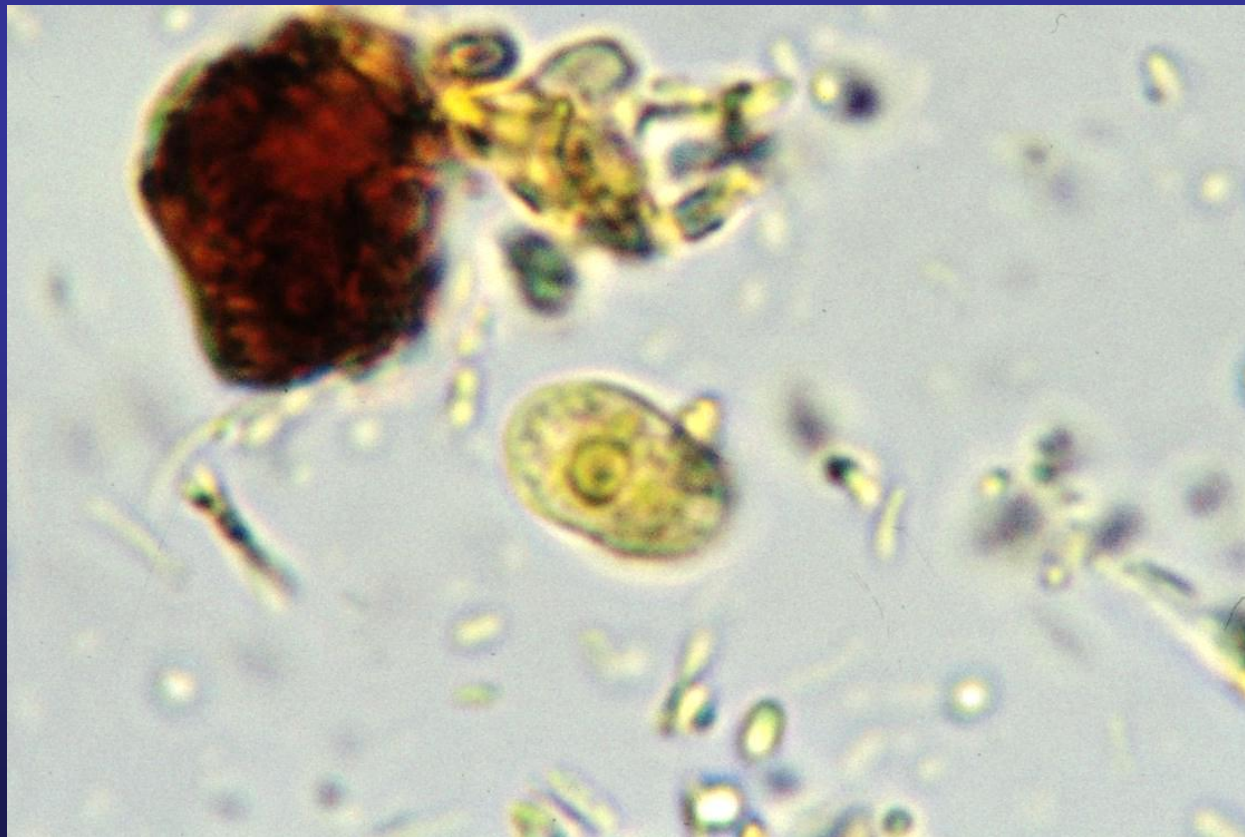
Small cyst with one visible nucleus. It has also been named *Entamoeba histolytica* small race. The nucleus shows the peripheral chromatin and a central karyosome (Lugol stain).





# *Entamoeba hartmanni*

Small oval cyst with one visible nucleus. It has also been named *Entamoeba histolytica* small race. The nucleus shows the peripheral chromatin and a central karyosome (Lugol stain).



# *Entamoeba coli*

Mature cyst with spongy cytoplasm in faeces. Four of the eight nuclei are visible in this plane. Note the coarse peripheral chromatin and the central karyosome of the nuclei (Lugol stain).



# *Entamoeba coli*

Large cyst ( $> 15\mu\text{m}$ ) in faeces with four (to six) visible nuclei (Lugol stain).



# *Entamoeba coli*

Large cyst ( $> 15\mu\text{m}$ ) in faeces with three nuclei visible  
(Lugol stain).



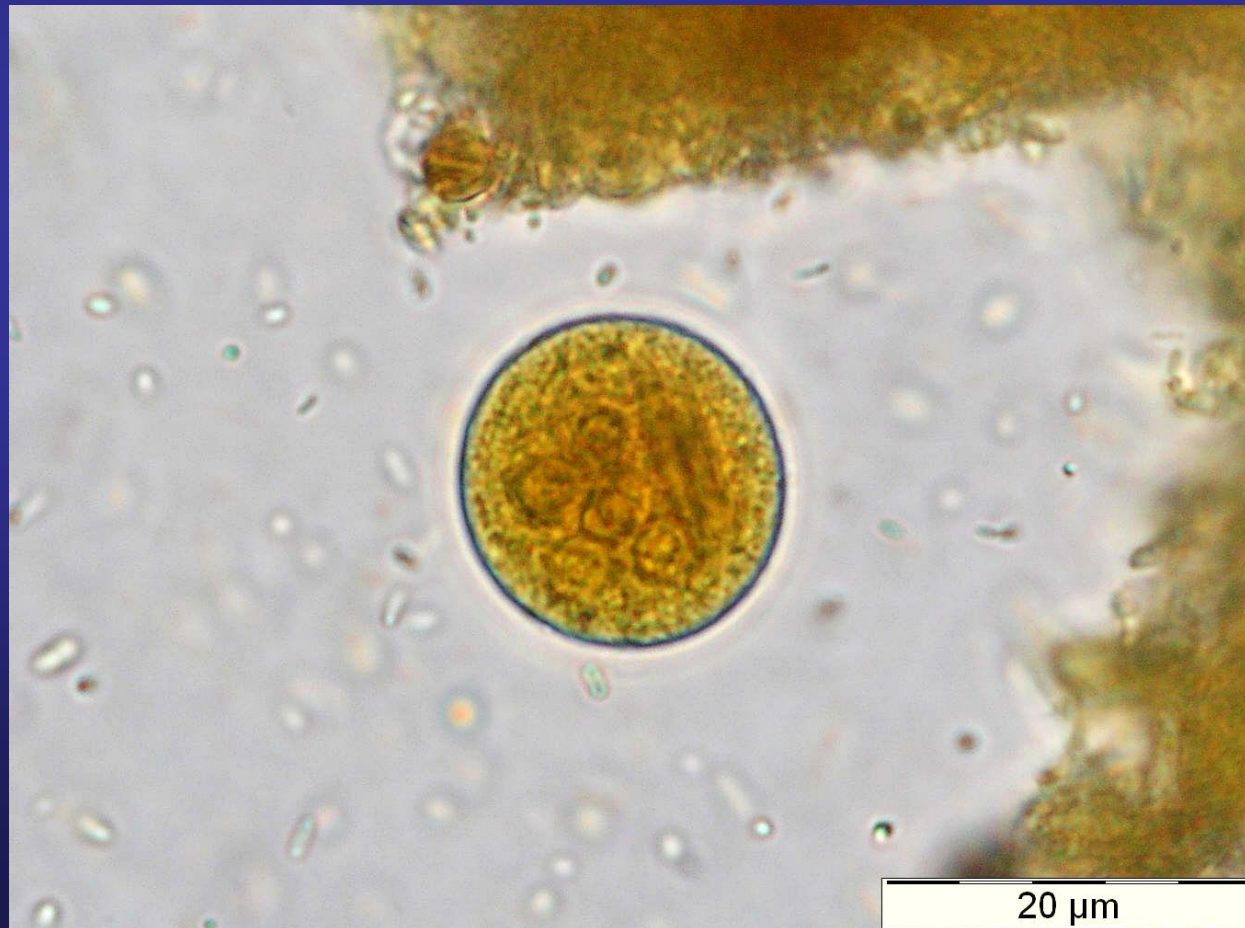
# *Entamoeba coli*

Large cyst (> 20 $\mu$ m) in faeces with four visible nuclei  
(Lugol stain).



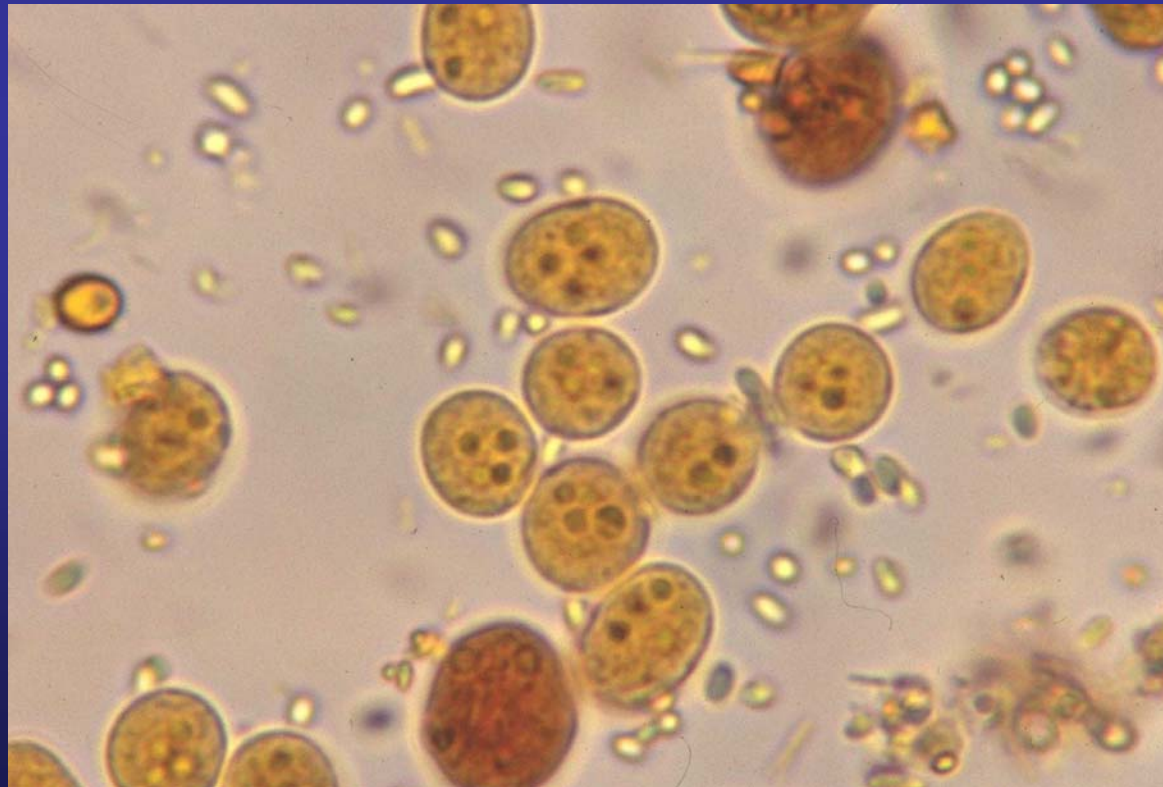
# *Entamoeba coli*

Large cyst in faeces with five nuclei and at the right a bundle of sharp chromatin bodies (Lugol stain).



# *Endolimax nana*

Many cysts are visible, each with one to four nuclei. The cysts are smaller than 10  $\mu\text{m}$  and contain four nuclei with a massive central karyosome. The two larger and darker cysts are *Giardia lamblia* (Lugol stain).



# *Endolimax nana*

Many cysts are visible with several nuclei. The cysts are smaller than 10  $\mu\text{m}$  and contain nuclei with a massive central karyosome (Lugol stain).





# *Endolimax nana*

Three cysts are visible with two to four nuclei. The cysts are smaller than 10  $\mu\text{m}$  and contain nuclei with a massive central karyosome (Lugol stain).



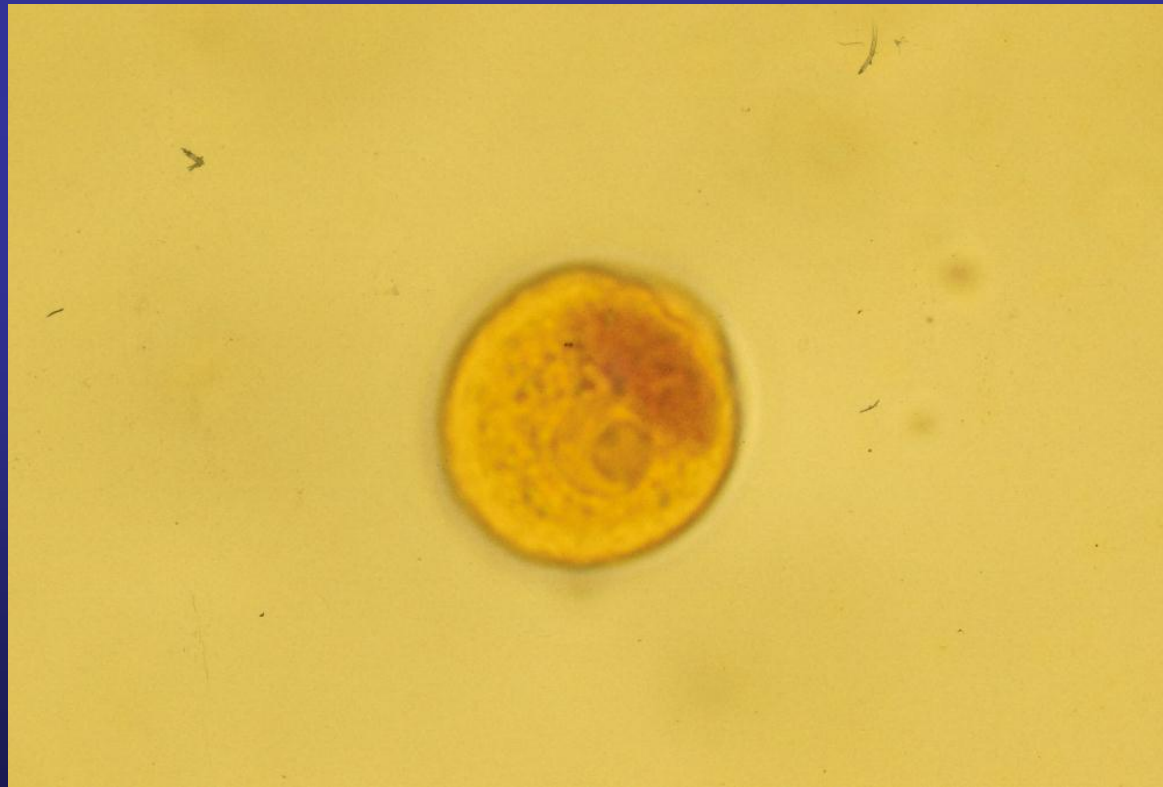
# *Endolimax nana*

Cyst with three visible nuclei. The cysts are smaller than 10  $\mu\text{m}$  and contain four nuclei with a massive central karyosome (Lugol stain).



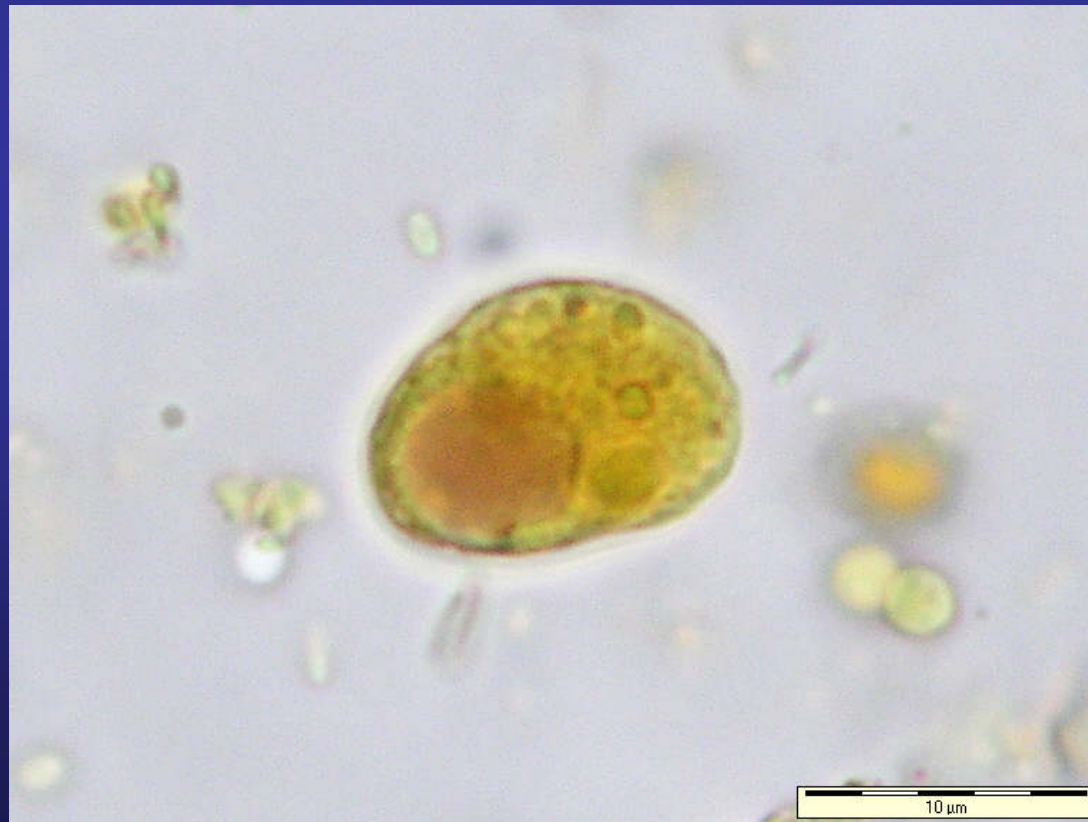
## *Iodamoeba bütschlii*

Mononucleate cyst with glycogen vacuole (dark brown with this stain) in faeces (Lugol stain).



