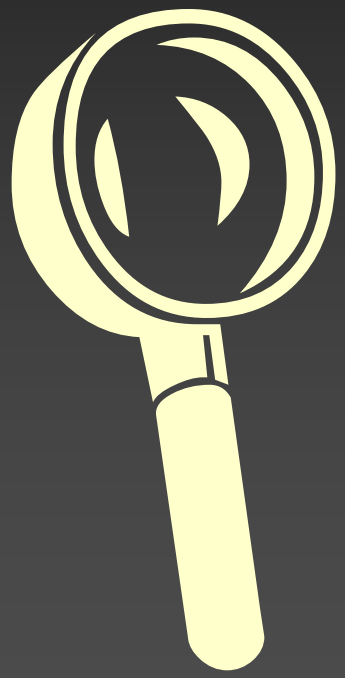
Institute for microbiology shows

TRACING THE CRIMINAL



Part eleven:
Criminals – parasites

For introduction…

Picture by: Petr Ondrovčík

Are you sure, that your new clone of giant bed-bug is my favourite birthday present?

Survey of topics

Parasites – introduction

Parasites – clinical description

Parasites – sampling

Parasites – diagnostic methods

Parasites – pictures

Parasites – introduction

Parasites classification

- Parasites are clinically important animals, not allways microscopical. They may be classified according to placing inside the organism, zoological criteria and other properties.
- Endoparasites (inner parasites):
  - Protozoa (amoebae, flagellates etc.)
  - Roundworms (pinworm, common roundworm,Trichuris, dog and cat roundworm)
  - Flukes (liver fluke, schistosoma)
  - Tapeworms (swine tapeworm, beef tapeworm, fish tapeworm, dwarf tapeworm)
- Ectoparasites are mostly various arthropods

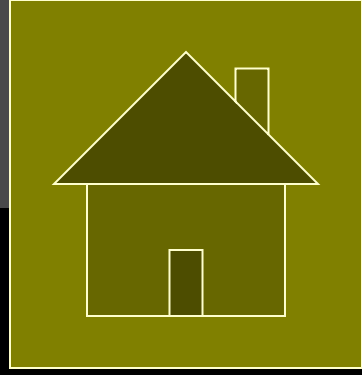
Historical term „worms“

- The term „worms“, or its latin equivalent „helmints“ was historically used for organisms with prolonged shape of body
- For practical reasons, we use this term sometimes even now
- Majority of them are **visible by a naked eye or a simple lens**. Some of them are quite large (e. g. 10 m in common tapeworm). Their eggs are microscopical

Flat worms and roundworms

- Zoologically, there are **at least two unrelated groups of organisms**
- **Flat worms (Plathelmintes)** are really flat at section. Among clinically important organismes we have here
  - **Flukes (Trematoda)** and
  - **Tapeworms (Cestoda)**
- **Roundworms (Nemathelmintes)** are round section. Group **Nematoda** belongs here

Another parasites classification



- It is also common to classify parasites according to organ systems:
  - Intestinal Parasites (from Lamblia to tapeworms)
  - Blood parasites (intra- a extraerytrocytal)
  - Urogenital parasites (e. g. Trichomonas)
  - Tissue parasites (e. g. Toxoplasma)

This classification is important also for diagnostics. Logically, in tissue parasites e. g. indirect diagnostics is more important: for direct diagnostics it is difficult to find a proper specimen to be taken

Parasites – clinical description

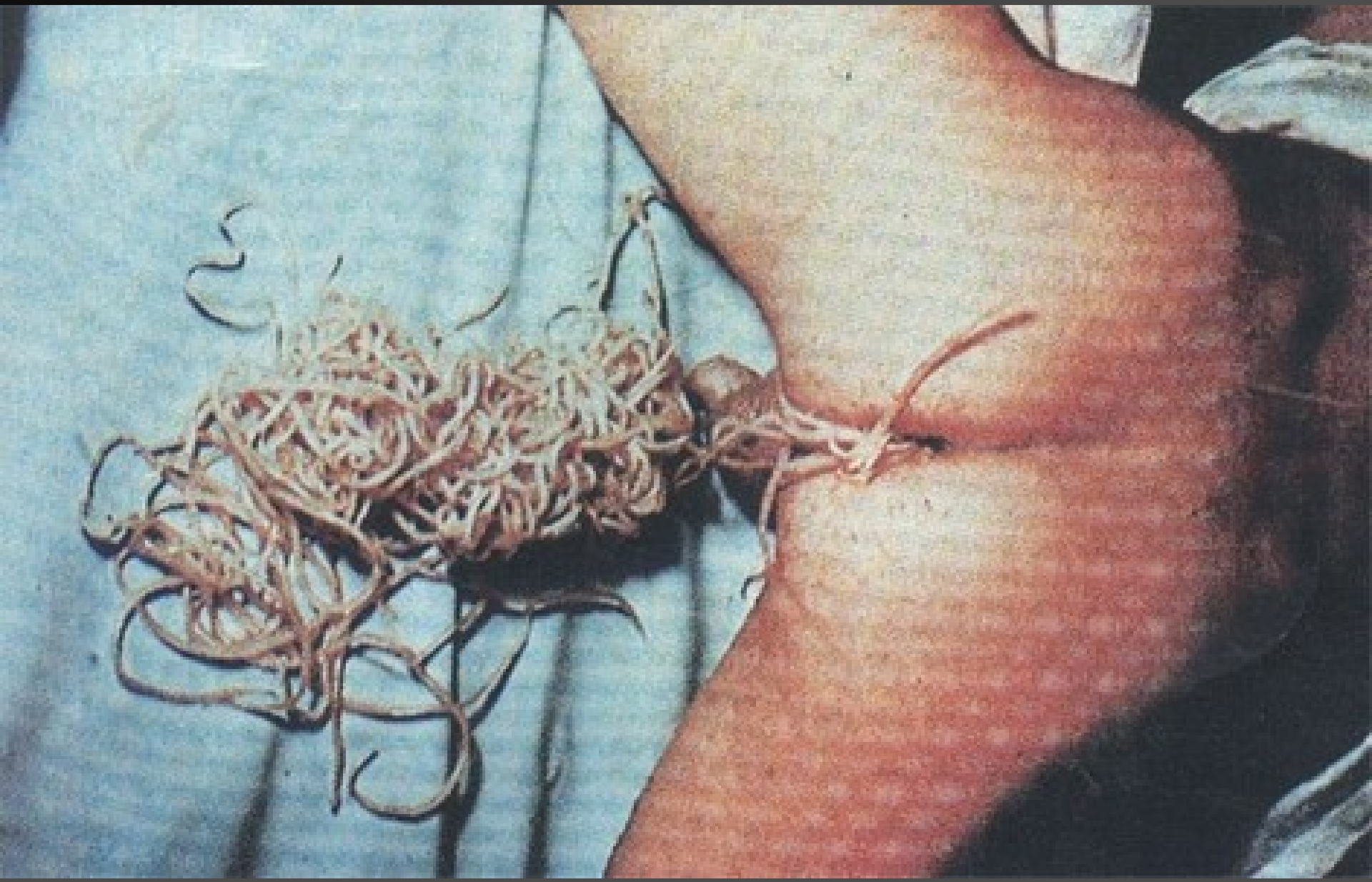
Story one

- Little Nicol all the time scraped her buttocks, and it was evident to parents and teachers that something is wrong. She was also inquiet and unconcentrated. So they placed a translucent tape on her anus and sent to the laboratory. And the result did not surprise anybody. Nicol started to use drugs and in a short time everything was in order again…

Causative agent was

- *Enterobius vermicularis* or pinworm. It is a tiny roundworm, that is present in the intestine. The eggs are present in perianal area. It is present mostly in children collectives. In small children autoinfections often occur.
- Another related roundworm is common rondworm – *Ascaris lumbricoides*. It is a little simillar to earthworm (*Lumbricus terestris*), but still slightly different. Roundwors may cause various problems, from allergic problems to mechanical obliteration of orifices of bile bladder and pancreas

Roundworms



www.sp01.com/micro/worms/imagepages/image1.htm.

Intestinal parazites

- Intestinal parazites are the most common ones. They may belong to any of endoparasital group. Some of them result in diarrhoeic diseases, some of them produce rather non-specific problems (dyspepsia, pruritus, tiredness…)
- **Most common ones:** Protozoa: amoebae (*Entamoeba histolytica),* flagellates (*Giardia intestinalis* = *Lamblia*). Nematoda: Pinworm, common roundworm and others. Trematoda: e. g. *Fasciolopsis buski*. Cestoda: common (swine and beef) tapeworm and other tapeworms

Non-pathogenous protozoa in the bowel

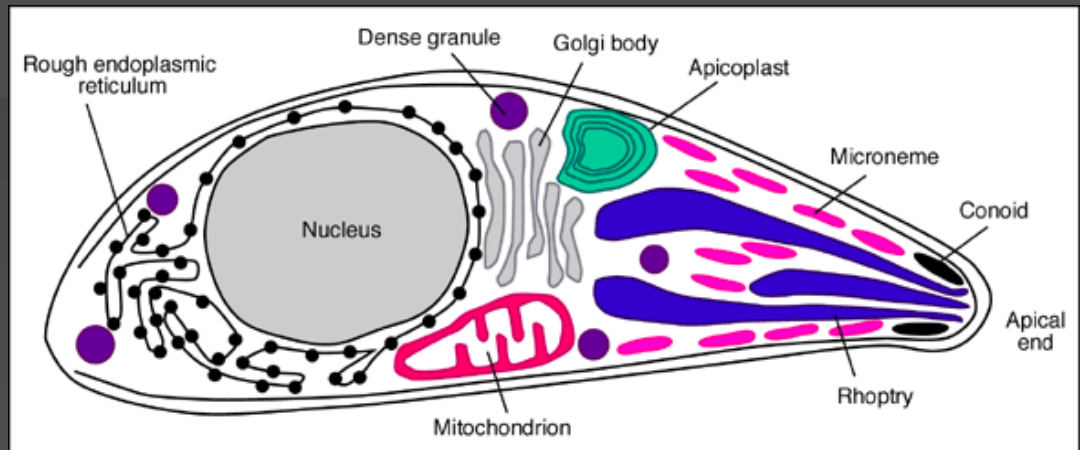
- **Not allways presence of parazites**, especially protozoa, **is a reason for treantment.** There are many species, that are considered to be rather non-pathogenous.
- The most important **non-pathogenous protozoa** are: *Entamoea dispar* (very simillar to pathogenous *E. histolytica!), Entamoeba coli, Entamoeba hartmanni, Endolimax nana, Iodamoeba buetschlii* and others.

Story two

- Bianca, who took care of several cats, had enlarged lymphonodes for some time, and long time it was a question, what is the reason. The throat swabs showed nothing, and also results ot other investigations were resultless.
- Bianca planned to have a child, and so she was affraid. And it was shown, that it is really so: the causative agent responsible for her lymphonode sydrome is really sometimes dangerous for pregnant women…

The causative agent was

- ***Toxoplasma gondii,*** a protozoon, that is transmitted by cats, although people say that people with dogs are in risk more than people with cats (they can wear the particles of cat faeces in their fur)
- In immunocompetent persons, majority of infections is **asymptomatical**



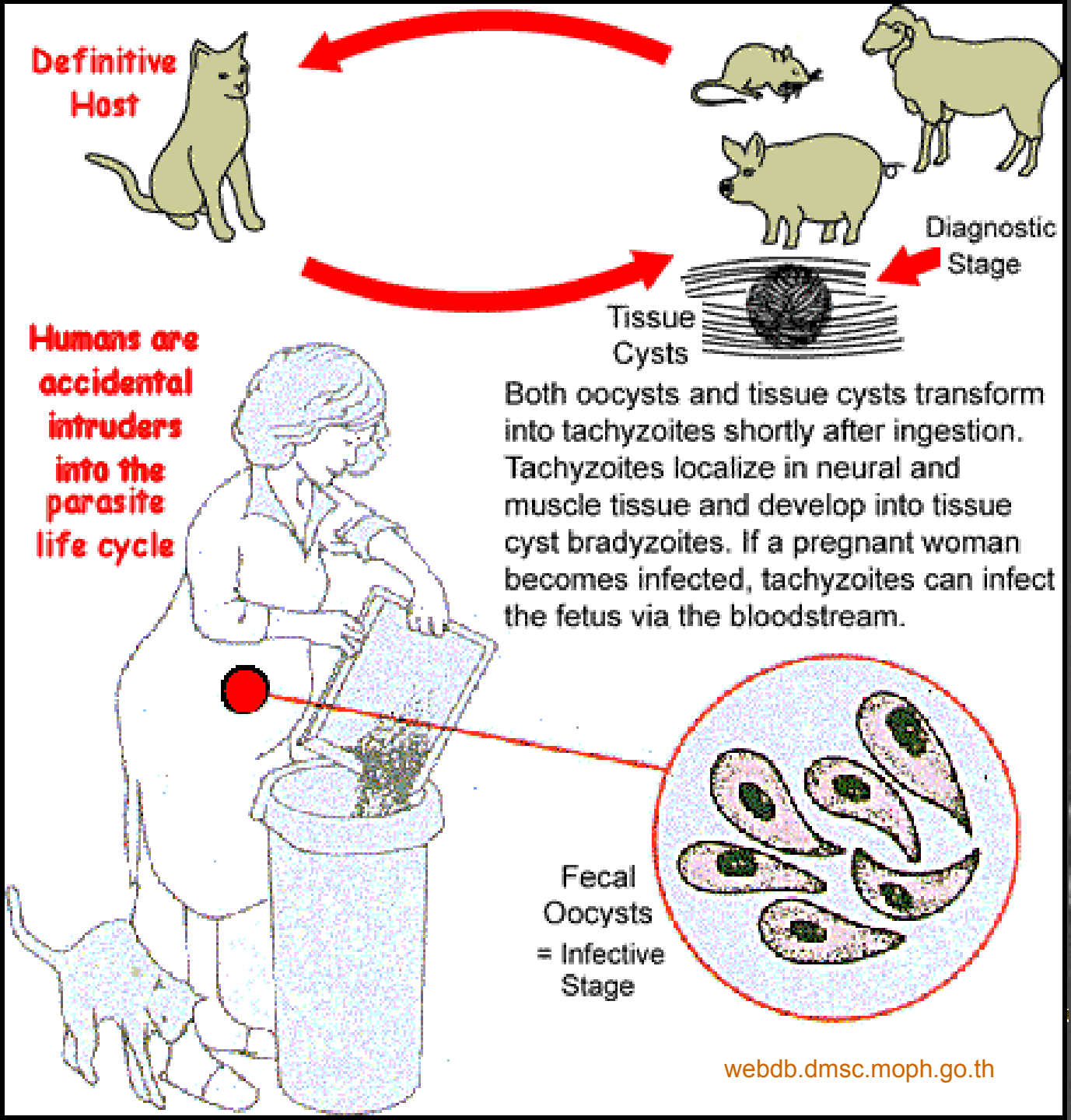
Ultrastructure of a *Toxoplasma gondii* tachyzoite

Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

http://fullmal.hgc.jp

In some persons e. g. a toxoplasma retinitis may occur…

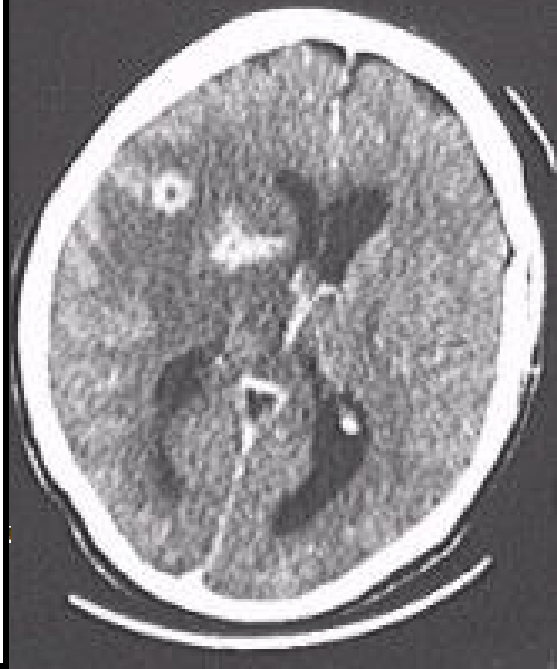
http://web.indstate.edu



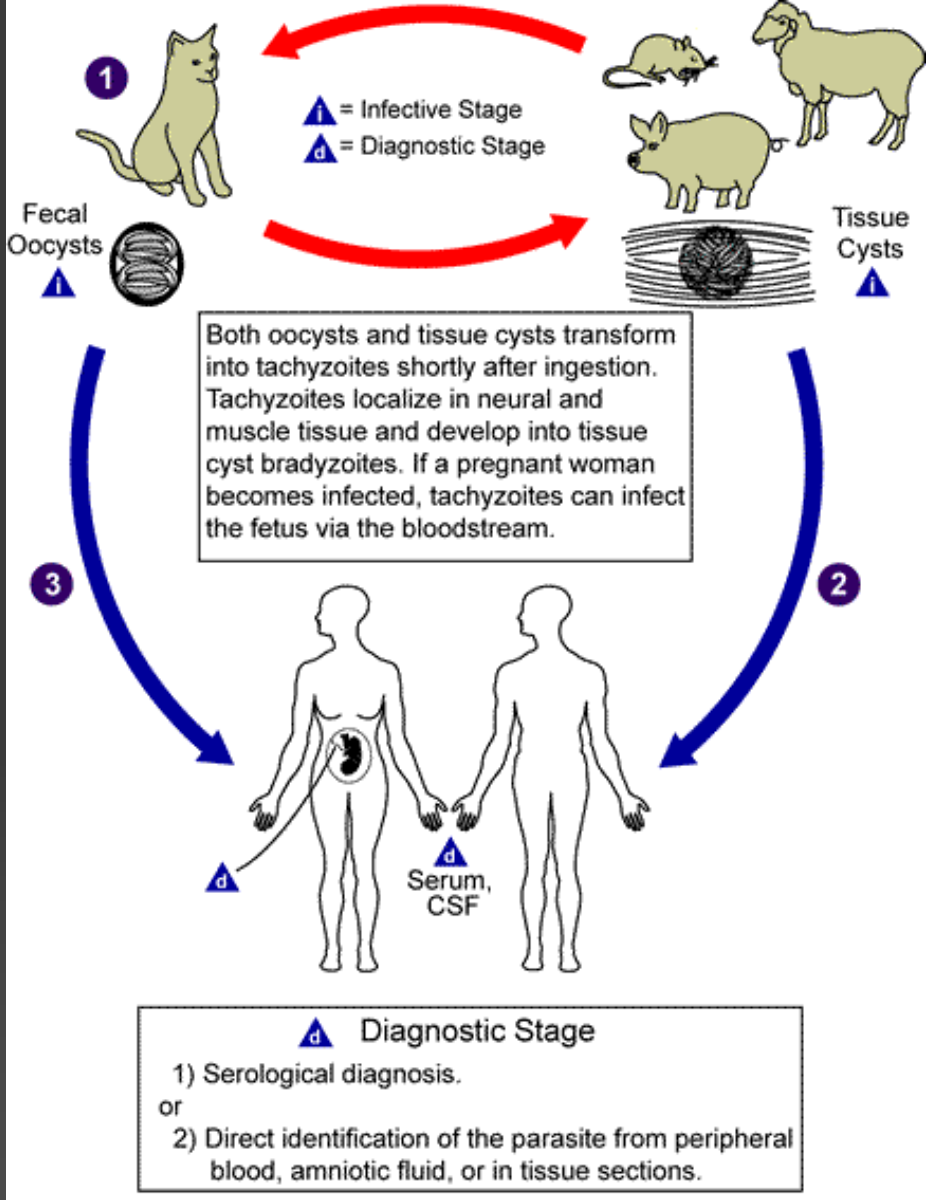
Toxoplasma life cycle

Down: toxoplasma cyst in brain

www.antoranz.net



Toxoplasma – life cycle



http://www.dpd.cdc.gov/dpdx/images/ParasiteImages/S-Z/Toxoplasmosis/Toxoplasma_LifeCycle.gif

Tissue parasites

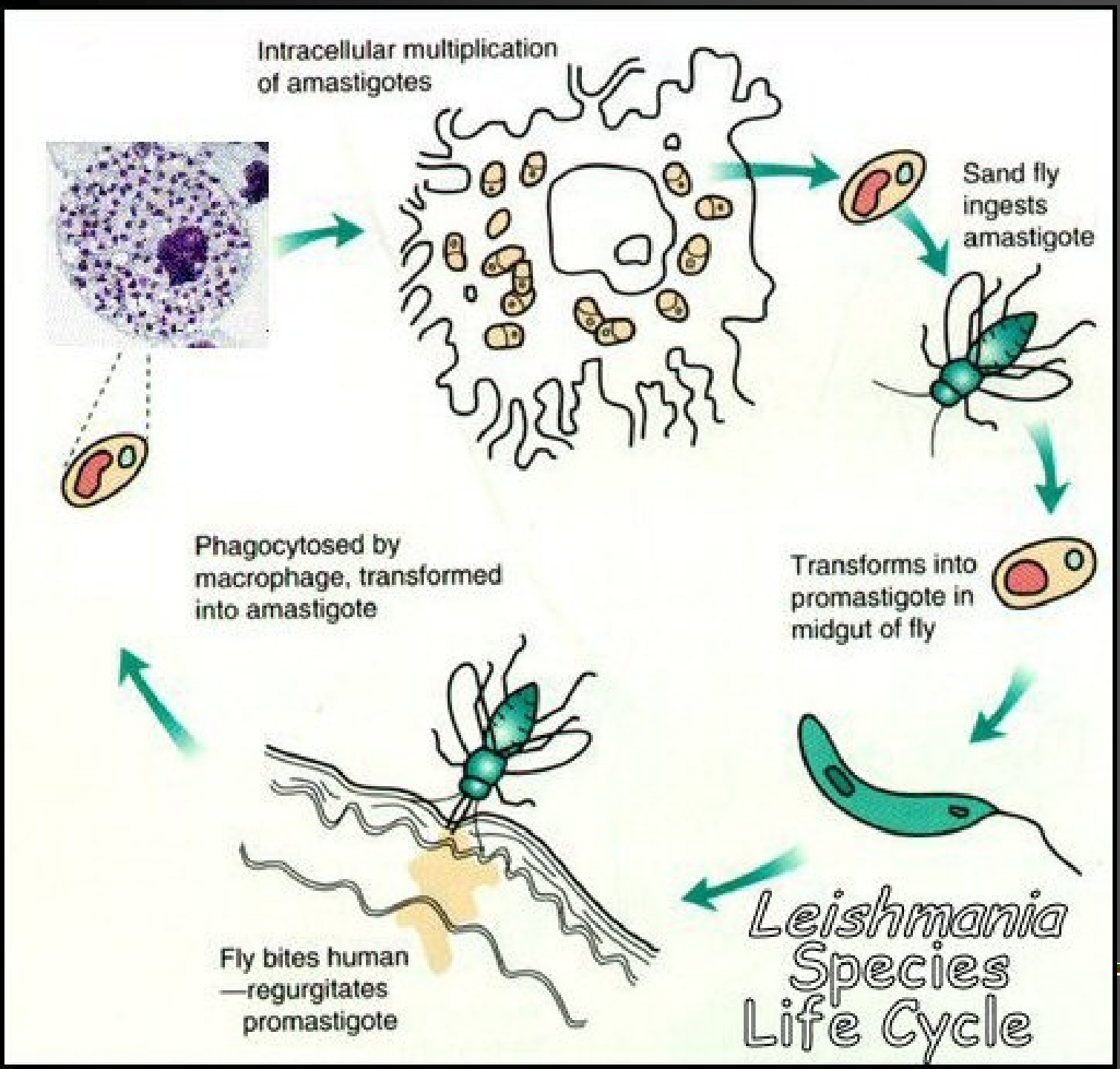
- Some parazites may live inside the organism.
  - Some **protozoa**, like toxoplasma, produce **parasital cysts.**
  - Some **nematodes** may be present in tissue, e. g. *Toxocara canis* or *T. cati*
  - Some **cestodes** (e. g. swine tapeworm) may produce **cysticercs** in the tissue
- **Symptomatology** is variable. In toxoplasmosis, cysts are clicinally quite mute. Cysticercs of tapeworms may be dangerous e. g. by pressing to important organs
- **Diagnostics** is difficult, because it is usually nothig to take for direct diagnostics.

Tropical and subtropical tissue parasites

- Among other important parasitoses there are **leishmanioses.** They occur in the whole tropical and subtropical band
- **Vector** is a tiny blood sucking insect *Phlebotomus*
- There exist **some twenty important species**, that may be classified into **“Old world“ leishmaniae and „New world“ leishmaniae,** and also to **skin, skin-mucosal and visceral**
- They may cause diseases **from skin mutilation to inner organ damage**, often lethal

http://web.indstate.edu/thcme/micro/parasitology

Leishmaniose



http://web.indstate.edu/thcme/micro/parasitology

Story three

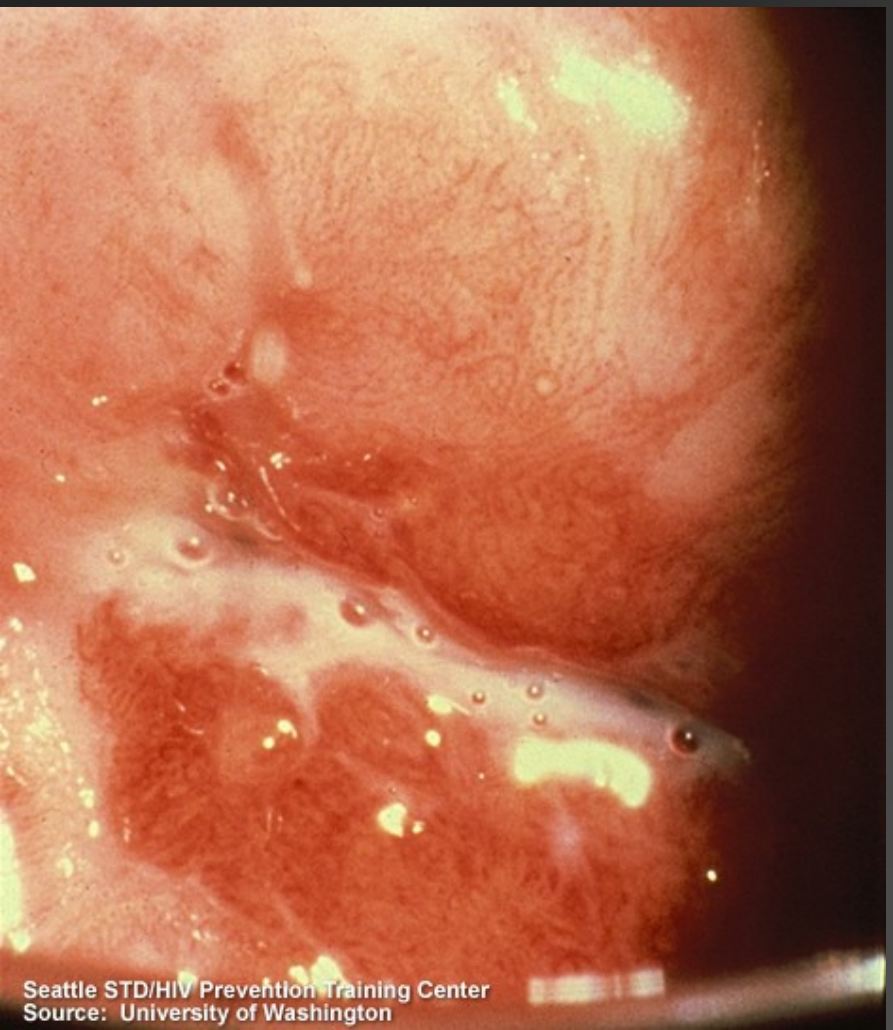
- **Joddie** had again some problems „there down“. Probably this was because she changed her lovers too often. This time bacteriological examination was of no help. So, **C. A. T. swab** was sent for examination, and so the result was found.

Causative agent was

- ***Trichomonas vaginalis*, a flagellate, that is transmitted sexually, although exceptionally other ways of transmission are possible, too**
- **Discharge** of typical smell and colour is typical
- In men, the disease is usually **asymptomatical**
- As treatment for anaerobic infections (metronidazol) is effective for trichomonosis, too, the number of trichomonad disease **decreases** in recent times.

Trichomonad discharge

depts.washington.edu



holebi.info/gids.php

So called „strawberry cervix“

webmedia.unmc.edu

Urogenital parasites

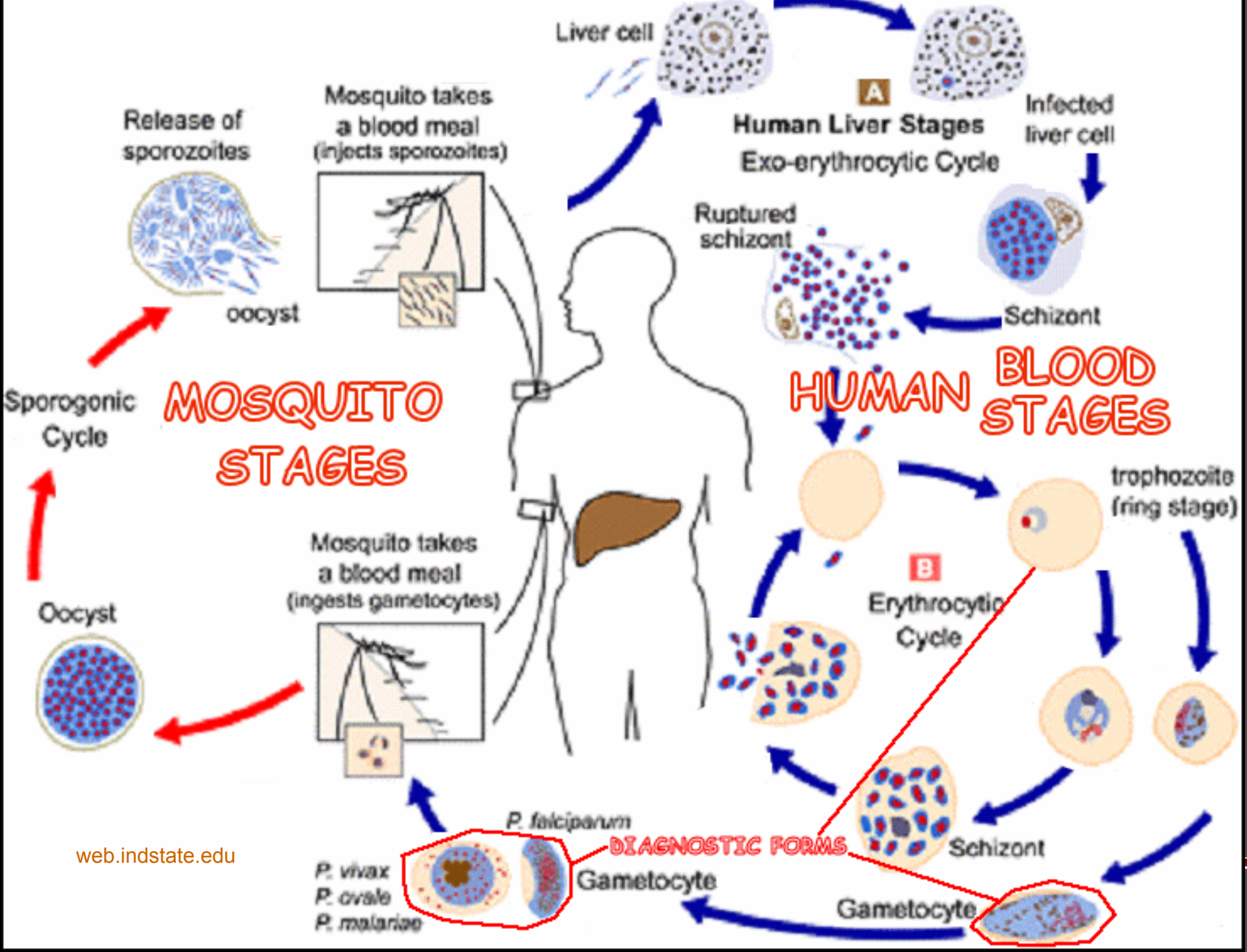
- Among **sexually transmitted** parasital diseases, trichomonosis is the only really important (if not discussing the pubic louse).
- Usually its diagnostic is done together with bacteriologica examination, as the ethiology is rarely clear at the first moment
- In the **urinary tract**, most important parasites are some flukes (schistosomas), i. e. Trematodes. Diagnostic is partially microbiological, partially histological.

