LABORATORY DIAGNOSIS OF PARASITIC INFECTIONS

STANDARD TECHNIQUES AND PITFALLS

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Stool

Stool processing

- Direct Fecal Smear (DFS)
- Kato-Katz Smear
- Kato Thick Smear

Concentration Technique

- Formalin Ether Concentration Technique (FECT)
- Merthiolate Iodine Formaldehyde Conc. Technique (MIFC)
- Stoll Concentration Technique

Stool Cultivation (Harada-Mori Method)

Kinyoun Stain

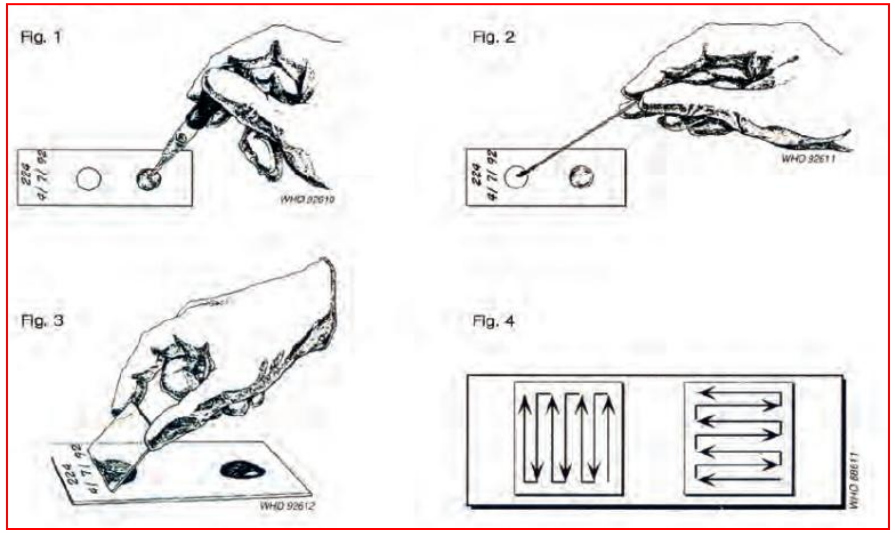
Most Commonly Used Laboratory Procedures

A. Direct Fecal Smears (Saline and iodine wet mount preparations)
B. Kato-Thick Technique
C. Kato-Katz Technique (Cellophane fecal thick smear)
D. Concentration Techniques (Formalin-ether/ethyl acetate sedimentation technique and Flotation technique)

Direct Fecal Smear (DFS)

Saline and Wet Mount preparations

- Easy to prepare
- Useful in the detection of trophic forms of amebae and flagellates
- Allows observation of the motility of parasites
- Helminth eggs and cysts of protozoa may be seen



(Source: WHO 1994)

1. Direct Fecal Smear (DFS)

- Requires taking small amounts of material from several parts of the stool specimen
- Detection of parasites may be affected by the thickness of the smear
- Not an efficient method for detecting cysts
- Low sensitivity in light infections

2. Kato-Katz (Cellophane covered thick smear)


Fig. 2

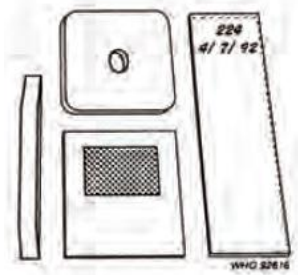



Fig. 3

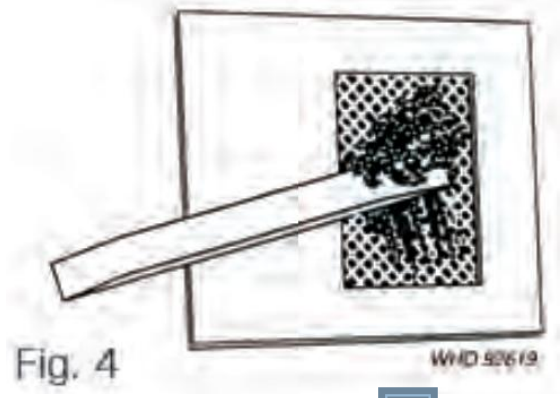
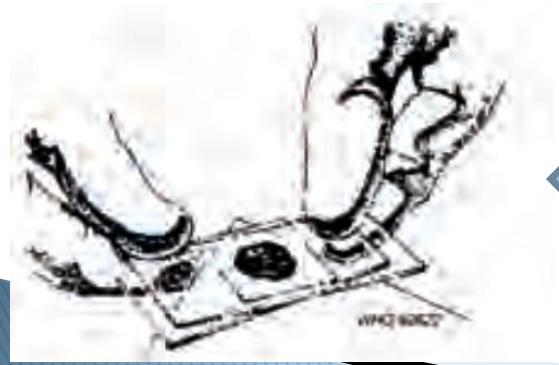


Fig. 4



2. Kato-Katz

- Qualitative and quantitative detection of worm eggs in stools (41.7 mg of stool)
- Useful in assessing the intensity of infection in schistosomiasis and common STH infections

  - Recommended by WHO for the quantification of STH eggs in the human stool (WHO, 2002)
  - Recommended for monitoring large-scale treatment programs implemented for the control of STH (Levecke *et al.*, 2011)
  - Can help in the visualization of *Schistosoma* miracidium.

Kato-Katz

- Consistency of the stool is the main determinant for sensitivity, i.e., drier stools yield higher egg counts than moist stools
- Can only be done on fresh formed stools and not on liquid and preserved samples
  - Sensitivity using a single smear is as low as 40% (Yu *et al.,* 1998)
  - Sensitivity increases with increasing number of specimens (Yu *et al.,* 2007)
  - Low sensitivity in light infections and in low prevalence areas (Leder, 2009)

Table 1. Mean value of the sensitivity and specificity of Kato-Katz stool examination technique for one, two or three stool samples collected from study participants living in 50 rice farming villages of the Province of Samar, the Philippines.