# *Iodamoeba bütschlii*

Cyst with glycogen vacuole (dark brown with this stain) and one massive nucleus in faeces (Lugol stain).

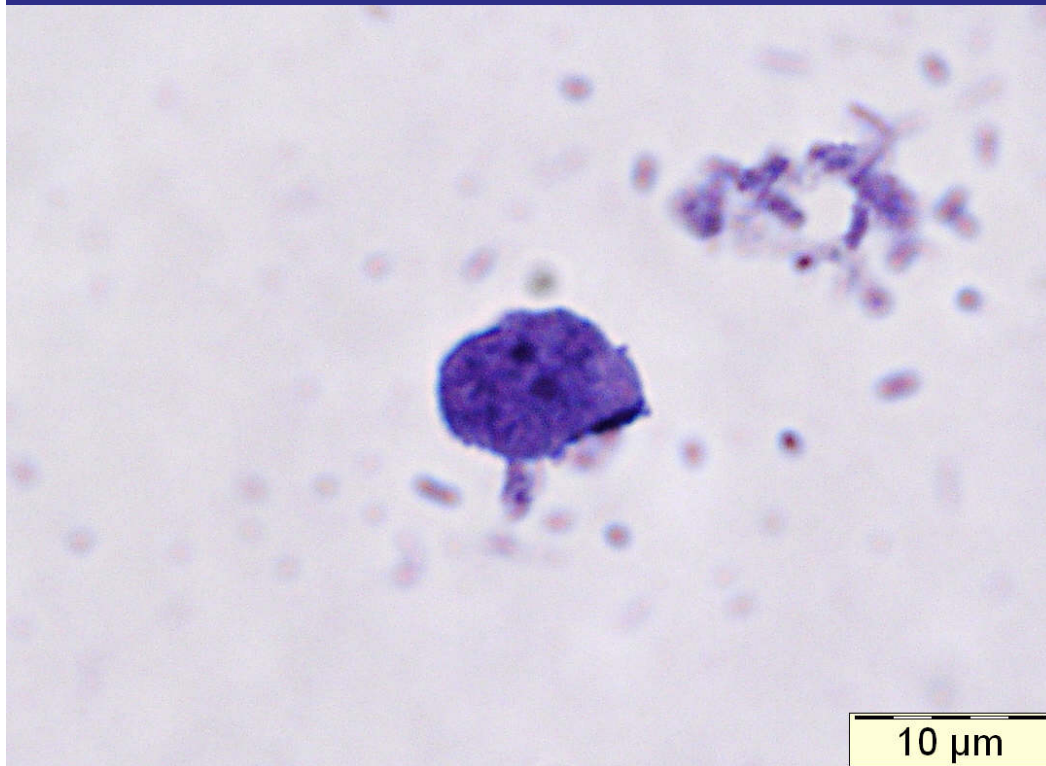


## *Iodamoeba bütschlii*

Cyst with glycogen vacuole (dark brown with this stain) and one massive nucleus in faeces (Lugol stain).



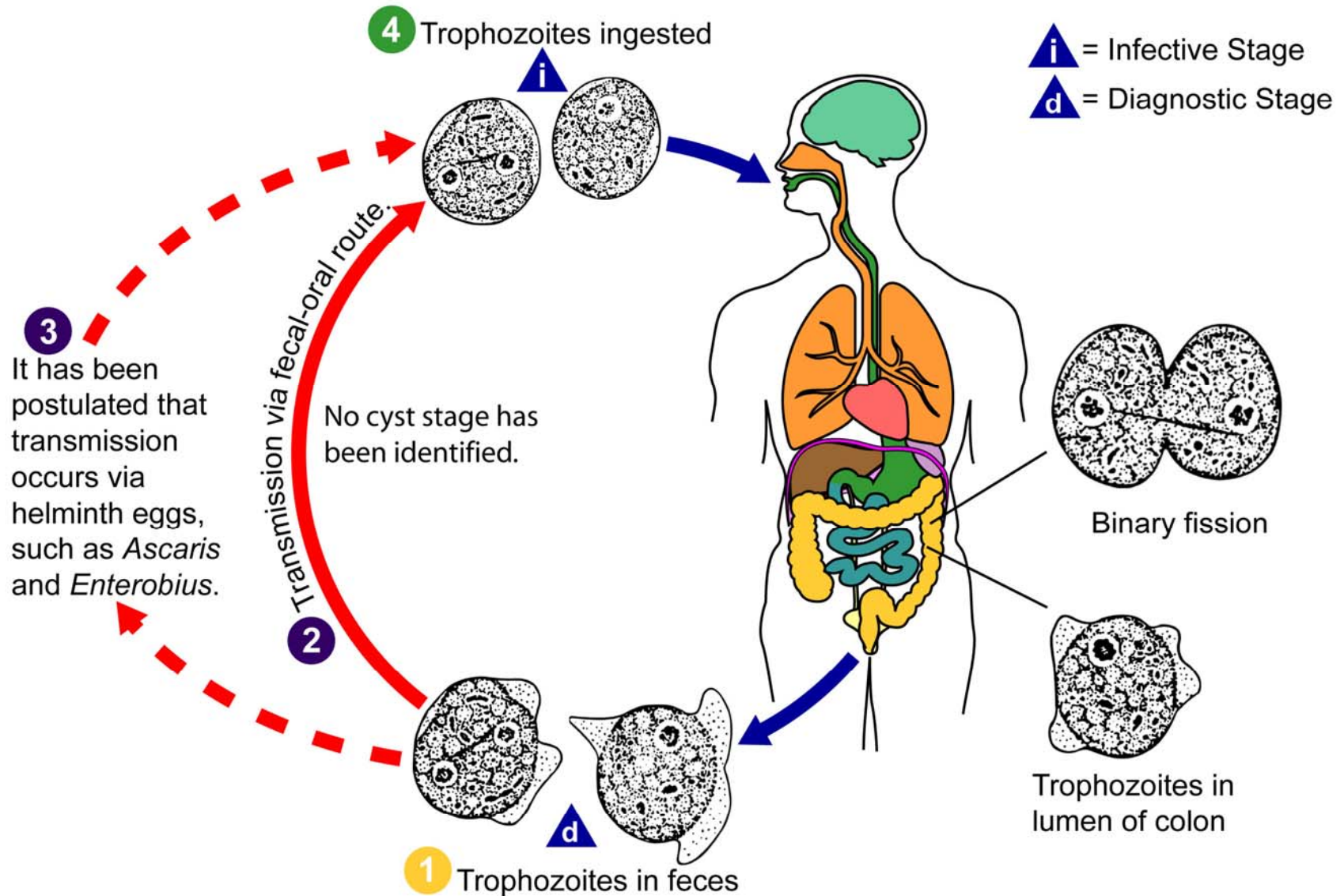
# *Dientamoeba fragilis*



- **Two nuclei.**
- “The unflagellated human flagellate “.
- Only (very labile) trophozoites, no cysts.
- Questionable enteric pathogen.
- Doxycycline, paromomycin, metronidazole (Sanford *et al.*, 2010).

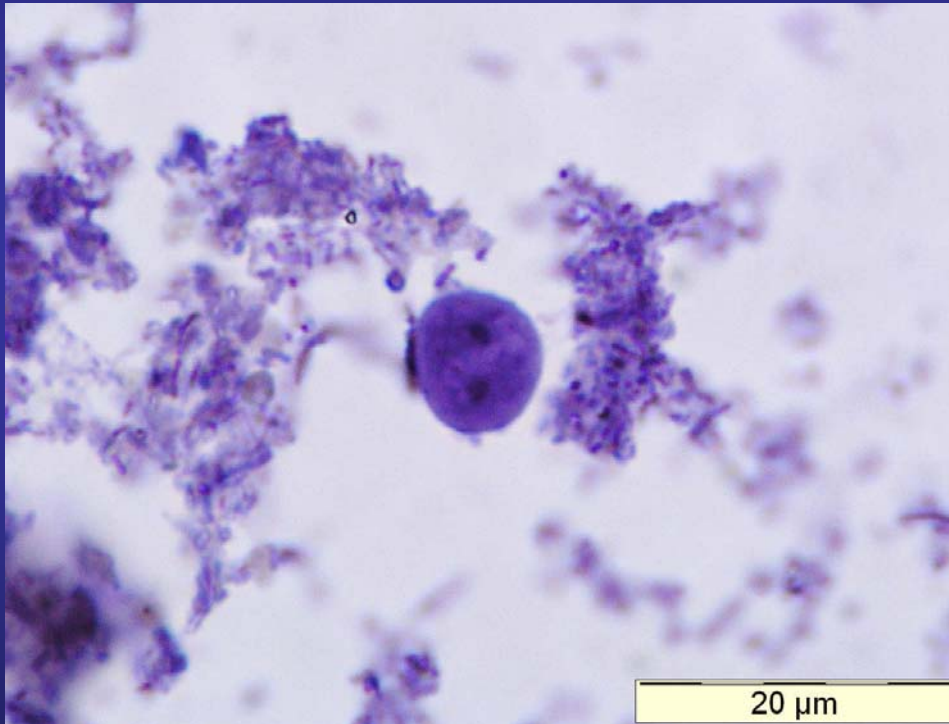
# Dientamoeba fragilis Infection

(*Dientamoeba fragilis*)



Courtesy CDC

# *Dientamoeba fragilis*



SAF fixative (sodium acetate acetic acid formalin) and iron hematoxylin stain have replaced the PVA (polyvinyl alcohol fixative with  $\text{HgCl}_2$ ) and trichrome stain.



# Preservatives

## (PVA)

- Polyvinyl alcohol
- “gold standard”
- Contains mercuric chloride = waste problem
- Trichrome or iron hematoxylin

## SAF

- Sodium acetate formalin
- Alternative, no waste problem
- Iron hematoxylin

# Sodium acetate acetic acid formalin (SAF)

- Sodium acetate 1.5 g
- Acetic acid, glacial 2.0 ml
- Formaldehyde, 37-40 % 4.0 ml
- Distilled water 92.0 ml



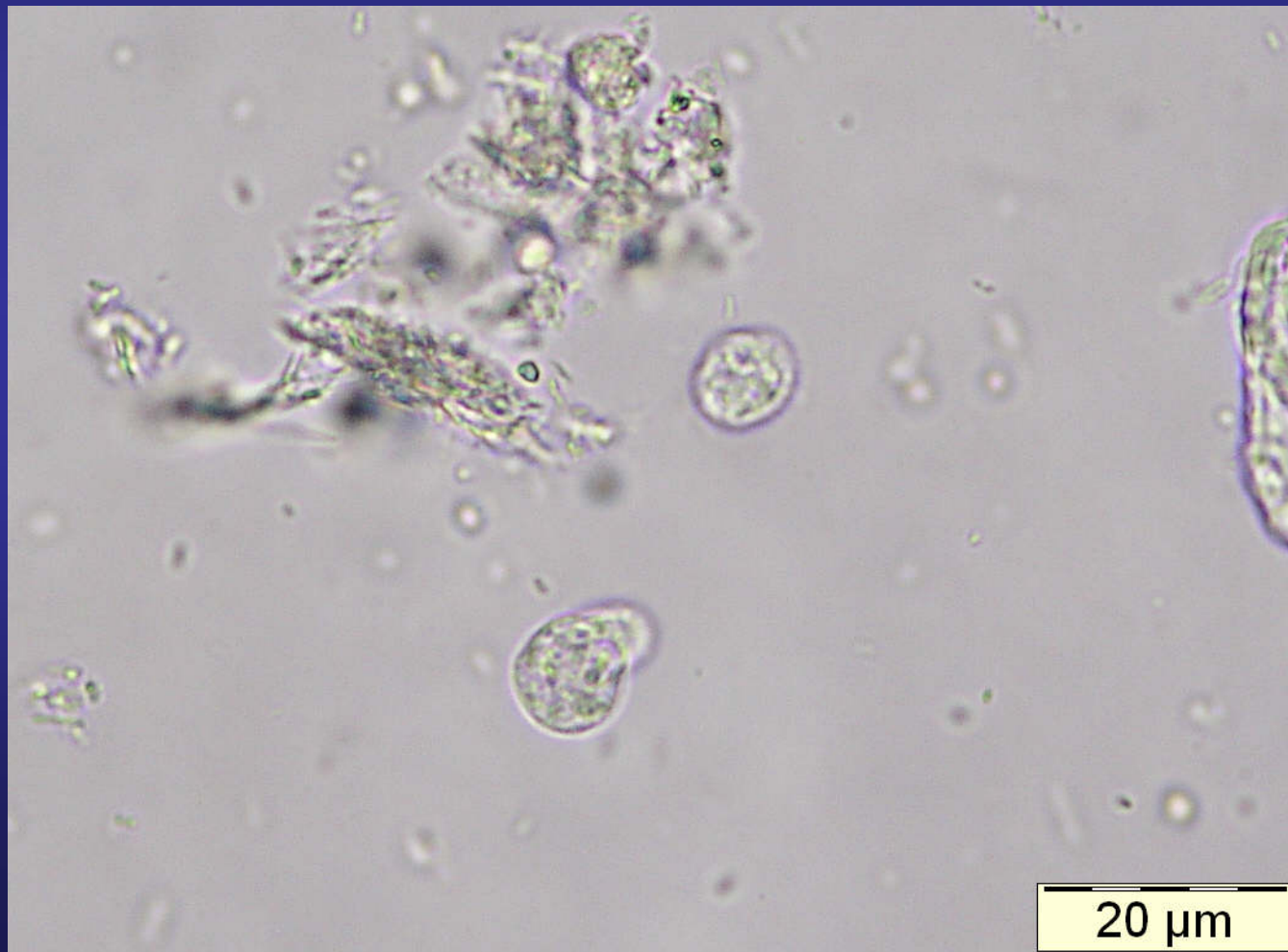
# *Dientamoeba fragilis*

In saline (Unstained).



# *Dientamoeba fragilis*

In saline (Unstained).

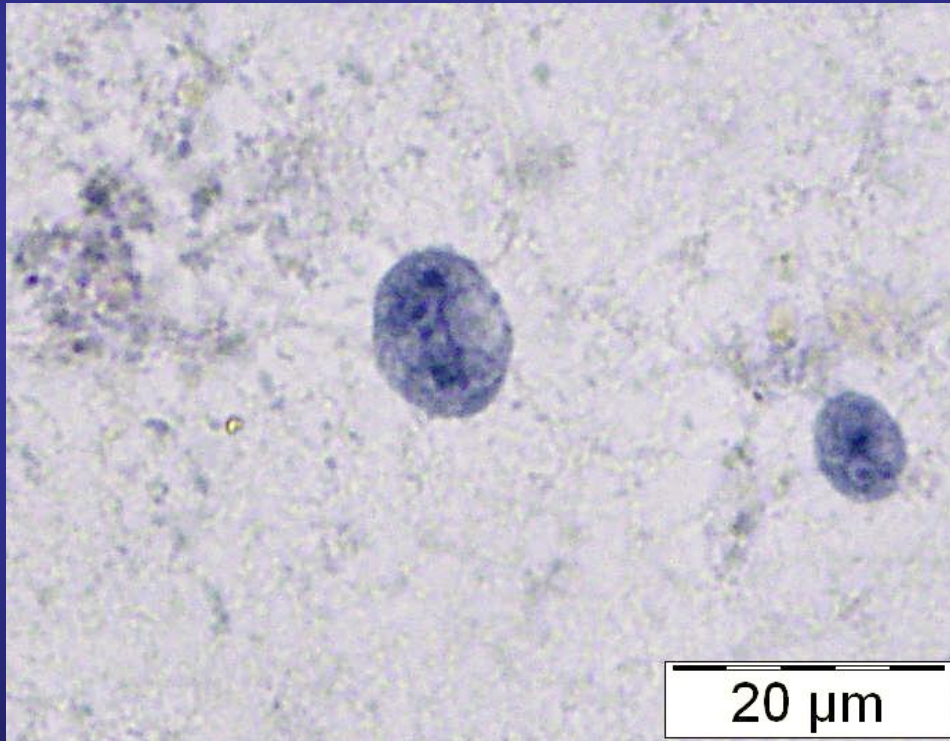


# *Dientamoeba fragilis*

In saline (Unstained).



# *Dientamoeba fragilis*

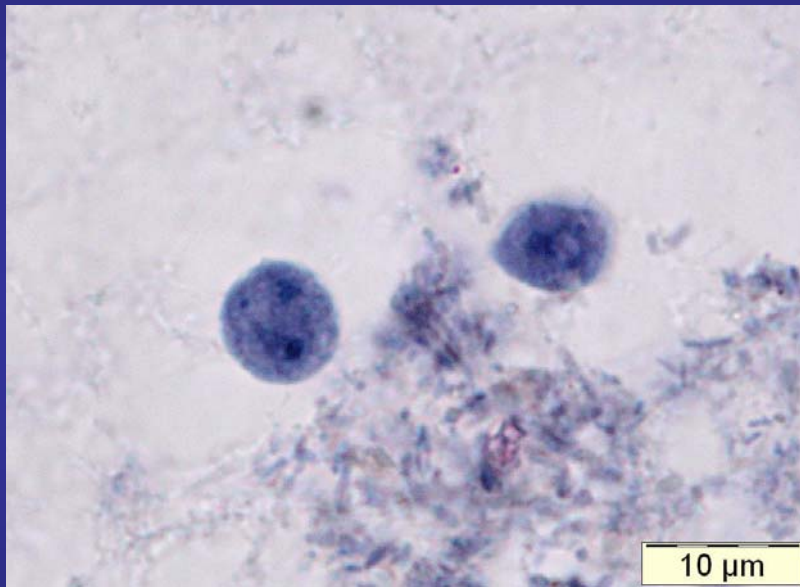


- From The Netherlands.
- In 247 unpreserved stool specimens: none.
- In 247 SAF-preserved stool specimens: 24.

