Laboratory diagnosis of parasitic diseases

Parasites and parasitism

- **PARASITE** - live orgamism living in or on, and having some metabolic dependence on another organism known as a **host**

- **PARASITISM** - a relationship in which one of the participants, the parasite, either harms its host or in some sense lives at the expense of the host

- **Protozoa:** unicellular organisms, e.g. Plasmodium (malaria)
- **Metazoa:** multicellular organisms, e.g. helminths (worms) and arthropods (ticks, lice)
- **An endoparasite:** "a parasite that lives within another living organism" e.g. malaria, Giardia
- **An ectoparasite:** "a parasite that lives on the external surface of another living organism" e.g. lice, ticks

Why study Parasitology?

- Many of these parasites are causative agents of major public health problems of the world.

- Recent estimates of prevalence of parasites in the world are:

Ascaris	1.5 billion
Hookworms	1.3 billion
Whipworms	1 billion
Filarial worms	657 million
Malaria	500 million
Schistosomes	210 million
Amebiasis	50 million
Taenia tapeworms	50 million
Clonorchis	20 million
Chagas' Disease	15 million

- These parasites cause varying morbidities and even mortalities

The burden of some major parasitic infections

Parasite	Diseases	No. people infected
Plasmodium	malaria	273 million
Soil transmitted helminths: • Roundworm (*Ascaris*) • Whipworm (*Trichuris*) • Hookworm (*Ancylostoma* and *Necator*)	 Pnemonitis, intestinal obstruction Bloody diarrhoea, rectal prolapse Coughing, wheezing, abdominal pain and anaemia	2 billion
Schistosoma	Renal tract and intestinal disease	200 million
Filariae	Lymphatic filariasis and elephantiasis	120 million
Trypanasoma cruzi	Chagas disease (cardiovascular)	13 million
African trypanosomes	African sleeping sickness	0.3 – 0.5 million
Leishamania	Cutaneous, mucocutaneous and visceral leishmaniasis	12 million; 2 million new cases/yr

Current disease portfolio from WHO report, 2001

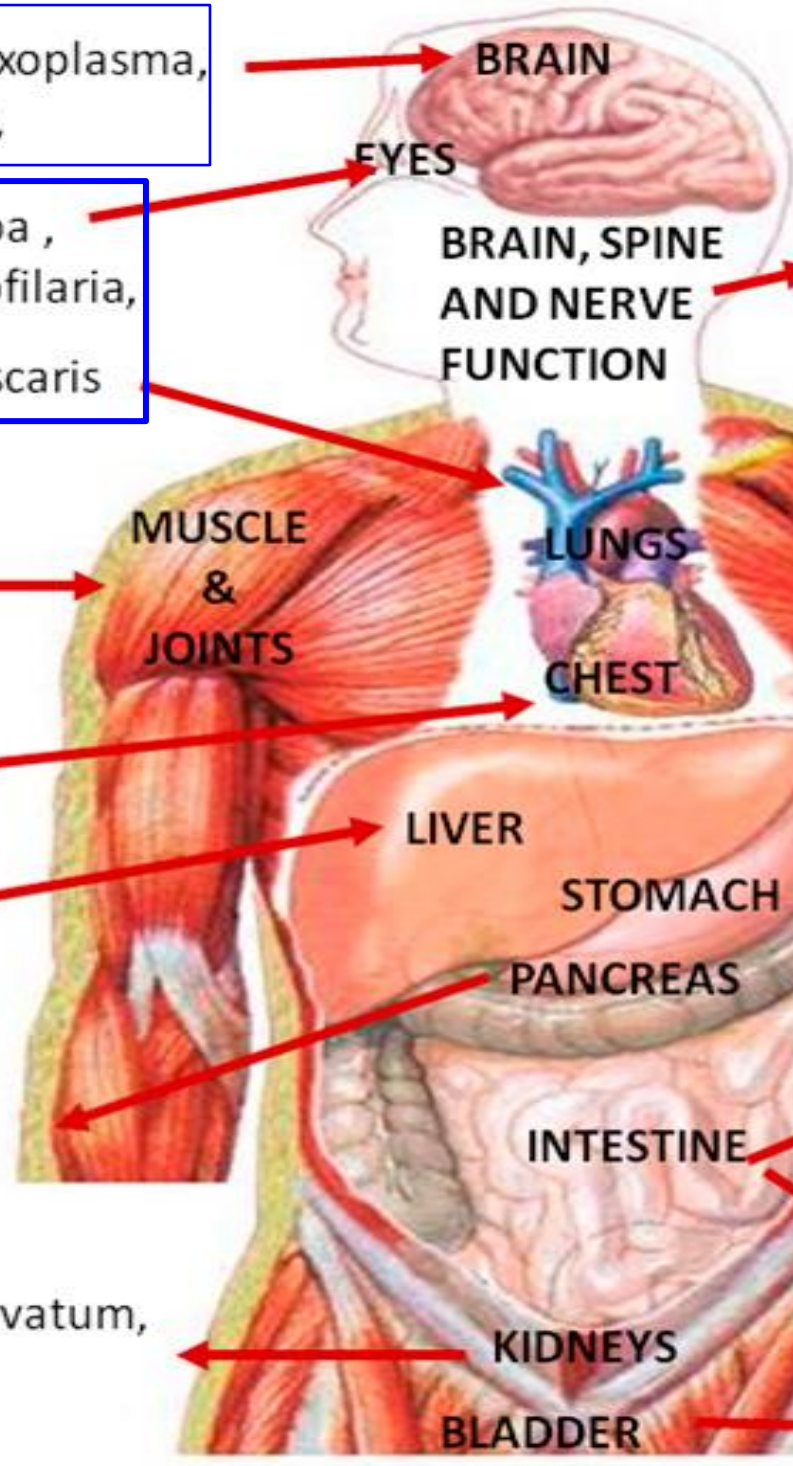
	TDR disease category	Disease burden DALYs* (thousands)			Deaths (thousands)		
		Total	Male	Female	Total	Male	Female
African trypanosomiasis	1	1,585	1,013	572	50	32	18
Dengue	1	433	286	147	12	8	4
Leishmaniasis	1	1,810	1,067	744	41	23	18
Malaria	2	40,213	19,237	20,976	1,080	522	558
Schistosomiasis	2	1,713	1,037	676	11	8	3
Tuberculosis	2	35,792	21,829	13,962	1,660	1,048	613
Chagas disease	3	680	360	320	21	12	9
Leprosy	3	141	76	65	2	2	1
Lymphatic filariasis	3	5,549	4,245	1,304	0	0	0
Onchocerciasis	3	951	549	402	0	0	0

Parasites
→ according to which site they inhabit

- **Intestinal and urogenital parasites**
  (protozoa and/or helminths)

- **Tissue and blood parasites**
  (protozoa and/or helminths)

PARASITES' FAVORITE LOCATION



Strongyloide, Ascaris, Toxoplasma, Echinostoma revolutum,

Trichinella, Loa loa , Toxoplasma, Dirofilaria,

Paragonimus, Ascaris

Trichinella, Ascaris, Strongyloide, Ancylostoma

Dirofilaria – Loa Loa

Clonorchis Fasciola

Eurytrema pancreaticum

Echinoporyphium recurvatum, Gastrothylax

Echinostoma revolutum, Macracanthorhynchus

Ascaris Ancylostoma

Gastrothylax

Fasciolopsis, Giardia, Entamoeba, Enterobius, Ascaris Tapeworm, Ancylostoma Trichinella

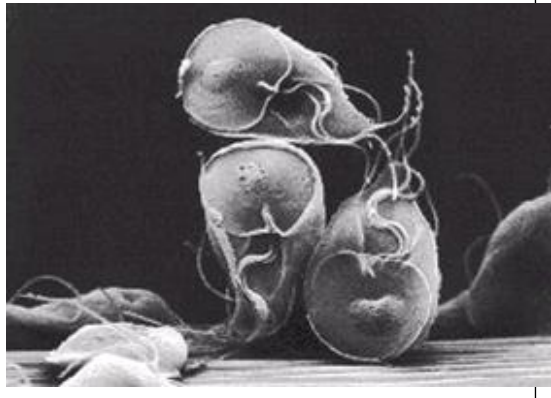
Schistosoma

Examples of important intestinal protozoa

Transmitted by the faecal-oral route and cause diarrhoea

Giardia lamblia: world-wide distribution, lives in the small intestine and results in malabsorption

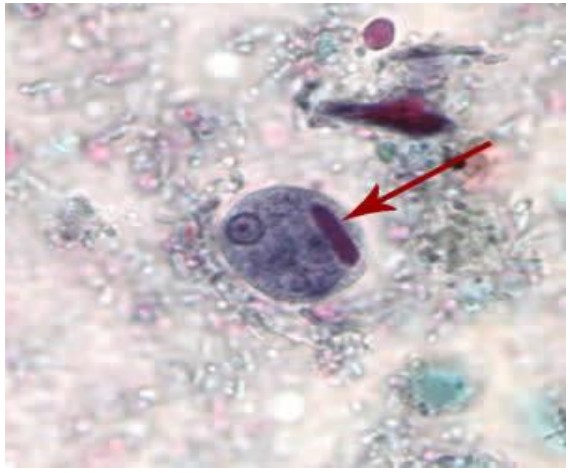
Entamoeba histolytica: may invade the colon and cause bloody diarrhoea – amoebic dysentery. Also causes amoebic liver abscess.



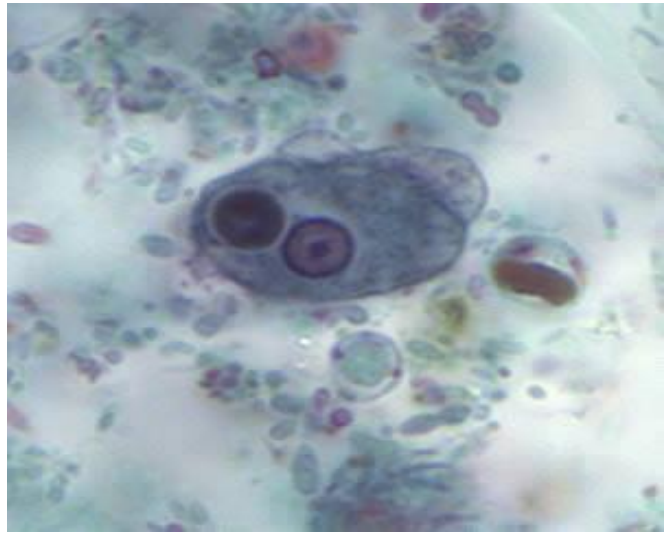
Cryptosporidium parvum: more prevalent in the immunocompromised

Cyclospora cyatenensis : parasitizes the small intestinal mucosa and may cause diarrhoea for several weeks

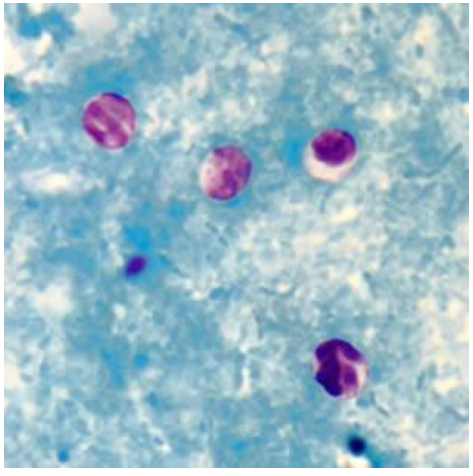
Balantidium coli: a large motile ciliated parasite that lives in the colon of pigs, humans and rodents and can lead to colonic ulceration



Cyst of *E. histolytica* stained with trichrome. Note the chromatoid body with blunt ends (red arrow)

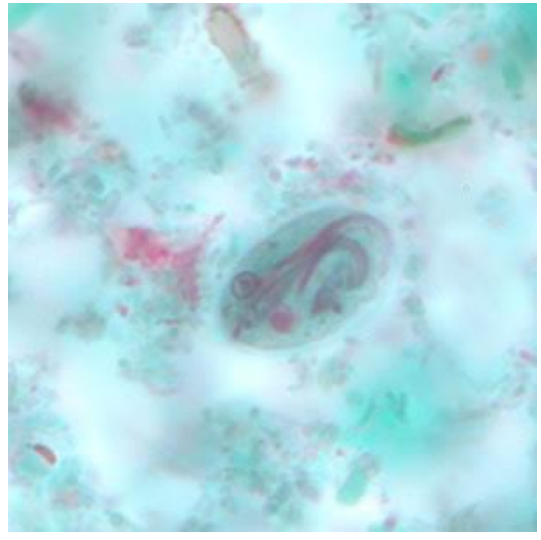


Trophozoites of *E. histolytica* with ingested erythrocytes stained with trichrome.