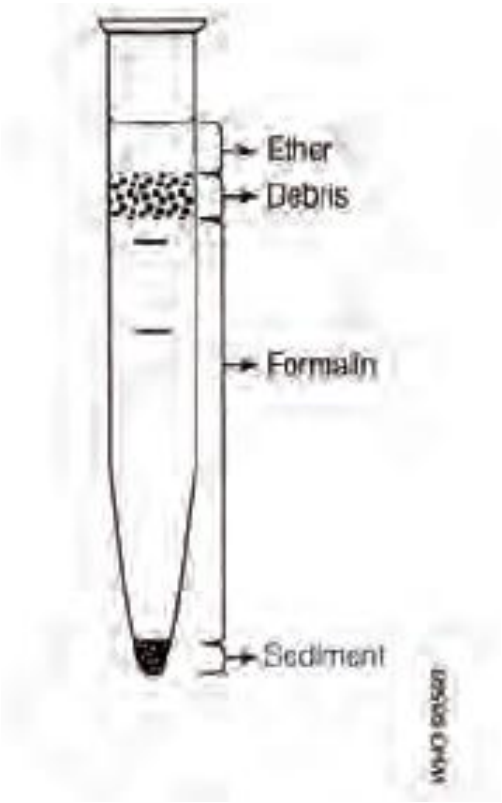
Test parameters	Number of stool samples	Helminth species		
		Ascaris	Hookworm	*Trichuris*
Sensitivity	1 stool sample	96.9 (96.1, 97.6)	65.2 (60.0, 69.8)	91.4 (90.5, 92.3)
	2 stool samples	99.9 (99.8, 99.9)	87.9 (84.0, 90.7)	99.3 (99.1, 99.4)
	3 stool samples	100.0 (100.0, 100.0)	95.8 (93.6, 97.2)	99.9 (99.9, 100.0)
Specificity	1 stool sample	96.1 (95.5, 96.7)	93.8 (92.4, 95.4)	94.4 (93.2, 95.5)
	2 stool samples	92.3 (91.2, 93.4)	87.9 (85.4, 91.0)	89.0 (86.8, 91.1)
	3 stool samples	88.7 (87.0, 90.3)	82.4 (79.0, 86.8)	84.0 (80.8, 87.0)

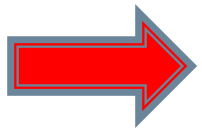
(MR Tarafder, *et al.,* 2010)

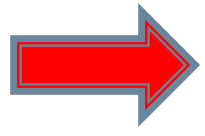
3. Kato-Thick Smear Technique

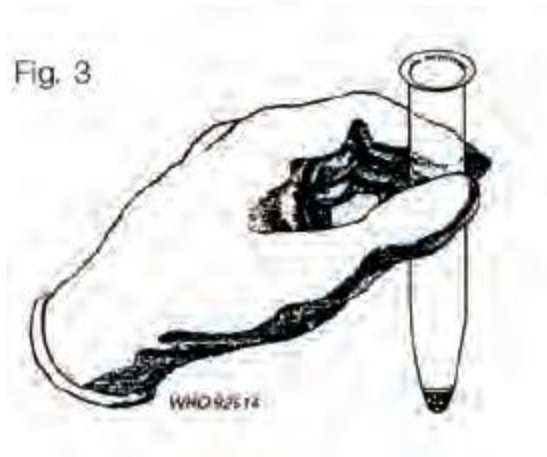
- Qualitative detection of worm eggs in stool
- Employs the examination of a standard 50-mg fresh stool

4. Formalin-Ether Concentration Technique









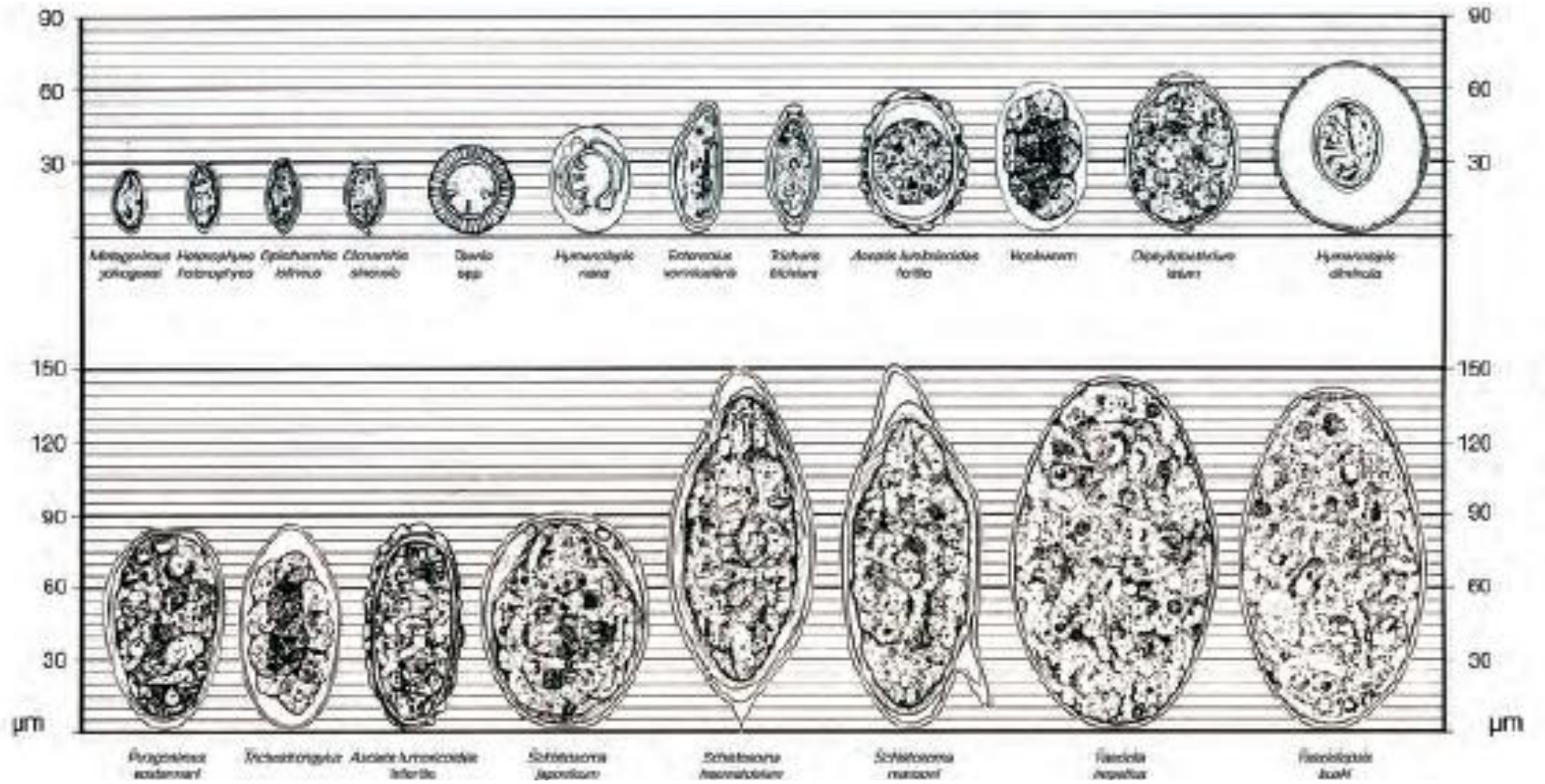
Formalin-Ether Concentration Technique

- Useful in the recovery of both helminth eggs and protozoan cysts; present in small numbers
- May be applied to formalin-preserved specimens
- Ethyl acetate may be used as a substitute to ether due to problems in storage and handling.