(Mank T. 1997. Thesis)

Stained with hematoxylin.

# *Dientamoeba fragilis*

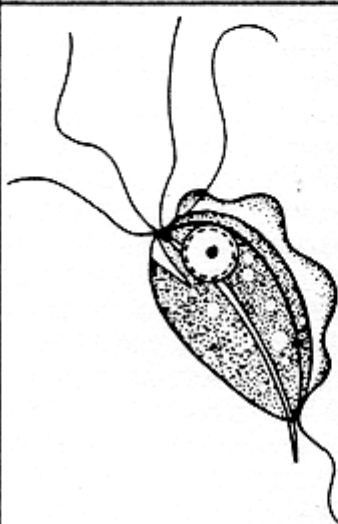
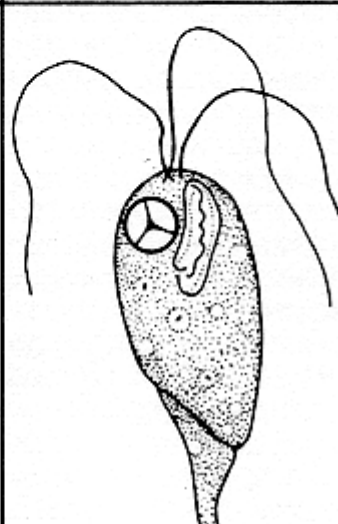
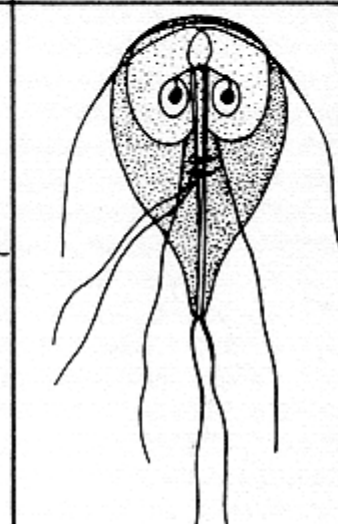
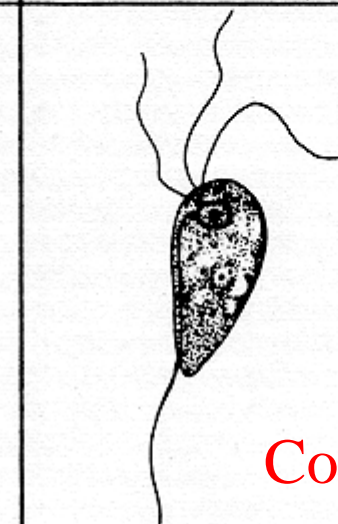
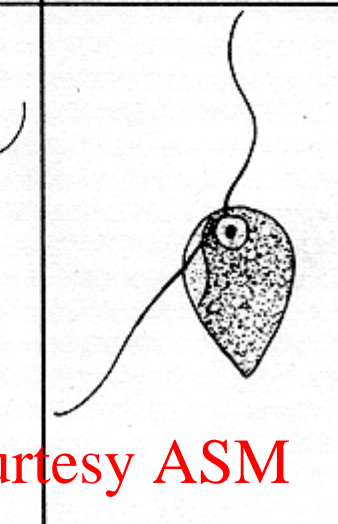


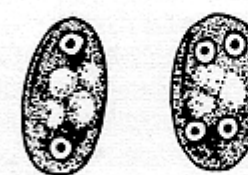



Stained with hematoxylin.

- In Brussels, Belgium.
- SAF-preserved stool specimens used.
- *D. fragilis* (6.3%) and *G. lamblia* (7.1%) in 448 patients.

Vandenberg O. *et al.* 2006. *Int J Infect Dis*:221, 2.

**FLAGELLATES**

|             | <i>Trichomonas hominis</i>  | <i>Chilomastix mesnili</i>  | <i>Giardia lamblia</i>  | <i>Enteromonas hominis</i>  | <i>Retortamonas intestinalis</i>  |
|-------------|---|---|---|---|---|
| Trophozoite |  |    |     |    |    |
| Cyst        | No cyst   |  |  |  |  |

Courtesy ASM

Scale:  
0 5 10  
μm

FIGURE 8 Flagellates found in human stool specimens. (From reference 4.)



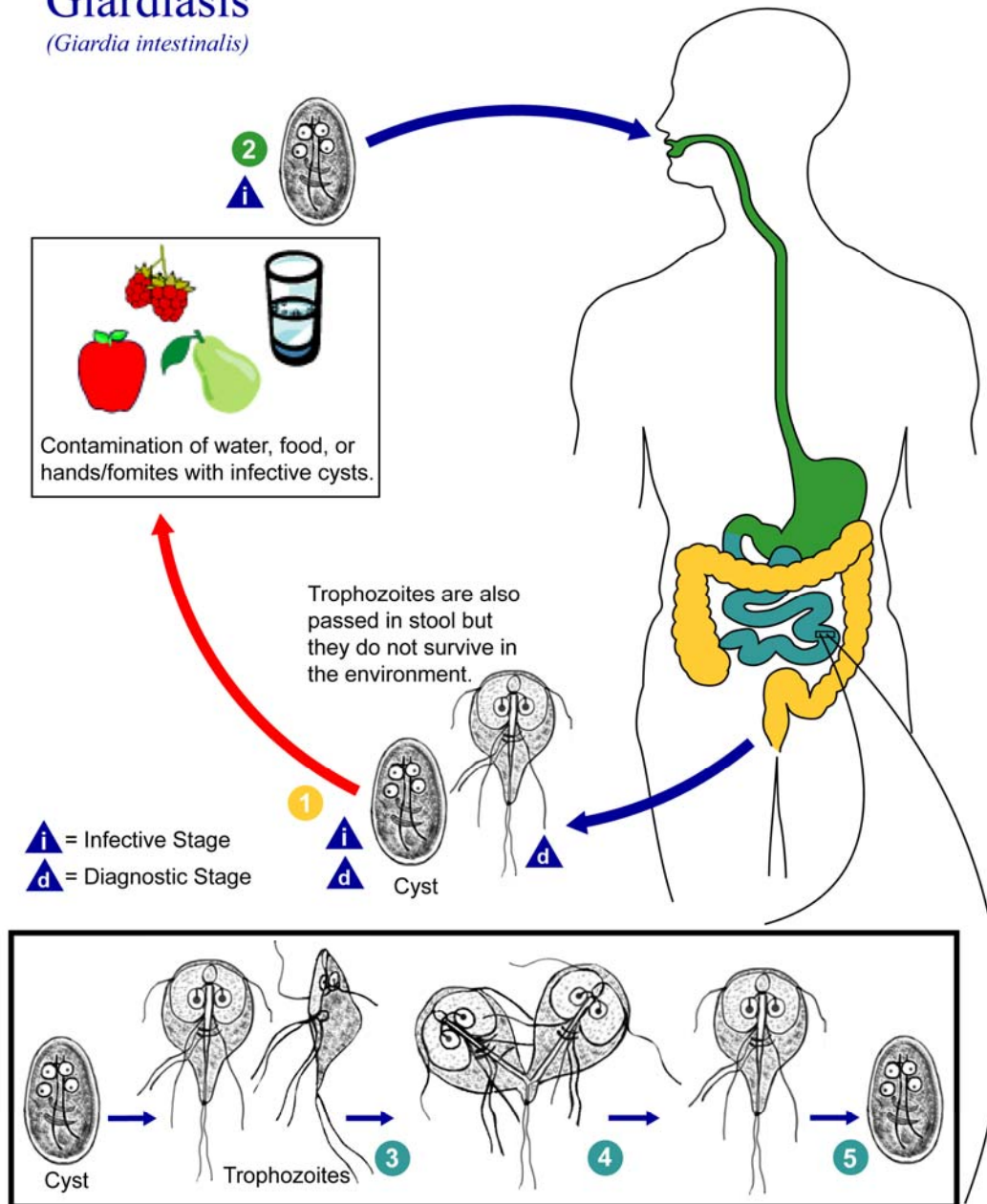
Antoon  
van  
Leeuwenhoek

*Giardia lamblia*  
1681



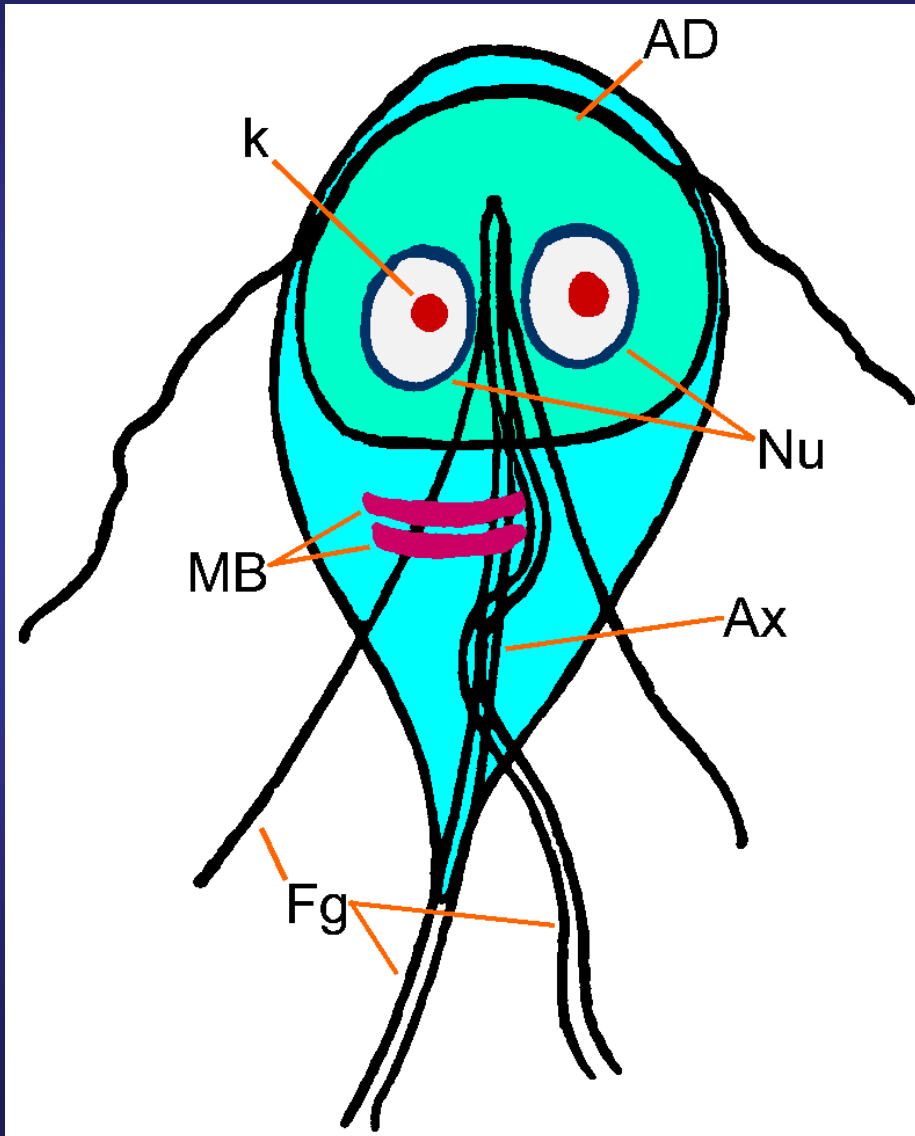
# Giardiasis

(*Giardia intestinalis*)



Courtesy CDC

# Trophozoite of *Giardia lamblia*

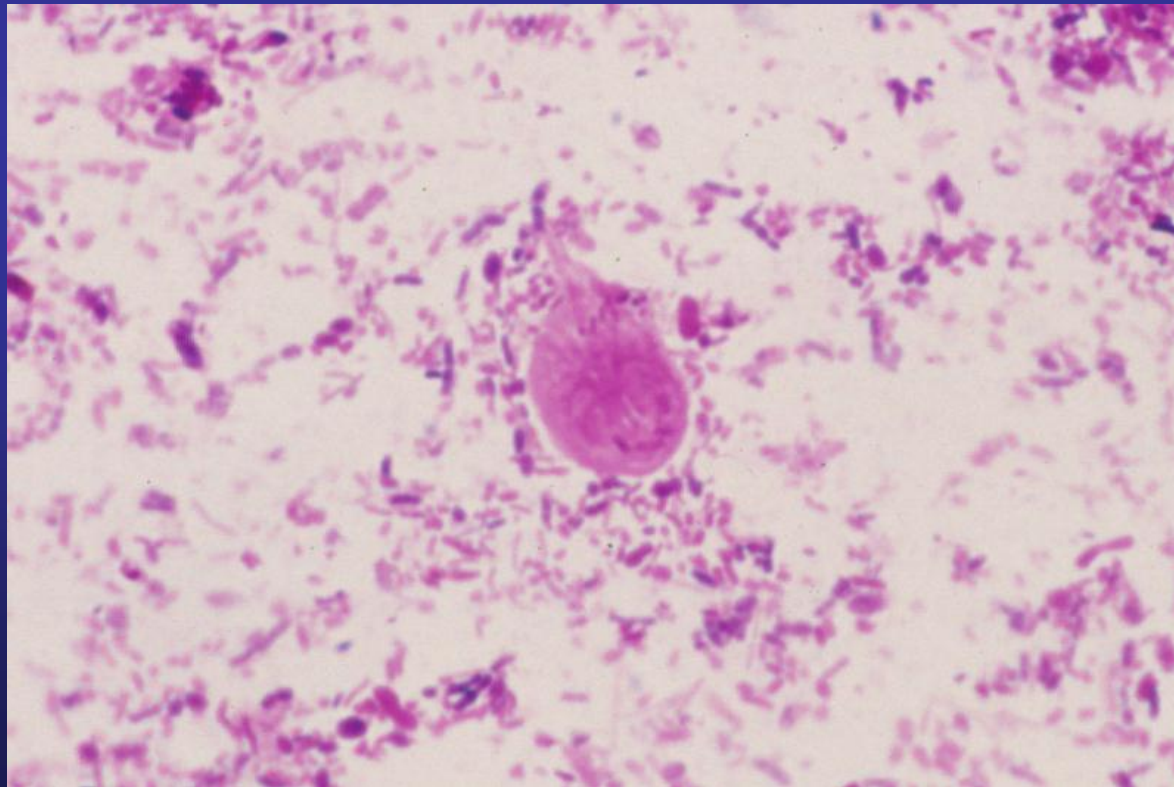


- Pear shape
- 12-15 x 5-10 x 2-4  $\mu\text{m}$
- 2 nuclei
  - large karyosome, no peripheral chromatin
- Fibrils (axonemes) evident
  - bilateral symmetry
- Pair of median bodies
- Adhesive disk (not always evident)
- 4 pair flagella
  - motility likened to a falling leaf

Courtesy Tulane

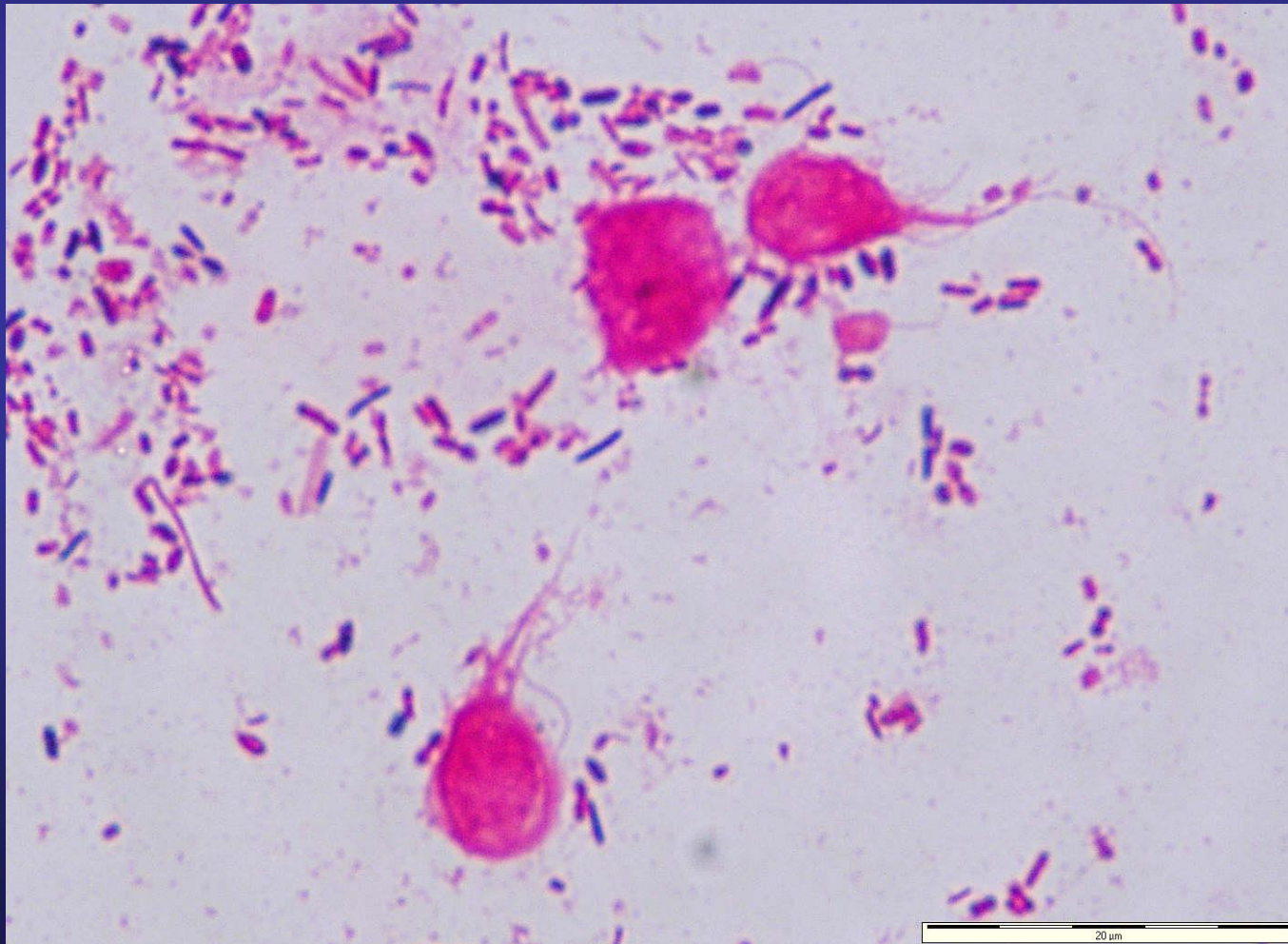
# *Giardia lamblia*

Trophozoite with two nuclei and several flagella in faeces (Gram stain).