Story four

- **Mr. Hiker** used to travel thoughout the world. After coming back from the last trip he started to have some probles, he caugth fever, then it finished, but **during three days** it started again. The GP sent him to **infection department**. They took his blood and made a smear to two slides, using two various methods. And really the causative agent was…

Causative agent was

- ***Plasmodium vivax*,** one of four species of malaric plasmodia
- **Malaria** is worldwide one of the most serious diseases. It causes ilness of many persons every day, including many coming from Europe.
- The worst course is in „tropical malaria“ or „malignant tertiane“, caused by ***P. falciparum.*** Milder are both „benign terciacas“, caused by ***P. vivax*** and ***P. ovale***. The quartane, caused by ***P. malariae***, is rare


Liver cell
A
Human Liver Stages
Exo-erythrocytic Cycle
Infected liver cell
Ruptured schizont
Schizont
Release of sporozoites
Mosquito takes a blood meal (injects sporozoites)
oocyst
Sporogonic Cycle
MOSQUITO STAGES
HUMAN BLOOD STAGES
trophozoite (ring stage)
B
Erythrocytic Cycle
Mosquito takes a blood meal (ingests gametocytes)
Oocyst
P. falciparum
DIAGNOSTIC FORMS
Schizont
P. vivax
P. ovale
P. malariae
Gametocyte
Gametocyte
web.indstate.edu

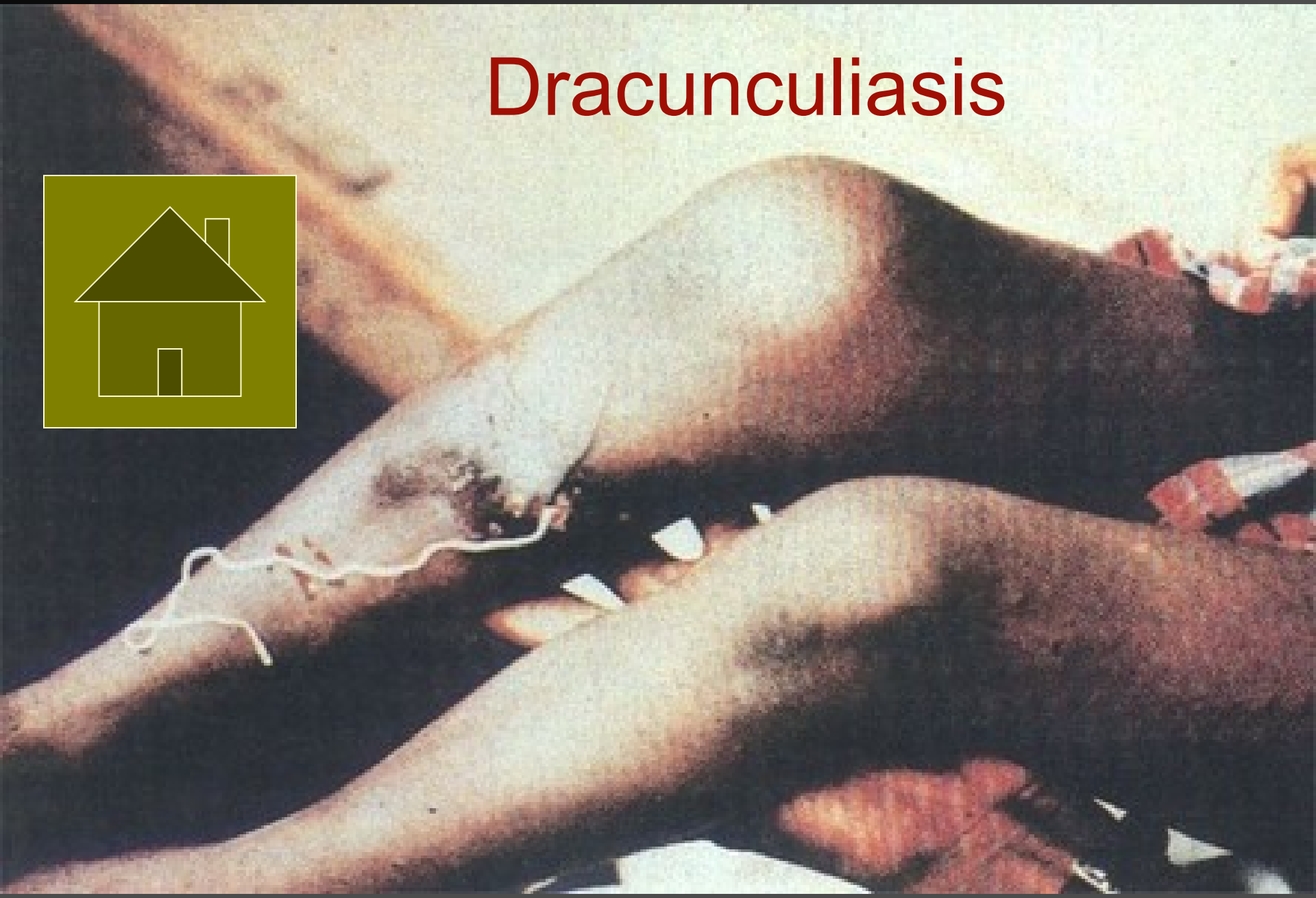
Bloostream parasites

- Among bloodstream parasites, of course, malarial plasmodia are the most important, but not the only ones.
- A **flagellate**, *Trypanosoma brucei* lives in bloodsteam, causing sleeping disease. *Trypanosoma cruzi* lives there too, producing Chagas disease
- **Nematodes** – filariae may live in bloodstream, too.
- **Symptomatology:** in all of them fever appeares, other symptomas are related to the agent

Elefantiasis

www.sp01.com/micro/worms/imagepages/image1.htm.

Dracunculiasis



www.sp01.com/micro/worms/imagepages/image1.htm.

Parasites – sampling

Sampling

- For **intestinal parasites** rectal swab is not sufficient, a bit of stool is needed (see more →)
- For ***Trichomonas*** either a slide for Giemsa staining is sent (alone or in pair with another one for Gram staining), or a C. A. T. swab
- For ***Acantamoeba*** used contact lenses are sent in their own fluid, eventually corneal scraping might be performed
- For **tissue parasites** serum is sent usually
- In **other** pararasites we sample ccording to situation (urine, content of a cyst)

Sampling for intestinal parasites

- To send stool for parasitological sampling (usually using Kato and Faust methods), we need **sample of stool sized like a hazel nut**. A vessel for sampling need not be sterile. Unlike virological examination the sample does not need low transport temperature
- *Specimen sized like a coconut (as sometimes some student say) is not recomended ☺*

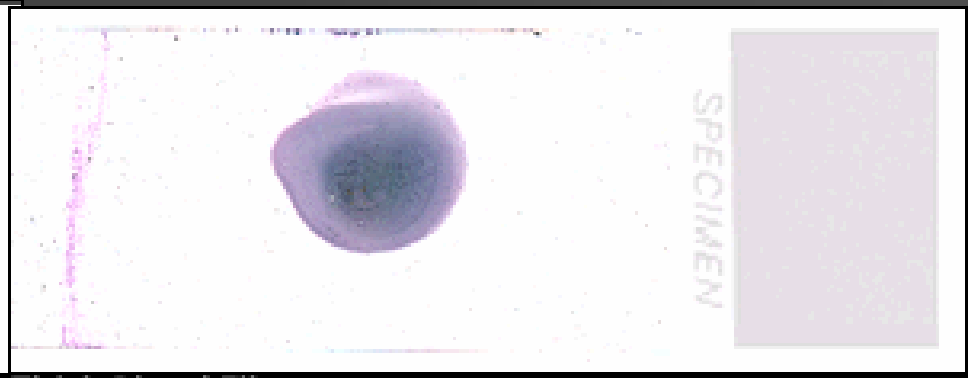
Sampling for pinworms

- For pinwoms, it is possible to send a stool specimen as for other intestinal parasites, but it is not optimal, because a better method exists
- It is Graham method, where a tape is placed around anal orifice, then removed, placed on a slide and sent to the laboratory (and here examined microscopically
- For technical reasons, the method is not very useful for adult persons, here classical stool specimen is sent usually

Sampling for blood parasites

- For blood parasites, it is recommended to send a thick smear and a thin smear. Thick smear is just a drop mixed by a corner of another slide, thin smear is spread to the slide by special movement. Thick smear is not fixated.

Thin Blood Film



Thick Blood Film

Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

Some more sampling methods

Search for	Used sampling procedure
toxoplasmosis	serum (for antibodies)
trichomonosis	C. A. T. swab, or smear
urinary schistosomosis	histological examination
giardiasis	duodenal juice (or stool)
acanthamoebiasis	used contact lens

C. A. T. swab for urethral and vaginal sampling for yeasts and Trichomonas

Parasites – diagnostic methods

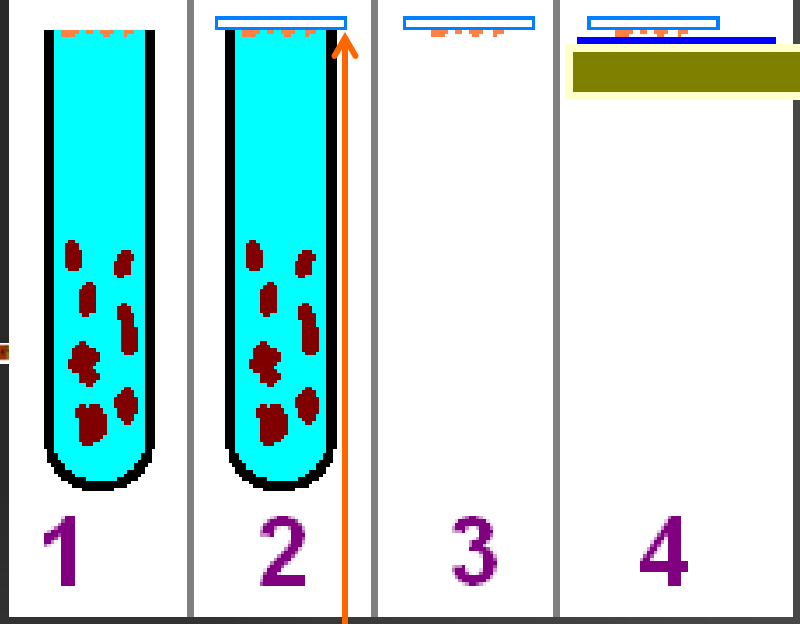
Parasites: diagnostical methods

- **Microscopy is important, either wet mount,** or **staining** (trichrom, Giemsa stain)
- **Culture** is rarelly used, in practice only in *Trichomonas* and *Acantamoeba.*
- **Among other direct methods** PCR is used recently
- **Indirect detection** is used in tissue parasitoses, mostly toxoplasmosis, larval toxocarosis etc.

Intestinal parasites diagnostics

- As a basis, we use methods based on **modified wet mount:**
  - **In Kato method** counterstain with malachite green is used, to make parasites better visible
  - **Faust method** is a concentration one (see later)
- **Graham method** is used in pinworms only (see later)
- **Wet mount „sensu stricto“ and stainded preparations (e. g. trichrom)** are used in increased suspicion for **intestinal protozoa** (either primarilly, or after seeing Faust and Kato)

Faust method



In the second halft, Kato is already prepared

- Principle: **stool is repeatedly mixed with $ZnSO_4$ solution, centrifugated and supernatant taken for the next step.** Finally, the solution is filled up to the top of the test-tube and covered by a coverslip. The parasites adhere to the coverslip from below. Then coverslip is removed and placed onto one halfth of a slide. On the second halft of the slide, Kato method is already prepared usually.

Methods for diagnostics of intestinal protozoa

- Helmint eggs are found directly in Faust and Kato methods. When something resembling cysts (of trophozoites) of protozoa is found, more methods are used. We use here
  - **Wet mount**, just stool mixed with a drop of saline, eventually a drop of Lugol solution is added after first observation to see better some structures
  - **Trichrom staining**. Fixation using alcohol-sublimate and further 70% alkohol, proper trichrom, 96% alcohol and carbolxylene. Or haematoxylin stain.
  - *for cryptosporidia eventually Ziehl Neelsen, or , in Czechia, Miláček staining (Mr. Miláček was a laboratory, assistant in parasitology in České Budějovice)*

Graham method in pinworm diagnostics

- The patient bends forward, stretches his/her buttocks, and now a **special transparent sticky tape** is sticked on his/her anus and mostly perianal rugae. Then the tape is removed again and sticked to a slide.
- **Transparency of the tape is crucial,** otherwise it is not possible to microscopy. (Nevertheless, some „experts“ send a non-translucent tape, or cover all the tape by a label with patient name)
- It is **easier than stool examination**. It is still used rather in children – adults use to have to hairy anus, so the method woudl be too painflul and difficult.

Practical microscopy in Faust, Kato and Graham methods

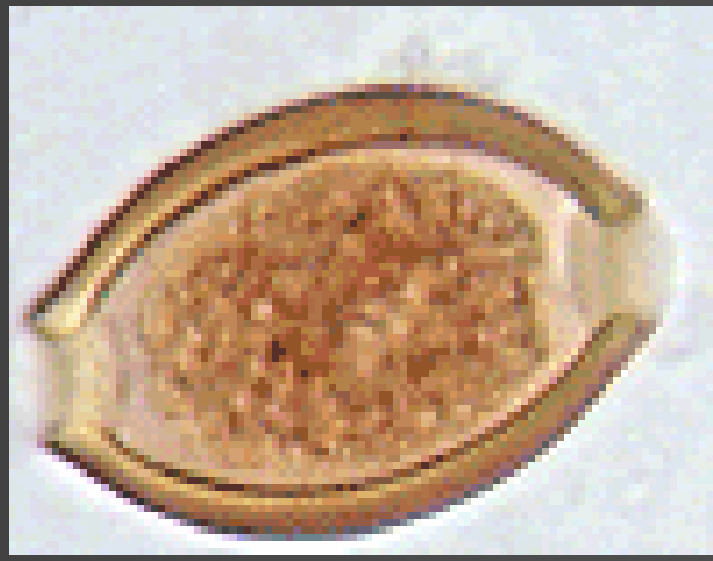
- **In all three methods we use no immersion, objectives 10×, 20×, 40×.**
- **Faust and Kato** methods are usually observed together, on the same slide
- **Graham method** result is microscopied just in the form how it comes to the laboratory, no preparation is needed.

Morphology of eggs of intestinal parasites

You should know at least these shapes to the examination

Pinworm

Enterobius



Trichuris

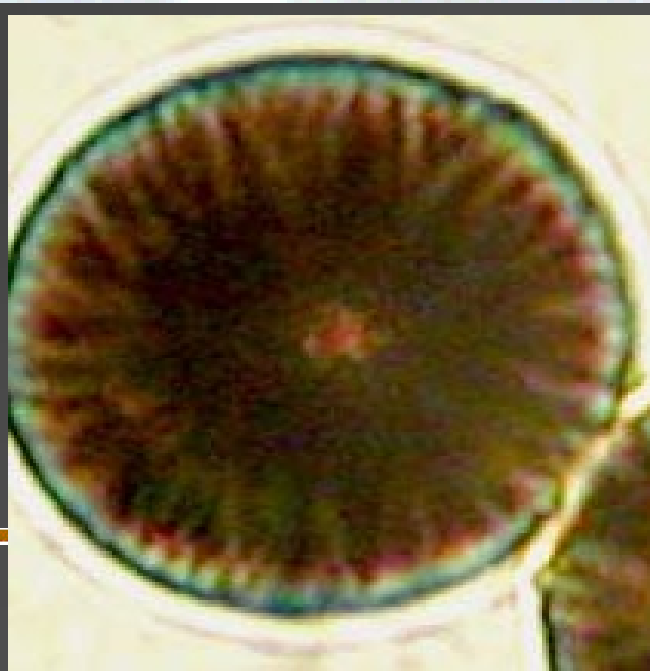
Roundworm

Ascaris

Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

Tapeworm

Taenia



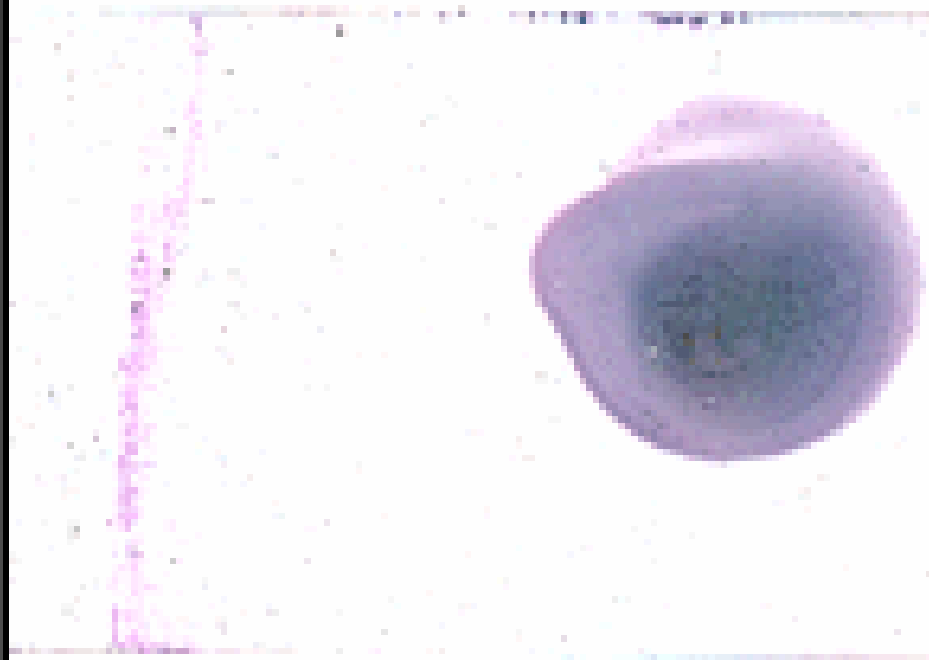
Diagnostics of blood parasites: thin smear and thick drop

- In diagnostics of blood parasites it is important to perform a smear using special methods of **thin smear and thick drop.**
- For both methods, fresh blood is used, of non-clotted blood, if the smear is not performed immediatelly. The thin smear is fixated, the thick drop is not. Both of them are **Giemsa stained.**
- Look at following pictures and short videoclips from a CD-ROM „Parazite Tutor“.

Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

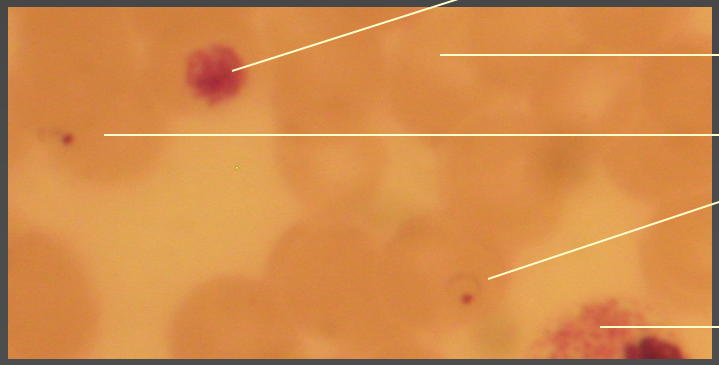
Thin smear

Thick drop



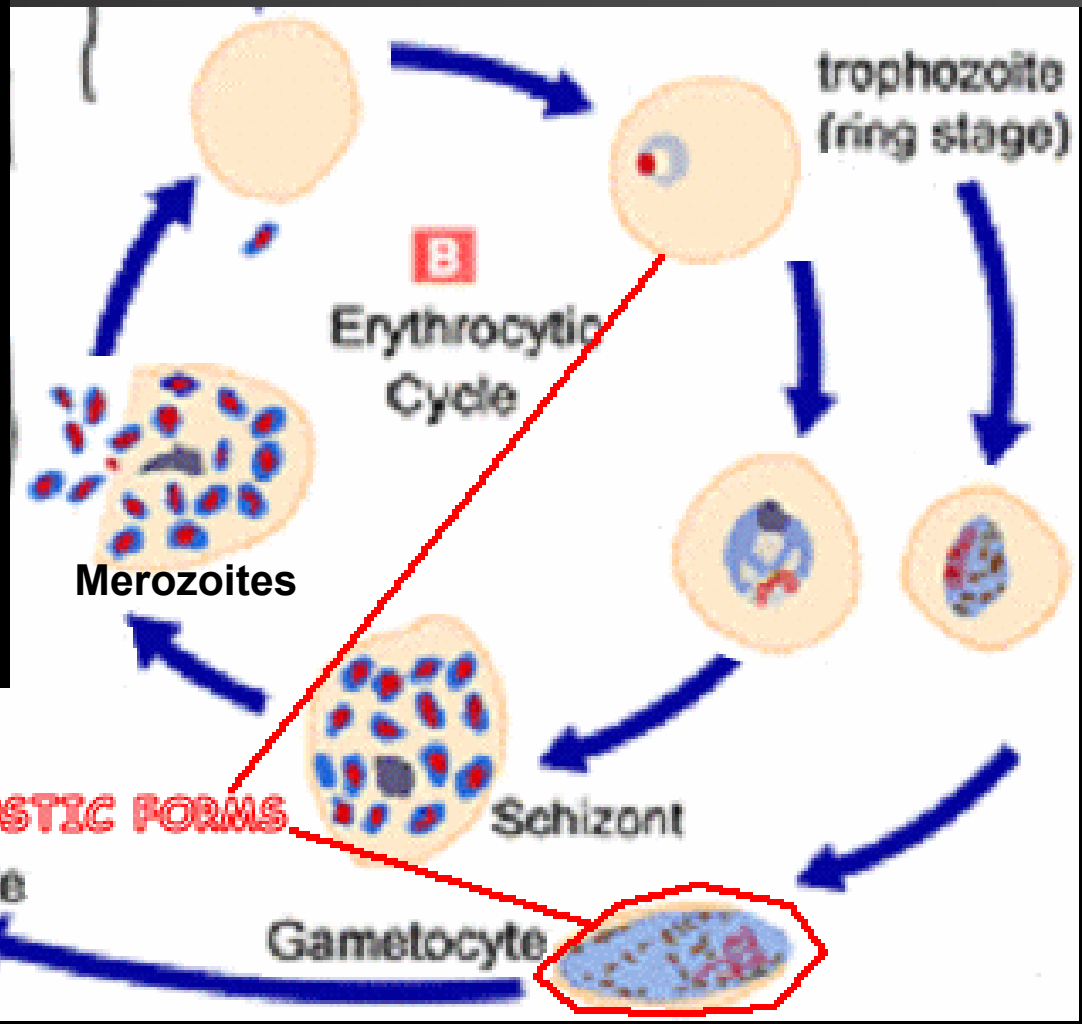
Microscopy of blood parasites – an example of a result

- We use immersion system, 100× objective. The preparation is usually Giemsa stained. On the picture below, we can see erythrocytes and young trophozoites of *Plasmodium falciparum*.



Erythrocytal stages of parasite, observable in the smears

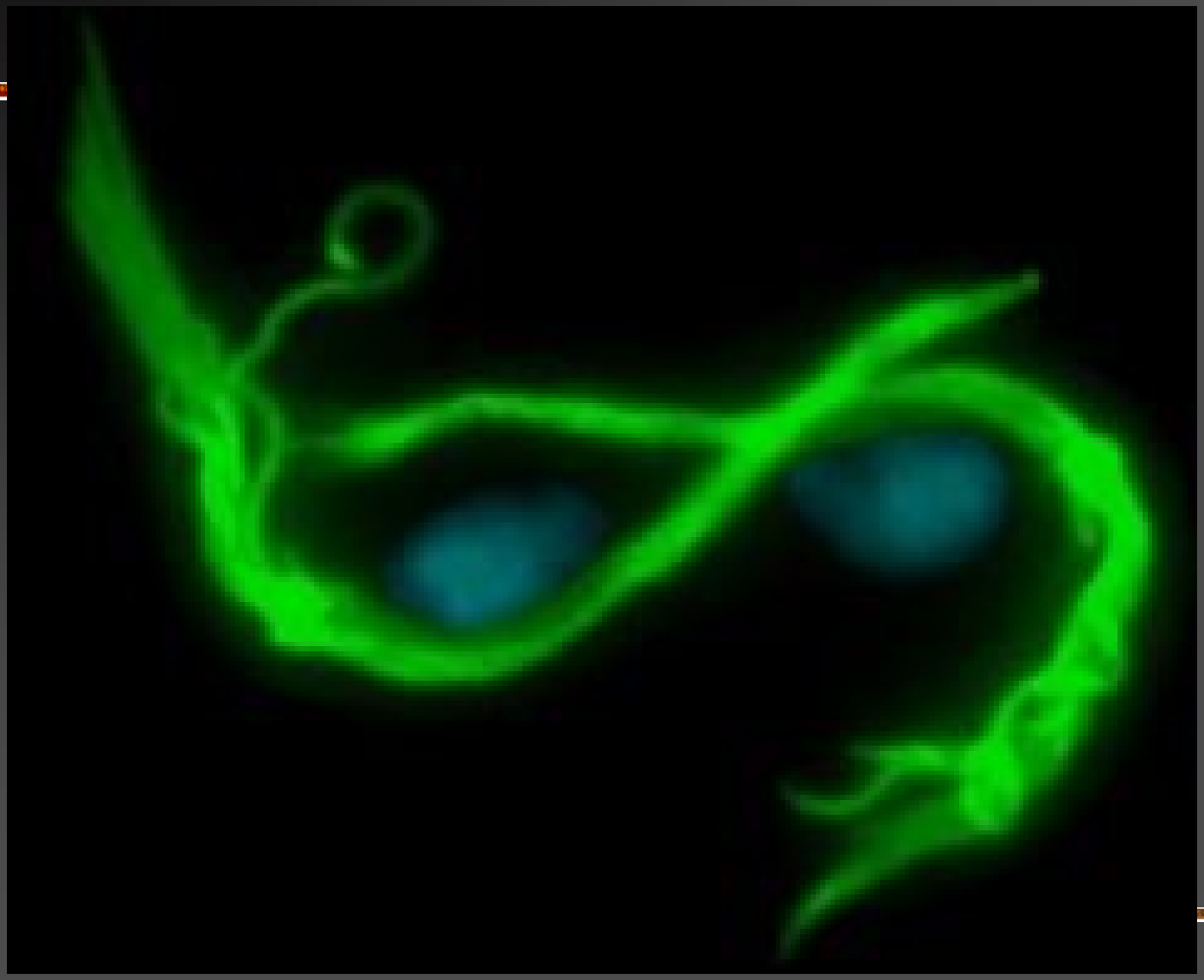
(Reduced to human erythocytal stages only, i. e. not liver stages and not mosquito stages)



Trichomonas diagnostics

- Trichomonads are recently diagnosed mostly using **culture-microscopical:**
  - A **C. A. T. swab** is performed
  - The medium is **cultured** overnight
  - A drop of medium **is microscopied like a wet mount**.
- The preparations **cannot be preserved**
- Therefore in our practicle we have the second possible way of diagnostics – **Giemsa stained smear on a slide**. When it is a part of „Microscopical appearance of vaginal microflora“ (MAVM), it is described as MAVM V.
- Other ways (e. g. fluorescence staining, see picture) are used rarelly

Trichomonas – fluorescence



http://www.ncl.ac.uk/microbial_eukaryotes/christophej_no_euml_l.html

Microscopical preparations of Trichomonas in MAVM (Giemsa)

- We use **immersion microscopy** (objective 100×, immersion oil)
- In some preparations we can see **yeasts, too**
- The pictures on Internet are usually ideal cases, oftend specially stained of computer adapted.

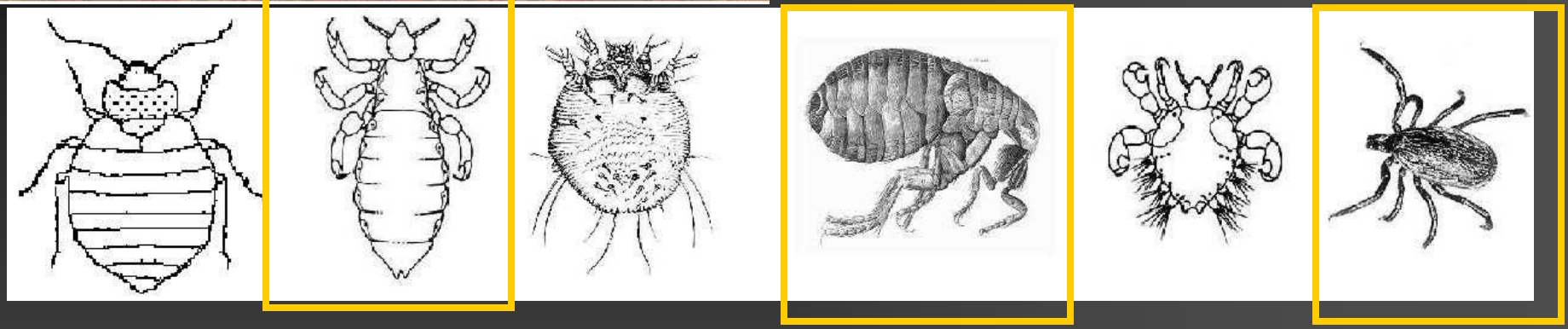
http://medschool.sums.ac.ir

Diagnostics of other parasital diseases

- In **ectoparasites** majority of diagnostics is non-microbiological (everything can be observed by a laik, eventually a dermatologist in case of *Sacroptes scabiei*)
- In **tissue parasites** serum for indirect diagnostics is sent usually (CFT, ELISA)
- In some cases, mostly tropical parasitioses, it is better to **consult sampling technique with a laboratory**

In some filarioses the sampling is recomended to perform during night only, or during day only.