  -It is more efficient than ether in the recovery of cestode eggs and *Giardia* cysts.
- Highly specific in detecting infection (97.4%) (Lier *et al.*, 2009)

Formalin-Ether Concentration Technique

- Requires 1 g of stool
- Sensitivity (low at 28.6%; Lier *et al.*, 2009)
- Special requirements
  - Trained staff
  - Centrifuge
  - Ether; Highly flammable
- Requires more time and effort to perform



In parasite diagnosis: Size, shape, organelles and defining feature, and variable reactivity with common stains

Objects Sometimes Mistaken for Helminth Eggs and Protozoan Cysts

- Plant hair
- Plant cells
- Seed fiber
- Starch granules
- Protein particles
- Vegetable cells
- Plant spring cells
- Fat droplets
- Oil droplets
- Pollen grains
- Arthropod eggs
- Fungus or yeast spores
- Air bubbles
- Scratches on slide

Pseudoparasites and Pitfalls

- Object that resembles a parasite or the egg of the parasite but neither a parasite nor parasitic in the host under consideration
- Syn. Pseudosymbiont

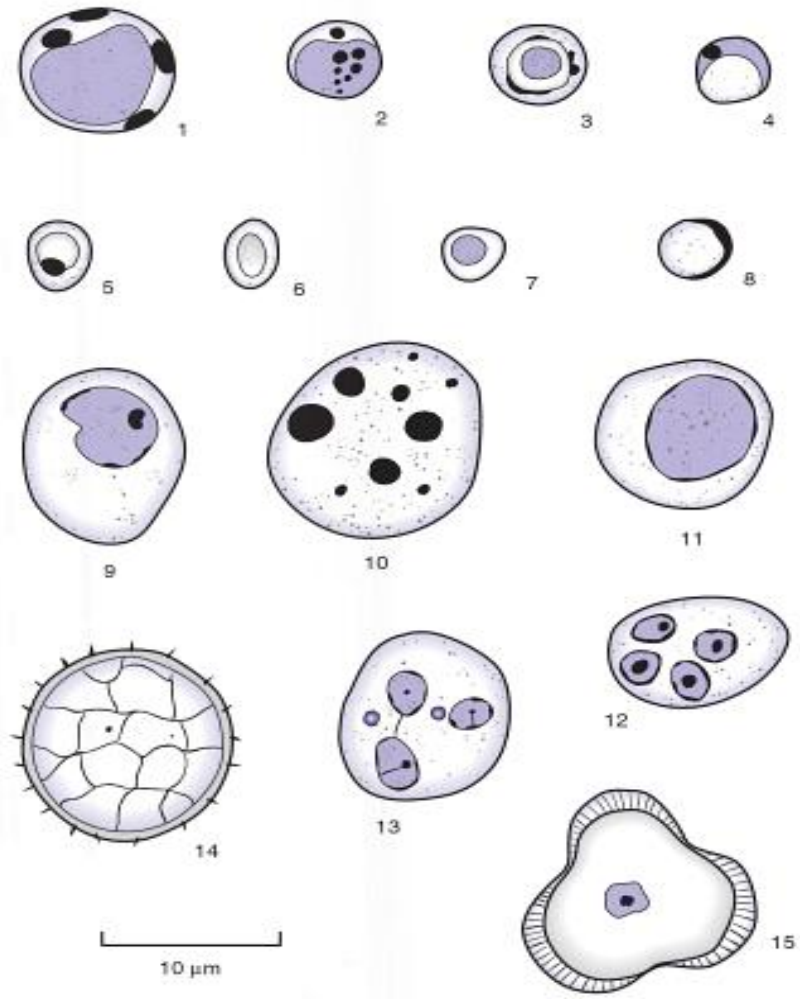
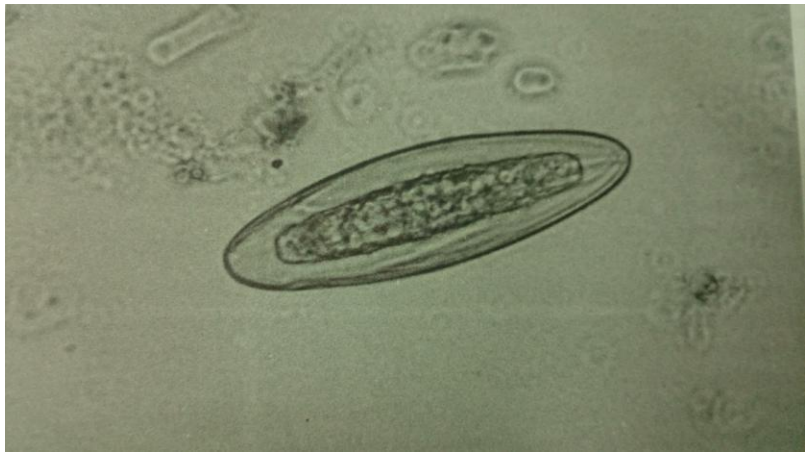


FIGURE 13-1 ■ Various structures that may be seen in stool preparations: *1–4, Blastocystis hominis; 5–8,* various yeasts; *9, 11,* squamous cells from rectal mucosa; *10,* deteriorated macrophage without nucleus; *12, 13,* polymorphonuclear leukocytes; *14, 15,* "pollen grains."