# *Giardia lamblia*

Trophozoites with several flagella in faeces (Gram stain).



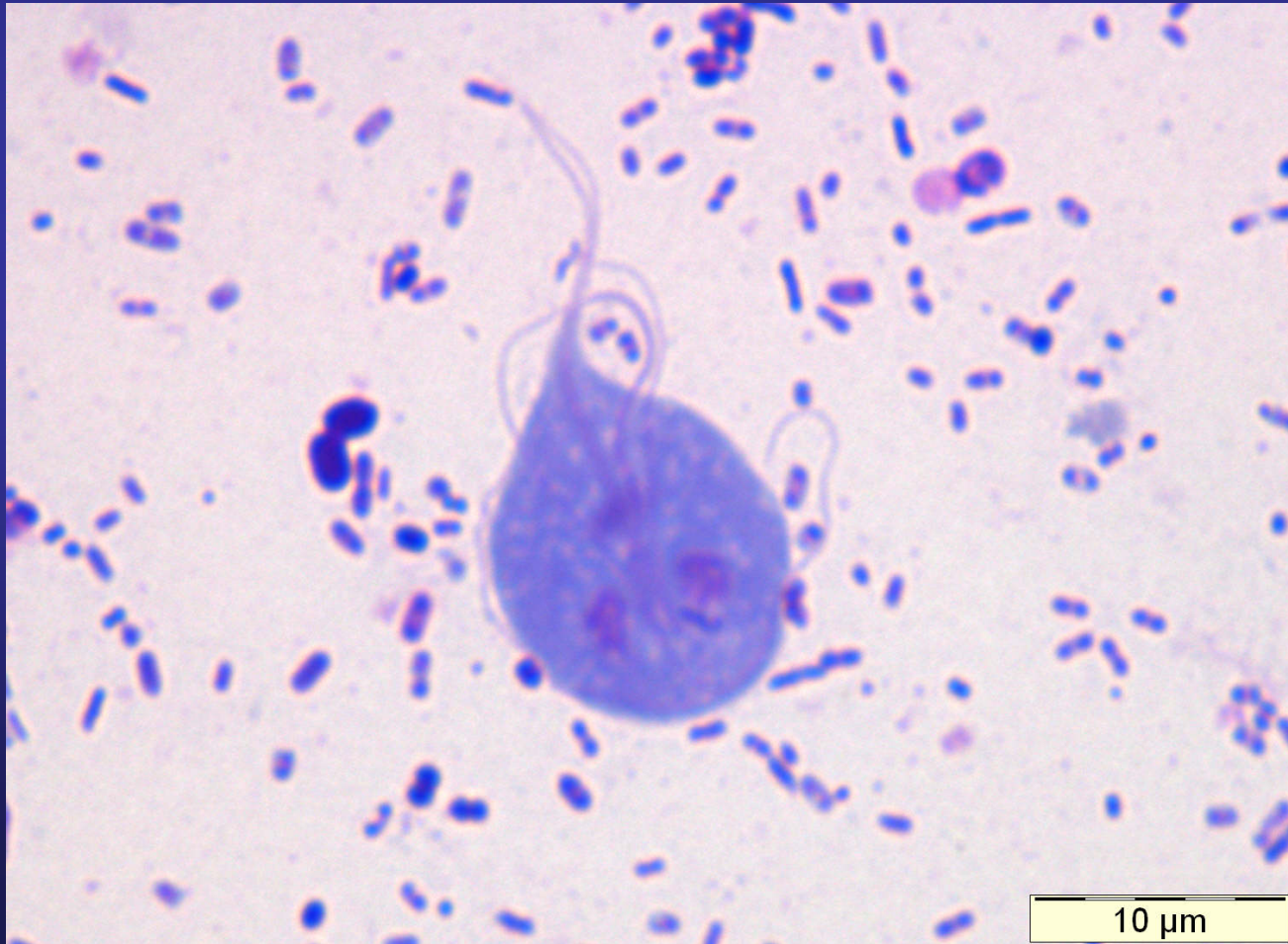
# *Giardia lamblia*

Trophozoite with adhesive (sucking) disk in faeces  
(Unstained).



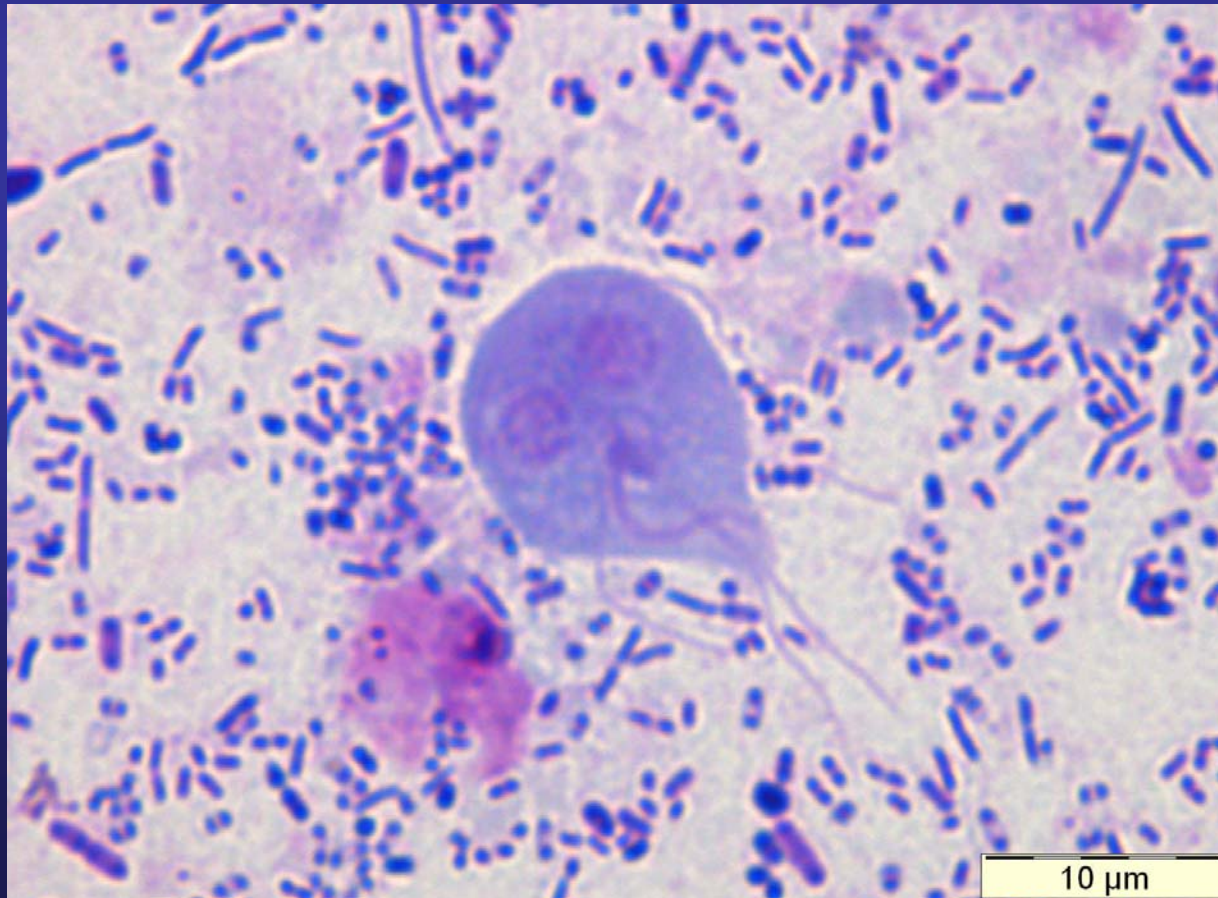
# *Giardia lamblia*

Trophozoite with two nuclei and several flagella  
(May-Grünwald-Giemsa stain)



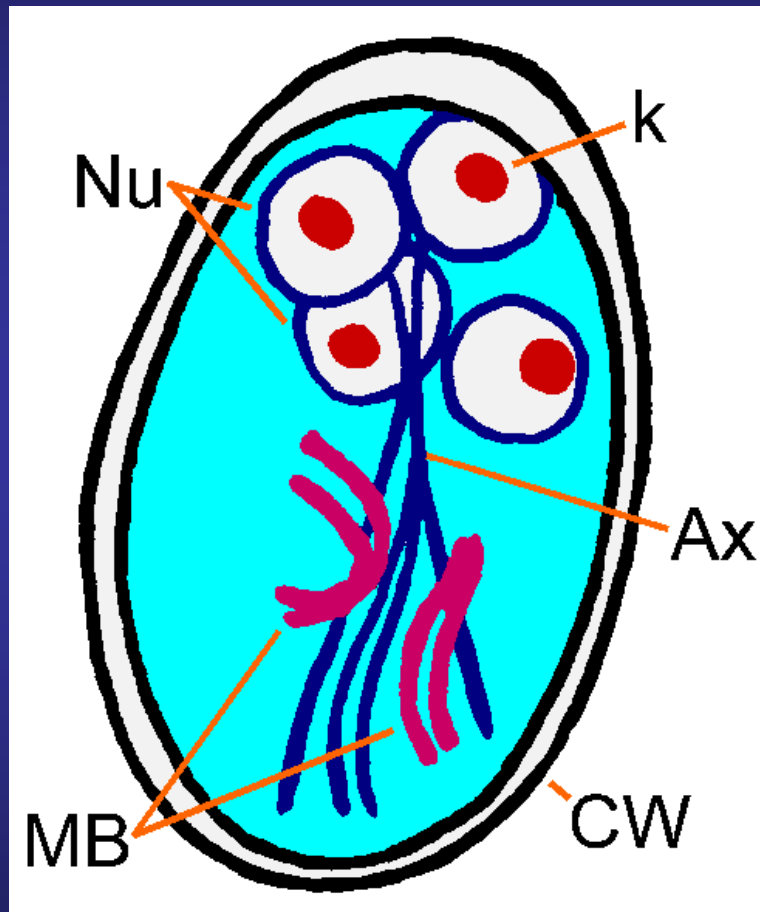
# *Giardia lamblia*

Trophozoite in faeces with two nuclei and several flagella. Length: 10 to 20  $\mu\text{m}$   
(May-Grünwald-Giemsa stain)





# Cyst of *Giardia lamblia*



- Oval shape
- 11-14 x 6-10  $\mu\text{m}$
- Distinct cell wall set apart from cytoplasm
- 4 nuclei at anterior end
  - large karyosome, no peripheral chromatin
- Fibrils (axonemes) evident
- Median bodies

Courtesy Tulane

# *Giardia lamblia*

Cyst with two visible nuclei and flagella (Lugol stain).



# *Giardia lamblia*

Cyst with two visible nuclei and flagella (Lugol stain).



# *Giardia lamblia*

Cyst with two visible nuclei and flagella in faeces collected with SAF (Unstained).



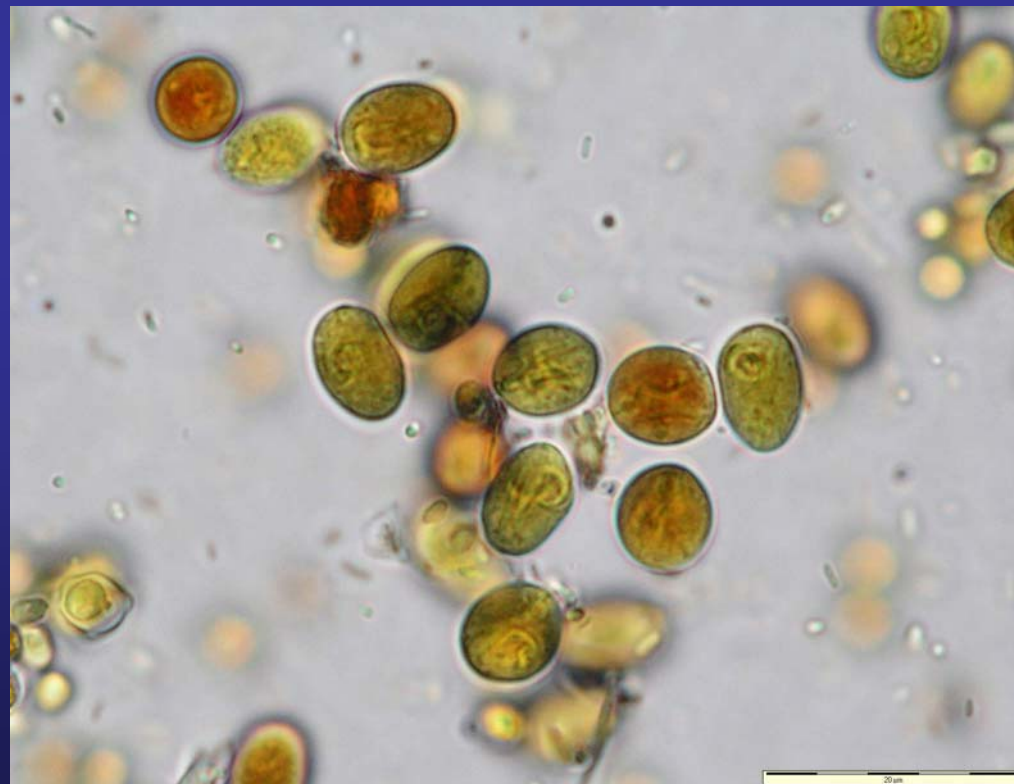
# *Giardia lamblia*

Two cysts with visible nuclei and flagella (Lugol stain).



# *Giardia lamblia*

Many cysts, round or oval, some with visible nuclei and flagella (Lugol stain).



# *Giardia lamblia*

Older shrunken cyst with two visible nuclei (Lugol stain).

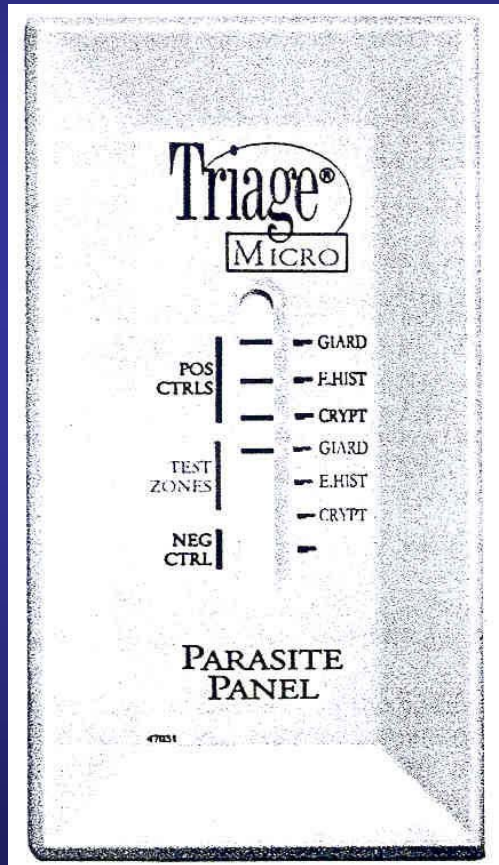


# *Giardia lamblia*: antigen detection by IF and ELISA

- Monoclonal antibodies: Merifluor (MERIDIAN) (*Cryptosporidium* and *Giardia*).
- 8/9 *Giardia* ELISAs are OK. (JCM, 1998, 1338).
- Triage parasite panel (BIOSITE) useful. (JCM, 2000, 3337; JCM, 2001, 334).
- One ELISA almost as sensitive as two microscopic examinations. (Mank T. 1997).