Cryptosporidium sp. oocysts stained with modified acid-fast.

G. duodenalis trophozoite stained with trichrome



G. duodenalis cyst stained with trichrome.

Examples of important systemic protozoa

Detected in the blood

- *Plasmodium*: the cause of malaria. There are 4 species that infect man: *P. falciparum, P. vivax, P. ovale and P. malariae*

- *Toxoplasma gondi:* transmitted by the ingestion of oocysts from cat faeces. Infection can lead to ocular problems and is also a cause of neonatal toxoplasmosis

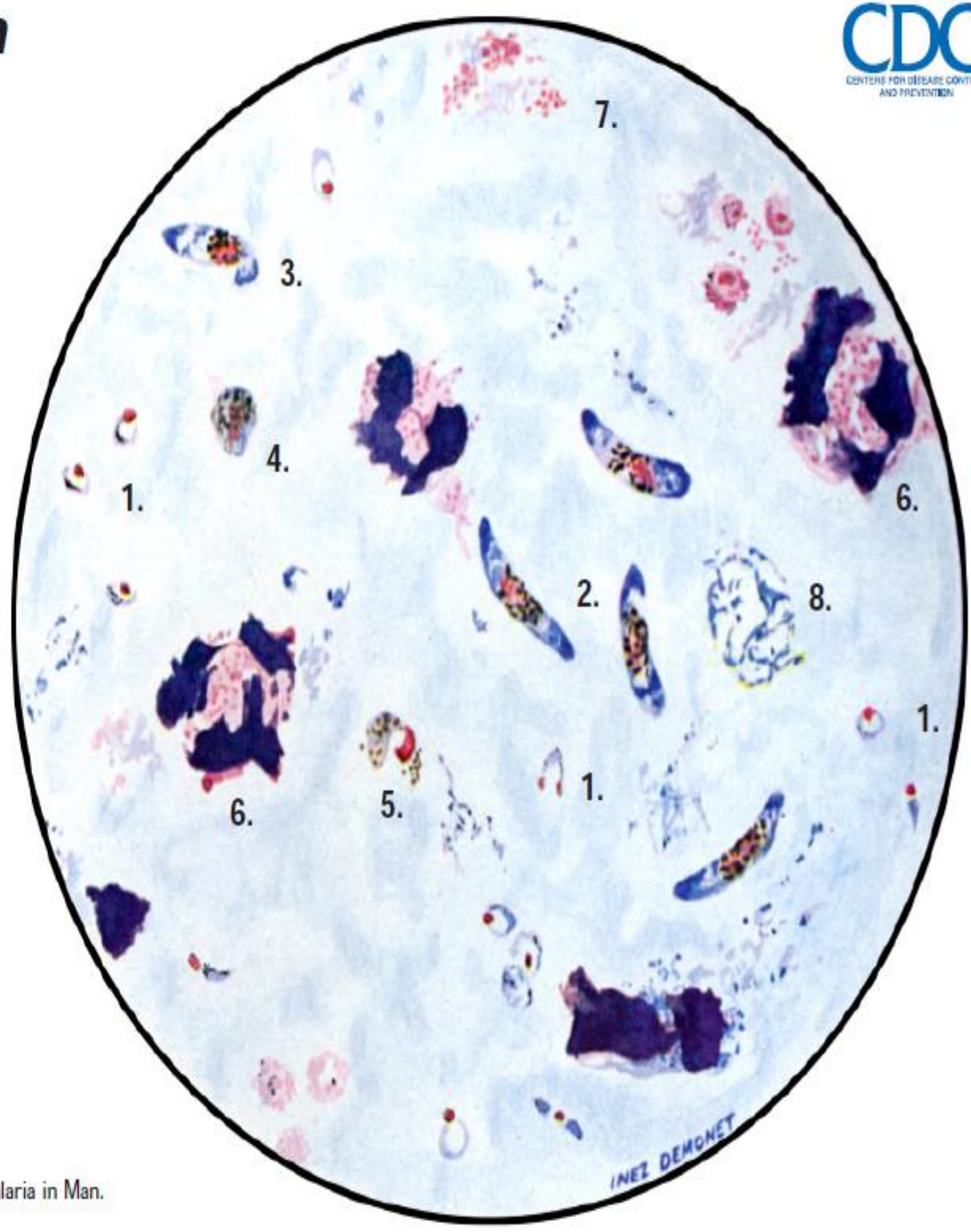
- *Leishmania*: transmitted by sand flies, can lead to visceral, cutaneous and mucocutaneous leishmaniasis

- Trypanosoma: haemoflagellates cause
  - In Africa - sleeping sickness (transmitted by the Tsetse fly)
  - In South America - Chagas disease (transmitted by the Reduviid bug)

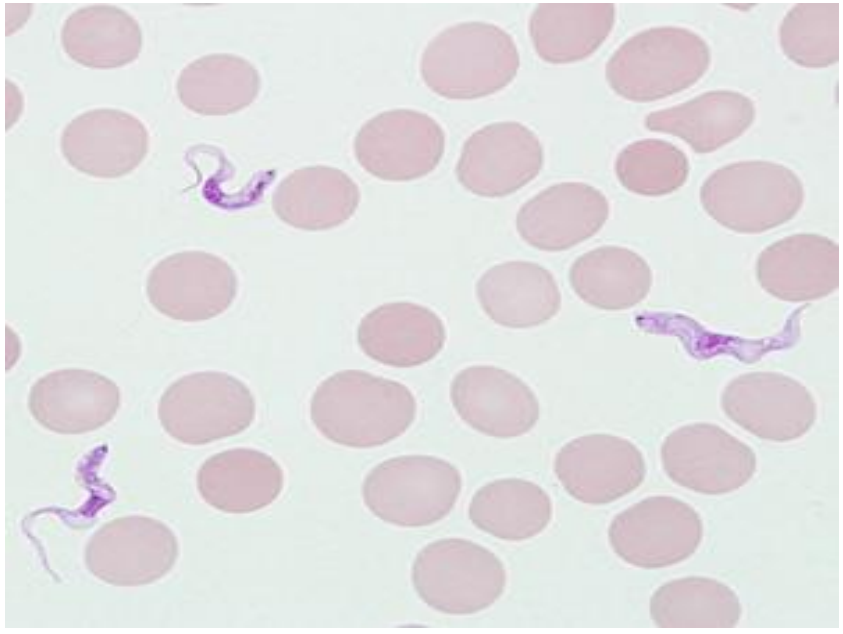
Typical lesion of cutaneous leishmaniasis

Plasmodium falciparum Blood Stage Parasites, Thick Blood Smears

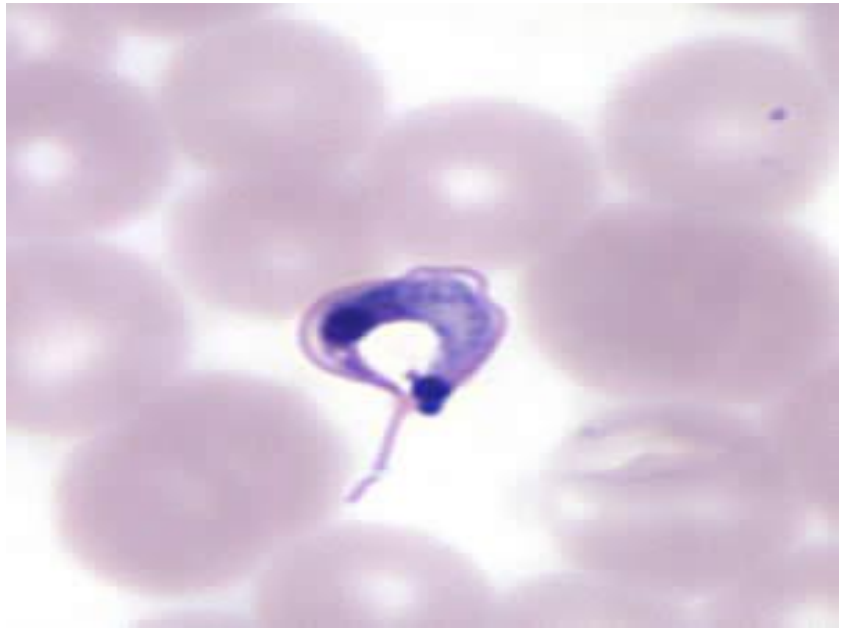
1: Small trophozoites.
2: Gametocytes – normal.
3: Slightly distorted gametocyte.
4: "Rounded-up" gametocyte.
5: Disintegrated gametocyte.
6: Nucleus of leucocyte.
7: Blood platelets.
8: Cellular remains of young erythrocyte.



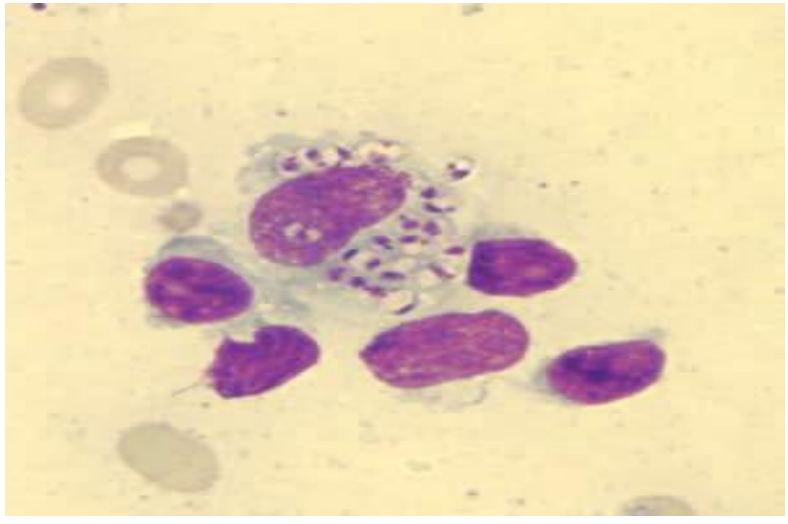
ion from: Wilcox A. Manual for the Microscopical Diagnosis of Malaria in Man. U.S. Department of Health, Education and Welfare, Washington, 1960.



Trypanosoma brucei ssp. in a thin blood smear stained with Giemsa.



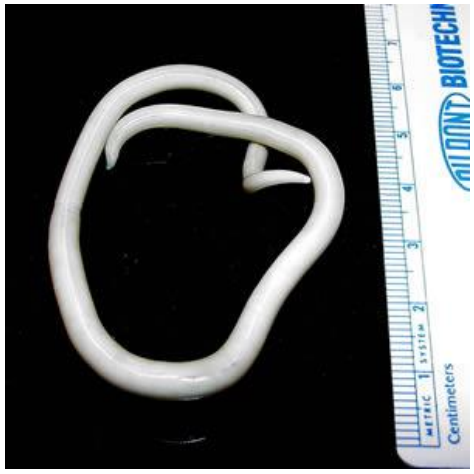
T. cruzi trypomastigote in a thin blood smear stained with Giemsa.