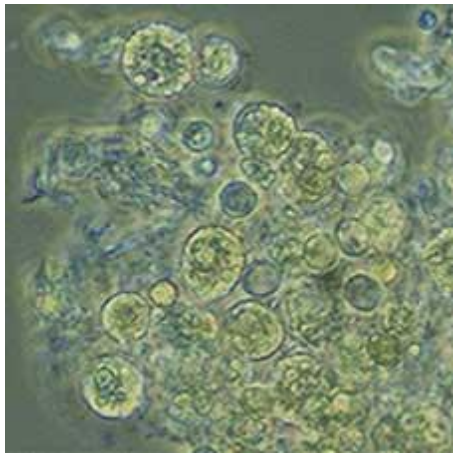
Pseudoparasite



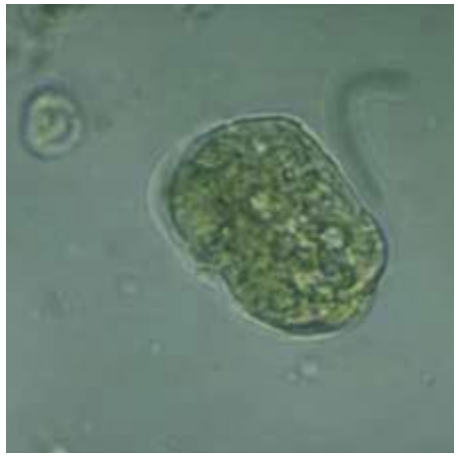
- *Psorospermium haeckelii*, or “Beaver body,” a protistan parasite of crayfish which may be found in human stools following the consumption of this delicacy. It is of no clinical significance
- (Original Magnification, x 400.)