# Antigen detection



- Good sensitivity and specificity
- In combination with conventional tests
- High cost  
(Triage = \$ 19.44/test)

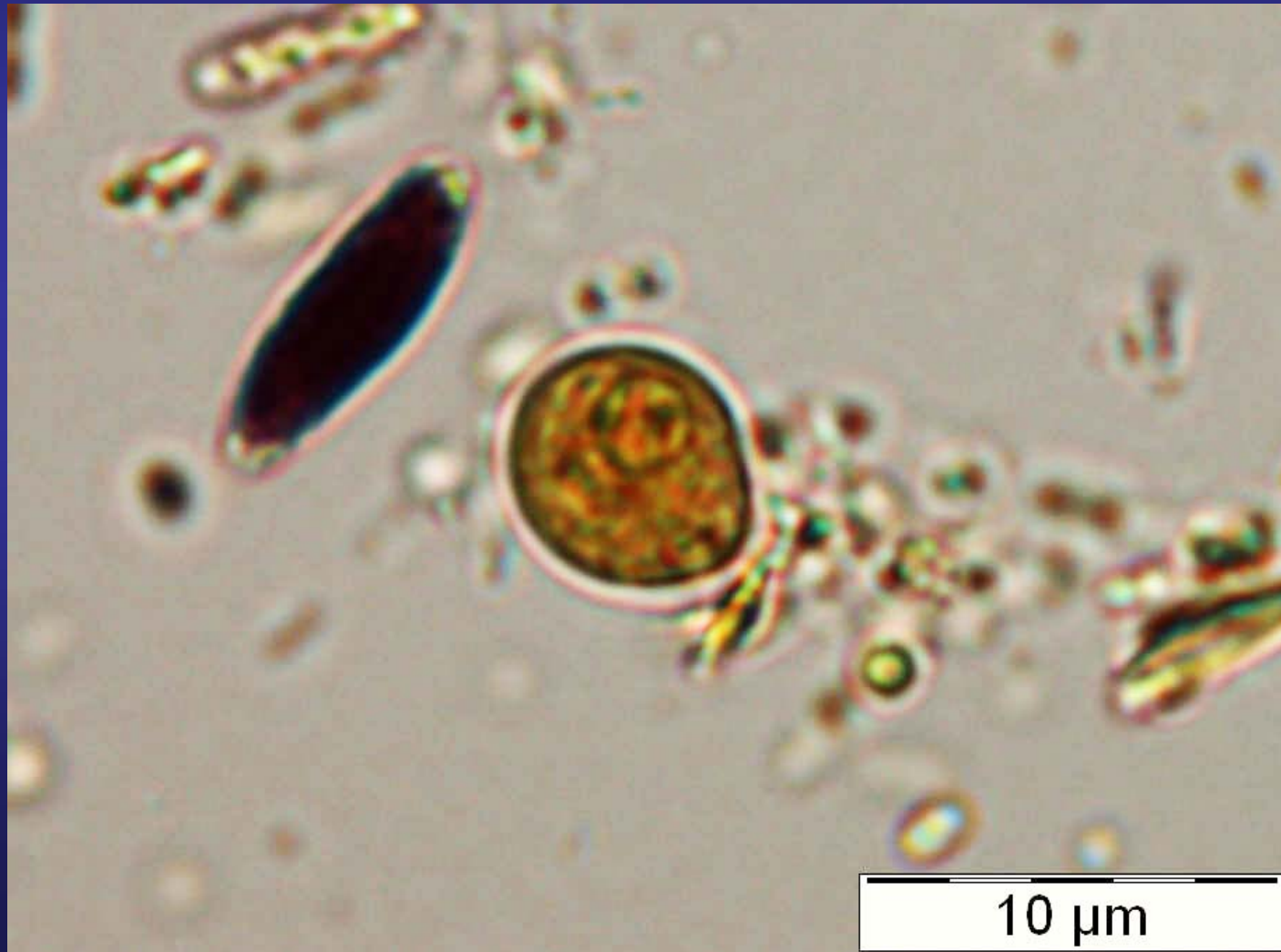
# *Chilomastix mesnili*

Pearshaped cyst with one nucleus (Lugol stain).



# *Chilomastix mesnili*

Pearshaped cyst with one nucleus (Lugol stain).



# *Chilomastix mesnili*

Pearshaped cyst with one nucleus (Lugol stain).



# *Chilomastix mesnili*

Two pearshaped small cysts with one nucleus (Lugol stain).



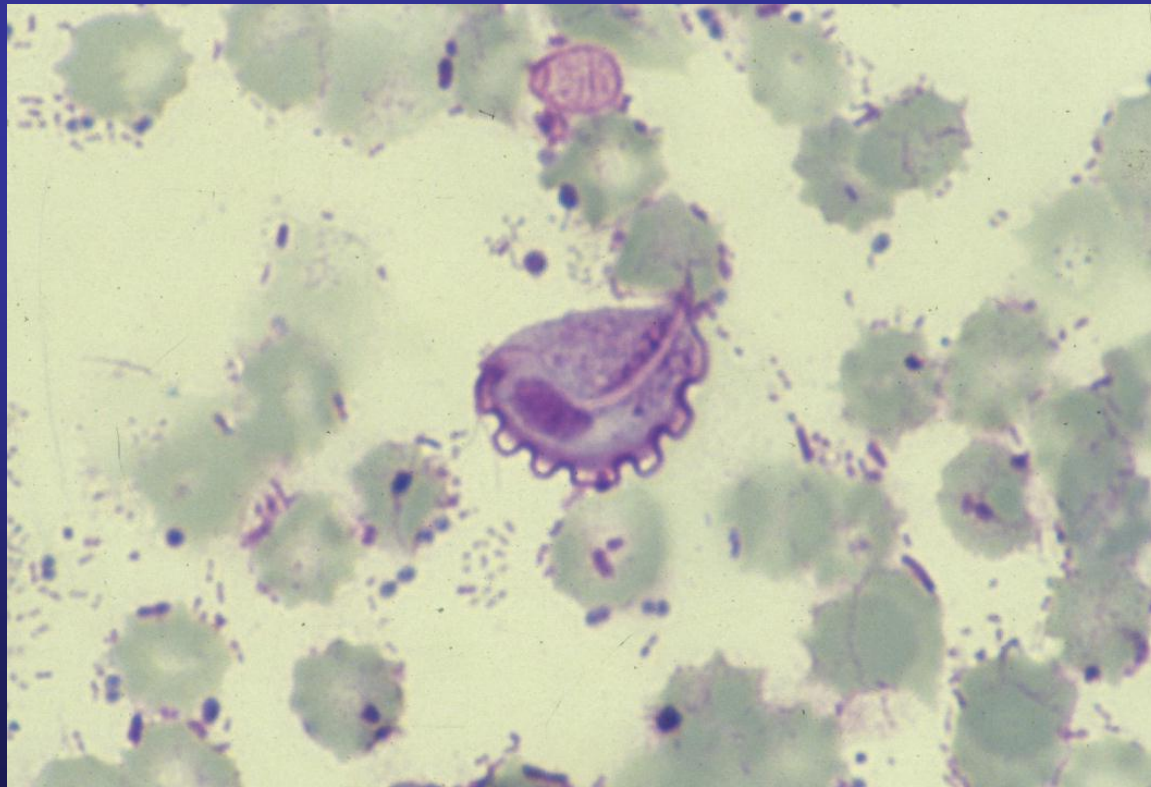
# *Chilomastix mesnili*

Trophozoite with one nucleus and flagella  
(Lugol stain).



# *Pentatrichomonas hominis*

In blood containing stool. Shown here are the long undulating membrane and the axostyle (8 to 15 $\mu$ m)  
(Osmic acid-Giemsa stain).



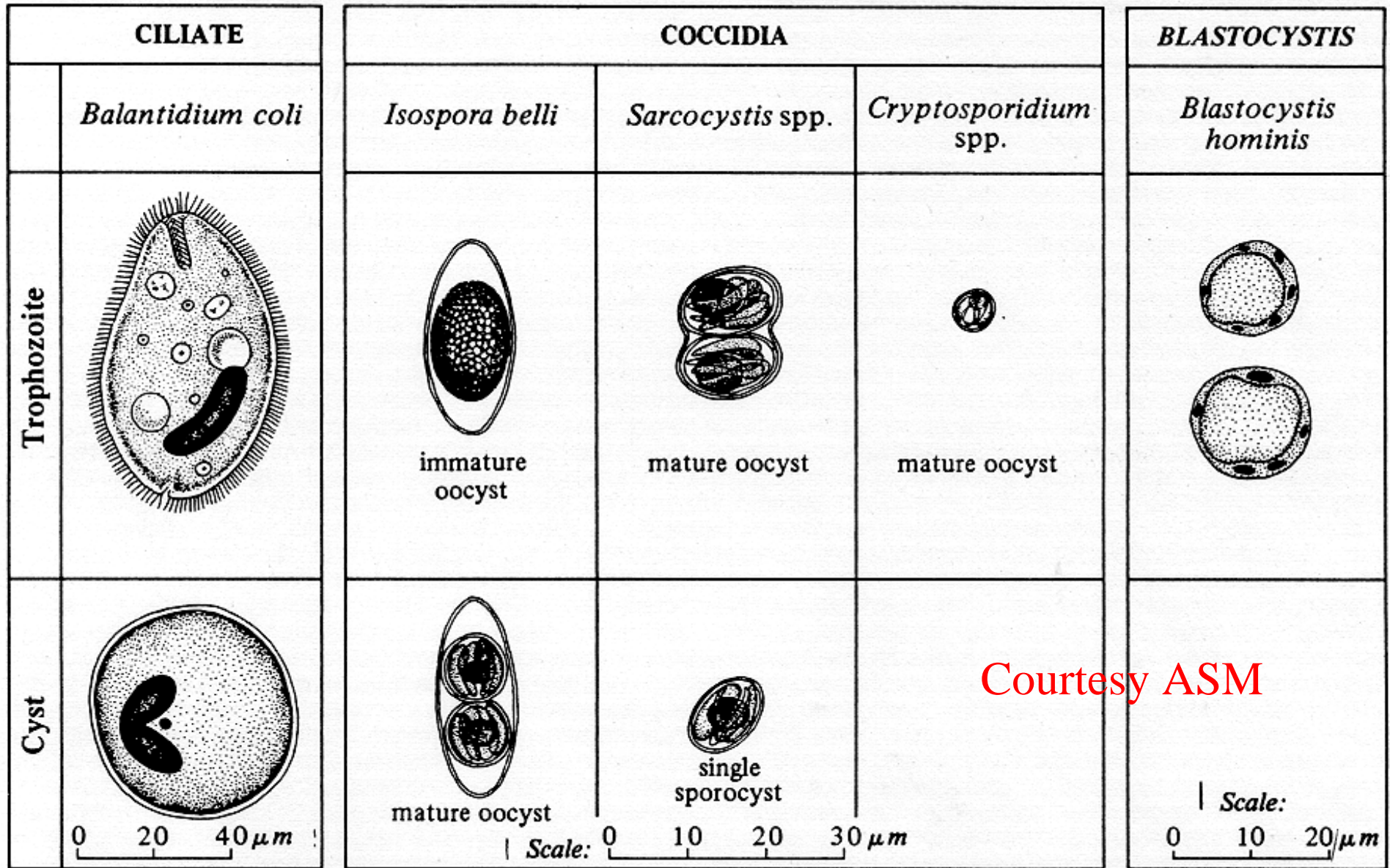


FIGURE 7 Ciliate, coccidia, and *Blastocystis hominis* found in human stool specimens. (From reference 4.)



# *Balantidium coli*

In stool. An ovoid ciliate, here as trophozoite, with clearly visible macro- and micronucleus, cystosome, and cilia. Size 60 to 80  $\mu\text{m}$  (Unstained).



# *Isospora belli*

Continued development of the oocyst occurs outside the body, to form two mature sporocysts, each containing four sporozoites, resembling *Sarcocystis* spp. Two immature ovoid oocysts in faeces. The oocyst contains one immature sporont (at the left), occasionally a dividing sporont (at the right) or two sporonts (not seen) (Unstained).



# *Isospora belli*

The oocyst contains a dividing sporont (Unstained).



# *Isospora belli*



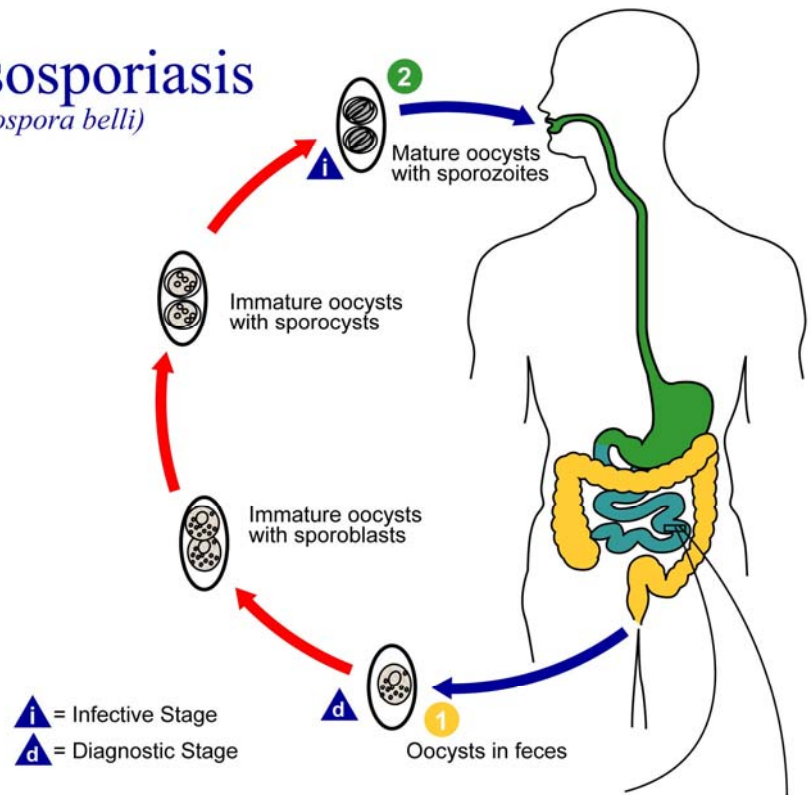
Two sporocysts with each  
four sporozoites.

- Human to human transmission.
- Eosinophilia may be present.
- Worldwide.
- Oocyst very pale and transparent.
- Wet-preparation examination preferred over the stained smear.
- dd. *Sarcocystis* spp.
- Cotrimoxazole (HIV).

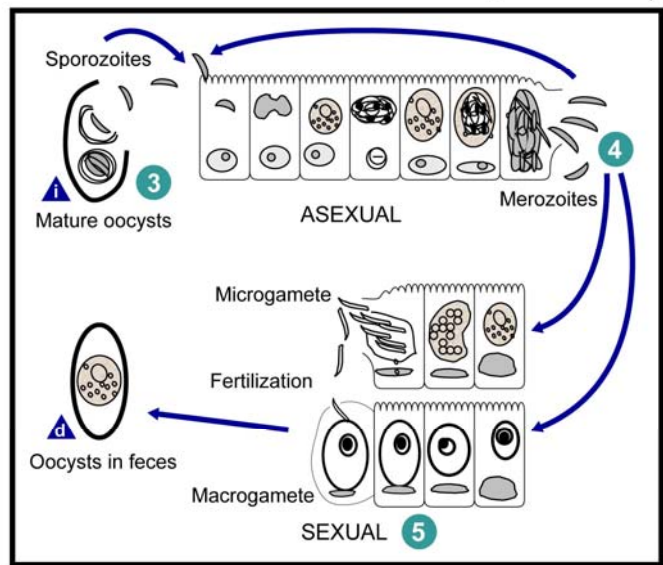
Fisk T.L *et al.* 2005.

# Isosporiasis

*(Isospora belli)*



**i** = Infective Stage  
**d** = Diagnostic Stage



Courtesy of CDC

# *Sarcocystis* spp.

Human contamination is mainly due to consumption of improperly cooked pork or beef meat. Mature sporocyst containing four sporozoites in faeces (Unstained).



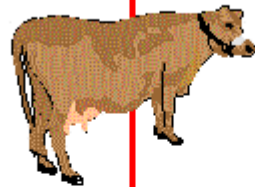
Fisk T.L *et al.* 2005.

# THE LIFE CYCLE OF *SARCOCYSTIS CRUZI*

The parasites infect the intestinal tissues of the host, reproduce asexually, and finally produce oocysts.



Oocysts are passed in the host's feces.

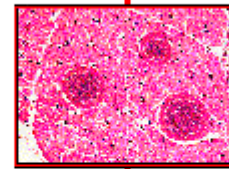


Oocysts are ingested by the intermediate host.

The definitive host is infected when it ingests bradyzoites in the tissue.



Zoitecysts, sarcocysts, or Miescher's tubules, filled with bradyzoites, form in the host's tissues.