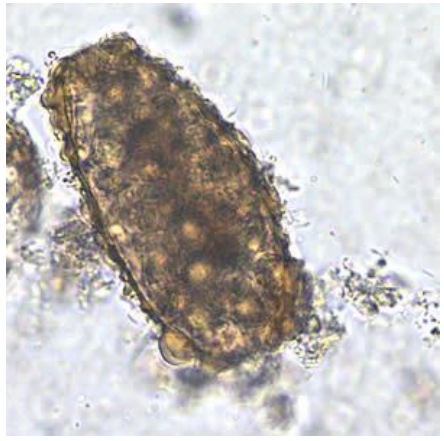


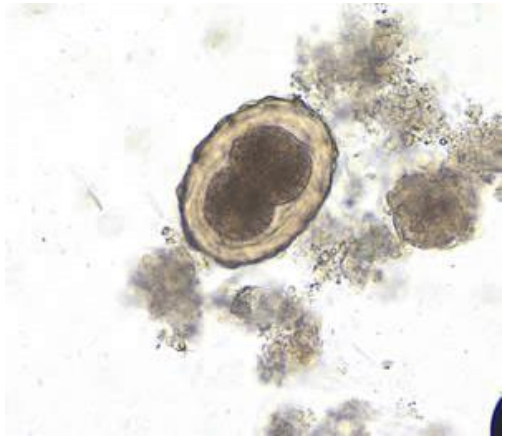
Leishmania sp. amastigotes in a Giemsa-stained tissue scraping

Examples of important intestinal nematodes

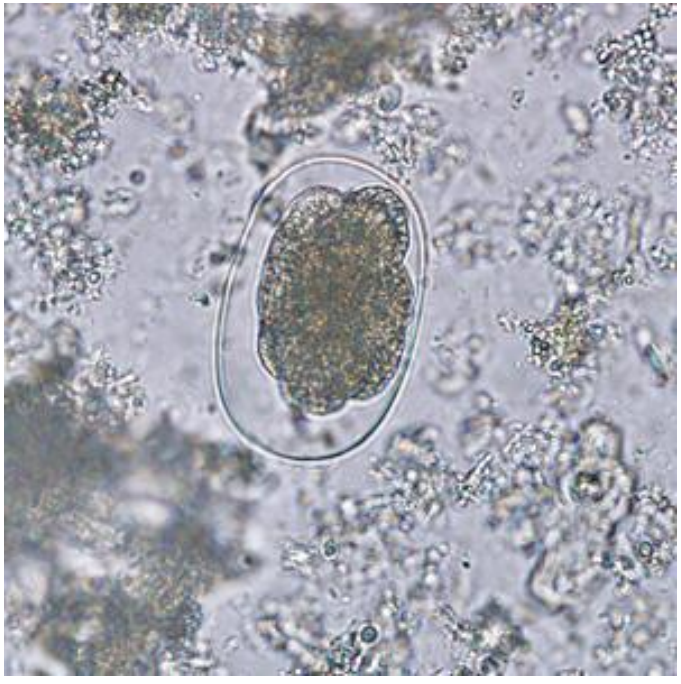
- *Trichuris* (whipworm)
  - A soil transmitted helminth
  - prevalent in warm, humid conditions
  - Can cause diarrhoea, rectal prolapse and anaemia in heavily-infected people
- *Ancylostoma* and *Necator* (hookworms)
  - A major cause of anaemia in the tropics
- *Strongyloides*
  - inhabits the small bowel
  - infection more severe in immunospressed people (e.g. HIV/AIDS, malnutrition, intercurrent disease)
- *Ascaris* (roundworm)
  - Found world-wide in conditions of poor hygiene, transmitted by the faecal- oral route
  - Adult worms lives in the small intestine
  - Causes eosinophilia
- *Enterobius* (pinworm or threadworm)
  - prevalent in cold and temperate climates but rare in the tropics
  - found mainly in children



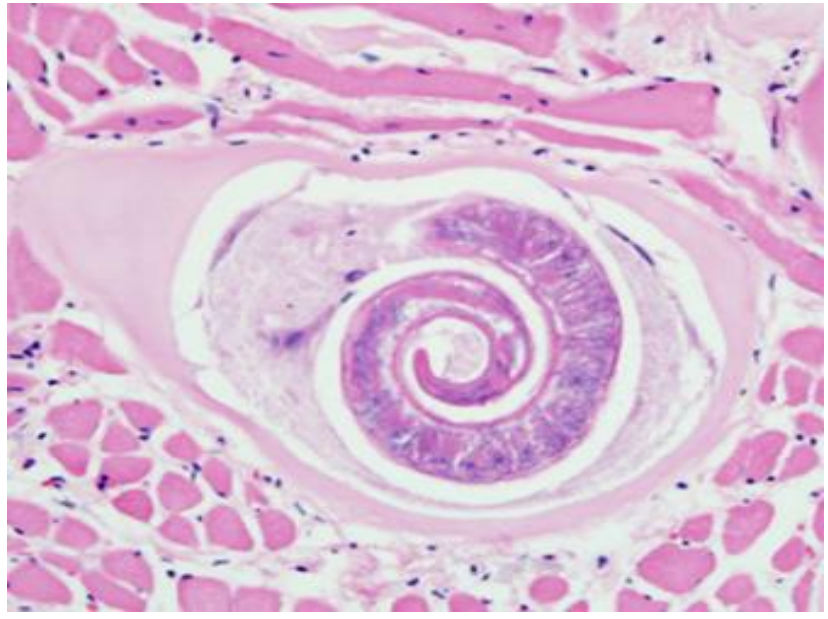




Adult female *A. lumbricoides*. Unfertilized egg of *A. lumbricoides* Fertilized egg



Hookworm egg in an unstained wet mount



Trichinella larva in tongue muscle of a rat, stained with hematoxylin and eosin

Examples of important systemic nematodes

Filaria worms including:

- ***Onchocerca volvulus* :**

Transmitted by the simulium black fly, this microfilarial parasite can cause visual impairment, blindness and severe itching of the skin in those infected

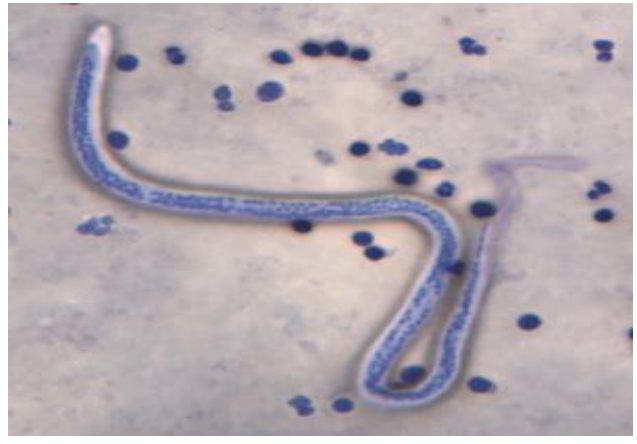
- ***Wuchereria bancrofti* :**

The major causative agent of lymphatic filariasis

- ***Brugia malayi* :**

Another microfilarial parasite that causes lymphatic filariasis

- Toxocara
  - A world-wide infection of dogs and cats
  - Human infection occurs when embryonated eggs are ingested from dog or cat faeces
  - It is common in children and can cause visceral larva migrans (VLM)



Microfilaria of *W. bancrofti* in a thick blood smear stained with Giemsa

Examples of important flatworms : cestodes

Intestinal :("tapeworms")

Taenia saginata

- worldwide
- acquired by ingestion of contaminated, uncooked beef
- a common infection but causes minimal symptoms

Taenia solium

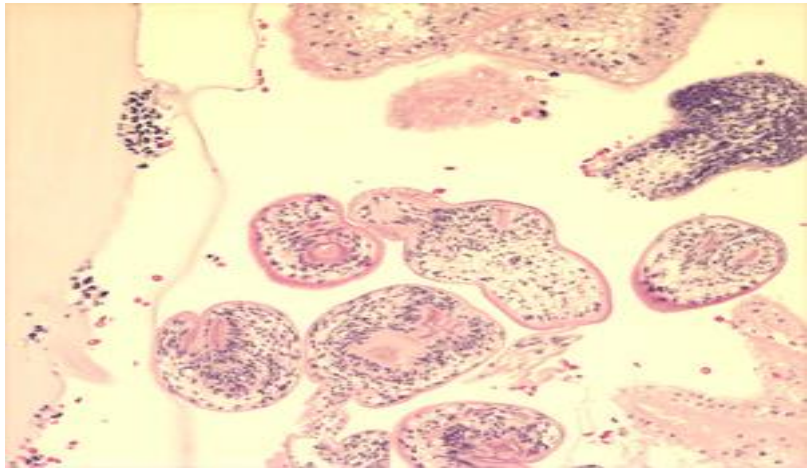
- worldwide
- acquired by ingestion of contaminated, uncooked pork that contains cystercerci
- Less common, but causes cystercicosis – a systemic disease where cysticerci encyst in muscles and in the brain – may lead to epilepsy

Systemic

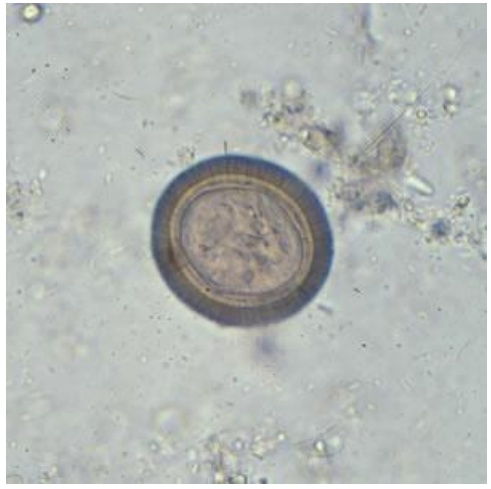
Echinococcus granulosus (dog tapeworm) and *Echinicoccus multilocularis* (rodent tapeworm)

Hydatid disease occurs when the larval stages of these organisms are ingested

The larvae may develop in the human host and cause space-occupying lesions in several organs, e.g. liver, brain



Protoscoleces in a hydatid cyst removed from lung tissue, stained with hematoxylin and eosin (H&E).



Taenia sp. egg in unstained wet mounts

Proglottid of T. saginata injected with India Ink

Examples of important trematodes (flukes)

Intestinal

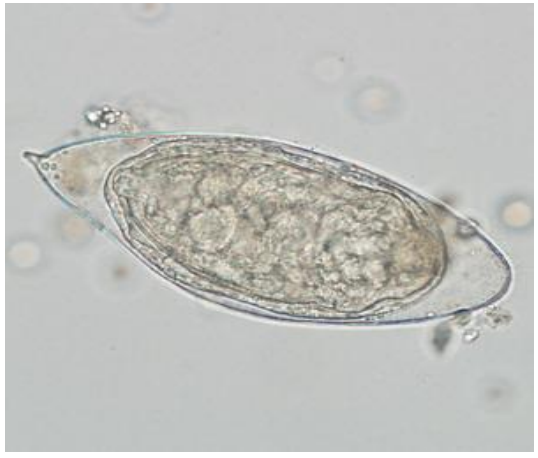
- *Fasciolopsis buski* : A common parasite of humans and pigs in South- east Asia. This parasite is one of the largest trematodes to infect man (8cm in length) and lives in the upper intestine. Chronic infection leads to inflammation, ulceration and haemorrhage of the small intestine

- *Paragonimus westermani* (lung fluke)- Widespread in the Far East and South east Asia, the parasite is acquired by ingestion of infective metacercariae in raw or pickled crustaceans

- ***Fasciola hepatica*** (liver fluke)- a parasite of sheep, humans become infected when ingest metacercariae that have encysted on watercress. The adult trematode lives in the intra-hepatic bile ducts of the liver. "Fascioliasis" can lead to severe anaemia in humans

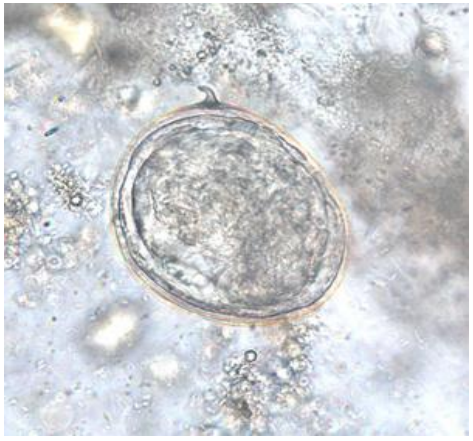
- *Clonorchis sinensis* (liver fluke): Widespread in China, Japan, Korea and Taiwan, this parasite is acquired by ingestion of infective metacercariae in raw or pickled fish

- *Schistosoma haematobium, S. mansoni* and *S. japonicum*

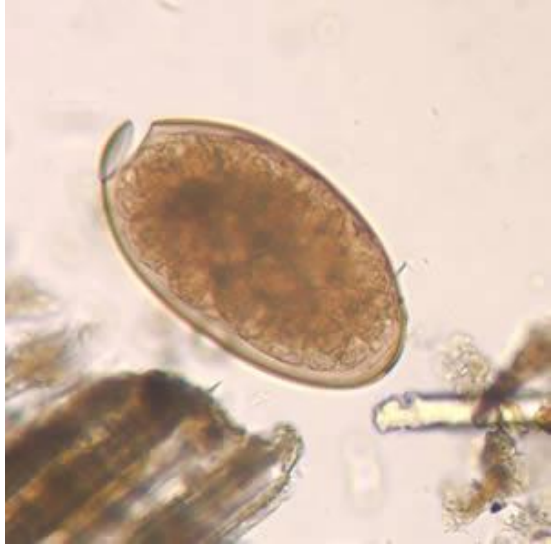


Egg of *S. haematobium* in a wet mount of a urine concentrate

Egg of *S. mansoni* in unstained wet mounts.



Egg of *S. japonicum* in an unstained wet mount of stool.