Algae

Tissue Cells



White Blood Cells
or Pus Cells



Macrophage

Epithelial cell

Plant Fiber and Hairs

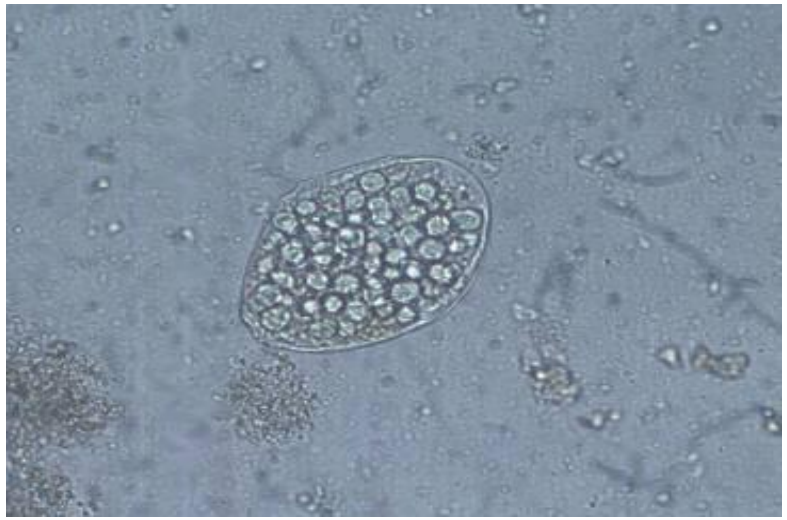
Plant fiber

Hair from Peach

Plant hair

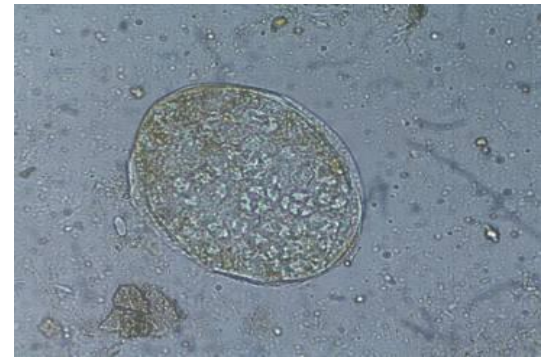
Strongyloides stercoralis

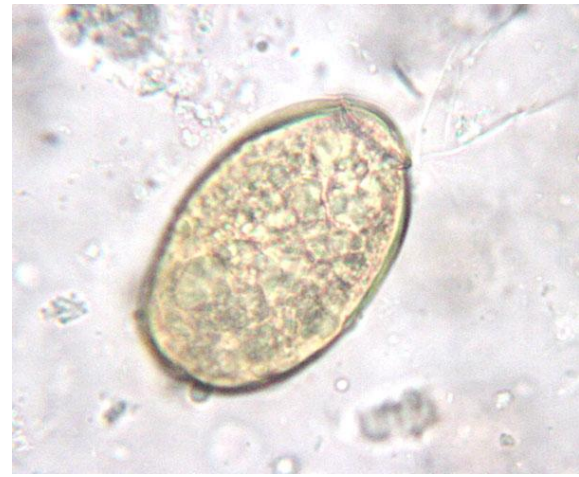
Vegetable Cells



300 x 268

Dipylidium caninum egg packets



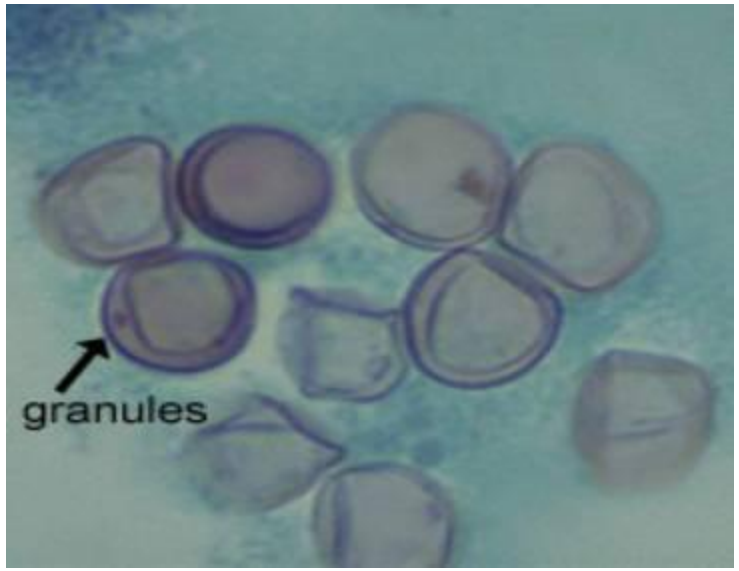


600 x 496

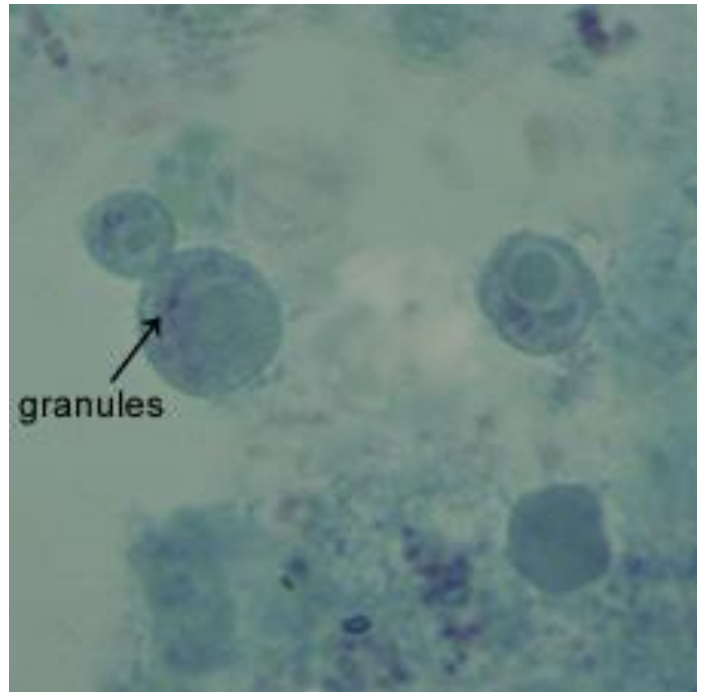
Paragonimus eggs

(Sources: http://www.phsource.us/ and www.nvcc.edu)

Red Blood Cells

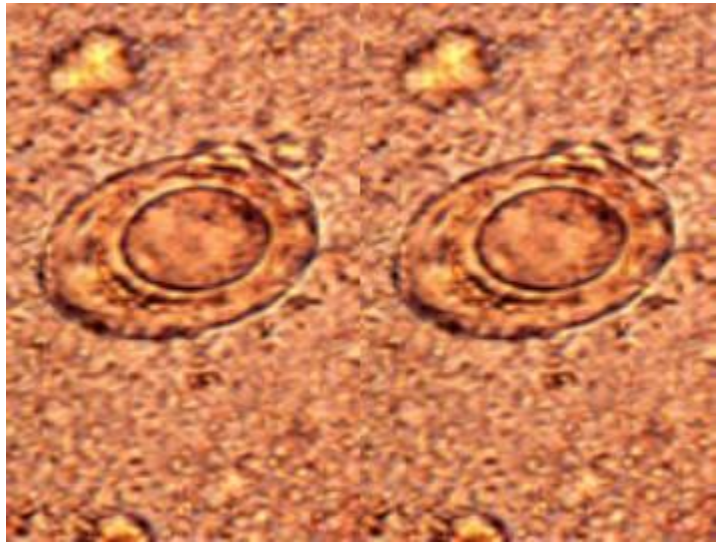


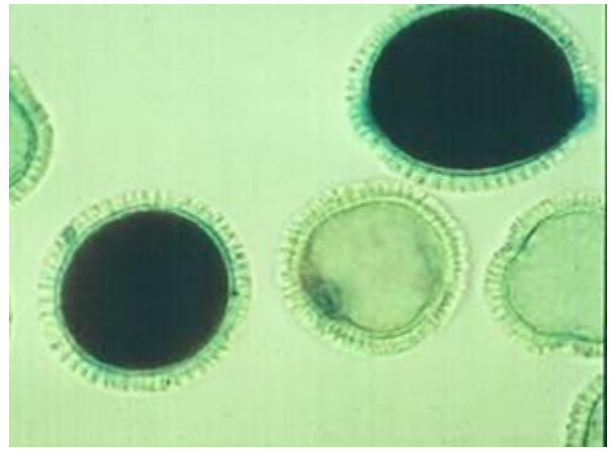
RBC's may appear to have a central body and a rim of cytoplasm or granules
(Source: University of Alberta)