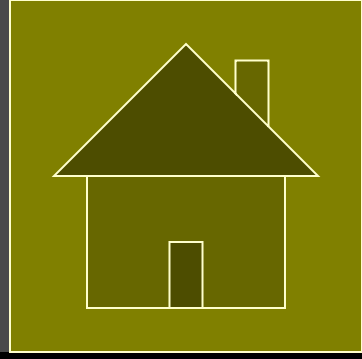
Recognizing ectoparasites



Bed bug Louse Itch mite Flea Crab louse Tick

Cimex Pediculus Sarcoples Pulex Phthirus Ixodes

Diagnostics of *Toxoplasma gondii* using serological tests

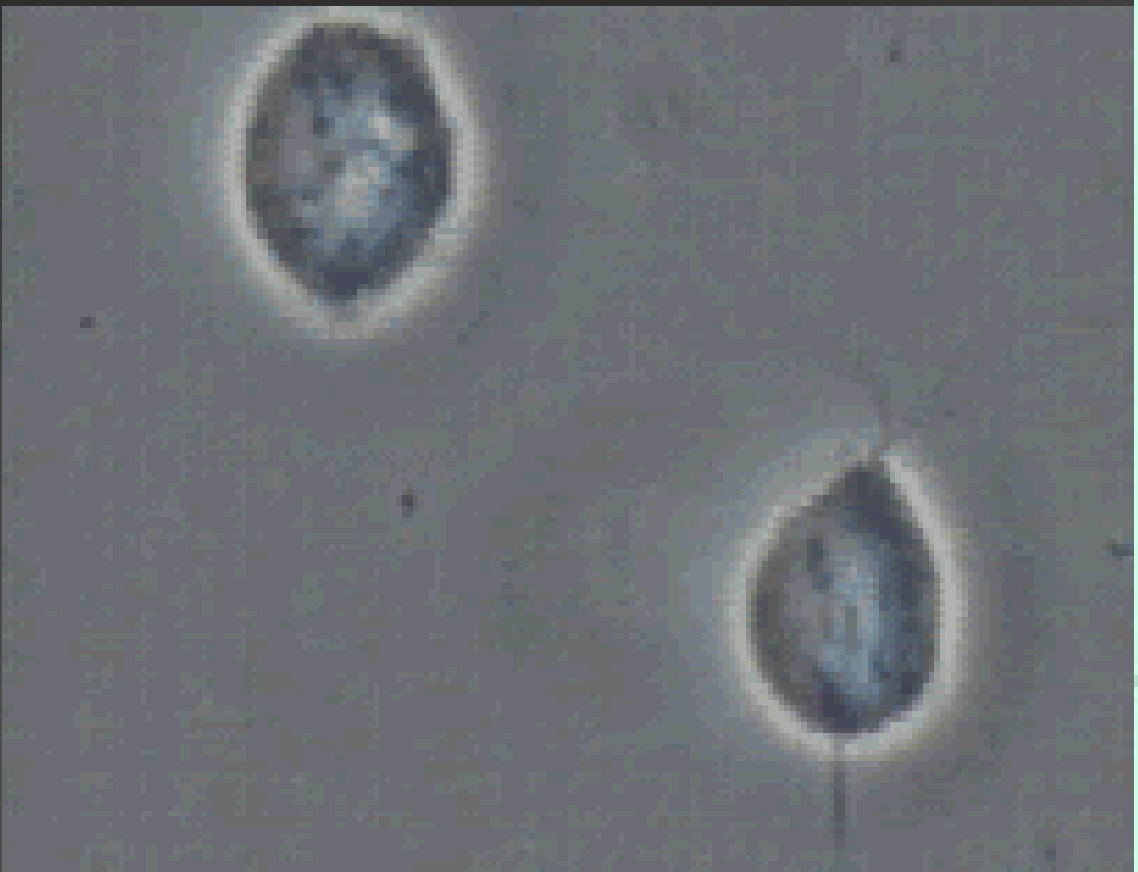


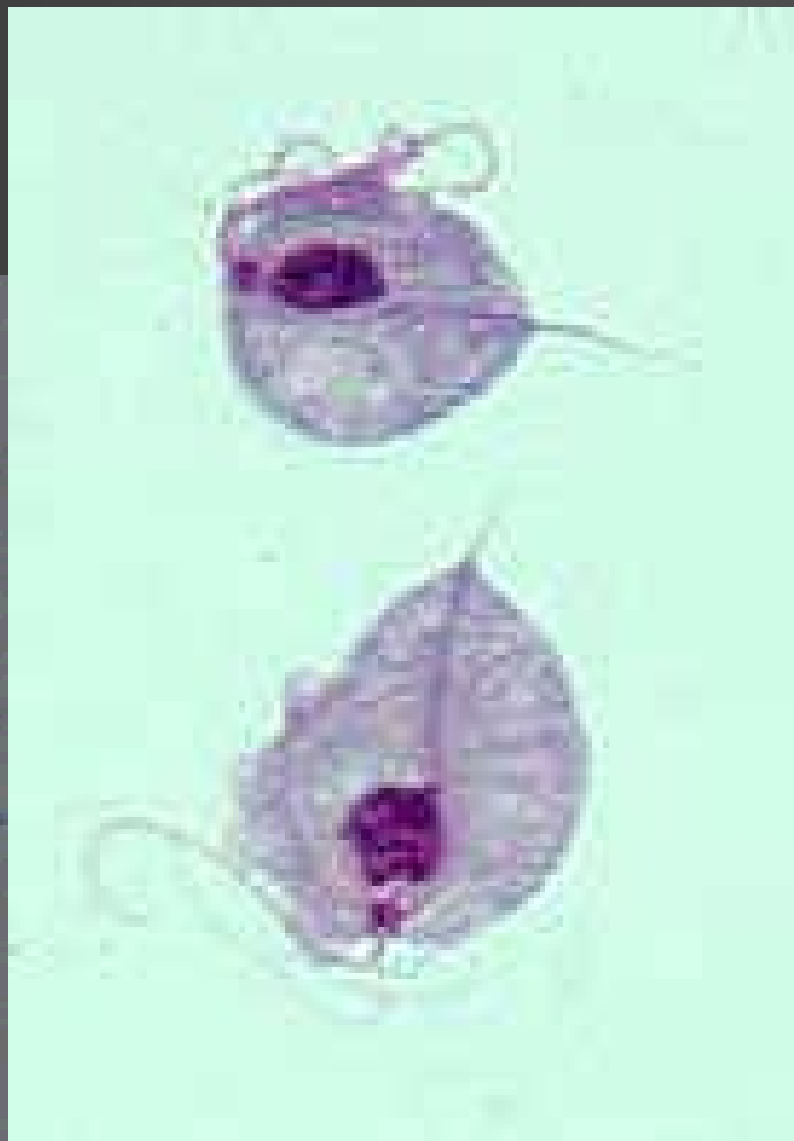
- In toxoplaxmosis, we often combine two tests.
- Complemet fixing test (CFT). It is performed like other complement fixing tests, see J09 practical
- ELISA, too, is like other ELISA reactions. One specific feature is that instead of IgM + IgG we mostly search for IgA + IgG antibodies. IgA antibodies are typical for recent infections, IgG for „status after“ an infection. Paradoxically, pregnant women with IgG + are in a better situation than IgG – (they are protected).

Parasites – pictures

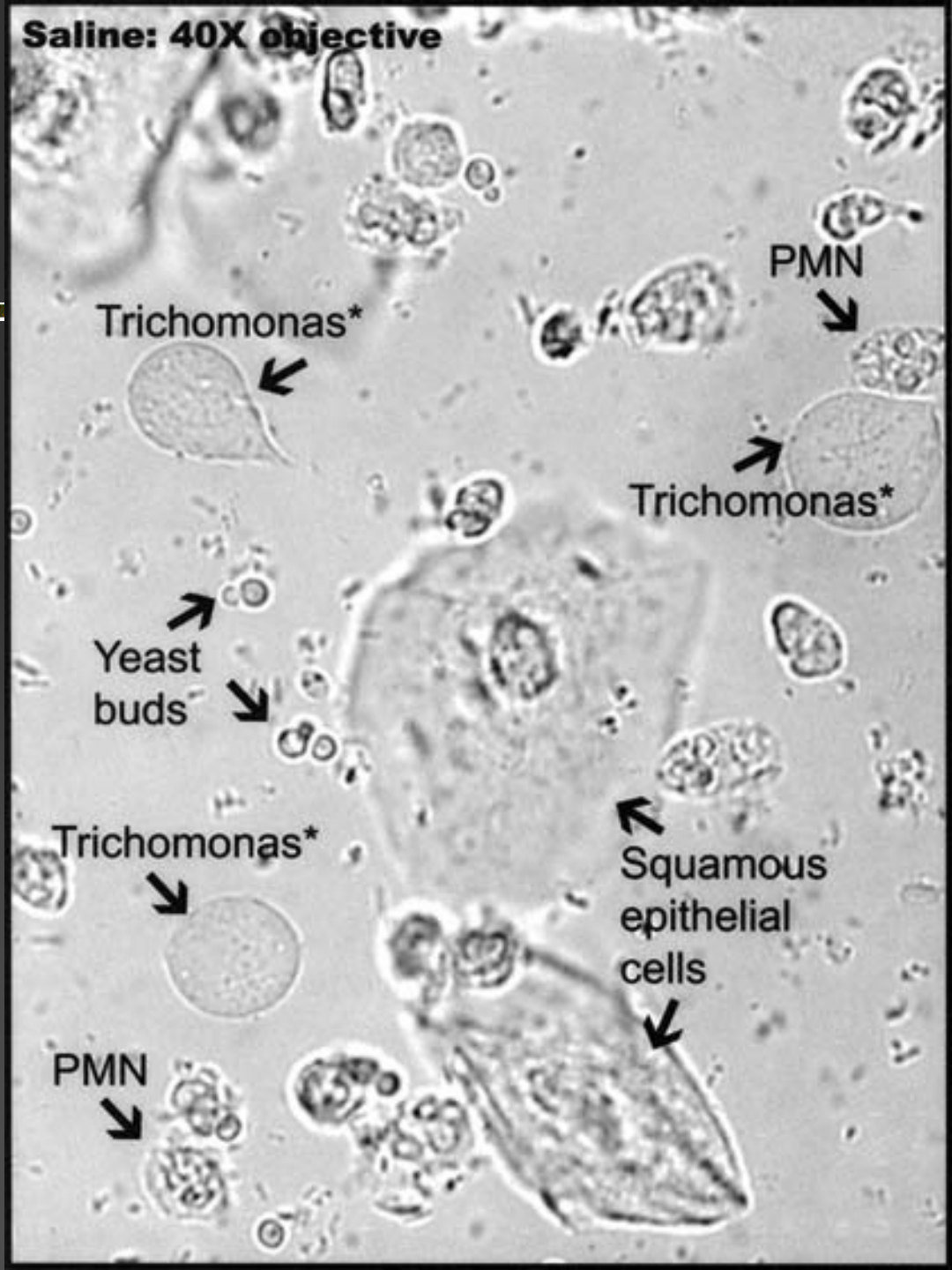
Trichomonas vaginalis

Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA



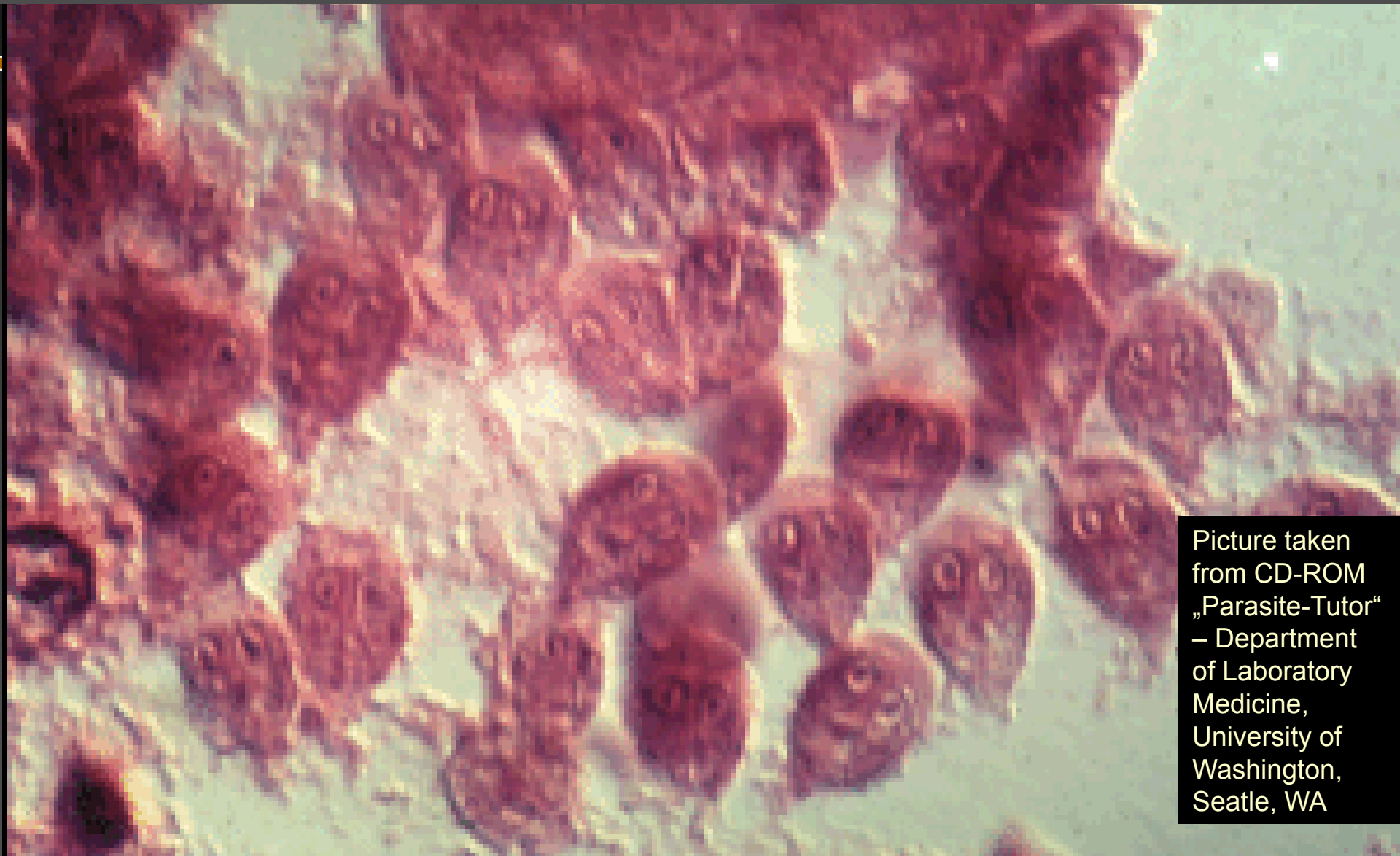


Trichomonas vaginalis



http://www.bu.edu/cme/std/images/slide7-vwp_g420.jpg

Giardia intestinalis (Lamblia) (trophozoites)

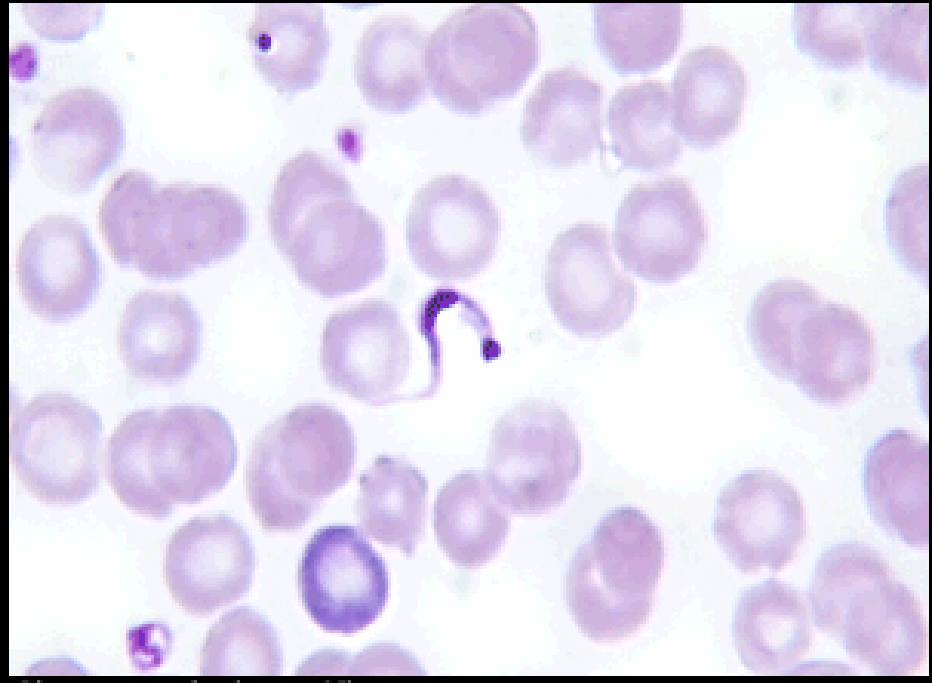


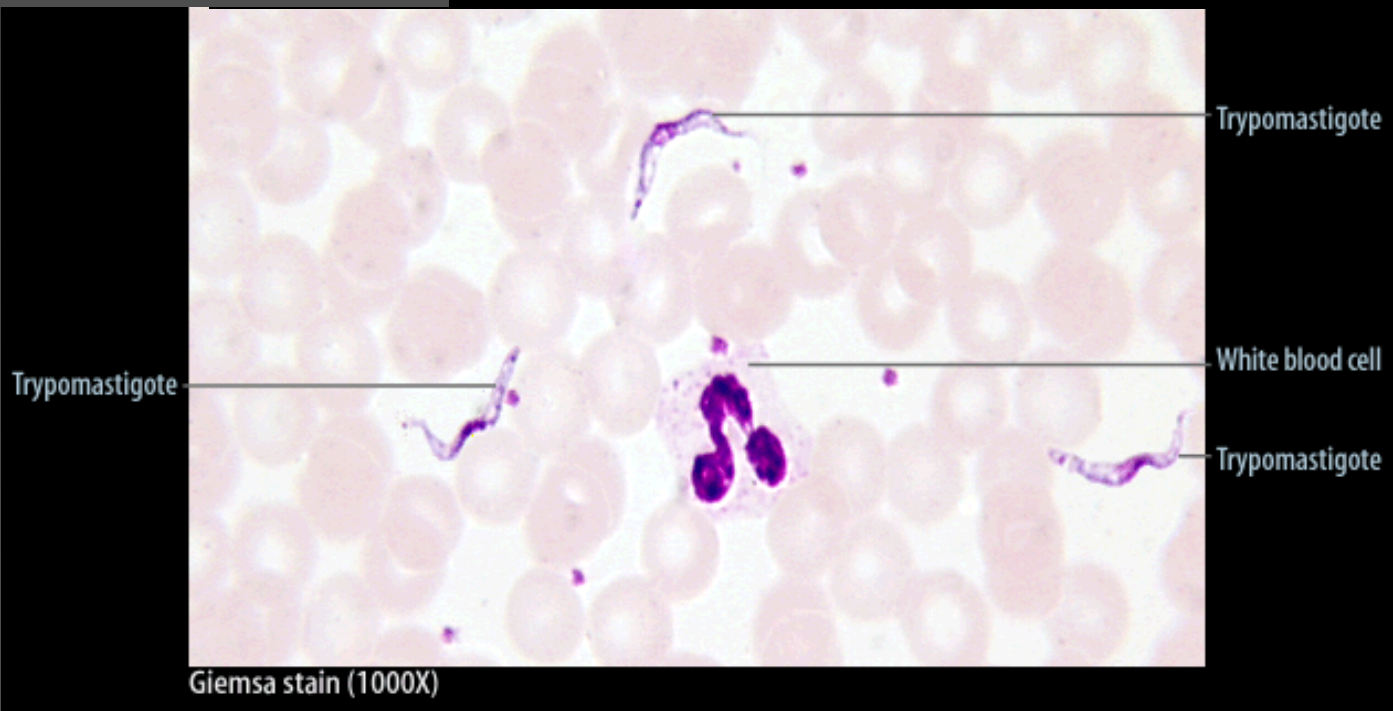
Picture taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

Giardia intestinalis – trophozoites

http://www.smittskyddsinstitutet.se/presstjanst/pressbilder/parasiter/

Trypanosoma cruzi (down), *Trypanosoma brucei* (up)