Egg of *F. hepatica* in an unstained wet mount

Diagnosis of Parasitic Infections

1. Clinical

2. Laboratory

Purpose of laboratory diagnosis :

- Confirmation of clinical suspicion
- Identification of unsuspected infection

Specimens

- Stool
- Blood
- Serum and plasma
- Others (anal swab, duodenal aspirate, sputum, urine, urogenital specimen)
- Tissues and aspirates

Stool examination

Sample collection:

- Sample is collected in clean, dry container
- Handled carefully
- Sometimes use preservative (10% formalin)
- Samples in some cases fresh(amoeba, ciliates)
- Liquid and soft stool examined within 15 min
- Not mixed with urine or disinfectant (as they will kill trophozoites)
- Specimens obtained by enema or laxatives are often positive for worm eggs or adult worm.

Examination of the stool sample:

Gross examination:

- Mucoid blood stained (acute amoebic dysentry), Parasites can be detected (nematodes, cestodes)

Microscopic examination:

- Saline mount
- Iodine Mount
- Thick smears – not commonly used
- Permanent stained smears
  - Iron hematoxylene
  - Whearley's trichrome stain
- Concentration methods
  - Floatation techniques
  - Sedimentation techniques

- Antigen detection
- Molecular diagnosis

Microscopic examination

Direct wet mount:

- Thin emulsion of small amount of faeces
- Few drops of saline
- Sometimes add lugol's iodine (nuclear details, glycogen vacuole in cyst)
- Protozoa (trophozoite), cyst, eggs and larva of helminths, crystals (charcot leyden)

Concentration methods

- Scanty parasites in the sample
- **Floatation** (eggs and cyst float , solution of high specific gravity)

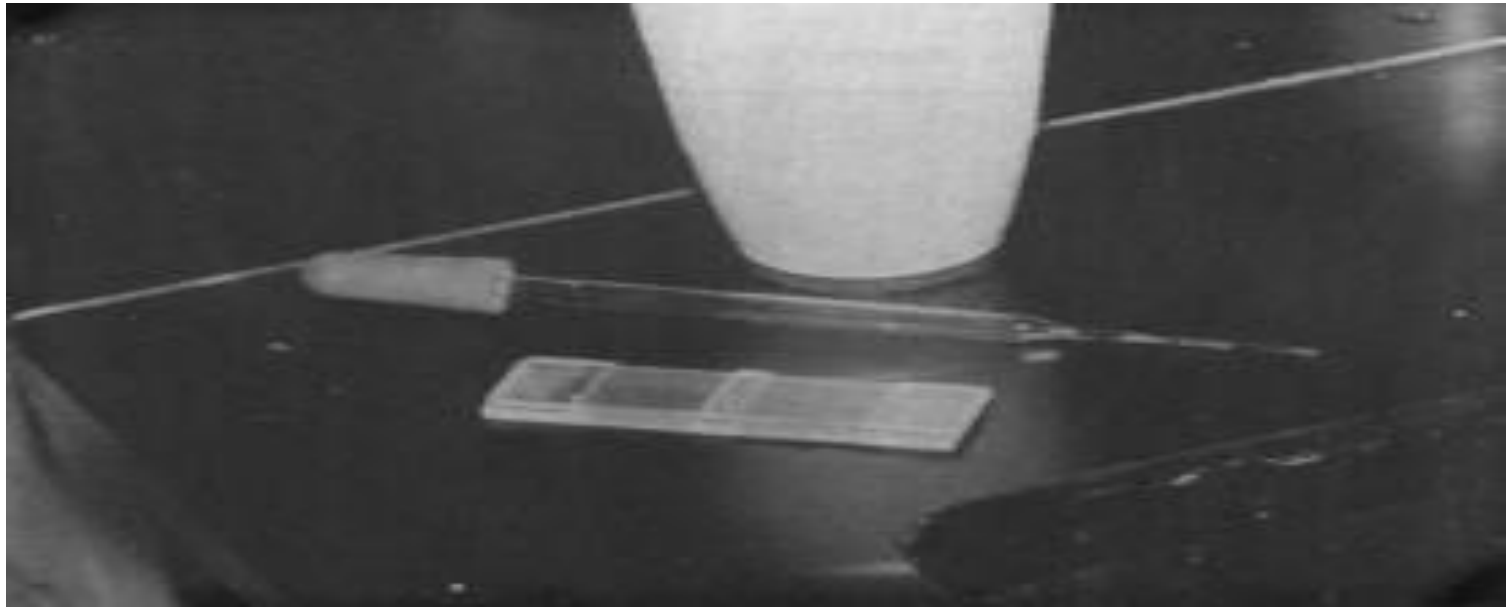
1. saturated sodium chloride (ascaris, hookworms)
2. Zinc sulphate centrifugation floatation (cyst, nematodes).

- **Sedimentation** (solution of low specific gravity):

  formol ether

  Egg count in 1 gram

Stoll's technique for counting helminth egg



3 gm stool and 42 ml water

0.15 ml on slid

Multiply result in 100

Number in 1 gm

Immunodiagnostic (antigen detection)

- Fresh or preserved stool samples are the appropriate specimens

Amebiasis

- EIA kits are commercially available for detection of fecal antigens for the diagnosis of intestinal amebiasis.
- These assays use monoclonal antibodies that detect the galactose-inhibitable adherence protein in the pathogenic *E. histolytica*.
- Several EIA kits for antigen detection of the *E. histolytica*/*E. Dispar (non-pathogenic amoeba)* group are available , but only the TechLab kit is specific for *E. histolytica*.

Cryptosporidiasis

Several kits are combined tests for *Cryptosporidium*, *Giardia*, and *E. Histolytica*.

DFA test identifies oocysts in concentrated or unconcentrated fecal samples by using a fluorescein isothiocyanate (FITC)-labeled monoclonal antibody is the most sensitive.

Giardiasis

DFA assays may be purchased that employ FITC-labeled monoclonal antibody for detection of *Giardia* cysts.

Organism	Kit name	Manufacturer - distributor[a]	Type of Test[b]
Cryptosporidium spp.	Crypto CELISA	Cellabs	EIA
	PARA-TECT™ Cryptosporidium Antigen 96	Medical Chemical Corporation	EIA
	ProSpecT Rapid	Remel	EIA
	ProSpecT	Remel	EIA
	Cryptosporidium	TechLab	EIA
	Cryptosporidium	Wampole	EIA
	Crypto CEL	Cellabs	IFA
	XPect Crypto	Remel	Rapid
Cryptosporidium spp./*Giardia duodenalis*	PARA-TECT™ Cryptosporidium/Giardia DFA 75	Medical Chemical Corporation	DFA
	Merifluor	Meridian	DFA
	ProSpecT	Remel	EIA
	Crypto/Giardia CEL	Cellabs	IFA
	ColorPAC*	Becton Dickinson	Rapid
	ImmunoCard STAT!*	Meridian	Rapid
	XPect	Remel	Rapid
Cryptosporidium spp./*Giardia duodenalis*/*Entamoeba histolytica*/*dispar*	Triage	BioSite	Rapid
Entamoeba histolytica	Entamoeba CELISA	Cellabs	EIA
	E. histolytica	Wampole	EIA
	E. histolytica II	TechLab	EIA
Entamoeba histolytica/*E. dispar*	ProSpecT	Remel	EIA
Giardia duodenalis	Giardia CELISA	Cellabs	EIA
	PARA-TECT™ Giardia Antigen 96	Medical Chemical Corporation	EIA
	ProSpecT	Remel	EIA
	Giardia II	TechLab	EIA
	Giardia	Wampole	EIA
	GiardiaEIA	Antibodies, Inc.	EIA
	Giardia CEL	Cellabs	IFA
	ProSpecT	Remel	Rapid
	Simple-Read Giardia	Medical Chemical Corporation	Rapid
Wuchereria bancrofti	Filariasis CELISA	Cellabs	EIA

Molecular diagnosis

(using stool sample)

If an unequivocal identification of the **parasite** can not be made, the stool specimen can be analyzed using molecular techniques such as polymerase chain reaction (PCR). PCR amplified fragments can be analyzed by using restriction fragment length polymorphisms (RFLP) or DNA sequencing if further characterization is needed.

- **Sample :**

Fresh stool should be kept cold or frozen till DNA extraction.

Samples collected in a preservative should be compatible with molecular detection (TotalFix, Unifix, modified PVA (Zn- or Cu-based), and Ecofix)

- **DNA Extraction** better using commercially available kits (Qiagen)

- **PCR analysis:**

Conventional PCR:

DNA is tested by PCR with diagnostic primers. Amplified DNA fragments are electrophoretically resolved on an agarose gel for analysis of results.

Real-Time PCR

The DNA amplification is monitored by measuring the fluorescence signal generated in the reaction vessel. The fluorescence signal is measured every cycle and is proportional to the amount of accumulated PCR product.

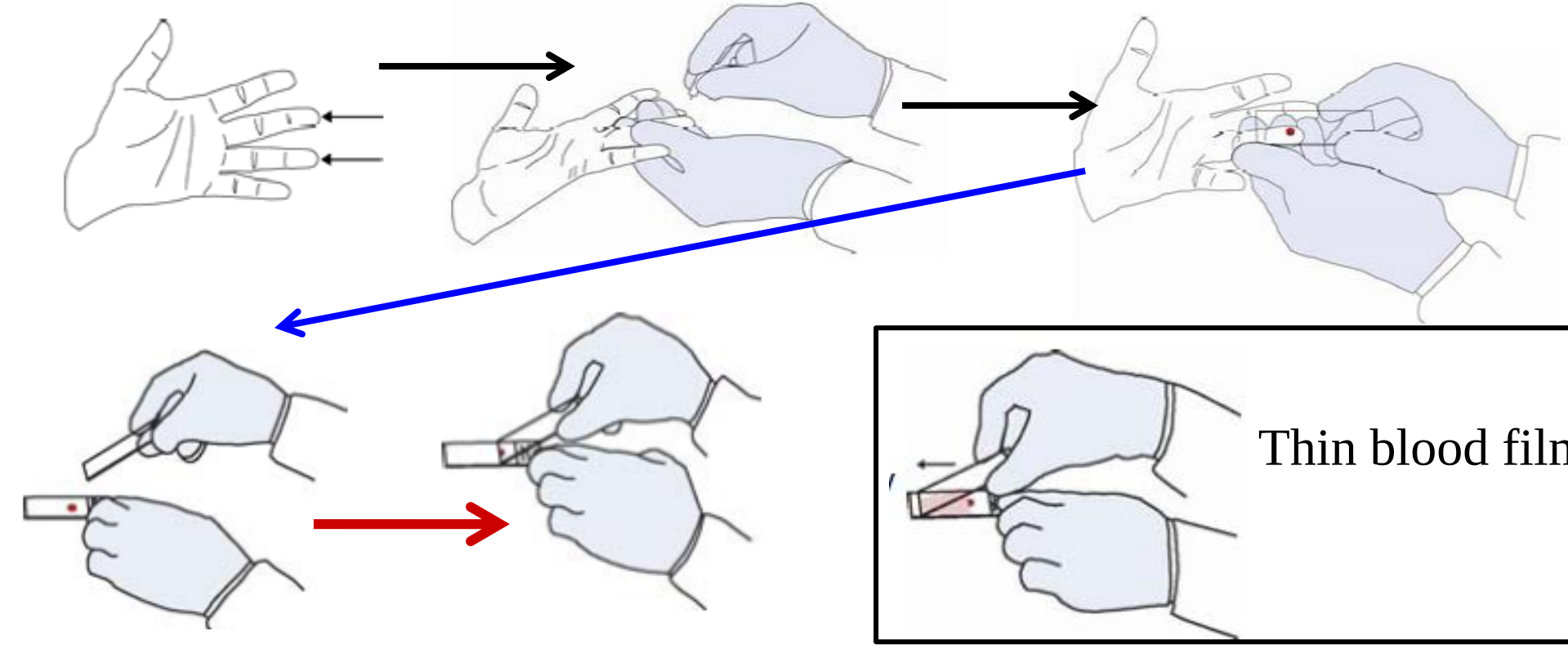
Blood examination

- Fresh capillary blood of finger or ear lobe
- Venous blood collected in EDTA (anticoagulant)

Blood sample will be used for :

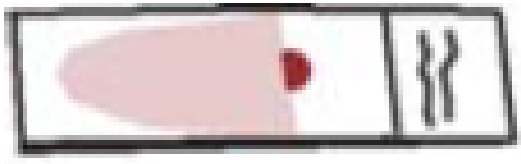
- Microscopic examination(Thin Smear, Thick smear, Wet mount for microfilaria).
- Molecular diagnosis
- Detection of parasite antigen
- Isolation of organisms
- Special tests