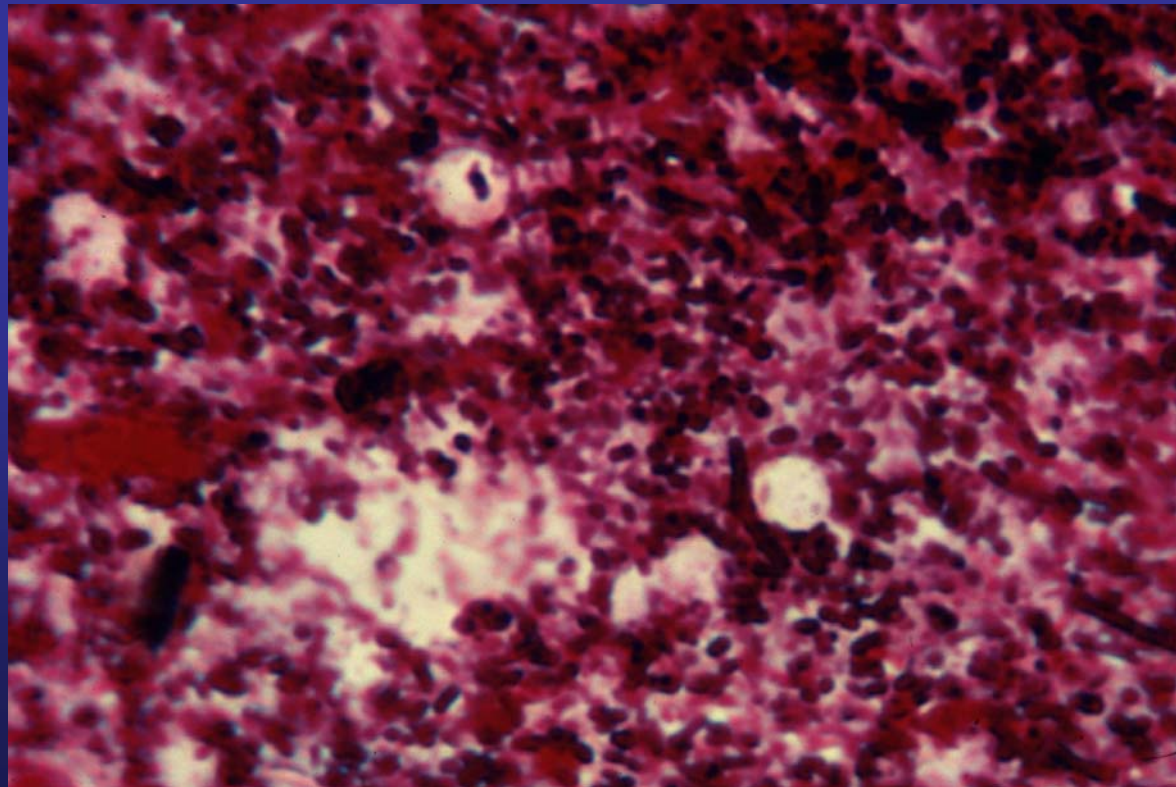
The oocysts excyst, and the parasites infect the host's tissues.

(Parasites and Parasitological Resources)

Courtesy The Ohio State University

# *Cryptosporidium parvum*

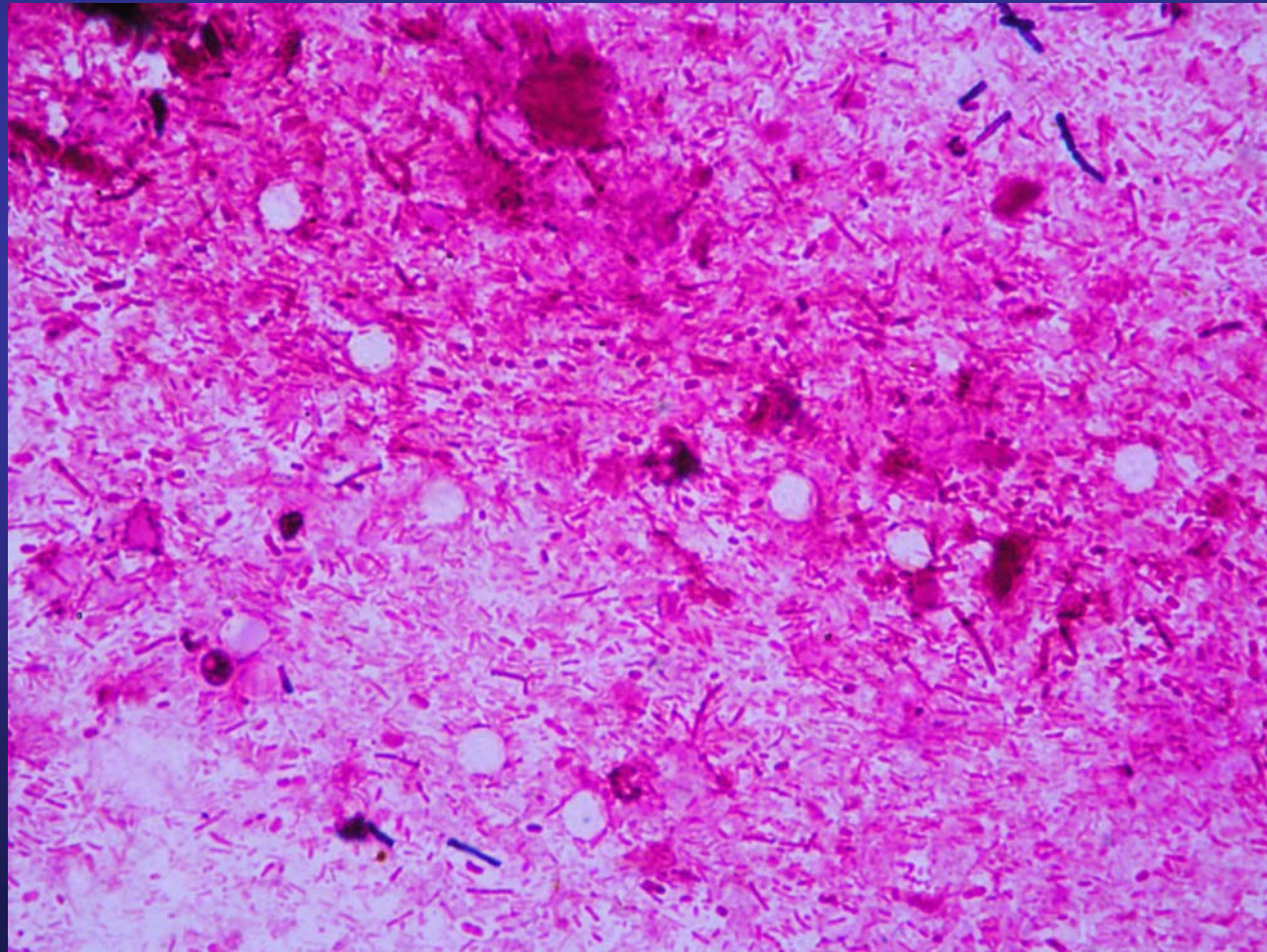
Several oocysts are seen as clear white holes in the densely coloured fecal mass (Gram stain).





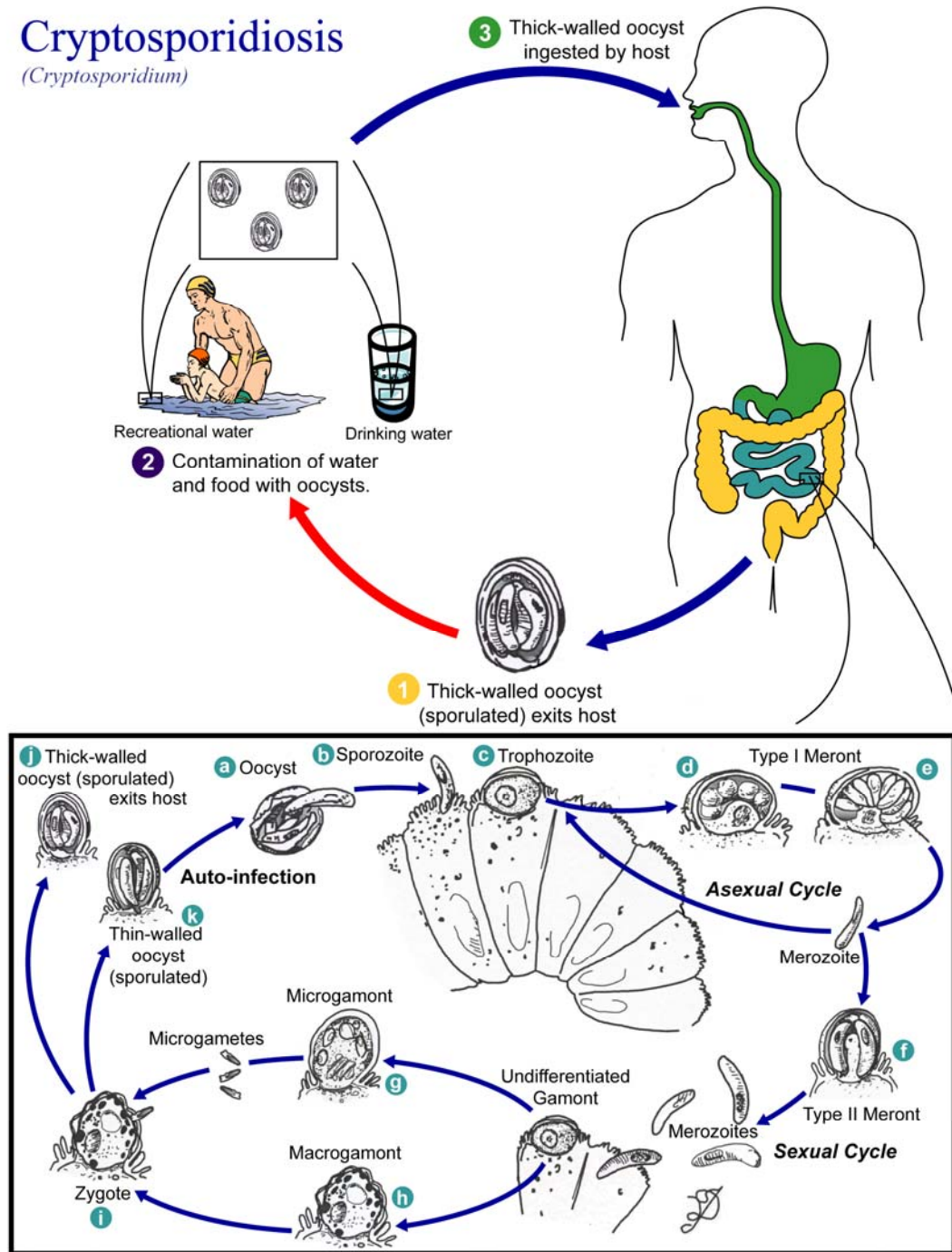
# *Cryptosporidium parvum*

Several oocysts are seen as clear white holes in the densely coloured fecal mass (Gram stain).



# Cryptosporidiosis

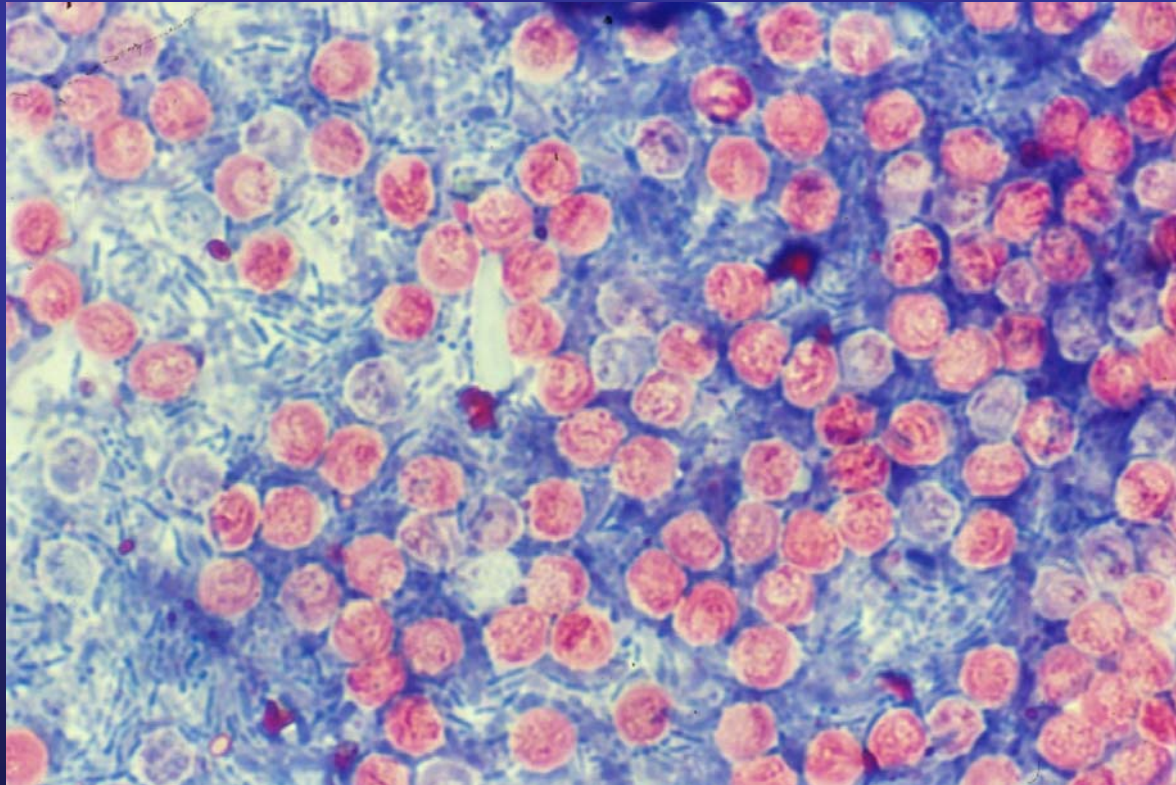
(*Cryptosporidium*)



Courtesy CDC

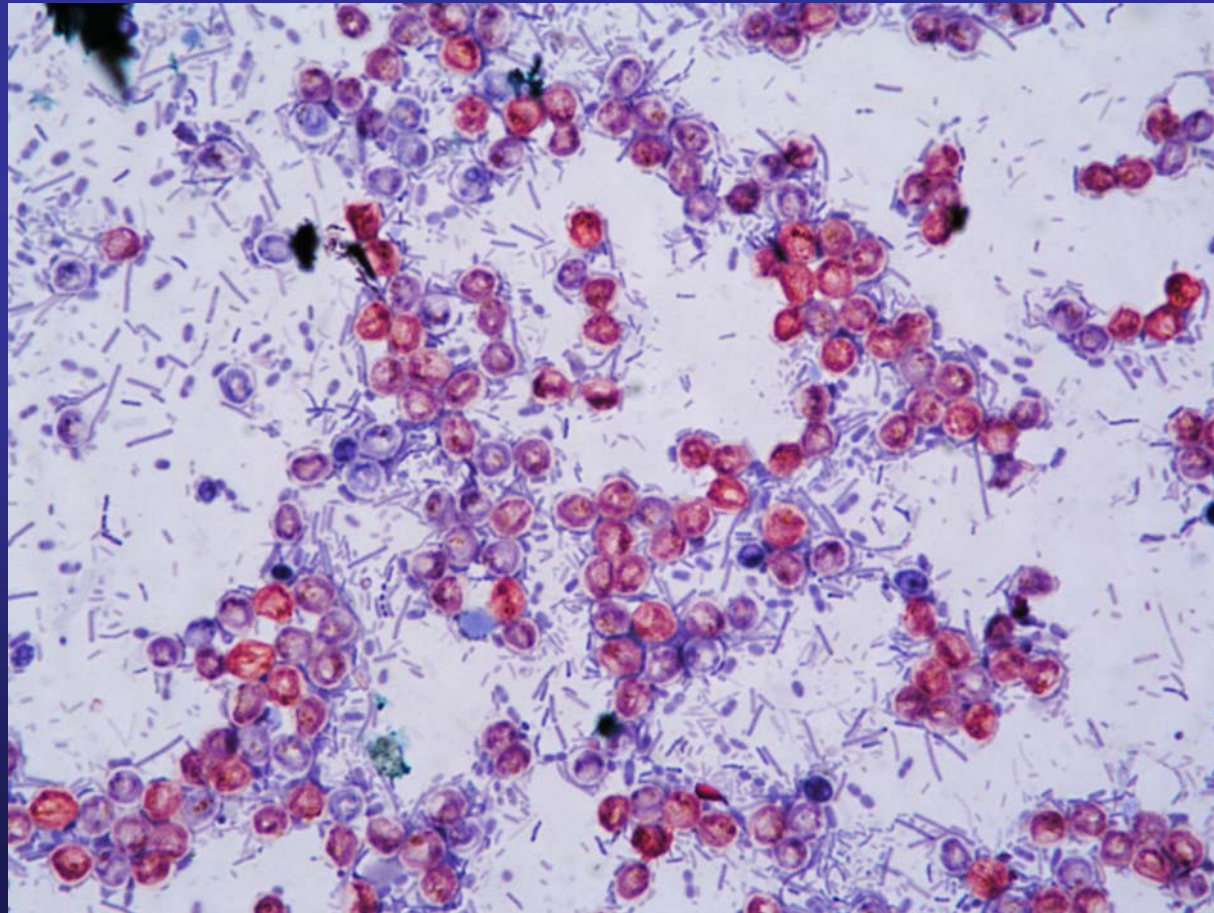
## *Cryptosporidium parvum*

Numerous oocysts containing four sporozoites are present. The bacteria are stained in blue by methyleneblue (Safranin stain).