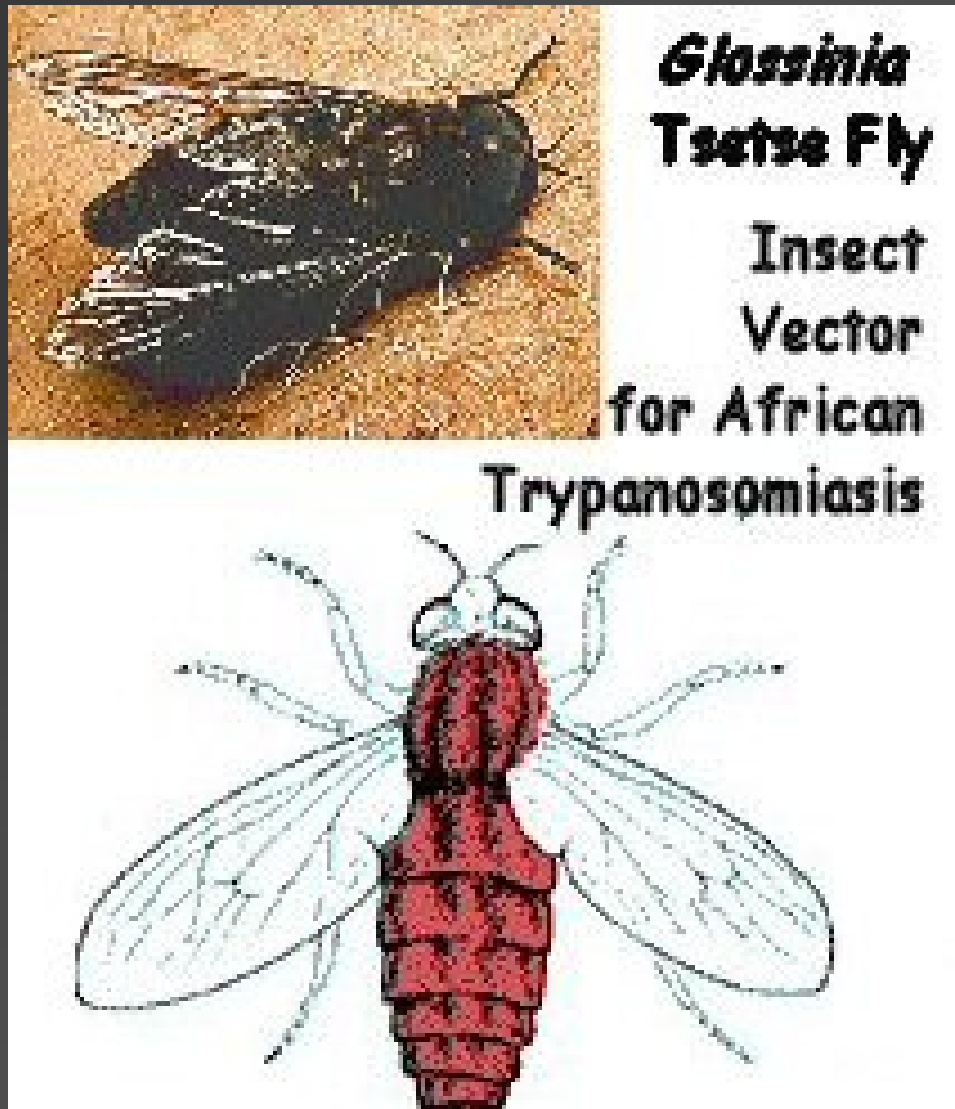


Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

Triatoma sp., vector of Chagas disease

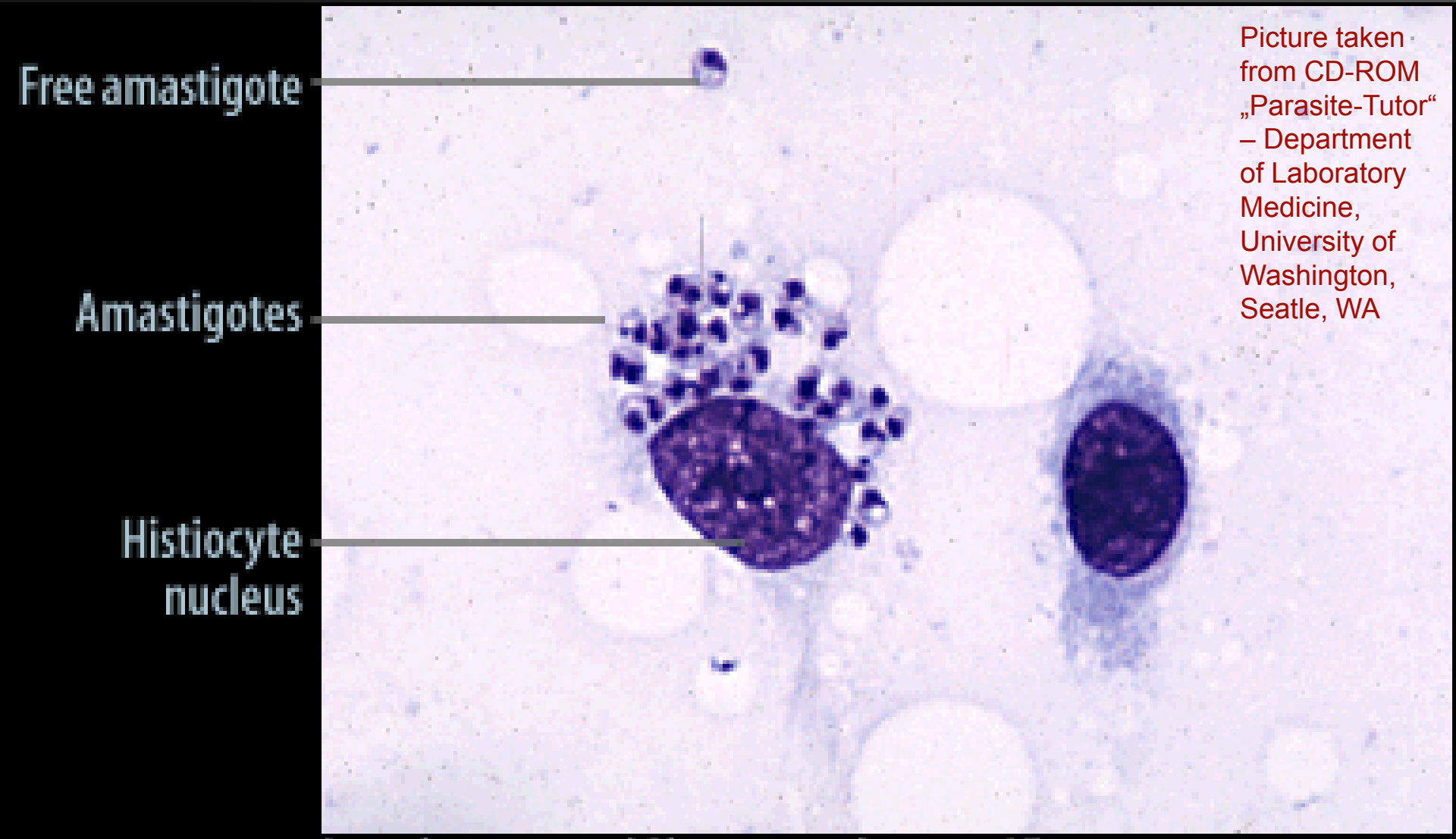
http://web.indstate.edu/thcme/micro/parasitology

Tse-tse *fly (Glossina)*, vector of sleeping disease

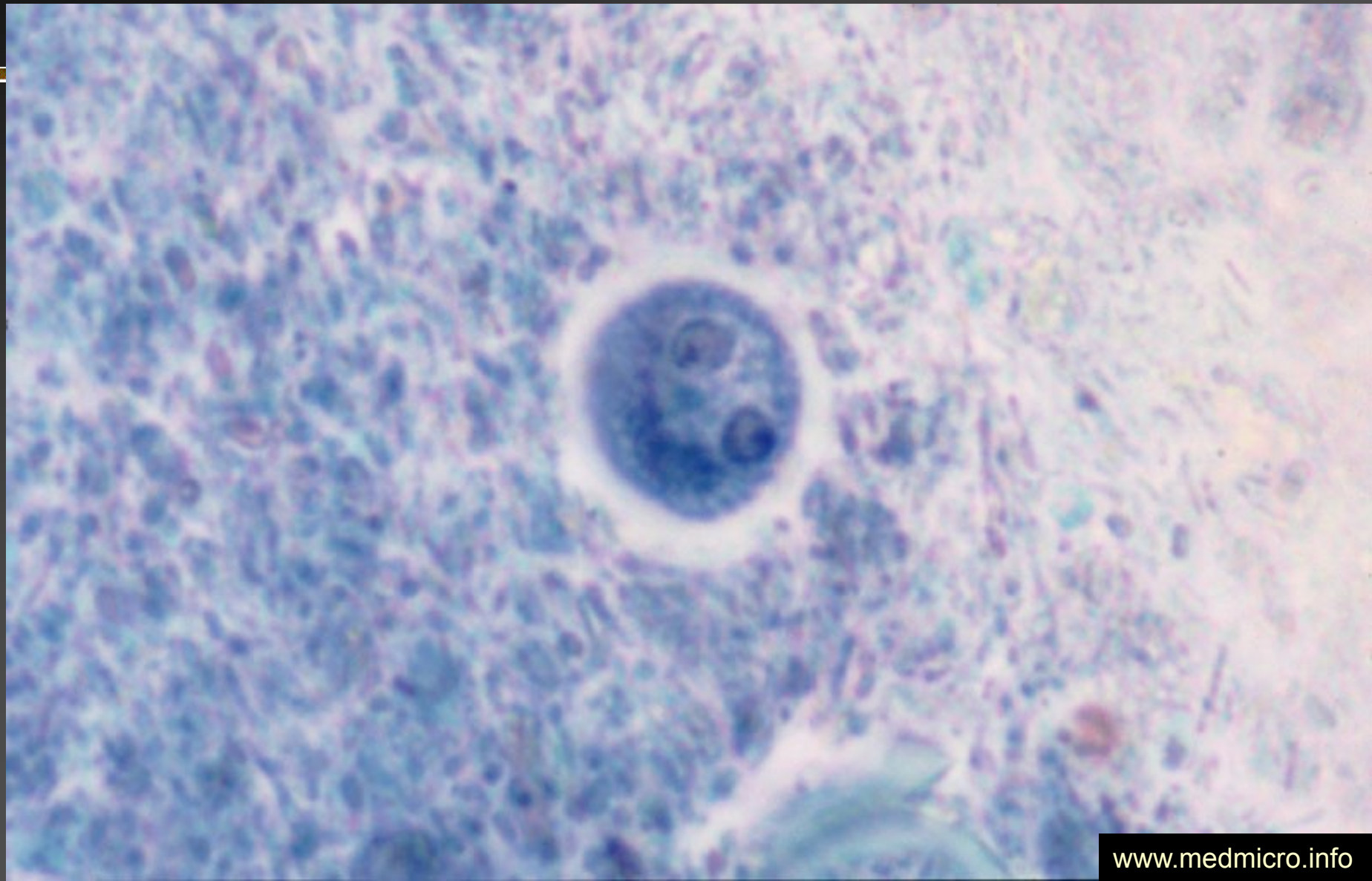


http://web.indstate.edu/thcme/micro/parasitology

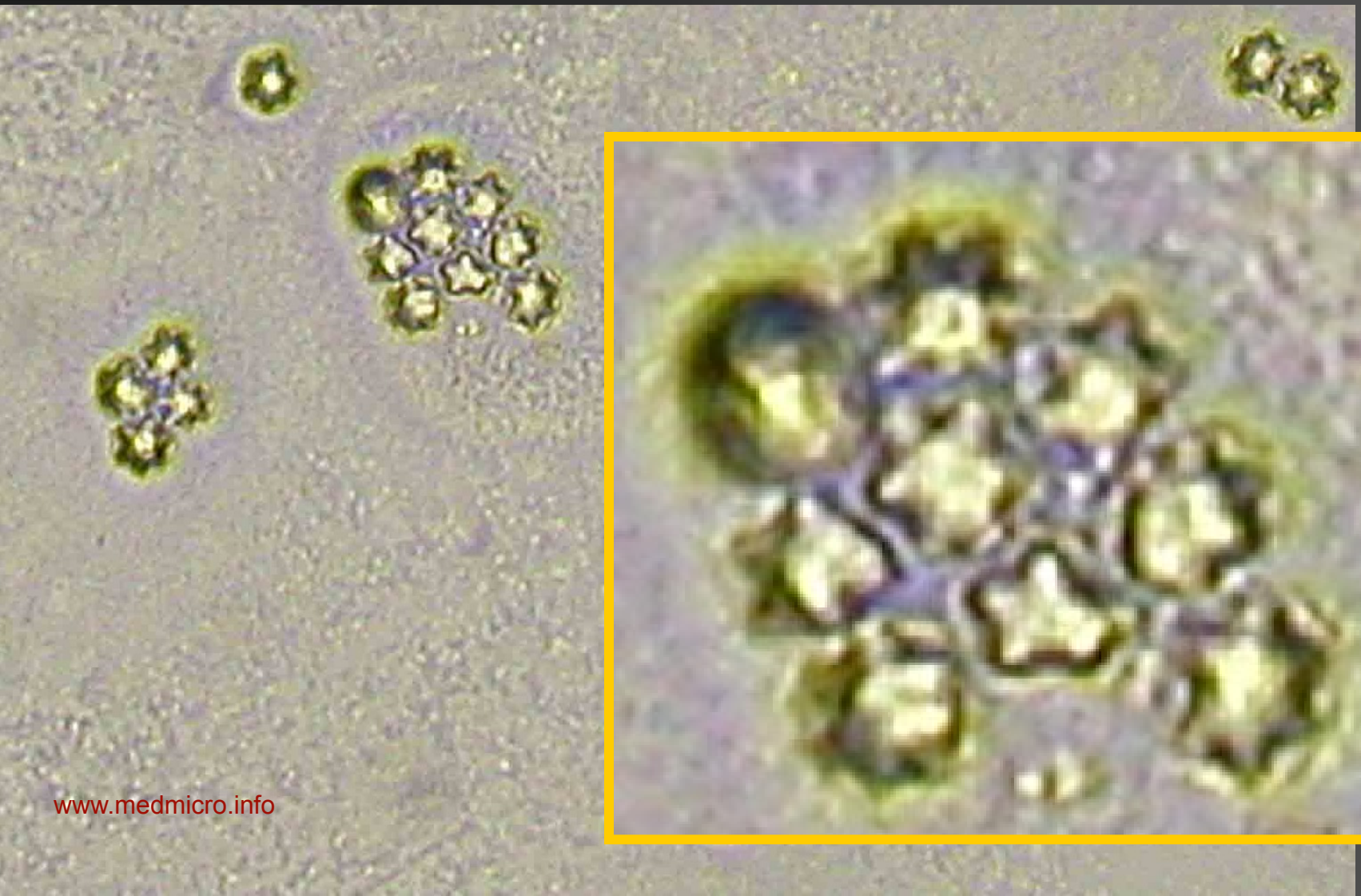
Leishmania sp.