Blood sample preparation for microscopic examination

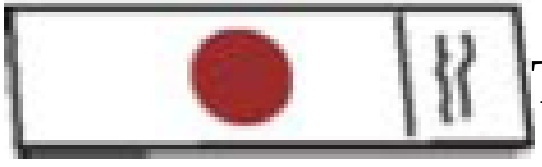


Thin blood film

Thick blood film



Thin blood film



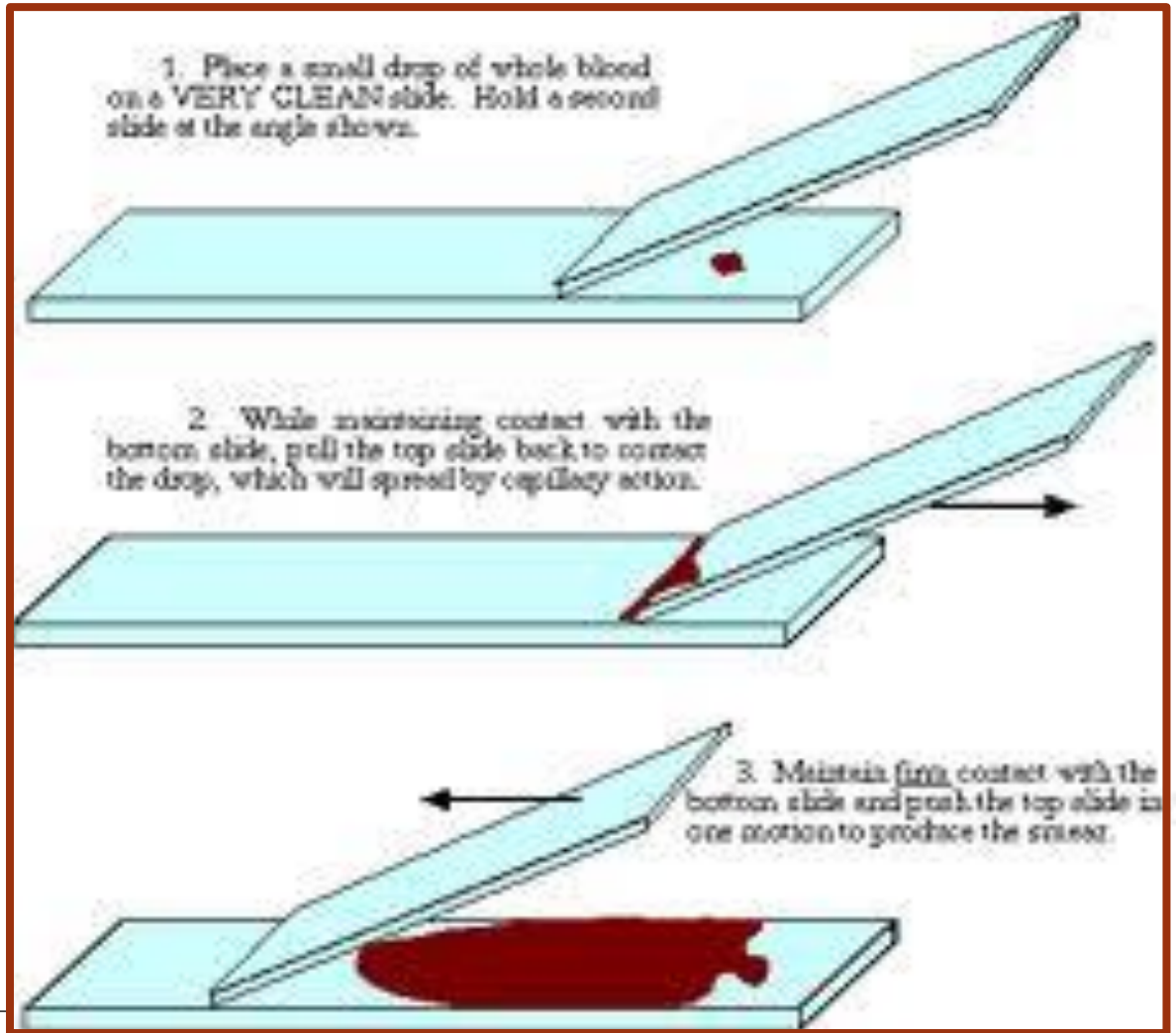
Thick blood film

Thick blood film

- Screen large amount of blood (light infection)
- Can be stained latter

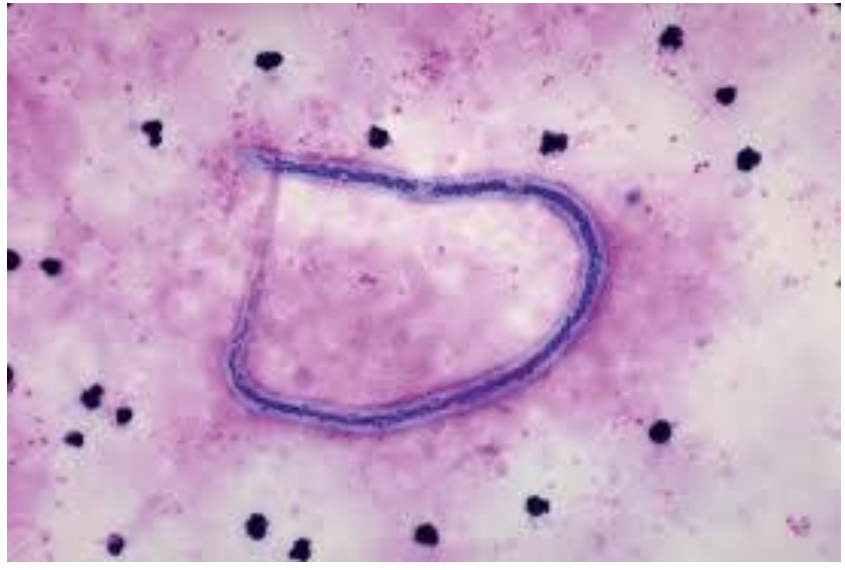
Thin blood film

In malaria Parasitized red blood cells and parasites
Definite species identification



Microfilaria

- Sample collection according to periodicity of microfilaria
- Concentration by sedimentation or membrane filtration (examine the filter)
- DEC provacation method



Microfilaria of *Wuchereria bancrofti*

Molecular diagnosis

(using blood sample)

- Collect a 1-5 ml blood sample in tube with EDTA.
- Blood can be collected on filter papers (e.g Whatman)
- DNA is extracted using DNA extraction kits

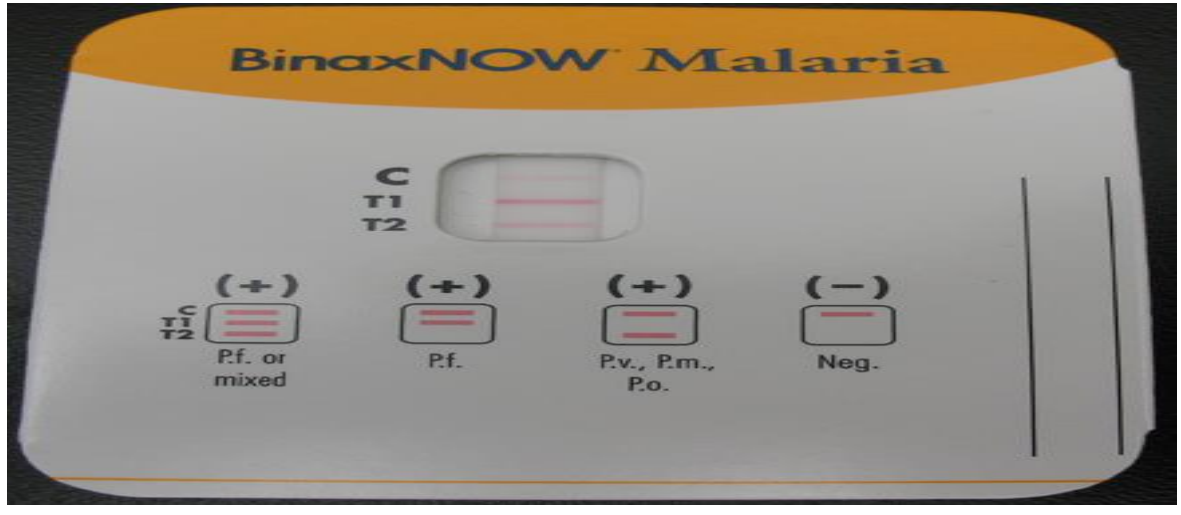
Species-specific diagnosis of malaria

Detection and speciation of *Plasmodium* is done with a two step nested PCR using the primers of Snounou et al 1993.

Detection of parasite antigens

(in blood sample)

- Rapid diagnostic tests for malaria employing immunochromatographic methods based on the detection of malarial antigens present in peripheral blood.
- only diagnose only *P. falciparum* malaria.
- Currently, the only available RDT for malaria in the United States is the BinaxNOW® Malaria Test.



Organism	Kit name	Manufacturer - distributor[a]	Type of Test[b]
Plasmodium	BinaxNOW® Malaria Test	Inverness Medical	Rapid (HRP2 and aldolase)
	Malaria-Ag	Cellabs	EIA
	OptiMal	Flow	Rapid (LDH)
	MAKROmed malaria test	MAKROmed Manufacturing, LTD	Rapid (HRP2)
	Paracheck Pf	Orchid	Rapid (HRP2)
	Visitect Malaria Pf	Omega Diagnostics LTD	Rapid (HRP2)
Wuchereria bancrofti	ICT Filariasis	Binax	Rapid
	Filariasis Ag-CELISA	Cellabs	EIA

Isolation of Organisms

(from blood)

- The diagnosis of *Leishmania* spp. is made by microscopic identification of the nonmotile, intracellular form (amastigote) in stained sections from lesions, and by culture of the motile, extracellular form (promastigote) on suitable media.

- Slides should be fixed and stained before they are sent unless reagents are not available.

- Serologic tests are also available to detect for anti-leishmanial antibodies; however, these tests are often not sensitive, particularly for diagnosing cutaneous leishmaniasis.

Special Tests: MQ Testing

- Mefloquine is recommended by CDC as a prophylactic against malaria.
- When individuals who are on mefloquine prophylaxis exhibit signs of malaria, blood samples are collected and analyzed for the presence of the drug.

- The drug is extracted from the blood and the concentration is determined by high-performance liquid chromatographic (HPLC) methods.
- Determining the level of mefloquine in the blood helps assess if the individual was adherent with his/her medication.

- This procedure is also useful to determine treatment failure due to a mefloquine resistant form of malaria.

Serum, plasma and others

Specimen Requirements

- Serum/plasma is required for all parasitic disease immunodiagnostic tests.
- A single sample is sufficient; acute and convalescent specimens are not necessary.
- CSF and eye fluids (vitreous or aqueous) are acceptable for selected diseases) but **MUST** be accompanied by a serum specimen.

Serum for all tests: 0.5 ml serum/plasma separated from RBCs .

CSF: 0.5 ml. Acceptable only for cysticercosis and baylisascariasis testing.

Eye fluids: 0.1 ml neat fluid (no washings). Acceptable only for toxocariasis

- **Serology – All tests available**
  - IHA
  - ELISA
  - CIEP
  - IF
  - CFT
- **More useful in**
  - Amoebiasis
  - Leishmaniasis
  - Malaria
  - Toxoplasmosis
  - Trichinosis
  - Filariasis
  - Echinococcosis

- **Skin Tests**

Specificity low, cross reactions common

Examples:

- Casoni's test
- Leishmanin test

Serum/Plasma Specimens

Antibody Detection Tests Offered at CDC

Disease	Organism	Test	Acceptable Specimens
Amebiasis	*Entamoeba histolytica*	Enzyme immunoassay (EIA)	Serum
Babesiosis	*Babesia microti* *Babesia* sp. WA1	Immunofluorescence (IFA)	Serum
Baylisascariasis	*Baylisascaris procyonis*	Immunoblot	Serum or CSF
Chagas disease	*Trypanosoma cruzi*	IFA	Serum
Cysticercosis	Larval *Taenia solium*	Immunoblot (Blot)	Serum, CSF
Echinococcosis	*Echinococcus granulosus*	EIA, Blot	Serum
Filariasis	*Wuchereria bancrofti* and *Brugia malayi*	EIA	Serum
Leishmaniasis	Leishmania braziliensis L. donovani L. tropica	IFA	Serum
Malaria	*Plasmodium falciparum* *P. malariae* *P. ovale* *P. vivax*	IFA	Serum
Paragonimiasis	*Paragonimus westermani*	Blot	Serum
Schistosomiasis	Schistosoma sp. S. mansoni S. haematobium S. japonicum	FAST-ELISA Blot	Serum
Strongyloidiasis	*Strongyloides stercoralis*	EIA	Serum
Toxocariasis	*Toxocara canis*	EIA	Serum, vitreous fluid
Trichinellosis (Trichinosis)	*Trichinella spiralis*	EIA	Serum