225 x 225
Blastocystis hominis

Pollen



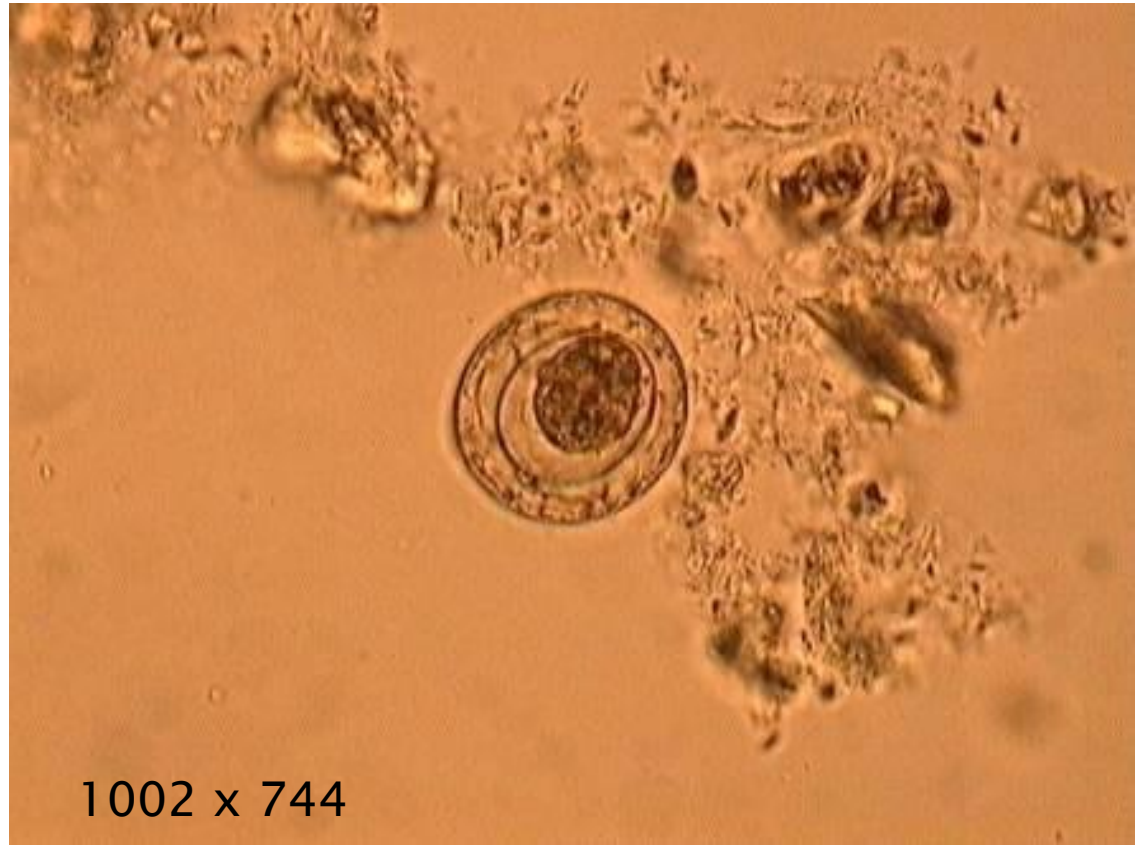


Geranium pollen cells
(Source: http://www.phsource.us/)

Taenia sp.

Pollen

Image illustrating pollen resembling a *Hymenolepis nana* egg. (Source: CDC)



Hymenolepis nana egg

Bee Pollen

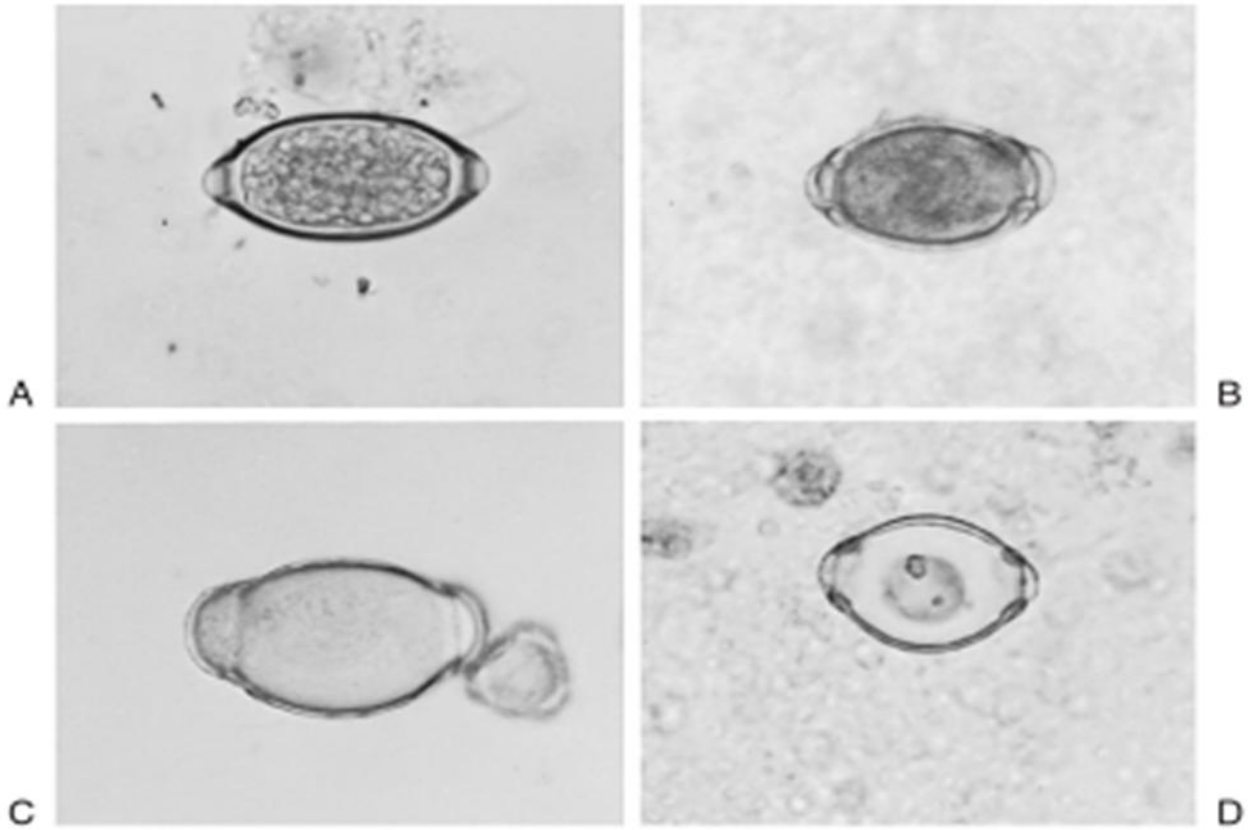
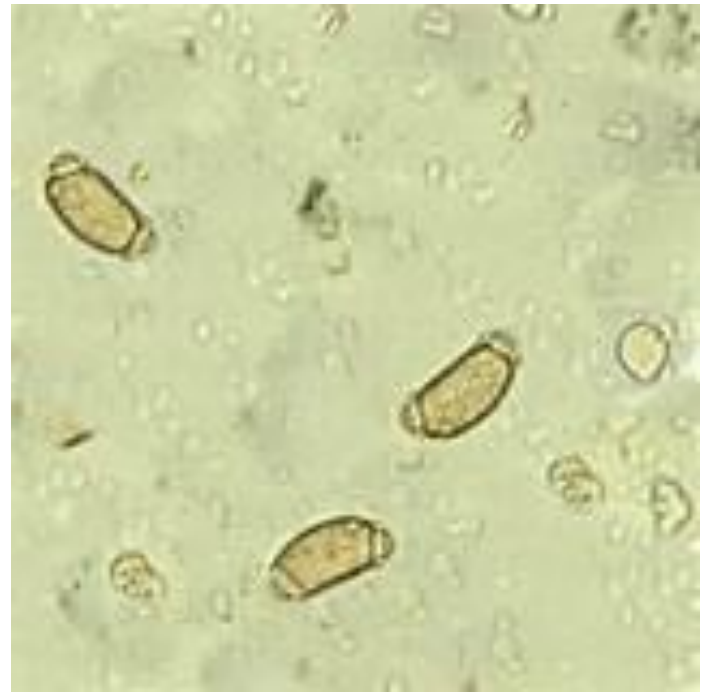