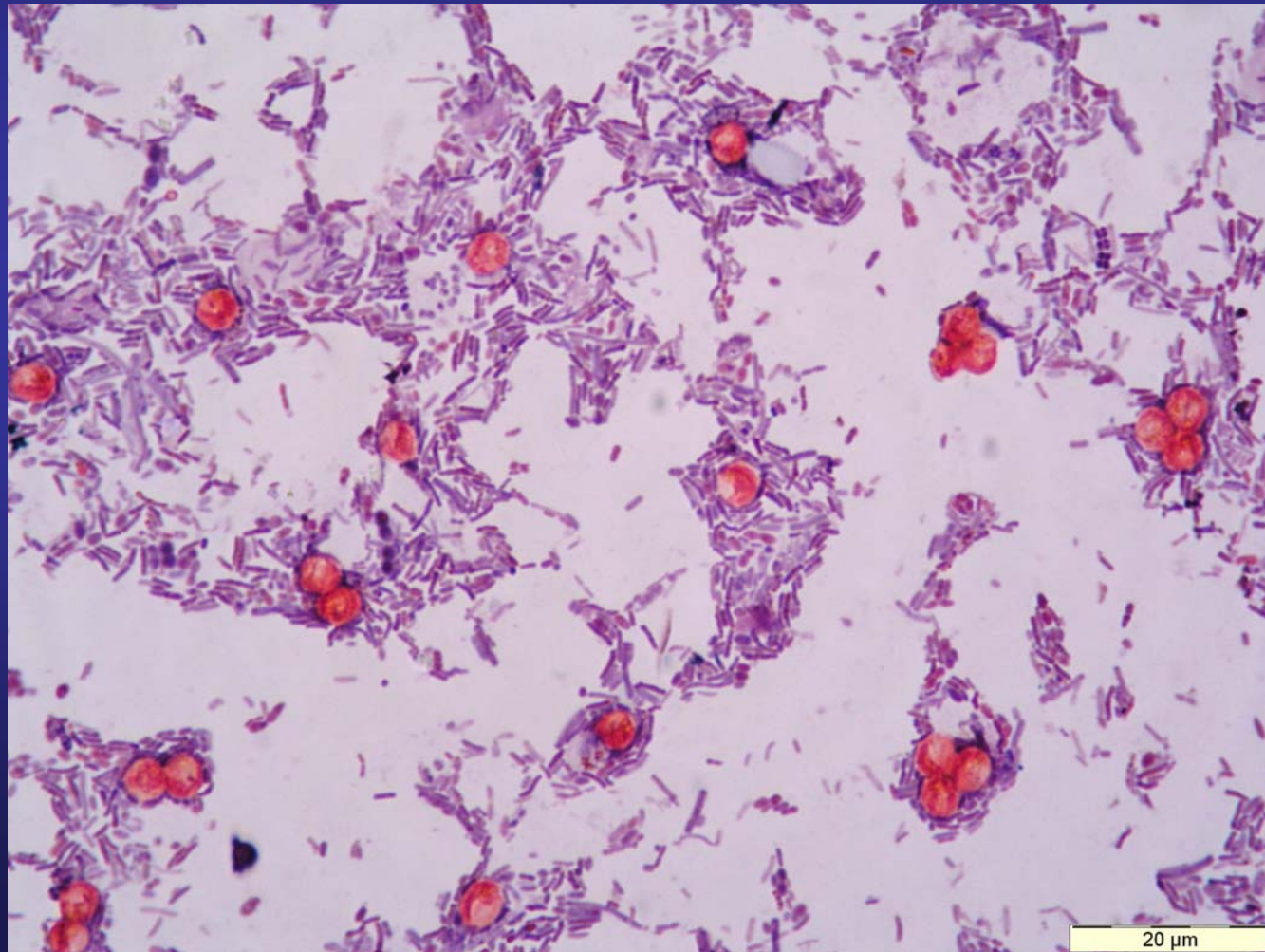
# *Cryptosporidium parvum*

Numerous oocysts. The bacteria are stained blue with methyleneblue (Safranin stain).



# *Cryptosporidium parvum*

Numerous oocysts. The bacteria are stained blue with methyleneblue (Safranin stain).



## *Cryptosporidium parvum*

Four oocysts containing four sporozoites are present.  
The bacteria are stained in blue by methyleneblue  
(Safranin stain).



# *Cryptosporidium parvum*

Oocyst containing four sporozoites  
(Modified Ziehl-Neelsen stain).



# *Blastocystis hominis*



(Gram stain)

- Contains a central-body form
- 5 - 30  $\mu\text{m}$
- 81 / 247 patients; 5 x more in SAF (The Netherlands)  
(Mank T., 1997. Thesis)



# *Blastocystis hominis*

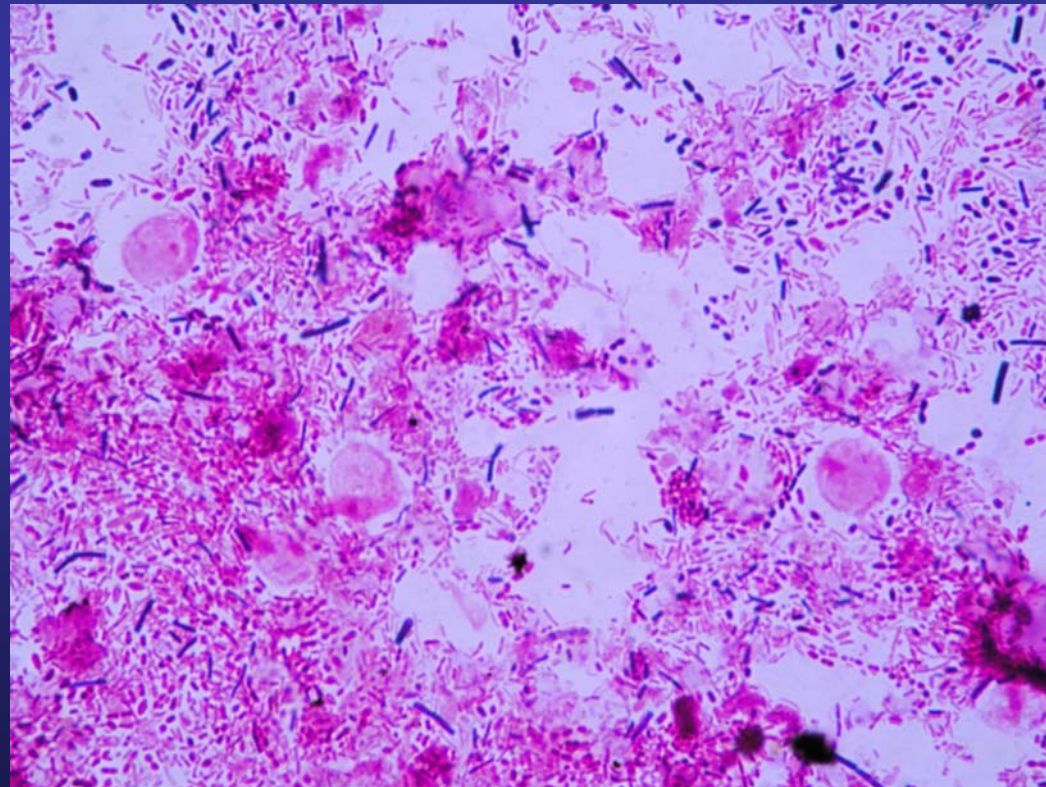


(Lugol stain)

- Belongs to the stramennoiles.
- Pathogenicity uncertain (HIV).
- Most prevalent human “protozoon”.
- Relatively labile (fixative recommended).

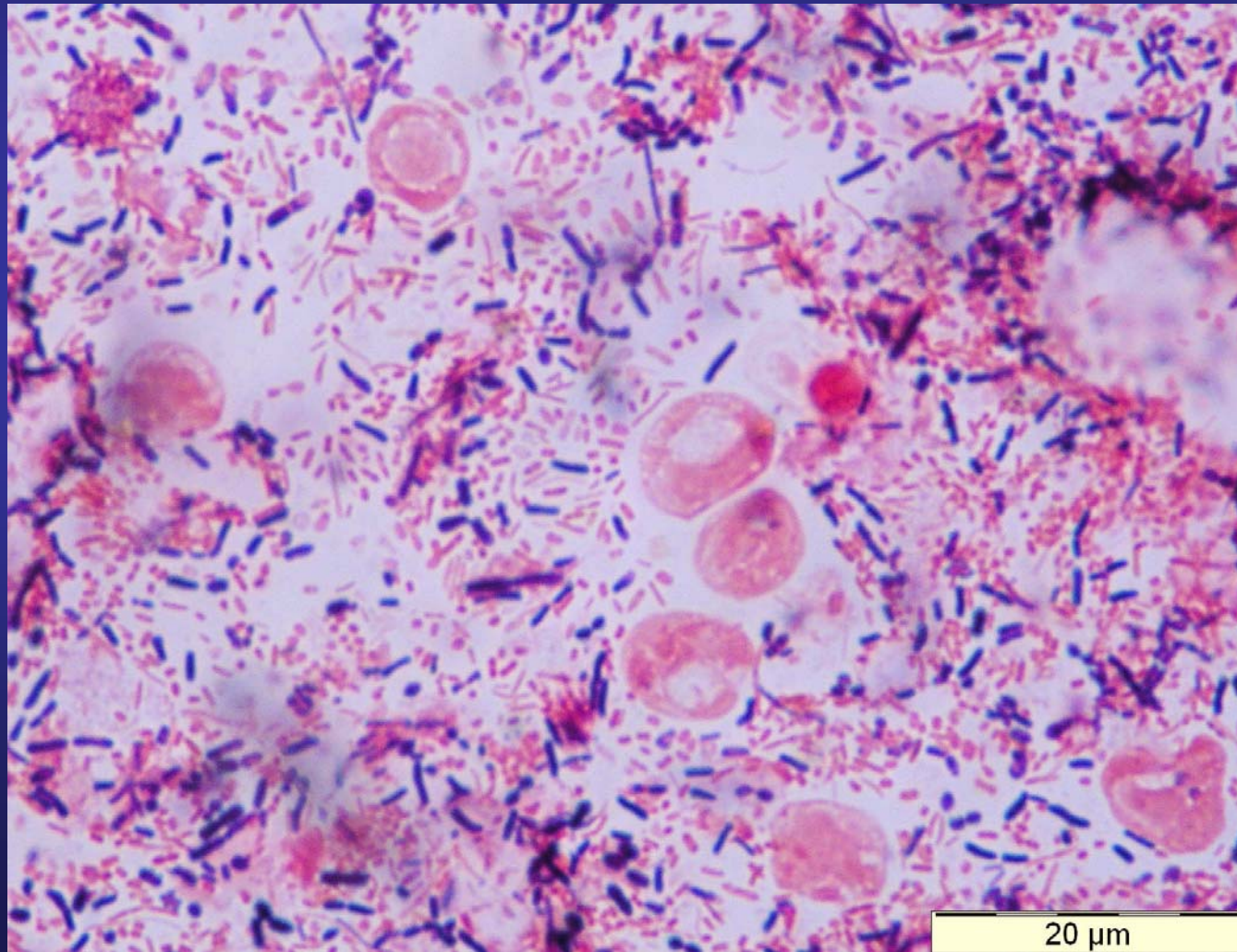
# *Blastocystis hominis*

Several cysts in a smear from faeces (Gram stain).



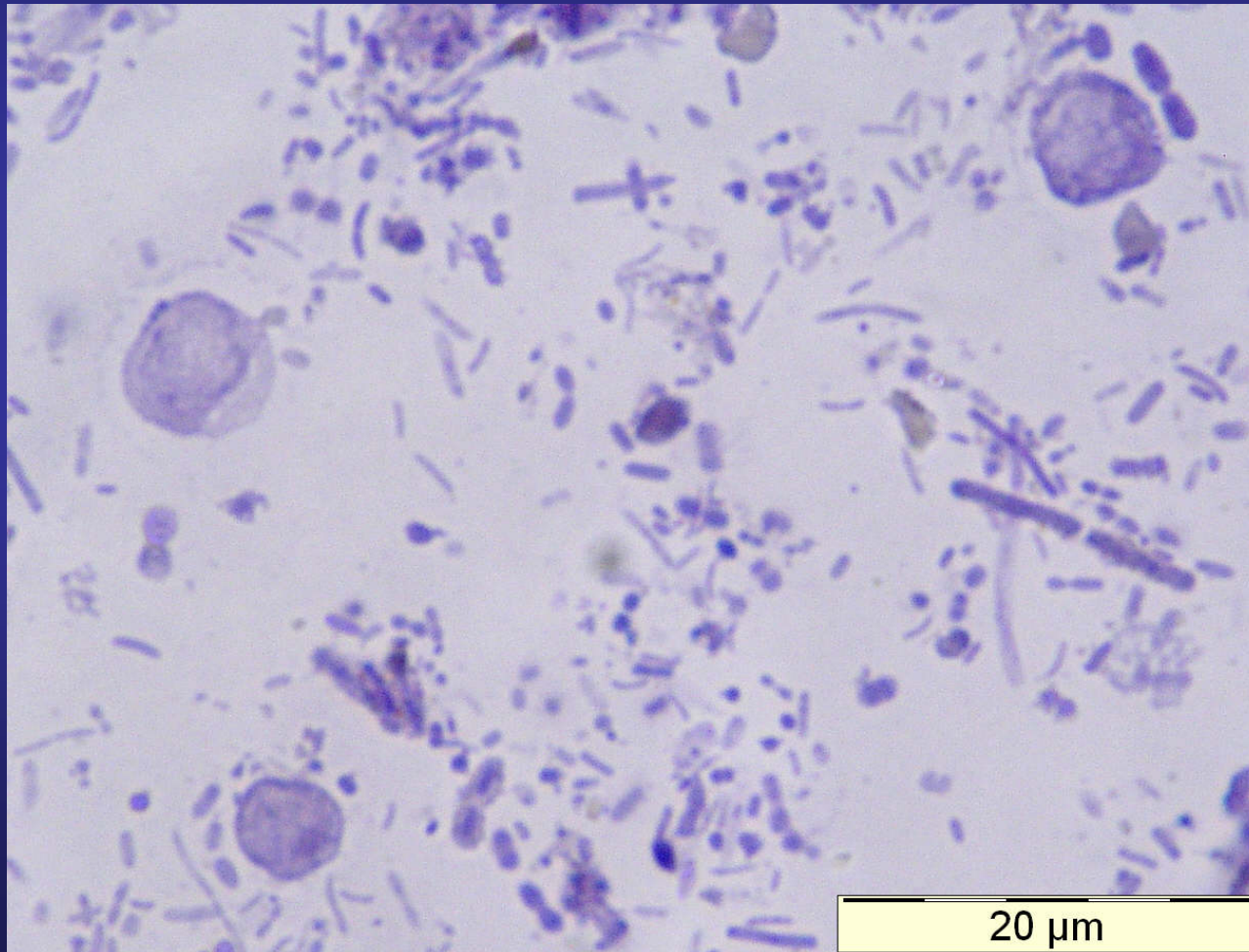
# *Blastocystis hominis*

Several cysts in a smear from faeces (Gram stain).



# *Blastocystis hominis*

Cysts in faeces (Hematoxylin stain).



# *Blastocystis hominis*

Cyst in faeces (Lugol stain).



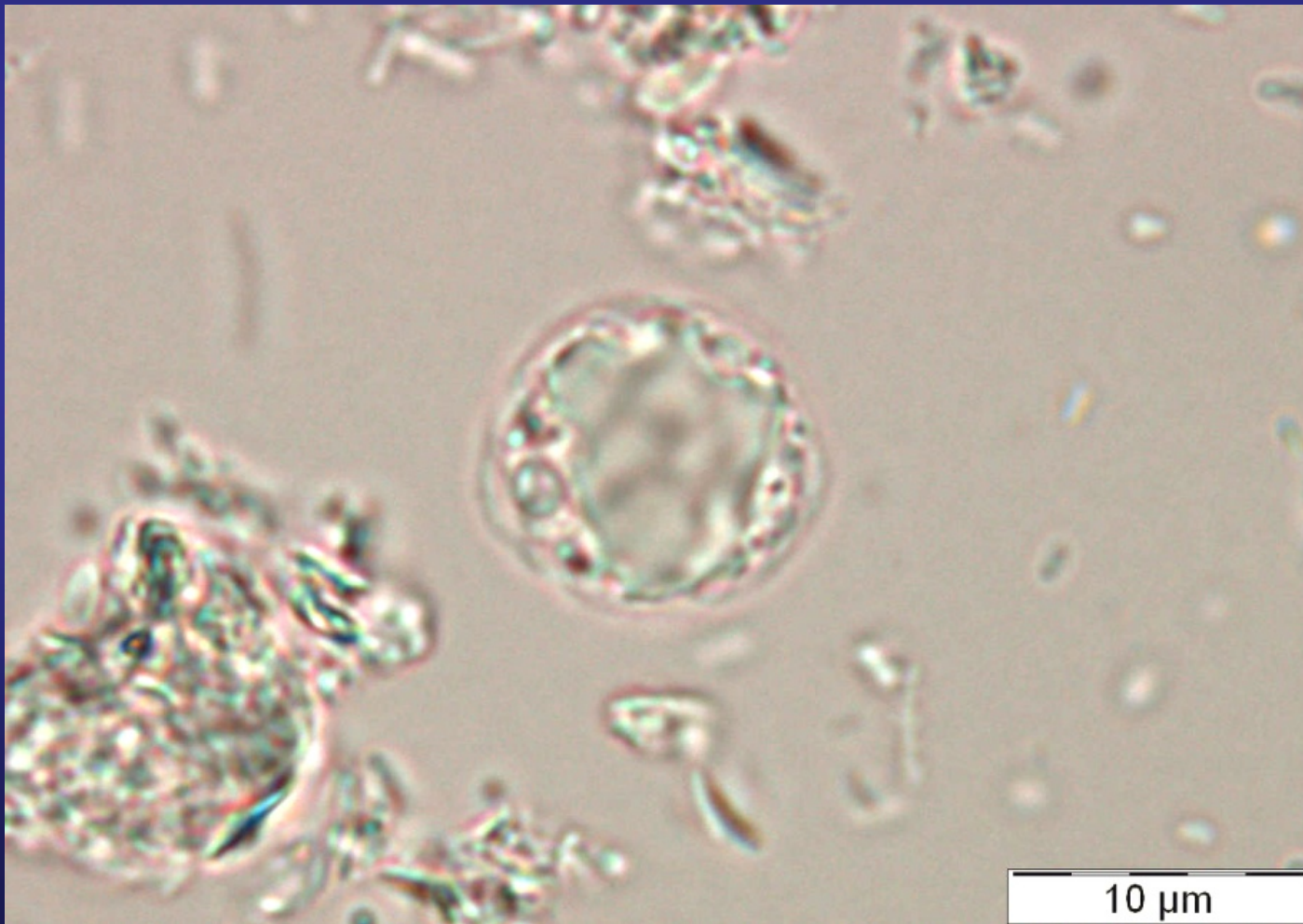
# *Blastocystis hominis*

Two cysts in faeces. At right the cyst reproduces by binary fission (Lugol stain).