Cultivation of parasites

Culture methods are used for :

- Amoeba
- Leishmania
- Trypanosoma
- Malarial parasite

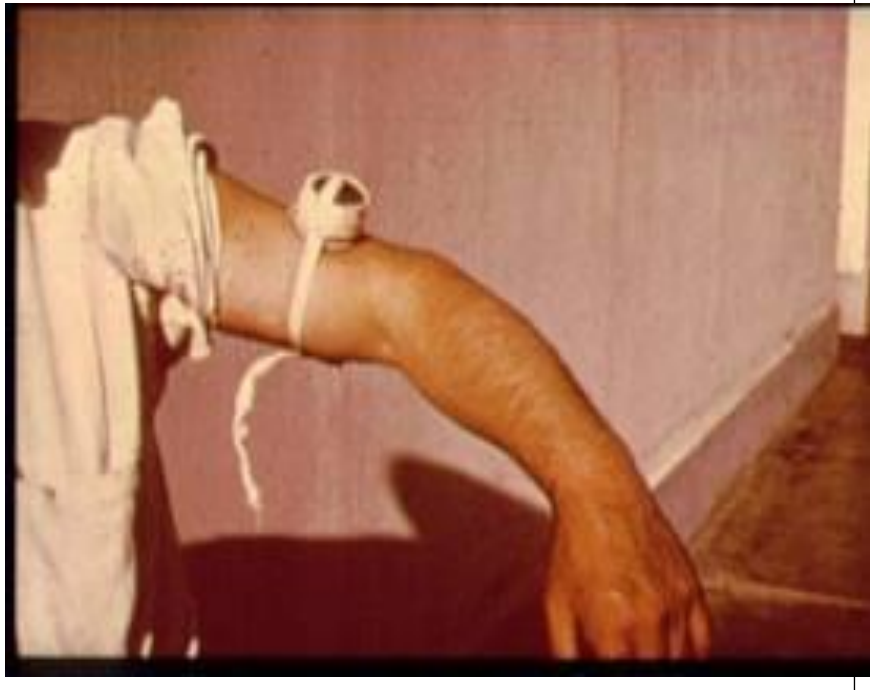
Animal inoculation

- Leishmania (young hamester)
- Trypanosomes (rat, mouse)
- Toxoplasma (all lab animals)

Xenodiagnosis:

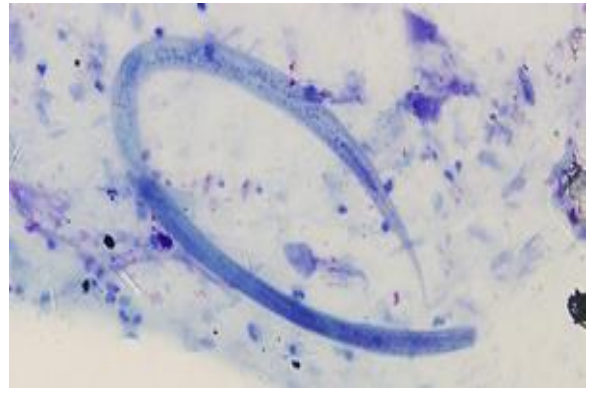
In chagas' disease

Vector infected experimentally



Sputum examination

Microscopic examination of sputum can identify:



- *Paragonimus westermani* eggs
- *Strongyloides stercoralis* larva
- *Ascaris lumbricoides* larvae
- hookworm larvae, and rarely *Entamoeba histolytica*.

❑ Sputum should be obtained from the lower respiratory passages not saliva.

❑Sputum specimens should be collected first thing in the morning.

A sputum sample can be examined in several ways:

- The unfixed specimen may be centrifuged and then the sediment examined as a direct wet mount.

- If the sputum is too viscous, an equal volume of 3% sodium hydroxide may be added, then centrifuge, and examine the sediment.

- The specimen can be preserved in 10% formalin and a formalin-ethyl acetate

- The specimen can be preserved in PVA if protozoa are suspected and stained with trichrome stain.

Vaginal swabs

- Demonstration of ***Trichomonas vaginalis*** trophozoites is usually done by preparing wet mounts made from vaginal swabs or scrapings.

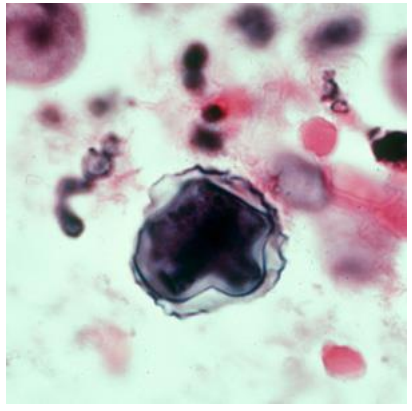
- If the specimen cannot be examined immediately, it should be preserved in PVA and stained smears examined later.

Commercially available tests for detection of *Trichomonas*

Organism	Kit name	Manufacturer - distributor[a]	Type of Test[b]
Trichomonas vaginalis	T. vag	Chemicon	DFA
	Quik-Trich	PanBio	LA

Tissue Specimens for Free-living Amebae (FLAs)

Tissue specimens, including biopsy, surgical or necropsy specimens, may be collected for the detection of free-living amebae (***Naegleria, Balamuthia,* and *Acanthamoeba***).



Acanthamoeba cyst

The desired specimens include:

- Tissue slides stained with hematoxylin and eosin (H&E).
- Unstained slides (for indirect Immunofluorescence, or IIF).
- Unfixed brain tissue or CSF for PCR.
- Unfixed corneal scrapings (for *Acanthamoeba*).
- Paraffin-embedded tissue block.

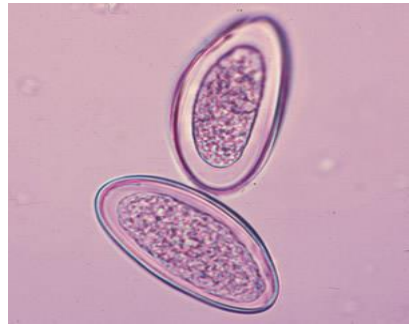
Cellulose Tape or Swube Tube Procedure for Demonstration of Pinworm Eggs

•The most reliable and widely used technique for demonstrating pinworm eggs (*Enterobius vermicularis*) is the cellulose tape or swube tube procedure.

•The adhesive part of the swube tube or tape is applied to the perianal area first thing in the morning.

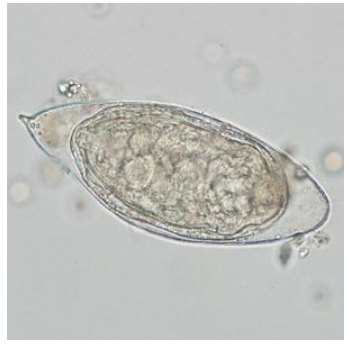
•Specimens should be collected on three consecutive mornings prior to bathing.

• If an infection is present, eggs and sometimes adult worms of *Enterobius vermicularis* will be present on the tape and can be seen under the microscope



E. Vermicularis egg

Urine Specimens



Urinary schistosomiasis

• Presence of *S. haematobium* eggs in urine is diagnostic for

•Eggs usually shed in the urine around midday, so an optimum urine specimen for diagnosis should be collected at noon.

• The specimen should be immediately centrifuged at 400 × *g* and the sediment examined by wet mount.

Trichomonas vaginalis

❑Motile trophozoites may also be found in the urine, especially in infected male patients.

❑Urine specimen should be centrifuged at 400 × *g*, the sediment mixed with a drop or two of saline, and examined by wet mount.

❑Temporary stains, such as methylene blue is helpful to see *T. Vaginalis*

Artifacts

•Cysts and trophozoites must be examined carefully in different fields of view and measurement is often essential. Objects such as epithelial cells and macrophages are around the same size as amoebic trophozoites: the latter may also move and contain red blood cells.

•White blood cells, plant and vegetable cells, fat globules, muscle fibers, pollen grains, yeasts cells and air bubbles may be confused with cysts or eggs.

• Air bubbles trapped under adhesive tape often resemble *Enterobius eggs.*

•*Plant hairs and fibers are easily confused with larvae*

•Earthworms may resemble roundworms.

•Eggs of *Heterodera, a parasitic nematode of root vegetables, may resemble hookworm eggs.*

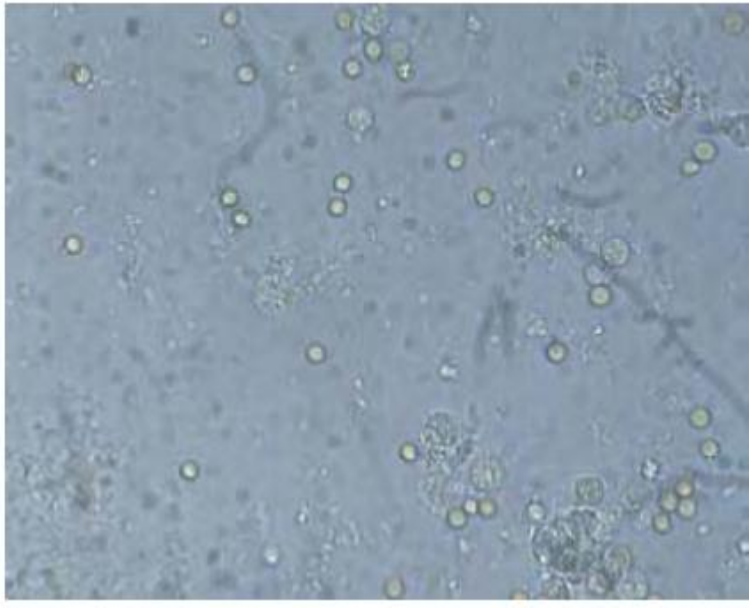
•Eggs originating from harmless mites in cereals or flour could be confused with hookworm ova

•A patient complaining of hematuria: we suspected *Schistosoma haematobium but, on* closer analysis, the eggs contained unidentified insects

Here we show examples of artifacts that may be confused for parasitic life stages.

Artifacts should be considered on the basis of size, shape, lack of organelles and defining feature, and variable reactivity with common

Artifacts



White blood cells can be mistaken for protozoan cyst (picture showing white blood cells)