Entamoeba histolytica, haematoxylin

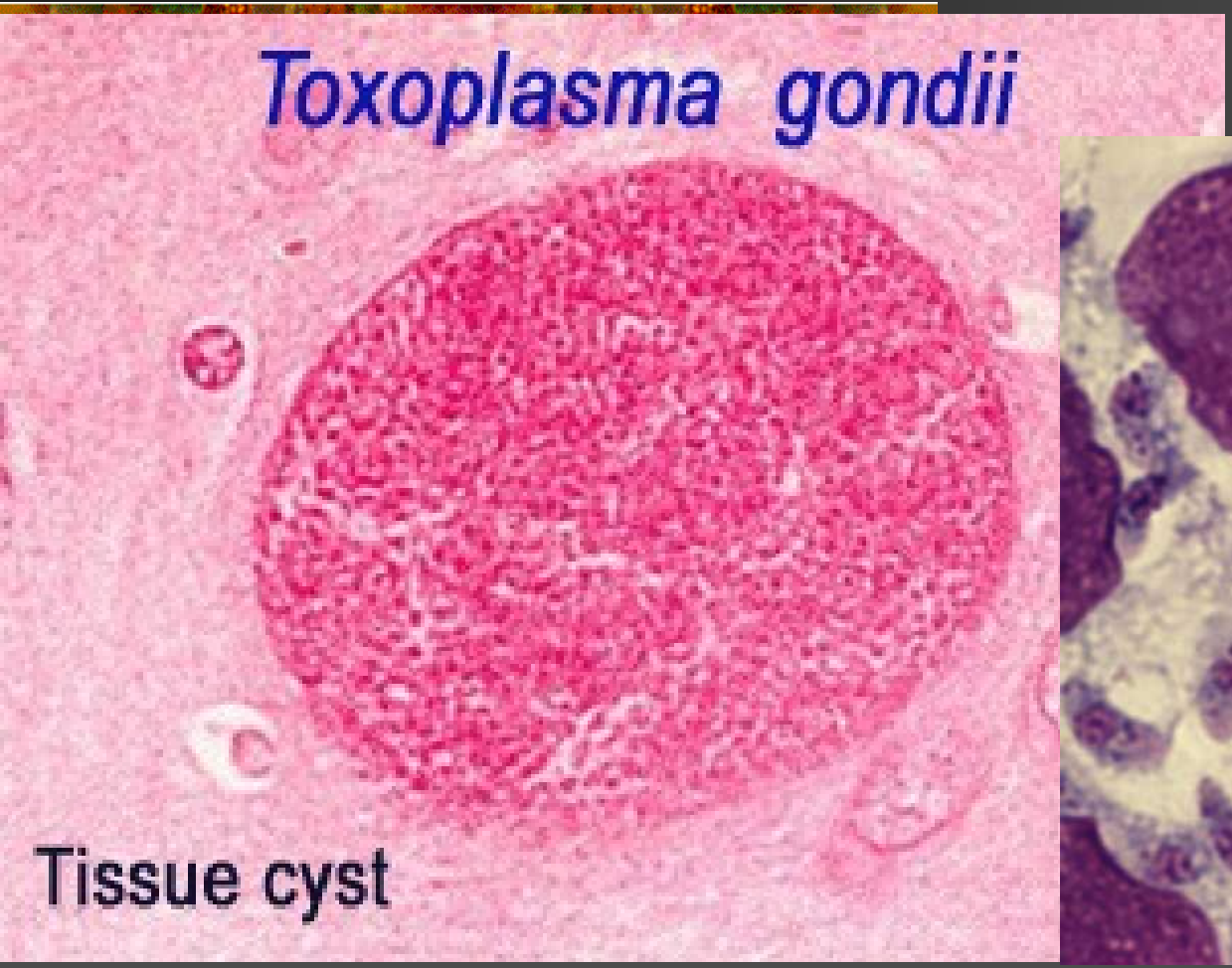


Acantamoeba sp.

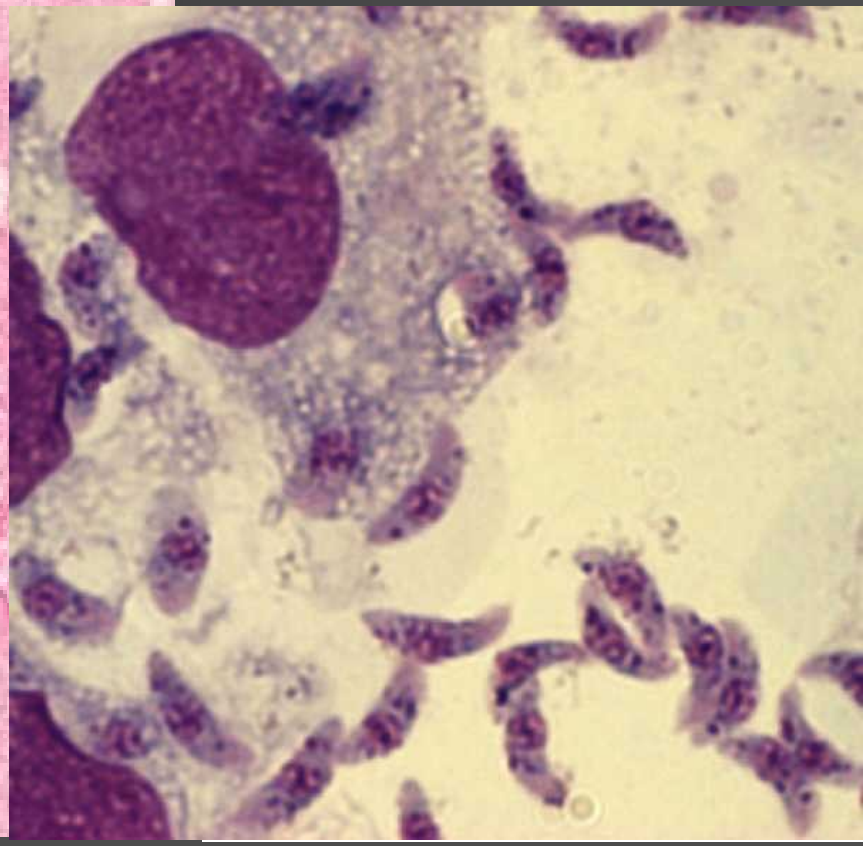


Toxoplasma gondii

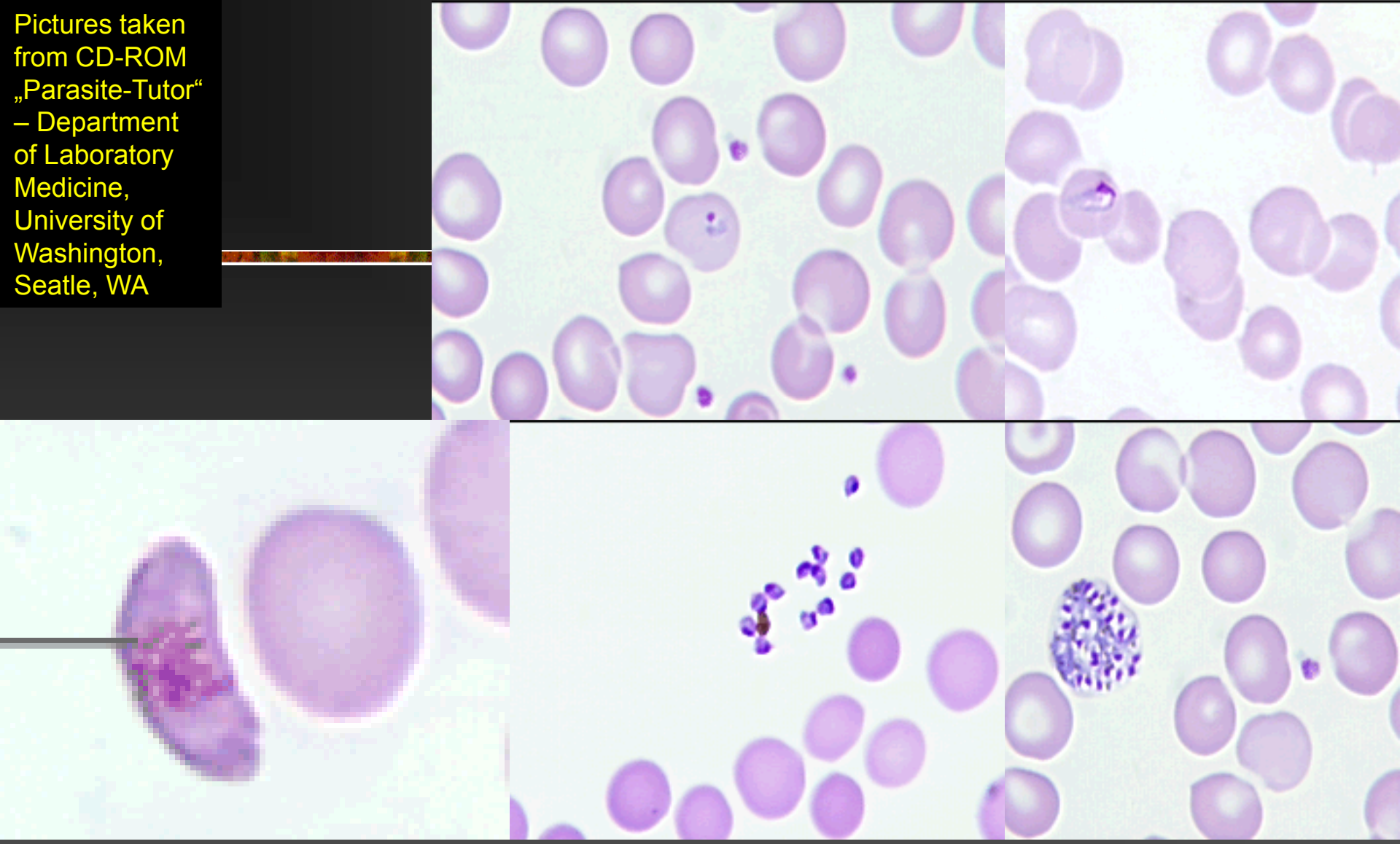
http://webdb.dmsc.moph.go.th/ifc_nih/applications/pics/Toxoplasma.jpg



http://www.smittskyddsinstitutet.se/upload/Analyser/ToxoplasmaSB.jpg



Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA



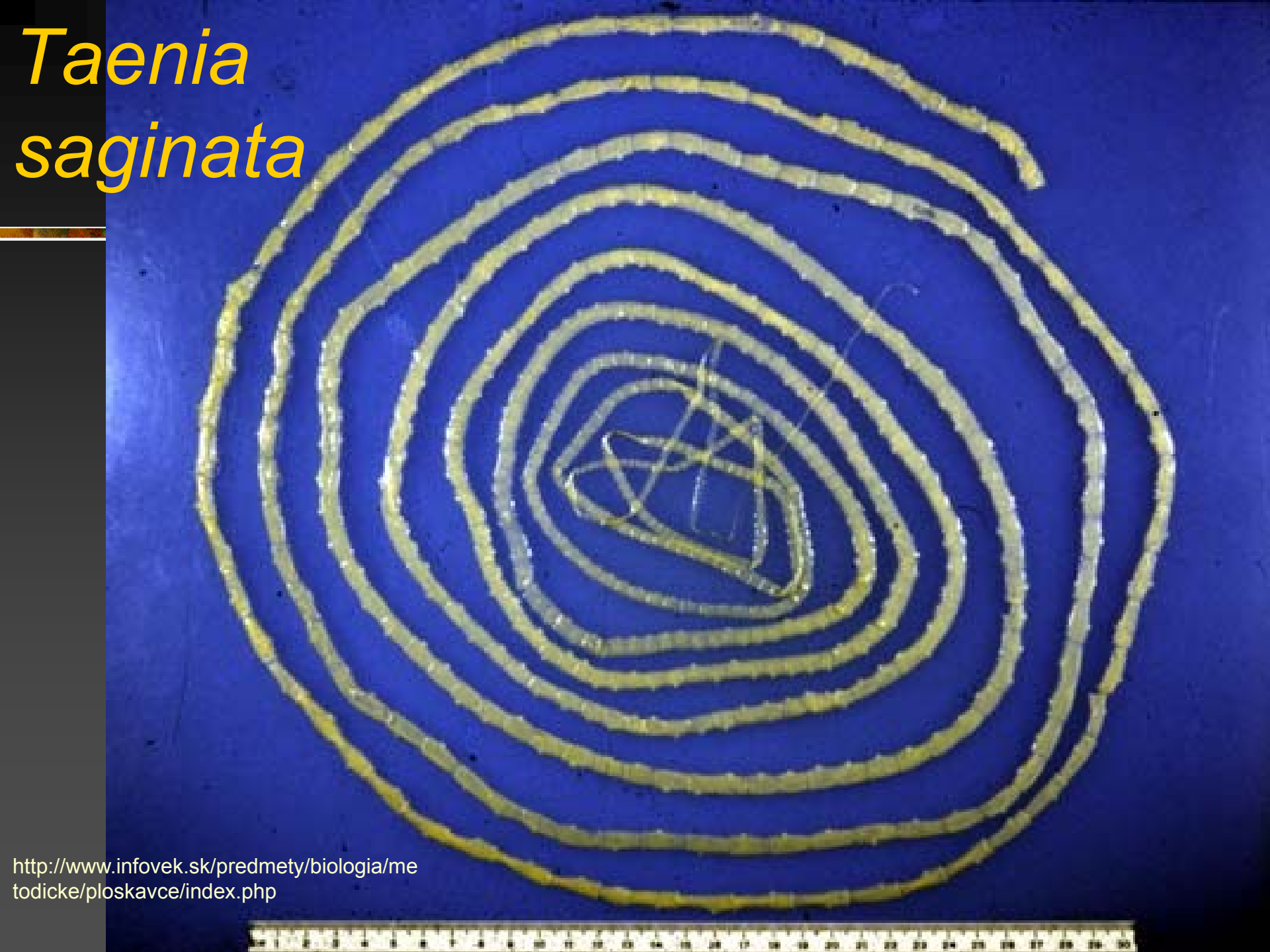
Various development stages of Plasmodia

Anopheles sp., vector of malaria

Anopheles mosquito (female)