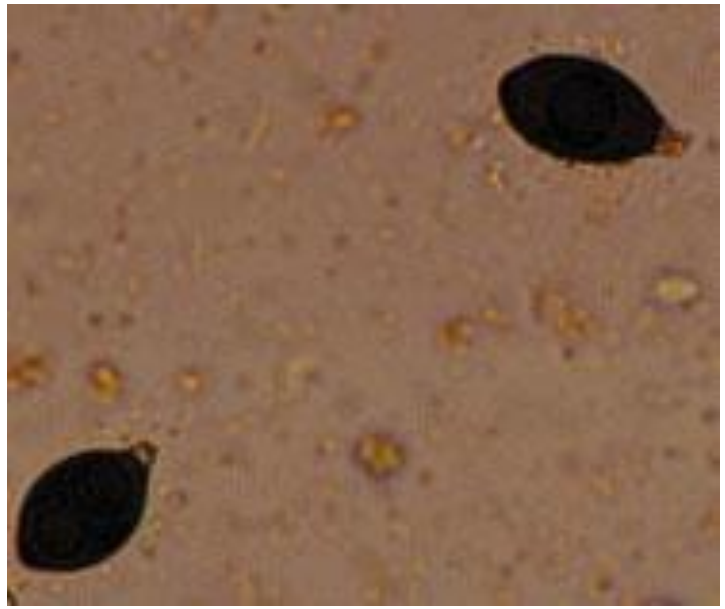
FIGURE 13-2 ■ A, *Trichuris* egg. B–D, Egglike objects from stools of persons taking "Australian bee pollen" dietary supplement.