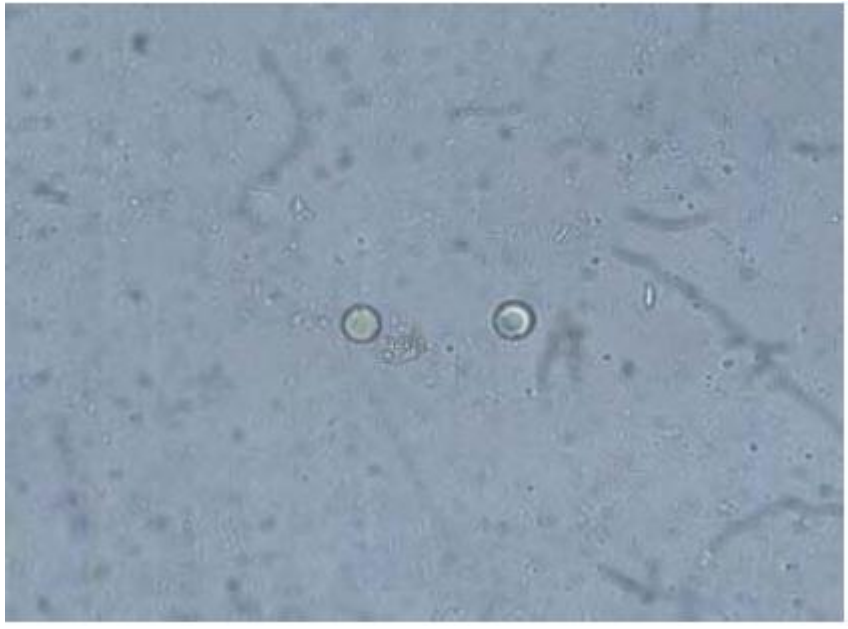
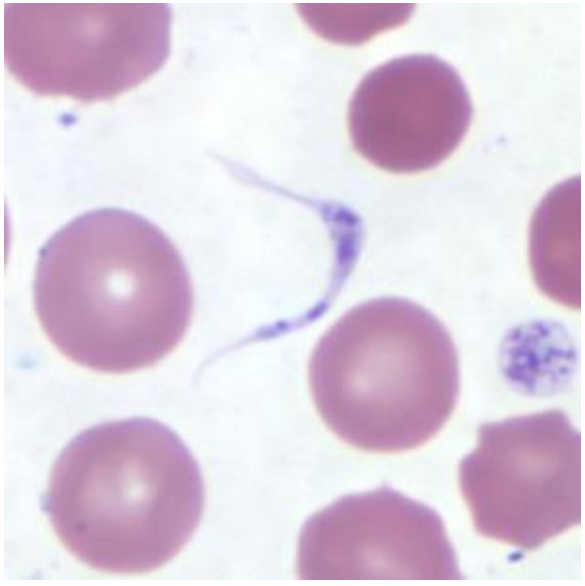
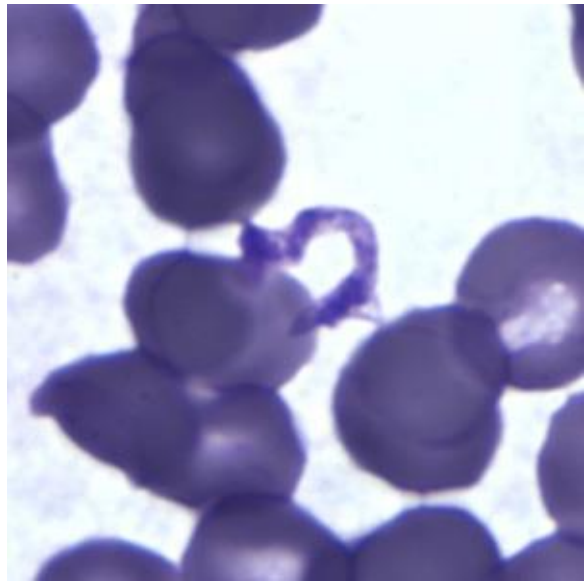
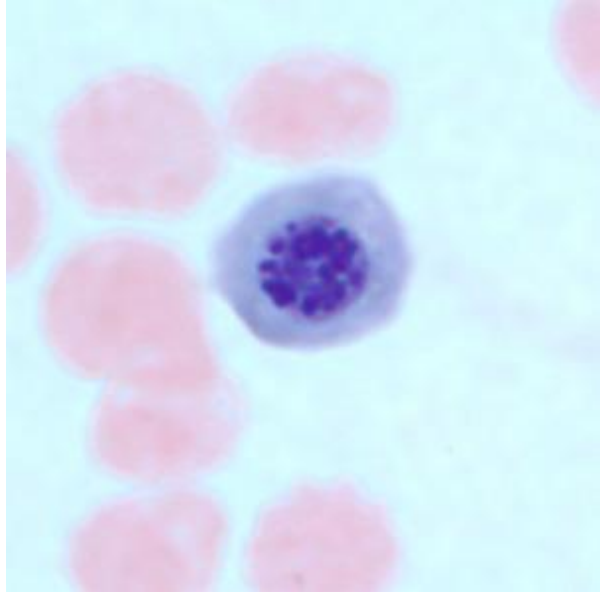


Image showing fat cells, which can be mistaken with protozoan cyst

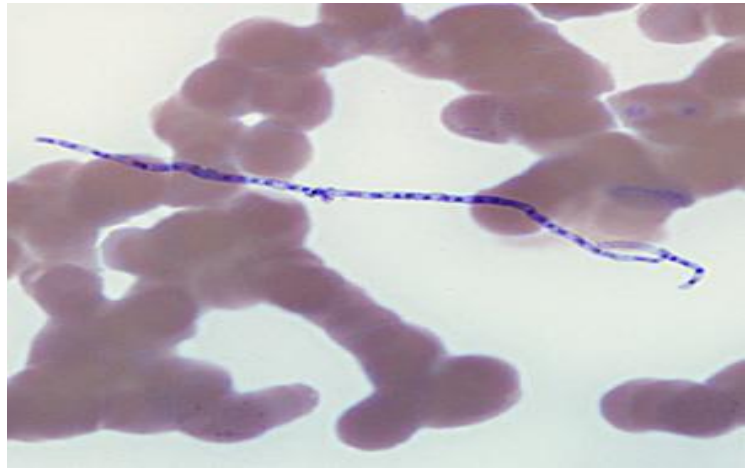




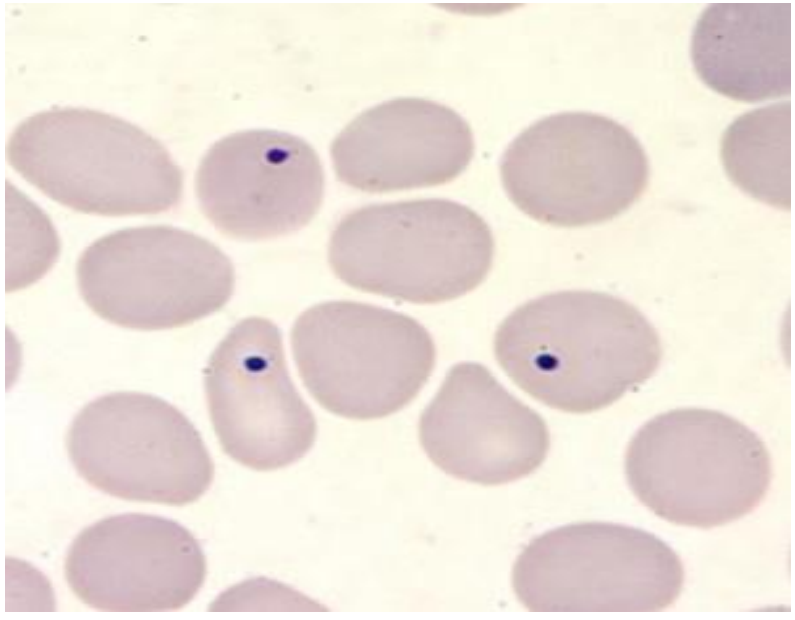
Platelets in thin blood smears. The nature of the platelets gives them the appearance of trypomastigotes of *Trypanosoma cruzi*.



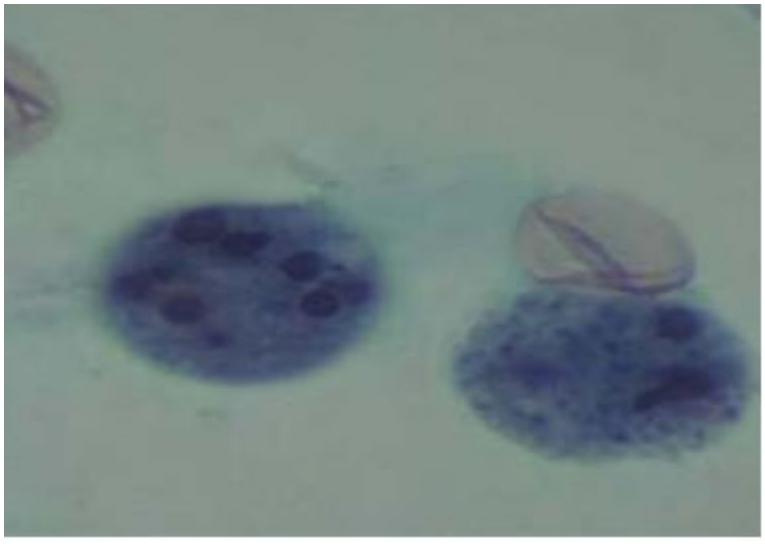
nucleated red blood cell in a thin blood smear, stained with Giemsa. may be confused for schizonts of *Plasmodium* spp.



Fungal spore of *Helicosporium* (or related). Such objects are air-borne contaminants in laboratories and may be mistaken for microfilariae in stained blood smears.



Howell-Jolly bodies in a thin blood smear, stained with Giemsa. They may be seen in splenectomized patients or patients with an otherwise non-functioning or atrophic spleen, and in patients with severe anemia or leukemia.



Trichrome-stained leukocytes can be mistaken with amoeba

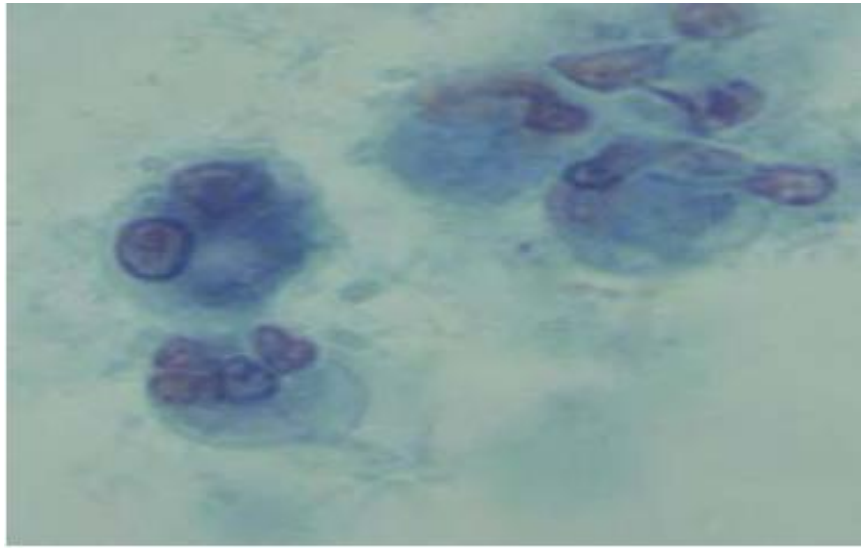


Image showing macrophage stained with trichrome, which can be easily mistaken with amoeba

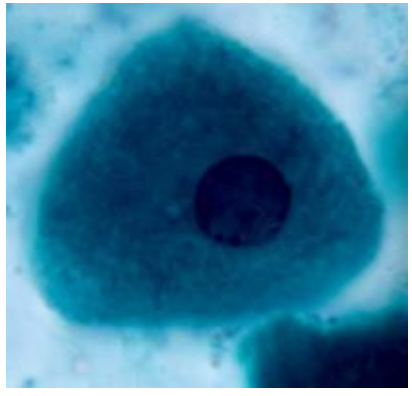
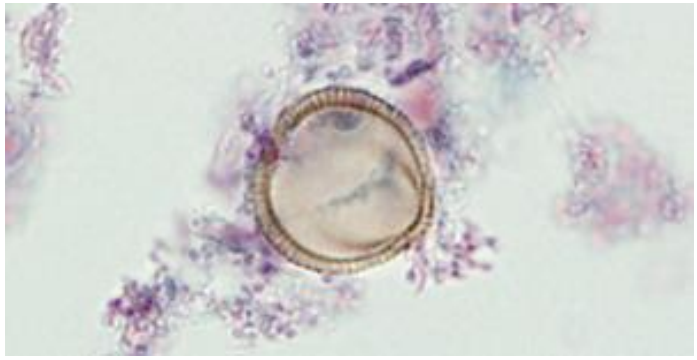
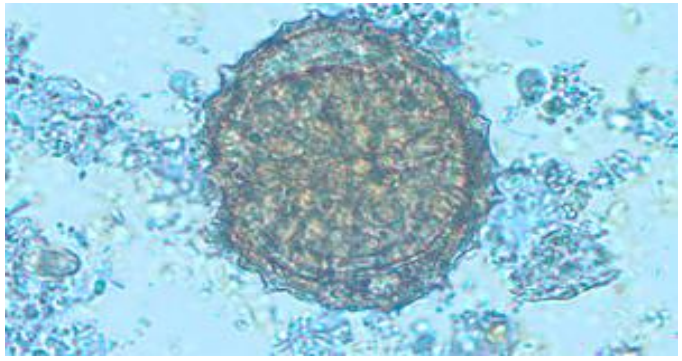


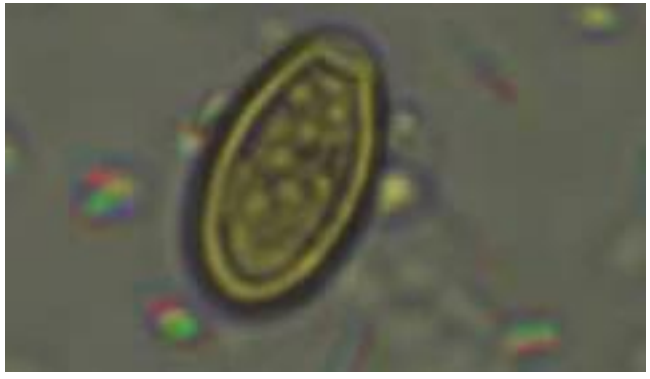
Image showing epithelial cell stained with trichrome, which can be easily mistaken with amoeba