Picture taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

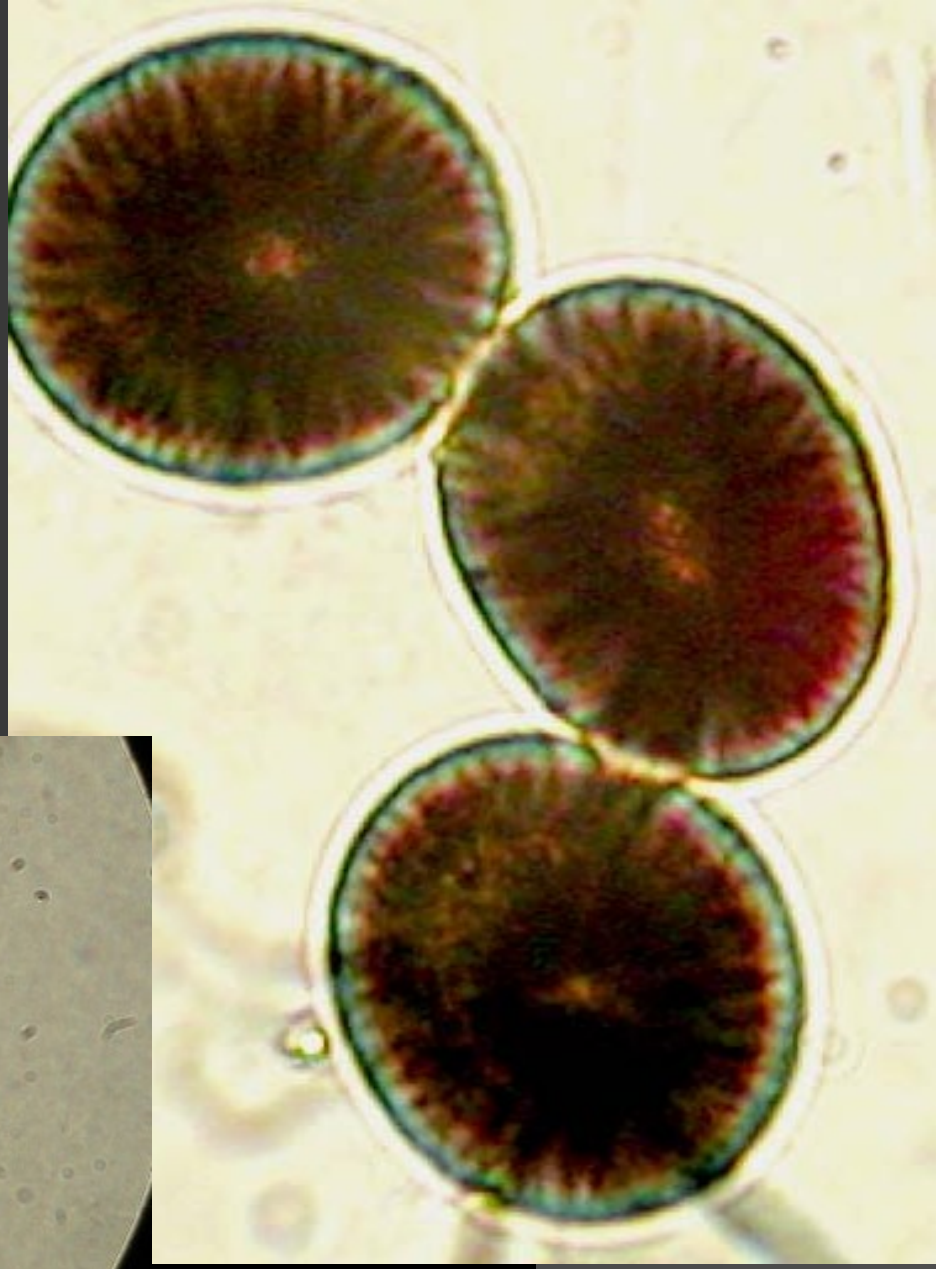
Taenia saginata



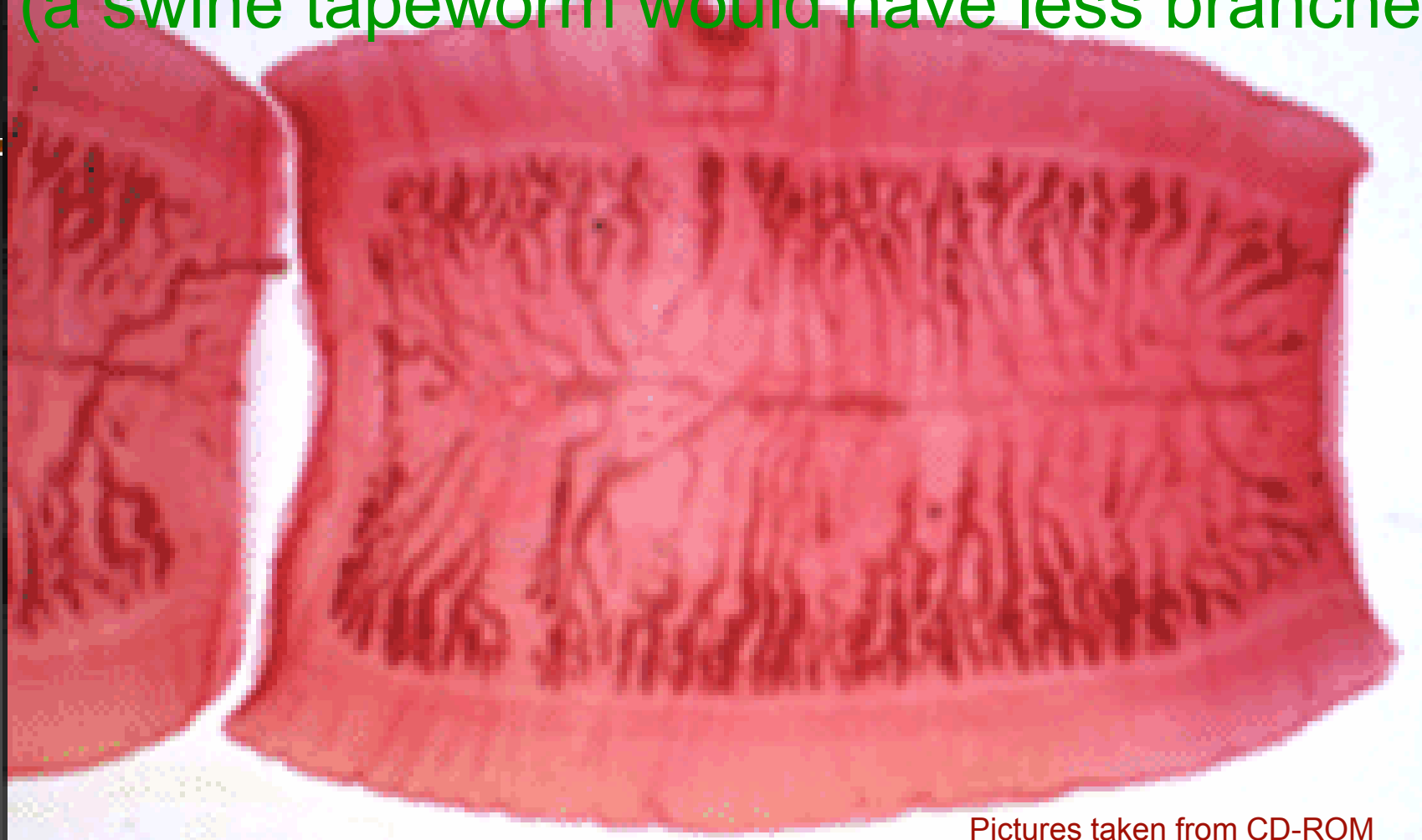
http://www.infovek.sk/predmety/biologia/metodicke/ploskavce/index.php

Eggs of tapeworms

Attention, eggs do not enable discriminate between *T. solium* and *T. saginata*, we would need proglotids for this!



Proglotid of a beef tapeworm
(a swine tapeworm would have less branches)



Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

Taenia saginata gravid proglottid (stained)

Pinworm with eggs

Pictures: Milada Dvořáčková a Ondřej Zahradníček

Comon roundworm eggs

Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA (left) amd www.medmicro.info (right)

Fertile egg (wet mount 400X)

Trichuris trichiura

Picture taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

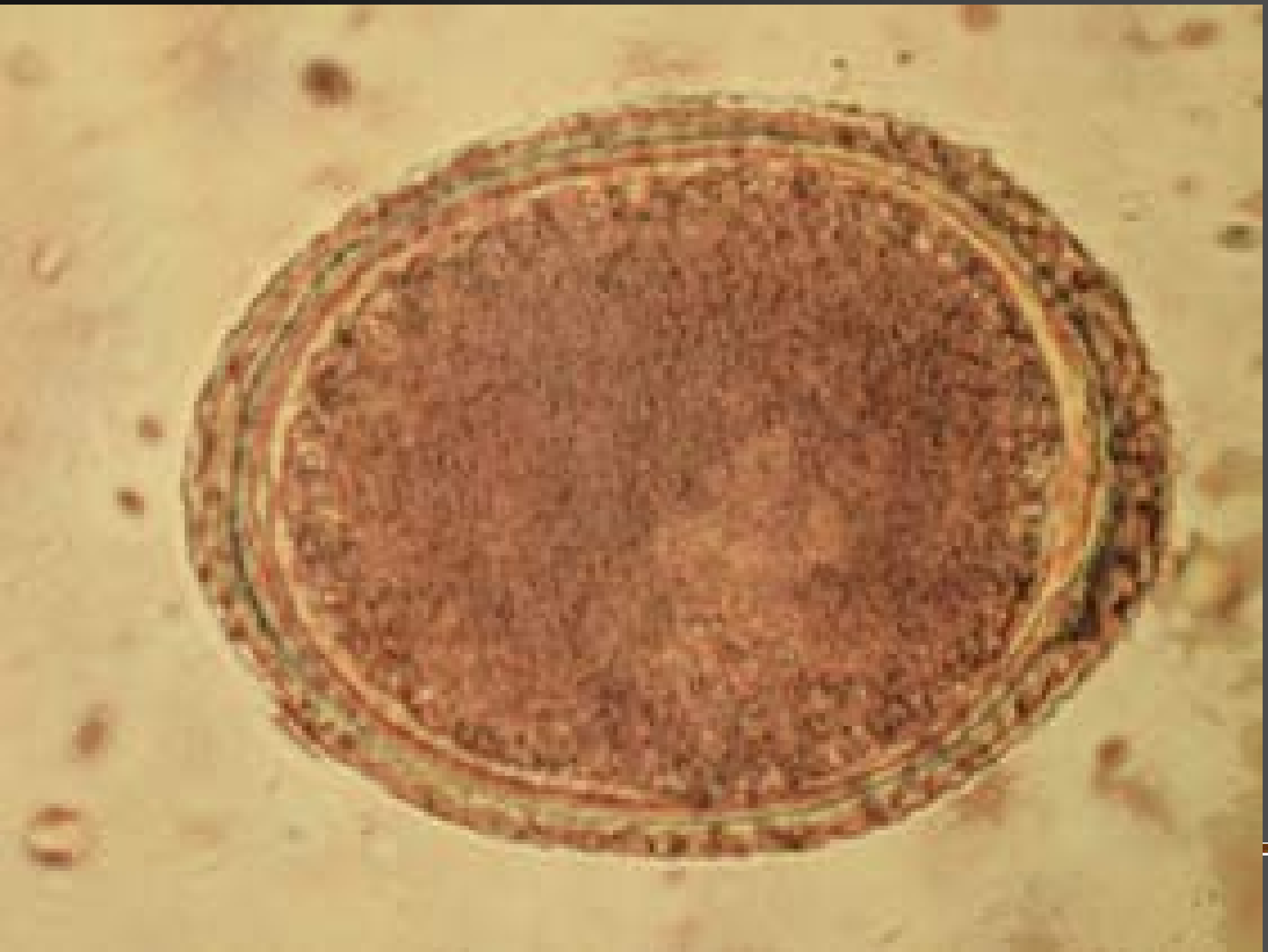
Toxocara canis

http://plpnemweb.ucdavis.edu/Nemaplex/Taxadata/Tcanis.htm

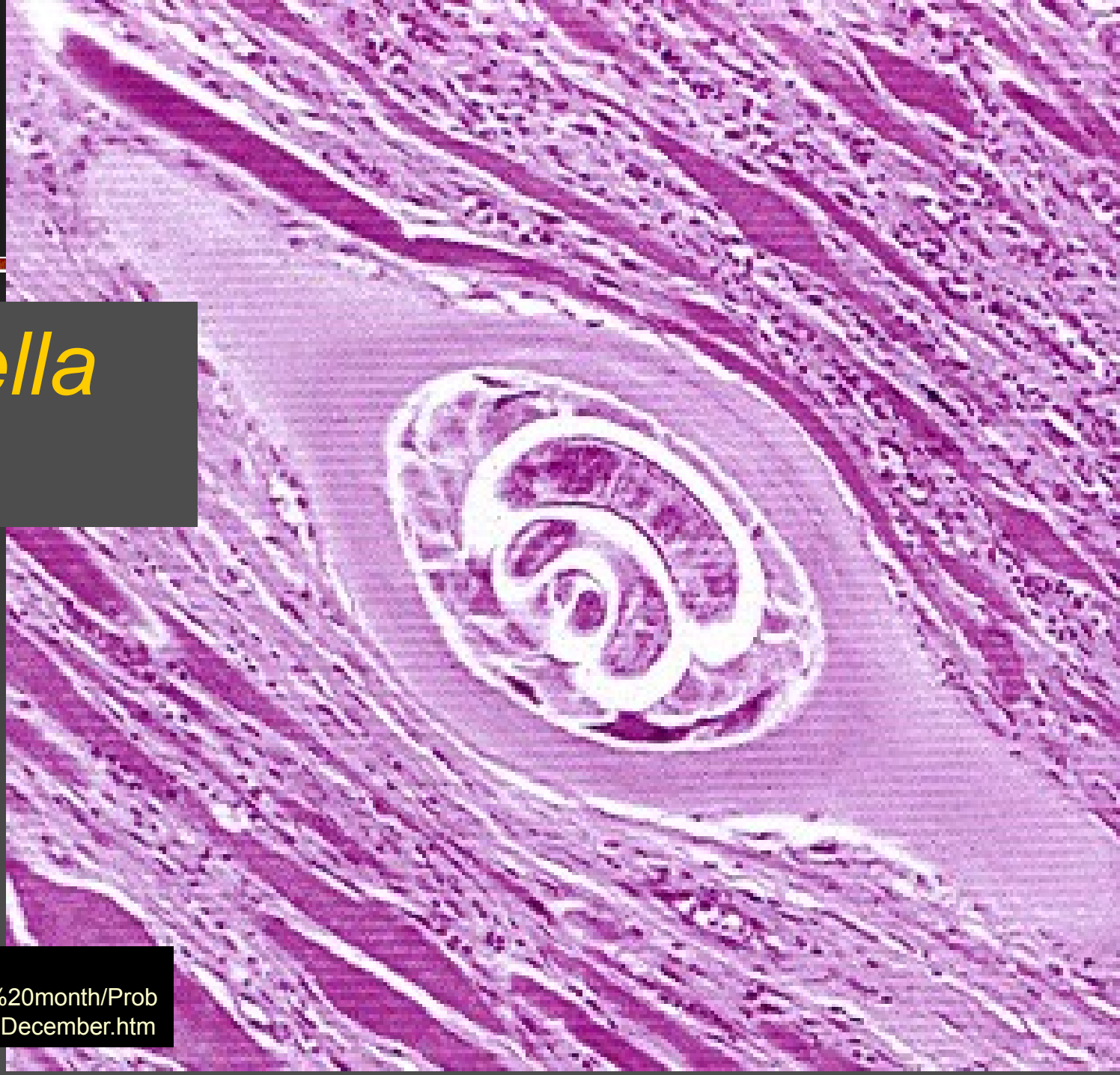
(from Parasite of the Month)

Toxocara canis

http://www.vet-doktor.de/ARCHIV/Gesundheit/Wurmprophylaxe/wurmprophylaxe.html



Trichinella spiralis



http://www.med-chem.com/Para/prob%20of%20month/Prob%20of%20Month%2012%20December.htm

Filariae

A – *Wuchereria bancrofti*

B – *Brugia malayi*

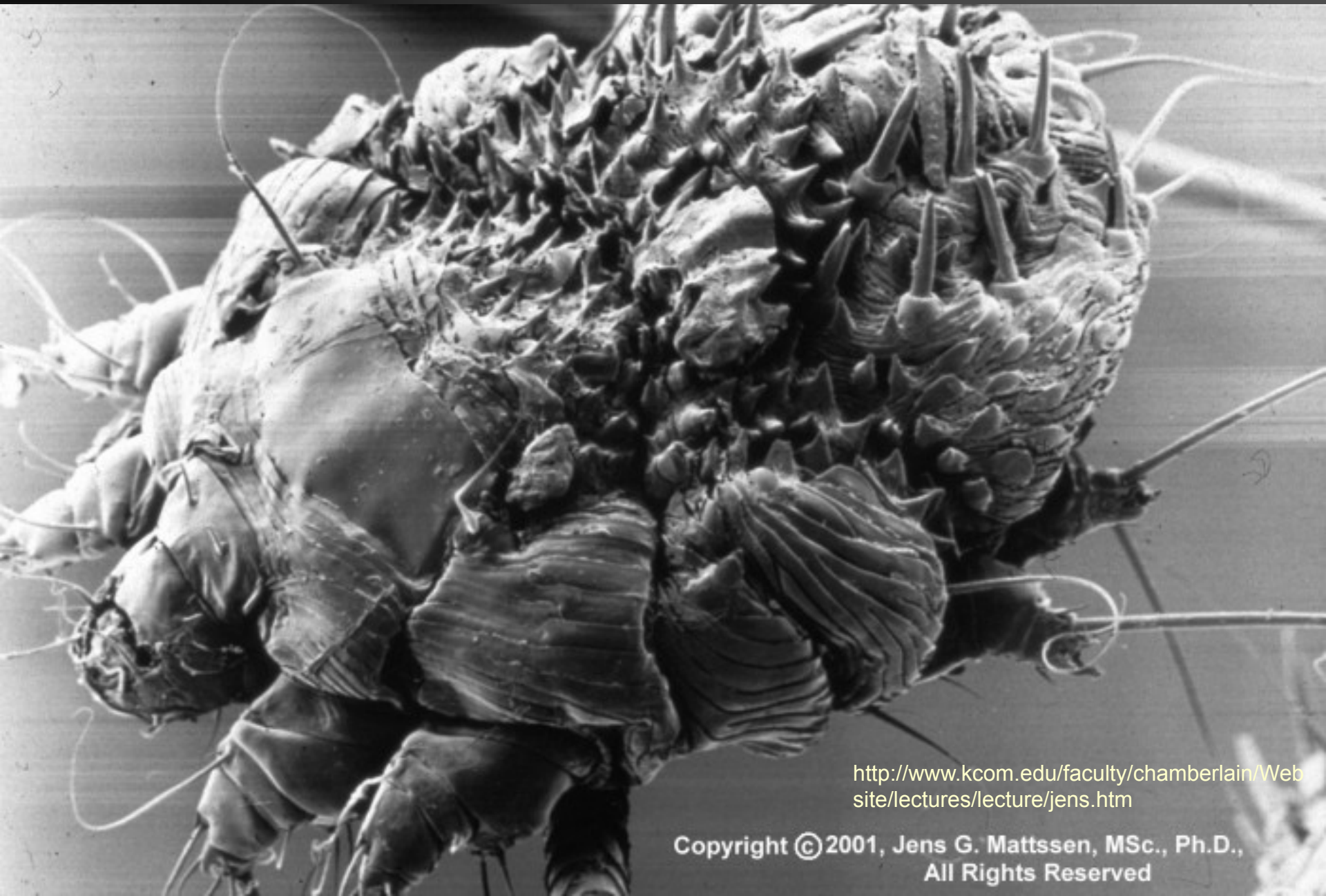
C – *Loa loa*

D – *Mansonella perstans*

E – *Mansonella ozardi*

Pictures taken from CD-ROM „Parasite-Tutor“ – Department of Laboratory Medicine, University of Washington, Seatle, WA

Sarcoptes scabiei



Pubic louse

CDC

http://www.ento.okstate.edu/ddd/insects/pubiclice.htm

Head louse with an egg

http://tn3-2.deviantart.com/300W/fs7.deviantart.com/i/2005/189/1/9/toxoplasma_by_chocko.jpg

The End

Picture on this page –
toxoplasmosis in artistic view