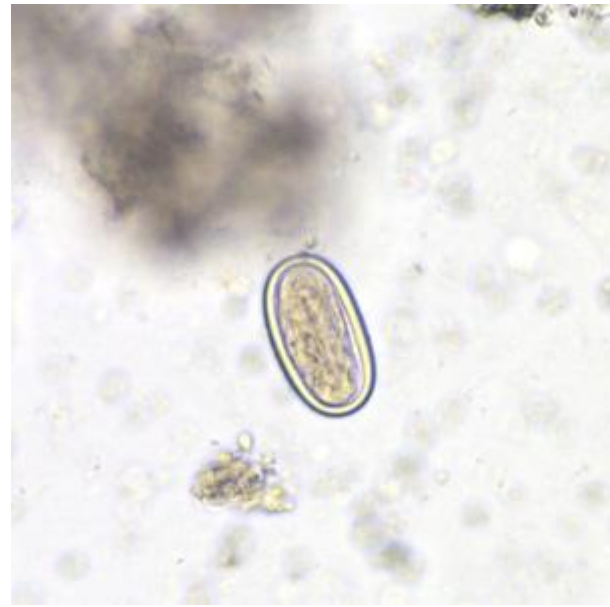
Source: Markell *et al*. 1992

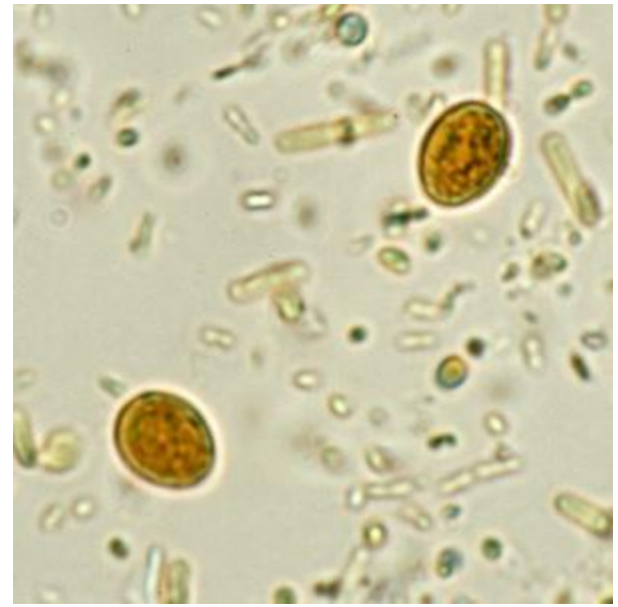


Source: http://www.cdc.com

Fungal Spores and Yeast Cells

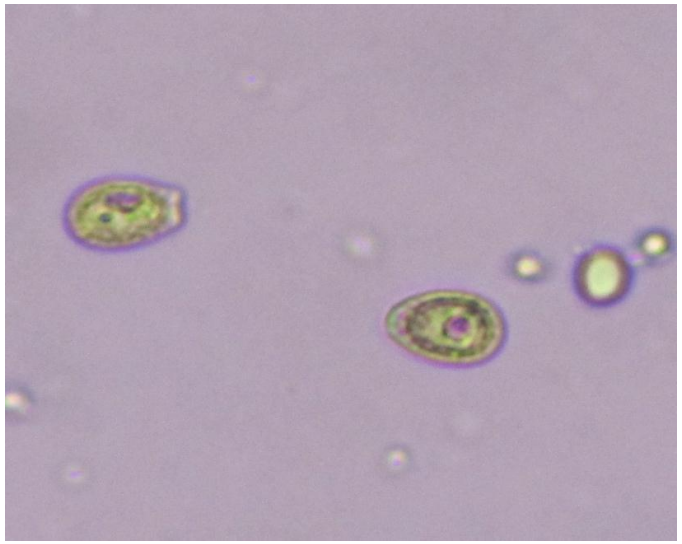


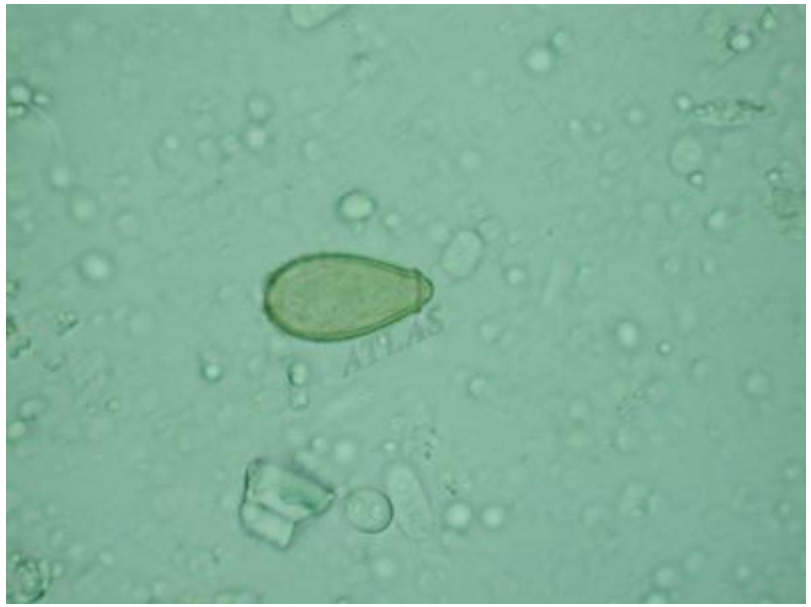




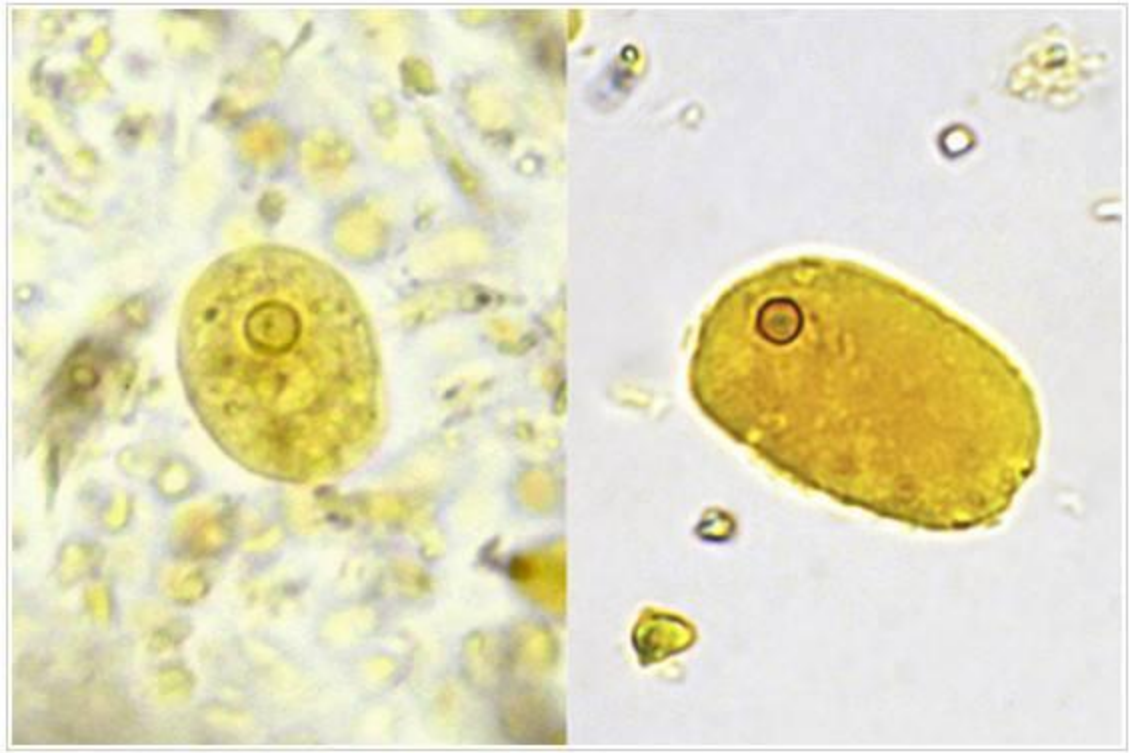
(Source: University of Pennsylvania School of Veterinary Medicine)

Spores



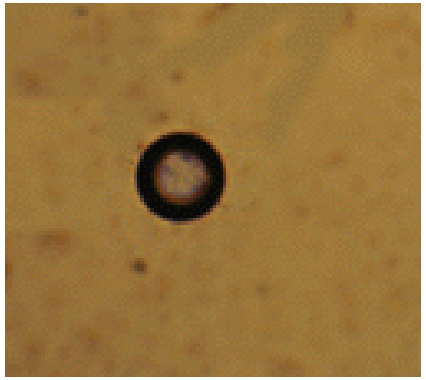


Heterophyid egg

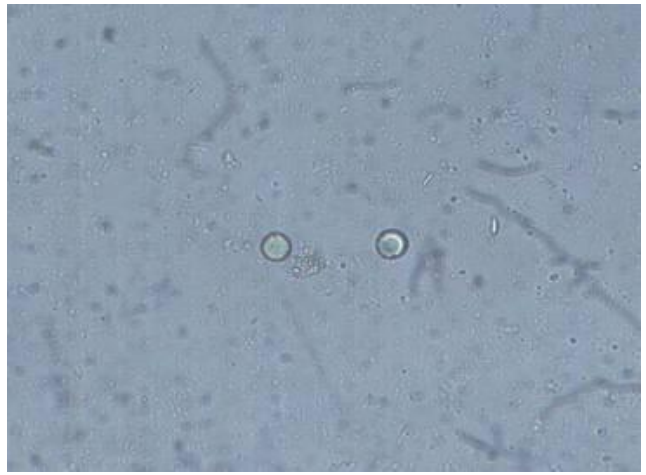


Iodine stain. Left, an *Entamoeba coli* trophozoite.
Right, this artefact is morphologically like a trophozoite, but the inside "nucleus" is actually superimposed fecal debris.

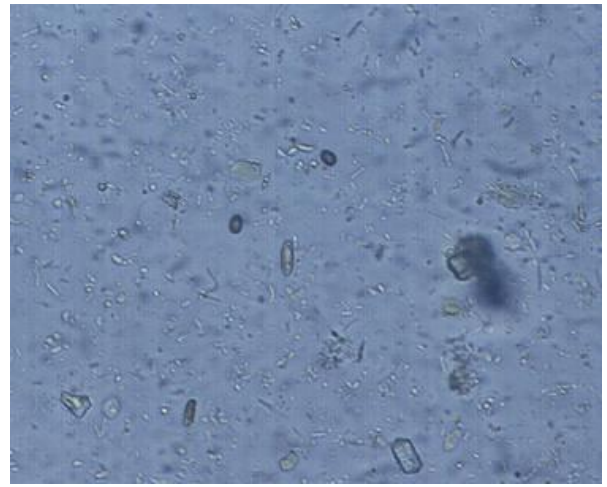
(Source: http://www.medical-labs.net/artifacts-in-stool-1710/)



Air Bubble



Fat globules



Arthropod eggs

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