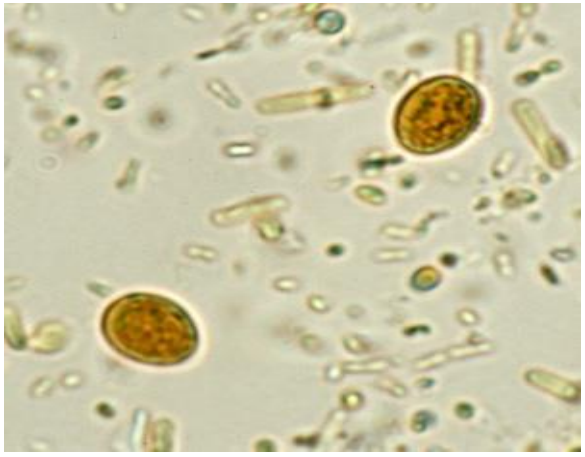
Pollen grain in a trichrome-stained stool specimen. In this focal plane, the grain looks like the striated egg of *Taenia* sp. However, notice the lack of refractile hooks



Pollen grain in a concentrated wet mount of stool. This grain looks very similar to the fertile egg of *Ascaris lumbricoides*

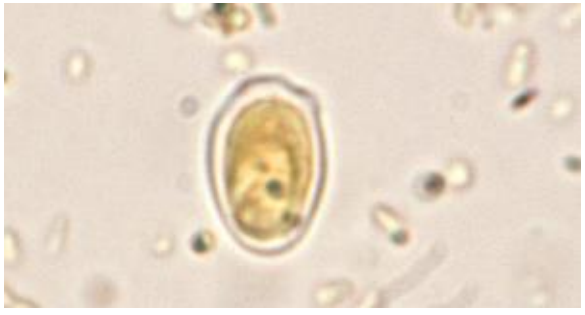


Possible pollen grain or algal or fungal spore in a concentrated wet mount of stool. Grains like this one resemble the operculated eggs of *Clonorchis*, *Metagonimus*. These grains are usually smaller than the trematode eggs

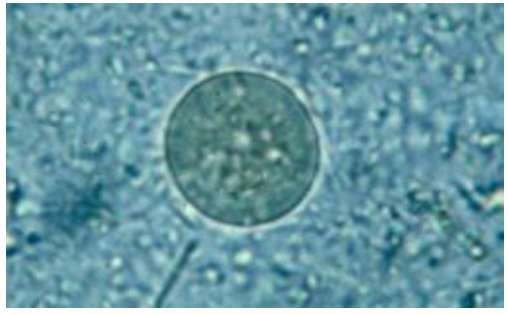


Yeast in an iodine-stained concentrated wet mount of stool. Yeast in wet mounts may be confused for *Giardia*.

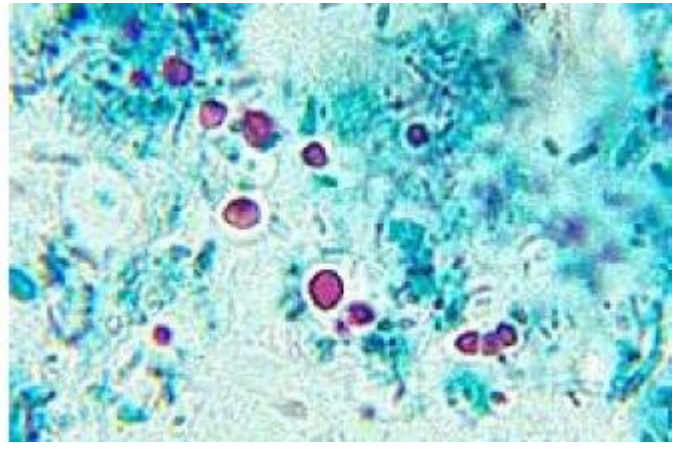
Spore of a morel mushroom. Such spores may be confused for helminth eggs, especially hookworm.



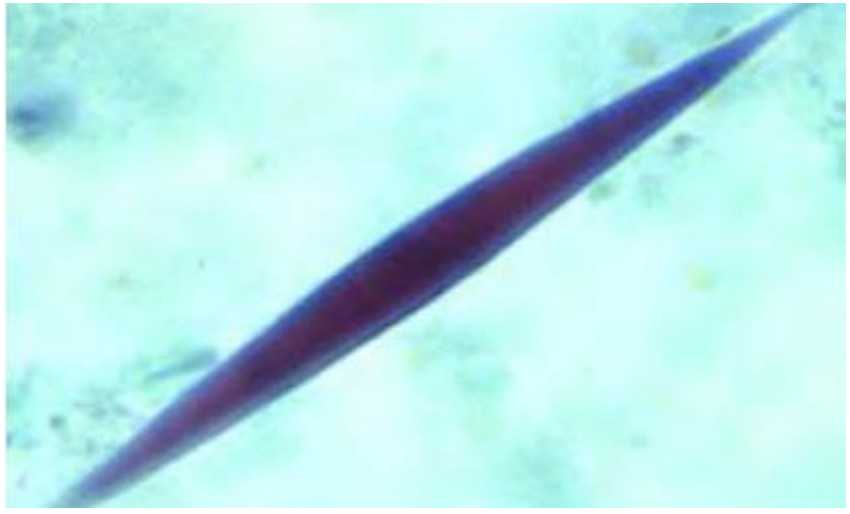
Fungal spore in concentrated wet mounts of stool. Such spores may be confused for protozoa such as *Giardia*



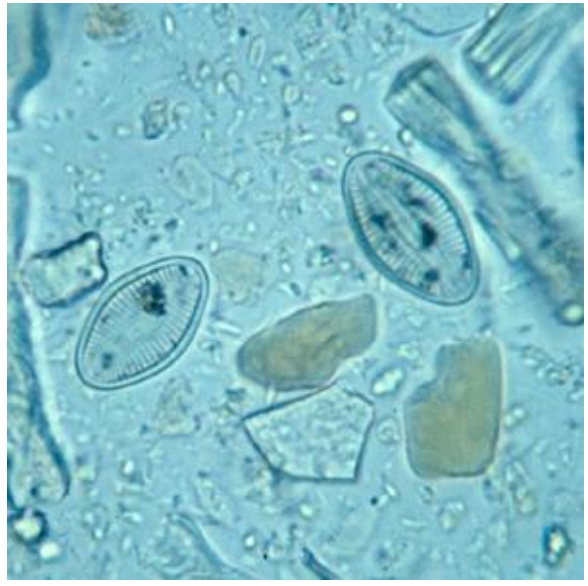
Fungal spore in a wet mount of stool. Such spores may be confused for the cysts of *Entamoeba*



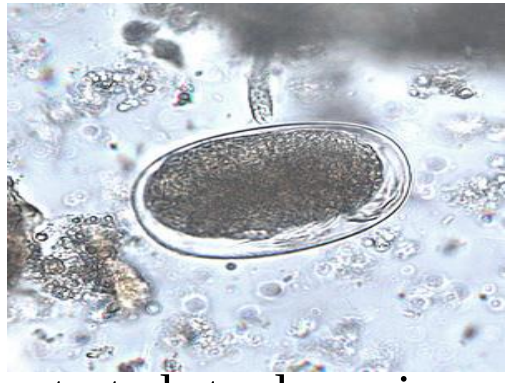
Yeast cells can be confused with cryptosporidium oocysts



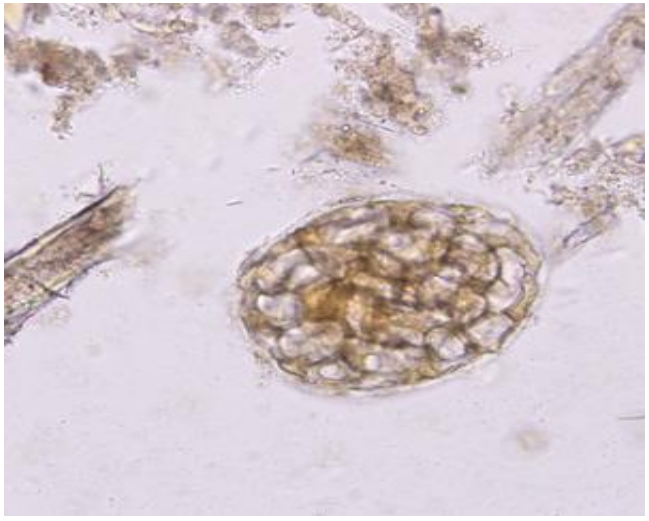
Charcot-Leyden crystal in the picture can be mistaken with hair



Diatoms do not specifically resemble any parasites of humans, but their size and shape and apparent structure are striking



Mite egg in a formalin-concentrated stool specimen. Mite eggs are similar to hookworm eggs but are usually larger (but not always). In this specimen, leg buds can be seen in the lower right area of the egg



Plant cell in stool. Such material can be common in stool and may be confused for helminth eggs, although they are larger than the eggs.

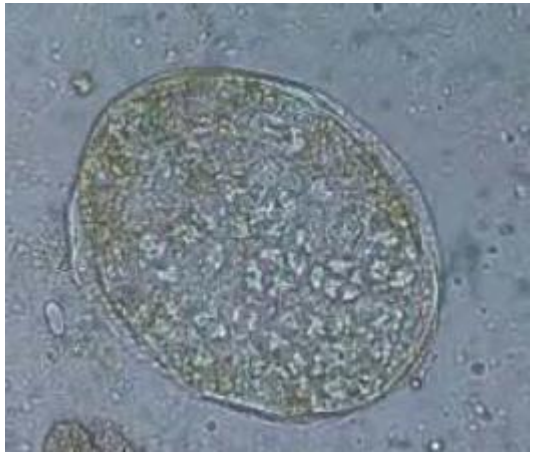
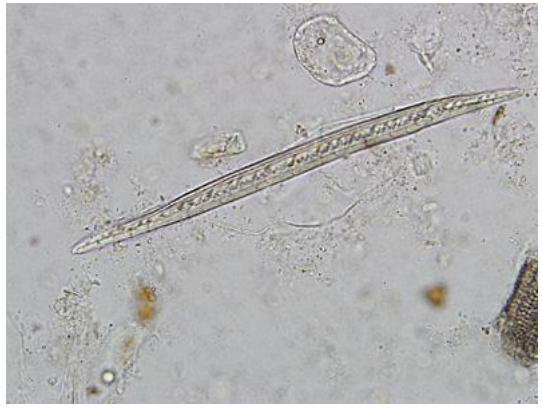
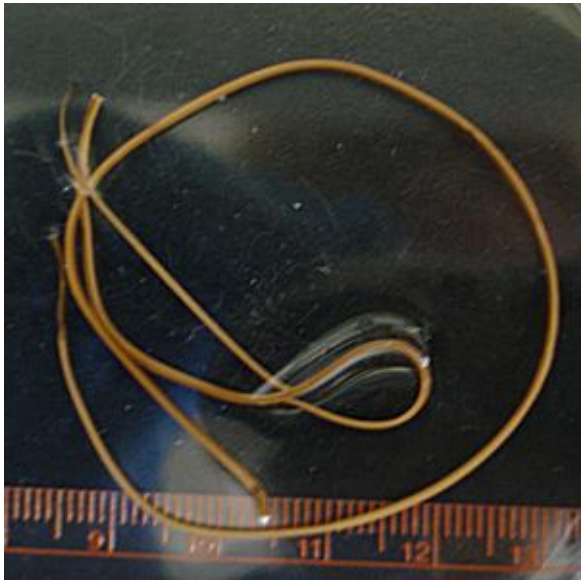


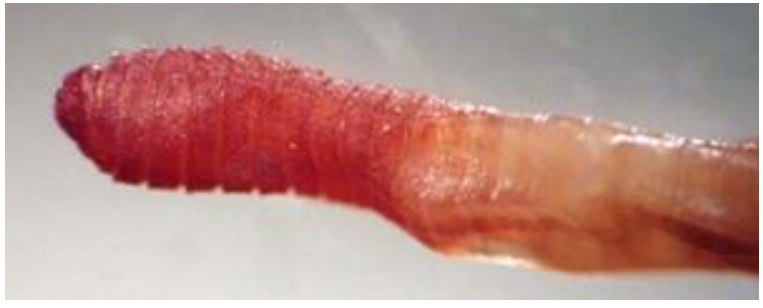
Image shown plant cell, which can be mistaken with paragonimus egg



Plant hairs can be common in stool and may be confused for the larvae of hookworm or *Strongyloides stercoralis*. However, they are often broken at one end, have a refractile center and lack the strictures seem in helminth larvae (esophagus, genital primordium)



Horsehair worms are parasites of insects and may be found in households and end up in toilets. As a result, they are often sent to the public health laboratories for identification.



Earthworms (*Lumbricus* and related) are commonly sent to the public health laboratories for identification. The presence of setae, segmentation, and a clitellum (red arrow) should distinguish them from parasitic helminths

Aquatic larvae of flies. The free-living aquatic larvae of various flies breed in standing water, including toilets, leading to the misconception they came from stool or urine. The presence of prolegs, a head capsule, breathing tubes (arrow), segmentation and/or setae will usually distinguish them from true parasitic worms.


THANK YOU