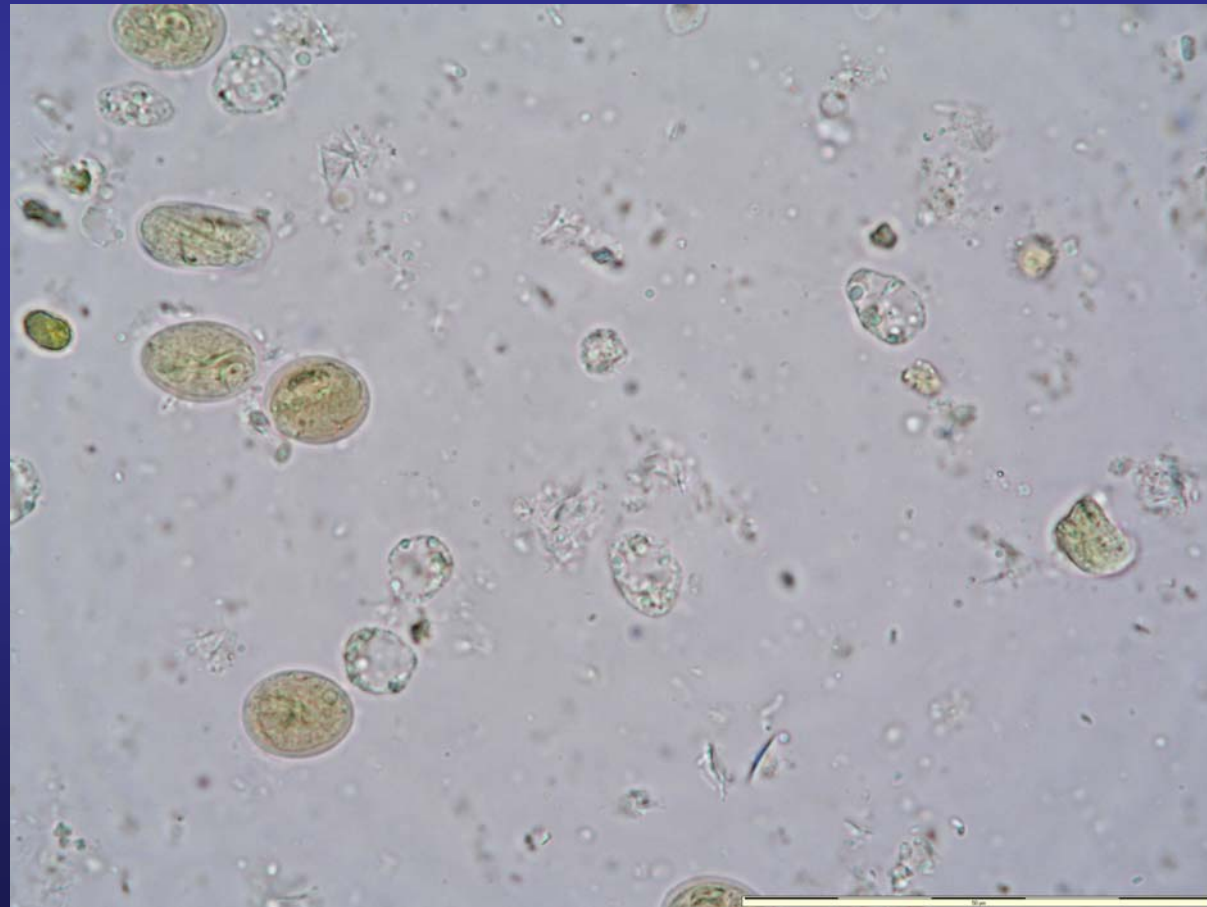
# *Blastocystis hominis*

Cyst in faeces collected with SAF (Unstained).



# *Blastocystis hominis*

Several cysts of *Blastocystis hominis* and of *Giardia lamblia* in faeces (Lugol stain).





# *Cyclospora cayetanensis*

- Blue-green algae, cyanobacterium-like bodies (CLB).
- Spherical oocyst (8-10  $\mu\text{m}$ ) containing small granules with a greenish shade, showing fluorescence under UV illumination.
- Do not stain with Lugol.
- Are acid fast with the Ziehl-Neelsen stain.

Lontie M. *et al.* Acta Clinica Belgica, 1995, 288.

# *Cyclospora cayetanensis*

Oocyst in faeces. Spherical oocyst (8-10  $\mu\text{m}$ ) containing small granules with a greenish shade, giving the aspect of a morula (Unstained).



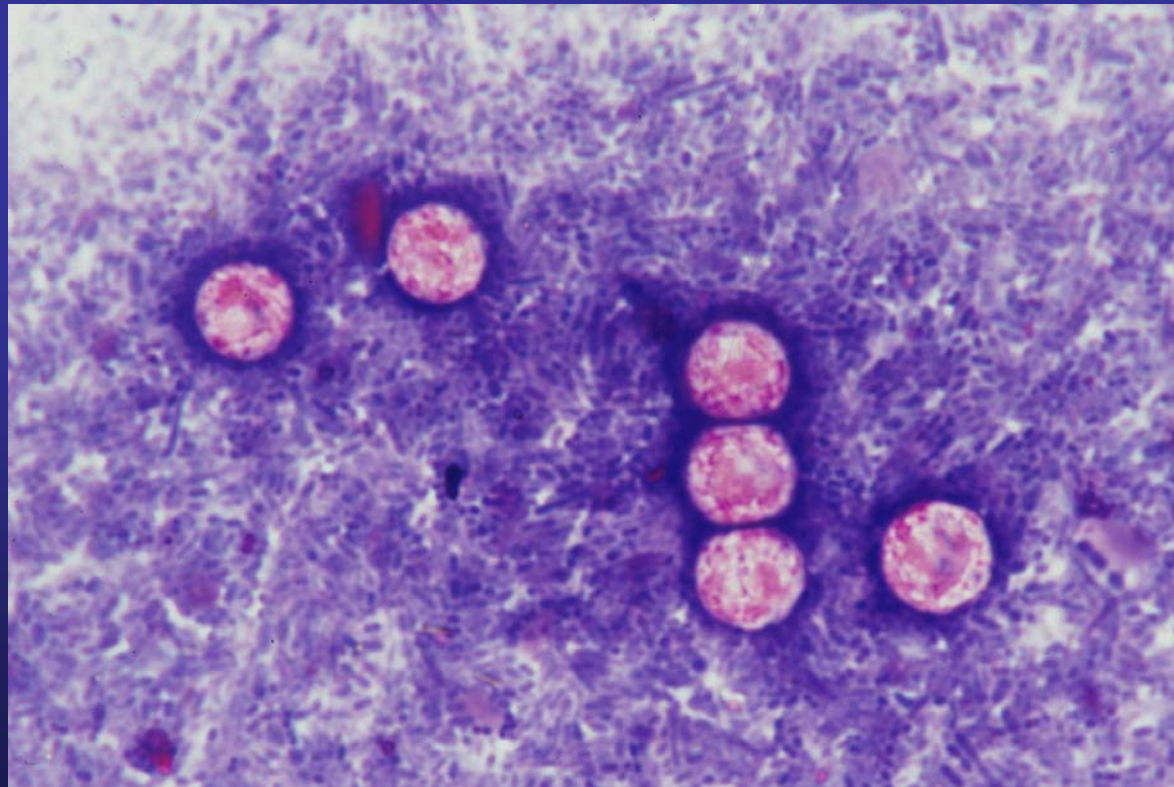
# *Cyclospora cayetanensis*

Two spherical oocysts in faeces (8-10  $\mu\text{m}$ ) containing small granules with a greenish shade, giving the aspect of a morula (Unstained).



# *Cyclospora cayetanensis*

Spherical oocysts (8-10  $\mu\text{m}$ ) containing small granules  
in faeces (Ziehl-Neelsen stain).



# Microsporidia

Immunosuppression (AIDS)

Intestine: *Enterocytozoon* spp., *Encephalitozoon*  
(*Septata*) spp.

Tissues: *Nosema* spp., *Encephalitozoon* spp.,  
*Pleistophora* spp., ...

Albendazole (GSK)

# Microsporidia: laboratory diagnosis

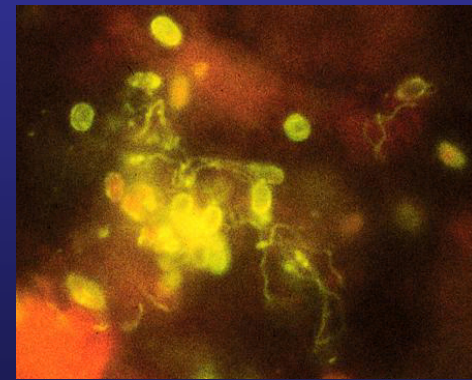
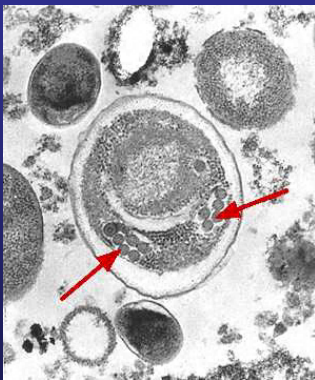
Very small spores (1 - 2  $\mu\text{m}$ )

Modified trichrome stain, Uvitex 2B, ...

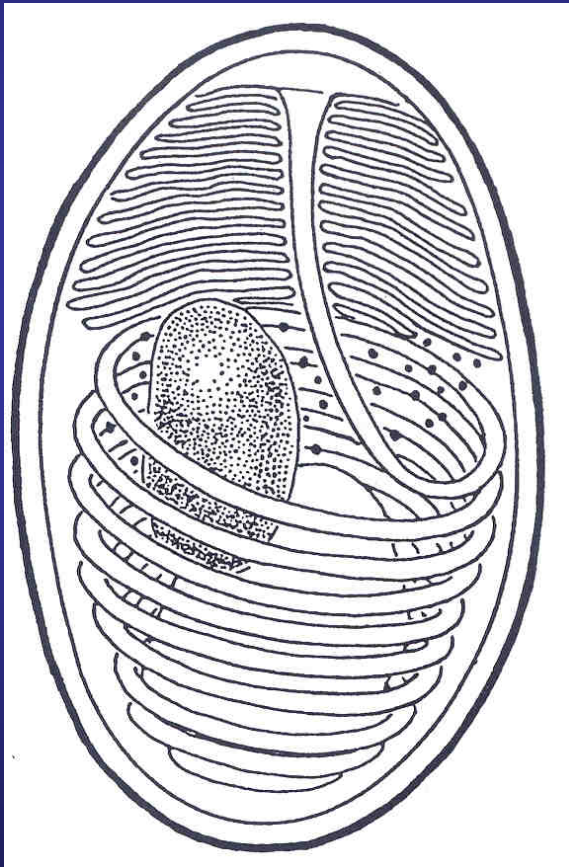
Transmission electron microscopy

Immunofluorescence assays

Molecular methods



# Microsporidia

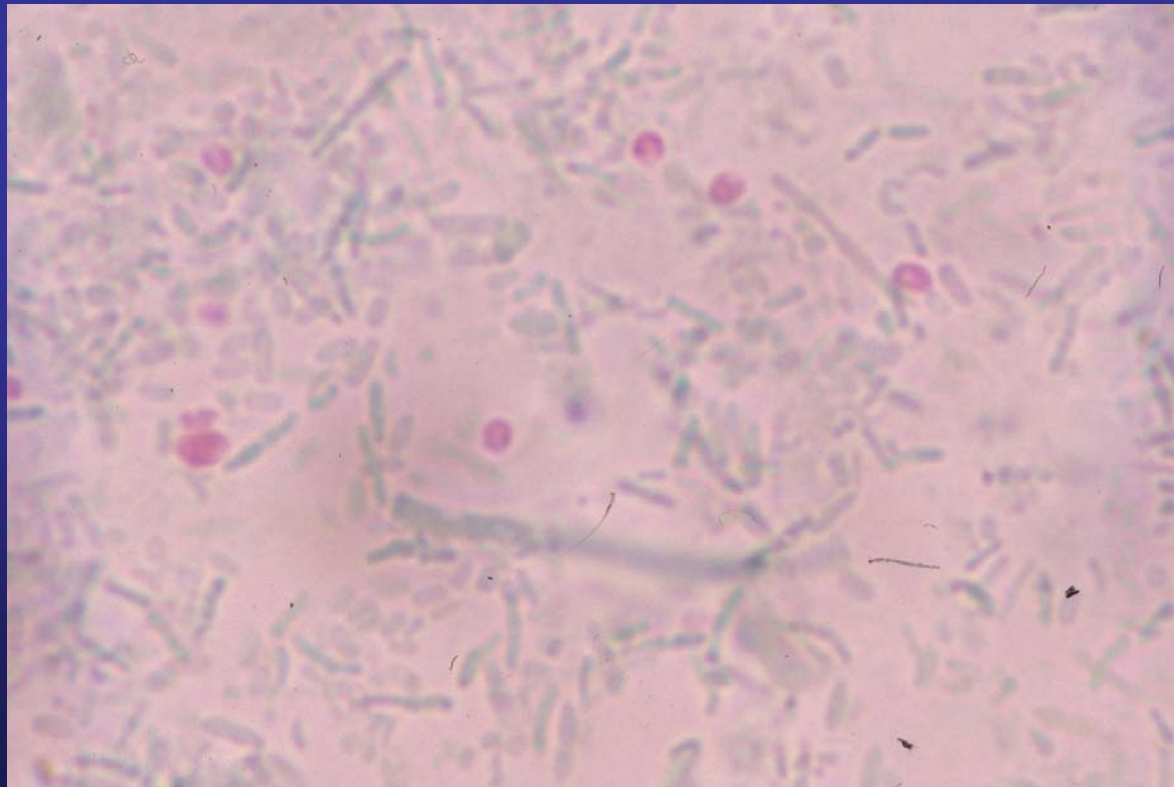


Spore containing a polar tubule, an extrusion mechanism for injecting the infecting spore content into host cells.

Courtesy Garcia L. 1999.

# Microsporidia

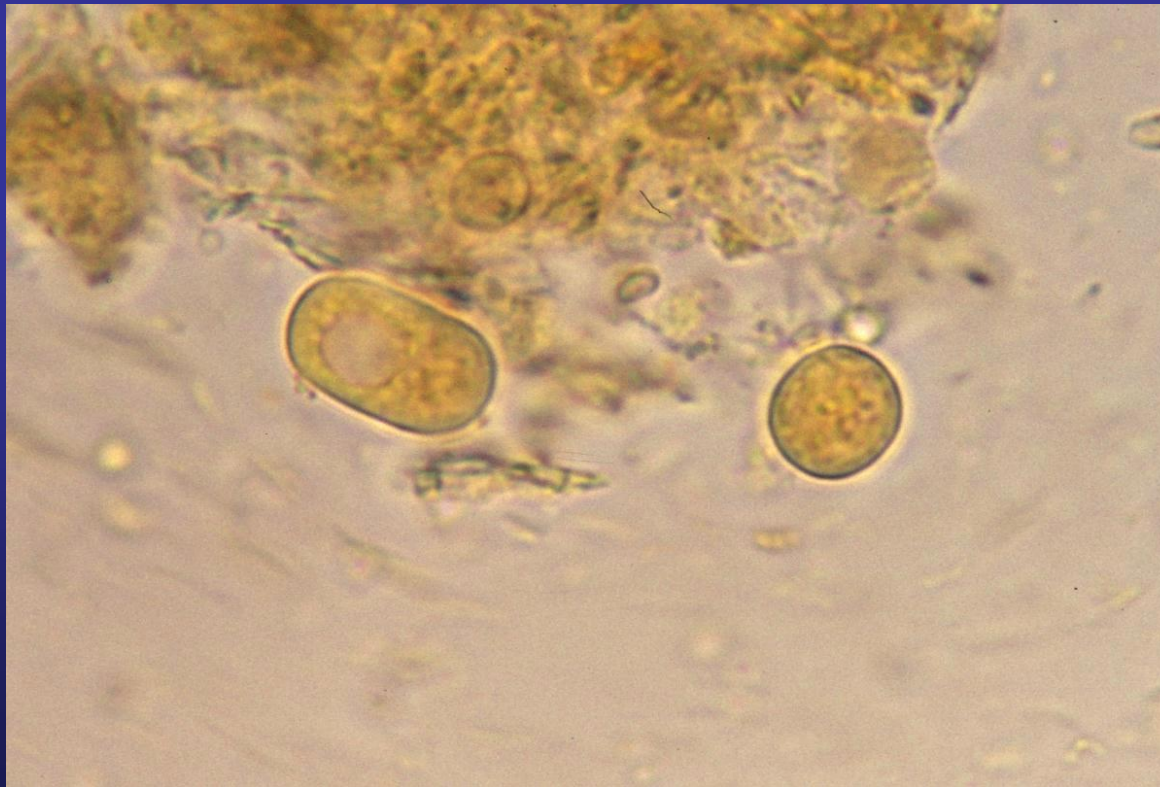
Small round to oval elements in faeces. They are only slightly larger than the light green bacteria (Trichrome stain).





# *Geotrichum candidum*

The round (blasto-)spores and the rectangular arthrospores of this fungus are sometimes mistaken in stool for protozoal cysts. *G. candidum* stains dark violet with the Gram stain and yellow-brown with Lugol (Lugol stain).



# *Geotrichum candidum*

*G. candidum* in culture. The round (blasto-)spores and the rectangular arthrospores of this fungus are sometimes mistaken in stool for protozoal cysts (Unstained).

